1-1-2002

The trophic transfer of Cd and Pb from navicula pelliculosa (Bacillariophyta) to Hyalella azteca (Amphipoda)

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The Trophic Transfer of Cd and Pb from *Navicula pelliculosa* (Bacillariophyta) to *Hyalella azteca* (Amphipoda)

by

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Bachelor of Science – Applied Chemistry and Biology Co-operative Program

Ryerson University, 2000

A thesis

presented to Ryerson University

in partial fulfillment of the

requirement for the degree of

Masters of Science

in the Program of

Environmental Applied Science and Management

Toronto, Ontario, Canada, 2002

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Abstract

The trophic transfer of Pb and Cd from *Navicula pelliculosa* (Bacillariophyta) to *Hyalella azteca* (Amphipoda).

Currently, information identifying the importance of food and water in the trophic transfer of metals for most aquatic organisms is limited, yet such information is essential for modeling metal movement within food webs. *Hyalella azteca* is a suitable organism to study the trophic transfer of metals since they represent a major, but potentially vulnerable component of the food web of many lakes. Since studies involving the trophic transfer are limited, the objective of this research was to determine the relative importance of food and water sources of Pb and Cd to these animals.

Hyalella azteca browses on the film of microscopic plants, animals and organic debris covering leaves, stems and other substrates. *Hyalella azteca* are epibenthic freshwater organisms that prefer foods high in protein. A major challenge of this study involved determining a substrate in which algae would grow and stay attached so *Hyalella azteca* could graze. Preliminary studies found that the diatom species, *Navicula pelliculosa* grew and adhered well to Teflon® surfaces and that *Hyalella azteca* grazed the diatoms from the surface of Teflon®. Thus, *Navicula pelliculosa* was grown in the presence of Pb and Cd concentrations and then fed to the organisms. No significant difference was found between organisms exposed to Pb and Cd from water and from water and food, indicating that Pb and Cd bioaccumulation from food is negligible when the dissolved inorganic fractions are buffered with ethylenediamine tetra-acetic acid (EDTA). The average $\log_{10}$ bioconcentration factor (BCF) for Cd was calculated to be 5.25 from water exposure and 5.49 from water and food exposure. The $\log_{10}$ BCF for Pb was calculated as 4.62 from water exposure and 4.59 from water and food exposure. From these results, it can be concluded that a food source containing Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd, had no noticeable effect on metal burdens to *Hyalella azteca*. Metal uptake by *Hyalella azteca* at the levels studied was found to be primarily from the dissolved phase.

Acknowledgements

This study was done it collaboration with Warren Norwood from the Canadian Centre for Inland Waters (CCIW). Uwe Borgmann, also from the CCIW, provided Ryerson with *Hyalella azteca* that enabled me the opportunity to conduct this research.

Funding was provided by the Toxic Substance Research Initiative (Environment Canada/Health Canada) and the Natural Sciences and Engineering Research Council of Canada.

I would also like to thank Warren Norwood further for his ongoing support throughout my thesis. I would also like to acknowledge Tara Patey for all her help in conducting the experiments, and Deborah Parent and Michael Twiss for their support and guidance.

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1.0 Introduction

Heavy metals such as lead and cadmium exist in the environment mainly from anthropogenic sources such as metal smelting and refining (Nriagu and Pacyna 1988). When metals such as Pb and Cd are released, they can be transported to the atmosphere, and subsequently to terrestrial and aquatic environments. Metals entering the aquatic environment may be partitioned into the solid phase by precipitation or absorption onto suspended particles, or they may be dissolved in the water column (Tessier et al. 1994). Therefore, aquatic organisms may accumulate metals by direct adsorption to body surfaces, ingestion and digestion of suspended particles (Wang and Fisher 1999), and through the membrane of the organism directly from the dissolved phase (Campbell 1995).

Numerous metals entering the aquatic environment are essential to life, but excess concentrations adversely affect water quality due to their toxicity. Since aquatic organisms are exposed to various metals within their environment, water quality guidelines have been designed to protect aquatic life. The procedure for the development of the Canadian Water Quality Guidelines (CWQG) was established from the publications and consultations with the following agencies: Water Quality Branch of Environment Canada; Provincial and Territorial Departments; the International Joint Commission; the United States Environmental Protection Agency (U.S. EPA) and the European Inland Fisheries Advisory Commission (EIFAC) (Canadian Water Quality Guidelines 1987). CWQG for aquatic life are not restricted to a particular (biotic) species, but species-specific information is provided in the rationale so that authorities may determine the appropriateness of the guidelines for the protection and enhancement of local species. The use of common indicator species (e.g. rainbow trout) provides useful information until data on native species are available (Canadian Water Quality Guidelines 1987). The total dissolved concentration of trace metals in water has been used to establish contamination levels in aquatic systems for the guidelines; however, these

measurements tend to be of limited use since they ignore the pollutant's availability to living organisms (Hare and Tessier 1998).

1.1 Metal speciation

The term "bioavailability" refers to the proportion of a contaminant present in the environment in forms that can be potentially assimilated by an organism (Hare 1992). The bioavailabilty of a given trace metal to an organism is related to its precise chemical form, which depends upon the pH of solution and metal-complexing ligands, including dissolved organic carbon (DOC) (Tessier et al. 1994). It is well-recognized that metal speciation influences the partitioning of metals between the solution and the solid phases (Buffle and DeVitre 1994). The toxicity and potential for bioavailability of trace metals are determined by the concentration of free-ion species [M^{Z+}]. In fact, many experiments have shown that the biological effects of trace metals are usually not related to total dissolved metal concentrations; rather, it is the free-metal ion activity that plays a pivotal role in the bioaccumulation of metals by aquatic organisms (Campbell 1995).

In natural water, only a small portion of the overall dissolved metal may be present as the free metal ion because metal ions form stable complexes with a large variety of inorganic and organic ligands, which in turn influence the bioavailabilty, toxicity and mobility of the metal (Mota and Dos Santos 1995). The inorganic dissolved complexes present in natural water are mainly chloro-, carbonato-, sulfato-, oxo-, and hydroxo complexes. Organic complexes are formed mainly from organic matter of biological origin and organic pollutants, which may have complexing properties. Organic matter is higher in rivers, lakes, and estuaries in comparison to open sea with concentrations ranging between 1 to 10 mg L^{-1} and 0.3 to 3 mg L^{-1}, respectively (Mota and Dos Santos 1995). Living organisms and their decomposition products contribute to the organic compounds released into natural waters. They are generally classified in two main categories: 1) organic compounds with a well-characterized structure and a low molecular weight (e.g. amino acids, hydroxy acids, monosaccharides), and 2) ill-defined organic compounds that cannot be fully

isolated but can be separated into different fractions of similar properties. These latter organic compounds comprise relatively large compounds (e.g., humic acids, polysaccharides, polypeptides, lipids, and protein) and smaller ones (e.g., fulvic acids and peptides with molecular weights < 10,000). Aquatic systems with high content of organic substances will therefore affect the bioavailability of the free-metal ion to aquatic organisms (Mota and Dos Santos 1995).

Metals transported to natural waters from land by surface water can be adsorbed onto mineral particles directly or can be adsorbed by organic coatings, which in turn, are absorbed to these particles. During transportation, sorbed metal species may be redistributed between the aqueous and solid phase due to the various chemical equilibria involved and the variation of the physiochemical conditions of the water (Mota and Dos Santos 1995).

As a result of the complexation of metals with inorganic and organic constituents and from sorption onto particulate matter occurring in the water, the concentration of the free metal ion in the water column is less bioavailable to aquatic organisms. Many direct and indirect methods have been development to measure metal speciation in natural waters.

1.2 Direct and indirect techniques for measuring metal speciation

1.2.1 Direct methods

The free ion concentration of metals can be determined from both direct and indirect chemical methods. With direct techniques, water samples are brought to the lab where metal speciation can be studied using separation and analytical techniques. Potentiometry, voltammetry (anodic stripping voltammetry; ASV), and chromatography are approaches that can obtain information on metal-ligand interactions. Potentiometry involves direct measurement of the free-metal ion, ASV involves measurement of direct labile electroactive metal forms, and chromatography involves the separation and measurements of organic forms of metals. Labile metal refers to the sum of the free-metal ion and complexes that are readily dissociated over time. Although these methods provide useful information on metal speciation, there are a number of disadvantages. Potentiometry often lacks the required sensitivity and

the ASV method is prone to interferences due to the adsorption of organic matter (Mota and Dos Santos 1995). Additionally, all three methods are limited in the metals that can be analysed.

Competitive ligand exchange is another direct method. This approach involves the addition of a known competing ligand to a water sample followed by a measurement after equilibration of the complex formed with the added ligand. This technique provides useful information on metal speciation in various aquatic media. However, manipulations are tedious and the interpretation is often ambiguous (Mota and Dos Santos 1995).

Diffusion gradient in thin films (DGT) is a relatively new technique that is currently being studied to determine its ability to directly measure the free-ion concentration. DGT quantifies the concentration of labile metal in the aquatic environment based on the flux of metal through a well-defined hydrogel (Zhang and Davison 1994). There are many advantages of using DGT for measuring trace metals in natural waters compared to the other direct methods previously stated including: 1) the device is easy to use; 2) it can provide information about the measured species by varying the thickness and the pore size of the diffusive gel layer; 3) it concentrates metals *in situ*; which allows an extremely low detection level, e.g., 4 picomolar; 4) many trace metals can be measured simultaneously; and 5) it yields time average concentration over the length of the deployment period (Zhang and Davison 1994, 1995). Since this technique is relatively new, further research is still required. Since constraints are associated with each method stated above, indirect methods are also available for determining metal speciation.

1.2.2 Indirect methods

Thermodynamic modelling involves calculating the speciation of trace metals from the total dissolved ligand and cation concentrations. This is an indirect method to determine speciation and its accuracy depends heavily on the ability to identify and quantify all ligands present in natural waters and on the quality of the thermodynamic database (Turner 1995). Two such chemical speciation models that can be used to calculate the concentration of various chemical species at chemical equilibrium are WHAM (Windermere Humic

Acid Model) and MINEQL+. WHAM is designed to calculate equilibrium chemical speciation in surface and ground water, sediments, and soils. The model is suitable for problems where the chemical speciation is dominated by organic matter (humic substances) (Tipping 1994). In comparison, MINEQL+ calculates the concentration of various chemical species at chemical equilibrium based on a database of species encountered in natural waters under standard conditions (Twiss et al. 2001).

The chemistry of natural waters is typically very complicated. Chemical constituents that are dissolved in water may form chemical complexes, precipitate as solid phases, de-gas from the system or adsorb onto particulate surfaces. All of these reaction pathways are affected by, and will affect, water quality parameters such as pH, alkalinity or ionic strength. MINEQL+ is a computer program that offers a way to understand these chemical interactions in a straightforward, unified manner (Schecher and McAvoy 1992). Indirect methods such as MINEQL+ are useful for conducting laboratory studies using chemically-defined media since they can be used to critically assess the biological response of a particular test organism to a given metal. To determine the availability of metals to an organism, it is critically important to consider metal speciation in the exposure media and MINEQL+ allows one to do this.

1.3 Free ion activity model (FIAM)

The free-ion activity model (FIAM) proposes that for aquatic organisms obtaining their metal from water, the biological response to metals, whether bioaccumulation or toxicity, is predicted best by the concentration of the free-ion (Morel 1983). It is the most powerful rational model that explains the biological effects of trace metals. It was initially developed to describe metal uptake by organisms from the dissolved phase (Morel 1983). The reaction of the free metal ion, M^{z+}, with a reactive site on the membrane of an organism (R-membrane), can be written as:

$$M^{z+} + \text{R-membrane} \xrightarrow{K_r} \text{MR-membrane} \quad (1)$$

where K_r is the equilibrium constant for the binding of a metal to the reactive site. The concentration of metal bound to the reactive site on a membrane is shown by:

$$[\text{MR-membrane}] = K_r \times [\text{R-membrane}]\,[M^{z+}] \quad (2)$$

The model assumes that the rate of metal uptake by an organism is limited by its rate of transport across membranes and not by the rate of equilibrium between dissolved metal and the reactive sites (Morel 1983, Tessier et al. 1994). From this, uptake will be proportional to [MR-membrane] and thus to M^{z+}. Likewise, the concentration of a metal in an organism, $[M_{org}]$, should be a function of the $[M^{z+}]$.

The FIAM has been used successfully to explain the biological effects of metal exposure on a variety of aquatic organisms in the laboratory (Pagenkopf et al 1974, Sunda and Guillard 1976, Sunda et al. 1978, Zamuda and Sunda 1982). In order to proclaim general applicability of the FIAM in nature, field validation was required.

A field study conducted by Hare and Tessier (1996) found that the free-ion activity model could be used to predict metal concentrations in animals in nature. They found that Cd concentrations in *Chaoborus punctipennis* were effectively predicted using the free Cd ion concentrations in lakes, providing that competition for biological uptake sites between hydrogen ions and free Cd ion, as well as Cd complexation by natural organic matter on the organism, was taken into account. These findings were crucial since this study showed that the original model, which was based on laboratory conditions, is applicable to the field. These findings suggest that the free-ion model provides an effective theoretical framework for use of animals as biomonitors of metal contamination in nature (Hare and Tessier 1996). Biomonitors are whole organisms or other biological parameters that are used to assess the degree of contamination in an ecosystem (Hare 1992).

1.4 Biotic Ligand Model (BLM)

An extension to the FIAM is the biotic ligand model (BLM). This model also describes metal uptake by aquatic organisms from the dissolved phase as does the FIAM however in addition to taking into account competition and complexation of the metal with other ions and ligands in solution, it also accounts for competition of ions at the biological receptor site in predicting toxicity to aquatic organisms. This is important to consider since cations associated with water hardness have shown to have the ability to reduce trace-metal toxicity. Pagenkopf (1983) explained this phenomenon by suggesting that hardness cations and trace metals compete for surface binding and uptake sites on the plasma membrane. The BLM calculates the level of accumulation of a metal at the site of action, the biotic ligand, taking into account all the chemical reactions that will lead to speciation of the metal into non-toxic forms and reduce bioavailability (DiToro et al. 2000).

The BLM provides a way to adjust water quality guidelines to site-specific conditions. This capability is particularly useful in settings where the environmental impacts of a metal have been incorrectly presumed, based on measured exposures levels, but where effects have not been demonstrated. This predictive capabilities of the BLM should prove to be useful in the evaluation of alternative watershed management control strategies, including the setting of discharge permit limits for metals (DiToro et al, 2000).

The BLM is currently under review by regulatory agencies and is being considered for use in the development of refined water quality criteria for copper and other metals in the United States, Europe, South America and elsewhere.

As stated above, both the FIAM and BLM were developed to describe metal uptake by organisms from water. However, consumer organisms may ingest metals from their food. Therefore, studying trace metal accumulation in aquatic organisms by food is equally as important as studying accumulation by water.

1.5 Trace metal uptake by aquatic organisms

There have been few studies conducted which examine trophic transfer of metals through the food chain of freshwater organisms. These studies are important to conduct since food chains consist of trophic levels linked in successive prey and predator relationships. Networks of these chains form complex food webs that route the supply, transfer and disposal of potentially toxic metals within ecological systems (Twiss et al. 1996, Nott 1998). Once metals are accumulated, either from the water or from their food, they can potentially react with the animal's biochemical machinery to produce measurable toxic effects at higher levels of the food chain, such as adverse impacts on behaviour or reproduction (Hare 1992). There are three levels of concern when considering the interaction of trace metals with aquatic organisms: 1) metal speciation in the external environment; 2) metal interactions with the biological membrane separating the organism from its environment; 3) metal partitioning with the organism and its biological effect (Campbell 1995). The biological effect of trace metals depends on where the metal binds within the organism. The general metal-organism interaction that occurs when metals are taken up from the aqueous phase will first be explained followed by accumulation.

1.5.1 Transport processes across biological membranes

Trace metals are in contact with cells through their cell wall (if present) and plasma (or cytoplasmic) membrane. The cell wall forms a structure through which trace metals can diffuse and to which they can bind. Plants, algae and fungi contain cell walls, whereas protozoa and animals do not. The structure and chemical composition of the cell wall differs among organisms, however it is generally a 30-to100 nm thick reticulated layer that consists mainly of polysaccharides (Tessier et al. 1994). It contains a mixture of chemical functional groups, with a predominance of carboxyl groups (Tessier et al. 1994). In contrast, the plasma membrane is much thinner (6-9 nm) than the cell wall and all living organisms contain it. The plasma membrane is composed of two layers of lipid molecules that isolate cells from the environment. It contains many specialized proteins, some of which can bind ions and polar molecules

(e.g. nutrients) from the environment and transport them into the cell (Tessier 1994). Selectivity of transport from and to the cell is generally due to the cytoplasmic membrane rather than the cell wall. There are four major transport routes in which metals transport across the plasma membrane of an organism and these include:

1) Carrier-mediated transport. Some proteins form a lipid soluble metal complex, which diffuses through the membrane, and the metal may be released in the cytosol (aqueous content of living cells). It is thought that most trace metals cross the membrane using this means of transport (Luoma 1983).

2) Transport through protein channels. Metal ions are transported within proteins that extend through the membrane that contains many hydrophobic groups (Simkiss 1989).

3) Passive diffusion. Metal forms that are non-polar can dissolve in the membrane and readily cross it (Phinney and Bruland 1994).

4) Endocytosis. A region of the membrane that can engulf a particle containing the metal and fuse to form an intracellular vesicle (Coombs and George 1978).

Therefore, the incoming metal encounters a wide range of potential binding sites. These binding sites are divided into two types; physiologically-inert sites and physiologically-active sites. The difference between the two types is that in the former, metals may "collect" without perturbing normal cell function. In contrast, metals affect cell metabolism at the physiologically active site. When the metal encounters the latter site, it may affect cell metabolism directly (e.g. if the binding site corresponds to a membrane-bound enzyme) or indirectly (if the bound metal is subsequently transported across the plasma membrane into the cell). Once a metal is within the cell, it may interact with a variety of intercellular sites that can have metabolic consequences (Campbell 1995).

Once a metal has entered the organism either through the aqueous phase or from a food source, the metal is transferred to sites of higher binding strength, which results in accumulation (Langston and Spence 1995). In order for accumulation not to cause ecotoxicological effects, an accumulated trace metal requires physiological detoxification. This typically occurs by the metal binding to high affinity sites in inorganic granules that are often phosphate-based, or in a detoxificatory protein such as metallothionein (MT) (Rainbow 1997). The nature and quantities of these ligands is dependent on the biological species.

1.5.2 Importance of depuration

Once a trace metal is accumulated in an organism by ingestion, it is important that metal in the intestinal tract be depurated prior to chemical analysis in order to prevent overestimation of truly biologically incorporated metal (Langston and Spence 1995). Defecation may increase or decrease apparent metal concentrations in amphipod (primarily marine aquatic species), depending on the relative concentrations of metal in the food source and the amphipods. For example, the presence of food in the gut usually lowers apparent Zn concentration in *Gammarus pulex* (food lower in Zn than tissue), but increases total Pb concentrations (food higher in Pb than tissue). Therefore, feeding on metal-rich food sources can in some cases radically alter analytical results if depuration of gut content is not conducted prior to tissue digestion (Langston and Spence 1995).

Trace metals accumulated by an organism from both the surrounding medium and its food source interact with various binding sites depending on the metal. Binding of the metal produces a response, which in turn gives the biological effect of the metal to the researcher. Studies have involved exposing aquatic organisms to aqueous metal concentrations in the absence of metal-contaminated food to determine the biological effect (Munger et al. 1999). Attempts to demonstrate metal uptake from food are few, since technical difficulties are involved in separating food and water as metal sources to the animal (Fisher and Reinfelder 1995). Several such studies have been conducted and the following is a summary of the current state of knowledge in this area.

1.6 Importance of food and water sources to freshwater organisms

The following are examples of previous studies that have determined the importance of food and water to three freshwater organisms.

Vighi 1981.

This laboratory study examined Pb accumulation at the different levels of a simple food chain consisting of primary producers (algae), primary consumers (crustacea), and secondary consumers (fish). The experimental design involved using an apparatus that consisted of four interconnecting compartments so that the organisms were bred in separate, but communicating, vessels (Fig. 1).

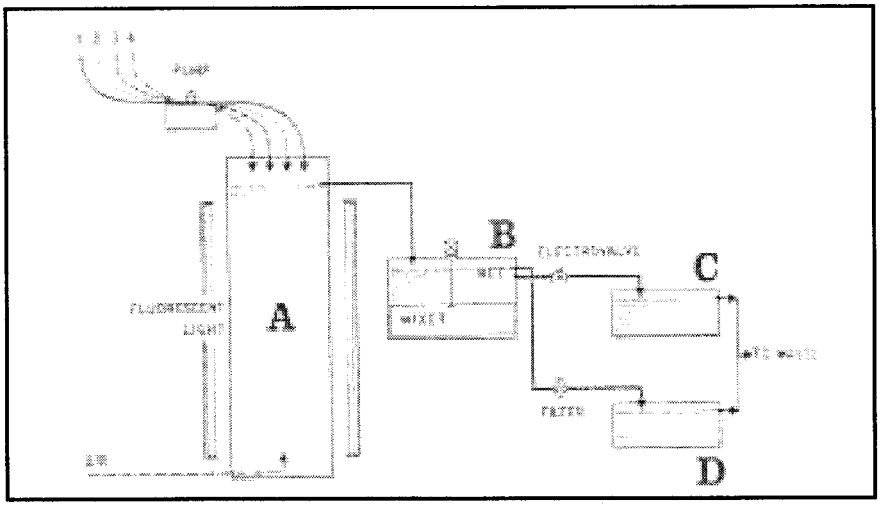

Figure 1. Schematic diagram of the experimental approach used in Vighi's (1981) study to evaluate both the accumulation of Pb at various levels of a simple food chain and the transfer between the three levels.

Compartment A consisted of the algae population *Selenastrum capricornutum*. Compartment B consisted of *Daphnia magna*, which fed on the algae from the first compartment. Compartments C and D were connected to Compartment B and consisted of fish (*Poecilia reticulata*). Compartment C contained fish and received water, algae and *Daphnia magna* from Compartment B and represented both a food and water source contaminated with Pb. Compartment D in comparison, received only contaminated water since the algae and *Daphnia magna* were filtered prior to entering this compartment.

