The water-sediment interactions for *Hyalella azteca* exposed to uranium-spiked or contaminated sediments and different overlying water chemistries

by

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ABSTRACT

In comparison with other metals such as Cd, Cu, Pb, Ni, and Zn, little is known about uranium (U) toxicity to *Hyalella azteca*. There is even no national U water or sediment quality guideline yet for the protection of aquatic life in Canada, despite Canada being home to some of the biggest U producers in the world. In this context, the aim of this research was to determine the toxic effects of U concentrations in the water and sediment to *H. azteca*, and if these relationships can be modelled. This thesis demonstrated that U bioaccumulation was mainly via the water phase rather than the sediment phase. It showed that U bioaccumulation measurements in *H. azteca* were more reliable indicators of U toxicity than U concentrations in the water or sediment. A water-bioaccumulation saturation model was satisfactory at describing this relationship. Overlying water chemistry was found not only to influence U bioaccumulation and toxicity in the *H. azteca* but also the desorption of U in the sediment into the overlying water. A water-sediment partitioning saturation model was also satisfactory at explaining these interactions.

Both body size and gut-content had an overall effect on U bioaccumulation in *H. azteca* exposed to water-only U concentrations in soft water. A saturation model was used not only to estimate the effect of gut-content on U bioaccumulation, but to predict the uptake and elimination rate constants for *H. azteca* exposed to water-only U concentrations.

A field study was conducted to determine if the saturation models developed and applied in the laboratory could be used in the field to quantify U bioavailability, bioaccumulation and toxicity to *H. azteca*. Unfortunately, U concentrations in the water and sediment were below concentrations needed to validate these models. However, toxicity, not related to U concentrations in the field, was observed at some field sites.

Overall this thesis not only encourages more work on U toxicity to *H. azteca*, but provides significant data and models to be used by risk assessors and regulators in the development of U water and sediment quality guidelines in the protection of aquatic environments in Canada.

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List of Abbreviations

10SAM	5-salt Standard Artificial Medium diluted to 10% with Nanopure® water
50SAM	5-salt Standard Artificial Medium diluted to 50% with Nanopure® water
α	Alpha, which represents max/$K_{0.5}$
AETE	Aquatic Effects Technology Evaluation Program
ANCOVA	Analysis of Co-Variance
ANOVA	Analysis of Variance
BDL	Below Detection Limit
BEN	Bentley Lake, near Bancroft, Ontario, Canada
BLM	Biotic Ligand Model
BOW	Bow Lake, near Bancroft, Ontario, Canada
$C_{BKG(s)}$	Background metal concentrations in the animal or sediment
CCME	Canadian Council of Ministers of the Environment
CI	Confidence interval
C_{sed}	Concentration of the metal in the sediment
(C) SQG	(Canadian) Sediment Quality Guideline
C_{TB}	Total body concentration of the metal
CTR	Centre Lake, near Bancroft, Ontario, Canada
C_w	Concentration of the metal in the water
CWQG	Canadian Water Quality Guidelines
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
DUN	Dunlop Lake, near Elliot Lake, Ontario, Canada
dw	Dry weight
E(B)C10	The effect concentration in water (or body) where 10% of the endpoint (e.g. growth) was decreased compared to the control
E(B)C25	The effect concentration in water (or body) where 25% of the endpoint (e.g. growth) was decreased compared to the control
E(B)C50	The effect concentration in water (or body) where 50% of the endpoint (e.g. growth) was decreased compared to the control
EC	Environment Canada
EDTA	Ethylenediaminetetra-acetic acid
ELT	Elliot Lake, Elliot Lake, Ontario, Canada
FUNPAR	A function parameter module used in SYSTAT
g	Growth rate
gutclear	A dummy variable set equal to 1or 0: 1 for 24 h gut-cleared animals and 0 for non-gut-cleared animals
HC	Health Canada
ICP-MS	Inductively coupled plasma-mass spectrometry
INT	Inlet Bay, upper part of Paudash Lake, near Bancroft, Ontario, Canada
ISQG	Canadian Interim Sediment Quality Guidelines
K	The concentration at which mortality reaches 0.5^{nw} or 0.5^{nb} times max
$K_{0.5}$	The half saturation constant
K_{Ca}	The binding strength for calcium to the ligand
k_e	Excretion rate constant
K_H	The binding strength for the hydrogen ion to the ligand
KI	The inverse of K
K_m	The binding strength of the metal to the site of toxic action or ligand
k_u	Uptake rate constant
L(B)C10	The lethal water or sediment (or body) concentration resulting in 10% mortality
L(B)C25	The lethal water or sediment concentration resulting in 25% mortality
L(B)C50	The lethal water or sediment concentration resulting in 50% mortality

LEL	Lowest Effect Level
LLRWMO	Low-Level Radioactive Waste Management Office
LOEL	Lowest Observable Effect Level
Loss	The fraction of metal lost in 24 h due to gut-clearance
L_T	The total bound plus free ligand (L)
m	Instantaneous mortality rate
M^{Z+}	Metal
max	Maximum accumulation in the animal or concentration in the sediment (above background concentration)
MCC	McCarthy Lake, near Elliot Lake, Ontario, Canada
MHSAM	Modified Hard 5-salt Standard Artificial Medium
MINTEQA2	A speciation program
MITHE-SN	Metals in the Human Environment Strategic Network
ML	Metal (M) binding to the site of toxic action or ligand (L)
MSSAM	Modified soft 5-salt Standard Artificial Medium
MOEE	Ontario Ministry of Environment and Energy
n	Sample size
N'	The number of surviving animals
N_o	The initial number of animals
nb	A constant based on background-corrected total body concentration
NIST	National Institute of Standards and Technology
NLET	National Laboratory for Environmental Testing
NONLIN	Non-linear regression
NSERC	Natural Sciences and Engineering Research Council of Canada
nw	A constant based on water concentration
OECD	Organisation for Economic Co-operation and Development
OMOE	Ontario Ministry of Environment
PEL	Probable Effect Level
PNEC	Predicted no effect concentration
PSQG	Provincial Sediment Quality Guidelines
PW	Pore water
PWQO	Provincial Water Quality Objectives
QKE	Quirke Lake, near Elliot Lake, Ontario, Canada
RES10	Restoule Lake; depth of 10 m
SAM	5-salt Standard Artificial Medium
SEL	Severe Effect Level
SID	Siddon Lake, near Bancroft, Ontario, Canada
SITE	Site overlying water
SLC	Screening Level Concentration
SSLC	Species Screening Level Concentration
SW	Surface water
SYSTAT	A statistical program
t	Time
TEL	Threshold Effect Level
TOC	Total organic carbon
TOM10	Tomiko Lake; depth of 10 m
TM-DWS	Trace Metal-Drinking Water Standard
TU	Toxic unit
US	Uranium size-standardized animals
V_{umax}	Maximum metal accumulation
W	Maximum wet weight of the animal
W'	The control or maximum wet weight of an animal when C_w=0

CHAPTER 1

INTRODUCTION

1.0 Introduction

1.1 Uranium in the Aquatic Environment

Uranium (U) is a non-essential metal naturally found in the environment, with two modes of toxicity: radiological and chemical (EC/HC, 2004; Sheppard et al., 2005). Chemical toxicity is considered to be more important than radiological toxicity due to the low specific activity for ^{238}U (1.24×10^4 Bq/g), the most abundant (99.3%) radioactive isotope in the environment (EC/HC, 2004; Sheppard et al., 2005).

Due to its significant value as a nuclear energy resource, U can enter the aquatic environment through anthropogenic activities such as the mining, milling and refining of U (EC/HC, 2004). Waterborne U concentrations in surrounding lakes near U operating facilities such as Rabbit Lake, McArthur River, Key Lake, Cluff Lake, and McClean Lake in Northern Saskatchewan, Canada, have been reported to range between 0.004 to 4 μmol U/L (EC/HC, 2004). Uranium concentrations in water near decommissioned mining sites at Elliot Lake and Bancroft, Ontario typically range from 0.004 to 0.2 μmol U/L (Clulow et al., 1998, OMOE, 2003).

Uranium concentrations in the sediment are usually in the range of 0.002 to 0.02 μmol U/g (Markich, 2002). The highest concentration reported in sediments impacted by U mining and milling in Canada was 80 μmol U/g dry weight (Hart et al., 1986; Cooley and Klaverkamp, 2000). Concentrations in sediments concentration near active U mines in Saskatchewan range from 0.02-20 μmol U/g dry weight, while 0.01 to 2 μmol U/g dry weight have been measured in sediments near former U mines in Ontario (OMOE, 2003; EC/HC, 2004). Uranium concentrations in the sediment vary depending on the particle size and mineral composition of the sediment, and the chemistry such as the pH, hardness and dissolved organic matter of the overlying water (Markich et al., 2002). Loss of U from water to sediment is affected by sediment type and is in the order of: organic sediment >clay> sand (Bird and Evenden, 1994).

1.2 Routes of Exposure and metal uptake

Aquatic organisms can be exposed to U through the water, sediment and/or diet. Depending on the route of exposure and the environmental conditions (temperature, pH, water and sediment chemistry, metal speciation, and metal distribution) there are two important pathways in which metals may enter and accumulate in an aquatic invertebrate such as freshwater amphipod, *Hyalella azteca*: the gills, via direct contact with the aqueous phase, and/or the gastrointestinal tract, via the ingestion of metal contaminated food or suspended sediment particles (Langston and Spence, 1995; Marsden and

Rainbow, 2004). The absorption of metals onto the exoskeleton of invertebrates may also contribute to metal uptake and accumulation. However, this type of metal uptake is considered not biologically available to play a physiological role within the animal (Rainbow and Dallinger, 1993; Rainbow, 2007).

Metal uptake at the gills occurs by the passive transport of dissolved metals from the aqueous phase into the haemolymph via intracellular high affinity protein carriers in the gills (Simkiss and Taylor, 1995). This metal uptake pathway at the gills includes most metals with the exception of the major ions (Na, K, Ca, and Mg) that rely on active pumps and metals bound as lipophilic organometallic compounds (e.g., Hg) that diffuse through a lipid membrane to gain entry into the animal (Rainbow and Dallinger, 1993; Simkiss and Taylor, 1995; Mardsen and Rainbow, 2004). Depending on the metal, once these dissolved metals are transported across the cell membrane, they may be further transferred to binding sites of higher metal-binding strengths in the gill (Langston and Spence, 1995). These metal-ligand complexes may be sequestered or excreted in the gills and/or further transported into the haemolymph, where the metals may be distributed to other tissues in the animal (Rainbow and Dallinger, 1993; Rainbow, 2007).

Freshwater invertebrates may also accumulate metal via ingestion of food and/or ingested sediment particles (Rand et al., 1995), however the mechanisms of this type of uptake route in invertebrates is less well known compared to metal uptake at the gills. Uptake at the gastrointestinal tract is thought to occur by dissolution of the particulate form of the metal from the foodstuff, followed by the absorption by the hepatopancreas and/or mid-gut of the gastrointestinal tract into the haemolymph and further transported to other tissues in the animal (Vonk, 1960). Metals in the aqueous phase are considered to be more bioavailable than metals associated with the solid phase, however higher metal concentrations in food and suspended sediment particles, does make the solid phase an important source of metal uptake by aquatic organisms (Langston and Spence, 1995). The bioavailability of the metal uptake from the diet will depend on the chemical form of the solid, the binding affinity of the metal to the solid, the presence of organic and inorganic particles in the solid, the particle size of the ingested solid, the feeding habits and the digestive process of the animal (Langston and Spence, 1995; Marsden and Rainbow, 2004).

1.3 Metal Regulation and excretion

After the metal is taken up, it may be transported, distributed, and/or sequestered within the animal and this may lead to elimination from the animal (Rainbow and Dallinger, 1993). The differences between metal uptake and excretion will determine metal bioaccumulation in the animal. To avoid potentially toxic effects of an influx of excess metal, the animal may be able to excrete the metal or store the metal in a soluble or insoluble form that is not bioavailable to cause adverse effects to the animal (Langston and Spence, 1995; Marsden and Rainbow, 2004). For example, the freshwater amphipod *H. azteca* can regulate Cu (Borgmann et al., 1993; Borgmann and Norwood, 1995) while the marine amphipod, *Allorchestes compressa* is able to regulate Cu and Zn, with some evidence of Cr regulation over a range of 0 to 100 µg Cr/L in the water (Ahsanullah and Williams, 1991). In some species there is a production of metal-binding ligands, such as metallothioneins, that bind the bioavailable metal into a non-toxic form (Langston and Spence, 1995). Chassard-Bouchaud (1982; 1983) found that U was in the form of an insoluble phosphate in the lysosomal system of the freshwater crayfish *Pontastacus leptodactylus,* and associated with calcium phosphate microgranules (spherocrystals) in the gill cuticle and hindgut epithelia of a marine crab, *Carcinus maenas,* when exposed to aqueous-phase U. These microgranules, which can eventually be eliminated from the animal, may act as temporary storage sites for U in the tissue and prevent adverse effects in the animal by binding the metal in a biologically unavailable form.

Knowledge of the uptake route, kinetics rate constants, bioaccumulation pattern, and relationship to the exposure concentration by the invertebrate of interest is required if these animals are to be used as biomonitors of metal bioavailability and toxicity in the environment. In other words, the bioaccumulated metal needs to increase with the exposure concentration, such that the metal cannot be fully regulated. When these assumptions are met, metal burdens in the *H. azteca* have been shown to be reliable indicators of metal bioavailability and toxicity (Borgmann and Norwood, 1997; Norwood et al., 2006). These bioaccumulation measurements of metals have also been shown to be better predictors of biological effects than concentrations in both the water and sediment, because the physicochemical factors (e.g. temperature, pH, metal speciation, and metal distribution) affecting metal bioavailability and toxicity in the environment are accounted for within the organism (Borgmann et al., 1991; Borgmann and Norwood, 1997; Borgmann, 2000).

1.4 Modifying factors affecting bioaccumulation

If not accounted for or controlled, factors such as growth and body size can contribute to variability in total metal bioaccumulation estimates due to their influence on the uptake kinetics, bioaccumulation and elimination of metals in the animal (Langston and Spence, 1995; Rand et al., 1995). If metal uptake is slow, growth can have an effect on body burden concentrations because a slow-growing animal may accumulate a higher concentration of a metal when compared to a faster growing animal, due to the dilution of the metal in the faster growing animal.. Body size can have a modifying effect due to the larger surface area to volume ratio and faster metabolic rate of smaller animals (Rainbow and Moore, 1986; Langston and Spence, 1995). What may be thought of as a sensitivity difference between organisms may actually be a body size effect on the kinetic rate constants due to the differences in surface area to volume ratios between organisms of different sizes (Rand et al., 1995). For instance, Markich (2003) found that the mean variability in valve movement responses in the freshwater (unionoid) bivalve *Velesunio angasi* exposed to waterborne U concentrations was reduced by 81% when corrected for body size, age and gender.

Gut-content can also contribute to total metal accumulation in the animal, even if there is not an effect on toxicity (Neumann et al., 1999). A high metal content in the gut can lead to a significant overestimation of bioaccumulation and metal bioavailability in the environment, which in turn can overestimate lethal body burden thresholds (e.g., lethal body burden causing 50% mortality, LBC50). For instance, Neumann et al. (1999) found that without gut-clearance real body concentrations of Pb and Zn were overestimated by 438 and 44%, respectively, in *H. azteca* exposed to Pb- or Zn-spiked sediments. This suggests that animals should be depurated prior to metal burden analysis, especially in sediment toxicity tests, so that the overestimation in total body bioaccumulation is avoided. Overall, when reporting bioaccumulation measurements and interpreting toxicity one must be sure to account and/or correct for the influence of modifying factors such as growth, body-size and gut-content which can contribute to variability in bioaccumulation measurements and toxicity estimates (Langston and Spence, 1995). Alternatively one could standardize test procedures, such as using same size animals and ensuring that animals were gut cleared, so that body size, growth and gut content do not influence the overall bioaccumulation measurements and toxicity estimates.

Water chemistry and the surface permeability of the animal can also influence metal bioaccumulation. Depending on their concentration, cation such as $Ca^{2+,}$ Mg^{2+}, Na^{+}, and H^{+} can compete with other metals such as Cd^{2+} and Pb^{2+} in the water for metal binding and uptake at a biological membrane (i.e., the gills), and/or with the ions in the animal (Borgmann, 2000; Pagenkopf, 1983). Anions such as OH^{-}, HCO^{-}_{3}, CO_3^{2-} , Cl^{-}, $S_2O_3^{2-}$,S^{2-},, and dissolved organic carbon in the water can also complex these metals and thus prevent uptake by the organism (Wood, 2008). For example, water pH can influence metal speciation and bioavailability in the water, and thereby metal bioaccumulation, by affecting the permeability and sensitive of the cell surface in the animal (Campbell and Stokes, 1985). At low pH, the hydrogen ion can compete for metal binding on a biological surface such as the fish gill, thus decreasing metal uptake (and toxicity) for the metals such as Cd and Zn, but not for the metal Pb (Pagenkopf, 1983; Bradley and Sprague, 1985a, b; Campbell and Stokes, 1985; Cusimano et al., 1986; Spry and Weiner, 1991). In the case of Pb, it appears that the effect of the hydrogen ion has more to do with its effect on metal speciation rather than with hydrogen ion competition at the biological surface (Campbell and Stokes, 1985; Spry and Wiener, 1991).

Low concentrations of Cd, Zn and Pb in the water have been associated with blocking Ca uptake in fish (Verbost et al., 1987, 1989; Hogstrand et al., 1995, 1996, Alsop and Wood, 1999; Rogers et al., 2003; Rogers and Wood, 2004), with higher concentrations of Ca in the water or food decreasing Cd and Pb uptake and accumulation (Varanasi and Gmur, 1978; Part et al., 1985; Franklin et al., 2005; Alves and Wood, 2006). This is because Cd (Verbost, 1987; Verbost, 1989), Pb (Varanasi and Gmur, 1978; Rogers et al., 2003; Rogers and Wood, 2004) and Zn (Spry et al., 1989; Hogstrand et al., 1995, 1996), are considered to be Ca-mimics or antagonists (Varanasi and Gmur, 1978; Verbost, 1987; Verbost, 1989; Spry and Weiner, 1991; Rogers et al., 2003; Rogers and Wood, 2004; Franklin et al., 2005) that compete at and/or are transported across the cell membrane using the same or similar channels to that of Ca. Similarly, Wright (1980) found that Cd uptake by freshwater amphipod, *Gammarus pulex* probably followed a similar uptake pathway to that of Ca, given that Cd uptake was significantly inhibited for animals exposed to dinitrophenol, a drug that inhibits active processes, and the negative relationship between the Cd uptake rate and Ca concentration in the animals.

The diet of animals can also influence metal uptake. For instance, if the animal is getting the proper metal ions such as Ca or Mg from the diet, the animal may be able to down-regulate transport channels at the gill surface, especially at contaminated waterborne sites, thus decreasing metal uptake of these ions and in turn of other toxic metals such as Cd and Pb into the animal. For instance, Varansai and Gmur (1978) found that coho salmon had reduced waterborne Pb concentrations in the gills when fed a Ca diet, while Alves and Wood (2006) suggested that an increase in dietary Ca may not only reduce Pb uptake at the rainbow trout intestine, but may down-regulate Ca and Pb uptake at the gills in order to maintain Ca ion-regulation in the fish.

Anionic ligands such as dissolved organic carbon have been shown to complex metals in the water and render them unavailable for uptake by the animal and in turn, reduce toxicity (e.g. Wang 1987; Spry and Weinger, 1991; Playle 1993 a,b; Paquin et al., 2002; Heijerick al., 2003; Clifford and McGeer, 2009). However, the presence of organic complexing agents in the water may facilitate metal uptake, such that lipophilic complexing agents that bind toxic metals (i.e., organometallic compounds) in the water may in turn be able to directly diffuse through the lipid membranes of the organism, accumulate and cause toxicity (Borgmann, 2000).

1.5 Uranium speciation and toxicity

The chemical speciation of U and uptake by organisms has been shown to be affected by the factors of the environment such as alkalinity, hardness, pH, and natural organic matter (Markich et al; 2000; Sheppard et al., 2005). Although U may occur in several oxidation states, the hexavalent (U^{6+}, $UO_2{}^{2+}$; uranyl ion) ions are the major and most stable form in oxidized waters because they readily form complexes with hydroxide, carbonate, phosphate or sulfate ions (Langmuir, 1978; Gascoyne, 1992; Markich, 2002; Sheppard et al., 2005). In anoxic waters and sediments, the tetravalent U (U^{4+}) is the major form (Markich, 2002). In the tetravalent form, U has a tendency to precipitate and remain immobile (Gascoyne, 1992). The uranyl and UO_2OH^+ ions have been suggested as the ions responsible for much of the toxicity of U (Markich, 2002). Markich et al. (2000) showed that the effect of U on the valve movements of the mussel *Velesunio angasi* was related to the activity of uranyl ion in solution. This relationship, however, was better explained using both UO22+ and UO_2OH^+ ions than with the UO_2^{2+} ion alone, with the formation of UO_2OH^+ ion being dependent on water alkalinity and the formation of uranyl ion being dependent on water hardness. In other words, an increase in alkalinity in the water will result in the formation of U ternary carbonate complexes which in turn decreases the UO_2OH^+ ion in solution, while an increase in Ca concentrations in the water, results in the formation of ternary U complexes with Ca that reduces the presence of UO_2^{2+} ion in solution..

Many studies (e.g. Tarzwell and Henderson, 1960; Parkhurst, 1984; Riethmuller et al., 2001; Charles et al., 2002; Borgmann et al., 2005) have shown that U toxicity decreases with increasing water hardness. In *H. azteca* exposed to U in one-week water-only experiments, U was found to be more toxic in soft water (hardness 18 mg $CaCO_3$/L; alkalinity 14 mg $CaCO_3$/L, pH 7.8) than hard water (water hardness 124 mg $CaCO_3$/L, alkalinity 84 mg $CaCO_3$/L, pH 8.3; Borgmann et al., 2005). The 7d- LC50 (lethal water concentration resulting in 50% mortality) was 0.02 mg U/L (measured) and 1.65 mg U/L (nominal) for the soft water and hard water, respectively (Borgmann et al., 2005). Tarzwell and Henderson (1960) reported a 96h-LC50 value of 3.1 mg U/L for fathead minnow (*Pimephales promelas*) exposed to uranyl nitrate at water hardness of 20 mg $CaCO_3$/L, total alkalinity of 20 mg $CaCO_3$/L, and pH 7.4. No toxicity was observed at a hardness of 400 mg $CaCO_3$/L /L, total alkalinity of 360 mg $CaCO_3$/L and pH 8.2. Poston et al. (1984) found that acute U toxicity to *Daphnia magna* decreased by a factor of 7.5 when water hardness and alkalinity were increased by approximately 1.5. They suggested that this decrease in U toxicity was due to the complexation of the uranyl ions with the carbonate ions. Charles et al. (2002) found a 5-fold decrease in U toxicity to *Chlorella* sp. when water hardness was increased 50-fold (8 to 400 mg $CaCO_3$/L), at a constant pH (7.0) and alkalinity (8 mg $CaCO_3$/L). It was suggested that the reduction in U toxicity was due to the competition between U and Ca for binding sites on the algal cell surface given that there was no significant difference in the speciation calculations of U with increasing water hardness.

Although the above studies have shown that U is more toxic in soft water than hard water, some of them have, however, failed to separate the effect of water hardness from that of alkalinity and pH (e.g. Tarzwell and Henderson, 1960; Parkhurst et al., 1984). For example, Parkhurst et al. (1984) reported a 96h LC50 for juvenile brook trout, *Salvelinus fontinalis,* to be 5.5 and 23 mg/L in soft (32 mg/L as $CaCO_3$; alkalinity 12 mg/L as CaCO3, pH 6.7) and hard (210 mg/L $CaCO_3$; alkalinity 54 mg/L as $CaCO_3$, pH 7.5) water, respectively. Thus, future studies are needed to elucidate the difference between alkalinity, pH and hardness so that the interpretations of U toxicity to an organism of interest are correct.

1.6 Environment Quality Guidelines

Currently there are no national environmental quality and management guidelines for the protection of aquatic life for U in Canada. This is unfortunate given that Canada is home to some of the richest U deposits in the world. The only national guideline for U is the Canadian drinking water quality guideline. The maximum acceptable concentration of U in the drinking water is set at 0.02 mg U/L (84 nmol U/L;HC, 2008). Ontario is the only province in Canada that has an emergency provincial interim water quality objective for U which is 0.005 mg/L (21 nmol U/L; OMOE, 1994). Care is required when interpreting this objective because it was derived using a limited data set (MOEE, 1994).

Of the studies available on U toxicity to freshwater organisms, half have used model organisms native to the Southern Hemisphere, mainly Australia (e.g. Bywater et al.,1991; Franklin et al., 2000; Markich et al., 2000; Charles et al., 2002; Hogan et al., 2005). These studies are not representative of species found in the temperate Northern Hemisphere and perhaps inappropriate to be relied upon, especially in cases where these species do not have similar uptake and toxicity patterns to native species, when setting and developing environment quality guidelines for U in Canada. The bulk of studies that use species native to North America (e.g. Tarzwell and Henderson, 1960; Parkhurst et al., 1984; Cooley and Klaverkamp, 2000; Pyle et al. 2001, 2002) have focused on fish models to evaluate U toxicity in freshwater environments. In order to develop a U water and sediment quality guideline that is representative and applicable to the holistic freshwater environment, one needs to evaluate the toxicity relationships of U not only in the water, but in U-contaminated sediment exposures to other potentially sensitive groups such as invertebrates.

Only a few studies have investigated U bioavailability and toxicity (LC50) to freshwater invertebrates such as *Daphnia magna* (e.g. Poston et al., 1984; Zeman et al., 2008), and *H. azteca* (Borgmann et al., 2005) via U waterborne exposures. The sublethal effects on survival, development time, growth and LC50 for *Chironomus ripanius* larvae (e.g. Dias et al., 2005, 2005) exposed to sediment-bound U have also investigated. In spite of the limited studies available in the literature on U toxicity to invertebrates, Sheppard et al. (2005) were able to derive a predicted no effect concentration (PNEC) for the chemical toxicity of U in freshwater invertebrates and benthos of 0.005 mg/L in water and 100 mg/g dry weight in sediment. These PNEC were derived using the most sensitive and ecologically relevant endpoint, such as the concentration where the endpoint was decreased by 25% compared to the control (EC25) for the most ecological relevant organism(s). The reliability of these PNEC values is low given the limited number of studies used to derive them. Therefore, more studies on U toxicity to a variety of ecologically relevant organisms and via different exposure routes (i.e. the sediment) are required if regulators want to develop and set appropriate water and sediment quality guidelines for U in Canada.

1.7 Project Objectives

The Biotic Ligand Model (BLM), which is a chemical–equilibrium based model, has gained popularity in North America in predicting metal toxicity (Paquin et al., 2002). The BLM considers how different factors in the environment, such as the concentration of the free-metal ions, complexation of free-metal ions with abiotic ligands and competition with other cations influence the extent to which a free-metal ion binds and accumulates on or in an organism (biotic ligand; Niyogi and Wood, 2004). An inherent problem of the BLM model, however, is in the limited types of chemical species and quality of the thermodynamic constants available to accurately predict the free ion speciation (Slaveykova and Wilkinson, 2005). Alternatively, a bioaccumulation saturation model has been shown to be successful at predicting and relating toxicity to the *H. azteca* exposed to the metals and metalloids, Cu, Cd, As, Co, Cr, Mn, Ni, Zn, Tl, Pb in water and sediment (Borgmann et al., 2004; Norwood et al., 2006). This model is analogous to the BLM in which toxicity is assumed to be a function of the amount of metal bound to a specific ligand (e.g. fish gill) that has become saturated on the organism (Paquin et al., 2002; Borgmann et al., 2004). This model is applicable only when the animal does not regulate or sequester the metal and shows a saturation relationship between the exposure and bioaccumulation of the metal (Borgmann and Norwood, 1997; Norwood et al., 2006). If properly developed and utilized, the bioaccumulation saturation model can be used to predict metal bioavailability and toxicity to invertebrates such as *H. azteca* exposed to U in the water. This model can also help explain the water-sediment interactions of *H. azteca* exposed to sediment bound U, if U binding to the sediment saturates. Regulators and risk assessors can in turn use these saturation models to develop appropriate U water and sediment quality guidelines.

The purpose of this thesis is to determine if a water–bioaccumulation saturation model or a water-sediment partitioning saturation model could be used to explain and quantify metal bioavailability, bioaccumulation and toxicity to a representative freshwater amphipod, *H. azteca,* exposed to U-spiked and contaminated field sediments with different overlying water chemistries. This amphipod is found throughout North America, has a short generation time and is easy to culture, identify and collect in the field (Borgmann and Munawar, 1989; Borgmann et al., 1989). It is sensitive to metals and commonly used in sediment toxicity tests (EC, 1997). The following objectives, grouped under the three headings, were set forth:

1. **Bioaccumulation and toxicity of U in sediments as affected by overlying water chemistry.** I first determined how water chemistry influences the bioavailability, bioaccumulation and toxicity of U to *H. azteca* exposed to spiked-U sediments by independently varying hardness, alkalinity and pH parameters in the overlying water. Second, I determined whether the water or sediment phase is the major exposure pathway of U bioaccumulation in *H. azteca* is a more reliable indicator of toxicity than concentrations in the water or sediment. Depending on the exposure pathway, the third objective was to determine U bioaccumulation saturates in the animal, and if a saturation model can be developed to explain metal bioavailability, bioaccumulation and toxicity to *H. azteca*. The last objective was to apply a water-sediment partitioning saturation model to explain the water-sediment interactions of U with different water chemistry (Chapter 2).

2. **Uranium kinetics and modifying factors.** I determined if there is the an effect on body size and gut-content on the U bioaccumulation and toxicity to *H. azteca* exposed to water-only U concentrations, given that these factors can influence the variability of metal bioaccumulation due to the kinetics of uptake and elimination. Another objective was to estimate the uptake and elimination rate constants to determine if steady-state is reached within a given exposure period to ensure that future designs and interpretation of freshwater U toxicity tests are correct (Chapter 3).

3. **Application of laboratory derived models in the field.** Determine if the water-bioaccumulation saturation model and water-sediment partitioning saturation model developed in the laboratory can be applied in the field to quantify and explain U bioavailability and toxicity under natural conditions (Chapter 4).

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CHAPTER 2

WATER-SEDIMENT INTERACTIONS FOR *Hyalella azteca* EXPOSED TO URANIUM-SPIKED SEDIMENT

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Abstract

Data on the toxicity of uranium in sediments to *Hyalella azteca* and the effect of overlying water chemistry are limited. This study exposed *H. azteca* to sediments spiked with U (0 to 10 000 µg U/g dry weight) and five different overlying waters, which varied independently in hardness and alkalinity. Water pH had a major effect on U uptake by *H. azteca*. Uranium toxicity was higher when overlying water pH was low, while desorption of U into the overlying water increased with increasing pH. There appears to be little effect of Ca on U uptake, other than its influence on U speciation. Experiments with caged animals indicate that U accumulation and toxicity occurs mainly through the dissolved phase rather than the solid phase. Uranium bioaccumulation is a more reliable indicator of U toxicity than U concentrations in water or sediment. Uranium bioaccumulation in *H. azteca* and U adsorption to sediment can be satisfactory explained using saturation models.

Keywords: Hyalella azteca; Uranium; Bioavailability; Toxicity; Biotic ligand model

2.0 Introduction

Uranium (U) is an interesting metal in that there are two modes of toxic action: chemical and radiological. The chemical form is generally of more concern, since the radioactivity of U has a low specific activity, 1.24 X 10^4 Bq/g for U-238, the most common isotope in natural ores (EC/HC, 2004). Due to its significant value as a nuclear energy resource, U can enter the aquatic environment through anthropogenic activities (EC/HC, 2004). An Environment Canada/ Health Canada (2004) report on releases from nuclear facilities concluded that U and U compounds from mines and mills were potentially chemically toxic and could cause immediate or long-term effects on the environment. Radionuclides, on the other hand were not considered to be toxic at the current concentrations released into the environment. However, the report did recommend that the release of any radionuclides from U facilities be regularly monitored to determine future risks associated with radiological exposure. Despite the concern that the chemical form of U in the environment may be toxic, there are currently no U water and sediment quality guidelines in Canada or the United States. This is impeding the management of aquatic environments near U mines and tailings.

The chemical speciation of U in water is complex and not fully understood. Chemical speciation of U is affected by hardness, pH and natural organic matter (Markich, 2002). Although U may occur in several oxidation states, the hexavalent uranyl ion (UO_2^{2+}) is the major and most stable form in oxidized waters. Chemical speciation models have shown that UO_2^{2+} and UO_2OH^+ ions may be the major species responsible for U toxicity (Markich et al., 2000) in aquatic organisms.

The toxicity of U in water varies with water chemistry. In *Hyalella azteca* exposed for 1 week in water-only experiments, there was 30-fold higher toxicity of U in soft water than hard water (Borgmann et al., 2005). Studies with fathead minnow, *Pimephales promelas* (Tarzwell and Henderson, 1960), green hydra, *Hydra viridissima* (Riethmuller et al., 2001) and *Chlorella* sp. (Charles et al., 2002) have also shown that U toxicity decreases with increasing water hardness. Currently, there are no published studies that address the toxic effects of sediment-bound U to the freshwater invertebrate *H. azteca;* a species commonly used in sediment toxicity tests and representative of invertebrates found in Canadian freshwaters (EC, 1997; Borgmann and Norwood, 1997a).

The Biotic Ligand Model (BLM), in which it is assumed that free metal causes toxicity when the metal binds and accumulates to a certain concentration at the site of action or a surrogate for that site on or in an organism, has gained popularity in North America as a tool to predict metal toxicity in the aquatic environment (Paquin et al., 2002). The BLM takes into consideration how different factors in water, such as complexation of free-metal ions with abiotic ligands and competition with other cations, can influence the extent to which a free-metal ion binds to a site of toxic action on an organism (Niyogi and Wood, 2004).

One way to account for the effects of different confounding factors (e.g., pH and hardness) on the speciation, bioavailability and toxicity of metals in water and sediments is to measure metal bioaccumulation by an organism. Metal body concentrations in *H. azteca,* have been shown to be a reliable indicator of metal toxicity for non-regulated and non-sequestered metals (Borgmann and Norwood, 1997a, 1999a; Norwood et al., 2006).

The purpose of this study was to investigate and compare the bioaccumulation, bioavailability, and toxicity of U to *H. azteca* exposed to U-spiked sediments with different overlying waters for 28 days. The objectives were to determine how overlying water chemistry affects the desorption of U from the sediment, if a saturation model like the BLM can be used to describe U bioaccumulation and toxicity to *H. azteca*, if pH and/or hardness are the main factor(s) influencing U speciation and toxicity, if the toxicity of U to *H. azteca* is via the solid phase (sediment) or dissolved phase (water) and if U bioaccumulation in *H. azteca* is a better indicator of U bioavailability and toxicity compared to U concentrations in water and sediment.

2.1 Theory

2.1.0 Metal Bioaccumulation

A mechanistic saturation model (Borgmann et al., 2004; Norwood et al., 2006) has been shown to be as good as, or better than, allometric equations (McGeer et al., 2003) at describing bioaccumulation of Cd, Cu, Hg, Ni, Pb, Ti, and Zn by *H. azteca*. This model is described by:

$$C_{TB} = \frac{\max C_w}{K_{0.5} + C_w} + C_{BKG} \tag{2.1}$$

where C_{TB} is the total body concentration of a metal, max is the maximum (above background concentration) accumulation of the metal by the organism, C_w is the concentration of the metal in water, $K_{0.5}$ is the half saturation constant (concentration at which the C_{TB} is halfway between the maximum accumulation and background concentrations), and C_{BKG} is the background C_{TB} in animals in the absence of any metal added to the water.

In the BLM (Di Toro et al., 2001, Paquin et al., 2002) metal binding to the site of toxic action can be described by:

$$[ML] = \frac{[L_T][M^{z+}]}{(1 + K_H[H^+] + K_{Ca}[Ca^{2+}] + \ldots)\,K_m^{-1} + [M^{Z+}]} \tag{2.2}$$

where L_T is the total (bound plus free) ligand ($[L_T]=[L] + [ML] + [HL] + [CaL] + \ldots$), K_m is the binding strength of the metal to L, K_H and K_{Ca} are the binding strengths of competing ions for the same site, and H^+ and Ca^{2+} are the concentration of hydrogen and free calcium ion. Other cations, such as potassium (K^+) or magnesium (Mg^+), could also appear in the model, depending on the metal and organism being tested, but are omitted here for simplicity. Eq. (2.2) is analogous to Eq. (2.1) (Borgmann et al., 2004). For instance, max may be equivalent to the number of binding sites on the biotic ligand, $[L_T]$. Alternatively C_{TB} might be a function of the ratio of metal uptake to metal excretion (Borgmann et al., 2004; Norwood et al., 2006). In this study, the max term is a function of ML, the rate of transport of metal into the organism by ML, and the inverse of the metal excretion rate.

The $K_{0.5}$ value can be expressed as:

$$K_{0.5} = a + b(H^+)^n + c(Ca) + \ldots \tag{2.3}$$

where $a = 1/K_m$, and b, and c are the products of a and the corresponding binding constants of the different cations. In Eq. (2.2) $n = 1$, however n can also be equal to other integer values if more than one hydrogen ion binds to the ligand. The value of n is the slope of the plot of log ($K_{0.5}$) versus log (H^+) if the other ions are held constant. Overall, Eq (2.3) results in a linear relationship between the $K_{0.5}$ value and all the major cations in the water, except possibly for H^+, on a free ion concentration basis.

2.1.1 Sediment

The binding of metal in water-sediment can also be explained by Eq. (2.1), but may, as in this study, require modification to include additional binding sites:

$$C_{sed} = \frac{max'C_w}{K'_{0.5} + C_w} + \frac{max''C_w}{K''_{0.5} + C_w} + C_{BKGS} \qquad (2.4)$$

where C_{sed} is the total metal concentration in the sediment, C_{BKGS} is the background concentration of C_{sed} in the absence of spiked metal, max' and max'' are the maximum (above background concentration) in the sediment, C_w is the concentration of the metal in water, $K_{0.5}$' and $K_{0.5}$'' are half saturation constant. The difference between Eqs. (2.1) and Eq. (2.4) is that in Eq. (2.4), C_{sed} is assumed to have two binding sites.

If the first binding site of the sediment is extremely weak, $K'_{0.5} >> C_w$, then Eq. (2.4) can be simplified to:

$$C_{sed} = \alpha C_w + \frac{max''C_w}{K''_{0.5} + C_w} + C_{BKGS} \qquad (2.5)$$

where "α" represents $max'/K_{0.5}'$; the initial slope of the saturation curve (Borgmann et al., 2004).

The inverse of α in Eq. (2.5) can also be related to major ion concentrations in a manner similar to $K_{0.5}$ in Eq. (2.3). In this case:

$$\frac{1}{\alpha} = a' + b'(H^+)^n + c'(Ca) + ... \qquad (2.6)$$

where *a*' = 1/K_m' and *b*', and *c*' are the products of *a*' and the corresponding binding constants of the different cations**.**

For the second binding site, the sediment $K_{0.5}$'' is related to the major ion concentration as described in Eq. (2.3):

$$K_{0.5} = a'' + b''(H^+)^n + c''(Ca) + ... \qquad (2.7)$$

2.1.2 Metal Toxicity and Bioaccumulation Relationships

Mortality in *H. azteca* is described using the mortality rate model (Borgmann et al., 2004):

$$m = \frac{-\ln(N'/No)}{t} - \frac{\ln(N/N')}{t} = m' + m'' \qquad (2.8)$$

where *m* is the mortality rate if mortality is constant and continuous, *N* is the number of surviving animals, N_0 is the initial number of animals, *N'* is the number of surviving control animals, *t* is time, *m'* is control mortality and *m''* is the mortality caused by the metal.

Mortality can also be described using the saturation models (Borgmann et al., 2004):

$$m = m' + \left[\frac{\max_{\mathrm{w}} \mathrm{C_w}}{\mathrm{K_w} + \mathrm{C_w}}\right]^{nw} \qquad (2.9a)$$

$$m = m' + \left[\frac{\max_{\mathrm{TBX}} \mathrm{C_{TB}}}{\mathrm{K_{TB}} + \mathrm{C_{TB}}}\right]^{nb} \qquad (2.9b)$$

where the $\max_{\mathrm{w}}$, $\max_{\mathrm{TBX}}$, $\mathrm{K_w}$, $\mathrm{K_{TB}}$, and $\mathrm{C_{TB}}$ (background-corrected) are analogous to max, $\mathrm{K_{0.5}}$, $\mathrm{C_W}$, and $\mathrm{C_{TB}}$ in Eq. (2.1).

At the LC50 (lethal concentration causing 50% survival) or LBC50 (lethal body concentration causing 50% survival), the $\max_{\mathrm{TBX}}$ constant in Eqs. (2.9a) and (2.9b) can be replaced with the LC50 or LBC50 giving (Borgmann et al., 2004):

$$m = m' + \left(\frac{\ln(2)}{t}\right)\left[\frac{\mathrm{C_w}((1/\mathrm{LC50}) + (1/\mathrm{K}))}{(1 + \mathrm{C_w}/\mathrm{K_w})}\right]^{nw} \qquad (2.10a)$$

$$m = m' + \left(\frac{\ln(2)}{t}\right)\left[\frac{\mathrm{C_{TB}}((1/\mathrm{LBC50}) + (1/\mathrm{K}))}{(1 + \mathrm{C_{TB}}/\mathrm{K_{TB}})}\right]^{nb} \qquad (2.10b)$$

If mortality reaches a maximum, then the K value represents the concentration at which mortality reaches 0.5^{nw} or 0.5^{nb} times max.

The effect of water chemistry on acute metal toxicity to invertebrates LC50 and LCB50 estimates can be predicted using the BLM model approach (De Schamphelaere and Janssen, 2002; Borgmann et al., 2004) by re-arranging Eq. (2.1):

$$C_w = \frac{K_{0.5}(C_{TB} - C_{BKG})}{\max - (C_{TB} - C_{BKG})} \tag{2.11}$$

and replacing C_{TB} with LBC50 and C_w with LC50 to give:

$$LC50 = \frac{K_{0.5}(LBC50 - C_{BKG})}{\max - (LBC50 - C_{BKG})} \tag{2.12}$$

If the LBC50, C_{BKG} and max are constant, then the LC50 is proportional to $K_{0.5}$ and can be described using an equation analogous to Eq. (2.3).

