# THE TOXICOLOGICAL EFFECTS OF THE MOUNT POLLEY TAILINGS IMPOUNDMENT BREACH ON FRESHWATER AMPHIPODS

**RAEGAN D. PLOMP**
**Bachelor of Science, University of Lethbridge, 2017**

A thesis submitted
in partial fulfilment of the requirements for the degree of

**MASTER OF SCIENCE**

in

**BIOLOGICAL SCIENCES**

Department of Biological Sciences

University of Lethbridge

LETHBRIDGE, ALBERTA, CANADA

# THE TOXICOLOGICAL EFFECTS OF THE MOUNT POLLEY TAILINGS IMPOUNDMENT BREACH ON FRESHWATER AMPHIPODS

RAEGAN D. PLOMP

Date of Defence: September 6, 2019

| | | |
|---|---|---|
| Dr. G. Pyle<br>Supervisor | Professor | Ph.D. |
| Dr. S. Wiseman<br>Thesis Examination Committee Member | Associate Professor | Ph.D. |
| Dr. R. Laird<br>Thesis Examination Committee Member | Associate Professor | Ph.D. |
| Dr. Laura Chasmer<br>Internal External Examiner<br>Department of Geography | Assistant Professor | Ph.D. |
| Dr. T. Russell<br>Chair, Thesis Examination Committee | Associate Professor | Ph.D. |

# Abstract

The bioavailability and toxicity of metals to amphipods is influenced by exposure route and co-toxic mechanisms. Metal bioavailability was studied in amphipods at sites affected by the 2014 Mount Polley Mining Corporation tailings impoundment breach. The area around the lake was also subjected to wildfire in 2017. Bioavailability was more correlated with sediment than waterborne metal concentrations with Cu correlating well with distance from the breach. Copper-sediment bioavailability to amphipods in combination with wildfire runoff was studied resulting in non-additive toxicity. *Hyalella azteca* exposed to Cu-enriched sediment with fire extract (FE) experienced a more-than-additive effect on survival and amphipod whole-body Cu concentration but no significant reduction in growth or acetylcholinesterase activity compared to the Cu-contaminated sediment or FE alone, respectively. The present study highlighted the importance of using biological vectors to assess toxicity, sediment-focused toxicity study following contamination events, and revealed the co-toxic effect of FE and Cu-enriched sediment.

# Acknowledgements

My acknowledgments must begin with my deepest thanks to my supervisor Dr. Greg Pyle for his guidance in this project and for always pushing me to achieve my best. Thank you to my supervisory committee members Dr. Steve Wiseman and Dr. Robert Laird, and my external examiner Dr. Laura Chasmer. Thank you to Dr. Jaimie Klemish for your endless encouragement, assistance with project planning and execution, and creation of the sampling site map. Thank you to the Quesnel River Research Center (QRRC) for your hospitality while I was completing my field work. Thank you to Michael Allchin for your assistance, accommodation, and boat driving while I was at the QRRC. Thank you to Lazlo Enyedy for your assistance with collecting water samples, boat driving, and knowledge of the Quesnel Lake area. Thank you to Dr. Ellen Petticrew for your invaluable advice while I was at the QRRC. Thank you to Dr. Ali Azizshirazi and Anne Willis for the preliminary sampling work done in the Quesnel Lake area that guided this project. Thank you to Dr. John Giesy for your expertise on polycyclic aromatic hydrocarbons. Thank you to Dr. Graham VanAggelen of the Pacific and Yukon Laboratory for Environmental Testing (PYLET) for providing *Hyalella azteca* to establish a culture in the Pyle laboratory. Thank you to Johane Joncas and the Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University for running my DOC and ICP-MS samples. Thank you to Sarah Bogart for your help with running the GF-AAS, establishing the *Hyalella azteca* culture, and project trouble-shooting. Thank you to Sean Everitt for trouble-shooting the AChE protocol. Thank you to Anne Willis, Lisa Bogart, Alicia Macdonald-Wilson, and Madison Gerbrandt for being excellent laboratory technicians. Thank you to James Nilsson for being an excellent field and laboratory technician.

I must thank the funding agencies who made this project possible. Thank you to the Natural Sciences and Engineering Research Council (NSERC). Thank you to Environment and Climate Change Canada. Thank you to the Environmental Damages Fund. Thank you to the Campus Alberta Innovation Program (CAIP).

I also need to thank my fellow lab-mates Parastoo Razmara, Dr. Ebi Lari, Sylvia Chow, Jody Heerema, Dylan Steinkey, Stina Lindh, Dr. Sina Volz, Sean Everitt, Lauren Zink, Sarah Bogart, and Dr. Jaime Klemish for your friendship and support during my time as a member of the Pyle lab. Finally, thank you to Mom, Dad, Allissa and Kailie for your unconditional love and for tolerating all my scientific mumbo jumbo over the past few years.

# TABLE OF CONTENTS

# List of Tables

**CHAPTER 2**

**CHAPTER 3**

# List of Figures

## CHAPTER 1

## CHAPTER 2

## CHAPTER 3

## List of Symbols and Abbreviations

| **Symbol** | **Definition** |
|---|---|
| **AChE** | Acetylcholinesterase |
| **AEA** | Aqueous extract of ash |
| **ANOVA** | Analysis of Variance |
| **APHA** | American Public Health Association |
| **BC** | British Columbia |
| **BCA** | Bicinchoninic acid assay |
| **BCENV** | British Columbia Ministry of Environment |
| **BCMOE** | British Columbia Ministry of Environment |
| **BLM** | Biotic ligand model |
| **CYP** | Cytochrome p450 |
| **DNA** | Deoxyribonucleic acid |
| **DO** | Dissolved Oxygen |
| **DOC** | Dissolved organic carbon |
| **DOLT-4** | Dogfish liver certified reference material for trace metals |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **FE** | Fire extract |
| **FTU** | Formazin turbidity units |
| **GF-AAS** | Graphite furnace atomic absorption spectrophotometer |
| **ICP-AES** | Inductively coupled plasma atomic emission spectrometry |
| **ICP-MS** | Inductively coupled plasma mass spectrometry |
| **LBC** | Lethal body concentration |
| **LC50** | Lethal concentration to kill 50% of the population |
| **MPMC** | Mount Polley Mining Corporation |
| **PAH** | Polycyclic aromatic hydrocarbon |
| **PCA** | Principle component analysis |
| **PCx** | Principle component (x represents a number) |
| **PHE** | Phenanthrene |
| **PHQ** | Phenanthrene quinone |
| **PYLET** | Pacific and Yukon Laboratory for Environmental Testing |
| **ROS** | Reactive oxygen species |
| **SAM** | Standard artificial media |
| **SLRS-6** | River water certified reference material for trace metals and other constituents |

# CHAPTER 1: Introduction

## Cu bioavailability to freshwater organisms

Copper (Cu) is a trace element that is commonly found in rocks and minerals. The mining of Cu is an essential aspect of today's economy due to its malleability, durability and conductivity which make it a valuable consumable. Growth in Cu production has been linear with human population size since the 1900s and is expected to continue to increase in the coming decades (Singer, 2017). In 2012, 17 million metric tonnes were consumed worldwide for use in electronics, construction, machinery, and consumer products (Kerr, 2014). The high demand for copper has increased the rate at which copper enters freshwater aquatic ecosystems through processes such as industrial wastewater. In addition to its economic value, Cu is an essential nutrient for all forms of plants and animals. Copper is necessary for many metabolic processes such as hemoglobin production, metabolism of glucose, and immune system response (Gaetke & Chow, 2003; Harris, 2001; Osredkar, 2011; Prohaska & Lukasewycz, 1989). These processes often exploit Cu's ability to convert between Cu(I) and Cu(II) that transport electrons and cause reactions that are important for biological function. Although Cu is an element required for biological function, exposure to Cu above biological requirements can lead to toxicity.

Organisms exposed to elevated concentrations of Cu can experience toxic effects when Cu is bioavailable. Most organisms posses mechanisms that protect against Cu toxicity that include regulated import and enhanced export into and out of the animal. The import of Cu has been hypothesized to involve high affinity Cu transporters that are members of the Ctr1 family of membrane proteins (Sinani et al., 2007). Many organisms use the cysteine-rich protein metallothionein to bind Cu and shuttle excess Cu out of the cell, therefore maintaining homeostasis (Roesijadi, 1996). When Cu concentrations are so high as to disrupt homeostasis, free Cu ions can promote the formation of reactive oxygen species (ROS) causing damage to lipids, proteins, and DNA (Gaetke & Chow, 2003). In the presence of reducing agents, Cu(II) is reduced to Cu(I) that allows the free electron to form hydroxyl radicals in the cell, a powerful ROS that is known to cause cell damage (Bremner, 1998; Lippard, 1999).

Historically, toxicological effect concentrations were based solely on total metal concentrations, or on dissolved or acid extractable metal concentrations (Niyogi & Wood, 2004). There has been increasing recognition that the total concentration of contaminants is not necessarily reflective of adverse toxicological effects in organisms. The toxicity of Cu has been shown to decrease with increasing water hardness (Borgmann et al., 2005; Chakoumakos et al., 1979; Howarth & Sprague, 1978; Pagenkopf et al., 1974) and dissolved organic carbon (DOC) (Al-Reasi et al., 2012; Gheorghiu et al., 2010; Giacomin et al., 2013) in various aquatic species. Erickson et al. (1996) found that individually increasing pH, hardness, DOC, sodium, and suspended solids all decreased Cu toxicity to larval fathead minnows (*Pimephales promelas*).

An approach known as the biotic ligand model (BLM) was developed to consider the influence of site-specific water chemistry on waterborne metals, such as Cu (Niyogi & Wood, 2004; Santore et al., 2001; Toro et al., 2001). The BLM predicts Cu toxicity to specific species as well as determining Cu species concentrations in water using input parameters of water chemistry that include pH, hardness, alkalinity, and DOC. Additionally, the BLM considers anion binding to form Cu-complexes that reduce Cu bioavailability. Water chemistry and Cu-complexes reduce Cu's ability to bind to the site of toxic action in biota, the biotic ligand (Paquin et al., 2002). The reduced binding ability of Cu occurs when Cu is bound to other materials and when cation present in the water compete for binding sites at the biotic ligand. The BLM can then predict Cu-species concentrations from dissolved Cu concentration, and estimate the Cu concentration that would result in toxic effects within a specified water body (Paquin et al., 2002).

### Copper in the environment: Cu mine disasters

Environmental Cu contamination is often the result of mine waste dumping, both intentional and accidental. Over the course of a century, Torch Lake (Michigan, USA) received over 200 million tonnes of tailings from the Cu industry with copper sediment concentrations now at an average of 1000 mg/kg (Cusack & Mihelcic, 1999). Ellenberger et al. (1994) found that yellow perch (*Perca flavescens*) egg masses reared in Torch Lake water had significantly longer hatching duration than those exposed to reference water.

Sudden and large influxes of Cu into the aquatic environment are relatively uncommon but they do occur in the case of mine tailing impoundment breaches. Serious failures have been defined as a release of at least 100 000 $m^3$ and/or loss of life while Very Serious failures are those that release at least 1 M $m^3$ and/or release distance >20 km and/or multiple loss of life (Bowker & Chambers, 2015). The incidence of severe impoundment failures seems to be on the rise as nearly 50% of all recorded Serious and Very Serious failures (from 1940-2010) have occurred since 1990, 16 out of 33 of which were Cu-related breaches (Bowker & Chambers, 2015). The average cost of the Very Serious failures since 1990 is $543 million ($2014) per breach (Bowker & Chambers, 2015). The increase in Serious and Very Serious failures has been attributed to the rising number of tailing impoundments that exceed 5 M $m^3$ (Bowker & Chambers, 2015).

Larger tailing impoundments have been on the rise due to decreasing ore quality, resulting in more waste products per tonne of processed ore (Bowker & Chambers, 2015). It is expected that the trend of increasing severe tailing impoundment breaches will continue into the coming decades (Bowker & Chambers, 2015).

Past Cu tailing impoundment breaches have varied in both size and severity. Buenavista del Cobre mine located in Sonora, Mexico experienced a Cu tailings impoundment breach that released 40 000 $m^3$ of tailings material in 2014 (Aparicio & Canales, 2017). While relatively small, the Buenavista del Cobre breach directly affected the health of 270 people and four water bodies. In 1998, the Aznalcollar mine in Spain experienced a larger tailings impoundment breach that released approximately 6 M $m^3$ of water and mud containing high concentrations of metals including Cu into the Agrio and Gaudiamar Rivers (Grimalt et al., 1999). In 2015, Mariana, Brazil experienced a massive tailings impoundment breach that occurred when the Fundão dam failed and released up to 62 M $m^3$ of iron ore tailings that were rich in Cu (Fernandes et al., 2016). The Fundão breach resulted in the death of 17 people, the destruction of 158 homes, and polluted 663 km of river.

The present study focused on a Very Serious tailings impoundment breach that occurred to the Mount Polley Mining Corporation (MPMC) Cu and Au mine located in British Columbia, Canada on August 4, 2014 (Figure 1.1). The breach resulted in the release of approximately 17 M $m^3$ of water and 8 M $m^3$ of tailings materials into Polley Lake, down Hazeltine Creek, and into the West Basin of Quesnel Lake (Petticrew et al., 2015). Quesnel Lake is a large, oligotrophic, y-shaped lake with three arms; West, North, and East. Initial waterborne Cu concentrations in Quesnel Lake’s West Basin were approximately 100 µg/L following the breach, but by May of 2015 Cu concentrations had generally dropped to below 6.6 µg/L (Imperial Metals, 2015). Upon entering

Figure 1. 1 Satellite images before (top) and after (bottom) the Mount Polley Mining Corporation tailings impoundment breach of August 4, 2014 (images from Google (n.d.)).

Quesnel Lake, the solid tailings Cu concentration range was approximately 100 – 700 mg/kg (SNC-Lavalin, 2014).

In Quesnel Lake immediately following the breach, there was a large turbidity plume of fine-particle sediment (<1 μm) that was over 1000 Formazin Turbitity Units (FTU) compared to pre-breach of <1 FTU in bottom waters (Imperial Metals, 2015; Petticrew et al., 2015). By May of 2015 turbidity had dropped to ~2 FTU but still remained above pre-breach turbidity readings (Graves et al., 2016). Despite the observed decrease in turbidity in the months following the breach Swan et al. (2014) observed that a significant proportion of total Cu was associated with the suspended fine particle sediment. This colloidal fraction persists in the water column at a depth of over 25 m, as evidenced by elevated Cu concentration at that depth relative to pre-breach conditions (Swan et al., 2014). Humidity cell and column testing has demonstrated that Cu bound to the find-particle sediment has a low potential to be bioavailable to aquatic organisms (SRK, 2015). The bound Cu is associated primarily with the silicate minerals and insoluble sulphides and when considering the alkaline conditions of Quesnel Lake, there is a low potential for Cu dissociation from the sediment as Cu ions. Despite the geochemical assessments, preliminary sampling done in the area suggests that Cu is bioavailable to organisms in Polley and Quesnel Lake (Azizishirazi and Pyle, unpublished).

## Climate change and wildfire effects on aquatic organisms

Variation in climate has occurred naturally over the course of the planet's history, but the unprecedented acceleration of rising global temperatures threatens to disrupt the functioning of natural ecosystems. Alteration in temperature (Webb et al., 2008), increased atmospheric carbon dioxide concentrations (IPCC, 2013), and frequency and severity of drought (Gillett et al., 2004; Milly et al., 2005) are all stressors that climate change influences. The frequency and severity of wildfire has also increased over time as a function of climate change (Abatzoglou & Williams, 2016). Westerling et al. (2006) observed earlier spring onset with higher spring and summer temperatures associated with increased wildfire since the mid-1980s. The temperature of the planet has increased by approximately 0.2°C per decade for the last three decades (Hansen et al., 2010), which continues to influence water cycles that can cause drought and subsequent wildfires (Dai, 2013). Globally, there has been an 18.7% increase in wildfire season length from 1979 – 2013 (Jolly et al., 2015). The economic cost of wildfire suppression has exceeded $1 billion dollars annually in Canada over the last decade and is expected to continue to rise with increased wildfire frequency (Goldammer, 2015). In 2017 and 2018 British Columbia, Canada, experienced the two worst fire seasons on record with 12 161 $km^2$ and 12 985 $km^2$ burned, respectively (British Columbia Wildfire Services, 2019). A part of the burn area of 2017 included the 1918 $km^2$ Elephant Hill wildfire (BC Wildfire Service, 2017) that burned through the Cariboo region, where the 2014 MPMC tailing impoundment breach occurred.

