Influence of Organic Matter on the

Bioavailability andToxicity of Nickel to the

Amphipod *Hyalella azteca*

A Thesis Submitted to the College of Graduate Studies
and Research in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in the
Toxicology Graduate Program
University of Saskatchewan
Saskatoon, SK, Canada

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Abstract

Understanding Ni speciation in solution and the partitioning of Ni between solution and solid sediment is important in determining Ni bioavailability and toxicity to *Hyalella azteca* in sediments. Water-only Ni toxicity tests were conducted to evaluate the effects of dissolved organic matter on Ni speciation and bioavailability. Test substances chemically and spectroscopically characterized for use in these tests included Little Bear Lake sediment, peat moss, and Suwannee River humic and fulvic acids. Nickel speciation, bioavailability and toxicity in the presence of dissolved organic matter was assessed via three methods: ion exchange measurements of the free Ni^{2+} ion, mathematical modeling using the Windermere Humic Aqueous Model (WHAM VI), and toxicity testing with *H. azteca*. It was found that the main bioavailable Ni species at the pHs tested (pH 8.10 - 8.33) was the free Ni^{2+} ion. This research also demonstrated that Ni may be significantly complexed, or largely free and labile, depending on the Ni:dissolved organic carbon ratio. Overall, the Ni:dissolved organic carbon ratio plays a greater role than either dissolved organic carbon source or fraction in determining Ni speciation and Ni bioavailability and toxicity to aquatic organisms.

Natural sediment was titrated with Ni under anaerobic conditions to evaluate the partitioning of Ni between solution and solid phase as pH varied (pHs 6, 7, 8). There was a noticeable increase in sediment Ni complexation with increasing pH.

To evaluate the influence of organic matter on Ni bioavailability and toxicity in sediments, 10-d toxicity tests (using *H. azteca*) were conducted with Ni spiked over a range of concentrations in both formulated and field-collected sediments. The total organic carbon content of sediment had a significant influence on Ni bioavailability to *H. azteca*. Formulated sediments with different amounts of organic carbon displayed a clear decrease in toxicity

with increasing organic carbon content at the same total Ni concentration. Results from both the formulated and natural sediment tests further indicated that toxicity was strongly correlated with pore-water Ni concentration, and that toxicity estimates based on pore-water Ni exposures were comparable to separate toxicity estimates for Ni in water-only tests. While excess acid-volatile sulfide in sediment appeared to predict the absence of acute Ni toxicity, it did not predict the absence of Ni bioaccumulation. This was potentially due to the presence of multiple pore-water Ni species (i.e., Ni^{2+}, $NiHS^+$) which were bioavailable in the sediment micro-environment of *H. azteca*.

Acknowledgements

First and foremost, I wish to thank my supervisor, Karsten Liber, for what has been a wonderful opportunity and grand adventure. Thank you for you for being my mentor, guide, and comrade.

I would like to express my gratitude to my advisory committee, Dr. Barry Blakley, Dr. P. M. Huang, Dr. Don Hamilton, and Dr. Hans Peterson, for their contributions during the course of my degree. I would also like to acknowledge my external examiner, Dr. David Mount, for his involvement and contribution to the thesis.

While I am grateful to a variety of people who have in some way provided me with technical information or assistance, special thanks go to Dr. Claude Fortin and Dr. Peter Campbell for the opportunity to study their ion exchange technique at INRS-Eau, University of Quebec. A special thank you also goes out to Barry Goetz (Agriculture Canada) who always had time to mentor me in Atomic Absorption Spectroscopy techniques no matter how many things he had on the burner (literally). Particular thanks to Ron DeVos for assistance during his Toxicology internship in 2002. Ron, you do Holland proud.

To my fellow students in the Toxicology program, I wish you all the best and thank you for your fellowship. A little frisbee goes a long way during trying times.

Finally, I would like to thank my family and close friends for their patience and encouragement. Without the support of my parents, Harold and Cora, brothers, Ian and Eric, sister, Karen, and beloved Uncle, Norris (Bud) Lindgren, I could not and would not have made it. A special thank you goes out to Kerrie Serben for not only field and laboratory assistance but for her patience, love, and moral support over the years.

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List of Abbreviations

AVS	acid-volatile sulfide
BLM	biotic ligand model
DOC	dissolved organic carbon
DOM	dissolved organic matter
EDTA	ethylenediaminetetraacetic acid
FA	fulvic acid
FTIR	Fourier transform infrared
HA	humic acid
IR	infrared
LBL FA	Little Bear Lake fulvic acid
LBL HA	Little Bear Lake humic acid
LC50	median lethal concentration
LOEC	lowest observed effect concentration
MATC	maximum acceptable toxicant concentration
NMR	nuclear magnetic resonance
NOEC	no observed effect concentration
OC	organic carbon
OM	organic matter
PWQC	pore-water quality criteria
SEM	simultaneously extracted metal
SQGs	sediment quality guidelines
SQC	sediment quality criteria
SR FA	Suwannee River fulvic acid
SR HA	Suwannee River humic acid
UV/VIS	ultraviolet/visible

Preface

This thesis describes the effects of organic matter on nickel (Ni) bioavailability and toxicity to *Hyalella azteca*, a common freshwater crustacean, and the methodologies used. Currently, one chapter has been published and six others are in the process of submission for publication in scientific journals. Some repetition of introductory material was therefore unavoidable.

Chapter 1 reviews background information, hypotheses, and objectives. Chapter 2 describes the validation of pore-water samplers (mini-peepers) designed for use in sediment toxicity tests (described in Chapter 8). Chapters 3 describes the isolation, fractionation, and characterization of dissolved organic matter test substances destined for use in water-only Ni tests (surrogates for pore-water exposures) which evaluated Ni speciation (Chapter 4) and Ni bioavailability and toxicity (Chapter 5) to *H. azteca*. Both the speciation and bioavailability data presented in Chapters 4 and 5 are derived from the same water-only Ni tests. Chapter 6 describes the titration of natural sediment with Ni for use in modeling predicted effects versus the actual toxic effects seen in formulated and natural sediment toxicity tests (Chapter 8). Chapter 7 describes a non-invasive sediment sampler (a mini-corer designed for use in sediment toxicity tests, Chapter 8) which is complementary to the mini-peeper described in Chapter 2.

CHAPTER 1

Introduction

1.1   Background

Sediments play a major role in most aquatic ecosystems. From single celled bacteria and algae to more complex organisms such as invertebrates and fish, a variety of plant and animal taxa depend upon aquatic sediment for habitat. It is therefore important that we develop the tools necessary for the protection of this resource.

While a variety of physical, chemical, and biological processes occur within sediment, its ability to accumulate trace-metals is of particular interest to government agencies and departments interested in developing guidelines for the protection of sediment quality (and hence environmental integrity). Whether contamination is from natural or anthropogenic sources, trace-metal concentrations in sediment may reach several orders of magnitude greater than that of the overlying water (Burton, 1991). Trace metals may concentrate to such a degree that sediments become toxic to benthic or epibenthic fauna. Concern over sediment contamination, and the associated need for monitoring, are the driving forces behind the development of sediment quality guidelines (U.S. EPA, 1994) or criteria which are technically defensible and have broad applicability (Ankley et al., 1996). These criteria are intended for use in preventing contamination of clean sediments and to aid in making regulatory and remediation decisions concerning already contaminated sediments (U.S. EPA, 1994). While water quality guidelines for metals have been in place for a

number of years, the development of sediment quality criteria (SQC) or guidelines (SQGs) is more recent. Essential to their development is the need to determine, for a given sediment, what concentration of metal will adversely affect the associated biota.

In evaluating metal partitioning in sediment, sediment amorphous sulfide content is thought to represent the main metal-binding phase for class B and borderline metals (Nieboer and Richardson, 1980) such that only metal in excess of reactive sulfide concentrations is thought to be bioavailable to the associated biota (U.S. EPA, 1994). This research studied the role of organic matter (in addition to AVS) in modifying the nickel (Ni) bioavailability and toxicity to *Hyalella azteca* (a common freshwater amphipod) in sediment. Relatively little is known about Ni complexation with sedimentary humic substances or about Ni bioavailability and toxicity in freshwater sediment.

1.2   Nickel

1.2.1   Sources (anthropogenic)

Nickel, which comprises approximately 0.008% of the earth's crust, is ubiquitous in soils and surface waters (National Academy of Sciences, 1975). Dissolved Ni in surface water is generally the result of the dissolution of primary bedrock materials, the deposition of particulate matter in rainwater, or the leaching of secondary soil phases (Boyle, 1981). North American background values of dissolved surface-water Ni are generally low and range from less than 1 to 10 µg/L (Stokes, 1981; Nriagu et al., 1996a). Elevated Ni concentrations in surface waters may also result from a variety of anthropogenic sources which include mining activities, smelting and refining, metal plating and manufacturing, nickel-cadmium battery disposal, and fossil-fuel refining and combustion (National Academy

of Sciences, 1975; Stokes, 1981; Nriagu and Pacyna, 1988). For example, Ni deposition from long-term smelting activity in the Sudbury area, Canada, has led to the elevation of Ni in the waters and sediments of some nearby lakes (Carignan and Nriagu, 1985). As a result of leaching from the surrounding metal-saturated soil, it is believed that elevated Ni levels in these watersheds will persist far into the future (Nriagu et al., 1996b). In northern Saskatchewan, Canada, Ni co-occurs in uranium deposits (Dahlkamp, 1993) and, as a result, Ni may be present in the near-field zone downstream of mine effluent or dewatering discharges (Cameco et al., 1995), or pose long-term water quality issues in flooded waste-rock storage pits (Dr. Richard Neal, pers. com.) such as those in the Key Lake area, Saskatchewan.

1.2.2   General aqueous Ni speciation

The divalent Ni^{2+} ion and its compounds predominate Ni speciation in most aqueous solutions (Latimer, 1952; Morel et al., 1973; Baes and Mesmer, 1976). Equilibrium computations by Morel et al. (1973) and measurements by authors such as Mandal et al. (2002) have shown that the free ion dominates Ni speciation in aerobic freshwaters in the pH range of 5 to 9. Naturally occurring inorganic ligands (e.g., CO_3^{2-}, OH^-, SO_4^{2-}, Cl^-) complex with Ni to a minor degree relative to the free ion concentration (Morel et al.,1973; Mandal et al., 2002).

Under anoxic aqueous conditions, Ni, similar to other metals having class B or borderline character (e.g., Cd, Cu, Pb, Zn; based on the classification of Nieboer and Richardson, 1980), is thought to readily form the relatively insoluble pure metal sulfide which readily precipitates from solution (e.g., Boulegue et al., 1982; Emerson et al., 1983;

Morse et al., 1987; Di Toro et al., 1991). Previous studies involving either anoxic saline waters (Jacobs et al., 1985), sulfide-rich ground water (Boulegue, 1977), marine sediment pore waters (Brooks et al., 1968; Presley et al., 1972), and formulated marine sediments (Oakley et al., 1980), have all found levels of dissolved class B and/or borderline metals to be well above those expected assuming an equilibrium between the precipitated pure metal sulfides and the dissolved species. Brooks et al. (1968) concluded from thermodynamic calculations that none of the metals considered (Ni included) could have existed in pore-water solution at the concentrations measured if they were bound as simple sulfides. Similarly, Oakley et al. (1980) found that the concentrations of dissolved metals measured in metal-spiked (Cu, Cd, Pb and Zn) formulated marine sediments were much higher than expected, assuming that pure metal sulfides were controlling metal solubility. As well, Jacobs et al. (1985) concluded that the observed metal profiles of Mn, Ni, Cu, Zn and Cd, across the O_2/H_2S interface in a marine basin were not the result of equilibrium between pure metal sulfide and the respective dissolved species. Authors have suggested that the formation of metal bisulfides and/or polysulfides may increase the solubility of metals beyond what the solubility product would indicate (Hemley, 1953; Krauskopf, 1956; Gardner, 1974). It was recently found that, for Ni, this may be due to the presence of bisulfide (HS^+), which complexes Ni to form $NiHS^+$ in anoxic seawater solutions (Luther et al., 1996).

It is hypothesized that metal enrichments in the interstitial waters may also be due to organic complexation (Nissenbaum and Swaine, 1976; Presley et al., 1972; Krom and Sholkovitz, 1978) which acts to dissolve or leach mineral phases. While oxygen containing functional groups are considered to be important in metal complexation, the high

concentration of nitrogen and sulfur in marine humic substances may greatly contribute towards the binding of trace metals (Nissenbaum and Swaine, 1976). Overall, while there is some debate as to the relative importance of organic versus inorganic mobilization of metals under anoxic conditions, inorganic sulfide ligands are thought to be of greater importance (Morse et al., 1987).

1.2.3   Aquatic toxicity

1.2.3.1 Bioavailability

While metal toxicity generally increases with increasing total dissolved metal concentration, for divalent cationic metals in aqueous solution the free metal ion is believed to represent the major bioavailable species and hence determine metal toxicity (Campbell, 1995; Morel, 1983). This does not mean that M^{2+} divalent cation is the only bioavailable form (e.g., MOH^{n-1} or MCl^{n-1} may also be bioavailable), but that ligand-complexed fractions (such as those bound to DOM) are less bioavailable relative to the free ion and therefore of less toxicological significance (Ankley et al., 1996). In reviewing the Free Ion Activity Model by Morel (1983), Campbell states that "In a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal. It is this reactivity that determines the extent of the metal's reactions with surface cellular sites, and hence its 'bioavailability'." Therefore, changes to metal speciation (i.e., the free ion concentration) can dramatically alter metal bioavailability and toxicity to aquatic organisms. Dissolved organic matter (DOM) is known to significantly affect the speciation of a number of divalent cationic metals (Hollis et al., 1997; Playle et al., 1993a; Town and Filella, 2002). While DOM is known to influence Ni speciation (e.g., Schnitzer and Skinner, 1967), the effect of DOM on Ni

bioavailability has been sparsely studied to date.

In addition to DOM, other water quality characteristics may modify Ni bioavailability. Increasing hardness (Ca^{2+} and Mg^{2+}) has been shown to reduce metal toxicity to aquatic organisms. For example, the sensitivity of *D. magna* to Ni appears to be strongly dependent on hardness, with no observed effect concentrations (NOECs) ranging from 10, 101, and 220 µg/L at water hardnesses of 51, 105, and 205 mg/L, respectively (U.S. EPA, 1986). Similarly, *C. dubia* 48-h Ni LC50s (median lethal concentrations) ranged from 81 to 400 µg/L with toxicity decreasing with hardness increasing from 50 to 253 mg/L as $CaCO_3$, respectively (Keithly et al., 2004). However, no hardness-dependent effect was noted on either *C. dubia* survival or reproduction under chronic Ni exposure conditions. The 96-h Ni LC50 for larval fathead minnows (*Pimephales promelas*) was found to increase from 0.45 mg/L to 2.27 mg/L as hardness was increased from 20 to 140 mg/L (Pyle et al., 2002). Cations such as Ca^{2+} and Mg^{2+} are believed to compete with trace metals (such as Ni) for uptake sites on aquatic organism respiratory surfaces. At lower pH values, H^+ cations are also thought to directly compete with trace metals for gill ligands. For example, Schubauer-Berigan et al. (1993) found the 48-h LC50 for 7-14-d-old *H. azteca* decreased from 2000 to 890 µg/L with increasing pH (6.3 to 8.3, respectively) at a relatively high hardness (300-320 mg/L as $CaCO_3$). The bioavailability and toxicity of a metal may also be altered by pH due to a direct influence on metal speciation.

Studies evaluating the above modifying factors of metal bioavailability and toxicity will ideally lead to the future development of a biotic ligand model (BLM) for Ni toxicity, similar to those BLMs being developed for other metals (see review by Paquin et al., 2002). In the BLM chemical equilibrium modeling predicts the concentration of a metal at the site

of action (the biotic ligand) and this concentration is in turn related to an acute toxicological response. This method of modeling not only takes metal speciation into account (such as complexation with inorganic and organic ligands) but also modifying factors such as cation competition (Ca^{2+}, Mg^{2+}, H^+) for binding sites on the biotic ligand (presumably the gills). If binding can be linked to uptake and hence total body burdens, a BLM may be able to predict, for a given total dissolved-Ni exposure, Ni body burden concentrations which are demonstrated to be predictive of organism health or impairment.

1.2.3.2 Freshwater toxicity

Summaries of Ni toxicity for a variety of aquatic organisms have previously been compiled in the literature (e.g., U.S. EPA, 1986; Keithly et al., 2004, as hardness normalized data). Generally, organism sensitivity can range from quite high (e.g., 81 µg/L; *C. dubia*; 48-h LC50; Keithly et al., 2004) to rather low for (e.g., 75.96 mg/L; *Hexagenia spp.*; 96-h LC50; Milani et al., 2003) depending upon the test organism and exposure conditions. Recently, Ni toxicity to *Hyalella azteca* has become more intensely studied. Keithly et al. (2004) found the *H. azteca* 96-h Ni LC50 to be 3.01 mg/L in water-only exposures at pH 7.7 - 8.0 and a hardness of 91 - 98 mg/L mg/L. Milani et al. (2003) found the 96-h Ni LC50 value of 3.60 mg/L at pH 7.5 - 8.5 and a hardness of 120 - 140 mg/L. Under sublethal exposures, *H. azteca* was found to be rather sensitive to Ni toxicity with a 14-d EC20 endpoint of 61µg/L and the NOEC and LOEC at 29 µg/L and 58 µg/L, respectively, at pH 8.0 - 8.3 and a hardness of 91 - 98 mg/L (Keithly et al. 2004). Ankley et al. (1991) found the 10-d LC50 for *H. azteca* to be 780 µg/L in water-only exposures (alkalinity, 45-46 mg/L as $CaCO_3$; hardness, 44-47 as CaCO3, pH 6.7-7.4), but commented that Ni was less toxic

in sediment pore-waters and speculated that this might have been due to the presence of modifying factors such as "hardness, dissolved organic carbon, etc." Longer-term spiked-sediment exposures by Milani et al. (2003) found the pore-water 28-d Ni LC50 for *H. azteca* to be 0.27 mg/L, the LC25 to be 0.17 mg/L, and the IC25 (inhibition concentration, 25%) for growth to be 0.11 mg/L.

Borgmann et al. (2001) and Keithly et al. (2004) have previously linked *H. azteca* total Ni body burden (referred to as tissue Ni) with lethality. Borgmann et al. (2001) reported that in a 28-d water-only test the calculated LA25 (lethal accumulation resulting in 25% mortality) for tissue Ni was 197 nmol/g (wet w.t.). The 14-d LA20 reported by Keithly et al. (2004) was 247 nmol/g (wet w.t.). It should be noted that the corresponding dissolved Ni exposure concentrations are remarkably low (8 µg/L and 37 µg/L; Borgmann et al., 2001 and Keithly et al., 2004, respectively; both values "hardness normalized' by Keithly et al., 2004).

1.2.3.3 Dissolved organic matter

1.2.3.3.1 Sources (allochthonous/autochthonous)

DOM results from the breakdown of terrestrial and/or aquatic plant, animal and microbial materials, and from the condensation reactions (polymerization) of smaller biomolecules (e.g., Wetzel, 1975). The relative contribution from either carbon source depends upon the nature of the watershed. The more recalcitrant fraction of DOM is comprised of humic substances (HSs) (Wetzel, 1975) which are operationally defined organic acids composed of a complex mixtures of large, biogenic, heterocyclic, refractory molecules occurring in all terrestrial and aquatic environments (Aiken et al., 1985; Perdue,

1998). Often comprising a significant proportion of the organic matter in surficial water (50-90%) and sediments (Jackson, 1975; Thurman and Malcolm, 1981; Pempkowiak et al., 1998), they play an important role in a number of aqueous processes which include (but are not limited to) carbon and nutrient cycling, geochemical weathering, trace metal complexation and sorption of nonpolar organic compounds (Woodwell et al., 1978; Jones et al., 1993; Rashid, 1971; Zhang et al., 1996; Penttinen et al., 1998; Gauthier et al., 1987). The typical range of DOM, measured as dissolved organic carbon (DOC; ~50% of DOM) in natural waters is from 2 to 10 mg/L (McKnight et al., 1983; Thurman, 1985), although swamps, marshes and bogs are known to be higher in DOC (10 to 60 mg/L DOC; Thurman, 1985). Sedimentary pore-water DOC concentrations (4 to 20 mg/L in oxic sediments, 10 to 390 mg/L in anoxic sediments; Thurman, 1985) are known to exceed the DOC of the associated overlying waters (Chin et al., 1998; Orem et al., 1986) due to release of DOC from the sediment solid-phase during degradation processes (Orem et al., 1986).

1.2.3.3.2 Operationally defined fractions

Based on solubility characteristics under acidic conditions, HSs are typically subdivided into either fulvic (FA) or humic acids (HA). Humic acids precipitate from solution at low pH whereas FAs remain in solution regardless of pH. Of the total HSs in surface waters, FAs typically account for the majority of the DOC (80%) with HAs accounting for the remainder (Thurman, 1985).

1.2.3.3.3 Dissolved organic matter influence on metal speciation, bioavailability and toxicity

While the functions of HSs in surficial water and sediments are of significant ecological and geochemical importance (see review by Jackson, 1975), the ability of humic substances to complex or sorb contaminants is of primary ecotoxicological importance. For example, fulvic and humic acids have been shown to form complexes with a variety of cationic divalent metals (e.g., Sholkovitz and Copland, 1981; Christl et al., 2001). These organic acids are rich in functional groups (COOH; phenolic and alcohol OH) (e.g., Oliver et al., 1983) which are thought to participate in metal complexation (e.g., Gamble and Schnitzer, 1973; Reuter and Perdue, 1977) and chelate metals at bidentate sites (Gamble and Schnitzer, 1973). Tipping and Hurley (1992) have compiled a list of pK_{MHA} values (a measure of binding strength) for various divalent cations and humic acids which indicates that binding strength increases in the order $Mg^{2+} < Ca^{2+} < Mn^{2+} < Cd^{2+} < Co^{2+} < Ni^{2+} \sim Zn^{2+} < Pb^{2+} < Cu^{2+} < VO^{2+}$. There is currently considerable debate as to the degree of influence DOM quality and source on metal complexation. Higgo et al., (1993) found that the binding strength values for six divalent cations (Ni included) and three FA sources were not significantly different despite their different source environments (source environments included a sandy aquifer, a peat bog, and peat bog/flood-plain silts). Modeling (WHAM V) of proton- and metal-binding data found that there were distinct differences between fulvic and humic acids, but that binding characteristics were not dependent upon source environment (Tipping 1998).

Until recently, information regarding Ni-DOM speciation and bioavailability was limited. While this gap is slowly being addressed by various researchers, the chemical speciation of Ni in metal-polluted waters and groundwaters remains poorly studied (Mandal et al., 2002; Hummel and Curti, 2003). Nickel interactions with humic substances from

various source environments (including recent sediments) and interactions with different DOM fractions, require further research. No information has been found involving pore water DOM fractions. The few studies performed to date have mainly involve low metal-to-ligand (Ni:DOC) ratios in efforts to determine conditional stability constants. While this information is useful for understanding Ni speciation at background levels, Ni speciation at Ni:DOC ratios of toxicological concern has received little attention.

While a number of studies have evaluated the toxicity of total Ni concentrations to various aquatic organisms, or the factors modifying Ni bioavailability (i.e., hardness or pH; see review by U.S. EPA, 1986), very little research has directly evaluated the effect of Ni speciation on bioavailability and toxicity. While there is abundant information in the literature suggesting that the predominant Ni species in freshwaters (pH 5-9) is the hydrated divalent cation (e.g., Morel et al., 1973), there is little information identifying Ni^{2+} as the main bioavailable Ni species. While studies have evaluated Ni speciation in the presence of DOM, aside from Spencer and Nichols (1983) and Mandal et al. (2002), no studies were located correlating the concentration of Ni^{2+} with a biological response via toxicity testing. A variety of studies evaluating the effects of dissolved organic matter (DOM) on transition metal speciation (e.g., Cd, Cu, Zn), have found that DOM generally reduces metal bioavailability in marine and freshwater systems (Sunda and Lewis, 1978; Meador, 1991; Playle et al., 1993a; Penttinen et al., 1998; Daly et al., 1990; Heijerick et al., 2003). Work involving Ni toxicity and DOM is virtually non-existent. Spencer and Nichols (1983) evaluated Ni bioavailability to two species of green algae in the presence of EDTA in synthetic media and linked Ni toxicity to the calculated free ion concentration. To date, Mandal et al. (2002) is the only study which has actually measured Ni^{2+} activity in toxicity

testing (contaminated surface water) and evaluated its effect on the algae *Pseudokirchneriella aubcapitata*. Their work involving the invertebrates *Daphnia magna* and *Hydra attenuata* was less successful in demonstrating a link between Ni speciation and a biological response. No studies have evaluated Ni bioavailability and organism response in the presence of pore-water DOM.

1.3   Sediment

1.3.1   General composition

Sediment can be described in general terms as a "...complex heterogenous mixture of different gaseous/liquid/solid, inorganic/organic, and living components derived from various sources and controlled by numerous physical, chemical, and biological variables (Reuther, 1998)."

1.3.2   Trace metals

Sediments are known to accumulate trace metals in aquatic ecosystems. This is particularly true of depositional sediments in close proximity to anthropogenic metal discharges. Such sediments may become so elevated in trace metals that they pose a risk to the associated biota. To better evaluate this risk, a more complete understanding of the processes affecting the bioavailability of metals (i.e., Ni) in sediment is required. For organisms closely associated with the sediment surface (or subsurface), the uptake of metals may result from exposure to Ni in the sediment pore water, overlying water, bulk sediment/food, or a combination of the three (Ankley, 1996; Lee et al., 2000a). For *H. azteca,* pore-water metal has been sown to be predictive of the presence and extent of metal

toxicity in sediments (Ankley et al., 1991, 1993; Hansen et al., 1996). Understanding Ni partitioning between the sedimentary solid and liquid phase is therefore essential in evaluating the bioavailability and toxicity of Ni to *H. azteca* in freshwater sediment.

1.3.3 Accumulative phases influencing nickel speciation/bioavailability

1.3.3.1 Equilibrium partitioning approach

In sediment, metals are partitioned between the solid and aqueous phase, with the solid phase usually having concentrations much greater than that of the interstitial water. The solid phase can be thought of as an assemblage of different metal scavenging moieties each in itself referred to as a 'phase'. Deutsch (1997) described a phase as "Each mineral type or other solid material with the same composition and properties...." The relative contribution of these different phases in partitioning metal is of much interest. While phase partitioning is more complex for metals than for nonionic organic contaminants (for which the main binding phase is organic carbon), it is believed that a method, similar to the equilibrium partitioning approach for nonionic organic chemicals in sediments, can be used to develop sediment quality criteria for metals (U.S. EPA, 1994). The equilibrium partitioning approach attempts to predict chemical bioavailability in sediment based on the partitioning of a chemical between the liquid and solid phases. A tool such as this would be important in sediment monitoring or in setting priorities for environmental remediation where financial resources may be limiting (U.S. EPA, 1994).

1.3.3.2 Acid-volatile sulfide

Microbial decomposition of organic matter typically results in sediments that are

anoxic under a thin oxic surface layer (Rhoads, 1974). For trace metals having class B or borderline character sulfide is considered to be the main binding phase in anaerobic sediment (Boulegue et al., 1982; Emerson et al., 1983; Morse et al., 1987). Low pore-water metal concentrations in such sediment result from the formation of the relatively insoluble metal sulfides (Di Toro et al., 1990; Yu et al., 2001). When molar concentrations of amorphous sulfide (referred to as acid-volatile sulfide or AVS; composed mainly of free sulfides, iron monosulfide, crystalline mackinawite, pyrrhotite, greigite, plus the sulfides of other divalent metals; Yu et al., 2001) exceed the summed molar concentration of the previously mentioned class B or borderline divalent cationic metals (referred to as simultaneously extracted metals or SEM), it has been well demonstrated that acute toxicity is unlikely (Di Toro et al., 1990, 1992; Berry et al., 1996; Hansen et al., 1996; Ankley et al., 1993; Liber et al., 1996). When SEM exceeds AVS, pore-water metal concentrations may increase dramatically with relatively little change in total sediment metal content (Di Toro et al., 1990). If metal activity is sufficiently elevated (elevated pore-water metal concentrations are predictive of elevated metal activity and hence toxicity in sediment) the sediment-associated biota may suffer from detrimental effects.

When the molar concentration of SEM is less than the molar concentration of AVS it has been well demonstrated that sediments are not acutely toxic (Berry et al., 1996; Hansen et al., 1996; Ankley et al., 1993). However, using SEM/AVS ratios to predict toxicity has met with less success. Acute metal toxicity does not always occur when SEM in a sediment exceeds AVS. For example, Berry et al., (1996) found that only 70% of sediments predicted to be toxic were actually toxic. Hansen et al., (1996) found that for sediments with SEM/AVS ratios >1, only 56.8% were actually toxic. These examples

suggest that other binding phases are important in determining metal bioavailability in sediment and that AVS alone may not be adequate for predicting acute toxicity (Ankley et al., 1993). One such additional binding phase is organic matter (OM) (Di Toro et al., 1990).

Previous Ni studies exposing aquatic organisms (including *H. azteca*) to Ni-spiked or contaminated sediments have found that when SEM/AVS <1, acute Ni toxicity is generally not observed (Di Toro et al., 1992; Ankley et al., 1991; Pesch et al., 1995). However, SEM/AVS ratios have been shown to be less predictive of the absence of Ni bioaccumulation in burrowing organisms such as *Lumbriculus variegatus* (Ankley et al., 1991) and the marine polychaete *Neanthes arenaceodentata* (Pesch et al., 1995). Pesch et al. (1995) found metal concentrations in *N. arenaceodentata* generally increased with increasing sediment metal concentration, SEM/AVS molar ratio, and pore-water metal concentrations. The occurrence of metal bioaccumulation at ratios less than 1 was suggested to be the result of release of Ni from oxidized metal sulfide (a result of bioturbation), uptake of metal from ingested sediment, or the adsorption to body surfaces. Under longer-term chronic exposure conditions, total SEM (Ni) has been suggested as contributing significantly towards the total metal body burden in suspension/deposit feeding clams (Lee et al., 2000a) rather than the SEM in excess of AVS or pore-water metal concentrations.

1.3.3.3 Organic matter (solid-phase)

1.3.3.3.1 Sources (allochthonous/autochthonous)

Organic matter in freshwater or marine sediments may derive from the decomposition of either terrestrial (allochthonous) or aquatic (autochthonous) plant and

animal sources. In sediment, OM may exist in the solid phase as surface coatings, typically concentrated on the finer particle surfaces, or as separate particles associated with the coarser size fractions (Horowitz and Elrick, 1987).

1.3.3.3.2 The effect of organic matter on metal bioavailability/toxicity

In addition to reactions with sulfide, the partitioning of metals in anoxic sediments may be influenced by the presence of other metal-binding phases such as OM (Di Toro et al., 1990; Mahony et al., 1996), typically characterized (quantified) as organic carbon (OC). The presence of OM, which provides additional metal-buffering capacity to sediments, explains why toxicity is not always observed at SEM/AVS ratios >1 or at positive [SEM−AVS] values (Di Toro et al., 1990; Hansen et al., 1996; Leonard et al., 1996a). While studies have evaluated the partitioning of metals to OM extracted from natural sediments (Fu et al. 1992), or metal partitioning to whole, natural sediments (Mahony et al., 1996), little work (aside from Besser et al. 2003) has specifically evaluated the influence of sediment OM on metal bioavailability and toxicity in sediments. No previous published study has evaluated the influence of both sediment OM and AVS on metal bioavailability and toxicity in sediments.

Since sedimentary OC content typically increases with decreasing sediment particle size (Horowitz and Elrick, 1987), OM may play an increasingly important role in metal speciation and bioavailability in finer sediments. Depositional areas occur where conditions are right for finer particles to settle from the water column. These finer particles have larger surface areas than those of coarse, sandy sediments. As a result, depositional sediments are capable of adsorbing significant quantities of trace metals directly, or through the

accumulation of geochemical coatings (OM, Fe and Mn oxyhydroxides) which will in turn act as trace element collectors (see review by Horowitz, 1991). Organic matter may exist as a surface coating or as a particulate, either of which may function as a trace-element collector.

OM is believed to be particularly important in sediments low in sulfide (Di Toro et al., 1990) and may also be an important binding phase in the oxic micro-environment surrounding many benthic organisms. It has been proposed that a relationship exists between the SEM that is in excess of AVS and the pore water metal activity $\{M^{2+}\}$, such that $[SEM] - [AVS] = K_{d,OC} f_{OC} \{M^{2+}\}$, where $K_{d,OC}$ is the partitioning coefficient between total dissolved metal concentration in the pore water and the organic carbon of the sediment, and f_{OC} is the weight fraction of OC in the sediment (U.S. EPA, 1994). More simply, metal in excess of the AVS capacity is partitioned between the OM fraction of the sediment and the pore water. However, further research is needed to fully understand the relative significance of OM metal binding in both anaerobic and aerobic sediments. The significance of OM binding could be especially important in aerobic (oxic) surface sediment where the AVS concentrations are low. Since most benthic organisms inhabit aerobic sediments, or at least oxic micro-environments within anaerobic sediment, the contribution of OM binding to metal bioavailability in sediment should be further investigated. Besser et al. (2003) found that by amending a formulated sediment with natural humus, pore-water metal concentrations were reduced (in addition to the associated toxicity to *H. azteca*) in Cu- and Cd-spiked sediments.

1.3.3.3.3 Other metal-binding phases

In aerobic sediments, metals associated with adsorption sites on the sediment govern the equilibrium of metals between the solid sediment and pore-water phases (Apte and Batley, 1995). In these sediments, both OM and iron and manganese oxides appear to play major roles in metal partitioning between the aqueous and solid phases (see review by Shea, 1988). The importance of these geochemical substrates is related to their large surface areas, high cation exchange capacities, and high surface charges (Horowitz and Elrick, 1987). While clay minerals also have these characteristics, clay may act as substrates for the precipitation and flocculation of other collectors such as OM and hydrous iron and manganese oxides (Horowitz, 1991). Clay minerals are therefore thought to be coated with materials that actually carry out the concentration of trace elements (Horowitz, 1991), although work by Nachtegaal and Sparks (2003) suggests that, while humic acid coatings may slow and alter the nature of the interaction of Ni with clay surfaces, Ni still forms a precipitate with the underlying surface.

While the anoxic conditions in the bulk sediment do not favour the formation of Mn or Fe oxides, these may exist in the aerobic sediments or soils and play a role in Ni binding or solubilization (depending upon redox conditions) (McKenzie, 1989). Tessier et al. (1979) found through sequential extractions that Ni from surficial sediments (not present as primary or secondary minerals) was mainly associated with iron and manganese oxides in sediments low in organic carbon. Larsen and Postma (1997) found Ni to have a strong association with Mn oxides in aquifer sediment. The high cation exchange capacity of organic matter (Rashid, 1974), its tendency to significantly cover other inorganic coatings (Davis, 1982), and the elevated OC content in depositional sediments may result in OM playing a major role in metal partitioning even in the presence of Fe and Mn oxides.

1.4   *Hyalella azteca*: A brief description

Hyalella azteca, also cited in the literature under the synonyms *Hyalella knickerbockeri* (Bate) or *H. dentata* (Smith) (Geisler 1944), is a common North and South American epibenthic organism. It is a small bodied member of the talitroidean amphipod family Hyalellidae (Bulycheva, 1957) (Environment Canada, 1997) which reaches sexual maturity in ~25-40 days and reaches a maximum length of ~7 mm (8 mg wet wt) in ~120 days, depending upon rearing conditions (Othman and Pascoe, 2001). *H. azteca* is thought to obtain its nutrition from the bacteria and algae associated with ingested sediment particles (Hargrave, 1970).

In recent years *H. azteca* has rapidly become routine in freshwater and estuarine sediment toxicity testing. Its short generation time, contact with the sediment and ease of culture make it a desirable animal model for assessing contaminated sediments (Environment Canada, 1997). *H. azteca* has also been shown to be very sensitive to metal exposure relative to other benthic organisms (Milani et al., 2003; Keithly et al., 2004). For example, Milani et al., 2003 found *H. azteca* to generally be the most sensitive to Cd, Cu and Ni toxicity compared to other benthic invertebrates such as (*Hexagenia* spp., *Chironomus riparious* and *Tubifex tubifex*). Keithly et al. (2004) found *H. azteca* to be relatively sensitive (under both acute and chronic exposures) to Ni (hardness normalized LC50 data) relative to other test organisms from previous studies.

1.5   Thesis hypotheses and objectives

1.5.1   Overall hypothesis

The overall null hypothesis was as follows:

Ho: *Organic matter (OM), including pore-water dissolved organic matter (DOM), is not an important binding phase for nickel and thus does not influence nickel bioavailability in sediment.*

This hypothesis was broken down into two separate (but related) components:

1.5.2 Hypothesis regarding dissolved organic matter

1.5.2.1 Hypothesis

I. Ho: *DOM does not influence the bioavailability/toxicity of nickel in depositional sediment pore water.*

1.5.2.2 Objectives

Using water-only experiments (used as surrogates for pore water exposure), ion exchange techniques, and chemical speciation modeling, the objectives were to determined whether:

- DOM influences nickel bioavailability/toxicity.

- different DOM fractions influence bioavailability/toxicity in the same manner (HA versus FA).

- pore-water DOM (Little Bear Lake sediment pore water fractions; HA and FA) modifies the bioavailability/toxicity of Ni in a manner similar to that of surface-water DOM (Suwannee River DOM) or DOM from a convenient source (peat).

- additional LBL pore water fractions (hydrophilic fractions) influence Ni bioavailability/toxicity.

1.5.3 Hypothesis regarding solid-phase organic matter

1.5.3.1 Hypothesis

II. Ho*: Solid phase organic matter does not influence nickel binding capacity in depositional sediment (OM is not an important binding phase).*

1.5.3.2 Objectives

Solid-phase Ni titrations and Ni bioavailability and toxicity sediment tests were used to;

- evaluate the partitioning of Ni in formulated sediments (differing in OC content only) and in natural sediments of various OC content.

- conduct whole sediment Ni toxicity tests with natural sediments and formulated sediments to determine if Ni bioavailability is affected by sediment OM content. The objective was to address the following questions: (i) in addition to sulfide, does solid-phase OM influence Ni bioavailability and toxicity in sediment?; (ii) relative to overlying-water Ni or total Ni, does pore-water Ni best represent the main source of bioavailable Ni for *H. azteca* in Ni-contaminated sediment?; and (iii) in the presence of excess AVS, does the prediction of a non-lethal response also mean the absence of Ni bioaccumulation?

CHAPTER 2

A dialysis mini-peeper for measuring pore-water metal concentrations in laboratory sediment toxicity and bioavailability tests

2.1 Introduction

In recent years, advances have been made in the understanding of metal bioavailability and toxicity in sediments. It is generally accepted that metal toxicity in sediment is not correlated with total metal content, but with the bioavailable metal fraction such as that in pore water (see review by (Luoma, 1989)). Work done with nonionic organic chemicals (Adams et al., 1985) and metals (Adams et al., 1985; Di Toro et al., 1990; Ankley et al., 1991; Hansen et al., 1996; DeWitt et al., 1996) has demonstrated a correlation between pore water contaminant levels and toxicity to benthic organisms.

Protocols have recently been developed to help standardize sediment toxicity testing in laboratory settings. Important to toxicity testing of metals is the accurate measurement of pore water metal concentrations. Using the Environment Canada *Chironomus tentans* testing protocol (Environment Canada, 1997) as an example, the termination of a 10-day toxicity test requires sieving of test sediments to retrieve all surviving test organisms. Destructive sampling of the sediment at the end of the test precludes most methods of pore water sampling. Pore water chemistry data are therefore often obtained from additional test replicates run concurrently. Animals are

generally included in these additional test beakers since benthic organisms can affect pore water metal partitioning through bioturbation (Peterson et al., 1996). It is assumed that there is no significant difference in pore water chemistry between toxicology beakers (those sieved) and chemistry beakers within any given treatment.

A popular device for sampling pore water *in situ* is the dialysis cell, also known as a "peeper" (Hesslein, 1976). A semi-permeable membrane separates a small chamber of distilled water or electrolyte solution from the surrounding pore water or overlying water. Solutes pass through the pores in the membrane allowing the cell to passively equilibrate with the surrounding solution. The equilibration period will depend upon sediment temperature, porosity, tortuosity, the diffusion coefficient of the substance of interest, and the extent to which the substance adsorbs to the solid sediment phase (Carignan, 1984). Equilibration times may vary from several days to several weeks depending upon the sediment characteristics and the dissolved species being investigated (Hesslein, 1976; Carignan, 1984) and overlying water quality.

We have modified Hesslein's 1976 peeper design (Hesslein, 1976) to create mini-peepers that are of a convenient size for use in laboratory sediment toxicity tests following recommended Environment Canada protocols (Environment Canada, 1997). Mini-peepers are designed for use directly in beakers containing sediment and test organisms. This allows for a direct correlation between pore water contaminant concentrations and contaminant bioaccumulation in test organisms rather than relying on the pore water chemistry data gathered from additional beakers. It also reduces the amount of sediment and number of test organisms needed for a test. In comparison to other pore water sampling techniques involving mechanical squeezing (Presley et al.,,

1967; Bender et al., 1987), gas pressurizing (Jahnke, 1988), and centrifugation followed by filtration of sediment (Sholkovitz, 1973; Emerson, 1976), peepers are simple to use, less time consuming, require little in terms of equipment (Carignan, 1984) and sediment handling, and should be less prone to experimental error.

This study was undertaken to evaluate the future use of mini-peepers in measuring sediment pore water metal concentrations in laboratory toxicity and bioavailability tests using field-collected or spiked sediment. Tests were conducted with nickel and zinc solutions, two size fractions of nickel-spiked sand, and two uncontaminated, field-collected ("natural") sediments spiked with nickel to determine the accuracy with which the mini-peepers measure pore water and overlying water metal concentrations and the time frames required for equilibration in different media.

2.2   Methods and materials

2.2.1   Mini-peepers

Construction of mini-peepers was based on the designs of Hesslein (1976) and Carignan (1984), but using much smaller dimensions. The samplers were cut from acrylic blocks that measure 57×13×12.5 mm (l×w×d) and are slightly pointed at the insertion end. Two small oblong chambers measuring 14×8×8 mm (l×w×d) with a volume of approximately 0.75 ml are machined 5 mm apart along the vertical axis (Figure 1). A semi-permeable membrane (polysulfone, 0.2 μm pore size) covering both cells is held in place by a thin (2 mm) face plate secured by small stainless steel screws. Solutes are able to pass through the pores in the membrane allowing the cell (filled with deionized water) to equilibrate with the surrounding pore or overlying water solution.
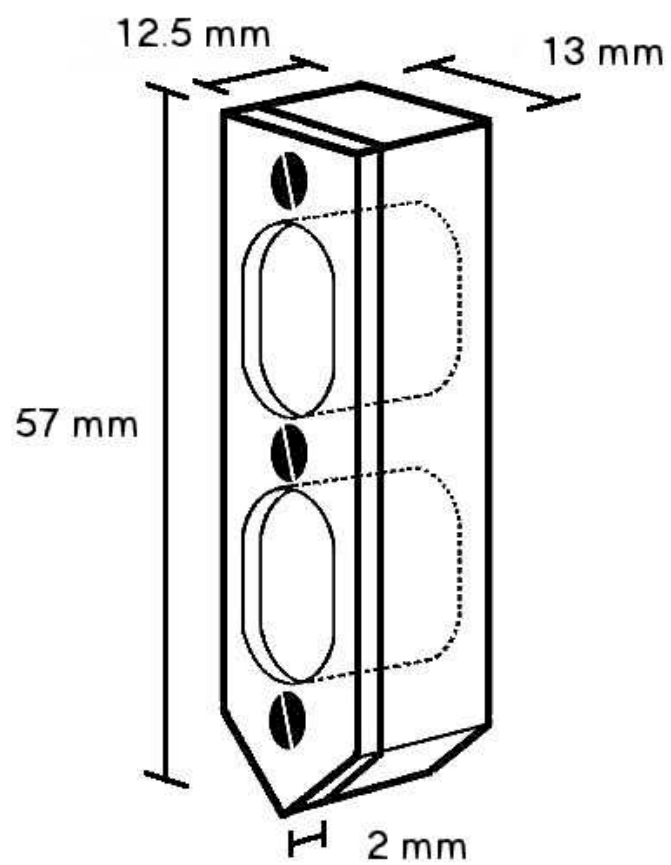
Figure 2.1.Schematic of mini-peeper.

Peeper cell volume was kept small relative to sediment pore water volume to minimize any dilution effects on pore water chemistry. The sediment surface area occupied by the peeper relative to the entire area of the test beaker was also kept to a minimum (6.6 %) since the presence of a peeper will decrease the surface area available for test organisms. All experiments were conducted in an environmental chamber at 23.5 ± 1°C.

2.2.2   Water-only experiments

A single cell in each mini-peeper was filled with deionized (Milli-Q™) water (pH 5.5) and covered with a 0.2-µm polysulfone membrane cut to fit. Twenty-four, 300-ml tall-form glass beakers were filled with 200 ml of deionized water spiked with 10 mg/L Ni in the form of $NiCl_2 \cdot 6H_2O$ (Strem Chemicals, Newburyport, MA, USA). At time 0, prior to the addition of the mini-peepers to the beakers, four mini-peepers and four replicate beakers with Ni solution were sampled. A single mini-peeper was then placed in each of the remaining 20 beakers. At periods

of 1, 6, 24, 48, and 96 h after addition of the peepers, the nickel solution in the beaker and the corresponding mini-peeper cell were sampled from four replicate beakers. Any water adhering to the outer peeper membrane surface was carefully removed using a pipette. The peeper samples

were drawn by piercing the membranes with an Eppendorf™ pipette fitted with a 1000-µl tip. A new tip was used for each sample. All samples were transferred to prewashed 8-ml Nalgene® bottles and acidified with 1 M distilled HNO_3 for preservation (pH<2.5). A second experiment was performed following the above procedure, but using 10 mg/L zinc, in the form of $ZnCl_2$ (BDH Chemicals, Vancouver, BC, Canada), instead of nickel.

2.2.3   Sand experiments

Two size fractions, coarse (425 - 850 µm) and fine (100 - 250 µm), of industrial, pre-sieved quartz sand (Unimin Corporation, Le Sueur, MN, USA) were thoroughly rinsed three times with distilled water and three times with deionized water, and allowed to air dry prior to use. The volumes of water required to saturate subsamples of the coarse and fine sand were divided by the total volumes of the saturated sand to calculate porosity. The coarse sand was saturated with a nickel chloride solution (30 mg/L Ni) and aged for 4 d prior to experimentation to allow the pore water nickel to equilibrate with the solid phase. The fine sand was saturated with a higher nickel concentration (120 mg/L Ni) and aged 3 d prior to experimentation. In both tests, approximately 100 ml of aged, saturated sand was added to each of 24, 300-ml tall-form glass beakers (total depth of ~3.5 cm). The beakers were gently tapped on a counter surface to compact the sand and displace air pockets. In each sand trial, single mini-peepers, prepared as described above, were inserted into the sand of 20 beakers until one chamber was above the sand (exposed to overlying water) and one chamber was below the sand-water interface (subsurface; exposed to pore water). After peeper insertion, a plastic disc with a diameter slightly less than that of the beaker was lowered onto the sand surface. A square hole cut in the centre of the disc allowed it to pass over the mini-peeper. Then, with minimum disturbance to the sand, deionized water (175 ml) was gently poured into the beaker and the disc gently lifted free. Four prepared mini-peepers, four beakers of nickel solution saturated sand, and four aliquots of deionized water were sampled prior to the insertion of the peepers to provide time 0 data. Subsamples of overlying water and pore water were analyzed for pH (Orion Model 370 pH meter) at time 0 and at the

end of the experiment (96 h). Four replicate samples for nickel analysis were collected

from the overlying water, pore water (isolated by vacuum filtration), and mini-peepers

(overlying and subsurface) at 1, 6, 24, 48, and 96 h. Composite overlying water samples

(6×1 ml drawn from around the mini-peeper) were collected prior to peeper removal

with an Eppendorf™ pipette fitted with a 1000-μL tip. The remaining overlying water in

each beaker was then siphoned down to the level of the sand before mini-peeper

removal. After removal, mini-peepers were carefully rinsed with deionized water to

remove any adhering sand. Any water adhering to the membrane was removed with a

pipette before the membrane was punctured and the samples retrieved as described

above. The saturated sand was scooped into Nalgene® filter holders and a vacuum line

applied to draw the pore water from the sand through a polysulfone filter (0.2 μm pore

size). Peeper, pore water, and overlying water samples were preserved with HNO_3 as

described in the water-only experiments.

2.2.4   Natural sediment experiments

Freshwater sediment used for experimentation was collected from two locations

in Little Bear Lake 54° 17' N, 104° 41' W), an oligotrophic lake in north-central

Saskatchewan, Canada, which is known to be free from significant metal contamination.

Using an Ekman grab sampler (15×15 cm), sediment samples were collected on August

16, 1998 (Sediment 1) and September 27, 1998 (Sediment 2). Sediments were placed in

20-L plastic pails and allowed to settle 1 h before the surface water was carefully

decanted. The sediment was brought back to the laboratory, homogenized, and

refrigerated (4°C) within 24 h of collection. Subsamples were oven dried overnight

(104°C) to determine the percent water content by weight. This value was in turn used to calculate sediment porosity (the volume of water in sediment divided by the total volume of sediment).

2.2.4.1 Trial 1

In this trial, 3.136 kg of Little Bear Lake Sediment 1 was spiked with 12.699 g of $NiCl_2 \cdot 6H_2O$ dissolved in 30 ml of deionized water (1 g Ni/kg wet sediment), shaken for 1 h, and allowed to equilibrate at ~4°C for 17 d in a sealed 14-L bucket. Prior to experimentation, the sediment was stirred with a large plastic spatula for 3 min. The experimental protocol was the same as described for the sand experiments, except that an extra set of beakers was added to create an 8-day sampling period (192 h). The final subsamples for pore water and overlying water were taken at this time. After removal of mini-peepers, the sediment in the test beakers was thoroughly stirred with a small stainless steel spatula before approximately 55 g were transferred to 50-ml Nalgene® tubes for centrifugation at 3800 rpm (1160g) for 15 min. The isolated pore water was then vacuum filtered through polysulfone membranes (0.2 μm pore size) and preserved as described above.

2.2.4.2 Trial 2

This trial followed the same methods and spiking protocol as used for Trial 1, except that Little Bear Lake Sediment 2 was used and that after shaking the spiked sediment for 1 h and aging it 10 d, it was then divided among the test beakers and aged another 10 d in the refrigerator (4°C) prior to experimentation. Peeper, pore water, and

overlying water sampling and pH measurements were conducted as described above.

2.2.5 Physical and chemical analyses

All nickel and zinc samples were analysed with an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 3100). Standards were made from those solutions used to spike the water, sand, or sediment. Subsamples of homogenized sediment and pore water were analyzed for organic carbon (OC) using a LECO-CR-12 carbon analyzer (Leco Corporation, St. Joseph, MI, USA) and for dissolved organic carbon (DOC) using a Shimadzu TOC-5050A analyzer (Shimadzu, Tokyo, Japan) with an ASI-5000A autosampler. Methods for the extraction and analysis of acid volatile sulfide (AVS) followed the procedure of Leonard et al. (Leonard et al., 1996b). Particle size analysis was performed following the pipette method (Percival and Lindsay, 1997). A list of physical and chemical characteristics of the Little Bear Lake sediments is provided in Table 2.1.

2.2.6 Statistics

All tests for statistical significance involved either unpaired t-tests ($\alpha = 0.05$), or Mann-Whitney Rank Sum tests for non-normal data, using SigmaStat® version 2.0 (Jandel Scientific Software, 1995).