2.2 Methods

2.2.0 Culturing

H. azteca were cultured as described by Borgmann et al. (1989) in dechlorinated Burlington tap water originating from Lake Ontario. Each culture container was supplied with 1 L of dechlorinated water (in mg/L: dissolved organic carbon = 0.6 ± 0.1; dissolved inorganic carbon = 18.7 ± 0.7; Cl = 28.2 ± 1.8; SO_4 = 42.0 ± 3.0; Ca = 36.5 ± 1.1; Mg = 9.0 ± 0.3; Na = 17.4 ±1.7; K= 1.7 ± 0.1, alkalinity (as $CaCO_3$) = 78.0 ± 2.8; hardness (as $CaCO_3$) = 127.9 ± 3.9, pH 7.9 to 8.2). Culture water was renewed weekly, with the young being separated from the adults in order to maintain a continuous supply of 0-1- week-old amphipods for the experiments. The culture and experimental animals were held in a walk-in incubator at 25 ± 1°C with a 16h light: 8h dark photoperiod. The animals that were used in the experiments were acclimated in their respective experimental water media (see below) for 3-7 days prior to the start of the experiments. Therefore, the initial age of the *H. azteca* at the start of the experiment was 3-14 days for the young and 14-15 weeks for the adults.

2.2.1 Sediment

Reference sediments from two Canadian Shield Lakes, Tomiko (TOM10, 46.06º N 79.80º W) and Restoule (RES10, 46.53º N 79.83º W), at a depth of 10 m, were used for U spiking. Sediments were collected in May of 1996 as described by Borgmann et al. (2001a). Equal volumes of TOM10 and RES10 were mixed together to produce a sufficient volume of sediment. Each sediment was composed of 56-59% clay, 41-44% silt, 6.7-8.8% organic carbon, 0% sand, and moisture content 83-87%. The sediments were not sieved or treated prior to spiking with U.

Sediments were spiked as described by Borgmann and Norwood, (1997b, 1999a). In short, a stock solution containing U (uranyl nitrate trihydrate, International Bio-Analytical Industries, Inc.) was diluted with Milli-Q® water to different nominal concentrations of U. For each nominal concentration, an equal amount of the diluted stock solution (250 mL) and reference sediment (250 mL) were mixed together in 500 mL bottles to achieve the nominal concentrations of 0.079-79 μmol U/g dry weight (dw). This procedure was repeated twice for each concentration.

The 500 mL bottles were attached to a rolling rotor and gently rolled at 4 rpm for a week. The bottles were removed from the rotor and stored at 4°C for 1 month to allow the U to bind and partition to the sediment. The two bottles with the same nominal concentrations were combined by first centrifuging the bottles for 20 min at 3540 rpm and then aspirating off ~250 mL of the overlying water. The two bottles of sediment were combined (500 mL of spiked sediment), and placed in a 4°C cold room until further use.

The concentrations of U in the sediment (Table 2.1) were determined by first drying each sediment sample for 1 week in a 60^{o}C oven and digesting the dried sediment with 70% nitric acid (high purity acid, omni*Trace*$^{®}$, E.M. Science) for 1 week at room temperature followed by 30% hydrogen peroxide (A.C.S. reagent, J.T. Baker) for 24 h. Each sample was then made up to a final volume with Milli-Q$^{®}$ water and analyzed by the National Laboratory for Environmental Testing (NLET), Burlington, Ontario using inductively coupled plasma mass spectrometry (ICP-MS; NLET, 2007).

2.2.2 Water Chemistry

Chronic 4-week (28 day) static, non-renewal experiments were conducted with five different overlying water treatments: SAM, moderately hard water with high alkalinity (5-salt Standard Artificial Medium: 1 mM $CaCl_2$, 1mM $NaHCO_3$, 0.25 mM $MgSO_4$, 50 μM KCl, 10 μM NaBr); MHSAM, hard water with low alkalinity (Modified Hard SAM: SAM with 90% of the $NaHCO_3$ replaced with NaCl); 50SAM, intermediate hard water with intermediate alkalinity (50% SAM: SAM diluted to 50% with Nanopure$^{®}$ water; Barnstead International, Iowa, USA); MSSAM, soft water with high alkalinity (Modified Soft SAM: same as SAM but with only 10% as much $CaCl_2$ and $MgSO_4$); and 10SAM, soft water with low alkalinity (10% SAM: SAM diluted to 10% with Nanopure$^{®}$ water (10SAM(1)) or 10SAM(2) with 0.1 mM of $NaHCO_3$ added to the water). SAM is similar to Lake Ontario water and 10SAM is similar to waters commonly found in Canadian Shield Lakes. The MHSAM has the same hardness and cation content as hard water (SAM) but the same pH and alkalinity as that of soft water (10SAM). The MHSAM water is similar to effluent that may flow from tailings that have been limed.

In initial experiments with 10SAM (1) there was a substantial decline in pH (to pH 5-6) in the overlying water after a 2-week equilibrium period. The overlying water in the 10SAM (1) treatment was replaced and allowed once again to equilibrate for two weeks. Since water renewal has the potential to flush bioavailable metals out of the test chamber (Ankley et al., 1993), a second experiment was completed with 10SAM (2). The addition of 0.1 mM $NaHCO_3$ to the original 10SAM (1) maintained a more constant pH, eliminating the need for water renewal. This allowed this treatment to be better compared with the other treatments where water renewal did not take place.

2.2.3 Experimental Set-up

Approximately 5 mL of sediment with a depth of 1 cm and a surface diameter of 3 cm was added to a polycarbonate Imhoff settling cone (Borgmann and Norwood, 1999b). One litre of overlying water was added to achieve a water-to-sediment ratio of 200:1 (Fig. 2.1). The use of settling cones with a high water to sediment ratio ensures a better quality of the overlying water (e.g. reduction in pH shifts) compared to standard static toxicity tests using beakers (Borgmann and Norwood, 1999b). The test chambers were covered with a plastic translucent lid and placed in a cone rack in a walk-in incubator at 25 ^{o}C. Each chamber was allowed to equilibrate for 2 weeks prior to the addition of the animals. The surface of the sediment was oxygenated by gentle aeration through a glass tube capped with a 200 µL polypropylene pipette tip. During the 2-week equilibrium period, the chambers were kept in the dark to prevent algal growth. Control treatments were done in triplicate, while the U-spiked treatments were done in duplicate.

After the initial 2-week equilibrium period in the dark, 15 *H. azteca* (3-14-day-old) were added to each chamber and exposed to the U-spiked sediment for 28 days under fluorescent lighting with a 16h light: 8h dark period. Ground TetraMin® fish flakes (Tetra Holding Inc., USA) were added to each test chamber three times per week at the following rates: week 1 and 2, 2 mg; week 3, 2.5 mg; and week 4, 5 mg.

Studies have shown that equilibration between metals in *H. azteca* and water is rapid (Borgmann and Norwood, 1999a, 1999b), allowing reasonable estimates of bioaccumulation to be obtained with 1-week exposures. Thus, for SAM (2) and 10SAM (2) treatments, 15 caged adult (14-15-week-old) *H. azteca* were suspended above the sediment mid-way through the 4-week tests (day 14) for 1-week (Fig. 2.1). Cages were made of 120 mL polypropylene specimen containers with the bottoms cut out and replaced with a 200 µm mesh. Each cage also contained a 2.5 x 2.5 cm of cotton gauze as a substrate for the animals. The purpose of the caged animals was to determine if U accumulation was from the dissolved (water) phase or the solid (sediment) phase, since non-caged animals were in contact with the sediment-water interface. These caged animals were fed 5.0 mg of ground TetraMin® fish flakes three times per week.

Two 10 mL overlying water samples were taken on days 0 and 28 from each chamber to determine total and dissolved U. Water samples were acidified to 1% with HNO_3 (high purity acid, omni*Trace*®, E.M. Science) and placed in a 4 ^{o}C cold-room pending analysis. For the dissolved U water samples, water was first passed through a 0.4 µm polycarbonate filter (Nuclepore® (PC) Polycarbonate, Whatman) prior to acidification. Ten milliliters of water samples were collected and used to measure pH (ATI ORION, Model 420A), ammonia (Aquarium Test for freshwater, NUTRAFIN®), conductivity (VWR Scientific, Model 1054) and dissolved oxygen (Thermo Orion, Model 805APlus) at the beginning and end of the experiments.

On day 28, 125 mL of overlying water were collected and measured for dissolved organic and inorganic carbon using a UV-persulfate TOC analyzer (Pheniox 8000™); 500 mL of water were collected and measured for the major ions Ca, Mg, Na, and K using an atom absorption spectrophotometer and Cl and SO_4 using ion chromatography. These water samples were all analyzed by the National Laboratory for Environmental Testing (NLET), Burlington, Ontario, Canada (NLET, 2007)

For the treatments involving exposure to caged animals, the cages were removed from the incubator on day 21. For all experiments the cones were removed on day 28. In both cases surviving amphipods were isolated by sieving and rinsing in clean water. The amphipods were counted and placed in a 120 mL plastic specimen container with 50 µM EDTA (Ethylenediaminetetra-acetic acid, BDH Chemicals, LTD.) and 5.0 mg TetraMin® food for 24 h to clear their guts. The gut-cleared amphipods were weighed as a group to provide a mean mass per container and dried for 48 h at 60°C. Groups of six dried amphipods (about 1-3 mg total dry mass) were digested as described in Borgmann and Norwood (1997b) and then analyzed for U concentrations. All U chemical analyses were done by the National Laboratory for Environmental Testing (NLET) using inductively coupled plasma-mass spectrometry (ICP-MS, NLET, 2007).

2.2.4 Statistical and Data Analysis

Statistical and model analyse were conducted using SYSTAT version 10.0 (Chicago, Illinois, USA). The survival data were converted to a mortality rate using Eq. (2.8) above. The mortality rates were fourth-root transformed, growth data were square-root transformed and all metal concentration data were logarithmically transformed to normalize the data and equalize variances before statistical analysis. Comparisons between treatments and groups were made by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test for differences among treatments and groups ($P<0.05$). The log-log relationships between U in the water, sediment and *H. azteca,* were obtained using the NONLIN (non-linear regression) module. The same module was used for calculating the 95% Wald confidence intervals, growth parameters and mortality rates. Curve fitting was performed using the combined 28-day data from all seven experiments to provide the best fit estimate of the overall relationship.

The LC50s in the water/sediment and the LCB50 in *H. azteca* were determined using the trimmed Spearman-Karber method (Hamilton et al., 1977). The measured U concentrations in each experiment were then divided by the respective LC50s to convert all water concentrations to toxic units (TU). The data for all experiments were then pooled and Eq. (2.10a) above was used to compute the LC50, LC25 and LC10 for the pooled data on a toxic unit basis by replacing the C_w term in Eq. (2.10a) with the TU data. Converting C_w into TU allowed pooling of the data from all experiments, thereby increasing the number of partial-effect points and providing a better estimate for the slope of the toxicity curve. The 1/K term was also replaced with KI, so that the inverse of K was estimated rather than K. This allows the 1/K estimate to be equal to 0 if K approaches infinity (Borgmann et al., 2004).

Growth after 4 weeks exposure was determined on a wet weight basis using the model described by Borgmann et al. (1998):

$$W = \frac{W'}{1 + a(\mathrm{C_w})^n} \tag{2.13}$$

where *W'* is the control or maximum wet weight when $C_w=0$, and *a* and *n* are constants. The FUNPAR module in SYSTAT was then used to compute the 10% (EC10), 25% (EC25) and 50% (EC50) growth effect from the estimated *a* and *n* values from Eq (2.13) as:

$$\mathrm{EC10} = \left(\frac{1/9}{a}\right)^{1/n}$$

$$\mathrm{EC25} = \left(\frac{1/3}{a}\right)^{1/n}$$

$$\mathrm{EC50} = \left(\frac{1}{a}\right)^{1/n}$$

where EC10, EC25 and EC50 = C_w in Eq. (2.13) with *W/W'* = 0.90, 0.75 and 0.5 respectively (Borgmann et al., 1998).

The EBC10, EBC25 and EBC50 were calculated using Eq. (2.1) with C_w replaced with EC10, EC25, and EC50, respectively, i.e.

$$\mathrm{EBC10} = \frac{\max \mathrm{EC10}}{\mathrm{K_{0.5}} + \mathrm{EC10}}$$

$$\mathrm{EBC25} = \frac{\max \mathrm{EC25}}{\mathrm{K_{0.5}} + \mathrm{EC25}}$$

$$\mathrm{EBC50} = \frac{\max \mathrm{EC50}}{\mathrm{K}_{0.5} + \mathrm{EC50}}$$

Uranium speciation was determined using stability constants from NIST Standard Reference Database 46, the OECD Nuclear Energy Agency (Guillaumont et al. 2003), and Dong and Brooks (2006) and compared to MINTEQA2 version 4.03 (U.S. EPA, Athens, Georgia, USA) for verification.

2.3 Results

2.3.0 Overlying Water Chemistry

Dissolved oxygen, ammonia and pH levels were consistent among the various experiments. The mean (95% CI) dissolved oxygen was 8.64 (0.58) mg/L for all experiments. Mean ammonia readings for all treatments were <0.1 mM, except for SAM (0.10 mM) and MSSAM (0.13 mM). Survival in the SAM and MSSAM treatments was, however, between 81 and 88%, suggesting that the increased ammonia levels did not contribute to toxicity. The LC50 of ammonia in tap water (similar to SAM) is 0.95 mM (Borgmann, 1994). Conductivity was consistent within overlying water treatments, but ranged from 482 (SAM) to 62 µS/cm (10SAM) among treatments.

Dissolved (0.4 µm filtered) U in the overlying water increased significantly ($p<0.05$) with increasing U concentrations in the sediment (Table 2.1). The release of U from the sediment into the overlying water increased with increasing pH. At pH around 8, there was a higher total dissolved U concentration in the overlying water compared to pH around 7 at the same sediment concentrations (Table 2.1).

2.3.1 Speciation Model

Uranium speciation estimates for the various treatments (Table 2.2), based on the model reported here, showed that at low pH 0.1-0.4% of U was in the free form, 53-61% was in the neutral carbonate complex, a lesser amount was present as dicarbonate, and 7-15% was in the form of one of two hydroxides. In addition, when there was a higher concentration of Ca (40 mg/L) in the water at a higher pH (7.97-8.00), approximately 62% was predicted in the $Ca_2UO_2CO_3$ form, while 68% was predicted as $CaUO_2(CO_3)_3$ at pH 7.23 and low Ca concentrations.

2.3.2 Bioaccumulation and Sediment Saturation Models

2.3.2.0 Bioaccumulation

A background term (C_{BKG}) was used in all saturation models (Table 2.3; Fig(s). 2.2 and 2.3) in order to fit the bioaccumulation data. These background terms were calculated from the geometric mean of the control samples in the absence of U exposure.

The bioaccumulation of U from water followed a dose-response relationship described by Eq. (2.1). The max and $K_{0.5}$ estimates and corresponding confidence limits (Table 2.3) for the different treatments were obtained using the NONLIN regression module in SYSTAT. The R^2 for all the treatments ranged from 0.86 to 0.98 (Table 2.3; Fig. 2.2). The max values among the different overlying water treatments were similar (Table 2.3). The half saturation ($K_{0.5}$) values, on the other hand, varied between the treatments, with the $K_{0.5}$ values being lower for treatments with a low pH and higher for treatments with a high pH. The same was true for the max/$K_{0.5}$ values, which represent the initial slope of the bioaccumulation curve. This agrees with the BLM where the $K_{0.5}$ value is dependent on the water chemistry, while the max term is independent of water chemistry.

These findings suggest that a U BLM model for *H. azteca* is feasible. Given that the max values among the different treatments were similar (Table 2.3), the geometric mean for max was calculated (73 nmol/g dw) and fixed in Eq. (2.1). The model was then re-run to estimate a second set of $K_{0.5}$ values (Table 2.3). The second set of estimated $K_{0.5}$ was used to calculate *a*, *b*, and *c* constants and *n* in Eq. (2.3).

The best fit in Eq. (2.3) was obtained when *n* was set equal to 4(Fig. 2.4). The *a*, *b* and the corresponding 95% confidence intervals were 4.2E-2 (1.6E-3-1.1) pmol/L and 2.17E+6 (4.5E+5-1.1E+7), respectively. The R^2 was equal to 0.91 (Fig. 2.4). For the $K_{0.5}$-free ion based on measured dissolved U and modelled percent free uranyl ion, a positive value for *c* could not be obtained. This suggests that there was no Ca effect on U uptake, other than its influence on U speciation.

2.3.2.1 Caged H. azteca

When U body concentrations were regressed against U water concentration for animals exposed to the water-only phase compared to animals exposed to both the dissolved and solid phase, the points overlapped (Fig. 2.3) in both the SAM (2) and 10SAM (2) treatments. This suggests that U is mainly accumulated via the water rather than the solid-phase sediment. When estimating the max and $K_{0.5}$ values using Eq. (2.1) for the caged adult animals (Table 2.3), the max and $K_{0.5}$ trends were similar to those animals exposed to the solid phase.

2.3.2.2 Sediment

The binding of metal to sediment can also be explained by a saturation model, but with inclusion of a second weak-binding site, Eq. (2.5). The max", $K_{0.5}''$ and max"/ $K_{0.5}''$ estimates are given in Table 2.4. The R^2 for the individual treatments ranged from 0.93 to 0.99. The $K_{0.}''$ values for the sediment to water interaction were lower for treatments of low pH relative to those with a high pH. The max" was also similar among the treatments, with a geometric mean of 7500 nmol/g dw. The α value, which represents the weak binding site on the sediment, was higher for the treatments with the lower pH than those with a higher pH. This matches the trend for max'/$K_{0.5}'$.

As was similarly done for the *H. azteca* bioaccumulation model, Eq. (2.1), the geometric mean of max for sediment (7500 nmol/g dw) was fixed in Eq. (2.5) and the model was re-run to estimate a second set of α and $K_{0.5}''$ values (Table 2.4). The second set of estimated α and $K_{0.5}''$ values were used to calculate the a' and b' constants in Eq. (2.6) for α, as well as the a'' and b'' constants in Eq. (2.7) for $K_{0.5}''$. The best fit for α in Eq. (2.6) was achieved when $n = 4$ and a' and b'[1] were equal to 0 and 170 (55-530), respectively. The R^2 was equal to 0.95.

The best fit in Eq. (2.7) for the sediment $K_{0.5}''$ was obtained when n was also equal to 4. The a'', b'' and the corresponding 95% confidence intervals for Eq. (2.7) were estimated to be 0 and 2.02E+5 (6.71E+4-6.08E+5), respectively. The R^2 was 0.96.

The ratio max"/$K_{0.5}''$ divided by α was also computed (Table 2.4). This ratio was similar among the treatments, suggesting that both the first weak binding site and the second binding site on the sediment are affected similarly by water chemistry (Table 2.4). This is not surprising since no value for a', a'', c' or c'' greater than 0 could be estimated, and since the best fit for n was equal to 4 in both Eqs. (2.6) and (2.7). Both $K_{0.5}''$ and $1/\alpha$ are, therefore, proportional to hydrogen ion to the fourth power.

2.3.2.3 Uranium Toxicity

The LC50 based on measured U in water, and corresponding confidence limits, were strongly dependent on overlying water chemistry (Table 2.5). The order of U toxicity was soft water, low alkalinity (10SAM)> hard water, low alkalinity (MHSAM)> soft water, high alkalinity (MSSAM)> intermediate hard water, intermediate alkalinity (50SAM)> hard water, high alkalinity (SAM). On average, U toxicity was higher when the overlying water pH was low. A higher concentration of Ca in the water did not have a major effect on U toxicity at circumneutral pH (Table 2.5).

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[1] In the published article the b' estimate was reported incorrectly. It should have been 170 and not 2.17E+6. All the appropriate changes were made throughout.

The LBC50 vaules for *H. azteca* were more consistent than LC50s between the different overlying waters and sediments (Table 2.5). This suggests that U bioaccumulation may be a better indicator of U bioavailability and toxicity than total U concentrations in the overlying water or the sediment.

When the dissolved U LC50s were compared on a free uranyl ion basis, there were more free ions in overlying waters of low pH compared to those of high pH (Table 2.5). In addition, a higher concentration of Ca in the overlying water (MHSAM) appeared to have only a minor effect in reducing the free uranyl ion concentration in the water. It appears that speciation of U in the water is affected primarily by pH, which in turns modulates U toxicity to *H. azteca.*

The LC50, LC25 and LC10 values obtained by fitting Eq. (2.10a) to the pooled data for U in water, respectively, on a toxic unit basis were 1.02, 0.74 and 0.56. This gave an LC25/LC50 and LC10/LC50 ratio of 0.73 and 0.55 for U in water. Applying Eqs. (2.1) and (2.5) gave an average L(B)C25/L(B)C50 and L(B)C10/L(B)C50 ratio of 0.87 and 0.76 for U in *H. azteca* and 0.79 and 0.65 for U in sediment, respectively.

Since U toxicity to *H. azteca* is relatively constant on a body concentration basis, and since bioaccumulation follows a saturation model, this saturation model can be applied to estimate the effect of the hydrogen ion (water chemistry) on U toxicity, i.e. the free ion LC50. Combining Eqs. (2.3) and (2.12) results in the relationship:

$$\mathrm{LC50_{Free}} = \frac{(a + b[\mathrm{H}]^4)(\mathrm{LBC50} - \mathrm{C_{BKG}})}{\max - (\mathrm{LBC50} - \mathrm{C_{BKG}})} \tag{2.14}$$

The exponent is expected to equal 4 because toxicity is a function of bioaccumulation and because bioaccumulation is best modelled assuming that four protons bind to each binding site. The observed free ion LC50s matched the predicted free ion LC50s, calculated using Eq. (2.14) with the *a* and *b* estimates from the bioaccumulation models and mean LBC50, reasonably well (Fig. 2.5a). The data for Fig. 2.5a were, however, too variable to clearly demonstrate a curvature at low hydrogen ion concentrations analogous to that seen in Fig. 2.4.

When the dissolved LC50 (nmol/L) is plotted against the H^+ (μmol H/L), it decreases with increasing H^+ (Fig. 2.5b). A single line cannot be drawn through these data because the speciation of U is dependent on Ca as well as pH. Nevertheless, the observed LC50s did match the predicted LC50s reasonably well (Fig. 2.5b).

Growth on a wet weight basis was modelled using Eq. (2.13). The estimated values for the *a* and *n* constants were, however, extremely variable. The exponent *n* ranged from 0.56 to 6.6 between experiments, with wide confidence limits. Both the median and geometric mean for *n* was 1.4. A second set of values for each experiment was, therefore, determined by fixing *n* to 1.4. The n value of 1.4 and the second set of *a* estimates were used to compute the EC10, EC25 (Table 2.5) and EC50 for growth. An EC25/EC50 and EC10/EC50 ratio of 0.46 and 0.21, respectively for U in the water was determined.

There was a growth effect of U on *H. azteca*. The EC25 followed a similar trend to the LC50 in that the EC25 was lower in overlying water of low pH than at high pH (Table 2.5). Although growth was a slightly more sensitive endpoint than survival, it was more variable. The confidence limits for the EC25 were considerably wider than for the LC50 (Table 2.5).

The EBC10, EBC25 and EBC50 could not be determined directly from bioaccumulated U using Eq. (2.13), because there were no surviving animals in which to measure U body concentrations at those concentrations causing mortality. Given that bioaccumulation followed a saturation model, Eq. (2.1) was applied to estimate the EBC10, EBC25 and EBC50. To do this C_w in Eq. (2.1) was replaced with the appropriate EC10, EC25 or EC50 value, the max was set to the geometric mean of 73 nmol U/g (Table 2.3) and the appropriate $K_{0.5}$ values (Table 2.3) were used.

EBC25/EBC50 and EBC10/EBC50 ratios of 0.68 and 0.41 for U in *H. azteca* were determined. Table 2.5 shows the EBC25 for *H. azteca* growth. The EBC25 shows a trend similar to that of the EC25, with EBC25 being more sensitive to overlying water chemistries of low pH than high pH. The EBC25 estimates were similar to those of the LBC50, suggesting that growth may be as sensitive as survival in predicting U toxicity in *H. azteca* exposed to spiked-U sediments for 28 days.

2.4 Discussion

Benthic organisms often accumulate most of their metal burdens from the overlying and/or pore water (Borgmann, 2000). That is the case in this study: *H. azteca* suspended in cages in overlying water (Fig. 2.3) showed the same bioaccumulation and toxicity of U as those exposed directly to U-spiked sediment. This suggests that the bioaccumulation of U by *H. azteca* is primarily through the dissolved phase rather than the sediment. This was also the case for studies with Cd, Ni and Pb (Borgmann et al., 1991; Borgmann and Norwood, 1999a; Borgmann et al., 2001b).

Since bioaccumulation is primarily via dissolved metal, the impact of metal-contaminated sediments on aquatic organisms involves two phases: desorption of metals from the sediment into the water, and uptake of the metals from the water into the animal. Many times the partitioning of metals from the sediment into the overlying water is influenced by the overlying water chemistry. That was the situation in this study, where the desorption of U from the sediment to the overlying water increased with increasing pH (Table 2.1). The higher concentration of U is probably due to a higher association of the uranyl ion with Ca-CO_3 complexes in water at increasing pH (Table 2.2). Speciation calculations, using recently published U stability constants (Dong and Brooks, 2006) suggest that the $Ca_2UO_2(CO_3)_3$ (~ 68% bound to uranyl ion) is more important at high Ca concentrations than the $CaUO_2(CO_3)_3^{2-}$ (~62% bound to uranyl ion), which appears to be more important at lower Ca concentrations with high pH (Table 2.2).

The saturation model, Eq. (2.1), which is analogous to the BLM, Eq.(2.2), was used to explain the dose-response relationship of U bioaccumulation in *H. azteca* via water (Fig. 2.2). The value of max for U for *H. azteca* was low when compared to other metals studies with *H. azteca* as the model organism. For instance, the geometric mean max of 73 nmol/g for this study was lower than those for As, Pb, Cd, Co, Cr and Hg (range 219-1760 nmol/g; Borgmann et al., 2004; Norwood et al., 2006). This suggests that a small amount of U bioaccumulation is enough to saturate the binding sites on *H. azteca* and therefore, contribute to toxicity.

Eq. (2.3) was useful for differentiating the effect of the hydrogen ion from that of hardness. The $K_{0.5}$ value, which is dependent on water chemistry, was determined to be a function of the hydrogen ion to the power of 4. The values of *a* (4.2E-14 mol/L) and *b* (2.17E+18) could be calculated (Fig. 2.4), but a positive value for *c* could not be obtained, suggesting that Ca does not play a major factor in reducing U uptake by *H. azteca.*

Since *a*=1/ K_m, the estimated log K for U was calculated to be 13.38. The log K for U is higher in comparison to chronic BLM studies on *Daphina magna* and rainbow trout for Cu (8.02) and Zn (5.50, De Schamphelaere and Janssen, 2004a; De Schamphelaere and Janssen, 2004b). However, the log K values for Cu and Zn cannot be realistically comparable because the log K values for Cu and Zn in the above chronic studies were by default set equal to the log K values estimated for acute studies. Log K values reported for Ag (7.3-10), Cu (7.4-8.0), Cd (8.6), Co (5.1), Pb (6.0) and Zn (5.3-5.5) for mostly acute studies (Niyogi and Wood, 2004) are all lower than our estimate of log K for U.

Since *b* (Eq. 2.3) = K_H/K_m, the log K value for H^+ calculated in this study is 31.7. However, this K value is for the binding of four, rather than just one, hydrogen ions to the ligand. It is not, therefore, comparable to the log K values for H^+ in the chronic Cu (6.7) and Zn (6.3) BLM models for *Daphina magna* and rainbow trout, respectively (De Schamphelaere and Janssen, 2004a; De Schamphelaere and Janssen, 2004b).

Fortin et al. (2007) found that the uptake of U by the green alga, *Chlamydomonas reinhardtii*, is influenced by pH: U uptake increased with increasing pH (5-7) despite a decrease (65-0.1%) in the proportion of calculated free uranyl ion concentration in solution. The authors suggested that the influence of pH is two-fold in that increasing pH reduces the proton concentration, thus decreasing competition for the physiologically active sites on the algal cells, while the complexation by carbonates and hydroxides reduces the free uranyl activity, thereby reducing U bioavailability. By comparison, for *H. azteca* the influence of pH on U uptake appears to be more dependent on U speciation than H^+ competition at ligand binding sites, given that there was a decrease in U uptake by *H. azteca* with increasing pH and decreasing free uranyl ions (Table 2.2).

The sediment-to-water saturation model, Eq. (2.5) was successful in explaining the interactions of U in the water with the sediment (Table 2.4). Both α and $K_{0.5}$'' were related to the effect of the hydrogen ion in the water-sediment saturation model as described by Eqs. (2.6) and (2.7), respectively. However, a value for a' and a'' could not be calculated. This is probably because the ligands in the sediment are likely almost all protonated, leaving limited amount of available free ligands. An experiment with overlying pH levels above 8, with fewer protons in solution and a higher proportion of free ligand, may be sufficient to determine these *a'* and *a''* values.

The ratio max"/$K_{0.5}$" divided by α was computed (Table 2.4) with similar values amongst treatments. This ratio indicates that both binding sites on the sediment are affected similarly by water chemistry (Table 2.4). This would be expected if a' and a'' are both negligible and if *n* in Eq. (2.6) equals *n* in Eq. (2.7).

Published studies on the toxicity of U to aquatic organisms are limited. The few studies that have looked at U toxicity to organisms such as fathead minnows (*P. promelas*), green hydra, *Chlorella* sp. and *H. azteca* (Tarzwell and Henderson, 1960; Riethmuller et al., 2001; Charles 2002) have all shown that increasing water hardness, decreases U toxicity. Borgmann et al. (2005) found that when *H. azteca* were exposed for 1-week to waterborne U concentrations, U toxicity to *H. azteca* was greater in soft water (hardness 18 mg $CaCO_3$/L; alkalinity 14 mg $CaCO_3$/L; pH 7.8) than in hard water (water hardness 124 mg $CaCO_3$/L, alkalinity 84 mg $CaCO_3$/L, pH 8.3). There was a 30-fold difference between the 7 day-LC50 for U in soft water (0.02 mg U/L measured) versus hard water (1.65 mg U/L; nominal; Borgmann et al., 2005). The same was true in this study where U was more toxic to *H. azteca* in soft than hard water (Table 2.5). There was an approximate 46-fold difference between hard water (SAM(1)) and soft water (10SAM(1)). These differences are largely driven by the presence of more free uranyl ion (less complexation) at low pH as stated above; inhibition of U uptake by hydrogen ions was not sufficient to fully overcome the effect of pH on U speciation (Table 2.5) and no inhibition of uptake by Ca ions could be detected after accounting for the effects of Ca on U speciation.

In this study, U toxicity expressed on a water basis was more variable than U toxicity expressed on either a sediment or body burden basis (Table 2.5). For instance, the chronic toxicity of U varied over 46-fold when expressed on a water basis versus 2.1 fold when expressed as a body burden. The same conclusion was reached in studies with sediments spiked with Cd, Ni, Pb, and Tl (Borgmann et al., 1991, Borgmann et al., 1998; Borgmann and Norwood, 1999a; Borgmann et al., 2001b), in which chronic toxicity of these metals was more constant when toxicity was expressed as a function of the metal bioaccumulated rather than water or sediment metal concentrations. For example, the chronic toxicity of Cd to *H. azteca* in dechlorinated tap water originating from Lake Ontario with and without the addition of complexing agents (humic acid and EDTA) or sediments varied by 36-fold when expressed as Cd measured in the overlying water, but only 2.6 fold when expressed as Cd measured in the body (Borgmann et al., 1991).

The importance of pH-dependent uranyl ion complexation in mediating U toxicity is reinforced when U toxicity is expressed on a free uranyl ion water basis at constant pH (Table 2.5). There was over a 3000-fold difference in U toxicity between overlying waters with low pH (10SAM (1)) compared to high pH (SAM(1)). However, the protective effect of Ca on the toxicity of total dissolved U at nearly constant high pH (MSSAM vs SAM) was not reflected in the toxicity on both a free uranyl ion and dissolved ion basis (Fig. 2.5a and 2.5b). Thus, the modest ameliorating effect of Ca on U toxicity appears to be due primarily to its effect on aqueous speciation, rather than the direct inhibition of U toxicity.

Unlike studies with Zn (Borgmann and Norwood, 1997b) and Pb (Borgmann and Norwood, 1999a) spiked sediments, U caused a reduction in growth (Table 2.5). This finding is similar to a previous study on *H. azteca* exposed to Cu-spiked sediment where growth was a sensitive indicator of metal toxicity (Borgmann and Norwood, 1997b).

Given that body concentrations are a good indicator of U toxicity to *H. azteca,* Eq. (2.12) can be used to predict the LC50 in water. When the predicted water LC50s estimates were compared to the observed LC50s using the trimmed Spearman-Kaber method, the predicted values were within a factor of 1.6 from the observed values (Table 2.5, Fig. 2.5a). This suggests that a saturation model can be used successfully to predict water LC50s, both in the lab (as done here) and in the field (if the effect of dissolved organic matter, if elevated, on speciation can be estimated).

Currently, sediment quality guidelines do not take overlaying water chemistry into account. This can be a problem for some metals, such as Cd (Nowierski et al. 2005) and, as shown in this study, for U. The current Canadian interim sediment quality guidelines (ISQGs) are not based on cause-and-effect relationships, but are derived from correlations between observed effects (i.e. decrease in benthic community) and sediment concentrations (CCME, 1999). An ISQG exceedance indicates that there is an increased likelihood that sediment toxicity is occurring, although this toxicity might not be caused by the metal for which the guideline is exceeded. Sediments are often contaminated by more than one metal and metal concentrations often correlate with one another (Borgmann, 2003). In addition, if the sediment has a strong binding affinity towards a metal, then a high total metal concentration in the sediment may be nontoxic to aquatic biota. On the other hand, sediments with a low binding capacity for metals may contain more bioavailable metal that may be toxic (Borgmann and Norwood, 1999a). This makes the prediction of effects difficult.

In this study we attempted to develop a true-cause effect SQG relationship given that (a) U concentration in *H. azteca* is a reliable indicator of U toxicity and bioavailability and that (b) Eqs. (2.1) and Eq. (2.5) for this study are both a function of the same U water concentration, C_w. In order to develop this model the predicted $LC50_w$ was first calculated using Eq. (2.12). Given that the geometric mean of max = 73 nmol/g (Table 2.2) and $LBC50\text{-}C_{BKG}$ = 43 nmol/g (Table 2.5), Eq. (2.12) was simplified to

$$LC50_w = K_{0.5w}\, 1.28 \tag{2.15}$$

Secondly, since, Eqs. (2.1) and (2.5) are both a function of the same C_w, the predicted $LC50_{sed}$ was determined by substituting C_w in Eq.(2.5) with $LC50_w$ in Eq. (2.15) to give:

$$LC50_{sed} = \alpha(K_{0.5w}1.28) + \frac{max''(K_{0.5w}1.28)}{K''_{0.5} + (K_{0.5w}1.28)} + C_{BKGS} \tag{2.16}$$

When Eqs. (2.15) and (2.16) were tested, both the predicted $LC50_w$ and the predicted $LC50_{sed}$ had similar values to those estimated using the trimmed Spearman-Karber method. The predicted values were within a factor of 2.1 of the observed values (Table 2.5, Fig. 2.5b). Thus a successful LC50 sediment model was generated in this study by comparing bioaccumulation of the metal in the tissue and in the overlying water with total metal concentration in the sediment. However, the ability of the current model to predict the bioavailability of U in field-collected sediments still needs to be determined, since there may be different pH/Ca relationships between U in overlying water and U in sediment in the field if the U is in a different form.

It appears from this study that saturation models can be successfully used to explain U desorption from the sediment to the water and U uptake by *H. azteca* and the resultant toxicity.

2.5 Conclusion

Overlying water chemistry (especially pH) affects U speciation in water, desorption of U from sediment, and U bioaccumulation by *H. azteca*. The influence of Ca on U uptake is negligible, except for its effect on U speciation in the overlying water. Uranium bioaccumulation was due primarily to the dissolved phase rather than the sediment solid phase. Uranium bioaccumulation in *H. azteca*, as well as water-sediment partitioning, can be satisfactorily explained using BLM type saturation models.

Uranium bioaccumulation in *H. azteca* was a better indicator of U toxicity than U concentrations in water and sediment. Growth and survival were both sensitive indicators of U toxicity for *H. azteca* exposed to U-spiked sediments.

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Table 2.1 Measured concentration of U in sediment and water

Nominal sediment (nmol U/g dw)	Measured sediment (nmol U/g dw)	Water concentration (nmol U/L)						
		10SAM (1)	10SAM (2)	MHSAM	50SAM	MSSAM	SAM (1)	SAM (2)
0	7.9	BDL	BDL	BDL	BDL	0.47	0.08	BDL
79	93	0.080		BDL			0.77	
790	630	0.89	1.5	0.80	2.0	7.7	14	6.1
1,400			2.8		3.6	14		23
2,500	3,300	3.7	6.1	3.3	8.7	23	40	50
4,400	4,500	12	10	6.0	15	79	100	93
7,900	8,600	16	34	15	43	66	160	210
14,000	12,000	26	54	26	62	190	470	890
25,000	19,000	61	150	79	310	400	660	940
44,000	34,000	130	220	160	480	920	1600	1000
79,000	59,000	280	780	310	1400	1600	3200	3200
	pH	6.9	7.2	7.2	7.6	8.0	7.9	8.0

BDL indicates below detection limit (0.08 nmol U/L). dw indicates dry weight. Acronyms for treatment differences in the composition of overlying water are defined in the text.

No value in the space indicates that this concentration was not analysed

Table 2.2 Uranium speciation estimates (%) for overlying water treatments based on model using stability constants were from NIST Standard Reference Database 46, the OECD Nuclear Energy Agency (Guillaumont et al. 2003), and Dong and Brooks (2006)

Complex	10SAM (1)	10SAM (2)	MHSAM	50SAM	MSSAM	SAM (1)	SAM (2)
UO_2^{++}	0.36	0.099	0.027	0.00068	0.000023	0.0000024	0.00000094
UO_2OH^+	15	7.4	2.0	0.12	0.011	0.00094	0.00042
$UO_2(OH)_2$	15	13	4.0	0.56	0.12	0.0093	0.0050
UO_2CO_3	61	53	16	2.3	0.49	0.038	0.020
$UO_2(CO_3)_2^{-2}$	6.8	19	9.7	6.2	7.7	0.56	0.41
$Ca_2UO_2(CO_3)_3$	0.13	1.1	42	45	16	62	62
$CaUO_2(CO_3)_3$	0.49	4.3	24	43	68	36	36
$MgUO_2(CO_3)_3$	0.010	0.091	0.52	0.93	1.5	0.77	0.77
Total	99	98	99	99	94	99	99
pH	6.9	7.2	7.2	7.6	8.0	7.9	8.0
Ca (mM)	0.10	0.10	1.0	0.50	0.10	1.0	1.0

Total concentration of UO_2^{++} used for the U speciation calculations was 1μM

Table 2.3 Maximum metal accumulation (max) and half saturation constant ($K_{0.5}$) estimates (95% confidence limits) and predicted $K_{0.5}$ for *Hyalella azteca* exposed to uranium spiked sediments for 28 days

Experiment	Estimates for juveniles					Prediction of *K*			Ions	
	max[a] (nmol/g)	$K_{0.5}$[a] (nmol/L)	BKG (nmol/g)	max/*K*	R^2	Dissolved $K_{0.5}$[b] (nmol/L)	$K_{0.5}$ free ion (pmol/L)	Pred $K_{0.5}$ ion[c] (pmol/L)	H^+ (µmol/L)	Ca (mol/L)
10SAM(1)	75 (42-140)	52 (24-110)	0.15	1.4 (1.1-1.9)	0.98	50	180	500	12E-02	1.0E-04
10SAM(2)	96 (15-610)	220 (26-1900)	0.090	0.44 (0.27-0.72)	0.88	160	160	50	6.9E-02	1.8E-04
MHSAM	69 (39-120)	51 (23-110)	0.14	1.4 (0.98-1.9)	0.97	55	15	26	5.9E-02	10E-04
50SAM	66 (34-130)	270 (120-630)	0.090	0.24 (0.18-0.34)	0.95	310	2.1	1.1	2.6E-02	4.9E-04
MSSAM	110 (14-820)	2100 (230-19 000)	0.050	0.050 (0.040-0.070)	0.94	1400	0.32	0.071	1.1E-02	1.2E-04
SAM (1)	54 (38-78)	610 (360-1000)	0.16	0.090 (0.070-0.12)	0.97	900	0.022	0.083	1.2E-02	9.9E-04
SAM (2)	58 (9.0-360)	3500 (410-31 000)	0.16	0.020 (0.010-0.030)	0.86	4700	0.044	0.064	1.0E-02	9.4E-04

Experiment	Estimates for caged adults				
	Max (nmol/g)	$K_{0.5}$ (nmol/L)	BKG (nmol/g)	Max/*K*	R^2
10SAM (2)	160 (20-1300)	740 (73-7500)	0.080	0.21 (0.14-0.33)	0.94
SAM (2)	78 (7-860)	2500 (140-42000)	0.10	0.030 (0.020-0.060)	0.87

[a] Estimated using $C_{TB} = \max C_w/(K_{0.5} + C_w)$.

[b] Estimated using max set to 73 (the geometric mean of the max estimates for the treatments).

[c] Estimated using predicted (pred) K (free ion) = $a + b(H^+)^n$; a=0.042 pmol/L; b=2.2E+06 pmol/L; n=4, H^+ units in µmol/L.

Table 2.4 Estimates for α, max and $K_{0.5}$ values for the model $C_{sed} = \alpha Cw + \text{max}''Cw/(K_{0.5}''+Cw) + C_{BKGs\ for\ the\ sediment}$

Experiment	α (nmol/L)	Max" (nmol/g)	$K_{0.5}$" (nmol/L)	C_{BKGS} (nmol/g)	Max"/$K_{0.5}$"	R^2	$K_{0.5}$"[a] (nmol/L)	Free $K_{0.5}$" (pmol/L)	Pred. $K_{0.5}$"[b] (pmol/L)	Dissolved α (nmol/L)	Free α (pmol/L)	Pred. α[c] (pmol/L)	Max"/$K_{0.5}$"[a]	Dissolved (Max"/$K_{0.5}$")/α
10SAM (1)	210(160-280)	6,000(2,800-13,000)	7.0 (2.0-28)	7.9	860 (430-2000)	0.98	9.0	34	46	200	56	26	830	4.2
10SAM (2)	84(61-120)	9, 400(5,500-16,000)	16 (7.0-36)	7.9	580 (410-870)	0.98	11	11	4.6	93	94	260	680	7.3
MHSAM	160(140-180)	9,700(7,700-12,000)	10 (6.0-13)	7.9	970 (920-1300)	0.99	6.0	1.6	2.4	180	660	490	1200	6.7
50SAM	34(26-44)	13,000(9,300-19,000)	31 (18-55)	7.9	430 (320-550)	0.99	12	0.083	0.097	45	6,600	12,000	620	14
MSSAM	33(19-57)	5,600(1,600-19,000)	28 (2.0-360)	7.9	200 (46-860)	0.93	47	0.011	0.0027	29	1,30,000	4,50,000	160	5.5
SAM (1)	18(14-22)	5,800(3,200-11,000)	57 (17-190)	7.9	100 (52-200)	0.98	89	0.0022	0.0039	17	6,80,000	3,10,000	84	4.9
SAM (2)	16(10-28)	5,500(1,000-30,000)	65 (2.0-1800)	7.9	85 (14-500)	0.93	110	0.0010	0.0020	15	16,00,000	5,90,000	68	4.5

[a] Using max = 7500, the geometric mean of the max estimates for the first set values.