The study involved two treatments. One treatment exposed all organisms to a Pb concentration of 23 nM and the second treatment exposed all organisms to 238 nM Pb. Both treatments ran for 4 weeks. The results from both treatments demonstrated that Pb accumulates in the trophic chain with a decreasing concentration factor from the lowest to the highest levels. From this study it was found that the Pb concentration factors for each organisms in the food chain exposed to 23 nM was 100 x 10^3 for *Selenastrum*, 5.0 x 10^3 for *Daphnia* and 3.6 x 10^3 for *Poecilia*. The concentration factors for the second treatment (238 nM) in comparison was lower than the first treatment with concentration factors of 29 x 10^3 for *Selenastrum*, 1.9 x 10^3 for *Daphnia* and 1.0 x 10^3 for *Poecilia*. It was also found from this study that for fish, food was the main factor responsible for Pb accumulation (Vighi 1981).

Munger and Hare 1997 and 1999.

Munger and Hare observed the relationship between predator and prey with respect to the trophic transfer of Cd in a laboratory study, published in 1997, and in a field study, published in 1999.

Their laboratory study was similar to Vighi's (1981) study in that it also observed a three-link planktonic food chain. The scope of this study was to determine if organisms at a lower level in the planktonic food web accumulate most of their metals from water under laboratory conditions. The study involved using a planktonic food chain composed of the larvae insect *Chaoborus punctipennis*, a crustacean prey item (*Ceriodaphnia dubia*), and the prey's algal food, the chlorophyte *Selenastrum capricornutum* (Fig. 2).

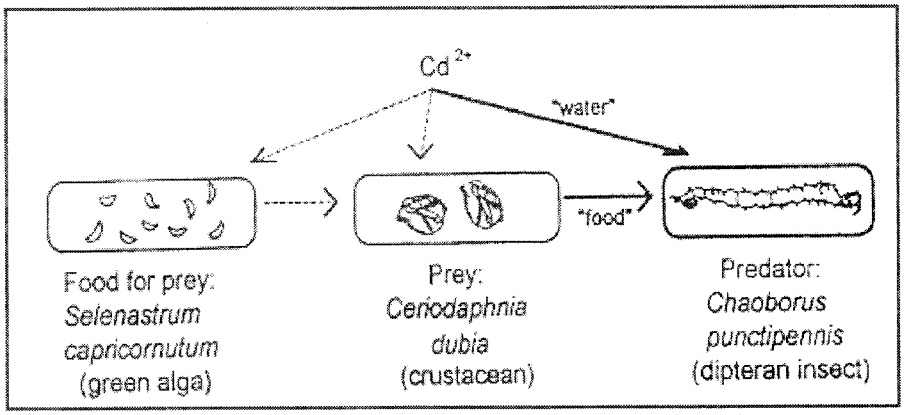

Figure 2. Illustration of the experimental food chain used in Munger and Hare's (1993) study to determine the importance of food and water as Cd sources to *Chaoborus punctipennis*.

The experimental design involved feeding the predator *Chaoborus* the crustacean prey, *Ceriodaphnia* that had been grown in the presence of Cd-rich food, *Selenastrum*. Two treatments were conducted. The first treatment involved exposing the predator to Cd in both food and water while the second treatment exposed the predator to Cd through food alone. Organisms at each level were exposed to a nominal [Cd^{2+}] of 10 nM (Munger and Hare 1997). From this study, no significant difference was found between the Cd concentration in larvae exposed to the metal in their food alone versus those exposed to Cd in both food and water, indicating that Cd bioaccumulation from water by *Chaoborus* larvae was negligible. Thus, these results suggested that the correlation is likely a consequence of Cd uptake from water by organisms at a lower level of the food chain (Munger and Hare 1997).

To confirm if the results could be applied in the natural environment, Munger and Hare conducted a similar experiment in the field (Munger and Hare 1999). The design of this study involved transferring larvae between low and high-Cd lakes. Larvae were held in mesh mesocosms that allowed the free passage of lake water but restricted the movement of planktonic crustaceans that represent a major food source. Zooplankton were collected from the lake and sieved through a mesh netting to eliminate those larger than the mouth of *Chaoborus,* and then fed to the predator. This study confirmed their laboratory

results since it was found that larvae in the natural environment consume Cd mainly from prey (Munger and Hare 1999).

The experimental designs of both Vighi (1981), and Munger and Hare's studies (1997, 1999) provide novel approaches to evaluate the biological impact of trace metals. Although the experimental designs of both these studies were structured differently, both found that with a three-link planktonic food chain, the predator obtains metals primarily from its prey.

Stephenson and Turner 1993.

Stephenson and Turner (1993) conducted a field study examining Cd dynamics in periphyton and *Hyalella azteca.* This study was conducted in experimental lakes in northwestern Ontario where both a Cd-contaminated and a pristine lake were examined. The study had 3 treatments:

Treatment 1

The first treatment involved collecting *H. azteca* from the Cd-contaminated lake and transferring them to the pristine lake where they were held in a cage and provided uncontaminated food that had been grown in the pristine lake. This treatment examined the loss of Cd by *H. azteca.*

Treatment 2

The second treatment involved collecting *H. azteca* from the pristine lake and then transferring them to the contaminated lake in a cage where Cd-contaminated food was provided. This treatment examined the uptake of Cd by *H. azteca* from food and water.

Treatment 3

The third treatment was conducted by collecting *H. azteca* from the pristine lake and placing them in a cage containing contaminated food and then submerging them in the pristine lake. This treatment examined the uptake of Cd by *H. azteca* from food.

In this study, it was found that the Cd concentration in food was not related to exposure concentrations of Cd in water. The $\log_{10}$ bioconcentration factor for *H. azteca* was calculated to be 5.51 (Stephenson and Turner 1993). Studies examining other two-member food chains are limited. The three studies

conducted by Vighi (1981), Munger and Hare (1997, 1999), and Stephenson and Turner (1993) are the three main studies that have contributed significantly to our understanding of Pb and Cd metal uptake from food in freshwater crustaceans.

From the three studies cited above, two recommendations are suggested to contribute further and thus enhance our current knowledge of the trophic transfer of trace metals. Firstly, in all three studies, only one metal was observed. Although these experiments are important, it would be more beneficial if more than one metal was introduced to the organism simultaneously so that one could determine if the presence of two metals behaves the same within the organism as if only one metal is present. Secondly, the concentrations of Pb used in Vighi's (1981) study and Cd from Munger and Hare's (1997) study were higher than that normally found in nature, with concentrations of 23 and 238 nM Pb, and 10 nM Cd used, respectively. Studying lower Pb and Cd concentrations needs to be conducted to determine if the same trend occurs. Based on previous research and the above suggestions, it is proposed that a study be conducted to determine the importance of food and water as sources of Pb and Cd to *H. azteca*.

1.7 *Hyalella azteca* for assessing bioaccumulation of Cd and Pb

Hyalella azteca is a species highly suited for assessing bioaccumulation of Cd and Pb since this organism is found in many freshwaters (Mathias 1971). Additionally, *H. azteca* is ecologically important (Copper 1965, Winnell and Jude 1987) since they are a vital food source to many fish (Borgmann et al. 1989). In addition, *H. azteca* are amenable to laboratory culture and toxicity testing, have a short generation time, and can easily be collected from natural systems (Stephenson and Mackie 1988, 1989, Borgmann and Munawar 1989, ASTM 1990). They are tolerant to a range of environmental conditions such as low dissolved oxygen concentrations and varying sediment grain size and salinity (Nebeker and Miller 1988, ASTM 1990, Nebeker et al. 1992, Suedel and Rodgers 1994). Also, accumulation of Pb and Cd in the dissolved phase is

independent of animal size, and Pb uptake kinetics suggest that steady state is attained within 4 days (MacLean et al. 1996).

H. azteca is widely used as a test organism in the laboratory since it can survive for prolonged periods (within the time frame of a bioaccumulation experiment; 8 days) without food, which allows for better control of trace metal speciation (Borgmann et al. 1991). Studies conducted with *H. azteca* mainly include toxicity test experiments and some of these studies include observing the relationship between Pb accumulation and toxicity under short-term exposures (MacLean et al. 1996), determining the relative toxicity of Cd and pentachlorophenol (Borgmann et al. 1989), and testing the toxicity and bioaccumulation of thallium (Borgmann et al. 1998).

1.8 Cd and Pb for assessing the trophic transfer of metals

Pb and Cd are important to study since they both are non-essential trace metals to *H.azteca* that can be potentially accumulated within the organism and thus can be transferred up the aquatic food chain. This can pose a threat to human life since *H. azteca* is an important food source to many fish and therefore these metals have the potential to be transferred from fish to humans.

In addition, *H. azteca* are sensitive to metals (Borgmann et al. 1989). Due to this characteristic, a biological response can easily be observed in laboratory experiments. Studies have shown that *H. azteca* are particularly sensitive to Cd in both water and sediments, and they have also found that *H. azteca* is sensitive to Pb. Borgmann et al. (1989) found increased mortality during chronic Cd exposure at 8 nM total dissolved Cd. With Pb, it was found that the lowest nominal concentration of Pb that was toxic after a six-week exposure was 482 nM total dissolved Pb (Borgmann et al. 1993).

Although Pb and Cd are not the only trace metals that *H. azteca* are exposed to in the natural environment, it is important that researchers begin to conduct experiments involving exposure to more than one metal from a natural food source. Therefore, studying both Pb and Cd together, will contribute to the previous studies conducted and will therefore enhance our current knowledge of the trophic transfer of metals.

1.9 Formulation of hypothesis

H. azteca are omnivorous feeders but prefer foods high in protein. *H. azteca* browse on the film of microscopic plants, animals and organic debris (aufwuchs) covering leaves, stems and other substrates (Pennak 1989). In the natural environment, *H. azteca* are exposed to numerous benthic algal species as a food source. Since *H. azteca* is an epibenthic freshwater organism, *Navicula pelliculosa* is a likely food source for *H. azteca* in nature and is therefore an ideal food source to study. In addition, the cell walls of diatoms are distinctive compared to other algae species in that silica, protein, and lipids compose the cell wall instead of cellulose and polysaccharides, as is the case with most algae. The diatom species *N. pelliculosa* is lipid-rich and this characteristic makes it an ideal food source for *H. azteca*.

The importance of food and water as Cd sources to *H. azteca* and *C. punctipennis* have shown that bioaccumulation from food was significant. Stephenson and Turner (1993) found that food was an important source of Cd to *H. azteca* at a relatively low Cd concentration of 0.86 nM in a field study. Munger and Hare (1993, 1997) also found bioaccumulation of Cd from food significant in both a laboratory and field study with the aquatic organism *C. punctipennis*. Although the Cd concentration used in the laboratory study was higher than in natural environments, the field study involved lower Cd concentrations, and the same results were obtained. Bioaccumulation of Pb was studied by Vighi (1981) and it was also found in this study that food was the main factor responsible for Pb accumulation in *Poecilia reticulata* at Pb concentrations of 23 nM and 238 nM. Since previous research has shown that Cd and Pb bioaccumulation from food occurred in three freshwater organisms, including *H. azteca*, it is predicted that bioaccumulation of both Cd and Pb in the same exposure medium will likely result in the same findings as previous studies.

1.10 Hypothesis

Since *H. azteca* naturally graze on algae, and previous studies with other freshwater organisms including *H. azteca, C. punctipennis and P. reticulata* have suggested that direct uptake of Cd and Pb from water was insignificant, it

is hypothesized that under laboratory conditions, the primary uptake route of Pb and Cd by *H. azteca* will be from food rather than from water.

1.11 Objective

The objective of this research will therefore be to determine the route of uptake at low ambient concentrations of Pb and Cd by *H. azteca.* This will be achieved by comparing the bioaccumulation of Pb and Cd in the presence of water and also in the presence of food and water. The experimental approach to this study will be similar to that described by Munger and Hare (1997) however, a two-link epibenthic food chain composed of *H. azteca* and the benthic pennate diatom species *N. pelliculosa* (food source) will be observed instead of a three-member planktonic food chain. The experimental design of this study will be slightly different than Munger and Hare's study. In this study *H. azteca* will be introduced to contaminated food and water in one treatment and contaminated water only in another. In comparison, Munger and Hare study exposed *C. punctipennis* to contaminated water and food in one treatment and contaminated food in another. Therefore, the difference between the two experiments is that food will be factored out in our study and water was factored out in Munger and Hare's study.

This study will first involve choosing a substrate to which *N. pelliculosa* will adhere and grow. After completion of preliminary studies, the first main experiment will involve exposing *H. azteca* to a range of aqueous Pb and Cd concentrations found in natural environments and in the Canadian Water Quality Guidelines (CWQG). The following experiments will then involve determining the importance of food and water as sources of Pb and Cd to *H. azteca.*

2.0 Methodology

2.1 Experimental design

In order to determine the importance of food and water as Pb and Cd sources for *H. azteca* the following preliminary studies were conducted. A major challenge with this study was to determine a substrate upon which algae would grow and stay attached so that *H. azteca* could graze upon them. Therefore, the first study involved determining a suitable substrate for the diatom *N. pelliculosa* (UTCC 33). The second study involved determining the number of days required for the substrate to contain sufficient *N. pelliculosa* biomass and the third study involved determining whether *H. azteca* would graze *N. pelliculosa* grown on the substrate.

Once these studies were complete, the first main experiment involved exposing *H.azteca* to various concentrations of Pb and Cd by water. This experiment was conducted to determine a concentration of Pb and Cd that will be used in the following experiments to assess the importance of food and water as Cd and Pb sources to *H. azteca.* The experiment involved feeding *H. azteca, N. pelliculosa* that had been previously grown in the presence of Pb and Cd (Fig. 3).

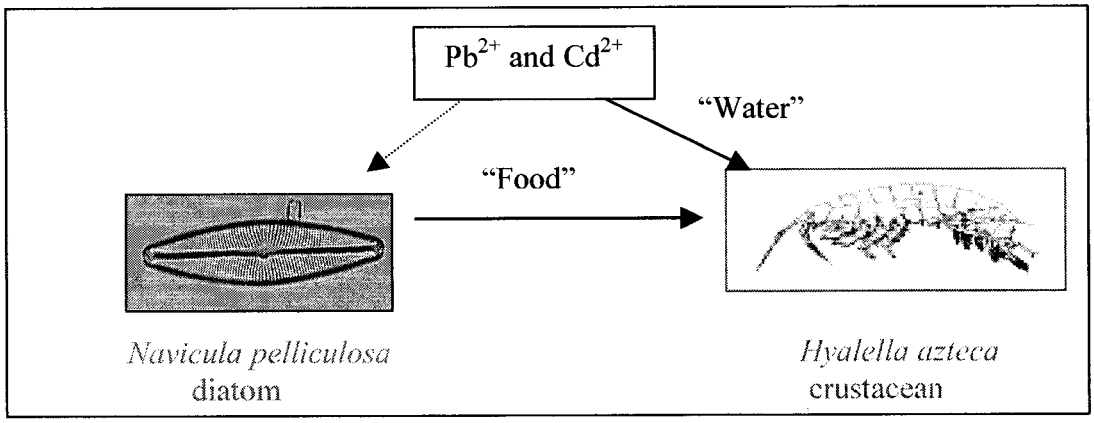

Figure 3. Diagrammatic representation of the experimental food chain indicating potential Pb and Cd sources at each trophic level. The experiments were designed to measure the relative importance of food and water sources (solid arrows) to *H. azteca*

The concentrations of Pb and Cd chosen to test the hypothesis are similar to those found in the natural environment. The total Pb and Cd

concentrations were buffered with 10 μM EDTA to achieve the desired inorganic Pb and Cd concentrations, which were calculated using the speciation program MINEQL+ (Version 4.5). Loss of metal from the buffer was <1.5 % for Cd and <0.1 % for Pb, indicating that the buffering capacity of EDTA was maintained throughout the three experiments (Refer to Appendix A for the calculated % inorganic metal accumulated by *H. azteca* during experimental exposures).

2.2 Preliminary studies

Study 1. Selection of substrate

A key criterion for determining a suitable substrate was one that was durable in both acid and acetone. This was important since the substrate was to be 1) soaked in dilute hydrochloric acid prior to use to remove metals; 2) immersed in acetone for chl-*a* measurements; and (3) placed in concentrated nitric acid at the end of an experiment for several days to digest the cells attached to the surface for metal analysis. It was also important that the surface be inert since it will be used to feed *H. azteca* a controlled concentration of metal in the form of Pb and Cd contaminated diatoms, therefore it was important that the surface of the substrate did not react with other metals that may be present in the medium.

From these criteria, plaques composed of Teflon® were chosen since they met the above criteria. Teflon® was tested to see if *N. pelliculosa* adhered to the surface by immersing the Teflon® plaques (area = 4.4 cm^2) in medium containing an inoculum of *N. pelliculosa* (UTCC 33). The diatoms were grown in medium consisting of 1 L of dechlorinated tap water amended with 10 μM EDTA, 12.5 μM silicate, 1 μM phosphate, 10 μM nitrate and Guilliard vitamin mix (Morel et al. 1975), which was sterilized by filtration using a 0.2-μm filter. The medium containing an inoculum of *N. pelliculosa* and the plaques were placed under a 16:8 h light:dark cycle for as many days as was required for sufficient growth to occur on the surface. Growth on the surface of the plaques were analyzed for chlorophyll-*a* (chl-*a*) by sampling plaques from the medium and rinsing them in trace metal-free FRAQUIL medium to remove loosely-

attached cells. Chl-*a* was measured by removing the plaques from medium with plastic tweezers and then gently rinsing them in FRAQUIL medium three times before they were immersed in 10 mL of 90% acetone (4°C) for 24 h (Welschmeyer 1994). After this time, chl-*a* was measured in the 90% acetone solution using a fluorometer (Turner design 700).

Study 2. Determine the number of days required for sufficient growth on substrate

Once it was established that *N. pelliculosa* readily adheres to Teflon® plaques, the next step was to determine the number of days required for the plaques to contain sufficient *N. pelliculosa* biomass to serve as a food source. A growth assay was therefore conducted and this study involved exposing fifty plaques in two types of medium. The first medium contained *N. pelliculosa* grown in 1 L of dechlorinated tap water medium (above). The second medium consisted of *N. pelliculosa* grown in 1 L of dechlorinated tap water medium (above) amended with the buffered Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd. The plaques were contained in a polyethylene container, covered with a plastic film, and placed under a 16:8 h light:dark cycle. Three plaques were sampled from each medium daily over 14 days to determine the growth rate. This was conducted by measuring chl–*a* on the plaques (Appendix B).

Study 3.Determine if H. azteca *graze* N. pelliculosa *grown on the substrate*

The next preliminary study involved determining if *H. azteca* graze *N. pelliculosa* grown on the plaques. To conduct this study, 2 *Hyalella* were given one 11-day-old plaque containing *N. pelliculosa* grown in the above medium containing no metals. *H. azteca* grazed on plaques for as many days as was required for complete removal of food. Growth of *N. pelliculosa* on the plaques over the same exposure period but not exposed to *H. azteca* was also measured so the grazing rate could be determined. Grazing rates were calculated as follows:

$$g_c = (\text{chl-}a_c - \text{chl-}a_o) / t \quad (3)$$

where: g_c = growth rate in control (µg/d)
chl-a_c = mass of chl-*a* on plaques in non-grazing treatment (µg)
chl-a_o = initial mass of chl-*a* on plaques (µg)
t = time (d)

$$r_{net} = (\text{chl-}a_F - \text{chl-}a_O) / t \quad (4)$$

where: r_{net} = net grazing rate (µg /d)
chl-a_F = mass of chl-*a* on plaques after 48 hours in grazing treatment (µg)
chl-a_o = initial mass of chl-*a* on plaques (µg)
t = time (d)

Once the growth rate in control (g_c) and net grazing rate (r_{net}) were calculated, the grazing rate (g_R) was calculated from:

$$g_R = r_{net} - g_c \quad (5)$$

2.3 Determination of Pb and Cd concentrations for use in bioassays

The selection of Pb and Cd concentrations for the first study (Experiment 1) which involved exposing *H. azteca* to various Pb and Cd concentrations, was determined from two sources: a study conducted by Hare and Tessier (1998) that reported total dissolved concentrations from numerous lakes in Canada; and the Canadian Water Quality Guidelines (1987). Hare and Tessier's (1998) study observed five impacted regions, two in Ontario and three in Quebec, where fifteen lakes were studied in Ontario and thirteen lakes were studied in Quebec. The findings from Hare and Tessier's study were used for this study since it provided a range of Pb and Cd concentrations found in both impacted and pristine areas.

Determining the Pb and Cd concentrations for Experiment 1 involved first averaging and categorizing the highest and lowest concentrations of Pb and Cd found in Hare and Tessier's (1998) study (Appendix C). From this, the inorganic Pb and Cd concentrations were chosen (Table 1 summarizes the inorganic concentrations selected).

Table 1. Pb and Cd concentrations selected for the bioassays

Source	Treatment number	[Pb] nM	[Cd] nM
Hare and Tessier (1998)	1	6.8	3.5
Hare and Tessier (1998)	2	136	0.39
Hare and Tessier (1998)	3	15	0.37
Canadian Water Quality Guideline (1987)	4	19.1	12.4

One treatment consisted of 6.8 nM Pb and 3.5 nM Cd, while another treatment consisted of a higher Pb concentrations (136 nM Pb) and lower Cd concentration (0.39 nM Cd) in comparison to Treatment 1. Treatment 4 consisted of a Cd concentration of 0.37 nM and a Pb concentration of 15 nM. The last treatment contained Pb and Cd concentrations found within the Canadian Water Quality Guidelines published in 1987 (CWQG), and included concentrations of 19.1 nM Pb and 12.4 nM Cd. The purpose of the CWQG is to protect freshwater aquatic life; therefore these concentrations were chosen to determine the effectiveness of these guidelines for the protection of *H. azteca* to Pb and Cd concentrations.

The inorganic concentrations of Pb and Cd were determined using the chemical speciation program MINEQL+ (Version 4.5) (Schecher and McAvoy 1992). Since the basal medium used for the experiments was dechlorinated tap water which originated in Lake Ontario, and the concentrations of Pb and Cd were chosen from various other lakes across Ontario and Quebec, the major ions associated with Lake Ontario had to be adjusted in the program to give the same proportion of ions and concentrations to achieve the free Pb and Cd concentrations in the selected lakes. Therefore, the average pH and Ca^{2+} concentrations from the range of lakes had to be determined (Appendix C). Once the average pH and Ca^{2+} concentrations were computed, the major ions associated with Lake Ontario were adjusted by dividing the initial concentration by a factor, which was calculated by dividing the initial [Ca^{2+}] ion of Lake Ontario water by the averaged [Ca^{2+}] ion for the specific treatment (Appendix C).