2.3 Results

2.3.1 Water-only experiments

Both nickel and zinc diffused rapidly into the mini-peeper cells achieving 95%

Table 2.1. Physical and chemical characteristics of natural sediments collected from Little Bear Lake, Saskatchewan, in August and September, 1998. Data are means ± SD (n = 3).

Sediment characteristics	Sediment 1 (Aug. 17, 1998)	Sediment 2 (Sept. 27, 1998)
Particle size (%)		
Sand (54 - 2000 µm)	66.5 ± 2.5	85.6 ± 0.1
Silt (2 - 53 µm)	18.4 ± 2.1	8.4 ± 0.1
Clay (<2 µm)	15.1 ± 0.4	6.0 ± 0.2
Pore water nickel (mg/L)	0.023 ± 0.008	0.005 ± 0.008
Water content (%)	77.5 ± 0.1	64.9 ± 0.1
Porosity	0.894 ± 0.001	0.821 ± 0.009
OC (%)	10.62 ± 0.5	4.08 ± 0.4
AVS (µmol/g d.w.)	20.17 ± 0.90	14.60 ± 0.15
pH	6.30 ± 0.03	7.16 ± 0.07

equilibration (based on mean values) by 24 h and 48 h for nickel and zinc, respectively (Figure 2.2).   There were no significant differences between the metal concentrations in peeper cells and the surrounding solutions by 48 h for nickel and 96 h for zinc. In the nickel experiment, the average coefficient of variation, *CV*, for nickel concentrations in peeper cells and surrounding water at both 48 and 96 h was 0.8 %. In the zinc experiment, the average *CV* for 48 and 96 h data was 2.3 % and 1.1 % for peeper and surrounding water data, respectively.

2.3.2   Sand experiments

Coarse sand: Nickel equilibration between the mini-peepers and pore water proceeded more slowly in sand than in the water-only experiment. Nickel equilibration was only 84% complete by 96 h (Figure 2.3a). Extrapolating from the graph, complete equilibration would have taken approximately 120 h. Overlying water results showed that nickel was slowly diffusing into the overlying water as the experiment progressed. The overlying peeper chambers showed concentrations very similar to those of the overlying water throughout the 96-h sampling period.   Mass balance data (not presented) revealed a small decrease (7.9 %) in aqueous nickel over the course of the experiment, suggesting minor nickel adsorption to the solid phase. Pore water initial and final pH values were 7.53 ± 0.03 and 7.66 ± 0.10, respectively.

At 24, 48, and 96 h, when the peeper and pore water nickel concentrations were the most similar, average *CVs* were 10.7 % and 8.1 % for the peeper and filtered pore water data, respectively.

Fine sand: Nickel equilibration was faster in the fine sand than in the coarse
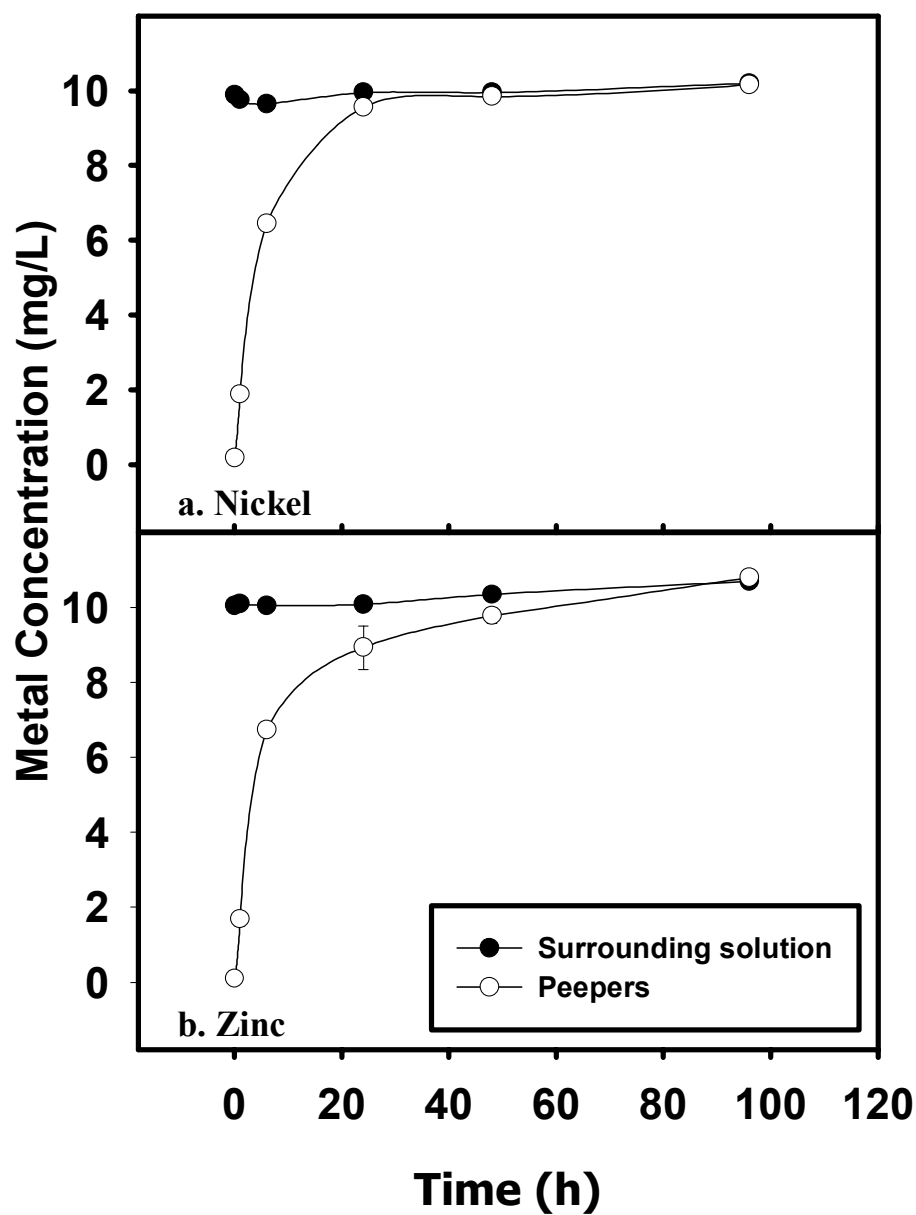
Figure 2.2. Equilibrium times for metal concentrations in mini-peeper cells and the surrounding metal solution in the absence of sediment. Data are means ± 1 SD.
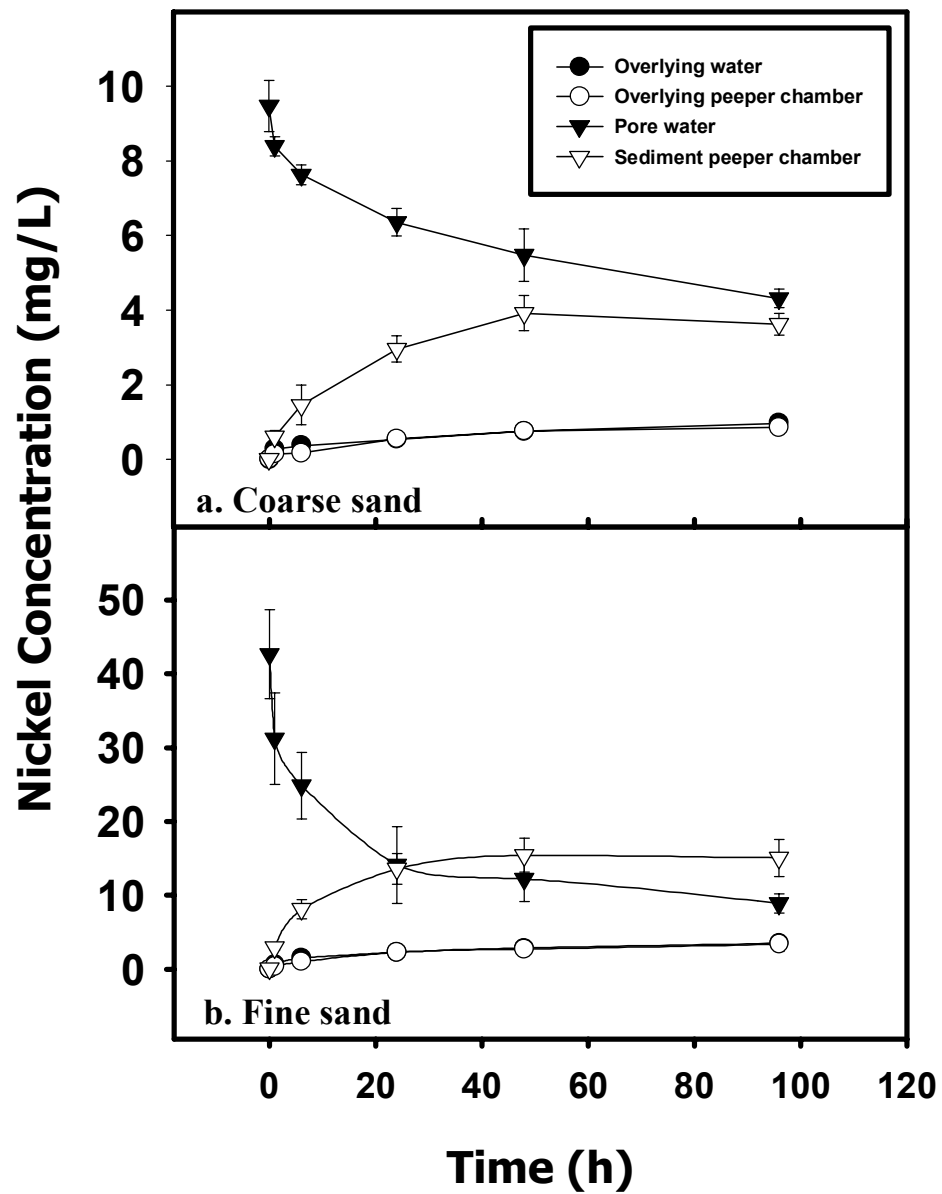
Figure 2.3. Nickel equilibration between mini-peeper cells and overlying water in nickel-spiked coarse sand (425-850 μm) and fine sand (100-250 μm). Data are means ± 1 SD.

sand. Equilibration in the fine sand occurred within 24 h (Figure 2.3b), but by 96 h the peeper showed an average concentration (69.6 %) greater than that of the pore water. Mass balance calculations showed a loss of 41% aqueous nickel over the first 24 h (data not presented) with no loss thereafter. At all sample times, the overlying peeper chambers were similar to the overlying water in nickel concentration. Pore water initial and final pH values were 7.13 ± 0.09 and 7.41 ± 0.18, respectively. At 24, 48, and 96 h the average *CVs* were 15.6 % and 25.4 % for the peeper and filtered pore water data, respectively.

2.3.3   Natural sediment experiments

Sediment 1: Subsurface peeper chambers were 88% equilibrated with the pore water by 24 h and not significantly different by 48 h (Figure 2.4a). There was a continuous decrease in pore water nickel and a subsequent increase in overlying water nickel concentration until the last sampling time (192 h) when nickel concentrations were nearly identical. Nickel concentrations were higher in the overlying peeper chamber than in the surrounding solution at 6, 24, 48, and 96 h, but not significantly different by 192 h. Mass balance data (not presented) showed a decrease (15%) in total nickel in solution within the first 6 h after which time the total mass of nickel in solution remained relatively constant. At 24, 48, 96, and 192 h, when the peeper and pore water concentrations were the most similar, the average *CVs* were 9.0 % and 4.8 % for the peeper and centrifuged/filtered pore water data, respectively.

Sediment 2: Spiking with 1 g Ni/kg wet sediment resulted in much higher pore water nickel concentrations in Sediment 2 than in Sediment 1. Despite the quantitative
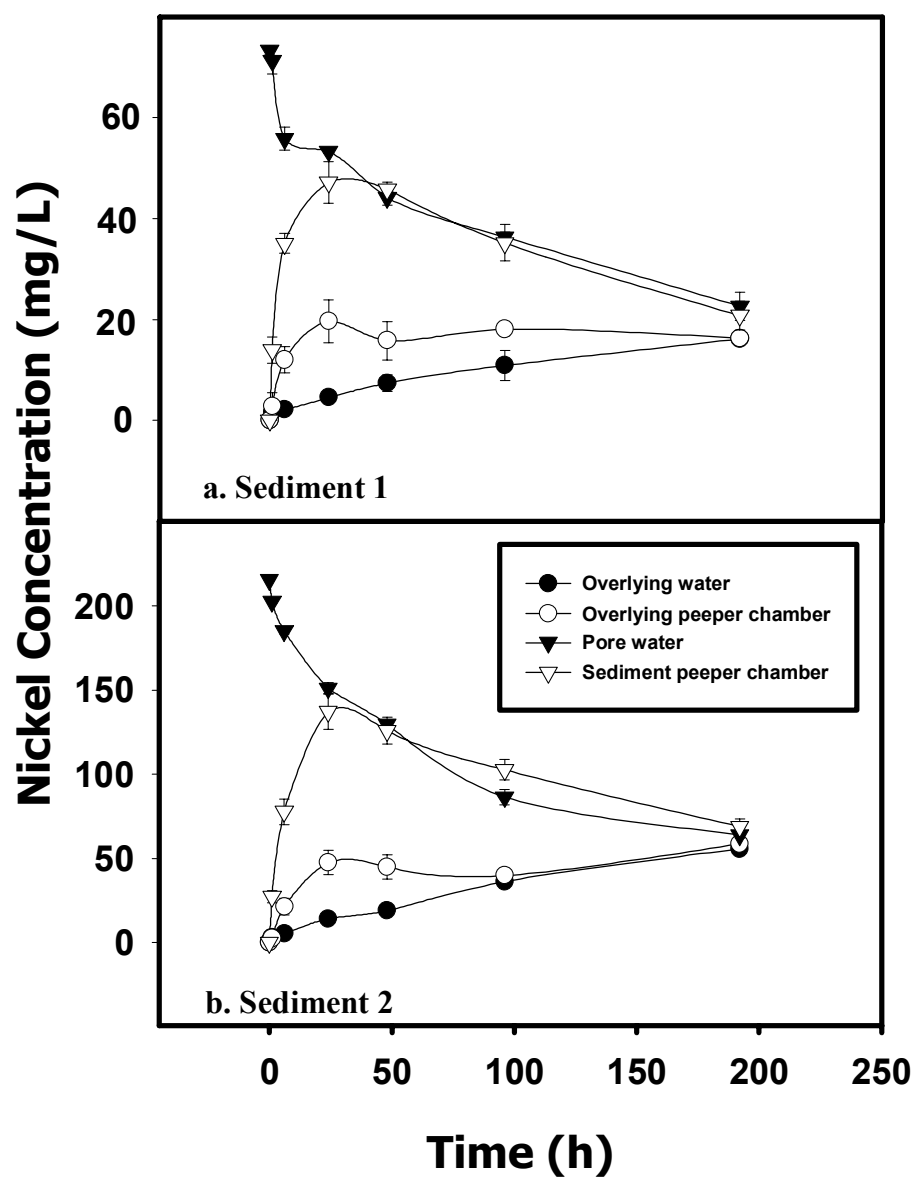
Figure 2.4. Nickel equilibration between mini-peeper cells and overlying water and sediment pore water in two nickel-spiked natural sediments. Data are means ± 1 SD.

difference, the equilibration patterns observed for Sediments 1 and 2 were strikingly

similar. There was no significant difference between the subsurface peeper and pore

water nickel concentration by 24 h. There was a continuous loss of nickel from the pore

water to the overlying water until the last sampling time when the concentration was

almost identical between the sediment and the overlying water. Compared to the

overlying water, the overlying peeper cells had consistently higher concentrations of

nickel until 96 h when there was no longer a significant difference between the two.

Mass balance data (not presented) showed a gradual decrease (19 %) in total aqueous

nickel over the first 48 h with a slight increase (8.1 %) over the last 96 h. At 24, 48, 96,

and 192 h, when the peeper and pore water concentrations were the most similar, the

average *CVs* were 7.8 % and 3.5 % for the peeper and centrifuged/filtered pore water

data, respectively.

2.3.4   Mini-peeper cell flux

Due to confounding effects, such as changes in metal partitioning to the solid

phase and metal diffusion into the overlying water, it was difficult to fully discern what

part the porosity of the media played in the equilibration of subsurface peeper cells.

Nickel concentrations in the test media for the 0 to 1 h period (taken as an average) and

peeper concentrations at 1 h were used to determine if the initial rate of equilibration was

correlated with sediment porosity. Because all experiments were conducted with

different nickel concentrations, a linear regression of peeper concentration versus

porosity was not appropriate. A linear regression of the nickel concentration in the

surrounding nickel solution (water-only experiment) or pore water nickel concentration

(sand and sediment experiments) versus the peeper cell concentration (both concentrations in mg/L) produced an adjusted r^2 value of 0.942 (Figure 2.5). This indicated that the higher the concentration of nickel surrounding the peeper, the greater the total flux of nickel into the peeper.

A linear regression of media porosity versus nickel flux (hr^{-1}) (expressed as a ratio of peeper cell concentration to the surrounding water or pore water concentration), produced an adjusted r^2 value of 0.879 (Figure 2.6). This indicated thatwhile absolute nickel concentrations in the surrounding media governed the flux rate into the peepers during the first hour of the experiments, the porosity of the surrounding medium strongly influenced the rate of peeper equilibration.

2.4   Discussion

2.4.1   Water-only experiments

Crank (1964) defined diffusion as "...the process by which matter is transported from one part of a system to another as a result of random molecular motions." In an aqueous solution of uncharged molecules, each individual molecule is continuously colliding with solvent molecules resulting in solute movement towards other regions, regardless of concentration. There is no preferred direction. If there are distinct regions of high and low molecular concentration, as would be the case with a peeper cell initially placed in a contaminant solution, the same proportion of solute molecules would diffuse out of the cell as would diffuse in (except at time 0 when there is no solute in the peeper cell). Because of a greater concentration of solute molecules surrounding the cell, there would be a resulting net movement of solute into the cell (Crank, 1964). The
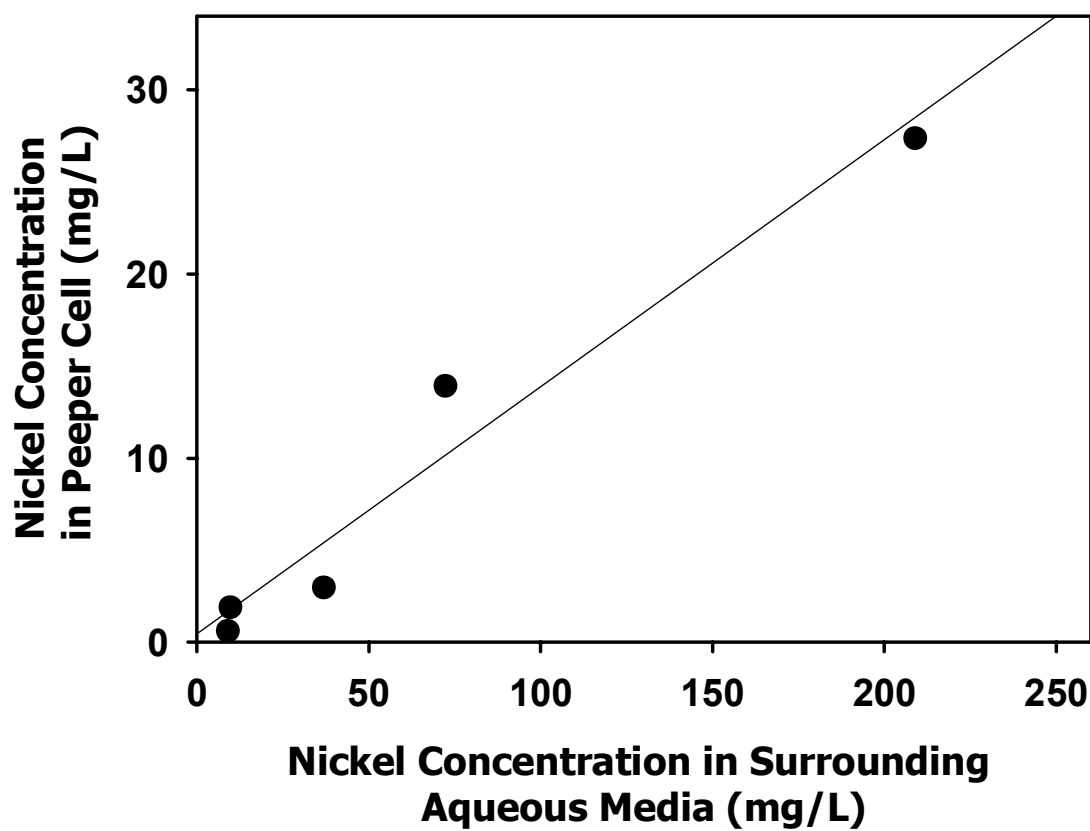
Figure 2.5. Regression of pore-water or surrounding water nickel concentration (mg/L) versus the peeper cell nickel concentration (mg/L) at 1 h.
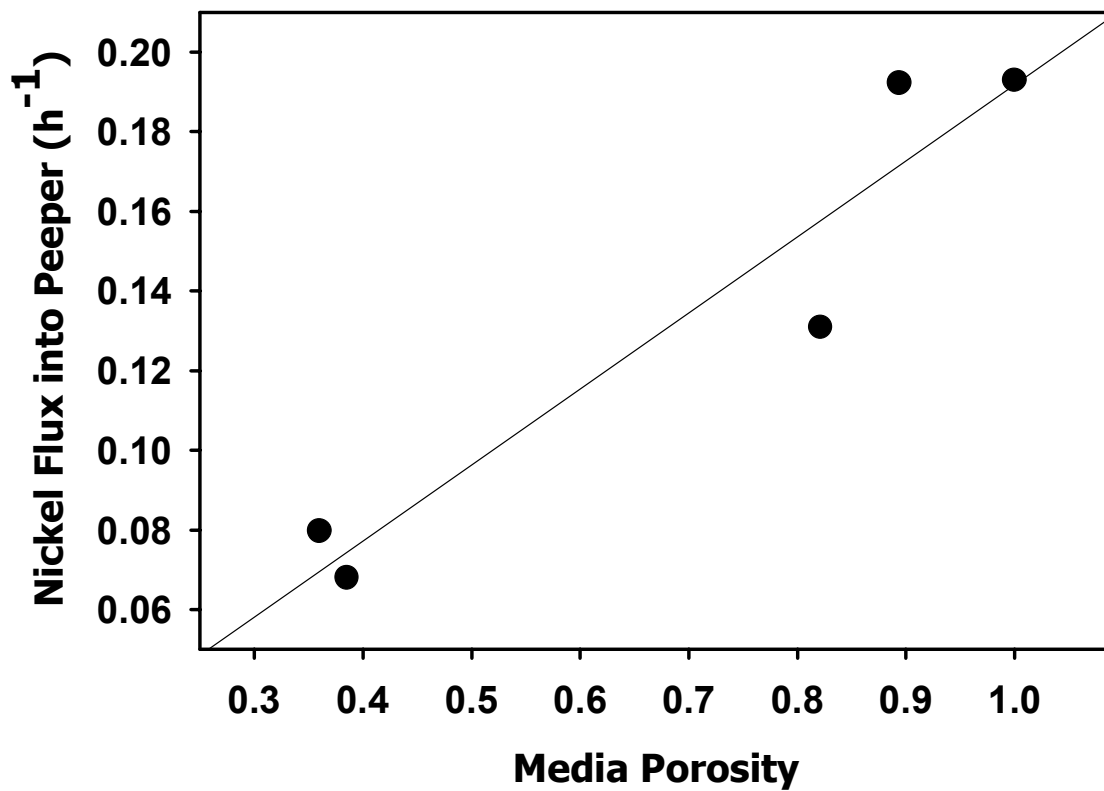
Figure 2.6 Regression of media porosity versus nickel flux (hr^{-1}, expressed as the ratio of peeper cell nickel concentration to the surrounding or pore-water nickel concentration in mg/L) for the first hour of all nickel tests.

equilibration rate of the peeper should, therefore, initially be rapid due to a high concentration gradient, but the net movement should begin to decrease as the number of molecules in the peeper increases. The total amount of molecules diffusing out of the cell increases, eventually approaching the amount diffusing in. This would result in a progressively more gradual equilibration rate between peeper and surrounding solution as seen in Figure 2.2.

With respect to solutions of charged particles such as nickel and zinc ions, movement is not only due to a concentration gradient, but also to an electrical field produced by the motion of the associated anion, Cl^- (Robinson and Stokes, 1959). The more mobile Cl^- ion will tend to diffuse faster than the hydrated metal ions (Li and Gregory, 1974) creating a minute electrical potential in the solution. In order to maintain electroneutrality, the positive and negative ions diffuse as a salt. This creates an increased rate of diffusion for the slower positive metal ion and a decreased rate of diffusion for the more mobile Cl^- (Robinson and Stokes, 1959; Li and Gregory, 1974).

Harper et al. (1997) found that by using the Einstein equation below (1), they could model equilibration time between peeper cells having various depths and a surrounding pore water metal solution.

$$t_e = L^2 / 2D_o \qquad (1)$$

Here L represents the depth of the peeper cell (cm), t_e is time (sec), and D_o is the tracer or self-diffusion coefficient (cm^2/sec). The equilibration time for the peeper cell is therefore dependant upon the D_o value and peeper cell depth (Harper et al., 1997). A list of some calculated D_o values for metal ions is given by Li and Gregory (1974). By calculating the D_o values for nickel and zinc salts (the formula for which is given by Van

Schaik et al. (1966)) adjusted to 23.5°C (the formula for which is given by Simpson and Carr, 1958) and assuming constant resupply surrounding the peeper face, we can calculate the theoretical time to equilibration in a 0.8 cm deep peeper cell.

From work by Harper et al. (1997), the times required for a peeper cell to achieve a mean concentration of 95% and 99% (in parenthesis) of that in the surrounding pore water is approximately $2.3t_e$ ($3.6t_e$), assuming no solute depletion surrounding the cell (complete resupply). At our experimental temperature of 23.5°C, the theoretical peeper equilibrium times are approximately 17.5 h (27.4 h) for nickel chloride and 16.9 h (27.1 h) for zinc chloride. The measured mean equilibration times were approximately 24 h (48 h) and 48 h (96 h) for nickel and zinc, respectively. These results suggest that resupply to the peeper cell was not complete and that there may have been a diffusion gradient around the cell face. Based on the theoretical diffusion coefficients for the Ni and Zn salts, $ZnCl_2$ should equilibrate almost as rapidly as $NiCl_2$. The reason for the longer equilibration time observed for $ZnCl_2$ is unclear.

2.4.2 Sand experiments

Diffusion for a given ion will always be slower in sediment pore water (D_s) than in overlying water (D_o), because the ions must follow a convoluted path around the sediment particles (Berner, 1980). The relationship can be expressed as:

$$D_s = D_o/\theta^2 \qquad (2)$$

where θ, tortuosity, is the average ratio of the actual distance a diffusing particle must travel through the sediment and the direct line distance in the direction of diffusion ($d\ell/dx$) (Harper et al., 1997). Molecular diffusion in sediments may be aided by

dispersion (slow groundwater flow), wave and current action, and bioturbation (Berner, 1980). While these forces can influence pore water solute movements *in situ*, they are not applicable to this laboratory study.

Solute diffusion into a peeper cell may create a surrounding zone of depletion. Conversely, desorption from the solid phase may resupply pore water metal concentrations surrounding the mini-peepers. When a trace element in solution is exposed to a solid surface, the element will be partitioned between the solution and solid phase by sorption reactions. At low solute concentrations, when surface binding sites are unsaturated, removal of pore water trace metal will result in desorption from the solid phase. This may resupply the pore water metal surrounding the peeper face thereby hastening the process of peeper equilibration (Harper et al., 1997). During our sand experiments, pore water nickel diffused into the overlying water. The concomitant drop in pore water metal concentration did not cause desorption from the solid phase, which would have been seen as an increase in total aqueous nickel (overlying water plus pore water). This suggests that desorption was not significantly influencing pore water metal concentrations in our sand experiments and that resupply to the peeper face was most likely the result of nickel diffusion through the sand and not desorption from the solid phase.

The coarse sand trial demonstrated that without desorption (resupply) from the sediment, equilibration relying upon diffusion proceeds more slowly in sediment than in a water-only situation. Solutes in sediment pore water will be slower to replenish areas of lower concentration because they must travel a convoluted path around the sediment particles. The slower resupply to the zone surrounding the face of the peeper results in a

slower peeper equilibration.

In the fine sand experiment, initial loss of aqueous nickel (adsorption to the solid phase), coupled with greater nickel diffusion into the overlying water, eliminated the slower final stages of equilibration seen in the water-only experiment. This brought the peeper cell and pore water nickel concentrations into equilibrium more rapidly than could be achieved by diffusion into the peeper alone. The rapid drop in pore water nickel in fine sand resulted not only in peeper equilibration by 24 h, but also in pore water concentrations lower than those of the peepers at 48 and 96 h. It appears that changing solute concentrations in low porosity sediments can result in longer equilibration times if resupply to the peeper is insufficient to maintain equilibration.

2.4.3   Natural sediment experiments

Sediment 1 and 2 appeared to have different nickel binding capacities. Sediment 2 had approximately three times more pore water nickel than Sediment 1 after spiking with the same amount of nickel. The differences in pore water nickel may have been attributable to different sediment aging protocols, but were more likely due to differences in primary binding phases such as organic carbon content, AVS, and particle size distribution (finer sediment has a greater cation binding capacity). The OC, AVS, clay, and silt contents in Sediment 2 were 2.6, 1.4, 2.5, and 2.2 times lower, respectively, than in Sediment 1.

According to Fick's First Law for sediments (Berner, 1980), the flux of solute from a sediment to the overlying water is:

$$J_s = - \phi D_s (\partial C / \partial x) \tag{3}$$

where J_s is the diffusional flux (mass per unit area of total sediment per unit time), $\partial C/\partial x$ is the concentration gradient in the sediment (the change in concentration with increasing sediment depth), and ϕ is porosity, the total volume of water in the sediment (assumed to be continuous) divided by the total volume of the sediment. Generally, natural sediments have higher porosities (0.894 and 0.821 for Sediment 1 and 2, respectively) than sand (0.36 and 0.39 for fine and coarse sand, respectively). According to Fick's First Law for sediments (3), this should result in a more rapid equilibration of the subsurface peeper cells as well as a more rapid diffusion of nickel into the overlying water. The results presented here show that the initial rate of peeper equilibration was correlated with sediment porosity (r^2=0.879), but factors altering pore water solute concentrations caused the equilibration to deviate from the equilibration curve seen in the water-only experiments. Again, as in the fine sand experiment, equilibration was rapid due to the added effect of the pore water nickel concentration rapidly decreasing to meet the increasing peeper cell concentration. Mass balance data showed that this was augmented by an initial loss of aqueous nickel in both natural sediments. One difference between the natural sediments and the fine sand was that the subsurface peepers reached equilibrium at a higher proportion of the initial pore water concentration in the natural sediments than in the sand (approximately 64 % for both Sediment 1 and 2 compared to 32% for the fine sand) indicating a more rapid resupply to the peeper face. Once equilibrated, this resupply was sufficient to maintain the subsurface peeper equilibrium with pore water. Carignan et al. (1985) found that equilibration of dialysis samplers (with 6 mm deep cells) occurred in less than a week for iron, zinc, nickel and organic carbon in sediment (porosity 0.77 to 0.70) at room temperature. The sediment had been

allowed to sit for three months and equilibrate with the overlying water prior to experimentation.

Until 96 and 192 h (Sediment 2 and 1, respectively) the overlying peeper cells in the natural sediment experiments showed higher concentrations of nickel than the corresponding composite overlying water samples. This likely reflects a diffusional nickel concentration gradient in the overlying water with the highest concentrations occurring at the sediment surface, an area likely sampled to a greater extent by the peeper than by pipetting.

2.4.4 Sampling precision

The average precision in pore water metal data (sand and sediment) obtained from replicate samples was generally the same when sampled using mini-peepers and when isolated by centrifugation/filtration (10.9 % ± 3.4 and 10.5 % ± 10.2, respectively). Considering their ease of use and their possible deployment in actual sediment toxicity test beakers, the use of mini-peepers appears to be a logical option for obtaining accurate and precise pore water contaminant data for sediment toxicity and bioavailability tests.

2.4.5 Recommendations on mini-peeper use

Care should be taken to allow metal-spiked sediment time to equilibrate within the test system and to allow inserted peepers to properly equilibrate with the sediment pore water prior to experimentation. This should reduce the chances of obtaining inaccurate measurements due to slow peeper equilibration in less porous sediments, or due to the presence of steep contaminant concentration gradients immediately above the

sediment surface. While this study did not explore the effect of peeper cell volume on the surrounding pore water chemistry, it is recommended that peeper cell volumes be kept small relative to the overall pore water volume to minimize the dilution factor. The influence of the peeper cell on surrounding pore water solute concentrations may need to be addressed in future research. While these experiments did not involve benthic organisms, mini-peepers have been successfully used in a number of sediment toxicity tests with various trace metals (Liber and White-Sobey, 2004; in review). Their use in measuring organic pore water contaminants remains to be investigated.

2.5   Conclusions

Given an appropriate equilibration period (approximately 96 h), mini-peepers appeared to work well for measuring sediment pore water and overlying water metal concentrations in laboratory tests. The period of equilibration is longer in low-porosity sediment, but appears to be well within the 10-d minimum period required for standardized sediment toxicity tests. Changes in aqueous metal concentrations (sorption reactions or diffusion into the overlying water) may affect the peeper equilibration period.

CHAPTER 3

Characterization of dissolved organic matter

3.1   Introduction

Humic substances are complex mixtures of large, biogenic, heterocyclic, refractory molecules occurring in all terrestrial and aquatic environments (Aiken et al., 1985; Perdue, 1998). Often comprising a significant proportion of the organic matter in surficial water and sediments (Jackson, 1975; Thurman and Malcolm, 1981; Pempkowiak et al., 1998), humic substances play an important role in a number of aqueous processes which include (but are not limited to) carbon and nutrient cycling, geochemical weathering, trace metal complexation and sorption of nonpolar organic compounds (Woodwell et al., 1978; Jones et al., 1993; Rashid, 1971; Zhang et al., 1996; Penttinen et al., 1998; Gauthier et al., 1987). While the functions of humic substances in surficial water and sediments are of significant ecological and geochemical importance (see review by Jackson, 1975), it is the ability of humic substances to complex or sorb contaminants that is of primary ecotoxicological importance.

Although not well understood, the structural characteristics (i.e., aromaticity, functional group content) of dissolved organic matter (DOM) may influence the extent to which contaminant sorption or complexation occur in solution (Gauthier et al., 1987; Schnitzer and Skinner, 1965). Past studies have found that humic substances from different source environments may vary in general chemistry (Malcolm, 1990) due to a

variety of interacting factors (see review by Vandenbroucke et al., 1985). These general differences may in turn affect the binding tendencies of various contaminants (Gauthier et al., 1987). To better understand contaminant bioavailability in soils, sediments and surface waters, it is therefore important to gain a better understanding of DOM chemistry and structural variability from these various sources.

Over the past several decades sedimentary organic matter has become more thoroughly characterized, especially with the advent of NMR technology. But while all types of sediment (estuarine, marine, freshwater) have begun to receive more attention, aside from the Laurentian Great Lakes area of North America, data on humic substances isolated from Canadian freshwater sediments appear to be sparse.

The objectives of this work were to chemically characterize humic and fulvic acids isolated from a northern Saskatchewan sediment and to compare their general structural and chemical attributes to those of (i) similar compounds extracted from commercially available peat moss, (ii) Suwannee River humic substances (purchased from the International Humic Substances Society, IHSS), and (iii) other DOC sources described in the open literature. For future research purposes, it is important to determine whether the latter two DOM sources are suitable analogues for sedimentary humic substances in metal toxicity testing. Without the use of chemical extractants (e.g., NaOH), pore-water DOM isolation and fractionation is time consuming and costly. Readily available (e.g., Suwannee River humic substances) or easily extractable (e.g., peat) pore-water DOM analogues are therefore desirable.

This research employed a variety of analytical and spectroscopic methodologies in attempts to evaluate both the nature (the general chemical and structural attributes)

and the source (allochthonous vs autochthonous) of Little Bear Lake sedimentary humic substances relative to Suwannee River and peat humic substances. Due to the heterogenous and ill-defined nature of humic substances and the limited information generated from any one type of analysis, a battery of methods was employed to better evaluate chemical and structural similarity of the DOM sources that would later be used in associated metal bioavailability studies.

3.2 Materials and methods

3.2.1 Dissolved organic matter isolation/fractionation

Little Bear Lake pore-water DOM

Freshwater sediment was collected from Little Bear Lake (LBL) (54° 17' N, 104° 41' W), an oligotrophic lake in north-central Saskatchewan, Canada, which is known to be free from significant metal contamination. Using an Ekman grab sampler (15×15 cm), sediment samples were collected from the same site on October 16, 1999, September 27, 2000, and June 16, 2001. On all sampling dates, sediments were placed in 20-L plastic pails and allowed to settle 1 h before the surface water was carefully decanted. The sediment was taken to the laboratory, homogenized, and refrigerated (4°C) within 24 h of collection. Pore water was collected by an initial centrifugation of sediment at a relative centrifugal force of 400 g (International Centrifuge model UV, International Equipment Co. Boston, MA, USA) for 20 min. Fine colloids were further removed via centrifugation twice at 17,200 g (Sorvall® Superspeed Refrigerated Centrifuge, Model RC5C, Kendro Laboratory Products, Newtown, CT, USA) for 15 min. The final supernatant was then passed through a series of filters (1.2 μm, glass microfiber filter,

VWR Scientific Products, West Chester PA, USA; 0.50 μm, Metrigard™ glass fiber filter, Pall Corporation, Ann Arbor, MI, USA; 0.45 μm, Supor®- 450 polyethersulfone membrane filter, Pall Corporation, Ann Arbor, MI, USA) ending with a filter pore size of 0.45 μm. This filtrate was stored in 4.5-L glass jugs and refrigerated until later fractionation into fulvic and humic acids.

Due to the low pore-water DOC content of Little Bear Lake sediment, limited quantities of humic substances were available for analysis. A dilute NaOH extraction was therefore carried out on the sediment to supply material for analyses requiring large quantities of test-substance. While extraction, concentration, and fractionation processes may all influence the amounts and possibly the nature of sedimentary and soil humic substances (Cronin and Morris, 1982; Hayes, 1985), a dilute NaOH extraction was chosen for its efficiency in extracting humic substances and for better comparison to other published studies. For this process, Little Bear Lake (LBL) sediment (3.0 kg wet weight) was placed in a plastic bucket under a N_2 atmosphere with 3 L of 0.1 M NaOH for 48 hours. The sediment was then thoroughly mixed and processed following the pore water isolation protocol described above. While this extraction process may have a slight oxidizing effect (Hayes et al., 1975), it has been reported that the elemental composition and infrared spectra are relatively unaffected (Vandenbroucke et al., 1985).

Fractionation protocols were similar to those described by Thurman and Malcolm (1981). Briefly, the filtered pore water or NaOH extract was acidified with 1 M HCl (BDH Inc., Toronto, ON, Canada) to pH 2 and passed through a pre-cleaned (cleaning procedure as described by Thurman and Malcolm, 1981), pre-acidified Amberlite® XAD-8 resin (40-60 mesh, Rohm and Haas Canada Inc., West Hill, ON, Canada) column. The

adsorbed humic substances were then eluted with 0.1 M NaOH (BDH Inc., Toronto, ON, Canada) and re-acidified to pH 1 to allow humic acid (HA) precipitation overnight. The following day the solution was centrifuged (12,000 to 22,500 g) for 15 min) and filtered (0.50 μm, pre-rinsed glass fibre filters) to remove the precipitated HAs. The HA filtrate was briefly rinsed with ultrapure water to remove excess NaCl and then redissolved in 0.1 to 1 M NaOH (depending upon the ease of dissolution). To further concentrate and to remove NaCl, the fulvic acid (FA) solutions were passed through a smaller XAD-8 column and rinsed with ultrapure water prior to elution. The final FA and HA solutions were passed through a cation exchange resin (AG® MP-50, Bio-Rad Laboratories, Hercules, CA, USA) in the hydrogen form to remove Na prior to lyophilization (Freezone® 4.5, Labconco Freeze Dry System Corporation, Kansas City, MO, USA). There was some difficulty in completely redissolving pore-water LBL HA in NaOH after the filtration process. The NaOH-extracted LBL HA was therefore rinsed with ultrapure water in a beaker to reduce the salt content. This LBL HA was not redissolved with NaOH nor passed through a cation exchange column prior to lyophilization as it tended to precipitate in the cation exchange resin.

Peat DOM

Peat moss DOM was chosen as a test substance due to the ease with which humic substances could be extracted with either water or NaOH. This allowed for the comparison of peat humic substances to those isolated from LBL pore-water or humic substances isolated via NaOH extraction of LBL sediment.

Briefly, dried, ground sphagnum peat moss (Sun Gro Horticulture, Seba Beach,

AB, Canada) with low trace element content (previously analyzed, data not presented) was placed in a plastic bucket. For each volume of dry, unsettled peat, an equal volume of ultrapure water was added, mixed thoroughly with a plastic spatula, and allowed to sit for 1 hour with occasional agitation. The solid peat was then squeezed by hand and the liquid extract sieved (100 µm) and centrifuged twice (17,200 g for 15 min) to remove coarse and fine colloidal material prior to filtration following the above methods. FA and HA fractions were obtained via the same fractionation protocol described above. Whole peat DOM (unfractioned) was obtained by passing the water-extracted peat DOM through the cation exchange resin prior to lyophilization.

Due to the low HA content of the water-extracted peat DOM, a NaOH extraction was carried out to increase HA yield. Peat (2 L dry, unsettled) was placed in a plastic bucket with 4 L of 0.1 M NaOH for 48 hours under N_2. The resulting DOM extract was similarly centrifuged, filtered and fractionated as described above for water-extractable peat.

Suwannee River humic substances

Suwannee River fulvic and humic acids were obtained from the International Humic Substances Society (St. Paul, MN, USA). The Suwannee River drains the Okeefenokee Swamp, a cypress wetland in southern Georgia, USA. These humic substances are thought to be primarily derived from the decay of terrestrial material (McKnight et al., 2001). They were chosen for this research because they are well characterized (data provided by the IHSS) and have become a common test substance in aquatic trace-metal research.

3.2.2 Dissolved organic matter characterization

Fulvic and humic acids are complex mixtures of molecules operationally defined based on their solubilities in acid or alkaline solutions. As a result, each fraction is chemically heterogenous and no single analytical test will completely describe both their general structure and chemistry. A battery of tests was therefore conducted to better evaluate the general differences between these DOM sources and fractions. For the sake of convenience, materials isolated without the use of NaOH will be referred to as water-extracted. Water-extracted materials (Suwanne River, LBL pore-water, peat) were analyzed using the following methods: elemental analysis, Fourier Transform Infrared spectroscopy (FTIR), fluorescence, $\delta^{13}C$, and COOH acidity (peat FA only). NaOH-extracted materials (LBL sediment, peat) were subjected to the following analyses: elemental analysis, ^{13}C-NMR, UV/VIS 272, and COOH acidity.

3.2.2.1 Elemental analysis

While elemental composition may be influenced by methods of isolation and purification (Hayes, 1985), the goal was to compare the general chemical composition amongst the three humic sources and fractions chosen. Elemental analysis was performed by Guelph Chemical Laboratories Ltd. (Guelph, ON, Canada) on LBL pore-water FA and HA, water-extracted peat FA, HA and unfractioned (whole) peat, as well as on NaOH-extracted LBL HA and peat FA and HA. Analyses of carbon, nitrogen, hydrogen and sulfur were performed with a Fisons/Carlo Erba CHNOS model EA1108 (Fisons Instruments USA, Denvers, MA, USA). Total phosphorus was measured via

ICP-AS spectroscopy (Liberty 110; Varian Inc., Mulgrave, Victoria, Australia). Ash

content was determined by ignition of samples at 750 °C in a Thermolyne Muffle

Furnace, model F47910 (Barnstead Thermolyne Instruments, Dubuque, IA, USA) for 2

h. Oxygen was calculated by subtraction after elemental composition was corrected for

moisture ($5.9 \pm 0.3\%$) and ash content (based on protocols of Huffman and

Stuber,1985).

3.2.2.2 FA/HA pore water composition

Due to the scarcity of data on characteristics of pore water DOM, it was

considered important to evaluate pore-water humic substance composition. FA and HA

pore-water fractions were subsampled and analyzed for DOC content using either a

Shimadzu TOC-5050A Total Organic Carbon Analyzer (Shimadzu, Tokyo, Japan) prior

to lyophilization, or with a Perkin-Elmer® 2400 CHN Elemental Analyzer (Perkin-

Elmer®, Norwalk, CT, USA) after lyophilization. Total FA and HA results were then

compared to the total carbon content of the original pore-water sample.

3.2.2.3 FTIR

Fourier Transform Infrared spectroscopy utilizes the absorption of infrared

radiation by matter. This can provide information concerning specific functional groups

or structural entities within a molecule. While complex mixtures of molecules such as

fulvic and humic acids yield simple spectra with broad bands, these spectra, considered

along with other measures, yield information regarding the net functional group content

of the various DOC sources (MacCarthy and Rice, 1985). Dried humic material (~1 mg)

was mixed thoroughly with 100 mg KBr (BDH Laboratory Supplies, Poole, England), compressed into a pellet, and placed into an infrared spectrometer (Model FTS-40, BIORAD Laboratories, Cambridge, MA, USA). The absorbance spectrum was then recorded for the region between 4000 cm^{-1} and 400 cm^{-1}. Spectra were obtained for the water-extracted FA and HA acid fractions for all three DOM sources. Interpretation of the absorption spectra were based on Bellamy (1975), MacCarthy and Rice (1985), Theng et al. (1966), Juo and Barber (1969), and Williams and Flemming (1980). The typical IR absorption bands are as follows: (i) a strong broad band at ~3400 cm^{-1} attributed to H-bonded OH groups; (ii) bands at 2934-2945 cm^{-1} attributable to aliphatic C-H bonds in methyl (CH_3) and/or methylene (CH_2) units; (iii) a pronounced peak at ~1720 cm^{-1} due to C=O stretching vibration due mainly to carboxyl groups; (iv) a shoulder at 1614-1629 cm^{-1} due to aromatic C=C vibrations in aromatic rings; (v) a peak at ~1400 cm^{-1} due to the O-H bending vibrations of alcohols or carboxylic acids; (vi) a peak at ~1220 cm^{-1} due to C-O stretching vibration and/or OH bending deformations of carboxyl groups and; (vii) a shoulder at 1067-1078 cm^{-1} due to C-O stretching in C-OH groups.

3.2.2.4 UV/VIS 272

Investigated by Gauthier et al. (1987), Traina et al. (1990) and Chin et al. (1994), absorbance in the 270-280 nm range is related to the degree of aromaticity of humic and fulvic acids, which in turn may indicate parent material source. Light absorbances (272 nm) were obtained using a DU® 640 Spectorphotometer (Beckman Coulter ™, Fullerton, CA, USA). All DOC solutions (40 - 50 mg C/L; similar to Gauthier et al.,

1987) were in a 0.2 M $NaNO_3$ solution of reconstituted water (described in Chapter 4, Table 4.1) adjusted to pH 7.0 ($\pm$ 0.1). Absorbance for this matrix was minimal since nitrate does not absorb radiation in this wavelength range (Chin et al., 1994). Solutions lacking DOM were used as blanks.

3.2.2.5 Fluorescence

Fulvic acids derived from either terrestrial or microbial parent materials contain fluorophores that differentially absorb visible and ultraviolet light. At an excitation wavelength of 370 nm, the ratio of the emission intensity at wavelengths of 450 and 500 nm has been found to differentiate between microbially derived fulvic acids (a ratio of ~1.9) and terrestrially derived fulvic acids (a ratio of ~1.4) (McKnight et al., 2001). The following methods are based on those of McKnight et al. (2001). Peat (water-soluble), Suwannee River FA and LBL FA (from pore water) were dissolved in ultrapure water and the pH adjusted to 2 with HCl. Final DOC concentrations were similar and were as follows: Peat FA, 17.4 $\pm$ 0.1; SRFA, 16.8 $\pm$ 0.1; and LBL FA, 15.5 $\pm$ 0.1 mg/L. The blank consisted of an ultrapure water sample (pH 2, DOC < 0.2 mg C/L). Fluorescence was measured using a SPEX 1680, 0.22m Double Spectrometer with a SPEX Fluorolog (Jobin Yvon Ltd, Edison, NJ, USA). Fluorescence intensity was measured at an excitation wavelength of 370 nm and at emission wavelengths between 400-700 nm at 1 nm increments. The fluorometer was set at a scan speed of 120 nm min^{-1} and a response time of 2 s. The intensity values of the emission spectra were adjusted by subtracting corresponding intensities of the blank.

3.2.2.6 NMR

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used to obtain semi-quantitative information about the carbon types in the humic substances. These samples included peat FA and HA, as well as LBL HA (all isolated via NaOH extraction). Similar to Benke et al. (1998), high-resolution solid-state ^{13}C-NMR spectra, with cross polarization (CP) and magic-angle spinning (MAS), were obtained at 90.6 MHz on a Bruker AM360WB spectrometer (Bruker Optics Inc., Billerica, MA, USA). The spectra were determined with a contact of 1.5 ms, a recycle delay of 1 s, a 90° pulse of 6.5 μs, and the number of scans ranged from 7,900 to 25,000. The chemical shift ranges used to estimate the relative carbon bond content (Table 3.1) were based on those of Knicker and Lüdemann (1995) and Dria et al. (2002). Regions were also integrated for COOH, aliphatic and aromatic content. The aliphatic region integrated included the region extending from 0 to 90 and one-fifth of the integrated region from 90 to 120. The aromatic portion consisted of the remainder of the 90 to 120 integrated region plus the 120 to 160 region. This methodology accounts for the contribution of anomeric carbon in carbohydrates to the 90-120 region typically assigned to aromatic carbons (Bates et al., 1991; Dria et al., 2002). The percent C contributed by COOH groups was determined from the integrated region of 160 to 190 (Hatcher et al., 1980; Malcolm, 1990).

3.2.2.7 $\delta^{13}C$

The $^{13}C/^{12}C$ isotopic ratio for DOM may reveal the parent material source. Isotopic analysis was performed on the water-soluble FA fractions in an attempt to

Table 3.1. ^{13}C NMR bond assignments for integrated regions of humic substances.

Integration regions (ppm)	Assignments and interpretations[a]
0-45	Paraffinic carbons from lipids and biopolymers
45-60	Methoxyl, mainly from lignin, and amino groups
60-90	Carbohydrate carbons
90-120	Carbohydrate and proton-substituted aromatic carbons
120-140	Carbon-substituted aromatic carbons
140-160	Oxygen substituted aromatic carbons
160-190	Carboxyl and aliphatic amide carbons
190-220	Aldehyde and ketone carbons

[a] Taken from Dria et al. (2002) and Knicker and Lüdemann (1995).

characterize the source materials of LBL sediment, peat and Suwannee River organic matter, and to compare the findings with previous isotopic work (e.g., Schiff et al., 1990). All ^{13}C analyses of dried fulvic acid samples were done on a Europa Scientific Gas/Solid/Liquid Preparation Module coupled to a tracer/20 Mass Spectrometer (PDZ Europa, Chesire, England) and reported relative to the International Atomic Energy Agency (IAEA, Vienna) certified calibrated standard materials as δ^{13}C, where

$$\delta^{13}C = [(R_{sample}/R_{standard})-1] \times 1000$$

and R equals the ^{13}C/^{12}C ratio in the combusted sample or standard. A more negative number means greater ^{12}C content or that ^{13}C is lighter in mass, whereas a more positive number indicates more ^{13}C relative to ^{12}C content.