[b] Predicted K (free ion) = $a'' + b''(H^+)^n$; $a''=0$, $b''=2.0E+05$ pmol, $n=4$.

[c] Predicted α (free ion) $=1/(a' + b'(H^+)^n$; $a'=0$, $b'=170$, $n=4$ H^+ units in μmol/L; in the published manuscript the formula was reported incorrectly (i.e. max'/a' + b'$(H^+)^n$), it is correct here.

Table 2.5 Trimmed Spearman-Karber LC50 estimates (95% confidence limits) for juvenile and adult *Hyalella* and EC50 for juvenile Hyalella exposed to spiked U-sediment for 28 days

Experiment	LC50 (juveniles)		Sediment (nmol U/g dw)	Free U ions [UO_2++]	LBC50 (adults)	LC50s model predicted		EC25 (juveniles)	EBC25 (juveniles)		
	Total Body (nmol U/g dw)	Water (nmol U/L)		(nmol U/L)	Total Body (nmol U/g dw)	Water[a] (nmol U/L)	Sediment[b] (nmol U/g)	Growth (nmol/L)	Growth[c] (nmol/g)	pH	Ca (mol/L)
10SAM (1)	32 (30-35)	61 (50-76)	19,000 (17,000-23,000)	22000 E-05		180	23,000	48 (26-87)	36	6.9	1.0E-04
10SAM (2)	43 (39-47)	200 (170-230)	27,000 (23,000-32,000)	20000 E-05	>120	64	23,000	87 (40-190)	26	7.2	1.8E-04
MHSAM	51 (43-61)	190 (130-270)	39,000 (29,000-54,000)	5000 E-05		130	23,000	120 (60-230)	50	7.2	10 E-04
50SAM	56 (52-61)	830 (720-950)	45,000 (42,000-48,000)	570 E-05		200	24,000	250 (150-400)	33	7.6	4.9E-04
MSSAM	27 (19-37)	560 (420-730)	22,000 (19,000-26,000)	13 E-05		400	48,000	190 (110-310)	9	8.0	1.2E-04
SAM (1)	45 (43-46)	2800 (2400-3300)	54,000 (47,000-62,000)	7.0E-05		4400	40,000	2500 (1200-5600)	54	7.9	9.9E-04
SAM (2)	>39	>3000	>59,000	2.8E-05	>110	8700	55,000	1100 (620-2000)	14	8.0	9.4E-04

[a] $LC50_{water}=K_{0.5water}*1.28$

[b] $LC50_{sediment}=\alpha*(K_{0.5water}*1.28) + ((max*K_{0.5water}*1.28)/(K_{0.5sediment} + K_{0.5water}*1.28)) + C_{BKGs}$

[c] Computed using EBC25=maxEC25/($K_{0.5}$ + EC25), where max is the geometric mean of 73 and $K_{0.5}$ is the estimated $K_{0.5}$ when max is equal to 73 (Table 2.3)

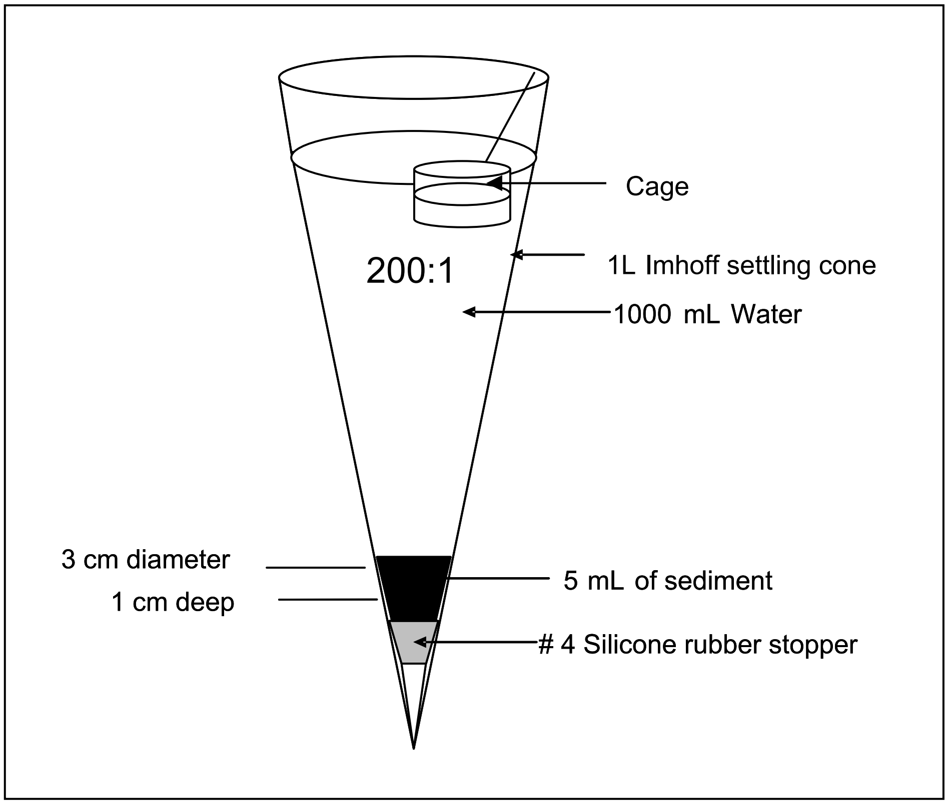

Fig. 2.1. Imhoff cone and cage experiment set-up**.**

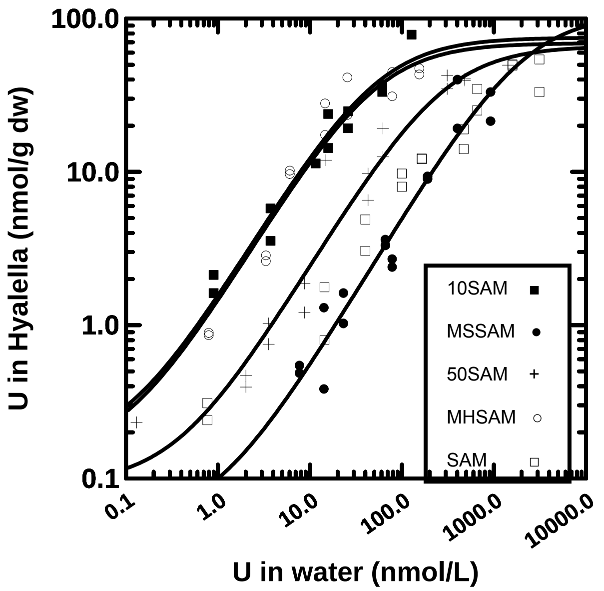

Fig. 2.2. U body concentration in *H. azteca* versus overlying water concentration. Lines represent the fitted saturation model C_{TB}= maxC_w/($K_{0.5}$ + C_w) + C_{BKG}. Since the line for SAM overlapped MSSAM, it was omitted for clarity.

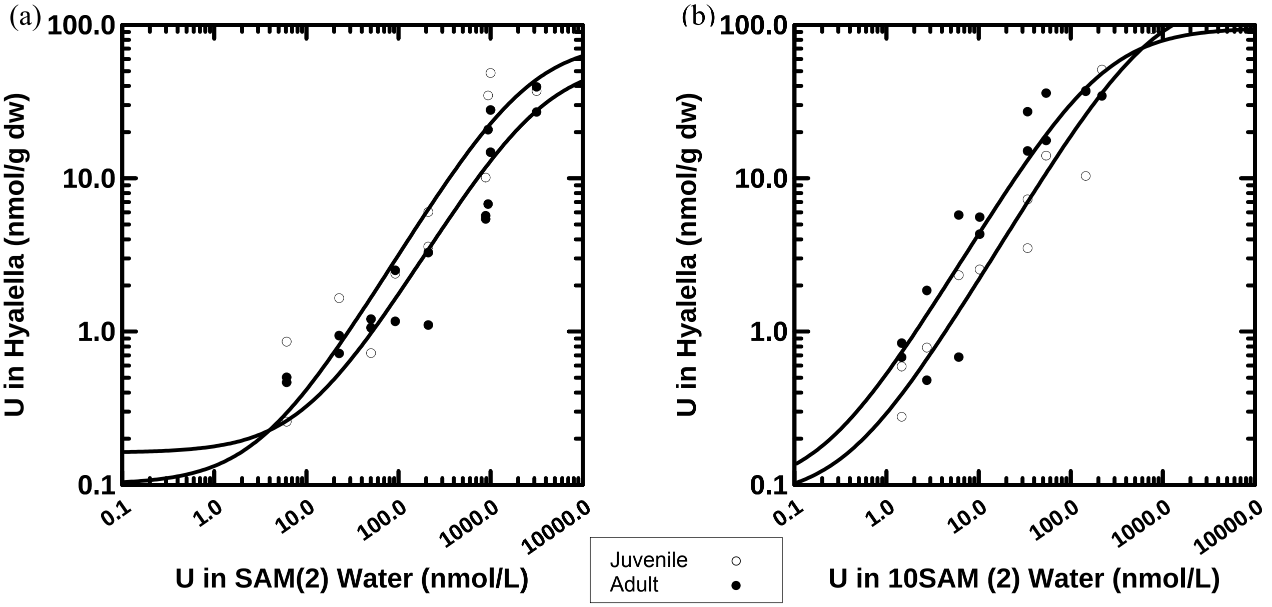

Fig. 2.3. U body concentration in juvenile (4 weeks exposure) versus caged adult *H. azteca* (1 week exposure) in the overlying water (a) SAM (2) and (b)10SAM(2).

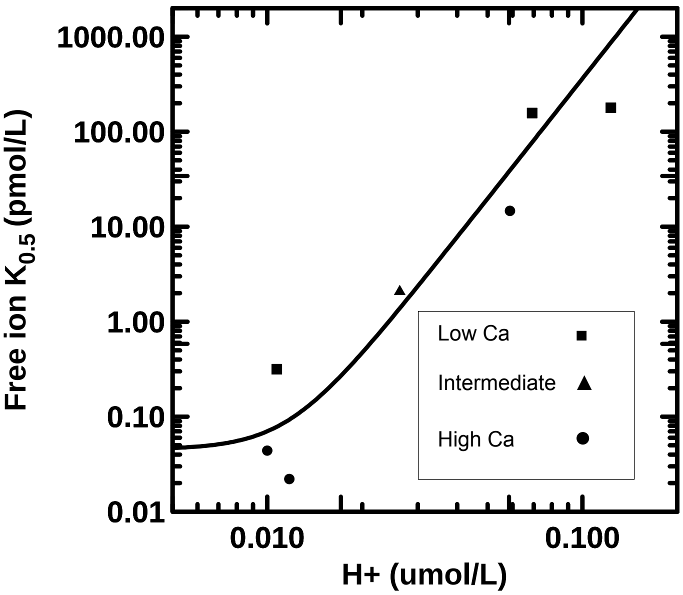

Fig. 2.4. Free ion $K_{0.5}$ versus hydrogen ion. Symbols represent overlying waters that had low Ca (10SAM and MSSAM) to intermediate Ca (50SAM) to high Ca (SAM and MHSAM). Fitted line represents the predicted $K_{0.5} = a + b(H^{+})^{4}$.

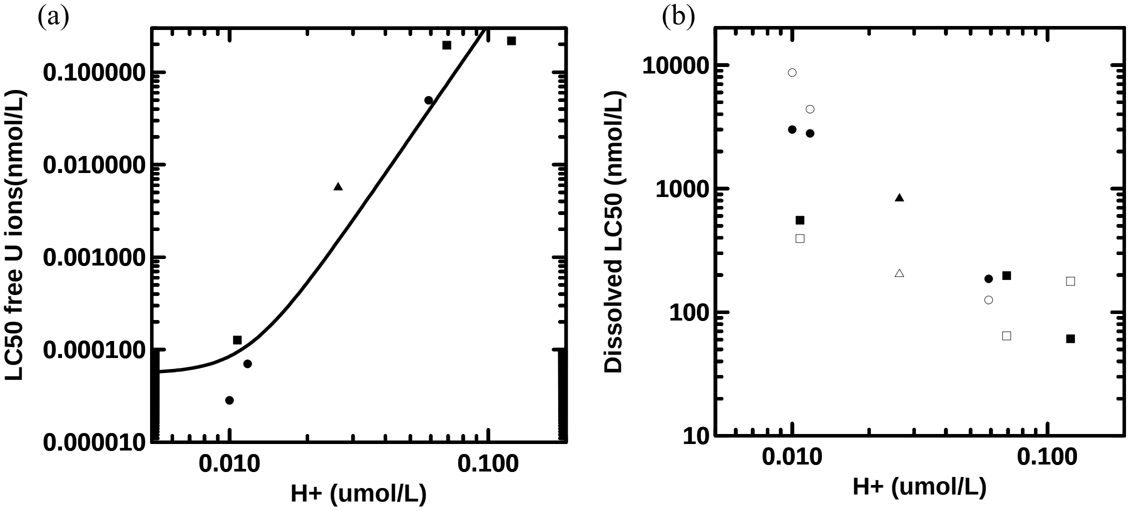

Fig. 2.5. LC50 free U ions versus H^+ (a), and LC50 dissolved U in water versus H^+ (b). Same legend as Fig. 2.4, with open symbols (b) representing predicted LC50 values using Eq. (2.14) and the fitted *a* and *b* estimates from the bioaccumulation model.

APPENDIX

Quality Assurance and Quality Control (QA/QC)

All U chemical analyses in terms of U concentrations in sediment, water and *H. azteca* were done by the National Laboratory for Environmental Testing (NLET) using inductively coupled plasma-mass spectrometry (ICP-MS, NLET, 2007). Quality assurance and quality control procedures were carried out by NLET, such as standard verifications to monitor sensitivity drift of the machine, control standards to confirm accuracy of the calibration curve, and reference materials to monitor accuracy and precision. A high-purity standard for U (High-Purity Standards, Charleston, South Carolina, USA) and an Environment Canada (National Laboratory for Environmental Testing at the National Water Research Institute, Environment Canada, Burlington, ON, Canada) certified trace elements drinking water standard (TM-DWS) were included with the sediment, water and *H. azteca* samples in order validate the ICP-MS calibration curve for U. The average percent recovery for the high-purity standard (n=3) and Environment Canada standard (n=6) from the true value was 97.4 and 99.8%, respectively. Method blanks were collected and processed at the same time as water and sediment and tissue digests were collected and processed, using the same containers, digestion or preservation techniques. Method blanks were measured, with U concentrations measured in the sediment, water and *H. azteca* being background corrected by subtracting by the blank measured value. This blank correction accounted for any potential U contamination that may have been present during the collection and processing of the samples. For the majority of the blank samples, U concentrations were below the ICP-MS detection level of 0.0005 μg/L.

Blanks and appropriate standard reference materials for the major ions (Ca, Mg, Na and K), DIC/DOC, and the anions, SO_4 and Cl measurements were analysed by NLET in accordance with their quality assurance and quality control procedures. Dissolved organic and inorganic carbon samples were measured on a UV-persulfate TOC analyzer (Pheniox 8000^{TM}). The major ions Ca, Mg, Na, and K were measured on an atom absorption spectrophotometer, while the anions, Cl and SO_4 were measured using ion chromatography.

pH measurements were measured using the Ross® Sure-Flow electrode (Thermo Scientific Inc., USA) with a filling solution of 3M KCl. A three buffer calibration of pH 4, pH, 7 and pH 10 (Canadawide Scientific LTD., Ottawa, Ontario, Canada) were used to calibrate the electrode. The buffers were at the same temperature as the samples when the electrode was calibrated.

Trimmed Spearman-Karber Method

The trimmed Spearman-Karber method (Hamilton et al., 1977) is able to estimate the LC50 and LBC50 from weighted averages of midpoints between concentrations on a logarithmic scale. The applied weight to each midpoint is the change in proportion of effect (i.e. mortality) between two concentrations. This method requires that the data be symmetrical, monotonic and includes both 0 and 100% effects (i.e. mortality). Trimming is used to try and correct for non-symmetry in the distribution curve (EC, 2005)..

EC (Environment Canada)., 2005. Guidance document on statistical methods for environmental toxicity tests. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario, pp. 241.

Table 2.A1 Relative ion concentrations of a 5-salt Standard Artificial Medium used to make up the different overlying water chemistries in chapter two

Experiment	**Ca & Mg**	**Na & K**	**HCO_3**
SAM[a]	1x	1x	1x
MHSAM[b]	01x	1x	0.1x
50SAM	0.5x	0.5x	0.5x
MSSAM	0.1x	1x	1x
10SAM	0.1x	0.1x	0.1x

[a] 1x= 1 mM $CaCl_2$; 1mM $NaHCO_3$; 0.25 mM $MgSO_4$; 50 μM KCl; 10 μM NaBr

[b] Contains 0.9 mM of NaCl

Table 2.A2 Overlying water chemistry measurements for the major ions, Cl, SO_4, DOC, DIC and alkalinity

			Major ions						
	Ca	Mg	Na	K	Cl	SO_4	DOC	DIC	Alkalinity
	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mg/L)	(mg/L)	mM
10SAM (2)	0.12(0.016)	0.031 (0.0030)	0.24 (0.022)	0.016 (0.010)	0.23 (0.023)	0.11(0.032)	2.3 (1.8)	2.7(1.3)	0.23 (0.11)
MSSAM	0.12 (0.013)	0.031 (0.0025)	1.3 (0.18)	0.073 (0.015)	0.33 (0.051)	0.12 (0.02)	2.9 (2.1)	13 (1.9)	1.1 (0.16)
SAM (2)	1.0 (0.052)	0.27 (0.011)	1.1 (0.057)	0.063 (0.010)	2.1 (0.09)	0.36 (0.02)	2.3 (1.4)	10 (1.1)	0.84 (0.10)

The overlying waters for the SAM (1), MHSAM, 50SAM and 10SAM(1) treatments were collected, but not measured for the major ions, Cl, SO_4, DOC, DIC and alkalinity.

For the 10SAM(2), MSSAM and SAM(2) treatments water samples were only submitted for samples that approximated the trimmed Spearman-Karber 4-week LC25 and LC50s estimates for the water. In other words, the sediments with the nominal concentrations of 2530 and 4430 nmol/g dw for both the 10 SAM and MSSAM treatments, and 7910 and 14 200 nmol/g dw for the SAM treatment.
n=4 with the 95% confidence interval in parentheses

Table 2.A3 The model input concentrations used to determine U speciation in Table 2.2

	10SAM (1)	10SAM(2)	MHSAM	50SAM	MSSAM	SAM (1)	SAM (2)
pH	6.9	7.2	7.2	7.6	8.0	7.9	8.0
Alkalinity (mM)	0.10	0.2	0.1	0.5	1.0	1.0	1.0
Ca (mM)	0.10	0.1	1.0	0.5	0.10	1.0	1.0
Mg (mM)	0.025	0.025	0.25	0.13	0.025	0.25	0.25
Na (mM)	0.10	0.20	1.0	0.51	1.0	1.0	1.0
K (mM)	0.0050	0.0050	0.050	0.025	0.050	0.050	0.050
Cl (mM)	0.21	0.21	3.0	1.0	0.25	2.1	2.1
SO_4 (mM)	0.025	0.025	0.25	0.13	0.025	0.25	0.25
Br (mM)	0.0010	0.0010	0.0010	0.0050	0.0010	0.010	0.010
UO_2^{++} (μM)	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The input values were based on the nominal concentrations used to make-up the different artifical overlying waters in Table 2.A1, with the exception of the pH values in which the measured values were used.
These nominals concentrations were within range of the measured values (Table 2.A2).

Table 2.A4 The thermodynamic (Log K) constants used for the U speciation estimates in Table 2.2

Ligand	Complex	Log K	Source
OH	$CaOH^+$	1.30	NIST 46
OH	KOH	0.214	NIST 46
OH	$MgOH^+$	2.60	NIST 46
OH	NaOH	0.100	NIST 46
OH	UO_2OH^+	8.75	NIST 46
OH	$UO_2(OH)_2$	15.8	NIST 46
OH	$UO_2(OH)_3^-$	21.7	NIST 46
OH	$UO_2(OH)_4^{-2}$	23.6	NIST 46
CO_3	$CaCO_3$	3.20	NIST 46
CO_3	$MgCO_3$	2.92	NIST 46
CO_3	$NaCO_3^-$	1.27	NIST 46
CO_3	UO_2CO_3	9.94	Guillaumont et al. (2003)
CO_3	$UO_2(CO3)_2^{-2}$	16.6	Guillaumont et al. (2003)
CO_3	$UO_2(CO_3)_3^{-4}$	21.8	Guillaumont et al. (2003)
$CaCO_3$	$Ca_2UO_2(CO_3)_3$	30.7	Dong and Brooks (2006)
$CaCO_3$	$CaUO_2(CO_3)_3$	27.2	Dong and Brooks (2006)
HCO_3	$CaHCO_3^+$	1.27	NIST 46
HCO_3	$MgHCO_3^+$	1.01	NIST 46
HCO_3	$NaHCO_3$	-0.25	NIST 46
$MgCO_3$	$MgUO_2(CO_3)_3$	26.1	Dong and Brooks (2006)
SO_4	$CaSO_4$	2.36	NIST 46
SO_4	HSO_4^-	1.99	NIST 46
SO_4	KSO_4^-	0.85	NIST 46
SO_4	$MgSO_4$	2.26	NIST 46
SO_4	$NaSO_4^-$	0.73	NIST 46
SO_4	UO_2SO_4	3.15	Guillaumont et al. (2003)
SO_4	$UO_2(SO_4)_2^{-2}$	4.14	Guillaumont et al. (2003)
SO_4	$UO_2(SO_4)_3^{-4}$	3.02	Guillaumont et al. (2003)
Cl	$CaCl^+$	0.629	NIST 46
Cl	KCl	-0.500	NIST 46
Cl	$MgCl^+$	0.600	NIST 46
Cl	NaCl	-0.500	NIST 46
Cl	UO_2Cl^+	0.170	Guillaumont et al. (2003)
Cl	UO_2Cl_2	-1.10	Guillaumont et al. (2003)

Ionic strength corrections were performed using the Davies equation (Serkiz, S.M., Allison, J.D., Perdue, E.M., Allen, H.E., Brown, D.S., 1996. Correcting errors in the thermodynamic database for the equilibrium speciation model MINTEQA2. Water Research 30, 1930-1933.)

Total UO_2^{++} concentration was 1.0 μM

The difference between the model used to estimate U speciation in Table 2.2 and the MINTEQ A2 version 4.03 (U.S. EPA, Athens, Georgia, USA) is that MINTEQ A2 version 4.03 does not include the updated stability constants for:

UO_2CO_3
$UO_2(CO3)_2^{-2}$
$UO_2(CO_3)_3^{-4}$
$Ca_2UO_2(CO_3)_3$
$CaUO_2(CO_3)_3$
$MgUO_2(CO_3)_3$
UO_2SO_4
$UO_2(SO_4)_2^{-2}$
$UO_2(SO_4)_3^{-4}$
UO_2Cl^+
UO_2Cl_2

which have become important in the prediction of U speciation (Dong and Brooks, 2006; Guillaumont et al., 2003).

CHAPTER 3

KINETICS OF URANIUM UPTAKE IN SOFT WATER AND THE EFFECT OF BODY SIZE, BIOACCUMULATION AND TOXICITY TO *Hyalella azteca*

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Abstract

The kinetics of uptake and the effect of body size on uranium (U) bioaccumulation and toxicity to *Hyalella azteca* exposed to water-only U concentrations in soft water were evaluated. The effect of body size on U bioaccumulation was significant with a slope of –0.35 between log body concentration and log body mass. A saturation kinetic model was satisfactory to describe the uptake rate, elimination rate and the effect of gut-clearance on size-corrected U bioaccumulation in *H. azteca*. The one-week lethal water concentrations causing 50% mortality for juvenile and adult *H. azteca* were 1100 and 4000 nmol U/L, respectively. The one-week lethal body concentration causing 50% mortality was 140 nmol U/g for juvenile *H. azteca* and 220 nmol U/g for adult *H. azteca.* One-week bioaccumulation studies that properly account for body-size and gut-clearance times can provide valuable data on U bioavailability and toxicity in the environment.

Keywords: Kinetics; uranium; *Hyalella azteca*; body size; toxicity

Capsule: Uranium accumulation by *H. azteca* approaches steady-state after one week but is strongly dependent on body size.

3.0 Introduction

In Canada uranium (U) concentrations in waters surrounding lakes near active and decommissioned U mines have been reported to range from 4.2-4200 nmol U/L (EC/HC, 2004; Clulow et al., 1998; OMOE, 2003). Mean U concentrations of 105 nmol U/g have been reported in the tissues of *Hyalella azteca* exposed to waterborne U concentrations near U mining operations (Robertson and Liber, 2007). Measurable U concentrations of 8-34 nmol U/g were also detected in composite samples of aquatic macroinvetrebrates collected near a U tailings site in Utah (Peterson et al., 2002).

Currently there are no national environmental quality guidelines for U to aid managers in the protection of aquatic life in Canada. The drinking water quality guideline of 0.02 mg/L (84 nmol/L) is the only comparable national guideline with the purpose of protecting the health of Canadians (HC, 2008). Ontario is the only province in Canada with a provincial interim water quality objective for U (0.005 mg/L, or 21 nmol/L) with the sole intention of protecting aquatic life 100% of the time (MOEE, 1994). However, caution needs to be used when applying this objective because it was set as an emergency value derived using the best, but limited, U toxicity studies with aquatic organisms, and was not subjected to a peer review (MOEE, 1994). This is unfortunate given that Canada, especially the province of Saskatchewan, is home to some of the largest U operation facilities and deposits in the world.

The freshwater amphipod *H. azteca* is a sensitive species commonly used in laboratory and field studies to evaluate metal toxicity (EC, 1997). Several authors (e.g. Borgmann et al., 1991; Norwood et al., 2006; Alves et al., 2008) have shown that non-essential metal concentrations in *H. azteca* provide a better prediction of metal toxicity to this invertebrate than metal concentrations in water and sediment because the physicochemical factors affecting metal bioavailability from the environment are taken into account (Borgmann and Norwood, 1995a).

Alves et al. (2008) found that U concentrations in *H. azteca* were more reliable indicators of toxicity than waterborne and sediment concentrations. Nevertheless, the influences of biological factors such as body size, which can affect metal concentrations in an animal (Langston and Spence, 1995), were not considered in that study. Studies with other animals and metals (e.g., Boyden, 1974, 1977; Strong and Luoma, 1981; Mubiana et al., 2006) have all shown a correlation of body size with metal concentrations in animals. Thus, the relationship between body size and U body concentration needs to be established if *H. azteca* is to be considered as a potential indicator species for U contamination in the environment.

The relationship between chronic U accumulation and toxicity of *H. azteca* exposed to U-spiked sediments has been determined (Alves et al., 2008). However, the exposure period needed for whole body U concentrations in *H. azteca* to reach steady state with the exposure media is currently unknown. Uptake studies with Cu, Zn and Pb (Borgmann and Norwood, 1995b; MacLean et al., 1996) have all shown that the steady-state between water and the tissue is reached in less than one week, making one-week accumulation studies feasible to predict long-term toxicity (Borgmann and Norwood, 1995b). If the kinetics of U are similar to the above metals, then short-term studies may be employed to quantify, evaluate, interpret and manage the potential impacts of U facilities and tailings to aquatic life in Canadian freshwaters.

The objectives of this study were: (1) to investigate the effect of body size on U accumulation in *H. azteca* exposed to waterborne U concentrations; (2) to evaluate U toxicity and bioaccumulation of juvenile and adult *H. azteca* exposed to water-only U concentrations for one week; (3) to determine if the kinetics of U for *H. azteca* exposed to waterborne concentrations for seven days versus 28 days is similar and can be explained using a saturation kinetic model; (4), to consider the effect of gut-clearance on metal accumulation, given that failure to gut-clear animals may overestimate the lethal body concentration causing toxicity (Neumann et al., 1999; Bartlett et al., 2004). All of these experiments were conducted in an artificial soft water medium similar to waters common to the Canadian Shield where the majority of U facilities and tailings are located.

3.1 Theory

3.1.0 Metal Bioaccumulation Model

The kinetics of U accumulation were modelled using

$$C_{TB} = \frac{V_{umax}}{(k_e + g)} \times \frac{C_W}{(K_{0.5} + C_W)}(1 - e^{-(k_e+g)t})(1 - loss \times gutclear) + C_{bkg} \quad (3.1a)$$

where C_{TB} (nmol/g) is the concentration of U in *H. azteca* as a function of time (*t*), C_W (nmol/L) is U concentration in water, and C_{bkg} is the background U concentration in the amphipods. When $C_W << K_{0.5}$ this is a first order one-compartment kinetic model similar to the ones that have often been used to describe bioaccumulation and elimination of metals via the water by various organisms (e.g., Xu and Pascoe, 1993; Borgmann and Norwood, 1995b; Lim et al., 1998). The rate of metal uptake ($k_u \times C_W$) is the product of the uptake rate constant (k_u, L/g/day) and the concentration of U in water. In Eq. (3.1a) $V_{umax}/K_{0.5}$ is equal to k_u at low C_W, while k_u equals V_{umax}/C_W as C_W approaches infinity. $K_{0.5}$, the (time-independent) half saturation constant, is the concentration of C_W at which the metal uptake rate equals $V_{umax}/2$ (Norwood et al., 2006). The rate of loss of U from amphipod tissues is the sum of the U excretion rate (k_e, per day) and growth dilution, described by the growth rate (*g*, per day).

At any given time when *t* is constant, Eq. (3.1a) can be simplified to

$$\mathrm{C_{TB}} = \frac{\mathrm{max} \times \mathrm{C_w}}{(\mathrm{K_{0.5}} + \mathrm{C_w})}(1 - \mathrm{loss} \times \mathrm{gutclear}) + \mathrm{C_{bkg}} \quad (3.1b)$$

where max = $(V_{umax}/(k_e + g))(1 - \mathrm{e}^{-(\mathrm{ke}+\mathrm{g})\mathrm{t}})$. This is Eq. (1) of Norwood et al. (2006) and Alves et al. (2008) and Eq. (2) of the Borgmann et al. (2004) study. At steady state ($t=\infty$ or large t, $\mathrm{e}^{-(ke+g)t} \sim 0$), and max becomes $V_{umax}/(k_e + g)$. Such a mechanistically based saturation model has been shown to describe the uptake of many metals well at steady-state, including U (Borgmann et al., 2004; Norwood et al., 2006; Alves et al., 2008). In Eq. (3.1a) *gutclear* is a dummy variable set equal to either 1 or 0: 1 for animals that have undergone a 24 h gut-clearance before digestion and 0 for non-gut-cleared animals. *Loss* is the fraction of U lost in 24 h due to gut-clearance (Norwood et al., 2006). Therefore (1 - *loss*× *gutclear*) = 1 for non-gut-cleared animals and (1 – *loss*) for gut-cleared amphipods. Inclusion of the (1 - *loss*× *gutclear*) term allows for the pooling of data from gut-cleared and non-gut-cleared amphipods into a single regression.

3.1.1 Mortality model

Given that the relationship between water and body concentration in this study followed a saturation curve, mortality in *H. azteca* was determined using the saturation model, similar to Eq. (3.1b) at steady-state and described in Borgmann et al., (2004):

$$m = m' + [\frac{\max \times C}{(K + C)}]^n \qquad (3.2)$$

where *m* is the instantaneous mortality rate (per week) computed by regressing ln(number of surviving animals) against time in weeks (Borgmann et al., 1998; Borgmann et al., 2004); *m'* is the mortality rate (per week) in control animals; max is a maximum analogous to the maximum for accumulation of the metal from the water by the organism; *C* is U in water (C_W) or the background-corrected total body concentration of U in the animal (C_{TBX}); *K* is a half saturation constant analogous to $K_{0.5}$ in Eq. (3.1a), *n* is a constant based on water (n_w) or background-corrected total metal body concentration (n_b).

The max term in Eq. (3.2) can be replaced with the LC50 (lethal water concentration causing 50% mortality) or the LBC50 (lethal body concentration causing 50% mortality) to give (Borgmann et al., 2004):

$$m = m' + \frac{\ln(2)}{t}[\frac{C(\frac{1}{L(B)C50} + \frac{1}{K})}{1 + \frac{C}{K}}]^n \qquad (3.3)$$

where *t* is the exposure time (in weeks) for any given LC50 or LBC50. *K* is equal to K_W for mortality based on U in water (LC50) or K_{TBX} for mortality based on U in *H. azteca* (LBC50). The same model was used to calculate the LC25, LBC25, LC10 and LBC10, by adjusting survival to correspond with 25% and 10% mortality for the L(B)C25 or L(B)C10 as follows:

$$m = m' + \frac{\ln(4/3)}{t}[\frac{C(\frac{1}{L(B)C25} + \frac{1}{K})}{1 + \frac{C}{K}}]^n \qquad (3.4)$$

$$m = m' + \frac{\ln(10/9)}{t}[\frac{C(\frac{1}{L(B)C10} + \frac{1}{K})}{1 + \frac{C}{K}}]^n \qquad (3.5)$$

Eqs. (3.2)-(3.5) are mathematically equivalent expressions, but allow computation of different L(B)C values.

3.2 Materials and Methods

3.2.0 Culturing

Hyalella azteca were cultured in dechlorinated Burlington City tap water from Lake Ontario as described by Borgmann et al. (1989). Water quality analyses as measured by the National Laboratory for Environmental Testing (NLET, 2007), Environment Canada, from January 2006 to January 2008 were (*n*=36, mean ± 95% CI in mg/L): dissolved organic carbon = 0.8 ± 0.1; dissolved inorganic carbon = 18 ± 0.5; Cl = 29 ± 3.3; SO_4 = 37 ± 1.6; Ca =36 ± 0.5; Mg = 8.6 ± 0.2; Na = 15 ± 0.8; K= 2.9 ± 0.8, alkalinity (as $CaCO_3$) = 76 ± 2.1; hardness (as $CaCO_3$) = 120 ± 1.1), pH 7.9-8.2. Culture water was renewed weekly with the young being separated from the adults in order to maintain a continuous supply of amphipods of known age. Animals were held in a walk-in incubator at 25 ± 1°C with a 16 h light: 8 h dark photoperiod. *H. azteca* in all experiments were acclimated to their experimental water medium for one week (7 days) prior to their use in the experiments.

3.2.1 Water chemistry and U water concentrations

All experiments were conducted in a 5-salt standard artificial medium (SAM: 1 mM $CaCl_2$; 1 mM $NaHCO_3$; 0.25 mM $MgSO_4$; 50 µM KCl; 10 µM NaBr) diluted to 10% (10SAM) with Nanopure® water (Barnstead International, Iowa, USA). The 10SAM medium was then spiked with uranyl nitrate trihydrate ($UO_2(NO_3)_2 \bullet 3H_2O$, International Bio-Analytical Industries, Inc.) to achieve nominal U concentrations of 0, 10, 18, 32, 40, 56, 100, 180, 250, 320, 560, 1000, 1800 nmol U/L for selected experiments.

3.2.2 Body size experimental set-up

A one-week (7 day) static, replicated, water renewal U-body size experiment was conducted in 5L of 10SAM in 7.5 L polycarbonate aquaria with 0, 100, and 250 nmol U/L. Each replicated aquarium contained approximately 365 acclimated *H. azteca* of varying size (0.01-0.10 mg dry weight), with the initial age of 1-22 weeks at the start of the experiment. Two 5 x 5 cm pieces of cotton gauze presoaked in distilled water were added to each container. The cotton gauze was used as a substrate for the *H. azteca*. Amphipods were fed ground TetraMin® fish flakes (Tetra Holding Inc, VA, USA, but made in Germany) at a rate of 5 mg three times per week.

The water exposure media were changed every two days in order to maintain constant U concentrations in the water and decrease the potential of U loss due to adsorption onto the walls of the test chambers (e.g., Borgmann et al., 1991; MacLean et al., 1996). In this experiment and throughout, 10 mL water samples were collected before and after water renewal and filtered with 0.4 µm (Nucleopore® (PC) Polycarbonate, Whatman) filter attached to a syringe for subsequent analysis of dissolved U. These filtered water samples were preserved by acidifying to 1% HNO_3 (high purity acid, omni*Trace*®, E.M. Science). Five milliliters unfiltered water samples were also collected before and after the water renewal for ammonia, conductivity, dissolved oxygen, and pH measurements. During every water renewal period, and after the addition of U to the water, test chambers were allowed to equilibrate for 3 h prior to the addition of animals and removal of water samples.

At the end of the seven day exposure period animals were sorted with half the amphipods being placed in 120 mL plastic specimen containers with 50 µM EDTA and 5 mg TetraMin® food for 24 h to clear their guts and remove any loosely-bound U from the surface of the animals. EDTA has been shown to complex U (Bhat and Krishnamaurthy, 1964) and remove U bound to the outside of the freshwater algal *Chlorella* (Franklin et al., 2000). EDTA has also been used in other studies (e.g., Borgmann and Norwood, 1995a; Neumann et al., 1999; Norwood et al., 2006) with *H. azteca* to complex other loosely bound metals (e.g. Cd, Cr, Co, Cu, Mn, Pb, and Zn) during gut-clearance to prevent re-absorption. The 0 and 24 h gut-cleared amphipods were each wet weighted as a group and then dried for 48 h in a 60°C oven to a constant weight. Animals of similar dry weights were pooled together prior to digestion. In some cases as many as 24 animals were grouped together for amphipods ranging in size from 0.01 to 0.05 mg dry weight and groups of two animals for amphipods between the sizes of 0.8-1 mg dry weight. Animals were digested using a modified procedure based on Stephenson and Mackie (1988), whereby 120 µL of 70% HNO_3 was added to each container and allowed to sit at room temperature for one week. This was followed by the addition of 100 µL of 30% H_2O_2 for 24 h. Each sample was then made up to a final volume of 5 mL with Nanopure® water.

3.2.3 Acute toxicity and bioaccumulation experiment

A one-week (7 day) static, replicated, water renewal concentration series test was conducted in 400 mL of 10SAM spiked with the nominal U concentrations of 0, 56, 100, 180, 320, 560, 1000, 1800 nmol/L in 500 mL polyethylene containers. Tests were initiated with 20 1-2-week juveniles and 15 4-6-week caged adult *H. azteca*. Cages were made out of 120 mL specimen cups with their bottoms cut out and replaced with a 200 µm mesh. Cages where placed in the overlying water and held in place with a clothes pin. The purpose of the caged adults was to have enough tissue and animals to determine if a bioaccumulation curve similar to Alves et al. (2008) could be reproduced.

A pre-soaked 5 x 5 cm piece of cotton gauze was added to each container and cage. Approximately 2.5 mg and 5 mg of ground TetraMin® fish food flakes were added to each container and cage, respectively, every second day. Water was also renewed every second day. On day seven, surviving amphipods were sorted and counted and placed into 120 mL specimen containers containing 50 µM EDTA and 5 mg of TetraMin® food for 24 h to clear their guts. Twenty-four hour gut-cleared amphipods were wet weighted and dried for 48 h in a 60°C oven. Groups of 5-6 dried amphipods were digested using the procedures described above.

3.2.4 Kinetic experimental set-up

3.2.4.0 Acute uptake and depuration time series

A one-week (7 day) static, replicated, water renewal U uptake experiment initiated with 4-6-week old acclimated *H. azteca,* and followed by a two-week (14 day) depuration period, was conducted in 1 L of 10SAM with and without U in a 2 L polyethylene container. Nominal U water concentrations of 0, 40, 100 and 250 nmol/L were used in the uptake experiment. For the depuration exposure period 50 μM EDTA in 10SAM in the absence of waterborne U concentrations was used. On day seven, animals were placed into 50 μM EDTA-10SAM solution. The use of EDTA in the depuration study allowed for the complexion of any potential U depurated from the animal and the removal of any strongly bound U from the surface of the animal. A 50 μM EDTA concentration was within the range tolerated by *H. azteca* (Borgmann and Norwood, 1995a). Water was renewed every two days for the uptake exposures and weekly for the depuration period.

Each container contained a 5 x 5 cm piece of cotton gauze with animals being fed 2.5 mg of ground TetraMin® fish food three times per week.

On day 0, 1, 2, 4, 7, 8, 9, 11, 14, and 21 six (6) animals were randomly sampled from each individual container. The amphipods were weighed as a group to provide a mean wet weight per container and then dried for 48 hours in a 60°C oven. The dried amphipods were digested as previously described in the above body-size experiment.

3.2.4.1 Chronic uptake time series

A four week (28 day) static, replicated, water renewal experiment was conducted in 5 L of 10SAM, with or without U, in a 7.5 L polycarbonate aquarium. The 10SAM medium was spiked with nominal U concentrations of 0, 10, 18, 32, and 56 nmol U/L. Forty-five, 4-6-week old acclimated *H. azteca* and two 5 x 5 cm pieces of cotton gauze presoaked in distilled water were added to each container. In this experiment water was renewed weekly.

Ground TetraMin® fish food flakes were added three times per week to each test container in the following amounts: 10 mg in week 0; 7.5 mg in week 1; 5.0 mg in weeks 2, 3, and 4.

On day 0, 1, 2, 4, 7, 14, 21, and 28, twelve (12) animals were randomly sampled from each individual container. Half of the amphipods were placed in 120 mL plastic specimen containers with 50 μM EDTA and 2.0 mg TetraMin® for 24 h to clear their guts. 24 h and 0 h gut-cleared amphipods were weighed as a group to provide a mean wet weight per container and then dried for 48 hours in a 60°C oven. Groups of six dried amphipods (about 1-3 mg total dry mass) were digested according to the above procedure described for the body size experiment.

3.2.5 Uranium analysis

Water and tissues were analyzed for U concentrations by NLET using inductively coupled plasma-mass spectrometry (ICP-MS; NLET, 2007

3.2.6 Statistical and data analysis

Statistical, data and model analyses were conducted using SYSTAT version 10.0 (Chicago, Illinois, USA) for Windows. All metal concentration data were logarithmically transformed, while the mortality rates were fourth-root transformed, to normalize data and equalize variances before statistical analysis. The log-log relationships between U in *H. azteca* and water were obtained using the NONLIN (non-linear regression) module. The FUNPAR command was used for calculating the 95% Wald confidence intervals. Curve fitting in the time series experiments was performed using the combined acute and chronic data in order to provide the best-fit estimate of the overall relationship.

3.3 Results

3.3.0 Water quality

Water quality parameters remained consistent between all experiments and containers. pH ranged between 6.7 and 7.2 (Table 3.1), while ammonia levels were below 0.06 mM. Conductivity measurements ranged between 37-59 μS/cm, whereas dissolved oxygen was between 7.1-9.4 mg/L. U water concentrations for the body size study averaged 95% of the target nominal U water concentrations (Table 3.1). For the concentration series (bioaccumulation and toxicity) experiment, U water concentrations were on average 62% of the target nominal U water concentrations, while approximately 64% and 54% of the target nominal U water concentrations were achieved in the acute and chronic time series, respectively (Table 3.1). The low U water concentrations for some of the treatments were probably due to the combination of U adsorption onto the test chamber walls, cotton gauze, food and/or uptake by the *H. azteca*.

