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Potential impact of northern resource development on aquatic biota: toxicity of chromium and rare earth element processing reagent

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Potential impacts of northern resource development on aquatic biota: toxicity of hexavalent chromium and a rare earth element processing reagent

By

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Abstract

Chromite and rare earth element development was identified in the 2015 Canadian Federal budget as a significant opportunity, however, key data gaps exist regarding the environmental concerns related to these resource developments. Chromium is essential in the production of stainless steel, and no suitable substitute is known. Rare earth elements (REEs) are a series of metals that are composed of 15 lanthanides, as well as scandium and yttrium. Uses for REEs range from electronic devices (i.e. cell phones, computers, televisions) to magnets and controlling nuclear reactors. While commercial production of REEs signify a great economic opportunity for Canada, key data gaps regarding chemicals involved in processing REEs has been identified. The first objective of this study was to determine the acute toxicity of hexavalent chromium (Cr (VI)) to the invertebrate species *Hyalella azteca* and to identify the potential mitigating influences of cations and dissolved organic matter (DOM). The second objective was to evaluate the acute toxicity of flotation reagent AERO 6493, conduct toxicity identification/reduction studies, and test REE processing wastewater toxicity to *H. azteca* and *Daphnia magna*. Standard methods were followed for both 48 h (*D. magna*) and 96 h (*H.* azteca) acute toxicity tests, in media with pH 7.3 and water hardness of 120 mg $CaCO_3$ /L (*D. magna*) and 60 mg $CaCO_3$ /L (*H. azteca*) for both objectives. For objective 1, effect of altering water chemistry on Cr (VI) toxicity to *H. azteca* was tested with additions of Ca (0.5-3.5mM), Na (0.5-3 mM), Mg (0.13-0.64mM), as well as additions of natural sources of DOC (from Pickle Lake and Luther Marsh) at concentrations of 5 and 12 mg DOC/L. No protective effect was observed with additions of Na^+, Pickle Lake and 2016 Luther Marsh DOC sources, but a significant protective effect was observed for 2015 Luther Marsh DOC, elevated Mg^{2+} and Ca^{2+} concentrations. For objective 2, LC50 was calculated based on survival/mortality for *H. azteca*

(2.6 E^{-16} % dilution of parent AERO 6493 compound for fresh, 3.9 E^{-18} % for aged) and immobilization for *D. magna* (2.0E^{-5} %). Acute REE processing wastewater toxicity was also calculated for *H. azteca* (2.44%) and *D. magna* (23.35%). Studies conducted to determine acute toxicity of Cr (VI) to *H. azteca* not only lead to an improved understanding of site-specific Cr (VI) toxicity, but also may help to improve the water quality guidelines for protection of aquatic life. As for the toxicity of REE processing reagent AERO 6493, dilutions anticipating the worst possible scenario was tested to invertebrate species. Calculated LC50s and EC50s of parent AERO 6493 and wastewater will help develop a better understanding of toxicity of chemicals incorporated in REE processing, as well as potential suggestions for risk assessment and remediation steps.

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List of Figures and Tables

Figure 1.1: Map of northeastern Ontario, Canada 12

Figure 2.1: Schematic representation of the biotic ligand model 20

Figure 2.2: Average mortality (%) of *H. azteca* to 96 h Cr (VI)$_{-D}$ exposure 32

Figure 2.3: 0 h nominal vs measured Cr (VI) water samples 33

Figure 2.4: 96 h nominal vs measured Cr (VI) of water samples 34

Figure 2.5: LC50 values (with upper 95% CI) for *H. azteca* exposure to Cr (VI)$_{-D}$ at various Pickle Lake DOC concentrations 35

Figure 2.6: LC50 values (with upper 95% CI) for *H. azteca* exposure to Cr (VI)$_{-D}$ at various Luther Marsh DOC concentrations 36

Figure 2.7: LC50 values (with upper 95% CI) for *H. azteca* exposure to Cr (VI)$_{-D}$ at different Ca concentrations 37

Figure 2.8: LC50 values (with upper 95% CI) for *H. azteca* exposure to Cr (VI)$_{-D}$ at different Na concentrations 38

Figure 2.9: LC50 values (with upper 95% CI) for *H. azteca* exposure to Cr (VI)$_{-D}$ at different Mg concentrations 39

Figure 3.1: Average mortality (%) of *H. azteca* to diluted parent AERO 6493 (non-heated).....58

Figure 3.2: Average mortality (%) of *H. azteca* to diluted parent AERO 6493 (heated) responses based on age of working stock solutions 59

Figure 3.3: Average mortality (%) of *D. magna* to diluted parent AERO 6493 60

Figure 3.4: Average mortality (%) of *D. magna* to diluted AERO 6493 supernatant 61

Figure 3.5: Average mortality (%) of diluted flotation wastewater to *D. magna*.....62

Figure 3.6: Average mortality (%) of diluted flotation wastewater to *H. azteca* 63

Table 2.1: Measured Cr (VI)$_{-D}$ concentration expressed as % of Cr (VI)$_{-T}$ 29

Table 2.2: Major speciation in RAM at 139 µg Cr (VI)-$_{D}$/L 30

Table 3.1: Ecotoxicity of the different component examples of AERO 6493 reagent 57

Table of Contents

Abstract 1

Chapter 1: Introduction **9**

1.1 Global market and resource development in Canada 10

1.2 Test organisms 13

1.3 Objectives 14

Chapter 2: Acute Cr toxicity **15**

Abstract 16

2.1 Introduction 17

2.1.1 Economic significance of chromium development 17

2.1.2 Chromium and related environmental concerns 17

2.1.3 Toxicity modifying factors (TMFs) 19

2.2 Objectives 21

2.3 Materials and methods 21

2.3.1 Hyalella azteca culturing 21

2.3.2 Acute Cr toxicity tests 22

2.3.3 Water chemistry tests 23

2.3.4 Sample analysis and statistics 24

2.4 Results 25

2.4.1 Acute Cr toxicity to H. azteca and influence of TMF 25

2.5 Discussion 25

2.6 Conclusion 28

Chapter 3: REE processing toxicity **40**

Abstract 41

3.1 Introduction 44

3.1.1 REEs and flotation processing reagents 44

3.1.2 TIE/TREs 45

3.2 Objectives 46

3.3 Materials and methods 47

3.3.1 Hyalella azteca and Daphnia magna culturing 47

3.3.2 Acute toxicity of AERO 6493 to H. azteca 48

3.3.3 Acute toxicity of AERO 6493 to D. magna 49

3.3.3.1 AERO 6493 tests 49

3.3.3.2 Toxicity identification/reduction tests 50

3.3.3.3 Wastewater experiments 50

3.3.4 Calculations of toxicity endpoints 51

3.4 Results 51

3.5 Discussion 52

3.6 Conclusion 56

Chapter 4: How this research is integrative **64**

References **66**

Chapter 1: Introduction

1.1 Global markets and resource development in Canada

Canada is one of many countries to export its mineral resources and partake in the world market. The market for mineral commodities rebounded in late 2009 following the global economic slowdown of 2008, showing a growing demand for mineral supplies around the world (Rhéaume and Caron-Vuotari, 2013). The countries that are dominant in the mining industry on a global scale include Australia, which produces and exports minerals like bauxite, coal, cobalt, copper, and China, who not only possess mineral resources and produces them, but also invests heavily in foreign industries to support the growing industrial sector (Government of Canada 2014; Rhéaume and Caron-Vuotari, 2013). Other notable regions also include Brazil, Argentina, Chile and Mexico, which are leading producers of base and precious metals, along with industrial minerals (USGS, 2011). Mining also occurs in the United States in varying degrees, operating in all 50 states, with Nevada, Arizona, Utah, Minnesota and Alaska ranked as the country's top producers (USGS, 2011).

Canada's mining industry produces 60 minerals and metals that constitute key raw materials, from daily used objects to advanced technologies (Natural Resources Canada, 2018). The extractive industrial sector, which combines mineral extraction with gas and oil extraction, contributed $124.8 billion to Canada's gross domestic production (GDP) in 2016 (The Mining Association of Canada, 2017). Looking specifically at the mineral extraction industry, the value of metals and minerals to Canada's economy has ranged between 2.7% and 4.5% of the country's GDP (The Mining Association of Canada, 2017). Canada's northern regions have an abundance of natural resources, and so the potential economic impacts of developing these regions also provide a great economic opportunity. In the Economic Action Plan of 2015, the Responsible Resource Development planned to dedicate $23 million over five years, starting in

2015-16, from Natural Resources Canada, to accelerate the technological innovation needed to separate and develop rare earth elements and chromite (Government of Canada, 2015).

Developing the chromite deposits located in northern Ontario's Ring of Fire (Figure 1) has the potential to make Canada a crucial global producer, processor and supplier of products that contain the metal chromium (The Canadian Institute of Mining, Metallurgy and Petroleum, 2018). Chromium is essential in the production of stainless steel, as the addition of chromium leads to corrosion and oxidation resistance (Johnson *et al*, 2006). Although other elements, such as nickel, may be added, chromium is an essential ingredient and no suitable substitute is known (Johnson *et al*, 2006; The Canadian Institute of Mining, Metallurgy and Petroleum, 2018). This presents a good opportunity for Canada to develop the chromite deposits it has, but chromite has not yet been mined in Canada. A crucial step before the development of chromium would involve an investigation of the element and potential environmental concerns.

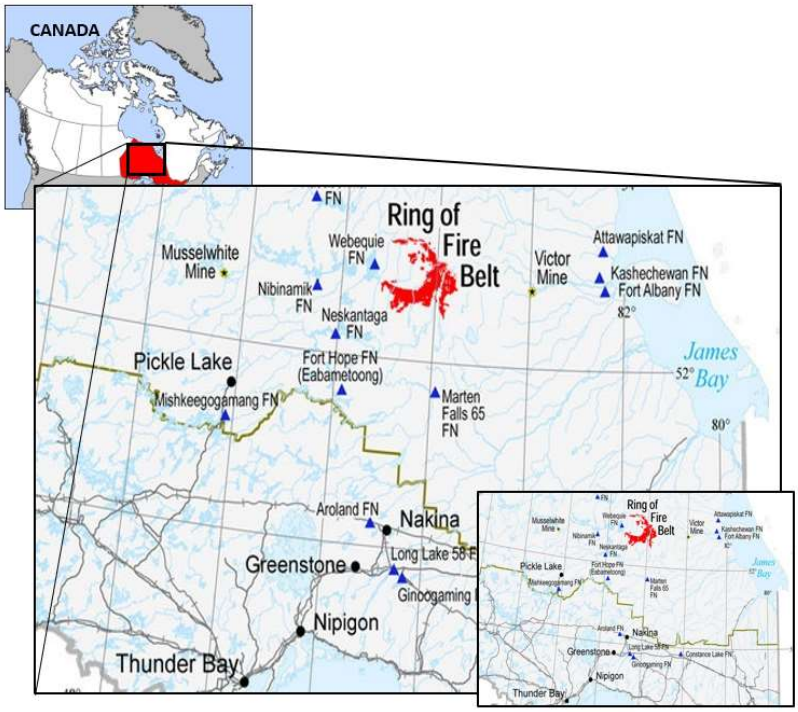

Figure 1.1. Map of northeastern Ontario, Canada. It shows Pickle Lake (NDOC source), Eabamet Lake, Eabametoong (source of *H. azteca)* around the Ring of Fire area.

Rare earth element (REE) mining and production also allows Canada with opportunities to enter the global market. According to the information presented at the Standing Committee on Natural Resources in June 2014, Canada has approximately 40-50% of the world's known REE resources, but no operating mines (Natural Resources Canada, 2016a). This makes Canada a strong candidate to become a substantial producer of REEs. However, the metallurgy for REE is a complex and resource intensive process, involving separation, refinement, alloying, and formation. As well, key data gaps exist with environmental issues associated with not just the REEs, but also the chemicals involved in the various stages of processing REEs.

1.2 Test organisms

Freshwater amphipod *Hyalella azteca* is a widely distributed species in North America (Borgmann, 1996; Environment Canada, 2013). This species resides in temperate lakes, ponds, slow flowing streams and in sediment. Freshwater amphipods including *H. azteca* are an important source of food for many species of fish, waterfowl, wading birds, salamanders and larger invertebrates (Environment Canada, 2013). *H. azteca* has been used extensively for both acute and chronic toxicity tests of contaminants associated with aquatic and terrestrial environments.

Commonly known as water fleas, *Daphnia magna* are freshwater microcrustaceans that belong in Order (or Suborder) Cladocera (Environment Canada, 1990). *Daphnia* populations can be found in a range of water bodies, from lakes to very small temporary pools, such as rock pools (Ebert, 2005). Daphnids are considered as an important link to many aquatic food chains, including for salmonids and other fish species in their juvenile stages (Environment Canada, 1990). Daphnids have been used extensively for evaluating acute lethality of test materials, as they have a relatively short life cycle, easy to culture in laboratory settings, and are sensitive to broad range of aquatic contaminants (Environment Canada, 1990).

1.3 Objectives

The goal of my Master of Science research is to develop a better understanding of the potential impacts of northern resource development on aquatic biota. This includes studying two issues related to exploitation of resources in the north, chromium toxicity and processing reagents for REE ores.

Objective I: Characterize the acute toxicity of chromium to the sensitive aquatic invertebrate *Hyalella azteca* and develop an understanding of the effect of water chemistry on toxicity.

Objective II: Understand the potential for the flotation processing reagent AERO 6493 promoter to cause impacts in aquatic environments. Acute toxicity of the reagent to *Hyalella azteca* and *Daphnia magna* was studied, as well as wastewater produced after its use in a bench-scale flotation test.

Chapter 2: Acute Cr Toxicity

Abstract

Ring of Fire is an area with large chrome deposit sites, as yet unexploited but anticipated for development in upcoming decades. The government of Ontario is interested in establishing a site-specific understanding of Cr toxicity to the aquatic biota for the anticipated development. The goals of this study were to evaluate the toxicity of Cr (VI) to the invertebrate *Hyalella azteca* and to characterize the potential toxicity mitigating influences of Ca, Na, Mg and dissolved organic matter (DOM). It was hypothesized that DOM complexation would reduce toxicity, but that cations (Ca, Mg, Na) would not compete for uptake (and toxicity) of Cr (VI). Standard methods were followed for 96 h acute toxicity tests, in reconstituted artificial medium (RAM) with pH 7.3 and water hardness of 60 mg $CaCO_3$. In RAM, the calculated LC50 was 139 µg Cr (VI) /L. Different sources of DOM (Pickle lake as northern source, Luther Marsh as southern source) were used to determine if toxicity responses will vary based location, as DOM composition differ based on the surrounding environment. Added concentrations up to 12 mg DOC/L from Pickle Lake and 12 mg DOC/L of DOM collected from Luther Marsh in 2016 did not have a significant effect in reducing Cr (VI) toxicity. 12 mg DOC/L of DOM collected from Luther Marsh in 2015 had a significant effect in reducing Cr (VI) toxicity. A significant effect in protection against Cr (VI) toxicity was observed at 1.5 and 3.5 mM of Ca^{2+} and Mg^{2+} at 0.38 and 0.64 mM. Increased Na concentrations up to 3 mM did not significantly alter LC50 values. This research determined the lethal median concentration of Cr (VI) which would cause lethality to *H. azteca*, as well as how altering water chemistry may influence toxicity responses. By better understanding the effect of toxicity modifying factors like water chemistry, it may help to improve water quality guidelines, as well as providing more information of Cr (VI) toxicity in the context of northern Canadian environments.

2.1 Introduction

2.1.1 Economic significance of chromium development

Various chromium compounds and chromium containing alloys are currently used in several industries. The largest application for chromium is in the production of stainless steel, as the inclusion of chromium provides corrosion resistance (Hjartarson *et al*, 2014). The development of the recently discovered chromite reserves in Ontario's Ring of Fire is highly likely in the foreseeable future, due to strategic importance of uninterrupted stainless-steel production in North America (Beukes *et al*, 2017). Chromite production could make Canada a significant global producer, processor and supplier, given the growing market for stainless steel (Hjartarson *et al*, 2014). It has been estimated that chromite reserves in the Ring of Fire could meet North American needs for several centuries (Chong, 2014). However, the Ring of Fire is in an area that forms the largest peatland, making it logistically and environmentally challenging for mining operations (Beukes *et al*, 2017). The development of chromate mining, along with infrastructure, in this environment will require meticulous planning to alleviate environmental impacts.