To determine the free [Pb^{2+}] and [Cd^{2+}] at the pH and alkalinity of the selected concentrations, MINEQL+ was first run in the absence of EDTA, Pb, and Cd (Appendix C). Once these concentrations were determined, MINEQL+ was run again using ions associated with Lake Ontario and 10 μM EDTA to determine the total Pb and Cd concentration at pH 8 to achieve inorganic Pb and Cd concentrations that were previously determined (Table 2) in a purely inorganic system. Hence, the inorganic concentrations of Pb and Cd were buffered using the chelator EDTA.

Table 2. Total Pb and Cd concentrations (mol/L) required to give the inorganic [Pb] and [Cd] for a Toronto City tap water (Lake Ontario) system at pH 8 and buffered with 10 μM EDTA.

Total	Treatment 2 Pb (6.8 nM), Cd (3.5 nM)	Treatment 3 Pb (136 nM), Cd (0.39 nM)	Treatment 4 Pb (15 nM), Cd (0.37 nM)	Treatment 5 Pb (19.1 nM), Cd (12.4 nM)
Pb concentration	8.50×10^{-6}	1.01×10^{-5}	9.70×10^{-6}	8.56×10^{-6}
Cd concentration	1.00×10^{-6}	7.00×10^{-9}	5.50×10^{-8}	1.29×10^{-6}

2.4 Culturing Technique for *H. azteca*

H. azteca, obtained from Dr. U. Borgmann, (NWRI; Canadian Centre for Inland Waters) were maintained under controlled growth conditions to obtain a continuous supply of *H. azteca* of known age for the experiments. *H. azteca* were cultured using dechlorinated City of Toronto tap water (originating in Lake Ontario, pH 8.0-8.7). The protocol used to culture *H. azteca* for our experiment was similar to Borgmann's (1989) study. Cultures were maintained in 1 L of water in a 2-L fluorinated low density polyethylene (FLDP) containers at 22°C under a 16:8 h light:dark cycle. The water was changed weekly for each container by separating the young *H. azteca* from the adults (4 weeks) through a filtering device that consisted of two sizes of mesh, 295 μm for collecting the young and 750 μm for collecting the adults. *H. azteca* were counted and then placed in clean containers consisting of 1 L of new water and cotton gauze. Approximately 20 *H. azteca* were added to each container and were fed approximately 5 mg of Tetra-Min® fish food 3 times a week.

2.5 Experiment 1 – *H. azteca* exposed to various concentrations of Pb and Cd by water alone

H. azteca (20 - 22 per container at 10-13 weeks old) were exposed to four Pb and Cd concentrations in 2 L of dechlorinated tap water buffered with 10 μM EDTA at 22°C, under a 16:8 h light:dark cycle. The test chambers used were 2-L polypropylene containers. Table 3 illustrates the total as well as the inorganic concentrations of Pb and Cd for each treatment.

Table 3. Total Pb and Cd concentrations (mol/L) and inorganic concentrations used for Experiment 1.

Treatment	[EDTA]	$[Pb]_{total}$	$[Cd]_{total}$	$[Pb]_{inorganic}$	$[Cd]_{inorganic}$
1 (control)	10.0×10^{-6}	0	0	0	0
2 (Pb 6.8 nM, Cd 3.5 nM)	10.0×10^{-6}	8.50×10^{-6}	1.00×10^{-6}	6.8×10^{-9}	3.5×10^{-9}
3 (Pb 136 nM, Cd 0.39 nM)	10.0×10^{-6}	10.1×10^{-6}	7.00×10^{-9}	136×10^{-9}	0.39×10^{-9}
4 (Pb 15 nM, 0.37 nM)	10.0×10^{-6}	9.70×10^{-6}	0.55×10^{-9}	15.0×10^{-9}	0.37×10^{-9}
5 (Pb 19.1 nM, Cd 12.4 nM)	10.0×10^{-6}	8.60×10^{-6}	1.30×10^{-6}	19.1×10^{-9}	12.4×10^{-9}
6 (control)	0	0	0	0	0

Note: The total concentrations of Pb and Cd were found to remain constant throughout the duration (7 days) of the experiment (Appendix C).

There were two different controls used for this experiment, one control consisted of 10 μM EDTA (Treatment 1) and the other with no added EDTA (Treatment 6). There was one replicate for each treatment in this experiment. The other 4 treatments contained the following Pb and Cd concentrations. Treatment 2 contained the inorganic Pb and Cd concentrations of 6.8 nM Pb and 3.5 nM Cd. Treatment 3 consisted of 136 nM Pb and 0.39 nM Cd, Treatment 4 consisted of 15 nM Pb and 0.37 nM, and Treatment 5 contained 19.1 nM Pb and 12.4 nM Cd. Solutions for each treatment were made individually and left for 24 hours before the start of the experiment to allow the solutions to equilibrate.

For this experiment, *H. azteca* were placed in plexi-glass cages (2.5 cm x 6.3 cm) containing 210 μm mesh. The experiment ran for 7 days with no food. At the end of the experiment, *H. azteca* were put in a solution of 10 μM EDTA for 10 minutes to remove Pb and Cd sorbed on their body surface before being dried, weighed, digested, and analyzed for Pb and Cd.

2.6 Experiment 2 – *H. azteca* exposed to Pb and Cd from food and water sources

H. azteca were exposed to the inorganic concentrations of 15 nM Pb and 0.37 nM Cd in 1 L of dechlorinated tap water mixed with 10 μM EDTA at 22°C, under a 16:8 light:dark cycle. Test chambers used for experiment 2 were 2-L polypropylene. No cages were used for this experiment. Four treatments were conducted (Table 4).

Table 4. Components associated with treatments in Experiments 2 and 3.

Treatment	Component			
	Hyalella	EDTA	*Navicula* (contaminated plaques)	Water containing Pb and Cd
1 (control)	X	X	-	-
2 (water exposure)	X	X	-	X
3 (water and food exposure)	X	X	X	X
4 (control for growth on plaques)	-	X	X	X

Two populations of *H. azteca* were used for Experiment 2, cultured *H. azteca,* which originated from the CCIW, and *H. azteca* from an aquarium, which consisted of *H. azteca* that originated from various laboratories. Treatments 1 through 3 contained 20 *H. azteca* per container. Solutions for each treatment were made individually and left for 24 hours before the start of the experiment to allow the solutions to equilibrate.

Cultured *H. azteca* and *H. azteca* collected from an aquarium were kept separate for each treatment. The ages of the cultured *H. azteca* ranged from 6 to 13 weeks old. Cultured and aquarium *H. azteca* were exposed to the same treatments. Therefore, there were two replicates for each treatment. With reference to Table 4, the second treatment exposed *H. azteca* to the inorganic concentrations of 15 nM Pb and 0.37 nM Cd by water alone. Treatment 3 exposed *H. azteca* to the same concentrations of Pb and Cd by water and also by food. *N. pelliculosa* was grown in the presence of 15 nM Pb and 0.37 nM Cd for 7 days before it was given as a food source for Treatment 3. Four plaques were placed in the container. The plaques were removed every second day and

replaced with new plaques in order to provide *H. azteca* with enough food. The control consisted of two replicates of 20 *H. azteca* of each population (cultured *H. azteca* and *H. azteca* from an aquarium) and were placed in 1 L of dechlorinated tap water mixed with 10 µM EDTA.

The duration of the experiment was 7 days. After day 6, *H. azteca* from all containers (including the controls) were removed and placed into new containers containing 1 L of dechlorinated tap water mixed with 10 µM EDTA to remove Pb and Cd sorbed on their bodies. *H. azteca* were supplied with 4 plaques of uncontaminated *N. pelliculosa* for 24 h to eliminate Pb and Cd in the gut. Material contained in the gut is external to the animal since it has not yet crossed a membrane into the organism. Therefore, depuration is important to conduct since it allows one to determine the amount of metal that has internalized in the organism.

2.7 Experiment 3 – *H. azteca* exposed to Pb and Cd from food and water sources

Experiment 3 was conducted in a similar manner to Experiment 2 in that it measured bioaccumulation of the same Pb and Cd exposure concentrations from water and from water and food. The differences between Experiments 2 and 3 was that Experiment 3 involved the use of cultured *H. azteca* instead of the two populations of *H. azteca* (cultured and aquarium) as in Experiment 2, and only 18 *H. azteca* were used per container for each treatment instead of 20 as in Experiment 2. The ages of *H. azteca* ranged from 6 to 19 weeks old and three replicates were conducted per treatment (3 with replicates with food and 3 without food). Solutions for each treatment were made individually and left for 24 hours before the start of the experiment to allow the solutions to equilibrate. Two replicates were conducted with the control and consisted of 18 *H. azteca* exposed to dechlorinated tap water containing 10 µM EDTA.

To determine if Pb and Cd in the media adsorbed to the sides of the container (polypropylene) during Experiments 1 through 3, 4-mL water samples were taken and preserved in 40 µL of nitric acid. Pb and Cd were analyzed using graphite furnace atomic absorption spectroscopy (GFAAS; AAnalyst 800, Perkin Elmer) (Appendix D).

2.8 Contamination of *N. pelliculosa* as a food source for *H. azteca*

Stock cultures of *N. pelliculosa* were maintained in dechlorinated tap water amended with 10 μM EDTA, Guilliard vitamin mix (Morel et al. 1975), trace metal mix, silicate, phosphate and nitrate (concentrations for the trace metal mix, silicate, phosphate and nitrates are described in Morel et al. 1975) at 21°C under a 16:8 light:dark cycle.

N. pelliculosa contaminated with Pb and Cd were used as a food for *H. azteca* and were grown on Teflon® plaques in a polyethylene container containing 1 L of medium consisting of an inoculum of *N. pelliculosa* mixed with dechlorinated tap water, 10 μM EDTA, trace metal mix, silicate, phosphate, nitrate and Pb and Cd stock. High levels of *N. pelliculosa* biomass (1.46 $\mu g/cm^2$) on the plaques were achieved after 7 days.

Pb and Cd concentrations in *N. pelliculosa* were measured prior to feeding them to *H. azteca,* and after two days of being exposed in the medium from Experiments 2 and 3. To measure the initial concentration of Pb and Cd per unit of chl-*a,* 4 plaques were selected at random from the pre-treatment culture. To measure the concentration of Pb and Cd that accumulated in *N. pelliculosa* over a two-day period, two containers were set-up during the course of the experiment. The medium within these containers was the same as Treatment 2 and 3 (buffered Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd). The containers consisted of 4 plaques and no *H. azteca.* The plaques were replaced every second day of the experiment in the container. Therefore, 2 plaques from each container were digested for metal analysis on the second day. The other 2 plaques were used to measure chl-*a*. Table 5 lists a summary of the daily procedures that were conducted throughout Experiments 2 and 3.

Table 5. Experimental protocols for Experiments 2 and 3.

Day	Treatment 1 (control)	Treatment 2 (water exposure)	Treatment 3 (water and food exposure)	Treatment 4 (Plaques only)
0	a) water sample and pH taken	a) water sample and pH taken	a) water sample and pH taken b) 4 plaques added to medium	a) water sample and pH taken b) 4 plaques added to medium
1	-	-	-	-
2	-	-	a) 4 plaques removed 4 plaques were measured for chl-*a* b) 4 new plaques added to medium	a) 4 plaques removed 2 plaques were measured for chl-*a* 2 plaques digested for metal analysis b) 4 new plaques added to medium
3	-	-	-	-
4	-	-	a) 4 plaques removed 4 plaques were measured for chl-*a* b) 4 new plaques added to medium	a) 4 plaques removed 2 plaques were measured for chl-*a* 2 plaques digested for metal analysis b) 4 new plaques added to medium
5	-	-	-	-
6	a) water sample and pH taken b) water was changed and 4 new plaques containing noncontaminated *N. pelliculosa* was added to allow the animals to depurate	a) water sample and pH taken b) water was changed and 4 plaques containing noncontaminated *N. pelliculosa* was added to allow the animals to depurate	a) 4 plaques removed - 4 plaques were measured for chl-*a* b) water sample and pH taken c) water was changed and 4 plaques containing noncontaminated *N. pelliculosa* was added to allow the animals to depurate	a) 4 plaques removed 2 plaques were measured for chl-*a* 2 plaques digested for metal analysis b) water was changed and 4 new plaques containing noncontaminated *N. pelliculosa* was added to allow the animals to depurate
7	a) *H. azteca* digested	a) *H. azteca* digested	a) *H. azteca* digested	b) *H. azteca* digested

Note:

- *H. azteca* were counted every 2 days for Experiment 3
- Growth/grazing rates were calculated from 0 to 2 days, 2 to 4 days, and 4 to 6 days for Experiments 2 and 3
- Metal concentrations were determined from 0 to 2 days, 2 to 4 days, and 4 to 6 days for Experiments 2 and 3

2.9 Digestion of *H. azteca* and *N. pelliculosa*

After completion of an experiment only *H. azteca* that survived and were motile and alert were placed into pre-weighed 1.5 mL micro-centrifuge tubes and oven-dried at 60°C for 72 hours. The number of *H. azteca* placed in each micro-centrifuge tube varied between 1 and 3. After drying, they were placed in a desiccator for 24 h to reach room temperature and then re-weighed using a micro-analytical balance (Mettler H2O) to determine the dry weight (refer to Appendix F for a comprehensive list of dry weights of *H. azteca* from each experiment). *H. azteca* were then digested with concentrated nitric acid for 6 days, followed by the addition of 30% hydrogen peroxide (Fisher Chemicals) for 24 hours and then diluted with de-ionized water (18 Ω-cm^{-1}; Milli-Q Academia + UV, Millipore Corporation) (Appendix F).

For Experiment 1, the volume of acid, peroxide, and water was based on the dry weight in an attempt to keep the ratio of dry weight to final volume more consistent. Therefore, the total volumes varied. For Experiments 2 and 3, the addition of concentrated nitric acid, peroxide and water was not based on the dry weight. The total final volume used for digestion for Experiments 2 and 3 was 1 mL. Therefore, to ensure there was no matrix interference, a standard addition was conducted using GFAAS with the largest and smallest dry weight using the same analytical method.

To ensure complete digestion of *H. azteca*, a slurry mixture of reference sample (TORT-2; National Research Council of Canada) was digested simultaneously with each experiment. To obtain similar weights to that of *H. azteca,* a slurry mixture was prepared by mixing a sample of TORT-2 with deionized water in a pre-cleaned plastic beaker. The mixture was continuously stirred for 1 h prior to the slurry being sampled to ensure the slurry was homogenized. The sample was dried at 60°C for 72 h before being digested.

To determine the concentrations of Pb and Cd in *N. pelliculosa* grown on the Teflon® plaques, each plaque was first rinsed in 10 µM EDTA prepared in dechlorinated tap water for 10 minutes. The plaques were transferred to trace metal-free FRAQUIL medium (Morel et al. 1975) then placed in an acid clean

15 mL polypropylene container, where concentrated nitric acid (Seastar®) was poured over the surface of the plaques containing *N. pelliculosa*. The cells were digested in concentrated nitric acid for at least 3 days before analysis.

2.10 Pb and Cd analysis by GFAAS

Water samples and digested tissue samples were analyzed for Pb and Cd using graphite furnace atomic absorption (GFAAS). Samples were analyzed on a plateau tube with modifiers of ammonium phosphate monobasic ($NH_4H_2PO_4$) and magnesium nitrate $Mg(NO_3)_2$ for Pb analysis and magnesium nitrate $Mg(NO_3)_2$ for Cd analysis. An electrode dischargeless lamp was used for Pb analysis and a hollow cathode lamp was used for Cd analysis. Samples were diluted to bring the Pb and Cd concentration in the digest into the working range of the machine (Refer to Appendix G for the analytical methods used to determine Pb and Cd concentrations in *H. azteca*, TORT-2 reference material, *N. pelliculosa,* and water samples).

2.11 Determination of grazing rates (g_r)

To determine the grazing rates of *H. azteca*, periphyton loss from the plaques grazed by *H. azteca* was measured throughout Experiment 2 and 3. Two plaques were removed from Treatment 4 (no grazing) after 48 h. In addition, four plaques were also removed from Treatment 3 (grazing) after 48 h. As previously stated, plaques were rinsed in FRAQUIL media to remove loosely attached periphyton and then individually placed in 90% acetone for 24 h for chl-*a* measurements using the TD-700 fluorometer (Turner Design) (Welschmeyer 1994).

2.12 Determination of Bioconcentration Factor (BCF)

To determine the bioconcentration factor of Pb and Cd for *H. azteca* the following equation was used:

$$BCF = [metal]_{organism} / [metal]_{water} \quad (6)$$

where: $[metal]_{organism}$ metal per dry weight of *H. azteca* (mol/kg)

$[metal]_{water}$ was computed by MINEQL+ and represented the concentration of the inorganic species of [Pb'] or [Cd'] (mol/L).

Therefore

$$BCF = L/kg \quad (7)$$

2.13 Statistical analysis

To test the hypothesis that the primary uptake route of Pb and Cd by *H. azteca* is from food rather than from water, statistical analysis was applied to the results. In order to determine if there is an effect of food bearing Pb and Cd on concentrations in *H. azteca*, the least significant difference (LSD) was computed and then added onto the calculated BCF that was determined for the *H. azteca* that were not fed during the course of the experiment. This was calculated by first conducting ANOVA on the $\log_{10}$ BCF tabulated for *H. azteca* from Treatment 2 and 3 from Experiments 2 and 3. From this analysis the mean square error (MSE) and the percentage points of the *F* distribution (*F*) were determined. The LSD was calculated using the following formula:

$$LSD = (2 \times F \times MSE/n)^{0.5} \quad (8)$$

where: MSE = mean square error

n = sample size

The calculated LSD was then added to the mean $\log_{10}$ BCF from Treatment 2 (*Hyalella* exposed to water alone).

$$LSD_{at\ 95\%} + \log_{10} BCF_{unfed\ mean} \quad (9)$$

The $\log_{10}$ BCF determined from the above calculation represents the BCF that is required in order to detect a statistically significant difference. Therefore, if the $\log_{10}$ BCF from Treatment 3 (*H. azteca* exposed to metal contaminated food) is

equal or greater to the above calculation (Eqn. 8), there was a significant effect from feeding.

2.14 Determination of the Potential Pb and Cd Accumulated by *H. azteca*

From Experiment 3, the potential metal accumulation over the duration of 6 days was calculated since *H. azteca* were counted in Treatment 3 (water and food exposure) on day 2, 4 and 6 of the experiment. The following formulae were used to calculate the potential metal accumulation for *H. azteca*.

$$C_a = g_R/H \quad (10)$$

where: C_a = amount of chl-*a* eaten per *H. azteca* (H) per day (µg chl-*a*/H/d)
g_R = grazing rate of chl-*a* per day (µg chl-*a*/day)
H = the average number of *H. azteca* in the treatment over a 2 day period

$$M_h = C_a \times M_b \quad (11)$$

where: M_h = mass of metal in *H. azteca* per day (mol/H/d)
C_a = amount of chl-*a* eaten per *H. azteca* per day (µg chl-*a*/ H/d)
M_b = mass of metal per unit of chl-*a* (mol/µg chl-*a*).

$$M_c = M_h \times t \quad (12)$$

where: M_c = mass of metal consumed by *H. azteca* (mol/H)
M_h = mass of metal in *H. azteca* consumed per day (mol/H/d)
t = time (d)

$$M_p = M_c \times D_h \quad (13)$$

where: M_p = potential metal accumulated (mol/kg)
M_c = mass of metal consumed per *H. azteca* in one day (mol/H)
D_h = average dry weight of an individual *H. azteca* (H/kg)

The sum of metal intake over a 2-day period from day 2, 4 and 6 were added together to give a total metal intake over 6 days from one experimental replicate.

3.0 Results

3.1 Preliminary studies

Preliminary studies showed that *N. pelliculosa* grew very well and adhered to the Teflon® plaques. Growth on plaques was stationary in both types of media after 7 days and remained stationary for an additional 7 days (Fig. 4).

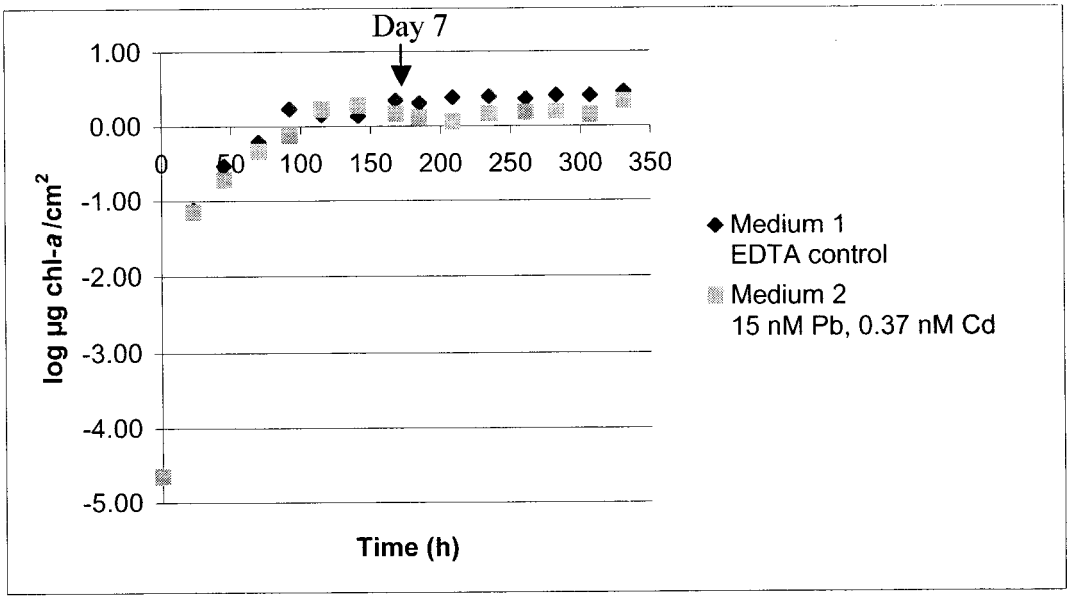

Figure 4. Growth curve of *N. pelliculosa* grown in the presence and absence of Pb and Cd. *N. pelliculosa* was grown in two types of medium simultaneously. Medium 1 contained dechlorinated tap water and 10 µM EDTA; Medium 2 contained dechlorinated tap water mixed with 10 µM EDTA and 15 nM Pb and 0.37 nM Cd.

This assay also showed that metal concentrations of 15 nM Pb and 0.37 nM Cd had no adverse affect on the growth of the diatom. In addition, it was also found that *H. azteca* graze on *N. pelliculosa* adhering to the Teflon® plaques, and that four days were sufficient for 2 *H. azteca* to clear the plaques containing *N. pelliculosa* with initial densities of 0.57 µg chl-*a*/cm^2.