Despite the negative economic consequences of wildfire, in many ecosystems wildfires play an essential role by promoting plant growth and maintaining grazing areas (Bowman et al., 2011). The increased inputs of nutrients into the environment following wildfire events can promote primary production; however, excessive growth can lead to eutrophication, such as massive algae blooms (Ranalli, 2004). Increased nutrient levels, particularly P, associated with wildfire have been shown to persist 6 to 7 years post-fire, causing changes in trophic interactions due to the associated large algae blooms (Silins et al., 2014, 2009).

Along with nutrients such as N and P, wildfires can release many other substances into the aquatic environment via runoff, such as ammonia, DOC and pyrogenic polycyclic aromatic hydrocarbons (PAHs) (Bladon et al., 2008; McEachern, et al., 2000; Olivella, et al., 2006). Increased input of DOC into freshwater environments can be beneficial, as DOC is well known to bind with toxicants and reduce toxicant bioavailability to aquatic organisms (Besser et al., 2003; Niyogi & Wood, 2004; Richards et al., 2001).

Pyrogenic PAHs can persist in the environment for extended periods of time potentially causing long-term effects (Abdel-Shafy & Mansour, 2016). Pyrogenic PAHs are organic compounds composed of two or more benzene rings formed through the incomplete combustion of organic material at high temperatures (Wang et al., 1999). Pyrogenic PAHs usually contain fewer rings (e.g. anthracene, pyrene, phenanthrene) and fewer alkylated substitutions than petrogenic PAHs (Wang et al., 1999). Although, there are PAHs that belong to both petrogenic and pyrogenic PAHs. The reduced number of rings allow pyrogenic PAHs to dissolve more easily than larger-ringed compounds making them more bioavailable to aquatic organisms (Olivella et al., 2006). *Daphnia magna, Hyalella azteca,* and *Chironomus riparius* exposed to sediment-bound fluoranthene yielded Lethal Concentration 50s (LC50s) of 10, 5, and 15 mg/kg dry weight, respectively (Verrhiest et al., 2001). Previous work done by Landrum et al. (2003) has found LC50 values of 1757 µg/L and 79.1 µg/L for *Diporeia* spp. exposed to naphthalene for 10 days and pyrene for 28 days, respectively.

Total post-wildfire runoff toxicity is studied with less frequency than the individual components that compose the runoff. Work done by Nunes *et al.* (2017)

exposing mosquitofish (*Gambusia holbrooki)* to wildfire surface runoff observed an increase in oxidative stress. Exposing the freshwater clam *Corbicula fluminea* to aqueous extract of ash (AEA) resulted in increased mortality and bioaccumulation of PAHs and metals (Silva et al., 2016). Silva et al. (2015) also exposed the alga *Pseudokirchneriella subcapitata* and *Lemna minor* to AEA that resulted a decrease in cell density and dry weight following acute exposure; however, similar deleterious effects were not observed for *Daphnia magna* exposed to AEA. While studying the toxicity of isolated wildfire runoff is of great importance, the interaction of wildfire runoff with other potential toxicants that are not components of the runoff remains largely unknown.

Contamination that occurs in natural environments seldomly occurs with just individual contaminants, rather, interactions with pre-existing contaminants can potentially exacerbate or ameliorate individual effects on freshwater biota. Most pre-existing regulatory guidelines are based on the risk of individual toxicants, often in combination with water quality parameters. However, most real-world exposures are in low doses to a range of contaminants (Giesy et al., 2002). Co-toxicity, therefore, is an important consideration when attempting to determine ecological risk of contamination events, such as the MPMC tailings breach in combination with wildfire runoff. Co-toxicity is a complex field and has been studied with metals with findings of less than predicted toxicity for individual metals (less-than-additive), the same as predicted toxicity (additive), and greater than predicted (more-than-additive toxicity) (Norwood et al., 2003).

Research done previously in our laboratory has demonstrated more-than-additive toxicity between PAHs and Cu. *Hyalella azteca* were exposed to individual PAHs, which can be constituents of wildfire runoff,  phenanthrene (PHE) and phenanthrene quinone

(PHQ) in combination with low concentrations of waterborne Cu (Gauthier et al., 2015). Amphipods experienced 26.9 ± 1.7% and 73.3 ± 13.3% greater mortality when exposed to Cu-PHE and Cu-PHQ mixtures, respectively, than could be explained by the individual toxicants alone (Gauthier et al., 2015). Gauthier (2016) also examined acetylcholinesterase (AChE) activity in amphipod following exposure to Cu and PHE. Acetylcholinesterase is a critical enzyme that regulates the concentration of acetylcholine at the synapse by hydrolyzing and inactivating acetylcholine. Exposure to Cu (Brown et al., 2004; Frasco et al., 2005; Haverroth et al., 2015) and PAHs (Kang & Fang, 1997; Kim et al., 1999) individually has been shown previously to reduce AChE activity in aquatic organisms. Gauthier et al. (2016) observed a reduction in AChE activity in *H. azteca* but exposure to Cu was not different from control and did not induce additive toxic effects when combined with PHE following a 24 h exposure period. While waterborne Cu and single PAHs have been shown to have a more-than-additive effect on toxicity to *H. azteca,* it is not known how a Cu-enriched sediment and wildfire runoff affect toxicity.

### ***Hyalella azteca* as an ideal test organism for sediment toxicity studies**

The freshwater amphipod *Hyalella azteca* is a standard test animal in toxicological studies for both water-only and sediment experimentation (Government of Canada, 2012). *Hyalella azteca* is an epibenthic, sediment-burrowing amphipod that feeds mostly on detritus material. The amphipod is distributed over most of North America making it an ideal species to preform relevant toxicity tests on. Nebeker et al. (1984) was the first to recommend *H. azteca* for sediment toxicity testing citing their intimate contact with sediments, high control survivability, and ease of rearing. The amphipod has been extensively used to assess field collected sediment and have provided reliable evidence of adverse biological contamination in sediments (reviewed by the Government of Canada, 2012). *Hyalella azteca* are also more sensitive to contamination in sediments relative to other freshwater organisms (Kemble et al., 1994; West et al., 1993).

## Research Objectives

The primary objective of this research was to determine if metals, particularly Cu, associated with the 2014 MPMC tailings breach are bioavailable to aquatic organisms at concentrations sufficient to cause toxicity by using a biotic vector. Given that wildfires threaten to alter the exposure environment, a secondary objective was to determine the extent to which wildfire runoff exacerbates or ameliorates Cu toxicity. To accomplish these objectives, experiments were conducted: (i) to determine if metals were bioavailable to laboratory and field amphipods, and if so, if the metals accumulate to concentrations sufficient to induce toxicity, (ii) to determine if bioavailable metals are primarily waterborne or sediment-bound derived, and (iii) to determine the single and combined effects of wildfire runoff and Cu-enriched sediment to *H. azteca* under controlled laboratory conditions.

# CHAPTER 2. Relative contribution of water or sediment to amphipod toxicity associated with a large copper mine tailings impoundment breach in British Columbia, Canada

## Introduction

On August 4, 2014 the Mount Polley Mining Corporation (MPMC) copper and gold mine located in British Columbia, Canada experienced a large tailings impoundment breach. The breach resulted in the release of approximately 25 M $m^3$ of water and solid tailings materials into Polley Lake, that then flowed down Hazeltine Creek, and into the West Basin of Quesnel Lake (Petticrew et al., 2015) (Figure 2.1). Polley Lake is situated alongside the east side of the mine site and is 6.17 km long x 0.65 km wide with a maximum depth of 35 m. Quesnel Lake is a large, deep, oligotrophic fjord lake with a surface area of 266 $km^2$ and volume of 41.8 $km^3$ (Petticrew et al., 2015). The breach raised the water level of Quesnel Lake and Polley Lake by approximately 7.7 cm and 170 cm, respectively (Golder Associates, 2016). The breach event also deposited material into Quesnel Lake creating a deposit approximately 0.6 km wide x 1.2 km long and 1-3 m deep at the mouth of Hazeltine Creek (Petticrew et al., 2015). A large turbidity plume of fine particle sediments (<1 μm) was also observed in Quesnel and Polley Lake post-breach (Imperial Metals, 2015; Petticrew et al., 2015).

The released tailings material was rich in metals and one of the main metals associated with the MPMC breach, and of potential concern, is Cu. The concentration of waterborne Cu found in the tailings impoundment before the breach was at a maximum of 64.1 μg/L (SNC-Lavalin, 2014). Copper concentrations found in Quesnel Lake, near the

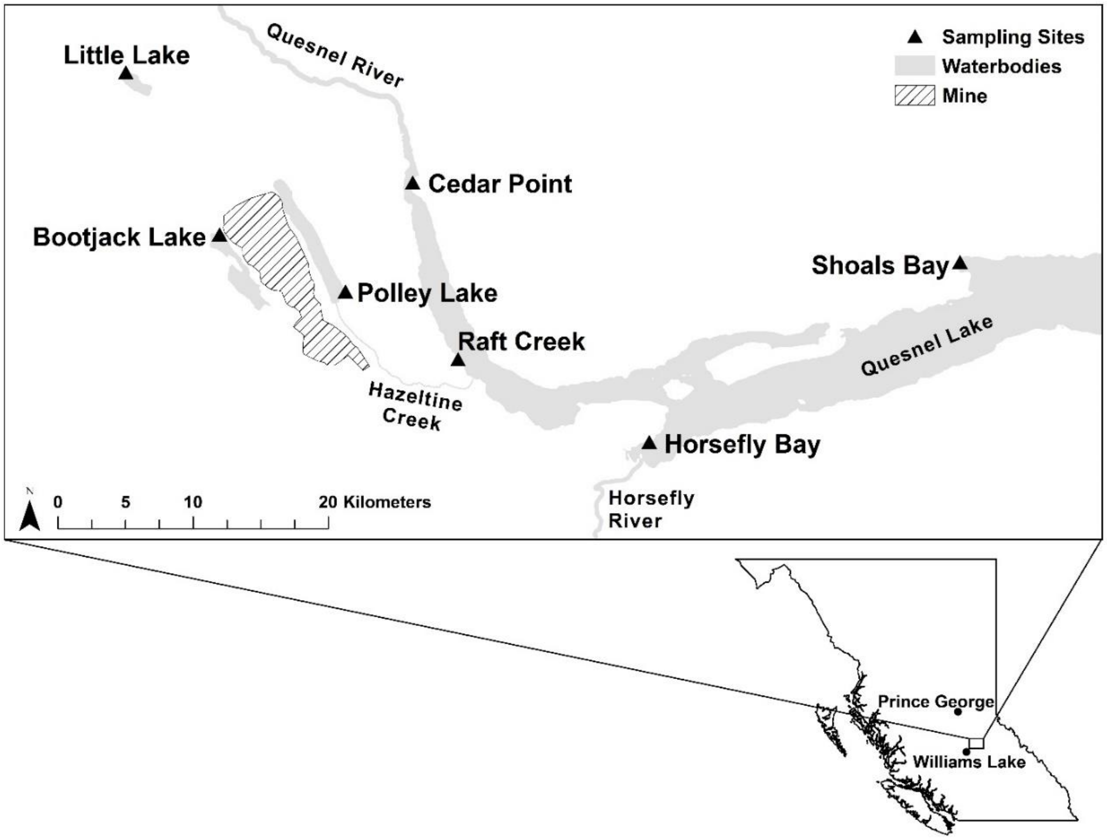

Figure 2. 1. Sampling site locations for the amphipod collection and the sediment and water used in the sediment exclusion study in Quesnel Lake, Polley Lake, Bootjack Lake and Little Lake.

mouth of Hazeltine Creek, immediately post-breach were as high as ~100 μg/L; however, by May of 2015 waterborne Cu concentrations had generally dropped to below 6.6 μg/L (Imperial Metals, 2015). Polley Lake Cu concentrations also decreased substantially by 2015 with concentrations at approximately 3 μg/L (Golder Associates, 2016).

The solid tailings material entering the affected lakes may have contributed to the initially high concentration of waterborne Cu measured post-breach. The solid tailings had a pre-breach average total Cu concentration of 931 mg/kg (SNC-Lavalin, 2014). Upon entering Quesnel Lake, concentrations of Cu in the sediment ranged from ~100 -

700 mg/kg Cu (Imperial Metals, 2015). Copper was also found to be associated with the suspended fine particle sediment (< 1 µm) (Swan et al., 2014). This colloidal fraction persists in the water column at a depth of over 25 m, as evidenced by elevated Cu concentration at that depth relative to pre-breach conditions (Swan et al., 2014).

Metal-contaminated water and sediment have been shown to contribute differentially contribute to freshwater invertebrate toxicology. Borgmann et al. (1991) studied Cd bioaccumulation in *H. azteca* and found that there was 2-2.6 fold higher Cd concentration in the animals exposed to Cd via sediment compared to water. *Hyalella azteca* exposed to sediment-bound Cu and Zn also demonstrated increased bioaccumulation compared to waterborne exposures for one-week and four-week exposures, respectively (Borgmann & Norwood, 1997). The difference in metal bioaccumulation could be due to differences in mode of toxicity or differences in Cu speciation between water and sediment.

The total concentration of Cu in water or sediment is not always a reliable indicator of toxicological effects due to differences in bioavailability. When Cu is in the aquatic environment, free bivalent and monovalent Cu cations are generally the most bioavailable forms to aquatic organisms. An excess of Cu ions can overwhelm an organism’s natural Cu homeostasis mechanisms resulting in oxidative damage (Gaetke et al., 2014). However, environmental Cu can also bind to organic material and fine sediments, reducing ionic Cu bioavailability (ATSDR, 2004). Geochemical assessments (humidity cell and column tests) of sediment from the MPMC breach suggest that Cu from the breach had a low potential for bioavailability by organisms due to high association (approximately 67%) with silicate minerals (SRK, 2015). The remaining 33% of Cu was found to be associated with insoluble sulphides, that would have a low potential for leeching due to the neutral or slightly alkaline nature of the tailings material and the affected water bodies (SRK, 2015). However, Byrne et al. (2018) demonstrated that Cu mobility at Hazeltine Creek in 2015 and 2016 could be occurring due to by copper sulphide weathering of streamside tailings material, and chemically through reductive dissolution of Cu-bearing Fe oxides. The ability that metals have to be dissolved or released from contaminated sediments is an important factor in determining bioavailability to biota, but this type of analysis fails to consider how biota themselves can affect metal bioavailability.

Mechanistically, waterborne Cu accumulation in aquatic organisms has been well described using the biotic ligand model (BLM) (Niyogi & Wood, 2004; Paquin et al., 2002; Santore et al., 2001). The BLM is able to predict toxicity in specific species by considering the ability that Cu has to bind at the site of toxic action (the biotic ligand e.g. gill) (Paquin et al., 2002). The BLM incorporates the concentrations of metal species with competing cations from site-specific ambient water chemistry to predict toxicity and subsequentially set short-term and long-term water quality criteria (Paquin et al., 2002). Additionally, the BLM can be used to determine the concentration of metal species dissolved in water by incorporating ambient water quality parameters (Paquin et al., 2002).

This study endeavors to test the bioavailability of metals in the water and sediment from the affected lakes using benthic freshwater amphipods. Amphipods interact directly with sediments making them an ideal study animal. It has been demonstrated that *H. azteca* have the ability to bioaccumulate Cu from contaminated sediment causing a reduction in survival and growth (Borgmann & Norwood, 1997; Milani et al., 2003). Additionally, preliminary sampling of amphipods performed in 2016 in Quesnel Lake demonstrated that whole-body amphipod Cu concentrations increased with decreasing distance from where the tailings material entered the lake, suggesting there is bioavailable Cu associated with the breach event (Azizishirazi and Pyle, unpublished).