2.1.2 Chromium and related environmental concerns

Chromium is a member of group 6 on the periodic table, the transitional metals. It is a hard, white, lustrous and brittle metal with a high melting point of 2000 ºC in its elemental form (Costa and Klein, 2006). The element exists primarily in either a trivalent (Cr (III)) or a hexavalent (Cr (VI)) redox state. Chromium also occurs in 2^+ oxidation state, but it is too unstable to occur in the environment (Kotaś and Stasicka, 2000) and very little is known about

its hydrolysis (Mohan and Pittman Jr, 2006). Hydrolysis of Cr (III) produces the mononuclear species $CrOH^{2+}$, $Cr(OH)_2^{+}$ $Cr(OH)_4^{-}$, the neutral species $Cr(OH)_3^{0}$, and both polynuclear species $Cr_2(OH)_2$ and $Cr_3(OH)_4^{5+}$ (Mohan *et al*, 2005; Mohan *et al*, 2006). The hydrolysis of Cr (VI) only produces neutral and anionic species, predominately CrO_4^{2-}, $HCrO_4^{2-}$, $Cr_2O_7^{2-}$ (Mohan *et al*, 2005; Mohan *et al*, 2006). At low pH and high chromium concentrations, $Cr_2O_7^{2-}$ predominates, while at a pH higher than 6.5, Cr (VI) exists in the form of CrO_4^{2-} (Mohan and Pittman Jr, 2006).

Mobility and bioavailability of chromium is dependent on its chemical form. In addition to insulin, Cr (III) is responsible for reducing blood glucose levels and has also been found to reduce blood cholesterol levels by diminishing the concentrations of low density lipoproteins in the blood (Mohan and Pittman Jr, 2006). In contrast, Cr (VI) species are more toxic than other oxidation states due to their strong oxidant power, high water solubility and ability to permeate cell membranes (Arzate-Cárdenas and Martínez-Jerónimo, 2011). Rudolf and Cervinka (2006) explained that determining the toxicity mechanisms of Cr (VI) are complex due to its intracellular chemistry, which includes different biochemical pathways and multiple targets. As Cr (VI) enters the cell through membrane anion carriers, it is reduced, resulting in products like Cr (V) and Cr (III). In addition, the reduction of Cr (VI) increases the production of reactive oxygen species, which can promote oxidative stress (Arzate-Cárdenas and Martínez-Jerónimo, 2011).

While Cr (VI) has several applications in varying industries, its introduction into aquatic environments poses a serious threat to fish and invertebrates. Chromium toxicity to aquatic biota is significantly influenced by abiotic variables, such as hardness, pH, and temperature (Eisler, 1988). In a study conducted by Park *et al* (2009), the combined effects of pH, hardness and DOC on acute Cr (VI) toxicity to *Daphnia magna* was investigated. It was determined that Cr (VI)

toxicity decreased at both pH 6 and 8, compared to pH 7. However, toxicity was not influenced by DOC concentrations, contrary to other metals. Cr (VI) toxicity also decreased with increase in hardness. Although no specific toxicity mechanisms for Cr have been identified for invertebrates, the acute symptoms of Cr exposure to fish include non-specific gill irritation responses to alterations in hematology and tissue histology to the specific inhibition of enzymes (Reid, 2012).

2.1.3 Toxicity modifying factors (TMFs)

Developing a better understanding of site-specific Cr toxicity also involves incorporating how toxicity responses to metals may vary depending on the underlying chemistry of water. Metal toxicity is not simply related to total metal concentrations, but also the free metal ion interaction with competing cations at the site of toxic action (Di Toro *et al.*, 2001). This relationship is summarized in the biotic ligand model (BLM) in Figure 2.1. Within the model, free metal ions can compete with other cations for key binding sites (biotic ligand) when sharing the same charge. This may result in the accumulation of metals at transport sites, preventing uptake of essential ions, and eventually leading to mortality of the organism. Therefore, cations that influence hardness can be protective because they compete with free metal ions for key binding sites of the organism (Niyogi and Wood, 2004).

Another factor known to influence toxicity responses to metals is the metal-ligand complexation. Natural organic matter is a heterogenous mixture of organic compounds, formed from the degradation of lignin rich plant materials and dead biomass (Al-Reasi *et al*, 2013). In aquatic systems, it occurs as particulate organic matter, as well as dissolved organic matter (DOM; defined as filtered through a 0.45μm membrane). DOM can be described based on the

composition of components such as humic acid, fulvic acids, carbohydrates, and amino acids (Al-Reasi *et al*, 2013). Overall, the specific composition of components will vary based on the environment itself. When present, the negatively charged groups on DOM will complex with free ion forms of metal, thereby reducing metal bioavailability and toxicity (Niyogi and Wood, 2004; Gillis *et al.*, 2010). This relationship can be seen in Figure 2.1.

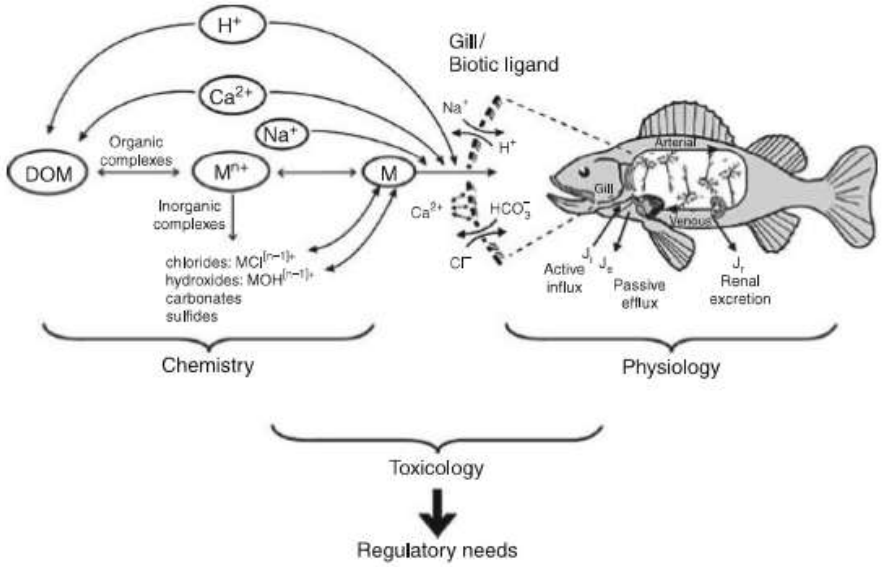

Figure 2.1. Diagram portraying the different interactions of free metal ion (M). The free metal ion is able to complex with organic and inorganic ligands, and compete with cations for key binding sites on the biotic ligand (from Paquin *et al*, 2002).

2.2 Objective

The objective of this study is to determine the acute toxicity of Cr (VI) to freshwater invertebrate species *H. azteca* and study how altering water chemistry may influence Cr toxicity by cation competition with Ca^{2+}, Na^{+}, Mg^{2+}, as well as organic complexation with DOC. It was hypothesized that cation additions will not reduce Cr (VI) toxicity to *H. azteca*, but presence of DOC may reduce Cr (VI) toxicity.

2.3 Materials and methods

2.3.1 Hyalella azteca culturing

Hyalella azteca were collected from Eabamet Lake, ON by Dr. W. Keller of the Ontario Ministry Environment in 2015 and were cultured in the lab for 2 years. Culturing followed the Environment Canada standardized Biological Test Method EPS 1/RM/33 Second Edition (Environment Canada, 2013). For culture and toxicity testing, the reconstituted aquatic medium (RAM) described by Vukov *et al.* (2016), which is a 50% dilution of that developed by Borgmann (1996) was used. The reconstituted medium was prepared with analytical grade $CaCl_2$, $NaHCO_3$, NaBr, KCl and $MgSO_4$ (Sigma-Aldrich, Mississauga, ON) at respective concentrations of 500, 500, 5, 25, 125 µM, to obtain a hardness of 60 mg of $CaCO_3$/L with pH of 7.3 ± 0.3. *H. azteca* cultures were maintained at the Centre for Cold Regions and Water Science research facility and held at 23°C ± 2 in a biochamber (LTCB-19 BioChamber, BioChambers Inc., Winnipeg, MB) with lighting at 600 to 800 lux and a 16:8 hour light:dark photoperiod. *H. azteca* were fed every two days with 5 mg of ground TetraMin® (Tetra Holding US, Inc, Made in Germany). Neonates between 0 and 7 days old were separated from cultures during weekly

water changes. A new fresh piece of cotton gauze was added to the beakers during weekly water changes.

2.3.2 Acute Cr toxicity tests

Chromium toxicity tests were carried out following the 96 h standard methods by Environment Canada (2013) with mortality as endpoint. The toxicity tests were conducted using neonates 2-9 days in age and incorporated up to 7 exposure concentrations with an unexposed control. Exposure solutions were created by dilution of a chromium stock solution (1000 mg/L, Sigma-Aldrich, Mississauga, ON) with RAM to achieve desired concentrations and pH adjusted to 7.3 ± 0.3 (measured using Radiometer E16M323 with pHC2701 electrode (ATI Scientific, Mississauga, ON)). Tests were conducted in duplicate or triplicate depending on neonate availability, with 10 neonates in 500 mL polyethylene beakers with 300 mL of test solution. The test solutions were equilibrated 24 h prior to test start. Each beaker had 10 cm by 5 cm piece of cotton gauze. At the beginning and end of the test, 15 ml water samples were taken, where one was filtered with a 0.45 µm membrane (polyethersulphone membrane, Fisher Scientific, Mississauga, ON) and the other was not. Both these water samples were used to determine the total (Cr (VI)-$_T$) and dissolved (Cr (VI)$_{-D}$) concentrations. Solution pH was also measured at the beginning and end of testing. Temperature and photoperiod during toxicity tests remained consistent to the culture conditions. At 96 h, the number of surviving and dead neonates were recorded. All water samples were acidified to 2% v/v with Trace Metals Grade HNO_3 (Fisher Scientific, Ottawa, ON). Total Cr (VI) concentrations were determined using unfiltered water samples (Cr (VI)$_{-T}$), while water samples filtered through 0.45 µm membrane were used to determine the dissolved Cr (VI) concentrations (Cr (VI)$_{-D}$). To calculate the change in Cr (VI)

exposure concentrations, Cr (VI)$_{-D}$ was divided by Cr (VI)$_{-T}$. This was calculated for measurements at 0 h and 96 h (Table 2.1). For calculating LC50 values, Cr (VI)$_{-D}$ measurements at 96 h were used.

2.3.3 Water chemistry tests

Initial toxicity tests were conducted in unmodified culture medium. Following this, the influence of DOM, Ca^{2+}, Mg^{2+}, and Na^{+} on toxicity responses were tested by systematically adjusting the concentration of one parameter, while keeping others constant. To determine the effect of DOM on acute chromium toxicity, DOM was added to test solutions at either 5 or 12 mg DOC/L. Testing was done with a northern source of DOM (Pickle Lake, ON, 51.4678° N, 90.1938° W) and a southern source (Luther Marsh, ON, 43.9618° N, 80.3996° W). DOM sources were concentrated using a portable reverse osmosis, followed by a resination step to remove any excess metals or cations prior to preservation at pH < 3. Rationale for using different DOM sources was to determine if toxicity response will vary based on location, as DOM composition differ based on the surrounding environment. Pickle Lake was chosen as it marks the northernmost point that is accessible to the Ring of Fire region via the Ontario provincial highway system. Luther Marsh was darker in colour, suggesting high aromatic and phenolic content, while Pickle Lake DOM was lighter in colour (low aromatic and phenolic content).

Pickle Lake DOM concentrate (184 mg DOC/L) was diluted to a concentration of either 5 or 12 mg DOC/L. Luther Marsh DOM source (concentrate of 220 mg DOC/L) was diluted to make 12 mg DOC/L. Water samples were collected as previously described with additional sampling (50 ml) at 0 h and 96 h, and these water samples were not acidified. To test the

influence of Ca on Cr (VI) toxicity, $CaCl_2$ was added to RAM when preparing test solutions. Tests were conducted at Ca^{2+} concentrations of 1.5 and 3.5 mM with a sufficient volume for all concentrations and replicates within tests to ensure that Ca concentrations were consistent within each test. Similarly, the influence of added Mg^{2+} (0.38 and 0.64 mM) was tested via additions of $MgSO_4$, while that of Na^+ (1.5 and 3 mM) by the additions of NaCl. Visual MINTEQ was used to determine the possible speciation and complexes at various water chemistry.

2.3.4 Sample analysis and statistics

Ca^{2+}, Mg^{2+}, Na^+, and Cr (VI) concentrations were determined using the inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8000, Perkin-Elmer Inc.) for both total Cr (VI) and Cr (VI) water samples filtered through 0.45 µm membrane at beginning and end of testing to measure the dissolved fraction. DOC concentrations were quantified by combustion catalytic oxidation with infrared CO_2 analysis using a total organic carbon analyzer was used (TOC-LCPH, Shimadzu Corporation). The median concentration that causes 50% mortality (LC50) over 96 h with 95% confidence intervals (CI) were determined by probit analysis using IBM® SPSS software (Armonk, NY). The effect of increased Ca^{2+} (or Mg^{2+}, Na^+ and DOC) on Cr toxicity were initially determined to be significant when the 95% CI did not overlap with the CI for tests with RAM only (i.e. no added Ca^{2+}). When CI overlap occurred, the Litchfield-Wilcoxon (1949) method was applied, as described in Environment Canada (2005).

2.4 Results

2.4.1 Acute Cr toxicity to H. azteca and influence of TMF

Table 2.1 summarizes the difference in Cr (VI)Using Visual MINTEQ, a chemical equilibrium model, the species and complexes present at the LC50 concentration (138 µg Cr $(VI)_{-D}$ /L) has been summarized in Table 2.2. Figure 2.3 and 2.4 also show a comparison of Cr $(VI)_{-D}$ and Cr $(VI)_{-T}$ at 0 h and 96 h. *H. azteca* mortality increased with increased concentrations of Cr (VI)-$_D$ at 96 h, shown in typical exposure response (Figure 2.2). Added concentrations up to 12 mg DOC/L from Pickle Lake did not have a significant effect in reducing Cr (VI) toxicity (Figure 2.5). 12 mg DOC/L of DOM collected from Luther Marsh in 2015 had a significant effect in reducing Cr (VI) toxicity, while 2016 did not significantly reduce toxicity (Figure 2.6). The influence of cationic competition on Cr (VI) toxicity was also studied. Cr (VI) toxicity was significantly reduced with elevated Ca^{2+} up to 3.5 mM (Figure 2.7). Additions of Na up to 3 mM did not significantly alter LC50 values (Figure 2.8). Additions of Mg significantly reduced Cr (VI) toxicity at both 0.38 and 0.64 mM compared to 0.16 mM (Figure 2.9).

2.5 Discussion

In this study, the acute toxicity of Cr (VI) to sensitive freshwater invertebrate *H. azteca* was determined and the potential effect of altered water chemistry on the toxicity responses were also explored. According to the BLM, acute toxicity of metals is described as accumulation at key binding sites of the biotic ligand (i.e. gills). Toxicity modifying factors, such as additions of cations (Ca^{2+}, Mg^{2+}, Na^{+}), can bind with the biotic ligand, thereby inhibiting free metal ion complexation (Paquin *et al*, 2002; Reid, 2012). Environmental ligands, such as DOM, are also

known to reduce metal toxicity. Study conducted by Ryan *et al* (2009) found DOC concentration, pH and water hardness significantly influenced copper toxicity to *D. magna*, which is consistent with results found in previous research done by Sciera *et al* (2004) and Erickson *et al* (1996) using fathead minnows, and De Schamphelaere *et al* (2002) using *D. magna*. Previous studies on Ag, Cd, Co, Cu, Pb, and Zn has shown that acute metal toxicity can be caused by inhibiting major cation transporters. For example, Cu^{2+} and Ag^{+} can inhibit Na transporters and prevent uptake of Na^{+} and Cl^{-} across fish gills, while Cd^{2+}, Co^{2+}, Pb^{2+}, and Zn^{2+} is able to affect Ca transporters and inhibit Ca^{2+} uptake (Niyogi and Wood, 2004).