3.2 Experiment 1 – *H. azteca* exposed to various concentrations of Pb and Cd from water only

From the four treatments in Experiment 1, it was found that *H. azteca* in Treatments 3 (Pb 136 nM, Cd 0.39 nM) and 4 (Pb 15 nM, 0.37 nM Cd) had the highest survival rates with a 65% and 55% survival, respectively. Although

these values are low, they are similar to Treatment 1, which was the control that contained 10 μM EDTA. Therefore, the data generated from Treatments 3 and 4 regarding the concentration of Pb and Cd accumulated in *H. azteca* can be compared since the health of the organisms were similar to that of the control. Treatment 2 (Pb 6.8 nM, 3.5 nM Cd) in comparison had no live *H. azteca* after 7 days and Treatment 5 (Pb 19.1 nM, 12.4 nM Cd) had only 5% survival (Table 6).

Table 6. Percent survival of *H. azteca* after a 7-day exposure to various metal concentrations of Pb and Cd by water in Experiment 1.

Experiment 1	
Treatment	% Survival
1 (control)	60
2 (Pb 6.8 nM, Cd 3.5 nM)	0
3 (Pb 136 nM, Cd 0.39 nM)	65
4 (Pb 15 nM, Cd 0.37 nM)	55
5 (Pb 19.1 nM, 12.4 nM)	5
6 (control)	77

Pb and Cd concentrations associated with Treatments 2 and 5 represented possible concentrations that are toxic to *H. azteca*. However, since only one replicate was conducted for each Treatment in Experiment 1, these findings should not be used to make any final conclusion regarding the effects that these concentrations have on *H. azteca* until the experiment is repeated with more replicates.

To determine the concentrations of Pb and Cd that accumulated in *H. azteca* during the course of the experiment, *H. azteca* were digested and analyzed. With respect to the concentrations of Pb in the medium for Treatments 3 and 4, the concentration of Pb decreased from Treatment 3 to 4 (136 nM to 15 nM respectively). With reference to Table 7, this difference in the Pb concentrations in the medium affected the amount that was accumulated by *H. azteca* since the concentration of Pb accumulated decreased as the concentration of Pb in the medium decreased. From Table 7, *H. azteca* from

Treatment 3 accumulated 3.41 x 10^{-3} mol/kg of *H. azteca,* and *H. azteca* from Treatment 4 accumulated 2.97 x 10^{-3} mol/kg *H. azteca.*

Table 7. Concentrations of Pb and Cd per *H. azteca* (mol/kg) after a 7-day exposure to various concentrations through water in Experiment 1. Values are mean ± standard deviation x 10^{3}; values in parentheses are sample size.

Treatment	Water exposure mol of Pb/kg *H. azteca*	Water exposure mol of Cd/kg *H. azteca*
3 - Pb (136 nM) Cd (0.39 nM)	3.41 ± 1.29 (7)	0.02 ± 0.01 (7)
4 - Pb (15 nM) Cd (0.37 nM)	2.97 ± 2.36 (9)	0.12 ± 0.09 (9)
5 - Pb (19.1 nM) Cd (12.4 nM)	0.67 (1)	0.56 (1)

The amount of Cd that accumulated in comparison to Pb seemed to be affected by the concentration of Pb present in the medium since it was found that when the Cd concentration remains constant (as in Treatment 3 and 4) the amount accumulated was different. It was seen that a higher concentration of Pb in the medium resulted in a lower accumulation of Cd by *H. azteca.* With reference to Table 7, the amount of Cd that accumulated in *H. azteca* from Treatment 3 was 0.02 x 10^{-3} (mol/kg *H. azteca*). The amount of Cd accumulated in Treatment 4 in comparison was higher than that observed in Treatment 3. The Pb concentration in the medium for Treatment 4 was 15 nM, and the concentration of Cd that accumulated was 0.12 x 10^{-3} mol/kg.

Treatment 5 contained a higher Pb and Cd concentration in the medium than Treatments 3 and 4, with concentrations of 19.1 nM Pb and 12.4 nM Cd. These concentrations seem to have affected the amount of Pb and Cd accumulated by *H. azteca* since the concentration of Pb accumulated was 0.67 x 10^{-3} mol/kg *H. azteca* and therefore was lower than the concentration of Pb accumulated from Treatments 3 and 4. With reference to Cd, the concentration accumulated by *H. azteca* from Treatment 5 was higher than the Cd concentrations from Treatments 3 and 4 since the concentration of Cd for Treatment 5 was 0.56 x 10^{-3} mol/kg *H. azteca.* Since these values represent the concentrations from the survival of one *H. azteca*, the results from this treatment will no longer be compared to Treatments 3 and 4.

3.3 Experiment 2 – *H. azteca* exposed to Pb and Cd from water and food sources

The percent survival between the two populations of *H. azteca* used for Experiment 2 varied. After a 6-day exposure period to 15 nM Pb and 0.37 nM Cd from water in one treatment and water and food in another, *H. azteca* collected from the aquarium had the highest survival in comparison to cultured *H. azteca*. The percent survival from each treatment for cultured *H. azteca* was higher (48%) in Treatment 2 (water exposure) than in Treatment 3 (food and water exposure) (33%). In contrast, percent survival of *H. azteca* from the aquarium was the same for both treatments after 6 days with 76% surviving (Table 8).

Table 8. Percent survival of two populations of *H. azteca* (cultured and aquarium) after a 6-day exposure to various treatments.

Cultured *H. azteca* Treatment	% Survival
1 (control)	52
2 (water exposure)	48
3 (food and water exposure)	33
Aquarium *H. azteca* Treatment	% Survival
1 (control)	95
2 (water exposure)	76
3 (food and water exposure)	76

The bioconcentration factor (BCF) was calculated for Pb and Cd for Treatment 2 (water exposure) and 3 (water and food exposure) for Experiments 2 and 3, which determined the importance of food and water as Pb and Cd sources to *H. azteca*. The BCF value for each sample was log transformed ($\log_{10}$) for each container and then averaged. BCF values for Experiments 2 and 3 are found in Appendix I.

The $\log_{10}$ bioconcentration factor (BCF) calculated for the two populations of *H. azteca* was found to be higher with aquarium *H. azteca* than cultured *H. azteca* for Pb (Table 9).

Table 9. Log_{10} bioconcentration factor (BCF) for the two different sets of *H. azteca* (cultured versus aquarium) after a 6-day exposure to 15 nM Pb and 0.37 nM Cd from water and from water and food (*N. pelliculosa*) in Experiment 2.

Replicate	Treatment 2 Water exposure Log_{10} BCF for Pb in *H. azteca*	Treatment 3 Water and Food exposure Log_{10} BCF for Pb in *H. azteca*
Cultured *H. azteca*	4.48 ± 0.39 (4)	4.58 ± 0.20 (2)
Aquarium *H. azteca*	5.17 ± 0.50 (6)	4.76 ± 0.32 (4)

Replicate	Treatment 2 Water exposure Log_{10} BCF for Cd in *H. azteca*	Treatment 3 Water and Food exposure Log_{10} BCF for Cd in *H. azteca*
Cultured *H. azteca*	4.88 ± 0.34 (4)	5.35 ± 0.03 (2)
Aquarium *H. azteca*	5.57 ± 0.53 (6)	5.45 ± 0.26 (4)

Note: Values are mean and ± standard deviation; values in parentheses are sample size.

Aquarium *H. azteca* had a larger BCF for Pb with respect to Treatment 2 (water exposure) than Treatment 3 (water and food exposure). In comparison, the log_{10} BCF for cultured *H. azteca* for Pb was similiar between the two treatments, 4.48 + 0.39 and 4.58 ± 0.19, respectively.

Cd accumulation by the aquarium *H. azteca* was greater than that for the cultured *H. azteca.* Treatment 2 (water exposure) for the two populations varied greatly with a log_{10} BCF for Cd for cultured *H. azteca* was 4.88 ± 0.34 and 5.57 ± 0.53 for aquarium *H. azteca.* In comparison to Treatment 3 (water and food exposure), the log_{10} BCF was greater than Treatment 2 for cultured *H. azteca.* This was different than what was observed for Pb since the BCF showed no difference between the two treatments. The average log_{10} BCF for Cd for Treatment 3 (food and water exposure) was smaller than Treatment 2 (water exposure) for *H. azteca* that were collected from the aquarium. The log_{10} BCF values were calculated as 5.45 ±0.26 and 5.57 ± 0.53, respectively. This trend was also found for Pb with the same population of *H. azteca.*

The results obtained from cultured *H. azteca* from the water exposure Treatment in Experiment 2 cannot be compared with Treatment 3 (15 nM Pb,

0.37 nM Cd) of Experiment 1, since *H. azteca* were handled differently at the end of the experiment. For Experiment 1, *H. azteca* were rinsed in 100 mL of 10 µM EDTA for 10 minutes and for Experiment 2, *H. azteca* grazed uncontaminated *N. pelliculosa* for 24 hr in 1 L of dechlorinated tap water containing 10 µM EDTA.

3.4 Experiment 3 – *H. azteca* exposed to Pb and Cd from water and food sources

The experimental set-up of Experiment 3 was similar to Experiment 2, however only cultured *H. azteca* were used and each treatment had three replicates. The average percent survival from the three replicates from Treatment 2 (water exposure) was lower than Treatment 3 (water and food exposure), 46% and 76 %, respectively (Table 10).

Table 10. Percent survival of cultured *H. azteca* after a 6-day exposure to 15 nM Pb and 0.37 nM Cd from water exposure and from a water and food exposure in Experiment 3.

Cultured *H. azteca* Treatment	% Survival Replicate 1	% Survival Replicate 2	% Survival Replicate 3	Average and Std. deviation
1 (control)	61	78	-	70 ± 12
2 (water exposure)	56	33	50	46 ± 12
3 (food and water exposure)	50	89	89	76 ± 22

To determine if there was a significant difference between the survivals of the three treatments, a t-test was used to compare the means. From, the results it was found that there was no significant difference (P=0.05) between Treatments 1 and 2, Treatments 1 and 3 and Treatments 2 and 3.

In comparison to Experiment 2, the percent survivals were different than that found in Experiment 3 since Treatment 2 (water exposure) in Experiment 2 was found to be 48 % and Treatment 3 (water and food exposure) had a percent survival of 33%. Although the survivorship was different between the two experiments, the $\log_{10}$ BCF for Experiments 2 and 3 were similar. The average $\log_{10}$ BCF from the three replicates for Treatment 2 in Experiment 3 (water

exposure) was 4.48 ± 0.42 for Pb (n = 3) and 5.38 ± 0.05 for Cd (n = 3) (Table 11).

Table 11. Log_{10} bioconcentration factor (BCF) for Pb and Cd for cultured *H. azteca* after a 6-day exposure to 15 nM Pb and 0.37 nM Cd from water and from water and food (*N. pelliculosa*) in Experiment 3.

Replicate	Treatment 2 Water exposure Log_{10} BCF for Pb in *H. azteca*	Treatment 3 Water and Food exposure Log_{10} BCF for Pb in *H. azteca*
1	4.80 ± 0.21 (7)	4.61 ± 0.25 (7)
2	4.63 ± 0.21 (6)	4.64 ± 0.13(7)
3	4.58 ± 0.16 (5)	4.53 ± 0.14 (7)
Average ± S.D	**4.67 ± 0.12**	**4.59 ± 0.06**

Replicate	Treatment 2 Water exposure Log_{10} BCF for Cd in *H. azteca*	Treatment 3 Water and Food exposure Log_{10} BCF for Cd in *H. azteca*
1	5.42 ± 0.21 (7)	5.61 ± 0.18 (7)
2	5.39 ± 0.12 (6)	5.53 ± 0.09(7)
3	5.32 ± 0.20 (7)	5.46 ± 0.08 (7)
Average ± S.D	**5.38 ± 0.05**	**5.53 ± 0.08**

Note: Values are mean and ± standard deviation; values in parentheses are sample size.

In comparison to Experiment 2, the log_{10} BCF for Treatment 2 (water exposure) for cultured *H. azteca* was 4.48 for Pb and 4.88 for Cd (Reference to Table 9). The average log_{10} BCF for Treatment 3 in Experiment 3 (water and food exposure) was also similar to Experiment 2 since the average log_{10} BCF for Experiment 3 was 4.59 for Pb (n = 3) and 5.53 for Cd (n = 3) and Experiment 2 obtained a log_{10} BCF of 4.58 (n = 1) for Pb and 5.35 for Cd (n = 1) for the cultured *H. azteca*.

The controls from all three experiments were similar, indicating that no detectable contamination occurred throughout the experiment. The results from all three experiments show that Pb was not detectable in *H. azteca* after exposure to 10 µM EDTA. However, Pb was detectable in *H. azteca* immersed in dechlorinated tap water containing no EDTA (Experiment 1) since 7.57 x 10^{-6} mol/kg *H. azteca* of Pb was found. These results showed that EDTA binds

to Pb, therefore making it unavailable to *H. azteca*. In contrast, Cd was detectable in all experiments. The average concentration of Cd in *H. azteca* was small since 4.45 ± 3.00 x 10^{-6} mol/kg *H. azteca* was detected (Table 12).

Table 12. Concentrations (mol/kg) of Pb and Cd in *H. azteca* in the controls from Experiments 1 through 3. Values are mean ± standard deviation x 10^{6}; values in parentheses are sample size.

Treatment	mol/kg of Pb in *H. azteca*	mol/ kg of Cd in *H. azteca*
Experiment 1		
EDTA control	1.05 ± 0.68 (6)	6.27 ± 5.60 (6)
Control with no EDTA	7.57 ± 6.38 (10)	9.45 ± 0.21 (10)
Experiment 2		
Control with Cultured Hyalella	-11.9 ± 13.6 (4)	2.79 ± 2.97 (4)
Control with Aquarium Hyalella	-0.81 ± 1.06 (7)	0.99 ± 0.78 (7)
Experiment 3		
EDTA control - replicate 1	-0.56 ± 0.70 (7)	4.07 ± 2.03 (7)
EDTA control - replicate 2	-0.48 ± 0.23 (7)	3.11 ± 0.46 (7)

Samples of similar weight of a certified reference sample (lobster hepatopancreas, TORT-2, National Research Council of Canada) confirmed the digestion efficiency for all experiments with a recovery of 117.7% ± 30 (n = 9) for Pb and a recovery of 109.5% ± 30 (n = 11) for Cd.

3.5 Concentration of Pb and Cd in the food source, *N. pelliculosa*

The concentrations of Pb and Cd in *N. pelliculosa* from Experiments 2 and 3 were similar, indicating *H. azteca* were exposed to the same concentration for both experiments. Table 13 illustrates that the concentration of Pb (mol of Pb/µg chl-*a*) after two days in the medium was 2.05 ± 0.62 x 10^{-9} for Experiment 2 and 3.49 ± 1.39 x 10^{-9} for Experiment 3.

Table 13. Concentrations of Pb (mol/μg of chl-*a*) in *N. pelliculosa* from Experiments 2 and 3. Values are mean ± standard deviation x 10^{9}; values in parentheses are sample size.

Set	Experiment 2 mol of Pb/μg chl-*a*	Experiment 3 mol of Pb/μg chl-*a*
Set 1 - day 0	0.78 ± 0.09 (3)	0.43 ± 0.02 (3)
Set 1 - day 2	1.34 ± 0.41 (4)	5.10 ± 0.55 (4)
Set 2 - day 0	1.50 ± 0.28 (4)	0.43 ± 0.07 (4)
Set 2 - day 2	2.31 ± 0.44 (4)	2.75 ± 0.22 (4)
Set 3 - day 0	0.33 ± 0.05 (4)	0.34 ± 0.02 (4)
Set 3 - day 2	2.50 ± 0.22 (4)	2.63 ± 0.20 (4)

***Note:**

Set 1 involved the plaques immersed in medium from day 0 to day 2 of the experiment
Set 2 involves the plaques immersed in the medium from day 2 to day 4 of the experiment
Set 3 involved the plaques immersed in the medium from day 4 to day 6 of the experiment

Similarly, the average concentrations of Cd (mol of Cd/μg chl-*a*) after two days was 18.6 ± 4.2 x 10^{-12} for Experiment. 2 and 19.8 ± 12.3 x 10^{-12} for Experiment 3 (Table 14).

Table 14. Concentrations of Cd (mol/μg of chl-*a*) in *N. pelliculosa* from Experiments 2 and 3. Values are mean ± standard deviation x 10^{12}; values in parentheses are sample size.

Set	Experiment 2 mol of Cd/μg chl-*a*	Experiment 3 mol of Cd/μg chl-*a*
Set 1 - day 0	0.81 ± 0.17 (3)	3.03 ± 0.23(4)
Set 1 - day 2	17.50 ± 3.03 (4)	33.30 ± 2.42 (4)
Set 2 - day 0	4.14 ± 0.35 (4)	3.59 ± 1.08 (4)
Set 2 - day 2	14.70 ± 4.75 (4)	16.80 ± 1.02 (4)
Set 3 - day 0	2.27 ± 0.65 (4)	2.09 ± 0.44 (4)
Set 3 - day 2	23.60 ± 4.16 (4)	9.29 ± 0.99 (4)

Since the Pb and Cd concentrations in the food source for both experiments have shown to be similar, the $\log_{10}$ BCF for Pb and Cd from Experiment 2 for cultured *H. azteca* can be added as another replicate to the BCFs obtained from Experiment 3 to determine if there is a statistical difference between the two treatments.

3.6 Statistical analysis

To test the hypothesis that the primary uptake route of Pb and Cd by *H. azteca* is from food rather than from water alone, the least significant difference (LSD) was computed. Based on a total of 4 BCF values for each treatment (Treatment 2 – water exposure, and Treatment 3 – water and food exposure) from Experiments 2 and 3, the values were first log transformed and then the mean square error (MSE) was computed for Pb (0.059) and Cd (0.034). The degrees of freedom for MODEL (v_1) were computed as 1 and the degrees of freedom for ERROR (v_2) were calculated as 4. Therefore, the F value corresponding to 1 and 4 is 5.99 (P = 0.05). These values are summarized in Table 15.

Table 15. Summary of values used to perform statistical analysis on the $\log_{10}$ bioconcentration factors from Experiments 2 and 3.

Pb	Treatment 2 Water exposure	Treatment 3 Water and food exposure	Cd	Treatment 2 Water exposure	Treatment 3 Water and food exposure
Sample size	4	4	Sample size	4	4
Average	4.62	4.59	Average	5.25	5.49
Variance	0.018	0.002	Variance	0.063	0.012
MSE	0.011	0.011	MSE	0.038	0.038
F (P = 0.05)	5.99	-	F (P = 0.05)	5.99	-
LSD at 95%	0.18	-	LSD at 95%	0.34	-

The LSD at 95% $(2 \times F \times \text{MSE}/n)^{0.5}$ was calculated as 0.18 for Pb and 0.34 for Cd. To determine if there was an effect from the presence of food, the LSD was added to the $\log_{10}$ BCF from Treatment 2. Based on the above calculation, 4.80 was calculated for Pb and 5.59 was calculated for Cd. Since

the average $\log_{10}$ BCF from the food and water treatment (Treatment 3) for Pb was lower (4.59) than 4.80, no difference was detected. The same result occurred for Cd since the average $\log_{10}$ BCF from the food and water treatment (Treatment 3) was also lower, 5.49 versus 5.59, respectively.

If the main route for Pb and Cd is from food, then Treatment 3 must be 2 times greater than uptake without food (Treatment 2) log 2 = 0.30. Therefore, if the hypothesis that the primary route of metal uptake by *H. azteca* is from food is true, then the $\log_{10}$ BCF from Treatment 3 must be greater than the mean $\log_{10}$ BCF from Treatment 2 + 0.30. To disprove the hypothesis, the $\log_{10}$ BCF for Treatment 3 must be smaller than Treatment 2 + 0.30. Since Treatment 3 is smaller than Treatment 2 + 0.30 for both Pb and Cd, (Treatment 3, 4.59 for Pb and 5.49 for Cd versus 4.92 for Pb and 5.55 for Cd) the hypothesis was disproved, but not by a statistically significant amount. To determine if uptake with food is statistically below 2 times uptake without food, one-way ANOVA on the $\log_{10}$ BCF for Treatment 2 + 0.30 was conducted against the $\log_{10}$ BCF for Treatment 3 for both Pb and Cd. The *P* value for Pb was calculated as 0.66 and 0.13 was calculated for Cd, therefore the amount is not significant since these values are greater than $P = 0.05$. This analysis shows that uptake from food is not statistically below 2 times the uptake without food. Therefore, these results suggest that uptake of Pb and Cd by *H. azteca* was from water.

3.7 Grazing rates from Experiments 2 and 3

Throughout Experiments 2 and 3, chl-*a* measurements were taken so grazing rates could be determined (Refer to Appendix H). Based on the results it was found that the grazing rates determined from both experiments were similar indicating that *H. azteca* ate approximately the same quantity of food in both experiments (Table 16).

Table 16. Grazing rates (μg/d) for Experiment 2 and 3

	Grazing rate		
Experiment 2	Container 1	Container 2	
Set 1 - day 0 to day 2	-3.51	-3.58	
Set 2 - day 2 to day 4	-3.21	-3.56	
Set 3 - day 4 to day 6	-0.82	-1.92	
Experiment 3	Container 1	Container 2	Container 3
Set 1 - day 0 to day 2	-1.80	-1.87	-1.64
Set 2 - day 2 to day 4	-3.05	-3.10	-3.15
Set 3 - day 4 to day 6	-2.96	-3.42	-3.27

3.8 Potential Pb and Cd concentrations in *H. azteca* from Experiment 3

During the duration of Experiment 3, *H. azteca* were enumerated when the plaques were renewed. Therefore, *H. azteca* were counted on day 2, 4 and 6 of the experiment. These population estimates were used to determine the potential metal accumulation over the duration of the experiment. From the calculations, the potential Pb that could be accumulated by *H. azteca* was 5.60 ± 0.53 x 10^{-3} mol of Pb/kg over a 6-day exposure period (Table 17).

Table 17. Potential Pb concentration (mol/kg) versus the actual concentration of Pb accumulated (mol/kg) from Experiment 3.