3.2.2.8 Acidity

Acidity measurements were made on NaOH-extracted humic substances following methods similar to Perdue et al. (1980). Due to limited sample quantities, peat FA and HA, and LBL HA were the only samples analysed for carboxyl acidity. Humic or fulvic acids were dissolved in a 0.1M NaCl solution (330-560 mg C/L) and allowed to sit overnight under a N_2 atmosphere at $24 \pm 1°C$. Aliquots (50 ml) were then titrated (under a N_2 stream) to pH 7.6 (Shuman 1990) with additions of 0.1 M NaOH.

3.3   Results

3.3.1   Elemental analysis

The elemental composition and atomic ratios for those humic substances analyzed are listed in Table 3.2. Elemental composition data found in the literature for

comparable environmental DOC sources are presented in Table 3.3. Consistent with the literature, C, O, H, N and S are the main elements present in LBL and peat humic substances. Of the fulvic acid fractions, Suwannee River and peat FA appear to be the most similar in elemental composition, while LBL FA is slightly lower in C and higher in O content. Little Bear Lake FA and HA were both higher in N and H content than the corresponding peat and Suwannee River humic fractions. This resulted in lower C/N and higher H/C ratios for LBL humic substances relative to the peat and Suwannee River fractions. The molar O/C ratio vs the H/C ratio graphed from Tables 3.2 and 3.3 has been in Figure 3.1 (a Van Krevelen plot). Data from Table 3.3 has been plotted as peatlands FA and sedimentary (lake) FA and HA. The sedimentary humic substances in general appear to have higher H/C ratios whereas the FAs seem to have higher O/C ratios.

3.3.2 FA/HA pore water composition

The FA and HA contents of pore water and water-soluble peat are given in Table 3.4. Pore-water humic substances make up ~27% of the total pore-water DOC and of that ~72% are FAs. Water-extracted peat DOC is 47% humic substances with >99% consisting of FAs.

3.3.3 FTIR

The FTIR spectra for SR, LBL pore water, and peat FAs are shown in Figure 3.2 (a) with the corresponding HAs shown in Figure 3.2 (b). While the magnitude of the peaks can not be compared directly, there is a qualitative similarity in the net functional

Table 3.2. Elemental analysis of Suwannee River, Little Bear Lake and peat humic substances.

Source of humic substances	Fraction	Elemental analysis (% by weight ± SD of the means)						Atomic ratios (± SD of the means)		
		C	H	O	N	P	S	C/N	H/C	O/C
Suwannee River[a]	Fulvic acid	52.44	4.31	42.2	0.72	<0.01	0.44	84.9	0.98	0.6
	Humic acid	52.55	4.4	42.53	1.19	<0.01	0.58	51.5	1	0.61
Little Bear Lake	Fulvic acid[b]	46.93 ±0.16	5.35 ±0.04	46.00 ±0.26	1.32 ±0.07	<0.01	0.39 ±0.06	41.6 ±2.2	1.36 ±0.02	0.74 ±0.01
	Humic acid[b]	31.53 ±0.53	5.33 ±0.05	59.49 ±0.58	3.58 ±0.08	0.17 ±0.02	<0.01	10.3 ±0.1	2.02 ±0.05	1.41 ±0.04
	Humic acid[c]	49.38± 0.22	7.25 ±0.31	37.69 ±0.50	5.67 ±0.04	NA	NA	10.2 ±0.1	1.75 ±0.07	0.57 ±0.01
Peat	Fulvic acid[b]	51.97 ±0.23	4.43 ±0.13	42.74 ±0.30	0.87 ±0.06	<0.01	<0.01	70.1 ±5.3	1.01 ±0.03	0.62 ±0.01
	Fulvic acid[c]	53.15 ±0.36	4.44 ±0.25	41.37 ±0.50	1.04 ±0.10	NA	NA	60.2 ±6.4	1.00 ±0.05	0.58 ±0.01
	Humic acid[c]	55.38 ±0.17	4.79 ±0.21	37.70 ±0.04	2.13 ±0.00	NA	NA	30.3 ±0.1	1.03 ±0.05	0.51 ±0.001
	Unfractioned[b]	37.28 ±0.04	4.85 ±0.07	54.26 ±0.05	3.60 ±0.09	0.11 ±0.02	<0.01	12.1 ±0.3	1.55 ±0.02	1.09 ±0.002

[a] Provided by or calculated from, information provided by the International Humic Substance Society (St. Paul, Mn, USA), moisture-corrected, ash-free.

[b] This study, moisture-corrected, ash-free, pore-water extraction.

[c] This study, moisture-corrected, ash-free, 0.1 M NaOH extraction.

Table 3.3. Elemental analysis of various humic substances found in the literature.

Source	Elemental analysis (% by weight ± SD of the means)				Atomic ratios (± SD of the means)		
	C	H	O	N	C/N	H/C	O/C
Peatland topsoil Fulvic acid[a]	43.5	4.3	50.8	1.6	26.2 ±3.1	1.25 ±0.13	0.86 ±0.16
Fulvic acid[b]	44.5	4.3	49.8	1.5	30.9 ±3.6	1.11 ±0.05	0.83 ±0.08
Lake Sediment Fulvic acid[c]	44.98 ±3.90	5.12 ±1.24	42.27 ±4.69	7.63 ±0.56	6.80 ±0.93	1.34 ±0.24	0.72 ±0.15
Fulvic acid[d]	51.75 ±1.47	6.55 ±0.72	38.96 ±1.34	2.74 ±1.22	26.20 ±11.43	1.51 ±0.21	0.57 ±0.03
Humic acid[c]	52.05 ±3.61	5.67 ±0.65	36.55 ±4.27	5.63 ±1.08	10.75 ±2.08	1.30 ±0.13	0.53 ±0.09
Humic acid[d]	54.85 ±1.39	5.19 ±0.60	36.42 ±1.51	3.54 ±1.69	23.65 ±15.33	1.13 ±0.15	0.50 ±0.03

[a] Fulvic acids isolated (water-soluble) from grassland topsoil in the Droemling fen area (intact peatlands), Germany (Kalblitz et al., 1999), ash-corrected.
[b] Fulvic acids isolated (water-soluble) from woodland topsoil in the Droemling fen area (intact peatlands), Germany (Kalblitz et al., 1999), ash-corrected.
[c] Sedimentary fulvic and humic acids isolated from 22 lakes, summarized from various authors in Ishiwatari (1985).
[d] Sedimentary fulvic and humic acids isolated from 10 lakes, Latvia (Klavin and Apsite, 1997). Atomic ratios are ± SE of the mean. Acid pretreatment (0.5 N HCl) and 0.5 N NaOH extraction.
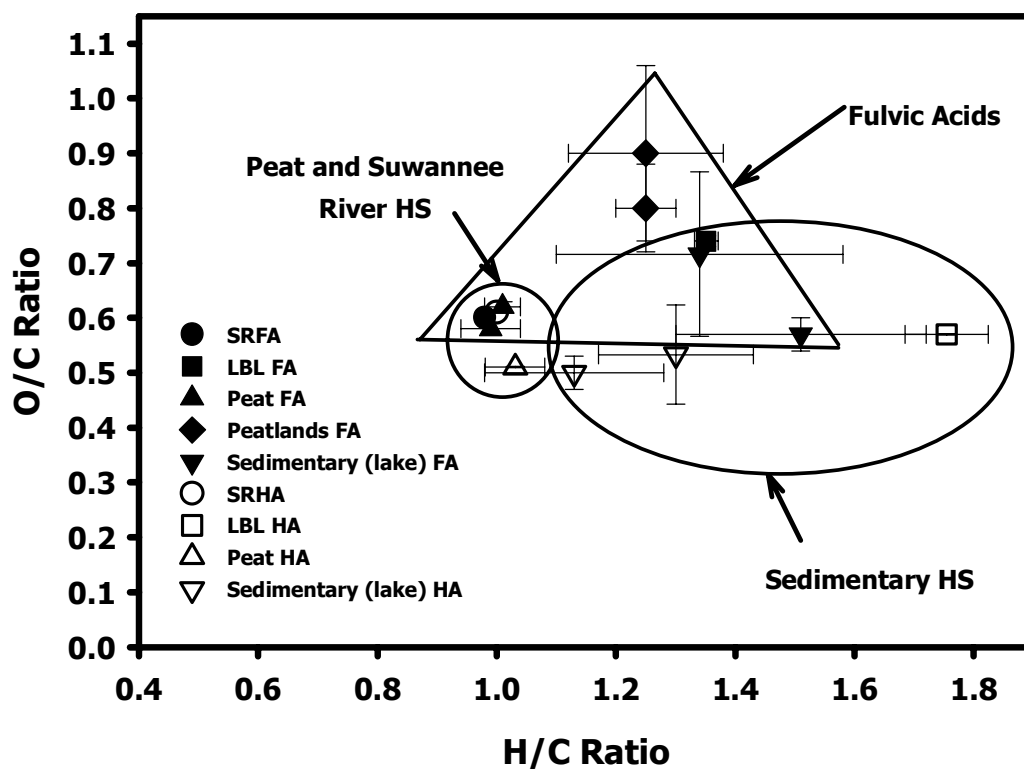
Figure 3.1. The O/C molar ratio versus the H/C molar ratio for humic substances (HS) described in the literature and isolated from Little Bear Lake (LBL), peat, and Suwannee River (SR). Data are means ± 1 SD.

Table 3.4. Fulvic and humic acid composition of Little Bear Lake pore water and water-extracted peat DOC.

	Humic Substances (% of total DOC ± 1 SD)	Relative Proportion of Total Humic Substances (± 1 SD)	
		FA (%)	HA (%)
Little Bear Lake pore water DOC[a]	26.5 ± 9.0	71.7 ± 5.2	28.3 ± 5.2
Peat DOC[a]	47.0 ± 15.0	99.4 ± 0.5	0.63 ± 0.5

[a] This study, water-extracted material.
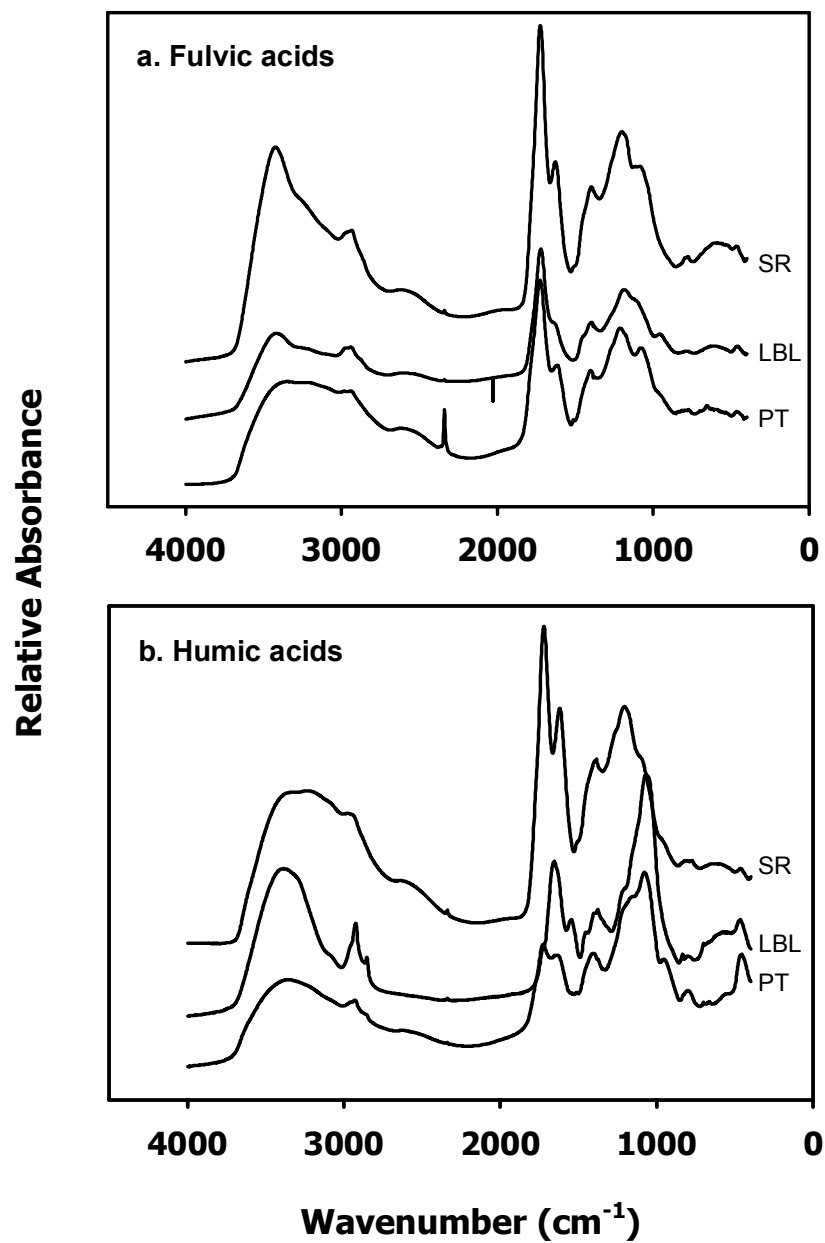
Figure 3.2. Fourier transform infrared spectra for Suwannee River (SR), Little Bear Lake pore-water (LBL), and peat (PT) fulvic and humic acids.

group content among the various FAs and HAs. The spectra are similar and show absorption bands previously reported to be characteristic of fulvic and humic acids (MacCarthy and Rice, 1985). The ratio of absorbances at 1650 cm^{-1} (C-H stretching in aromatic rings) and 2950 cm^{-1} (C-H stretching in methyl and methylene groups) is used as a relative measure of aromaticity (Pempkowiak, 1998). The calculated ratios for the FA fractions are given in Table 3.5. According to this measure, SR FA has the highest aromaticity followed by LBL FA and water-soluble peat. It appeared that there was salt contamination in the peat and LBL HA samples used for FTIR analysis. Due to the possible reduction or elimination of HA spectra peaks (1720, 1220) as a result of salt formation, the HA spectra were not used to evaluate aromaticity.

3.3.4   UV/VIS 272

The absorptivities (L·mg^{-1}·cm^{-1}) for the various test substances at 272 nm, and similar values for selected substances from the literature, are listed in Table 3.5. Both peat and LBL HAs appear to be more aromatic relative to the FA fraction. The LBL HA and FA solutions were lower in absorptivity (less aromatic) than their peat counterparts. All HAs isolated were more aromatic than the marine humic acids reported in the literature.

3.3.5   Fluorescence

The fluorescence spectra for the fulvic acids of peat, Suwannee River and LBL pore-water, at an excitation wavelength of 370 nm, are presented in Figure 3.3. The intensity of fluorescence per unit C has been shown to be variable and unreliable as an

Table 3.5. IR and UV-VIS spectroscopic properties of humic substances.

Humic material	IR spectra ratios[a] (A_{1650}/A_{2950})	UV-VIS spectra[b] (absorptivity[c])
SRFA	1.55	
Peat HA	n.p.	57.4
Peat FA	1.26	41.2
LBL HA	n.p.	43.6
LBL FA	1.48	31.2
Marine sedimentary humic acids[d]	-[g]	2.6-10.1
Soil humic acids[e]	-	12.0-46.0
Soil fulvic acids[f]	-	10.0-16.0

[a] All water-extracted or water soluble.
[b] All 0.1 M NaOH extracted.
[c] Absorptivity values are given in units of $(L \cdot mg^{-1} \cdot cm^{-1}) \times 10^3$.
[d] Gauthier et al. (1987). Total of 6 sites.
[e] Gauthier et al. (1987). Total of 5 sites.
[f] Gauthier et al. (1987). Total of 3 sites.
[g] Not reported.
n.p. = not performed.
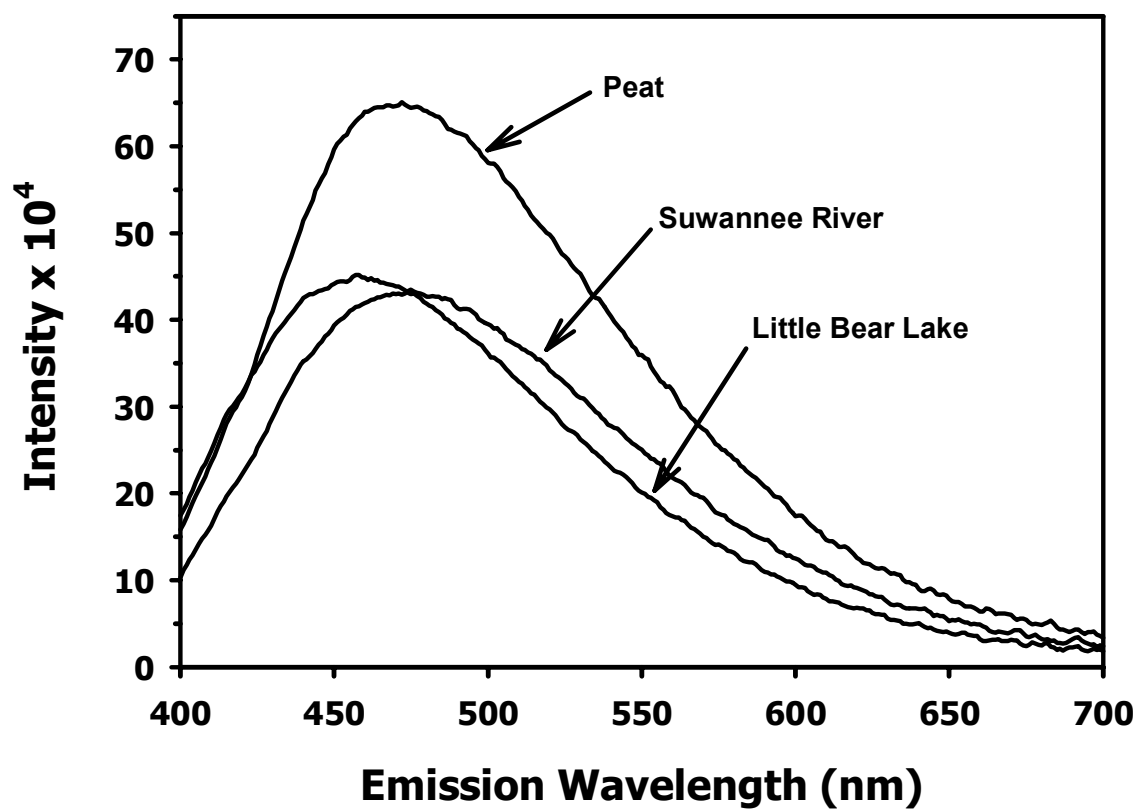
Figure 3.3. Fluorescence spectra for Suwannee River, Little Bear Lake pore water, and peat fulvic acids at an excitation wavelength of 370 nm.

indicator of chemical characteristics (McKnight et al., 2001) and is therefore ignored. The fluorescence indices (the ratio of emission intensity [450 nm/500 nm] at 370 nm excitation) and peak wavelengths are listed in Table 3.6. The greater the index ratio, the lower the aromatic content. The fluorescence index was highest for LBL FA while peat FA and SR FA were similar.

3.3.6 NMR

The CP-MAS ^{13}C-NMR spectra for peat FA and HA and LBL HA are given in Figure 3.4. Relative to the peat fractions (which appear to be very similar), the LBL HA is noticeably different in the allocation of C bonds. The various integrated spectral regions expressed as a percent of the total carbon are listed in Table 3.7. Values from the literature (Malcolm, 1990) for Suwannee River FA and HA are also listed in Table 3.8. The peat HA is lower in COOH content (24.1%) and slightly more aliphatic (+11.6%) relative to the FA fraction. The aromatic content is similar for the peat fractions. Relative to peat HA, the LBL HA spectrum is much lower in COOH (-33.8%) and in aromatic content (-33.8%), and higher in aliphatic C content (+24.1%, due mainly to paraffinic content in the 0-45 region). From table 3.8 data, the C distribution for peat FA is comparable to SR FA for aromatic and carboxyl content and peat HA appears similar to SR HA and soil HA for carboxyl content, but is lower in aromatic C content.

3.3.7 δ^{13}C

The results of the δ^{13}C analysis were similar for the three fulvic acids tested and are as follows; LBL FA = -27.73 ± 0.01; SR FA = -27.99 ± 0.04; peat FA = -26.73 ±

Table 3.6. Fulvic acid fluorescence indices.

	DOC (mg/L ± 1 SD)	Fluorescence index[a]	Peak wavelength (nm)
Little Bear Lake fulvic acid[b]	15.5±0.1	1.24	457
Suwannee River fulvic acid	16.8±0.1	0.99	475
Peat fulvic acid[c]	17.4±0.1	1.02	471

[a] The ratio of DOC fluorescence emission intensities at 450 nm and 500 nm using a 370 nm excitation wavelength.
[b] Isolated from pore water.
[c] Water-extracted.
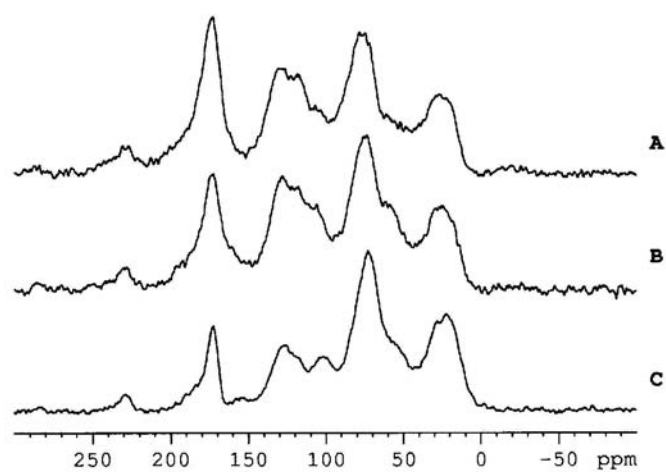
Figure 3.4.The CP-MAS ^{13}C-NMR spectra for (A) peat fulvic acid, (B) peat humic acid and (C) Little Bear Lake humic acid. All 0.1 M NaOH extracted.

Table 3.7. ^{13}C-NMR integrated spectral areas for peat and Little Bear Lake humic substances. Values are a percent of the total integrated area.

Region (ppm)	Peat FA	Peat HA	LBL HA
190-220	4.5	2.7	0.9
160-190	19.9	15.1	10
160-140	4.1	4.3	2.5
140-120	13.4	13.7	9.6
120-90	15.2	16.1	13.4
90-60	22.3	24.5	30.5
60-45	5.5	7.4	8.9
45-0	15	16.1	24.3

Table 3.8. ^{13}C-NMR integrated spectral areas for peat, Little Bear Lake, Suwannee River and soil humic substances. Values are the percent C of the total integrated area between 0 and 220 ppm.

Region (ppm)	Peat FA	Peat HA	LBL HA	SR FA[a]	SR HA[a]	Soil FA[a]	Soil HA[a]
Aliphatic	47.4	52.9	69.7	-[b]	-	-	-
Aromatic	28.3	29.3	19.4	25	38	21.7	36.7
Carboxyl	19.9	15.1	10	18	14	15.8	15.3

[a] Data from Malcolm (1990).
[b] Not provided

0.02.

3.3.8   Acidity

The results of direct potentiometric titrations for carboxylic acidity are given in
Table 3.9, along with Suwannee River data from Oliver et al. (1983). Water-soluble
peat FA had a higher COOH functional group content than the NaOH-extracted FA,
although both were similar to SR FA. The NaOH-extracted FA was 32% higher in
COOH acidity than the HA fraction. Little Bear Lake HA had the lowest COOH
content of the three HA sources.

3.4   Discussion

Humic substances often represent a significant proportion of the DOC in surface
waters (Thurman and Malcolm, 1981). While humic substances are also abundant in
sediments, their contribution to pore-water DOC composition is often ignored. Most
studies involve the sampling of pore-water for total DOC measurements, or perform
humic substance extractions from the solid phase. The 'natural' FA/HA composition of
the pore-water environment is poorly studied. This study found that humic substances
contribute significantly to LBL pore-water DOC content (26.5 ± 9.0 %) and could
therefore play an important role in metal or nonpolar organic chemical speciation in
pore-water.

Among the various humic sources and fractions studied there appear to be broad
chemical similarities. Qualitatively, the FTIR spectra illustrate this similarity and show
absorption bands reported to be characteristic of fulvic and humic acids (MacCarthy and

Table 3.9. Carboxylic acidity (NaOH titration) of fulvic and humic acids from three sources. Data are means ± 1 SD.

Source	Fraction	COOH acidity (μeq/mg C)
Suwannee River[a]	FA	11
	HA	8.2
Peat	FA[b]	12.9±0.1
	FA[c]	11.1±0.1
	HA[c]	7.3±0.6
Little Bear Lake	HA[c]	3.7±0.1

[a] Oliver et al. (1983).
[b] Water-extracted.
[c] NaOH extracted.

Rice, 1985). There are also strong similarities in the major bands present in the ^{13}C NMR spectra of the humic acids. It is proposed that these similarities in source and fraction are due to the similar functional group contents resulting from partial degradation of plant components (Wershaw et al., 1990). The differences between these groups lie mainly in relative abundance. Quantitatively, there appear to be subtle differences between the source and fraction of humic substances. Similar to the findings of Klavins and Apsite (1997), HAs (regardless of origin) were found to have lower O/C ratios (lower O, higher C) than the corresponding FA fraction. Humic acids from surface waters or sediments are generally known to have a higher C content than the FA fractions (Vanderbroucke et al., 1985; Klavins and Apsite, 1997). The higher O content in FA has been linked with higher functional group content (Klavins and Apsite, 1997). In this study, NaOH-extracted peat HA was found to be 32% lower in COOH acidity relative to the corresponding FA. Similarly, the ^{13}C-NMR results found peat HA to have ~24% lower COOH content than the FA fraction. Other published data support that FA fractions tend towards higher acidity than the corresponding HA fractions. For example, from a variety of source environments, Oliver et al. (1983) found a 40% higher carboxyl content in the FA fraction compared to the HA fraction. Other studies utilizing ^{13}C-NMR analysis (e.g., Cook and Langford, 1998) have also found FA to be higher in carboxyl functional group content relative to the corresponding HA fraction.

It is likely that the water-extracted peat FA was higher in acidity than the NaOH-extracted FA because it represented material that was more soluble. The solubility of humic substances is known to be directly related to functional group content (Oliver et al., 1983). High molecular weight organic matter dissolved in anoxic pore waters is the

partially degraded portion of labile organic matter in the sediment (Orem et al., 1986).

Degradation may reduce molecular weight and increase functionality allowing degraded

material to dissolve in pore water (Orem et al., 1986). Pore-water DOM may therefore

be more acidic relative to NaOH-extracted material. This may have implications for

modeling of metal speciation in pore-waters using NaOH-extracted materials as

analogues.

Understanding the source of sedimentary material is important in interpreting the

chemistry of the associated humic substances relative to those from other environments.

While the DOM sources evaluated here are to some extent the result of aquatic

degradation processes, this study attempted to evaluated the extent of influence of the

parent materials on the chemical nature of the final organic material (i.e., whether the

parent material was allochthonous, autochthonous, or a combination of the two). From

the variety of methods employed it would appear that there are chemical and structural

differences due to the source environments of these humic substances. For example, the

higher nitrogen content in the sedimentary humic substances (likely due to greater

autochthonous material input) is consistent with previous studies where the atomic N/C

ratio is higher in lake sediment than in soil (Ishiwatari, 1985) or peat (Ishiwatari, 1972).

The higher H/C ratio for sedimentary humic substances (see Figure 3.1) is due the

greater aliphatic content in sediment relative to terrestrial sources (Ishiwatari, 1972;

Klavins and Apsite, 1997). While sedimentary humic substances have been found to be

higher in aliphatic content relative to terrestrial carbon sources (Hatcher et al., 1980),

peat also has a significant aliphatic portion in its structure (Hatcher, et al., 1980). The

aliphatic content in both peat and sediment organic matter may be the result of

degradation of algal or bacterial remains (Orem and Hatcher, 1987). Little Bear Lake

HA may be higher in parafinic content (0-45 region in ^{13}C-NMR) due to greater algal

and microbial input relative to the peat. The similarity in elemental composition between

the peat and Suwannee River FAs could be due to their terrestrial origin and similar

degradation processes.

The less involved spectroscopic methods used to evaluate the sources and nature

of these humic substances generally compare their relative degree of aromaticity.

Microbially derived organic matter is lower in aromaticity than terrestrial organic matter,

which is more abundant in lignin (Hatcher et al., 1980). The greater absorption of light

at 272 nm indicates higher aromatic content. Our results (Table 3.5) suggest that the

water-extracted (pore-water) LBL humic substances are less aromatic than their peat

counterparts, but are more aromatic than marine sedimentary humic substances produced

autochthonously (Gauthier et al., 1987). The fluorescence ratio of emission intensity

(450 nm/500 nm at 370 nm excitation) is also used as an indicator of aromaticity. It was

found by McKnight et al. (2001) that indices for microbially-derived FAs were ~1.9,

while terrestrially derived material (such as Suwannee River FA) was ~1.4. While the

fluorescence indices in this study (Table 3.6) are lower than those of McKnight et al.

(2001) and therefore not comparable, the lower fluorescence indices for peat and SR FA

suggest they are more aromatic than LBL FA and are likely the result of significant

terrestrial organic matter input. Again, LBL FA appears to be more autochthonous in

composition relative to peat and Suwannee River FA.

While the range of DOC precursors such as terrestrial C_3 plants (predominant in

temperate regions) and aquatic phytoplankton overlap in δ^{13}C signature (Schiff et al.,

1990), this study attempted to determine which parent-material source predominated in the three humic substances investigated. Temperate lake DOM composition may be influenced by leachates from the soil and plants of the surrounding watershed, as well as from algae and bacteria in the water column and sediment (McKnight et al., 1994). Organic matter predominantly derived from planktonic sources (such as marine sedimentary humic acids) have $\delta^{13}C$ values in the −20 to −26 % range (Nissenbaum and Kaplan, 1972; Cronin and Morris, 1982; Deines, 1980). Soil humic acid (terrestrial input) has a $\delta^{13}C$ value closer to the −25 to −26 % range which falls within the $\delta^{13}C$ range of carbon for most trees and temperate-zone plants (Nissenbaum and Kaplan, 1972). Schiff et al. (1997) found that a number of Canadian Precambrian streams and groundwaters north of Toronto, Canada, were confined to a narrow $\delta^{13}C$ range from −25.5 % to −28.6 % with the majority around −27.0 %, and probably resulted from significant terrestrial input. Our results are similar to these values and those previously found for streams, wetlands, soil percolates and groundwater from central Ontario (Schiff et al., 1990). Little Bear Lake lies in a northern temperate area where the terrestrial vegetation is comprised predominantly of C_3 plants having $\delta^{13}C$ values averaging −28 % (O'Leary, 1988). The $\delta^{13}C$ content of LBL sedimentary DOM is similar to that of peat and Suwannee River humic substances and from this measure appears to be predominantly terrestrial in origin. However, care must be taken in this interpretation. There is no distinct break in the $\delta^{13}C$ values for autochthonously or allochthonously derived sediment organic matter, the $\delta^{13}C$ ranges for Canadian lake sediments are not well studied, and there are processes that, in principle, could affect $\delta^{13}C$ signatures (Deines, 1980). For example, sediment $\delta^{13}C$ not only shows a

correlation with parent material, but also with latitude (Deines, 1980). Overall, our $\delta^{13}C$ results do not suggest the domination of algal precursors in the formation of LBL lake sedimentary FA, but do not preclude their contribution.

Implications for contaminant partitioning and bioavailability

As mentioned previously, general chemical and structural differences between DOM sources and fractions (such as aromaticity) may affect the binding tendencies of various contaminants (Gauthier et al., 1987). Because carbon source influences the chemistry and structure of sedimentary organic matter, sedimentary humic substances will differ depending upon the autochthonous and/or allochthonous C input during sedimentary diagenesis. Partitioning models which consider total C content only may need to further consider the "quality" of the organic matter (i.e., aromaticity) which has been demonstrated to strongly influence contaminant complexation (Gauthier et al., 1987). Due to predominantly terrestrial origins (and hence higher aromaticity), Suwannee River humic substances may therefore not be the optimal analogues to use when evaluating hydrophobic organic contaminant partitioning to sedimentary humic substances (either dissolved or solid-phase).

Similar to hydrophobic organic contaminants, metal complexation may also be influenced to various degrees by differences in the structural nature of organic matter. For example, previous studies have suggested aromatic salicyclate and phthalate-like structures are important in metal complexation (Schnitzer and Skinner, 1965; Schnitzer, 1969). Leenheer et al. (1998) present NMR evidence to suggest that the abundance of an oxy-succinic acid structures conferred greater complexation ability to a specific metal-

binding fraction of SR FA. Differences in the relative abundance of such structures between DOC sources or fractions may translate into differences in metal complexation. Through Ni complexation work (see Chapter 4) it was found that, while there was little difference between the same DOC fraction from different sources, the HA fraction bound Ni to a greater extent than the FA fraction from the same DOC source. These results are similar to the findings of Shuman and Cromer (1979) who found that, for the humic substances of a North Carolina coastal lake, the conditional stability constants for Cu were higher for the HA relative to the FA fraction. Christl et al. (2001) also reported Cu as being more strongly bound by HA relative to the corresponding FA, although this difference was reduced at higher Cu concentrations. The difference in binding was attributed to chemical structure rather than to total functional group content. It was suggested that there is a higher probability of adjacent carboxyl and phenolic groups on the HA aromatic rings and that this results in the formation of stronger complexes with Cu^{2+} for HAs than for FAs. The higher functional group content of the FAs may compensate for these structural differences at higher metal concentrations (Christl et al., 2001).

Given the diversity of possible arrangements of functional groups on the DOC backbone, the preponderance of a particular general arrangement of functional groups (rather than total functional group content) may confer a greater binding to the HAs. It is possible the there may be a greater abundance of this general type of functional group coordination in HAs relative to FAs. While our HA NMR data do not show the same peak shifts reported by Leenheer et al. (1998) as indicating a greater content of oxysuccinic-type functional group arrangements, it would seem that functional group

arrangement (DOM structure) is somewhat more important than functional group content.

3.5   Conclusions

While all humic substances analyzed appeared to have similar functional groups present, they differed in relative functional group content with source and fraction. Little Bear Lake sedimentary humic substances were higher in aliphatic content and lower in aromaticity and carboxyl group content than peat and Suwannee River humic substances. The results from the various analytical methods employed suggest a higher autochthonous content in LBL sediment, or at least degradation processes somewhat different from those of peat and SR HS sources. There was a strong similarity between our NaOH-extracted peat humic substances and Suwannee River humic substance which suggests that peat FA and HA may serve as an economical source of Suwannee River DOM analogue. For pore-water DOM, a more thorough study should be undertaken to evaluate spatial and seasonal fluctuations in humic substances. Overall, our data suggest that LBL sediment DOM likely consisted of a mixed input from terrestrial and autochthonous carbon sources. With regard to metal-binding potential, water-soluble FAs have a higher carboxyl group content and may have a higher complexation capacity relative to NaOH-extracted FA material. The higher aromatic content of peat and Suwannee River humic substances (or other terrestrially derived humic substances) may confer greater sorptive capacities, relative to sedimentary humic substances, for nonpolar organic contaminants. Possibly due to differences in functional group arrangement, the HA fraction is able to complex metals such as Cu and Ni to a greater extent than the

corresponding FA fraction.

CHAPTER 4

Nickel speciation in the presence of dissolved organic matter

4.1   Introduction

Nickel (Ni), which comprises approximately 0.008% of the earth's crust, is ubiquitous in soils and surface waters (National Academy of Sciences, 1975). Dissolved Ni in surface water is generally the result of the dissolution of primary bedrock materials, the deposition of particulate matter in rainwater, or the leaching of secondary soil phases (Boyle, 1981). North American background values of dissolved surface-water Ni are generally low and range from less than 1 to 10 μg/L (Stokes, 1981; Nriagu et al., 1996a). Elevated Ni concentrations in surface waters may result from a variety of anthropogenic sources which include mining, smelting and refining, metal plating and manufacturing, nickel-cadmium battery disposal, and fossil-fuel refining and combustion (National Academy of Sciences, 1975; Stokes, 1981; Nriagu and Pacyna, 1988). For example, nickel deposition from long-term smelting activity in the Sudbury area, Canada, has led to the elevation of nickel in the waters and sediments of some nearby lakes (Carignan and Nriagu, 1985). As a result of leaching from the surrounding metal-saturated soil, it is believed that elevated Ni levels in these watersheds will persist far into the future (Nriagu et al., 1996b). In northern Saskatchewan, nickel co-occurs in uranium deposits (Dahlkamp, 1993) and, as a result, Ni may be present in the near-field zone downstream of mine effluent or dewatering discharges (Cameco et al., 1995).

The divalent Ni^{2+} ion and its compounds predominate nickel speciation in most aqueous solutions (Latimer, 1952; Morel et al., 1973; Baes and Mesmer, 1976). Equilibrium computations by Morel et al. (1973) and measurements by authors such as

Mandal et al. (2002) have shown that the free ion dominates Ni speciation in aerobic freshwaters in the pH range of 5 to 9. Naturally occurring inorganic ligands (e.g., CO_3^{2-}, OH^-, SO_4^{2-}, Cl^-) complex with Ni to a minor degree relative to the free ion concentration (Morel et al.,1973; Mandal et al., 2002).

While toxicity generally increases with increasing total dissolved metal concentration, many studies have shown that for divalent cationic metals the free (hydrated) ion is usually the most bioavailable/toxic metal form (see review by Campbell, 1995). Therefore, changes in metal speciation (i.e., the free ion concentration) can dramatically affect bioavailability and toxicity to aquatic organisms. Dissolved organic matter (DOM) is known to significantly affect the speciation of a number of divalent cationic metals (e.g., Hollis et al., 1997; Playle et al., 1993a; Town and Filella, 2002).

The typical range of dissolved organic carbon (DOC; ~50% of DOM) in natural waters is from 2 to 10 mg/L (McKnight et al., 1983; Thurman, 1985), although swamps, marshes and bogs are known to be higher in DOC (e.g., 10 to 60 mg/L DOC; Thurman, 1985). Sedimentary pore-water DOC concentrations (4 to 20 mg/L in oxic sediments, 10 to 390 mg/L in anoxic sediments; Thurman, 1985) are known to exceed the DOC of the associated overlying waters (Chin et al., 1998; Orem et al., 1986) due to release of DOC from sediment during degradation processes (Orem et al., 1986). Dissolved organic matter is thought to result from both the breakdown of terrestrial and/or aquatic plant, animal and microbial tissues, and from the condensation reactions (polymerization) of smaller biomolecules (e.g., Wetzel 1975). The more recalcitrant fraction of DOM is comprised of humic substances (HSs) (Wetzel 1975). These are operationally defined organic acids composed of large, refractory compounds occurring in DOM as a complex heterogenous mixture (Aiken et al., 1985). They are known to comprise a significant proportion (50-90%) of coloured surface waters DOC (Thurman, 1985). Based on solubility characteristics, HSs are typically subdivided into either fulvic (FA) or humic acids (HA). Of the total HSs in surface waters, FAs typically account for the majority of

the DOC (80%) with HAs accounting for the remaining 20% (Thurman, 1985). In aqueous systems, HSs play an important role in metal speciation. They are rich in functional groups (COOH; phenolic and alcohol OH) (e.g., Oliver et al., 1983) which are thought to participate in metal complexation (Gamble and Schnitzer, 1973; Reuter and Perdue, 1977). Fulvic and humic acids have been shown to form complexes with a variety of cationic divalent metals (Sholkovitz and Copland, 1981; Christl et al., 2001).

Until recently, information regarding nickel-DOM interactions was limited. While this gap is slowly being addressed by various researchers, the chemical speciation of nickel in metal-polluted waters remains poorly studied (Mandal et al., 2002). Nickel interactions with humic substances from various source environments, and interactions with different DOM fractions, require further research. The studies performed to date have focussed mainly upon low metal-to-ligand (Ni:DOC) ratios in efforts to determine conditional stability constants. While this information is useful for understanding Ni speciation at background levels, Ni speciation at Ni:DOC ratios of toxicological concern has received little attention.

The objective of this research was to evaluate Ni speciation in the presence of DOM from various environmental sources (surface water, peat, sediment) and fractions (FA, HA) at Ni concentrations toxicologically relevant to the benthic crustacean *Hyalella azteca*. This animal is common to North American freshwaters and over the last decade has become a popular test organism in sediment contaminant research. This research aimed to address the following questions: (i) does DOM influence Ni speciation and hence Ni bioavailability and toxicity to *H. azteca*?; (ii) does the environmental source of DOM (i.e., pore-water, surface-water, peat) or composition (FA vs HA) differentially affect nickel speciation; and (iii) when investigating metal speciation in sediment pore water, is surface water DOM a suitable analogue for pore-water or sedimentary DOM?

4.2   Materials and methods

4.2.1   Ion exchange technique: theory

To evaluate the effects of DOM on Ni speciation in solution, a method was required to measure the free, hydrated Ni^{2+} ion. Unlike other divalent cationic metals such as Cu^{2+} and Cd^{2+}, no probe currently exists which is capable of measuring aqueous Ni^{2+}. A miniaturized ion exchange technique (IET) for measuring Cd^{2+} (Fortin and Campbell, 1998) was therefore adapted to measure Ni^{2+}. Briefly, the ion exchange technique involved passing a sample of metal solution (with high sodium content) through a small quantity of ion exchange resin contained within a miniature column. Within a short period of time (8 min), an equilibrium is established between the free and bound metal ions in solution such that the concentration of Ni in the influent and effluent equalize. Concentrations of nickel bound to the resin (which are eluted with a strong acid) vary proportionally to the concentration of the free metal ion in the original sample. Once a distribution coefficient is established for samples having a known nickel speciation composition (such as in a synthetic test media where speciation can be accurately modelled), the free metal ion concentration can be calculated in samples having the same bulk composition but unknown speciation due to the addition of other ligands (i.e., FA or HA). The calculation of the free metal ion $[M^{z+}]$ in the unknown solution can be performed using equation 1,

$$[M^{z+}] = ([M_{Elute}] \times V)/(\lambda_o \times m_r) \qquad (1)$$

where $[M_{Elute}]$ is the concentration of metal in the eluant having a volume V (mass of the eluant divided by its density), m_r is the mass of the resin, and $\lambda_o = [R_z M]/[M^{z+}]$, the ratio of resin-bound metal (mg Ni/g resin) to free metal ion concentration in the original solution for which the metal speciation is known (as calculated by WHAM, Model VI; Tipping, 1998). The brief contact time between sample and resin limits the dissociation of metal from other potentially labile metal species (i.e., metal-DOM complexes) and

subsequent binding to the resin (Apte and Batley, 1995; Donat et al., 1994). As a result, this method yields a measure determined predominantly by the free Ni^{2+} ion activity. The reader is referred to the work of Fortin and Campbell (1998) and Cantwell et al. (1982) for a more detailed discussion of ion exchange theory.

4.2.2   Ion exchange technique: reagents and methods

Determination of the free Ni^{2+} ion concentration in the various test solutions was accomplished through a combination of computer modeling using the Windermere Humic Aqueous Model (WHAM, Model VI; Tipping, 1998) and ion exchange measurements. Various combinations of Ni and DOC concentrations were made using synthetic water. The component concentrations for the synthetic test water are listed in Table 4.1. In the processing of each test sample for Ni^{2+} analysis, a miniature column (Teflon TFE tubing; Cole-Parmer, vernon Hills, IL, USA) containing ~7-8 mg of cation exchange resin (Dowex® 50WX8, 50-100 mesh; Supelco, Bellefonte, PA, USA) was rinsed (sequentially) with the following solutions: ultrapure water (18.2 MΩ resistivity; NANOpure® Diamond™ Life Science (UV/UF) Ultrapure Water System; Barnstead/Thermolyne, Dubuque, IA, USA) (4 min); 0.1 M NaOH (2 min); a repeat of ultrapure water (4 min); synthetic test-water (no nickel) having 0.2 M $NaNO_3$ (4 min); the test sample containing Ni and 0.2 M $NaNO_3$ (with or without DOM) (8 min); a short plug of ultrapure water to rinse the tubing and resin of unbound Ni (5 sec); and finally, 2 ml of 0.1 M HNO_3 (double-distilled). Excluding the 0.1 M NaOH and acid elutions, all solutions were of similar pH (previously adjusted with either NaOH or HCl). Excluding the acid elution, all steps were executed using at a flow rate of 5 ml/min using a peristaltic pump (Gilson Minipuls3, Villiers, France) and plastic tubing (Elkay purple-purple tubing; Elkay Products, Inc., Shrewsbury, MA, USA). The HNO_3 elution was performed at a flow rate of 0.5 ml/min. In previous work (data not presented), the time

Table 4.1. Synthetic water composition.

Component	Concentration (M)	Manufacturer
$NaHCO_3$	$1.71 \cdot 10^{-3}$	Sigma Chemical Co., St. Louis, MO, USA.
$CaSO_4 \cdot 2H_2O$	$5.21 \cdot 10^{-4}$	Alfa Aesar®, Ward Hill, MA, USA.
$MgSO_4$	$7.45 \cdot 10^{-4}$	Alfa Aesar®, Ward Hill, MA, USA.
KCl	$8.02 \cdot 10^{-5}$	BDH Inc., Toronto, ON, Canada

required for column equilibration ($[Ni_{T, influent}] = [Ni_{T, effluent}]$) was determined to be 8 min.

Analysis of dissolved Ni solutions ($NiCl_2 \cdot 6H_2O$, Strem Chemicals, Newburyport, MA, USA; or $Ni(NO_3)_2 \cdot 6H_2O$, BDH Inc., Toronto, ON, Canada) was performed using either an atomic absorption flame or furnace spectrometer (Septa AA 50B and Spectra 220Z with a GTA 110Z, respectively; Varian Australia Pty Ltd, Mulgrave, Australia). Nickel atomic absorption standard solution (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) was used as a quality check on all standard curves. Quality check readings averaged <5% difference from nominal Ni concentrations for both flame and furnace. Dissolved organic carbon was measured using a Shimadzu TOC-5050A Total Organic Carbon Analyzer (Shimadzu, Tokyo, Japan). All ion exchange measurements were conducted at room temperature ($20 \pm 1°C$). Dissolved organic carbon was added to synthetic media after dissolution with a minimum amount of 0.1 M NaOH. In Experiments 1 and 2 (described below), the hardness and alkalinity were 125 ± 3 mg/L and 90 ± 6 mg/L, respectively, expressed as $CaCO_3$. In Experiments 3 and 4 the hardness and alkalinity were 147 ± 8 mg/L and 101 ± 6 mg/L, respectively, expressed as $CaCO_3$.

The methods for dissolved organic matter isolation and fractionation have been described previously in Chapter 3. Test materials were obtained from surface waters (Suwannee River fulvic and humic acids; SR FA and SR HA), sedimentary pore-water (Little Bear Lake; LBL) and water-extracted peat (Experiments 1-3), as well as via 0.1 M NaOH extractions of peat and Little Bear Lake sediment (Experiment 4).

4.2.3 Statistics

Statistics were performed using SigmaStat® 3.0 (SPSS Inc., 2003). One-way analysis of variance was used to detect statistical differences in Ni^{2+} concentration among the various solutions. When the data were normally distributed with homogeneity of

variance, statistically different solutions were identified using a Bonferroni *t*-test for multiple comparisons and Dunnett's Method for multiple comparisons versus a control group. In the event of unequal variance, Dunn's method for multiple comparisons was employed.

4.2.4   Experiment 1: Acute Ni concentration + DOC

To evaluate the effect of representative surface water DOC concentrations (~10 mg/L DOC; Reuter and Perdue, 1977) on an acutely toxic dissolved Ni concentration (5 mg/L; 85.1 µM), Ni solutions were mixed with various fractions of DOM from different environmental sources. The chosen Ni concentration lies near the 96-h LC50 for *Hyalella azteca* in water-only exposures (see Chapter 5). Test substances included SR FA, SR HA, LBL FA, LBL HA, peat FA, peat HA, and whole (unfractionated) peat DOM. Nominal DOC concentrations (based on the addition of elementally-characterized test material) were 10 mg/L for all test substances. The pH was 8.24 ± 0.02 and the solutions were aged a minimum of 3 h prior to addition to the ion exchange column. All control and treatment solutions had three replicates and all solutions were mixed and stored in pre-cleaned low-density polyethylene bottles (LDPE).

4.2.5   Experiment 2: Acutely to chronicly toxic Ni concentrations + DOC

This experiment was conducted with nominal Ni concentrations ranging from acutely toxic to *H. azteca* (5 mg/L; 85.1 µM), to slightly above background surface-water values (0.02 mg/L; 0.34 µM). The DOC values were again representative of average surface waters (~10 mg/L). Each Ni concentration tested had an associated control solution (no DOC) for the calculation of λ_o. All control and treatment solutions had three replicates. Three DOC materials were investigated: peat FA [12.3 ± 0.7 mg/L], SR HA [8.1 ± 0.6 mg/L], and SR FA [10.3 ± 0.1 mg/L]. The pH was $8.16 \pm$

0.04 and all solutions were aged a minimum of 3 h prior to addition to the ion exchange column.

4.2.6   Experiment 3: Sublethal Ni concentration + low DOC

This experiment was conducted at a total Ni concentration of 0.2 mg/L (3.4 μM), a value slightly above the no-observable-effect-concentration (10-day NOEC) for exposure of *H. azteca* to pore-water nickel in natural sediment (see Chapter 8). After mixing and pH adjustment (8.33 ± 0.02), 60 ml of each replicate solution was poured into 80-ml glass beakers and aged overnight in a controlled-environment chamber (16:8 h light:dark cycle, 24 ± 1°C) prior to the addition of ten, 7-14-d old *H. azteca* in each of five replicates (the tissue data from which are presented in Chapter 5). All beakers were covered with glass to minimize evaporation. After a 48 h exposure period, the animals were removed and 50 ml of each solution placed in a 60-ml LDPE sample bottle with $NaNO_3$ ($[Na^+]$ = 0.2 M) and refrigerated until IET analysis. The DOC sources and fractions investigated (all expressed as mg C/L) included LBL FA (11.0 ± 0.1 mg/L), LBL HA (3.0 ± 0.1), SR FA (11.9 ± 0.3 mg/L), SR HA (12.7 ± 0.6 mg/L), un-fractionated (whole) peat (11.6 ± 0.1 mg/L), and peat FA (11.4 ± 0.3, 6.1 ± 0.1 and 2.7 ± 0.1 mg/L).

4.2.7   Experiment 4: Sublethal Ni concentration + a range of DOC

Total Ni concentrations were kept constant at 0.5 mg/L (8.51 μM), a value slightly above the 10-day pore-water LOEC values for *H. azteca* (see Chapter 8) in natural sediment toxicity tests. To represent a broader range of surface or pore-water HS concentrations (a wider Ni:DOC ratio), the DOC values ranged from very low in control solutions (~0.6 ± 0.1 mg/L) to rather elevated (~132.7 ± 6.1 mg/L) in the highest treatments. Due to the high concentrations of DOC required for this experiment, the

peat and LBL humic substances were isolated via 0.1 M NaOH extraction (see Chapter 3). While most solutions were very similar in chemistry, the highest DOC solutions had conductivities slightly higher than the control solutions (474 ± 14 µS/cm vs 422 ± 2 µS/cm) due to the addition of NaOH during pH adjustment (which increased Na content by ~75.0 %). Therefore, a second set of controls (with NaCl) were added to match the conductivity of the highest DOC solutions. The average pH was 8.10 ± 0.03. Similar to Experiment 3, all solutions were aged overnight before use in 2-day *H. azteca* exposures (the tissue data from which are presented in Chapter 5). Test solutions were processed similar to those of Experiment 3 for Ni speciation measurements.

4.3   Results

4.3.1   Experiment 1

At 5 mg/L Ni (Figure 4.1), there were only minor differences in the free ion concentrations among the various solutions and there were no statistical differences between the control solution (no DOC) and the various DOC solutions except for peat FA (12.6% less Ni^{2+} compared to the control). While Ni^{2+} represented the main Ni species present (84 %) in the control solution, $NiCO_3$ (8 %) and $NiHCO_3^+$ (5 %) were also predicted (using WHAM VI) to be present.