Cr (VI) toxicity was only influenced by DOC concentrations up to 12 mg/L from Luther Marsh collected in 2015. Two different sources were compared, including Luther Marsh, which is an aromatic group enriched source that has been shown to offer strong protection against metal toxicity. These results are generally similar to the study conducted by Stackhouse and Benson (1988), where the influence of humic acid (dissolved organic material present in soils) on Cr (VI) toxicity to *Daphnia pulex* was examined. While there were significant decreases in Cr (VI) toxicity observed between control to 50 mg humic acid /L at 24 and 48 h exposures, by 72 h of exposure, no significant differences in Cr (VI) LC50 values were observed (Stackhouse and Benson, 1988). Reported 96 h LC50 with 5 mg humic acid/L was almost identical to the 96 h LC50 with 50 mg humic acid/L. Another study done by Park *et al*. (2009) with *Daphnia magna* observed similar trends, where Cr (VI) toxicity was also not influenced by DOC concentrations. The possible explanation to why DOC did not interact Cr (VI) may be due to that the hydrolysis of Cr (VI) produces only neutral and anionic species, predominately $Cr(OH)_3^0$, CrO_4^{2-}, $HCrO_4^{2-}$, and $Cr_2O_7^{2-}$ (Mohan and Pittman Jr., 2006). This suggests that additions of DOC concentrations may not significantly interact with the species produced.

As little information exists in literature on the toxicity modifying factors of Cr (VI), the effect of increased Ca, Na, and Mg concentrations on Cr (VI) toxicity were studied. The increase in Na concentrations did not reduce Cr (VI) toxicity to *H. azteca*, while the addition of Ca and Mg showed an increase of LC50 values. The effect of elevated Ca^{2+} up to 3.5 mM on reducing Cr (VI) toxicity may be due to improving the overall wellness of the organism, rather than mitigating Cr (VI) toxicity (Cairns and Yan, 2009). For additions of Na, it could be suggested that it may not compete with Cr (VI) for key binding sites, as the products of Cr (VI) hydrolysis are neutral and anionic species (Mohan and Pittman Jr., 2006). As CrO_4^{2-} is the dominant Cr (VI) species at which the tests were conducted, it could be assumed that CrO_4^{2-} is what may be responsible for causing toxicity. The Mg concentrations added in RAM and additional amounts for increased Mg concentration tests were in salt form, $MgSO_4$. CrO_4^{2-}, along with other metal oxyanions like selenate and molybdate, are structurally similar to sulfate, and therefore may compete with sulfate for transport, as well as intracellular binding sites although this has yet to be established (Bridges and Zalups, 2010; Reid, 2012). The increased concentrations of Mg (in the form of $MgSO_4$), also meant the increased concentration of sulfate, which may be the factor reducing Cr (VI) toxicity. To investigate this proposed theory, Visual MINTEQ, a chemical equilibrium model that can be used to calculate metal speciation and complexes in natural water parameter of choice, was used to determine how Cr (VI) speciation may change depending on adjusting concentration of one parameter (i.e. Ca^{2+}, in the form of $CaCl_2$), while keeping others constant. Calculated outputs from the software for elevated concentrations of Ca^{2+}, Mg^{2+}, and Na^{+} showed a change in composition of CrO_4^{2-}, suggesting that it may be a combination of CrO_4^{2-} and other speciation and/or complexes that is responsible for causing toxicity.

2.6 Conclusions

In this study, acute toxicity of Cr (VI) to *H. azteca* was determined and found Cr (VI) to be similar to what was reported in Borgmann *et al*. (2005). Results indicated Na^{+}, Pickle Lake and 2016 Luther Marsh DOC did not have a protective effect on Cr (VI) toxicity. However, additions of Ca^{2+} and Mg^{2+} had a significant effect on reducing Cr (VI) toxicity to *H. azteca*. These results are somewhat consistent with the results from Park *et al* (2009), where they observed that DOC did not have a protective effect and noted that hardness (in the form of $CaCO_3$) was the most important parameter in determining Cr (VI) toxicity to *D. magna*. While this study provided more information on Cr (VI) toxicity, more research is required to fully understand the influence of water chemistry on Cr (VI) toxicity.

Table 2.1: Cr (VI) nominal and measured dissolved ($Cr(VI)_{-D}$) concentrations expressed as a % of measured total ($Cr(VI)_{-T}$). Cr (VI) solutions were made in RAM.

Nominal (µg/L)	$Cr(VI)_{-D}$ / $Cr(VI)_{-T}$ (%)	
	0 h	96 h
0	86.4	94.3
50	96.9	99.1
100	99.0	99.4
200	99.1	99.5
300	98.6	98
500	99.7	98

Table 2.2: Major species in RAM at 139 µg Cr (VI)-$_D$/L. These species were determined by using Visual MINTEQ.

Species	Concentration
Br^{-1}	0.000005
Ca^{+2}	0.0012969
$CaCl^{+}$	2.4587E-06
$CaCO_3$ (aq)	0.00017791
$CaCrO_4$ (aq)	8.0887E-07
$CaHCO_3^{+}$	0.000001814
$CaOH^{+}$	2.7988E-06
$CaSO_4$ (aq)	0.000017291
Cl^{-1}	0.001022
CO_3^{-2}	0.00015577
$Cr_2O_7^{-2}$	2.898E-18
CrO_3Cl^{-}	8.218E-23
$CrO_3SO_4^{-2}$	4.1392E-22
CrO_4^{-2}	1.8605E-06
H^{+1}	5.6902E-11
H_2CO_3* (aq)	1.8217E-08
H_2CrO_4 (aq)	6.5068E-21
HCO_3^{-}	0.00015537
$HCrO_4^{-}$	2.5578E-10
HSO_4^{-}	3.9952E-13

Species	Concentration (mol/L)
K^{+1}	0.000029964
KCl (aq)	1.3617E-08
$KCr_2O_7^-$	4.4224E-22
$KCrO_4^-$	1.5635E-10
KOH (aq)	6.7772E-09
KSO_4^-	1.5858E-08
Mg^{+2}	0.00011108
$Mg_2CO_3^{+2}$	4.261E-09
$MgCl^+$	3.3375E-07
MgCO3 (aq)	0.000007705
MgHCO3+	1.3062E-07
MgOH+	4.5589E-06
MgSO4 (aq)	1.1848E-06
Na+1	0.00050818
NaCl (aq)	2.3606E-07
NaCO3-	1.2441E-06
NaCrO4-	3.5467E-09
NaHCO3 (aq)	3.6856E-08
NaOH (aq)	8.1459E-08
NaSO4-	2.2284E-07
OH-	0.00014995
SO4-2	0.00010629

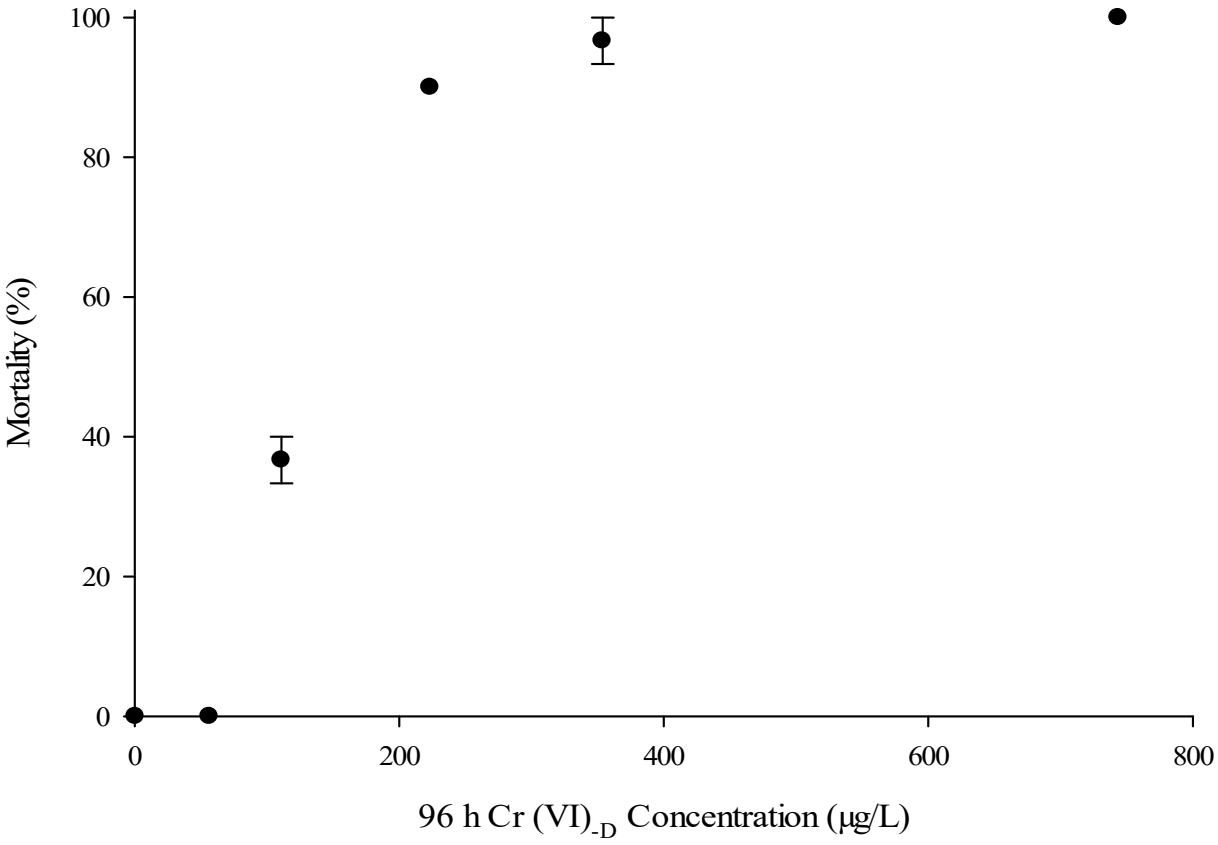

Figure 2.2. Exposure response curve showing average percent mortality of *H. azteca* in 96 h acute Cr toxicity test in RAM. The tests consisted of 10 organisms per concentration, done in triplicate. Calculated LC50 value was 138µg/L.

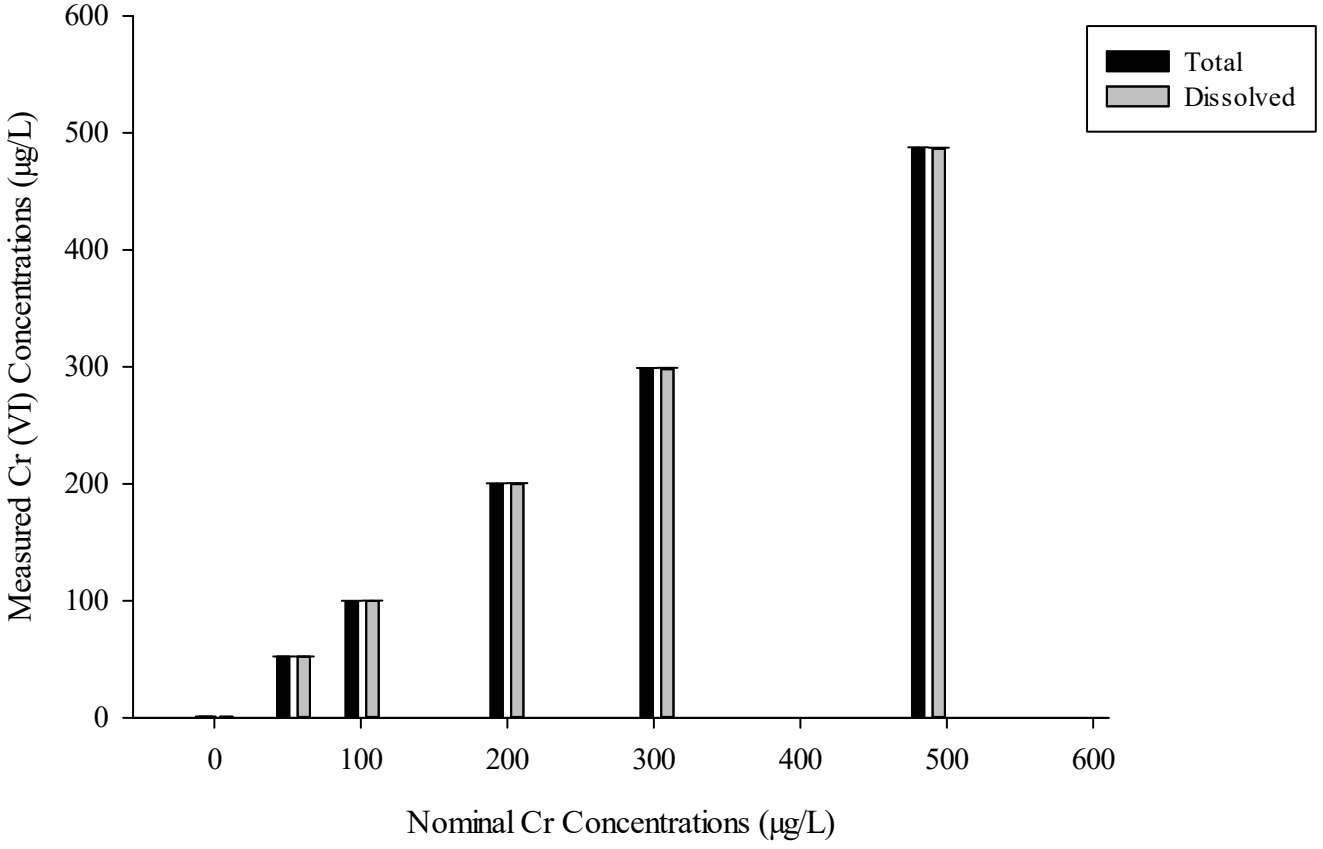

Figure 2.3. 0 h comparison of nominal Cr (VI) concentrations to measured water samples. Measured water samples included total Cr (VI) concentrations (total, $Cr(VI)_{-T}$) and water samples filtered through 0.45 µm membrane (dissolved, $Cr(VI)_{-D}$). Error bars indicate standard errors.

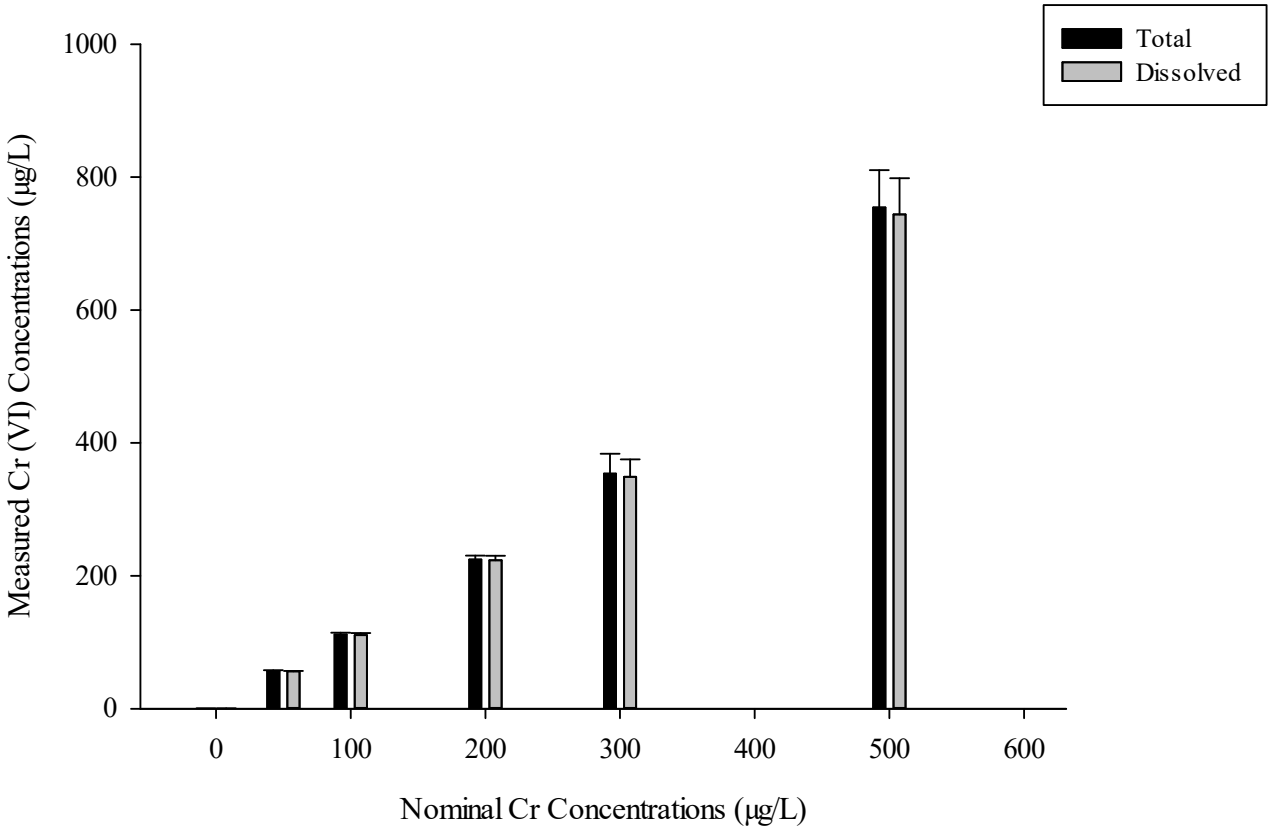

Figure 2.4. 96 h comparison of nominal Cr (VI) concentrations to measured water samples. Measured water samples included total Cr (VI) concentrations (total, Cr $(VI)_{-T}$) and water samples filtered through 0.45 µm membrane (dissolved, Cr $(VI)_{-D}$). Error bars indicate standard errors.