The objective of this research was to determine if metals, particularly Cu, associated with the 2014 MPMC tailings breach, are bioavailable to aquatic organisms at concentrations sufficient to cause toxicity. To accomplish this objective: (i) laboratory-reared *H. azteca* were used in a sediment exclusion study, using water and sediment collected from four sites in Quesnel Lake as well as Polley, Bootjack, and Little Lake. A sediment exclusion study was used to determine if there is toxicity associated with the MPMC tailings impoundment breach and if so, whether that toxicity is waterborne or sediment derived by controlling access that *H. azteca* have to the sediment. (ii) Water, sediment, and amphipods were collected for metal concentration analysis from the same seven sites to determine if site metal concentrations corroborated with the above-mentioned sediment exclusion study.

## Materials and Methods

### *Study sites*

The MPMC Cu and Au mine is located approximately 51 km northeast of Williams Lake, British Columbia, Canada. The MPMC mine sits between Polley Lake to the east and Bootjack Lake to the west (Figure 2.1). Bootjack Lake has a surface area of 2.68 km$^2$ and average depth of 7.5 m. Bootjack Lake is fed primarily by Morehead Creek and water flows out of Bootjack Creek. Polley lake is a eutrophic lake with a surface area of 4.53 $km^2$ and average depth of 18 m. Water from Polley lake flows out of Hazeltine Creek into Quesnel Lake. Quesnel Lake is a large, oligotrophic glacial lake with a catchment area of 6200 $km^2$, supporting three major basins fed by the Horsefly River near the west basin, the Mitchell River in the north basin, and Niagara Creek in the east basin. Water from Quesnel Lake flows out to the Quesnel River, that then flows into the Fraser River before it reaches the Pacific Ocean. Consequently, both the Quesnel River and Quesnel Lake represent important habitat for migratory fish species, such as sockeye salmon (*Oncorhynchus nerka*). Quesnel Lake is the deepest fjord lake in the world, with a maximum depth of approximately 610 m in the east basin and an average depth of 157 m. It is the fifth deepest lake in British Columbia and the deepest lake in the Cariboo region where it resides.

Sampling sites were selected based on preliminary sampling for the presence of amphipods and site proximity to the tailings breach (Figure 2.1). All sampling sites were within the littoral zone in approximately one meter of water. The study site at Polley Lake was located at the southern end of the lake by a small campground near the Hazeltine Creek discharge where the tailings material flowed out of Polley Lake and into Quesnel Lake. The Bootjack Lake site was located at the northern end of the lake represented a lake that was affected by regular mining activities owing to its close proximity to the Mount Polley mine, but one that did not receive any tailings material from the breach event. The Little Lake study site, located on the northern edge of the lake, was unaffected by both mine activities and the breach event, so it served as a reference site for the sites not located within Quesnel Lake. Within Quesnel Lake four sites were chosen at varying radial distances from where the tailings material entered the lake. The nearfield site, Raft Creek, was in the West Basin of Quesnel Lake immediately adjacent (~0.8 km) to where the tailings material discharged into Quesnel Lake and represented the most contaminated site. The Cedar Point site was located near a recreation area in the West Basin of Quesnel Lake, close to the outflow of Quesnel Lake into the Quesnel River. The Cedar Point site was approximately 9.1 km from the mouth of Hazeltine Creek, where tailings material discharged into Quesnel Lake. The Horsefly Bay site was located near the mouth of the Horsefly River, which drains a metal-rich, actively mined watershed immediately north of Quesnel Lake. It is located within an inlet approximately 11.9 km from where the tailings were discharged into Quesnel Lake, and is considered to be outside of the West Basin. Shoal's Bay was located outside of the West Basin in the main channel of the lake. It was approximately 25.3 km from the Hazeltine discharge point and considered to be an in-lake reference site. Preliminary sampling of water and sediment at Shoal's Bay indicated that it remained relatively unaffected by the tailings discharge into the west basin of Quesnel Lake.  The most affected study site from the breach was Raft Creek, followed by Cedar Point, then Horsefly Bay, and Shoal Bay (Figure 2.1).

### *Hyalella azteca culturing method*

Amphipods were acquired from Environment and Climate Change Canada, Pacific and Yukon Laboratory for Environmental Testing (PYLET) in April of 2017. Amphipods were cultured at the Alberta Water and Environmental Science Building at the University of Lethbridge in standard artificial medium (SAM; Borgmann, 1996), in 40 L tanks with 30 L of culture water. Water was kept at 23°C and was constantly aerated. The culture was held at a 16:8 h light/dark photoperiod using full spectrum fluorescent lights at 645 lux. Cotton gauze cut into 3x3 cm squares were used as an artificial habitat substrate in each tank. Eighty percent water changes were conducted weekly on the tanks.

Each culture tank had 120 mg of ground Tetramin® Tropical fish flake food (Tetra US; #77101) added three times a week to maintain culture size. Any uneaten food was removed the next day. Prior to the start of the test, juveniles were removed from the tanks using a filter stack of 600 µm (adults) and 215 µm mesh (juveniles).

### *Sediment Exclusion*

Water and sediment were collected from each sampling site mentioned above in 2018 from May 31 – June 5. Water was collected under the water's surface in acid-washed plastic buckets, triple rinsed with site water. Lids were attached to sample buckets underwater to exclude surface debris. Before collecting water samples, any disturbed sediments were allowed to settle before a sample was collected. Sediment was collected by pushing an acid-washed bottomed-out bucket into the sediment and then removing the overlying water and collecting the sediment. Water and sediment were used in the sediment exclusion experiment within two weeks post-collection.

A sediment exclusion experiment was designed to determine the relative contribution between overlying water or sediment as a potential source of toxicity to laboratory reared *H. azteca*. The fourteen-day experiment was performed following Canadian Environmental Protection Service Biological Test Method protocols with some adjustments (Government of Canada, 2012). Laboratory-reared *H. azteca* of mixed age (50% juvenile, 50% adult) were randomly exposed to sediment and water collected from the seven sites described above. Each site had two treatments where test animals either had access to sediment or did not have access to sediment. Sediment exclusion treatments had a 0.45 µm filter barrier that allowed *H. azteca* to interact only with potential contaminants that were dissolved in the overlying water. Sediment-access treatments had a 250 µm mesh barrier that allowed interaction with site sediments while preventing test animals from burrowing into the substrate, potentially making them unrecoverable at the end of the test. Both laboratory controls were composed of culture water and sand, with one control having the 0.45 µm barrier while the other having the 250 µm barrier. The mesh barriers were fixed to one end of a 7.62 cm glass tube with aquarium-grade silicon that was then placed in a 300 mL beaker containing 50 mL of sediment and 200 mL of test water (Figure 2.2) (Government of Canada, 2012). All test vessels were randomly placed in water baths and each vessel received 12 randomly selected animals, added two animals at a time. Replicates were kept at 23±1°C, aerated continuously, and with a photoperiod of 16:8 hour light: dark at 500 lux (Government of Canada, 2012). Every replicate was fed ad libitum with Tetramin® tropical fish food flakes a total of six times on non-consecutive days over the duration of the exposure. After the exposure, animals were transferred to clean culture water to depurate for 24 h. Animals were then rinsed in a 50 µM ethylenediaminetetraacetic acid (EDTA) solution for one minute to remove surface-bound metals from the amphipods' carapace (Norwood et al., 2007). Immediately following EDTA rinsing, animals were dried until constant weight was achieved. Average weight per animal was determined by weighing all surviving amphipods from a replicate and dividing by the number of surviving animals in the replicate.

### *Whole-body amphipod Cu concentration analysis*

The concentration of Cu present in the whole-body of *H. azteca* from the sediment exclusion study was measured since Cu was identified as a major contaminant of concern following the MPMC breach of 2014. Amphipod-Cu concentration of whole-body *H.*

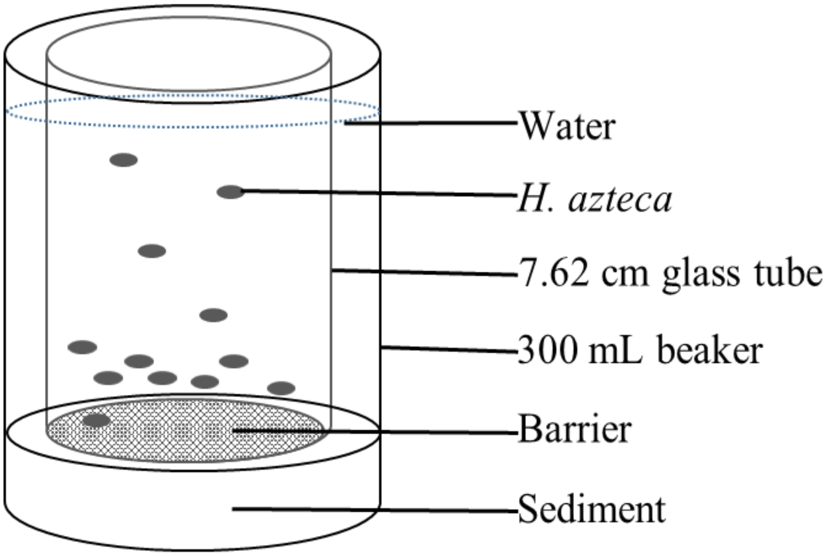

Figure 2. 2. Test vessel used in the sediment exclusion study. *Hyalella azteca* were contained in a glass tube with a barrier of either 0.45 µm or 250 µm mesh to exclude access or allow access to the sediment, respectively.

*azteca* was determined using a graphite furnace atomic absorption spectrophotometer (GF-AAS, Agilent Technologies, USA). Dried amphipod replicates were randomly combined in duplicate in order to obtain sufficient tissue quantities for testing. Samples were prepared following methods described by Lindh et al. (2019) with some adjustments. Animals were digested in concentrated nitric acid (trace metal grade; Fisher Scientific; Canada) at a 1:10 ratio (tissue mass (mg): acid (µL)) for 3 h at 80°C. Acid-only blanks were digested in triplicate alongside the tissue to confirm that no Cu contamination took place during the digestion procedure. Certified reference material was also digested simultaneously (DOLT-4 dogfish liver; National Research Council Canada). Following digestion, samples were cooled to room temperature and diluted at a ratio of

1:2 (initial tissue mass (mg): Millipore water (mL)). Supernatant was then pipetted off and used for Cu analysis.

The GF-AAS analysis was performed as previously described by Lindh et al. (2019). Samples were run on an Agilent 240 FS atomic absorption spectrometer with a GTA 120 graphite tube atomizer and a Cu hollow cathode lamp (Agilent Technologies: catalog no. 5610101400). Machine specifications during runs were as follows: wavelength 324.8 nm, slit width 0.5 nm, and a step was added to the burn profile (step 9, temperature 2300°C, time 4.0 s, flow 0.3 L/min) to reduce previous sample carryover. Otherwise, the GF-AAS was run according to the manufacturer specifications. Samples were diluted as necessary with Millipore water to fall within the calibration range. Certified reference material, SLRS-6 (National Research Council Canada), was run approximately every 10 samples to ensure the accuracy of the analysis (mean percentage of recovery 94.2%) along with a blank (mean blank 0.65 µg/L). Approximately every 20 samples, analytical duplicates were run to ensure analytical consistency (mean difference 2.4%). Measured Cu concentrations were adjusted by weight of the amphipod, dilution, and by blank concentration.

### *Sediment exclusion water quality*

Water quality measurements were taken on day 0, day 7, and at the end of the experiment on day 14 (Table 2.1). Temperature and DO were monitored every other day. Dissolved oxygen and temperature were measured via a YSI Pro20 Instrument (YSI, USA) dissolved oxygen probe (model# PRO 20). A Traceable conductivity meter (VWR, Canada) was used to measure conductivity. The pH was analyzed using a pH meter

Table 2. 1. Water quality for H. azteca exposed to a sediment exclusion experiment (B in sample title indicates no sediment access) using water and sediment collected from Little, Bootjack, Polley, and Quesnel Lake (±SD)[a].

| **Sample** | **Temp (°C)** | **DO (% $O_2$)** | **pH[b]** | **Alkalinity (mg/L as $CaCO_3$)** | **Hardness (mg/L as $CaCO_3$)** | **Conductivity (µS/cm)** | **DOC (mg/L)** |
|---|---|---|---|---|---|---|---|
| Lab Control | 22.5±0.5 | 89.9±0.7 | 8.0 (8.0-8.1) | 109±14 | 163±5 | 540±16 | 0.6 |
| Lab Control-B | 23.2±0.7 | 90.8±3.6 | 8.0 (8.0-8.1) | 111±16 | 160±8 | 460±53 | 0.4 |
| Little Lake | 23.2±0.6 | 90.2±3.5 | 8.2 (8.2-8.3) | 75±20 | 125±13 | 430±171 | 7.8 |
| Little Lake-B | 23.3±0.5 | 91.2±2.5 | 8.2 (8.2-8.2) | 77±18 | 121±10 | 420±163 | 7.5 |
| Bootjack Lake | 22.5±0.5 | 87.9±5.6 | 8.0 (7.8-8.1) | 85±17 | 116±30 | 169±64 | 6.4 |
| Bootjack Lake-B | 23.0±0.7 | 85.7±6.0 | 7.8 (7.8-7.8) | 100±2 | 114±27 | 168±64 | 6.2 |
| Polley Lake | 22.3±0.3 | 87.9±5.6 | 8.1 (8.1-8.1) | 96±3 | 140±16 | 244±28 | 6.3 |
| Polley Lake-B | 23.0±0.3 | 92.8±3.6 | 8.1 (8.1-8.2) | 98±4 | 142±18 | 244±29 | 5.9 |
| Shoals Bay | 22.4±0.3 | 96.1±2.5 | 7.8 (7.8-7.9) | 47±6 | 85±9 | 122±21 | 2.4 |
| Shoals Bay-B | 23.0±0.8 | 88.7±4.0 | 7.8 (7.8-7.9) | 50±8 | 84±11 | 132±30 | 2.1 |
| Horsefly Bay | 22.9±0.2 | 90.8±3.6 | 7.8 (7.8-7.9) | 93±10 | 74±33 | 177±92 | 4.5 |
| Horsefly Bay-B | 23.5±0.3 | 92.5±5.2 | 7.9 (7.8-7.9) | 98±5 | 73±33 | 186±100 | 4.3 |
| Cedar Point | 22.5±0.5 | 87.6±3.5 | 8.1 (8.1-8.1) | 68±19 | 91±19 | 131±11 | 2.8 |
| Cedar Point-B | 23.0±0.7 | 87.7±3.7 | 8.1 (8.0-8.1) | 63±12 | 91±18 | 138±16 | 2.4 |
| Raft Creek | 22.8±0.8 | 92.0±1.9 | 8.1 (8.1-8.1) | 59±9 | 105±10 | 181±57 | 1.7 |
| Raft Creek-B | 23.0±0.8 | 93.4±2.1 | 8.1 (8.1-8.1) | 61±11 | 103±7 | 196±71 | 1.6 |

[a] The data for pH, alkalinity, hardness, and conductivity had n=3, temperature and dissolved oxygen (DO) values had n=7, dissolved organic carbon (DOC) had n=1.

[b] pH water quality reported as median (range)

(Accumet™ Excel XL15 pH meter; Fisher Scientific, Canada). Alkalinity and hardness were measured using the titration method described by the American Public Health Association (APHA, 1989). Water samples for DOC were filtered and stored at -20°C and analyzed by the Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University (Thunder Bay, Ontario).

### *Metal concentration analysis of field collection of water, sediment, and amphipods*

Water, sediment, and amphipods were collected from the seven sites within and from around Quesnel Lake from May 31 – June 5, 2018. All equipment was triple rinsed with site water before being used to collect samples. Water samples were collected on site in triplicate and filtered through 0.45 μm filters. The water samples were preserved with nitric acid to 5% (trace metal grade; Fisher Scientific; Canada). Sediment samples were also collected in triplicate and were preserved by freezing at -20°C. Amphipods were collected from the littoral zone at each sampling site in water no greater than one meter deep using sweep nets. Collected animals were placed into acid washed Nalgene bottles that were filled with site water and aerated. Upon return to the laboratory, amphipods were randomly separated into six evenly numbered replicates and euthanized with 70% ethanol. Replicates from a single site had the same number of animals but between sites numbers varied based on animal abundance (ranged from 372-780 animals per site). Amphipod replicates were placed into individual glass vials and dried until constant weight was achieved.