Potential metal ingested from food				Estimated accumulation from food	Actual accumulation from food	Actual accumulation from water
Pb	**Accumulation**				**Pb**	**Pb**
Replicate	Day 2	Day 4	Day 6	Sum of Pb accumulated over a 6 day exposure period (mol of Pb/kg)	Treatment 3 Food and Water Exposure (mol of Pb/kg)	Treatment 2 Water Exposure (mol of Pb/kg)
1	1.81 x 10^{-3}	2.41 x 10^{-3}	2.23 x 10^{-3}	6.18 x 10^{-3}	0.69 x10^{-3}	1.01 x 10^{-3}
2	1.82 x 10^{-3}	1.72 x 10^{-3}	1.94 x 10^{-3}	5.48 x 10^{-3}	0.68 x 10^{-3}	0.70 x 10^{-3}
3	1.65 x 10^{-3}	1.75 x 10^{-3}	1.74 x 10^{-3}	5.14 x 10^{-3}	0.53 x 10^{-3}	0.60 x 10^{-3}
			Average	**5.60 x 10^{-3}**	**0.64 x 10^{-3}**	**0.77 x10^{-3}**
			Std. Deviation	**0.53 x 10^{-3}**	**0.09 x 10^{-3}**	**0.21 x 10^{-3}**

In comparison to the actual concentration of Pb per organism for both treatments, the potential was higher since the actual concentration of Pb accumulated for Treatment 3 (water and food exposure) was $0.64 \pm 0.09 \times 10^{-3}$ mol of Pb/kg.

With respect to Cd, the potential concentration of Cd per organism for the three treatments was $53.6 \pm 43.6 \times 10^{-6}$ mol of Cd/kg. The actual concentration was higher for Treatment 3 than the potential with a concentration of $132.0 \pm 28.4 \times 10^{-6}$ mol of Cd/kg (Table 18).

Table 18. Potential Cd concentration (mol/kg) versus the actual concentration of Cd accumulated (mol/kg) from Experiment 3.

Potential metal ingested from food				Estimated accumulation from food	Actual accumulation from food	Actual accumulation from water
Replicate	Day 2	Day 4	Day 6	Sum of Cd accumulated over a 6 day exposure period (mol of Cd/kg)	Treatment 3 Food and Water Exposure (mol of Cd/kg)	Treatment 2 Water Exposure (mol of Cd/kg)
1	11.8×10^{-6}	1.31×10^{-6}	78.9×10^{-6}	104.0×10^{-6}	163.0×10^{-6}	106.0×10^{-6}
2	11.9×10^{-6}	10.5×10^{-6}	6.84×10^{-6}	29.3×10^{-6}	127.0×10^{-6}	94.8×10^{-6}
3	10.7×10^{-6}	10.7×10^{-6}	6.15×10^{-6}	27.6×10^{-6}	107.0×10^{-6}	85.0×10^{-6}
			Average	**53.6×10^{-6}**	**132.0×10^{-6}**	**95.3×10^{-6}**
			Std. Deviation	**43.6×10^{-6}**	**28.4×10^{-6}**	**10.5×10^{-6}**

4.0 Discussion

The hypothesis for this study stated that under laboratory conditions, the primary uptake route of Pb and Cd by *H. azteca* would be from food rather than from water. From the results, the hypothesis was disproved since no difference was found between *H. azteca* exposed to the buffered Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd from water versus those exposed to same concentration of Pb and Cd from water and food. Since the calculated BCF values from the Treatment exposing *H. azteca* to Pb and Cd by food and water was not twice as large as the water exposure treatment, the results suggest that main uptake route was from water rather than from food. From our laboratory study, Teflon® plaques were found to be an excellent substrate to use for determining the importance food in the uptake of metals since it was found that *N. pelliculosa* adhered and grew on the surface, and *H. azteca* grazed the algae.

Experiments 2 and 3 determined the importance of food and water. The BCF were log transformed from each experiment and statistical analysis was performed to determine if there was a significant effect from the exposure from food. For Pb, the $\log_{10}$ BCF for Treatment 2 (water exposure) was calculated as 4.62, and 4.59 was calculated for Treatment 3 (food and water). The $\log_{10}$ BCF for Cd was calculated as 5.25 and 5.49 for Treatments 2 and 3. Although the BCF value for Cd from Treatment 3 (food and water) appears to be larger than Treatment 2 (water exposure), there was no difference found between the two treatments at both $P = 0.05$ and using the least significant difference (LSD) method. There was also no difference found between the two treatments for Pb.

The results from the present study were compared with Stephenson and Turner's (1993) field study to see if they are in agreement. Their study was similar to the present one in that it involved determining the primary route of uptake of Cd by *H. azteca* and it used an environmentally realistic low ambient concentration of Cd (0.86 nM), which was similar to the buffered inorganic Cd concentrations examined here. Different results were found between the two studies. Stephenson and Turner (1993) found that *H. azteca* derive Cd primarily from food rather than from water. These results are not in agreement with those

obtained from our study since the opposite was found. There are several possible explanations for this discrepancy.

4.1 Explanations for differences with previous studies

Firstly, the medium used for these two studies were different. Stephenson and Turner's (1993) study was conducted in soft water lakes and the medium used in our experiment was dechlorinated tap water, which originated from Lake Ontario and therefore was representative of hard water. It has been found that cations associated with water hardness such as Ca^{2+} and Mg^{2+} have the ability to reduce trace-metal toxicity. Pagenkopf (1983) explained this phenomenon by suggesting that hardness cations and trace metals compete for surface binding and uptake sites on the plasma membrane. Since the presence of Ca^{2+} ions was greater in the present study than in the earlier study (Stephenson and Turner 1993), Cd accumulation may have been reduced due to competition between Ca^{2+} and Cd^{2+} for binding and uptake sites. The concentration of Ca^{2+} ions in the lakes studied by Stephenson and Turner (1993) was measured at 2.22 mg L^{-1} for the Cd-contaminated lake and 3.38 mg L^{-1} for the pristine lake. In contrast, the concentration of Ca^{2+} reported in Lake Ontario is 40 mg L^{-1} (Borgmann et al. 1991). Due to these appreciable differences in Ca^{2+} concentrations between the two studies, this may have resulted in different Cd accumulation in *H. azteca* and therefore represents one possible reason why the outcome between the two studies were different.

Another explanation why the results may have differed may be due to the approach in which they used to determine the primary route of Cd since uptake and depuration were treated as a single, rather than as multiple processes. With reference to Stephenson and Turner's (1993) study, depuration was conducted by transferring *H. azteca* collected from the Cd contaminated lake to the pristine lake. *H. azteca* were exposed to Cd in the contaminated lake for approximately 2 months before being transferred. Uptake of Cd in comparison was conducted from two treatments. Treatment 2 involved transferring *H. azteca* collected from the pristine lake to the Cd contaminated lake for 11 days where they were fed Cd-contaminated food. Treatment 3

involved feeding *H.azteca* collected from the pristine lake, Cd-contaminated food. The results from these studies were then used in a model to determined Cd dynamics in *H. azteca,* and thus determined the importance of food and water as a Cd source to *H. azteca*. It was found from these studies that depuration rates measured in *H. azteca* transferred to the pristine lake could not be reconciled with the faster rates required to fit the observed uptake curves from the *H. azteca* that were taken from the pristine lake and transferred to the Cd-contaminated lake. Treating uptake and depuration as single processes may account for the differences in results since in our experiment uptake and depuration were treated as multiple processes and therefore were more controlled. In the present study, *H. azteca* from all treatments in Experiments 2 and 3, depurated for 24 h before they were digested.

One explanation why problems were seen when the results from the three treatments were combined into the Stephenson model may have been due to the fact that *H. azteca* were not depurated at the end of the exposure periods. As previously stated, it is important that metal in the intestinal tract is depurated prior to chemical analysis to prevent overestimation of truly biologically incorporated metal (Langston and Spence 1995). Thus, perhaps the Cd concentrations found in Stephenson and Turner's (1993) study may have been overestimated since *H. azteca* that fed on Cd-contaminated food did not depurate prior to digestion. After studying the uptake rate of Cd for 11 days in the contaminated lake (Treatment 2) and the uptake from contaminated food in the pristine lake (Treatment 3), the organisms should have been depurated in the pristine lake. Depuration rates should have been calculated from each treatment and these values should have been used in the model to determine Cd dynamics in *H. azteca* for Treatments 2 and 3.

Another area in which Stephenson and Turner's (1993) study could have been further improved was in determining the grazing rates of periphyton by *H. azteca*. In their study, grazing rates were determined by giving 30 *H. azteca* 3 tiles containing periphyton, which had been grown in the pristine lake, for 48 hours in a test container. This grazing rate was then used to determine the

assimilation efficiency with which *H. azteca* absorbed Cd from natural periphyton. The calculated assimilation efficiency was found to be higher than reported in the literature. In order to determine if the assimilation efficiency was a true representation of how much Cd was absorbed, grazing rates should have been determined from the tiles that were grazed during the experiment, since the behaviour or response of organisms can change depending on the situation or environment in which they reside. Since grazing rates were not determined from the treatments, the value determined from the laboratory experiment could have overestimated how much Cd was absorbed since the periphyton used in Treatments 2 and 3 in the field contained Cd. Therefore, grazing rates should have been determined from the treatments in the field since grazing rates may differ when the organism is exposed to Cd in food versus a food source with no appreciable metal content.

Another reason that may explain why the results from our study differed from Stephenson and Turner's (1993) study include the age/size of *H. azteca* that was used in the two studies. Since metal residues in organisms are a function of net accumulation based on weight, any change in body size or condition can potentially influence body concentrations (Langston and Spence 1995). Relationships between metals and growth are often described by one of three scenarios: concentrations increase, decrease, or are independent of size/age. The effects of growth are highly species-, metal- and sometimes site-specific (Langston and Spence 1995). Therefore, knowing the age/size of the organism can be beneficial when interpreting metal concentrations.

In our study, *H. azteca* of known ages (6 to 17 weeks) were used. However, in Stephenson and Turner's (1993) study, the ages of the *H. azteca* were not known since they were collected from the lake using a sweep net. It has been observed with amphipods that Cu, Fe, Pb and Zn appear to be affected similarly by size, with smaller individuals exhibiting the highest metal concentration (Rainbow and Moore 1986). One explanation for this is the large surface area: to volume ratio and faster metabolic rate of smaller invertebrates (Langston and Spence 1995).

Many studies have been conducted with various organisms, including *H. azteca*, to determine the effect of body size on metal accumulation. One component of the study by MacLean et al. (1996) observed the effects of *H. azteca* body size on Pb accumulation at a high aqueous Pb concentration (480 nM). Stephenson and Mackie (1989) in addition, examined the effects of *H. azteca* size on Cd accumulation from three softwater lakes in Central Ontario. The results from these studies indicated a small effect of body size on Pb accumulation and no effect of Cd accumulation since body size was independent of Cd concentrations. These results indicated that Pb and Cd accumulation is independent of age/size in aqueous environments. However, further studies are needed to determine if the presence of food and/or other metals affect bioaccumulation in different ages and sizes of *H. azteca*. Therefore, it is possible that different ages/sizes of *H. azteca* were used in the two experiments and this could have also contributed to the different results obtained.

Another reason that may explain the differences in the results between our study and Stephenson and Turner's (1993) study is the duration of exposure and food source used for both studies. The duration of exposure to Pb and Cd from food and water in our experiment was 6 days and only *N. pelliculosa* was used as a food source. Stephenson and Turner (1993) conducted their experiment for 11 days and the food source was a combination of various types of algal assemblages including Cyanophyceae, Diatomae, and Chlorophyceae. The most abundant taxa on the tiles included *Anabaena*, *Lyngbya*, *Navicula* and *Bulbochaete* (Stephenson and Turner 1993). The exposure period chosen for our experiment was based on the duration that *H. azteca* are able to survive in the absence of food since one treatment included exposing *H. azteca* to metals from water in order to separate the two routes of uptake. Six days were sufficient for our experiment since MacLean et al. (1996) found that mean body concentrations of Pb in *H. azteca* started to level off after the fourth day in a aqueous Pb concentration of 480 nM. With respect to Cd, it has been found that small freshwater invertebrates reached equilibrium with Cd concentrations in

solution in five days or less (Poldoski 1979, Wright 1980 and Dressing et al. 1982, Neumann and Borgmann 1997). From the above studies, and the fact that *H. azteca* can survive up to 8 days without food, a period of 6 days was chosen for the present experiments. From our results, uptake of Cd from food was not found to be the primary route of uptake for *H. azteca,* as was seen with Stephenson and Turner (1993). Therefore, perhaps *H. azteca* in the present study required a longer exposure time in the presence of food in order to reach internal steady state.

In addition to the exposure duration, the food source used in our study may also be a reason for the different results obtained between our study and Stephenson and Turner's (1993). It was stated by Munger et al. (1999) that conclusions of many laboratory studies should be accepted with caution for many reasons including: 1) Cd concentrations in artificial exposure media often largely exceed those at even highly contaminated sites; 2) a consumer's food and the consumer itself are often exposed to different Cd concentrations; 3) Cd speciation in water, and thus Cd bioavailability, are usually not controlled; 4) food is usually not exposed to Cd for a sufficient length of time to reach an internal steady state; 5) consumers are stressed by unnatural experimental conditions; and 6) a natural mixture of food is not given to the consumers. With regards to our study, all the above conditions were met. However, the food source in our experiment was limited to one algal species and undoubtedly some traces of bacteria and fungi. Although this is likely a food source for *H. azteca* under field conditions, it might not be the sole source. Stephenson and Turner (1993) provided a natural mixture of food in their study since tiles were grown in the lake and contained a variety of algal species. Due to different structures and chemical compositions that exist between algal species (e.g. cell wall, versus those with no cell wall), metal concentrations, and availability via digestion from the prey items likely vary. Therefore, perhaps more Cd was available in one species compared to other species in Stephenson and Turner's (1993) study. This could therefore have provided more Cd and thus increased the accumulation from food.

One last explanation that may explain why our results did not correspond with Stephenson and Turner's (1993) field study may be due to the presence of Pb. In our study, *H. azteca* were exposed to 15 nM and 0.37 nM Cd in both water and the food source. In comparison to Stephenson and Turner study (1993), only Cd was studied. Since the present study involved determining the importance of food and water with two metals (Pb and Cd), this may have influenced the results. The presence of another metal, such as Pb, may produce competition for binding and uptake sites on the plasma membrane between Cd.

To date, Stephenson and Turner's (1993) field study and our laboratory study represent the only two experiments that have determined the importance of metals in food and water sources to *H. azteca.* Thus, more studies are needed to further our knowledge of the transfer of metals from the environment to *H. azteca* and how the presence of other metals affect accumulation.

The explanations stated above attempt to understand why our results did not agree with Stephenson and Turner's (1993) field study. Although the outcome was not the same between the experiments, they both represent the first experiments that have observed the importance of food and water as a Cd sources to *H. azteca* in the field and in the laboratory. These studies therefore have furthered our knowledge on the transfer of metals through the food chain. This research has also contributed to the knowledge of accumulation of two metals from food and water since Pb was studied in addition to Cd.

4.2 Detoxifications of trace metals

The accumulation of nonessential metals, such as Pb and Cd, by an organism, either from their food or water, can follow two basic detoxification strategies to control the intracellular speciation of a metal. These include 1) regulating the intracellular concentrations of metal by either preventing the metal from entering or expelling the metal once it has been taken up, or 2) by synthesising ligands of the appropriate affinity that can bind the metal and effectively remove it from reactions that may be deleterious to cellular functioning (Mason and Jenkins 1995). The mechanism, which the organism

employs, depends upon the species and the metal. Pb and Cd are both classified as class B metals which means they form complexes with sulfide, organosulfides, nitrogen and oxygen. However, complexes with sulfur are more common and stable (Mason and Jenkins 1995). The classification of metal is important since it influences bioaccumulation. For example, since Pb and Cd are both class B metals, they compete with each other for entry into the organism. The main pathway in which these metals can enter an organism is through the calcium channel in the cell membrane (Simkiss and Taylor 1995). The Ca^{2+} channel can generate large, open channel transfer rates of 10^6 ions per second and is highly selective over Na^+ and K^+ channels and Mg^{2+} transport (Simkiss and Taylor 1995). Since this channel is selective, competition occurs between ions and this was seen between Pb and Cd in Experiment 1, which involved exposing *H. azteca* to various aqueous concentrations of Pb and Cd. With reference to Table 7, the amount of Cd that accumulated by *H. azteca* in comparison to Pb seemed to be affected by the concentration of Pb present in the medium. It was found that when the Cd concentrations remained constant, the amount of Cd accumulated was different. It was seen that a higher concentration of Pb in the medium resulted in a lower accumulation of Cd by *H.azteca.*

4.3 Pb and Cd exposure to *H. azteca* by water

From Experiment 1, it was found that the concentration of Pb or Cd that accumulated per kg of *H. azteca* varied between the treatments. One possible explanation for this may be the competition between Pb and Cd for binding and uptake sites on the membrane. Competition may have resulted due to the different concentrations of Pb and Cd used for each treatment. This can been seen from the results found between Treatments 3 (136 nM Pb, 0.39 nM Cd) and 4 (15 nM Pb, 0.37 nM Cd). Although the Cd concentration was the same in both treatments, the Cd concentration per kg of *H. azteca* from Treatment 3 and Treatment 4 yielded different Cd concentrations. The concentration of Cd in *H. azteca* in Treatment 3 was 0.21×10^{-4} mol of Cd/ kg *H. azteca*. Treatment 4 in comparison produced 1.16×10^{-4} mol of Cd/ kg *H. azteca*. Since the

concentration of Pb was significantly greater in Treatment 3 versus Treatment 4, it can be speculated that the available sites on the membrane were limited and thus reduced the accumulation of Cd. The presence of other metals is not the only competition that occurs for binding and uptake sites. Water hardness ions such as Ca^{2+}, which were previously mentioned, also compete for binding sites. Since the medium used in our experiment was representative of hard water (40 mg/L Ca^{2+}), Ca^{2+} cations were present and were therefore also competing with Pb and Cd for binding sites. The selectively for Ca^{2+} occurs through two or more high affinity sites (Simkiss and Taylor 1995), therefore Ca^{2+} will be selected first before Pb^{2+} or Cd^{2+}. Since Ca^{2+} occupies a significant portion of the binding sites, there are limited sites for Pb and Cd. Therefore competition between water hardness ions in addition to an increase of Pb^{2+} ions might explain why *H. azteca* accumulated less Cd in Treatment 3 than in Treatment 4. A higher Pb concentration in the medium affected the amount of Pb accumulated by *H. azteca* since the Pb concentration in *H. azteca* (mol of Pb/kg) for Treatment 3 (136 nM Pb) was larger than Treatment 4 (15 nM Pb), 3.41×10^{-3} and 2.97×10^{-3}mol of Pb/kg, respectively.

4.4 Pb and Cd exposure to *H. azteca* by water and by water and food

Experiments 2 and 3 determined the importance of water and food as Pb and Cd sources to *H. azteca* at concentrations of 15 nM Pb and 0.37 nM Cd. The bioconcentration factor was calculated and used to compare the results obtained from these experiments since one concentration of Pb and Cd was used. BCF values were not used in Experiment 1 since BCF is a ratio of the concentration of a given metal in an organism, to that in its environment. Since different concentrations of metal in the water were present in Experiment 1, this would have contributed to BCF values that were not a true representation of the concentration, since low concentrations of a metal in water can increase the BCF value for a given accumulation of metal.

To determine the importance of food and/or water as sources of Pb and Cd to *H. azteca*, the BCF values were compared between the two treatments for one metal. If the BCF from the treatment containing food and water was higher

than the water exposure treatment, it appears that the organism accumulates the metal from its food. From the results, the BCF values were the same for Pb between the two treatments and therefore the presence of food had no effect on accumulation. However, the BCF for Cd for Treatment 3 (food and water exposure) was larger than Treatment 2 (water exposure) strongly suggesting some Cd was accumulated from the food source. At a buffered Cd concentration of 0.37 nM, there was not a significant effect since no statistical difference was detected between the two exposures.

One explanation as to why Cd may have accumulated more in *H. azteca* from the food rather than Pb may be due to the metal binding proteins, metallothioneins, associated with *H. azteca*. Metallothionein is widely perceived as fulfilling a detoxifying role of borderline and class B metals by sequestering intracellular Cd, however its presence may drive the process of further accumulation (in metallothionein producers) by effectively binding Cd internally, thus maintaining the diffusion gradient that further supports the passive entry of Cd (Langston and Spence 1995). These intracellular metal binding proteins are a class of sulfhydryl-rich, low molecular weight proteins that are capable of binding divalent ions such as Cd and Hg and therefore decreasing their toxicity to the organism (Chan 1998). Although Pb is classified as a Class B metal, it does not bind to metallothionein (Chan 1998). Therefore, since metallothionein has a great affinity for Cd this could explain why an increase in Cd concentration was found in *H. azteca* after being exposed to Pb and Cd from both the food and water yet not for Pb.

Another possible explanation that may have resulted in the Cd concentrations being higher in Treatment 3 than Treatment 2 may be the location in which the metal resided within the food source. It has been found that cellular distribution of a metal in an animal's food can strongly influence its assimilability in the animal after it is ingested (Fisher and Reinfelder 1995). The extent to which a metal is absorbed by the gut tissue of an organism depends on the degree to which the metal is bound to soluble cytosolic components in the algal cells consumed as food (Reinfelder and Fisher 1991). For diatoms, it has

been found that Pb remains bound to the surface of diatom cells upon digestion by a predator and is not assimilated by the organism (Fisher et al. 1983, Schulz-Baldes and Lewin 1976). In contrast, Cd has been found in the cytoplasm of algal cells and therefore is assimilated by the organism (Reinfelder and Fisher 1991, Li 1980). Therefore, trace metals that are concentrated on phytoplankton surfaces, such as Pb, will pass through the gut of the animal (Michaels and Flegal 1990). Metals, like Cd, associated with the cytoplasm of the algal cell, are likely to be assimilated by the organism. This is due to the gut containing the bulk of the RNA precursors of the metal-binding proteins that are induced by exposure to Cd in food (Hare 1992). Since Cd is found in the cytoplasm of algal cells and metallothionein has a strong affinity for Cd, this may explain why a larger BCF for Cd was found when *H. azteca* was exposed to Cd from food and water exposure versus water alone. This may also explain why the BCF value did not change for Pb between the two treatments since Pb largely remained bound to the surface of diatom cells, and therefore was not assimilated in the organism.

4.5 Potential Pb and Cd accumulated by *H. azteca*

To determine if the actual Pb and Cd concentrations found in Experiment 3 was the maximum potential that *H. azteca* could accumulate from food, a mass balance equation was used to determine the potential accumulation of Pb and Cd. With reference to the results for Pb, it was found that the potential accumulation over a 6-day period is 5.60×10^{-3} (mol of Pb/kg of *H. azteca*). In comparison to the actual concentration found in Treatment 3, the potential is higher since a concentration of 0.64×10^{-3} mol of Pb/kg of *H. azteca* was determined (Table 17). It has been shown that aquatic organisms accumulate Pb mainly from the dissolved phase (Michaels and Flegal 1990), and based on the results found in our study, this trend was found within *H. azteca* since the actual concentration of Pb from Treatment 2 (water exposure) was greater than Treatment 3 (water and food exposure), 0.77×10^{-3} mol of Pb/kg of *H.azteca* versus 0.64×10^{-3} mol of Pb/kg of *H. azteca,* respectively.