3.3.1 Body size effect

Whole-body U concentrations in both non-gut-cleared and 24 h gut-cleared *H. azteca* were higher in smaller animals than larger animals when exposed to either 100 or 250 nmol/L water-only U concentrations (Fig. 3.1). The relationship between body concentration and body mass for the pooled data can be described using the log regression relationship of the power funtion:

$$C_{TB} = a\,(B_m)^b(1\text{-}loss \times gutclear) \qquad (3.6)$$

where C_{TB} is the total U body concentration in the animal, a is a constant, B_m is the body weight and b is the overall slope of the relationship (Rainbow and Moore, 1986). The (1-*loss* × *gutclear*) term is equivalent to the term in Eq. (3.1a) and therefore permits the pooling of the gut-cleared and non-gut-cleared animals. The total loss of the metal eliminated in 24 h by gut-cleared as compared to non-gut-cleared animals was 72% (i.e., *loss* = 0.72, *gutclea*r = 0 or 1 in Eq. (3.6).

The overall slope (b, Eq. 3.6) of the relationship was negative, indicating that the U-body concentration decreases with increasing body weight of the animals. This relationship was significant (ANCOVA, P <0.05) for the pooled data (Fig. 3.1) with an R^2 of 0.85 and a slope (b; 95% confidence interval) of -0.35 (-0.44 to -0.26). The relationship between U-body concentration and body mass using only the 24 h gut-cleared animals was also significant (ANCOVA, P <0.05) with the b coefficient estimate of -0.35 (-0.45 to -0.25). This relationship was also significant when using only the non-gut-cleared animals, giving a b estimate of -0.35 (-0.51 to -0.18). Therefore, in order to increase the power of the model the gut-cleared and non-gut-cleared animals were pooled using the (1-*loss* × *gutclear*) term.

The slope was not significantly different for *H. azteca* exposed to 100 versus 250 nmol U/L waterborne U (ANCOVA, P = 0.83). The estimated value of a for *H. azteca* exposed to the 100 and 250 nmol U/L treatment was 160 (130-190) and 350 (290-420), respectively. Overall this study suggests that *H. azteca* body size needs to be taken into account before one can properly explain U bioaccumulation.

3.3.2 One-week bioaccumulation

Given that there was a body size effect on U bioaccumulation, body size was standardized to 0.22 mg dry weight for the concentration series and time series experiments. This was the geometric mean for body dry weight when all the animals from all experiments except the body size experiment were pooled.

Size-standardized U bioaccumulation was explained using the bioaccumulation saturation model, Eq. (3.1b) (Fig. 3.2; Table 3.2). However, due to high variability in the adult bioaccumulation data R^2 was only 0.53 (Fig. 3.2).

The size-standardized adult and juvenile bioaccumulation data, when plotted on the same graph, overlapped completely (Fig. 3.2). This suggests that the juveniles may have a similar uptake curve to the adults. Given that there were no surviving juveniles at nominal U concentrations > 320 nmol U/L in the water (Fig. 3.2), and hence no tissue to measure U accumulation in the animals, and that the juveniles follow a similar uptake, the adult $K_{0.5}$ of 560 (Table 3.2) was fixed in the juvenile bioaccumulation saturation model to estimate max. This relationship produced an R^2=0.92 and a max of 210 nmol/g. The predicted max for the young *H. azteca (*210 nmol/g) is 12.5% lower than the computed max of 240 nmol/g for the adult *H. azteca* (Table 3.2), since the mean accumulation at lower U concentrations in water was 12.5% lower in the young than the adult *H. azteca*.

3.3.3 Kinetics

3.3.3.0 Acute study

The total loss of metal eliminated in 24 h for gut-cleared versus non-gut-cleared animals in one week was 79%, similar to the 72% loss observed in the size experiment.

With the exception of the body size and depuration experiments, data from all one-week exposure experiments with the corrected gut-cleared (i.e., by dividing body concentration by 1-loss x gutclear) and non-gut-cleared adult *H. azteca* were pooled. Pooling the data not only increased the intermediate water concentrations used in the different experiments, but provided an overall best model fit to the data. Pooling these data was feasible because the *H. azteca* came from the same genetic stock (i.e., culture), were approximately the same age in each experiment (i.e., 4-6 weeks old) and were all exposed using the same water medium (i.e., 10SAM). For visual display purposes (Figs. 3.3-3.5), total U concentration in the non-gut-cleared and corrected gut-cleared *H. azteca* was corrected further by dividing by the term $C_w/(K_{0.5} + C_w)$. This correction factor accounted for the different U waterborne concentrations to which these animals were exposed.

A growth constant of 0.023/day (Table 3.3) was calculated using the pooled data by regressing ln(dry weight) against time (day). The growth rate constant was fixed in Eq. (3.1a) when estimating metal accumulation ($V_{u\max}$) and the elimination rate constant k_e (Table 3.3).

The corrected U bioaccumulation for *H. azteca* exposed to water U concentrations of 10-1800 nmol/L from the pooled studies for one week showed a rapid accumulation within the first day, followed by a more gradual increase in the remainder of the uptake phase (Fig. 3.3). Depuration was fairly rapid within two weeks in the absence of U water concentrations (Fig. 3.4). The depuration curve was estimated based on animals previously exposed to U waterborne concentrations of 40-250 nmol/L in the acute uptake time series study. For the other experiment (i.e., chronic uptake time series) a true depuration period of more than 24 h was not done. The value of k_e based on the depuration experiment was equal to 0.29 (0.25-0.33), with an R^2=0.94 (Table 3.3; Fig. 3.3). Uptake was estimated to reach 86% of steady state in one week, based on this k_e.

Given that the juvenile and adult *H. azteca* fit the same bioaccumulation curve (Fig. 3.1; Eq. 3.1b) in the bioaccumulation toxicity experiment when $K_{0.5}$ was set to 560 nmol/L, $K_{0.5}$ was also set to 560 in the saturation kinetic model (Eq. 3.1). The estimate of k_e = 0.29 was used to estimate V_{umax}, and in turn $V_{umax}/K_{0.5}$, the value of k_u at low C_W, in the acute and chronic study. The value of V_{umax} was estimated to be 810 (650-1000) giving a $V_{umax}/K_{0.5}$ of 1.4 (Table 3.3; Fig. 3.3).

3.3.3.1 Chronic study

The full 28 day bioaccumulation data set, pooled with the seven day data from the acute uptake time series, was modelled in the same way as described above for data to day seven. The total loss of metal in 24 h for gut-cleared versus non-gut-cleared animals in four weeks was 76%. The corrected bioaccumulation of U in non-gut-cleared and corrected gut-cleared *H. azteca* pooled data demonstrated a quick accumulation within the first four days (Fig. 3.5). The value of V_{umax} was estimated to be 880 (740-1100) with a $V_{umax}/K_{0.5}$ of 1.6 when $K_{0.5}$ and k_e were fixed to 560 and 0.29, respectively (Table 3.3; Fig. 3.5). These values were similar to the V_{umax} and $V_{umax}/K_{0.5}$ of 810 and 1.4 estimated in the acute study. Bioaccumulation reached 99.98% of steady state in the chronic study, based on the one compartment model. However, a slow further increase in U accumulation after day 14, beyond that predicted by the one-compartment model, is suggested by the data (Fig. 3.5).

3.3.4 Mortality

The mortality model based on the saturation model (Eqs. 3.3-3.5) was used to estimate the LC50, LC25, LC10 and LBC50, LBC25 and LBC10. The LC50 values, 1100 and 4000 nmol/L for juveniles and adults, respectively (Table 3.4), differed more between young and adult than did the LBC50 values (140 and 220 nmol/g, respectively, Table 3.5). The same was true for the LC25 and LC10 values. The fit of the model for the adults was poor (R^2=0.61) when compared to the juveniles (R^2=0.92) when estimating the LC50 to LC10 values. The same was true for the LBC50-LBC10 estimates.

3.4 Discussion

3.4.0 Body size

Factors such as growth and body size can contribute to variability in total metal body concentrations if not taken into account (Langston and Spence, 1995). In this study there was a significant negative correlation between metal body concentration and body mass (Fig. 3.1). In Fig. 3.1 the relatively few data points for the body size <0.1 mg dw may be influencing the overall significance of the slope. However when these data are removed, there is still a significant (P<0.05) negative correlation (-0.36) between metal body concentration and body mass. This suggests that the smaller animals had a higher body concentration of U than the larger animals. The body size relationship remains even after gut-clearance which results in a 72% loss of total metal. It may be that the majority of the bound U is strongly adsorbed to the integument of the *H. azteca,* since small animals have a larger surface to volume ratio than large animals (Rainbow and Moore, 1986). Alternatively, U might be absorbed internally and the abundance of uptake sites for U might be proportional to surface area. In either case, this study indicates that, for U uptake by the freshwater amphipod *H. azteca*, researchers need to standardize for body size before reporting U bioaccumulation parameters such as lethal body concentrations (i.e., LBC50), especially for field experiments where different sized *H. azteca* are present. For example, the importance of correcting for body size was evident in a study with the freshwater (unionoid) bivalve *Velesunio angasi* exposed to waterborne U concentrations, whereby the mean variability in valve movement responses was reduced by 81%, when size, age and/or gender were taken into account (Markich, 2003).

3.4.1 Bioaccumulation

In this study a bioaccumulation saturation model, Eq. (3.1a), was used to predict U bioaccumulation in size-standardized juvenile and adult *H. azteca* exposed to water-only U concentrations for one week (Table 3.2; Fig. 3.2). These U bioaccumulation estimates (Table 3.2) compared well with Alves et al. (2008) who used the same saturation model to predict U bioaccumulation via the water in *H. azteca* exposed to U-spiked sediment for 28 days (Fig. 3.2). In fact, the bioaccumulation saturation curve went through all the data points when the max (73 nmol/g) and $K_{0.5}$ (106 nmol/L, the geometric mean for 10SAM treatments) estimated by Alves et al., (2008) were used and compared to data from this study (Fig. 3.2).

3.4.2 Kinetics

As observed in the Borgmann and Norwood (1995b) study, where Cu and Zn kinetics were better predicted using a saturation model, the same was true for this study with U. The uptake of U by *H. azteca* was fast, with 89% and 99.98% of the steady state being reached by day seven and day 28 for the acute and chronic uptake time series studies, respectively. The time to steady state for U by the *H. azteca* was slower compared with the metals Cu (4 days), Zn (<4 days) and Pb (4 days), but faster than the compound tributyltin (14 days, Borgmann and Norwood, 1995b; MacLean et al., 1996; Bartlett et al., 2004).

The acute and chronic $V_{umax}/K_{0.5}$ estimates for U by the *H. azteca* (Table 3), equal to k_u at low C_W, is similar to the k_u for Pb of 1.5/day for *H. azteca* exposed to one-week waterborne Pb concentrations (MacLean et al., 1996). The initial four-day k_u estimate with Cu (0.73/day) and Zn (3.5/day) is also similar (Borgmann and Norwood, 1995b). The only difference between the kinetics of Pb, Cu, Zn, and U is that both Pb and Zn have higher k_e rates, 0.76 and 0.68, respectively, than that of Cu (0.16) and U (0.29).

Bioaccumulation, and hence uptake rate (if k_e is constant), levels off above approximately 1000 nmol/L (Fig. 3.2). This is higher than U concentrations found in most natural waters (Clulow et al., 1998, OMOE, 2003, EC/HC, 2004). Uranium uptake appeared to level off by day seven in the seven-day experiment (Fig. 3.3). However, the chronic uptake of U by *H. azteca* did not completely level off by day 28, as would be expected based on the k_e of 0.29 obtained in the depuration experiment (Fig. 3.5). This suggests the presence of another slower compartment that may be storing U during long term exposure. Unfortunately, the data from this study were not sufficient to fully model this slower compartment. However, Chassard-Bouchaud (1983) found that U was associated with calcium phosphate microgranules (spherocrystals) in the gill cuticle and hindgut epithelia of marine crabs (*Carcinus maenas)* when exposed to water-only U concentrations for 14 days. Similarly, Chassard-Bouchaud (1982) found that U was in the form of an insoluble phosphate in the lysosomal system of the freshwater crayfish *Pontastacus leptodactylus* when exposed to U concentrations in water. These microgranules, which can eventually be eliminated out of the animal, may act as temporary storage sites for U in the tissue. This may be the same for *H. azteca* given that Graf and Michaut, (1977) found that spherocrystals similar to those in the marine crab are present in amphipods of the genus *Orchestia*.

3.4.3 Gut-clearance

An understanding of the effect of gut-clearance on whole body metal concentration is important so that the overestimation of true body metal concentrations is avoided in non-gut-cleared animals, especially following sediment exposure, and when comparing lethal body concentrations (e.g., LBC50s) in water and sediment exposed animals. Gut-clearance is not always necessary in water-only exposures, but it is for sediment exposures. Estimates of LBC50s for both non-gut-cleared animals (when conducting water-only tests) and gut-cleared animals (for comparison to metal bioaccumulation in exposures to contaminated sediments) are, therefore, needed. Neumann et al. (1999) and Bartlett et al. (2004) have both demonstrated how gut-contents significantly contribute to total metal (e.g., Pb, Cd, Zn and Cu) and tributyltin body concentrations in *H. azteca* exposed to contaminated sediments. For instance, Neumann et al. (1999) found that non-gut-cleared *H. azteca* exposed to spiked sediments had initial total body concentrations of Pb, Zn and Cd that were 438, 44, and 12% above the true body concentration, respectively. Hence, gut-clearance is necessary in sediment exposures. However, true excretion from the body also occurs during the gut-clearance period. In this study 72-79% of total U loss from the body in 24 h was estimated in the various experiments. This loss is higher than that for the metals and metalloids As (34%), Co (13%), Cr (4%) and Mn (48%), also in water-only exposures (Norwood et al., 2006). The U excretion rate measured after gut-clearance (0.29/day, Table 3.2) would be expected to result in a 24% loss of total body U after 24 h, far less than the 72-79% loss observed. A reason for the additional 48-55% loss may be the presence of a second fast true body component as well as gut-content.

Although no U was added to the food in this study, U could have adsorbed onto the food and accumulated in the gut. The diet, depending on the animal, metal and type of food sources, may have a significant or even dominant effect on metal accumulation in aquatic animals compared to the dissolved phase (Wang and Fisher, 1999). Stephenson and Turner (1993) reported that approximately 58% of the Cd concentration in field collected *H. azteca* was due to Cd absorption from the food. Borgmann et al. (2007) investigated the relative contribution of food collected in the field and water to the bioaccumulation of 27 metals and metalloids in caged *H. azteca* near rivers affected by metal mining and found that Cd, Cu and Se were the only metals in which the food had a significant effect on whole body accumulation. Despite there being a 6.5 fold concentration range of U in the food in the Borgmann et al. (2007) study, there was no effect of U concentrations in the food on total U concentration in the *H. azteca.* This suggests that U in the diet was not bioavailable in that study. No attempt was made in this study to measure U uptake from food.

3.4.4 Toxicity

The mortality model based on the saturation model was used to predict the sensitivity of *H. azteca* exposed to waterborne U concentrations for one week (Tables 3.4 and 3.5). The LC50 estimate for the juveniles was approximately 13 times higher than the one-week juvenile LC50 (88 nmol/L) conducted in water similar to 10SAM (Borgmann et al., 2005). We are not able to explain these differences between our study and Borgmann et al. (2005), especially since the latter study did not report toxicity as total body concentration (e.g. LBC50).

At the LC50 concentration of 4000 nmol/L for the adults (Table 3.4), U bioaccumulation was starting to level off (Fig.3.2). Uranium bioaccumulation was not determined for juveniles above 320 nmol/L and thus it cannot be claimed that accumulation leveled off at 1100 nmol/L, the LC50 concentrations for the juveniles. However, if accumulation in the juvenile *H. azteca* was also reaching a maximum, this may partly explain why the LBC50 estimates were more similar between the juveniles and adults when reported on a body concentration basis than a waterborne basis.

3.5 Conclusion

Uranium bioaccumulation, kinetics, and toxicity can be explained successfully using a saturation model. However, when predicting U bioaccumulation and toxicity for *H. azteca*, a body size correction is required. Given that 89% of steady state was reached within one week, based on the one-compartment model, one week U bioaccumulation tests are feasible to predict the bioavailability and impact of U contamination in the water to *H. azteca.* As long as U water concentrations are below levels resulting in saturation of uptake, U concentrations in *H. azteca* will reflect current U levels in the environment, and *H. azteca* are likely to be good monitors of U bioavailability in the field.

Acknowledgements

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Table 3.1 Mean measured waterborne U concentrations and pH for the different series of experiments in this study

Body Size Experiment (7 days)

Nominal	Measured nmol/L	pH
0	0.13 ± 0.29	7.1 ± 0.25
100	110 ± 20	7.1 ± 0.14
250	280 ± 50	7.1 ± 0.08

One-week toxicity study

Nominal	Measured nmol/L	pH (uptake)
0	0.20 ± 0.16	7.0 ± 0.30
56	43 ± 19	7.0 ± 0.23
100	88 ± 34	7.0 ± 0.21
180	150 ± 73	7.0 ± 0.23
320	270 ± 140	7.1 ± 0.22
560	580 ± 240	7.1 ± 0.15
1000	1100 ± 370	7.0 ± 0.18
1800	1900 ± 670	6.9 ± 0.22

Acute kinetic study (7 days)

Nominal	Measured nmol/L	pH (uptake)	pH (depuration)
0	0.12 ± 0.31	7.4 ± 0.68	6.37 ± 0.55
40	37 ± 14	7.4 ± 0.78	6.20 ± 0.23
100	65± 19	6.9 ± 0.20	6.13 ± 0.22
250	190 ± 51	6.9 ± 0.22	6.02 ± 0.22

Chronic kinetic Study (28 days)

Nominal	Measured nmol/L	pH
0	0.01 ± 0.02	6.9 ± 0.18
10	5.4 ± 0.02	6.9 ± 0.20
18	10 ± 2.2	7.0 ± 0.16
32	19 ± 3.4	6.9 ± 0.19
56	60 ± 6.3	6.9 ± 0.20

Values represented as mean ± standard deviation
n=19 for chronic U water measurements
n=14 for acute U water measurements
n=12 for the one week toxicity U water measurements
n=12 for the body size experiment

Table 3.2 Maximum metal accumulation (*max*), half saturation constant ($K_{0.5}$), background metal accumulation (C_{bkg}), and the 95% confidence interval in parentheses for metal accumulation fitted to a water-bioaccumulation saturation curve[a,b]

	max (nmol/g)	$K_{0.5}$ (nmol/L)	$max/K_{0.5}$ (L/g)	C_{bkg} (nmol/g)	R^2
Adults	240 (100-560)	560 (160-2000)	0.46 (0.24-0.74)	0.15	0.53
Juveniles[c]	210 (150-210)	560 (fixed)	0.37 (0.32-0.45)	0.07	0.85

[a] model $C_{TB} = (\max \text{ x} C_w / (K_{0.5} + C_w)) + C_{bkg}$

[b] These estimates are based on 24 h gut-cleared animals; 0 h gut-cleared animals were not sampled in this particular study

[c] The max for the juveniles is an extrapolated value based on $max/K_{0.5}$ and computed using a fixed $K_{0.5}$ of 563 estimated using the adult *H. azteca* standardized to a body size of 0.22 mg dry weight

Table 3.3 Kinetic model[a] estimates (95% confidence interval in parentheses)

	Acute		Chronic
	Uptake	Depuration	Uptake
g	0.023 (0.015-0.035)	0.023 (0.015-0.035)	0.023 (0.015-0.035)
Duration (day)	7	14	28
C_{bkg} (nmol/g dw)	0.09	0.12	0.09
V_{umax}	810 (650-1000)	1300 (1000-1700)	880 (740-1100)
$K_{0.5}$[b] (fixed)	560	560	560
k_e[c] (fixed)	0.29	0.29 (0.25-0.33)	0.29
R^2	0.85	0.94	0.82
$V_{umax}/K_{0.5}$	1.4		1.6
loss (in 24h)	0.79 (0.73-0.86)		0.76 (0.70-0.82)
steady-state (%)	89%	99%	99.98%

[a] model $C_{TB}=(V_{umax}/(k_e+g))(C_w/(K_{0.5}+C_w))(1-e^{-(ke+g)t})(1\text{-}loss \times gutclear) + C_{bkg}$

[b] The fixed $K_{0.5}$ is based on the estimated value from the bioaccumulation study

[c] The fixed k_e is based on the estimated value from depuration study

[d] Percent of steady-state=$(1- e^{-(ke+g)t}) \times 100$

Table 3.4 K_w, exponent for water (n_w; Eq.3.3), LC50, LC25 and LC10 (nmol/L), and the 95% confidence interval in parentheses estimates for *H. azteca* exposed to waterborne U concentration in soft water for one week

	K_w	n_w	LC50	LC25	LC10	R^2
Adults	660	3.7	4000 (210-77000)	2100 (1200-3600)	1200 (780-1900)	0.61
Juveniles	1600	2.8	1100 (860-1300)	540 (430-680)	300 (230-390)	0.92

Table 3.5 K_{TBX} , exponent for total body (n_b ; Eq.3.3), LBC50, LBC25, LBC10 (nmol/g), and the (95% confidence interval in parentheses) for H. azteca exposed to waterborne U concentrations in soft water for one week

	K_{TBX}	n_b	LBC50	LBC25	LBC10	R^2
Adults	10,000	6.4	220 (130-350)	190 (160-220)	160 (140-180)	0.61
Juveniles	-1400	2.8	140 (130-150)	100 (92-120)	74 (63-87)	0.92

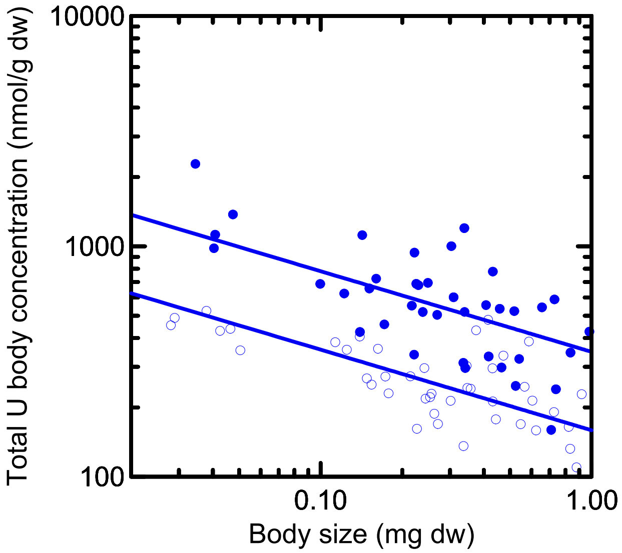

Fig. 3.1 Total U body concentration versus body size in *H. azteca* exposed to 100 (○) and 250 (●) nmol U/L for one week (R^2=0.85, in both treatments). Data includes the pooled non-gut-cleared and corrected gutclear animals. Gut-cleared animals were divided by (1-*loss* × *gutclear*) to correct for total loss of U concentration in 24h.

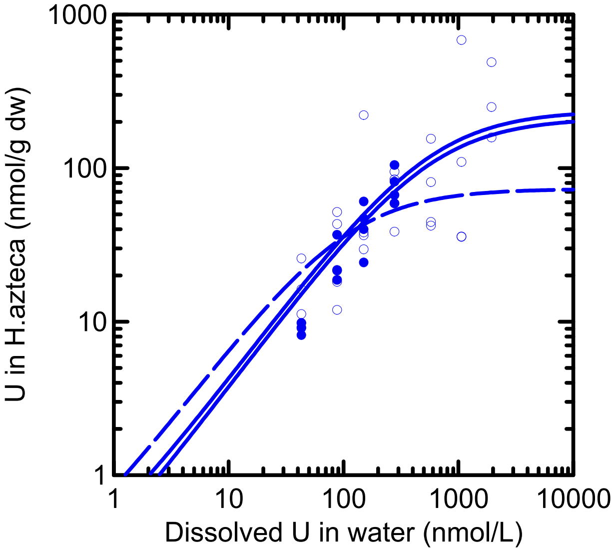

Fig. 3.2 Bioaccumulation saturation curve for juvenile, R^2=0.85 (●) and adult, R^2=0.53 (○) *H. azteca* exposed to waterborne U concentrations of 56-1800 nmol/L for one week in the acute toxicity-bioaccumulation experiment. The solid line represents the bioaccumulation saturation curve (Eq.(3.1)), using the max and $K_{0.5}$ parameters estimated in this study (Table 3.2); the dash line represents the bioaccumulation saturation curve (Eq.(3.1a)) estimated using the geometric mean *max* of 73 nmol/g and $K_{0.5}$ of 106 nmol/L (for the 10SAM treatment) in the Alves et al. (2008) study.

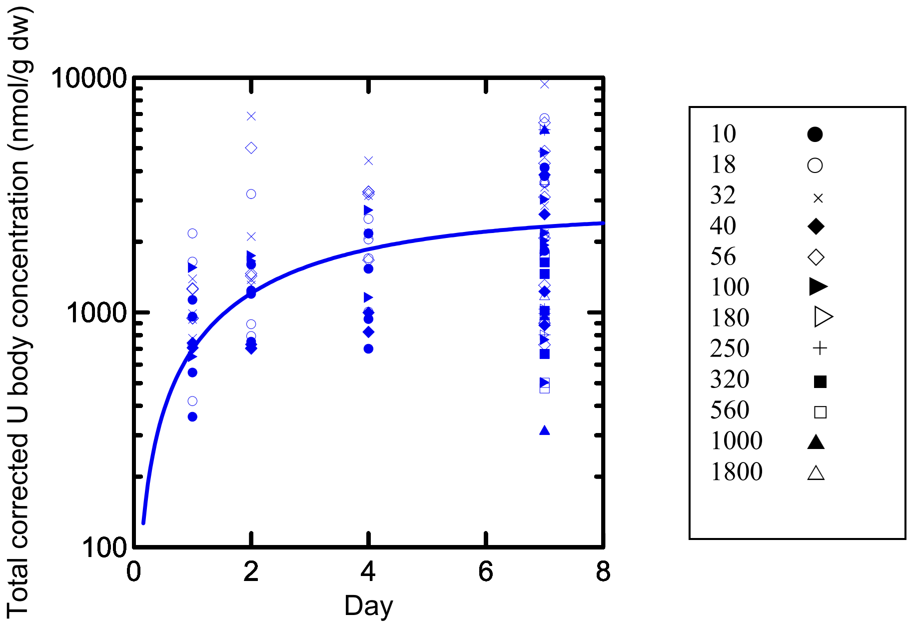

Fig. 3.3 Corrected total corrected body concentration (nmol U/g dry weight) versus time (day) for pooled non-gut-cleared and corrected 24h gut-cleared (i.e. by dividing the body concentration by1-*loss* × *gutclear*) *H. azteca* exposed to the pooled waterborne U concentrations of 10-1800 nmol/L from various experiments for one week (R^2=0.85). The corrected concentration in *H. azteca* were further divided by $C_w/(K_{0.5} + C_w)$ to account for differences in U water concentrations.

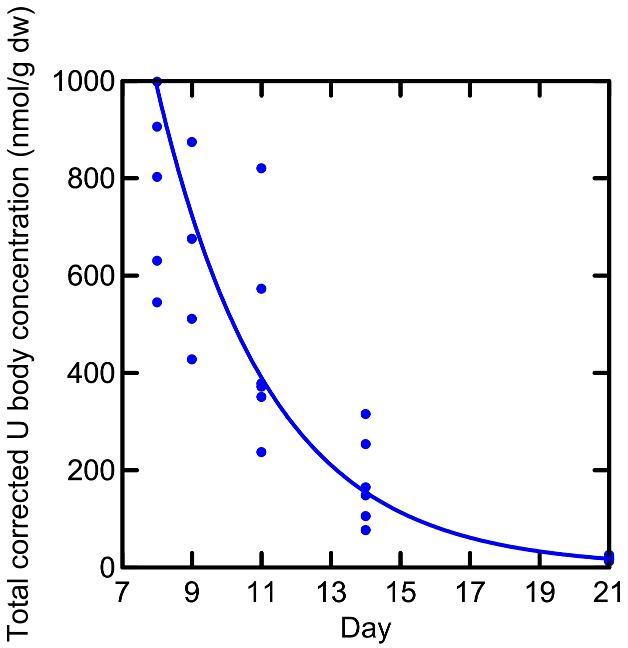

Fig. 3.4 Depuration curve for total corrected U-body concentration versus time (day) for *H. azteca* in the absence of waterborne U concentrations (R^2=0.94). *H. azteca* were exposed for one week to U waterborne concentrations of 40-250 nmol/L (i.e., acute uptake time series experiment) prior to depuration. Corrected body burden in *H. azteca* were divided by $C_w/(K_{0.5} + C_w)$ to account for differences in water U concentrations.

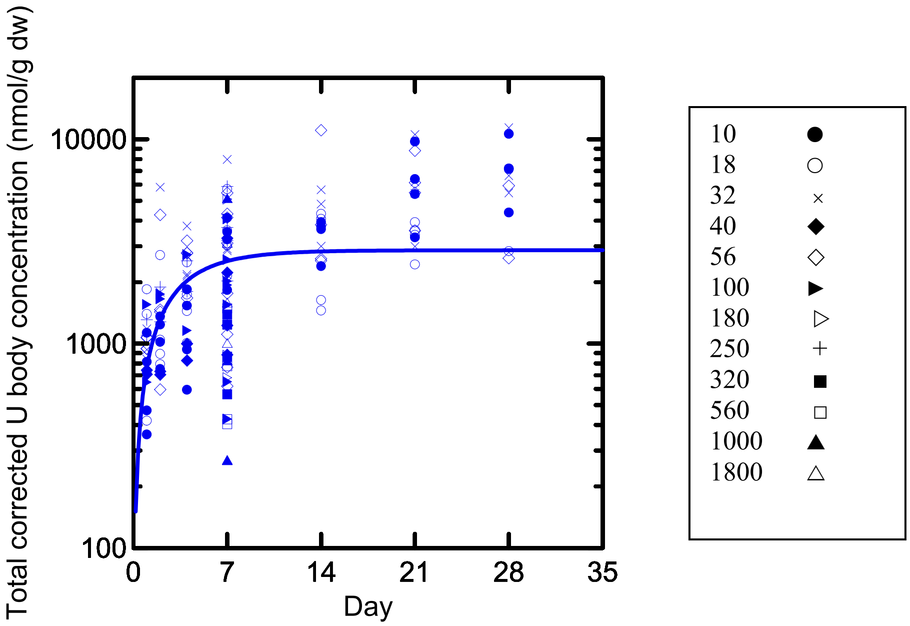

Fig. 3.5 Total corrected U-body concentration versus time (day) for non-gut-cleared and corrected gut-cleared animals (i.e., $1\text{-}loss \times gutclear$) *H. azteca* exposed to pooled waterborne U concentrations of 10-1800 nmol/L from various experiments for four weeks (R^2=0.82). Corrected body concentration in *H. azteca* were divided by $C_w/(K_{0.5} + C_w)$ to account for differences in U water concentrations.

APPENDIX

Qualiy Assurance and Quality Control (QA/QC)

Quality assurance and quality control procedures were carried out by NLET, such as standard verifications to monitor sensitivity drift of the machine, control standards to confirm accuracy of the calibration curve, and reference materials to monitor accuracy and precision (ICP-MS, NLET, 2007). A high-purity standard for U (High-Purity Standards, Charleston, South Carolina, USA) and an Environment Canada (National Laboratory for Environmental Testing at the National Water Research Institute, Environment Canada, Burlington, ON, Canada) certified trace elements drinking water standard (TM-DWS) were included with the water and *H. azteca* samples in order validate the ICP-MS calibration curve for U. The average percent recovery for the high-purity standard (n=6) and Environment Canada standard (n=6) from the true value was 99.5 and 108%, respectively. Method blanks were collected and processed at the same time as water and sediment and tissue digests were collected and processed, using the same containers, digestion or preservation techniques. Method blanks were also measured. Uranium concentrations in the water and *H. azteca* were background corrected, by subtracting by the blank measured value. This blank correction accounted for any potential U contamination that may have been present during the collection and processing of the samples. For the majority of the blank samples, U concentrations were below the ICP-MS detection level of 0.0005 μg/L.

Blanks and appropriate standard reference materials for the major ions (Ca, Mg, Na and K), DIC/DOC, and the anions, SO_4 and Cl measurements were analysed by NLET in accordance with their quality assurance and quality control procedures. Dissolved organic and inorganic carbon samples were measured on a UV-persulfate TOC analyzer (Pheniox 8000^{TM}). The major ions Ca, Mg, Na, and K were measured on an atom absorption spectrophotometer, while the anions, Cl and SO_4 were measured using ion chromatography.

CHAPTER 4

THE CONCENTRATION OF URANIUM AND 26 OTHER METALS IN THE WATER, SEDIMENT, AND TISSUES OF *Hyalella azteca* EXPOSED TO SEDIMENT AND SITE WATER COLLECTED NEAR FORMER URANIUM MINING DISTRICTS: ELLIOT LAKE AND BANCROFT, ONTARIO, CANADA

Abstract

The purpose of this study was to determine if a water-bioaccumulation model using the freshwater amphipod *Hyalella azteca* as the model organism, and a water-sediment saturation model developed in a previous study, could be applied to quantify Uranium (U) bioavailability under natural conditions. The evaluation and bioavailability of 26 other metals were also assessed in the sediment, water and *H. azteca,* in order to distinguish between toxicity, if any, caused by U exposure versus those caused by the presence of others metals in the environment. The water-bioaccumulation saturation model could not be validated in this study due to U concentrations in *H. azteca* and overlying water being close to background levels. Similarly, U concentrations in the sediment were close to background concentrations for the majority of the sites making it difficult to test the water-sediment partitioning saturation model. For some sites where metal concentrations in the sediment and water were above background concentrations, the water-sediment partitioning saturation model under-predicted U binding to the sediment when compared to the observed field concentrations. Toxicity did take place at some sites, but this toxicity was not due to U, but rather to Cd. This study emphasized the use of metal bioaccumulation when conducting field risk assessments to determine metal bioavailablitiy in the aquatic environment.

4.0 Introduction

There have been many studies that have focused on bioaccumulation of uranium (U) and U-series radionuclides, mainly radium, in many species near Elliot Lake, a former U mining district in Ontario, Canada (e.g., Clulow et al., 1991; Clulow et al., 1992; Clulow et al., 1996; Clulow et al., 1998a; Clulow et al., 1998b). However, the chemical toxicity of U, which is considered to be more toxic than the radiological toxicity (EC/HC, 2004) has received little attention in the field and in aquatic invertebrates. Of the studies that are currently available, Alves et al. (2008) found that for *Hyalella azteca*, a sensitive freshwater amphipod, U bioaccumulation was mainly via the water rather than the sediment when *H. azteca* were exposed to U-spiked sediments for 28 days (Alves et al., 2008). The bioaccumulation of this animal was found to be a more reliable indicator of U toxicity than concentrations in the water and sediment (Alves et al., 2008). However, Alves et al. (Chapter 3) cautioned that body-size needs to be taken into account when reporting U accumulation and toxicity in these animals, given the presence of a significant negative relationship between U and body size when *H. azteca* were exposed to water-only U concentrations for one week.

Water chemistry such as pH can influence U bioaccumulation and toxicity in some species. For instance, Markich et al. (2000) found that the valve movement response of a freshwater bivalve, *Velesunio angasi,* was dependent on pH, whereby the bivalves were more sensitive to U concentrations at a low water pH (5.0). By applying a water-bioaccumulation and water-sediment partitioning saturation model, Alves et al. (2008) were able to determine that water pH not only affects U speciation in the water, but the dissolution of U from the sediment into the water and the uptake of U by *H. azteca*. In fact, the half-saturation constant for U partitioning or accumulation in their saturation model varied strongly with the hydrogen ion concentration to the fourth power, suggesting the presence of four binding sites for hydrogen ion on the sediment or *H. azteca*.

Although, Alves et al. (2008) was able to successfully apply these saturation models in a practical laboratory setting, their validity in the field still need to be demonstrated. If these models are successfully validated in the field, they will be essential monitoring tools for environmental regulators and industry in the management of aquatic environments near U mines and tailings management areas. Therefore, the goal of this study was to first determine if U is bioavailable to *H. azteca* exposed to field sediments and waters near decommissioned U mining and tailings management areas in Ontario, and if the saturation models employed by Alves et al. (2008) can be applied to quantify U concentrations under natural conditions. The evaluation and bioavailability of 26 other metals (i.e. Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, V and, Zn) were also assessed in the sediment, water and *H. azteca*, in order to distinguish between toxicity, if any, caused by U exposure versus toxicity caused by presence of others metals.

4.1 Theory

4.1.0 Metal Bioaccumulation

A saturation model (Borgmann et al., 2004; Alves et al., 2008) has been shown to describe the bioaccumulation of metals such as Cd, Cu, Pb, Ni and U by *H. azteca*; this model is described by:

$$C_{TB} = \frac{\max C_W}{K_{0.5} + C_W} + C_{bkg} \tag{4.1}$$

where C_{TB} is the total body concentration of a metal, max is the maximum (above background concentration) accumulation of the metal by the organism, C_W is the concentration of the metal in water, $K_{0.5}$ is the half saturation constant (concentration at which the C_{TB} is halfway between the maximum accumulation and background concentrations), and C_{bkg} is the background C_{TB} in animals in the absence of any added metal to the water.

The $K_{0.5}$ value for U is expressed as:

$$K_{0.5} = a + b(\mathrm{H}^+)^4 \tag{4.2}$$

where $a = 1/K_m$ and K_m is the binding strength of the metal to the binding site (ligand), and b is the product of a and the corresponding binding constants to the hydrogen ion. The estimated values for the a and b constants predicted from the Alves et al. (2008) study are shown in Table 4.1. In Eq. (4.2), 4 is the slope of the plot of log ($K_{0.5}$) versus log (H^+) if the other ions are held constant. This slope also represents the number of hydrogen binding sites (i.e. four bindings sites) binding to the ligand.

4.1.1 Water-sediment partitioning

The binding of U in water to sediment is also described using a saturation model, similar to Eq. (4.1), but with an inclusion of an additional binding site (Alves et al., 2008):

$$C_{sed} = \frac{\max' C_W}{(K_{0.5}' + C_W)} + \frac{\max'' C_W}{(K_{0.5}'' + C_W)} + C_{bkgs} \tag{4.3}$$

where C_{sed} is the total metal concentration in the sediment, *max'* and *max''* are the maximum (above background concentration) in the sediment, $K_{0.5}'$ and $K_{0.5}''$ are half saturation constants, analogous to $K_{0.5}$ in Eq. (4.1) and C_{bkgs} is the background concentration of C_{sed} in the absence of any added metal to the sediment.

If the first binding site of the sediment is weak, $K_{0.5}' >> C_w$, then Eq. (4.3) can be simplified to (Alves et al., 2008):

$$C_{sed} = \alpha C_W + \frac{\max'' C_W}{(K_{0.5}'' + C_W)} + C_{bkgs} \tag{4.4}$$

where α represents $max'/K_{0.5}'$; the initial slope of the saturation curve (Borgmann et al., 2004; Alves et al., 2008).

The inverse of α and binding to the second binding site are also related to the hydrogen ion concentration similar to $K_{0.5}$ in Eq. (4.2) as follows:

$$\frac{1}{\alpha} = a' + b'(\mathrm{H}^+)^4 \tag{4.5}$$

and

$$K_{0.5}'' = a'' + b''(\mathrm{H}^+)^4 \tag{4.6}$$

where $a' = 1'/K_m'$, $a'' = 1''/K_m''$; b' and b'' are the products of a' and a'', respectively, and the corresponding binding constants to the hydrogen ion, while K_m' and K_m'' are the binding strengths of the metal to binding site. The estimated values for the constants a', a'', b', and b'' from Alves et al. (2008) are shown in Table 4.1.

4.2 Materials and Methods

4.2.0. Study area(s)

4.2.0.1 Elliot Lake, Algoma District

The City of Elliot Lake is located on the Canadian Shield in Northern Ontario, within the Serpent River watershed, north of Lake Huron and between Sudbury and Sault Ste. Marie (Fig. 4.1). Uranium mining and milling operations flourished during the mid-1950's to 1960's and then again from the 1970's to the 1990's with a total of 12 decommissioned mines, 11 associated decommissioned mills, and 10 decommissioned tailings management areas near Elliot Lake (EC/HC,2004; LLRWMO, 2004; Minnow Environmental Inc., 2005).

Five lakes near Elliot Lake were sampled: Ten Mile (TNM), Dunlop (DUN), Quirke (QKE), Elliot (ELT), and McCarthy (MCC) Lake for U concentrations in the sediment, water and *H. azteca* (Fig. 4.2). Ten Mile and Dunlop were selected as reference lakes because they were both upstream of any U decommissioned mines/mills and tailings management areas (Fig. 4.2; EC/HC, 2004). Quirke and Elliot Lake were chosen because at one time there were a total of seven and three operating mines, respectively, near the vicinity of these lakes (LLRWMO, 2004; Minnow Environmental Inc. 2005; Fig. 4.2). Currently, Quirke Lake, which is the largest water body in the Serpent River watershed, receives treated effluent from three tailings management areas (EC/HC, 2004). McCarthy Lake, which is downstream of all the decommissioned mines/mills and tailings management areas, receives the combined flow from all the above lakes sampled (EC/HC, 2004). In terms of lakes, McCarthy Lake is a good representative of the total U concentrations within the Serpent River watershed.

4.2.0.2 Bancroft, Hasting County

The town of Bancroft, on the southern edge of the Canadian Shield in Eastern Ontario, is located on the York River, north-east of Toronto and west of Ottawa (Fig. 4.1). Uranium mining and milling occurred during the late 1950's to mid-1960's and then resumed again in late 1970's to early 1980's with a total of two decommissioned mines, a decommissioned mill and three decommissioned tailings management areas (OMOE, 2003; LLRWMO, 2004; Fig. 4.3). Five Lakes near Bancroft were sampled: Centre (CTR), Siddon (SID), Bentley (BEN), Bow (BOW), and the upper part of Paudash Lake, Inlet (INT) Bay, for U concentrations in the sediment, water and *H. azteca*. Centre and Siddon Lake were chosen as reference lakes, given their locations upstream of any decommissioned mines or mill, and tailings management areas (Fig. 4.3). Both Bentley and Bow Lake were adjacent to a decommissioned mill and a tailings management area, whereas Inlet Bay was downstream of a decommissioned mine and a tailings management area (Fig. 4.3). All of these lakes empty into the Crowe River system, which forms part of the Lake Ontario watershed (OMOE, 2003).