Collected water, sediment, and amphipods samples were sent to the Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University for metal analysis using inductively coupled plasma mass spectrometry (ICP-MS) for water or inductively coupled plasma atomic emission spectroscopy (ICP-AES) for sediment and amphipods. The dried amphipods and sediment samples were digested in a 3:1 ratio of $HNO_3$: HCl nitric acid in closed Teflon vessels using microwave-assisted acid digestion using Mars Xpress (US EPA, 2007). Water samples were digested similarly but just used $HNO_3$. Following digestion, the amphipod samples were diluted to 25 mL and the sediment and water samples were diluted to 40 mL, with deionized water. Supernatant from the water, sediment, and amphipod digest were analyzed. The original ICP-MS and ICP-AES results measured the concentration of 14 metals for water, 29 metals for sediment, and 14 metals for field-collected amphipods.

Analysis of water was carried out on an Elan DRC-e device (PerkinElmer SCIEX; USA). A 1 ppb quality control was run to gauge the accuracy of the analysis (mean recovery 94.2%). Analytical duplicates were run to ensure consistent machine measurements (mean difference 0.6%). Analysis of sediment and whole-body amphipods was carried out on a Varian Vista Pro ICP-OES device (Varian, Inc., USA). A 1 ppb quality control was run for machine accuracy (mean recovery for sediment and amphipods 92.1% and 89.8%, respectively). Analytical duplicates were run for sediment and amphipods with a mean difference of 2.9% and 4.9%, respectively.

Site water quality (dissolved oxygen (DO), temperature, conductivity, and pH) was measured on-site with a Professional Plus Multiparameter Instrument (YSI, USA) (Table 2.2). Water samples were collected in falcon tubes and taken back to the laboratory to measure water hardness and alkalinity using the titration method described by the American Public Health Association (Table 2.2) (APHA, 1989). The DOC samples were filtered and frozen at -20°C and analyzed by the Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University.

### *Statistical Analysis*

For the sediment exclusion study, survival, weight, and amphipod-Cu concentrations were analyzed via two-way analysis of variance (ANOVA) to determine the response *H. azteca* have to exposure to site water and sediment, depending on sediment access. A post-hoc Tukey’s test was used to determine differences if the two-way ANOVA resulted in significant differences. Further, correlations were determined between *H. azteca* whole-body Cu concentrations and survival or weight.

Table 2. 2. Water quality (±SD) for sampling sites in Little, Bootjack, Polley, and Quesnel Lake[a]

| **Sample** | **Temp (°C)** | **DO (% $O_2$)** | **pH** | **Alkalinity (mg/L as $CaCO_3$)** | **Hardness (mg/L as $CaCO_3$)** | **Conductivity (µS/cm)** | **DOC (mg/L)** |
|---|---|---|---|---|---|---|---|
| Little Lake | 15.5 | 97.0 | 7.36 | 104±3.1 | 123±2.1 | 212.6 | 7.2±0.1 |
| Bootjack Lake | 13.1 | 88.2 | 7.75 | 43±1.5 | 87±2.5 | 87.0 | 6.1±0.1 |
| Polley Lake | 13.7 | 97.3 | 7.86 | 96±2.0 | 158±3.1 | 238.3 | 5.3±1.0 |
| Shoals Bay | 9.6 | 98.2 | 7.74 | 51±2.5 | 89±2.4 | 78.9 | 2.0±0.1 |
| Horsefly Bay | 11.0 | 84.9 | 7.72 | 100±7.8 | 44±2.0 | 62.1 | 4.2±0.1 |
| Cedar Point | 9.3 | 98.7 | 7.79 | 54±1.5 | 78±2.5 | 94.5 | 2.5±0.1 |
| Raft Creek | 7.0 | 96.2 | 7.89 | 51±2.1 | 104±2.5 | 83.9 | 2.0±0.4 |

[a] Data for temperature, dissolved oxygen (DO), pH, and conductivity had n=1, because measurements were taken *in situ* at the time of sampling. Alkalinity, hardness, and dissolved organic carbon (DOC) had n=3.

A principle component analyses (PCA) ordination was performed on the ICP-MS and ICP-AES datasets. The PCA assisted with visualizing the variability of metal concentrations among sites. Metals/metalloid removed because they were below detection limits from the water dataset were Cd, Co, and Se, sediment had Ar, Cd, Mo, Pb, Sb, Se, Sn, and Tl removed, and amphipods had Ar, Ni, Se, and V removed. For sites within Quesnel Lake, Pearson correlations were used to detect associations between individual metals that were measured in both the water and sediment in relation to whole-body amphipod metal concentrations. The metals that Pearson correlations were performed on were Al, Ca, Cr, Cu, Fe, Mg, Na, and Zn.

For water, sediment, and amphipods collected from Quesnel, Little, Polley, and Bootjack Lake, a one-way ANOVA was used to compare Cu concentrations among sites followed by a post-hoc Tukey's test if significant differences were found. All the statistical analyses were performed using R, version 3.2.5 (R Core Team, 2018). Mean differences in all tests were considered to be statistically significant when $p \leq 0.05$.

## Results

### *Sediment exclusion*

*Hyalella azteca* survived the entire 14-d exposure in control treatments and showed no significant mortality (Figure 2.3A). However, animals that were allowed access to Polley Lake sediment experienced a reduction in survival of 12.5% compared to controls ($F_{7,80}$=3.35, p=0.003. Animals exposed to Raft Creek sediment, regardless of sediment-access or not, experienced 15.3% and 12.5% higher mortality than controls. It was found that amphipod growth was not affected by ability to access to the sediment ($F_{2,80}$=0.72, p=0.65). Growth was affected by site but only between sites in Quesnel Lake (Horsefly Bay, Cedar Point, and Raft Creek) in comparison to Little Lake, the outside Quesnel Lake reference site ($F_{7,80}$=5.46, p=0.02) (Figure 2.3B). Amphipod whole-body Cu concentration was also unaffected by access to the sediment ($F_{2,32}$=1.09, p=0.39) but was affected by which site the water and sediment was collected from ($F_{7,32}$=7.75, p=0.009) (Figure 2.3C). Animals that were exposed to water and sediment from Bootjack and Polley Lake had increased Cu accumulation compared to laboratory controls but not to the reference site Little Lake. Within Quesnel Lake, there is increasing Cu accumulation with decreasing distance from where the tailing material from the 2014 MPMC tailing impoundment breach entered the lake. Animals exposed to Raft Creek water and sediment experienced some of the highest Cu bioaccumulation which correlates with the reduction in survival observed in the present study. A similarly high Cu

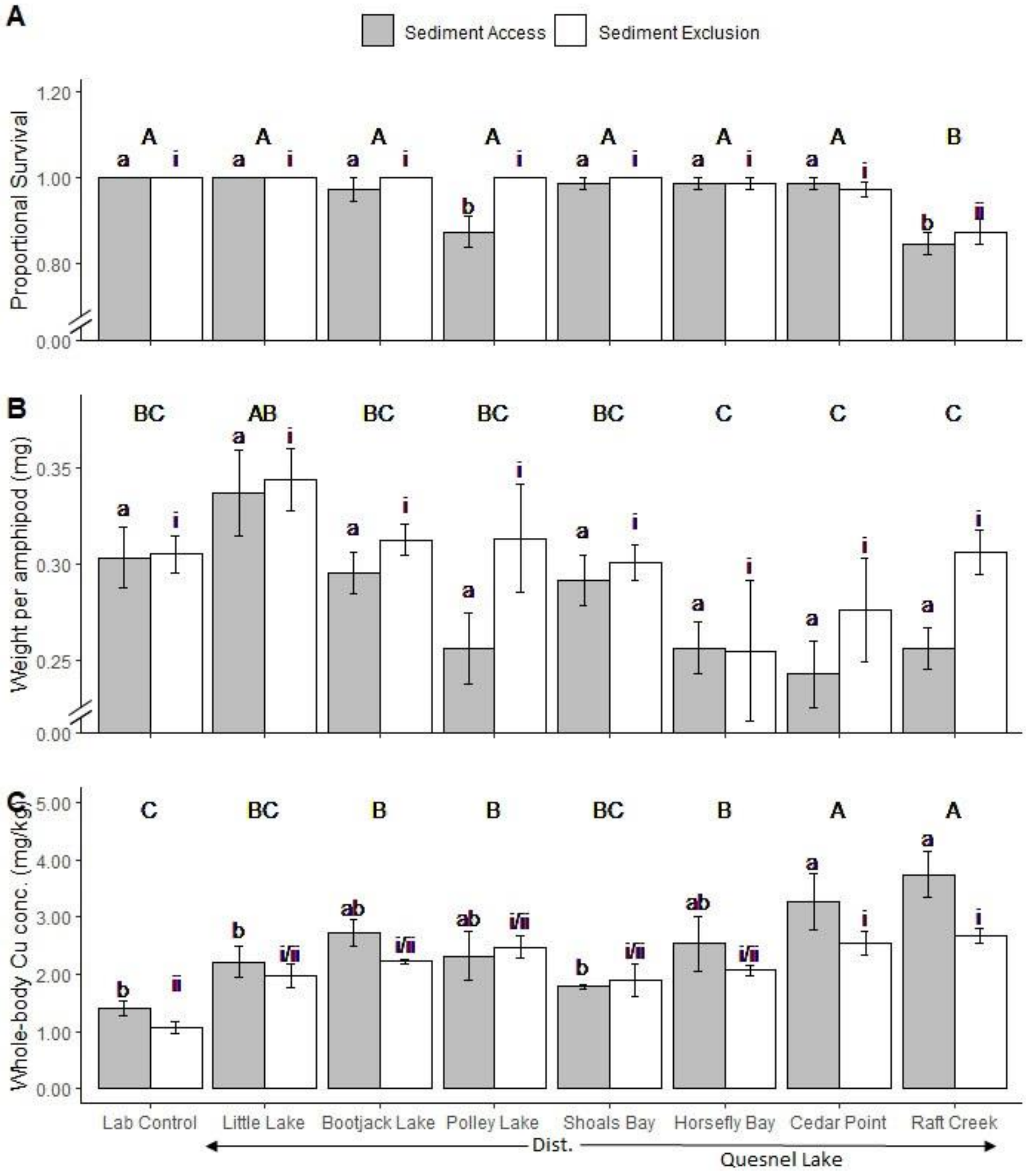

Figure 2. 3. Mean (A) survival, (B) growth, and (C) whole-body Cu concentration ± SE for *H. azteca* exposed in a sediment exclusion experiment with water and sediment collected from Little, Bootjack, Polley and Quesnel Lake in relation to distance (Dist.) from the MPMC mine. Uppercase letters, asterisks, lowercase letters, and roman numerals denote significant differences among sites, exclusion within site, sediment access, and sediment excluded treatments, respectively ($p \leq 0.05$). Survival and growth n=8, *H. azteca*-Cu concentration, n=4.

accumulation was not observed in amphipods exposed to Polley Lake sediment to account for the reduction in survival. Oppositely, Cedar Point *H. azteca* had high Cu bioaccumulation but no accompanying effect on survival. The correlation between whole-body Cu concentration and survival was weakly negative (R=-0.57, p=0.02) as was whole-body Cu and weight (R=-0.48, p=0.06).

### *Site water, sediment, and amphipod ICP-MS and ICP-AES analysis*

Average water, sediment, and whole-body amphipod metal concentrations are shown in Table 2.3, Table 2.5, and Table 2.6, respectively. The only water-metal found to be above British Columbia Ministry of the Environment BCENV approved and working water quality guidelines (BCMOE, 2017, 2018) (for metals that have guidelines) was Al in Polley Lake, Horsefly Bay, and Raft Creek (Table 2.4). Sediment-metals found in the study sites above BCENV working sediment quality guidelines (BCMOE, 2017) (for metals that have guidelines) were; Cu in Bootjack Lake, Polley Lake, and Raft Creek, Mn in Polley Lake and Raft Creek, and Ni in Bootjack Lake, Cedar Point and Raft Creek.

Since a PCA was used on the total metal concentrations for water, sediment, and whole-body amphipods, a scree analysis was performed to determine the principle components that contained the most information. It was found that only the first three components contained significant information on metal concentration with total variability explained of 95.5% for water (Table 2.7), 91.6% for sediment (Table 2.8), and 95.7% (Table 2.9) for whole-body amphipod Cu-concentrations. For water, the ordination in the planes of PC1 and PC2 resulted in distinct clusters of sites (Figure 2.4). One cluster included Cedar Point and Raft Creek (i.e. near-field of the directly affected area in

Table 2. 3. Total metal concentration in water samples (n=3) collected from Little, Polley, Bootjack and Quesnel Lake.

| **Sample Site** | | **Al (mg/L)** | **As (µg/L)** | **Ca (mg/L)** | **Cr (µg/L)** | **Cu (µg/L)** | **Fe (mg/L)** | **Mg (mg/L)** | **Na (mg/L)** | **Ni (µg/L)** | **V (µg/L)** | **Zn (µg/L)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection Limit | | 0.03 | 0.06 | 0.01 | 0.16 | 0.22 | 0.01 | 0.01 | 0.05 | 0.20 | 0.20 | 0.25 |
| Little Lake | Mean | ND | 0.35 | 30.53 | 0.78 | 1.19 | ND | 5.31 | 11.21 | 0.35 | 1.23 | 1.74 |
| | SEM | - | 0.02 | 0.32 | 0.01 | 0.05 | 0.00 | 0.01 | 0.04 | 0.08 | 0.08 | 0.09 |
| Bootjack Lake | Mean | ND | 0.30 | 14.23 | 0.22 | 1.64 | 0.02 | 2.49 | 2.56 | 0.47 | 0.45 | 2.43 |
| | SEM | - | 0.01 | 0.07 | 0.00 | 0.06 | 0.00 | 0.02 | 0.08 | 0.01 | 0.03 | 0.71 |
| Polley Lake | Mean | 0.07 | 0.69 | 40.29 | 0.42 | 3.53 | 0.01 | 5.54 | 8.98 | 0.46 | 1.54 | 3.07 |
| | SEM | 0.01 | 0.02 | 0.32 | 0.03 | 0.02 | 0.00 | 0.03 | 0.18 | 0.05 | 0.02 | 0.63 |
| *Quesnel Lake* | | | | | | | | | | | | |
| Shoals Bay | Mean | ND | 0.09 | 16.39 | 0.18 | 0.53 | 0.01 | 2.03 | 1.15 | 0.64 | 0.34 | 1.12 |
| | SEM | - | 0.01 | 0.61 | 0.01 | 0.04 | 0.00 | 0.14 | 0.42 | 0.05 | 0.01 | 0.24 |
| Horsefly Bay | Mean | 0.16 | 0.27 | 12.37 | 0.25 | 6.10 | 0.05 | 1.86 | 1.93 | 1.02 | 0.58 | 5.05 |
| | SEM | 0.12 | 0.04 | 0.94 | 0.05 | 1.20 | 0.01 | 0.05 | 1.53 | 0.27 | 0.12 | 4.91 |
| Cedar Point | Mean | ND | 0.39 | 20.68 | 0.30 | 0.84 | 0.01 | 2.45 | 1.15 | 0.61 | 0.52 | 1.43 |
| | SEM | - | 0.04 | 0.08 | 0.05 | 0.08 | 0.00 | 0.01 | 0.07 | 0.07 | 0.03 | 0.05 |
| Raft Creek | Mean | 0.09 | 0.12 | 19.41 | 0.24 | 0.99 | 0.01 | 2.25 | 1.61 | 0.63 | 0.51 | 2.59 |
| | SEM | 0.05 | 0.01 | 0.14 | 0.02 | 0.07 | 0.00 | 0.02 | 0.39 | 0.03 | 0.01 | 0.70 |
| BCENV guideline | | 0.05 | 5.00 | - | - | ≤2-4.9* | 0.35 | - | - | 25-135* | - | 7.5-58* |