With respect to Cd, the potential accumulation from food over a 6-day exposure period was calculated to be 53.6 x 10^{-6} mol of Cd/kg of *H. azteca.* In comparison to the actual concentration found in Treatment 3 (water and food exposure), the potential was lower since a concentration of 132 x 10^{-6} mol of Cd/kg of *H. azteca* was achieved for this treatment. One current mechanism that has been proposed to explain why Cd accumulation occurs is that of the action of metal binding proteins, metallothioneins. However, the role of the bioaccumulation rate resulting from metallothionein has not yet been verified. Thus, further research is required to determine the role of these proteins in *H. azteca* after ingestion of Cd from food since it was found in our study that Cd accumulation in *H. azteca* does increase in the presence of Cd containing food. This was seen since in the present study, the actual concentration was lower in the water exposure treatment than the food and water treatment with concentrations of 95.3 x 10^{-6} mol of Cd/kg of *H. azteca* for water exposure and 132 x 10^{-6} mol of Cd/kg of *H. azteca* for water and food exposure.

5.0 Conclusion

In our study it was found that Pb and Cd bioaccumulation in *H. azteca* from the food source *N. pelliculosa* was negligible when the dissolved inorganic fractions are buffered with 10 μM EDTA. The average $\log_{10}$ BCF value for Cd demonstrated an increase in the presence of food, however after calculating the least significant difference it indicated that there was no significant difference from the presence of food at a concentration of 15 nM Pb and 0.37 nM Cd. These results were not in agreement with a previous field study with *H. azteca* conducted by Stephenson and Turner (1993) since it was found food was the main route of uptake at a relatively low Cd concentration of 0.86 nM. It was however found in the present study that *H. azteca* do accumulate Cd from food, however it was found that the amount they accumulated was not that much greater than those organisms exposed to just Pb and Cd by water. Due to the different findings from these two studies, no solid conclusion can be made about the importance of food and water as Pb and Cd sources to *H. azteca*. Differences in the results may have occurred for a variety of reasons such as the use of different media to conduct the experiments, different ages/sizes of *H. azteca*, duration of the experiments and different food sources. Therefore, the following recommendations are suggested to shed light on the trophic transfer of metals to *H. azteca*.

6.0 Recommendations

In this study, a method was established to feed benthic organisms a natural food source under controlled laboratory conditions. Therefore, it is recommended that our study be repeated for a longer duration than 6 days to determine if the exposure period affects the results. It is recommended that an 11-day exposure period be used since this was the time period Stephenson and Turner (1993) used in their study.

In addition to increasing the duration of the experiment, it is also recommended that different algae species be studied to determine if uptake of Cd and Pb changes with different food sources. Perhaps the physical characteristics of the algae species affect how metals are assimilated in an organism. It has been suggested that trace metals, ingested with phytoplankton lacking cell walls, are more likely to be retained in the animal, thereby increasing the probability that the metal will be assimilated (Fisher and Reinfelder 1995).

It is also recommended that further experiments be conducted using more than two metals. It was seen in Experiment 1, which involved exposing *H. azteca* to various aqueous Pb and Cd concentrations, that introducing more than one metal could increase the competition for binding and uptake sites on the membrane. In addition, it was also seen in Experiments 2 and 3, which determined the importance of food and water as Pb and Cd sources to *H. azteca,* how accumulation in the organism can increase in the presence of food. Therefore, studying different metals in combination with Pb and Cd through these two exposure routes would further enhance our knowledge of how *H. azteca* respond to a variety of other metals in presence and absence of food.

To determine the importance of Pb and Cd as food and water sources to *H. azteca*, our study exposed *H. azteca* to one concentration each of Pb and Cd, 15 nM and 0.37 nM, respectively. These concentrations were derived from a study conducted by Hare and Tessier (1998) that measured total metal concentrations in 27 lakes across Ontario and Quebec. Our research marks the first laboratory experiment that studied the importance of food and water with

two metals within *H. azteca*. However, more studies are required at low environmental concentrations to show the effects that different concentrations may have on the organism. Therefore, one last recommendation is to continue to study the importance of food and water as Pb and Cd sources to *H. azteca*, both in the field and in the laboratory, at lower ambient concentrations to determine if the same trend is seen as in our study.

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8.0 Appendix

Appendix A: Inorganic fraction Pb and Cd accumulated by *H. azteca* in Experiments 1 through 3.

Purpose: To determine if the buffer, ETDA, was maintained throughout Experiments 1 through 3.

Method: The inorganic fraction of Pb and Cd accumulated by *H. azteca* during Experiments 1 through 3 was calculated by dividing the concentration of metal in the organism by the total metal concentration in the medium.

Results:

Table A1. Inorganic Pb and Cd accumulated by *H. azteca* from Experiment 1.

Treatment	% $Pb_{inorganic}$ accumulated by *H. azteca*	% $Cd_{inorganic}$ accumulated by *H. azteca*
3 (Pb 136 nM and Cd 0.39 nM)	0.04	0.40
4 (Pb 15 nM and Cd 0.37 nM)	0.02	0.15
5 (Pb 19.1 nM and 12.4 nM)	0.01	0.03

Table A2. Inorganic Pb and Cd accumulated by *H. azteca* from Experiment 2.

Treatment Cultured *H. azteca*	% $Pb_{inorganic}$ accumulated by *H. azteca*	% $Cd_{inorganic}$ accumulated by *H. azteca*
2 (water exposure)	0.03	0.28
3 (food and water exposure)	0.04	0.83

Treatment Aquarium *H. azteca*	% $Pb_{inorganic}$ accumulated by *H. azteca*	% $Cd_{inorganic}$ accumulated by *H. azteca*
2 (water exposure)	0.10	1.48
3 (food and water exposure)	0.07	1.06

Table A3. Inorganic Pb and Cd accumulated by *H. azteca* from Experiment 3.

Treatment	**% $Pb_{inorganic}$ accumulated by *H. azteca***	**% $Cd_{inorganic}$ accumulated by *H. azteca***
2 (water exposure)	0.01	0.28
3 (food and water exposure)	0.02	0.65

Appendix B: Growth assay with *N. pelliculosa* on Teflon® plaques.

Purpose: To determine if the Teflon® plaques had sufficient biomass of *N. pelliculosa* for a food source for *H. azteca,* and if the growth was stationary after 7.

Method: The growth assay was conducted be growing *N. pelliculosa* on acid clean Teflon® plaques in the presence and absent of Pb and Cd. The control medium was prepared by mixing 1 L of dechlorinated tap water with 10 μM EDTA, 12.5 μM silicate, 1 μM phosphate and 10 μM nitrate and Guilliard vitamin mix (Morel et al. 1975). The medium was sterilized by filtration using a 0.2 μm-pore size filter.

The medium containing Pb and Cd consisted of the inorganic concentrations of 0.37 nM Cd and 15 nM Pb. The medium was prepared by first mixing 1 L of dechlorinated tap water with 1 mL of guillard vitamin mix, 1 mL of trace metal stock, 1 mL of 0.01 M EDTA and 10 ml's of silicate, phosphate, nitrate The solution was mixed several times before the addition of 1.929 mL of 4.3e-03 Mol/L $Pb(NO_3)_2$ and 0.0609 mL of 8.896e-04 Mol/L $CdCl_2\ 2^{1/2}H_20$ was added. The media was sterilized by filtration using 0.2 μM filter.

In two polyethylene containers, 50 plaques were immersed in both mediums and covered with clear saran wrap to avoid air dust contamination and left under a 16:8 h light:dark cycle. Three plaques were randomly selected from each medium daily for 15 days. The plaques were rinsed in FRAQUIL medium (Morel et al. 1975) and then placed in 10 mL of 90% acetone for 24 h. Chl-*a* measurements were then taken after 24 h using the fluorometer (Turner design 700).

Results:

Table B1. Biomass of *N. pelliculosa* grown on Teflon® plaques. *N. pelliculosa* was inoculated in two mediums simultaneously. Medium 1 contained dechlorinated tap water and 10 µM EDTA; Medium 2 contained dechlorinated tap water mixed with 10 µM EDTA, 15 nM Pb and 0.37 nM Cd.

Medium 1: EDTA control		**Medium 2: 15 nM Pb and 0.37 nM Cd**	
Time (h)	**log µg chl-a/cm^2**	**Time (h)**	**log µg chl-a/cm^2**
0	-4.64	0	-4.64
23	-1.11	23	-1.14
45	-0.52	45	-0.71
70	-0.21	70	-0.33
92	0.23	92	-0.12
115	0.15	115	0.23
141	0.14	141	0.28
168	0.35	168	0.17
185	0.31	185	0.11
209	0.38	209	0.06
235	0.39	235	0.16
261	0.36	261	0.18
283	0.41	283	0.19
307	0.41	307	0.15
331	0.46	331	0.34

Appendix C: Determination of Pb and Cd concentrations for Experiment 1.

Purpose: To determine the Pb and Cd concentrations to be used for Experiment 1.

Method: The selection of Pb and Cd concentrations for Experiment 1 was determined from a study conducted by Hare and Tessier (1998) and from the Canadian Water Quality Guidelines, which were published in 1987 (CCREM).

Determining the Pb and Cd concentrations for Experiment 1 first involved averaging and categorizing the highest and lowest Pb and Cd concentrations from Hare and Tessier (1998) study (Table C1a and C1b).

Table C1a. Total dissolved Cd concentrations (nM) from 27 lakes in Canada (Hare and Tessier 1998).

Low [Cd] nM	High [Cd] nM
0.38	4.9
0.19	2.18
0.24	1.63
0.32	1.93
0.27	2.23
0.54	2.37
0.2	3.14
0.25	1.13
0.42	1.43
0.29	1.19
0.31	7.12
0.32	
0.67	
0.15	
0.15	
0.4	
Average	Average
0.32	2.7

Table C1b. Total dissolved Pb concentrations (nM) from 27 lakes in Canada (Hare and Tessier 1998).

Low [Pb] nM	Highest [Pb] nM	High [Pb] nM
0.9	9.9	1.7
0.2	14.1	1.2
0.9	12	1.5
		1.5
		1
		2.1
		1.9
		1.4
Average	Average	Average
0.67	12	1.5

Once the concentrations of Pb and Cd were categorized, the choosen concentrations were determined based on the averages found from Tables C1a and C1b. Table C2 summarizes the concentrations of Pb and Cd.

Table C2. Summary of the Pb and Cd concentrations chosen from Hare and Tessier's study (1998).

Source	Treatment	[Pb] nM	[Cd] nM
Hare and Tessier (1998)	Low Pb, High Cd	0.67	2.7
Hare and Tessier (1998)	High Pb, Low Cd	12	0.32
Hare and Tessier (1998)	Low Pb, Low Cd	1.5	0.32
Canadian Water Quality Guidelines (198	High Pb, High Cd	19	11.5

Once the concentrations were chosen, the inorganic concentrations of Pb and Cd for each treatment were determined using the chemical speciation program MINEQL+ (Version 4.5). Since the basal medium used for the experiments was dechlorinated tap water that originated from Lake Ontario, and the concentrations of Pb and Cd were chosen from various other lakes across Ontario and Quebec, the major ions associated with Lake Ontario had to be adjusted in the program to give the same proportion of ions and concentrations. Therefore, the average pH and calcium concentration from the range of lakes had to be determined (Tables C3 and C4).

Table C3a. The average pH values associated with the Cd concentration selected from the various lakes (Hare and Tessier 1998).

Lake in range of [Cd]			
Cd 0.32 nM		**Cd 2.7 nM**	
pH	**mol/L**	**pH**	**mol/L**
6.04	9.12×10^{-7}	4.79	1.62×10^{-5}
6.37	4.27×10^{-7}	5.86	1.38×10^{-6}
6.76	1.74×10^{-7}	6.67	2.14×10^{-7}
5.5	3.16×10^{-6}	5.81	1.55×10^{-6}
5.98	1.05×10^{-6}	4.62	2.40×10^{-5}
5.94	1.15×10^{-6}	6.38	4.17×10^{-7}
6.02	9.55×10^{-7}	7.11	7.76×10^{-8}
6.12	7.59×10^{-7}	7.27	5.37×10^{-8}
7.4	3.98×10^{-8}	7.1	7.94×10^{-8}
7.26	5.50×10^{-8}	6.58	2.63×10^{-7}
7.24	5.75×10^{-8}	4.58	2.63×10^{-5}
6.55	2.82×10^{-7}		
6.18	6.61×10^{-7}		
7.18	6.61×10^{-8}		
6.63	2.34×10^{-7}		
5.65	2.24×10^{-6}		
	Average		Average
	7.64×10^{-7}		6.41×10^{-6}
pH = -log[H+]	6.10	pH = -log[H+]	5.19

Table C3b. The average pH values associated with the Pb concentrations selected from the various lakes (Hare and Tessier 1998).

Lake in range of [Pb]					
Pb 1.5 nM		**Pb 12 nM**		**Pb 0.67 nM**	
pH	**mol/L**	**pH**	**mol/L**	**pH**	**mol/L**
6.04	9.12×10^{-7}	4.79	1.62×10^{-5}	6.58	2.63×10^{-7}
6.76	1.74×10^{-7}	4.58	2.63×10^{-5}	7.27	5.37×10^{-8}
5.98	1.05×10^{-6}			6.55	2.82×10^{-7}
5.94	1.15×10^{-6}				
6.12	7.59×10^{-7}				
6.38	4.17×10^{-7}				
7.24	5.75×10^{-8}				
6.18	6.61×10^{-7}				
	Average		Average		Average
	6.47×10^{-7}		2.13×10^{-5}		2.00×10^{-7}
pH = -log[H+]	6.19	pH = -log[H+]	4.67	pH = -log[H+]	6.70

Table C4a. The average Ca^{2+} concentrations associated with the Cd concentrations selected from the various lakes (Hare and Tessier 1998).

Cd 0.32 nM		**Cd 2.7 nM**	
[Cd] (nM)	**Ca^{2+} (M)**	**[Cd] (nM)**	**Ca^{2+} (M)**
0.38	9.50×10^{-5}	4.9	1.37×10^{-4}
0.19	1.07×10^{-4}	2.18	6.90×10^{-5}
0.24	1.17×10^{-4}	1.63	1.04×10^{-4}
0.32	4.70×10^{-5}	1.93	9.70×10^{-4}
0.27	8.40×10^{-5}	2.23	5.20×10^{-4}
0.54	5.20×10^{-5}	2.37	1.06×10^{-4}
0.2	1.13×10^{-4}	3.14	2.45×10^{-4}
0.25	5.20×10^{-5}	1.13	1.88×10^{-4}
0.42	4.10×10^{-4}	1.43	4.90×10^{-5}
0.29	1.97×10^{-4}	1.19	8.90×10^{-5}
0.31	2.33×10^{-4}	7.12	7.70×10^{-5}
0.32	8.90×10^{-5}		
0.67	5.20×10^{-5}		
0.15	5.00×10^{-4}		
0.15	9.70×10^{-5}		
0.4	4.70×10^{-5}		
Average		Average	
0.32	1.43×10^{-4}	2.66	2.32×10^{-4}

Table C4b. The average Ca^{2+} concentrations associated with the Pb concentrations selected from the various lakes (Hare and Tessier 1998).

Pb 1.5 nM		Pb 12 nM		Pb 0.67 nM	
[Pb] (nM)	**Ca^{2+} (M)**	**[Pb] (nM)**	**Ca^{2+} (M)**	**[Pb] (nM)**	**Ca^{2+} (M)**
1.7	9.40×10^{-5}	9.9	1.37×10^{-4}	0.9	8.90×10^{-5}
1.2	1.17×10^{-4}	14.1	7.70×10^{-5}	0.2	1.88×10^{-4}
1.5	8.40×10^{-5}			0.9	8.90×10^{-5}
1.5	5.20×10^{-5}				
1	5.20×10^{-5}				
2.1	1.06×10^{-4}				
1.9	2.33×10^{-4}				
1.4	5.20×10^{-4}				
Average		Average		Average	
1.54	1.57×10^{-4}	12	1.07×10^{-4}	0.67	1.22×10^{-4}

Once the average pH and Ca^{2+} concentrations were computed, the major ions associated with Lake Ontario were adjusted by dividing the initial concentration by a factor, which was calculated by dividing the initial Ca^{2+} ion concentration of Lake Ontario water by the averaged Ca^{2+} ion concentration for the specific treatment (Table C5).

Table C5. The adjustment of the major ions associated with each Treatment in Experiment 1.

	Treatment 2 Pb (6.8 nM) Cd (3.5 nM)	**Treatment 3 Pb (136 nM) Cd (0.39 nM)**	**Treatment 4 Pb (15 nM) Cd (0.37 nM)**	**Treatment 5 Pb (19.1nM) Cd (12.4 nM)**
Average pH	5.95	5.4	6.15	8.0
Average Ca^{2+}	1.78×10^{-4}	1.27 x 10-4	1.50 x 10-4	9.50×10^{-4}
Adjustment factor	5.34	7.48	6.33	1.00
Ion [ ] adjusted				
Ca^{++}	1.78×10^{-4}	1.27×10^{-4}	1.50×10^{-4}	9.50×10^{-4}
Mg^{++}	6.75×10^{-5}	4.81×10^{-5}	5.68×10^{-5}	3.60×10^{-4}
Na^{+}	$1.09 \text{ x-}10^{-4}$	7.75×10^{-5}	9.16×10^{-5}	5.80×10^{-4}
K^{+}	$8.24 \text{ x-}10^{-6}$	5.88×10^{-6}	6.95×10^{-6}	4.40×10^{-5}
HCO_3^{-}	$3.43 \text{ x-}10^{-4}$	2.45×10^{-4}	2.89×10^{-4}	1.83×10^{-3}
SO_4^{2-}	$6.18 \text{ x-}10^{-5}$	4.41×10^{-5}	5.21×10^{-5}	3.30×10^{-4}
Cl^{-}	1.39×10^{-4}	9.89×10^{-5}	1.17×10^{-4}	7.40×10^{-4}

To determine the free $[Pb^{2+}]$ and $[Cd^{2+}]$ at the pH and alkalinity of the selected concentrations, MINEQL+ was ran in the absence of EDTA, Pb, and Cd (Table C6).

Table C6. Inorganic $[Pb^{2+}]$ and $[Cd^{2+}]$ for each treatment.

	Treatment 2 Pb (6.8 nM), Cd (3.5 nM)	**Treatment 3 Pb (136 nM), Cd (0.39 nM)**	**Treatment 4 Pb (15 nM), Cd (0.37 nM)**	**Treatment 5 Pb (19.1 nM), Cd (12.4 nM)**
PPb	9.2	7.93	8.85	8.76
PCd	8.58	9.5	9.51	8.02

Once the free ion concentrations were determined, MINEQL was ran again using the concentrations of the major ions associated with Lake Ontario and 10 μM EDTA to determine the total Pb and Cd concentration at pH 8 to achieve inorganic Pb and Cd concentrations (Table C7).

Table C7. $[Pb]_{total}$ and $[Cd]_{total}$ at pH 8 to give the inorganic $[Pb^{2+}]$and $[Cd^{2+}]$ concentrations.

Total	Treatment 2 Pb (6.8 nM), Cd (3.5 nM)	Treatment 3 Pb (136 nM), Cd (0.39 nM)	Treatment 4 Pb (15 nM), Cd (0.37 nM)	Treatment 5 Pb (19.1 nM), Cd (12.4 nM)
Pb concentration	8.50×10^{-6}	1.01×10^{-5}	9.70×10^{-6}	8.56×10^{-6}
Cd concentration	1.00×10^{-6}	7.00×10^{-9}	5.50×10^{-8}	1.29×10^{-6}

Appendix D: Sorption of Pb and Cd to the experimental polypropylene containers.

Purpose: To determine if Pb and Cd absorbed to the sides of the polypropylene containers during the experiment.

Method: Water samples were analyzed for Pb and Cd using GFAAS. The analytical method used to analyze Pb using GFAAS was, 15 µL sample, 10 µL $NH_4H_2PO_4$, and 5 µL $Mg(NO_3)_2$. The samples were analyzed for Pb under the calibration curve of the standards 10 nM and 500 nM Pb. The method used for Cd analysis in comparison, was 10 µL of sample and 10 µL $Mg(NO_3)_2$. Samples were analyzed under the calibration curve of the standards 2 nM and 50 nM Cd. All samples, except for the controls, were diluted in a 0.4 M HNO_3 matrix.

Results:

Experiment 1

Table D1. Concentrations of Pb in water samples from each Treatment in Experiment 1.

Day (D)	Treatment 1 (nM)	Treatment 2 (nM)	Treatment 3 (nM)	Treatment 4 (nM)	Treatment 5 (nM)	Treatment 6 (nM)
D0	2.787	8704.8	10552	11227	9345.9	0.926
D1	3.789	8534.6	10412	10529	9157.1	4.434
D3	2.313	9140.4	10278	10423	9362.7	2.933
D4	3.03	8620.5	10497	10569	9388.3	2.336
D5	1.648	9057.6	10557	11485	9528.3	1.867
D6	2.014	9031.5	10892	10923	9624.1	0.823
D7	2.304	9119.2	10809	11490	9710.7	1.893
Expected concentrations		8500	10100	9700	8600	

Table D2. Concentrations of Cd in water samples from each Treatment in Experiment 1.

Day (D)	Treatment 1 (nM)	Treatment 2 (nM)	Treatment 3 (nM)	Treatment 4 (nM)	Treatment 5 (nM)	Treatment 6 (nM)
D0	0.12	1502.1	9.7854	82.818	1954.9	0.1061
D1	0.48	1469.2	9.8121	81.706	1920	0.0843
D3	0.34	1481.5	9.5273	82.868	1917.7	0.4857
D4	0.28	1501.1	10.184	87.892	1938.6	0.4322
D5	0.29	1524.2	10.177	86.015	2009.4	0.2842
D6	0.18	1470.7	10.356	82.924	1961.3	0.2361
D7	0.42	1468.1	10.279	87.664	2008.7	0.5541
Expected concentrations		1000	7	55	1300	

Experiment 2

Table D3. Concentrations of Pb in water samples from Treatments1 and 3 in Experiment 2.