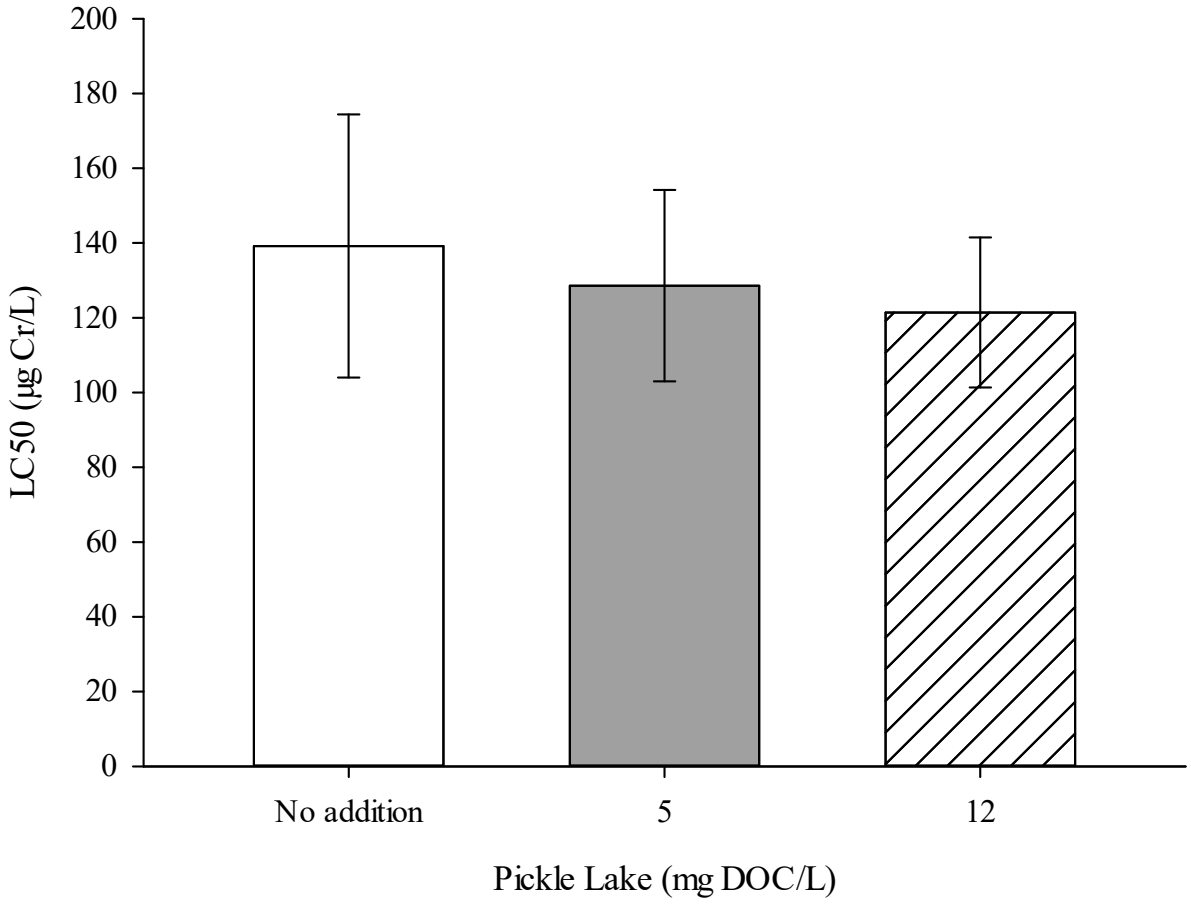

Figure 2.5. The measured 96 h LC50 values for $Cr(VI)_{-D}$ exposure to *H. azteca* with varying concentrations of Pickle Lake DOC. Error bars indicate 95% confidence intervals. There was no significant effect of DOC on Cr toxicity.

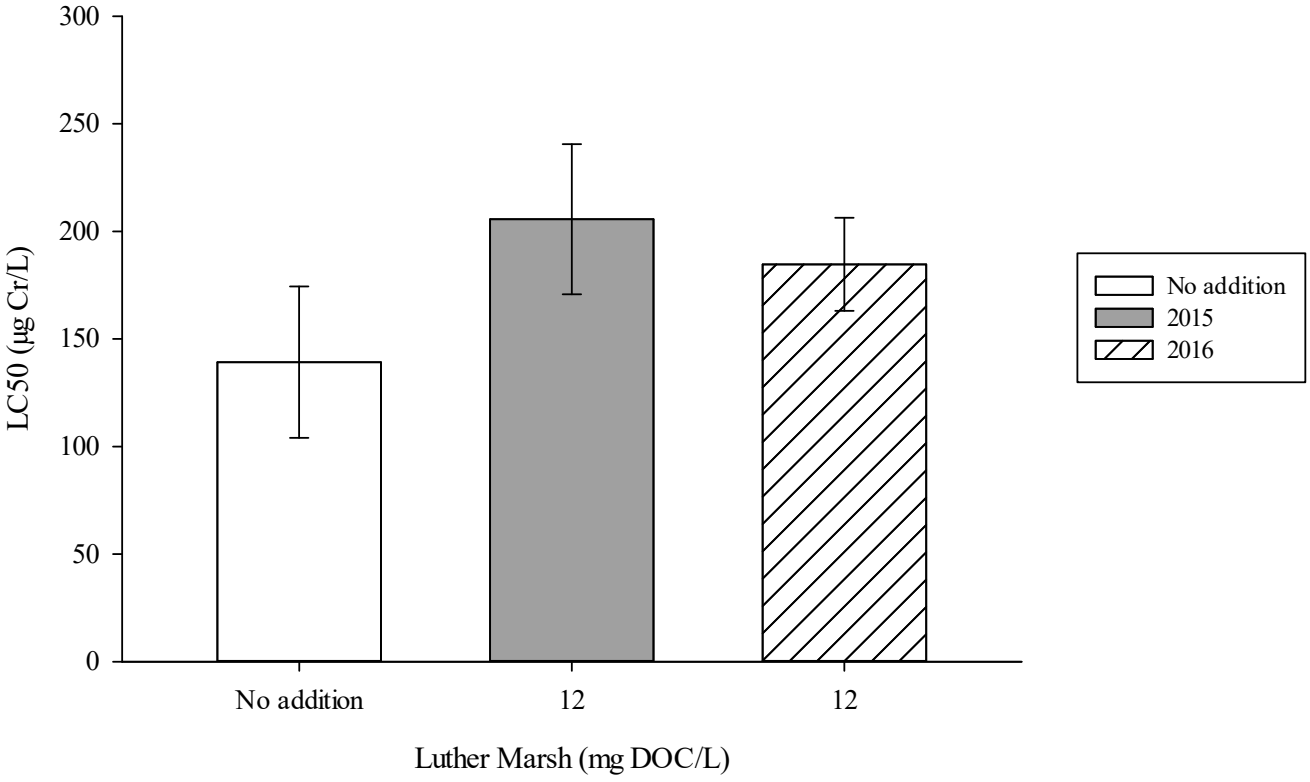

Figure 2.6. The measured 96 h LC50 values for Cr (VI)$_{-D}$ exposure to *H. azteca* at no addition and 12 mg DOC/L from Luther Marsh. While from the same location, samples were collected at different times, as indicated by the legend. Error bars indicate 95% confidence intervals. There was a significant effect of DOC when using 2015 Luther Marsh source on Cr (VI) toxicity.

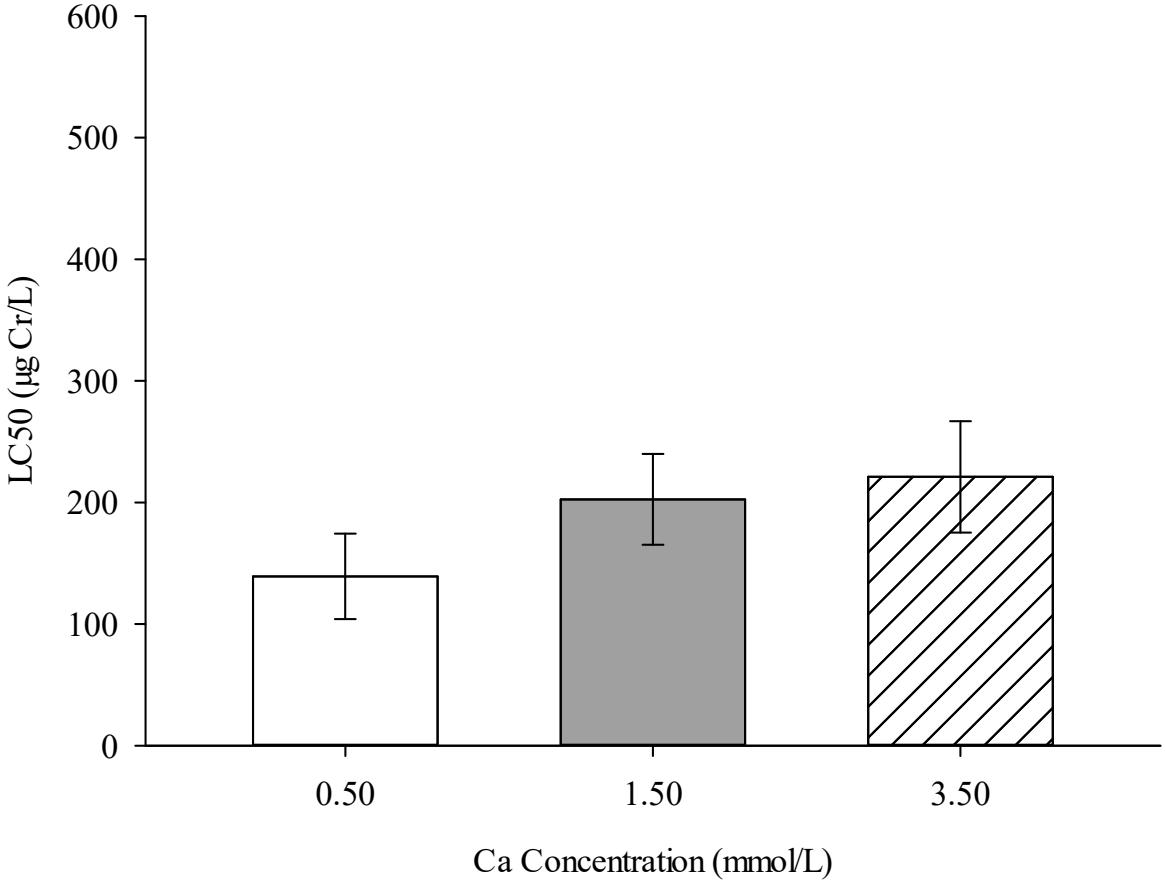

Figure 2.7. The 96 h LC50s of Cr (VI)$_{-D}$ exposure to *H. azteca* at different Ca concentrations. The first bar represents Ca concentration in culture medium. Results are shown for all measured Cr (VI) and Ca concentrations and error bars indicate 95% confidence intervals. Additions of Ca did have a significant effect in reducing Cr (VI) toxicity.

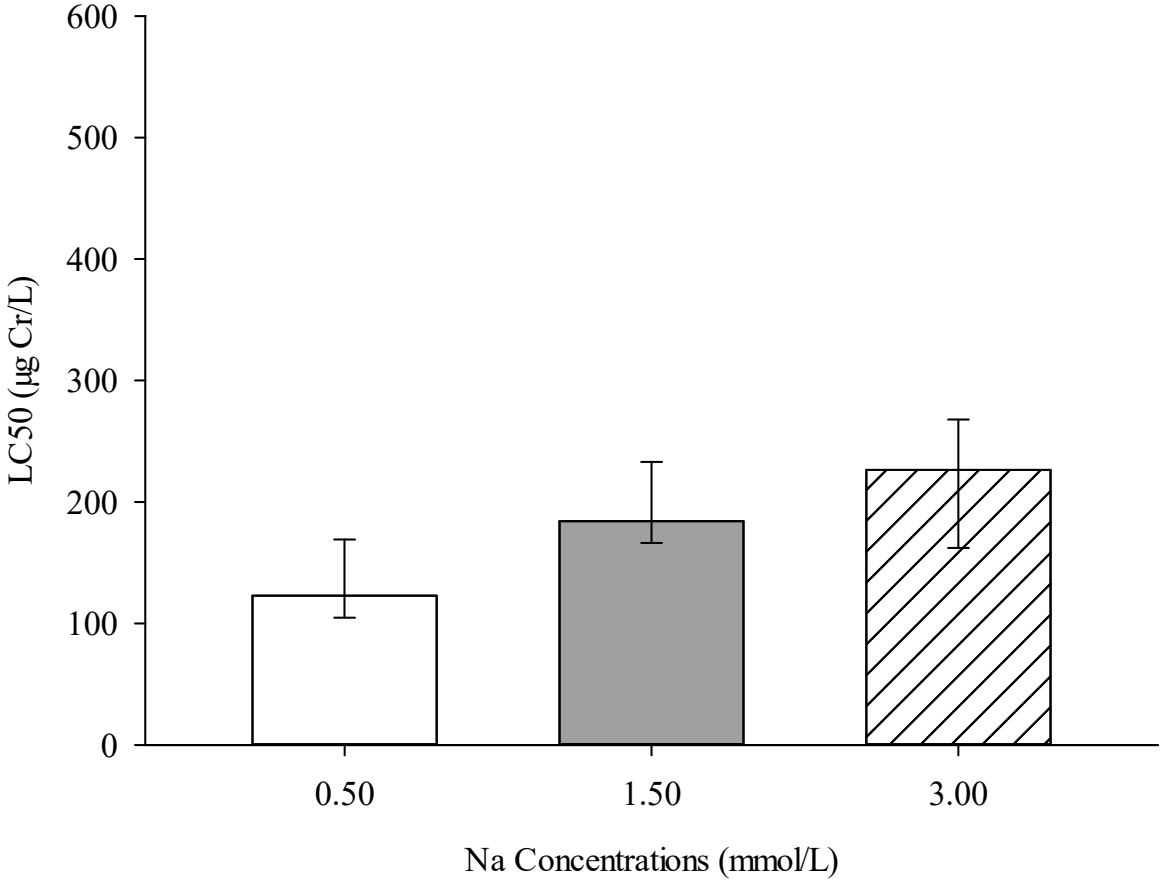

Figure 2.8. 96 h LC50 of Cr (VI)$_{-D}$ exposure to *H. azteca* at increasing concentrations of Na. Results are shown for all measured Cr (VI) and Na concentrations and error bars indicate 95% confidence intervals. Addition of Na did not have a significant effect in reducing Cr (VI) toxicity.