*Note:* BCENV guidelines were from the approved and working guidelines (BCMOE, 2017, 2018), ND = not detected
*Guideline values calculated using the range of hardness found at the sample sites. See Table 2.4 for exact values

Table 2. 4. Exact BCEVN guidelines concentrations for metals adjusted to site specific water hardness*

| **Sample Site** | **Cu (µg/L)** | **Ni (µg/L)** | **Zn (µg/L)** |
|---|---|---|---|
| Little Lake | 4.92 | 112 | 10.0 |
| Bootjack Lake | 3.48 | 85.9 | 7.5 |
| Polley Lake | 6.32 | 135 | 58.5 |
| *Quesnel Lake* | | | |
| Shoals Bay | 3.56 | 87.5 | 7.5 |
| Horsefly Bay | 1.76 | 25.0 | 7.5 |
| Cedar Point | 3.12 | 79.1 | 7.5 |
| Raft Creek | 4.16 | 98.5 | 18.0 |

*Water hardness taken from Table 2.2

Table 2. 5. Total metal concentrations (mg/kg) in sediment (n=3) collected from Little, Polley, Bootjack and Quesnel Lake.

| Sample site | | Al | Ba | Be | Ca | Co | Cr | Cu | Fe | K | Mg | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection Limit | | 0.03 | 0.10 | 0.04 | 0.06 | 0.20 | 0.03 | 0.05 | 0.05 | 1.0 | 0.20 | 0.05 |
| Little Lake | Mean | 7583 | 22.9 | 0.17 | 6340 | 6.92 | 11.09 | 24.67 | 11569 | 310.4 | 5813 | 279.3 |
| | SEM | 300 | 0.7 | 0.00 | 307.3 | 0.10 | 0.76 | 0.65 | 467 | 9.6 | 107.1 | 10.9 |
| Bootjack Lake | Mean | 6049 | 55.7 | 0.14 | 3679 | 9.86 | 53.10 | 51.97 | 14888 | 309.5 | 13161 | 299.6 |
| | SEM | 348 | 3.4 | 0.01 | 209.3 | 0.58 | 9.85 | 0.82 | 345 | 19.8 | 1559 | 4.8 |
| Polley Lake | Mean | 18059 | 40.3 | 0.25 | 7683 | 11.23 | 11.20 | 38.55 | 19543 | 318.3 | 9088 | 473.1 |
| | SEM | 528 | 10.2 | 0.01 | 159.2 | 0.29 | 1.08 | 1.54 | 1133 | 11.0 | 504.9 | 20.0 |
| *Quesnel Lake* | | | | | | | | | | | | |
| Shoals Bay | Mean | 2227 | 8.5 | 0.05 | 659.3 | 1.91 | 3.80 | 2.68 | 6089 | 302.6 | 1472 | 44.6 |
| | SEM | 161 | 0.6 | 0.01 | 79.06 | 0.02 | 0.32 | 0.32 | 278 | 9.7 | 21.3 | 1.0 |
| Horsefly Bay | Mean | 5906 | 18.0 | 0.13 | 16187 | 6.28 | 17.49 | 22.34 | 12567 | 467.7 | 3410 | 174.2 |
| | SEM | 350 | 1.5 | 0.01 | 774.9 | 0.37 | 1.23 | 2.12 | 523 | 39.8 | 21.4 | 5.1 |
| Cedar Point | Mean | 7155 | 11.9 | 0.10 | 14012 | 7.69 | 20.98 | 25.02 | 15724 | 215.7 | 6537 | 230.7 |
| | SEM | 176 | 0.6 | 0.00 | 342.8 | 0.22 | 0.84 | 2.95 | 652 | 3.1 | 191.1 | 6.7 |
| Raft Creek | Mean | 1762 | 79.9 | 0.24 | 16151 | 11.56 | 13.83 | 42.68 | 19239 | 854.3 | 9248 | 469.7 |
| | SEM | 942 | 2.3 | 0.01 | 681.6 | 0.43 | 0.90 | 0.75 | 1453 | 85.2 | 434.4 | 29.9 |
| BCEVN guideline | | - | - | - | - | - | 37.30 | 35.70 | 21200 | - | - | 460 |

| Sample site | | Na | Ni | P | Pb | S | Si | Sr | Ti | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection Limit | | 0.20 | 0.2 | 1.0 | 1.0 | 1.0 | 2.0 | 0.20 | 2.0 | 0.40 | 0.03 |
| Little Lake | Mean | 1474 | 10.7 | 793.6 | ND | 94.9 | 628.4 | 45.5 | 140.0 | 26.7 | 32.39 |
| | SEM | 92 | 0.7 | 15.37 | - | 1.5 | 32.0 | 4.3 | 11.67 | 0.9 | 1.16 |
| Bootjack Lake | Mean | 1205 | 82.4 | 585.5 | 14.5 | 139.4 | 547.2 | 35.4 | 192.5 | 23.0 | 35.91 |
| | SEM | 53 | 12.3 | 9.89 | 6.07 | 18.1 | 53.0 | 1.9 | 11.75 | 1.0 | 0.55 |
| Polley Lake | Mean | 6635 | 9.7 | 1448 | 7.69 | 126.9 | 703.2 | 60.7 | 292.5 | 46.4 | 41.19 |
| | SEM | 446 | 0.4 | 87.22 | 2.56 | 7.5 | 15.4 | 13.2 | 25.6 | 0.7 | 2.40 |
| *Quesnel Lake* | | | | | | | | | | | |
| Shoals Bay | Mean | 1066 | 6.74 | 259.9 | ND | 13.6 | 256.8 | 3.8 | 80.4 | 4.1 | 11.71 |
| | SEM | 39 | 0.73 | 32.29 | - | 1.7 | 12.3 | 0.6 | 1.6 | 0.2 | 0.64 |
| Horsefly Bay | Mean | 1003 | 13.4 | 279.6 | ND | 144.3 | 581.1 | 75.8 | 236.6 | 22.7 | 21.93 |
| | SEM | 97 | 0.9 | 12.9 | - | 42.1 | 12.8 | 4.1 | 5.6 | 0.6 | 1.03 |
| Cedar Point | Mean | 858 | 18.8 | 512.4 | 7.20 | 107.7 | 726.6 | 74.3 | 187.7 | 31.9 | 32.91 |
| | SEM | 7 | 0.9 | 62.9 | 2.40 | 21.6 | 32.4 | 4.1 | 9.0 | 1.5 | 1.79 |
| Raft Creek | Mean | 1495 | 18.8 | 1049. | ND | 150.1 | 794.2 | 132.1 | 301.2 | 44.4 | 36.11 |
| | SEM | 112 | 1.1 | 27.28 | - | 27.1 | 6.7 | 7.3 | 6.1 | 1.2 | 1.70 |
| BCENV guideline | | - | 16.0 | - | 35.0 | - | - | - | - | - | 123.00 |

*Note:* BCENV guidelines were taken from both the approved and working guidelines, ND = not detected

Table 2. 6. Total metal concentrations (mg/kg) in whole-body amphipods (n=6) collected from Little, Polley, Bootjack and Quesnel Lake.

| **Sample site** | | **Al** | **Ca** | **Cd** | **Co** | **Cr** | **Cu** | **Fe** | **Mg** | **Na** | **Zn** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection Limit | | 1.50 | 0.50 | 0.75 | 0.50 | 0.80 | 0.10 | 0.25 | 0.50 | 1.00 | 0.20 |
| Little Lake | Mean | 391.2 | 86864 | ND | ND | 0.80 | 61.31 | 900.9 | 1725 | 4562 | 66.7 |
| | SEM | 70.2 | 1896 | - | - | 0.00 | 1.01 | 200.0 | 44.6 | 227 | 2.8 |
| Bootjack Lake | Mean | 241.5 | 86335 | ND | ND | 3.49 | 77.78 | 467.6 | 1643 | 6051 | 70.3 |
| | SEM | 50.2 | 1846 | - | - | 2.43 | 2.50 | 109.4 | 32.4 | 325 | 4.53 |
| Polley Lake | Mean | 393.7 | 86050 | ND | ND | 0.93 | 69.72 | 997.3 | 1505 | 5185 | 62.3 |
| | SEM | 81.1 | 3135 | - | - | 0.13 | 2.65 | 181.5 | 21.9 | 516 | 0.49 |
| *Quesnel Lake* | | | | | | | | | | | |
| Shoals Bay | Mean | 117.5 | 80740 | ND | ND | ND | 57.56 | 523.9 | 1208 | 4618 | 64.3 |
| | SEM | 29.5 | 2163 | - | - | - | 1.53 | 202.2 | 33.8 | 340 | 1.59 |
| Horsefly Bay | Mean | 291.7 | 85296 | ND | ND | ND | 64.45 | 568.1 | 1502 | 4942 | 67.6 |
| | SEM | 41.2 | 1645 | - | - | - | 1.13 | 64.8 | 29.9 | 354 | 1.45 |
| Cedar Point | Mean | 419.3 | 77434 | 1.06 | 0.73 | 1.93 | 71.19 | 831.7 | 1433 | 4396 | 65.6 |
| | SEM | 137.9 | 2606 | 0.07 | 0.23 | 0.51 | 1.40 | 266.2 | 82.0 | 397 | 0.52 |
| Raft Creek | Mean | 435.2 | 84366 | ND | ND | 1.17 | 80.03 | 562.1 | 1519 | 5181 | 63.7 |
| | SEM | 131.9 | 1798 | - | - | 0.37 | 1.52 | 189.8 | 52.5 | 466 | 1.01 |

*Note:* ND = not detected

Quesnel Lake), Shoals Bay (i.e. Quesnel Lake reference) and Bootjack Lake (near-mine site). Another cluster included Little Lake (i.e. Polley and Bootjack Lake reference) and Polley Lake (i.e. directly affected). Horsefly Bay (i.e. far-field site in Quesnel Lake) was on its own. Magnesium had the highest positive eigenvalue loading on PC1, whereas Ni had the highest negative loading (Table 2.7). On PC2, the highest negative loading was for Cu and there were no positive loadings. Chromium had the highest loading on PC3, whereas As had the highest negative loading.

The clustering of sites along the planes of PC1 and PC2 for water-metal concentrations can be attributed to certain metals. The metals that account for the most variability along the plane of PC1 are Ca and Mg (correlation of 0.90 and 0.94, respectively) which agrees with the hardness measured at each site. Little Lake and Polley

Table 2. 7. Results of principle component analysis on the concentration of metals in water from Quesnel, Polley, Bootjack, and Little Lake.

| Water | PC1 | PC2 | PC3 |
|---|---|---|---|
| Variance explained (%) | 54.0 | 35.4 | 6.0 |
| Cumulative explained variance (%) | 54.0 | 89.4 | 95.5 |
| | | | |
| *Eigenvectors* | | | |
| Al | -0.220 | -0.401 | 0.052 |
| As | 0.263 | -0.266 | **-0.590** |
| Ca | 0.367 | -0.152 | -0.262 |
| Cr | 0.325 | -0.122 | **0.644** |
| Cu | -0.133 | **-0.472** | -0.068 |
| Fe | -0.272 | -0.352 | 0.224 |
| Mg | **0.385** | -0.172 | 0.040 |
| Na | 0.360 | -0.195 | 0.323 |
| Ni | **-0.358** | -0.202 | 0.017 |
| V | 0.337 | -0.283 | -0.064 |
| Zn | -0.177 | -0.442 | -0.010 |

*Note.* Principle components shown were selected based on scree analysis. Boldface values represent the highest and lowest loadings for each component.

Lake have the highest hardness values (Table 2.2) and are clustered together in the positive PC1 direction while Horsefly has the lowest hardness and is in the negative direction of PC1. It is most likely that hardness accounts for the clustering of sites on PC1. The clustering on PC2 is the most influenced by Cu and Zn (correlation of 0.93 and 0.87, respectively) but the sites do not seem to cluster strongly according to these metals. The lack of strong Cu and Zn clustering on PC2 is most likely due to the low total variance explained by PC2 of 17.2% giving PC2 less influence on clustering patterns than PC1.

Table 2. 8. Results of principle component analysis on the concentration of metals in sediment from Quesnel, Polley, Bootjack, and Little Lake.

| Sediment | PC1 | PC2 | PC3 |
|---|---|---|---|
| Variance explained (%) | 62.6 | 17.2 | 11.6 |
| Cumulative explained variance (%) | 62.6 | 79.9 | 91.6 |
| | | | |
| Eigenvectors | | | |
| Al | 0.254 | 0.185 | -0.135 |
| Ba | 0.225 | -0.129 | 0.089 |
| Be | 0.260 | 0.107 | -0.125 |
| Ca | 0.142 | **0.222** | **0.448** |
| Co | **0.278** | -0.090 | -0.028 |
| Cr | 0.065 | -0.508 | 0.116 |
| Cu | 0.240 | -0.282 | 0.008 |
| Fe | **0.274** | 0.008 | -0.010 |
| K | 0.147 | 0.164 | 0.343 |
| Mg | 0.212 | -0.344 | -0.081 |
| Mn | 0.274 | 0.026 | -0.128 |
| Na | 0.140 | 0.135 | **-0.498** |
| Ni | 0.053 | **-0.523** | 0.068 |
| P | 0.229 | 0.115 | -0.345 |
| S | 0.236 | -0.102 | 0.237 |
| Si | 0.248 | 0.090 | 0.126 |
| Sr | 0.215 | 0.187 | 0.353 |
| Tl | 0.258 | 0.101 | 0.088 |
| V | 0.268 | 0.128 | -0.058 |
| Zn | 0.254 | -0.111 | -0.152 |

*Note.* Principle components shown were selected based on scree analysis. Boldface values represent the highest and lowest loadings for each component.

Table 2. 9. Results of principle component analysis on the concentration of metals in whole-body amphipods collected from Quesnel, Polley, Bootjack, and Little Lake.

| Amphipods | PC1 | PC2 | PC3 |
|---|---|---|---|
| Variance explained (%) | 37.2 | 26.3 | 6.6 |
| Cumulative explained variance (%) | 37.2 | 63.5 | 95.7 |
| | | | |
| Eigenvectors | | | |
| Al | -0.125 | -0.286 | **0.544** |
| Ca | 0.419 | 0.153 | 0.345 |
| Cd | -0.383 | -0.401 | -0.078 |
| Co | **-0.381** | -0.403 | -0.095 |
| Cr | 0.233 | **-0.473** | **-0.245** |
| Cu | 0.191 | -0.445 | 0.099 |
| Fe | -0.250 | -0.016 | 0.508 |
| Mg | 0.273 | -0.237 | 0.425 |
| Na | **0.456** | -0.174 | -0.055 |
| Zn | 0.284 | -0.251 | **-0.242** |

*Note.* Principle components shown were selected based on scree analysis. Boldface values represent the highest and lowest loadings for each component.