Day (D)	Treatment 1 (nM)	Treatment 3 (nM)
D0	3.504	10225
D6	3.82	10613
Expected concentration		9700

Table D4. Concentrations of Cd in water samples from Treatments 1 and 3 in Experiment 2.

Day (D)	Treatment 1 (nM)	Treatment 3 (nM)
D0	0.048	82.37
D6	0.041	81.3
Expected concentration		55

Experiment 3

Table D5. Concentrations of Pb in water samples from each Treatment in Experiment 3.

Day (D)	Treatment 1 (nM)	Treatment 2 (nM)	Treatment 3 (nM)	Treatment 4 (nM)
D0	4.204	9748.9	9875.2	100080
D6	3.981	9642.3	9984.5	9485
Expected concentrations		9700	9700	9700

Table D6. Concentrations of Cd in water samples from each Treatment in Experiment 3.

Day (D)	Treatment 1 (nM)	Treatment 2 (nM)	Treatment 3 (nM)	Treatment 4 (nM)
D0	-0.788	77.8	81.8	78.6
D6	-0.552	82.6	85.9	79.4
Expected concentrations		55	55	55

Appendix E: Dry weights (mg) of *H. azteca*.

Purpose: To determine the dry weights of *H. azteca* from each experiment.

Method: At the end of each experiment, *H. azteca* that survived (motile and alert), were placed in pre-weighed 1.5 mL micro-centrifuge tubes using an acid clean plastic pipette. The animals were dried at 60^{O}C for 72 h and then placed in a desiccator for 24 h to reach room temperature before being re-weighed using a micro analytical balance (Mettler H2O).

Results:

Table E1. Dry weights (mg) of *H. azteca* from Experiment 1.

Sample I.D	Treatment 1	Treatment 3	Treatment 4	Treatment 5	Treatment 6
A	0.45	1.31	0.96	0.72	0.47
B	0.81	1.02	0.65		0.63
C	0.99	0.60	0.40		1.05
D	0.55	0.13	1.21		0.35
E	0.44	0.37	0.24		1.18
F	0.44	0.61	0.64		0.38
G		0.20	0.51		0.41
H		0.61	0.34		0.06
I			0.55		0.16
J					0.84

Table E2. Dry weights (mg) of *H. azteca* from Experiment 2.

	Control		Water		Food and Water	
Sample I.D	Cultured	Aquarium	Cultured	Aquarium	Cultured	Aquarium
A	0.10	2.80	**-0.05**	0.29	3.18	3.04
B	0.73	1.36	2.50	1.21	0.58	1.24
C	0.50	4.37	2.83	4.88	3.23	4.33
D	0.43	4.98	1.76	5.04		3.71
E		3.80	2.20	3.32		**22.82**
F		4.05		0.40		
G		2.67				
H						

Table E3. Dry weights (mg) of *H. azteca* from Experiment 3.

Sample I.D	Control		Water			Food and Water		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep.3
A	1.24	1.50	1.17	1.24	1.27	0.83	1.98	0.97
B	1.94	1.61	0.84	0.77	1.33	1.29	2.33	2.61
C	1.33	1.43	1.24	0.92	**0.00**	0.49	1.52	1.55
D	0.82	2.03	0.69	1.14	0.44	0.78	1.60	1.42
E	0.45	1.48	0.33	0.65	**-0.01**	0.75	1.44	1.83
F	1.17	1.88	0.46	0.58	0.50	0.60	1.37	1.19
G	0.58	1.50	0.56	0.69	0.33	0.56	0.64	0.72

*Note: Rep. = replicate

Weights **highlighted** were excluded in the calculation of BCF

Appendix F: Digestion of *H. azteca*

Purpose: Digest tissue samples of *H. azteca* for Pb and Cd analysis using GFAAS.

Method: *H. azteca* were digested with concentrated nitric acid for 6 days, followed by the addition of 30% hydrogen peroxide for 24 h and then Milli Q-water.

For Experiment 1, the volume of acid, peroxide and water were based on the dry weights in an attempt to keep the ratio of dry weight to final volume more consistent. The number of *H. azteca* per micro-centrifuge tube ranged from 1 to 2. Table F1 (provided by Warren Norwood) illustrates the volumes of each reagent required for a specific dry weight.

Table F1: Volume of HNO_3, H_2O_2 and H_2O required for the digestion of *H. azteca.*

Dry wt (mg)	HNO_3 (µL)	H_2O_2 (µL)	H_2O (µL)	Total (mL)
<0.751	13	10	477	0.5
0.751-1.500	25	20	955	1
1.501-2.250	38	30	1432	1.5
>2.250	50	40	1910	2

For Experiments 2 and 3 the number of *H. azteca* added to each tube varied between 2 to 3. The addition of concentrated nitric acid, peroxide and water for these experiments, was not based on the dry weight of *H. azteca* as in Experiment 1, since the dry weights were greater than 1.50 mg. Therefore, for Experiments 2 and 3 the same volume of acid, peroxide and water was added to each micro-centrifuge tube to achieve a final volume of 1 mL. The volumes added were, 125 µL of HNO_3, 100 µL of H_2O_2 and 725 µL of H_2O for a final volume of 1.0 mL. Standard additions were conducted using GFAAS (AAnalyst 800) with the largest and smallest dry weight after complete digestion to

determine if there was a matrix interference using the same method for the two samples.

Appendix G: Analytical Methods using GFAAS for Pb and Cd analysis with digested tissue samples and water samples.

Purpose: To develop analytical methods using graphite furnace atomic absorption (GFAAS) to determine Pb and Cd concentrations in *H. azteca*, Tort 2: reference material, *N. pelliculosa* and water samples.

Method: Water samples and digested tissue samples were analyzed for Pb and Cd using graphite furnace atomic absorption (AAnalyst 800, Perkin Elmer). Samples were analyzed on a plateau tube with modifiers of ammonium phosphate monobasic ($NH_4H_2PO_4$) and magnesium nitrate $Mg(NO_3)_2$ for Pb analysis and magnesium nitrate $Mg(NO_3)_2$ for Cd analysis. The electrode dischargeless lamp was used for Pb analysis and hollow cathode lamp was used for Cd analysis.

Results:

Analytical methods developed for digested tissue samples

H. azteca and TORT 2; Reference material

Experiment 1

The analytical method developed for Pb analysis using GFAAS for Experiment 1 for *H. azteca* and TORT-2 sample was 25 µL of sample, 10 µL $NH_4H_2PO_4$, and 5 µL $MgNO_3$. Samples were analyzed under the calibration curve of the standards 10 nM and 500 nM Pb. For Cd analysis the method of 13 µL of sample and 5 µL $MgNO_3$ was used and the analysis was done under the calibration curve of the standards 2 nM and 50 nM Cd. Digested tissue samples of *H. azteca* from Treatments 3 and 4 were diluted ten times before analysis. Samples from Treatment 5 however, were diluted fifty times.

Before the analysis was conducted, standard additions were conducted with two samples of digested *H. azteca* tissue to determine any matrix interference. Sample was diluted ten times before Pb and Cd was added to the each sample. To determine matrix interference 0, 100 and 400 nM Pb was added to three samples and for Cd, 0, 10 and 40 nM was added to three other

samples. Standard additions were also conducted on the reference material to also determine if there was matrix interference.

Experiments 2 and 3

The analytical method developed for Pb analysis using GFAAS for Experiments 2 and 3 for *H. azteca* and TORT-2 tissue sample was 15 µL of sample, 10 µL $NH_4H_2PO_4$, and 5 µL $MgNO_3$. Samples were analyzed under the calibration curve of the standards, 10 nM and 500 nM Pb. For Cd analysis the method of 10 µL of sample and 10 µL $MgNO_3$ was used and the analysis was done under the calibration curve of the following standards 2 nM and 50 nM Cd. With exception to the controls, all digested tissue samples of *H. azteca* and the reference material were diluted ten times before they were analysed for Pb and Cd. Standard additions were also conducted and the method used was the same as above.

To ensure that the GFAAS was functioning properly between analyses for each experiment, slopes generated from the standard curves were compared for Pb and Cd (Table G1).

Results:

Table G1. Corresponding slopes and correlation coefficients from the GFAAS analysis of Pb and Cd in digested tissue samples of *H. azteca* from Experiments 1 through 3.

(a) Experiment 1

	Pb analysis		Cd analysis
Slope	0.00067	Slope	0.00156
Correlation Coefficient	0.99999	Correlation Coefficient	0.99983

(b) Experiment 2

	Pb analysis		Cd analysis
Slope	0.00040	Slope	0.00191
Correlation Coefficient	0.99996	Correlation Coefficient	1

c) Experiment 3

Pb analysis		Cd analysis	
Slope	0.00044	Slope	0.00276
Correlation Coefficient	1	Correlation Coefficient	0.99995

N. pelliculosa

The method developed for Pb and Cd analysis in *N. pelliculosa* using GFAAS for Experiments 2 and 3 was determined as follows. For Pb analysis, the method used was 20 µL of sample, 10 µL $NH_4H_2PO_4$, and 5 µL $Mg(NO_3)_2$. Samples analyzed under the calibration curve of the standards 100 nM and 600 nM Pb. The method used for Cd analysis was 30 µL of sample and 5 µL $Mg(NO_3)_2$. These samples were analyzed under the calibration of curve of the standards 10 and 30 nM Cd. Plaques that were 7 days old were diluted twenty times before analysis, and plaques that were 11 days old were diluted fifty times. Standard additions were also performed with samples to ensure no matrix interference. These were conducted by adding 0 and 500 nM of Pb to two samples, and 0, 5 and 15 nM of Cd to three other samples.

Again, to ensure that the GFAAS was functioning properly between analyses for each experiment, slopes generated from the standard curves were compared for Pb and Cd for Experiment 2 and 3. Table G2 illustrates the slopes produced from Experiments 2 and 3.

Table G2. Corresponding slopes and correlation coefficients from the GFAAS analysis of Pb and Cd in digested samples of *N. pelliculosa* from Experiments 2 and 3.

(a) Experiment 2

Pb analysis		Cd analysis	
Slope	0.00053	Slope	0.00846
Correlation Coefficient	0.99925	Correlation Coefficient	0.99224

(b) Experiment 3

Pb analysis		Cd analysis	
Slope	0.00056	Slope	0.00848
Correlation Coefficient	0.99874	Correlation Coefficient	0.99007

Analytical methods determined for the analysis of Pb and Cd concentrations in water samples from Experiments 1 through 3

Water samples

At the beginning of each experiment 4 mL of water was sampled from each container and preserved in 40 µL of concentrated nitric acid. For Experiment 1, water samples were taken daily as well as the temperature and pH. For Experiments 2 and 3, water samples were taken at the beginning (day 0) and end (day 6) of the experiment. The temperature and pH was recorded at the beginning and end of the experiment. Water samples were refrigerated until analysis.

Pb and Cd concentrations in the water samples were analyzed using GFAAS. The method used to analyze Pb was 15 µL sample, 10 µL $NH_4H_2PO_4$, and 5 µL $Mg(NO_3)_2$. The method used for Cd analysis was 10 µL of sample and 10 µL $Mg(NO_3)_2$. The samples were analyzed for Pb under the calibration curve of the standards 10 nM and 500 nM Pb. Cd, in comparison was analyzed under the calibration curve of the standards 2 nM and 50 nM Cd. All samples, except for the controls, were diluted in a 0.4 M HNO_3 matrix.

Appendix H: Chlorophyll *a* analysis from Experiments 2 and 3 with calculations on grazing rates.

Purpose: Determine grazing rates from Experiments 2 and 3.

Method: Chlorophyll *a* readings were taken using a fluorometer (TD-700: Turner design). Before the plaques were used for an experiment, four plaques were sampled to determine the initial biomass. On days 2, 4 and 6 of the experiment, the plaques were removed from the test chambers and chlorophyll-*a* measurements were taken. Two plaques were removed from the test chambers that contained no *H. azteca*, to determine the growth of *N. pelliculosa* on the plaque over the two-day grazing period. Contaminated plaques were placed in the test chambers on days 0, 2 and 4. On day 6, *H. azteca* in all containers were given noncontaminated plaques.

Before chlorophyll *a* readings were taken, each plaque was rinsed in 20 mL of FRAQUIL media and then stored in 10 mL of 90% acetone for 24 hours in the refrigerator (Welschmeyer 1994).

Results:

Table H1: Chl-*a* measurements from Experiment 2.

Set 1
day 0 to day 2

day 0		day 2 - plaques after grazing			day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-*a* (µg/L)	Container 2 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	578.60	1	16.31	3.96	1	748.0
2	547.80	2	18.84	6.44	2	624.70
3	1051.40	3	21.85	2.71	3	797.0
4	659.0	4	16.52	2.37	4	712.90
Average	**709.20**	**Average**	**18.38**	**3.87**	**Average**	**720.65**
Std. Dev.	**232.90**	**Std. Dev.**	**2.60**	**1.8**	**Std. Dev.**	**72.70**

Set 2
day 2 to day 4

day 0		day 2 - plaques after grazing			day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-*a* (µg/L)	Container 2 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	768.40	1	186.15	32.32	1	820.20
2	764.0	2	109.24	77.81	2	610.90
3	664.0	3	42.58	8.46	3	718.30
4	728.0	4	88.86	32.37	4	708.50
Average	**731.10**	**Average**	**106.71**	**37.74**	**Average**	**714.48**
Std. Dev.	**48.30**	**Std. Dev.**	**59.90**	**29.0**	**Std. Dev.**	**85.50**

Set 3
day 4 to day 6

day 0		day 2 - plaques after grazing			day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-*a* (µg/L)	Container 2 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	278.40	1	449.20	9.68	1	445.80
2	330.90	2	289.20	150.55	2	564.30
3	301.30	3	268.50	17.20	3	439.50
4	434.40	4	208.40	155.76	4	420.40
Average	**336.25**	**Average**	**303.83**	**83.30**	**Average**	**467.5**
Std. Dev.	**68.90**	**Std. Dev.**	**102.80**	**80.8**	**Std. Dev.**	**65.40**

Table H2: Chl-*a* measurements from Experiment 3.

Set 1
day 0 to day 2

day 0		day 2 - plaques after grazing				day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-*a* (µg/L)	Container 2 Chl-*a* (µg/L)	Container 3 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	315.40	1	7.80	7.07	71.34	1	398.80
2	324.40	2	9.71	9.97	4.97	2	382.0
3	284.90	3	80.00	4.93	102.76	3	397.90
4	341.70	4	9.13	22.45	50.34	4	364.90
Average	**316.60**	**Average**	**26.66**	**11.11**	**57.35**	**Average**	**385.90**
Std. Dev.	**23.80**	**Std. Dev.**	**35.60**	**7.80**	**41.03**	**Std. Dev.**	**16.0**

Set 2
day 2 to day 4

day 0		day 2 - plaques after grazing				day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-a (µg/L)	Container 2 Chl-*a* (µg/L)	Container 3 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	419.70	1	12.98	13.56	9.80	1	679.90
2	461.90	2	35.44	23.69	56.84	2	590.40
3	452.30	3	105.28	25.69	32.80	3	577.0
4	529.30	4	33.69	38.70	7.56	4	778.30
Average	**465.80**	**Average**	**46.85**	**25.41**	**26.75**	**Average**	**656.40**
Std. Dev.	**46.0**	**Std. Dev.**	**40.30**	**10.30**	**23.10**	**Std. Dev.**	**93.20**

Set 3
day 4 to day 6

day 0		day 2 - plaques after grazing				day 2 - no grazing	
Plaque	Chl-*a* (µg/L)	Plaque	Container 1 Chl-*a* (µg/L)	Container 2 Chl-*a* (µg/L)	Container 3 Chl-*a* (µg/L)	Plaque	Chl-*a* (µg/L)
1	617.50	1	110.70	30.56	71.37	1	561.80
2	651.60	2	77.49	17.77	24.80	2	676.50
3	432.40	3	153.60	23.45	49.37	3	864.70
4	612.0	4	128.49	31.80	80.76	4	738.40
Average	**578.40**	**Average**	**117.60**	**25.90**	**56.60**	**Average**	**710.40**
Std. Dev.	**98.90**	**Std. Dev.**	**32.0**	**6.50**	**24.90**	**Std. Dev.**	**126.30**

Table H3: Calculation of grazing rates from the food and water treatment in Experiment 2.

Set 1 – day 0 to day 2

µg/chl-*a*		µg	Container 1 (µg)	Container 2 (µg)
Before feeding	A	7.09		
After 48 hours of feeding	B		0.18	0.039
Control (No feeding for 48 hrs)	C	7.21		

Growth in 2 days (g)		
Growth (C-A) µg chl –*a*	D	0.12
Therefore growth per day (D/2 days) µg/d	E	0.06

Net Grazing (For Container 1)			**Net Grazing (For container 2)**		
Net Grazing (r_{net}) per day for container 1 (B - A/2) µg/d	F	-3.45	Net Grazing (r_{net}) per day for container 2 (B - A/2) µg/d	G	-3.53
Grazing Rate (r_g)			**Grazing Rate (r_g)**		
grazing rate = r_{net} – g			grazing rate = r_{net} - g		
(F–E) µg/d		**-3.51**	(G-E) µg/day		**-3.58**

Set 2- day 2 to day 4

µg/chl-*a*		µg	Container 1 (µg)	Container 2 (µg)
Before feeding	A	7.31		
After 48 hours of grazing	B		1.07	0.38
Control (No grazing)	C	7.49		

Growth in 2 days (g)		
Growth (C-A) µg chl -*a*	D	0.18
Therefore growth per day (D/2 days) µg/d	E	0.09

Net Grazing (For Container 1)			**Net Grazing (For Container 2)**		
Net Grazing (r_{net}) per day for Container 1 (B–A/2) µg/day	F	-3.12	Net Grazing (r_{net}) per day for Container 2 (B-A/2) µg/day	G	-3.47
Grazing Rate (r_g)			**Grazing Rate (r_g)**		
			grazing rate = r_{net} - g		
(F–E) µg/day		**-3.21**	(G-E) µg/day		**-3.56**

Set 3 – day 4 to day 6

µg/chl-*a*		µg	Container 1 (µg)	Container 2 (µg)
Before feeding	A	3.36		
48 hours after grazing	B		3.04	0.83
Control (No grazing)	C	4.68		

Growth in 2 days (g)		
Growth (C-A) µg chl -*a*		1.31
Therefore growth per day (D/2 days) µg/d	D E	0.66

Net Grazing (For Container 1)			**Net Grazing (For Container 2)**		
Net Grazing (r_{net}) per day for container 1 (B–A/2) µg/d	F	-0.16	Net Grazing (r_{net}) per day for container 2 (B-A/ 2) µg/d	G	-1.26
Grazing Rate (r_g)			**Grazing Rate (r_g)**		
Grazing rate = r_{net} – g (F-E) µg/d		**-0.82**	grazing rate = r_{net} - g (G-E) µg/day		**-1.92**

Appendix I: Bioconcentration Factors for Experiment 1, 2, and 3

Purpose: To determine the ratio of the concentration of Pb and Cd in *H. azteca* to that in its environment.

Method: The following equation was used to calculate the bioconcentration factor of Pb and Cd in *H. azteca*.

$$BCF = [metal]_{organism} / [metal]_{water}$$

where: $[metal]_{organism}$ moles of metal per kg of *H. azteca* (mol/kg)
$[metal]_{metal}$ [Pb'] or [Cd'] equals the sum of the inorganic species in the medium (mol/L)

Results:

Experiment 1.

Table I1. Summary of the values used to calculate the BCF for Pb in all treatments.

Experiment 1.
Treatment 3 – Pb 136 nM, Cd 0.39 nM

[Pb'] (mol/L)
1.36e-7

Treatment 3 Pb (136 nM), Cd (0.39 nM)	**# of *H. azteca* per tube**	**Mean [Pb]**	**Weight of *H. azteca* (kg)**	**Volume of extract (L)**	**Mol of Pb**	**[Pb] mol/ *H. azteca* (kg)**	**BCF for Pb**	**Log_{10} BCF**
3a	2	5719.8	1.31E-06	0.001	5.72E-09	4.37E-03	3.21E+04	4.51
3b	1	2548.2	1.02E-06	0.001	2.55E-09	2.50E-03	1.82E+04	4.26
3c	2	4446.2	6.00E-07	0.0005	2.22E-09	3.71E-03	2.71E+04	4.43
3e	1	2777.2	3.70E-07	0.0005	1.39E-09	3.75E-03	2.74E+04	4.44
3f	1	2657.7	6.10E-07	0.0005	1.33E-09	2.18E-03	1.59E+04	4.20
3g	1	2180.3	2.00E-07	0.0005	1.09E-09	5.45E-03	3.98E+04	4.60
3i	1	2322.1	6.10E-07	0.0005	1.16E-09	1.90E-03	1.39E+04	4.14
Total	**11**				**Average**	**3.41E-03**	**2.49E+04**	**4.37**
					Std. Dev	**1.29E-03**	**9.41E+03**	**0.17**

Treatment 4 – Pb 15 nM, Cd 0.37 nM

[Pb'] (mol/L)
1.5 e-08

Treatment 4 Pb (15 nM), Cd (0.32 nM)	**# of *H. azteca* per tube**	**Mean [Pb]**	**Weight of *H. azteca* (kg)**	**Volume of extract (L)**	**Mol of Pb**	**[Pb] mol/ *H. azteca* (kg)**	**BCF for Pb**	**Log_{10} BCF**
4a	2	1985.3	9.60E-07	0.0005	9.93E-10	1.03E-03	6.87E+04	4.84
4b	2	3972.8	6.50E-07	0.0005	1.99E-09	3.06E-03	2.04E+05	5.31
4c	1	6618.5	4.00E-07	0.0005	3.31E-09	8.27E-03	5.51E+05	5.74
4d	1	1053.2	1.21E-06	0.001	1.05E-09	8.70E-04	5.80E+04	4.76
4e	1	1452.2	2.40E-07	0.0005	7.26E-10	3.03E-03	2.02E+05	5.31
4f	1	1960.7	6.40E-07	0.0005	9.80E-10	1.53E-03	1.02E+05	5.01
4g	1	4401.2	5.10E-07	0.0005	2.20E-09	4.31E-03	2.87E+05	5.46
4h	1	2477	3.40E-07	0.0005	1.24E-09	3.64E-03	2.43E+05	5.39
4i	1	1091	5.50E-07	0.0005	5.46E-10	9.92E-04	6.61E+04	4.82
Total	**11**				**Average**	**2.97E-03**	**1.24E+07**	**5.18**
					Std. Dev	**2.36E-03**	**9.80E+06**	**0.32**

Treatment 5 – Pb 19.1 nM, Cd 12.4 nM

[Pb'] (mol/L)
1.91e-8

Treatment 5 Pb (19.1 nM), Cd (12.4 nM)	**# of *H. azteca* per tube**	**Mean [Pb]**	**Weight of *H. azteca* (kg)**	**Volume of extract (L)**	**Mol of Pb**	**[Pb] mol/ *H. azteca* (kg)**	**BCF for Pb**	**Log_{10} BCF**
5a	1	969.49	7.20E-07	0.0005	4.85E-10	6.73E-04	**3.52E+04**	**4.55**

Table I2. Summary of the values used to calculate the BCF for Cd in all treatments for Experiment 1.