4.3.2   Experiment 2

Nickel speciation results from Experiment 2, where a fixed DOC concentration was titrated with Ni, are plotted in Figure 4.2. The Ni^{2+} content in the three different DOC solutions has been plotted as a percentage of the Ni^{2+} content in solutions without added DOC. Similar to
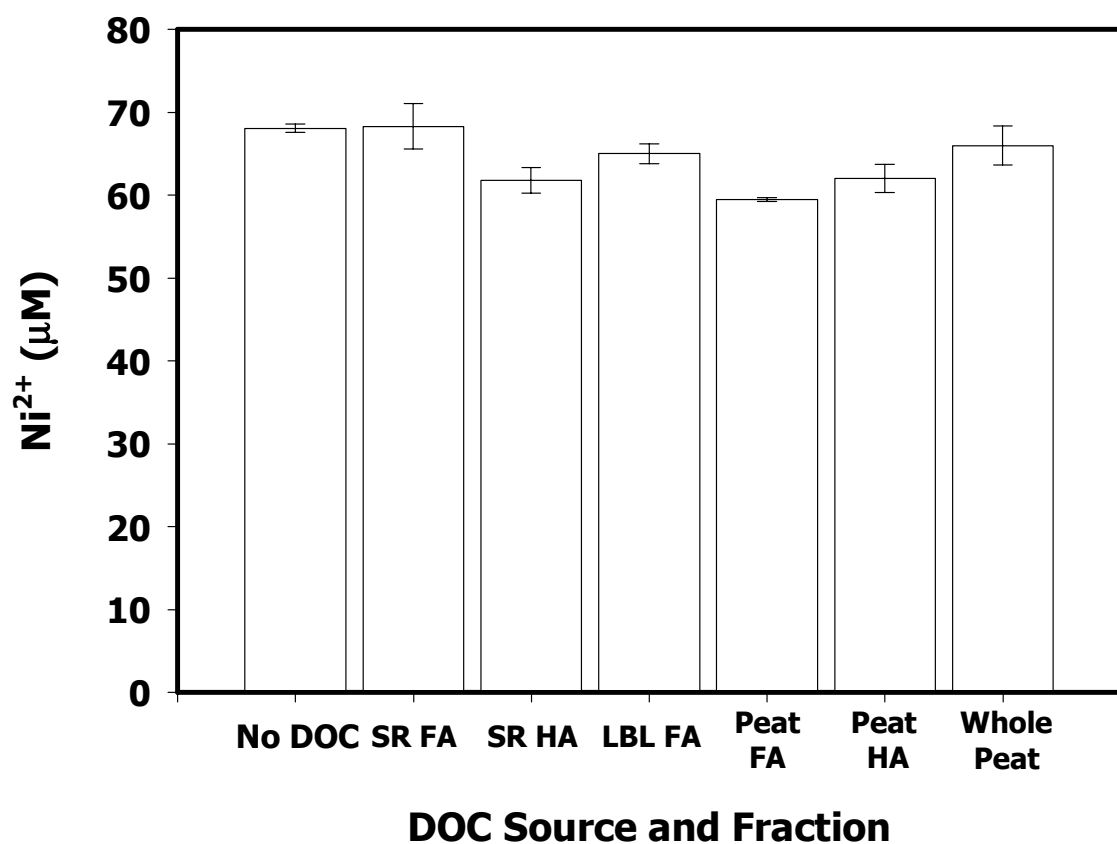
Figure 4.1. Ni^{2+} (µM) in various 5 mg/L total Ni (85.2 µM) and 10 mg/L Suwannee River fulvic acid (SR FA), Suwannee River humic acid (SR HA), Little Bear Lake fulvic acid (LBL FA), peat fulvic acid (Peat FA), peat humic acid (Peat HA), and whole (unfractioned) peat DOC solutions. Data are means ± 1 SD.
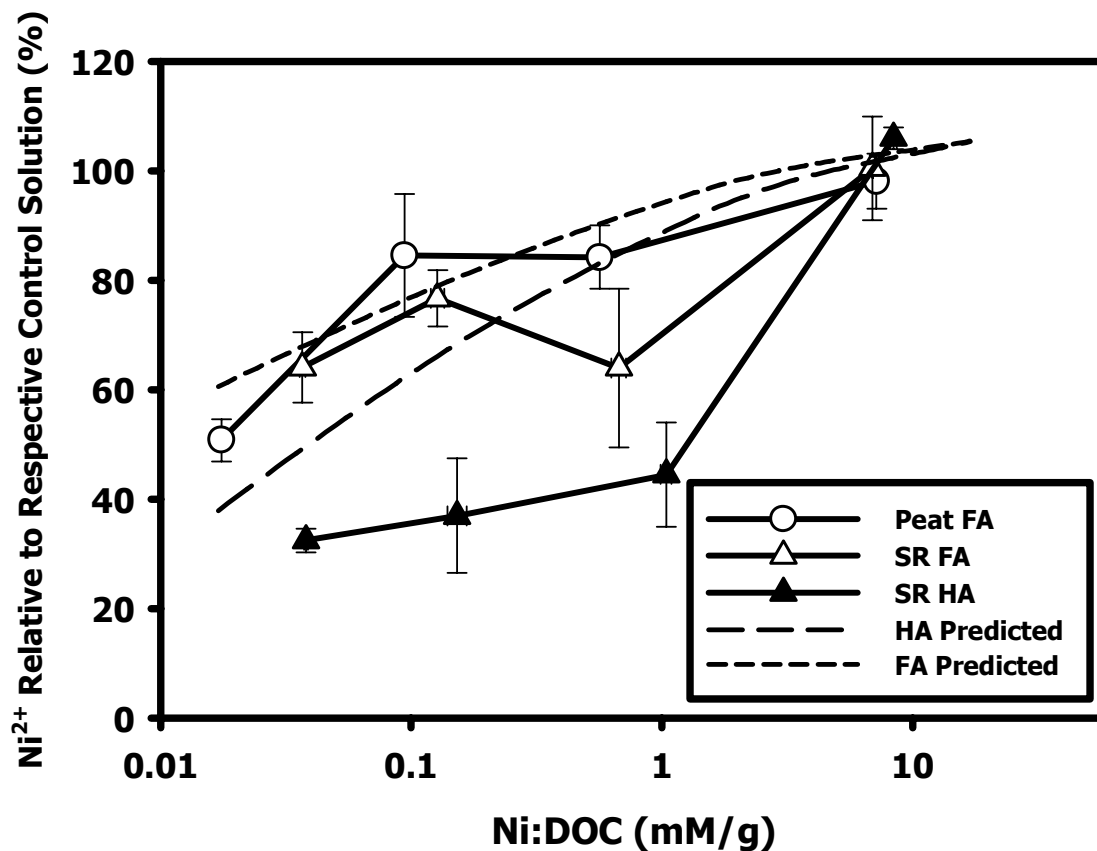
Figure 4.2. Percent free Ni²⁺ in fulvic acid (FA) and humic acid (HA) solutions relative to control solutions lacking humic substances at a range of Ni:DOC ratios. Dashed lines are model predictions using WHAM VI. Data are means ± 1 SD.

the results of Experiment 1, for the highest Ni:DOC ratios tested ($\sim$5 mg/L Ni and $\sim$10 mg/L DOC) there was little discernable difference between the Ni^{2+} content of the different DOC solutions. As the Ni:DOC ratio decreased, the differences in free Ni ion concentration increased. In those solutions were the reduction in Ni^{2+} was discernable, SR HA reduced the measured Ni^{2+} to a greater extent than the peat FA and SR FA solutions. The WHAM VI simulations seemed to adequately predict the percent change in free Ni^{2+} content for FAs, but underestimate the complexation of Ni with HAs, especially at Ni:DOC ratios between 0.1 and 1 mM/g.

4.3.3   Experiment 3

There was a statistical difference in Ni^{2+} concentration between the control and all treatment solutions, as well as in Ni^{2+} content among the various $\sim$11-12 mg/L DOC solutions (Figure 4.3; bars no. 2, 4, 5, 6, 7). The only combinations of solutions not statistically different are LBL FA vs SR FA and SR FA vs Peat FA. While a number of DOC solutions were statistically different in free Ni^{2+} ion content, the only solution with appreciably different Ni speciation was the SR HA which had $\sim$50 % less free Ni^{2+} than the SR FA solution. Although peat FA solutions were diluted from 11.4 (100%) to 2.7 (25%) mg/L, there was no significant decrease in free ion with decreasing DOC. Whole peat (unfractioned) reduced the free ion content to levels intermediate of SR FA and SR HA solutions. The WHAM VI prediction for Ni^{2+} in the presence of FA (11.5 mg/L) was similar to the measured value for LBL pore-water FA, but higher than peat FA and SR FA. The measured value for SR HA was much less ($\sim$50%) than the WHAM predicted value for HA (12.7 mg/L DOC).

4.3.4   Experiment 4

At every concentration of DOC, regardless of source and fraction, the Ni^{2+}
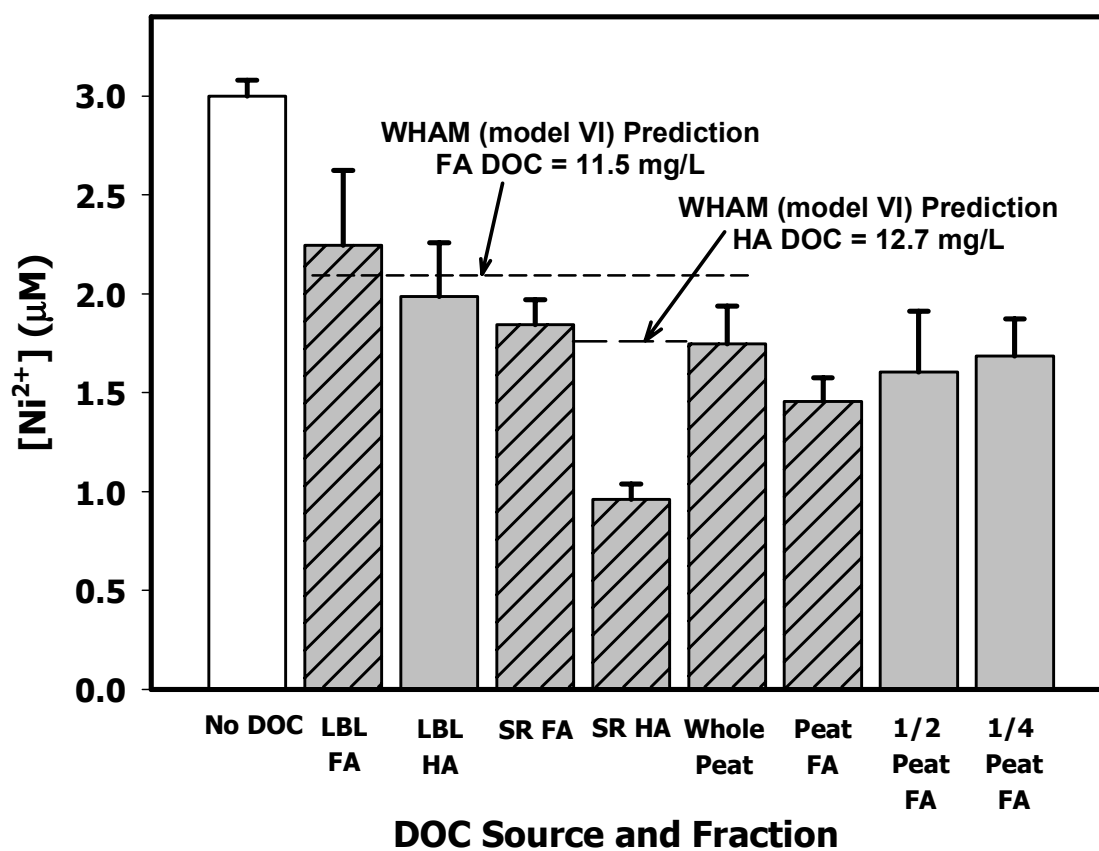
Figure 4.3. Free Ni^{2+} concentration in 200 μg/L (3.41 μM) total nickel solutions having various additions of DOC. Data are means ± 1 SD. For x-axis abbreviations, see Figure 4.1.
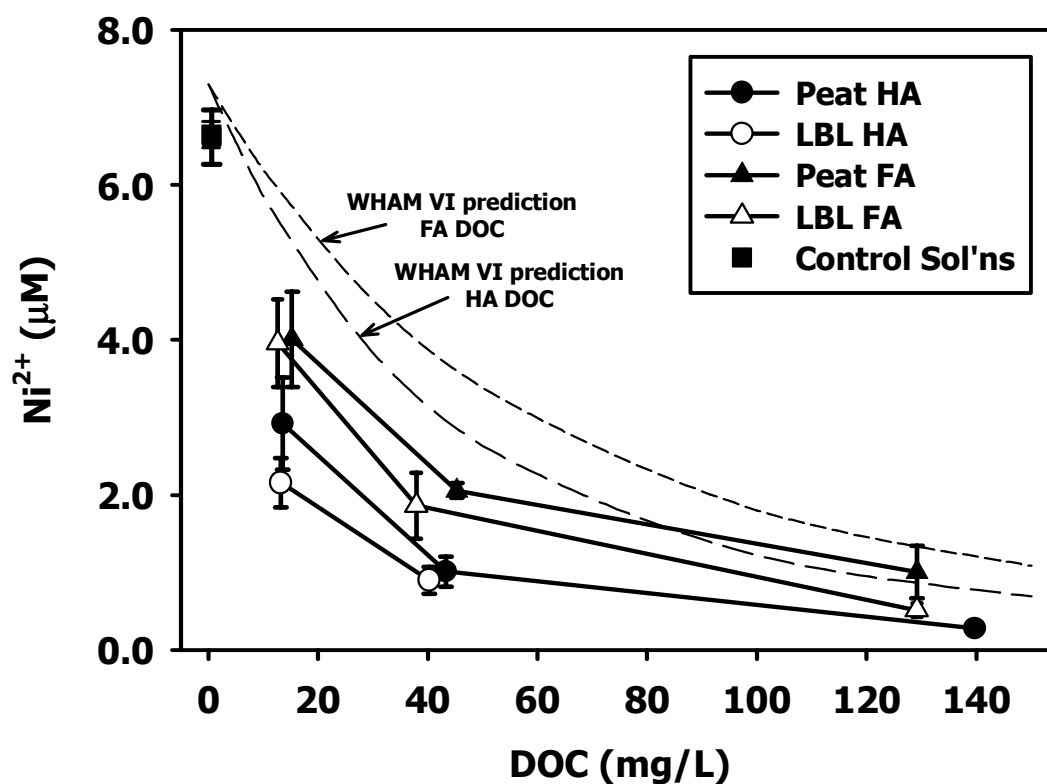
Figure 4.4. The change in free Ni²⁺ concentration (μM) with increasing DOC (mg/L) in *Hyalella azteca* water-only exposure solutions having a fixed total Ni concentration (8.52 μM). Data are means ± 1 SD. For legend abbreviations, see Figure 4.1.

concentrations were significantly different from the respective control solutions (Figure 4.4). Relative to one another, peat FA and LBL FA were not significantly different, nor were peat HA and LBL HA solutions for any given DOC concentration. At all DOC concentrations tested, the FA solutions were always significantly higher in free-ion content than their associated HA solutions. In the presence of added FAs and HAs, the measured Ni^{2+} concentrations were consistently lower than the predicted concentrations except at the highest DOC concentrations, where measured and predicted concentrations were similar.

Figure 4.5 is an amalgamation of all data from Experiments 2 and 4 and is expressed as the measured Ni^{2+} free-ion concentration versus total Ni:DOC ratio. For a given Ni:DOC ratio, the FA fraction complexes less Ni than the HA fraction. The WHAM VI predictions for FA and HA solutions are similar to the measured values at the highest DOC concentrations tested, but overestimated Ni^{2+} content at lower DOC values.

4.4   Discussion

Divalent cationic metal ions are known to complex with DOM (e.g., Hollis et al., 1997; Playle et al., 1993a; Town and Filella, 2000). Since the free metal ion is generally thought to be the most bioavailabile metal species, DOM therefore decreases metal bioavailability and toxicity to aquatic organisms. This study aimed to evaluate whether DOM significantly influences Ni speciation (and hence Ni bioavailability and toxicity) at total Ni concentrations known to impair the biology of the amphipod *H. azteca*.

There are currently a number of studies (some fairly recent) evaluating Ni-DOM interactions in freshwater and marine systems. These studies generally involve Ni concentrations at low background values. Since these levels are not harmful to aquatic organisms, higher Ni:DOC ratios are required in order to evaluate Ni-DOM interactions
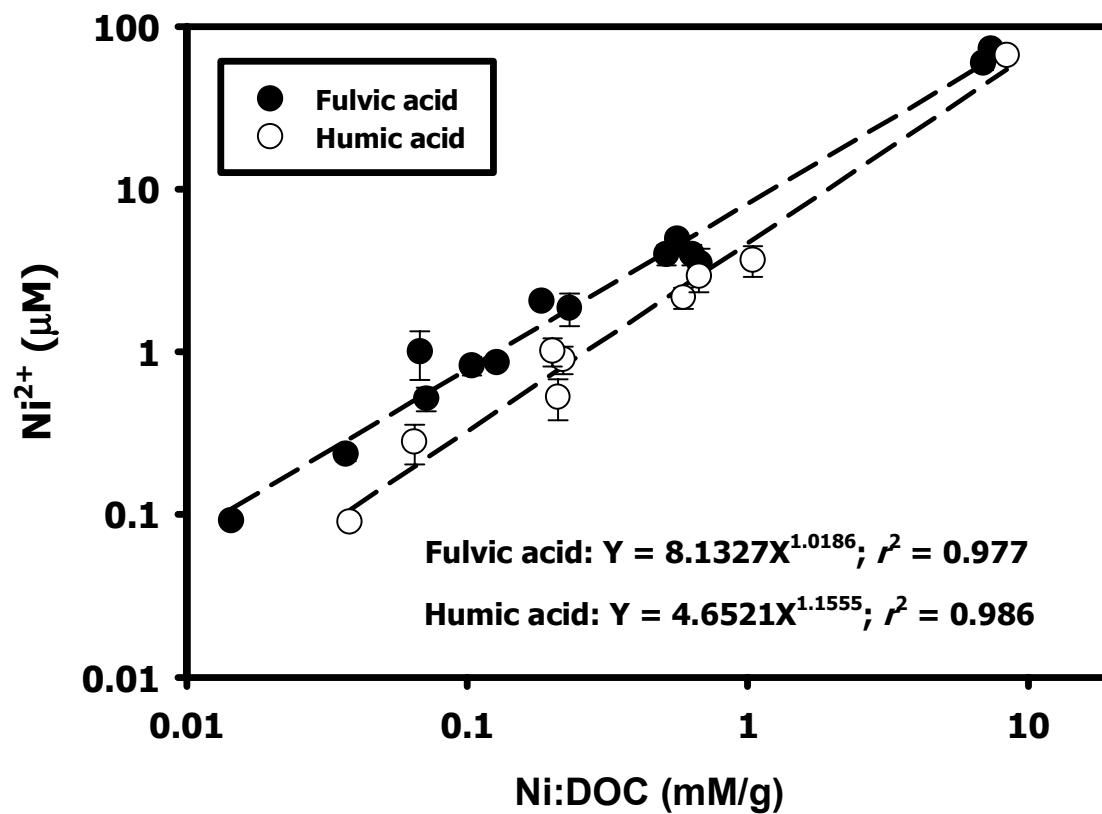
Figure 4.5. Free Ni^{2+} versus the Ni:DOC ratio (expressed as mM Ni/g DOC) in synthetic water solutions: data (means ± 1 SD) are pooled from Experiments 2 and 4.

at Ni concentrations toxic to aquatic organisms. The results shown in Figure 4.1 demonstrate that, at a representative surface water DOC concentration (~ 10 mg/L), regardless of source or fraction, DOC does little to alter Ni speciation at total Ni levels approximating the 96-h LC50 for *H. azteca*. In other words, at Ni:DOC ratios ≥ 8.51 mM/g, environmentally relevant surface water concentrations of DOC play an insignificant role in Ni speciation. In aqueous systems, the addition of a complexing agent at molar concentrations much lower than the metal ion to be complexed (i.e., at a high metal:DOC ratio) means that the added ligand has little effect on metal speciation (Lerman and Childs, 1974). While high Ni:DOC ratios mean little or no changes to Ni speciation, as the ratio decreases the free ion content becomes measurably reduced. For example, at a ratio of ~ 1 mM/g the decrease in the free ion concentration lies between ~ 16 and 55% (depending upon DOC source and fraction). Near the lower end of the Ni:DOC ratios tested here ($\sim 3.7 \times 10^{-2}$ mM/g) the decrease in free Ni^{2+} ranged from ~ 36 to 68%. This ratio corresponds to ~ 10 mg/L DOC and a total Ni concentration of ~ 22 µg/L, a total Ni level just below that found to reduce *Daphnia magna* reproduction by 16% over a three-week period (30 µg/L; Biesinger and Christensen, 1972). Dissolved organic carbon may therefore significantly reduce the bioavailability and toxicity of Ni to crustaceans at low total Ni levels when long-term sublethal endpoints are measured.

In aqueous metal research, can one humic substance (i.e., surface water FA) serve as an analogue for another (i.e., pore-water FA)? Some research has sought to replace natural organic material with mixtures of simple organic acids (e.g., dipicolinic, oxalic and malonic acids; MacRae et al., 1999) although there are concerns over replacing natural ligands with synthetic ones which may not behave similarly under test conditions. Other research, using a commercially available humic acid to create artificial pore-waters, completely ignored any differences potentially arising from DOC source (Boucher and Watzin, 1999). No previous studies were found evaluating the effect of environmental DOC source on Ni bioavailability. The results presented in Figures 4.3

and 4.4 show that DOC source plays a minor role in Ni speciation relative to Ni:DOC ratio. Whether obtained from surface water, peat, or sediment, FAs complex Ni similar to another. This agrees with previous research involving Cu binding to FA isolated from lakes, rivers, estuaries and soils (Playle et al., 1993a; Cabaniss and Shuman 1988; McKnight et al., 1983) where pH, alkalinity, cation binding and ionic strength were cited as being more important factors in predicting the free Cu ion concentration than the binding differences between the FAs from different environments. Therefore, at metal concentrations of toxicological concern, surface water FA (e.g., SR FA) or peat FA appear to provide suitable analogues for sedimentary FA (e.g., LBL FA).

It has been stated before that DOM is a heterogenous mixture of ill-defined organic molecules (Aiken et al., 1985; Perdue, 1998). Operationally defined entities (such as fulvic and humic acids) have emerged as the result of attempts to better understand the general chemical composition of DOM. This study found that, similar to the WHAM VI simulations, the HA fraction complexes more Ni than the corresponding FA fraction at a given Ni:DOC ratio (see Figure 4.5). These results are similar to the findings of Shuman and Cromer (1979) who found that, for humic substances from a North Carolina coastal lake, the conditional stability constants for Cu were higher for the HA than the FA fraction. Christl et al. (2001) also reported that Cu was more strongly bound at pH 6 and 8 by HA relative to the corresponding FA fraction, although these differences were reduced at higher Cu concentrations. Chemical structure was thought to be involved in these differences rather than total functional group content. It was suggested that there is a higher probability of carboxyl and phenolic groups occurring adjacent to one another on the HA aromatic rings and this results in the formation of stronger complexes with Cu^{2+} for HAs than for FAs. The higher functional group content of the FAs may compensate for any structural differences at high Cu concentrations (Christl et al., 2001). While the chemical characterization data for DOM in Chapter 3 do not provide any clues as to whether or not this is the case, data

presented in Chapter 5 suggest that, although there are differences in the measured Ni^{2+} content in similar concentrations of FA and HA fractions, they do not result in significant differences in the bioavailability of Ni to *H. azteca*.

While Ni^{2+} represents the main bioavailable Ni species (see Chapter 5), Ni complexed to DOM may also be, at least partially, labile and hence bioavailable. Ignoring all other factors potentially modifying Ni bioavailability such as water hardness, alkalinity, competing metal cations, pH and salinity, one must also consider the lability of Ni-DOM complexes. At a low metal:DOC ratio there are ample complexation sites on organic matter for metal complexation. Not only are free metal ion concentrations significantly reduced under these conditions (see Figure 4.2), but bonding is stronger and therefore less labile in the presence of competing biological ligands (i.e., gill surfaces; Playle et al., 1993b). Under low-nickel conditions in wastewater effluents, surface runoff and marine waters (total Ni $\leq$100 nM range), reported log *K* values ranged from 12 to > 17 (Donat et al., 1994; Xue et al., 2001; Sedlak et al., 1997). Strong Ni-binding ligands in lake water were suggested to potentially result from aliphatic algal exudates and algal breakdown products, and humic substances (Achterberg et al., 1997). A continuum of conditional stability constants is thought to exist between metal and DOM such that these constants are dependant upon the metal:DOC ratio (Playle et al., 1993b). At low total Ni concentrations (as in the case of most environmental Ni-FA binding), Ni preferentially binds to high affinity ligands on HSs (Cabaniss, 1990). The chemical nature of these "high affinity" sites for Ni is not yet known. Whether these binding sites involve specific functional groups present in low concentrations (e.g., S or N functional groups), functional groups arranged in a particular structural conformation (i.e., high-affinity chelation sites), or possibly a combination of the two, is unknown. High affinity organic ligands in ground, river and lake waters have a very low total Ni complexation capacity (e.g., 13-100 nM; Xue et al., 2001) which would be exceeded at Ni levels well below toxic thresholds.

At high metal:DOC ratios (i.e., at toxic metal concentrations), low-affinity sites (with a high complexation capacity) are predominantly involved in metal complexation (Playle et al., 1993b; McKnight et al., 1983). While the measured free-ion concentration is reduced at higher metal:DOC ratios, the weak complexation may mean that the labile Ni-DOC fraction is significant. Mandal et al. (2002) found that in the presence of a high-affinity cation exchange resin, a significant percent of Ni-DOM will dissociate depending upon the Ni:DOC ratio. The free Ni plus labile fraction, and the corresponding dissociation rate coefficients, increased with increasing Ni:DOC ratio. Therefore, in the presence of high-affinity biological ligands, weakly DOM-bound metals are potentially bioavailable to aquatic organisms. The relationship between the free ion and bioavailability/toxicity may therefore be weakened at higher metal:DOC ratios due to the bioavailability of labile metal-DOM complexes. This is dealt with at greater lengths in Chapter 5.

In attempting to predict Ni speciation in the presence of DOM one must remember that the interaction of a humic substance with a divalent cationic metal is variable (Gamble and Schnitzer, 1973). Not only does the Ni-DOM stability constant depend upon the Ni:DOC ratio, but also on other conditional factors such as pH, ionic strength, salinity and concentrations of competing divalent cations, such as Ca^{2+} and Mg^{2+} (Tipping, 1993; Hummel et al., 1995; Schnitzer, 1971; Lores and Pennock, 1998). Over the last few decades, mathematical models incorporating these factors have become increasingly sophisticated at predicting metal speciation in aqueous systems. While the application of models to natural waters requires further research and refinement, modeling with well-defined synthetic media should yield less ambiguous results. The more recent version of the WHAM (model VI) has been designed to better predict speciation in the presence of competing cations such as H^+, Ca^{2+} and Mg^{2+}. The research described here found that, in synthetic media of moderate hardness and alkalinity, this model provides an adequate prediction of Ni speciation for water-extracted peat FA and

SR FA, but potentially overestimates the free Ni^{2+} ion concentration for HAs. For NaOH extracted peat, LBL FA and LBL HA, the model predictions were similar to the measured values at lower Ni:DOC ratios, but overestimated Ni^{2+} at higher ratios.

4.5   Conclusions

While a representative surface-water DOC concentration (~10 mg/L) did not affect Ni speciation at a total Ni concentration lethal to *H. azteca*, DOC is able to significantly influence Ni speciation at lower, sublethal Ni levels. The degree of influence on speciation is determined primarily by the Ni:DOC ratio. Environmental source of DOC had a small effect on Ni speciation. Dissolved organic matter fraction also affected Ni speciation with the HA fraction complexing Ni to a greater extent than the FA fraction. From a practical perspective, Suwannee River and peat humic substances appear to be suitable analogues for pore-water or sedimentary DOM when evaluating metal bioavailability in sediments. In synthetic media, WHAM (model VI) appears to best predict Ni^{2+} concentrations for water-extracted FAs, but underestimates the degree of Ni complexation with SR HA. For NaOH-extracted humic substances, the model predictions were most similar to the measured values at lower Ni:DOC ratios, but overestimated Ni^{2+} at higher ratios.

Chapter 5

The influence of dissolved organic matter on nickel bioavailability and toxicity to

Hyalella azteca in water-only exposures

5.1   Introduction

While a variety of physical, chemical, and biological processes occur within sediments, their ability to behave both as a sink and source of trace-metals makes them of ecotoxicological interest. In recent years, advances have been made in understanding metal bioavailability and toxicity in sediments. For organisms closely associated with surface sediments, the uptake of metals may result from exposure to the overlying water, the sediment pore water, sediment/food ingestion, or a combination of the three (Ankley, 1996; Lee et al., 2000a). For divalent cationic metals in aqueous solution, the free metal ion is believed to represent the major bioavailable species and hence determine metal toxicity (Campbell, 1995; Morel, 1983). In reviewing the Free Ion Activity Model described by Morel (1983), Campbell states that "In a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal. It is this reactivity that determines the extent of the metal's reactions with surface cellular sites, and hence its 'bioavailability'."

From a variety of studies evaluating the effects of dissolved organic matter (DOM) on transition metal speciation (e.g., Cd, Cu**,** Zn), it has been found that DOM

generally reduces metal bioavailability in marine and freshwater systems (Sunda and Lewis, 1978; Meador, 1991; Playle et al., 1993a; Penttinen et al., 1998; Daly et al., 1990; Heijerick et al., 2003). Divalent cationic metal ions are thought to complex with DOM at bidentate sites (Gamble and Schnitzer, 1973), thereby becoming less available for uptake by aquatic organisms. While a number of studies have evaluated the toxicity of total nickel (Ni) concentrations to various aquatic organisms, or the factors modifying Ni bioavailability (i.e., hardness or pH; see review by U.S. EPA, 1986), very little research has directly evaluated the influence of Ni speciation on bioavailability and toxicity. No studies appear to have evaluated Ni bioavailability in the presence of pore-water DOM. While there is abundant information in the literature suggesting that the predominant Ni species in freshwaters (pH 5 - 9) is the hydrated divalent cation (e.g., Morel et al., 1973), there is little information identifying Ni^{2+} as the main bioavailable Ni species. While studies have evaluated nickel speciation in the presence of DOM, aside from Spencer and Nichols (1983) and Mandal et al. (2002), no studies have correlated the concentration of Ni^{2+} with a biological response via toxicity testing. Spencer and Nichols (1983) evaluated Ni bioavailability to two species of green algae in the presence of EDTA (ethylenediaminetetraacetic acid) in synthetic media and linked Ni toxicity to the calculated free ion concentration. To date, Mandal et al. (2002) is the only study which has actually measured Ni^{2+} activity in toxicity testing (contaminated surface water) and evaluated its effect on the algae, *Pseudokirchneriella subcapitata*. Their work involving *Daphnia magna* and *Hydra attenuata* was less successful in demonstrating a link between Ni speciation and a biological response.

The objective of this research was to evaluate Ni bioavailability in the presence of

different fractions (fulvic acid, FA; humic acid, HA) of DOM from various

environmental sources (surface water, peat, sediment) at Ni concentrations

toxicologically relevant to the benthic crustacean, *Hyalella azteca*. This animal is

common to North American freshwaters, and over the last decade has become a popular

test organism in sediment contaminant research. Specifically, this research aimed to

address the following questions: (i) does DOM influence Ni bioavailability and toxicity to

H. azteca?; (ii) does the environmental source of DOM (i.e., pore water, surface water,

peat) or composition (FA vs HA) differentially affect Ni bioavailability?; and (iii) does

surface water or peat-extracted DOM modify Ni bioavailability in a manner similar to

that of pore-water DOM? Ultimately, we wished to determine whether commercially

available DOM (Suwannee River humic substances), or economical sources (peat humic

substances), are suitable analogues for pore-water or sedimentary DOM. This would

simplify future pore-water metal-DOM research. Other studies have used simple organic

compounds to simulate metal-DOM complexation (e.g., mixtures of dipicolinic, oxalic

and malonic acids; MacRae et al., 1999), but there have been some concerns raised as to

the validity of making such analogies (McLaughlin, 1998). Simple analogues may not

behave te same way as much more complex natural DOM molecules, which are

essentially a heterogenous soup of ligands having a wide range of complexation

constants.

Three sources of DOM were used during experimentation: Little Bear Lake

(LBL) sedimentary DOM, peat leachates, and Suwannee River (SR) humic substances.

Nickel bioavailability and toxicity was assessed via three methods; ion exchange

measurements of the free Ni^{2+}, mathematical modeling using the Windermere Humic

Aqueous Model (WHAM, Model VI; Tipping, 1998), and toxicity testing with *H. azteca*. Results from the first two approaches are presented in Chapter 4. The following information focuses primarily upon the toxicity testing component, but also incorporates information from Chapter 4 where appropriate.

5.2   Materials and methods

5.2.1   General methods

In order to evaluate the effect of DOM on Ni bioavailability and toxicity, amphipods (*H. azteca*) were exposed to various concentrations of Ni (corresponding to lethal and sublethal levels) and DOM. The DOM sources and fractions included Suwannee River (humic and fulvic acids), peat (humic and fulvic acids, hydrophilic fraction, and unfractioned peat) and Little Bear Lake sediment (humic and fulvic acids). The details of the DOM sampling, extraction, isolation and fractionation protocols are provided in Chapter 3. Dissolved organic carbon (DOC) content was measured using a Shimadzu TOC-5050A Total Organic Carbon Analyzer with an ASI-5000A autosampler (Shimadzu, Tokyo, Japan). Solid DOC was added to test solutions after dissolution with a minimum amount of 0.1 M NaOH prior to addition to the test matrix. Dissolved organic carbon samples were stored in 25-ml plastic vials (Richards Packaging, Edmonton, AB, Canada) and frozen until analysis. All toxicity tests were conducted in well-aerated synthetic (reconstituted) water. Dissolved oxygen and water temperature were measured at the start and end of each test (Dissolved Oxygen Meter, Model 835, Orion Research Inc., Beverly, MA, USA). The component concentrations for the synthetic water test media were listed previously in Table 4.1. All experiments were

conducted in an environmental chamber (16:8-h light:dark cycle, $24 \pm 1°C$). All water

quality measurements were performed on a minimum of three samples taken pre and

post-test and stored in 25-ml vials which were refrigerated until analysis (within 48-h).

Solution pH was measured with an Orion Model 370 pH meter (Beverly, MA, USA).

Hardness and alkalinity were measured using a HACH Digital Titrator (model 16900,

Hach Company, Loveland, CO, USA). Conductivity of all control solutions (μS/cm)

was measured with an Orion Model 170 Conductivity Meter. Unless otherwise

mentioned, the average water quality readings for all tests conducted were as follows:

hardness, 137 ± 6 mg/L as $CaCO_3$; alkalinity, 90 ± 5 mg/L as $CaCO_3$; conductivity, 480

± 49 μS/cm; temperature, $22.4 \pm 0.5°C$; and dissolved oxygen, 8.0 ± 0.2 mg/L. Samples

were collected for Ni analysis from the pre- and post-test solutions, stored in 8-ml LDPE

bottles, and acidified with HNO_3 to pH<2.5. Analysis of Ni solutions was performed

using either an atomic absorption flame or furnace spectrometer (Septa AA 50B and

Spectra 220Z with a GTA 110Z, respectively; Varian Australia Pty Ltd, Mulgrave,

Australia). A Ni atomic absorption standard solution (Aldrich Chemical Company, Inc.,

Milwaukee, WI, USA) was used as a quality check on all standard curves. Quality check

readings averaged <5% difference from nominal Ni concentrations for both flame and

furnace. The measurement of free Ni^{2+} was accomplished via an ion exchange technique

described previously in Chapter 4. This methodology kept the contact time between the

test solution and ion exchange resin to a minimum thereby limiting the dissociation of Ni

carbonate and Ni-DOM complexes (Donat et al., 1994).

5.2.2   Water-only toxicity tests

5.2.2.1 Acute lethality tests

It was determined from initial range-finding tests with *H. azteca* (data not presented) that good control survival was not possible beyond 48 h without the addition of food. Metals may complex with food thereby creating the potential for both a dietary and respiratory metal exposure. While the dietary contribution to Ni uptake in an acute exposure scenario would likely be small relative to the free-ion uptake across the gills, food was withheld to prevent this potentially confounding factor. Adding fine feed (such as liquified fish flakes) would also alter the DOM content.

Nickel in the form of $NiCl_2 \cdot 6H_2O$ (Strem Chemicals, Newburyport, MA, USA) and $Ni(NO_3)_2 \cdot 6H_2O$ (BDH Inc., Toronto, ON, Canada) was used in making test solutions, the nominal concentrations of which were 0, 1, 5, 30, and 100 mg/L. A total of four DOC concentrations were tested (measured values ranging from ~0 to 40 mg/L) to evaluate the effect of DOM on Ni bioavailability at acutely toxic Ni concentrations. For these acute exposures, the test substances included water-extracted whole (unfractioned) peat DOM, peat FA, hydrophilic peat DOM, and Suwannee River FA and HA. All test solutions were adjusted with 0.1 M HCl and 0.1 M NaOH to reach a pH (pH 8.31 ± 0.08) similar to Saskatoon (SK, Canada) municipal water (used for culturing test organisms). All Ni-DOM solutions (60 ml in 80-ml glass beakers; four replicates per Ni concentration) were allowed to equilibrate in the test chamber for a minimum of 3 h (though most were left overnight) prior to the addition of eight *H. azteca* (7-14 d old) per replicate. To reduce test variability, for each DOM source/fraction, all four DOC test concentrations were tested on the same day with animals from the same cohort. All tests were performed twice. Upon completion of one SR FA and one SR HA test, the

animals were processed (as described below) and analyzed for total tissue Ni. To

determine the incipient LC50, one 48-h test (peat FA) was extended to 96 h by feeding a

Tetramin® (Melle, Germany) slurry to the animals at 48 h.

5.2.2.2 Sublethal exposures tests

Two 48-h experiments were conducted at total Ni concentrations that were

sublethal to *H. azteca*. The first sublethal experiment (see Experiment 3, Chapter 4) was

conducted with a total Ni concentration of 0.2 mg/L (3.4 μM) and a DOC concentration

representative of many surface waters (~10 mg/L). This Ni concentration was slightly

above the no observed effect concentration (10-d NOEC) for exposure of *H. azteca* to

the pore-water of uncontaminated field-collected sediments spiked with Ni (see Chapter

8). After mixing and pH adjustment, 60 ml of test solution were added to 80-ml glass

beakers and placed in a test chamber overnight prior to addition of ten, 7-14-d old *H.*

azteca (five replicate beakers per treatment and control). All beakers were covered with

glass sheets to minimize evaporation. After 48 h, the animals were removed (the details

of which are described below) and 50 ml of each solution placed in a 60-ml LDPE

sample bottle with $NaNO_3$ (final $[Na^+] = 0.2$ M) and refrigerated until free-ion analysis

was performed (presented in Chapter 4). The DOC sources and fractions investigated

included Suwannee River FA (SR FA; 11.9 ± 0.3 mg/L DOC) and HA (SR HA; $12.7 \pm$

0.6 mg/L DOC), peat FA (11.4 ± 0.3, 6.1 ± 0.1 and 2.7 ± 0.1 mg/L DOC), and LBL FA

(11.0 ± 0.1 mg/L DOC) and HA (3.0 ± 0.1 DOC), as well as whole, un-fractionated peat

(11.6 ± 0.1 mg/L DOC). The average solution pH of this first sublethal experiment was

8.31 ± 0.03.

In a second sublethal exposure experiment (see Experiment 4, Chapter 4), the total Ni concentration was kept constant at 0.5 mg/L (8.51 µM), a value slightly above the average 10-d pore-water lowest observed effect concentration (LOEC) for *H. azteca* in uncontaminated field-collected sediments spiked with Ni (see Chapter 8). To represent a broad range of both surface and pore-water DOC concentrations (a wider Ni:DOC ratio), the DOC concentration was varied from very low in control solutions (0.6 ± 0.1 mg/L) to high in some test solutions (132.7 ± 6.1 mg/L). Due to the high concentrations of DOC required for this experiment, the peat and LBL humic substances were isolated via 0.1 M NaOH extractions (see isolation and fractionation protocols, Chapter 3). The average pH for this experiment was 8.10 ± 0.03 (adjusted with either NaOH or HCl) and all solutions were aged overnight in the test chamber prior to the addition of animals (12 animals × 4 replicates per treatment). While most solutions were very similar in chemistry, the highest DOC solutions had conductivities slightly higher than the control solutions (474 ± 14 µS/cm vs 422 ± 2 µS/cm) due to the addition of NaOH during pH adjustment (which increased Na content by ~75.0 %). Therefore, a second set of controls (with NaCl) were added to match the conductivity of the highest DOC solutions. Upon test completion (48-h), the animals were removed (the details of which are described below) and 50 ml of each solution placed in a 60-ml LDPE sample bottle with $NaNO_3$ (final $[Na^+] = 0.2$ M) and refrigerated until free-ion analysis was performed (presented in Chapter 4). Only three of the four replicates were analysed for free-ion content.

5.2.2.3 Tissue Ni analysis

EDTA rinse

Upon completion of some acute toxicity tests (SR FA, SR HA) and both sublethal exposure experiments, the surviving animals were transferred into 250 ml of clean synthetic water. The animals were then transferred (with ~1 ml of synthetic water) into 5 ml of a 1 mM EDTA solution (Sigma Chemical Co., St. Louis, MO, USA) and left for 15 min. This EDTA "wash" was performed to reduce surface-adsorbed Ni relative to the total Ni body burden. Other authors have washed *H. azteca* with a more dilute EDTA solution for longer periods of time (e.g., Neumann et al., 1999), but because no gut purging was required in these experiments, the rinse period was kept brief to limit tissue Ni depuration. After the EDTA wash, animals were transferred onto a mesh screen (~100 μm), triple rinsed with ultrapure water, pipetted into small pans, and dried overnight at 60 °C. The animals were then weighed and stored until digestion.

Tissue digestion and analysis

The tissue Ni analysis methodology was based upon protocols provided in Neumann et al. (1999) for metals analysis of *H. azteca*. Each replicate of dried, pre-weighed animals was digested in 50 μl of 70% nitric acid at room temperature for 6 d. Digestion vessels consisted of pre-cleaned (5% nitric acid, overnight), 2-ml polyethylene cuvettes (Elkay® Elrean, Costelloe, CO, USA) which were sealed with Parafilm "M"®(American National Can ™, Chicago, IL, USA) to limit evaporation. On the sixth day of digestion, 40 μl of 30% H_2O_2 (BDH Inc., Toronto, ON, Canada) were added. On the seventh day, ultrapure water was added to reach a final volume of 500 μl. Tissue samples were analyzed on or soon after the 8th day. Blanks were used to determine

background Ni readings from digestion reagents. Tissue analysis was performed on a

Varian SpectraAA 220Z graphite furnace atomic absorption spectrometer with Zeeman

background correction. Tissue reference material was obtained from the Canadian

National Research Council (TORT-2, lobster hepatopancreas) and digested and analyzed

for Ni as a quality control check.

5.2.3. Statistics

Median lethal concentrations (LC50 values) were calculated using the trimmed

Spearman-Karber method (Hamilton et al., 1977). Other statistical analyses were

performed using SigmaStat® 3.0 (SPSS Inc., 2003). One-way analysis of variance was

used to determine statistical differences in free Ni^{2+} concentration and *H. azteca* tissue Ni

concentrations among the various treatments and control. When the data were normally

distributed with equal variance, statistically different treatments were identified using

Dunnett's Method for multiple comparisons versus the control group. In the event of

unequal variance (as with Experiment 3 tissue data), the data were log_{10}-transformed

prior to analysis.

5.3 Results

5.3.1. Acute lethality tests

5.3.1.1. Mortality

The 48-h Ni LC50 values in the presence of whole peat, peat hydrophilic fraction

and peat FA (all water-extracted) are shown in Figure 5.1a. Suwannee River FA and SR

HA LC50 values are presented in Figure 5.1b. The average 48-h Ni LC50 value for all
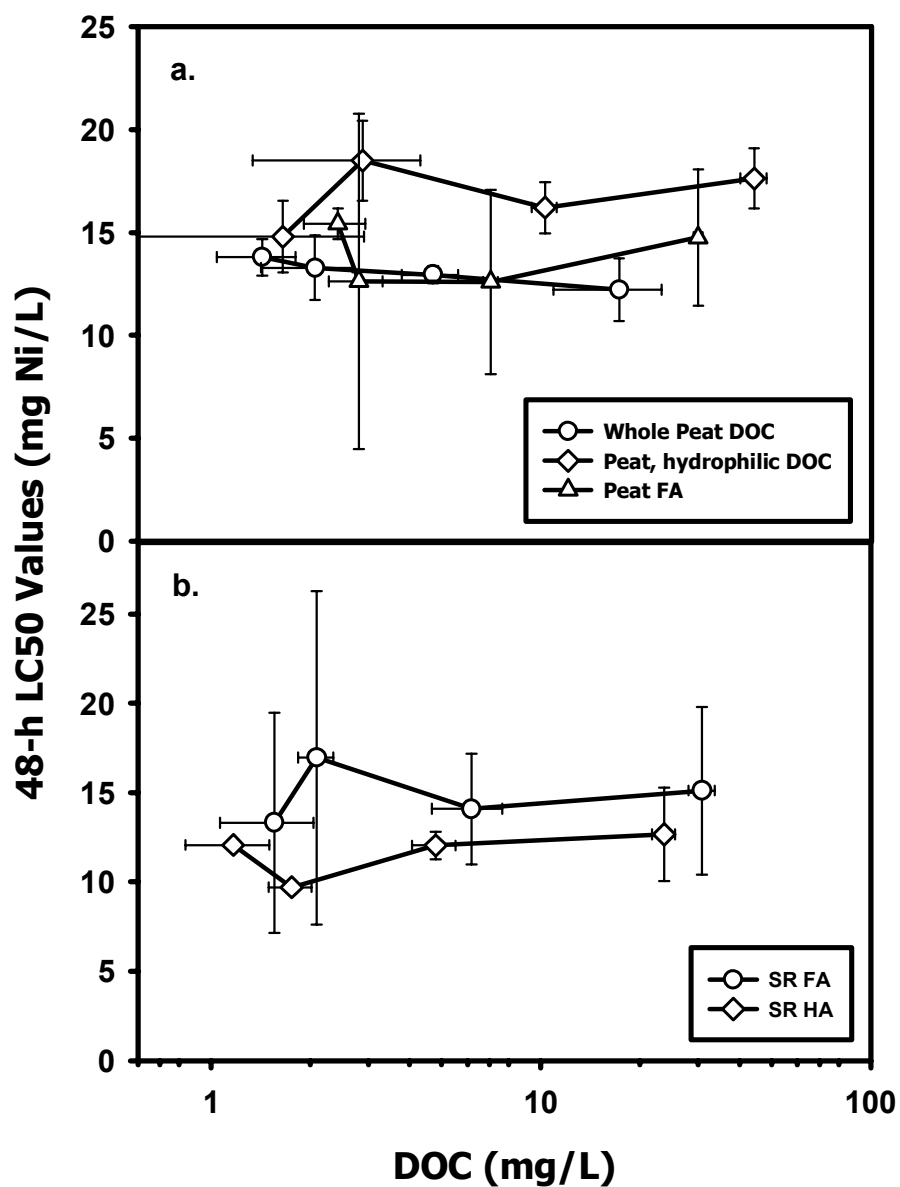
Figure 5.1. 48-h Ni LC50 values for *Hyalella azteca* at different DOC concentrations: (a) peat dissolved organic carbon and (b) Suwannee River fulvic acid (SR FA) and humic acid (SR HA). Error bars represent 1 standard deviation.

tests combined (listed in Appendix A) was 14.0 ± 2.2 mg/L. Increasing DOC (from 1 to 40 mg/L DOC) had no clear effect on the 48-h Ni LC50 for *H. azteca*. Within the range of DOC concentrations tested, the various sources and fractions did not differentially affect acute Ni toxicity.

5.3.1.2 Tissue residues

The tissue residue (Ni) concentrations of amphipods surviving the SR FA and SR HA 48-h LC50 experiments are shown in Figures 5.2a and b, respectively. There were insufficient animals available for accurate analysis of the 30 mg/L Ni solutions. Generally, tissue Ni concentrations increased with increasing total Ni in the exposure solution. For the 1 and 5 mg/L Ni solutions in tests with both Suwannee River FA and HA, tissue Ni concentrations were not significantly reduced by increasing DOC. The only significant difference noted in tissue Ni was in the SR HA control groups where the highest DOC solution yielded a significantly lower tissue Ni concentration relative to the lowest DOC solution.