For sediment, the ordination in the planes of PC1 and PC2 resulted in two distinct clusters (Figure 2.5). Polley Lake and Raft Creek (i.e. near-field and directly affected sites) clustered together. Little Lake (i.e. Polley and Bootjack Lake reference), Horsefly Bay (i.e. far-field site in Quesnel Lake) and Cedar Point (i.e. near-field site in Quesnel Lake) resulted in a second cluster. Bootjack Lake was on its own as well as Shoals Bay. Iron and Co had the highest positive eigenvalues loading on PC1 and there were no negative loadings (Table 3.5). On PC2, the highest negative loading was for Ni whereas the highest positive loading was for Ca. Ca had the highest loading on PC3, whereas Na had the highest negative loading. Site clustering along the planes of PC1 and PC2 for

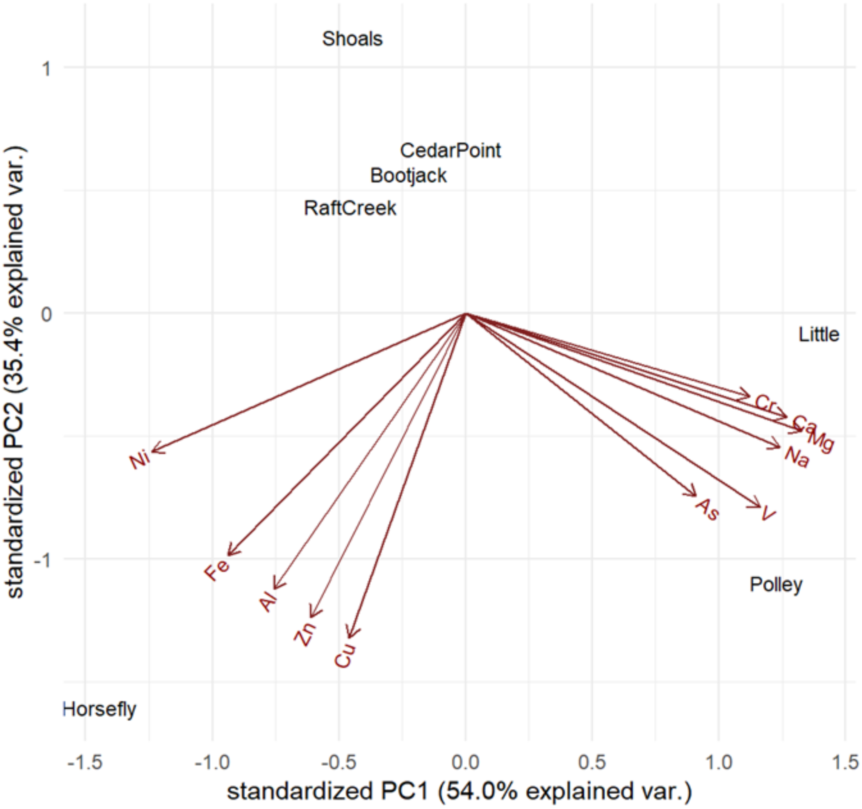

Figure 2. 4. Principle component analysis (PCA) ordination plot of water-metal concentrations in the plane of PC1 and PC2. Each label represents a study site in Polley, Little, Bootjack, or Quesnel Lake (Raft Creek, Cedar Point, Horsefly Bay, and Shoals Bay). Metal concentrations are represented by vectors with vector length proportional to correlation coefficients, and vector direction indicative of the direction of maximum correlation.

sediment-metal concentrations can be attributed to by a large range of metals. PC1 is highly correlated with many metals including Co, Fe, Mn, V, Be, Ti, and Al all correlating at greater than or equal to 0.90 and Cu at 0.85. The highest correlation for PC2 were Ni and Cr at 0.97 and 0.94, respectively, which were especially high in Bootjack Lake compared to the other sampling sites.

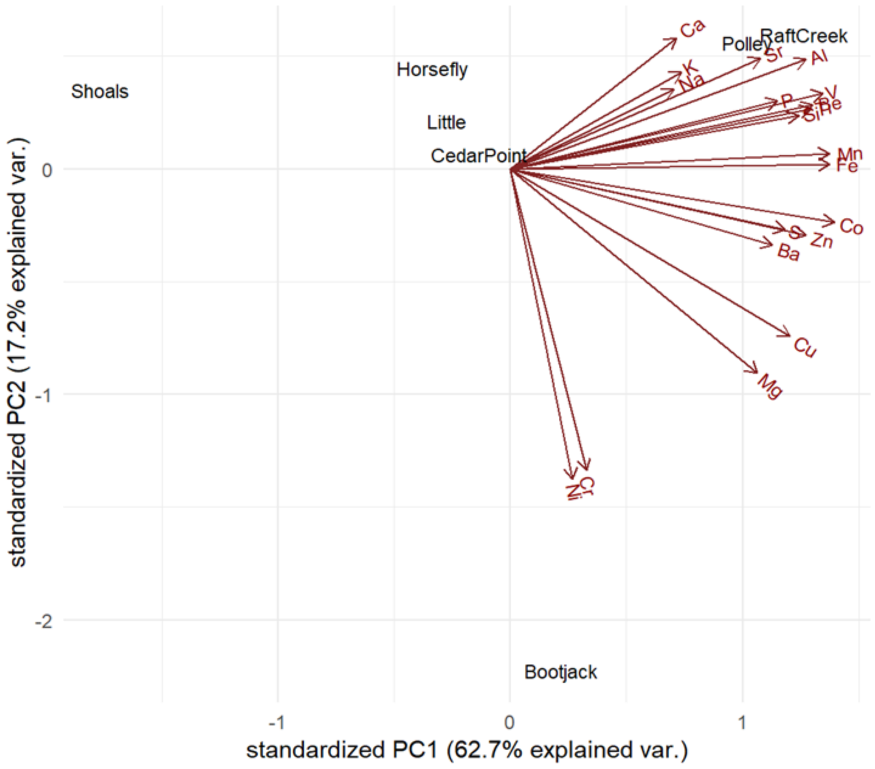

Figure 2. 5. Principle component analysis (PCA) ordination plot of sediment metal concentrations in the plane of PC1 and PC2. Each label represents a study site in Polley, Little, Bootjack, or Quesnel Lake (Raft Creek, Cedar Point, Horsefly Bay, and Shoals Bay). Metal concentrations are represented by vectors with vector length proportional to correlation coefficients, and vector direction indicative of the direction of maximum correlation relative to the ordination plane.

For whole-body amphipod metal concentration, the ordination in the planes of PC1 and PC2 resulted in one distinct cluster (Figure 2.6): Polley Lake and Raft Creek (i.e. near-field and directly affected sites), Little Lake (i.e. Polley and Bootjack Lake reference), and Horsefly Bay (i.e. far-field site in Quesnel Lake). Bootjack (near-mine site), Cedar Point (i.e. near-field site in Quesnel Lake) and Shoals Bay (i.e. Quesnel Lake reference) were on their own. Sodium had the highest positive eigenvalue loading on PC1 and whereas Co had the highest negative loading (Table 6). On PC2, the highest negative loading was for Cr and there were no positive loadings. Al had the highest loading on

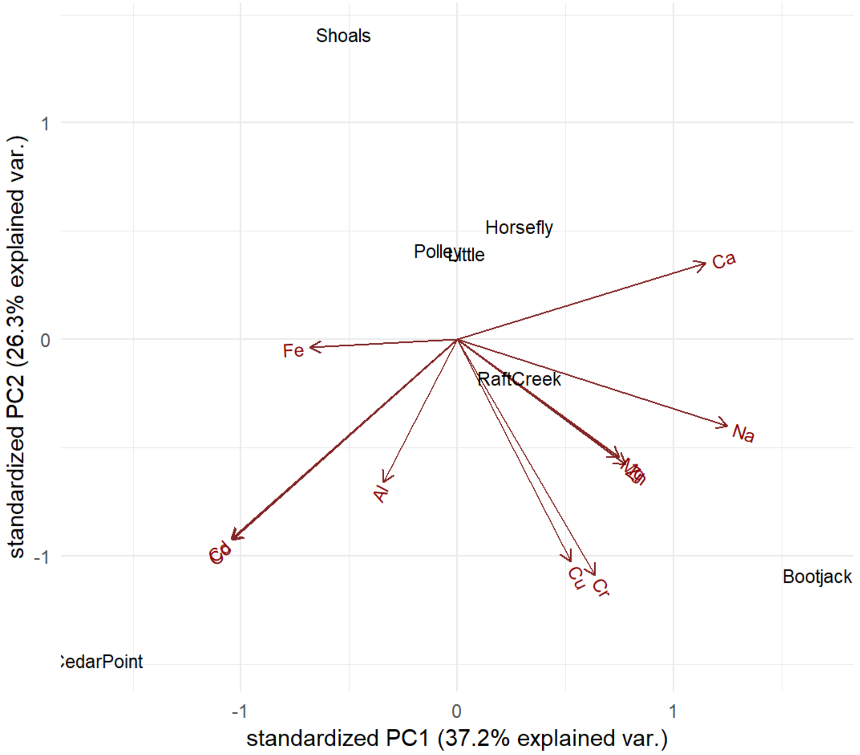

Figure 2. 6. Principle component analysis (PCA) ordination plot of whole-body amphipod metal concentrations in the plane of PC1 and PC2. Each label represents a study site in Polley, Little, Bootjack, or Quesnel Lake (Raft Creek, Cedar Point, Horsefly Bay, and Shoals Bay). Metal concentrations are represented by vectors with vector length proportional to correlation coefficients, and vector direction indicative of the direction of maximum correlation relative to the ordination plane.

PC3, whereas Cr had the highest negative loading.

The clustering of sites along the planes of PC1 and PC2 for whole-body amphipod were mostly attributed to by fewer metals than for water or sediment. The metals most associated with PC1 were Na and Ca at 0.88 and 0.80 as a correlation, respectively. Correlation for PC2 were generally lower than PC1 with Cr at 0.77 and Cu at 0.72 as the most highly correlated metals along PC2.

To investigate the correlation of whole-body amphipod metal concentrations with water or sediment, Pearson correlations were carried out with water and sediment metal concentrations found in Quesnel Lake (Table 2.10). High correlation was found between whole-body amphipod-Cu concentrations and sediment-Cu concentrations. In contrast, amphipod-Cu and water-Cu concentration correlated at -0.20. The high amount of difference found between the correlation values is indicative of bioavailable sediment-Cu rather than water-Cu. The same could be said for Al, with high sediment correlation and low water correlation. Sodium in the sediment and water was found to also highly correlate with amphipod-Na concentrations which could be facilitating Cu uptake in the animals.

Table 2. 10. Pearson correlation coefficients (r) between whole-body amphipod metal concentrations and water or sediment metal concentrations from Quesnel Lake.

| Metal | Water | Sediment |
|---|---|---|
| Al | 0.14 | **0.77** |
| Ca | -0.64 | 0.36 |
| Cr | **0.81** | 0.66 |
| Cu | -0.20 | **0.97** |
| Fe | -0.21 | 0.37 |
| Mg | 0.15 | 0.71 |
| Na | **0.77** | **0.84** |
| Zn | 0.73 | -0.16 |

*Note.* Boldface value represents the high correlation values

The potential for Cu to be bioavailable to amphipods was further explored since it was identified as a metal of potential concern and had high correlation between whole-body amphipod Cu concentrations and sediment-Cu concentrations. Amphipod whole-body Cu concentrations were found to differ among study sites ($F_{6,35}$=21.83, p< 0.001) with the highest Cu concentrations found in Bootjack Lake (i.e. near-mine site), Polley Lake, and Raft Creek (i.e. directly affected and near-field sites) and lowest in Little Lake, Shoals Bay (i.e. reference sites) and Horsefly Bay (i.e. far-field site) (Figure 2.7C). In agreement with the Pearson correlation test, amphipod-Cu concentrations were much more reflective of sediment concentrations than water concentrations (Figure 2.7). Among the Quesnel Lake sampling sites, whole-body amphipod-Cu and sediment Cu concentrations decreased with increasing distance from where the tailings material entered the lake in 2014. Water Cu concentrations within the Quesnel Lake sampling sites did not exhibit a similar pattern. Polley Lake had increased water, sediment, and amphipod Cu concentrations compared to Little Lake. Of note, Cu concentrations were highest in Bootjack Lake ($F_{6,14}$=105.9, $p< 0.001$) and Horsefly Bay ($F_{6,14}$=58.14, $p< 0.001$) in sediment and water, respectively; neither of which received direct input from the tailings breach.

## Discussion

A short-term laboratory sediment exclusion study has demonstrated that when laboratory raised amphipods were exposed to water and sediment from sites affected by the 2014 MPMC tailings impoundment breach, there were resulting toxicological effects. The *H. azteca* that were allowed access to Polley Lake sediment, and animals exposed to Raft Creek regardless of their ability to access the sediments, showed higher mortality than controls and reference sites. There was, however, no effect of sediment access or site on growth between relevant sites.

Specifically, in the sediment exclusion study we found that Cu from water and

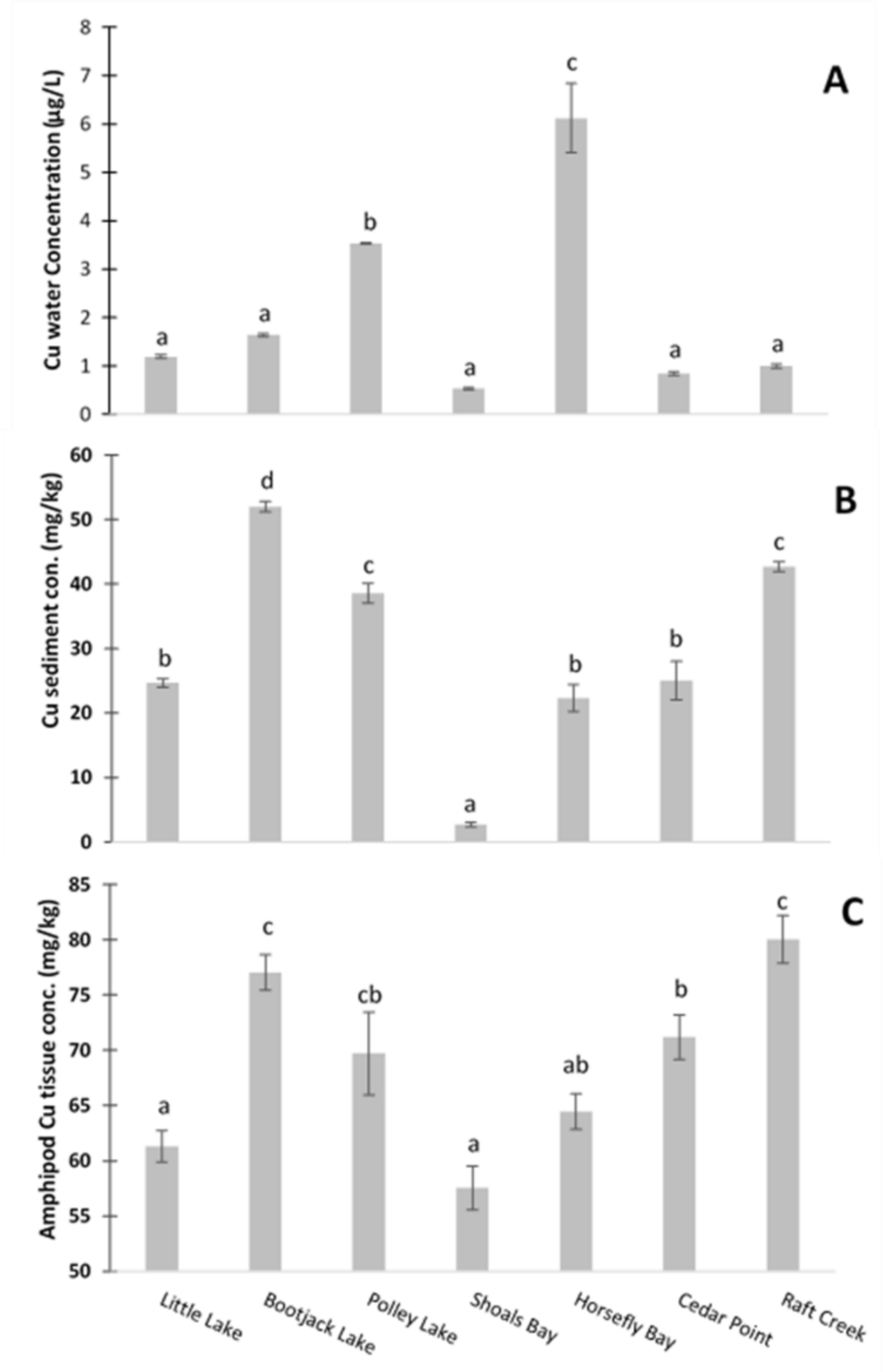

Figure 2. 7. Mean Cu concentration (± SEM) for (A) water, (B) sediment, and (C) whole-body amphipod Cu concentration collected from sites in Little, Bootjack, Polley, and Quesnel Lake (Shoals Bay, Horsefly Bay, Cedar Point, and Raft Creek) measure via ICP-MS. Water and sediment n=3, amphipod-Cu concentration, n=6. Letters denote significant differences ($p < 0.05$).

sediment collected from Quesnel and Polley Lake is bioavailable to amphipods. Sediments appeared to be more relevant for explaining the whole-body amphipod metal load than water. In the sediment-exclusion study, Cu was found to increase in whole-body *H. azteca* in all contaminated sites relative to laboratory controls. Of the non-reference sites, the most impacted sites from the 2014 MPMC tailing impoundment breach within Quesnel Lake, Cedar Point and Raft Creek, were also found to have 19.2% and 28.4% higher amphipod-Cu concentrations relative to the reference site, Shoals Bay. Borgmann and Norwood (1997) previously demonstrated that *H. azteca* experience 4 week 50% lethal body concentration (LBC 50) of 1.30 and 3.84 µmol/g for Cu. When specifically examining the toxicological potential of Cu, the reduction in survival observed in the present study could be attributed to an imbalance in Cu homeostasis leading to Cu ion initiated Fenton and Fenton-like reactions that lead to oxidative stress causing cell damage and eventual mortality (Gaetke et al., 2014). Whole-body amphipod Cu concentrations were not seen to increase in Polley Lake in conjunction with the observed decrease in survival for *H. azteca* that had sediment access. Previous work done by Borgmann et al. (1993) has shown that *H. azteca* can regulate Cu when exposed to a spiked sediment-Cu concentrations as high as 95 mg/kg. It may be that sediment-bound Cu in Polley Lake is not at a high enough concentration to disrupt the natural Cu homeostasis in the animal. Since the tailings material consisted of several potential contaminants it is possible that a contaminant other than Cu could account for the discrepancy in the survival results.