4.2.1 Sediment

Sediments were collected near Elliot Lake in August 2007 and Bancroft in October 2007. A mini-PONAR grab was used to collect approximately 250 mL of sediment at each sampling site (Table 4.2). For each individual lake, sediments were collected at the deepest part of the lake and at 20 meters for lakes near Elliot Lake and 10 meters for lakes near Bancroft. Samples were placed into 250 mL acid-washed polypropylene containers. Sediment samples were stored in a 4°C cooler and transported to Burlington, Ontario, Canada, Centre for Inland Waters Research, Environment Canada. Sub-samples of the sediments were freeze dried and analyzed for particle size using the procedure of Duncan and LaHaie (1979) and total organic carbon using the LECO®CR-12 carbon determinator (LECO Corp, MI, USA). Other sub-samples of the sediment were dried in a 60°C oven for one week and analyzed for moisture content before being digested and analyzed for the 27 metals: Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V, and Zn by the National Laboratory for Environmental Testing (NLET), Environment Canada, Burlington, Ontario, Canada, using inductively coupled plasma-mass spectrometry (ICP-MS, NLET, 2007). Sediment digestions were carried out as described by Alves et al. (2008) for the *H. azteca*. The leftover sediments were placed in a 4°C walk-in incubator prior to their use in the cone experiments (see below).

4.2.2 Field water

Ten liters of surface water at the deepest part of each sampled lake were collected along with field blanks (Nanopure® water). Water temperature, conductivity, pH, and dissolved oxygen were measured with a submersible YSI Datasonde probe (YSI 6600D-multi-parameter water quality monitor, YSI Environmental System, OH, USA). Ammonia was analyzed using an assay kit (Aquarium Test for freshwater, NUTRAFIN®, Quebec, Canada).

Water was collected for dissolved inorganic carbon and dissolved organic carbon (DIC/DOC), alkalinity, hardness, and the major ions: Ca, Mg, Na, and K measurements at the deepest part of the lake. For the DIC/DOC water was collected in glass containers with no head space. Filtered (0.45 µm Supor® (PES) membrane ion chromatography Acrodisc®, Pall Corporation, Ann Arbor, MI) and unfiltered water were collected and acidified to 1% HNO_3 with 70% supra trace HNO_3. Water samples were stored in a 4°C cooler and transported to Burlington, Ontario were they were kept in a 4°C walk-in incubator pending analysis.

4.2.3 Benthic collection and other samples

Hyalella azteca and periphyton were collected, where present, near the shoreline (Table 4.2). *Hyalella azteca* were collected by disrupting the surface layer of the sediment for one minute and skimming the layer with a D-net. The animals were placed into a 10 L bucket with lake water and transported to Burlington. These animals were counted, gut-cleared for 24 h in dechlorinated water originating from Lake Ontario, dried, digested, using the procedure described in Alves et al. (2008), and analyzed for the above mentioned 27 metal concentrations by NLET (NLET, 2007). These field collected *H. azteca* were used only as an indication of their presence in the lake and as a measurement of metal concentrations in the field for these animals. They were not used in the cone experiments (see below).

Periphyton was collected by scraping off rocks and logs near the shoreline (Table 4.2). The periphyton was placed in a 1 L acid-wash polypropylene container with lake water and stored in a 4°C cooler while in the field and in a 4°C walk-in incubator once in the laboratory. Sub-samples of the periphyton were dried and digested as described in Alves et al. (2008) for *H. azteca* and then analyzed for the 27 metals by NLET (NLET, 2007). The leftover periphyton was used as food source for the *H. azteca* as a side experiment in the cone study (see below).

4.2.4 Culturing

Hyalella azteca were cultured using the procedures of Borgmann et al. (1989), but in polyethylene containers rather than glass containers. Each culture was supplied with 1L of dechlorinated Burlington City tap water originating from Lake Ontario (from July 07 -Dec 07 in mg/L (n=9)): dissolved organic carbon = 1.1 ± 0.2 ; dissolved inorganic carbon = 18.3 ± 0.8 ; Cl = 26.9 ± 1.3; SO_4 = 39.1 ± 2.1 ; Ca = 35.4 ± 0.5 ; Mg = 8.7 ± 0.1 ; Na =14.5 ± 0.8 ; K = 3.6 ± 1.9, alkalinity (as $CaCO_3$) = 76.3 ± 3.1; hardness (as $CaCO_3$) = 124.3 ± 1.6, pH 7.9 to 8.2). Culture water was renewed weekly with the young being separated from the adults in order to maintain a continuous supply of zero to one-week-old-amphipods. The culture and experimental animals were held in a walk-in incubator at 24 ± 1°C with a 16h light: 8h dark photoperiod. Animals used in the experiments were acclimated in a 5-salt artificial medium (see below) for one week prior to the start of the experiments. The initial age of juvenile *H. azteca* used in the cone studies at the start of the experiments was 7-14 days old.

4.2.5 Experiment water chemistry

Chronic four-week (28 day) static, non-renewal experiments were conducted using sediments from each site with both site water and a moderately hard water with high alkalinity, 5-salt Standard Artificial Medium (SAM): 1 mM $CaCl_2$; 1mM $NaHCO_3$; 0.25 mM $MgSO_4$; 50 µM KCl; 10 µM NaBr. The SAM is similar to Lake Ontario water with a pH of about 8. Given that Canadian Shield waters are usually soft, with a lower pH around 7, the inclusion of SAM in this study was to provide some variability to the water chemistry in the study and to observe if there were any consistent patterns in terms U partitioning from field sediment and decrease in U bioaccumulation in *H. azteca* with an increase in overlying water pH as reported in Alves et al., (2008).

Experiments were carried as described by Alves et al. (2008), but with the exclusion of caged animals. In short, within 4-7 days of sediment collection, approximately 5 mL of sediment with a depth 1 cm and surface diameter of 3 cm was added to a polycarbonate Imhoff settling cone. One liter of SAM or site overlying water was added to each cone in order to achieve a water to sediment ratio of 200:1. Each cone was allowed to equilibrate for two weeks. After the two-week equilibrium period 15 acclimated *H. azteca* in SAM were added to each cone and exposed to the field sediments and the different overlying waters for 28 days with a 16h light: 8 h dark period at 24 ± 1°C. Amphipods were fed ground TetraMin$^{®}$ fish flakes (Tetra Holding Inc., U.S.A) or natural periphyton collected in the field three times per week at the following rates: 2 mg for week 1 and 2; 2.5 mg for week 3, and 5 mg for week 4.

Ten milliliters of filtered (0.45 µm) overlying water samples were collected on day zero and 28 from each chamber for the measurement of the dissolved metal concentrations, Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V, and Zn in the water. Water samples were acidified to 1% with HNO_3 (high purity acid, omniTrace$^{®}$, E.M. Science) and placed in a 4^{o}C cold-room pending analysis. Ten milliliter water samples were collected and used to measure pH (ATI ORION, Model 420A), ammonia (Aquarium Test for freshwater, NUTRAFIN$^{®}$), conductivity (VWR Scientific, Model 1054) and dissolved oxygen (Thermo Orion, Model 805APlus) at the beginning and end of the exposure periods.

On day 28, 125 mL of overlying water was collected and measured for dissolved organic and inorganic carbon using a UV-persulfate TOC analyzer (Pheniox 8000^{TM}), while 500 mL of water was collected and measured for the major ions Ca, Mg, Na, and K using an atom absorption spectrophotometer and Cl and SO_4 using ion chromatography. These water samples as well as the field collected waters were analyzed by NLET (NLET, 2007).

Surviving amphipods were sieved and rinsed in clean City of Burlington tap water. The amphipods were counted and placed in a 120 mL plastic specimen container with 50 μM EDTA (Ethylenediaminetetra-acetic acid, BDH Chemicals, LTD.) made-up in SAM and 5.0 mg of TetraMin for 24 h to clear their guts. The gut-cleared amphipods were weighed as a group to provide a mean mass per container and dried for 48 h at 60°C. Groups of six dried amphipods were digested as described in Alves et al. (2008) and analyzed for the 27 metal concentrations by NLET (NLET, 2007).

4.2.6 Quality Assurance and Quality Control (QA/QC)

Blanks and appropriate standard reference materials for major ions, DIC/DOC, SO_4 and Cl measurements were analysed by the National Laboratory for Environmental Testing (NLET) in accordance with their quality assurance and quality control procedures.

All U chemical analyses in terms of the 27 metals (Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, V and, Zn) concentrations in sediment, water *H. azteca* and periphyton were done by the NLET using inductively coupled plasma-mass spectrometry (NLET, 2007). Quality assurance and quality control procedures were carried out by NLET, such as standard verifications to monitor sensitivity drift of the machine, control standards to confirm accuracy of the calibration curve, and reference materials to monitor accuracy and precision (ICP-MS, NLET, 2007). Method blanks and field blank were collected at the same time as the water was collected and the sediment and tissue digests were processed, using the same containers, digestion or preservation techniques. Measured metal concentrations in the sediment, water, *H. azteca* and periphyton were background corrected by subtracting these values by the method blank and field blank measurements. This blank correction accounted any potential metal contamination that may have been present during the collection and processing of the samples.

A high-purity standards certified reference material for trace elements (High-Purity Standards, Charleston, South Carolina, USA) and an Environment Canada (National Laboratory for Environmental Testing at the National Water Research Institute, Environment Canada, Burlington, ON, Canada) certified trace elements drinking water standard (TM-DWS) were analysis along side the sediment, water, *H.* azteca, and periphyton samples in order to validate the ICP-MS calibration curve for the different elements. These standards were acidified to 1% with HNO_3 (high purity acid, omni*Trace*®, E.M. Science). Two additional certified sediment reference materials (SUD-1, collected from Ramsey Lake near Sudbury, Ontario, and WQB-3, a mixture of Hamilton Harbour and Lake Ontario sediments, National Laboratory for Environmental Testing at the National Water Research Institute, Environment Canada, Burlington, ON, Canada) processed in the same manner as the sediment digests were included to validate the ICP-MS calibration curve for the different elements when analysing the sediment samples. The Institute for National Measurement Standards National Research Council of Canada certified reference material, Tort-2, lobster, hepatopancrease (National research council of Canada, Ottawa, Canada), digested using the *H. azteca* digestion method, was used to validate the ICP-MS calibration curve for the different elements during the *H. azteca* samples analyses. The mean percent recovery for the high-purity standard (n=4) and Environment Canada certified standards (n=4) for a suite of certified metals was within 11% and 7%, respectively, of the certified standard values. The mean percent recovery for the two certified reference sediments, SUD-1 (n=2) and WQB-3 (n=4), were both within 8% of the certificate reference value for the certified metals. The mean recovery for Tort-2 (n=6) was between 2% of the certified value among the different certified metals.

4.2.7 Statistics and data analysis

All statistical tests were performed using SYSTAT version 10.0 (Chicago, Illinois, USA). Survival data was arcsine transformed to approximate the normal distribution. Comparisons in normalized data between the treatments and the lakes were made using a nested analysis of variance (ANOVA) where treatments were nested within the lakes, followed by a Tukey's pairwise comparison for differences among the treatments and lakes ($P<0.05$).

4.3 Results

4.3.0 Surface water chemistry in field

Dissolved oxygen concentrations were higher in the lakes sampled near Bancroft than near Elliot Lake. Water temperature was about 10°C higher for water collected from Elliot Lake area compared to Bancroft area (Table 4.3). This may explain why the dissolved oxygen concentrations were higher for the Bancroft region. given that more oxygen can dissolve in cold water compared to warm water. pH readings for the Elliot Lake sites (7.3-8.5) were more variable than those from Bancroft sites (7.7 to 7.9; Table 4.3).

Conductivities were on average lower (28-170 μS/cm) at sites near Elliot Lake compared to those at Bancroft (46-540 μS/cm; Table 4.3). This is not surprising since the major ions Ca, Mg, Na and K, as well as the Cl, and SO_4 were higher in the Bancroft waters in comparison to sites near Elliot Lake (Table 4.3). Dissolved organic carbon (DOC) was consistent between the two studies ranging between

1.7 to 3.6 mg/L for the Elliot Lake sites and 3.5 to 5.6 mg/L for the Bancroft sites. Dissolved inorganic carbon (DIC) was on average three times higher for waters in the Bancroft region compared to the sites in the Elliot Lake district (Table 4.3), thus explaining the higher alkalinity measurements in the Bancroft region (Table 4.3). The high conductivity at some sites (e.g., MCC1, QKE1, BEN1, BOW1; Table 4.3) may be associated with the liming of the tailings that took place in the past and/or is currently taking place at some of the tailings management areas near Elliot Lake (e.g. QKE Lake).

4.3.1 Overlying water chemistry in the cone experiments

Dissolved oxygen in the overlying water was on average higher for the Elliot Lake study (7.9-8.3 mg/L) compared to the Bancroft study (7.3-7.7; Table 4.4); but overall was consistent within each study. pH was maintained around 8.0 for *H. azteca* exposed to the overlying SAM water in all studies. The site waters: SID, BEN, BOW and INT were also maintained around pH of 8.0 in the four-week cone experiments for *H. azteca* fed the TetraMin or periphyton diet (Table 4.4). The overlying water for the TNM, DUN, MCC, and ELT sites were around circumneutral pH for animals fed a TetraMin or periphyton diet (Table 4.4). The same was true of the overlying water from the QKE site for animals fed periphyton. For animals reared on a TetraMin diet for the CTR and QKE sites, pH was around 6.1 and 6.2, respectively (Table 4.4).

Conductivity ranged between 430-480 μS/cm in cones with the SAM overlying water. Similar to the observations in the field waters, the major ion and conductivity measurements were on average lower for site waters collected near Elliot Lake compared to sites near Bancroft (Table 4.3; Table 4.4). For the BEN and BOW sites, conductivity was higher than the SAM overlying water. This is not surprising given that major ions Ca and Mg were higher than those in the SAM water for these sites, also explaining the higher hardness levels at these sites (Table 4.4).

Dissolved organic carbon was on average 2-3 times lower in the SAM overlying waters, compared to the site waters. The only exception to this was QKE-SAM (10 mg/L) where DOC was 2.5 times higher compared to the site waters (4.1 mg/L; Table 4.4). With the exception of the BEN and BOW sites, the opposite was true for DIC and alkalinity measurements, whereby these measurements where on average higher for the SAM versus site waters (Table 4.4).

4.3.2 Metal concentrations in field collected lake water samples

Dissolved metal and metalloid concentrations for the surface water collected at the various lakes in the Elliot Lake and Bancroft regions are reported in Table 4.5. The dissolved metals and metalloids concentrations in the surface water were compared to available freshwater Ontario Provincial Water Quality Objectives (PWQO) for Ag, Al, As, B, Be, Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, Se, Tl, U, V, Zn and Canadian Water Quality Guidelines (CWQG) for Ag, Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Se, Tl and Zn, which both report these values on a total metal concentration basis (MOEE,1999; CCME,1999). Comparing dissolved versus total metal concentrations values should not affect the overall intent of the guidelines, which is to protect all forms and aspects of aquatic life and life stages during any duration of metal exposure via the water (MOEE, 1999; CCME, 1999), especially given that the dissolved or the free ion form of the metal is usually associated with causing adverse effects to the organism. In other words, if the dissolved form exceeds the total metal concentration values of the guidelines this suggests that the environmental conditions may not be appropriate for supporting aquatic life (MOEE, 1999).

For the CWQG, the guidelines are derived using the lowest observable effect level (LOEL) based on chronic studies using a non-lethal endpoint (e.g. growth and reproduction) for the most sensitive life cycle/stage of the most sensitive native Canadian aquatic species studied, and then multiplying this value by an uncertain safety factor of 0.1. Alternatively, the guideline may be set by using the median lethal (LC50) or median effective concentration (EC50) from an acute study for the most sensitive species and then multiplying by an acute/chronic ratio (CCME, 1999). If there are limited data for a particular contaminant an interim guideline is derived (CCME, 1999). The PWQG are derived in a similar manner (OMOE, 1992).

The majority of the dissolved metal and metalloid concentrations in the surface water collected in the field were well below the set PWQO and CWQG, except for the metals B, Cd and U (Table 4.5). Boron concentrations exceed the PWQO of 18500 nmol/L by 1.8 fold only at BEN1 (Table 4.5). Boron concentrations were also close to PWQO guideline for BOW1 (17000 nmol/g), but did not exceed this value (Table 4.5). Cadmium concentrations were at the CWQG interim value of 0.04 nmol/L, based on a hardness of 10 mg/L as $CaCO_3$, for TNM (0.04 nmol/L) and DUN (0.03 nmol/L), and exceeded the CWQG interim value of 0.07 nmol/L for Cd at ELT (0.09 nmol/L; Table 4.5).

The highest dissolved U concentrations in the water were found at the BOW1 (130 nmol/L), BEN1 (110 nmol/L) and QKE1 (4.1 nmol/L; Table 4.5). For the BOW1 and BEN1 sites, these U concentrations exceeded the PWQO of 21 nmol/L, by 6 and 5 fold, respectively (Table 4.5).

Centre Lake, a reference lake in the Bancroft area, did have elevated Fe levels of 3500 nmol/L in its surface water. However, these concentrations were approximately 1.5 times lower than both the PWQO and CWQG of 5400 nmol/L for Fe (Table 4.5).

Dunlop Lake, a reference lake in the Elliot Lake area, had elevated concentrations of Cr (15 nmol/L) compared to the other lakes in the study. However, this value was below the PWQO and CWQG for Cr (VI) of 19 nmol/L and Cr (III) 170 nmol/L (Table 4.5). Chromium redox speciation was not measured in the water so it cannot be concluded if the dissolved fraction of Cr was in the Cr (VI) or

Cr (III) form, with the Cr (VI) form being more toxic at lower concentrations than Cr(III). Although, there are no guidelines for Sr, this metal was between 10-200 times elevated at BEN1 and BOW1 compared to the other sites sampled in this study (Table 4.5).

4.3.3 Metal concentrations in overlying water of the cone experiments

Metal concentrations in the site or SAM overlying waters in the cone experiment are shown in Table 4.6. The overlying water and *H. azteca* (see below) data were pooled together for the different sediment sampling sites within each lake for the sediment cone toxicity tests. This was done due increase the sample size..

The metals concentrations of Al, Ga, Mn, Pb and V concentrations were often higher in the SAM overlying water when compared to the site waters collected near the Elliot Lake and Bancroft areas. Selenium was always higher in the SAM overlying water with the Bancroft area collected sediments, while U concentrations were higher in the SAM overlying waters with the sediments collected near the Elliot Lake area (Table 4.6). The higher concentrations of these metals in the SAM overlying water are probably due to the complexation of these metals to carbonates with increasing pH and hardness in the water. Based on the available PWQO and the CWQG, the majority of the metals and metalloids concentrations in the overlying SAM and site waters were below the guidelines except for Cd concentrations in the overlying SAM water at DUN, and Al, B, Cd, Cu, and U at various overlying site waters near the Elliot Lake and Bancroft areas (Table 4.6). Al concentrations exceeded the CWQG at the DUN, QKE and CTR-site when adjusting for pH (<6.5; Table 4.6). For instance, Al concentrations were four times higher than the CWQG at CTR-site.

Boron concentrations exceeded the PWQG at BEN and BOW sites for animals fed the TetraMin or periphyton diets (Table 4.6). Cadmium concentrations exceeded the CWQG for most sites in Elliot Lake area in the overlying site water and only the CTR-site near Bancroft after accounting for hardness (Table 4.6). Cadmium concentrations exceeded the CWQG when adjusted for hardness for DUN-SAM. Similarly, Cu concentrations in the overlying site water exceeded the PWQG and CWQG at some sites near Elliot Lake when adjusted for hardness (Table 4.6). For example Cu concentrations exceeded the PWQG and the CWQG by 2.5 and 1.3 times, respectively at the ELT-site (Table 4.6). Uranium concentrations only exceeded the PWQG at the BEN and BOW Lake using the overlying water sites and for animals fed the TetraMin or periphyton diets (Table 4.6).

4.3.4 Sediment concentrations and analysis

Sediments at the deepest part of QKE Lake were not collected due to unsafe weather conditions (i.e. too windy) at the time of collection. Sediments at 20 meters for QKE were obtained but not measured for total organic carbon and particle size due to a potential risk of radionuclide exposure to the operator during analysis. Organic carbon was always higher than the inorganic carbon for all sites, with sediments sampled near Bancroft having a higher total carbon measurements compared to the Elliot Lake area sites (Table 4.7). The presence of a higher density of cottages and smaller lake area and lake depth or the smaller watershed to smaller lake area ratio near the Bancroft sampling sites compared to those near Elliot Lake area may partly explain the higher organic content at Bancroft (Table 4.2; Table 4.7; Fig.4.2-4.3).

In terms of the particle size distribution (Table 4.7), the majority of the lakes in the Elliot Lake area were classified as clayey silt, with the deepest site at DUN1 being sandy silt. All lakes near Bancroft were classified as silty clay (Table 4.7). Moisture content in the sediments ranged between 80-95% for all sites (Table 4.7).

Metal concentrations in the sediments for the different lakes are shown in Table 4.8. The concentrations for the metals and metalloids: As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the sediments were compared to available freshwater Ontario Provincial Sediment Quality Guidelines (PSGQ): Severe Effect Level (SEL) and Lowest Effect Level (LEL; Jaagumagi, 1992). The SEL is the concentration of the metal in the sediment that is detrimental (i.e. may cause death) to 95% of benthic species, while the LEL is the metal concentration in the sediment which 5% of benthic species cannot tolerate (i.e. there is a 5% biological (toxic) response to the benthic species; Jaagumagi, 1992). These levels were derived using the Screening Level Concentration (SLC) approach, an effects-based approach, developed by Neff et al. (1986), whereby a frequency distribution of the metal concentrations collected at different field sites and in co-occurrence with the presence of benthic species is used to calculate a species SLC (SSLC) using the 90th percentile, where 90% is the metal concentration that can be tolerated by a specific species. From here another frequency distribution is generated using the SSLC for each species, with the 5th percentile of this SSLC frequency distribution being set as the LEL and the 95th percentile being set as the SEL (Neff et al., 1986).

Sediment metal concentrations were also compared to the national freshwater Canadian Sediment Quality Guidelines (CSQG) Interim Sediment Quality Guideline (ISQG) and Probable Effect Level (PEL) for the available metals and metalloids As, Cd, Cr, Cu, Pb, and Zn. The ISQG (or Threshold Effect Level (TEL)) is the concentration at which adverse biological (toxic) effects, such as death, rarely occur to aquatic species. It is derived by taking the geometric mean of the 15th percentile concentration of a biological effect (i.e. survival, growth, reproduction, etc.) data set and the 50th percentile concentration of a biological no-effect data set (CCMC, 1999). The ISQG is derived only when data are available, but limited in terms of the sediment type and/or characteristics, given that these factors can modify and influence metal bioavailability and toxicity to aquatic species (CCME, 1999). The PEL, on the other hand, is the level at which adverse biological (toxic) effects frequently take place to aquatic species. This level is calculated by taking the geometric mean of the 50th percentile concentration of the biological effect data set and the 85th percentile concentration of the biological no-effect data set (CCME, 1999).

The metals Al, Fe, and Mn were consistently higher on a molar basis compared to other 24 metals at both the Elliot Lake and Bancroft sampling regions (Table 4.8). For the metals Fe and Mn, the majority of these high concentrations were found in lakes near decommissioned mines, more so for the Elliot Lake region compared to the Bancroft region (Table 4.8). These elevated Fe and Mn concentrations may be associated with the solubilization of these metals that probably took place in the past when the U ore was acid leached (Dreesen et al., 1982).

Currently there are no PSQG and CSQG for the metal Al. According to PSQG, Fe levels for the Elliot Lake region were all above the LEL (380 μmol/g dw), with two sites in this region, notably MMC2 and QKE1, exceeding the SEL (780 μmol/g dw), by 1.4 and 5.8 times, respectively (Table 4.8). Iron levels near the Bancroft region only exceed the PSQG-LEL at BOW sites (Table 4.8). All other sampling sites near Bancroft were below the PSQG-LEL (Table 4.8).

Manganese concentration in the sediments, exceeded the PSQG SEL of 20 μmol/g dw for the majority of the sites, except at DUN2, MCC1, ELT1, QKE1, CTR1 and CTR2 (Table 4.8). The PSGQ LEL of 8.4 μmol/g dw for Mn was reached at all the sites near Bancroft and Elliot Lake, except for CTR2 (Table 4.8).

For both the Elliot Lake and Bancroft regions As concentrations were at or above both the CSQG-ISQG (0.08 μmol/g dw) and PSQG-LEL (0.08 μmol/g dw) for all sample sites (Table 4.8). The CSQG-PEL, however, was at or exceeded the DUN1, ELT1, QKE1 and CTR1 sites, while DUN1 and QKE1 exceeded the PSQG-SEL for As, by 1 and 2 times, respectively (Table 4.8). Similarly, Cd concentrations exceed or where at the CSQG-ISQG (0.01 μmol/g) and PSQG-LEL (0.01 μmol/g) for all sites except the QKE1 sites, which was below the ISQG and LEL value (Table 4.8). Only the DUN1, a reference site, exceeded the CSQG-PEL for Cd. No site exceeded the PSQG-SEL (0.09 μmol/g dw) for Cd (Table 4.8).

The DUN1 site also exceeded the CSQG-PEL (0.44 μmol/g) for Pb (Table 4.8). Mean Pb concentrations exceeded both the PSQG-LEL (0.15 μmol/g dw) and CSQG-SEL (0.17 μmol/g dw) at all sediment sampling sites (Table 4.8). Only QKE1 and BOW1 exceeded the PSQG-SEL (1.21 μmol/g dw) by approximately 1.7 and 4.0 times, respectively for Pb. In terms of the CSQG-PEL (0.44 μmol/g dw), DUN1, QKE1, CTR1, SID1, SID2, and BOW1 and BOW2 exceeded this level for Pb (Table 4.8).

Interestingly, sediments from the reference lake, TNM, had high concentrations of metals present, most notably Cr and Ni at the TNM1 Site (Table 4.8). The concentrations of Cr and Ni at the TNM1 site were on average 10 and 27 times higher, respectively, compared to concentrations measured in sediments at different sampling sites in the Elliot Lake region (Table 4.8). The mean Cr concentration in the sediment (7.9 μmol/g dw; Table 4.8) was approximately 4.6 times higher than the CSQG-PEL of 1.7 μmol/g dw. Overall, Cr concentrations were above the PSGQ LEL of 0.50 μmol/g, and below the PSQG SEL of 2.1 μmol/g dw for all other sampling sites near Elliot Lake (Table 4.8). Chromium levels, however, were below PSGQ LEL for all sampling sites in the Bancroft region (Table 4.8).

The mean Ni concentrations were at or above the PSQG LEL of 0.27 μmol/g dw for all sites in the Elliot Lake region and the BOW, SID and BEN sites near Bancroft (Table 4.8). The TNM1 was the only site that exceeded the PSQG SEL of 1.28 μmol/g dw for Ni (Table 4.8). The mean Ni concentration for the TNM1 sediment was approximately 14 times higher than PSQG-SEL (Table 4.8). No available CSQG were available for Ni.

Mean Cu concentrations were above both the PSGQ-LEL (0.25 μmol/g dw) and CSQG-ISQG (0.56 μmol/g dw) at all sampling sites, except for the INT and CTR2 sites where mean Cu concentrations in the sediment were below the CSQG-ISQG. Only the TNM2 and QKE1 exceeded the PSQG-SEL (1.73 μmol/g dw) for Cu by approximately 2 and 1 times, respectively. No sites exceeded the CSQG-PEL (3.1 μmol/g dw) for Cu in this study.

The mean Zn concentrations were all above the PSQG-LEL (1.84 μmol/g dw) and the CSQG-ISGQ (1.88 μmol/g dw), but on average 4.2 and 1.6 times below the PSQG-SEL (12.5 μmol/g dw) and CSQG-PEL (4.82 μmol/g dw), respectively (Table 4.8).

Uranium concentrations in the sediment were between 10-80 times higher for ELT2, QKE1, BOW1 and BOW2 sites when compared to the references lakes (Table 4.8). Although there are currently no sediment quality guidelines for U in Ontario or Canada, the severe effect level (SEL) value of 14.3 μmol/g dw for U derived in the Thompson et al. (2005) study using the SLC approach for U released from U mining and milling activities in Northern Saskatchewan and Ontario was not exceeded at any of the study area sites. But the LEL of 0.13 μmol/g for U estimated in that study was exceeded at ELT2, QKE1, BEN1, BOW1 and BOW2 sites (Table 4.8; Thompson et al. 2005).

Thompson et al (2005) also derived the LEL and SEL for the metals Mo, Se and V. When the Mo, Se and V concentrations in the sediment in this study were compared to the Thompson et al. (2005) estimates, Mo concentrations only exceeded the LEL of 0.09 μmol/g dw at TNM1, SID1, SID2, BEN2, BOW1 and BOW2, with no site breaching the SEL of 5.6 μmol/g dw. Nevertheless, the LEL of 0.01

and 0.54 μmol/g dw for Se and V, respectively, were exceeded at all sites in the Bancroft and Elliot Lake area (Table 4.8; Thompson et al., 2005), with the SEL of 0.06 μmol/g dw and 1.5 μmol/g dw for Se and V, respectively, being exceeded at QKE1, and TNM2 for Se and MCC1, MCC2 and ELT1 for V (Table 4.8; Thompson et al., 2005).

4.3.5 Metal concentrations in field Periphyton and TetraMin

The metals Al, Fe and Mn were the highest compared to the other 24 metals analyzed in the periphyton collected near the Elliot Lake and Bancroft sites (Table 4.9). The confidence intervals in Table 4.9 were very wide making it very difficult to determine reliable metal concentrations estimates in the periphyton. Metal concentrations in the TetraMin were very low in comparison to those measured in the periphyton (Table 4.9). For example, U concentrations in the TetraMin were about 200 times lower than that for the lowest U concentrations measured in the periphyton collected at DUN3 (Table 4.9).

4.3.6 Metal Concentrations in H. azteca in the field

Hyalella azteca were collected at all sites except QKE Lake. The absence of the *H. azteca* at QKE Lake was probably due to the sparse macrophytes along the shoreline at this site. This is not the ideal habitat to find *H. azteca* due to limited food source and coverage from predation (Strong, 1972). Out of the 27 metal concentrations measured in these animals for the Elliot Lake region, the metals Al, Be, Cr, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Sb, V, and Zn were found to be the highest in the tissues of the *H. azteca* collected at TNM (Table 4.10). *Hyalella azteca* collected from MCC Lake had the highest metal concentrations in their tissues for the metals Ag, Bi, Co, La, Rb, Se, Tl and U when compared to the other lakes in this region (Table 4.10). For instance, the concentrations of Fe and Pb measured in the *H. azteca* at TNM were about four times higher than the metal concentrations measured in *H. azteca* collected at DUN, MCC and ELT Lake (Table 4.10). Similarly, Ni and V concentrations were about six and three times, respectively higher at TNM compared to the other Lakes in the Elliot Lake region (Table 4.10). In addition, Co concentrations in *H. azteca* at MCC Lake were about three times higher compared to the other sites near Elliot Lake. Interestingly, *H. azteca* collected at DUN, a reference lake, had Cd concentrations that were on average three times higher than Cd concentrations at other sites within the Elliot Lake region (Table 4.10).

In comparison to the Elliot Lake sites, metal concentrations in *H. azteca* collected near Bancroft were on average lower, except for the metals B, Li and Sr. Both B and Li in *H. azteca* at the Elliot Lakes sites were below detection limits (BDL; Table 4.10). The highest metals concentrations in the *H. azteca* collected in the Bancroft region were found at CTR, a reference lake, for the metals Cd, Co, Mn, Pb, Sb, and Se. Cadmium and Mn concentrations were six times and three times, respectively higher in *H. azteca* collected from CTR in comparison to the *H. azteca* collected at SID, BEN, BOW, and INT. The metals B, Sr, U, V and Zn were the highest in *H. azteca* collected at BEN, while the metals Be, Cu, Fe, La, Li, were the highest at BOW. The metals Cr, Mo, Rb, and Tl were reported to be the highest in the *H. azteca* collected at INT (Table 4.10).

Alves et al. (Chapter 3) found that there was a significant relationship between body size and U bioaccumulation. Table 4.10 reports both standardized (US) and non-standardized U-body concentrations in *H. azteca*. Body size was standardized to 0.20 mg dw, the geometric mean when the dry weights of all the animals collected in the field from both study areas were pooled together. U concentrations in the standardized and non-standardized animals did not vary by much (Table 4.10). This may be because the animals at these sites were similar in size. The three highest U concentrations reported in the *H. azteca* from highest to lowest were collected at BEN (2.5 nmol/g), MCC (1.9 nmol/g) and BOW Lake (1.5 nmol/g; Table 4.10).

4.3.7 Sediment Toxicity Tests: Metal Bioaccumulation and survival

For the majority of sites, metal concentrations in *H. azteca* exposed to the SAM water were on average lower than those exposed to the site waters (Table 4.11). *Hyalella azteca* exposed to the overlying site water and fed TetraMin or periphyton diets had approximately the same or lower total metal concentrations, on average, in their bodies when compared to total metal concentrations measured in the field collected *H. azteca* (Table 4.10; Table 4.11). The exception to this was for the metals and metalloids concentrations of Ag, As, Ba, Cd and Fe in the Bancroft region, whereby these concentrations were consistently higher in the *H. azteca* used in the sediment toxicity tests than the field collected *H. azteca* (Table 4.10; Table 4.11). In addition, Cd bioaccumulation in the *H. azteca* used in the cone sediment toxicity tests for the DUN-site was the same as that measured in the field collected *H. azteca*, at around 430 nmol/g dw, a Cd body concentration that was close to the four-week lethal body concentration causing 25% mortality (LBC25) effect level of 640 nmol/g dw derived in Borgmann et al. (2004; Table 4.10; Table 4.11).

Uranium concentrations in the cultured *H. azteca* were also standardized to 0.30 mg dw, the geometric mean when the dry weights for all the animals from both study areas were pooled (Table 4.11). U concentrations in the standardized and non-standardized animals did not change by very much. Uranium bioaccumulation in *H. azteca* (Table 4.11) was on average between 2-20 times lower than those of the *H. azteca* collected in the field (Table 4.10).

Animals fed the periphyton diet had on average about the same or higher total metal concentrations in their bodies than animals reared on TetraMin diet at both the Elliot Lake and Bancroft study regions (Table 4.11). Due to the wide variation in the confidence value and the small sample size (n=1), in some cases (Table 4.10; Table 4.11), a significant difference in total metal accumulation between animals fed the different diets could not be inferred. The same was true when trying to determine if there was a difference between metal accumulation in *H. azteca* exposed to the overlying site waters versus those exposed to the overlying SAM waters (Table 4.11).

For *H. azteca* exposed to the overlying SAM water and fed TetraMin, survival was maintained between 90-98% for all sites (Fig.4.4). Survival for the site waters was at or below 50% for all lakes, except at BEN (82%), BOW (72%), and MCC (55%) for animals reared on TetraMin (Fig.4.4).

Mean survival for animals exposed to overlying site water and fed contaminated periphyton was at or below 60% (Fig. 4.4). For ELT, MCC and INT, mean survival was similar between those fed TetraMin versus those fed the contaminated periphyton (Fig. 4.4). Metal concentrations in the *H. azteca* were also approximately the same for animals fed the TetraMin versus periphyton diet at these sites (Table 4.11). For QKE-SITE mean survival (23%) was higher in animals fed the periphyton diet than those fed the TetraMin diet (7%). Survival was around 13% and 27% for animals fed periphyton and exposed to site water from BEN and BOW, respectively. These mean survivals were about 6.3 and 2.7 fold lower than for animals exposed to site water and fed the TetraMin diet. When comparing the metal body concentrations in the animals fed the periphyton versus those fed the TetraMin for the BEN and BOW sites, metal body concentrations were similar except for the metals Al and As, which were on average about four times higher in animals fed the contaminated periphyton (Table 4.11).

4.4 Discussion

4.4.0 Bioaccumulation and sediment to water saturation models for U

Uranium concentrations measured in cultured *H. azteca* exposed to waters and sediments collected in the field at purportedly contaminated sites, with the exception of BEN and BOW, were at or close to the mean background concentration (95% confidence interval) of 0.12 (0.03) nmol/g dw measured in *H. azteca* exposed to reference overlying waters and sediments (Table 4.11; Table 4.12). This mean background concentration in *H. azteca* was similar to the background concentrations of 0.09 to 0.16 nmol/g and 9.2 to 13 nmol/g in *H. azteca* reported by Alves et al. (2008) and Roberston and Liber (2007), respectively.

Uranium concentrations at some sites (Table 4.10; Table 4.11) were also close to the background U water concentrations of 0.03 to 0.99 nmol/L measured at the reference sties in this study and in the Alves et al. (2008) study, where background concentrations in the overlying water ranged between BDL to 0.47 nmol/L (Table 4.10; Table 4.11). This may explain, why at some sites such as MCC, the observed versus predicted ratios were close to unity when applying the water-bioaccumulation saturation model proposed by Alves et al. (2008; Table 4.12). Taken together, the low concentrations of U in the *H. azteca* and water in this study are not sufficient to properly test the water-bioaccumulation saturation model. The U bioaccumulation and water data should be significantly higher than background concentrations. For example, U concentrations in *H. azteca* and water in this study were on average 90 times lower than mean LBC10 for *H. azteca* (29 nmol/g) and 50 times lower than the mean LC10 (72 nmol/L) estimated in Alves et al. (2008) study using 10SAM; a water medium that was more sensitive to U toxicity.

Only the BEN and BOW sites had measured U concentrations in field collected *H. azteca* and *in situ* lake water, were high enough to test the water-bioaccumulation saturation model, using a max of 73 nmol/g and the a' and b' estimates of 0.042 pmol/L and 2.02E+05 from the Alves et al. (2008) study (Table 4.1; Table 4.12). The observed/ measured ratios were 0.9 and 0.3 for the BEN and BOW Lakes, respectively. This suggests that the model could potentially be used in a first-tier risk assessment given that the observed accumulation matched the predicted accumulation within a factor of 3.3.

The bioaccumulation-saturation model was tested using data from Robertson and Liber (2007), who exposed caged *H. azteca in situ* to surface and pore water for 4 days near an active U operation (Rabbit Lake) in Saskatchewan, Canada. Uranium concentrations in the Roberston and Liber (2007) study were significantly above background levels in the water and *H. azteca* and within the U concentrations used to derive that the water-bioaccumulation model in Alves et al. (2008) study. The overall observed/predicted ratios were within a factor of 4.6 using the Robserton and Liber study after adjusting for steady-state (Table 4.12; Chapter 3). The water-bioaccumulation model under-predicted U bioaccumulation in the *H. azteca* at all sites, except the Horseshoe Pond surface water site (Table 4.12). This can be problematic, despite the observed/predicted ratios being within a factor of 4.6, because this would result in an under-estimation of U toxicity in the *H. azteca* by a factor of up to 4.6. Nevertheless, the water-bioaccumulation saturation model may be a potentially good tool in a first-tier risk assessment to predict toxicity in *H. azteca* exposed to U concentrations in the water phase (pore or surface water).

Diet may have a significant effect on metal accumulation in aquatic animals (Wang and Fisher, 1999) and thus affect the water-bioaccumulation saturation model predictions. Borgmann et al. (2007) investigated the relative contribution of food collected in the field and water to the bioaccumulation of 27 metals and metalloids in caged *H. azteca* near rivers affected by metal mining and found that Cd, Cu and Se were the only metals in the food that had a significant effect on whole body accumulation. Despite there being a 6.5 fold concentration range of U in the food in the Borgmann et al. (2007) study, there was no effect of U concentrations in the food on total U concentration in the *H. azteca*. This is most likely the case in this study given that U bioaccumulation in the *H. azteca* was quite constant between animals fed the TetraMin versus the periphyton diet after accounting for body-size (Table 4.11) and given that U concentrations of up to 1100 nmol/g were reached in the periphyton at some sites (e.g. QKE and BOW; Table 4.9). For example, the *H. azteca*/periphyton ratio was 3.0E-03 for the QKE and BOW sites whereby U concentrations in periphyton was 1100 nmol/g dw and U concentrations in size-standardized (US) *H. azteca* were around 2.8 (Table 4.9; Table 4.11). This suggests that U in the periphyton was not bioavailable to *H. azteca*. It also indicates that U in the diet did not have an influence on the water-bioaccumulation saturation model predictions.

The water-sediment partitioning saturation model proposed by Alves et al. (2008) under-predicted U concentrations in the sediment when using U water concentrations measured in the overlying SAM water, and for most sites using the site overlying water, while over-predicting U concentrations in the sediment using the lake water (Table 4.12). However, the water-sediment saturation model cannot be tested critically with the majority of naturally contaminated sediments collected in this study, except for the BEN and BOW sites using the lake water, and the BOW site using site water, because U concentrations in the overlying and lake waters, as mentioned above, were low when compared to the Alves et al. (2008) study. When testing the water-sediment partitioning model, the observed/predicted ratio was 2.0 and 1.1 for the BEN and BOW sites, respectively, using U concentrations measured in the site overlying water (Table 4.12). However, the observed/predicted ratio was 0.03 for the BEN and 0.35 for the BOW site when using U concentrations in the lake water to test the water-sediment partitioning model. This suggests, at least for the U concentrations measured in the lake water, that the uranyl ion used to spike the sediments in the Alves et al. (2008) study had a higher binding to the spiked-sediments than in the field sediments. This is more likely due to U being more available to bind during the sediment spiking procedure than in the field. Taken together, this suggests that the water-sediment saturation model derived in the Alves et al. (2008) study cannot be used with natural contaminated sediments to predict toxicity because the partitioning of U from field sediments into water will be less than predicted using the water-sediment saturation model, and thus toxicity will be under-estimated.

Although U redox speciation in the sediment was not determined, the sediment surface was oxygenated for two weeks prior to the start of the experiment and throughout. Thus, there is reason to believe that the surface-bound U would most likely be in the U(VI) form, given U(VI) is the major form of U in oxic environments, whereas U(IV) is the major form of U in anoxic environments (Markich, 2002). However, if U was still in the U(IV) form, this may explain the lower U concentrations in the SAM water and sites with similar pH (Table 4.5; Table 4.6), because the U(IV) can easily precipitate and remain immobile (Markich, 2002) in the sediment. However, the low concentrations of U dissolved from the sediment to the SAM water and to sites of similar pH may also be due to the mere fact that U concentrations in the sediment were low and potentially not available to partition into the overlying water at higher pH (8).

When measured U concentrations above background levels in the surface water from the Robertson (2006) study were used to test the water-sediment partitioning saturation model, the observed/predicted ratios were within a factor of four for these sites (Table 4.12). However, for the sites using U concentrations above background in the pore water (Robertson, 2006), the observed/predicted ratios were around 0.04 and 0.1 (Table 4.12). Thus, the water-sediment partitioning saturation model appears to work for U concentrations in the surface water, but not for U concentrations measured in the pore water. Given that the observed concentrations of U in the sediment (Csed) were the same using the surface versus the pore water, a plausible reason as to why the water-sediment partitioning saturation model predicted higher U concentrations in the sediment using the pore water is a higher concentration of DOC or humic substances in the pore water than the surface water. The DOC may be heavily complexing the U (Markich, 2002) and thus increasing U concentrations in the pore water. For example DOC concentrations in this study were on average ten times lower in the surface water, when compared to the pore water in the Robsertson (2006) study (Tables 4.3, 4.4). Taken together, this suggests that the water-sediment portioning model may be an appropriate first-tier assessment tool for regulators to quantify U concentrations and predict sediment toxicity under natural contaminated sediments with above background U surface-water, but not pore water measurements.