Treatment 3 – Pb 136 nM, Cd 0.39 nM

[Cd'] (mol/L)
3.93e-10

Treatment 3 Pb (136 nM), Cd (0.39 nM)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
3a	2	36.48	1.31E-06	0.001	3.65E-11	2.79E-05	7.10E+04	4.85
3b	1	22.02	1.02E-06	0.001	2.20E-11	2.16E-05	5.50E+04	4.74
3c	2	26.56	6.00E-07	0.0005	1.33E-11	2.21E-05	5.62E+04	4.75
3e	1	20.46	3.70E-07	0.0005	1.02E-11	2.76E-05	7.02E+04	4.85
3f	1	21.49	6.10E-07	0.0005	1.07E-11	1.76E-05	4.48E+04	4.65
3g	1	7.21	2.00E-07	0.0005	3.61E-12	1.80E-05	4.58E+04	4.66
3i	1	14.87	6.10E-07	0.0005	7.43E-12	1.22E-05	3.10E+04	4.49
Total	**11**				**Average**	**2.10E-05**	**5.34E+04**	**4.71**
					Std. Dev	**5.64E-06**	**1.43E+04**	**0.13**

Treatment 4 – Pb 15 nM, Cd 0.37 nM

[Cd'] (mol/L)
3.71e-10

Treatment 4 Pb (15 nM), Cd (0.37nM)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
4a	2	98.67	9.60E-07	0.0005	4.93E-11	5.14E-05	1.39E+05	5.14
4b	2	207.93	6.50E-07	0.0005	1.04E-10	1.60E-04	4.32E+05	5.64
4c	1	262.43	4.00E-07	0.0005	1.31E-10	3.28E-04	8.85E+05	5.95
4d	1	85.05	1.21E-06	0.001	8.50E-11	7.03E-05	1.90E+05	5.28
4e	1	47.79	2.40E-07	0.0005	2.39E-11	9.96E-05	2.69E+05	5.43
4f	1	122.39	6.40E-07	0.0005	6.12E-11	9.56E-05	2.58E+05	5.41
4g	1	175.8	5.10E-07	0.0005	8.79E-11	1.72E-04	4.64E+05	5.67
4h	1	14.93	3.40E-07	0.0005	7.47E-12	2.20E-05	5.94E+04	4.77
4i	1	49.87	5.50E-07	0.0005	2.49E-11	4.53E-05	1.22E+05	5.09
Total	**11**				**Average**	**1.16E-04**	**1.16E+05**	**5.37**
					Std. Dev	**9.41E-05**	**9.40E+04**	**0.35**

Treatment 5 – Pb 19.1 nM, Cd 12.4 nM

[Cd'] (mol/L)
1.24E-08

Treatment 5 Pb (12.4 nM), Cd (12.4 nM)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
5a	1	800.65	7.20E-07	0.0005	4.00E-10	5.56E-04	**4.38E+04**	**4.64**

Experiment 2.

Table I3. Summary of the values used to calculate the BCF for Pb in Treatment 2 (water exposure) for Experiment 2 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Pb'] (mol/L)
1.5e-08

Treatment 2 Water Exposure (cultured)	**# of *H. azteca* per tube**	**Mean [Pb]**	**Weight of *H. azteca* (kg)**	**Volume of extract (L)**	**Mol of Pb**	**[Pb] mol/ *H. azteca* (kg)**	**BCF for Pb**	**Log_{10} BCF**
2a-b	2	3132.87	2.50E-06	0.001	3.13E-09	1.25E-03	8.35E+04	4.92
2a-c	2	1220.92	2.83E-06	0.001	1.22E-09	4.31E-04	2.88E+04	4.46
2a-d	2	951.24	1.76E-06	0.001	9.51E-10	5.40E-04	3.60E+04	4.56
2a-e	2	307.05	2.20E-06	0.001	3.07E-10	1.40E-04	9.30E+03	3.97
Total	**8**				**Average**	**5.91E-04**	**3.94E+04**	**4.48**
					Std. Dev	**4.7E-04**	**3.2E+04**	**0.39**

Treatment 2 Water Exposure (aquarium)	**# of *H. azteca* per tube**	**Mean [Pb]**	**Weight of *H. azteca* (kg)**	**Volume of extract (L)**	**Mol of Pb**	**[Pb] mol/ *H. azteca* (kg)**	**BCF for Pb**	**Log_{10} BCF**
2b-a	3	3431.07	2.9E-07	0.001	3.43E-09	1.18E-02	7.89E+05	5.90
2b-b	3	4390.02	1.21E-06	0.001	4.39E-09	3.63E-03	2.42E+05	5.38
2b-c	3	3268.77	4.88E-06	0.001	3.27E-09	6.70E-04	4.47E+04	4.65
2b-d	3	4071.57	5.04E-06	0.001	4.07E-09	8.08E-04	5.39E+04	4.73
2b-e	2	3531.97	3.32E-06	0.001	3.53E-09	1.06E-03	7.09E+04	4.85
2b-f	2	1902.47	4.00E-07	0.001	1.9E-09	4.76E-03	3.17E+05	5.50
Total	**16**				**Average**	**3.79E-03**	**2.53E+05**	**5.17**
					Std. Dev	**4.28E-03**	**2.85E+05**	**0.50**

Table I4. Summary of the values used to calculate the BCF for Pb in Treatment 3 (water and food exposure) for Experiment 2 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Pb'] (mol/L)
1.5 e-08

Treatment 3 Food and Water Exposure (cultured)	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
3a-b	2	2483.1	3.18E-06	0.001	2.48E-09	7.81E-04	5.21E+04	4.72
3a-d	2	1337.1	3.23E-06	0.001	1.34E-09	4.14E-04	2.76E+04	4.44
Total	**4**				**Average**	**5.97E-04**	**3.98E+04**	**4.58**
					Std. Dev	**2.59E-04**	**1.73E+04**	**0.20**

Treatment 3 Food and Water Exposure (aquarium)	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
3b-a	3	1773.7	3.04E-06	0.001	1.77E-09	5.83E-04	3.89E+04	4.59
3b-b	3	3147.5	1.24E-06	0.001	3.15E-09	2.54E-03	1.69E+05	5.23
3b-c	3	3033.9	4.33E-06	0.001	3.03E-09	7.01E-04	4.67E+04	4.67
3b-d	2	1948.8	3.71E-06	0.001	1.95E-09	5.25E-04	3.50E+04	4.54
Total	**11**				**Average**	**1.09E-03**	**7.25E+04**	**4.76**
					Std. Dev	**9.70E-04**	**6.47E+04**	**0.32**

Table I5. Summary of the values used to calculate the BCF for Cd in Treatment 2 (water exposure) for Experiment 2 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Cd'] (mol/L)
3.71 e-10

Treatment 2 Water Exposure (cultured)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
2a-b	2	147.47	2.50E-06	0.001	1.47E-10	5.90E-05	1.59E+05	5.20
2a-c	2	64.43	2.83E-06	0.001	6.44E-11	2.28E-05	6.14E+04	4.79
2a-d	2	78.68	1.76E-06	0.001	7.87E-11	4.47E-05	1.21E+05	5.08
2a-e	2	22.44	2.20E-06	0.001	2.24E-11	1.02E-05	2.75E+04	4.44
Total	**8**				**Average**	**3.42E-05**	**9.22E+04**	**4.88**
					Std. Dev	**2.18E-05**	**5.89E+04**	**0.34**

Treatment 2 Water Exposure (aquarium)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
2b-a	3	232.60	2.90E-07	0.001	2.33E-10	8.02E-04	2.16E+06	6.34
2b-b	3	264.59	1.21E-06	0.001	2.65E-10	2.19E-04	5.90E+05	5.77
2b-c	3	201.83	4.88E-06	0.001	2.02E-10	4.14E-05	1.12E+05	5.05
2b-d	3	250.19	5.04E-06	0.001	2.5E-10	4.96E-05	1.34E+05	5.13
2b-e	2	193.28	3.32E-06	0.001	1.93E-10	5.82E-05	1.57E+05	5.20
2b-f	2	134.61	4.00E-07	0.001	1.35E-10	3.37E-04	9.08E+05	5.96
Total	**16**				**Average**	**2.51E-04**	**6.77E+05**	**5.57**
					Std. Dev	**2.95E-04**	**7.95E+05**	**0.53**

Table I6. Summary of the values used to calculate the BCF for Cd in Treatment 3 (water and food exposure) for Experiment 2 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Cd'] (mol/L)
3.71e-10

Treatment 3 Food and Water Exposure (cultured)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
3a-b	2	276.6	3.18E-06	0.001	2.77E-10	8.70E-05	2.35E+05	5.37
3a-d	2	256.5	3.23E-06	0.001	2.57E-10	7.94E-05	2.14E+05	5.33
Total	**4**				**Average**	**8.32E-05**	**2.24E+05**	**5.35**
					Std. Dev	**5.35E-06**	**1.44E+04**	**0.03**

Treatment 3 Food and Water Exposure (aquarium)	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
3b-a	3	249.6	3.04E-06	0.001	2.5E-10	8.21E-05	2.22E+05	5.35
3b-b	3	324.2	1.24E-06	0.001	3.24E-10	2.61E-04	7.05E+05	5.85
3b-c	3	333.5	4.33E-06	0.001	3.33E-10	7.70E-05	2.08E+05	5.32
3b-d	2	272.4	3.71E-06	0.001	2.72E-10	7.34E-05	1.98E+05	5.30
Total	**11**				**Average**	**1.23E-04**	**3.33E+05**	**5.45**
					Std. Dev	**9.20E-05**	**2.48E+05**	**0.26**

Experiment 3.

Table I7. Summary of the values used to calculate the BCF for Pb in Treatment 2 (water exposure) for Experiment 3 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Pb'] (mol/L)
1.5e-08

Treatment 2 Replicate 1 Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
1b-a	2	727.07	1.17E-06	0.001	7.3E-10	6.21E-04	4.14E+04	4.62
1b-b	2	615.78	8.4E-07	0.001	6.2E-10	7.33E-04	4.89E+04	4.69
1b-c	2	731.40	1.24E-06	0.001	7.3E-10	5.90E-04	3.93E+04	4.59
1b-d	1	1170.07	6.9E-07	0.001	1.2E-09	1.70E-03	1.13E+05	5.05
1b-e	1	276.69	3.3E-07	0.001	2.8E-10	8.38E-04	5.59E+04	4.75
1b-f	1	409.04	4.6E-07	0.001	4.1E-10	8.89E-04	5.93E+04	4.77
1b-g	1	1112.57	5.6E-07	0.001	1.1E-09	1.99E-03	1.32E+05	5.12
Total	**10**				**Average**	**1.01E-03**	**7.00E+04**	**4.80**
					Std. Dev	**5.57E-04**	**3.71E+04**	**0.21**

Treatment 2 Replicate 2 Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
2b-a	1	681.49	1.24E-06	0.001	6.8E-10	5.50E-04	3.66E+04	4.56
2b-b	1	725.57	7.7E-07	0.001	7.3E-10	9.42E-04	6.28E+04	4.80
2b-c	1	797.91	9.2E-07	0.001	8.0E-10	8.67E-04	5.78E+04	4.76
2b-d	1	1117.07	1.14E-06	0.001	1.1E-09	9.80E-04	6.53E+04	4.82
2b-e	1	185.09	6.5E-07	0.001	1.9E-10	2.85E-04	1.90E+04	4.28
2b-f	1	324.88	5.8E-07	0.001	3.2E-10	5.60E-04	3.73E+04	4.57
Total	**6**				**Average**	**6.97E-04**	**4.65E+04**	**4.63**
					Std. Dev	**2.76E-04**	**1.84E+04**	**0.21**

Treatment 2 Replicate 3 Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
3b-a	2	815.48	1.27E-06	0.001	8.2E-10	6.42E-04	4.28E+04	4.63
3b-b	2	477.72	1.33E-06	0.001	4.8E-10	3.59E-04	2.39E+04	4.38
3b-d	1	346.01	4.4E-07	0.001	3.5E-10	7.86E-04	5.24E+04	4.72
3b-f	1	205.95	5E-07	0.001	2.1E-10	4.12E-04	2.75E+04	4.44
3b-g	1	266.68	3.3E-07	0.001	2.7E-10	8.08E-04	5.39E+04	4.73
Total	7				**Average**	**6.02E-04**	**4.01E+04**	**4.58**
					Std. Dev	**2.08E-04**	**1.39E+04**	**0.16**

Table I8. Summary of the values used to calculate the BCF for Pb in Treatment 3 (water and food exposure) for Experiment 3 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Pb'] (mol/L)
1.5e-08

Treatment 3 Replicate 1 Food and Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
1c-a	2	822.99	8.30E-07	0.001	8.23E-10	9.92E-04	6.61E+04	4.82
1c-b	2	1185.07	1.29E-06	0.001	1.19E-09	9.19E-04	6.12E+04	4.79
1c-c	1	566.92	4.90E-07	0.001	5.67E-10	1.16E-03	7.71E+04	4.89
1c-d	1	365.42	7.80E-07	0.001	3.65E-10	4.68E-04	3.12E+04	4.49
1c-e	1	537.71	7.50E-07	0.001	5.38E-10	7.17E-04	4.78E+04	4.68
1c-f	1	239.94	6.00E-07	0.001	2.40E-10	4.00E-04	2.67E+04	4.43
1c-g	1	134.18	5.60E-07	0.001	1.34E-10	2.40E-04	1.60E+04	4.20
Total	**9**				**Average**	**6.99E-04**	**4.66E+04**	**4.61**
					Std. Dev	**3.41E-04**	**2.27E+04**	**0.25**

Treatment 3 Replicate 2 Food and Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
2c-a	3	926.9	1.98E-06	0.001	9.27E-10	4.68E-04	3.12E+04	4.49
2c-b	3	1863.5	2.33E-06	0.001	1.86E-09	8.00E-04	5.33E+04	4.73
2c-c	2	1168.5	1.52E-06	0.001	1.17E-09	7.69E-04	5.12E+04	4.71
2c-d	2	610.2	1.60E-06	0.001	6.10E-10	3.81E-04	2.54E+04	4.41
2c-e	2	1198.5	1.44E-06	0.001	1.20E-09	8.32E-04	5.55E+04	4.74
2c-f	2	1039.1	1.37E-06	0.001	1.04E-09	7.58E-04	5.06E+04	4.70
2c-g	2	470.4	6.40E-07	0.001	4.70E-10	7.35E-04	4.90E+04	4.69
Total	**16**				**Average**	**6.78E-04**	**4.52E+04**	**4.64**
					Std. Dev	**1.77E-04**	**1.18E+04**	**0.13**

Treatment 3 Replicate 3 Food and Water Exposure	# of *H. azteca* per tube	Mean [Pb]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Pb	[Pb] mol/ *H. azteca* (kg)	BCF for Pb	Log_{10} BCF
3c-a	3	429.9	9.70E-07	0.001	4.30E-10	4.43E-04	2.95E+04	4.47
3c-b	3	1006.6	2.61E-06	0.001	1.01E-09	3.86E-04	2.57E+04	4.41
3c-c	2	1111.0	1.55E-06	0.001	1.11E-09	7.17E-04	4.78E+04	4.68
3c-d	2	439.6	1.42E-06	0.001	4.40E-10	3.10E-04	2.06E+04	4.31
3c-e	2	1421.9	1.83E-06	0.001	1.42E-09	7.77E-04	5.18E+04	4.71
3c-f	2	646.9	1.19E-06	0.001	6.47E-10	5.44E-04	3.62E+04	4.56
3c-g	2	387.4	7.20E-07	0.001	3.87E-10	5.38E-04	3.59E+04	4.55
Total	**16**				**Average**	**5.31E-04**	**3.54E+04**	**4.53**
					Std. Dev	**1.70E-04**	**1.13E+04**	**0.14**

Table I9. Summary of the values used to calculate the BCF for Cd in Treatment 2 (water exposure) for Experiment 3 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Cd'] (mol/L)
3.71e-10

Treatment 2 Replicate 1 Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
1b-a	2	60.89	1.17E-06	0.001	6.09E-11	5.20E-05	1.40E+05	5.15
1b-b	2	91.13	8.4E-07	0.001	9.11E-11	1.08E-04	2.93E+05	5.47
1b-c	2	65.70	1.24E-06	0.001	6.57E-11	5.30E-05	1.43E+05	5.16
1b-d	1	123.08	6.9E-07	0.001	1.23E-10	1.78E-04	4.81E+05	5.68
1b-e	1	25.17	3.3E-07	0.001	2.52E-11	7.63E-05	2.06E+05	5.31
1b-f	1	60.31	4.6E-07	0.001	6.03E-11	1.31E-04	3.54E+05	5.55
1b-g	1	81.86	5.6E-07	0.001	8.19E-11	1.46E-04	3.94E+05	5.60
Total	**10**				**Average**	**1.06E-04**	**2.87E+05**	**5.42**
					Std. Dev	**4.85E-05**	**1.31E+05**	**0.21**

Treatment 2 Replicate 2 Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
2b-a	3	98.62	1.24E-06	0.001	9.86E-11	7.95E-05	2.15E+05	5.33
2b-b	3	81.48	7.7E-07	0.001	8.15E-11	1.06E-04	2.86E+05	5.46
2b-c	3	108.03	9.2E-07	0.001	1.08E-10	1.17E-04	3.17E+05	5.50
2b-d	3	131.33	1.14E-06	0.001	1.31E-10	1.15E-04	3.11E+05	5.49
2b-e	2	61.60	6.5E-07	0.001	6.16E-11	9.48E-05	2.56E+05	5.41
2b-f	2	32.44	5.8E-07	0.001	3.24E-11	5.59E-05	1.51E+05	5.18
Total	**16**				**Average**	**9.48E-05**	**2.56E+05**	**5.39**
					Std. Dev	**2.36E-05**	**6.38E+04**	**0.12**

Treatment 2 Replicate 3 Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
3b-a	2	95.67	1.27E-06	0.001	9.57E-11	7.53E-05	2.03E+05	5.31
3b-b	2	68.15	1.33E-06	0.001	6.81E-11	5.12E-05	1.38E+05	5.14
3b-d	1	36.43	4.40E-07	0.001	3.64E-11	8.28E-05	2.23E+05	5.35
3b-f	1	27.27	5.00E-07	0.001	2.73E-11	5.45E-05	1.47E+05	5.17
3b-g	1	53.11	3.30E-07	0.001	5.31E-11	1.61E-04	4.34E+05	5.64
Total	**7**				**Average**	**8.50E-05**	**2.29E+05**	**5.32**
					Std. Dev	**4.45E-05**	**1.20E+05**	**0.20**

Table I10. Summary of the values used to calculate the BCF for Cd in Treatment 3 (water and food exposure) for Experiment 3 in medium consisting of the inorganic Pb and Cd concentrations of 15 nM Pb and 0.37 nM Cd.

[Cd'] (mol/L)
3.71e-10

Treatment 3 Replicate 1 Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
1c-a	2	133.31	8.3E-07	0.001	1.33E-10	1.61E-04	4.33E+05	5.64
1c-b	2	213.74	1.29E-06	0.001	2.14E-10	1.66E-04	4.47E+05	5.65
1c-c	1	111.32	4.90E-07	0.001	1.11E-10	2.27E-04	6.13E+05	5.79
1c-d	1	107.91	7.80E-07	0.001	1.08E-10	1.38E-04	3.73E+05	5.57
1c-e	1	202.57	7.50E-07	0.001	2.03E-10	2.70E-04	7.29E+05	5.86
1c-f	1	57.12	6.00E-07	0.001	5.71E-11	9.52E-05	2.57E+05	5.41
1c-g	1	48.72	5.60E-07	0.001	4.87E-11	8.70E-05	2.35E+05	5.37
Total	**9**				**Average**	**1.63E-04**	**4.41E+05**	**5.61**
					Std. Dev	**6.66E-05**	**1.80E+05**	**0.18**

Treatment 3 Replicate 2 Food and Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
2c-a	3	250.96	1.98E-06	0.001	2.51E-10	1.27E-04	3.42E+05	5.53
2c-b	3	384.40	2.33E-06	0.001	3.84E-10	1.65E-04	4.45E+05	5.65
2c-c	2	205.88	1.52E-06	0.001	2.06E-10	1.35E-04	3.65E+05	5.56
2c-d	2	135.91	1.6E-06	0.001	1.36E-10	8.49E-05	2.29E+05	5.36
2c-e	2	177.80	1.44E-06	0.001	1.78E-10	1.23E-04	3.33E+05	5.52
2c-f	2	199.78	1.37E-06	0.001	2.00E-10	1.46E-04	3.93E+05	5.59
2c-g	2	67.51	6.4E-07	0.001	6.75E-11	1.05E-04	2.85E+05	5.45
Total	**16**				**Average**	**1.27E-04**	**3.42E+05**	**5.53**
					Std. Dev	**2.62E-05**	**7.06E+04**	**0.09**

Treatment 3 Replicate 3 Food and Water Exposure	# of *H. azteca* per tube	Mean [Cd]	Weight of *H. azteca* (kg)	Volume of extract (L)	Mol of Cd	[Cd] mol/ *H. azteca* (kg)	BCF for Cd	Log_{10} BCF
3c-a	3	122.98	9.7E-07	0.001	1.23E-10	1.27E-04	3.42E+05	5.53
3c-b	3	272.79	2.61E-06	0.001	2.73E-10	1.05E-04	2.82E+05	5.45
3c-c	2	200.76	1.55E-06	0.001	2.01E-10	1.30E-04	3.49E+05	5.54
3c-d	2	111.96	1.42E-06	0.001	1.12E-10	7.88E-05	2.13E+05	5.33
3c-e	2	188.07	1.83E-06	0.001	1.88E-10	1.03E-04	2.77E+05	5.44
3c-f	2	121.37	1.19E-06	0.001	1.21E-10	1.02E-04	2.75E+05	5.44
Total	**14**				**Average**	**1.07E-04**	**2.90E+05**	**5.46**
					Std. Dev	**1.87E-05**	**5.03E+04**	**0.08**