The results of the field work collections corroborate with the results of the sediment-exclusion study. The present study has revealed similarities in sediment-Cu concentrations and Cu concentrations in whole-body amphipods of both field and laboratory-raised amphipods. The pattern of the sediment-Cu concentrations closely correlates with whole-body amphipod Cu concentrations whereas water-Cu concentrations do not. Within Quesnel Lake, amphipod-Cu concentrations of field collected amphipods and laboratory-raised *H. azteca* increases with decreasing distance from where the tailings entered Quesnel Lake, as does sediment Cu concentrations. The increasing sediment and amphipod-Cu concentrations in relation to distance from where the tailings material entered the lake suggests that Cu associated with the solid tailings from the MPMC tailing impoundment breach is bioavailable to amphipods. Although recent geochemical analyses have suggested that Cu was unlikely to be bioavailable owing to high association with silicate minerals and insoluble sulphides (SRK, 2015), the results of this study suggest otherwise. Ingestion of contaminated sediments by benthic amphipods may contribute to metal bioavailability since the acidic environment of the gut could release sediment-bound Cu into the readily bioavailable Cu ion. It could also be that, as Byrne et al. (2018) suggests, weathering of bound Cu sulphides and reductive dissolution of Cu-bearing Fe oxides observed in Hazeltine Creek is occurring in the remaining tailings material in Quesnel and Polley Lake.

While Cu was a focus for the sediment exclusion study there were several metals associated with the 2014 MPMC tailings breach, and the surrounding geology, that could account for the toxicological effects observed in the experiment. The Cariboo region has had mines for over a century refining Cu, Ag, Au, and Mo (Britton, 2016). The Quesnel Lake area is located upon some of the highest ranked metallic mineral potential areas in the province of British Columbia so a high correlation between many metals in the sediment is not unexpected (Hashmi et al., 2015). The results of the present study indicate more similarities between whole-body amphipod metal concentrations and sediment-metal than water-metal concentrations. Similar results have been found in the mine tailings impacted Torch Lake (MI, USA) that has much higher metal concentrations in the sediment than in overlay water (Cusack & Mihelcic, 1999). Total metals found in the sediment of the present study are most strongly associated with the most affected sites, Polley Lake and Raft Creek. The high metal sediment concentrations could be contributed to by the fine particle colloid material present in the waterbodies after the tailings impoundment breach. Karathanasis (1999) demonstrated that negatively charged colloids enhanced metal transport by 5 – 50 fold over control treatments. A similar transportation of metals could be occurring in Quesnel and Polley Lake from deep water deposited tailings material. The colloidal material could be transported up into the littoral zone during seasonal turnover where amphipods can interact with it.

Correlation between environmental metal concentration and whole-body amphipod metal concentrations suggests that metals in the study lakes are bioavailable. There was high correlation among field amphipod-metal concentrations and waterborne Cr and Na concentrations. Correlations were also high for whole-body amphipod metal concentrations and sediment- metal concentrations for Al, Cu, and Na at the study sites suggesting that these metals are bioavailable. The observed reduction in survival for the sediment exclusion study could be attributed to these metals, especially when considering their combined toxicity. Previous work done by Kamunde et al.(2003) has shown that when the uptake of Na increases at the gill of rainbow trout (*Oncorhynchus mykiss*), the uptake of Cu also increased due to the use of the same membrane transport protein. Since Quesnel Lake is an oligotrophic waterbody, it is likely that freshwater organisms in the lake experience elevated uptake of Na to account for the loss of body ions to the water, contributing to the high amphipod whole-body Cu concentrations observed in the present study. Other cations, such as Al and Cr could be experiencing the same co-uptake mechanism, accounting for their elevated concentrations found in the field collected amphipods. Shuhaimi-Othman (2008) previously exposed adult *H. azteca* to metal contaminated field sediment and found that Ni and, similar to the present study, Cu was found to bioaccumulate more than the other metals found in the sediment.

Similar to the present study, metal accumulation correlations between field and laboratory-raised amphipods have been found for animals exposed to sediment from the Upper Clark Fork River in Montana (Ingersoll et al., 1994). Ingersoll et al. observed that while the pattern of amphipod-Cu concentration correlated strongly between field and laboratory-raised animals, laboratory-raised animals had drastically lower whole-body Cu concentrations than those collected from the field. The difference in field and laboratory animal Cu concentrations in the present study could be partially attributed to the naturally higher sediment-Cu concentrations found at the study sites compared to the low Cu conditions that laboratory animals are cultured in. However, differences in exposure routes must also be considered. Laboratory-raised amphipod's primary route of metal exposure was through the sediment while field collected animals may be exposed through sediment, overlying water, and food (Ingersoll et al., 1994). The ingestion of metal-bound material could contribute to an increase in amphipod-metal concentrations when metal dissociates in the acidic environment of the gut causing the release and uptake of cations into the tissues of the organism.

Interestingly, several of Bootjack Lake's amphipod-metal concentrations were similar to, or higher than, the concentrations found for the amphipods in the breach-affected Polley Lake. Several of Bootjack Lake's sediment-metal concentrations were also even higher than Polley Lake's. Bootjack Lake's above BCENV guideline sediment-

Cu, Cr, and Ni concentration may be high due to the mining operations occurring near its banks and/or the naturally high metal concentrations found in the area. Windblown metal-rich particles from mined material along with mine-waste runoff could be deposited into Bootjack Lake creating a waterbody rich in metals resulting in high amphipod-metal concentrations. The metal concentrations in Polley Lake may be residual from the breach event, despite remediation efforts, which may have led to the amphipod-metal concentrations similar to those in Bootjack Lake.

Little Lake was found to be clustered with other contaminated sites such as Cedar Point and Polley Lake for sediment-metal concentrations and amphipod-metal concentrations, respectively. It is not clear why this is the case since Little Lake was not impacted by the 2014 MPMC tailings breach. Historical mining and its associated waste may have influenced the lake's metal concentration There is evidence of a historical massive subaerial flow east of Little Lake as the result of volcanic eruption in the distant past (Bailey, 1990). This eruption could have deposited metals into the sediment where Little Lake now lies, leading to increased sediment-metal concentrations and subsequent whole-body amphipod metal concentrations.

The only waterborne metal above BCENV guidelines was Cu in Horsefly Bay (BCMOE, 2018). However, there is not a correspondingly high whole-body Cu concentration in Horsefly Bay amphipods or in *H. azteca* from the sediment exclusion study compared to the other sites. Attributing the high waterborne Cu concentration to the MPMC tailings impoundment breach does not seem likely given the lower concentration of waterborne Cu found at sites closer to the breach event such as Raft Creek. The Horsefly River, that flows into Horsefly Bay, is metal rich where it drains into Quesnel Lake likely explains the high Cu concentrations found in the water of Horsefly Bay. The waterborne metal inflow into Horsefly Bay is most likely in a form that is not readily bioavailable to amphipods as is evident with the higher DOC present in Horsefly Bay compared to the other within Quesnel Lake sites.

Certain amphipods, such as *H. azteca*, are often used as representatives for other freshwater invertebrates such as nematodes, oligochaetes and *Chironomus* species (Amiard-Triquet & Berthet, 2015). Results from the present study suggest that other species found in the study lakes may be similarly affected by the metals found in the sediment. Amphipods, and other macroinvertebrates, are an important part of trophic interactions in freshwater systems as they represent an important food source for fish such as salmon and trout, among others. There could be severe long-term consequences if wild populations of invertebrates are experiencing the same levels of mortality as those found in the sediment-exclusion study for Polley Lake and Raft Creek. There is also the potential for metals from MPMC tailings impoundment breach to transfer through various tropic levels. Previously, Zn has been shown to potentially magnify in the freshwater fish *Rutilus ylikiensis* from eating invertebrates in Lake Pamvotis, Greece (Papagiannis et al., 2004). Copper has also been shown to accumulate in predatory polychaete worms feeding on other polychaete worms exposed to Cu-rich sediments (Rainbow et al., 2006). Croteau et al. (2005), also demonstrated that Cd could biomagnified from algae to invertebrates to fish.

### *Conclusions*

From the correlating results of sediment metal concentrations, site whole-body amphipod-metal concentration, and the laboratory-raised *H. azteca* sediment exclusion experiment, metals are bioavailable in Quesnel Lake and Polley Lake in a pattern that is congruent with increasing distance from where the tailing from the MPMC tailing impoundment breach of 2014 entered the lake. The results of this study are also suggestive that sediment is the primary source of bioavailable metals. These findings have implications for future restoration efforts in the waterbodies affected by the MPMC tailings impoundment breach of 2014 along with other metal mine waste contaminated lakes. The present study also highlights the importance of sediment-based toxicity testing in the event of tailing impoundment breaches. It is recommended that additional studies should be conducted on (i) the sub-lethal effect that bioaccumulation of metals from sediment has on wild populations of amphipods and other freshwater invertebrates, and (ii) the ability of bioaccumulated metal to facilitate the trophic transfer of metal from invertebrates to fish and possibly to humans in freshwater environments.

# CHAPTER 3. The single and combined effects of wood-fire runoff and sediment-bound copper on the freshwater amphipod, *Hyalella azteca*

## Introduction

Wildfires have been increasing in frequency and severity as a result of climate change (Gillett et al., 2004; Westerling et al., 2006). Increased average yearly temperatures, reduced rainfall, in combination with an earlier onset of spring all increase wildfire risk (Gillett *et al.*, 2004; Westerling et al., 2006). In 2017 and 2018 British Columbia, Canada has experienced the two worst fire seasons on record with 12 161 km$^2$ and 12 985 km$^2$ burned, respectively (British Columbia Wildfire Services, 2019). The trend of worsening wildfire seasons is expected to continue with climate change, so the impact of wildfire runoff on the condition of freshwater is of concern. Ash and burnt material that wash into freshwater ecosystems could be leading to potentially hazardous contamination.

Wildfires can release nitrogen, phosphorus, ammonia, DOC, and pyrogenic polycyclic aromatic hydrocarbons (PAHs) into the aquatic environment via runoff (Bladon et al., 2008; McEachern, et al., 2000; Olivella, et al., 2006). Increased nutrient concentrations, particularly P, associated with wildfire have been shown to persist 6 to 7 years post-fire causing changes in trophic interactions due to large algae blooms (Silins et al., 2014, 2009). Increased input of DOC into freshwater environments could be beneficial as DOC is well known to ameliorate toxicants, such as metals, by sequestering them from the water column and reducing their bioavailability to aquatic organisms (Besser et al., 2003; Niyogi & Wood, 2004; Richards et al., 2001). The released pyrogenic PAHs persist in the environment potentially causing long-term effects (Abdel-Shafy & Mansour, 2016).

The individual components released by wildfire have been frequently studied; however, the study of total post-wildfire runoff toxicity is a recently developing field. Previously, Nunes et al. (2017) exposed mosquitofish (*Gambusia holbrooki)* to post-fire surface runoff that resulted in an increase in oxidative stress but no associated oxidative damage. Silva et al. (2015) exposed *Pseudokirchneriella subcapitata* and *Lemna minor* to an aqueous extract of wood ash (AEA) which caused a decrease in microalgae density and dry weight, respectively, for both primary producers following acute exposure. However, no deleterious effects were observed for *Daphnia magna* exposed to AEA (Silva et al., 2015). Exposure of the freshwater clam *Corbicula fluminea* to AEA resulted in increased mortality and bioaccumulation of PAHs and metals (Silva et al., 2016). While studying the toxicity of isolated wildfire runoff is of great importance, the interaction of wildfire runoff with other potential toxicants remains largely unknown. Contamination that occurs in natural environments often interacts with pre-existing contaminants to form mixtures that can potentially exacerbate or ameliorate the contaminant's individual effects. Mixture toxicology has been studied in metals with findings of less-than-additive, additive, and more-than-additive results for metal co-toxicity (Norwood et al., 2003). Co-toxicity, therefore, is an important consideration when attempting to determine ecological risk of contamination events.

Previous research in our laboratory demonstrated that Cu toxicity is enhanced in the presence of certain hydrocarbon contaminants, which can be constituents of wildfire runoff, such as the pyrogenic PAHs phenanthrene (PHE) and phenanthrene quinone (PHQ) in *Hyalella azteca* (Gauthier *et al.*, 2015). Amphipods exposed to Cu-PHE and

Cu-PHQ mixtures experienced 26.9 ± 1.7% and 73.3 ± 13.3% more mortality, respectively, than could be explained by the toxicity of the individual toxicants alone (Gauthier et al., 2015). While there are studies on single PAH and metal interactions, there is a gap in our understanding of how total wildfire runoff affects Cu toxicity.

The combination of Cu and wildfire runoff could occur in a natural system. The 2014 Mount Polley Mining Corporation (MPMC) Cu and Au mine experienced a large tailings impoundment breach that released approximately 17 M $m^3$ of Cu rich water 8 M $m^3$ of solid tailings into Polley Lake, down Hazeltine Creek and into Quesnel Lake (Petticrew et al., 2015). Quesnel Lake is a large (surface area 266 $km^2$), deep (maximum 610 m), oligotrophic fjord lake located in the Cariboo region of British Columbia, Canada (Petticrew et al., 2015). Copper was identified as a potential contaminant of concern following the breach with waterborne concentrations as high as ~100 µg/L but dropped to below 6.6 µg/L by May of 2015 (Imperial Metals, 2015). The solid tailings initial Cu concentration in Quesnel Lake was as high as 700 mg Cu/kg (Imperial Metals, 2015). While restoration efforts have taken place, solid tailings from the breach remain in the affected lakes. In 2017, the Elephant Hill wildfire swept through the Cariboo region where the mine is located. The Cu-rich solid tailings released from the MPMC tailing impoundment breach in combination with the wildfire runoff from the 2017 Elephant Hill wildfire could interact, increasing Cu toxicity to freshwater organisms. An opposing hypothesis is one of Cu amelioration where DOC from wildfire runoff could reduce Cu toxicity via competitive binding to the site of toxic action (Niyogi & Wood, 2004).

The objective of the present study was to determine the extent to which wildfire runoff exacerbates or ameliorates Cu sediment toxicity. To accomplish this objective, *H. azteca* were exposed for 14 days under controlled laboratory conditions to a Cu-enriched sediment in combination with increasing concentrations of an artificially created wildfire runoff.

## Methods

### *Culturing Method*

*Hyalella azteca* were cultured as was described in Chapter 2.

### *Artificial wildfire runoff preparation*

Balsam fir and Fraser fir trees were burned to create ash and burnt material. Coniferous tree species were selected to be relevant to the subalpine fir- and Engelmann spruce-dominant forest found around the MPMC breach area. A total of 52.80 kg of Balsam fir and 8.07 kg of Fraser fir were burned in an outdoor fire pit until the fire extinguished itself approximately 6 hours after ignition. The fir trees had been reduced to ash and fragments of not completely burnt trunk. The ash and burnt material was then combined with culture water at a 1:4 v/v ratio (Silva et al., 2015). The mixture was stirred daily for one week prior to use and continuously aerated. The aged mixture was filtered through aquarium filter wool to remove large fragments of debris. The resulting fire extract (FE) test water was then diluted with culture water to create a FE concentration gradient of 0, 12.5, 25, 50, 75 and 100%.