The data set used in this study and from other published sources are not adequate to sufficiently validate the above saturation models. A further development of the water-sediment partitioning saturation model may need to include other water chemistry parameters such as DOC in the water especially in instances were U concentrations in the pore water, rather than the surface water, are needed to predict U concentrations in the sediment.

4.4.1 Overall risk assessments for metals

The approach proposed in the Aquatic Effects Technology Evaluation program (AETE; ESG, 1999), a Canadian government-industry program that reviewed appropriate technologies for evaluating the impacts of mine effluents on the aquatic environment, can be used to assess the impacts of metals in the Elliot Lake and Bancroft regions. This is achieved by addressing the four questions: (1) Are contaminants getting into the system? (2) Are contaminants bioavailable? (3) Is there a measurable response? (4) Are the contaminants causing this response?

4.4.1.0 Are the contaminants getting into the system?

The concentrations of the metals in the water and sediment demonstrate that metals above background levels are present in the lakes sampled at Elliot Lake and Bancroft (Table 4.5-4.6; Table

4.8). However, sediment profiles were not conducted in this study to quantify temporal and spatial trends in contamination due to natural, pre-mining, post-mining and current metal concentrations. The sampling device used in this study, the mini-polar grab, does not sample from a defined depth. Thus, the sediment in these grab samples is most likely a mixture of surface and pre/post-industrial sediment that are variable and not equally distributed. Therefore, it cannot be concluded that metals that were measured in the sediment collected in this study were from recent metal depositions. As such, the metal and metalloids measured in the sediments are not sufficient to definitively answer the question "are the contaminants getting into the system?" in this study.

Consequently, measured metals and metalloids concentrations in the surface water can be useful to answer the question "are contaminants getting into the system?" When the geometric mean of the metals and metalloids measured in the field collected water (Table 4.5) near Elliot Lake and/or Bancroft were compared to the metal and metalloids concentrations measured in the lakes surrounding the decommissioned mines/mills and tailings management areas, it appears that some metals and metalloids may still be getting into the system. For instance, the metals and metalloids Al, As, B, Ba, Be, Ga, La, Li, Mo, Ni, Rb, Se, Sr, Tl, U , V and Zn measured at the various contaminated sites near Elliot Lake area were elevated when compared to the reference lakes, TNM and DUN (Table 4.5). For the Bancroft area the metals and metalloids B, Cr, Ga, Li, Mo, Ni, Rb, Sr, and U were higher in the contaminated lakes than the reference lakes, CTR and SID (Table 4.5). It is suggested that the higher levels of the metals and metalloids in the contaminated sites versus those of the reference lakes (Table 4.5) may be due to metals/metalloids run-off from the decommissioned U mine/milling and tailings management areas or potentially the desorption of the metals/metalloids from the sediment, given that the majority of the elevated contaminates at these sites (e.g. As, Mo, Se and V) are mostly associated with U mining and processing activities. The use of statistics could be inferred here to determine if there were any significant difference between metals concentrations in the contaminated and the reference lake. However, the sample size (n) was three for each individual site near Elliot Lake and two for the Bancroft region making the statistics unreliable.

Interestingly, the metals Al, Co, Fe, La and Li were between 8-25 times and 10-50 times higher in the water at CTR Lake when compared to the reference lake (i.e. SID), and the contaminated sites (i.e. BEN, BOW, and INT; Table 4.5), respectively, for the Bancroft study area. These high metals at CTR Lake could not be explained, especially given that the sampling site for this reference lake was upstream of the tailings management area and the decommissioned mine (Fig. 4.3).

4.4.1.1 Are contaminants bioavailable?

The metals As, Fe, Mn, Cd, Cr, Pb, Ni, Zn and U exceeded the PSQG-PEL and the CSQG-ISQG at some sites (e.g. TNM, DUN, BEN, BOW; Table 4.5), but this does not guarantee that these metals are bioavailable to cause an adverse effects to benthic organisms. The sediment compositions for the majority of sites in this study were composed mainly of clay and silt particles (Table 4.7). Clay sediments, for example, are known to retain metals more strongly than sandy sediment thus rendering some metals to be less bioavailable to cause adverse effects to organisms. Complexation of U to organic carbon and Fe and Mn oxides in aerobic sediments and to sulfides associated with Fe in anaerobic sediments also influence metal bioavailability (Sibley et al., 1996). The elevated levels of Fe and Mn in the sediments at the majority of sites in this study compared to the other metals analyzed, may have limited the bioavailability of other metals. This is because Fe and Mn oxides are considered major scavengers of other metals such as Cu, Pb, Ni and Zn under aerobic conditions (Tessier et al., 1996).

The influence of overlying water chemistry may also influence metal bioavailability in the sediment, especially when the sediment concentrations are below the SQG. For instance, Alves et al. (2008) found that with increasing overlying water pH (around 8), the desorption of U from the sediment into the overlying water increased. However, the majority of U in the overlying water was not bioavailable because the uranyl ion was probably bound as a calcium-carbonate complex (~62-68%).

A more direct way to answer the question "are the contaminants bioavailable" is to measure metal bioaccumulation in the animal. This is because the effects of sediment and water chemistry on metal bioavailability, speciation and uptake in the animal are all taken into account (Borgmann et al., 1991; Borgmann et al., 1998; Borgmann and Norwood, 1999; Borgmann, 2000; Alves et al., 2008). When comparing metal concentrations measured in *H. azteca* collected in the field (Table 4.10) versus cultured *H. azteca* used in the sediment cone toxicity tests, (Table 4.11), metal concentrations measured in the field were often higher than *H. azteca* measured in the cone sediment toxicity tests. However, these differences were not significant, given the wide variance between field collected *H. azteca* and cultured *H. azteca* (Table 4.10; Table 4.11). Thus it appears that some contaminants are bioavailable, because they were bioaccumulated in these animals.

What is interesting is that some metals appeared to be just as bioavailable, and sometimes more, at some of the reference sites when compared to the study sites. A case in point is Cd bioaccumulation at the DUN site for *H. azteca* collected in the field and in the sediment toxicity tests (Table 4.10; Table 4.11). At this site Cd concentrations in the *H. azteca* were the same at around 430 nmol/g dw, approximately 2.6 and 9.3 times higher than metal concentrations measured in the field-collected and sediment-test *H. azteca*, respectively, from the other contaminated sites near Elliot Lake area (Table 4.10; Table 4.11). Similarly, the same was true for CTR lake, a reference lake, whereby Cd bioaccumulation in the *H. azteca* collected in the field (9.9 nmol/g dw) or in the sediment toxicity tests (69 nmol/g) was about 6 and 22 times, respectively higher than in the tests with contaminated sites

(Table 4.10; Table 4.11). Overall, this suggests that there does not seem to be any impact due to mining, i.e. the reference lakes had higher metal concentrations than the contaminated sites, but some metals are nevertheless bioavailable at some of the sites, given that metal concentrations were measured in the *H. azteca* (Table 4.10; Table 4.11).

4.4.1.2 Is there a measurable response?

A benthic community structure analysis may confirm the presence of adverse effects to organisms under natural conditions. However, it cannot quantify or determine a specific cause-effect relationship between the organism and the metal concentrations. A more direct way to determine if the metal concentrations in the sediment and water are toxic to animals is to conduct a sediment toxicity test that exposes organisms to these contaminated sediments and waters. The only drawback of sediment toxicity tests are that the test conditions do not necessary reflect the conditions found in the field. Hence, a benthic community analysis or an *in situ* bioassay alongside a toxicity test may be required (OMOE, 2003).

Although, a benthic community analysis was not conducted in this study, sediment toxicity tests were. It terms of a measurable response via the sediment toxicity tests, there was no effect (i.e. mortality was greater than 90%) when animals were exposed to the natural contaminated sediments and the SAM overlying water (Fig.4.4). However, there were some effects (i.e. mortality) using the site overlying water, but this toxicity was not significant different ($P < 0.05$) between the contaminated sites and reference sites (Fig. 4.4). Thus, it cannot be claimed that this measurable response was due to past mining operations.

4.4.1.3 Are the contaminants causing this response?

The CSQG and PSQG are based on associative information, spiked-sediment toxicity data and field data, and not on a site specific basis, thus making it difficult to identify and evaluate a cause-effect relationship between a metal or metals and the biota (CCME,1999; Jaagumagi, 1992). They also do not take into consideration the effect of water chemistry. Therefore, comparisons of sediment metal concentrations with sediment quality guidelines may predict that an effect may be taking place at the site of interest, but not the cause of this effect. The same is true when comparing metal concentrations in the water to water quality guidelines.

If the critical body concentrations (i.e. LBC25) that cause toxicity for the different metals are known (Tables 4.10-4.11; Table 4.13) it may be possible to identify the metal or metals that are causing the measurable response. Studies (Borgmann et al., 1991; Borgmann et al., 1998; Borgmann and Norwood, 1999; Borgmann, 2000; Alves et al., 2008) with *H. azteca* and the metals Cd, Pb, Tl, and U have shown that metal bioaccumulation is a more reliable indicator of metal toxicity than water or sediment concentrations for metals that are not regulated or sequestered by the animal. For example, Alves et al. (2008) found that when *H. azteca* were exposed to different water chemistries, varying hardness and alkalinity independently, that the LC50 measurement in the water varied over 50 fold, while on a body concentration basis it varied only 2 fold. However, body concentrations cannot be successfully employed for metals that are able to be regulated such as Cu and Zn, or stored, because there may not be a clear relationship between bioaccumulation and toxicity (Borgmann, 2000).

According to Table 4.13, and ignoring the possible physiological regulation of Cu and Zn, one might suspect that there could be some form of adverse effect or toxicity taking place for the TNM-SAM and QKE-SAM treatments based on the calculated one-week LBC25 measured/effect ratio of 0.78 for Cu at TNM and 0.70 for Zn at QKE Lake. However, the sediment toxicity tests show no toxicity to *H. azteca* exposed to the SAM overlying waters from these sites and all other sites near the Elliot Lake and Bancroft region for the SAM overlying water (Fig. 4.4). For *H. azteca* whereby Cu and to an extent Zn concentrations are regulated by these animals, a bioaccumulation-toxicity relationship may not be suitable (Borgmann and Norwood, 1995; Borgmann, 2000). In these situations the overlying metal water concentrations and the critical water concentration from toxicity tests may provide additional information (Borgmann, 2000). In this case, both the Cu and Zn concentrations at the TNM-SAM and QKE-SAM were well below the LC25 and LC50 effect concentrations for Cu and Zn in the water (Table 4.13; Table 4.14).

Survival was around 72 and 82% for the BOW and BEN Lake, respectively, for *H. azteca* exposed to the site waters and fed TetraMin (Fig. 4.4). Despite U concentrations in the water exceeding the PWQO of 21 nmol/L, by 5 and 6 fold for BEN and BOW, respectively, the high pH (~8.0) and hardness (200-280 mg/L $CaCO_3$; Table 4.6) at these sites could have limited the bioavailability of this metal. For instance U bioaccumulation in the *H. azteca* (Table 4.11) was well below the LBC10 of 32 nmol/g for a similar water chemistry for *H. azteca* exposed to U-spiked sediments (Alves et al., 2008).

The low survival for CTR (8%) and QKE (7%) for animals reared on TetraMin and exposed to site overlying water may be partly explained by the low pH of 6 in the water, a pH value which the *H. azteca* do not tolerate (Table 4.4; Fig. 4.4). The concentrations of Al, Cd, and Pb in the site overlying water at CTR Lake and Cd, and Mn concentrations in the site overlying water for QKE Lake (Table 4.6) may have also contributed to the measurable response. The measured/effect LC50 ratio based on one-week toxicity tests and compared to the metals Al, Cd, and Pb measured at CTR Lake was 0.23, 0.19, and 0.22, respectively. The measured/effect one-week LC50 was 0.44 and 0.39 for Cd and Mn, respectively at QKE-site (Table 4.6; Table 4.14)

Cadmium bioaccumulation (69 nmol/g dw) in *H. azteca* exposed to CTR-SITE overlying water was approximately 0.11 the LBC25 of 637 nmol/g dw for Cd in soft water, while the measured/LBC25 effect ratio was 0.14 for Pb (Table 4.13). There is currently no LBC25 estimate for Al for *H. azteca*. Based on the available published data, it appears that the metals Cd and Pb may have contributed to the measurable response at CTR-SITE.

Cadmium and Mn bioaccumulation in the *H. azteca* exposed to the site overlying water at QKE were about 20 and 29 times lower than the four-week LBC25 of 637 and 4400 nmol/g for Cd and Mn, respectively (Table 4.11). The metal body concentrations for the metals Co, Cr, and Ni were elevated in the *H. azteca* exposed to QKE-SITE (Table 4.11). Although the water hardness was about 75 mg/L as $CaCO_3$ at QKE (Table 4.4), the measure/ LBC25 effect ratio was calculated to be 0.81, 0.41, and 0.79 for the metals Co, Cr and Ni, respectively, using a four-week LBC25 estimated in water hardness of 130 mg/L as $CaCO_3$ (Table 4.13). It may be suggested that the metals Co, Cr, and Ni may be exerting some adverse effects to the animals exposed to QKE water (Fig. 4.4).

Although there was a 50% survival of *H. azteca* exposed to the SID overlying site water and fed TetraMin, this measurable response could not be explained. Iron levels in the *H. azteca* (15000 nmol/g dw) were about 1.4 times higher at SID-SITE when compared to CTR-SITE and about 8 times higher at SID lake when compared to the other lakes in this region (Table 4.11). Gillan et al. (2004) found that the amphipod *Urothoe poseidonis* living in the burrows of *Echinocardium cordatum* frequently had Fe-encrusted coatings on their appendages and sternites in the winter. However, in the summer they were not coated with Fe. The uncoated amphipods in the summer were suggested to be related to the death of the Fe-covered amphipods (Gillan et al. 2004). The same can be taking place here, whereby Fe accumulates on the surface of the animals to an extent that it blocks physiology activities on the surface of the animals or prevents the animals from doing daily activities such as gathering and eating, thus explaining the 50% survival at SID-SITE (Fig. 4.4).

The low survival for the DUN-SITE (27%; Fig. 4.4) may be associated with Cd and Zn concentrations in the water given the measured/effect (LC50) ratio of 0.92 and 0.35, respectively for this site (Table 4.14). Cadmium bioaccumulation in the surviving *H. azteca* from the sediment toxicity tests (430 nmol/g) and those collected in the field (also 430 nmol/g) for the DUN-SITE were on average three times higher than other Cd concentrations measured at other sites (Table 4.10; Table 4.11). The measure/ LBC25 effect ratio was 0.68 for Cd at DUN-SITE (Table 4.14). Thus for the DUN-SITE it appears that toxicity may be partially due to Cd concentrations in the water, given that the bioavailability and toxicity of Cd to the *H. azteca* is primarily due to the dissolved metal (Warren et al., 1998).

Zinc bioaccumulation could have also contributed to a measurable response given a measure/ LBC25 effect ratio of 1.2 (Table 4.11; Table 4.13). However, this body concentration was similar to those for the animals in the SAM overlying water, where survival was greater than 90% (Fig. 4.4). This is probably because Zn may be partially regulated in the animal (Borgmann and Norwood, 1995) and thus not a good metal to use a bioaccumulation-toxicity relationship on (Borgmann, 2000). In fact, both Cu and Zn concentrations, which may be regulated by the *H. azteca,* were close or above the four-week LBC25 effect of 2200 nmol/g and 1800 nmol/g for Cu and Zn, respectively, for amphipods exposed to the site water (Table 4.11; Borgmann, 2004). However, these body concentrations were no different to Cu and Zn body concentrations measured in the *H. azteca* exposed to the SAM water, where survival was greater than 90% (Table 4.11; Fig. 4.4), and where measured Cu and Zn in the site overlying water were about 11-660 and 10-1200 fold lower than the four-week LC50 estimates for Cu and Zn, respectively, in hard water (Borgmann et al., 2004; Table 4.6).

The low survival at TNM (40%), ELT (37%) and MCC (55%; Fig. 4.4) for *H. azteca* fed TetraMin and exposed to the site overlying water could not be explained. However, Cd water concentrations were about the same (~0.30 nmol/L; Table 4.6) at these sites. The one-week LC50 measure/effect ratio was on average about 0.23 for these waters using the one-week LC50 of 1.3 nmol/L for Cd estimated in Borgmann et al. (2005; Table 4.14). When bioaccumulation measurements for the metals Co and Cr were compared to the four-week LBC25 estimates from Norwood et al. (2007) for TNM, the measure/ LBC25 effect was 0.61 and 0.23 for Co and Cr, respectively. Similarly, the measure/LBC25 effect measurement for Co was 0.24 for ELT. For INT the low survival of 40% could not be explained by the available data.

For ELT, MCC, and INT, mean survival was similar between those fed the TetraMin versus those fed the periphyton diets (Fig. 4.4). Metal concentrations in the *H. azteca* were also approximately the same for animals fed the TetraMin versus periphyton diet at these sites. For QKE mean survival (23%) was higher in animals fed the periphyton diet versus those fed the TetraMin diet (7%). Metal bioaccumulation for the metals Co, Cr, Mn and Ni were apparently higher in the animals fed the TetraMin diet than those fed the periphyton diet (Table 4.11) at QKE. The four-week LBC25 measure/effect ratio using the estimate (280 nmol/g dw) was 0.78 for animals fed the TetraMin diet (Table 4.11; Borgamann et al. 2004) at the QKE. Similarly, for Co and Cr, the four-week LBC25 measured/effect ratio was 0.81 and 0.42, respectively (Table 4.11; Borgmann et al., 2004). It could be suggested that Co, Cr and Ni were some of the metals that may have contributed to toxicity present at QKE for animals fed the TetraMin. However, toxicity could not be explained for those animals fed the periphyton diet.

Survival was around 13% and 27% for animals fed periphyton and exposed to site overlying water from BEN and BOW, respectively. These mean survivals were less than the mean survival measurements for the animals fed the TetraMin diet and exposed to the site water at BEN (82%) and BOW (72%) sites. When comparing the metal body concentrations in the animals fed the periphyton versus those fed the TetraMin for these sites, metal body concentrations were similar except for the metals Al and As, which were on average about four times higher in animals fed the contaminated periphyton (Table 4.11). The four-week LBC25 measured/effect ratio, using the LBC25 estimated in hard-water (83 nmol/g) for As was 0.45 and 0.37 for BEN and BOW, respectively (Table 4.14). A four-week LBC25 is not known for Al for *H. azteca*. One could suggest that the metals As and Al from the water and food were the contaminants responsible for this response, but the concentrations of these metals were comparable to the metal concentrations measured in the site water and periphyton for the other sites (Table 4.6; Table 4.9). The overall low survival in all the animals fed periphyton diets at all sites may be the result of the poor nutritional quality of periphyton and not the metals in the environment. In other words, periphyton may not have provided the adequate nutrition for growth and survival of *H. azteca*, whereas the TetraMin diet is specially formulated to provide the proper nutrition for this species.

Overall, the sediment toxicity tests do show that toxicity does exist at some sites in the Elliot Lake and Bancroft regions, however, the contaminants causing this measurable response could not be predicted for all sites. The only metal strongly implicated in contributing to toxicity is Cd at DUN. Low pH of about 6.0 at some of the sites (i.e. CTR-SITE and QKE-SITE) may have also contributed to the poor survival of the *H. azteca*. The sediment toxicity tests suggest that toxicity in these regions was not due to U concentrations in the sediment, water and *H. azteca* (Tables 4.6, 4.8, 4.11)..

4.5 Conclusion

A water-bioaccumulation model could not be properly validated to quantify metal concentrations in the *H. azteca* under natural conditions because most water and body concentrations of U were low and often close to background. The water-sediment binding model was suggested to be an appropriate model to predict sediment toxicity using natural contaminated sediments and surface U water measurements. It appears that metals Al, As, B, Ba, Be, Ga, La, Li, Mo, Ni, Rb, Se, Sr, Tl, U, and V may be entering the aquatic environment at some sites when compared to the reference lakes. Some of these metals were also bioavailable to the *H. azteca* when measured on a body concentration basis at some of the sites. There was toxicity present in this study, but it was not due to U concentrations in the sediment, water or bioaccumulation by the *H. azteca*. At some sites toxicity may have been partially due to the bioaccumulation of the metals Cd, Cr, Co or Ni by *H. azteca*, or to low pH. For other sites it could not be inferred if this toxicity was due to metal concentrations in the sediment, water or *H. azteca*.

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Table 4.1 Table of coefficients[a] for Eq.(s) 2, 5 and 6

Constant	Estimate
max (nmol/g dw)	73
a (pmol/L)	0.042
b	2.2E+06
a' (pmol/L)	0
b'	170
max" (nmol/g dw)	7500
a" (pmol/L)	0
b"	2.0E+05

[a] Estimates from Alves et al. (2008)

H^+ units in μmol/L

Table 4.2 Sampling locations of field collected sediment, water, *H .azteca* and periphyton

Location	Site ID	Laditude (N)	Longitude (W)	Depth (m)	Collection type[a]
Elliot Lake Area					
Ten Mile Lake[b]	TNM1	46 ° 31' 23"	82° 45' 55"	100	Sediment/water
(TNM)	TNM2	46 ° 31' 28"	82° 45' 55"	20.0	Sediment
	TNM3	46 ° 30' 15"	82° 48' 25"	0.30	Periphyton/*H.azteca*
Dunlop Lake[b]	DUN1	46 ° 29' 40"	82° 42' 32"	46.5	Sediment/water
(DUN)	DUN2	46 ° 29' 35"	82° 42' 18"	20.0	Sediment
	DUN3	46 ° 29' 18"	82° 42' 44"	0.30	Periphyton/*H.azteca*
McCarthy Lake	MCC1	46 ° 19' 42"	82° 26' 30"	29.5	Sediment/water
(MCC)	MCC2	46 ° 19' 39"	82° 26' 33"	20.0	Sediment
	MCC3	46 ° 19' 37"	82° 26' 40"	0.60	Periphyton/*H.azteca*
Elliot Lake	ELT1	46 ° 23' 34"	82°40' 55"	30.6	Sediment/water
(ELT)	ELT2	46 ° 23' 41"	82° 40' 56"	20.0	Sediment
	ELT3	46 ° 23' 50"	82° 40' 52"	0.80	Periphyton/*H.azteca*
Quirke Lake	QKE1	46 ° 27' 42"	82° 33' 38"	20.0	Sediment/water
(QKE)	QKE2	46 ° 30' 08"	82° 33' 08"	0.30	Periphyton
Bancroft Area					
Centre Lake[b]	CTR1	45° 00' 35"	78° 02' 54"	17.4	Sediment/water
(CTR)	CTR2	45° 00' 37"	78° 02' 51"	10.0	Sediment
	CTR3	45° 00' 39"	78° 02' 55"	0.70	Periphyton/*H.azteca*
Siddon Lake[b]	SID1	45° 01' 44"	77° 54' 22"	18.2	Sediment/water
(SID)	SID2	45° 01' 45"	77° 54' 26"	10.0	Sediment
	SID3	45º 01' 46"	77° 54' 29"	0.30	Periphyton/*H.azteca*
Bentley Lake	BEN1	45° 01' 35"	77° 54' 47"	11.5	Sediment/water
(BEN)	BEN2	45° 01' 35"	77° 54' 49"	10.0	Sediment
	BEN3	45° 08' 33"	77° 54' 56"	0.30	Periphyton/*H.azteca*
Bow Lake	BOW1	45° 01' 02"	77° 55' 29"	15.2	Sediment/water
(BOW)	BOW2	45° 01' 00"	77° 55' 31"	10.0	Sediment
	BOW3	45° 01' 05"	77° 54' 35"	0.60	Periphyton/*H.azteca*
Inlet Bay	INT1	44° 58' 49"	78° 01' 40"	15.7	Sediment/water
(INT)	INT2	44° 58' 57"	78° 01' 47"	10.0	Sediment
	INT3	44° 59' 08"	78° 01' 54"	0.50	Periphyton/*H.azteca*

[a] Field *H. azteca* were also collected for background metal analysis in the field

[b] Reference Lake

Table 4.3 Field collected water chemistry measurements

Field		Dissolved				Major Ion							as $CaCO_3$	
	Temp	O_2	pH	Cond.	Ca	Mg	Na	K	Cl	SO_4	DOC	DIC	Alk.	Hardness
	(°C)	(mg/L)		(μS/cm)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
TNM1	22	8.9	8.1	28	3.5	0.44	0.52	0.17	0.25	4.9	1.7	1.1	4.6	11
DUN1	23	8.3	8.5	30	3.3	0.53	0.97	0.20	0.82	5.0	2.6	0.80	3.3	10
MCC1	23	8.8	8.3	140	18	1.6	3.5	1.8	4.4	41	3.2	2.3	9.6	51
ELT1	23	8.5	8.2	93	5.7	1.1	9.5	0.45	14	8.0	3.6	2.3	9.6	19
QKE1	21	8.3	7.3	170	22	1.7	2.9	2.4	3.6	56	2.3	1.1	4.6	63
CTR1	13	10	7.9	46	3.6	0.67	4.5	0.35	6.6	4.6	5.3	1.8	7.5	12
SID1	15	12	7.8	280	24	5.1	24	2.0	51	13	3.5	12	48	80
BEN1	14	10	7.7	540	83	17	12	2.7	17	210	4.1	16	65	280
BOW1	14	11	7.7	440	57	13	14	2.3	22	140	3.5	14	60	200
INT1	13	10	7.8	110	14	2.4	5.3	0.61	7.7	12	5.6	7.0	29	44

n=1 for all field measurements

Table 4.4 Overlying water chemistry measurements for the cone experiments

Elliot Lake Area

	Dissolved					Major Ion						as $CaCO_3$	
	O_2 (mg/L)	pH	Cond. (μS/cm)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	Cl (mg/L)	SO_4 (mg/L)	DOC (mg/L)	DIC (mg/L)	Alk. (mg/L)	Hardness (mg/L)
TNM-SAM	7.9 (0.18)	8.0 (0.13)	450 (25)	47 (6.7)	7.5 (1.1)	29 (4.7)	2.8 (0.5)	90 (11)	31 (4.0)	2.3 (0.59)	12 (1.8)	48 (7.3)	150 (21)
TNM-Site	7.9 (0.16)	7.0 (0.25)	42 (7.8)	4.9 (0.38)	0.6 (0.02)	1.2 (0.04)	0.77 (0.03)	1.0 (0.17)	6.9 (0.67)	4.1 (0.29)	2.2 (1.1)	9.0 (4.5)	15 (1.0)
DUN-SAM	8.1 (0.17)	8.0 (0.06)	440 (15)	43 (3.4)	6.8 (0.49)	27 (1.9)	2.6 (0.13)	82 (5.8)	30 (2.2)	2.1 (0.10)	10 (1.2)	43 (4.9)	140 (10)
DUN-Site	8.1 (0.21)	6.7 (0.46)	50 (8.7)	5.4 (0.52)	0.76 (0.04)	1.7 (0.11)	0.79 (0.10)	1.8 (0.17)	11 (2.4)	4.6 (0.20)	0.75 (0.88)	3.1 (3.7)	17 (1.5)
MCC-SAM	8.1 (0.29)	7.9 (0.06)	430 (16)	43 (0.20)	6.7 (0.15)	27 (0.69)	2.6 (0.08)	82 (2.0)	31 (2.7)	2.1 (0.59)	9.6 (0.39)	40 (1.6)	140 (1.1)
MCC-Site	8.2 (0.33)	6.9 (0.26)	160 (8.4)	21 (2.7)	1.9 (0.19)	4.5 (0.47)	2.5 (0.30)	5.8 (0.65)	50 (7.1)	5.0 (0.0)	1.1 (0.98)	4.6 (4.1)	59 (7.4)
MCC-P	8.2 (0.30)	7.0 (0.26)	170 (17)	22	1.8	4.2	2.2	5.3	56	4.2	1.5	6.0	62
ELT-SAM	8.1 (0.28)	8.1 (0.03)	440 (17)	46 (0.69)	7.2 (0.21)	28 (0.98)	2.7 (0.14)	89 (3.4)	32 (0.69)	2.1 (0.78)	9.7 (1.1)	40 (4.5)	140 (2.6)
ELT-Site	8.1 (0.32)	6.9 (0.48)	110 (8.6)	7.4 (0.04)	1.3 (0.05)	11 (0.49)	0.98 (0.03)	17 (0.49)	13 (1.4)	5.5 (0.49)	0.95 (0.10)	4.0 (0.41)	24 (0.10)
ELT-P	8.2 (0.51)	7.3 (0.02)	110 (4.2)	6.6	1.2	11	0.6	15	11	4.2	1.8	7.5	21
QKE-SAM	8.2 (0.48)	8.1 (0.06)	470 (41)	45	6.8	27	3.4	83	42	10	10	42	140
QKE-Site	8.3 (0.84)	6.2 (0.69)	200 (21)	27	2.1	3.9	3.2	5.0	70	4.1	0.3	1.2	75
QKE-P	8.3 (0.53)	6.7 (0.18)	190 (13)	25	1.9	3.4	2.6	4.2	69	2.6	0.6	2.5	71.0

n=8 for all sites, except for those indicated below for all reading except the major ions reading, n=2

n=4 for QKE-SAM, QKE-Site, MCC-P, ELT-P, BEN-P and BOW-P cone experiment sites for all reading except major ions, n=1

All data reported as mean (95% confidence interval)

SAM-indicates the artificial overlying water media

Site-indicates overlying site water

P-indicates amphipods fed periphyton and exposed to overlying site water

Table 4.4 Overlying water chemistry measurements for the cone experiments (continued)

Bancroft Area													
	Dissolved					Major Ion						as $CaCO_3$	
	O_2 (mg/L)	pH	Cond. (µS/cm)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	Cl (mg/L)	SO_4 (mg/L)	DOC (mg/L)	DIC (mg/L)	Alk. (mg/L)	Hardness (mg/L)
CTR-SAM	7.6 (0.24)	7.9 (0.20)	450 (18)	43 (2.5)	7.0 (0.38)	27 (1.3)	2.8 (0.40)	84 (3.6)	36 (7.0)	2.8 (0.59)	3.9 (0.98)	16 (4.1)	140 (7.7)
CTR-Site	7.4 (0.26)	6.1 (0.74)	94 (20)	5.7 (1.7)	0.91 (0.22)	5.7 (0.66)	1.1 (0.29)	8.6 (0.96)	15 (6.8)	6.7 (1.6)	1.7 (0.49)	6.9 (1.2)	18 (2.9)
SID-SAM	7.7 (0.15)	8.0 (0.11)	460 (24)	48 (0.98)	7.6 (0.05)	29 (0.59)	3.0 (0.06)	91 (3.2)	45 (6.1)	3.0 (0.78)	7.3 (0.20)	30 (0.47)	150 (1.5)
SID-Site	7.5 (0.23)	8.0 (0.12)	350 (22)	27 (1.1)	5.6 (0.12)	27 (0.49)	2.7 (0.20)	58 (0.39)	26 (9.1)	4.9 (0.98)	6.6 (2.8)	27 (6.8)	91 (1.8)
BEN-SAM	7.5 (0.15)	8.0 (0.16)	460 (22)	47 (1.3)	7.7 (0.20)	28 (0.29)	2.8 (0.15)	87 (0.59)	39 (6.4)	2.6 (0.49)	7.9 (0.0)	33 (0.0)	150 (2.3)
BEN-Site	7.7 (0.18)	8.0 (0.19)	650 (26)	92 (3.0)	19 (0.49)	15 (0.29)	3.5 (0.05)	20 (0.39)	240 (11)	5.9 (0.0)	9.9 (4.5)	41 (11)	310 (5.5)
BEN-P	7.5 (0.28)	8.2 (0.12)	670 (68)	91	19	14	3.0	19	240	5.0	14	56	310
BOW-SAM	7.5 (0.10)	8.0 (0.14)	480 (39)	53 (7.9)	8.4 (1.3)	31 (4.2)	3.1 (0.53)	96 (14)	62 (13)	2.6 (0.29)	5.7 (3.4)	23 (8.3)	170 (15)
BOW-Site	7.4 (0.08)	8.1 (0.12)	540 (26)	69 (2.7)	15 (0.49)	17 (0.49)	3.3 (0.27)	26 (0.78)	190 (4.9)	4.3 (0.59)	8.2 (1.7)	34 (4.0)	230 (5.1)
BOW-P	7.4 (0.15)	8.2 (0.06)	550 (52)	77	17	18	3.1	28	210	4.7	12	50	260
INT-SAM	7.4 (0.08)	7.9 (0.21)	450 (21)	44 (1.3)	7.0 (0.30)	27 (0.89)	2.7 (0.04)	85 (3.1)	37 (5.7)	2.6 (0.78)	6.6 (5.9)	27 (14)	140 (2.6)
INT-Site	7.3 (0.12)	7.7 (0.17)	150 (11)	17 (0.98)	2.9 (0.26)	7.0 (0.50)	1.3 (0.05)	11 (0.99)	25 (6.8)	7.4 (0.20)	4.7 (3.8)	19 (9.3)	54 (2.0)
INT-P	7.4 (0.09)	7.8 (0.07)	140 (15)	17	2.9	6.4	1.1	9.8	28	7.1	4.9	20	54

n=8 for all sites, except for those indicated below for all reading except the major ions reading, n=2

n=4 for QKE-SAM, QKE-Site, MCC-P, ELT-P, BEN-P and BOW-P cone experiment sites for all reading except major ions, n=1

All data reported as mean (95% confidence interval)

SAM-indicates the artificial overlying water media

Site-indicates overlying site water

P-indicates amphipods fed periphyton and exposed to overlying site water

Table 4.5 Twenty-seven metal analysis (nmol/L) for filtered (0.45 μm) water collected in the field

	Elliot Area						Bancroft Area						PWQO^{a}	CWQG^{b}
	TNM1	DUN1	Mean*	MCC1	ELT1	QKE1	CTR1	SID1	**Mean***	BEN1	BOW1	INT1	(nmol/L)	(nmol/L)
Ag	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.01 (0.01)	BDL	BDL	0.93	0.93
Al	140 (26)	160 (40)	150(23)	350 (43)	300 (74)	150 (25)	*460 (0.0)*	61 (11)	170(230)	9.3 (3.6)	20 (18)	93 (7.2)	560-2800^{c,a}	190-3700^{e}
As	2.1 (0.28)	2.0 (0.15)	2.1(0.17)	6.1 (0.41)	2.2 (0.08)	4.0 (0.20)	2.8 (0.00)	2.1(0.39)	2.4(0.45)	2.9 (0.39)	1.6 (0.00)	2.5 (0.13)	67^{c}	67
B	400 (6.0)	410(30)	410(14)	970 (30)	520 (12)	1300 (20)	390 (0.0)	1700 (27)	800(720)	<u>**34000 (180)**</u>	17000(90)	1300 (120)	<u>**19000^{c}**</u>	
Ba	0.06 (0.001)	0.09 (0.001)	0.07(0.01)	0.13 (0.001)	0.09 (0.002)	0.30 (0.003)	0.08 (0.00)	0.37 (0.002)	0.17(0.16)	0.25 (0.001)	0.21 (0.01)	0.11 (0.001)		
Be	BDL	BDL	0.04(0.05)	0.11 (0.00)	0.15 (0.07)	0.18 (0.07)	0.44 (0.00)	BDL	0.22(0.19)	0.33 (0.00)	0.72 (0.11)	0.49 (0.11)	1200-120000^{f}	
Bi	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL		
Cd	0.04 (0.02)	0.03 (0.03)	0.03(0.01)	0.02 (0.02)	<u>**0.09 (0.17)**</u>	0.07 (0.06)	BDL	BDL	BDL	BDL	BDL	BDL	0.89-4.5^{c,g}	0.15^{c,h}
Co	BDL	0.11 (0.13)	0.01(0.08)	0.23 (0.20)	0.22 (0.19)	0.09 (0.08)	*1.00 (0.03)*	0.04 (0.02)	0.01(0.08)	0.26 (0.02)	0.08 (0.13)	0.09 (0.00)	15	
Cr	6.3 (9.9)	15 (26)	3.3(13)	3.6 (2.9)	3.3 (2.2)	11 (14)	BDL	BDL	BDL	3.9 (1.1)	BDL	2.1 (0.37)	19-170^{d,i}	19-170^{i}
Cu	6.6 (8.3)	2.5 (2.2)	2.2 (4.3)	8.4 (9.3)	6.7(6.4)	7.4 (11)	BDL	BDL	BDL	BDL	BDL	BDL	16-79^{c,j}	32-63^{k}
Fe	59 (62)	310 (490)	75(250)	150 (97)	88 (47)	170 (120)	*3500 (53)*	420 (37)	1200(1800)	340 (12)	330 (350)	780 (32)	5400	5400
Ga	BDL	BDL	BDL	0.02 (0.01)	0.02 (0.01)	BDL	0.06 (0.01)	0.03 (0.00)	0.04(0.02)	0.09 (0.00)	0.02 (0.03)	0.05 (0.01)		
La	0.02 (0.01)	0.02 (0.01)	0.02(0.004)	0.12 (0.05)	0.07 (0.03)	0.18 (0.02)	*0.59 (0.01)*	0.05 (0.00)	0.17(0.31)	0.38 (0.02)	0.40 (0.42)	0.42 (0.01)		
Li	BDL	4.8 (9.4)	BDL	450 (9.0)	29 (0.0)	630 (9.0)	29 (0.0)	180 (14)	72(88)	350 (0.0)	530 (0.0)	100 (0.0)		
Mn	0.91 (0.63)	16 (26)	2.1 (13)	35 (57)	32 (51)	12 (5.9)	*860 (2.0)*	46 (0.0)	200(460)	1400 (4.0)	300 (4.0)	180 (1.0)		
Mo	0.10 (0.03)	0.10 (0.01)	0.13(0.02)	2.0 (0.1)	0.40 (0.03)	2 .0 (0.1)	0.06 (0.04)	4.6 (0.01)	1.7(2.3)	7.0 (0.09)	17 (0.10)	3.8 (0.21)	420^{c}	760
Ni	2.6 (1.8)	2.3 (1.3)	2.2(1.0)	9.0 (0.95)	7.5 (1.2)	9.4 (1.7)	0.68 (0.33)	2.2 (0.00)	1.2(0.88)	6.0 (0.33)	7.5 (2.3)	3.4 (0.33)	430	430-2600^{l}

Table 4.5 Twenty-seven metal analysis (nmol/L) for filtered (0.45 μm) water collected in the field (continued)

	Elliot Area						Bancroft Area						PWQO[a]	CWQG[b]
	TNM1	DUN1	Mean*	MCC1	ELT1	QKE1	CTR1	SID1	**Mean***	BEN1	BOW1	INT1	(nmol/L)	(nmol/L)
Pb	0.25 (0.36)	BDL	0.02(0.19)	0.14 (0.22)	0.38 (0.23)	0.21 (0.10)	0.46 (0.08)	0.72 (0.14)	0.57(0.17)	0.23 (0.00)	0.22 (0.19)	0.21 (0.09)	4.8-24[c,m]	4.8-34[n]
Rb	6.5 (0.08)	7.3 (0.13)	6.9(0.35)	77 (1.1)	14 (0.5)	110 (1.3)	12 (0.0)	48 (0.23)	24(21)	48 (0.23)	64(0.46)	19 (0.0)		
Sb	0.38 (0.08)	0.35 (0.02)	0.36(0.02)	0.35 (0.02)	0.35 (0.03)	0.38 (0.07)	0.27 (0.03)	0.19 (0.02)	0.23(0.05)	0.16 (0.02)	0.21 (0.02)	0.32 (0.02)	170[c]	
Se	0.42 (0.08)	0.38 (0.00)	0.40(0.04)	0.59 (0.07)	0.38 (0.14)	0.72 (0.08)	0.25 (0.00)	0.06 (0.12)	0.20(0.08)	0.19 (0.12)	0.25 (0.00)	0.32 (0.12)	1300	13
Sr	110 (0.0)	130 (1.5)	120(7.5)	350 (3.3)	260 (0.00)	380 (4.7)	390 (0.0)	2100 (11)	900(960)	22000 (0.0)	10000 (0.0)	780 (8.9)		
Tl	0.01 (0.003)	0.03 (0.00)	0.01(0.01)	0.2 (0.03)	0.2 (0.1)	0.2 (0.02)	0.09 (0.01)	0.07 (0.00)	0.08(0.01)	0.08 (0.00)	0.06 (0.05)	0.10 (0.01)	1.5[c]	3.9
U	0.01 (0.01)	0.02 (0.01)	0.01(0.004)	2.4 (0.15)	2.4 (0.04)	4.1 (0.11)	0.12 (0.01)	1.8 (0.03)	0.48(0.98)	**110 (1.0)**	**130 (160)**	3.9 (0.28)	**21[c]**	
V	0.42 (0.01)	0.43 (0.11)	0.42(0.05)	1.2 (0.09)	0.88 (0.14)	0.45 (0.08)	1.7 (0.02)	1.3 (0.15)	1.5(0.27)	1.7 (0.04)	1.2 (0.33)	1.5 (0.04)	120[c]	
Zn	22 (7.1)	6.2 (9.1)	11(8.9)	13 (2.1)	67 (71)	87 (72)	21 (4.0)	21 (28)	18(12)	11 (1.0)	5.7 (7.3)	37 (19)	310[c]	460

BDL indicates below detection limit

Data reported as mean (95% confidence Interval); n=3 for Elliot Lake Area; n=2 for Bancroft Area

*Geometric mean for reference lake waters near the Elliot Lake or Bancroft area

[a] Provincial Water Quality Objective (MOEE, 1999)

[b] Canadian Water Quality Guideline (CCME, 1999)

[c] Intermin guideline

[d] 56 nmol/g at pH 4.5-5.5; 2800 nmol/L at pH >6.5 to 9.0

[e] 190 nmol/L at pH<6.5; 3700 nmol/L at pH ≥ 6.5

[f] 1200 nmol/g for hardness <75 $CaCO_3$ mg/L; 12000 nmol/L at >75 mg/L as $CaCO_3$

[g] 0.89 nmol/L for hardness 0-100 mg/L as $CaCO_3$; 4.5 for hardness >100 mg/L as $CaCO_3$

[h] $10^{\{0.86[\log(\text{hardness})]-3.2\}}$

[i] 19 nmol/L for Cr(VI); 170 nmol/L Cr(III)

[j] 16 nmol/L for hardness of 0-20 mg/L as $CaCO_3$; 79 nmol/L for hardness > 20 mg/L as $CaCO_3$

[k] 32 nmol/L for hardness of 0-120 mg/L as $CaCO_3$; 47 nmol/L for hardness120-180 mg/L as $CaCO_3$; 63 nmol/L for hardness >180 mg/L as $CaCO_3$

[l] 430 nmol/L for hardness of 0-60 mg/L as $CaCO_3$; 1100 nmol/L for hardness 60-120 mg/L as $CaCO_3$; 1900 nmol/L for hardness 120-180 mg/L as $CaCO_3$; 2600 nmol/L for hardness of >180 mg/L as $CaCO_3$

[m] 4.8 nmol/L for hardness of <30 mg/L as $CaCO_3$; 15 nmol/L for hardness 30-80 mg/L as $CaCO_3$; 24 nmol/L for hardness >80 mg/L as $CaCO_3$

[n] 4.8 nmol/L for hardness of 0-60 mg/L as $CaCO_3$; 9.7 nmol/L for hardness 60-120 mg/L as $CaCO_3$; 19 nmol/L for hardness 120-180 mg/L as $CaCO_3$; 34 nmol/L for hardness of >180 mg/L as $CaCO_3$

Indicates that the value has reached or exceeded the water quality guideline

Highlighted values indicate that the metals in the contaminated lakes are higher than the metals/metalloids concentrations measured in the geometric mean reference lakes for the respective area.