5.3.2. Sublethal exposure tests

5.3.2.1 Experiment 3: Sublethal Ni concentration + low DOC

The free Ni^{2+} measurements from Experiment 3, Chapter 4, and the associated tissue Ni concentrations are presented in Figure 5.3a and b, respectively. There were statistically significant differences in the measured Ni^{2+} concentrations between the control solution (synthetic water, no DOC) and all DOC treatments (average reduction in $Ni^{2+} = 43.6 \pm 12.6\%$ relative to the control), as well as among most of the DOC
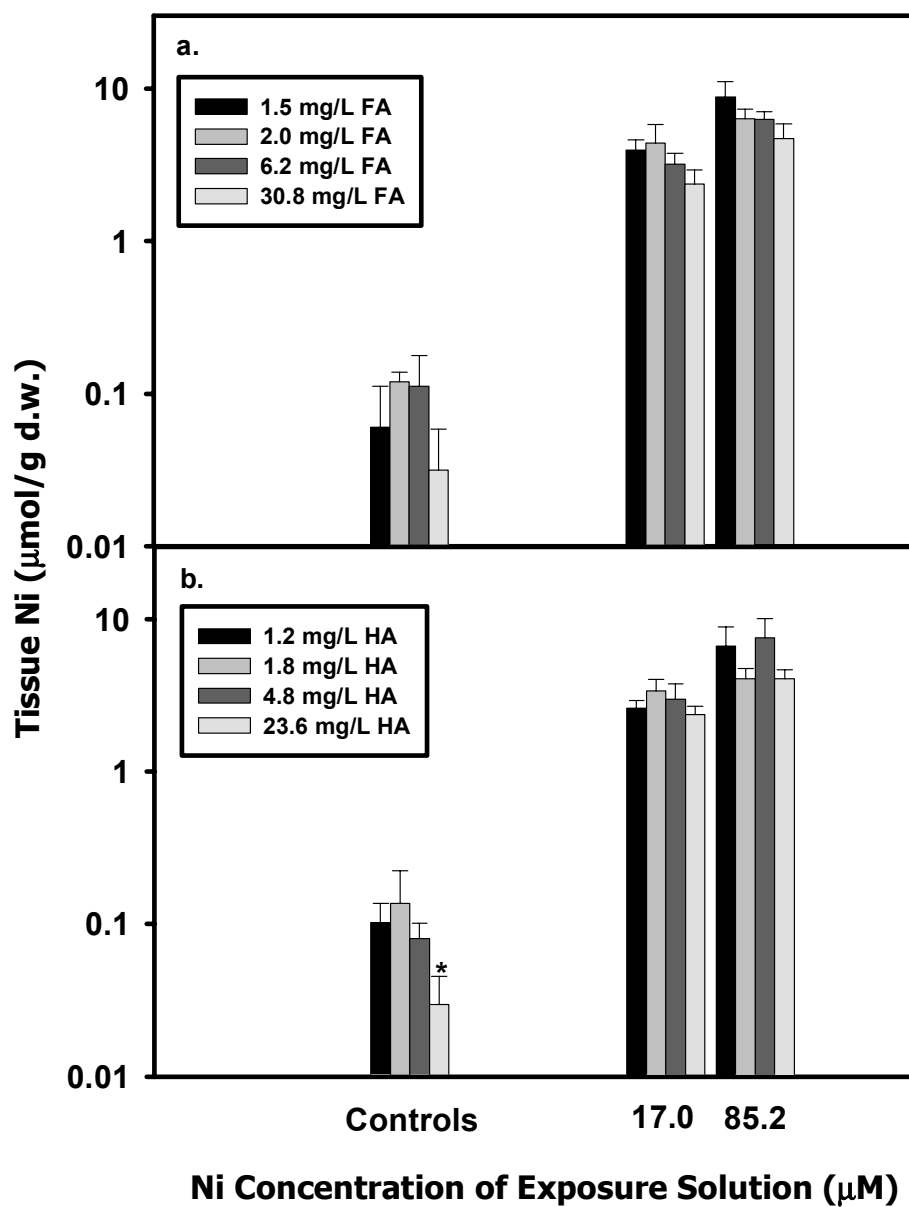
Figure 5.2. Ni tissue residues in *Hyalella azteca* (from Figure 5.1b) after 48-h exposure in Ni solutions containing (a) Suwannee River fulvic acid (FA) and (b) Suwannee River humic acid (HA). Error bars represent 1 standard deviation.
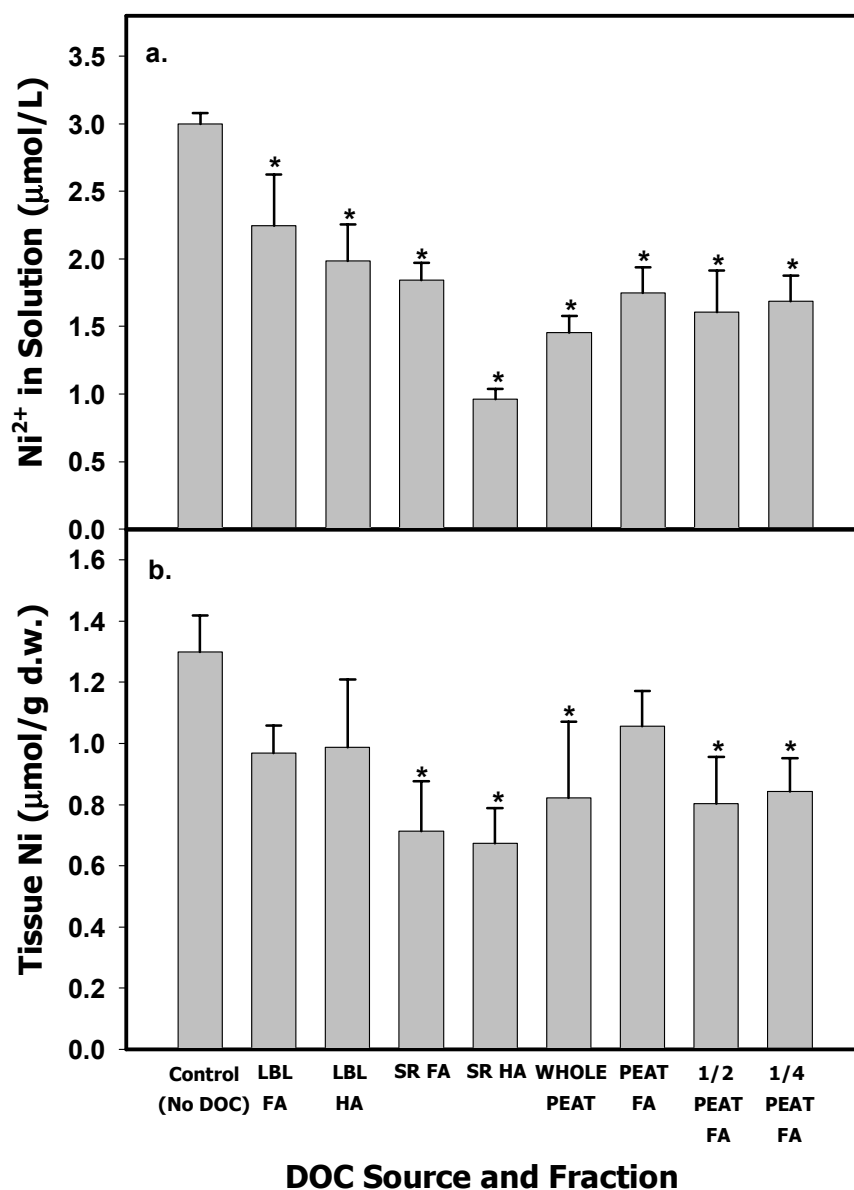
Figure 5.3. (a) Ni^{2+} concentration (µM) in different 3.41 µM total-nickel solutions containing various DOC sources and fractions, and (b) the corresponding *Hyalella azteca* tissue Ni concentrations (µmol/g d.w.) in 48-h water-only exposures. An asterisk denotes a significant difference relative to the control solution or tissue. Error bars represent 1 standard deviation.

solutions. The only combinations of solutions not statistically different were the LBL FA vs SR FA and SR FA vs peat FA. For tissue Ni, the LBL FA, LBL HA, and peat FA tissues were the only treatments not significantly different from the control, (average reduction for all treatments = $33.9 \pm 10.4\%$ relative to the control). For solutions with similar DOC concentrations (11.6 ± 0.6 mg/L; LBL HA and the two lowest peat FA solutions were excluded), the average reduction in tissue Ni was $42.2 \pm 11.2\%$ relative to the control. While SR HA had a much lower measured Ni^{2+} free ion concentration ($\sim 50\%$ lower) than the SR FA solution, the respective tissue Ni concentrations were not significantly different. A regression of tissue Ni (μmol/g) versus free Ni^{2+} (μM), both $\log_{10}$ - transformed, yielded an r^2 of 0.681, where log tissue Ni = -0.167 + 0.556[log Ni^{2+} concentration].

5.3.2.2 Experiment 4: Sublethal Ni concentration + a range of DOC

Free Ni^{2+} measurements and total tissue Ni concentrations from the second sublethal exposure experiment (at elevated DOC) are presented in Figures 5.4a and b, respectively. There was no significant difference between the two sets of controls (with or without added NaCl) although animals in the control solution with higher conductivity had somewhat lower in tissue Ni content, partly due to one low replicate reading. The following analyses were performed relative to the control solution without added NaCl. There were statistically significant decreases in Ni^{2+} concentrations (average Ni^{2+} reduction = $51.0 \pm 13.4\%$) and, excluding the peat FA solutions, in tissue Ni content (average tissue Ni reduction = $35.1 \pm 7.4\%$) at the lowest DOC concentration (~ 14 mg/L DOC) relative to the control solution (no added DOC). At all higher DOC
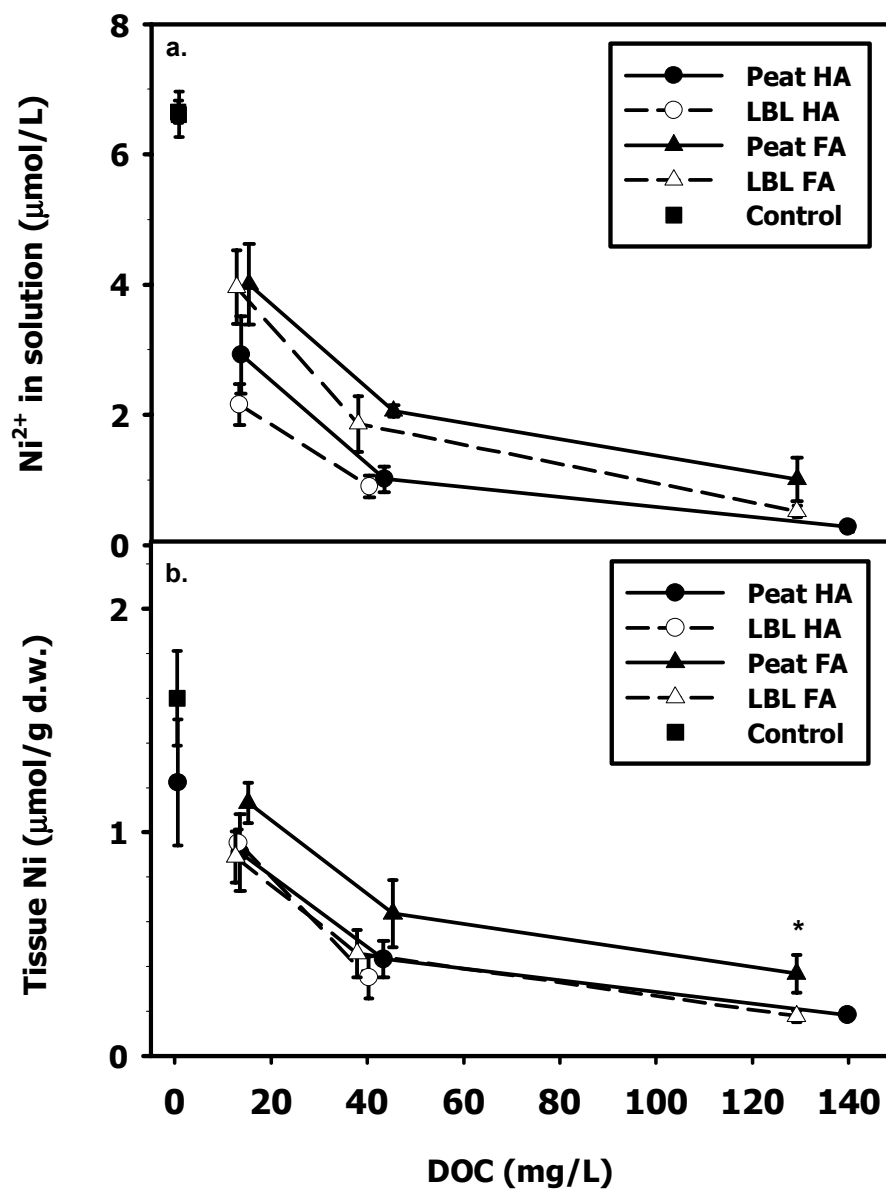
Figure 5.4. (a) Ni^{2+} concentrations in different 8.52 μM total Ni solutions containing a range of DOC concentrations, and (b) the corresponding *Hyalella azteca* **tissue Ni concentrations (μmol/g d.w.) in 48-h water-only exposures. Error bars represent 1 standard deviation**.

concentrations, the tissue Ni levels were well below those of control solutions. At the highest DOC concentrations (~130-140 mg/L), the solution Ni^{2+} and associated tissue Ni reductions were $91.0 \pm 5.6\%$ and $84.8 \pm 6.7\%$, respectively, relative to the controls. While tissue Ni concentrations from the peat FA solutions were higher than those for the corresponding HA solutions, the statistical differences between the Ni^{2+} concentrations in the FA and HA solutions (for most DOC concentrations, see Chapter 4) did not materialize in the tissue Ni data, except for peat at the highest DOC concentration (denoted with an asterisk).

Regressions of tissue Ni versus solution Ni:DOC ratio ($r^2 = 0.906$) and tissue Ni versus the solution Ni^{2+} ($r^2 = 0.931$) are plotted in Figures 5.5a and b, respectively. The Ni:DOC ratio data in Figure 5.5a and both tissue Ni and solution Ni^{2+} data in Figure 5.5b have been $\log_{10}$ -transformed. These results indicate that, for a given set of exposure conditions (i.e., similar pH, synthetic media), there is a very strong relationship between the free Ni^{2+} ion concentration (as measured by IET) and total tissue Ni in *H. azteca*, and that the free Ni ion concentration is strongly influenced by the Ni:DOC ratio.

5.4   Discussion

The majority of Ni-DOM speciation research to date has involved evaluating the complexation of Ni at low background DOM concentrations (low Ni:DOC ratios). It has been pointed out by other recent authors (Mandal et al., 2002) that few researchers have evaluated the nature of Ni-DOC interactions at Ni levels considered to pose a toxicological risk to aquatic organisms.

5.4.1   Acute Ni tests

In 48-h Ni exposure tests with DOC concentrations relevant to most North American surface waters (see Figure 5.1), regardless of source or composition (fraction), DOC does not significantly reduce acute Ni toxicity to *H. azteca*. The LC50 values in this study did not increase with increasing DOC level indicating that the Ni concentrations necessary for *H. azteca* mortality within 48-h were greater than what could be significantly complexed by the DOC. It has been shown previously that the addition of a complexing agent at concentrations lower than that of the metal ion to be complexed (i.e., a high metal:DOC ratio) results in little or insignificant effects on the metal ion speciation (Lerman and Childs, 1973; Chapter 4). The concentrations of DOC found in most surface waters are therefore insignificant relative to the concentration of Ni required for short-term *H. azteca* lethality. As the duration of a lethality test is increased, the LC50 diminishes until it reaches what is termed the incipient LC50 (when the LC50 becomes independent of time). These *H. azteca* experiments were limited to 48 h due to poor control survival beyond this time frame without addition of food (a decision made to avoid the confounding factors stated previously). Since the incipient LC50 lies beyond 48 h for *H. azteca*, one peat-FA trial was continued until 96 h (with feeding at 48 h) to evaluate the effect of DOC on acute Ni toxicity at lower total-Ni concentrations (while ignoring any confounding factors). From this experiment it was determined that the 96-h LC50 (4.2 ± 1.2 mg/L; which did not reach the incipient LC50) remained unaffected by increasing DOC. Therefore, changes to the bioavailability and toxicity of Ni at lethal concentrations will not likely manifest unless tests use extremely elevated DOC concentrations, a longer exposure duration (to reach lower, incipient
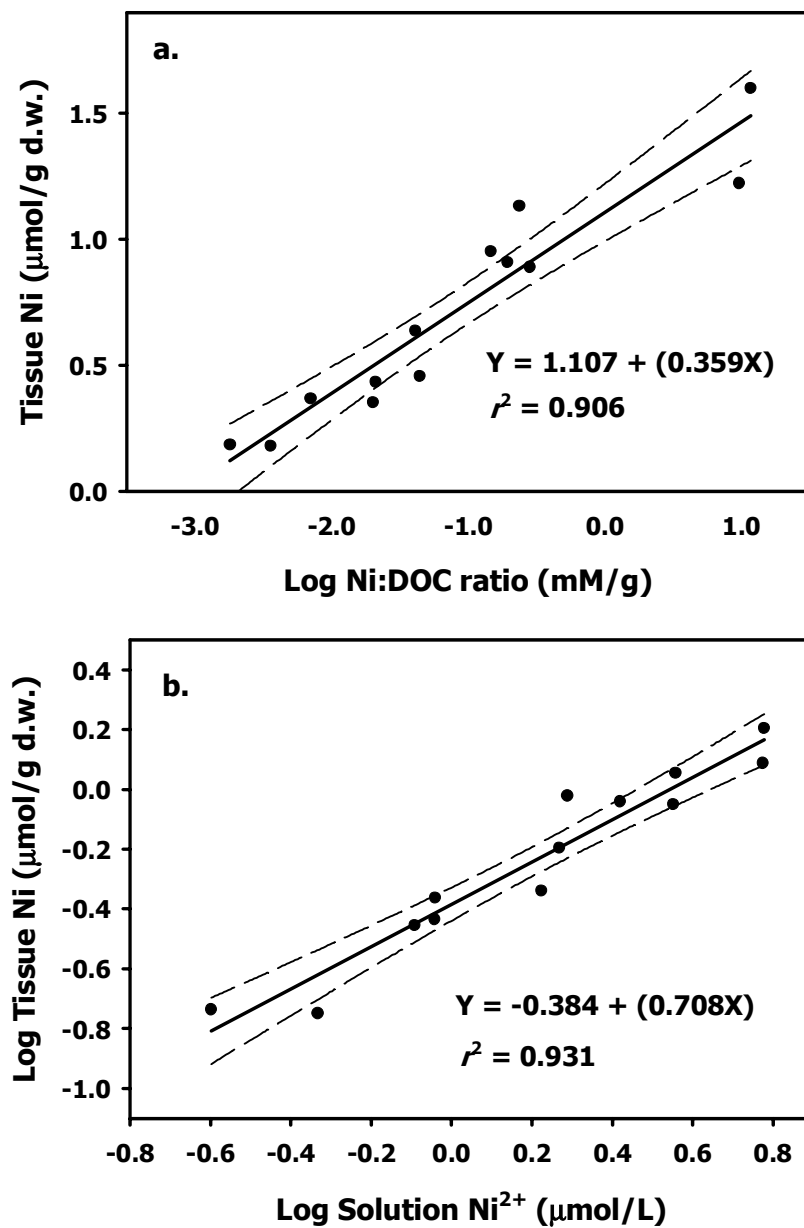
Figure 5.5. *Hyalella azteca* tissue Ni concentration (μmol/g d.w.) versus (a) Ni:DOC ratio (mM/g), and (b) Ni^{2+} concentration (μmol/L) in 48-h water-only exposures.

LC50 total-Ni concentrations), or a test organism much more sensitive than *H. azteca* to short-term acute Ni toxicity. Each approach would decrease the Ni:DOC ratio, thus possibly allowing for a measurable change in Ni speciation in the presence of DOC and hence a measurable alteration in the biological response.

In support of the above interpretation, total Ni body burden (Figures 5.2a and b) does not appear to be significantly reduced by DOC at the Ni concentrations used in this acute toxicity study. These results further suggest that lower Ni:DOC ratios are required before changes in bioavailability will manifest itself as tissue Ni reductions. Extrapolating from the sublethal results discussed below, a statistical difference should occur in the average 48-h and 96-h LC50 tissue Ni concentrations for *H. azteca* (a 35% reduction) at ~385 and ~115 mg/L DOC, respectively. While these DOC values are unlikely to occur in natural surface waters, they may possibly occur in some sedimentary interstitial waters (Thurman, 1985).

5.4.2 Sublethal Ni tests

In 48-h tests, the bioavailability of Ni to *H. azteca* at sublethal exposure concentrations (≤ 500 µg/L) was reduced by environmentally realistic additions of DOC, regardless of DOC source and fraction. At Ni concentrations (200 µg/L and 500 µg/L, respectively) between the *H. azteca* 10-d NOEC and LOEC (see Chapter 8) and with DOC at representative surface-water concentrations, the free Ni^{2+} concentration was significantly reduced relative to control solutions (averaging ~44% and ~51% at 200 and 500 µg Ni/L, respectively; Figures 5.3a and 5.4a). There were corresponding reductions in tissue Ni concentration in almost all DOC solutions regardless of source, fraction, or

DOC concentration (Figures 5.3b and 5.4b; averaging ~34 and ~35% at 200 and 500 µg Ni/L, respectively). While the SR HA solution in Experiment 4.3 (Figure 5.3a) had ~50 % less free Ni^{2+} relative to the SR FA solution, there was no significant difference between the two in total tissue Ni (Figure 5.3b). This suggests that while differences existed in the measured free ion content, the DOM-bound Ni in the SR HA solutions was more labile relative to the Ni-SR FA complexes, thereby making up for the lower free ion concentration. The measured Ni^{2+} was also significantly different between the LBL FA and LBL HA fractions (Figure 5.4a), but this difference was also absent in the corresponding Ni tissue data. While peat HA, LBL FA and LBL HA solutions all had very similar tissue Ni values at similar DOC concentrations (Figure 5.4b), the peat FA solutions consistently yielded higher tissue Ni levels (23.4 to 103.3%) relative to the average of the other three test substances.

As discussed in Chapter 4, DOC source does not appear to differentially affect Ni speciation to a significant degree. From the above results, we can also say that DOC source does not appear to significantly affect Ni bioavailability. The differences found in the free Ni^{2+} concentrations for FAs and HAs, but absent in the corresponding tissue Ni concentrations, may be due to a portion of the HA-bound Ni being relatively labile and contributing to the *H. azteca* body burden. In metal-DOM interactions, there is a continuum of complexation strengths varying from very high at low metal:DOC ratios, to very low at high metal:DOC ratios (Playle et al., 1993b). At high metal:DOC ratios even the weakest binding sites on DOM are able to sequester metal. This weakly-bound metal, while not contributing to the measured Ni^{2+} activity, remains labile in the presence of competing ligands such as those found on the surface of gills (fish or invertebrate).

Mandal et al. (2002) also demonstrated that as Ni:DOC ratio decreases, so to does the dissociation rate coefficient. Ni-DOM lability is therefore reduced at lower Ni:DOC ratios due to stronger complexation.

At the lowest Ni:DOC ratios tested (i.e., Figure 5.4; 500 µg/L and ~130-140 mg/L DOC), the reduction in free Ni, relative to the control solutions, and tissue Ni were dramatic (~91 and ~85%, respectively). In pore-water environments, where DOC may be elevated relative to the overlying water, DOC may significantly reduce the bioavailability of Ni at concentrations known to be toxicologically significant to benthic organisms. While there exists a large amount of surface water DOC data and some studies involving marine sediments (see Thurman, 1985), the literature is far from comprehensive with respect to average freshwater sedimentary DOC concentrations within those layers inhabited by benthic organisms. More research is required to fill this information gap. This research demonstrates that Ni could be significantly complexed, or largely free and labile, depending on the Ni:DOC ratio.

While it is generally presumed that the main bioavailable Ni species in aquatic systems is the free Ni^{2+} ion, only two studies to date have tested this assumption. Work by Spencer and Nichols (1983) evaluated the effect of the free Ni^{2+} ion concentration (modeled, not measured) on the growth of two species of green algae. They manipulated Ni speciation through the addition of two chelators (EDTA; NTA, nitrilotriacetic acid) to chemically defined media containing a fixed Ni concentration (10.2 µM) and found that the growth of both alga species was independent of total Ni and inversely related to the modeled Ni^{2+} ion concentration. Mandal et al. (2002) came to a similar conclusion using growth inhibition of the freshwater alga,

Pseudokirchneriella subcapitata, as the biological response. Growth inhibition of the alga was highly correlated with the free plus labile Ni concentrations in surface waters collected near ore processing and smelting operations near Sudbury, Canada. As well, they found that the higher the Ni:DOC ratio in the sample, the more labile the complexed metal. As a result, the Ni-DOM dissociation rate coefficient was also correlated with algal inhibition. The authors tried to make similar links between Ni^{2+} and biological effects in animal models (*Daphnia magna* and *Hydra attenuata*), with limited success. From Figures 5.5a and 5.5b it can be seen that there is indeed a strong relationship between measured Ni^{2+} (IET) and total Ni tissue levels for *H. azteca*, and that Ni^{2+} is dependant upon the Ni:DOC ratio. While a portion of DOM-bound Ni may be labile (as mentioned above), these results demonstrate that the free Ni^{2+} ion appears to represent the main bioavailable Ni species for aquatic organisms exposed in Ni contaminated systems.

While other factors affect Ni bioavailability and toxicity (i.e., other competing metals, hardness cations such as Ca^{2+} and Mg^{2+}, monovalent cations such as Na^+, K^+ and H^+, and competing inorganic ligands such as OH^- and CO_3^{2-}), when all media components are kept constant, the Ni:DOC ratio appears to play a major role in Ni speciation and hence Ni bioavailability and toxicity. Tissue Ni concentrations provided a reliable measure of Ni bioavailability within the 48-h time frame used here and these results suggest that tissue Ni (whole animal) may provide a good measure of Ni bioavailability to *H. azteca* regardless of the presence of modifying factors.

5.5   Conclusions

The main bioavailabile Ni species at the pHs tested (8.10 to 8.31) is the free Ni^{2+} ion. At acutely toxic Ni concentrations, the bioavailability of Ni to *H. azteca* appears to be unaffected by representative surface water DOC concentrations (1.2 to 39.5 mg/L). At Ni concentrations sublethal to *H. azteca* ($\leq$500 µg/L), the bioavailability of Ni is significantly reduced in the presence of representative surface water DOC concentrations regardless of source or fraction. Dissolved organic matter fraction (i.e., FA and HA) differentially affects Ni speciation, but has little or no effect on overall Ni bioavailability, under the conditions used here, as measured by total tissue Ni levels. This research demonstrated that Ni may be significantly complexed, or largely free and labile, depending on the Ni:DOC ratio. Overall, the Ni:DOC ratio plays a greater role than either DOC source or fraction in determining Ni speciation and hence bioavailability and toxicity to aquatic organisms. Whole animal (*H. azteca*) Ni levels appear to provide a sensitive measure of Ni bioavailability and toxicity in short-term water-only exposures.

Chapter 6

Nickel partitioning in formulated and natural freshwater sediments

6.1   Introduction

Sediments are known to accumulate trace metals in aquatic ecosystems. This is particularly true of depositional sediments in close proximity to anthropogenic metal discharges. Such sediments may become so elevated in trace metals that they pose a risk to the associated biota. To better evaluate this risk, a more complete understanding of the processes affecting the bioavailability of metals, such as nickel, in sediment is required. For organisms closely associated with surface sediments, the uptake of metals may result from exposure to the overlying water, the sediment pore water, sediment/food ingestion, or a combination of the three (Ankley, 1996; Lee et al., 2000a). It has been found for the common benthic crustacean, *Hyalella azteca,* that pore-water metal is an indicator of metal activity that correlates well with animal exposure in sediment (Ankley et al., 1991, 1993; Hansen et al., 1996). Understanding nickel partitioning between the sedimentary solid and liquid phases is therefore important in evaluating the bioavailability and toxicity of nickel to *H. azteca* in freshwater sediment.

Depositional areas occur where conditions are right for finer particles to settle from the water column. These finer particles have larger surface areas (on a fixed volume basis) than those of coarse, sandy sediments. As a result, depositional sediments

are capable of adsorbing significant quantities of trace metals directly, or indirectly through the accumulation of geochemical coatings (organic matter, iron and manganese oxyhydroxides) which will in turn act as trace element collectors (see review by Horowitz, 1991). Organic matter, which may exist as a surface coating or as a particulate, is one such trace-element collector. Since sedimentary organic carbon (OC) content typically increases with decreasing sediment particle size (Horowitz and Elrick, 1987), organic matter may play an increasingly important role in metal speciation and bioavailability in finer sediments.

Microbial decomposition of organic matter typically results in sediments that are anoxic under a thin oxic surface layer (Rhoads, 1974). Under these anoxic conditions, class B or borderline divalent cationic metals such as Cd, Cu, Pb, Zn and Ni (as classified by Nieboer and Richardson, 1980) readily form metal sulfides. As long as amorphous sulfide concentrations are in excess of the total trace-metal concentration (on a molar basis), these metals will occur predominantly as insoluble metal sulfides (Di Toro et al., 1990; Yu et al., 2001). Attempts to assess sediment toxicity based simply on acid volatile sulfide (AVS) and the metals (SEM) simultaneously extracted in cold acid (SEM/AVS ratio, or SEM in excess of AVS), while generally good at predicting non-toxic sediments, have proven less successful in identifying toxic sediments. In anoxic sediments, it is thought that metals in excess of sulfide may complex with organic matter (Mahony et al., 1996). This is thought to further buffer organisms against metal toxicity. Organic matter is believed to be particularly important in sediments low in sulfide (Di Toro et al., 1990), but it may also be an important binding phase in the oxic micro-environment surrounding many benthic organisms.

Due to the methods and reagents employed, traditional sequential extraction procedures will not differentiate between metal bound to AVS and to organic matter in sediments (Mahony et al., 1996). Metal partitioning in natural, freshwater sediment has therefore been investigated via the Anoxic Sequential Batch Titration (ASBT) method. Previously developed to evaluate metal sorption under anaerobic conditions (Mahony et al., 1996), this method allows for the determination of both AVS-bound and non-AVS bound metal.

The objectives of the research presented here were to determine the partitioning of Ni between the solid and aqueous phases in natural sediments, and to estimate the total sediment Ni concentrations required to elicit lethal and sublethal responses from *H. azteca* in 10-d sediment toxicity tests. In addition, a natural, field-collected sediment high in OC and low in AVS was used to evaluate Ni complexation to organic matter over a range of pH levels. The results were later used to better interpret both natural and formulated sediment toxicity testing results for *H. azteca* (described in Chapter 8).

6.2 Materials and methods

6.2.1 Natural sediment collection and storage

The collection and storage of natural sediments was described in Chapter 2, Section 2.2.4. These three sediments were collected from Little Bear Lake, Saskatchewan on June 16, 2001, and stored at 4°C until used in the present experiments in 2003. Sediments were designated as sediment A, B and C. Sediment D was created by combining sediments B and C on a 1:1 dry weight basis.

6.2.2 Sediment properties

Sediment organic carbon content was measured using a LECO-12 carbon analyzer (Leco, St. Joseph, MI, USA). Particle size distribution was calculated following the pipette method (Percival and Lindsay, 1997). Pore-water was isolated from archived sediments (A, B and C) via centrifugation at a relative centrifugal force of 12, 000 g for 15 min under a nitrogen atmosphere. The pore-water pH was then measured (Orion Model 370 pH meter, Beverly, MA, USA) under a nitrogen stream. The pH for sediment D was assumed to be intermediate (the geometric mean) of its constituent sediments (B + C).

6.2.3 ASBT nickel titrations

The ASBT protocol followed was similar to that of Mahony et al. (1996). Briefly, aliquots of sediment A (high OC, low AVS) were titrated with Ni under anoxic conditions at pH 6, 7 and 8. All test solutions were prepared by adding Goode Buffers (designed to be non-reactive with metals) to synthetic water. The buffers (at a 0.005 M concentration) were as follows: MES (2-(N-morpholino)ethanesulfonic acid, sodium salt) pKa 6.1, Sigma M-3885; MOPS (3-(morpholino)propane sulfonic acid, sodium salt), pKa 7.2, Sigma M-9381; HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethane-sulfonic acid, sodium salt), pKa 7.5, Sigma H-2393. The composition of the synthetic water used in all experiments was described in Chapter 4, Table 4.1. Each buffer solution was adjusted with NaOH or HCl to the desired pH (6, 7 or 8). For each sediment sample, exactly 24.6 g of wet sediment (1.5 g dry wt) was added to each 250-ml Florence flask under a N_2 atmosphere. Deoxygenated (DO <0.2 mg/L), Ni-spiked

buffer solution was then added to each treatment flask along with reconstituted water for a final volume of 100 ml (pore water volume was included in the calculations) and mixed gently before addition to the flask trains. The sediment was spiked with Ni solutions ranging from 0 to 2.18 mmol/L (145.3 μmol Ni/g dry sediment) added as $Ni(NO_3)_2 \cdot 6H_2O$ (BDH Laboratory Supplies, Poole, England). All treatments were performed in duplicate. A total of four control flasks (no added Ni) were used at each pH for total AVS determination. All flasks were gently bubbled with N_2 for 6 h after which time the N_2 was stopped and the flasks allowed to settle for 5-10 min in a glove box under a N_2 atmosphere. A sample of overlying water was pipetted off (25 ml) and filtered (0.45 μm pore size, polysulfone) for total dissolved Ni analysis. The remainder of the overlying water was then gently decanted and the sediment thoroughly mixed and divided into two pre-weighed sample bottles, one for dry weight and one for AVS analysis. Nickel analysis was performed using either a flame or furance AA spectrometer (Septa AA 50B or Spectra 220Z with a GTA 110Z, respectively; Varian Australia Pty Ltd, Mulgrave, Australia).

6.2.4 AVS Procedure

AVS analysis followed the protocol of Brouwer and Murphy (1994) which was later modified by Leonard et al. (1996b). Each sample for AVS analysis was weighed and added to a clean, dry, 500-ml diffusion bottle along with a 100 ml aliquot of deoxygenated (DO <0.2 mg/L) ultrapure water (used to rinse the sediment from the sample bottle). Sulfide-antioxidant-buffer (SAOB, 10 ml) composed of 0.2 M NaOH, 0.1 M L-ascorbic acid (AnalaR®, BDH Inc., Toronto, ON, Canada) and 0.1 M EDTA

(Sigma®, Sigma Chemical Co., St. Louis, MO, USA) was added to a 25-ml vial suspended above the sample inside the diffusion bottle. The lid of the diffusion bottle was then secured and 10 ml HCl (36.5-38.0%, AnalaR®) added through a hole in the diffusion bottle lid. The hole was then immediately sealed with a rubber stopper and the bottles mixed briskly on a multi-head magnetic stirring system. After 1 h, the 25-ml vial was removed from the diffusion bottle and sealed with a snap lid. The concentration of sulfide in the SAOB solution was measured immediately against freshly prepared standards using an Orion Model 9416 Silver/Sulfide Half-Cell Electrode with a Model 90-02 Double Junction Reference Electrode (Orion Research Inc, Beverly, MA, USA). If analysis was not performed immediately, the samples were stored under a N_2 atmosphere until processed (<6 h). Sulfide standard QC checks were performed in duplicate each day with a lead perchlorate ($Pb(ClO_4)_2 \cdot 3H_2O$) titration of the 0.001 μM sulfide standard. A QC check of the lead perchlorate solution was performed with a flame AA spectrometer using lead standards made from $Pb(NO_3)_2$. The lead perchlorate solutions were $101.9 \pm 4.5\%$ of the nominal concentration. The sulfide detection limit was 0.03 μmol S/ml when 10 ml of SAOB were used. Acid volatile sulfide digestions of sediment C were performed to determine the efficiency of the AVS protocol over time. Similar to other authors (e.g., van Griethuysen et al., 2002), not all AVS digested was absorbed by the SAOB at 1 h. At 1 h, only 73.7% of the total AVS was absorbed by the SAOB (using measued AVS concentrations at 3 h as 100 % AVS). The measured AVS values were therefore adjusted accordingly.

The metal solutions resulting from the cold acid extractions of the natural and formulated sediments described below (the SEM) were filtered and analyzed for total Ni.

Only sediments from the toxicity tests were analyzed for SEM. Since SEM has previously been shown to be unaffected by digestion time (van Griethuysen et al., 2002) and since Ni concentrations in the SEM were similar to the nominal spiked Ni values in this study (see Chapter 6), one hour was deemed to be adequate for accurate SEM (Ni) digestion.

6.2.5 Toxicity testing

Natural sediments

Toxicity testing protocols are described in detail in Chapters 7 and 8. Briefly, 10-d spiked sediment toxicity tests using *Hyalella azteca* were conducted using the four freshwater sediments described above. These sediments varied substantially in AVS and OC content (Table 6.1). Each toxicity test had five Ni treatments (spiked as $Ni(NO_3)_2 \cdot 6H_2O$) and one set of control sediment, all of which had six replicates. Simultaneously extracted metal and AVS were sampled from three replicates at the start and three replicates at the termination of each test in each set of control and treatment beakers using the methods described in Chapter 7. The AVS values presented in Table 6.1 for each sediment are from the controls.

Formulated sediments

A detailed description of the construction and properties of the formulated sediment is provided in Wang and Liber (unpublished manuscript). Briefly, a locally obtained soil (dried and pulverized), calcite ($CaCO_3$), dolomite ($CaMgCO_3$), dry ground peat, and deionized water were mixed such that only the peat (OC) content was varied

Table 6.1. Physical and chemical characteristics of natural sediments collected from Little Bear Lake, Saskatchewan, Canada. Data are means ± 1 standard deviation.

Sediment characteristic	Natural sediment			
	A	B	C	D
Particle size (%)				
Sand (54 - 2000 μm)	17.7±1.9	88.3±0.6	22.8±0.5	52.8±2.2
Silt (2 - 53 μm)	20.0±1.7	4.2±0.6	23.9±1.8	13.4±0.1
Clay (<2 μm)	62.3±3.6	7.4±1.1	53.3±1.2	33.8±2.1
Water content (%)	90.4±0.2	40.6±0.2	81.1±0.4	74.0±0.5
OC (%)[a]	18.31±0.27	0.70±0.06	14.25±0.11	7.53±0.02
AVS[b] (μmol/g d.w.)	0.73±0.13	1.80±0.67	44.05±15.90	27.87±5.48
pH[c]	6.99±0.02	7.41±0.03	6.55±0.04	6.98±0.61

[a] Organic carbon.
[b] Acid-volatile sulfide.
[c] pH was measured in pore water isolated from archived sediment.

among sediments.

For a single batch of formulated sediment, 5.0 kg of soil were placed in a large mixing bowl. Peat (previously soaked overnight in deionized water, triple rinsed and hand-squeezed) was then added (for a desired total OC content) along with calcite and dolomite (each as 0.5% of the total dry weight). Mixing (5-6 min) was accomplished using a large bakery-style dough mixer sealed with plastic sheeting. All formulated sediments were allowed to age for 14 d in a sealed plastic bucket at room temperature (~20°C) prior to spiking. The formulated sediments discussed in this chapter are designated Sediments 1, 2 and 3 and were constructed with increasing OC content (Table 6.2).

6.2.6   Statistics

Median lethal concentrations (LC50s) values were calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977). The highest sediment-Ni concentration resulting in no significant difference in the final average weight per animal, relative to the control, was designated as the no observed effect concentration (NOEC). The next greater Ni concentration was designated the lowest observed effect concentration (LOEC). Calculation of the NOEC and LOEC were performed using SigmaStat® 3.0 (SPSS Inc., 2003). One-way analysis of variance was used to determine statistical differences in *H. azteca* average weight among the various treatments and the control. When the data were normally distributed with equal variance, statistically different treatments were identified using Dunnett's Method for multiple comparisons versus a control group. In the event of unequal variance, Dunn's method for multiple

Table 6.2. Physical and chemical characteristics of formulated sediments varying

in OC content. Data are means ± 1 standard deviation.

Sediment characteristic	Formulated sediment		
	1	2	3
Particle size (%)[a]			
Sand (54 - 2000 µm)	50	50	50
Silt (2 - 53 µm)	33	33	33
Clay (<2 µm)	17	17	17
Water content (%)	25.8±0.1	32.7±0.2	43.2±0.1
OC[b] (%)	1.61±0.14	3.20±0.32	6.76±0.02
AVS[c] (µmol/g d.w.)	nd[d]	nd	nd
pH[e]	7.3	7.3	7.3

[a] From Wang and Liber (unpublished manuscript) before the addition of peat.
[b] Organic carbon.
[c] Acid-volatile sulfide.
[d] Not detected. Detection limit = 0.03 µmol/g.
[e] From Wang and Liber (unpublished manuscript).

comparisons was employed. All analyses were performed with $\alpha = 0.05$.

6.2.7 Partitioning equations

Following the method of Mahony et al. (1996), the sorbed nickel fraction (C_s) was determined by calculating the difference between the total metal added (C_T) and the measured aqueous metal fraction (C_W) (equation 1).

$$C_s = C_T - C_W \tag{1}$$

The total nickel sorbed to sediment particles can be considered a combination of sulfide-bound metal and metal bound to non-sulfide components (equation 2).

$$C_s = C_{s,AVS} + C_{s,non\text{-}AVS} \tag{2}$$

The first term in this equation can be solved by assuming that the nickel bound to AVS is approximately equal to the total measured AVS concentration in each test (equation 3).

$$C_{s,AVS} = [AVS] \tag{3}$$

Sulfides have been shown to successfully compete with other common sedimentary ligands to form strong (insoluble) metal complexes with class B metal ions (Emerson et al., 1983). The reaction has also been demonstrated to be rapid for cadmium, achieving thermodynamic equilibrium within minutes to hours (Di Toro et al., 1990). The non-AVS-sorbed Ni fraction can therefore be calculated by combining equations 2 and 3 (equation 4).

$$C_{s,non\text{-}AVS} = C_S - [AVS] \tag{4}$$

The non-AVS-sorbed nickel concentration can then be normalized by the OC fraction where f_{oc} is the fraction of OC relative to the total dry weight of the sediment (equation

5).

$$C_{s,OC} = C_{s,non\text{-}AVS} / f_{oc} \tag{5}$$

For simplicity, Freundlich isotherms were used to express the relationship

between non-AVS-bound Ni and dissolved Ni. Due to the high OC content in the Ni-

titrated test-sediment (described later), it was assumed that Ni was predominantly bound

to OC. Metal binding in excess of AVS in anoxic sediments has previously been

attributed to sediment organic carbon for class B metals such as Cd, Cu and Pb (Mahony

et al., 1996). The relationship between OC-bound and dissolved Ni is shown in

equation 6, were C_w equals the dissolved Ni concentration (μmol/L) where m and n are

constants.

$$C_{s,OC} = mC_w{}^n \tag{6}$$

In anoxic sediment Ni will preferentially bind to AVS ($C_{s,AVS}$) until this pool is

exhausted. After that, Ni will then partition between pore water and binding sites on the

organic matter. Substituting equations 5 and 6 into equation 2, the total Ni (μmol/g)

predicted to elicit toxicity (C_s or C_{SQC}) is therefore

$$C_s \text{ or } C_{SQC} = C_{s,AVS} + mC_{PWQC}{}^n f_{oc} \tag{7}$$

where SQC stands for sediment quality criteria and PWQC stands for pore-water quality

criteria.

To evaluate the accuracy of predicting Ni bioavailability and toxicity to *H. azteca*

using the above Ni partitioning calculations, 10-d pore-water Ni LC50, LOEC and

NOEC values from spiked formulated and natural sediment toxicity tests (see Chapter 8)

were entered into each equation as though they were pore-water quality criteria (C_{PWQC}).

The total sedimentary Ni (μmol/g) required to achieve these criteria (referred to as the

predicted LC50, LOEC or NOEC) was calculated for each sediment. The predicted LC50, LOEC or NOEC Ni values were then compared to the actual toxicity-testing results (referred to as the actual LC50, LOEC or NOEC values).

6.3   Results

6.3.1   Sediment characteristics

The physical and chemical characteristics in Table 6.1 and 6.2 show that, while lower in water content, the formulated sediments characteristics were similar to, or fell within, the ranges measured in the Little Bear Lake natural sediments. The particle size distribution for the formulated sediments is from the earlier work by Wang and Liber (manuscript) which thoroughly characterized sediments constructed via this methodology. It has been assumed that the particle size ranges from Wang and Liber (unpublished manuscript) are consistent with the sediments constructed in this study since exactly the same constituents were used. In the natural sediments, particle size distribution ranged from coarse (sediment B) to fine (sediment A). The overall particle size distribution in the formulated sediments was most similar to Sediment D. In the formulated sediments, the OC content ranged from 1.61 to 6.76 % of the total d.w. OC in the natural sediments ranged from very low (0.70%) in sediment B to high (18.31%) in sediment A. Acid volatile sulfide ranged from undetectable in the formulated sediments to very high in sediment C (44.05 µmol sulfide/g d.w.). The pH was close to neutral for all formulated and natural sediments.

6.3.2   Anoxic sequential batch titrations

Figure 6.1a shows the total sorbed nickel (calculated from equation 1) versus the total dissolved nickel (measured) in natural sediment A. As pH increased so did the binding of nickel to the sediment solid phases. The total AVS concentration in the control solutions was 0.84 ± 0.14 µmol/g d.w. for all three ASBT tests combined. Figure 6.1b shows the total non-AVS-sorbed Ni (equation 4) versus the total dissolved nickel. This plot is similar to Figure 6.1a, but does not include binding due to AVS. The final plot (Figure 6.1c) shows the non-AVS-sorbed nickel concentration normalized by the OC fraction (equation 5). Again, as pH increased, so did the partitioning of Ni to the solid-phase OC.

Figure 6.2 is a log-log plot of bound Ni (µmol/g OC) versus total dissolved nickel (µmol/L); the relationship is approximately linear for the range of Ni concentrations tested. For simplicity, Freundlich isotherms were therefore used to express the relationship between bound and dissolved Ni for each pH tested. The data points presented at bottom-left have not been included in the isotherms for any pH since these data are skewed to the right as a result of background pore-water Ni concentrations present prior to Ni-spiking (measured in toxicity-test control sediment; Chapter 8). These dissolved-Ni concentrations were also well below the toxic range of interest. The Freundlich isotherms (equation 6) for each pH data set yielded the equations and r^2 values given in Figure 6.2, were Y is bound Ni (µmol/g OC) and X equals the dissolved Ni concentration (µmol/L).
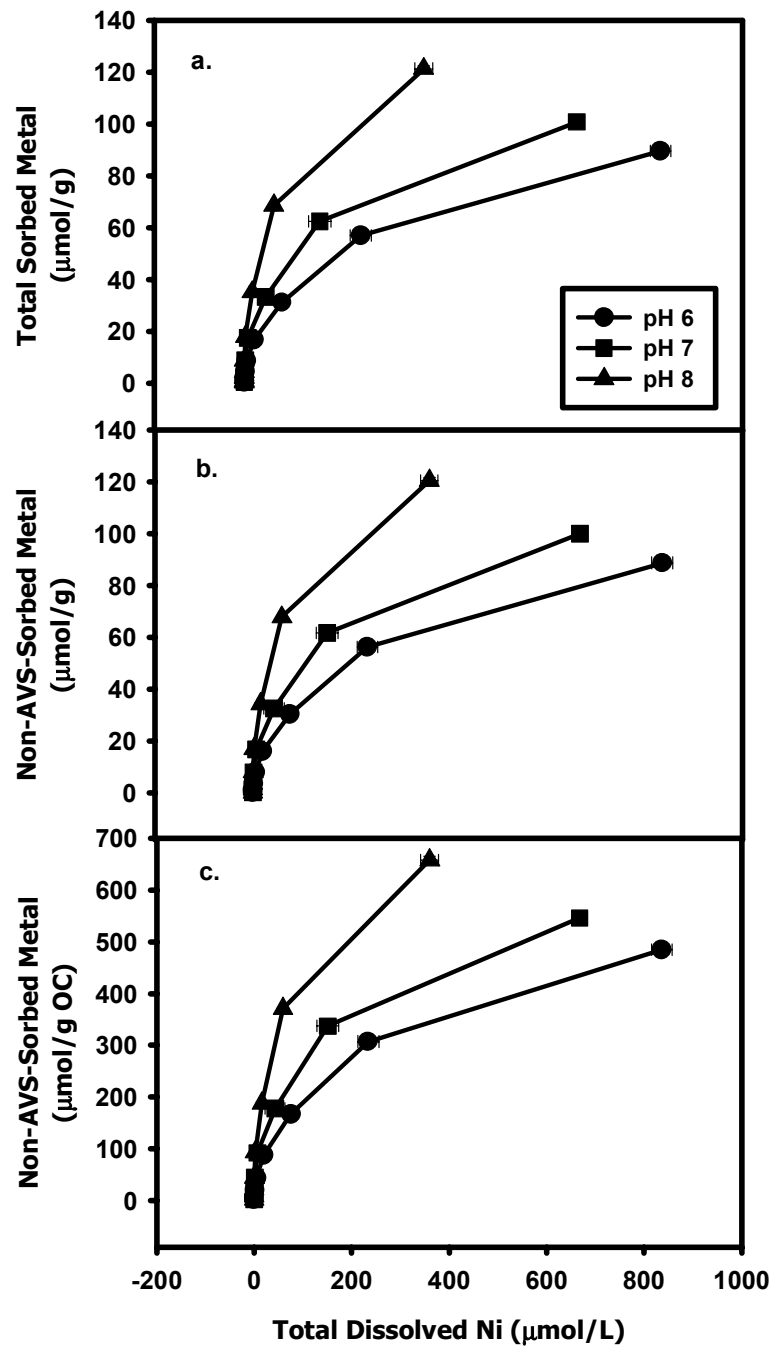
Figure 6.1. Isotherm plots of sorbed versus total dissolved nickel in natural sediment at pH 6, 7 and 8. Total sorbed nickel is plotted in (a), non-AVS-sorbed nickel in (b), and organic carbon normalized non-AVS-sorbed nickel in (c). Data are means ± 1 standard deviation.
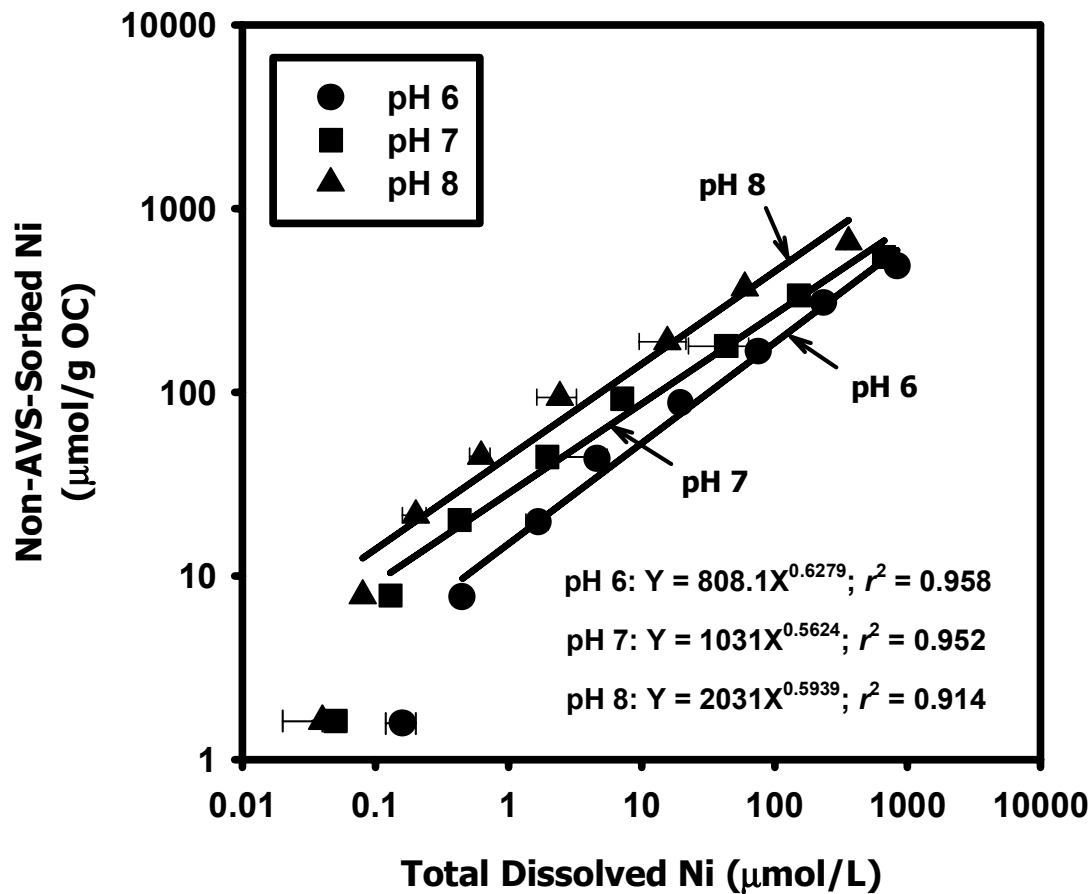
Figure 6.2. Non-linear regressions of sorbed Ni (μmol/g OC) versus total dissolved Ni (μmol/L) in sediment titrations at pH 6, 7 and 8. Data are means ± 1 standard deviation.