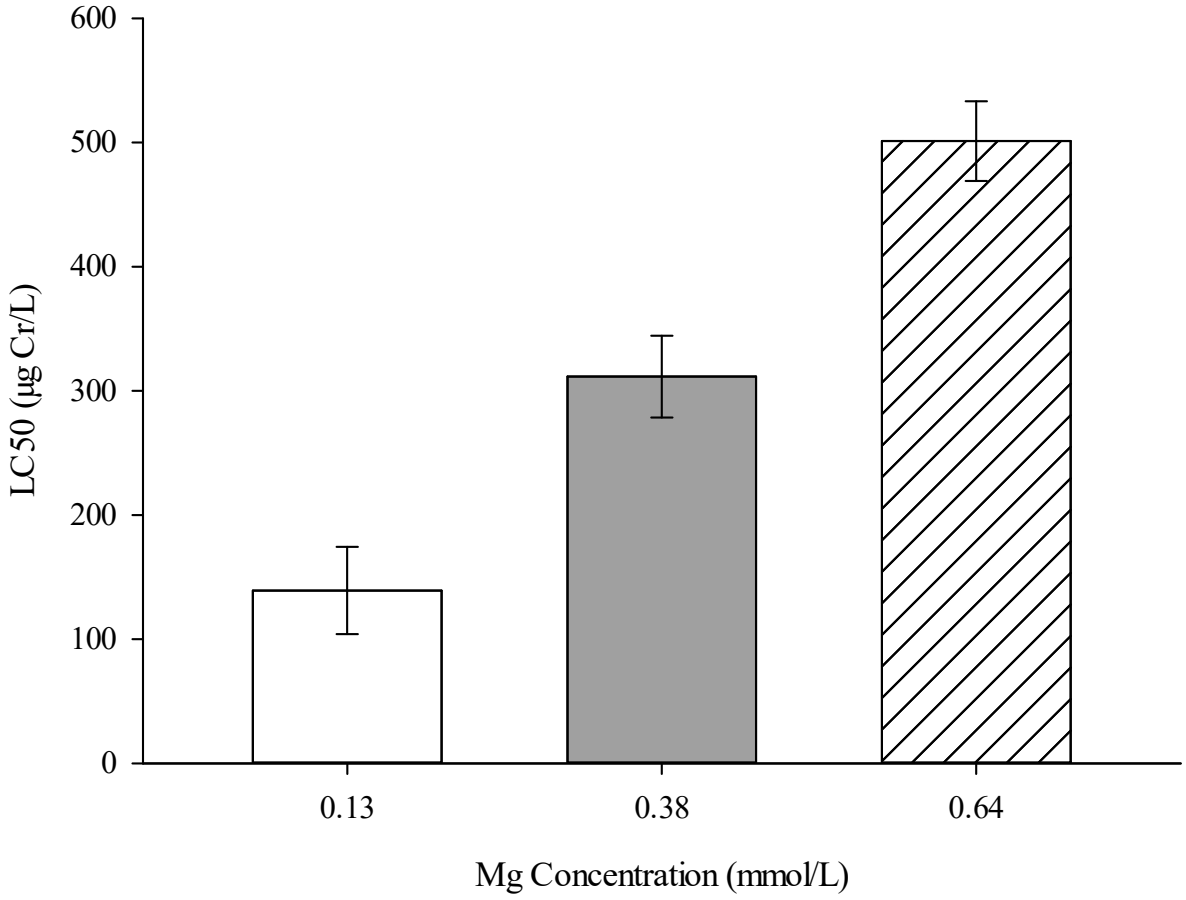

Figure 2.9. 96 h LC50 of Cr (VI)$_{-D}$ exposure to *H. azteca* at increasing concentrations of Mg. First bar of the graph represents Cr (VI) LC50 in culture medium. A significant effect was observed with additions of Mg.

Chapter 3: REE processing toxicity

Abstract

There is a growing environmental concern with the development of REE mining and processing. Although there are some recent studies on the toxicity of individual REEs, little is known about the environmental issues associated with REE processing. The goal of this study was to evaluate the toxicity of flotation reagents used in REE processing to the invertebrate species *Hyalella azteca* and *Daphnia magna*. Testing procedures followed the Environment Canada standard test method for *H. azteca* and *D. magna*. Serial dilutions of parent AERO 6493 solution were used to create test solution. For *H. azteca*, the calculated 96 h LC50 was a dilution of $2.6E^{-16}$ % of parent AERO 6493 stock solution with exposure solutions prepared from fresh working stock solutions (< 24 h old). When working stock solutions were aged for 14 d (or longer) before exposure solutions were created, the 96 h LC50 was $3.9E^{-18}$ % dilution of parent AERO 6493 solution. For *D. magna*, calculated 48 h EC50 for tests created using fresh working stock solutions was a dilution of $2.0E^{-6}$ % of parent AERO 6493. Attempts at toxicity identification evaluation (TIE) involved testing AERO 6493 supernatant toxicity to *D. magna*, which resulted in 100 % immobilization from dilutions of 10^{-14} to 1% of AERO 6493 supernatant. Wastewater received from pilot plant study testing the effectiveness of AERO 6493 as a flotation reagent for REE extractions was also tested. For *D. magna*, the 48 h EC50 was a dilution of 23.35 % of original wastewater, while for *H. azteca*, the 96 h LC50 was a dilution of 2.44 % of original wastewater. Lastly, testing with various dilutions of filtered through 0.45 µm membrane was also conducted to *D. magna*. When exposed to diluted wastewater that had been filtered, 100 % mobilization of *D. magna* was observed in all dilutions. Exposures were conducted to mimic the flotation apparatus, as well as suggested dilutions that are environmentally relevant if parent AERO 6493 and/or wastewater were released. This study will

overall contribute to developing a better understanding of the potential for flotation processing reagent AERO 6493 to cause toxicity to the aquatic biota.

3.1 Introduction

3.1.1 REEs and flotation processing reagents

Mine production of REE as rare earth oxides has been consistently increasing since 1960 (Natural Resources Canada, 2016c). From 1965 to 1985, United States was the dominant global rare earth producer. In the early 2000s, China became the largest producer of rare earths and controlled both their own rare earth resources and the supply chain (Natural Resources Canada, 2016c). The prices of REE increased up to ten-fold in 2010-2011, which encouraged the development of REE projects in Canada and other countries (Natural Resources Canada, 2016b). However, it was identified in numerous reports (Government of Canada, 2014; USGS, 2016; Natural Resources Canada, 2016b; Natural Resources Canada, 2016c) that China is dominating manufacturing, producing close to 85% of the world's REE. Even with competition, it was identified that Canada has an opportunity to turn its rare earth deposits into an economic advantage and secure its own supply of REEs for the future (Government of Canada, 2014).

The commercial production of REEs in Canada signifies a great economic opportunity. REE minerals are recovered from various types of mineral deposits, including bästnasite, monazite and xenotime by froth flotation (Natural Resources Canada, 2016b). The currently operating flotation circuits generally receive high grade ores, containing one or two of the listed minerals. Rare earth flotation can be a difficult process, as minerals containing REEs are extensively dispersed and inter-grown with several carbonate, phosphate and oxide minerals (Zinck, 2013). In comparison to the current REE projects in other countries where the flotation circuits use high, coarse grained ore, the Canadian REE projects intend to use Canadian ores that are typically finer grained and a lower grade (Natural Resources Canada, 2016b). The Canadian REE projects also aim to collect a wider variety of minerals, sometimes several from a single

deposit (Natural Resources Canada, 2016b). Natural Resources Canada was directed in the Federal Budget of 2015 to support the acceleration and development of a rare earth element mining industry in Canada (Natural Resources Canada, 2016a). In 2016, a literature review identified that although there are studies on the toxicity of individual REEs, there are a lack of studies focusing on the environmental issues associated with reagents and chemicals involved in REE processing.

Rare earth element minerals are recovered by froth flotation processes from various types of deposits, including monazite and xenotime (Natural Resources Canada, 2016b). Flotation is a physical process to separate particles of various minerals by utilizing the difference in their surface properties (Boulanger *et al*, 2016). The mineral, in its pulp phase, enters the flotation cells, where it is then mixed with flotation processing reagents with constant aeration (Boulanger *et al*, 2016). The product of the flotation process would be a froth rich layer containing the mineral of interest, as well as some undesirable minerals with similar chemical properties. Water is also included in the product, but can be removed from the froth and recycled back into the flotation circuit (Natural Resources Canada, 2016b).

There are three general types of flotation reagents: collectors, regulators, and frothers. Collectors are surfactants that reduce the stability of the hydrating layer of the mineral which allows the rare earth mineral to float to the surface to be collected in the froth (Natural Resources Canada, 2016a). In rare earth mineral flotation, the use of ionizing collectors is most common. Regulators help control the flotation process by modifying the action of the collector. Different types of regulators include activators, depressants, dispersants and pH modifiers, which work with the various physical and chemical properties of the mineral of interest. Lastly, frothers, like collectors, are also surfactants that aid flotation by the formation and preservation of small

bubbles, helping with the formation of froth. The most common chemical families of frothers include aliphatic alcohols and propylene glycols (Natural Resources Canada, 2016a).

The report published in 2016 by Natural Resources Canada highlight the process related chemicals, potential contaminants in waste streams, and potential by-products that are environmentally relevant chemicals of potential concern (COPCs). Out of these COPCs, the chosen flotation processing reagent of interest for this study was AERO 6493 promoter. It is alkyl hydroxamate based, composed of alkyl alcohol, monocarboxylic fatty acids, and quaternary ammonium compounds. The major component of the chemical is alkyl alcohol, which makes up 30-60% of the mixture. Currently, AERO 6493 promoter is used for the removal of coloured impurities from kaolin and for oxide copper recovery (Cytec Industries, 2002). Some examples of toxicity of AERO 6493 constituents to the aquatic biota include specific compounds, such as methanol for alkyl alcohol. In this sense, there are some acute toxicity data generated on the individual example components of AERO 6493, but there is no data available on the mixture's toxicity to freshwater aquatic organisms.

3.1.2 Toxicity reduction evaluations and toxicity identification evaluations

Standard toxicity test protocols provide guidance on a dose-response based framework used for measuring and assessing aquatic biological effects of toxic substances. Toxicity identification evaluations (TIEs) and toxicity reduction evaluations (TREs) rely on standard toxicity test protocols to improve understandings of cause and mechanisms of toxic action of complex substances, mixtures such as effluents. As part of TREs, TIE approach is divided into three phases. Phase I contains methods to characterize physical or chemical nature of the

constituents, such as solubility, volatility and filterability without specifically identifying the toxicity of components (U.S. EPA, 1991). The first phase results are intended to assist with identifying potentially toxic components in the substance, but the results generated can also be used to develop possible treatment methods to remove toxicity without specific identification of toxic components. Typical tests in Phase I include initial toxicity assessment, pH adjustment tests, filtration, and solid phase extraction tests. Phase II (U.S. EPA, 1993a) provides methods to identify the specific components if they are non-polar organics, metals, or ammonia. The first two phases are intended for acutely toxic effluents, but effluents causing chronic toxicity can also be evaluated using these methods. As well, the manipulations in Phase I characterizations and Phase II identifications might suggest to inaccurate conclusions about the cause of toxicity. Therefore, Phase III is highly recommended and used to confirm the suspected component (U.S. EPA, 1993b). Phase III identification is applicable even if the toxicant was not identified using Phases I and II.

3.2 Objectives

The objective of this study is to determine REE processing reagent AERO 6493 toxicity to freshwater invertebrates *H. azteca* and *D. magna*. As little information is known on REE processing toxicity, toxicity tests were conducted mimicking flotation conditions, as well as dilutions that were environmentally relevant. It is difficult to hypothesize the how toxic AERO 6493 would be to aquatic invertebrates, as it is a proprietary compound. Based on what is known about hydroxamic acid compounds, however, it was hypothesized that it would be very toxic to both *H. azteca* and *D. magna*.

3.3 Materials and methods

3.3.1 Hyalella azteca and Daphnia magna culturing

Hyalella azteca were collected from Eabamet Lake, ON by Dr. W. Keller of the Ontario Ministry of Environment in 2015 and were cultured in the lab for 2 years. Culturing followed the Environment Canada standardized Biological Test Method EPS 1/RM/33 Second Edition (Environment Canada, 2013). For culture and toxicity testing, the reconstituted aquatic medium (RAM) described by Vukov *et al.* (2016), which is a 50 % dilution of the medium based on Borgmann (1996) was used. The reconstituted medium was prepared with analytical grade $CaCl_2$, $NaHCO_3$, NaBr, KCl and $MgSO_4$ (Sigma-Aldrich, Mississauga, ON) at respective concentrations of 500, 500, 5, 25, 125 μM, to obtain a hardness of 60 mg of $CaCO_3$/L with pH of 7.3 ± 0.3 (measured using Radiometer E16M323 with pHC2701 electrode (ATI Scientific, Mississauga, ON)). *H. azteca* cultures were maintained at the Centre for Cold Regions and Water Science research facility and held at 23 °C ± 2 in a biochamber (LTCB-19 BioChamber, BioChambers Inc., Winnipeg, MB) with lighting at 600 to 800 lux and a 16:8 hour light:dark photoperiod. *H. azteca* were fed every two days with 5 mg of ground TetraMin® (Tetra Holding US, Inc, Made in Germany). Neonates between 0 and 7 days old were separated from cultures during weekly water changes. A new fresh piece of cotton gauze was added to the beakers during weekly water changes.

Daphnia magna were obtained from Aquatic Research Organisms Inc. and followed culturing methods from the environment protection series biological test method EPS 1/RM/11 (Environment Canada, 1990). For culture and toxicity testing, artificial medium (AM) described by Borgmann (1996) was used. The artificial medium had a hardness of 120 mg of $CaCO_3$/L with pH of 7.3 ± 0.3. *D. magna* cultures were maintained at the Centre for Cold Regions and

Water Science research facility and held at 23 °C ± 2 in a biochamber (LTCB-19 BioChamber, BioChambers Inc., Winnipeg, MB) with lighting at 400 to 800 lux and a 16:8 hour light:dark photoperiod. Upon arriving to the research facility, *D. magna* were acclimated to the AM for a week. Each culture was stored in a glass beaker with 1L of AM per 20 adult *D. magna* (Environment Canada, 1990). Culture medium was replaced with fresh medium three times a week, and neonates were kept each time. Cultures of three successive ages were kept on hand to ensure there would always be available adults known to be 2 to 5 weeks old to supply neonates for tests (Environment Canada, 1990). *D. magna* were fed a mixture of 4 mL of algae (30 % *Chlorella vulgaris* and 70 % *Pseudokirchneriella subcapitata*, Aquatic Research Inc., Hamptons, NH) and 1 mL of YCT (*Saccharomyces cerevisiae*, cereal leaf and troutchow, Aquatic Research Inc., Hamptons, NH) after every watcher change.

3.3.2 Acute toxicity of AERO 6493 to Hyalella azteca

Standard static 96 h water-only toxicity tests (Environment Canada, 2013) were conducted with neonates exposed to one of seven AERO 6493 dilutions, plus an unexposed control. Temperature and photoperiod during tests remained consistent to the culture conditions and mortality was the endpoint. The parent stock solution of AERO 6493 was provided by Natural Resources Canada. Initial testing involved diluting the parent stock solution at room temperature and allowing it to equilibrate for 24 h prior to starting the test. After these preliminary tests and upon the advice of Natural Resources Canada, all solutions involved in preparation or testing were warmed up to 35 °C in a water bath and thoroughly mixed using a magnetic stir plate. To simulate the preparation of AERO 6493 prior to the the flotation process (J. Chaulk, Natural Resources Canada, pers. Comm). The effect of solution aging on toxicity

responses was also studied by testing solutions prepared fresh (< 24 h) and made at least 14 d prior. All solutions were diluted with AM and adjusted to pH of 7.3± 0.1 and prepared 24 h prior to starting the exposures. The tests were done in duplicate or triplicate depending on the availability of neonates, with 10 neonates in 500 mL polyethylene beakers with 300 mL of test solution. Each beaker had a 10 cm by 5 cm piece of cotton gauze. Dead and surviving neonates were counted and recorded after 96 h of exposure.

3.3.3 Acute toxicity of AERO 6493 to Daphnia magna

3.3.3.1 AERO 6493 tests

Aquatic toxicity tests of 48 h duration without medium renewal following Environment Canada's standard methods (Environment Canada, 1990) were done with *Daphnia* neonates < 24 h old. Neonates were exposed to one of seven AERO 6493 dilutions, plus an unexposed control. Temperature and photoperiod during tests remained consistent to the culture conditions, with immobilization as the endpoint. For *Daphnia* tests, all solutions were prepared at 35 °C in a water bath and thoroughly mixed using a magnetic stirrer. These tests started with dilution ranges where an appropriate response curve was observed with *H. azteca*. Working stock solutions and exposure solutions were created following the same methodology as described above for *H. azteca*. Solutions were adjusted to a pH of 7.3± 0.1 and prepared 24 h prior to starting the exposures. The tests were conducted in duplicate or triplicate depending on the availability of neonates, with 10 neonates in 300 mL polyethylene beakers with 150 mL of test solution. Endpoint for these tests were immobilization.