Highlighted italic values indicate that the metals/metalloids in the reference lakes are higher than the contaminated sites

Table 4.6 Measured metal concentrations (nmol/L) in the overlying water for *H. azteca* exposed to field collected sediments in the cone experiments

Elliot Lake Study Area

Metal	TNM-SAM	TNM-Site	DUN-SAM	DUN-Site	MCC-SAM	MCC-Site	MCC-P	ELT-SAM	ELT-Site	ELT-P	QKE-SAM	QKE-Site	QKE-P	PWQO^{a} (nmol/L)	CWQG^{b} (nmol/L)	one-week (effect) LC50 (nmol/L)	four-week (effect) LC50 (nmol/L)	four-week (effect) LC25 (nmol/L)
Ag	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.93	0.93	2.3^{o}		
Al	700 (480)	280 (150)	870 (290)	**220 (160)**	910 (600)	800 (1100)	170 (25)	830 (610)	210 (20)	150 (40)	200 (44)	**180 (290)**	15 (3.6)	560-2800^{c,d}	190-3700^{e}	3300^{o}		
As	3.8 (4.8)	5.3 (3.6)	6.9 (5.5)	7.0 (4.9)	1.6 (1.9)	7.9 (2.3)	5.9 (0.39)	2.7 (3.9)	3.9 (2.3)	1.3 (0.52)	1.8 (2.0)	5.3 (4.5)	3.7 (0)	67^{c}	67	7800^{o}; 6500^{p}	5600^{q}	4300^{q}
B	28 (15)	520 (37)	14 (12)	520 (26)	49 (16)	1100 (86)	1300 (130)	21 (26)	620 (57)	620 (120)	170 (310)	1500 (150)	1500 (190)	19000^{c}		270 000^{o}		
Ba	0.12 (0.04)	0.11 (0.02)	0.20 (0.06)	0.23 (0.05)	0.11 (0.04)	0.20 (0.05)	0.28 (0.17)	0.12 (0.04)	0.15 (0.03)	0.16 (0.08)	0.24 (0.19)	0.38 (0.08)	0.43 (0.17)					
Be	BDL	0.03 (0.05)	BDL	0.39 (0.47)	BDL	0.17 (0.21)	0.17 (0.11)	BDL	0.22 (0.13)	0.22 (0.22)	BDL	0.22 (0.43)	0.22 (0.22)	1200-120000^{f}		7400^{o}		
Bi	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL					
Cd	0.30 (0.07)	**0.33 (0.34)**	**0.46 (0.11)**	**1.2 (0.92)**	0.33 (0.15)	**0.24 (0.17)**	**0.08 (0.04)**	0.16 (0.04)	**0.32 (0.06)**	**0.10 (0.01)**	0.25 (0.43)	**0.57 (0.94)**	0.04 (0.04)	0.89-4.5^{c,g}	**0.15**^{c,h}	1.3^{o}; 14^{p}	4.6^{r}; 7.8^{t}	3.2^{r}-6.2^{t}
Co	1.7 (2.2)	1.2 (1.9)	1.0 (0.78)	0.20(0.21)	1.4 (0.66)	0.56 (0.63)	0.61 (0.77)	0.95 (0.89)	0.53 (0.39)	0.31 (0.38)	1.10 (0.65)	1.2 (1.9)	0.19 (0.07)	15		270^{o}; 1000^{p}	180^{q}	68^{q}
Cr	1.4 (0.47)	2.0 (1.2)	0.78 (0.11)	1.2 (0.10)	0.83 (0.29)	1.7 (0.53)	6.3 (3.8)	0.65 (0.16)	2.0 (0.23)	5.3 (5.3)	0.27 (0.23)	1.2 (0.42)	0.07 (2.2)	19-170^{d,i}	19-170^{i}	60^{o}; 2600^{p}	730^{q}	240^{q}
Cu	32 (18)	***63 (56)***	52 (37)	***37 (11)***	28 (17)	30 (8.6)	27 (18)	39 (27)	**40 (13)**	*22 (6.2)*	47 (45)	28 (26)	13 (4.2)	*16-79*^{c,j}	**32-63**^{k}	570^{o}; 1400^{p}	720^{r}	440^{r}
Fe	40 (7.5)	96 (75)	38 (9.1)	59 (50)	68 (64)	440 (770)	130 (120)	52 (36)	83 (33)	65 (11)	280 (360)	2200 (3800)	180 (240)	5400	5400			
Ga	0.09 (0.05)	0.06 (0.04)	0.19 (0.10)	0.04 (0.03)	0.20 (0.11)	0.10 (0.09)	0.03 (0.03)	0.11 (0.09)	0.04 (0.02)	0.007 (0.01)	0.07 (0.01)	0.09 (0.13)	0.01 (0.01)					
La	0.02 (0.02)	0.11 (0.07)	0.04 (0.01)	0.14 (0.09)	0.03 (0.03)	0.58 (0.76)	0.15 (0.11)	0.04 (0.04)	0.42 (0.25)	0.17 (0.01)	0.08 (0.09)	1.8 (2.4)	0.77 (0.21)			130^{o}		
Li	BDL	14 (0)	BDL	32 (7.1)	14 (12)	420 (28)	500 (71)	BDL	43 (12	36 (14)	36 (71)	690 (57)	700 (71)			94000^{o}		
Mn	210 (210)	310 (600)	130 (47)	7.4 (11)	520 (600)	37 (49)	1.6 (0.54)	96 (120)	22 (40)	5.7 (12)	450 (880)	670 (1200)	12 (4.8)			1700^{o}; 3100^{p}	200 000^{q}	150 000^{q}
Mo	1.9 (0.85)	1.2 (0.78)	1.9 (1.0)	0.60 (0.52)	1.9 (0.90)	2.1 (0.62)	1.3 (0.77)	1.1 (0.75)	0.66 (0.18)	0.38 (0.11)	0.94 (1.2)	0.79 (1.3)	0.54 (0.70)	420^{c}	760			
Ni	18 (4.1)	72 (100)	26 (9.7)	30 (4.7)	21 (5.6)	17 (5.7)	13 (2.7)	14 (5.3)	23 (8.4)	16 (11)	30 (24)	96 (130)	20 (7.2)	430	430-2600^{l}	1300^{o}; 2300^{p}	580^{r}	400^{r}
Pb	0.61 (0.06)	0.24 (0.19)	0.81 (0.22)	0.14 (0.11)	0.69 (0.14)	0.50 (0.64)	0.16 (0.01)	0.64 (0.16)	0.68 (0.23)	0.37 (0.02)	1.2 (1.3)	0.60 (0.88)	0.21 (0.19)	4.8-24^{c,m}	4.8-34^{n}	4.8^{o}; 53^{p}	59^{r}	36^{r}
Rb	11 (0.31)	12 (0.19)	12 (0.10)	12 (0.10)	12 (0.10)	12 (0.04)	12 (0.10)	12 (0.03)	12 (0.03)	12 (0.04)	12 (0.03)	12 (0.03)	12 (0.03)					
Sb	0.33 (0.20)	0.68 (0.13)	1.1 (0.52)	1.2 (0.32)	0.47 (0.26)	0.74 (0.22)	0.53 (0.03)	0.51 (0.31)	0.69 (0.13)	0.50 (0.07)	0.43 (0.48)	0.45 (0.04)	0.33 (0.02)	160^{c}		5600^{o}		
Se	1.5 (0.78)	2.4 (1.2)	0.95 (0.12)	1.3 (0.23)	BDL	0.92 (0.10)	0.82 (0.12)	0.16 (0.19)	0.92 (0.26)	0.63 (0)	0.82 (1.4)	1.1 (0.75)	0.76 (0.00)	1300	13	520^{o}; 4700^{p}		
Sr	200 (58)	160 (47)	210 (49)	210 (54)	210 (49)	430 (69)	440 (47)	220 (52)	330 (49)	300 (31)	200 (110)	480 (120)	430 (44)					
Tl	0.11 (0.04)	0.21 (0.11)	0.14 (0.09)	0.38 (0.17)	0.08 (0.02)	0.25 (0.10)	0.19 (0.01)	0.09 (0.04)	0.17 (0.06)	0.13 (0.005)	0.21 (0.12)	0.58 (0.58)	0.36 (0.22)	1.5^{c}	3.9	59^{o}; 230^{p}	78^{r}	51^{r}
U	0.54 (0.22)	0.05 (0.03)	0.25 (0.05)	0.03 (0.02)	0.29 (0.10)	0.51 (0.21)	0.83 (0.10)	4.4 (4.9)	2.1 (1.5)	1.7 (0.14)	*14 (8.9)*	2.3 (1.5)	2.6 (2.1)	*21*^{c}		88^{o}	200^{s}	95^{s}; 2100^{u}
V	19 (15)	16 (12)	24 (11)	8.4 (2.2)	17 (13)	13 (9.3)	2.9 (2.5)	16 (13)	10 (6.8)	3.1 (2.6)	0.54 (0.37)	0.22 (0.08)	0.11 (0.02)	120^{c}		7200^{p}		
Zn	49 (34)	65 (11)	78 (29)	*300 (220)*	190 (240)	98 (63)	62 (26)	89 (95)	130 (33)	52 (55)	22 (3.9)	200 (160)	67 (14)	*310*^{c}	460	860^{o}; 3400^{p}	3100^{r}	2500^{r}

Table 4.6 Measured metal concentrations (nmol/L) in the overlying water for *H. azteca* exposed to field collected sediments in the cone experiments (continued)

Bancroft Study Area

Metal	CTR-SAM	CTR-Site	SID-SAM	SID-Site	BEN-SAM	BEN-Site	BEN-P	BOW-SAM	BOW-Site	BOW-P	INT-SAM	INT-Site	INT-P	PWQO^{a} (nmol/L)	CWQG^{b} (nmol/L)	one-week (effect) LC50 (nmol/L)	four-week (effect) LC50 (nmol/L)	four-week (effect) LC25 (nmol/L)
Ag	BDL	0.002 (0.01)	BDL	BDL	BDL	0.01 (0.001)	0.01(0.003)	BDL	BDL	BDL	BDL	BDL	BDL	0.93	0.93	2.3^{o}		
Al	750 (660)	**750 (770)**	180 (130)	120 (79)	270 (150)	140 (110)	31 (11)	290 (240)	310 (290)	64 (55)	580 (440)	200 (76)	190 (3.2)	560-2800^{c,d}	**190-3700^{e}**	3300^{o}		
As	4.3 (2.2)	6.4 (2.4)	2.9 (1.9)	5.2 (1.4)	2.2 (1.0)	4.3 (0.63)	3.3 (0.53)	1.7 (1.1)	2.2 (0.57)	1.9 (0.21)	3.4 (1.5)	5.1 (2.1)	3.6 (0.74)	67^{c}	67	7800^{o}; 6500^{p}	5600^{q}	4300^{q}
B	BDL	500 (81)	110 (89)	1800 (110)	1500 (360)	*37000 (1700)*	*37000 (4700)*	890 (270)	*18000 (1100)*	*19000 (4500)*	67 (47)	1400 (150)	1400 (300)	*19000^{c}*		270 000^{o}		
Ba	140 (33)	190 (9.1)	160 (66)	410 (36)	82 (31)	300 (14)	340 (95)	110 (44)	240 (22)	290 (110)	140 (52)	160 (9.7)	200 (62)					
Be	0.25 (0.05)	1.2 (0.90)	0.22 (0.13)	0.41 (0.13)	0.35 (0.06)	0.62 (0.06)	0.56 (0.054)	0.50 (0.37)	1.2 (0.60)	0.68 (0.11)	0.18 (0.18)	0.45 (0.13)	0.61 (0.02)	1200-120000^{f}		7400^{o}		
Bi	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL					
Cd	0.17 (0.10)	**0.24 (0.26)**	0.16 (0.08)	0.07 (0.05)	0.08 (0.05)	0.06 (0.01)	0.05 (0.03)	0.13 (0.07)	0.06 (0.02)	0.08 (0.06)	0.24 (0.14)	0.04 (0.02)	0.04 (0.04)	0.89-4.5^{c,g}	**0.15^{c,h}**	1.3^{o}; 14^{p}	4.6^{r}; 7.8^{t}	3.2^{r}-6.2^{t}
Co	2.3 (1.9)	3.4 (5.7)	0.93 (0.79)	0.39 (0.16)	0.19 (0.16)	0.33 (0.03)	0.36 (0.20)	0.71 (0.17)	0.40 (0.12)	0.28 (0.05)	1.1 (0.86)	0.35 (0.12)	0.43 (0.37)	15		270^{o}; 1000^{p}	180^{q}	68^{q}
Cr	1.5 (0.83)	1.1 (0.25)	1.1 (1.1)	0.95 (0.70)	1.5 (0.56)	0.68 (0.27)	0.86 (0.71)	0.50 (0.34)	0.20 (0.13)	0.37 (0.10)	3.5 (4.5)	1.2 (0.39)	1.9 (0.12)	19-170^{d,i}	19-170^{i}	60^{o}; 2600^{p}	730^{q}	240^{q}
Cu	BDL	8.0 (5.7)	BDL	4.1 (7.8)	5.1 (12)	14 (22)	1.1 (2.0)	2.7 (11)	1.1 (1.4)	1.1 (11)	2.6 (9.0)	19(21)	9.6 (8.7)	16-79^{c,j}	32-63^{k}	570^{o}; 1400^{p}	720^{r}	440^{r}
Fe	55 (75)	260 (280)	27 (32)	170 (150)	33 (15)	24 (16)	14 (1.1)	30 (32)	38 (10)	34 (19)	110 (100)	200 (130)	47 (8.7)	5400	5400			
Ga	0.43 (0.25)	0.11 (0.04)	0.32 (0.14)	0.28 (0.13)	0.71 (0.30)	0.46 (0.12)	0.23 (0.14)	0.20 (0.02)	0.09 (0.04)	0.07 (0.004)	0.28 (0.10)	0.12 (0.01)	0.08 (0.02)					
La	0.06 (0.07)	0.74 (0.83)	0.05 (0.06)	0.08 (0.07)	0.11 (0.08)	0.16 (0.06)	0.18 (0.008)	0.37 (0.09)	0.45 (0.13)	0.32 (0.11)	0.12 (0.08)	0.26 (0.20)	0.23 (0.04)			130^{o}		
Li	BDL	31 (7.8)	BDL	150 (10)	BDL	340 (18)	350 (52)	5.9 (11)	540 (35)	570 (140)	BDL	130 (17)	130 (36)			94000^{o}		
Mn	4.8 (0.94)	BDL	3.2 (1.6)	BDL	5.1 (7.0)	1.7 (6.1)	0.59 (6.7)	23 (37)	7.5 (12)	BDL	9.1 (4.0)	BDL	4.3 (11)			1700^{o}; 3100^{p}	200 000^{q}	150 000^{q}
Mo	1.9 (1.3)	0.98 (0.45)	6.2 (3.9)	11 (3.1)	7.9 (2.4)	15 (2.0)	13 (0.33)	6.7 (2.1)	24 (3.2)	21 (0.05)	2.2 (1.0)	5.1 (1.0)	4.3 (1.1)	420^{c}	760			
Ni	9.0 (4.5)	19 (6.0)	16 (6.7)	10 (4.2)	6.8 (2.7)	13 (4.7)	9.0 (4.6)	19 (4.8)	19 (8.3)	11 (12)	13 (6.9)	8.6 (2.2)	14 (14)	430	430-2600^{l}	1300^{o}; 2300^{p}	580^{r}	400^{r}
Pb	0.11 (0.05)	1.1 (1.7)	0.09 (0.03)	0.08 (0.02)	0.12 (0.02)	0.08 (0.05)	0.08 (0.03)	0.15 (0.06)	0.16 (0.14)	0.11 (0.13)	0.20 (0.04)	0.17 (0.12)	0.06 (0.03)	4.8-24^{c,m}	4.8-34^{n}	4.8^{o}; 53^{p}	59^{r}	36^{r}
Rb	5.7 (3.7)	17 (4.7)	7.1 (4.8)	50 (5.4)	5.2 (3.0)	48 (4.1)	47 (6.5)	11 (4.8)	69 (7.7)	74 (19)	6.9 (2.9)	24 (5.8)	25 (9.2)					
Sb	0.73 (0.41)	0.88 (0.44)	0.39 (0.28)	0.56 (0.36)	0.40 (0.10)	0.56 (0.14)	0.60 (0.23)	0.09 (0.03)	0.22 (0.03)	0.26 (0.10)	0.30 (0.15)	0.51 (0.13)	0.49 (0.25)	160^{c}		5600^{o}		
Se	4.3 (1.4)	2.1 (1.5)	8.8 (4.4)	5.8 (2.4)	5.7 (2.2)	2.5 (0.55)	2.3 (1.6)	10 (5.0)	2.4 (0.85)	2.8 (0.15)	7.2 (3.3)	3.7 (2.6)	3.0 (1.4)	1300	13	520^{o}; 4700^{p}		
Sr	350 (70)	530 (73)	390 (140)	2200 (110)	780 (150)	22000 (900)	23000 (2700)	580 (100)	10000 (430)	11000 (2500)	340 (62)	910 (86)	880 (110)					
Tl	0.12 (0.07)	0.11 (0.09)	0.07 (0.05)	0.05 (0.04)	0.01 (0.01)	0.01 (0.01)	0.02 (0.01)	0.02 (0.04)	0.04 (0.03)	0.02 (0.003)	0.07 (0.06)	0.05 (0.01)	0.09 (0.10)	1.5^{c}	3.9	59^{o}; 230^{p}	78^{r}	51^{r}
U	0.11 (0.07)	0.10 (0.06)	0.40 (0.17)	0.99 (0.81)	1.5 (0.33)	*66 (38)*	*88 (18)*	11 (7.3)	*150 (63)*	*190 (0.90)*	1.6 (0.84)	1.8 (1.3)	3.4 (1.8)	*21^{c}*		88^{o}	200^{s}	95^{s}; 2100^{u}
V	9.4 (7.1)	5.9 (3.9)	5.8 (7.4)	6.8 (6.8)	41 (20)	32 (8.5)	15 (18)	0.35 (0.31)	0.82 (0.29)	0.93 (0.59)	7.4 (6.1)	4.1 (0.37)	1.9 (0.71)	120^{c}		7200^{p}		
Zn	47 (30)	190 (120)	40 (22)	53 (36)	26 (9.2)	33 (13)	2.6 (2.6)	11 (5.2)	2.6 (5.6)	7.2 (18)	91 (78)	22 (9.0)	20 (26)	310^{c}	460	860^{o}; 3400^{p}	3100^{r}	2500^{r}

n=4 for all sites, except for those indicated below for the cone experiments

n=2 for QKE, MCC-P, ELT-P, BEN-P and BOW-P cone experiment sites

All data reported as mean (95% confidence interval)

SAM-indicates the artificial overlying water media

Site-indicates overlying site water

P-indicates cones fed periphyton and exposed to overlying site water

[a] Provincial Water Quality Objective (MOEE, 1999)

[b] Canadian Water Quality Guideline (CCME, 1999)

[c] Intermin guideline

[d] 560 nmol/g at pH 4.5-5.5; 2800 nmol/L at pH >6.5 to 9.0

[e] 190 nmol/L at pH<6.5; 3700 nmol/L at pH ≥ 6.5

[f] 1200 nmol/g for hardness < 75 CaCO$_3$ mg/L; 12000 nmol/L at >75 mg/L as CaCO$_3$

[g] 0.89 nmol/L for hardness 0-100 mg/L as CaCO$_3$; 4.5 nmol/L for hardness >100 mg/L as CaCO$_3$

[h] $10^{\{0.86[\log(\text{hardness})]-3.2\}}$ in ug/L

[i] 19 nmol/L for Cr(VI); 170 nmol/L Cr(III)

[j] 16 nmol/L for hardness of 0-20 mg/L as CaCO$_3$; 79 nmol/L for hardness > 20 mg/L as CaCO$_3$

[k] 32 nmol/L for hardness of 0-120 mg/L as CaCO$_3$; 47 nmol/L for hardness120-180 mg/L as CaCO$_3$; 63 nmol/L for hardness >180 mg/L as CaCO$_3$

[l] 430 nmol/L for hardness of 0-60 mg/L as CaCO$_3$; 1100 nmol/L for hardness 60-120 mg/L as CaCO$_3$; 1900 nmol/L for hardness 120-180 mg/L as CaCO$_3$; 2600 nmol/L for hardness of >180 mg/L as CaCO$_3$

[m] 4.8 nmol/L for hardness of <30 mg/L as CaCO$_3$; 15 nmol/L for hardness 30-80 mg/L as CaCO$_3$; 24 nmol/L for hardness >80 mg/L as CaCO$_3$

[n] 4.8 nmol/L for hardness of 0-60 mg/L as CaCO$_3$; 9.7nmol/L for hardness 60-120 mg/L as CaCO$_3$; 19 nmol/L for hardness 120-180 mg/L as CaCO$_3$; 34 nmol/L for hardness of >180 mg/L as CaCO$_3$

[o] Data source from Borgmann et al. (2005) using the most sensitive measurements for metals added as atomic absorption standards or anion salts. Water hardness of 18 mg/L as CaCO$_3$

[p] Data source from Borgmann et al. (2005) in with a water hardness of 120 mg/L as CaCO$_3$

[q] Data source from Norwood et al. (2006) in hard water with a water hardness of 120 mg/L as CaCO$_3$

[r] Data source from Borgmann et al. (2004) using the saturation model estimates. Average water hardness was ~ 130 mg/L as CaCO$_3$

[s] Data source from Alves et al. (2008) in a water hardness of ~ 13 mg/L as CaCO$_3$

[t] Data source from Borgmann et al. (2004) using the saturation model estimates with a water hardness of ~ 13 mg/L as CaCO$_3$

[u] Data source from Alves et al. (2008) in a water hardness of ~ 130 mg/L as CaCO$_3$

Indicates that the value has reached or exceeded the CWQG

Indicates that the value has reached or exceeded the PWQG

Table 4.7 Sedimentology

Composition (%)								
Site	Moisture	Organic carbon	Inorganic carbon	Total carbon	Sand	Silt	Clay	Classification
TNM1	89	8.0	0.14	8.1	0.48	60	39	Clayey Silt
TNM2	92	8.3	0.25	8.6	0.69	62	37	Clayey Silt
DUN1	80	9.5	0.40	9.9	0.37	65	35	Sandy Silt
DUN2	90	2.9	0.26	3.2	9.0	67	24	Clayey Silt
MCC1	82	5.2	0.16	5.4	0.19	61	39	Clayey Silt
MCC2	83	3.8	0.11	3.9	0.53	53	47	Clayey Silt
ELT1	89	7.0	0.03	7.0	0.40	58	41	Clayey Silt
ELT2	89	6.6	0.14	6.7	1.7	63	36	Clayey Silt
QKE1	92	NM	NM	NM	NM	NM	NM	NM
CTR1	88	19	0.46	20	0.39	29	71	Silty clay
CTR2	94	13	4.2	17	0.71	35	65	Silty clay
SID1	93	13	2.4	16	0.54	41	58	Silty clay
SID2	95	17	3.3	20	0.35	29	71	Silty clay
BEN1	95	20	3.2	23	0.27	29	71	Silty clay
BEN2	94	19	4.2	24	0.31	28	72	Silty clay
BOW1	93	8.0	0.73	8.7	0.31	44	56	Silty clay
BOW2	92	6.5	0.13	6.7	0.43	44	56	Silty clay
INT1	88	13	0.13	14	0.11	35	64	Silty clay
INT2	91	13	0.19	13	0.18	38	62	Silty clay

n=1

NM indicates not measured

Table 4.8 Twenty-seven metal analysis (μmol/g dw) for sediment collected in the field

	Elliot Lake Area									PSQG (μmol/g dw)[a]		CSQG (μmol/g dw)[b]	
	TNM1	**TNM2**	**DUN1**	**DUN2**	**MCC1**	**MCC2**	**ELT1**	**ELT2**	**QKE1**	**LEL**	**SEL**	**ISQG**	**PEL**
Ag	0.003	0.002	0.004	0.001	0.003	0.003	0.003	0.003	0.01				
Al	680	320	820	550	990	1400	1000	980	270				
As	**0.22**	**0.11**	***0.46***	**0.10**	**0.16**	**0.16**	**0.23**	**0.13**	***0.80***	**0.08**	***0.44***	0.08	0.23
B	0.55	0.26	0.23	0.14	0.53	0.71	0.79	0.52	0.26				
Ba	0.86	1.4	1.5	0.92	1.2	1.8	1.2	1.2	1.0				
Be	0.11	0.21	0.16	0.09	0.10	0.14	0.12	0.13	0.04				
Bi	0.002	0.002	0.006	0.002	0.003	0.003	0.003	0.002	0.06				
Cd	**0.02**	**0.02**	**0.05**	**0.01**	**0.01**	**0.02**	**0.02**	**0.01**	0.004	**0.01**	***0.09***	0.01	0.03
Co	0.88	0.36	0.26	0.19	0.46	0.61	0.49	0.76	0.40				
Cr	***7.9***	**0.81**	**0.70**	**0.48**	**1.0**	**1.5**	**1.1**	**0.82**	**0.61**	**0.50**	***2.1***	0.72	1.7
Cu	***1.5***	***2.3***	***1.3***	**0.59**	***1.3***	**0.89**	**0.65**	**0.75**	***1.7***	**0.25**	***1.7***	0.56	3.1
Fe	**370**	**390**	**430**	**290**	***730***	***1100***	***690***	***660***	***4500***	**380**	***780***		
Ga	0.04	0.07	0.08	0.06	0.12	0.18	0.11	0.09	0.11				
La	0.25	0.50	0.39	0.27	0.40	0.51	0.38	0.49	0.86				
Li	0.29	1.2	0.36	1.1	2.6	4.3	1.9	1.6	0.08				
Mn	***37***	***24***	***27***	**12**	**15**	***42***	**14**	***24***	**12**	**8.4**	***20***		
Mo	0.59	0.03	0.04	0.01	0.02	0.03	0.02	0.02	0.08				
Ni	***18***	**0.60**	**0.97**	**0.34**	**0.78**	**0.88**	**0.58**	**0.68**	**0.60**	**0.27**	***1.3***		
Pb	**0.25**	0.09	***0.86***	**0.28**	**0.34**	**0.29**	**0.33**	**0.36**	***2.0***	**0.15**	***1.2***	0.17	0.44
Rb	0.10	0.16	0.12	0.09	0.26	0.39	0.20	0.16	0.03				
Sb	0.004	0.001	0.007	0.001	0.002	0.002	0.003	0.002	0.010				
Se	0.03	0.06	0.05	0.02	0.02	0.02	0.02	0.02	0.06				
Sr	0.22	0.27	0.35	0.18	0.33	0.53	0.38	0.32	0.07				
Tl	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001				
U	0.02	0.05	0.02	0.02	0.02	0.04	0.07	0.41	0.81				
V	0.56	0.90	0.90	0.70	1.5	2.0	1.4	1.2	0.80				
Zn	**2.8**	**2.9**	**4.4**	**2.3**	**3.0**	**4.3**	**2.8**	**3.0**	**3.1**	**1.8**	***13***	1.9	4.8

[a] Provinical Sediment Quality Guideline for the Porvince of Ontario; LEL-lowest effect level; PEL-Probable effects Level (Jaagumag

[b] Canadian Sediment Quality Guideline; ISQG-Intermin Sediment Quality Guideline; PEL-Probable Effects Level (CCME, 1999)

Indicates that the value is close (within 25%) or exceeds the PSQG LEL

Indicates that the value is close (within 25%) or exceeds the PSQG PEL

Indicates that the value is close (within 25%) or exceeds the CSQG ISQG

Indicates that the value is close (within 25%) or exceeds the CSQG PEL

Table 4.8 Twenty-seven metal analysis (μmol/g dw) for sediment collected in the field (continued)

	Bancroft Area										PSQG (μmol/g dw)[a]		CSQG (μmol/g dw)[b]	
	CTR1	**CTR2**	**SID1**	**SID2**	**BEN1**	**BEN2**	**BOW1**	**BOW2**	**INT1**	**INT2**	**LEL**	**SEL**	**ISQG**	**PEL**
Ag	0.004	0.001	0.002	0.002	0.001	0.001	0.002	0.002	0.002	0.001				
Al	450	350	280	360	290	250	900	440	550	550				
As	**0.25**	**0.08**	**0.21**	**0.12**	**0.09**	**0.09**	**0.11**	0.07	**0.10**	**0.09**	**0.08**	***0.44***	0.08	0.23
B	0.57	0.22	0.77	1.8	13	17	9.6	9.1	1.3	1.2				
Ba	1.6	0.84	1.9	1.9	1.2	0.91	2.2	1.4	1.6	1.3				
Be	0.09	0.07	0.10	0.11	0.13	0.10	2.8	0.80	0.14	0.13				
Bi	0.004	0.002	0.002	0.002	0.002	0.002	0.001	0.001	0.002	0.001				
Cd	**0.02**	**0.01**	**0.02**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**	**0.02**	**0.02**	**0.01**	***0.09***	0.01	0.03
Co	0.25	0.16	0.19	0.26	0.17	0.15	0.23	0.60	0.22	0.21				
Cr	0.26	0.23	0.28	0.37	0.33	0.27	0.43	0.24	0.33	0.38	**0.50**	***2.1***	0.72	1.7
Cu	**0.59**	**0.34**	**0.72**	**0.74**	**0.72**	**0.60**	**0.87**	**0.69**	**0.39**	**0.38**	**0.25**	***1.7***	0.56	3.1
Fe	96	29	210	72	71	63	**360**	**500**	69	74	**380**	***780***		
Ga	0.07	0.05	0.07	0.08	0.06	0.05	0.29	0.14	0.07	0.07				
La	0.49	0.36	1.2	1.5	1.4	0.95	13	9.0	0.98	0.86				
Li	0.50	0.59	0.60	0.94	0.90	0.74	4.4	2.5	1.3	1.9				
Mn	***9.7***	4.1	***36***	***22***	***30***	***20***	***25***	***54***	***43***	***21***	**8.4**	***20***		
Mo	0.06	0.02	0.29	0.12	0.08	0.15	0.24	0.39	0.03	0.04				
Ni	**0.21**	0.17	**0.28**	**0.32**	**0.28**	**0.24**	**0.71**	**0.76**	0.20	0.20	**0.27**	***1.3***		
Pb	***0.86***	**0.39**	**0.42**	**0.45**	**0.32**	**0.32**	***4.9***	**0.73**	**0.31**	**0.20**	**0.15**	***1.2***	0.17	0.44
Rb	0.07	0.07	0.05	0.09	0.08	0.07	0.28	0.25	0.08	0.08				
Sb	0.011	0.006	0.009	0.007	0.005	0.005	0.006	0.004	0.005	0.004				
Se	0.03	0.02	0.04	0.04	0.04	0.04	0.02	0.02	0.02	0.02				
Sr	0.62	0.59	0.58	1.4	4.6	4.2	3.0	2.3	0.65	0.70				
Tl	0.002	0.001	0.002	0.002	0.002	0.001	0.003	0.001	0.002	0.002				
U	0.03	0.02	0.08	0.09	0.15	0.11	3.3	1.2	0.14	0.12				
V	1.3	0.67	0.73	0.68	1.3	0.99	1.1	0.74	1.2	1.0				
Zn	**3.0**	**2.0**	**2.6**	**3.2**	**2.5**	**2.4**	**2.8**	**1.6**	**3.6**	**3.6**	**1.8**	***13***	1.9	4.8

[a] Provinical Sediment Quality Guideline for the Porvince of Ontario; LEL-lowest effect level; PEL-Probable effects Level (Jaagumagi, 1992)

[b] Canadian Sediment Quality Guideline; ISQG-Intermin Sediment Quality Guideline; PEL-Probable Effects Level (CCME, 1999)

Indicates that the value is close (within 25%) or exceeds the PSQG LEL

Indicates that the value is close (within 25%) or exceeds the PSQG PEL

Indicates that the value is close (within 25%) or exceeds the CSQG ISQG

Indicates that the value is close (within 25%) or exceeds the CSQG PEL

Table 4.9 Twenty-seven metal analysis for periphyton (nmol/g dw) collected in the field and TetraMin fish flakes

	TNM3	DUN3	MCC3	ELT3	QKE2	CTR3	SID3	BEN3	BOW3	INT3	TetraMin
Ag	0.27 (0.05)	0.29 (0.05)	0.61 (0.75)	0.26 (0.08)	0.72 (0.55)	0.65 (0.55)	0.10 (0.04)	0.28 (0.15)	0.37 (0.10)	0.79 (0.94)	0.96 (0.07)
Al	140000 (21000)	330000 (45000)	180000 (190000)	120000 (31000)	170000 (110000)	590000 (520000)	160000 (37000)	180000 (51000)	66000 (27000)	27 0000 (320000)	35000 (3500)
As	48 (6.6)	28 (3.0)	87 (99)	30 (7.0)	130 (110)	63 (61)	13 (7.0)	20 (13)	34 (8.4)	93 (100)	32 (2.6)
B	660 (210)	120 (93)	2000 (2500)	1600 (280)	700 (930)	2700 (3000)	1900 (1600)	2200 (1700)	12000 (4500)	12000 (11000)	520 (5.2)
Ba	1900 (440)	1000 (150)	4200 (5200)	2000 (330)	3300 (3800)	3400 (2600)	1600 (1200)	1200 (320)	2800 (1000)	5900 (6500)	97 (6.6)
Be	47 (3.6)	47 (7.1)	66 (78)	42 (9.6)	82 (75)	0.001 (0.001)	0.004 (0.003)	0.004 (0.003)	0.007 (0.005)	0.006 (0.006)	0.53 (0.03)
Bi	0.96 (0.10)	0.87 (0.17)	1.6 (1.9)	0.66 (0.16)	4.6 (3.6)	1.3 (1.3)	0.16 (0.04)	0.27 (0.17)	0.43 (0.14)	0.67 (0.86)	0.27 (0.49)
Cd	11 (3.7)	6.3 (2.4)	18 (23)	6.8 (0.97)	12 (13)	8.4 (4.1)	0.64 (0.37)	1.7 (0.65)	3.7 (1.8)	8.6 (9.1)	3.5 (0.1)
Co	77 (22)	86 (9.9)	1800 (2200)	740 (270)	1000 (820)	270 (200)	70 (15)	120 (46)	140 (47)	380 (470)	2.2 (0.32)
Cr	200 (22)	210 (24)	190 (190)	170 (46)	210 (140)	360 (320)	120 (21)	150 (64)	97 (36)	240 (290)	13 (16)
Cu	320 (35)	220 (47)	820 (1200)	210 (44)	590 (530)	510 (440)	210 (58)	240 (92)	340 (100)	360 (400)	130 (5.1)
Fe	160000 (15000)	230000 (26000)	330000 (360000)	160000 (41000)	450000 (330000)	970000 (850000)	150000 (42000)	270000 (120000)	60000 (190000	860000 (1100000)	3300 (170)
Ga	33 (3.1)	45 (4.5)	39 (44)	19 (4.6)	36 (31)	140 (130)	28 (4.9)	47 (14)	19 (8.7)	67 (88)	2.6 (0.25)
La	83 (15)	42 (8.7)	460 (520)	130 (29)	750 (730)	200 (170)	180 (39)	500 (250)	890 (310)	670 (810)	0.45 (0.09)
Li	490 (180)	510 (190)	1300 (1400)	430 (120)	530 (220)	2400 (2100)	1100 (220)	1600 (440)	340 (180)	2200 (2800)	16 (5.1)
Mn	19000(6200)	16000 (3500)	190000 (220000)	540000 (8600)	170000 (200000)	110000 (73000)	14000 (6800)	91000 (28000)	130000 (48000)	500000 (630000)	590 (15)
Mo	5.0 (0.82)	5.1 (0.93)	15 (18)	3.4 (0.69)	92 (72)	28 (32)	3.7 (2.3)	12 (4.6)	35 (12)	50 (56)	5.0 (0.31)
Ni	340 (82)	190 (21)	1500 (2000)	510 (92)	1100 (1200)	280 (250)	140 (42)	210 (84)	470 (100)	540 (620)	18 (0.95)
Pb	120 (18)	81 (19)	500 (590)	88 (17)	580 (450)	160 (140)	21 (9.9)	62 (29)	79 (29)	240 (300)	1.8 (0.37)
Rb	43 (1.9)	36 (3.1)	84 (88)	35 (10)	63 (55)	270 (250)	100 (34)	78 (51)	55 (22)	210 (250)	81 (2.4)
Sb	1.2 (0.41)	0.65 (0.55)	1.4 (2.0)	0.82 (0.14)	1.5 (1.9)	3.6 (3.2)	0.32 (0.22)	0.61 (0.26)	1.70 (0.54)	2.9 (3.3)	0.10 (0.02)
Se	24 (3.2)	13 (0.62)	18 (18)	7.0 (2.0)	30 (28)	13 (11)	2.7 (1.6)	5.2 (1.0)	16 (9.0)	27 (32)	11 (1.1)
Sr	330 (73)	200 (33)	230 (250)	250 (53)	140 (120)	1300 (1300)	490 (72)	1800 (810)	4000 (940)	1000 (880)	1500 (68)
Tl	0.45 (0.29)	0.27 (0.03)	1.5 (1.8)	0.38 (0.06)	2.2 (2.4)	3.1 (0.81)	0.28 (0.06)	0.34 (0.18)	0.61 (0.37)	2.3 (2.0)	0.06 (0.01)
U	6.4 (1.2)	4.0 (0.85)	220 (280)	110 (28)	1100 (1000)	13 (13)	3.6 (1.6)	62 (23)	1100 (770)	370 (390)	0.20 (0.01)
V	280 (25)	390 (48)	350 (370)	180 (46)	250 (180)	1600 (1400)	210 (52)	350 (110)	210 (73)	590 (730)	7.8 (0.59)
Zn	2200 (330)	1200 (260)	4800 (6200)	1700 (320)	3000 (2800)	3400 (2100)	640 (260)	780 (260)	1100 (360)	4100 (4500)	1100 (33)

All data reported as mean (95% confidence interval); n=3

Table 4.10 Twenty-seven metal analysis (nmol/g dw) for *H.azteca* in the field

	Elliot Lake Area				Bancroft Area					four-week LBC25
	TNM3	DUN3	MCC3	ELT3	CTR3	SID3	BEN3	BOW3	INT3	(nmol/g)
Ag	0.84 (0.74)	1.5 (0.33)	3.6 (0.64)	1.5 (0.20)	0.002 (0.0004)	0.0004 (0.0003)	0.001 (0.0005)	0.0006 (0.0001)	0.0023 (0.001)	
Al	21000 (27000)	12000 (8800)	5100 (4700)	7400 (7900)	1100 (1300)	3000 (4800)	1300 (49)	550 (290)	420 (260)	
As	20 (4.8)	23 (2.4)	26 (2.3)	28 (4.9)	BDL	BDL	BDL	BDL	BDL	83[b]
B	BDL	BDL	BDL	BDL	13 (170)	BDL	380 (490)	190 (100)	110 (160)	
Ba	540 (400)	1700 (230)	690 (120)	1400 (210)	510 (420)	230 (310)	BDL	BDL	BDL	
Be	0.15 (0.29)	0.08 (0.16)	BDL	BDL	BDL	BDL	0.19 (0.47)	0.58 (0.73)	0.33 (0.09)	
Bi	0.01 (0.01)	BDL	0.08 (0.16)	BDL	0.03 (0.01)	0.10 (0.17)	0.01 (0.005)	0.006 (0.009)	0.02 (0.02)	
Cd	180 (110)	**430 (79)**	140 (6.0)	190 (19)	9.9 (0.86)	1.6 (1.1)	1.9 (0.23)	1.5 (0.26)	1.5 (0.59)	**300**[c]; 640[d]
Co	16 (8.2)	9.6 (3.6)	40 (6.4)	12 (4.5)	7.9 (0.91)	4.1 (1.4)	5.1 (3.8)	3.6 (0.37)	2.7 (0.62)	90[b]
Cr	63 (59)	11 (4.1)	29 (5.0)	32 (26)	4.7 (2.1)	1.2 (0.71)	2.3 (0.21)	BDL	7.5 (7.7)	150[b]
Cu	**1800 (370)**	1300 (210)	1600 (80)	1600 (160)	1200 (81)	1300 (170)	1100 (220)	1300 (25)	1100 (260)	2200[c]
Fe	18000 (22000)	4100 (1500)	5500 (3900)	4500 (4600)	870 (260)	1000 (220)	430 (120)	1700 (490)	610 (100)	
Ga	1.8 (1.5)	0.94 (0.35)	1.0 (0.36)	0.92 (0.31)	0.86 (0.04)	0.97 (0.35)	0.91 (0.06)	0.74 (0.08)	0.94 (0.10)	
La	3.1 (2.7)	0.81 (0.32)	5.8 (2.2)	3.3 (2.0)	0.98 (0.13)	0.61 (0.12)	0.92 (0.24)	1.5 (0.3)	0.84 (0.66)	
Li	BDL	BDL	BDL	BDL	140 (110)	110 (115)	2.4 (35)	140 (93)	140 (120)	
Mn	2100 (910)	1300 (460)	1700 (830)	1100 (85)	1100 (140)	410 (66)	440 (300)	340 (74)	530 (450)	44000[b]
Mo	14 (5.0)	7.6 (1.5)	8.0 (0.61)	5.1 (1.1)	8.3 (0.88)	8.5 (0.33)	7.2 (1.1)	8.3 (0.63)	10 (0.71)	
Ni	720 (1200)	58 (6.7)	150 (140)	130 (230)	18 (12)	25 (39)	12 (10)	5.3 (1.3)	8.0 (3.5)	280[c]
Pb	26 (27)	5.1 (1.2)	9.8 (11)	7.1 (9.9)	3.6 (0.51)	BDL	BDL	BDL	BDL	81[c]
Rb	280 (53)	260 (33)	350 (20)	220 (13)	290 (8.3)	210 (15)	190 (45)	200 (5.9)	310 (16)	
Sb	3.3 (4.3)	1.1 (0.65)	1.5 (1.5)	0.86 (1.2)	0.15 (0.15)	BDL	0.04 (0.11)	0.11 (0.07)	0.10 (0.14)	
Se	22 (18)	22 (13)	23 (3.6)	14 (3.4)	22 (7.9)	13 (6.2)	16 (10)	8.8 (4.7)	17 (6.4)	
Sr	880 (390)	1800 (160)	1100 (110)	1900 (94)	4200 (300)	2800 (250)	7600 (6200)	4300 (730)	1500 (980)	
Tl	BDL	BDL	0.26 (0.51)	BDL	0.28 (0.04)	0.23 (0.03)	0.27 (0.004)	0.27 (0.029)	0.52 (0.003)	360[c]
U	0.47 (0.23)	0.39 (0.10)	1.9 (0.23)	1.4 (0.2)	0.23 (0.02)	0.29 (0.008)	2.5 (2.0)	1.5 (0.80)	1.0 (0.51)	33[e]; 37[f]
US[a]	0.44 (0.08)	0.38 (0.19)	1.7 (0.64)	1.2(0.53)	0.47 (0.13)	0.66 (0.22)	5.1 (2.3)	3.0 (1.7)	2.0 (0.50)	33[e]; 37[f]
V	16 (21)	4.7 (1.6)	5.6 (3.0)	5.1 (3.4)	1.4 (0.38)	0.94 (0.24)	4.6 (2.8)	1.1 (0.49)	0.68 (0.37)	
Zn	***7200 (9300)*** [f]	***1700 (130)*** [f]	***2700 (1500)*** [f]	***2700 (2700)*** [f]	970 (64)	890 (40)	1100 (9.0)	950 (92)	890 (160)	1800[c]

n=4, except for Ten Mile and Bow, n=3 and Bentley and Inlet, n=2

[a] US indicates animals that have been standarized (95% confidence interval) to 0.20 (0.12) mg dw