6.3.3 Ni toxicity predictions vs actual test results

The predicted LC50 values (μmol/g; based on predicted pore-water Ni concentrations) for the four natural sediments (A-D), at the three tested pHs, have been plotted in Figure 6.3 as closed symbols. The actual total-Ni LC50 values (from the toxicity tests described in Chapter 8) are plotted as open symbols at the pore-water pH values measured from the archived sediment subsamples. Also in Figure 6.3 are the predicted and actual LOEC and NOEC values. Since all of the Ni treatments in sediment B had a significant effect on average weight per animal (relative to the controls), a NOEC value could not be derived for that sediment. Due to an observed decrease in AVS with increasing total Ni concentrations (an observation discussed further in Chapter 7), all calculations for the prediction of the LC50, LOEC and NOEC endpoints were based upon the AVS concentrations measured in the corresponding Ni-treated sediments in the toxicity tests. The AVS concentrations for the predicted LC50 values were estimated from measurements for the two nearest actual endpoints. The predicted LC50, LOEC and NOEC values for sediments A (see Figure 6.3) were similar to the respective actual values (although somewhat higher at 36.4%, 39.5%, and 96.8% greater that the actual levels, respectively). The predicted LC50 and LOEC values for sediment B were similarly close to the actual values (28.6% less and 73.8% more than the actual levels, respectively). While the predicted LC50 for sediment C was close to the actual value (which was 15.2% lower), the remaining predicted LC50, LOEC and NOEC values for sediments C and D were all substantially higher than the actual values. Interestingly, the predicted NOEC values in sediments C and D were similar to or greater than the predicted corresponding LOEC values. While this is counter intuitive, it results from the
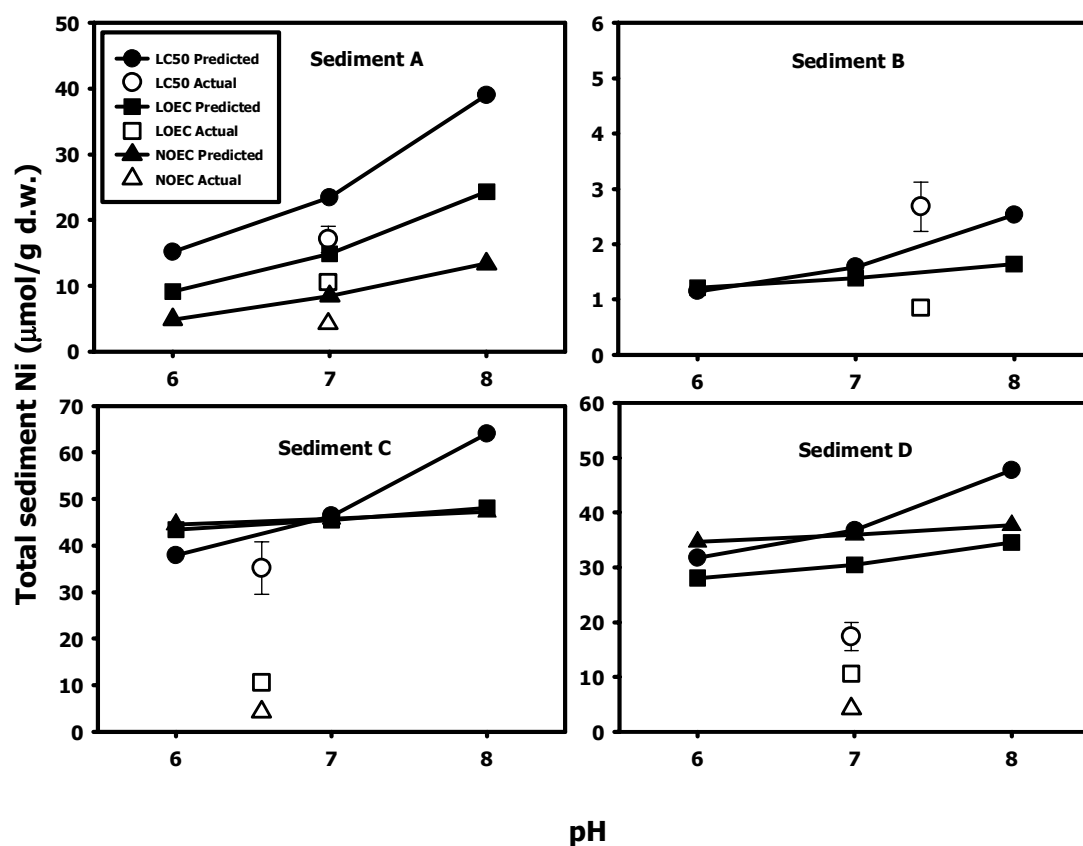
Figure 6.3. Predicted and actual total sediment Ni concentrations (μmol/g d.w.) required to reach pore-water Ni levels corresponding to 10-d *Hyalella azteca* LC50, LOEC, or NOEC values in natural sediments A to D. Data are means ± 1 standard deviation.

decrease in AVS observed with increasing Ni (see Chapter 7, Figure 7.2). If the AVS concentrations used in the calculations were at a fixed value for all Ni-treatments of a given sediment, the magnitude of the predicted endpoint Ni concentrations would be as follows: LC50 > LOEC > NOEC.

The total sediment Ni (μmol/g) concentration predicted to result in LC50, LOEC and NOEC endpoints in the formulated sediments (based on predicted pore-water Ni concentrations) have been plotted in Figure 6.4 as closed symbols. The actual values are shown as open symbols at a pH of 7.3. Since all of the total-Ni concentrations tested in sediment 3 had an effect on the average weight per animal (relative to the controls), a NOEC value could not be calculated. The predicted LC50, LOEC and NOEC values for sediments 1 to 3 were similar to the actual values. However, there appeared to be an upward shift in the predicted LC50 and LOEC values relative to the actual values as OC content increased. While the sediments were buffered with calcite and dolomite, pore-water pH may have decreased with increasing OC (peat) content. The nominal pH of 7.3 may therefore overestimate the true pH especially in sediment 3.

SEM (in this case Ni) in excess of AVS is thought to represent the Ni fraction available for complexation with OC. The actual LC50, LOEC and NOEC values were therefore calculated based not only on total sedimentary metal (μmol/g), but also on SEM concentration (μmol/g) in excess of AVS. These values were then compared to the SEM levels predicted to have similar biological effects. The results are graphed in Figure 6.5, and appear similar to the previous set of plots in Figure 6.3. The predicted LC50, LOEC and NOEC values based on excess SEM for sediments A and B were reasonably similar to the actual values. The predicted LC50, LOEC and NOEC values
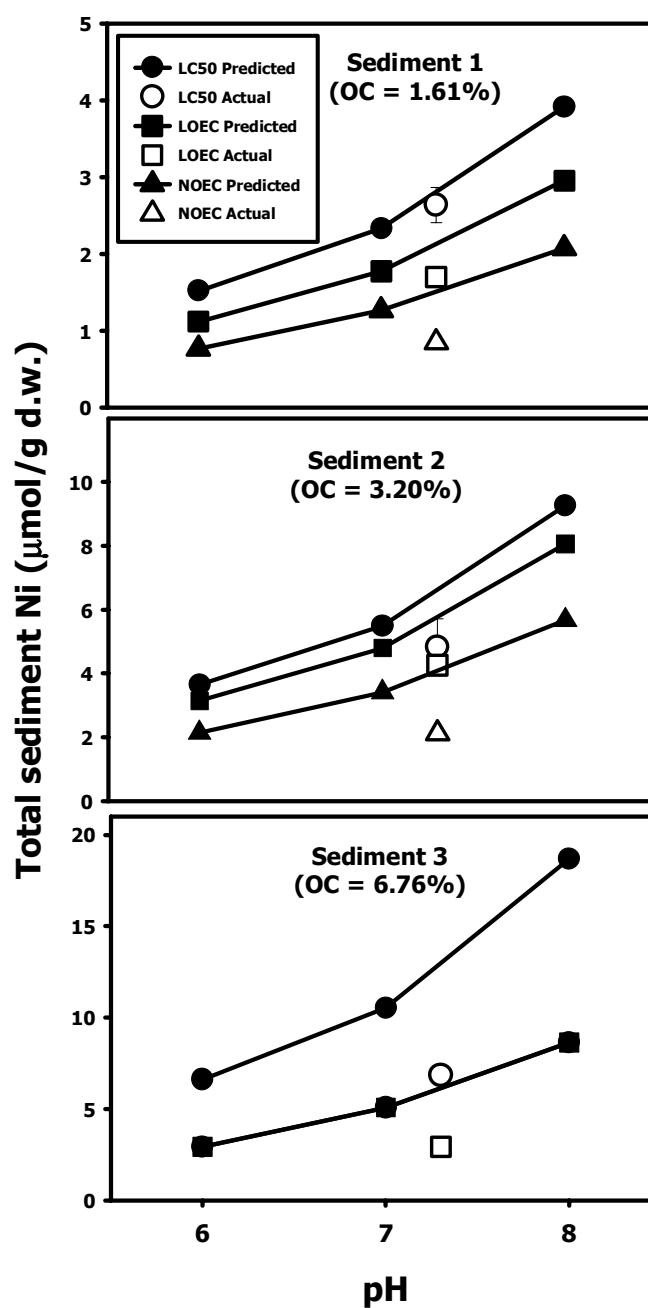
Figure 6.4. Predicted and actual total sediment Ni concentrations (μmol/g d.w.) required to reach pore-water Ni levels corresponding to 10-d *Hyalella azteca* LC50, LOEC, and NOEC values in formulated sediments varying in organic carbon (OC) content. Actual data are means ± 1 standard deviation.
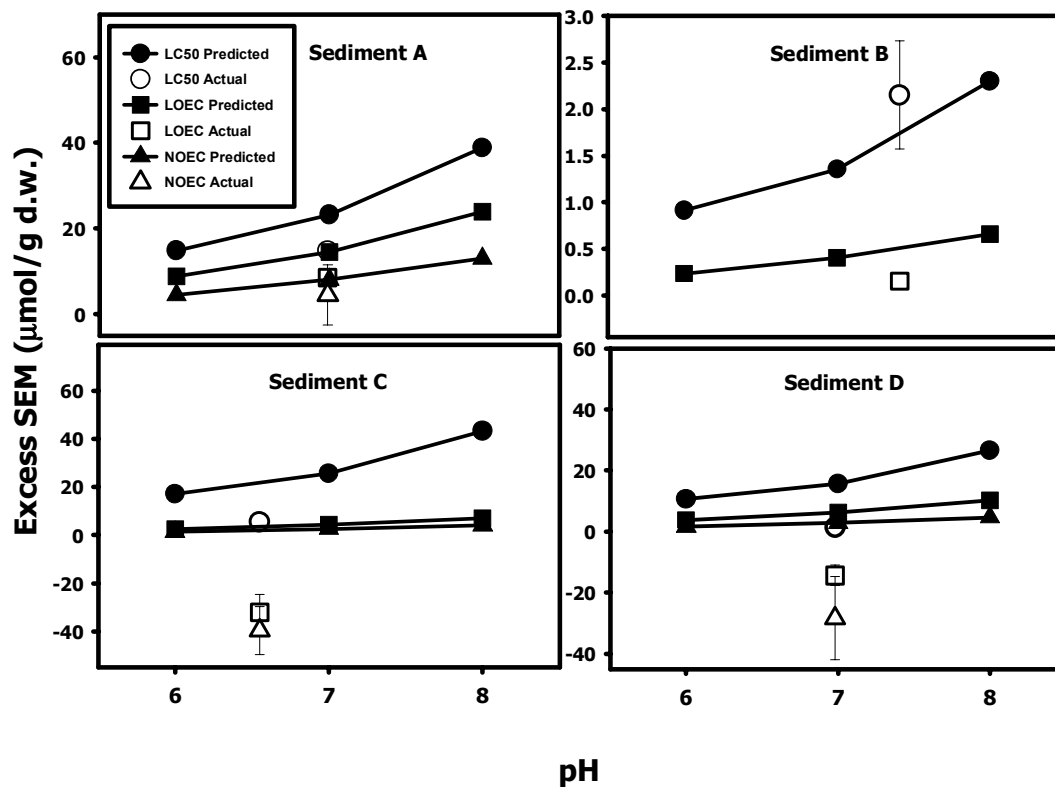
Figure 6.5. Predicted and actual total sediment SEM (Ni) concentrations in excess of AVS required to reach pore-water levels corresponding to 10-d *Hyalella azteca* LC50, LOEC, and NOEC values in natural sediments A to D. Actual data are means ± 1 standard deviation.

for sediments C and D were all generally higher than the actual values.

6.4   Discussion

Previous studies have identified Ni as one of a number of divalent cationic metals for which acute toxicity to benthic organisms in depositional sediments is strongly influenced by AVS content (Ankley et al., 1991; Di Toro et al., 1992; Pesch et al., 1995; Berry et al., 1996). Upon addition to anoxic sediment containing AVS, these metals are thought to preferentially form insoluble metal sulfides displacing Fe and Mn in the process (Di Toro et al., 1990). Once AVS content is exceeded, these metals are then thought to be available for complexation with OC, as was the case for Cd, Cu and Pb in the sediment titrations of Mahony et al. (1996). Therefore, the presence of additional binding phases, such as OC, helps to explain why SEM/AVS molar ratios may exceed 1, or SEM - AVS can exceed 0, without acute metal toxicity being observed. While other Ni-binding phases may be present within a sediment (e.g., carbonates; Yu et al., 2001), the high cation exchange capacity of organic matter (Rashid, 1974)**,** its tendency to significantly cover other inorganic coatings (Davis, 1982), and the very high OC content (18.3%) in our titrated sediment, meant that Ni complexation in excess of AVS content could reasonably be attributed to OC. The data presented in Figure 6.1 demonstrates that sediments low in AVS but high in OC may complex significant quantities of Ni under anaerobic conditions. Organic matter content will therefore significantly influence nickel bioavailability and toxicity in sediments low in AVS (discussed further in Chapter 8).

Within the range of Ni concentrations tested, the log-log plot of bound Ni

(µmol/g OC) versus total dissolved Ni (µmol/L) was approximately linear for those

concentrations above the influence of background dissolved Ni (Figure 6.2). Similar to

other trace-metal research (i.e., Mahony et al., 1996), increasing pH was found to

significantly increase metal partitioning to the sediment solid phase. Increased

partitioning to organic matter with increasing pH has also been reported for Ni binding

to organic colloids (Cantwell and Burgess, 2001) and to dissolved organic carbon

(Christensen and Christensen, 2000). Carboxylic and phenolic functional groups present

in humic acids (which are abundant in sediment and peat) deprotonate at higher pH

(Benedetti et al., 1996). This results in an increased number of potential metal-binding

sites. The increase in Ni binding between pH 6 and 8 demonstrates that small changes to

the pore-water pH (the interstitial environment) can dramatically affect Ni speciation and

therefore Ni bioavailability within sediment. Similarly, the pH has been found to be the

main factor modifying metal partitioning in soils (Peijnenburg et al., 2001).

Given the total sediment Ni concentration and its binding affinity to OC at a

given pH, sediments A and B in Figure 6.3 and sediments 1 through 3 in Figure 6.4

demonstrate that Ni bioavailability and toxicity in low-AVS sediment are reasonably

predictable. The predicted LC50, LOEC and NOEC values for all of these sediments

were relatively similar to the actual values (within a factor of 1.8 ± 0.9) indicating the an

equilibrium partitioning approach to assessing the potential for acute and chronic

sediment toxicity can work. Conversely, bioavailability appears to be less predictable in

those sediments in which AVS plays a greater role in the overall metal partitioning.

Aside from the LC50 value in sediment C, all of the measured toxicity endpoints in

sediments C and D are well below the predicted levels. It would seem that the presence

of elevated concentrations of AVS reduce the predictability of Ni bioavailability and toxicity based on its partitioning to AVS and OC in sediments.

The central caveat in the SEM:AVS equilibrium partitioning approach to estimating metal toxicity in sediments is that the metal of interest is in equilibrium with the metal sulfide phase. Due to the relative insolubility of the metal-sulfide complex, pore-water metal (a predictor of sediment-metal toxicity) should be well below toxic concentrations when AVS is in excess of SEM. However, a true equilibrium between the metal-sulfide and the respective dissolved species in pore water does not appear to exist in either nature or the laboratory. Previous studies involving anoxic saline waters (Jacobs et al., 1985), sulfide-rich ground water (Boulegue, 1977), marine sediment pore waters (Brooks et al., 1968; Presley et al., 1972), or formulated marine sediments (Oakley et al., 1980), have all found levels of dissolved class B and/or borderline metals to be well above those expected assuming an equilibrium between the precipitated sulfides and the dissolved species. Brooks et al. (1968) concluded from thermodynamic calculations that none of the metals considered (Ni included) could have existed in solution in the concentrations measured if they were bound as simple sulfides. Similarly, Oakley et al. (1980) found that the concentrations of dissolved metals measured in metal-spiked (Cu, Cd, Pb and Zn), formulated marine sediments were much higher than would have been expected assuming that pure metal sulfides were controlling metal solubility. As well, Jacobs et al. (1985) concluded that the observed metal profiles of Mn, Ni, Cu, Zn and Cd, across the O_2/H_2S interface in a marine basin were not the result of equilibrium between pure metal sulfide and the respective dissolved species. The lack of an equilibrium between precipitated sulfides and dissolved species in sulfidic ground

water in a study by Boulegue (1977) was believed to be due to enhanced metal solubilization by complexation with polysulfide ions and/or thiosulfate. Other authors have also suggested that the formation of metal bisulfides and/or polysulfides may increase the solubility of metals beyond what the solubility product would indicate (Hemley, 1953; Krauskopf, 1956; Gardner, 1974). More recently, Ni has been found to form $NiHS^+$ in anoxic seawater solutions (Luther et al., 1996).

While sulfides have been found to dominate the solid-phase partitioning of Ni (Nissenbaum and Swaine, 1976), metal enrichments in the interstitial waters may also be due to organic complexation (Nissenbaum and Swaine, 1976; Presley et al., 1972; Krom and Sholkovitz, 1978) which acts to dissolve or leach mineral phases. While oxygen containing functional groups are considered to be important in metal complexation, the high concentration of nitrogen and sulfur in marine humic substances may greatly contribute towards the binding of trace metals (Nissenbaum and Swaine, 1976). Overall, while there is some debate as to the relative importance of organic versus inorganic mobilization of metals under anoxic conditions, polysulfide complexes are thought to be of greater importance (Morse et al., 1987).

The lower observed toxicity values compared to predicted values may also result from the competition of Fe^{2+} with Ni^{2+} for binding sites on OC. During the formation of the more insoluble NiS, Fe^{2+} is liberated from amorphous FeS. Competition from liberated Fe^{2+} would reduce the partitioning of Ni to OC in sulfidic sediments and thereby reduce the predicted protective effects of OC. The competitive effects of displaced Fe^{2+} were not evaluated in the anoxic titrations involving low-AVS sediment.

Based on the previous works cited above, it would appear that the spiked Ni did

not achieve equilibrium with NiS in sediments C and D. While pore-water Ni concentrations did show a dramatic increase once total Ni exceeded AVS, pore-water Ni at all SEM:AVS ratios was measured at concentrations well above those expected (detailed further in Chapter 8). While excess AVS means that the dissolved Ni concentrations will be relatively low (and hence acute toxicity is not seen), excess AVS does not mean the complete absence of Ni from pore water or the absence of Ni activity. While the assumption of equilibrium with the corresponding metal-sulfide may appear valid for less soluble class B metals such as Cd, this may not be the case for borderline metals such as Ni. In the presence of abundant or excess AVS, Ni can be present in the pore water (possibly as $NiHS^+$) and can be bioavailable to *H. azteca* (discussed in Chapter 8).

In addition to expressing sedimentary metals as total concentrations, they may also be expressed as SEM in excess of AVS. Figure 6.5 shows that, similar to other Ni studies involving amphipods (e.g., Ankley et al., 1991; Di Toro et al., 1992), acute toxicity (LC50) is not seen when AVS is in excess of SEM. High mortality was seen in all sediments only when SEM exceeded AVS. Toxicity tests relying solely upon mortality therefore appear to agree with SEM:AVS approach. However, the LOEC and NOEC endpoints indicate that, while a large percentage of spiked Ni will associate with AVS in anoxic sediment, Ni speciation is more complex and attempts to apply the SEM:AVS method to more subtle Ni toxicity endpoints (e.g., growth and tissue Ni) may require a more detailed understanding of transitional metal behaviour in anaerobic sediment and at the oxic-anoxic boundary.

Application to sediment quality guidelines

This study showed that organic matter had a substantial effect of Ni bioavailability and toxicity, especially in high OC, low AVS sediments. A substantial shift in the partitioning of Ni to the solid phase occurred in the formulated sediments when OC content was increased. Similar to our findings for Ni, previous work by Mahony et al. (1996) and Besser et al. (2003) have found that Pb, Cd and Cu partitioning is also influenced by sedimentary OC content. As suggested by Ankley et al. (1996) and reiterated by Besser et al. (2003), it would seem that equilibrium partitioning-based criteria (or guidelines) can be improved through the evaluation of metal complexation to sedimentary organic matter.

The SEM:AVS ratio or SEM in excess of AVS has proven to be very predictive of non-acutely toxic sediments (see review by Ankley et al., 1996). The present research has shown that while excess AVS appears to predict the absence of acute Ni toxicity, it does not always predict the absence of chronic toxicity endpoints such as impaired growth. This may be due to the solublization of Ni resulting from the formation bisulfide complexes. Further research should therefore be conducted with a broader range of pore-water matrixes to better evaluate Ni binding under a variety of environmental conditions. Since metals rarely occur singly under field conditions, it would seem appropriate to suggest that future research also be applied to sediments contaminated with multiple class B and borderline metals, or with other cationic metals (such as Fe) which may compete with SEM in excess of AVS for OC binding sites, to better simulate contaminated field conditions.

6.5   Conclusions

In addition to AVS, organic matter influences Ni partitioning in sediments and thereby directly affects Ni bioavailability and toxicity to *H. azteca*. Partitioning to the solid sediment phase was found to increase with increasing pH (pH 6 to 8). Using the equilibrium partitioning approach, lethal and non-lethal toxicity concentrations were predictable in low AVS sediments. Due to a hypothesized lack of equilibrium between the spiked Ni and the associated sediment NiS, and the possible competition of Fe^{2+} with Ni^{2+} for binding sites on OC, equilibrium predictions overestimated the combined protective effects of AVS and OC in the mid to high AVS sediments. While excess Ni toxicity to *H. azteca* only occurred in sediments with SEM concentrations in excess of AVS, chronic effects such as reduced weight occurred even in the presence of excess AVS. Equilibrium partitioning based sediment quality criteria (or guidelines) can be improved through the evaluation of metal complexation to sedimentary organic matter, but further research is required.

Chapter 7

A non-destructive method for sampling SEM and AVS from laboratory sediment

toxicity tests

7.1   Introduction

Sediments are known to behave as both sink and source of trace metals. Various

studies have found that total sediment metal concentration is a poor measure of metal

bioavailability and toxicity. Pore water metal concentration appears to be a better

predictor of metal exposure to the associated sediment-burrowing biota (e.g., Swartz et

al., 1985; Ankley et al., 1993). Work has therefore progressed to evaluate metal

partitioning between the sedimentary solid and aqueous phases. For sulfide-forming

metals (i.e., Cd, Cu, Pb, Ni and Zn), the concentration of acid volatile sulfide (AVS) is

considered to be a major determinant of speciation in anoxic sediment (Di Toro et al.,

1990). Trace metals released during AVS digestion are referred to as the simultaneously

extracted metals (SEM). Interpretation of sediment toxicity test results relies heavily

upon the SEM:AVS ratio, or the SEM in excess of AVS. If the SEM concentration

does not exceed the binding capacity of AVS, the sediments are not expected to be toxic.

If SEM exceeds AVS, sediments may or may not be toxic, depending upon the

abundance of additional binding phases such as organic carbon (OC).

Standard upon completion of all sediment toxicity testing is the retrieval of the

surviving organisms via wet-sieving. This process is destructive and precludes sampling

the sediment for chemical characterization. To compensate for this limitation, the current approach is to have separate chemistry (i.e., SEM:AVS, total metals, pore-water metals) and biology/toxicology (i.e., survival, average weight, bioaccumulation) replicates. Researchers are therefore often faced with the issue of how to balance the need for adequate replication with logistical constraints. In reviewing the literature it was apparent that, with the growing interest in *H. azteca* as a benthic animal model, and in the effects of AVS on metal bioavailability, a non-destructive methodology was required for the measurement of SEM and AVS directly from the same test vessels in which the animals were exposed. This is especially important in bioavailability studies where even small differences between a biological and chemical replicate could make test results very difficult to interpret (e.g., when SEM:AVS ratios are near 1.0).

This research describes a simple method for obtaining both SEM:AVS and biological data from the same sediment test replicates without compromising either the precision and accuracy of the chemical data, or affecting the associated amphipod growth and survival data. The methodology described here was also developed to be complementary to a previously developed method for pore-water collection (Doig and Liber, 2000).

7.2   Materials and methods

7.2.1   Natural sediment toxicity tests

Core tubes (mini-corers) were used to obtain SEM:AVS data from toxicity tests with Ni-spiked sediments. Tests were also conducted to assess the influence of organic matter on Ni toxicity and bioavailability (see Chapters 6 and 8); toxicity testing protocols

are described in detail in Chapter 8. Briefly, 10-d spiked sediment toxicity tests using *H. azteca* were conducted on four freshwater sediments (A-D) spiked with nickel (Ni). Using an Ekman grab sampler (15×15 cm), sediments A-C were collected from Little Bear Lake (54° 17' N, 104° 41' W), an oligotrophic lake in north-central Saskatchewan, Canada, which is known from previous analysis to be free from significant metal contamination. Sediments were placed in 20-L plastic pails and allowed to settle 1 h before the surface water was carefully decanted. The sediment was brought back to the laboratory, homogenized, and refrigerated (4°C) within 24 h of collection. Sediments B and C were also combined on a 1:1 dry weight basis to create sediment D.

Each 10-d toxicity test was comprised of one set of controls and five Ni treatments (spiked as $Ni(NO_3)_2 \cdot 6H_2O$), each consisting of six replicates. After sediments were spiked and thoroughly mixed using a paint shaker for 1 h, the sediments were aged for 12 d at 4°C. Aliquots (for a total of 80 ml) were then added to 300-ml tall-form beakers (Figure 7.1) equipped with mesh screens for use in a flow-through test system modified from Benoit et al. (1993). Mini-peepers (previously described in Chapter 2; Doig and Liber, 2000) were carefully placed in the beakers along with 100 ml of overlying water. The mini-peepers were offset from the beaker center to provide space for the SEM:AVS core-tubes. The assembled beakers were then aged a further 4 d at 4°C prior to their addition to the test system. At this time, core tubes for SEM:AVS sampling (described below) were placed in the beakers and the sediments aged another 4 d prior to the addition of ten, 7-14 d old *H. azteca*. At the beginning of each toxicity test (immediately prior to the addition of animals), one core sample was taken from each of three replicates (mini-peepers were removed from the remaining three replicates). No
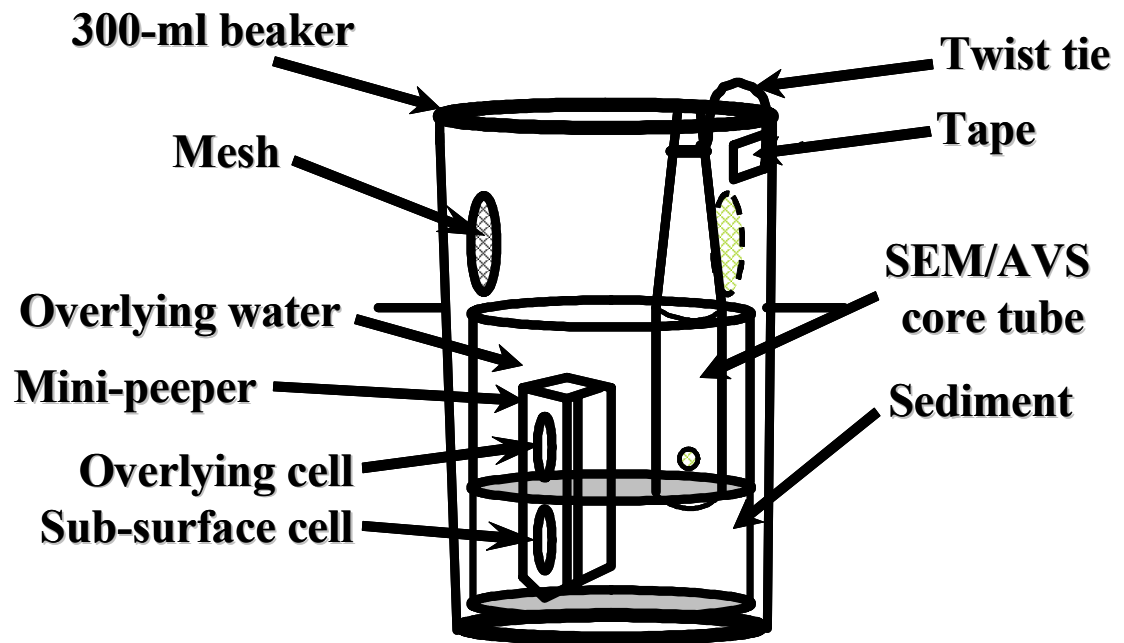
Figure 7.1. Diagram of STIR system test vessel with the associated SEM:AVS core sampler and mini-peeper.

one replicate had both the mini-peeper and mini-corer removed on the same day. This was done to minimize disturbance of the sediment surface in each of the beakers. On day 10, the remaining three core tubes in all treatments were sampled, but additionally rinsed this time with water from a squirt bottle prior to their removal from the test vessels. This detached any animals potentially adhering to the tube surface. All surviving organisms were retrieved via wet-sieving (425 µm mesh) of the sediment. Survival was recorded before the animals were thoroughly rinsed (see the more detailed description in Chapter 8), gut purged for 12 h and then dried overnight at 60°C for average weight determination.

7.2.2   AVS sampling precision and accuracy

To prevent the accidental removal of test animals during SEM:AVS sampling, the mini-corers (as described below) prevent *H. azteca* from disturbing the sediment inside the core tube. The burrowing activities of benthic organisms can potentially reduce AVS concentrations in surficial sediments (Peterson et al., 1996). The sediment AVS concentration measured via the mini-corers may therefore be elevated relative to the sediment outside of the core tube due to the absence of bioturbation.

To evaluate the influence of mini-corers on SEM and AVS measurements, 10-day sediment tests with sediment C (high in AVS) were setup following the above methods for toxicity testing (complete with 10 animals per replicate). Nickel was not added to this sediment and it was therefore not aged prior to addition to the test beakers. Six replicate beakers had mini-corers in place (as described below) for the duration the 10-d test. Using these mini-corers, SEM:AVS was sampled on day 10. Six separate

beakers had no mini-corers in place during the test but were sampled using mini-corers on day 10. At day 10 of the experiment, the AVS concentrations in beakers with and without mini-corers were analyzed (as described below) and compared.

7.2.3   SEM:AVS mini-corers

The SEM:AVS mini-corers were constructed using 10-ml (~17 mm outer diameter at the base) modified Finntip® (Thermo Labsystems, Helsinki, Finland) polypropylene pipet tips. The broad end (base) of the pipet tip was trimmed so that only the smooth portion of the tube remained. The narrow end was clipped to snugly allow for the insertion of a 30-ml plastic syringe with a slip tip (VWR, Scientific products,West Chester, PA, USA). A 0.5-cm dia. hole was melted ~1.7 cm above the broad end of the tube. This hole was covered with a fine nytex mesh (100 μm) and secured with silicon sealant (GE Sealants and Adhesives, Huntsville, NC, USA). This allowed overlying water inside the tube to equilibrate with the surrounding overlying water during automated water renewals, while preventing *H. azteca* from entering the tube. After the silicon cured, tubes were acid bathed (1 M HCl) overnight and soaked for 2 d in ultrapure water.

As described above, one core tube was placed into each replicate beaker 4 d prior to the addition of animals. This was done by gently pressing the broad end of the tube deep enough into the sediment surface to prevent *H. azteca* from burrowing underneath (~0.5 cm). To keep the tubes upright, one end of a plastic twist tie (with a loop) was placed over the tip and the other end of the tie taped to the side of the beaker. Core sampling was conducted by pressing the tubes vertically to the bottom of the beakers,

inserting the slip tip of the syringe in the narrow end of the core tube, and creating a slight negative pressure. The tube (with the syringe attached) was then twisted and gently withdrawn from the sediment while allowing the overlying water to flow past the tube and fill the hole created in the sediment. This prevented the collapse of the surrounding sediment and disruption of the associated mini-peeper (to be removed on day 10). The core tubes were then placed over an empty beaker, inverted, and the overlying water drawn off using the syringe. Once this overlying water was discarded, the syringe was re-inserted into the core tube and used to create a positive pressure and extrude the sediment from the broad end. The sediment samples were kept in 25-ml plastic vials, securely sealed and placed under a N_2 atmosphere for subsequent SEM:AVS analysis.

7.2.4   SEM:AVS analysis

AVS analysis followed the protocols of Brouwer and Murphy (1994) which were later modified by Leonard et al. (1996b). Under a N_2 atmosphere, each sediment core sample was mixed and subsampled for dry weight analysis (dried at 60°C overnight). The remaining bulk of the core sample was then weighed and added to a clean, dry, 500-ml diffusion bottle along with 100 ml of deoxygenated (DO <0.2 mg/L), ultrapure water. Sulfide-antioxidant-buffer (SAOB, 10 ml) composed of 0.2 M NaOH, 0.1 M L-ascorbic acid (AnalaR®, BDH Inc., Toronto, ON, Canada), and 0.1 M ethylenediaminetetraacetic acid (EDTA; Sigma®, Sigma Chemical Co., St. Louis, MO, USA) was added to a 25-ml plastic vial suspended above the sediment sample inside the diffusion bottle. The lid of the diffusion bottle was then secured and 10 ml of 12 M HCl added through a hole in the

diffusion bottle lid. The hole was then immediately closed with a rubber stopper and the bottles mixed briskly on a multi-head magnetic stirring system using 5-cm magnetic stir bars previously placed inside the diffusion bottles. After 1 h, the plastic vial containing the SAOB was removed from the diffusion bottle and immediately capped. The SAOB solution was then diluted to a volume of 30 ml and the sulfide promptly measured against freshly prepared standards using an Orion Model 9416 Silver/Sulfide Half-Cell Electrode with a Model 90-02 Double Junction Reference Electrode (Orion Research Inc, Beverly, MA, USA). If analysis was not performed immediately, the samples were stored under a N_2 atmosphere until processed (within 6 h). Sulfide standard QC checks were performed in duplicate each day via a lead perchlorate $(Pb(ClO_4)_2 \cdot 3H_2O)$ titration of the 0.001 μM sulfide standard. A QC check of the lead perchlorate solution was performed on a flame atomic absorption spectrometer using and lead standards made from $Pb(NO_3)_2$. The lead perchlorate solutions were $101.9 \pm 4.5\%$ of the nominal concentration. The sulfide detection limit for this method was 0.03 μmol S/ml when 10 ml of SAOB was used.

Digestions of sediment C were performed to determine the efficiency of the AVS digestion protocol as a function of time. Similar to the findings of other authors (van Griethuysen et al., 2002), not all of the digested AVS was absorbed by the SAOB in 1 h. At 1 h only $73.7 \pm 5.1\%$ of the total AVS was absorbed by the SAOB (using measured AVS concentrations at 3 h as 100 % AVS). Therefore, AVS values were adjusted accordingly.

The metal solutions resulting from the cold acid extractions (the SEM) were filtered and analyzed for total Ni. Since SEM has previously been shown to be

unaffected by digestion time (van Griethuysen et al., 2002), and since Ni concentrations in the SEM were similar to the nominal Ni values used in this study, 1-h SEM digestion results were not adjusted.

7.2.5   Statistics

Since three of the six replicate mini-corers in each treatment were removed prior to the addition of animals in the Ni toxicity tests, the effects of the mini-corers on *H. azteca* survival and average weight could be evaluated. Unpaired and paired *t*-tests or Wilcoxon Signed Rank tests were performed depending on whether the data were normally or non-normally distributed, respectively. Differences in control animal growth was observed between toxicity-test sediments. Growth data from each toxicity test was therefore averaged for comparison in paired *t*-tests.

To assess potential influence of the mini-corers on AVS concentrations, an unpaired *t*-test was performed on the AVS data. All statistics were performed using SigmaStat® 3.0 with $\alpha = 0.05$ (SPSS Inc., 2003).

7.3   Results

7.3.1   Biological endpoints

Total survival and average weight per amphipod from control and Ni-treated sediments are presented in Table 7.1. For both the control and Ni-treated sediments, there was no significant effect of mini-corers on *H. azteca* survival. Survival of test animals was therefore not impaired by the presence of the mini-corers during the test, nor were animals accidentally removed by the SEM:AVS sampling on day 10. For the

Table 7.1. Survival (%) and average weight (µg/animal d.w.) data for *Hyalella azteca* from 10-d Ni-spiked sediment toxicity tests with or without the presence of mini-corers. AVS data are from separate unspiked 10-d sediment tests. Data are means ± 1 standard deviation.

Assessment endpoint or measurement	SEM:AVS mini-corers		
	absent[a]	present[a]	relative difference (%)
Toxicity tests			
Control sediments			
Survival[b]	99.2 ± 2.9	97.5 ± 4.5	1.7
Average weight[c]	1.25 ± 0.20	1.24 ± 0.28	1.2
Ni-spiked sediments			
Survival[d]	96.7 ± 6.7	97.7 ± 6.3	1.0
Average weight[e]	1.01 ± 0.21	1.06 ± 0.24*	5.4
Mini-corer chemistry			
AVS (µmol/g d.w.)	35.49 ± 2.48	34.18 ± 3.99	3.7
AVS (*CV* %)	7.0	11.7	

[a] For the toxicity tests; the three replicates from which the mini-corers were removed prior to the addition of test animals are listed as corers "absent". The three replicates in which the mini-corers were present throughout the test (and removed on day 10) are identified as "present". For the mini-corer chemistry replicates; the six replicates without mini-corers are listed as "absent". The six replicates in which the mini-corers were present throughout the test (and removed on day 10) are identified as "present".
[b] $n = 12$ for each group
[c] $n = 4$ for each group
[d] $n = 30$ for each group
[e] $n = 10$ for each group
* Significantly different from beakers with mini-corers absent.

control sediments, there was no significant affect on average weight per animal as a result of the presence of the mini-corers during the tests. In the Ni-treated sediments (Ni concentrations at or below the LOEC), there was a significant affect ($p = 0.048$) on animal weight, with animals in the replicates having the mini-corers present being 5.4 % larger.

7.3.2   Chemical endpoints

In the test evaluating the effect of the mini-corers on AVS, the average AVS concentration in the beakers (Table 7.1) with mini-corers present for 10-d was not significantly different from those beakers without mini-corers. The *CV*s for the beakers with mini-corers absent or present (six replicates each; Table 7.1) was low at 7.0 % and 11.7 %, respectively.

While the data presented in Figures 7.2 and 7.3 are not directly related to the evaluation mini-corers for sampling toxicity-test AVS, they are included to illustrate the need for adequate SEM:AVS sampling in spiked-sediments. The AVS measurements for the various control and Ni-treated sediments are given in Figure 7.2. While each control and Ni-treatment showed comparatively little change in AVS from day 0 to 10, there was a noticeable decrease in AVS with increasing total Ni. The *CV*s from the toxicity tests (three replicates per treatment) were 17.4 ± 3.0 % and 22.0 ± 3.7 % for day 0 and day 10 AVS data, respectively. Occasionally, individual samples were lost prior to analysis. Sets of AVS lacking all three replicates were not included in the calculations.

The concentrations of SEM from the various control and treatment sediments are given in Figure 7.3. Day 10 SEM samples for sediment B were lost and therefore not
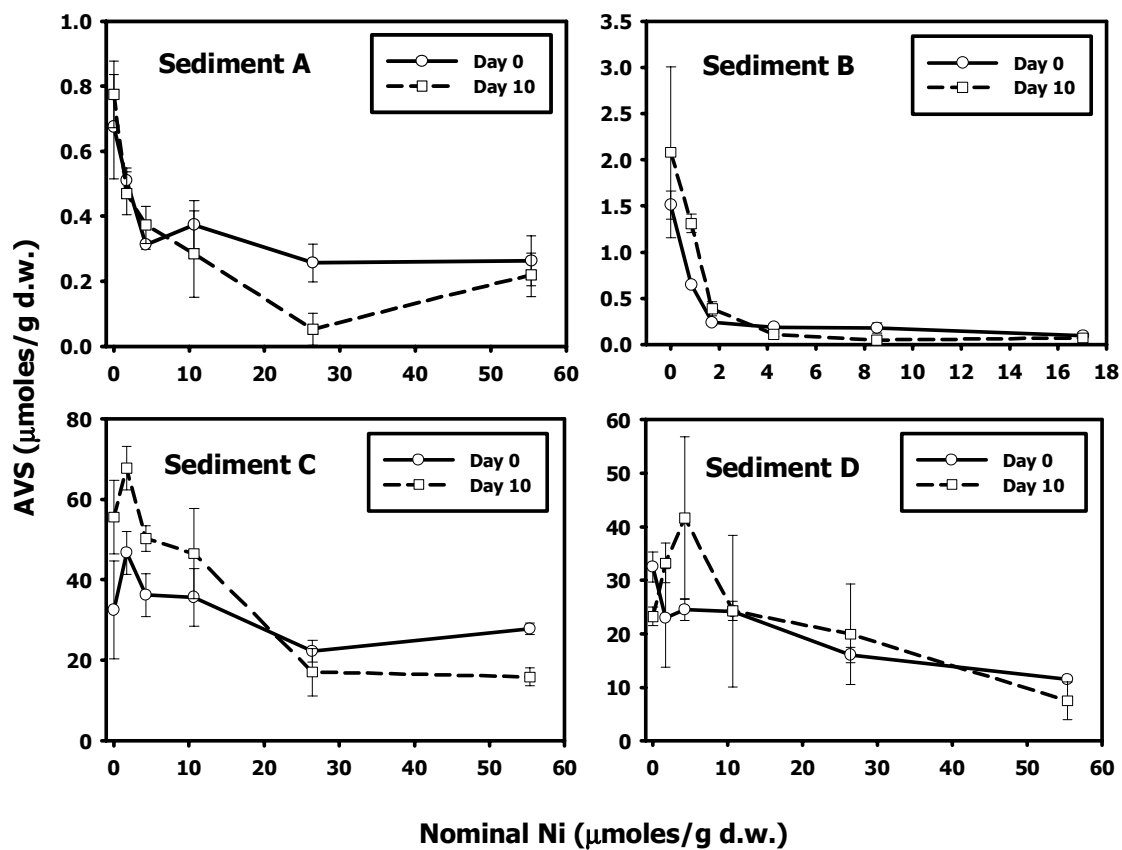
Figure 7.2. Acid volatile sulfide (AVS) concentration (µmoles/g d.w.) versus the total nominal Ni (µmoles/g) in four, Ni-spiked sediment toxicity tests. Data are means ± 1 SD.
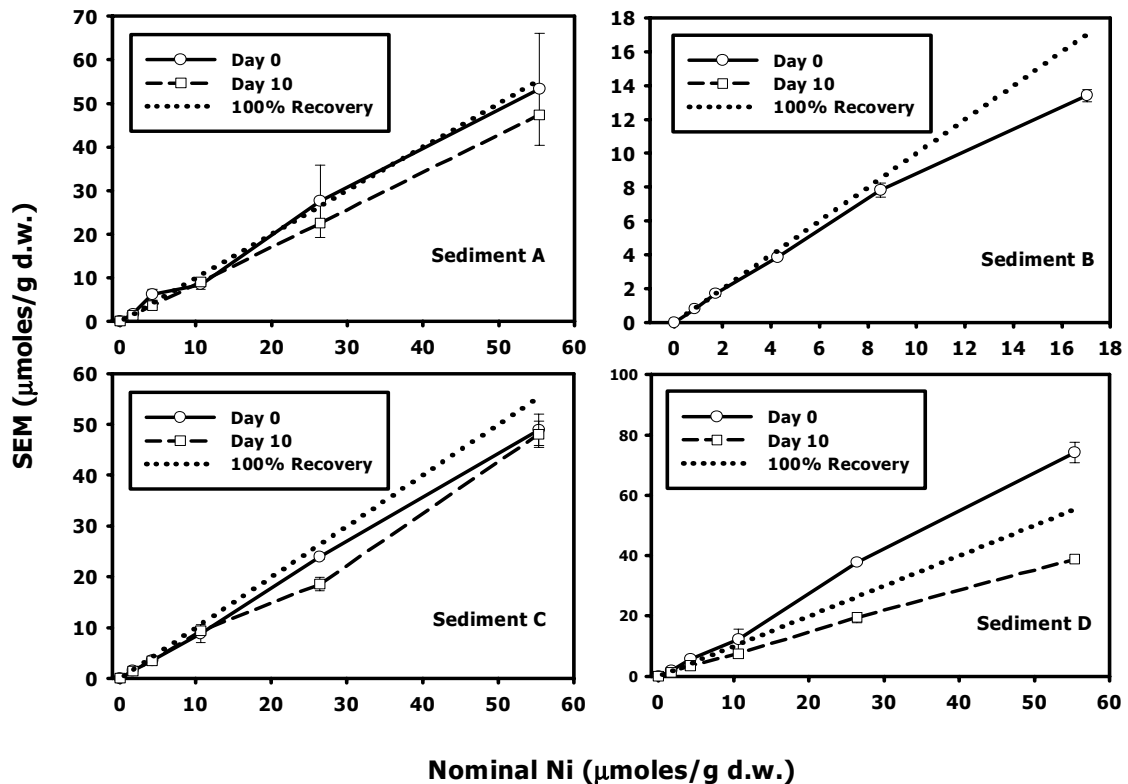
Figure 7.3. The measured SEM concentration (μmoles/g d.w.) versus total sediment Ni (nominal; μmoles/g d.w.) in four Ni-spiked sediment toxicity tests. The dotted lines represent the theoretical SEM$_{Ni}$ concentrations present in the spiked sediment. Data are means ± 1 standard deviation.

presented. Total SEM recovered was similar to the nominal, spiked concentrations (94.1 ± 7.7%), although there was some variability in the two highest SEM concentrations in sediment D. The average *CV* among the three replicate SEM samples per Ni-treatment was 10.7 ± 7.3 % and 3.1 ± 2.1 % for day 0 and day 10 data, respectively, for all tests combined. Sets without all three replicates were not included in the calculations.

7.4   Discussion

Due to logistical constraints, past laboratory sediment toxicity testing have often employed little or no replication of AVS and SEM measurements. *Hyalella azteca* sediment toxicity testing protocols typically involve aliquots of contaminated or spiked sediment placed in a glass test vessel in a flow-through system followed by introduction of the organisms of interest (e.g., Ankley et al., 1991; Ankley et al., 1993; Leonard et al., 1995; Hansen et al., 1996). While overall replication usually ranges from 4 - 6 vessels per treatment, these are often subdivided into single replicates for initial and final chemical analysis, with the remaining replicates for biological/toxicological analysis. Overall, initial and final sediment chemistry (if collected) is often unreplicated and the biological data reduced to a minimal number of replicates (e.g., 1 to 4). The results presented in Table 7.1 demonstrate that upon test completion SEM:AVS was successfully sampled directly from the toxicology test vessels using the mini-corer without any associated mortality or changes in the average weight of test animals. Mini-corers also did not appear to influence AVS concentration. While the burrowing activity of *H. azteca* may alter the AVS concentration in the surficial micro-environment, burrowing activities were apparently not extensive enough to alter the AVS

concentrations in the bulk sediment sample.

Figure 7.2 demonstrates that an inverse relationship exists between Ni concentration in sediment and AVS. This has previously been noted in other studies (Di Toro et al., 1992; Pesch et al., 1995; Berry et al., 1996). Copper has also been shown to have an inverse relationship with AVS, while Cd, Pb and Zn do not (Berry et al., 1996). Conversely, Figure 7.3 demonstrates that SEM remained similar to the nominal concentrations. Ni, as well as other cations, have been shown to catalyze the oxidation of H_2S ($Ni^{2+} > Co^{2+} > Mn^{2+} > Cu^{2+} > Fe^{2+} > Ca^{2+} = Mg^{2+}$) in the presence of oxygen (Chen et al., 1972; Snavely and Blount,1969). Nickel was found to be by far the most effective catalyst (Snavely and Blount,1969). This may explain why AVS decreased with increasing total Ni (Figure 7.2) but SEM remains similar to nominal Ni concentrations (Figure 7.3). The variable and sometimes unpredictable nature of AVS under laboratory conditions therefore highlights the need for adequate SEM:AVS sampling in spiked-sediment toxicity testing.

Overall, the mini-corer methodology was developed for use with small organisms occupying the uppermost surficial layer of sediment. When using the mini-corer it has been assumed that the presence of *H. azteca* minimally influences the bulk properties of the sediment and therefore does not result in significant alteration in SEM and AVS. This assumption may not hold true under finer scales of resolution (i.e., <0.5 cm) where the burrowing habits of *H. azteca* may alter the sediment micro-environment redox potential. Organisms that burrow more deeply into the sediment are unsuited for use with this methodology since they may be removed or harmed by the mini-corer sampling process. Organisms that burrow more extensively may also dramatically alter the bulk

sediment redox characteristics surrounding the core tubes thereby making the cores less accurate in measuring SEM:AVS and less representative of the bioturbated sediment.

7.5   Conclusions

This core-tube method allowed for sampling of both bulk SEM:AVS and surviving animals directly from the same beakers in sediment toxicity tests, and was shown to have negligible effect on animal survival or growth. Acid volatile sulfide sampling precision was comparable whether the mini-corers were present in test vessels for 10 d (CV = 11.7 %), or simply used as core tubes to collect sediment on day 10 (CV = 7.0 %). While this approach to sampling SEM and AVS appears to be suitable for tests involving *H. azteca*, use with other benthic invertebrates may depend upon the size and habits of the specific test organism.

Chapter 8

The significance of organic matter in modifying nickel bioavailability and toxicity to *Hyalella azteca* in sediment

8.1   Introduction

Depositional sediments may act as both a trace-metal sink and source in aquatic ecosystems. If sufficiently bioavailable, accumulated sedimentary trace-metals may pose a risk to associated biota. In recent years, advances have been made in understanding the factors governing metal bioavailability and toxicity in sediments. Work involving a number of divalent cationic metals (Di Toro et al., 1990; Ankley et al., 1991; Hansen et al., 1996; DeWitt et al., 1996; Lee et al., 2000a; Besser et al., 2003) has demonstrated that, depending upon the organism and the specific test conditions, metal toxicity in sediment may be correlated with either the total sediment metal content, the bioavailable metal fraction in pore water, or the metal concentration in the overlying surface water. Metal exposure and uptake for *Hyalella azteca,* a common benthic crustacean, has been found to correlate well with pore-water metal concentrations (Ankley et al., 1993). If we are to understand sedimentary metal bioavailability and toxicity to *H. azteca*, it is therefore important to evaluate the partitioning of metals between the solid and aqueous sedimentary compartments (i.e., one must adopt an equilibrium partitioning approach).

For trace metals having Class B or Borderline character (e.g., Cd, Cu, Pb, Ni, Zn; based on the classification of Nieboer and Richardson, 1980), sulfide is considered to be

the main binding phase in anoxic sediment (Boulegue et al., 1982; Emerson et al., 1983; Morse et al., 1987). Low pore-water metal concentrations in such sediment result from the formation of relatively insoluble metal sulfides (Di Toro et al., 1990). When molar concentrations of such amorphous sulfide (referred to as acid-volatile sulfide or AVS) exceeds the summed molar concentration of the above mentioned divalent cationic metals (referred to as simultaneously extracted metals or SEM), it has been well demonstrated that acute toxicity is unlikely to occur (Di Toro et al., 1990, 1992; Berry et al., 1996; Hansen et al., 1996; Ankley et al., 1993; Liber et al., 1996). When SEM exceeds AVS, pore-water metal concentrations may increase dramatically with essentially no change in total sediment metal content (Di Toro et al., 1990). If sufficiently elevated, pore-water metals may be detrimental to the sediment-associated biota.

In addition to reactions with sulfide, the partitioning of metals in anoxic sediments may be influenced by the presence of other metal-binding phases such as organic matter (OM) (Di Toro et al., 1990; Mahony et al.,1996), typically characterized as organic carbon (OC). The presence of OM, which provides additional metal-buffering capacity to sediments, may explain why toxicity is not always observed at SEM:AVS ratios >1, or at positive [SEM] – [AVS] values (Di Toro et al., 1990; Hansen et al., 1996; Leonard et al., 1996a). While studies have evaluated the partitioning of metals to OM extracted from natural sediments (Fu et al., 1992), or metal partitioning to whole, natural sediments (Mahony et al., 1996), little work (aside from Besser et al., 2003) has specifically evaluated the influence of sediment OM on metal bioavailability and toxicity in sediments. No previous published study has directly evaluated the influence of both

sediment OM and AVS simultaneously on metal bioavailability and toxicity in sediments.

Using Ni-spiked sediments (both formulated and field-collected), the objectives of the present research were to address the following questions. (i) In addition to sulfide, does OM influence Ni bioavailability and toxicity in sediment? (ii) Relative to overlying-water Ni or total Ni, does pore-water Ni better predict the bioavailability of Ni for *H. azteca* in Ni contaminated sediment? (iii) In the presence of excess AVS (relative to SEM), does the prediction of a non-lethal response also mean the absence of Ni bioaccumulation in *H. azteca*?