3.3.3.2 Toxicity identification/reduction tests

Toxicity tests using the TIE/TRE approach started by mimicking the environmentally relevant dilution, approximately 1.5 mL of AERO 6493 in 1 L of water based on the worst-case scenario that 100% of AERO 6493 used in processing ends up in effluent (R. Cameron, Natural Resources Canada, pers comm.). Two different mixtures were created, where one beaker held 1.5 mL of AERO 6493 in 1 L AM, while the other held 1.5 mL of AERO 6493 in 1 L of Milli-q water. Mixtures were adjusted to a pH of 11 ± 0.1 with the addition of KOH (Sigma-Aldrich, Mississauga, ON) and left for 72 h to allow for precipitation to occur. The AERO 6493 and Milli-q mixture had to be centrifuged further for 2 h at 800 rpm to allow for separation (Sorvall ST8, Thermo Fisher Scientific). Supernatants of both mixtures were collected and diluted further with AM depending on the desired dilutions. Other portions of solution preparation were made as described in AERO 6493 toxicity testing for *D. magna*.

3.3.3.3 Wastewater experiments

Wastewater received from a pilot plant study testing the effectiveness of AERO 6493 as a flotation reagent for extraction of REEs, conducted by Tesfaye Ngeri at Natural Resources Canada, was also tested. The basic preliminary characterization of wastewater involved recording qualitative observations of the wastewater (colour, odour, turbidity, presence of solids), and measuring the general water chemistry (pH, conductivity, turbidity) prior to dilution. Initial preliminary testing included exposing undiluted wastewater to 10 daphnid neonates. After the preliminary tests, a dilution series from 0.03125 % to undiluted wastewater was used to

determine toxicity to *D. magna*. A test with filtered wastewater was also conducted to determine if it would alter responses.

3.3.4 Calculations of toxicity endpoints

The toxicity endpoints for *H. azteca* were mortality at 96 h and immobilization at 48 h for *D. magna*. As AERO 6493 is a proprietary compound, reported exposure dilutions are nominal. Toxicity values are expressed as proportions (as %) of the parent AERO 6493 material. For example, 10^{-2} % is 1 part parent material with 9,999 parts of AM culture solution. Based on these endpoints, the median concentration that causes 50 % mortality (LC50) and immobilization (EC50) with 95 % confidence interval (CI) were calculated by probit analysis using IBM® SPSS software (Armok, NY).

3.4 Results

Numerous tests were conducted to determine the appropriate exposure dilution range where the response did not result 100 % mortality in all exposure dilutions. Tests with dilutions of parent AERO 6493 without any manipulations were conducted with *H. azteca*, summarized in Figure 3.1. In these tests, solutions were prepared and tested at 21 ± 2 °C, as solutions involved in flotation would most likely to be collected in tailings for a long time (and not warmed up). Tests conducted with warming up all solutions to 35 °C to mimic flotation process are summarized in Figure 3.2. During these tests, variability in toxicity responses depended on the age of working stock solutions. When exposure solutions were prepared from fresh working stock solutions (< 24 h old), the LC50 for *H. azteca* was a dilution of $2.6E^{-16}$ % of parent AERO

6493 stock solution (Figure 3.2). When working stock solutions were aged for 14 d (or longer) before exposure solutions were created, the LC50 was $3.9E^{-18}$ % dilution of parent AERO 6493 solution (Figure 3.2). As for *D. magna*, the calculated EC50 for tests conducted with fresh working stock solutions (< 24 h old) warmed up to 35 °C was a dilution of $2.0E^{-6}$ % of parent AERO 6493 solution (Figure 3.3). As for the TIE/TRE exposures, diluted parent AERO 6493 supernatant to *D. magna* showed 100 % immobility from dilution ranges of 10^{-14} to 1 % (Figure 3.4). Dilutions of wastewater received from the pilot plant were also explored. For *D. magna*, the calculated EC50 was a dilution of 23.35 % of original wastewater (Figure 3.5), and dilution of 2.44 % of original wastewater for *H. azteca* (Figure 3.6). Lastly, a series of dilutions from 0.03125 to 75 % of original wastewater were filtered through a 0. 45µm membrane and exposed to *D. magna*. No immobility was observed in the filtered dilution range (Figure 3.6).

3.5 Discussion

Based on the safety data sheet provided by NRCan, the different groups of the flotation reagent AERO 6493 are alkyl alcohol (30-60 %), monocarboxylic fatty acids #2 (1-5 %), fatty acids, C6-12, Me esters (0-1 %), and quaternary ammonium compounds di-C8-10-akyldimethyl, chlorides (0-1 %). While some aquatic toxicity data is provided on the safety data sheet for the different components, there were no references included. The safety data sheet does not list all the chemicals due to confidential proprietary information. This makes it difficult to fully understand and identify what specific component is causing toxicity. As the exact chemical components of listed groups were not specified, an example of each component was chosen to identify the known toxicities of each examples. For all the different examples, the known ecotoxicity to aquatic biota has been summarized in Table 3.1. Out of these components of

AERO 6493, quaternary ammonium compounds are used in disinfectants, biocides and detergents (Krezinger *et al*, 2007; Zhang *et al*, 2015), with reported *D. magna* EC50 of 91 µg/L (Di Nica *et al*, 2017).

Toxicity tests involving the different age of working stock solutions to *H. azteca* was conducted to determine if there would be a difference in responses. Exposure solutions created using fresh working stock solutions (< 24 h) was more toxic than working stock aged for 14 d or longer. It could be speculated that as AERO 6493 is an organic compound, when diluted in RAM, it could have potentially degraded. The potential explanation for the variability of the toxicity responses to both aged and fresh stock solutions could be due to possible dilution errors carried over each serial dilution.

Exposures of diluted parent AERO 6493 to *D. magna* (48 h) and *H. azteca* (96 h) demonstrated greater sensitivity in the latter. However, exposures were not conducted under the same water chemistry. Tests conducted with *D. magna* had double the concentration of $CaCl_2$, $NaHCO_3$, NaBr, KCl and $MgSO_4$ compared to *H. azteca*. In particular, Ca is the principal cation of minerals that help form the structural components of carapaces (Cairns and Yan, 2009). As crustacean zooplankton are very sensitive to Ca levels due to their heavily calcified exoskeleton and repeated molting (Cairns and Yan, 2009), elevated Ca levels in *D. magna* cultures and test solution may have contributed to improving their overall wellness.

The attempted TIE (Phase I) approach involved pH adjustment of already diluted parent AERO 6493 to assist in the identification of the potentially toxic components in AERO 6493. Adjustment of pH was done as it is one modification included in TIE techniques for characterizing the cause of toxicity (U.S. EPA, 1991). Exposures conducted with AERO 6493 supernatant to *D. magna* resulted in 100 % mortality, even at dilution ranges where surviva; was

previously observed in tests with dilutions of parent AERO 6493. Dilutions of parent AERO 6493 were done in both Milli-q and AM, as increasing to pH 11 would promote the precipitation of salts in the AM mixture. Supernatant collected from AM mixture was tested because definite precipitation was observed (even though it may just be the salts from AM). It could be speculated that when diluted AERO 6493 was left for 72 h to allow for precipitation and centrifuged afterwards, some components of AERO 6493 may have precipitated out of solution. Based on the results, it could be suggested that the supernatant tested could include the portion of AERO 6493 that is responsible for causing toxicity, but narrowing down which component would require further testing.

Testing the pilot plant wastewater toxicity is relevant, as wastewater would contain possibly the most environmentally relevant dilution of parent AERO 6493. While it is important to know the toxicity of parent AERO 6493 to invertebrates, it would also be relevant to know what the toxicity responses would be when testing the mixture that would include parent AERO 6493 reagent after it has been applied to the flotation process. While it is difficult to know what or how much of the reagent ends up as a part of the wastewater, it is likely that very low amounts of AERO 6493 would be present in the wastewater based on the effectiveness of AERO 6493 as a flotation reagent for REE extraction. It is also important to note that parent AERO 6493 is already diluted before being added into the flotation process, so it is even more diluted within the wastewater itself.

When ending the exposures, the separation of wastewater and culture medium was observed. Similar to the toxicity of parent AERO 6493, *H. azteca* was much more sensitive to the wastewater than *D. magna*. To speculate as to why *H. azteca* was more sensitive to the wastewater, it is important to know that as benthic invertebrates, *H. azteca* like to stay at the

bottom of test beakers, which is also where the wastewater would settle when given some time to do so. As separation of the wastewater and culture medium was observed at the end of testing for both invertebrates, it could be likely that *H. azteca* may be exposed to the wastewater fraction more than *D. magna*. This idea is also supported by the results from exposure conducted with diluted wastewater filtered through a 0.45 µm membrane to *D. magna*, as 100% survival was observed. It could be suggested that the solid/total fraction of wastewater is more toxic than the dissolved fraction.

Although there has been some research conducted on the ecotoxicity of mining flotation reagents to aquatic biota (i.e. study on the toxicity of mining reagents to rainbow trout conducted by Ruber and Leduc, 1975), there is limited information available on the potential environmental issues associated to REE mining with a focus on the chemicals associated with REE processing. Even with known ecotoxicity of mining flotation reagents, it is difficult to compare them, as flotation toxicity is not well researched. The report published by Natural Resources Canada (2016a) includes a list of chemicals, compounds, and products and classifies each substance for its hazardous properties based on the globally harmonized system of classification and labeling of chemicals (GHS). Out of these chemicals and compounds, some of the COPCs identified as a high hazard to the aquatic biota include ammonia, sodium sulphide, copper, tallow amine, and hydroxamic acid (Natural Resources Canada, 2016a). AERO 6493 is a hydroxamic acid based compound and was chosen because it was classified as highly hazardous to the aquatic biota in the report. This was primarily due to the persistence of the substance, as it has low solubility in water. Conducting toxicity exposures at concentrations (or dilutions) that may be encountered in receiving waters near sites of mining operations is of importance as they provide more information on the potential toxicity responses if unscheduled releases occur. While there are

studies conduced on REE processing chemicals, the focus is on the efficiency of the reagents rather than the toxicity aspect.

3.6 Conclusion

In this study, acute toxicity of flotation reagent AERO 6493 to *H. azteca* and *D. magna* was determined. *H. azteca* was much more sensitive to parent AERO 6493 compared to *D. magna*, with calculated LC50 for *H. azteca* was a dilution of 10^{-16} to 10^{-18} % of parent AERO 6493 and dilution of 10^{-6} % of parent AERO 6493 solution for *D. magna*. As for TIE approach, dilution range from 10^{-14} to 1 % of pH adjusted AERO 6493 supernatant resulted in 100 % immobilization to *D. magna*, suggesting that the supernatant is more toxic compared to the untreated parent AERO 6493 to *D. magna*. Toxicity responses to pilot plant wastewater exposure showed similar response, where *H. azteca* was more sensitive than *D. magna*. Filtering the wastewater through a 0.45 µm membrane showed 100% survival for *D. magna*. Overall, it has been identified that AERO 6493 flotation reagent is highly toxic to invertebrate species *H. azteca* and *D. magna*.

Table 3.1. Reported endpoint concentrations organized for different examples of component that makes up AERO 6493. Information on the percent composition of AERO 6493 was on the safety data sheet, provided from NRCan.

% of AERO 6493	Substance type	Example component	CAS # of example	Organism	Endpoint Type	Endpoint concentration	Reference
30-60	Alkyl alcohol	Methanol	67-56-1	*D. magna*	48 h EC50	14 500 mg/L	Poirier *et al*, 1986
		Ethanol	64-17-5			12 318 mg/L	Takahashi *et al*, 1987
2-10%	Monocarboxylic fatty acid	Oleic acid	112-80-1	Fathead minnow	96 h LC50	205 mg/L	Mattson *et al*, 1976
		Linoleic acid	463-40-1	Green algae	96 h LC50	1.66 mg/L	Kamaya *et al*, 2003
0-1%	Fatty acids ME esters	10 surfactants	-	*D. magna*	48 h EC50	Units reported µM	Sandbacka *et al,* 2000
0-1%	Quaternary ammonium compounds	Quaternary ammonium compounds, di-C8-10-alkyldimethyl, chlorides	68424-95-3	*D. magna*	48 h EC50	91 µg/L	Di Nica, 2017

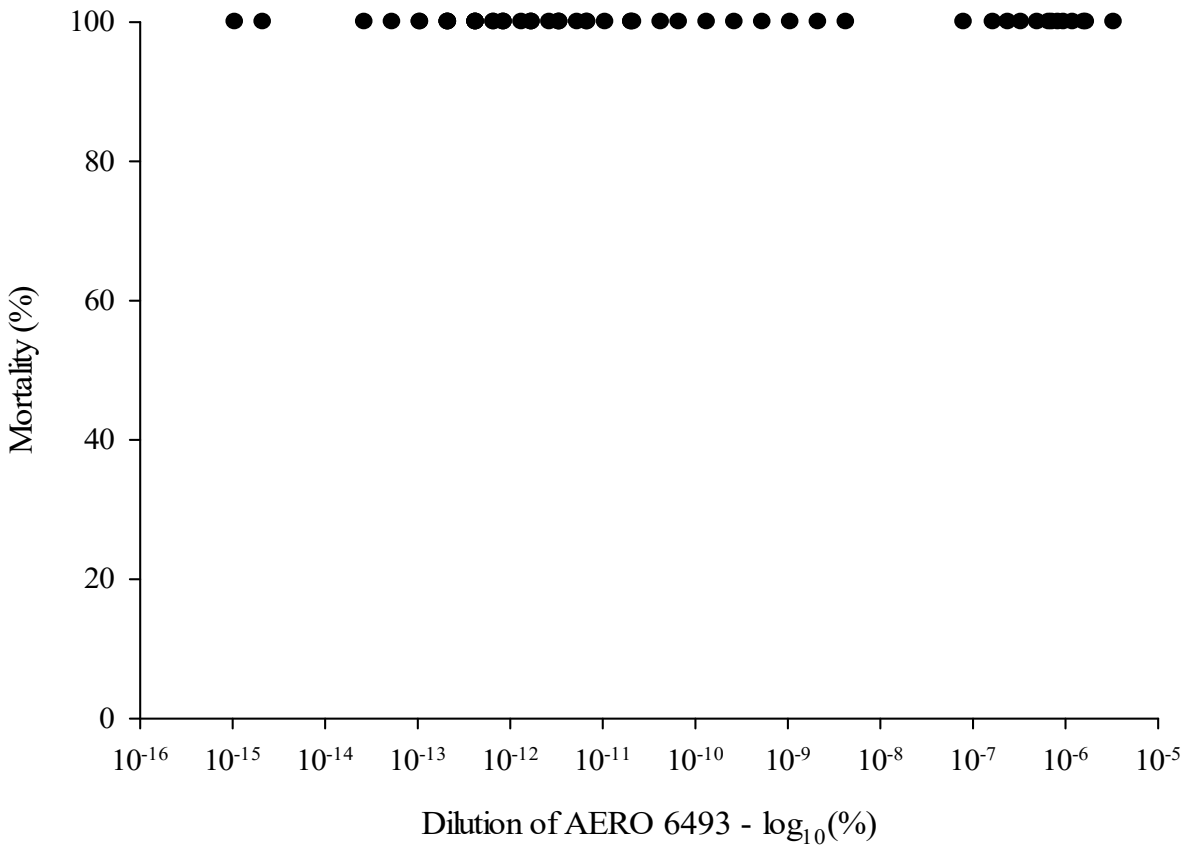

Figure 3.1. Concentration and response curve showing mortality of *H. azteca* when exposed to various dilutions of parent AERO 6493 in culture medium, pH adjusted to 7.3. The tests conducted did not involve warming up any solutions. Initial dilution tests showed AERO 6493 is very toxic.