[b] Data source from Norwood et al. (2007) using the 24 h gut cleared corrected estimates. Water hardness was ~120 mg/L as $CaCO_3$

[c] Data source from Borgamann et al. (2004) using the saturation model estimates. Average water hardness was ~ 130 mg/L as $CaCO_3$

[d] Data source from Borgmann et al. (2004) using the saturation model estimates in a water hardness of ~ 13 mg/L as $CaCO_3$

[e] Data source from Alves et al. (2008) in a water hardness of ~ 130 mg/L as $CaCO_3$

[f] Data source from Alves et al. (2008) in a water hardness of ~ 13 mg/L as $CaCO_3$

Table 4.11 Metal concentrations in *H. azteca* exposed to SITE or SAM overlying water, field collected sediments and fed TetraMin or periphyton for four weeks

Elliot Study Area														four-week LBC25
Metal	TNM-SAM	TNM-SITE	DUN-SAM	DUN-SITE	MCC-SAM	MCC-SITE	MCC-P	ELT-SAM	ELT-SITE	ELT-P	QKE-SAM	QKE-SITE	QKE-P	(nmol/g)
Ag	0.56 (0.22)	0.73 (0.93)	0.96 (0.29)	BDL	1.4 (0.38)	BDL	0.47 (0.03)	1.0 (0.38)	1.7 (1.5)	1.5 (1.1)	0.49 (0.19)	BDL	1.8	
Al	1600 (640)	2200 (2500)	1200 (880)	1300 (100)	1200 (570)	710 (330)	1700 (1500)	1500 (800)	2400 (2300)	1600 (220)	1300 (1500)	900	2900	
As	1.2 (3.1)	BDL	2.2 (3.1)	BDL	BDL	BDL	BDL	1.6 (4.0)	BDL	BDL	BDL	BDL	BDL	83^a
B	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.0 (16)	BDL	BDL	BDL	BDL	BDL	
Ba	160 (42)	590 (320)	230 (83)	1300 (310)	250 (42)	630 (300)	1500 (260)	260 (18)	720 (580)	1300 (470)	230 (100)	790	1500	
Be	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Bi	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Cd	22 (7.9)	82 (83)	34 (14)	430 (260)	11 (2.5)	37 (17)	24 (14)	9.4 (2.0)	81 (84)	37 (4.7)	6.7 (4.3)	32	28	300^b; 640^c
Co	30 (33)	55 (100)	21 (7.8)	13 (6.8)	15 (5.4)	7.9 (13)	36 (6.9)	19 (4.3)	22 (28)	39 (1.6)	14 (2.2)	73	14	90^a
Cr	3.9 (2.1)	34 (30)	3.5 (0.71)	56 (7.1)	15 (21)	26 (32)	19 (0.60)	9.1 (10)	17 (9)	31 (16)	5.8 (2.0)	61	14	150^a
Cu	1700 (350)	2200 (2000)	1600 (140)	1500 (380)	1400 (140)	1700 (430)	1700 (340)	1400 (190)	1500 (410)	1500 (36)	1400 (150)	1200	1700	2200^b
Fe	920 (200)	1300 (770)	1200 (310)	1700 (540)	960 (240)	1800 (540)	1500 (690)	1300 (380)	1600 (530)	1100 (430)	1300 (330)	3400	3300	
Ga	0.95 (0.07)	1.2 (1.3)	0.94 (0.07)	BDL	0.93 (0.06)	0.67 (0.84)	0.55 (0.11)	0.90 (0.14)	0.67 (0.93)	0.44 (0.03)	0.83 (0.3)	1.9	0.45	
La	0.31 (0.10)	0.49 (0.08)	0.28 (0.02)	0.85 (0.46)	0.23 (0.04)	2.1 (1.7)	2.2 (0.43)	0.41 (0.11)	3.7 (2.1)	0.60 (0.34)	0.36 (0.18)	70	7.6	
Li	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Mn	350 (150)	5900 (11000	500 (200)	1400 (1200)	620 (240)	1800 (1700)	660 (100)	380 (240)	700 (380)	450 (420)	200 (45)	1500	270	44000^a
Mo	3.4 (0.5)	3.2 (3.0)	3.4 (0.26)	BDL	4.2 (1.8)	0.10 (4.9)	0.68 (1.5)	4.3 (0.60)	2.0 (4.2)	0.98 (0.20)	1.0 (0.56)	BDL	0.53	
Ni	22 (11)	37 (45)	22 (3.0)	52 (24)	39 (19)	52 (53)	30 (4.8)	17.0 (3.8)	36 (16)	38 (18)	25 (2.4)	220	33	280^b
Pb	0.88 (0.45)	2.0 (1.2)	1.3 (0.56)	8.2 (6.6)	1.1 (1.3)	2.1 (1.6)	1.6 (0.26)	1.3 (0.72)	6.0 (5.8)	2.2 (1.5)	1.2 (0.72)	69	1.3	81^b
Rb	43 (4.0)	240 (160)	45 (3.2)	220 (54)	120 (14)	430 (70)	440 (27)	51 (3.1)	220 (30)	220 (5.7)	70 (55)	490	380	
Sb	0.06 (0.10)	0.21 (0.40)	0.05 (0.02)	0.28 (1.3)	0.20 (0.35)	0.06 (0.12)	BDL	0.10 (0.11)	BDL	0.12 (0.05)	0.02 (0.03)	2	0.47	
Se	8.9 (1.3)	5.5 (4.7)	9.0 (0.78)	BDL	6.3 (0.88)	8.9 (2.1)	8.9 (2.1)	8.0 (0.64)	4.9 (4.9)	14 (0.04)	10 (3.4)	BDL	29	
Sr	260 (6.5)	1800 (1200)	270 (6.6)	1500 (440)	260 (28)	750 (160)	820 (38)	290 (21)	1500 (800)	1300 (140)	240 (23)	700	460	
Tl	0.87 (0.11)	2.8 (3.4)	1.3 (0.20)	2.2 (2.2)	0.66 (0.14)	0.62 (0.44)	0.33 (0.26)	0.88 (0.22)	1.2 (1.0)	0.73 (0.05)	1.8 (0.66)	BDL	0.83	360^b
U	0.14 (0.02)	0.17(0.14)	0.10 (0.04)	0.02 (0.02)	0.12 (0.03)	0.13 (0.11)	0.61 (0.31)	0.29 (0.23)	0.56 (0.91)	0.53 (0.17)	0.49 (0.22)	0.88	3.1	33^d; 37^e
Us	0.15 (0.02)	0.14 (0.11)	0.11 (0.05)	0.01 (0.02)	0.13 (0.04)	0.15 (0.09)	0.40 (0.17)	0.32 (0.26)	0.43 (0.68)	0.39 (0.10)	0.52(0.30)	0.51	2.7	33^d; 37^e
V	2.0 (0.69)	2.4 (0.76)	1.5 (0.27)	0.35 (0.69)	1.2 (0.36)	2.0 (1.4)	2.5 (0.64)	1.2 (0.30)	1.3 (1.4)	4.1 (1.1)	0.28 (0.08)	BDL	BDL	
Zn	970 (46)	1300 (950)	950 (13)	2100 (1100)	960 (70)	1300 (210)	1100 (90)	1000 (82)	1400 (320)	1600 (640)	1280 (260)	2100	1700	1800^b

Table 4.11 Metal concentrations in *H. azteca* exposed to SITE or SAM overlying water, field collected sediments and fed TetraMin or periphyton for four weeks (continued)

Bancroft Study Area														four-week LBC25
Metal	CTR-SAM	CTR-SITE	SID-SAM	SID-SITE	BEN-SAM	BEN-SITE	BEN-P	BOW-SAM	BOW-SITE	BOW-P	INT-SAM	INT-SITE	INT-P	(nmol/g)
Ag	2.4 (0.32)	2.6	1.6 (0.58)	1.2 (0.40)	0.71 (0.07)	0.71 (0.13)	1.2	0.52 (0.14)	0.46 (0.03)	0.54	2.2 (0.17)	1.8 (0.50)	2.6 (0.01)	
Al	670 (400)	210	720 (490)	780 (640)	510 (390)	490 (170)	2400	830 (470)	570 (220)	2000	680 (310)	1200 (2100)	1700 (450)	
As	8.6 (1.0)	3.2	8.1 (3.0)	9.5 (1.4)	6.0 (1.1)	10 (1.7)	37	7.9 (2.5)	7.7 (2.2)	31	7.1 (1.3)	8.7 (6.1)	22 (0.81)	83[a]
B	6.2 (15)	BDL	13 (8.7)	BDL	30 (28)	160 (30)	110	4.2 (12)	150 (23)	BDL	2.6 (16)	48 (150)	75 (130)	
Ba	230 (57)	1100	270 (90)	720 (180)	160 (64)	150 (52)	260	220(120)	280 (85)	570	230 (88)	470 (110)	970 (140)	
Be	0.10 (1.0)	1.1	BDL	0.36 (0.60)	0.39 (0.20)	BDL	BDL	BDL	0.57 (0.39)	0.51	0.36 (0.25)	0.10 (0.58)	BDL	
Bi	0.02 (0.005)	0.1	0.02 (0.01)	0.02(0.01)	0.01 (0.002)	0.01 (0.01)	0.03	0.01 (0.01)	0.01 (0.01)	0.02	BDL	BDL	0.01 (0.01)	
Cd	7.4 (5.7)	69	5.5 (4.1)	4.2 (2.5)	2.8 (1.4)	2.4 (1.2)	4.7	1.8 (0.88)	1.4 (0.45)	3.1	3.4 (1.1)	4.4 (1.2)	4.9 (1.5)	300[b]; 640[c]
Co	6.2 (2.3)	14	5.5 (1.3)	4.9 (1.1)	3.6 (3.0)	2.3 (0.80)	2.8	4.8 (1.4)	4.1 (1.5)	4.8	6.7 (1.7)	4.2 (1.9)	3.9 (0.44)	90[a]
Cr	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	150[a]
Cu	1200 (96)	1400	1400 (320)	1200 (410)	1200 (180)	1400 (140)	1300	1000 (100)	1200 (53)	1400	1300 (79)	1300 (19)	1200 (57)	2200[b]
Fe	1600 (590)	11000	2100 (2100)	15000 (21000)	1300 (410)	1700 (1600)	1800	4100 (3200)	2200 (670)	1400	1700 (1400)	4400 (7800)	BDL	
Ga	1.1 (0.04)	1	1.2 (0.07)	1.2 (0.06)	1.1 (0.09)	1.1 (0.05)	0.89	1.0 (0.11)	1.1 (0.07)	0.89	1.1 (0.04)	1.2 (0.05)	0.89 (0.09)	
La	0.55 (0.26)	3.5	0.73 (0.54)	0.33 (0.07)	1.3 (1.3)	0.59 (0.17)	0.33	6.4 (1.3)	5.7 (1.6)	3.6	1.1 (0.22)	1.1 (0.15)	0.75 (0.60)	
Li	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Mn	86 (31)	1500	110 (76)	270 (240)	220 (140)	260 (94)	32	270 (250)	550 (180)	BDL	360 (270)	330 (350)	390 (130)	44000[a]
Mo	4.1 (0.13)	BDL	5.8 (1.2)	3.4 (2.4)	3.7 (0.49)	5.0 (0.79)	2.2	3.7 (1.5)	4.0 (1.9)	0.02	4.4 (0.48)	4.2 (0.01)	3.4 (1.3)	
Ni	46 (91)	BDL	27 (40)	BDL	1.9 (4.2)	35 (44)	BDL	22 (27)	59 (91)	6	4.9 (3.1)	6.2 (5.5)	BDL	280[b]
Pb	0.85 (0.30)	11	0.33 (0.22)	BDL	0.29 (0.10)	0.04 (0.12)	BDL	0.05 (0.27)	0.21 (0.25)	BDL	0.27 (0.51)	0.52 (0.58)	0.30 (0.37)	81[b]
Rb	38 (7.6)	180	40 (11)	240 (31)	31 (2.9)	170 (13)	150	61 (10)	310 (27)	320	46 (5.7)	220 (2.3)	280 (0.66)	
Sb	0.15 (0.03)	0.06	0.17 (0.07)	0.26 (0.12)	0.16 (0.07)	0.11 (0.09)	0.22	0.05 (0.03)	0.10 (0.15)	0.36	0.10 (0.02)	0.20 (0.20)	BDL	
Se	13 (1.0)	15	12 (2.2)	12 (4.2)	11 (1.6)	9.9 (2.0)	18	12 (2.2)	6.1 (7.8)	11	10 (4.1)	8.7 (2.3)	30 (0.77)	
Sr	430 (53)	3800	420 (80)	2700 (240)	820 (100)	9200 (1400)	6300	550 (36)	6400 (900)	5700	460 (88)	2100 (92)	2000 (170)	
Tl	1.2 (0.18)	2	0.82 (0.14)	0.57 (0.21)	0.17 (0.07)	0.17 (0.07)	0.12	0.18 (0.04)	0.22 (0.07)	0.19	1.1 (0.23)	0.84 (0.16)	1.4 (0.06)	360[b]
U	0.04 (0.002)	0.01	0.14 (0.06)	0.19 (0.08)	0.19 (0.12)	1.6 (0.19)	0.49	1.1 (0.67)	2.7 (1.0)	4.2	0.39 (0.10)	0.31 (0.10)	0.95 (0.05)	33[d]; 37[e]
Us	0.05 (0.003)	0.004	0.16 (0.06)	0.18 (0.08)	0.24(0.18)	2.1(0.31)	0.46	1.3 (0.76)	3.0 (1.4)	2.9	0.49 (0.06)	0.34 (0.14)	0.42 (0.05)	33[d]; 37[e]
V	0.49 (0.39)	BDL	0.15 (0.38)	0.13 (1.6)	0.97 (0.42)	2.5 (1.1)	1.5	0.24 (0.15)	0.35 (0.17)	BDL	0.79 (0.18)	1.1 (0.25)	0.64 (0.26)	
Zn	900 (39)	740	890 (43)	730 (240)	840 (28)	910 (14)	720	890 (12)	900 (160)	420	900 (41)	920 (170)	960 (27)	1800[b]

US indicates animilas that have been standarized (95% confidence interval) to 0.30 (0.04) mg dw

n=4 for all sites, except for those indicated below for the cone experiments

n=2 for QKE-SAM, MCC-P, ELT-P, cone experiment sites

n=1 for QKE-SITE, QKE-P, CTR-SITE, BEN-P, BOW-P

All data reported as mean (95% confidence interval)

SAM-indicates the artifical overlying water media

SITE-indicates overlying site water

P-indicates amphipods fed periphyton and exposed to overlying site water

[a] Data source from Norwood et al.(2007) using the 24h gut cleared corrected estimates. Water hardness was ~120 mg/L as $CaCO_3$

[b] Data source from Borgamann et al. (2004) using the saturation model estimates. Average water hardness was ~ 130 mg/L as $CaCO_3$

[c] Data source from Borgmann et al. (2004) using the saturation model estimates in a water hardness of ~ 13 mg/L as $CaCO_3$

[d] Data source from Alves et al. (2008) in a water hardness of ~ 130 mg/L as $CaCO_3$

[e] Data source from Alves et al. (2008) in a water hardness of ~ 13 mg/L as $CaCO_3$

Table 4.12 Model predictions for the water-bioaccumulation and water-sediment partitioning saturation models using field collected U concentration data

Water-Bioaccumulation Saturation Model

	SAM overlying water and cultured *H. azteca* (cone experiment)						Site overlying water and cultured *H.azteca* (cone experiment)						Lake water and field *H. azteca* (Field measurements)					
	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed[a] (nmol/g)	Predicted[b] (nmol/g)	Observed/ Predicted	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed[a] (nmol/g)	Predicted[b] (nmol/g)	Observed/ Predicted	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed[a] (nmol/g)	Predicted[b] (nmol/g)	Observed/ Predicted
ELT	4.4	8.1	2.3E-07	0.32	0.13	2.5	2.1	6.9	3.4E-01	0.43	1.40	0.3	2.4	8.2	8.0E-07	1.2	0.52	2.3
MCC	0.29	7.9	2.3E-06	0.13	0.12	1.1	0.51	6.9	3.4E-01	0.15	0.43	0.3	2.4	8.3	5.3E-08	1.7	0.49	3.4
QKE	14	8.1	4.3E-07	0.52	0.2	2.6	2.3	6.2	6.8E+00	0.51	0.15	3.4	4.1	7.3	2.0E-02		4.7	
BEN	1.5	8.2	1.0E-07	0.24	0.15	1.6	66	8.0	1.2E-08	2.1	0.13	16.2	110	7.7	2.5E-05	5.1	5.4	0.9
BOW	11	8.0	1.4E-06	1.3	0.27	4.8	150	8.1	2.4E-07	3.0	0.59	5.1	130	7.7	4.0E-05	3.0	9.2	0.3
INT	1.6	7.9	5.9E-06	0.49	0.18	2.7	1.8	7.7	3.0E-04	0.34	0.85	0.4	3.9	7.8	5.7E-05	2.0	1.4	1.4
Unknown Pond (SW)[d]													490	6.3	5.7E+00	100 (71)	22	4.6 (3.2)
Horseshoe Pond (SW)[d]													370	6.6	1.7E+00	34 (23)	40	0.9 (0.6)
Unknown Pond (PW)[d]													19000	7.0	4.0E-02	81 (56)	80	1.0 (0.7)
Horseshoe Pond (PW)[d]													6200	6.8	4.3E-01	150 (100)	79	1.9 (1.3)

Water-Sediment Partitioning Saturation Model

	SAM overlying water (cone experiment)						Site overlying water (cone experiment)						Lake water (Field measurements)					
	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed (nmol/g dw)	Predicted[c] (nmol/g dw)	Observed/ Predicted	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed (nmol/g dw)	Predicted[c] (nmol/g dw)	Observed/ Predicted	Measured U in water (nmol/L)	pH	UO_2^{2+} (%)	Observed (nmol/g)	Predicted[c] (nmol/g)	Observed/ Predicted
ELT	4.4	8.1	2.3E-07	240	140	1.7	2.1	6.9	3.4E-01	240	1400	0.2	2.4	8.2	8.0E-07	240	540	0.44
QKE	14	8.1	4.3E-07	810	440	1.8	2.3	6.2	6.8E+00	810	74	11	4.1	7.3	2.0E-02	810	3800	0.21
BEN	1.5	8.2	1.0E-07	130	70	1.9	66	8.0	1.2E-08	130	64	2.0	110	7.7	2.5E-05	130	4600	0.03
BOW	11	8.0	1.4E-06	2300	450	5.1	150	8.1	2.4E-07	2300	2100	1.1	130	7.7	4.0E-05	2300	6600	0.35
INT	1.6	7.9	5.9E-06	130	150	0.9	1.8	7.7	3.0E-04	130	960	0.1	3.9	7.8	5.7E-05	130	1400	0.09
Unknown Pond (SW)[e]													520	6.1	1.1E+01	16000	4500	3.6
Horseshoe Pond (SW)[e]													430	6.6	1.7E+00	14000	18000	0.8
Unknown Pond (PW)[e]													19000	7.0	4.0E-02	16000	450000	0.04
Horseshoe Pond (PW)[e]													6200	6.8	4.3E-01	14000	260000	0.1

[a] Observed values are based on animals size-standardized (95% Confidence intervals) to 0.30 (0.04) mg dw and 0.20 (0.12) mg for the cone experiment and field measurements, respectively

[b] Bioaccumulation of U in for the cultured and field collected *H. azteca* was predicted using Eq. (1), where $C_{TB} = \max{}^*C_W/(K_{0.5} + C_W) + C_{bkg}$ and the values in Table 4.1

Lake water refers to U concentrations measured in the field collected surface water (Table 4.5) and not used in the cone experiments

The mean C_{bkg} term was 0.12 ± 0.03; 0.13 ± 0.06, and 0.49 ± 0.10 nmol/g for cultured or field collected *H. azteca* exposed to the overlying SAM, site and lake water, respectively, for the control sites

[c] Predicted concentration of U in the sediment was predicted using Eq.(4), where $C_{sed}=\alpha{}^*C_W + \max''{}^*C_W/(K_{0.5}'' + C_W) + C_{bkgs}$ and the values in Table 4.1. The mean CBKGS was 42 ± 21 nmol/g dw

McCarthy Lake concentrations in the sediment were below background concentrations. Thus the water-sediment partitioning saturation model was not tested doe this Lake.

[d] Using Data from the Robertson and Liber (2007) study, SW indicates *H.azteca* exposed to surface water; PW indicates; H. azteca exposed to the pore water, animals were exposed for 4-days; $C_{bkg} = 9.24$ nmol/g

[e] Using data from Robertson (2006); $C_{bkgs} = 546$ nmol/g

[f] Adjusted for steady state based on the data from Chapter 3, where percent of steady-state (69%)= $(1-e^{-ket})100$; ke=0.29, t=4 and growth (*g*) is assumed to be negligible; values in brackets indicated the observed U concentration measured the *H. azteca in* Robertson and Liber (2007) study prior to steady state adjustments

Table 4.13 Measure/Effect ratios for the maximum mean measured metal concentrations for different metals in the overlying water (nmol/L) and *H. azteca* (nmol/g dw) after four-week sediment toxicity tests, and available effect LC25 (nmol/L) estimates in the water and effect LBC25 (nmol/g dw) estimates in *H.azteca*

	Maximum measured metal concentration in overlying water				**Maximum measured metal accumulation in *H. azteca***			
Metal	Site	Measure	Effect (LC25)	Measure/Effect	Site	Measure	Effect (LBC25)	Measure/Effect
As-hard	DUN-SAM	6.9	4300^{a}	0.002	BEN-SITE	10	83^{a}	0.12
Cd-hard	DUN-SAM	0.46	3.2^{b}	0.14	DUN-SAM	34	300^{b}	0.11
Cd-soft	DUN-SITE	1.2	6.2^{b}	0.19	**DUN-SITE**	430	640^{b}	**0.68**
Co-hard	CTR-SAM	2.3	68^{a}	0.03	**TNM-SAM**	30	90^{a}	**0.33**
Cr-hard	INT-SAM	3.5	240^{a}	0.01	MCC-SAM	15	150^{a}	0.10
Cu-hard	DUN-SAM	52	440^{b}	0.12	**TNM-SAM**	1700	2200^{b}	**0.78**
Mn-hard	MCC-SAM	520	150000^{a}	0.004	MCC-SAM	620	44000^{a}	0.01
Ni-hard	QKE-SAM	30	400^{b}	0.08	BOW-SITE	59	280^{b}	0.21
Pb-hard	QKE-SAM	1.2	36^{b}	0.07	DUN-SAM **(QKE-SITE)**	1.3 (69)	81^{b}	0.02 **(0.85**)
Tl-hard	QKE-SAM	0.21	51^{b}	0.004	QKE-SAM	1.8	360^{b}	0.005
U-hard	BOW-SITE	140	2100^{c}	0.07	BOW-SITE	2.7	33^{c}	0.08
U-soft	QKE-SITE	2.3	95^{c}	0.02	ELT-SITE	2.1	37^{c}	0.06
Zn-hard	MCC-SAM	190	2500^{b}	0.08	QKE-SAM	1280	1800^{b}	**0.70**

soft indicates soft water, with a water hardness around 18 mg/L , but not more than 120 mg/L $CaCO_3$, Borgmann et al., 2005

hard indicates a hard water similar to SAM with a hardness of around 120 mg/L $CaCO_3$ or higher, Borgmann et al., 2005

[a] Data source from Norwood et al., 2007 based on four-week water-only experiments

[b] Data source from Borgmann et al., 2004 based on four-week water-only or sediment experiments

[c] Data source from Alves et al., 2008 based on four-week sediment experiments

Table 4.14 Mesure/effect ratios for the maxmium mean measured metal concentrations for different metals in different overlying waters (nmol/L) after four-week sediment toxicity tests, and available one-week effect LC50 (nmol/L) estimates for water

	Maximum Mean measured metal concentration in the overlying water			
Metal	Site	Measure	Effect[a]	Measure/Effect
Al-soft	BEN-SITE	800	3300	0.24
Cd-hard	DUN-SAM	0.46	14	0.03
Cd-soft	DUN-SITE	1.2	1.3	**0.92**
Cr-soft	INT-SAM	2	60	0.03
Cu-hard	DUN-SAM	52	1400	0.04
Cu-soft	TNM-SITE	63	570	0.11
Mn-hard	MCC-SAM	520	3100	0.17
Mn-soft	QKE-SITE	670	1700	**0.39**
Ni-soft	QKE-SITE	96	1300	0.07
Pb-hard	QKE-SAM	1.2	53	0.02
Pb-soft	CTR-SITE	1.1	4.8	0.23
U-soft	QKE-SITE	2.3	88	0.03
Zn-hard	MCC-SAM	190	3400	0.06
Zn-soft	DUN-SITE	300	860	**0.35**

soft indicates soft water, with a water hardness around 18 mg/L , but not more than 120 mg/L $CaCO_3$, Borgmann et al., 2005.

hard indicates a hard water similar to SAM with a hardness of around 120 mg/L $CaCO_3$ or higher, Borgmann et al., 2005.

[a] Data source from Borgmann et al., 2005; one-week LC50 in water-only experiments

For the following metals the measured/effect ratio for one-week LC50 was
<0.01: Ag, As, B, Be, Co, Cr-hard, La,Li, Ni-hard, Sb, Se, Tl, U-hard, and V.

Fig. 4.1 Location of sampling sites (Natural Resources Canada, 2002)

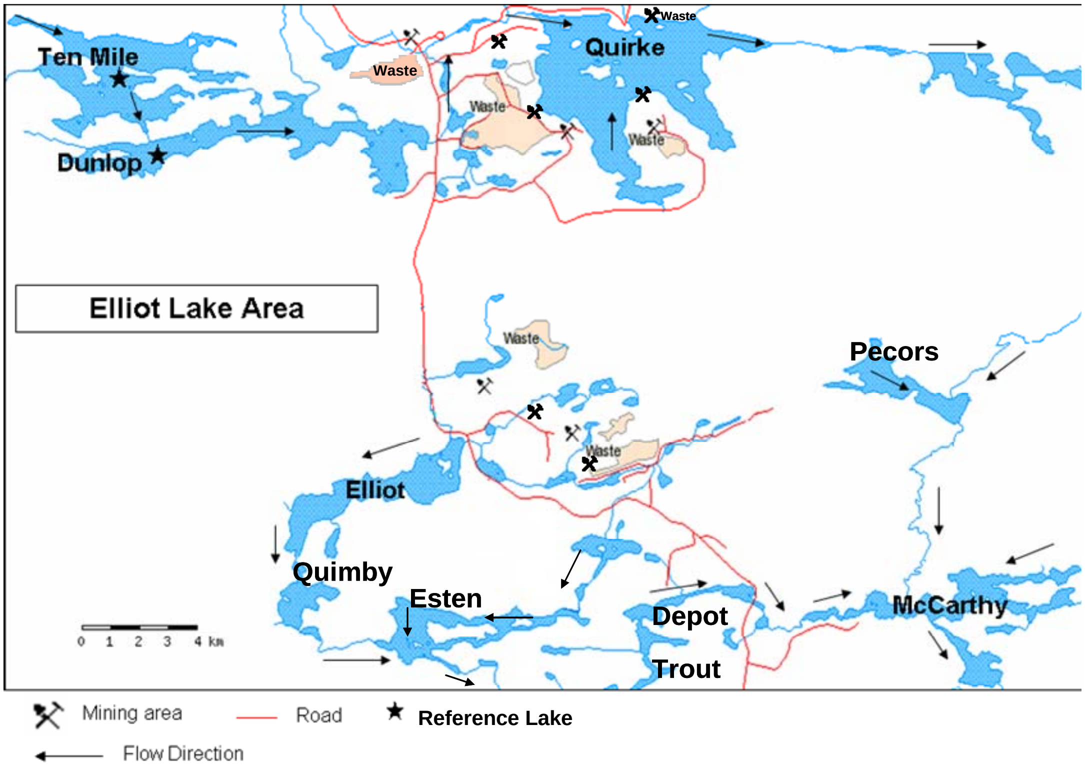

Fig. 4.2 Map of Elliot Lake and sampling site locations (Minnow Environmental Inc., 2005; Natural Resources Canada, 2009)

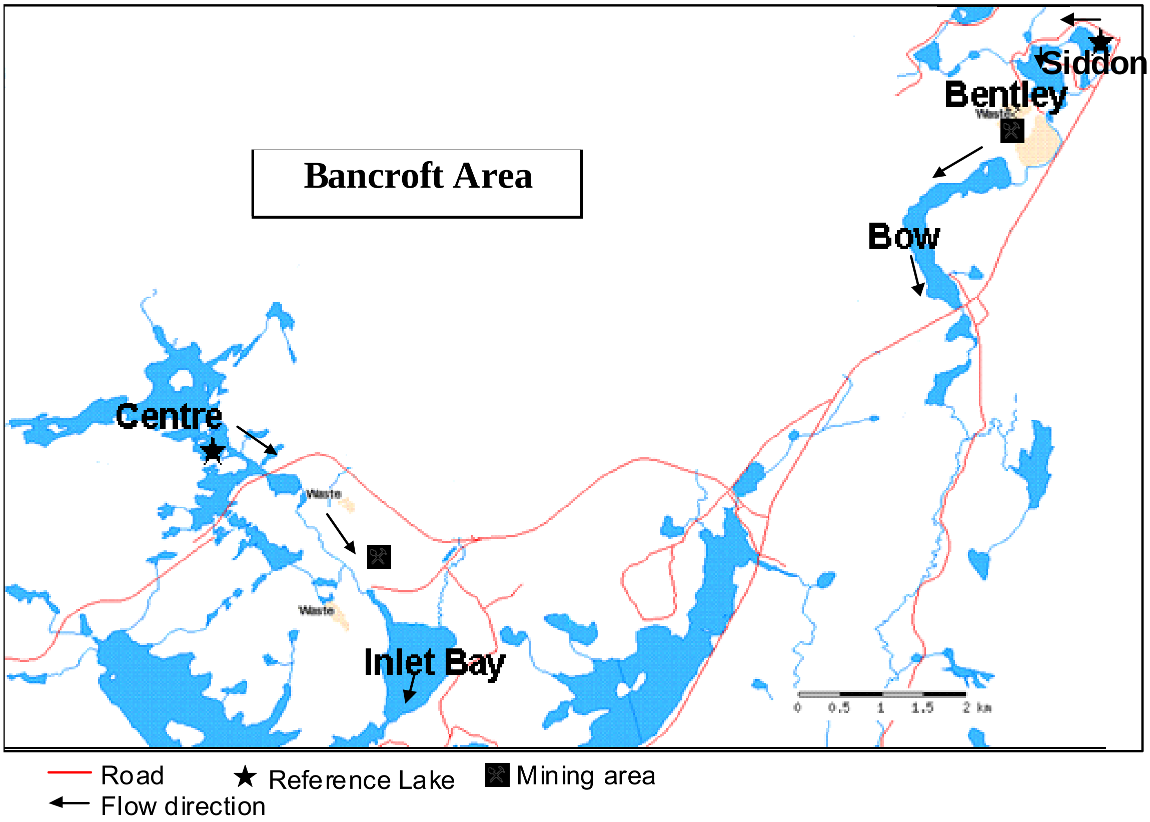

Fig. 4.3 Map of Bancroft and sampling site locations (OMOE, 2003; Natural Resources Canada, 2009)

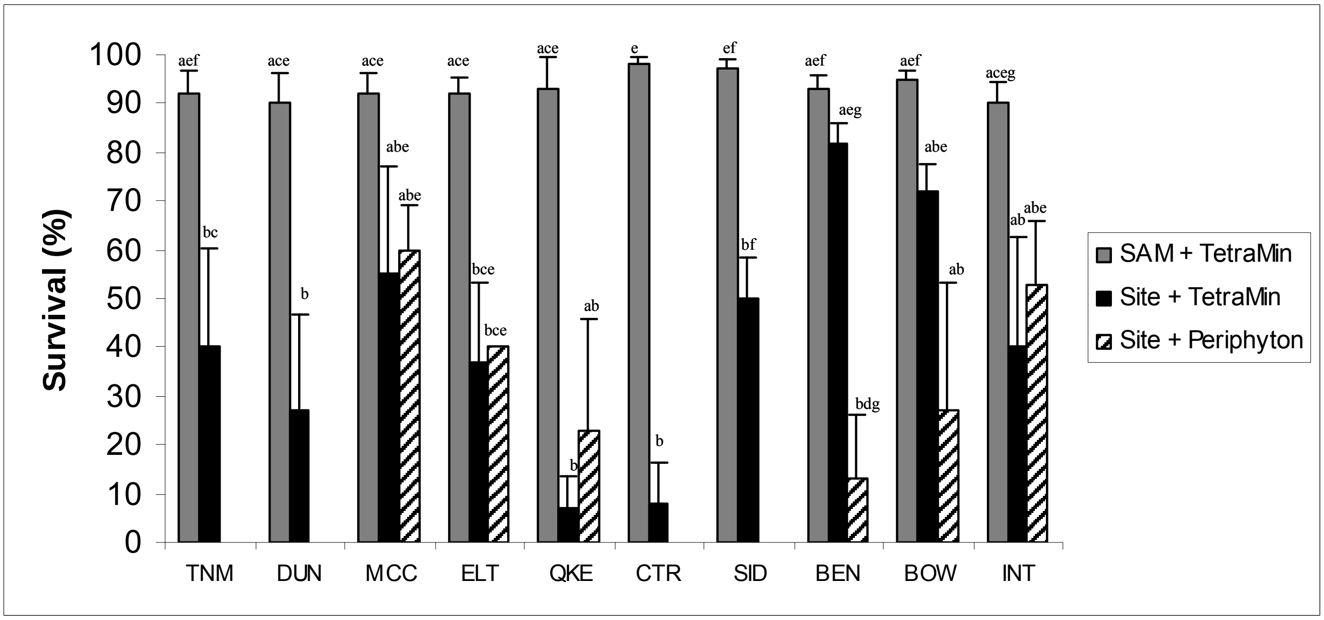

Fig. 4.4 Mean survival for *H. azteca* fed TetraMin or periphyton and exposed to overlying SAM or site water for the sediment toxicity tests. Data is represented as mean ± 95 % confidence interval. For all treatments n=4, except for the periphyton treatments where n=2. Values sharing the same lower case letter are not significantly different from the other treatment values (P<0.05). Comparsions between the treatments and lakes were maded by an nested ANOVA followed by Tukey's multiple pairwaise comparison test for differences among treatments and lakes (P<0.05).

APPENDIX

Table 4.A1 Survival data (95% Confidence Interval) and pH values for Fig. 4.4

Lake	**SAM + TetraMin**	**pH**	**Site + TetraMin**	**pH**	**Site + Periphyton**	**pH**
TNM	92 (9.8)	8.0	40 (40)	7.0		
DUN	90 (13)	8.0	27 (40)	6.7		
MCC	92 (8.2)	7.9	55 (44)	6.9	60 (19)	7.0
ELT	92 (6.3)	8.1	37 (32)	6.9	40	7.3
QKE	93 (13)	8.1	7 (13)	6.2	23 (46)	6.7
CTR	98 (3.3)	7.9	8 (16)	6.1		
SID	97 (3.8)	8.0	50 (17)	8.0		
BEN	93 (5.3)	8.0	82 (8)	8.0	13 (26)	8.2
BOW	95 (3.3)	8.0	72 (11)	8.1	27 (52)	8.2
INT	90 (8.4)	7.9	40 (46)	7.7	53 (26)	7.8

CHAPTER 5

Summary and Conclusions

It was shown that overlying water chemistry affects Uranium (U) bioavailability, bioaccumulation and toxicity to *H. azteca* exposed to U-spiked sediments for 28 days (Chapter 2). Water pH, rather than calcium, predominantly affects the dissolution of U from the sediment and U bioavailability and uptake by *H. azteca*. It was determined that Ca affects U accumulation through its effect on speciation rather than through direct competition with U for uptake. The desorption of U from the sediment into the overlying water increased with pH from 7 to 8. The increased dissolution of U from the sediment into the overlying water at higher pH was probably due to the complexation of the uranyl ion with Ca-CO_3 in the overlying water. Approximately 68% of U was estimated to be in the form of $Ca_2UO_2(CO_3)_3$ and 62% in the form of $CaUO_2(CO_3)_3^{2-}$ complexes in overlying waters containing high and low Ca concentrations, respectively, at higher pH. Despite the higher U concentrations in overlying water at higher pH, toxicity was higher in animals exposed to U-spiked sediment concentrations at lower pH. This was because at low pH (~7) there was the presence of proportionately more UO_2^{2+} ions, which are considered to be responsible for U toxicity, compared to the overlying waters with a high pH (~8). On average, approximately 0.16 and 8.2% of U was estimated to be in the form, UO_2^{2+} and UO_2OH^+, respectively, at low pH compared to 8.8E-06 % as UO_2^{2+} and 4.1E-3 % as UO_2OH^+ at higher pH in the overlying waters.

Experiments with caged animals suggest that U bioaccumulation and toxicity is mainly via water rather than the sediment phase (Chapter 2). Uranium bioaccumulation was found to be a more reliable indicator of U toxicity than U concentrations in water or sediment, with the growth and survival parameters being equally sensitive indicators of U toxicity for *H. azteca* exposed to U-spiked sediments.

Given that U bioaccumulation was mainly via the water phase, a water-bioaccumulation model was satisfactory to explain U bioaccumulation. It was estimated that the half-saturation constant for U accumulation varied strongly with pH (H^+ to the fourth power). The water-sediment interaction was also satisfactorily explained using a saturation model, with U in the sediment also being a function of the H^+ to the power of 4 (Chapter 2).

The effect of body size on U bioaccumulation was determined to be significant, with a slope of -0.35 between log body concentration and log body mass for *H. azteca* exposed to water-only U concentrations in soft water for seven days (Chapter 3). The effect of gut-clearance on whole-body concentrations in *H. azteca* was also substantial, with an approximate 72-79% loss of total U-body concentrations in 24h. This loss in total U concentrations by *H. azteca* may be due either to the elimination of the gut contents or a fast excretion compartment. However, the data were not sufficient to model this fast compartment. Overall, this demonstrates that body size needs to be standardized and gut-contents accounted for in *H. azteca* so that the total variability in U bioaccumulation is reduced and toxicity estimates are accurate.

A saturation kinetic model was used to predict the uptake rate, elimination rate and the effect of gut-clearance on whole U body concentration in *H. azteca* exposed to acute (7 days) and chronic (28 days) waterborne U concentrations in soft water (Chapter 3). The uptake of U by *H. azteca* was fast, with 89.0% and 99.98% of the steady-state being reached by day seven and day 28, respectively, in uptake time series studies. Given that steady state was approached in most experiments, this indicates that U concentrations in *H. azteca* will reflect current U levels in the environment and that *H. azteca* are likely to be good monitors of U contamination in the field. However, it should be noted that the chronic uptake of U by *H. azteca* did not completely level off by day 28. This suggests that there may be another slower compartment for U in *H. azteca*. Unfortunately, the data from this study were not sufficient to model this slower compartment.

Although a U water-bioaccumulation saturation model was successfully applied in the laboratory to predict U bioavailability, toxicity and bioaccumulation, this model could not be properly validated under natural conditions near former U mining districts because U concentrations in *H. azteca* and water were at or close to background concentrations (Chapter 4). When the U water-bioaccumulation saturation model was applied using U concentrations in the water and *H. azteca* above background from published studies, the predicted concentrations of U in the *H. azteca* were within a factor of five when compared to the observed U concentrations in the *H. azteca*. This suggests that the water-bioaccumulation saturation model may be a useful tool for regulators as part of a first-tier risk assessment (Chapter 4).

The water-sediment partitioning saturation model using waterborne U concentrations above background concentrations under-predicted U concentrations in the sediment when compared to the observed field concentrations, except for the tests using the lake overlying water, where U concentrations in the sediment were mostly over-predicted when compared to the field observations. When the U water-sediment partitioning saturation model was applied using U concentrations in the surface water above background from published studies, the predicted concentrations of U in the sediment were within a factor of four when compared to the observed U concentrations in the sediment (Chapter 4). Overall this demonstrates that the water-sediment partitioning saturation model can be used in a first-tier risk assessment to predicted U toxicity and quantify U concentrations using natural contaminated sediments and surface waters concentrations above background levels (Chapter 4).

Toxicity in terms of mortality did occur at some sites in U mining areas, but this toxicity was not due to U concentrations in the environment (Chapter 4). Based on a preliminary assessment of 26 other metals in the aquatic environment, toxicity to the *H. azteca* at some sites appeared to be due to Cd concentrations in the water and the bioaccumulation of the metals Cd, Cr, Co or Ni by *H. azteca* or due to low pH. At other sites it could not be inferred if toxicity was the result of metal concentrations in the sediment, water or bioaccumulation by the *H. azteca*.

This thesis demonstrates the influence of overlying water chemistry on U bioavailability, bioaccumulation and toxicity to *H. azteca* and how *H. azteca* can be an indicator of U toxicity in the environment. It also shows how the water-bioaccumulation saturation model and the -sediment partitioning saturation model can be used as an efficient first-tier assessment tool for regulators to quantify U concentrations and toxicity in the field for *H. azteca* and predict toxicity using natural contaminated sediments. The data set used in this study and from other published sources may not have been adequate to sufficiently validate the above saturation models. A larger sample size may be required to further test these two saturation models, especially the water-sediment partitioning model, for quantifying U in the field. Further development of the water-sediment partitioning saturation model may need to include other water chemistry parameters such as dissolved organic carbon in instances were U concentrations in the pore-water are needed to predict U concentrations in the sediment. Presently, Canada has a national drinking water and soil quality guidelines for the protection of human health and terrestrial organisms (i.e., www. ccme.ca). It is hoped that the findings of this thesis will be incorporated into future water and sediment quality guidelines and regulations, given the limited data on U toxicity to native North American invertebrates species and the fact that there are currently no national water or sediment quality guidelines in the protection of freshwater aquatic life in the environment in Canada, which is home to some of the world’s biggest U producing mines and deposits.