8.2 Materials and methods

8.2.1 General methods

Freshwater amphipods (*Hyalella azteca*, 7-14 d old), obtained from an in-house culture maintained at the Toxicology Centre, University of Saskatchewan (SK, Canada), were exposed to Ni-spiked sediments in 10-d toxicity tests following methods similar to U.S. EPA (2000). Toxicity endpoints included survival, average weight per animal and whole-organism Ni concentration (referred to as tissue Ni). The Ni-spiked sediments included formulated sediments, constructed to vary in total OC content (1.61 - 6.76%), and natural sediments collected from the field.

8.2.1.1 Formulated Sediments

A more detailed description of the construction and properties of the formulated sediment is provided in Wang and Liber (unpublished manuscript). Briefly, a locally obtained "organic" soil (dried and pulverized), calcite ($CaCO_3$), dolomite ($CaMgCO_3$),

ground re-hydrated peat (<2 mm), and deionized water were mixed together. Different sediments varied only in peat content (OC). For each batch of formulated sediment, 5.0 kg of soil was first placed in a large mixing bowl. Peat (soaked overnight in deionized water, then triple rinsed and hand-squeezed) was added (for a desired OC content) along with calcite and dolomite (each as 0.5% of the total dry weight). These components were then mixed for several minutes with one half of the total water. If the sulfide content was to be amended, iron ($FeSO_4·7H_2O$) and sulfide ($Na_2S·XH_2O$) were separately dissolved in deoxygenated water (making up the remainder of the total sediment water content) and added individually (iron first) to the sediment (one a 1:1 molar basis). All of the components combined were then mixed under a nitrogen atmosphere (5-6 min). Prior to spiking with Ni, all formulated sediments were aged for 14 d in sealed plastic buckets at room temperature (~20°C). The formulated sediments were designated as sediments 1 through 6. Sediments 1 to 3 were constructed with increasing OC content. Sediments 4 and 5 were identical to sediments 1 and 2, respectively, except that these sediments were spiked with 10 μmol sulfide/g of sediment dry weight (d.w.). Sediment 6 was constructed identical to sediment 2, except that it was spiked with 30 μmol sulfide/g d.w.

8.2.1.2 Field-collected sediments

Ten-d Ni-spiked sediment toxicity tests were conducted with four field-collected freshwater sediments designated A, B, C and D. Using an Ekman grab sampler (15×15 cm), sediments A, B and C were collected from three sites in Little Bear Lake (54° 17' N, 104° 41' W), an oligotrophic lake in north-central Saskatchewan, Canada, which was

known to be free from significant metal contamination. These sediments were placed in 20-L plastic pails and allowed to settle 1 h before the surface water was carefully decanted. The sediment was brought back to the laboratory, homogenized, sieved to remove coarse debris (≥ 5 mm), and refrigerated (4°C) within 24 h of collection. Sediments B and C were combined on a 1:1 dry weight basis to create sediment D.

8.2.2 Toxicity test protocol

Sediments (formulated or natural) were placed in 1-L Nalgene® bottles and spiked with Ni stock solution, $Ni(NO_3)_2\cdot 6H_2O$, previously diluted with ultrapure water to a total volume of 6 ml. The control sediments received 6-ml blanks of ultrapure water. After spiking, each bottle was thoroughly stirred, capped and shaken on a Wrist Action Shaker (Burrell® Corporation, Pittsburgh, PA, USA) for 1 h. The bottles were then aged at 4°C for 14 d. The sediments were then mixed on the shaker for 1 h and aliquots of each control and treatment sediment (~80 ml) divided separately into six replicate beakers equipped with mesh screens for use in a modified STIR system (modified from Benoit et al., 1993). At this time, each beaker was equipped with a mini-peeper (previously described in Chapter 2; Doig and Liber, 2000) and 100 ml of overlying water. The mini-peeper dialysis cells were covered with a semi-permeable polysulfone membrane (0.45 μm pore size). The beakers were then aged 4 d (at 4°C) prior to their addition to the modified STIR system housed in a test chamber (16:8-h light:dark cycle, 24±1°C). Upon addition to the STIR system, the AVS:SEM mini-corers (previously described in Chapter 7) were carefully placed into each beaker adjacent to the mini-peepers. The beakers were then aged another 4 d (except sediment 6, which was aged

11 d) in the modified STIR system prior to the addition of ten, 7-14 d old *H. azteca*

upon test initiation. The overlying water was automatically, partially renewed (~30%) at

6-h intervals (charcoal-filtered and aerated Saskatoon, SK, municipal water). All

individual test beakers were without aeration, with the exception of sediment 6 which

had excessively low DO levels (<2.5 mg/L) prior to test initiation. Each test beaker

received 5 mg (d.w.) of a pureed Tetramin® fish food (Tetra, Melle, Germany) slurry

daily as a food source which was gently pipetted over the sediment surface. Upon test

termination (day 10), all animals were retrieved via wet-sieving of the sediment (425-μm

mesh) and survival recorded. To remove any adhering sediment or test solution, all

animals were rinsed in 250 ml of clean, municipal water prior to transfer to another 80 ml

beaker. Here the animals were allowed to gut-purge for 12 h in the presence of 5 mg

(d.w.) of added Tetramin® slurry. To reduce the potential contribution of adsorbed Ni

relative to the total Ni body burden, all animals were transferred (with ~1 ml of

municipal water) into 5 ml of a 1 mM EDTA (ethylenediaminetetra-acetic acid) solution

(Sigma Chemical Co., St. Louis, MO, USA) for 15 min. After the EDTA wash, the

animals were transferred to a mesh screen (100 μm), and triple rinsed with ultrapure

water. They were then rinsed from the mesh into a watch glass, pipetted into small

plastic petri dishes, and dried overnight at 60 °C. The animals were subsequently

weighed to an accuracy of 0.01 mg and stored dry at room temperature (~20°C) in 2-ml

polyethylene cuvettes (Elkay® Elrean, Costelloe, CO, USA) sealed with Parafilm

"M"®(American National Can ™, Chicago, IL, USA) until digestion. The tissue

digestion protocols were previously described in Chapter 5.

8.2.3 Chemical measurements

8.2.3.1 Overlying water

All sampling for water or sediment chemistry occurred within a 2-h period prior to a water renewal. Dissolved oxygen and temperature were measured daily (Dissolved Oxygen Meter, Model 835, Orion Research Inc., Beverly, MA, USA) in three replicates of each control and Ni-treatment.

Other overlying-water quality measurements (excluding Ni) included, pH, hardness, alkalinity, conductivity, ammonia and dissolved organic carbon (DOC). Samples for these measurements (collected at the start of day 0 and the end of 10) were composites consisting of replicates 1 + 2, 3 + 4 and 5 + 6 from each of the control and Ni-treatments. The exception to this sampling regime was overlying-water DOC which was only sampled from the control and two Ni-treatments (mid and high Ni content). Dissolved organic carbon was measured using a Shimadzu TOC-5050A Total Organic Carbon Analyzer with an ASI-5000A autosampler (Shimadzu, Tokyo, Japan). Ammonia was measured using an Orion Aquafast II spectrometer and its associated reagents. Day 0 and 10 results, for the above mentioned measurements, were combined to obtain an overall average. Hardness and alkalinity were measured using a HACH Digital Titrator (model 16900, Hach Company, Loveland, CO, USA). Conductivity of all control solutions (μS/cm) was measured with an Orion Model 170, Conductivity Meter.

For pore-water and overlying-water Ni measurement, mini-peepers were removed (after the above mentioned water quality sampling) from three replicates of each set of control and Ni-treatment beakers (day 0 and day 10). Mini-peeper samples were pipetted into 8-ml Nalgene® bottles and acidified with HNO_3 to pH <2. Analysis of

dissolved Ni solutions was performed using either an atomic absorption flame or furnace spectrophotometer (Septa AA 50B or Spectra 220Z with a GTA 110Z, respectively; Varian Australia Pty Ltd, Mulgrave, Australia). A Ni atomic absorption standard solution (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) was used as a quality check on all standard curves. Quality check solution readings averaged <5% difference from nominal Ni concentrations for both flame and furnace. Possibly due to diffusion from the sediment and dilution from overlying-water renewal, d-10 pore-water and overlying water Ni concentrations were always lower than d-0 Ni concentrations (mean decrease = 14.2 ± 31.5 % and 71.5 ± 10.1 % for the formulated and natural sediments, respectively. Day 0 and 10 results for dissolved Ni were combined for an overall arithmetic average.

8.2.3.2 Pore water

Water quality measurements for pore water (taken exclusively from the subsurface peeper cells) included Ni, total hardness and DOC. Subsamples for pore-water DOC analysis were removed prior to acidification. Hardness was analysed for only the control and two Ni treatments (mid and high Ni-spiked sediments). Day 0 and 10 pore-water DOC replicates were separately pooled and diluted to obtain adequate volumes for DOC analysis. The pore-water results (day 0 and 10) were averaged to provide an overall concentration for both DOC and Ni for each control and Ni-treatment. Pore water from archived samples of unspiked sediment were isolated (via centrifugation) and analyzed under a N_2 atmosphere for pH. The pH for sediment D was assumed to be intermediate (the geometric mean) of its constituent sediments (B + C).

8.2.3.3 Sediment

Particle size analysis was performed on subsamples of archived sediments using the pipette method (Percival and Lindsay, 1997). At the start of day 0 and the end of day 10, after mini-peeper sampling, mini-cores (for SEM and AVS analysis) were removed from three replicates in each set of control and Ni-treated sediments. Day 0 and 10 AVS and SEM results were combined for an overall average. Details of the SEM:AVS sampling and analysis methods were as previously described in Chapter 7. Subsamples of homogenized sediment from two control replicates were dried and analysed for total OC using a LECO-CR-12 carbon analyzer (Leco Corporation, St. Joseph, MI, USA).

8.2.4 Statistics

Median lethal concentration (LC50) values were calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977). One-way ANOVA was used to detect statistical differences in the average weight per animal among the respective control and Ni treatments, and to derive no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values. When the data were normally distributed with equal variance, statistically different treatments were identified using Dunnett's Method for multiple comparisons versus the control group. In the event of unequal variance, a Kruskal-Wallis one-way ANOVA followed by Dunn's method for multiple comparisons was employed (SigmaStat® 3.0; SPSS Inc., 2003). To strengthen the comparison of various chemical and biological measurements, not only were the data graphed in a manner similar to previous studies (e.g., Di Toro et al., 1990; Berry et al.,

1996; Hansen et al., 1996), but also compared as log-log or arcsine-log transformed regressions.

8.3   Results

8.3.1. Sediment and water quality

The physical and chemical sediment characteristics listed in Table 8.1 show that, while lower in water content, the formulated sediments characteristics were similar to, or fell within, the ranges measured for the natural sediments.   In the natural sediments, particle size distribution ranged from coarse (sediment B) to fine (sediment A). The particle size distribution for the formulated sediments was most similar to natural sediment D. In the formulated sediments, the OC content ranged from 1.61 to 6.76 % of the total d.w. and the AVS ranged from undetected (<0.03 µmol sulfide/g d.w.) to 17.52 µmol sulfide/g d.w. While sediments 4 and 5 were spiked with 10 µmol sulfide/g d.w., the sulfide appeared to have largely oxidized. Sediment 6, spiked with 30 µmol sulfide/g d.w., was the only sulfide-spiked sediment with a substantial sulfide content (17.52 µmol sulfide/g d.w.). The OC content in the natural sediments ranged from 0.70% (sediment B) to 18.31% (sediment A), and the sulfide content ranged from low (sediment A; 0.73 µmol sulfide/g d.w.) to very high (sediment C; 44.05 µmol sulfide/g d.w.). Natural sediment pore-water pH ranged from 6.55 to 7.41. Pore-water DOC ranged from 268 to 460 mg/L and 33 to 135 mg/L in the formulated and natural sediments, respectively.

The overlying-water chemistry (Table 8.2) in the formulated sediment tests was generally similar to that for the natural sediment tests. Due to experimental error, day 10 hardness, alkalinity, conductivity and pH were not analysed for sediment 6, so values

Table 8.1. Physical and chemical characteristics of formulated sediments and natural sediments (from Little Bear Lake, SK, Canada). Data are means ± 1 standard deviation.

Sediment characteristic	Sediment test					
	1	2	3	4	5	6
Formulated sediments						
Particle size (%)[a]						
Sand (54 - 2000 µm)	50	50	50	50	50	50
Silt (2 - 53 µm)	33	33	33	33	33	33
Clay (<2 µm)	17	17	17	17	17	17
Water content (%)	25.8±0.1	32.7±0.2	43.2±0.1	27.4±0.5	40.2±0.7	42.6±3.2
OC (%)	1.61±0.14	3.20±0.32	6.76±0.02	1.69±0.13	4.28±0.29	3.83±0.30
AVS (µmol/g d.w.)	nd[b]	nd	nd	0.04±0.00	0.28±0.37	17.52±1.37
Pore-water pH[e]	7.3	7.3	7.3	7.3	7.3	7.3
Pore-water DOC (mg/L)[d]	268±29	276±22	384±66	450±21	460±56	333±61
Natural sediments			A	B	C	D
Particle size (%)						
Sand (54 - 2000 µm)			17.7±1.9	88.3±0.6	22.8±0.5	52.8±2.2
Silt (2 - 53 µm)			20.0±1.7	4.2±0.6	23.9±1.8	13.4±0.1
Clay (<2 µm)			62.3±3.6	7.4±1.1	53.3±1.2	33.8±2.1
Water content (%)			90.4±0.2	40.6±0.2	81.1±0.4	74.0±0.5
OC (%)			18.31±0.27	0.70±0.06	14.25±0.11	7.53±0.02
AVS (µmol/g d.w.)			0.73±0.13	1.80±0.67	44.05±15.90	27.87±5.48
Pore-water pH[e]			6.99±0.02	7.41±0.03	6.55±0.04	6.97[f]
Pore-water DOC (mg/L)[d]			55±15	135±65	34±9	33±9

[a] The particle size distribution is from the earlier work by Wang and Liber (unpublished manuscript) which thoroughly characterized sediments constructed via this methodology. It has been assumed that the particle size ranges from Wang and Liber are consistent with the sediments constructed in this study.
[b] nd = not detected; <0.03 µmol/g d.w.
[c] Data from Wang and Liber, unpublished manuscript.
[d] Average pore-water DOC data taken from sediment tests.
[e] pH was measured in pore water isolated from archived sediment.
[f] For comparison purposes, pH was assumed to be the geometric mean of the two parent sediments (B and C).

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Table 8.2. Overlying-water chemistry data from Ni-spiked formulated and natural sediment toxicity tests involving *Hyalella azteca*. Data are means ± 1 standard deviation across all control and Ni-treatments.

Sediment	DO[a] (mg/L)	DOC[b] (mg/L)	Hardness[c] (mg/L)	Alkalinity[c] (mg/L)	Conductivity (µS)	pH	Ammonia (mM)
Formulated sediment							
1	6.0±0.7	10.1±0.1	143±7	89±11	562±51	7.87±0.05	–
2	5.9±0.7	8.9±1.4	123±38	97±8	581±67	7.96±0.03	0.071±0.015
3	4.7±0.9	12.3±0.1	175±32	91±7	612±95	8.06±0.10	0.013±0.01
4	5.6±1.2	17.4±2.0	108±5	120±7	675±60	8.32±0.02	0.020±0.006
5	5.6±0.9	23.3±2.3	207±16	121±9	860±134	8.23±0.02	0.028±0.006
6	7.6±0.1	17.2±3.9	101±22	85±12	1028±229	7.89±0.20	0.0149±0.004
Natural sediment							
A	5.6±0.6	2.6±0.4	132±3	64±7	426±23	8.17±0.08	0.072±0.022
B	5.3±0.7	3.5±0.1	136±9	87±2	472±67	8.24±0.04	0.094±0.034
C	4.8±0.5	2.5±0.3	134±4	71±21	453±26	8.00±0.02	0.153±0.037
D	4.9±0.6	2.9±0.5	140±9	72±17	459±26	7.93±0.03	0.118±0.056

[a] DO = dissolved oxygen.
[b] DOC = dissolved organic carbon.
[c] Expressed as $CaCO_3$.

represent day 0 data only. The average water temperature for all formulated and natural

sediment tests combined was $20.8 \pm 1.1°C$. Dissolved oxygen levels in all sediment tests

were well above levels shown to be safe in 10-d tests with *H.azteca* (Irving et al., 2004).

The overlying-water pH ranged from 7.87 to 8.32 and 7.93 to 8.24 in the formulated and

natural sediments, respectively. Ammonia concentrations ranged from 0.013 ± 0.010

mM to 0.071 ± 0.015 mM and 0.072 ± 0.022 mM to 0.153 ± 0.037 mM in all formulated

and natural sediments, respectively. These concentrations were well below the lowest

levels (1 mM total ammonia) shown to cause growth impairment in 10-week, water-only

tests involving *H. azteca* (Borgmann, 1994) under similar pH conditions (pH 8.0 - 8.4).

The elevated overlying-water DOC values in the formulated sediment tests were due to

diffusion of DOC from the DOC-rich pore waters. Conductivity was similarly higher in

the overlying water of the formulated sediments, again due to the diffusion of solutes

from the pore-water. Formulated sediments 4 through 6 were noticeably higher in

conductivity due to the addition of iron and sulfide salts.

8.3.2   Amphipod survival

Amphipod mortality after 10-d exposure has been plotted against total sediment

Ni concentration in Figure 8.1. Concentration-response regression equations of the

appropriate arsine-root transformed (survival) and log-transformed data (total Ni, pore-

and overlying-water Ni, tissue Ni, average weight per animal) are listed in Table 8.3.

While toxicity thresholds for the various formulated sediments (Figure 8.1a) were

somewhat variable, mortality generally increased with increasing total Ni ($r^2 = 0.771$,

$p<0.001$). In the sediments having only the OC content manipulated (sediments 1

Table 8.3. Various regressions of data from Ni-spiked sediment toxicity tests with *H. azteca*. Survival data were arcsine-root transformed. All remaining data (total Ni, pore- and overlying-water Ni, tissue Ni, average weight) were log-transformed.

Endpoint	Dependent variable	Independent variable	r^2 (p)	Formula
Survival				
Formulated sediments[a]	Mortality (%)	Total Ni[d]	0.771 (<0.001)	Y = 0.258 + 0.954X
	Mortality (%)	Pore-water Ni	0.784 (<0.001)	Y = -0.230 + 0.652X
	Pore-water Ni[c]	Total Ni	0.896 (<0.001)	Y = 0.804 + 1.396X
	Overlying Ni[c]	Pore-water Ni	0.975 (<0.001)	Y = -0.900 + 1.067X
Natural sediments	Mortality (%)	Total Ni	0.483 (<0.001)	Y = -0.0227 + 0.801X
	Mortality (%)	Pore-water Ni	0.719 (<0.001)	Y = 0.157 + 0.444X
	Pore-water Ni	Total Ni	0.460 (<0.001)	Y = 0.-4.096 + 1.519X
	Overlying Ni	Pore-water Ni	0.969 (<0.001)	Y = -1.072 + 0.837X
Average weight				
Formulated sediments[b]	Average weight[e]	Total Ni	0.772 (<0.05)	Y = 2.057 - 0.545X
	Average weight	Pore-water Ni	0.784 (<0.05)	Y = 2.690 - 0.684X
	Pore-water Ni	Total Ni	0.807 (<0.05)	Y = 0.949 + 0.722X
Natural sediments	Average weight	Total Ni	0.129 (>0.05)	-
	Average weight	Pore-water Ni	0.751 (<0.001)	Y = 2.062 - 0.160X
	Pore-water Ni	Total Ni	0.302 (<0.05)	Y = -0.169 + 1.202X
Tissue Ni				
Formulated sediments	Tissue Ni[f]	Total Ni[a]	0.326 (<0.05)	Y = 0.890 + 0.712X
	Tissue Ni	Total Ni[b]	0.123 (>0.05)	-
	Tissue Ni	Pore-water Ni[a]	0.724 (<0.001)	Y = -1.303 + 0.549X
	Average weight	Tissue Ni[b]	0.283 (>0.05)	-
Natural sediments	Tissue Ni	Total Ni	0.276 (<0.05)	Y = -0.819 + 0.628X
	Tissue Ni	Pore-water Ni	0.886 (<0.001)	Y = -0.962 + 0.363X
	Average weight	Tissue Ni	0.842 (<0.001)	Y = 1.685 - 0.353X

[a] Formulated sediments 1 through 6.
[b] Formulated sediments 1 through 3.
[c] All pore-water and overlying-water Ni concentrations were expressed as µmole/L.
[d] All total (sedimentary) Ni concentrations were expressed as µmole/g (d.w.).
[e] Average weight (µg) per animal (d.w.).
[f] All tissue Ni concentrations were expressed as µmol/g (d.w.).
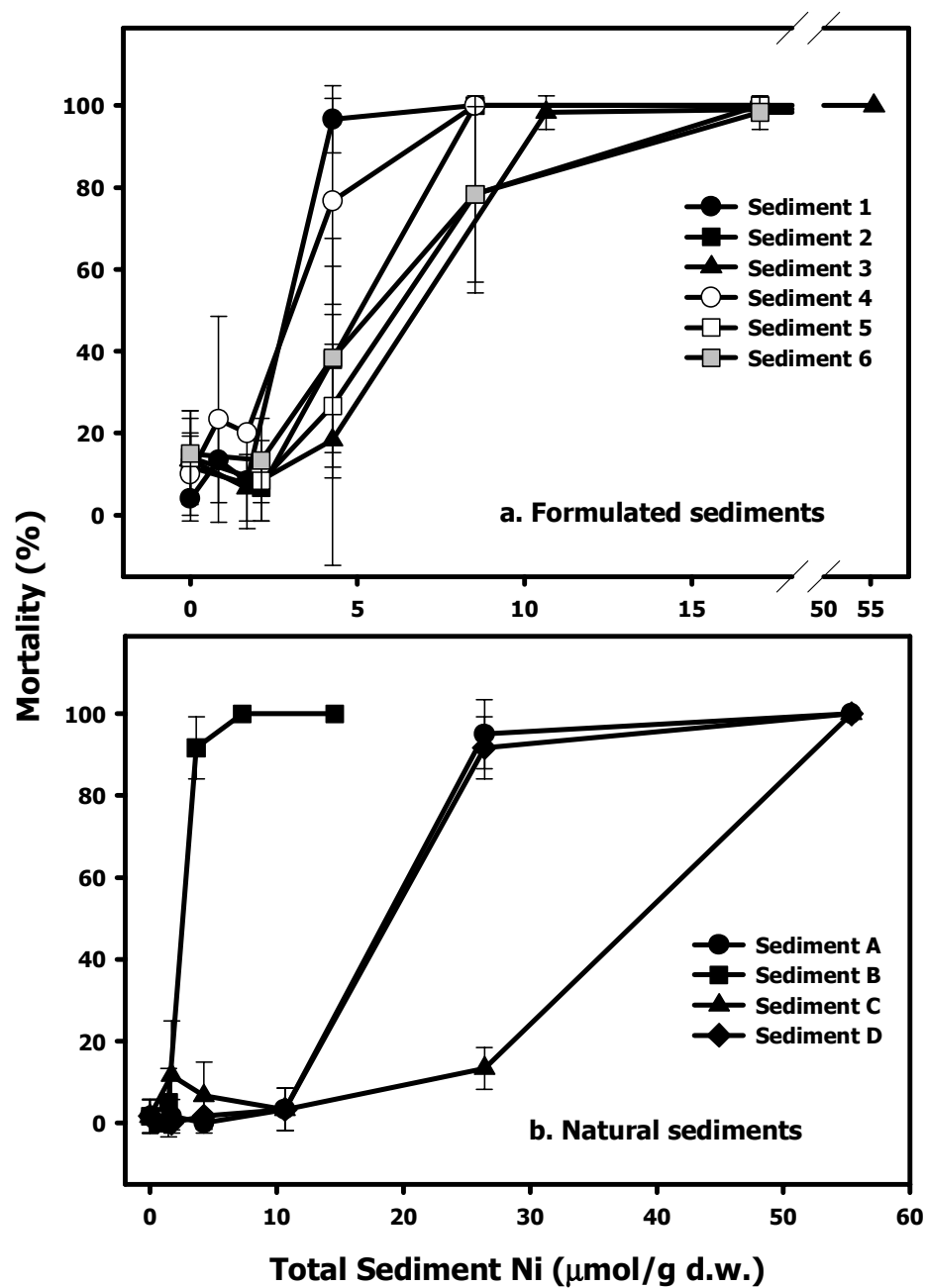
Figure 8.1. *Hyalella azteca* mortality (%) versus total sediment Ni (μmol/g d.w.) in 10-d Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.

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through 3), the LC50 (Table 8.4) increased (from 2.64 to 6.85 µmol/g d.w.; 155 to 402 mg/kg) with increasing OC. The LC50 also increased in sediment 6 (5.98 µmol/g), which was constructed similar to sediment 2 (LC50 = 4.83 µmol/g; d.w.), but with higher sulfide. The mortality of *H. azteca* in Ni-spiked, natural sediments (Figure 8.1b) also generally increased with increasing total Ni (r^2 = 0.483, p<0.001). Relative to the formulated sediments, natural sediments had a broader range in total-Ni LC50 values (2.67 - 35.14 µmol/g, or 157 to 2062 mg/kg; Table 8.5). Again, these values increased with increasing sedimentary OCand AVS content.

Variability in the concentration-response relationships for both formulated and natural sediments was reduced by plotting *H. azteca* mortality against the mean pore-water Ni concentration (Figure 8.2). Mortality (%) (arcsine-root transformed) versus pore-water Ni (log-transformed) had an r^2 value of 0.784 (p < 0.001) and 0.719 (p<0.001) for the formulated and natural sediments tests, respectively. There was a strong correlation between pore-water Ni and total Ni (r^2 = 0.896, p <0.001) for all formulated sediments, indicating that pore-water Ni strongly co-varied with total Ni. This correlation was weaker in the natural sediments (r^2 = 0.460, p<0.001).

The overall average pore-water Ni LC50 values for formulated sediments 1 to 6 and the four natural sediments were 42.9 ± 12.2 µmol/L (2.52 ± 0.72 mg/L) and 45.2 ± 16.7 µmol/L (2.65 ± 0.98 mg/L), respectively (Tables 8.4 and 8.5). These values are lower than the mean 96-h LC50 previously generated in water-only experiments (71.6 ± 20.4 µmol/g, see Chapter 5).

Ni concentrations in the overlying peeper cells were approximately one order of magnitude lower than those of the pore-water peeper cells, but the two were very

Table 8.4. Toxicity endpoints from Ni-spiked formulated sediment toxicity tests with *Hyalella azteca*.

Formulated sediment	LC50[a]		LOEC[b]			NOEC[b]		
	Total Ni[c] (μmol/g)	Pore-water Ni (μmol/L)	Total Ni[c] (μmol/g)	Pore-water Ni (μmol/L)	Tissue Ni (μmol/g)	Total Ni[c] (μmol/g)	Pore-water Ni (μmol/L)	Tissue Ni (μmol/g)
1	2.64 (2.53-2.76)	29.1 (27.6-30.8)	1.7	16.63 ±0.33	0.651 ±0.165	0.85	8.27 ±0.98	0.146 ±0.044
2	4.83 (4.41-5.30)	41.1 (36.5-46.3)	4.26	31.14 ±1.85	1.068 ±0.056	2.13	15.49 ±0.28	0.244 ±0.061
3	6.85 (6.64-7.07)	34.8 (33.2-36.3)	1.7	9.48 ±1.67	0.058 ±0.012	N/A[d]	N/A	N/A
4	3.01 (2.53-3.57)	41.7 (33.0-52.8)	N/A[e]	N/A	N/A	N/A	N/A	N/A
5	6.31 (5.69-6.99)	64.6 (56.0-74.6)	N/A[e]	N/A	N/A	N/A	N/A	N/A
6	5.98 (5.34-6.71)	46.3 (39.0-55.0)	4.26	26.62 ±22.72	0.141 ±0.070	2.13	14.18 ±14.49	0.143 ±0.036

[a] LC50 values are presented with the 95% confidence intervals in parenthesis.

[b] LOEC and NOEC (growth) pore-water and tissue data are means ± 1 standard deviation.

[c] Based on nominal exposure concentrations.

[d] Sediment 3 did not have a NOEC.

[e] Poor control animal growth in sediments 4 and 5 resulted in no LOEC or NOEC. strongly correlated ($r^2 = 0.975$, $p<0.001$; $r^2 = 0.969$, $p <0.001$ for the formulated and natural sediments, respectively).

Table 8.5. Toxicity endpoints from Ni-spiked natural sediment toxicity tests with *Hyalella azteca*.

	LC50[a]		LOEC[b]			NOEC[c]		
	Total Ni (μmol/g)	Pore-water Ni (μmol/L)	Total Ni (μmol/g)	Pore-water Ni (μmol/L)	Tissue Ni (μmol/g)	Total Ni (μmol/g)	Pore-water Ni (μmol/L)	Tissue Ni (μmol/g)
Natural sediment								
A	17.12 (16.15-18.14)	22.1 (19.8-24.7)	10.65	8.53 ±5.06	0.294 ±0.051	4.26	2.54 ±1.64	0.130 ±0.027
B	2.67 (2.46-2.91)	52.6 (43.8-63.2)	0.85	4.39 ±5.87	0.102 ±0.019	N/A[c]	N/A	N/A
C	35.14 (32.45-38.09)	45.1 (36.3-56.0)	10.65	1.20 ±1.09	0.100 ±0.010	4.26	0.41 ±0.50	0.101 ±0.020
D	17.43 (16.20-18.74)	61.0 (50.4-73.6)	10.65	9.26 ±12.66	0.240 ±0.026	4.26	1.92 ±2.55	0.105 ±0.021

[a] LC50 values which are presented with the 95% confidence intervals in parenthesis.
[b] LOEC and NOEC pore-water and tissue data are means ± 1 standard deviation.
[c] Sediment B did not have a NOEC.
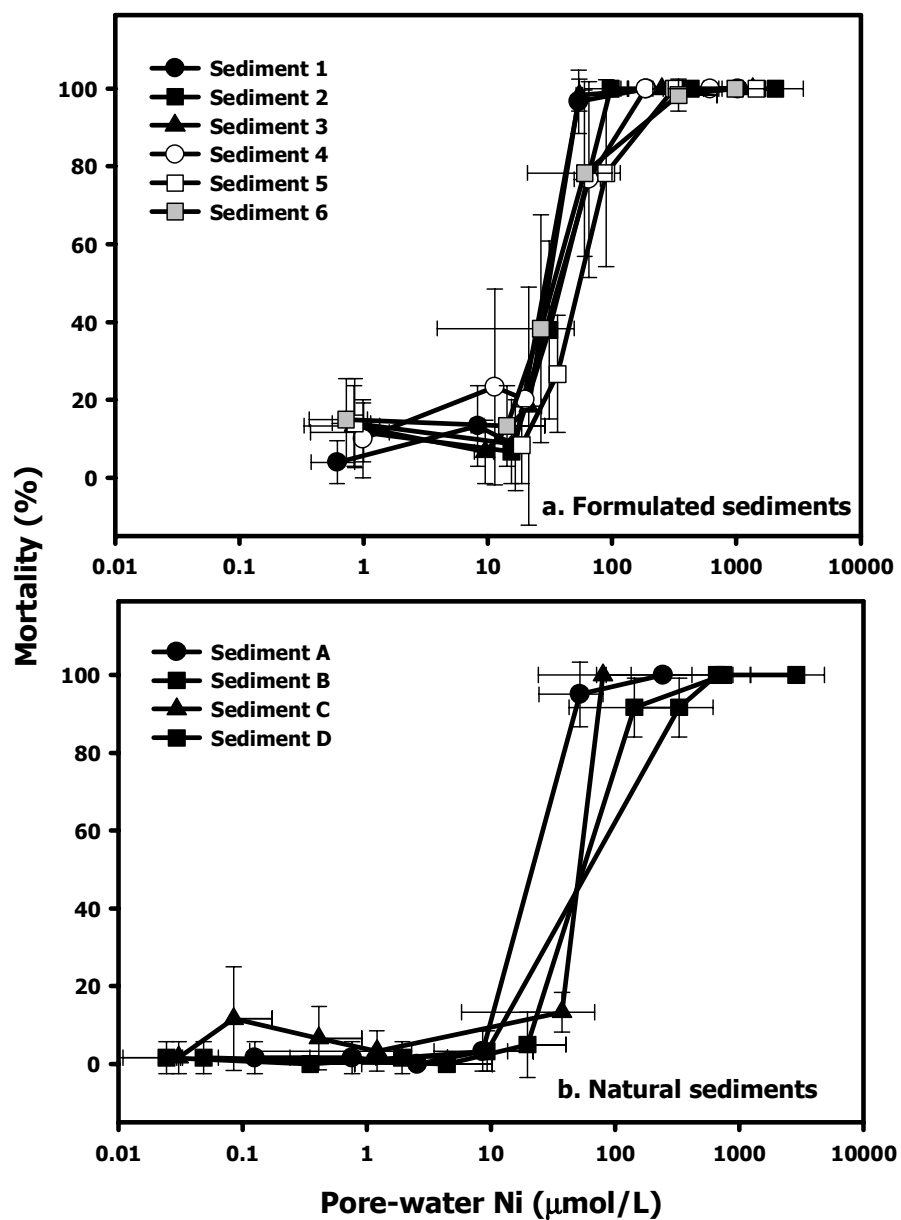
Figure 8.2. *Hyalella azteca* **mortality (%) versus pore-water Ni (μmol/L) in 10-d Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.**

strongly correlated ($r^2 = 0.975$, $p<0.001$; $r^2 = 0.969$, $p <0.001$ for the formulated and natural sediments, respectively). Because the overlying-water Ni concentrations were well below Ni concentrations found to cause mortality in water-only tests of similar duration (discussed later), pore-water Ni has been presented as the appropriate measure of toxicologically-relevant Ni in the general micro-environment of *H. azteca*.

Pore-water hardness in both the formulated and natural sediments was generally found to be positively correlated with total Ni concentration in the sediment (presumably due to cation displacement). The hardness at the Ni LC50, LOEC and NOEC values was therefore calculated from a regression of total hardness versus total spiked-Ni (Table 8.6) in each of the sediment tests (regressions not presented). A multiple linear regression was computed to account for the effects of DOC and hardness on LC50 and LOEC variability, but no statistically significant correlation was found for either the formulated or natural sediments.

Amphipod mortality versus $[SEM_{Ni}]-[AVS]$ has been graphed in Figure 8.3. The absence of AVS in formulated sediments 1 to 5 dictated that mortality could only occur at positive $[SEM_{Ni}]-[AVS]$ values. In sediment 6 and all natural sediments, mortality generally occurred when SEM_{Ni} concentrations were in excess of AVS (SEM_{Ni} - AVS > 0). The shift in the toxicity response to the right of 0 (especially evident in sediment A) suggests that sediments may have a Ni complexation capacity greater than that provided by AVS.

8.3.3 Amphipod average weight

While variable, there was an overall reduction in the average weight of *H. azteca*

Table 8.6. Estimated pore-water hardness at selected toxicity endpoints in Ni-spiked formulated and natural sediment toxicity tests with *Hyalella azteca*. Hardness at each endpoint was calculated from a linear regression of hardness versus total sediment Ni (µmol/g d.w.).

Sediment	LC50[a] Hardness (mg/L as $CaCO_3$)	LOEC[a] Hardness (mg/L as $CaCO_3$)	NOEC[a] Hardness (mg/L as $CaCO_3$)
Formulated			
1	751	676	607
2	729	698	586
3	543	443	-
4	1325	-[b]	-
5	966	-	-
6	875	851	821
Natural			
A	243	241	239
B	304	260	-
C	350	273	253
D	457	422	390

[a] Median lethal concentration (LC50); lowest-observed-effect-concentration (LOEC); no-observed-effect-concentration (NOEC).
[b] NOEC or LOEC value could not be calculated
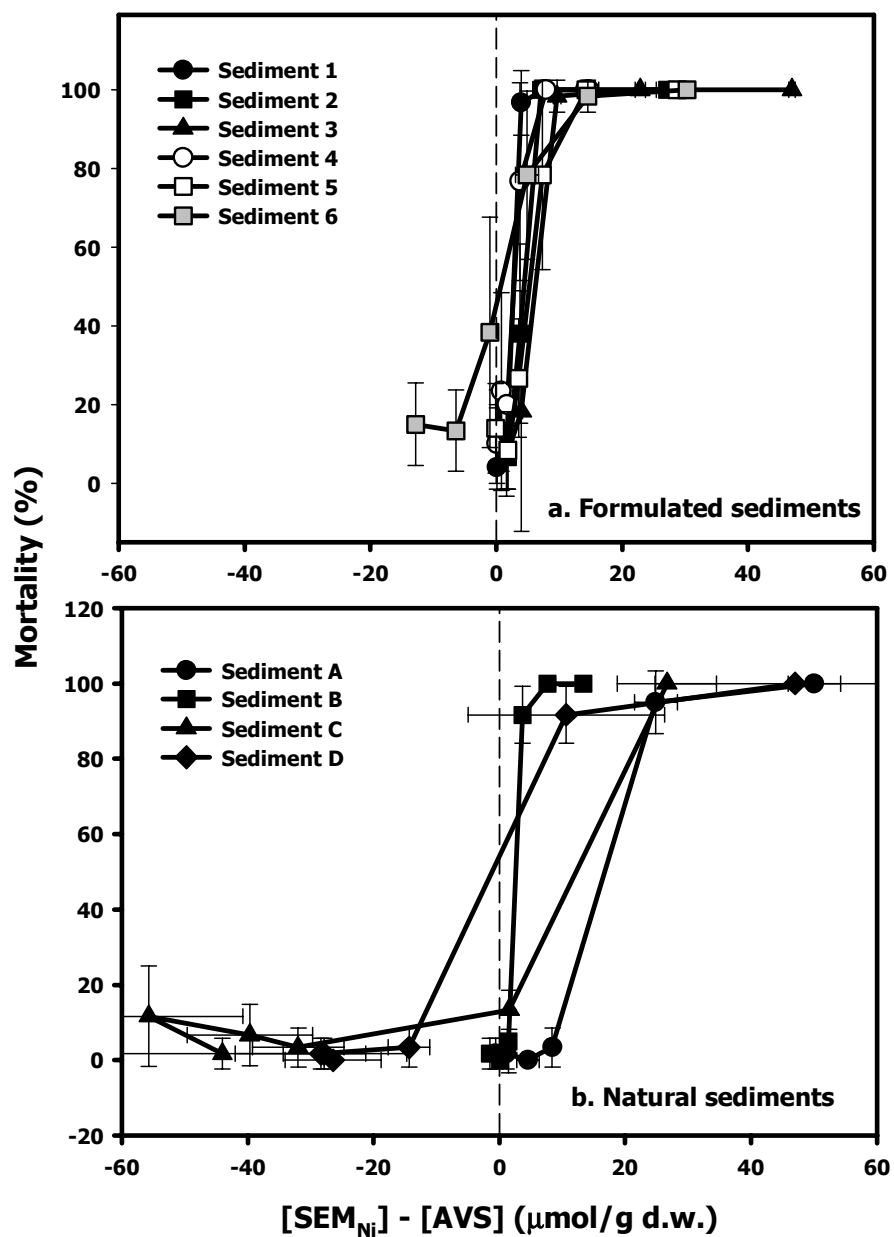
Figure 8.3. *Hyalella azteca* mortality (%) versus [SEM$_{Ni}$] - [AVS] (µmol/g d.w.) for 10-d Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.

with increasing total sediment Ni concentration (Figure 8.4). Sediments 4 and 5 (and to a lesser extent sediment 6) had much poorer control-animal growth relative to the sediments without sulfide amendments (sediments 1 through 3). As a result, NOEC and LOEC values could not be calculated for sediments 4 and 5. When omitting those sediments having iron and sulfide amendments, there was a strong negative correlation between average weight per animal and total sediment Ni ($r^2 = 0.772$, $p<0.05$). This correlation was not significant for the natural sediments ($p>0.05$). Since it was unlikely that Ni influenced animal weight in the control sediments and sediments with Ni concentrations below the NOEC, these sediments were not included in any regression calculations relating to average weight.

When evaluating *H. azteca* average weight against pore-water Ni concentration, there appeared to be a threshold above which the average weight significantly decreased (8.27 - 15.49 μmole/L and 0.41 - 2.54 μmol/L) in both the formulated and natural sediments, respectively (Figure 8.5). This threshold can be expressed as the maximum acceptable toxicant concentration (MATC; Suter et al., 1990) which is the geometric mean of the LOEC and NOEC pore-water Ni values. The pore-water Ni MATC was 16.84 ± 7.24 μmole/L and 3.19 ± 2.17 μmol/L for the formulated and natural sediments, respectively. Overall, average weight per animal was strongly correlated with pore-water Ni ($r^2 = 0.784$, $p<0.05$; $r^2 = 0.751$, $p<0.001$) in formulated sediments 1 to 3 and all naturals sediments, respectively. However, there was generally a strong correlation between pore-water Ni and total Ni ($r^2 = 0.807$, $p < 0.05$) indicating that pore-water Ni strongly co-varied with total Ni in the formulated sediments. The correlation between pore-water Ni and total Ni in the natural sediments was moderate ($r^2 = 0.302$, $p<0.05$),
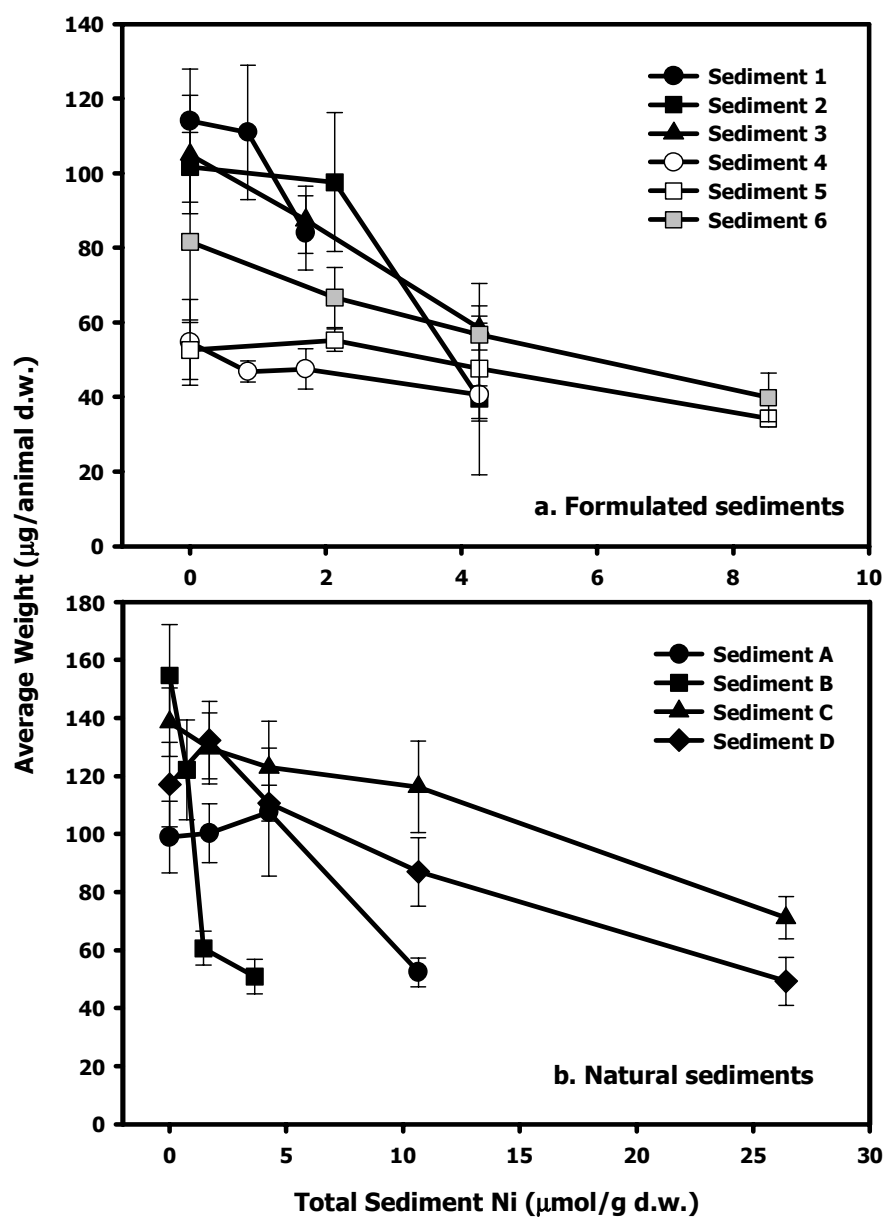
Figure 8.4. *Hyalella azteca* average weight (μg/animal d.w.) versus total sediment Ni (μmol/L) in Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.

Figure 8.5. *Hyalella azteca* average weight (μg/animal d.w.) versus pore-water Ni (μmol/L) in Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.

indicating low direct covariance.

The LOEC and NOEC values (for growth), based on total and pore-water Ni concentrations, are listed in Table 8.4 and 8.5. The formulated sediment average pore-water Ni LOEC value (sediments 1 through 3) was 19.08 ± 11.04 µmol/L (1.12 ± 0.65 mg/L) with an average reduction in weight per animal of $34.8 \pm 23.3\%$. The corresponding average pore-water Ni NOEC was 11.88 ± 5.11 µmol/L (0.70 ± 0.30 mg/L). The natural sediment average pore-water Ni LOEC was 5.85 ± 3.77 µmol/L (0.34 ± 0.22 mg/L) with an average reduction in the weight per animal of $27.5 \pm 13.63\%$. The average pore-water Ni NOEC value was 1.62 ± 1.09 µmol/L (0.10 ± 0.06 mg/L).

As shown by the asterisks denoting LOEC data points in Figure 8.6, even in the presence of AVS concentrations in excess of SEM, sediments 1, C, and D, demonstrated significant decreases (30.4%, 16.1%, 25.8%, respectively) in the weight per animal relative to their respective controls.

8.3.4   Ni bioaccumulation in *H. azteca*

While there was a general trend of increasing tissue Ni concentration with increasing total sediment Ni across sediments (Figure 8.7), the overall correlation was moderate ($r^2 = 0.326$, $p<0.05$) and weak ($r^2 = 0.276$, $p<0.05$) for the formulated and natural sediments, respectively. For formulated sediments 1 through 3 only, the correlation was not statistically significant ($p>0.05$). When plotted against pore-water Ni concentration (Figure 8.8), tissue Ni concentrations displayed a strong correlation ($r^2 = 0.724$; $p<0.001$; $r^2 = 0.886$, $p<0.001$) in the formulated and natural sediments,
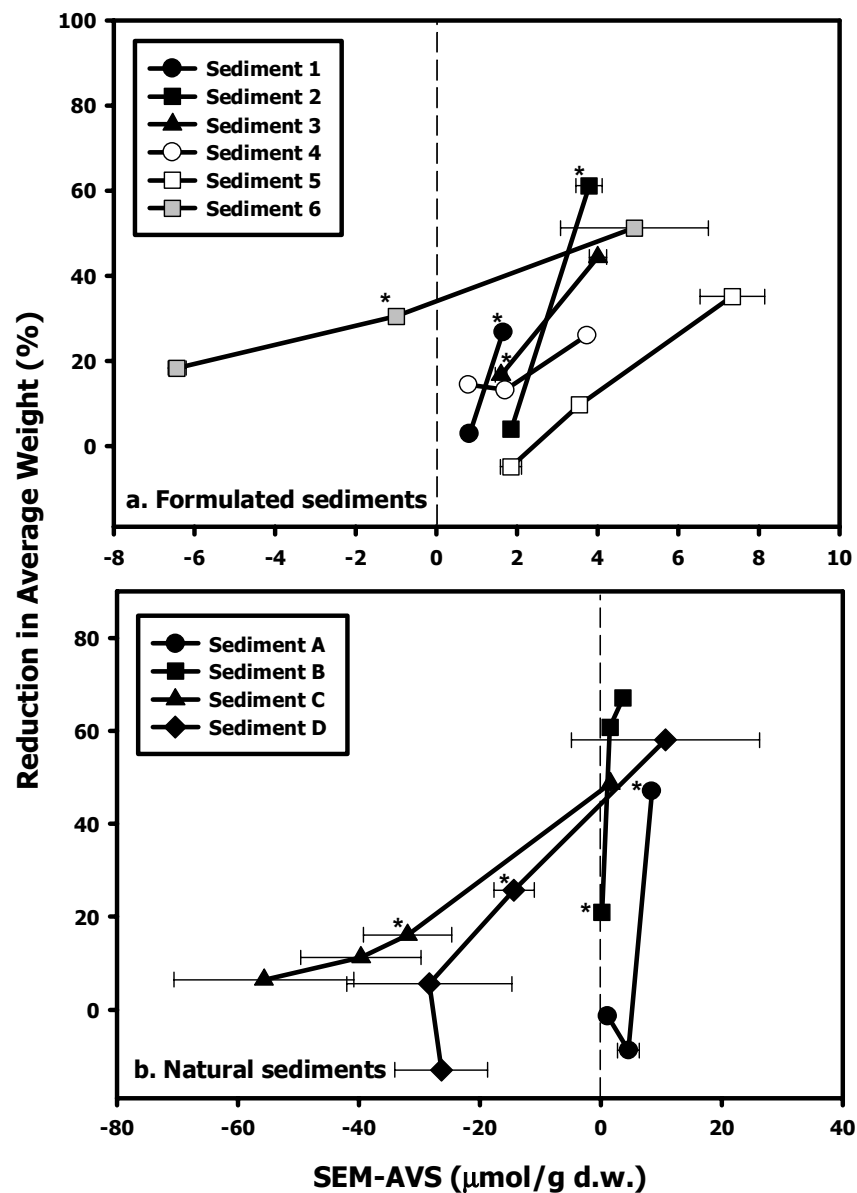
Figure 8.6. Reduction in *Hyalella azteca* average weight (%) relative to control values plotted against [SEM]‒[AVS] (µmol/g d.w.) in Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Asterisks denote the lowest statistically significant reduction in weight (LOEC) relative to the corresponding control. Data are means ± 1 standard deviation.
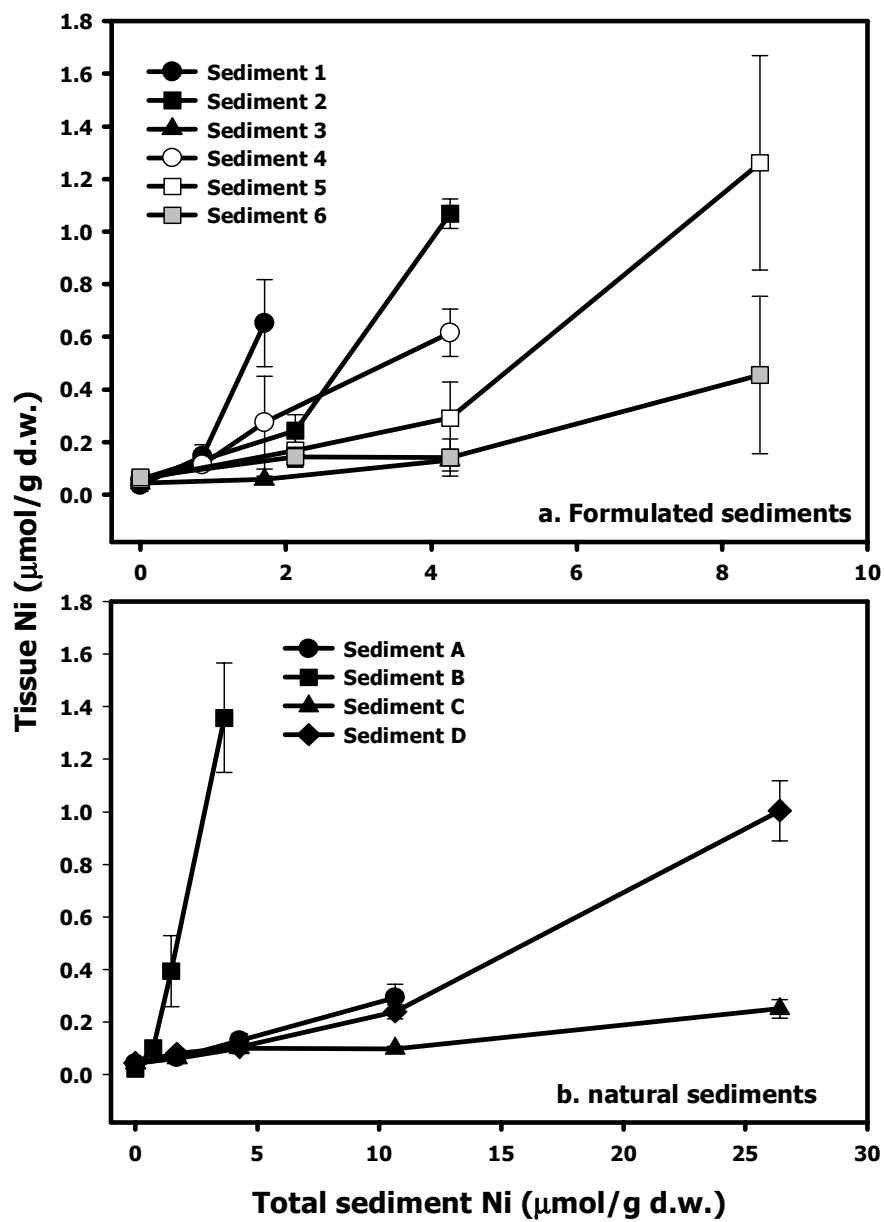
Figure 8.7. *Hyalella azteca* tissue Ni (μg/g d.w.) versus total sediment Ni
(μmol/g d.w.) in Ni-spiked formulated (a) and natural (b) sediment toxicity
tests. Data are means ± 1 standard deviation.