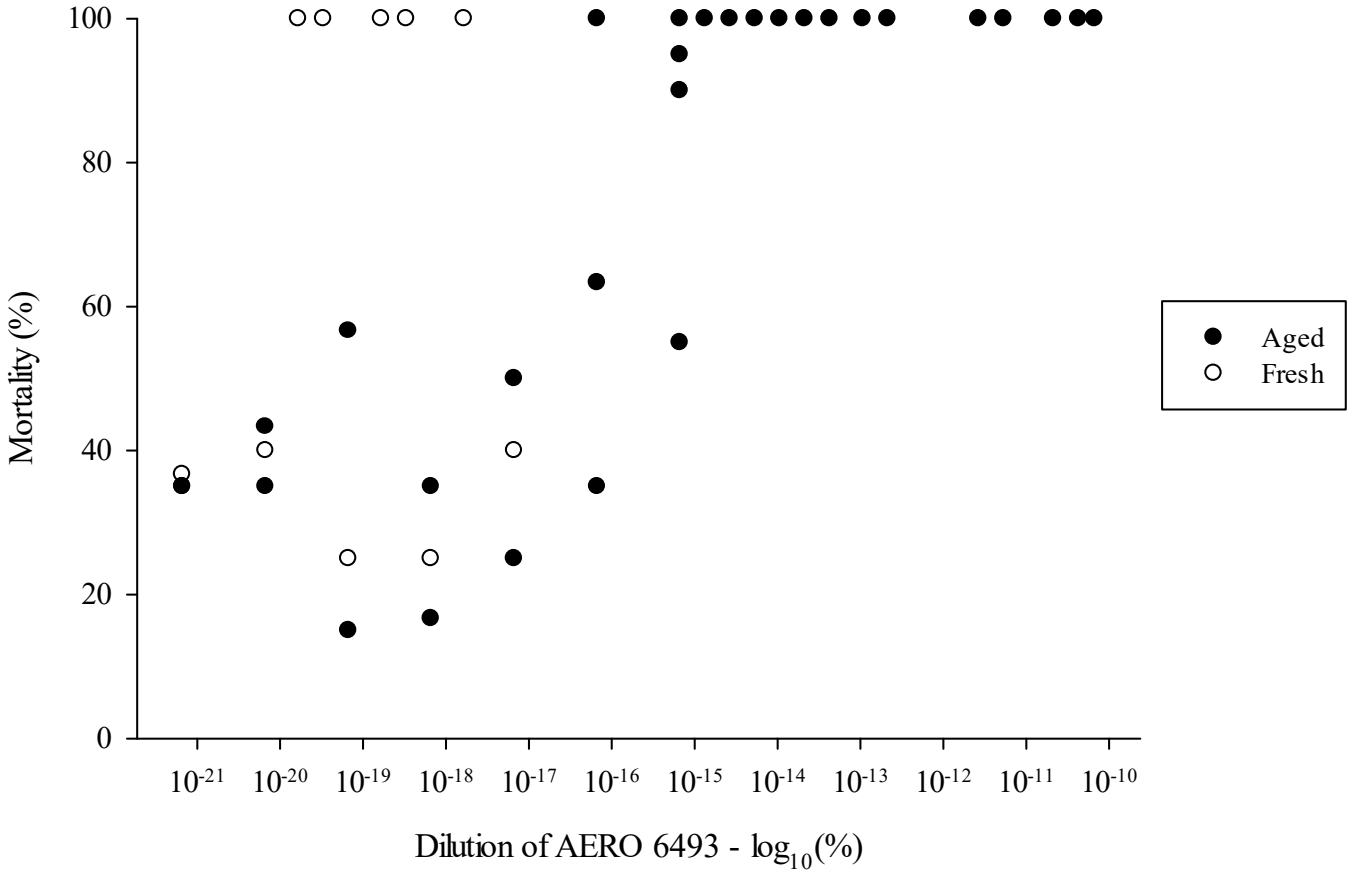

Figure 3.2. *H.azteca* mortality responses to 96 h acute exposure with various dilutions of parent AERO 6493. Tests conducted with fresh (created 24 h prior) and aged (created at least 14 d prior) working stock solutions have both been plotted to illustrate the differences in responses. Testing for these experiments involved warming up all test solutions to mimic flotation conditions where AERO 6493 would be used. Calculated LC50 were 2.6 E^{-16} % for fresh, 3.9 E^{-18} % for aged.

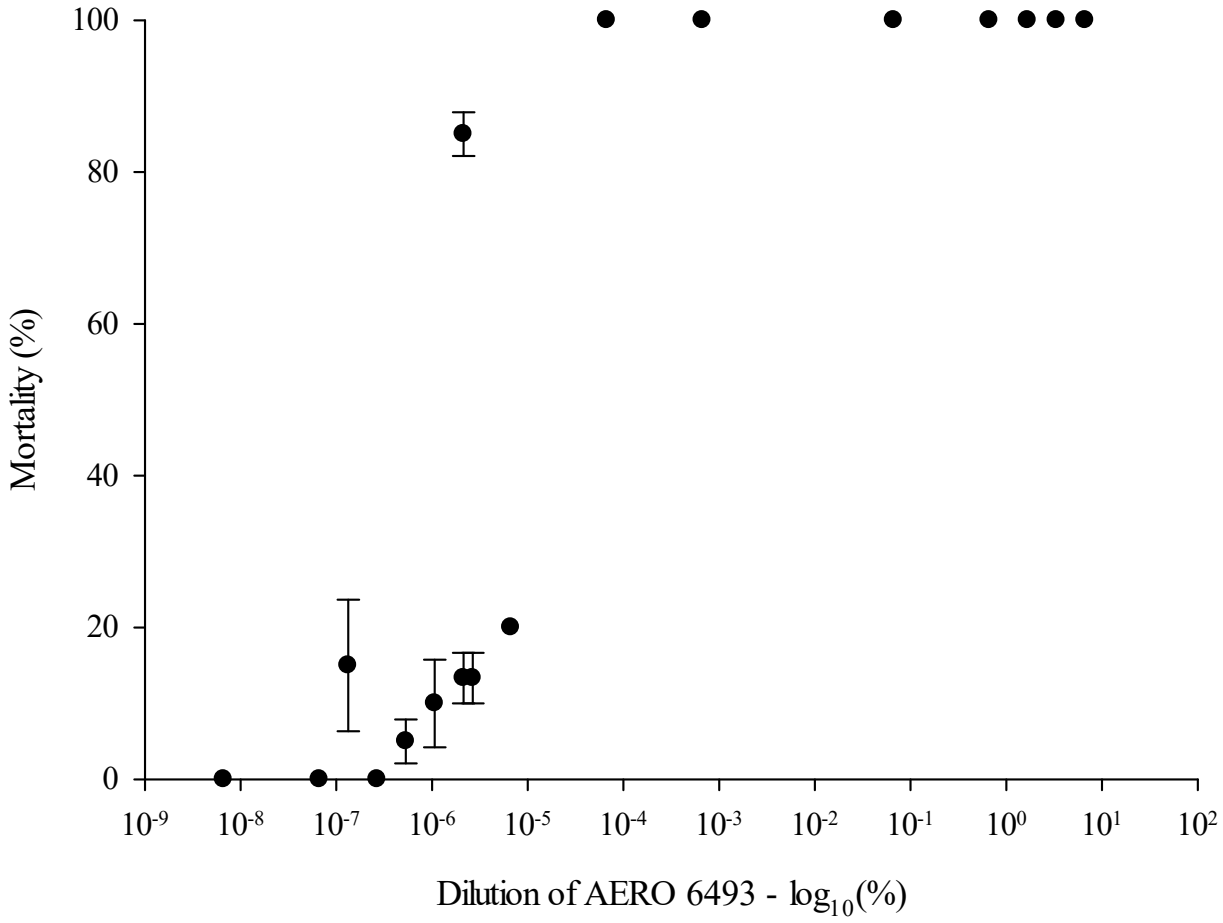

Figure 3.3. Toxicity of parent AERO 6493 to *D. magna*. Based on these tests, it was noted that AERO 6493 was not as toxic to *D. magna* as it was to *H. azteca*. Calculated EC50 was 2.0 E^{-6} %.

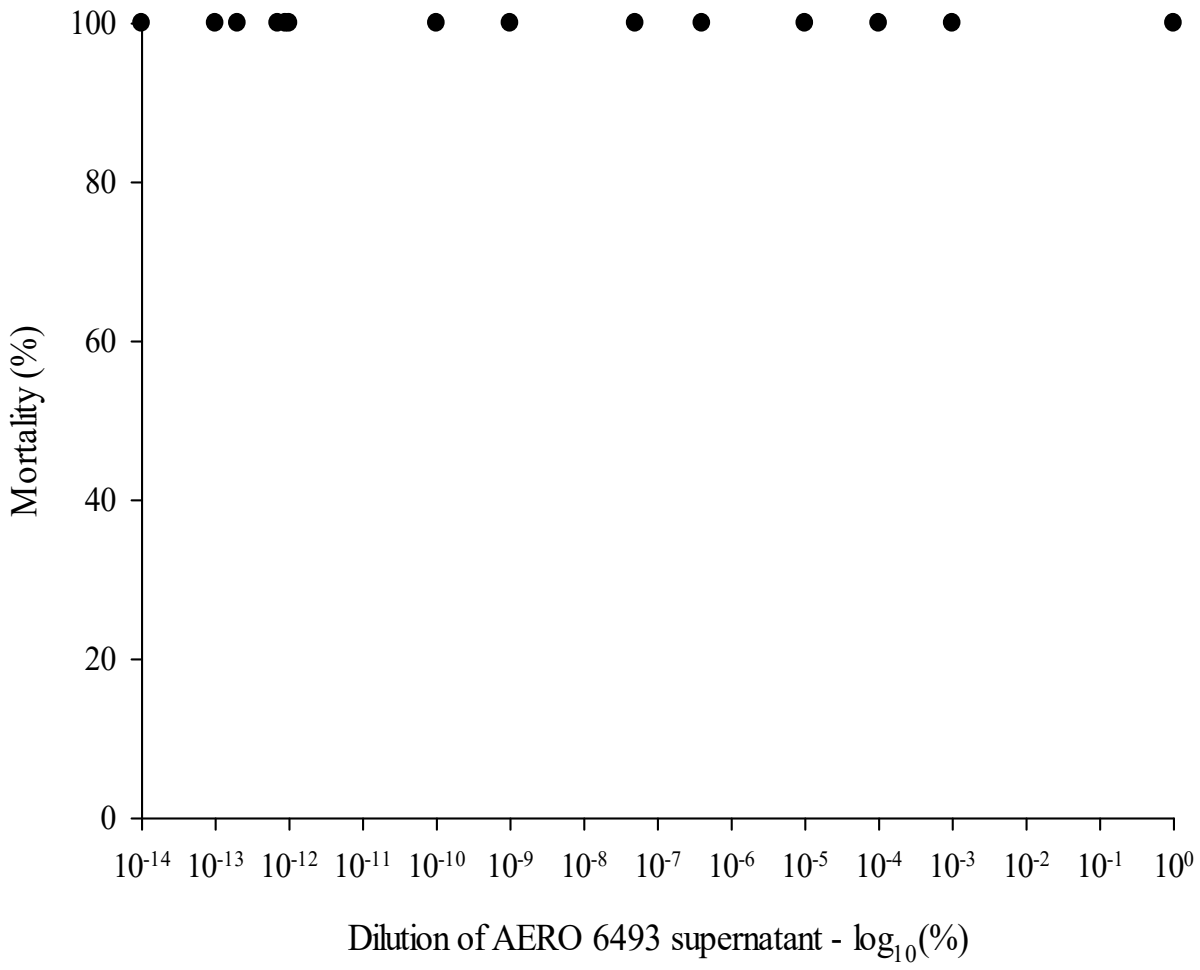

Figure 3.4. 48 h exposures of various diluted parent AERO 6493 supernatant (from Milli-q) to *D. magna*.

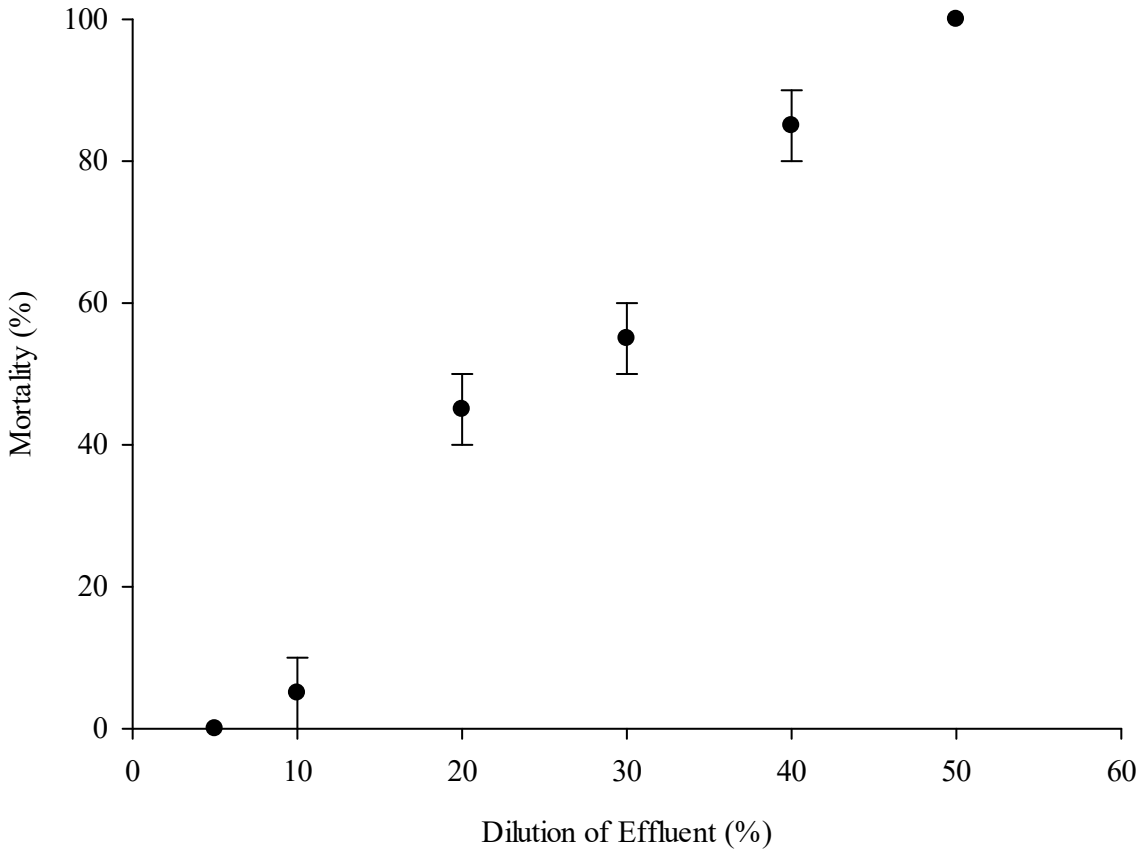

Figure 3.5. 48 h exposure of various dilutions of wastewater to *D. magna*. The calculated EC50 was 23.3 %. It is important to note that parent AERO 6493 is already diluted in the wastewater itself, so dilutions shown here cannot be compared to the previous tests. It is hard to identify what or how much of the original parent compound ends up in the effluent after AERO 6493 processing reagent has been mixed in the separation process.