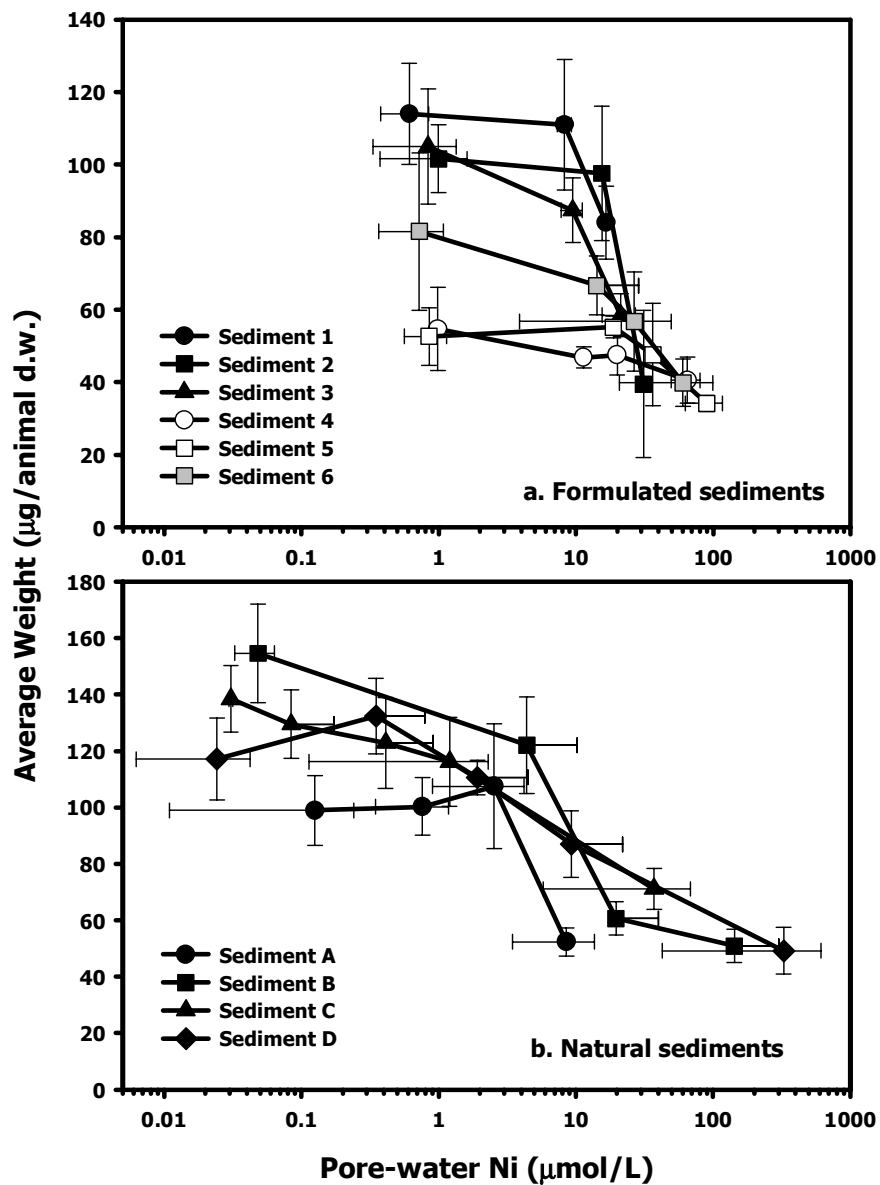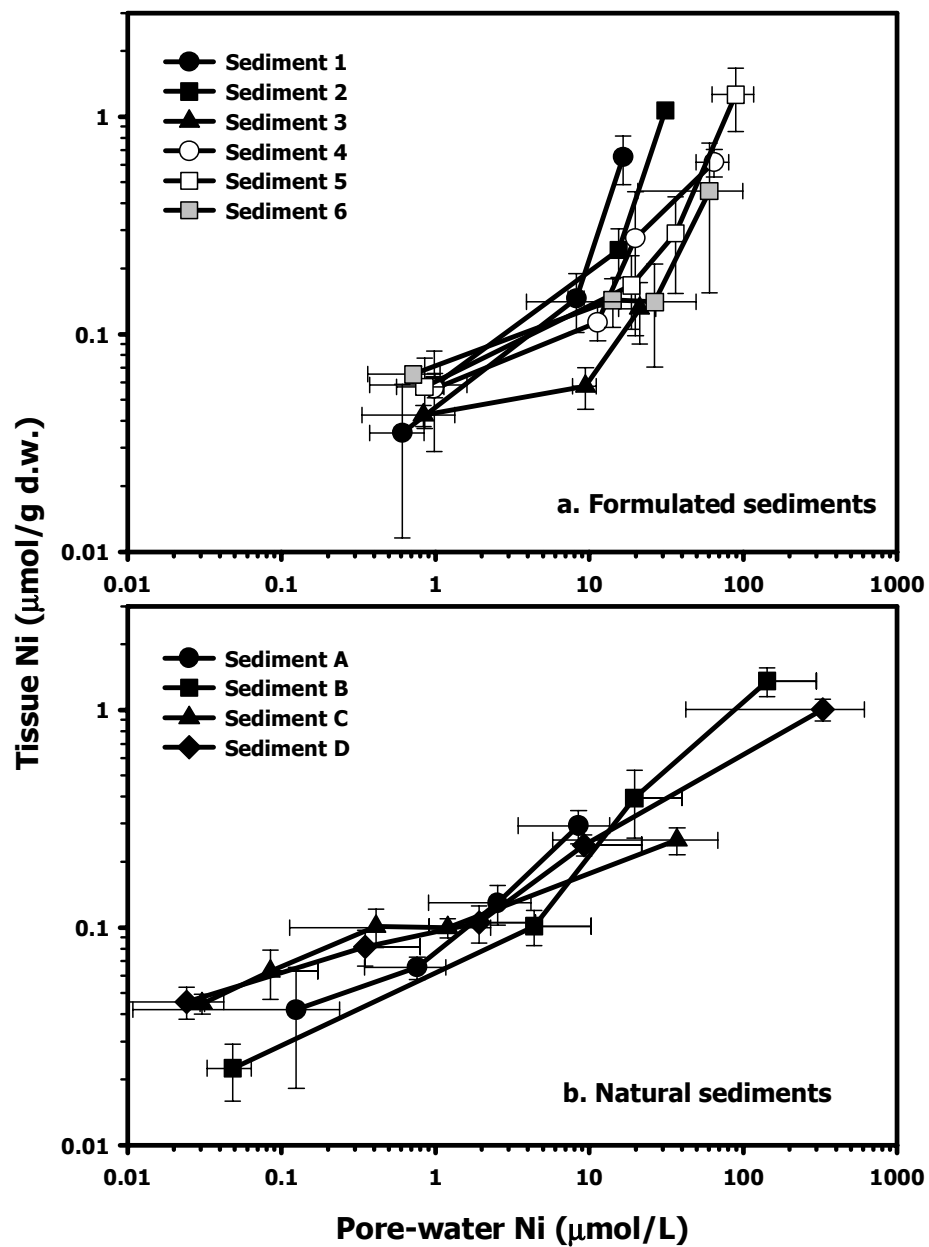**Figure 8.8.** *Hyalella azteca* tissue Ni (μg/g d.w.) versus pore-water Ni (μmol/L) in Ni-spiked formulated (a) and natural (b) sediment toxicity tests. Data are means ± 1 standard deviation.

respectively. Both pore-water Ni (μmol/L) and tissue Ni (μg/g d.w.) were also evaluated against $[SEM_{Ni}] - [AVS]$ (Figure 8.9) for the natural sediments. Even in the presence of excess AVS, pore-water Ni (Figure 8.9a) was not only quantifiable, but continued to increase with increasing SEM (i.e., with increasing total Ni). Tissue Ni (Figure 8.9b) showed a similar trend with definite tissue Ni accumulation at $[SEM_{Ni}]$ $- [AVS]$ levels less than 0.

The tissue-Ni LC50 was 0.553 ± 0.149 μmol/g d.w ($n = 2$) and 0.621 ± 0.150 μmol/g d.w ($n = 2$) for the formulated and natural sediment tests, respectively. The tissue-Ni NOEC for survival was 0.295 ± 0.070 μmol/g d.w. ($n = 4$; 7.5 ± 5.0 % mortality) in the natural sediments and 0.289 ± 0.190 μmol/g d.w. ($n = 6$; 19.7 ± 11.8 % mortality) in formulated sediments.

Animal weight and tissue Ni have both been plotted against pore-water Ni in Figure 8.10. Lines of best fit have been included to highlight the trends in the data. As pore-water Ni increased, there was a corresponding increase in tissue Ni resulting in a decrease in average weight. While average weight showed no measurable correlation with tissue Ni ($p>0.05$; log transformed data) in sediments 1 through 3 (excluding the controls and Ni-treatments less than the NOEC), it was strongly correlated with tissue Ni ($r^2 = 0.849$; $p<0.001$) in the natural sediments. Although plotted in Figure 8.10a, the data from formulated sediments 4 through 6 were excluded from this regression since factors other than Ni (discussed below) appeared to influence amphipod growth in these sediments. Data above the corresponding pore-water Ni LC50 have been excluded from the trend line plotted for natural sediments in Figure 8.10b. The obvious relationship between tissue Ni concentration and the average weight of surviving animals suggests

-204-
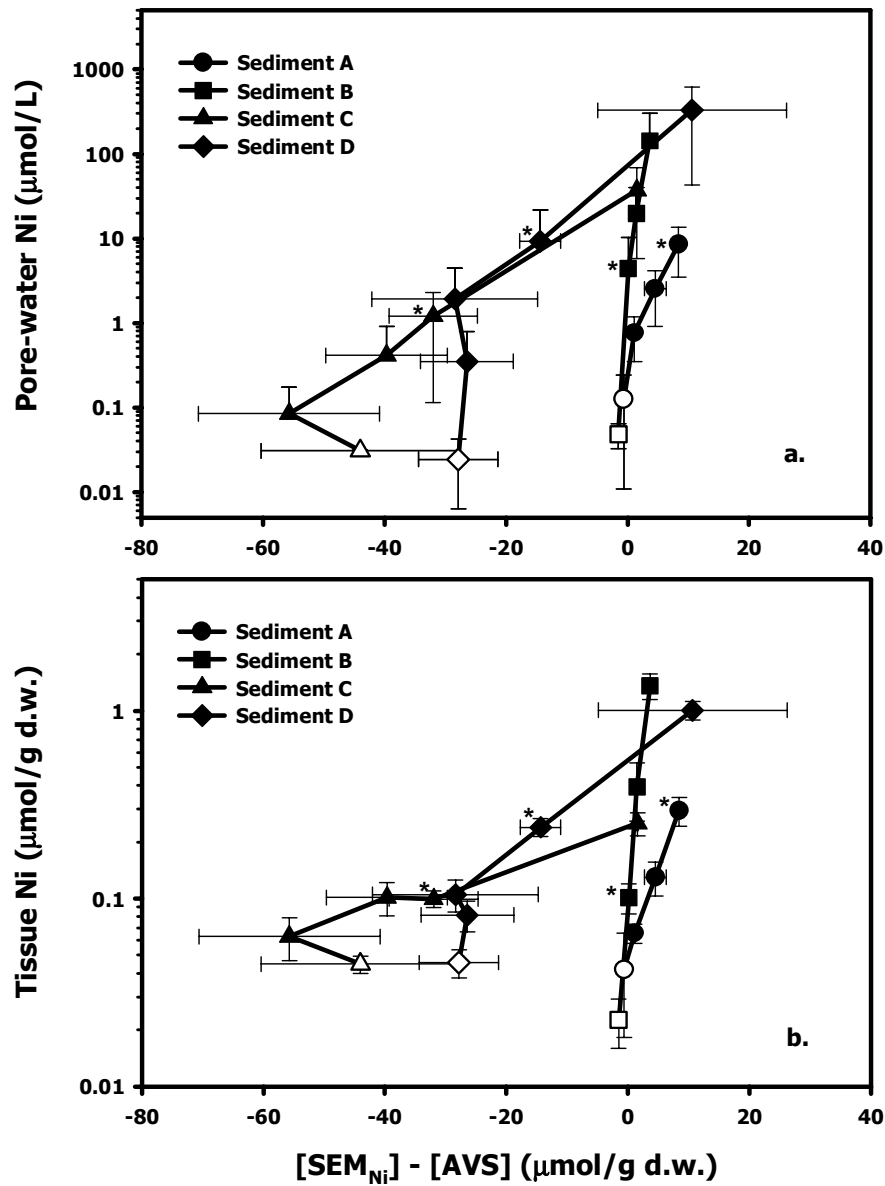
Figure 8.9 Pore-water Ni (a) and tissue Ni (b) versus [SEM$_{Ni}$] −[AVS] in 10-d Ni-spiked natural sediment toxicity tests. Asterisks denote pore-water and tissue Ni LOEC values. Control sediment data (represented by open symbols) are included for comparison. Data are means ± 1 standard deviation.
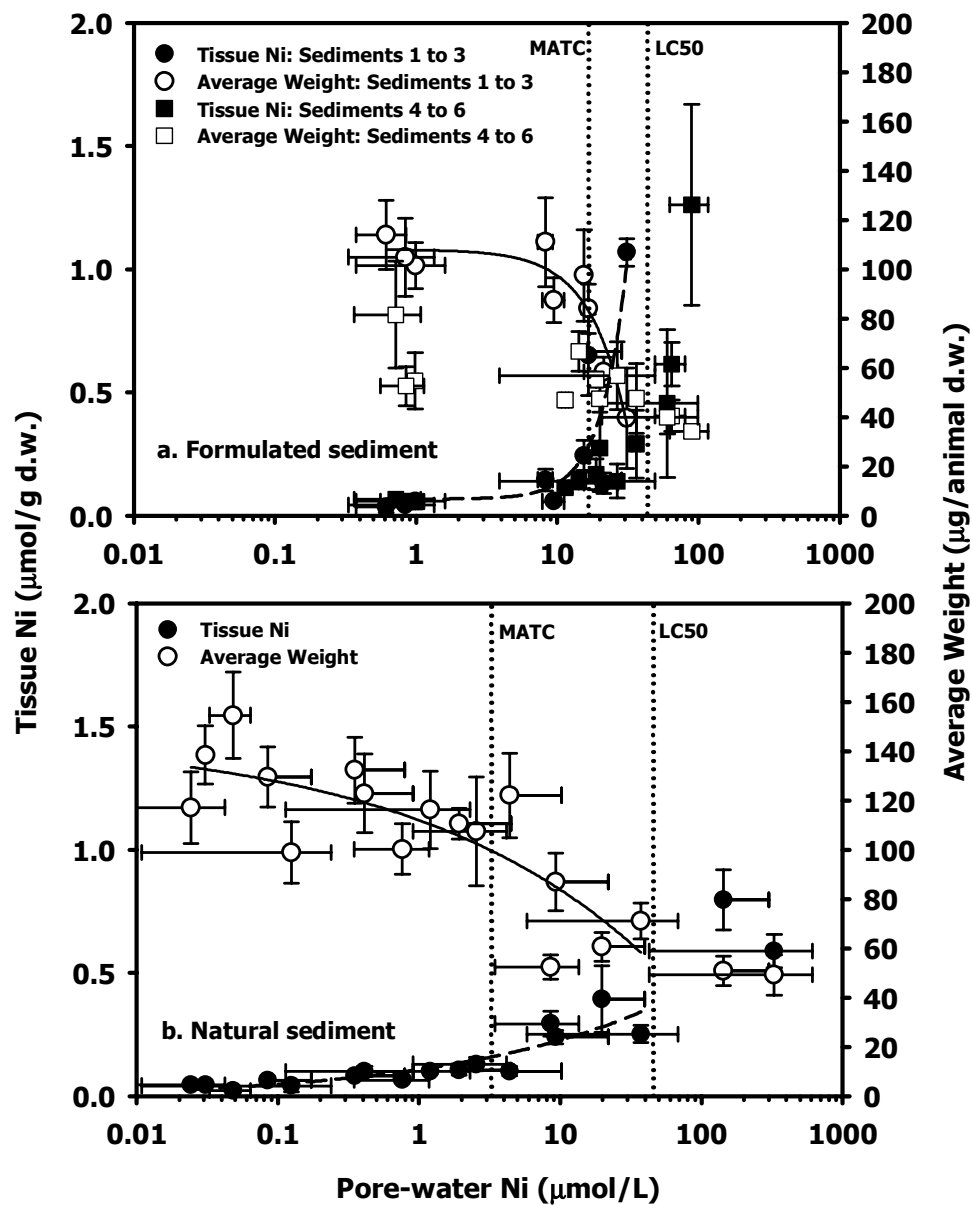
Figure 8.10. Tissue Ni concentration and average weight per animal versus pore-water Ni concentration in Ni-spiked formulated (a) and natural (b) sediments. Vertical lines represent the maximum acceptable toxicant concentration (MATC) and 10-d LC50 for pore-water Ni. Data are means ± 1 standard deviation.

that there is a tissue-Ni threshold above which animal growth is significantly affected. The MATC for tissue Ni was 0.409 ± 0.143 μmol/g d.w. and 0.152 ± 0.048 μmol/g d.w. for those formulated (sediments 1 and 2) and natural sediments, respectively, having both a LOEC and NOEC based on growth.

8.4   Discussion

8.4.1   Amphipod survival

In nature, field-collected sediments are extremely variable in their physical and chemical properties (Suedel and Rodgers, 1991). It is inherently difficult to locate sediments that are similar in most respects and differ in only a single characteristic of interest (such as OC and AVS content). Formulated sediments provide a strong method to better understand the biogeochemical factors affecting Ni bioavailability and toxicity in natural sediments. For example, formulated sediments 1 through 3 (Figure 8.1), which were constructed to increase in OC content alone (1.61, 3.20 and 6.76%, respectively), showed a corresponding 2.6-fold increase in the total-Ni LC50. Sedimentary Ni toxicity, based on total Ni concentration, was therefore significantly altered by the manipulation of OC content. Relative to the formulated sediments, total-Ni LC50 values ranged more widely (2.67 to 35.14 μmol/g, or 13.2-fold) in the natural sediments. In comparison to sediment B, the higher LC50 values in sediments A, C and D were due to the higher OC and AVS content, both of which provide buffering against Ni toxicity. The corresponding pore-water LC50 values (for formulated sediment 1 through 3 and natural sediments) remained relatively constant (Table 8.4), showing far less variability (0.3 and 2.8-fold,, respectively) than the LC50 values based on total Ni. Results from the present

study indicated that, while correlations between mortality and total Ni showed a moderate to strong r^2 value (especially for the formulated sediments), the regressions of mortality versus pore-water Ni yielded stronger relationships, particularly for the natural sediments (the overall r^2-value increased from 0.483 to 0.719; the increase in the r^2-value for the formulated sediments was marginal).

The strong correlation between overlying and pore-water Ni, found in both formulated and natural sediment tests, indicated that dissolved Ni diffused out from the pore water over the duration of the tests. This covariance resulted in the positive correlations between amphipod mortality and overlying-water Ni (data not presented) to appear similar to the relationships of mortality versus pore-water Ni. However, given that; the 10-d LC50 values (42.9±12.2 and 45.2±16.7 µmol/L in the formulated and natural sediment tests, respectively) were intermediate to the previously derived 96-h water-only LC50 value (71.6 µmol/L, see Chapter 5; 61.3 µmol/L, Milani et al., 2003) and the 10-d water-only LC50 value (13.3 µmol/L) of Ankley et al. (1991); there was a strong correlation between amphipod mortality and pore-water Ni; and that the overlying-water Ni levels were too low to account for the lethal effects seen in these tests, pore-water Ni appears to represent the strongest predictor of Ni exposure and uptake in these sediments. Studies by Hansen et al. (1996) and Ankley et al. (1991) have also identified pore-water (Cu and Ni, respectively) as a strong predictor of metal toxicity for *H. azteca* exposed to metal-spiked sediments.

Despite differences in pore-water characteristics (i.e., DOC and hardness), the average pore-water Ni LC50 values were similar in both sets of sediments (Table 8.4). Within each set of formulated and natural sediments, pore-water DOC and hardness

appears to have only a minor influence upon acute pore-water Ni toxicity. Previous work (Chapter 5) suggests that, at the pore-water Ni:DOC ratios seen in these tests, the protective effects of DOC would be subtle and therefore easily masked by other confounding influences such as dissolved Fe or differences in pH.

Previous studies (e.g., Di Toro et al., 1990) have found that the partitioning of metal between the solid and aqueous sediment phases is strongly influenced by AVS. Studies specifically involving Ni-contaminated sediments (spiked or field-collected) and benthic organisms have found Ni toxicity to be dependent upon the SEM:AVS ratio rather than total Ni content (Ankley et al., 1991; Di Toro et al., 1992; Pesch et al., 1995). Theoretically, once AVS complexation is saturated, barring the presence of other metal-binding phases, pore-water metal concentrations should increase sharply with further addition of metal (Di Toro et al., 1990). Data presented here show that significant amphipod mortality occurred when SEM_{Ni} concentrations exceeded AVS (Figure 8.3). Sediment A, containing low AVS but high OC content, buffered acute Ni toxicity until total $[SEM_{Ni}]$ $-[AVS]$ exceeded 8.4 µmol/g. Sediment B, in contrast, having low AVS and minimal OC content, displayed Ni toxicity at SEM_{Ni} levels marginally in excess of AVS (3.66 µmol/g). Organic carbon, therefore, can significantly affect the Ni buffering capacity of sediments having little or no AVS. Organic carbon may also play a metal-binding role in sediments with mid to high AVS content (such as sediments C and D), but the absolute contribution towards metal complexation, relative to AVS, may be minor. The quantification of Ni binding to OC was detailed in Chapter 6.

8.4.2 Amphipod average weight

While final weight per animal generally decreased with increasing total Ni content (Figure 8.4), there was a wide range in average weight for any given total-Ni concentration in both the formulated and natural sediment tests. For example, animal weight was dramatically reduced in sediment B at total Ni concentrations much less than those observed to reduce weight in the other natural sediments. In those sediments, the elevated OC (sediment A), or the combined effect of OC and AVS (sediments C and D), served to buffer the organisms from Ni toxicity until (relative to sediment B) much higher total-Ni concentrations were reached. Growth trends were somewhat obscured in the formulated sediments due to poorer growth of control animals for sediments 4 through 6. These sediments received iron and sulfide amendments during their construction and these, or other constituents, may have negatively affected *H. azteca* growth. Dissolved iron was thought to have reduced the survival of *H. azteca* in the metal-spiked, formulated sediments of Gonzalez (1996). Future work involving formulated sediments may need to further investigate the effects of iron and sulfide salt amendments on amphipod growth. When removing sulfide-amended sediments from the analysis, there was a strong relationship between average animal weight and total Ni (r^2 = 0.772). Average weight versus pore-water Ni (Figure 8.5) also showed a strong correlation (r^2 = 0.784). These findings are the result of the strong covariance between pore-water Ni are total Ni (r^2 = 0.807) in the formulated sediments. Theoretically, if two sediments have only minor physical and chemical differences, the partitioning of a metal within each (between the solid and aqueous phases) should be reasonably similar. While formulated sediments 1 through 3 were constructed to increase incrementally in OC

content, the physical and chemical similarities between these sediments resulted in the strong covariance of pore-water Ni and total Ni. For the natural sediments which had more varied physical and chemical characteristics, the metal partitioning was less similar and the correlation between pore-water Ni and total Ni was weak ($r^2 = 0.302$). The strong correlation between average weight and pore-water Ni ($r^2 = 0.728$) in the natural sediments, therefore, supports the premise that pore-water, rather than total Ni, represents the better predictor of Ni uptake for *H. azteca* in acute/subacute spiked-sediment toxicity tests. Care should therefore be taken when conducting metal-spiked sediment experiments. Sediments with similar characteristics will partition metals similarly resulting in a stronger covariance between total metal and pore-water metal. If pore-water metal is not sampled (as often seems to occur), it may appear as though the main route of exposure is via ingestion of total metal, whether or not this is actually the case.

Pore-water Ni concentrations between the geometric mean of the NOEC and LOEC values appear to represent a toxicity threshold (discussed further below) above which animal growth is significantly impaired (Figure 8.5). The difference in pore-water threshold values between the two sets of sediments may be related to differences in the pore-water micro-environment (i.e., hardness, DOC, Fe, pH) which, while not significantly correlated with weight, may have had subtle influences upon Ni speciation and bioavailability.

The asterisks in Figure 8.6 (denoting the LOEC values) demonstrate that while excess AVS appears to adequately predict the absence of acute toxicity, statistically significant reductions in amphipod growth may still occur. For example, in sediment 6,

at an $[SEM_{Ni}] - [AVS]$ difference of -1.00 µmol/g, the reduction in weight per animal

was 30.4%. Similarly in sediments C and D at $[SEM_{Ni}] - [AVS]$ differences of -32.0 and

-14.4 µmol/g, respectively, the corresponding weight per animal was reduced by 16.1%

and 25.8% relative to the respective controls. These results suggest that, while not

present at lethal concentrations, pore-water Ni was present (even in the presence of

excess AVS) and Ni was sufficiently bioavailable so as to impair the growth of *H. azteca*

(discussed further below).

8.4.3   Nickel bioaccumulation in *H. azteca*

As previously mentioned, amphipod growth was impaired even in the presence of

excess AVS. Although pore-water Ni concentrations in natural sediments C and D were

relatively low when AVS exceeded SEM content, pore-water Ni increased with

increasing SEM_{Ni} content. While the greatest increases in tissue Ni occurred at or above

$[SEM_{Ni}] - [AVS] = 0$, there were measurable increases in tissue Ni (strongly correlated

with increased pore-water Ni) at all $[SEM_{Ni}] - [AVS]$ values (including negative values).

In some instances, pore-water Ni was sufficiently elevated so as to cause growth

impairment.

Bioaccumulation of divalent cationic metals in invertebrates in the presence of

excess AVS has been noted previously. Pesch et al. (1995), in 10-d toxicity tests

involving *Neanthes arenaceodentata* in contaminated Hudson River sediments and

metal-spiked marine sediments, found that tissue metal concentrations (Cd and Ni)

generally increased with increasing sediment metal concentration, increasing SEM:AVS

molar ratio, and increasing pore-water metal concentrations. The presence of metal in

the worms at SEM:AVS ratios less than 1 was thought to be the result of metal release of from oxidized metal sulfide (a result of burrowing), metal uptake from ingested sediment, or metal adsorption to body surfaces. Working with metal-spiked sediments and bivalves, Lee at al. (2000a) found Ni to be significantly bioaccumulated (above control tissue concentrations) even in the presence of excess AVS. Bioavailability was found to be significantly related to total SEM (for *Macoma balthica*) rather than to [SEM] −[AVS] or pore-water metal concentrations. In 28-d, field-collected sediment exposures, Ingersoll et al. (1994) found bioaccumulation of Cu and Zn in *H. azteca* at SEM:AVS ratios ≤1. While the animals were not depurated prior to analysis, it was felt that the gut contents did not significantly contribute towards whole-body metal content.

If pore-water Ni is present, and it is bioavailable to *H. azteca*, questions remain as to the nature of the Ni speciation. The main bioavailable form of aqueous Ni is thought to be the divalent cation (see Chapter 5). Due to the strong tendency for Ni sulfide for form under anoxic conditions (Di Toro et al., 1990), free Ni^{2+} should exist at only very low, non-toxic concentrations in the presence of excess AVS. Calculations based on the work of Di Toro et al. (1992) show that, in the presence of excess AVS, Ni^{2+} should not have been present at the pore-water Ni concentrations found in sediments C and D (the predictions are two orders of magnitude lower). One possible explanation for this discrepancy is that Ni is present in the pore-water as soluble sulfide compounds which fit the definition of "dissolved" (<0.45 μm). Ni has been found to form $NiHS^+$ in anoxic seawater solutions (Luther et al., 1996). While stable in the anoxic layer, $NiHS^+$ may diffuse into the micro-environment of *H. azteca* and oxidize. This would result in the release of bioavailable Ni^{2+} for uptake across respiratory

surfaces. While the bioaccumulation of Ni would occur in the surficial sediment layer as a result of exposure to Ni^{2+}, uptake would covary with the bulk, anoxic pore-water Ni content. Soluble $NiHS^+$ itself has been found to be relatively labile and hence potentially bioavailable to organisms occupying sulfidic environments (Luther et al., 1996). Solid-phase NiS may also oxidize in the micro-environment of *H. azteca* thereby releasing bioavailable Ni^{2+}, but that would not account for the strong correlation seen between tissue Ni and pore-water Ni. The formation of metal sulfides may also stabilize AVS in the surficial sediment layer to some degree (Lee et al., 2000b) thereby hindering the oxidation process. On the other hand, Ni^{2+} has been shown to have a catalytic effect on the oxidation of H_2S (Snavely and Blount, 1969) and may therefore produce an opposite effect.

Overall, Ni speciation and associated effects upon Ni bioavailability/toxicity in the micro-environment of *H. azteca*, relative to the bulk sediment, require further consideration and research. As suggested by Pesch et al. (1995), a finer-scale of AVS and dissolved-metal sampling resolution would provide greater insight into the details of sedimentary metal exposure. Differences in the pore-water composition (hardness, DOC, Fe, pH) may also have influenced Ni bioavailabiltiy and toxicity, although available data were too limited to draw conclusive links.

Many metals (e.g., Cd, Pb, Hg, Zn) have been found to be poorly or non-regulated in *H. azteca* (Borgmann et al., 1991,1993). *H. azteca* appears to also poorly regulate internal Ni concentrations with tissue Ni increasing with increasing pore-water Ni. Both Borgmann et al. (2001) and Keithly et al. (2004) have previously linked elevated *H. azteca* total-Ni body burdens with lethality. The tissue-Ni LC50 values

found for both the formulated and natural sediments (131.2 ± 35.5 nmol/g and 147.0 ± 35.3 nmol/g, respectively, expressed as wet w.t.), are similar to the 28-d water-only LA25 (lethal accumulation resulting in 25% mortality) reported by Borgmann et al. (2001; 197 nmol/g wet w.t.) and the 14-d LA20 reported by Keithly et al. (2004; 247 nmol/g wet w.t.). With respect to growth, tissue Ni increased until reaching a critical toxicity threshold above which growth was impaired. While growth of animals in some formulated sediments was affected by factors other than Ni (sediments 4 through 6), a critical toxicity threshold appears in both sets of sediment data (0.409 ± 0.143 μmol/g d.w. and 0.152 ± 0.048 μmol/g d.w., for formulated and natural sediments, respectively). This threshold represents the point where the protective internal homeostatic mechanisms are overrun allowing free Ni^{2+} to bind to, and interfere with, the more sensitive cellular constituents (Maison and Jenkins, 1995). The difference in threshold values between the formulated and natural sediments may be partially explained by the growth dilution effect (Rainbow and Moore, 1986). The animals in the natural sediments grew larger than in the formulated sediments resulting in dilution of tissue Ni.

In general, amphipods are believed to hold potential as biomonitors of environmental metal pollution since they have generally been found to poorly regulate whole-body metal concentrations (Rainbow and White, 1989). Furthermore, *H. azteca* may be useful as a biomonitor since total-Ni body burden appears to provide a good measure of site-specific Ni bioavailability.

8.5 Conclusions

This study lead to a number of conclusions concerning Ni bioavailability and

toxicity to *H. azteca* in Ni-spiked freshwater sediments. First, under acute/subacute test conditions, pore-water Ni best predicted Ni toxicity represented and Ni bioaccumulation. In addition to AVS, the presence of sediment organic matter provided increased protection against acute Ni toxicity. While excess AVS appeared to predict the absence of acute Ni toxicity, it did not predict the absence of Ni bioaccumulation. Even in the presence of excess AVS, pore-water Ni was elevated above the controls, it was bioavailable, and, above certain toxicity thresholds (for both pore-water Ni and tissue-Ni), it impaired the growth of *H. azteca*. In addition, multiple pore-water Ni species (i.e., Ni^{2+}, $NiHS^+$) are potentially present and bioavailable in the micro-environment of *H. azteca*. Overall, for a given sediment, the partitioning of Ni between sediment binding phases, such as AVS and OM, and the presence of modifying factors in the pore-water (e.g., hardness, DOC, Fe, pH), will determine the bioavailability and toxicity of Ni.

Chapter 9

Discussion

9.1   Introduction

Sediments are important components in both freshwater and marine ecosystems. In recent years, attempts have been made to better understand the bioavailability and toxicity of metals in sediments with the overall objective of developing site-specific sediment quality guidelines (SQGs), or equilibrium partitioning-based sediment guidelines (ESGs), for the protection of aquatic life. This research was conducted to evaluate the role both dissolved and solid-phase organic matter play in influencing Ni bioavailability and toxicity in freshwater sediment. Until recently, Ni had received little attention in the literature relative to other base metals such as Cd or Cu. The following is a brief summary of findings from the water-only and spiked-sediment research previously presented in this thesis, followed by a discussion how this research fits into the current state of knowledge in metal ecotoxicology and its applicability to field situations (e.g., deriving site- or sediment-specific environmental quality guidelines).

9.2   Water-only research

The benthic animal model chosen for this research was *Hyalella azteca*. This organism is common to North American freshwaters, and over the last decade has become a popular test organism in sediment contaminant research. Researchers have

previously found pore-water metal concentrations to be important in predicting sediment

toxicity to *H. azteca* (e.g., Ankley et al., 1993). Therefore, to evaluate the influence of

DOM on Ni speciation and bioavailability in pore water, surrogate water-only Ni studies

were conducted. The Ni-DOM studies performed prior to this have mainly focussed

upon low metal-to-ligand (Ni:DOC) ratios in efforts determine conditional stability

constants. While this information is useful for understanding Ni speciation at

background Ni levels, Ni speciation at Ni:DOC ratios of toxicological concern has

received little attention. Furthermore, at study inception, it was not known whether

DOM influenced Ni bioavailability to *H. azteca*, whether DOM source or fraction

differentially affected Ni speciation and hence bioavailability, or whether DOM isolated

from sediment pore-water would bind Ni similarly to surface water DOM (i.e.,

Suwannee River humic substances supplied by the International Humic Substances

Society) or peat DOM, both of which are desirable for use as analogues of sediment

organic matter. Additionally, if there were detectable differences in Ni binding, could

these be due to measurable chemical or structural differences in the DOC?

Nickel bioavailability and toxicity was assessed via three methods: ion exchange

measurements of the free Ni^{2+} ion, mathematical modeling using the Windermere Humic

Aqueous Model (WHAM, model VI; Tipping, 1998), and toxicity and bioaccumulation

testing with *H. azteca*. It was found that, under acute 48-h Ni exposures, Ni speciation

as well as toxicity (measured as the LC50; 14.0 ± 2.2 mg/L) were not significantly

altered by representative surface water DOC concentrations (~10 mg/L). At such

elevated Ni:DOC ratios there was more metal in solution than could be significantly

complexed by the DOC ligands. Under lower, Ni concentrations (≤ 500 µg/L), it was

found that the bioavailability of Ni (measured as Ni accumulation in *H. azteca*) was significantly reduced in the presence of representative surface-water DOC concentrations regardless of DOC source or fraction (i.e., FA and HA). At elevated DOC concentrations, such as those potentially found in pore-water environments, Ni bioavailability (measured as Ni bioaccumulation) was dramatically reduced. The decrease in Ni accumulation was strongly correlated with the free Ni^{2+} ion concentration. Whole animal Ni concentrations provided a good measure of Ni bioavailability in short-term water-only exposures. With respect to DOC chemistry and structure, while the same DOM fraction might be very different in functional group content or aliphatic/aromatic composition, environmental source of DOM had little apparent effect on Ni speciation and bioavailability at sublethal Ni concentrations. Overall, the Ni:DOC ratio plays a greater role in determining Ni speciation and hence bioavailability and toxicity to aquatic organisms than either DOM source or fraction. Surface-water DOM such as Suwannee River humic substances or peat humic substances may therefore serve as suitable analogues for pore-water humic substances in Ni research.

9.3   Sediment

While previous attempts to assess sediment toxicity based simply on the SEM:AVS ratio or SEM in excess of AVS, were generally good at predicting non-toxic sediments, they proved to be less successful in identifying toxic sediments. This was most likely due to the presence of other binding phases such as OM (e.g., Mahony et al., 1996). The sediment component of this research was designed to investigate the

influence of OM (in addition to AVS) on the partitioning of Ni between the solid and aqueous phases in formulated and natural sediments, and to evaluate how this in turn affected Ni bioavailability and toxicity to *H. azteca*. It was hoped that this research would aid in the future development of site-specific sediment quality guidelines based on an equilibrium partitioning approach.

To appropriately proceed with this research, novel sediment methodologies were first required. Formulated sediments (using peat as a carbon source) were constructed following the protocol of Wang and Liber (unpublished manuscript) so that individual sediment characteristics, such as OC and/or AVS content, could be altered to evaluate the subsequent effects on Ni bioavailability and toxicity. Destructive sampling (i.e., sieving) is typically required at the end of sediment toxicity tests to retrieve all surviving test animals. This precludes the sampling of pore-water or SEM:AVS directly from those test vessels. Such data must therefore be obtained from additional, chemistry-only replicates, run concurrently. With the logistical constraints typically faced when conducting research (as in this study), there is a strong need for non-destructive sampling methodologies. A classical peeper (dialysis cell) field design was thus modified to create mini-peepers that are of convenient size for direct pore-water sampling in laboratory sediment toxicity and bioavailability tests. Complimentary to this, a mini-corer was developed to provide SEM:AVS data directly from those same toxicity test vessels, again eliminating the need for additional, chemistry-only replicates. While the mini-corer appears to be suitable for tests involving *H. azteca*, use with other animal models may depend upon size and habits of the specific test organism. *H. azteca* does not burrow deeply into the surficial sediment making the mini-corer suitable for SEM:AVS sampling

without impacting animal recovery.

Complimentary to toxicity testing, sediment titrations were performed under anoxic conditions to evaluate Ni partitioning to sediment OM. A natural, field-collected sediment high in OC and low in AVS was used to evaluate Ni complexation to organic matter over a range of pH. It was found that OM strongly influenced Ni partitioning. Ni partitioning to OM was also found to be significantly influenced by pH, with complexation increasing with increasing pH (from pH 6 to 8).

In Ni-spiked formulated and natural sediment toxicity tests it was determined that, similar to other studies, pore-water Ni represented the best predictor of Ni exposure and accumulation for *H. azteca*. In addition to AVS, the presence of OM (solid-phase) provided increased protection against acute Ni toxicity, particularly in low-AVS sediments. As predicted by the SEM:AVS model (Di Toro et al., 1990), acute toxicity was generally seen only at SEM concentrations in excess of AVS. However, while excess AVS appeared to predict the absence of acute Ni toxicity, it did not predict the absence of Ni accumulation. Even in the presence of ample AVS, pore-water Ni was present, it was bioavailable and, above certain toxicity thresholds (for both pore-water Ni and tissue-Ni), it impaired the growth of *H. azteca*. It is hypothesized that multiple pore-water Ni species (i.e., Ni^{2+}, $NiHS^+$) were present in anaerobic sediment pore-water and may have yielded bioavailable Ni^{2+} in the micro-environment of *H. azteca*.

Using an equilibrium partitioning approach incorporating both [SEM] −[AVS] and OC content, lethal and non-lethal toxicity test endpoints were predictable in low AVS sediments. Due to a lack of equilibrium between dissolved pore-water Ni and the pure metal sulfide, and the possible competition of liberated Fe^{2+} with Ni^{2+} for binding

sites on organic matter, toxicity predictions (based on sediment OC and AVS content) overestimated the combined protective effects of AVS and OC in the sediments containing mid to high (27.87 - 44.05 μmol/g d.w.) AVS concentrations. Overall, it was found that equilibrium partitioning-based guidelines can be improved through the incorporation of metal complexation to sedimentary OM (in addition to AVS), although further research is required.

9.4   Discussion

Fixed metal concentrations, based upon empirical analysis of matching chemical and biological data, have previously been proposed for use as sediment quality guidelines (SQGs) (Long and MacDonald, 1998). Basing SQGs on fixed Ni concentrations holds little predictive power, unless sediments are similar in their general physical and chemical characteristics. As the characteristics of various sediments diverge, so to does metal partitioning behavior. An equilibrium partitioning approach (ESGs) involving the [SEM] –[AVS] normalization of toxicity data accounts for site-specific differences in sediment characteristics. Although limitations to basing ESGs on AVS are recognized, the inclusion of metal complexation to OM (particularly in low AVS sediment) appears to further refine the predictive capabilities for acute toxicity.

From this and previous research, it could be argued that the use of AVS to derive SQGs may not be easy given its redox sensitive nature. Acid volatile sulifde is known to vary spatially, with sediment depth, and with season (Ankley et al., 1994). In marine sediments, as AVS increases during the summer and fall, and the reduction boundary moves upwards towards the sediment surface, adsorbed metals, such as Ni, may be

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released into the pore or overlying waters as Mn oxides are reduced (Shaw et al., 1990). Krantzberg (1994) hypothesized that the reduction of precipitated Fe and Mn hydroxides in contaminated Hamilton Harbour sediments, Lake Ontario, result in greater metal bioavailability and toxicity in the fall rather than the spring. Overall, metal bioavailability may be seasonally problematic, either through the oxidation of metal sulfides or reduction of scavenging phases such as Mn oxides. Basing SQGs on an unstable or potentially transient binding phase therefore requires greater study. For example, under prolonged resuspension events (such as dredging or storm events), AVS in surface sediments may decrease dramatically and thereby affect metals partitioning (Simpson et al., 1998).

For undisturbed sediments, the [SEM] – [AVS] normalization procedure makes the assumption that an equilibrium exists between dissolved Ni and the formation of the pure metal sulfide (NiS). While an equilibrium may be achieved to a greater degree for class B metals, it was found that this does not appear to hold true for Ni (a borderline metal). While acute toxicity predictions appear unaffected by this (pore-water Ni is relatively low in the presence of excess AVS), excess AVS does not predict the absence of Ni bioaccumulation and more subtle, long-term toxicity endpoints such as growth or reproduction. Elevated sediment Ni concentrations, even in the presence of excess AVS, could therefore have serious impacts in the field where exposures occur over the long-term.

The apparent catalytic action of Ni on the oxidation of AVS further complicates predicting the behavior of metals in sediments. Most often contaminated sediments contain a mixture of metals rather than individual metals such as Ni. As sediment Ni

concentrations increase singularly, or in combination with a number of metals, AVS has been found to decrease (Di Toro et al., 1992; Berry et al., 1996; Pesch et al., 1995). Since Ni is less insoluble than a number of other metal-sulfide forming metals (e.g., Cu, Cd, Pb), at SEM concentrations exceeding AVS (possibly in the aerobic surficial sediment layer) free Ni^{2+} may catalyze the oxidation of AVS. Therefore, Ni released by the formation of more insoluble metal sulfides may decrease AVS concentrations thereby liberating more metals. It is unknown whether this process occurs in field sediments contaminated with multiple metals.

The current limitation of the [SEM] – [AVS] normalization approach is that guidelines based on AVS will only apply to those metals known to readily complex with sulfide under anoxic conditions. Class A metals and metaloids (e.g., U, Se, As), which do not readily bind with sulfide, are not considered. If SEM metals exceed AVS, liberated SEM metals (such as Ni) may compete for binding sites on organic matter or Fe and Mn oxides with these other elements, thereby further complicating toxicity predictions.

While WHAM (Model VI; Tipping, 1998) appears to adequately predict Ni speciation for pure FA and HA solutions, it is unknown what fraction of the pore-water DOC is active in complexing Ni (i.e., behaves as pure FA or HA). While pore-water humic substance composition was quantified, this research did not evaluate the Ni-binding behavior of pore-water DOM as a whole (except at high Ni:DOC ratios where the effect on Ni bioavailability was minimal; data not presented). In surface waters, the fraction of DOM that behaves like isolated FA may be relatively high (40-80%; Tipping, 1998), but no information exists concerning pore-water DOM. Further research

involving isolated, whole, pore-water or surface water DOM may be necessary to apply these Ni-DOM complexation results to the sediment pore-water matrix. The water-only research demonstrated that DOM is capable of influencing Ni speciation and hence bioavailability and toxicity at the Ni and DOC concentrations found in the 10-d sediment toxicity tests. In order to apply the water-only research findings to more complex environments such as sediment pore-waters (or even surface waters), other bioavailability-modifying factors such as hardness and pH need to be more thoroughly evaluated both individually and in combination with DOC. While the data generated here were too limited to establish a relationship between pore-water Ni, hardness, DOC and Ni bioaccumulation in the sediment toxicity tests, a relationship likely exists. The development of a biotic ligand model (BLM) for Ni toxicity in sediments, similar to those BLMs being developed for surface water using other aquatic organisms (see review by Paquin et al., 2002), would help predict/model Ni toxicity in sediment pore-water environments. In the BLM, chemical equilibrium modeling predicts the concentration of a metal at the site of action (the biotic ligand) and this concentration is in turn related to an acute toxicological response. This method of modeling not only takes metal speciation into account (such as complexation with inorganic and organic ligands), but also modifying factors such as cation competition (Ca^{2+}, Mg^{2+}, H^+) for binding sites on the biotic ligand (generally the respiratory surfaces). If binding can be linked to uptake and hence total body burdens, a BLM for *H. azteca* may be able to predict, for a given total dissolved-Ni exposure, Ni body burden concentrations (which this research has demonstrated to be predictive of growth impairment).

In order to evaluate the effects of sediment Ni on *H. azteca* (or any benthic

organism) in a BLM, it is critical to properly evaluate organism exposure. *H. azteca* are small crustaceans which appear to occupy a micro-environment near the sediment-water interface. From this research it was determined that immediately above and below this interface dissolved Ni concentrations varied by one order of magnitude. Similarly, factors known to directly or indirectly influence Ni bioavailability and toxicity, such as DOC and hardness, also displayed a gradient across this boundary. While the mini-peeper cells sampled the pore water and overlying water separately, *H. azteca* occupies the surficial sediment environment between the two. Nickel exposure may therefore be intermediate between the high Ni concentrations in pore-water and the much lower concentrations in the overlying water. Metal exposure may also depend upon the daily behavior of this organism and the diffusional characteristics of the sediment test system. Further work is therefore required to better evaluate the micro-environment of small bodied epibenthic organisms, such as *H. azteca*, and to better characterize metal exposure. The habitat of *H. azteca* also needs to be evaluated in relation to the redox boundary which, in fine grained sediments, may exist within 1 or 2 mm of the sediment surface (Rhoads, 1974; as well as this study), and in relation to the diffusional boundary layer which exists at the sediment-water interface (Boudreau and Guinasso, 1982). Finer-scale vertical sampling would also provide better measures of water quality variables (e.g., pH, hardness, DOC) known to affect Ni bioavailability and toxicity in the exposure medium.

While high dissolved metal concentrations mean that the main bioavailable fraction is the free metal ion, this does not preclude other routes of uptake. At lower, sublethal dissolved metal concentrations and elevated metal concentrations in the food

source, dietary Ni may contribute significantly to the total metal body burden. Dietary Cd has been reported to account for as much as 58% of the daily uptake in *H. azteca* under chronic exposure conditions (Stephenson and Turner, 1993). While dietary Ni is likely a minor contributor to total Ni body burdens in *H. azteca*, relative to free ion uptake under acute exposure conditions, more research is required to specifically address dietary versus aqueous Ni uptake, especially under chronic, environmentally-relevant conditions.

Because amphipods have generally been found to poorly regulate body metal concentrations, they are believed to possess potential as biomonitors of environmental metal pollution (Rainbow and White, 1989). *H azteca* appears to be sensitive to Ni toxicity and appears to poorly regulate internal Ni concentrations. Since critical Ni body burdens were estimated for survival and growth, total-Ni body burden appears to provide a good measure of site-specific Ni bioavailability. Borgmann et al. (1991) have previously linked Cd body burdens to survival in *H. azteca*. While longer-term tests (i.e., 4-week growth; Borgmann et al, 2001) may be required to determine the critical body burdens relevant to *H. azteca* in the field, *H. azteca* appears to be suitable for monitoring bioavailability of multiple metals. Under field conditions, non-toxicological stresses (such as predation) will also need to into taken into consideration when estimating chronic body-burden thresholds. Gentile et al. (1982), found that a combination of Ni exposure and predation drove a mysid shrimp population towards extirpation at total dissolved Ni concentrations that were lower than would be required in the absence of predation.

Overall, there is a need to recognize that a myriad of complexities exist in the

physical and geochemical nature of sediments. While the equilibrium partitioning

approach to establishing metal criteria in sediments appears to hold promise as a

diagnostic tool for the protection and assessment of freshwater and marine sediments,

much work remains to be done in addressing the various issues discussed above.

Chapter 10

10. References

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Appendices

Appendix A: Average 48-h LC50 (± 1 standard deviation) results from duplicate *Hyalella azteca* Ni toxicity tests with various DOM sources and fractions in reconstituted water. Data are means ± 1 standard deviation.

				48-h Ni LC50 (mg/L)					
DOC (mg/L)	Whole peat	DOC (mg/L)	Peat hydrophilic DOC	DOC (mg/L)	Peat FA	DOC (mg/L)	SR FA	DOC (mg/L)	SR HA
0.8±0.2	13.80±0.88	0.9±0.9	14.79±1.74	1.5±0.3	15.43±0.75	1.6±0.5	13.32±6.16	1.2±0.3	12.07±0.29
1.2±0.4	13.30±1.56	1.8±1.0	18.49±1.94	1.7±0.4	12.63±8.15	2.1±0.3	16.95±9.33	1.8±0.3	9.71±0.28
3.1±0.7	12.94±0.42	7.5±0.7	16.20±1.24	4.9±0.3	12.60±4.48	6.2±1.5	14.08±3.10	4.8±0.7	12.04±0.78
13.5±5.5	12.22±1.52	39.5±4.1	17.62±1.46	25.4±0.9	14.76±3.33	30.8±2.8	15.12±4.69	23.6±1.9	12.66±2.62