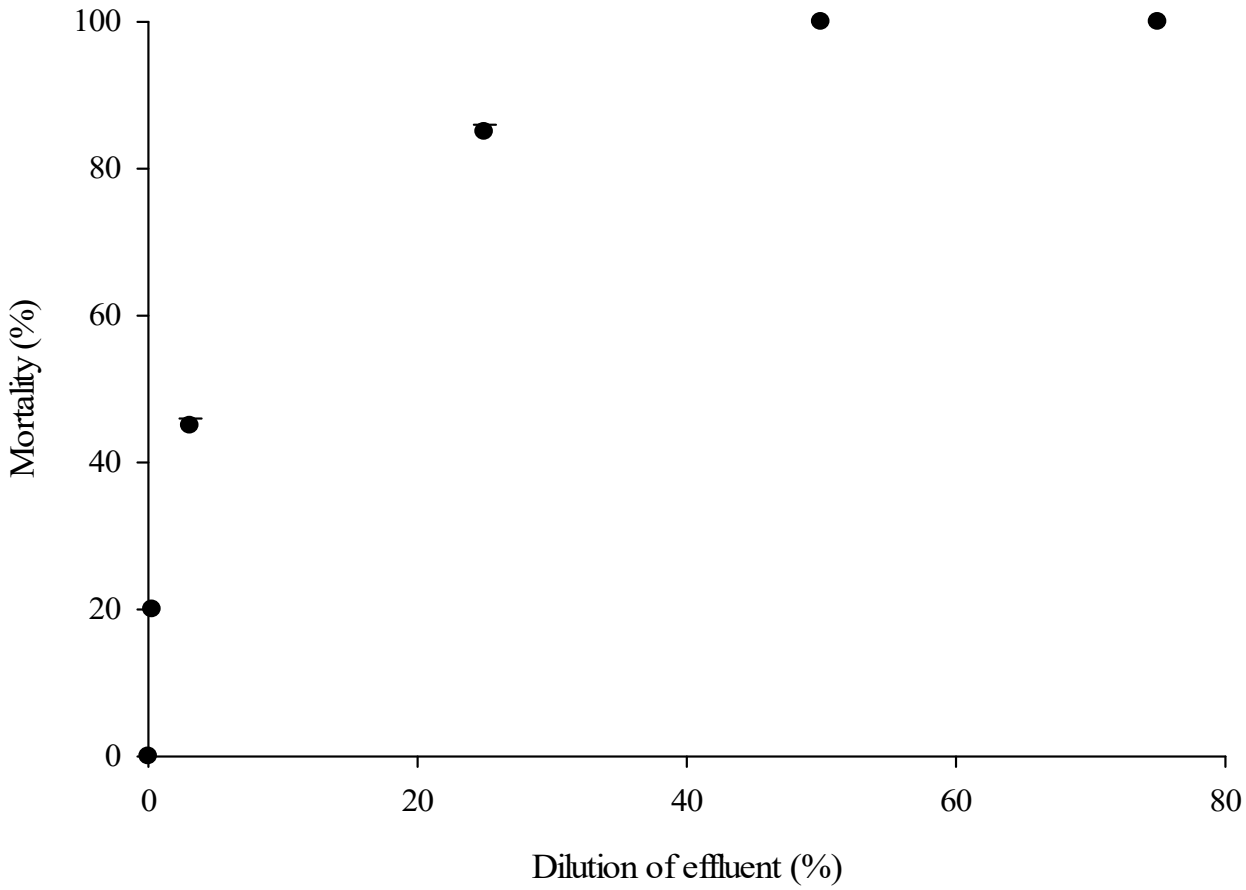

Figure 3.6. 96 h exposure of various dilutions of wastewater to *H. azteca*. Based on this test, the calculated LC50 was 2.44 %. As parent AERO 6493 is already diluted within the wastewater, it is hard to calculate how much of parent AERO 6493 is present once it has been added to the separation process.

Chapter 4: How this research is Integrative

4.1 Integration of numerous fields of science

This research was integrative, as it involved not only a biological aspect, but also chemical aspects regarding toxicity of chromium and rare earth element processing reagents. The potential impact of northern resource development on aquatic biota was assessed by studying the acute toxicity of Cr (VI) and processing reagent AERO 6493 to freshwater invertebrate species. Studying Cr (VI) toxicity involved conducting tests to living organisms, but also studying how biological responses may relate to the total Cr (VI) concentrations obtained from chemical analyses. Other chemical aspects also included incorporating various water hardness ions and DOM as potential toxicity modifying factors of Cr (VI). This meant studying the different speciation of Cr (VI) in water, and how its interaction with different ions present in the aquatic environment may result in reducing toxicity responses. This aspect also meant working with a software (geochemical speciation model) to determine the possible Cr (VI) concentrations and species present in the water at various tested parameters. As for AERO 6493 toxicity project, it incorporated TIE/TRE method with AERO 6493 itself, as well as testing effluent toxicity. Understanding the toxicity of the different chemicals involved in REE processing and its effluents provides useful information for industries and policy-makers regarding possible remediation strategies to overcome any ecological stresses from possible contamination. The research conducted also highlights the gap of limited information on REE processing.

References

Arzate-Cárdenas, M.A. and Martínez-Jerónimo, M.J., 2011. Age-altered susceptibility in hexavalent chromium-exposed *Daphnia schodleri* (Anomopoda: Daphniidae): integrated biomarker response implementation. Aquatic Toxicology. 105: 528-534.

Beukes, J.P., du Preez, S.P., van Zyl, P.G., Paktunc, D., Fabritius, T., Päätalo, M., 2017. Review of Cr (VI) environmental practices in the chromite mining and smelting industry-Relevance to development of the Ring of Fire, Canada. Journal of Cleaner Production. 165: 874-889.

Bridges, C.C., and Zalups, R.K., 2010. Chapter 3: Ionic and molecular mimicry and the transport of metals. In: Zalups, R.K., and Koropatnick, J., editors. Cellular and Molecular Biology of Metals. Florida: CRC Press. p. 241-294.

Brix, K.V., Volosin, J.S., Adams, W.J., Reash, R.J., Carlton, R.G., McIntyre, D.O., 2001. Effects of sulfate on the acute toxicity of selenate to freshwater organisms. Environmental Toxicology and Chemistry. 20: 1037-1045.

Borgmann, U., 1996. Systematic analysis of aqueous ion requirements of *Hyalella azteca*: a standard artificial medium including the essential Bromide ion. Archives of Environmental Contamination and Toxicology. 30: 356-363.

Boulanger J.F., Bazin, C., Turgeon, K., 2016. Literature Review of the Concentration of Rare Earth Elements (REE) through Flotation Processes. Ottawa, ON: CanmetMining, Natural Resources of Canada. Report No.: NRCan Bid #5000019519.

Cairns, A. and Yan, N., 2009. A review of the influence of low ambient calcium concentrations on freshwater daphniids, gammarids, and crayfish. Environmental Reviews. 17: 67-79.

The Canadian Institute of Mining, Metallurgy and Petroleum, 2018. Canadian rare earth elements and chromite: R&D program. Westmount, QC: CIM.

Chong, J., 2014. Chapter 2: Context. Resource Development in Canada: A Case Study on the Ring of Fire. Ottawa, Canada: Library of Parliament. Publication No. 2014-17-E. p. 1-6.

Costa, M. and Klein, C.B., 2006. Toxicity and carcinogenicity of chromium compounds in humans. Critical Reviews in Toxicology. 36: 155-163.

Cytec Industries, 2002. Flotation reagent fundamentals. Mining Chemicals Handbook. Revised Edition. Woodland Park, NJ: Cytec Industries Inc. p. 87-101.

De Schampheleare, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for *D. magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environmental Science and Technology. 36: 48-54.

Di Nica, V., Gallet, J., Villa, S., Mezzanotte, V., 2017. Toxicity of Quaternary Ammonium Compounds (QACs) as single compounds and mixtures to aquatic non-target microorganisms: experimental data and predictive models. Ecotoxicology and Environmental Safety. 142: 567-577.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic Ligand Model of the acute toxicity of metals: 1. Technical basis. Environmental Toxicology and Chemistry. 20: 2383-2396.

Dinker, M.K. and Kulkarni, P.S., 2015. Recent Advances in Silica-Based Materials for the Removal of Hexavalent Chromium: A Review. Journal of Chemical and Engineering Data. 60: 2621-2540.

Eisler, R., 1986. Chromium hazards to fish, wildlife and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report. 85(1.6).

Erickson, R.J., Benoit D.A., Mattson, V.R., Nelson, H.P. Jr., Leonard, E.N., 1996. The effects of water chemistry on the toxicity of copper of fathead minnows, Environmental Toxicology and Chemistry. 15: 181-193.

Environment Canada, 1996. Section 4: Universal test procedures. Biological Test Method: Acute Lethality Test using *Daphnia* spp. Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/11. May 1996 amendments. p. 14-25.

Environment Canada, 2005. Method development and applications section. Guidance document on statistical methods for environmental toxicity tests. Ottawa, ON. Report EPS 1/RM/46. June 2007 amendments. p. 138-140.

Environment Canada, 2013. Biological test method: test for survival and growth in sediment and water using the freshwater amphipod *Hyalella azteca*. Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/33, 2nd Edition. p. 29-42.

Government of Canada, 2014. Part II: Rare earth elements global market. The rare earth elements industry in Canada- Summary of evidence. Ottawa, ON: Standing Committee on Natural Resources. 41st Parliament, Second Session. p. 5-11.

Government of Canada, 2015. Budget Plan, Chapter 3.5- Growing trade and expanding markets [internet]. [Cited 2018 June 13]. Available from https://budget.gc.ca/2015/docs/plan/ch3-5-eng.html

Gillis, P.L., McGeer, J.C., Mackie, G.L., Wilkie, M.P., Ackerman, J.D., 2010. The effect of natural dissolved organic carbon on the acute toxicity of copper to larval freshwater mussels (*Glochidia)*. Environmental Toxicology and Chemistry. 29: 2519-2528.

Hansen, L.D., Maier, K.J., Knight, A.W., 1993. The effect of sulfate on the bioconcentration of selenate by *Chironomus decorus* and *Daphnia magna*. Archives of Environmental Contamination and Toxicology. 25: 72-78.

Hjartarson, J., McGuinty, L., Boutillier, S., Majernikova, E., 2014. Beneath the Surface: Uncovering the economic potential of Ontario's Ring of Fire. Ontario Chamber of Commerce, ISBN: 978-1-928052-02-9.

Johnson, J., Schewel L., Gradel, T.E., 2006. The contemporary anthropogenic chromium cycle. Environmental Science and Technology. 40: 7060-7069.

Kamaya, Y., Kurogi, Y., Suzuki, K., 2003. Acute toxicity of fatty acids to the freshwater green alga *Selenastrum capricornutum*. Environmental Toxicology. 18: 289-294.

Kotaś, J. and Stasicka, Z., 2000. Chromium occurrence in the environment and methods of its speciation. Environmental Pollution. 107: 263-283.

Kreuzinger, N., Fuerhacker, M., Scharf, S., Uhl, M., Gans, O., Grillitsch, B., 2007. Methodological approach towards the environmental significance of uncharacterized

substances- quaternary ammonium compounds as an example. Science Direct. 125: 209-222.

Maier, K.J., Foe, C.G., Knight, A.W., 1993. Comparative toxicity of selenate, selenite, seleno-DL-methionine and seleno-DL-cystine to *Daphnia magna*. Environmental Toxicology and Chemistry. 12: 755-763.

Mattson, V.R., Arthur, J.W., Walbridge, C.T., 1976. Acute toxicity of selected organic compounds to fathead minnows. Environmental Research lab, Duluth, Minnesota. EPA-600/3-76-097.

The Mining Association of Canada, 2017. Facts and figures of the Canadian mining industry [internet]. Mining Association of Canada; [Cited 2018 June 10]. Available from https://mining.ca/sites/default/files/documents/Facts-and-Figures-2017.pdf.

Mohan, D., Singh, K.P., Singh, V.K. 2005. Removal of hexavalent chromium from aqueous solution using low-cost activated carbons derived from agricultural waste materials and activated carbon fabric cloth. Industrial and Engineering Chemistry Research. 44: 1027-1042.

Mohan, D., Singh, K.P., Singh, V.K. 2006. Trivalent chromium removal from wastewater using low cost activated carbon derived from agricultural waste material and activated carbon fabric cloth. Journal of Hazardous Materials. 135: 280-295.

Mohan, D. and Pittman Jr., C.U., 2006. Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. Journal of Hazardous Materials. 137: 762-811.

Natural Resources Canada, 2018. Canadian mineral production [internet]. Government of Canada; [Cited 2018 June 13]. Available from https://www.nrcan.gc.ca/mining-materials/publications/17722.

Natural Resources Canada, 2016a. Literature review of the potential ecotoxicological effects arising from rare earth element (REE) processing. Ottawa, ON: Natural Resources Canada. Report No.: 1546601.

Natural Resources Canada, 2016b. Literature review of the concentration of rare earth elements (REE) through flotation processes. Ottawa, ON: Natural Resources Canada. Report No.: NRCan Bid Number 5000019519.

Natural Resources Canada, 2016c. The state of global rare earth industry: a review of market, production, processing and associated environmental issues. Ottawa, ON: Natural Resources Canada. Report No.: NRC-EME-55766.

Niyogi, S. and Wood, C.M., 2004. Biotic Ligand Model, a flexible tool for developing site-specific water quality guidelines for metals. Environmental Science and Technology. 38: 6177-6192.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., Wu, K.B., 2002. The biotic ligand model: a historical overview. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 133: 3-35.

Park, E.J., Jo, H.J., Jung, J., 2009. Combined effects of pH, hardness and dissolved organic carbon on acute metal toxicity to *Daphnia magna*. Journal of Industrial and Engineering Chemistry. 15: 82-85.

Poirier, S.H., Knuth, M.L., Anderson-Buchou, C.D., Brooke, L.T., Lima, A.R., Shubat, P.J., 1986. Comparative toxicity of methanol and N,N-Dimethylformamide to freshwater fish and invertebrates. Bulletin of Environmental Contamination and Toxicology. 37: 615-621.

Reid, S.D., 2012. Chapter 8: Molybdenum and Chromium. In: Wood, C.M., Farrell, A.P., Brauner, C.J., editors. Homeostasis and Toxicology of Essential Metals: Volume 31A. USA: Elsevier. p. 376-417.

Rhéaume, G. and Caron-Vuotari, M., 2013. The future of mining in Canada's North. The Conference Board of Canada, Centre for the North.

Rudolf, E. and Cervinka, M., 2006. The role of intracellular zinc in chromium (VI) induced oxidative stress, DNA damage, and apoptosis. Chemico-Biological Interactions. 162: 212-217.

Ryan, A.C., Tomasso, J.R., Klaine, S.J., 2009. Influence of pH, hardness, dissolved organic carbon concentration, and dissolved organic matter source on the acute toxicity of copper to *Daphnia magna* in soft waters: implications for the biotic ligand model. Environmental Toxicology and Chemistry. 28: 1663-1670.

Sandbacka, M., Christianson, I., Isomaa, B., 2000. The acute toxicity of surfactants on fish cells, *Daphnia magna* and fish- a comparative study. Toxicology in Vitro. 14: 61-68.

Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S., 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*. Environmental Toxicology and Chemistry. 20: 2397-2402.

Sciera, K.L., Isley, J.J., Tomasso, J.R., Klaine, S.J., 2004. Influence of multiple water quality characteristics on copper toxicity to fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry. 23: 2900-2905.

Stackhouse, R.A., Benson, W.H., 1988. The influence of humic acid on the toxicity and bioavailability of selected trace metals. Aquatic Toxicology. 13: 99-108.

Takahashi, I.T., Cowgill, U.M., Murphy, P.G., 1987. Comparison of ethanol toxicity to *Daphnia magna* and *Ceriodaphnia dubia* tested at two different temperatures: static acute toxicity test results. Bulletin of Environmental Contamination and Toxicology. 39: 229-236.

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluation: Phase I Toxicity Characterization Procedures. U.S. Environmental Protection Agency, Duluth, MN. EPA/600/6-91/003, 2nd edition.

U.S. EPA, 1993a. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. U.S. Environmental Protection Agency, Duluth, MN. EPA/600/R-92/080.

U.S. EPA, 1993b. Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. U.S. Environmental Protection Agency, Duluth, MN. EPA/600/R-92/081.

USGS, 2016. Mineral commodities: Rare earths. Mineral Commodity Summaries 2016. Reston, VA: U.S. Geological Survey. p. 134-136.

USGS, 2011. The mineral industries of Latin America and Canada. Mineral Yearbook: Latin America and Canada. Reston, VA: U.S. Geological Survey.

Vukov, O., Smith, D.S., McGeer, J.C., 2016. Acute dysprosium toxicity to *Daphnia pulex* and *Hyalella azteca* and development of the biotic ligand approach. Aquatic Toxicology. 170: 142-151.

Vutukuru, S.S., 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. International Journal of Environmental Research and Public Health. 2: 456-462.

Zhang, C., Cui, F., Zeng, GM., Jiang, M., Yang, ZZ., Yu, Z, 2015. Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. Science of the Total Environment. 518-519: 352-362.

Zinck, J., 2013. Realizing Canada's Rare Earth Elements Resource Potential: R&D Perspective. Canadian Institute of Mining, Metallurgy and Petroleum Westmount, Canada: MET SOC. p. 25-35.