### *Copper-enriched sediment preparation*

Copper-enriched sediment at a concentration of 225 mg Cu/kg was used in this experiment as preliminary experimentation revealed that this concentration did not reduce survival but did reduce growth in *H. azteca*. By selecting a Cu-exposure concentration that induces sub-lethal toxicity, we could determine if co-exposure with FE exacerbates or ameliorates toxicity associated with the Cu-enriched sediment. The Cu-enriched sediment was created by making a 2.21 mg/L solution of $CuSO_4$ $5H_2O$ (purity >98.5%; VWR International; catalog no. BDH3312-2) in $ddH_2O$. The Cu solution was combined with bentonite clay at a ratio of 2 mL of solution: 5 grams of bentonite. The mixture was then manually stirred until all clay was saturated with solution and thoroughly combined. The mixture than was left overnight before being used in the toxicity test.

### *Toxicity Test*

To investigate the possible interaction of Cu-enriched sediment and FE, a chronic fourteen-day sediment toxicity test was performed using *H. azteca* following Canadian Environmental Protection Service Biological Test Method protocols with some adjustments (Government of Canada, 2012). All tests were run with randomly selected mixed-age *H. azteca* (50% neonates, 50% adults). All tests were run at 23±1°C with a 16:8 h light/dark photoperiod and were lit with full-spectrum fluorescent bulbs emitting light at 500 lux. All tests were carried out in 300 mL tall form glass beakers filled with 50 mL of Cu-enriched sediment and 200 mL of test water. Animals were exposed to varying concentrations of FE and a single concentration of 225 mg Cu/kg bound sediment. There was a total of 11 treatments: laboratory control, bentonite control, 12.5% FE, 50.0% FE, 100.0% FE, 225 mg Cu/kg, 225 mg Cu/kg + 12.5% FE, 225 mg Cu/kg + 25.0% FE, 225 mg Cu/kg + 50.0% FE, 225 mg Cu/kg + 75.0% FE, and 225 mg Cu/kg +100.0% FE. Laboratory control consisted of culture water with a 3 cm x 3cm square of 70 µm mesh to serve as a substrate. The bentonite control consisted of culture water with bentonite clay prepared as described above for the Cu-enriched sediment but without the addition of Cu salt. Twelve mixed-aged *H. azteca* randomly assigned to each replicate. Half of the animals were juveniles (<9 days old) and half were adults (>4 weeks). Animals were fed ad libitum with finely ground Tetramin® Tropical fish food flakes six times throughout the experiment on non-consecutive days. Following the toxicity test, animals were transferred to clean culture water and left for 24 hours to depurate the contents of their guts. After depuration the *H. azteca* were rinsed for one minute in a 50 μM ethylenediaminetetraacetic acid (EDTA) solution made using culture water to remove bound metals from the body surface of the amphipods (Norwood et al., 2007). Animals were then transferred to pre-weighed aluminum weigh boats, rinsed with culture water for one minute to remove any remaining EDTA, and then the culture water was removed using a pipette. Animals were then dried for 48 hours at 60 °C and weighed.

### *Whole Body Cu Analysis*

To determine the concentration of Cu in the whole-body of the exposed *H. azteca,* animals were analysed via GF-AAS as was described in Chapter 2. The certified reference material, SLRS-6 had mean percentage of recovery 94.1%, mean blank was 0.55 µg/L. The analytical duplicates had a mean difference of 1.0%.

### *Acetylcholinesterase analysis*

Acetylcholinesterase activity was measured using methods described by Bartlett *et al.* (2016). A minimum of five amphipods were used in each replicate. Amphipods were homogenized in 500 μL homogenizing buffer (Tris buffer: Invitrogen; catalog no. 15504020) at pH 8 with 1% v/v Triton X-100 (Alfa Aesar; catalog no. J62289) and centrifuged at 10,000 g for 10 min at 4ºC. The resulting supernatant was used in the assay. Triplicate additions to the microplate were as follows: 40 µL of homogenizing buffer (blank), electric eel cholinesterase (enzyme standard; Sigma-Aldrich; catalog no. C3389) or supernatant, 250 µL Ellman's reagent (5,5'-dithiobis[2-nitrobenzoic acid];Thermo Scientific; catalog no. 22582) and 10 µL of acetylthiocholine iodide (Sigma-Aldrich; catalog no. A5751). Absorbance was measured on a microplate reader (Varioskan Flash; Thermo Scientific; USA) at 405 nm in two-minute intervals for 30 min.

Protein quantification of the amphipod supernatant was measured using a bicinchoninic acid assay (BCA) as described by Bartlett *et al.* (2016). Triplicate additions to the microplate were as follows: 25 µL of homogenizing buffer, bovine albumin protein standards (0, 100, 250, 500, and 1000 µg/mL; Sigma-Aldrich; catalog no. A7906), electric eel cholinesterase or homogenate supernatant were added with 200 mL of the BCA working reagent. The plate was incubated at room temperature for 2 h and absorbance was measured on a microplate reader (Varioskan Flash; Thermo Scientific; USA) at 562 nm. The protein concentrations were calculated using the standard curve made from the bovine serum standards.

Acetylcholinesterase activity was calculated using the equation:

$$Specific\ Activity = \frac{A \times R_{Vol} \times 1000}{E \times PL \times H_{Vol} \times PR}$$

where specific activity is in µmol/min/g protein, A is the change in absorbance per minute, $R_{Vol}$ is the reaction volume (300 mL), 1000 is a unit conversion factor (g to mg), E is the extinction coefficient for 5,5 -dithiobis[2-nitrobenzoic acid] (1.36 104 1/M cm), PL is the pathlength (0.875 cm), $H_{vol}$ is the homogenate volume (500 mL), and PR is protein in the homogenate (mg/mL).

### *Water Quality*

Water quality was measured at the beginning (day 0), halfway point (day 7), and at the end (day 14) of the experiment in one replicate of each treatment (Table 2.1). Dissolved oxygen and temperature were measured every other day using a dissolved oxygen probe (YSI Pro20 dissolved oxygen meter; YSI). The pH was measured using a pH meter (accumet™ Excel XL15 pH meter; Fisher Scientific). Conductivity was measured using a Traceable conductivity meter (VWR, catalog no. 89094-762). Hardness and alkalinity were measured using the titration method described by the American Public Health Association (APHA, 1989). Dissolved organic carbon samples were filtered and stored at -20°C and were analyzed by Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University.

### *Statistical Analysis*

A one-way ANOVA followed by a post-hoc Tukey's test was used to determine significant differences among treatments. The total whole-body Cu data set was log-transformed to meet assumptions of homogeneity. Additionally, the more-than-additive accumulation of whole-body Cu concentration of Cu+FE was analyzed using a one-sample singled tailed t-test where the mean of the Cu and the FE exposure were combined and compared with the Cu+12.5% FE exposure. All of the data were analyzed using R (R

Core Team, 2018). Mean differences were considered to be statistically significant different when $p < 0.05$.

## Results

### *Survival*

Percent survival of the controls in both laboratory and bentonite controls was not affected by the experimental conditions (Figure 3.1). All exposures that contained FE concentrations greater than 12.5%, including those with and without Cu-enriched bentonite, experienced 100% mortality by the end of the experiment. Survival in the 12.5% FE and the 225 mg/kg Cu-enriched sediment exposures did not differ from control. However, when 12.5% FE is combined with the 225 mg/kg Cu-enriched sediment there was a 41% reduction in survival ($F_{4,31}$=45.89, $p<0.001$) (Figure 3.1).

### *Weight*

Weight could only be compared among treatments that did not result in complete mortality. The controls and 12.5% FE exposure had greater per organism weight than the 225 mg Cu/kg and the 225 mg Cu/kg + 12.5% FE exposures ($F_{4,31}$=23.7, $p<0.001$) (Figure 3.2). However, the weight per organisms in the surviving exposures that contained Cu-enriched sediment did not differ from each other (Figure 3.2).

### *Amphipods whole-body Cu concentration*

Results from the Cu GF-AAS analysis revealed differences in whole-body amphipod Cu concentrations between treatments ($F_{4,12}$=110.8, $p<0.001$). All exposures

Table 3. 1. Average water quality parameters (±SD) in water samples for toxicity test using H. azteca exposed to a Cu-enriched sediment and artificially created fire extract (FE)[a]

| **Treatment** | **Temp (°C)** | **DO (% $O_2$)** | **pH [b]** | **Alkalinity (mg/L as $CaCO_3$)** | **Hardness (mg/L as $CaCO_3$)** | **Conductivity (µS/cm)** | **DOC (mg/L)** |
|---|---|---|---|---|---|---|---|
| Laboratory Control | 22.6±0.5 | 96.3±4.3 | 7.88 (7.83-7.91) | 57±7.8 | 182±19 | 456±39 | 3.3 |
| Bentonite Control | 23.7±0.1 | 92.8±0.7 | 8.42 (7.91-8.57) | 71±18 | 152±42 | 819±68 | 14.7 |
| Cu | 23.2±0.5 | 91.5±1.9 | 8.43 (7.90-8.56) | 100±31 | 124±66 | 856±102 | 15.0 |
| 12.5% FE | 22.2±0.2 | 88.9±1.5 | 8.46 (8.41-8.51) | 259±18 | 87±19 | 1022±177 | 10.1 |
| 50.0% FE | 22.3±0.2 | 90.6±1.7 | 8.78 (8.61-8.91) | 634±51 | 107±30 | 2443±133 | 41.6 |
| 100.0% FE | 22.3±0.2 | 91.4±2.9 | 9.16 (8.88-9.18) | 1234±12 | 133±42 | 4013±90 | 80.3 |
| Cu+12.5% FE | 23.5±0.3 | 91.5±4.7 | 8.64 (8.41-8.68) | 278±3.6 | 85±30 | 1190±322 | 23.2 |
| Cu+25.0% FE | 23.4±0.5 | 91.9±6.9 | 8.81 (8.51-8.86) | 349±56 | 71±15 | 1627±304 | 36.9 |
| Cu+50.0% FE | 23.1±0.8 | 92.6±5.7 | 9.02 (8.70-9.03) | 691±70 | 72±15 | 2563±237 | 58.5 |
| Cu+75.0% FE | 22.9±0.5 | 91.3±1.1 | 9.07 (8.88-9.07) | 927±23 | 67±18 | 3257±32 | 74.4 |
| Cu+100% FE | 23.7±0.0 | 88.3±0.9 | 9.17 (8.92-9.20) | 1260±99 | 66±7.0 | 3953±38 | 96.3 |

[a] The data given for pH, alkalinity, hardness, and conductivity had n=3, temperature and dissolved oxygen (DO) had n=7, dissolved organic carbon (DOC) had n=2.

[b] pH water quality reported as median (± range)

that were not controls had increased concentrations of Cu (Figure 3.3). This increased Cu was greatest in amphipods exposed to Cu+FE which was also greater than the concentration of Cu in animals exposed to Cu or FE individually (Figure 3.3). In fact, a more-than-additive increase in whole-body Cu concentration was found for animals exposed to Cu+FE than to animals exposed Cu or FE individually ($t_3$=2.30, p=0.05).

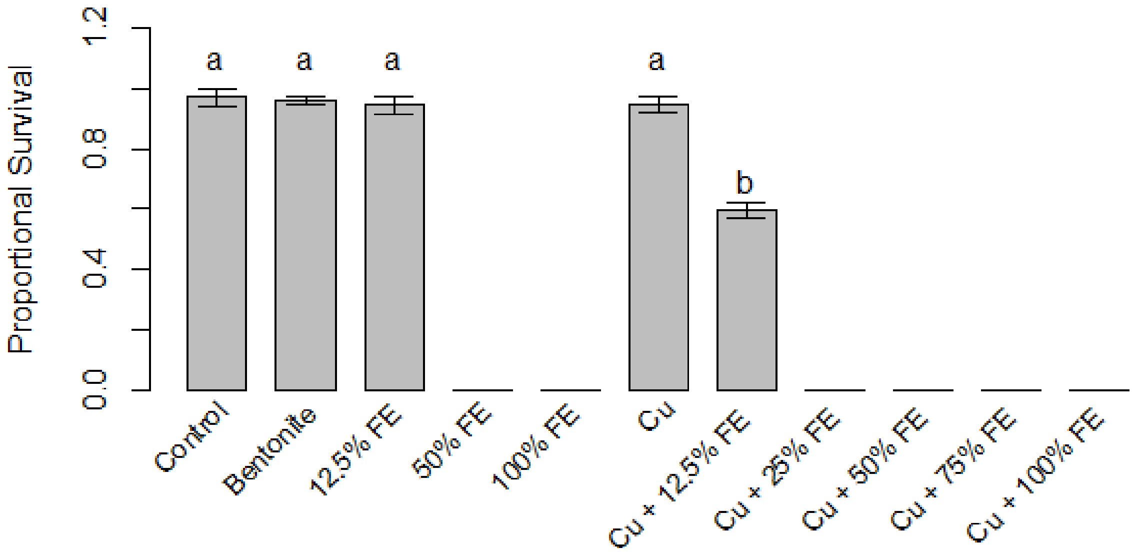

Figure 3. 1. Mean of proportional survival ± standard error for surviving *H. azteca* following exposure to various percent concentrations of fire extract (FE), and 225 mg Cu/kg sediment (Cu). Replication of laboratory control and FE exposures was n=6. Replication of bentonite, Cu, and Cu+FE exposures was n=8. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$).

*Acetylcholinesterase assay*

Acetylcholinesterase activity was found to differ among treatments ($F_{4,10}=32.44$, $p<0.001$). Mean measured amphipod AChE activity did not differ between laboratory and bentonite controls (Figure 3.4). Exposure to either FE or Cu+FE resulted in a similar reduction in AChE activity compared to controls; however, the activity between animal exposed to FE or Cu+FE did not differ from each other (Figure 3.4). There was also an increase in AChE activity found for amphipods exposed to 225 m/kg Cu-enriched

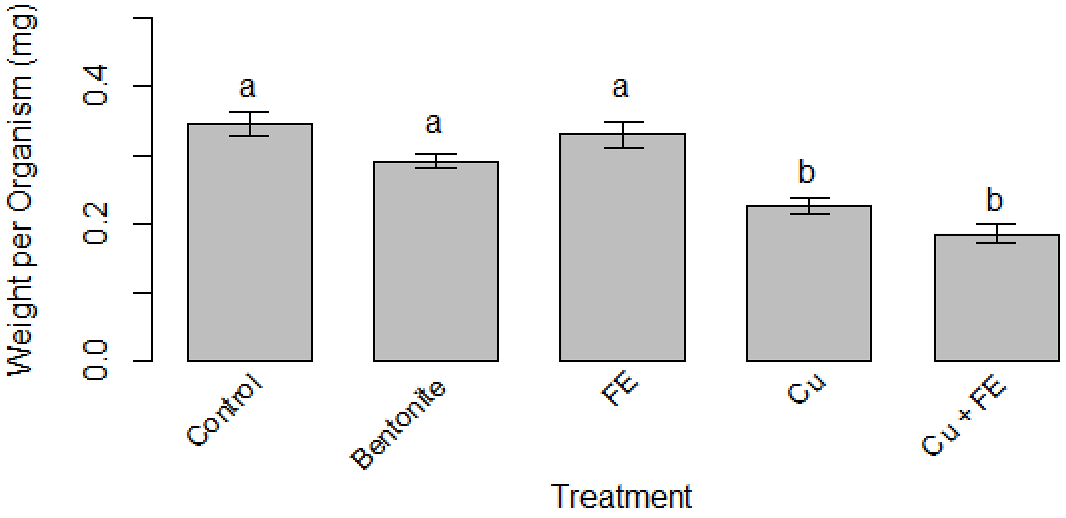

Figure 3. 2. Mean dry weight per organism ± standard error for *H. azteca* following exposure to 12% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment bound Cu-12% FE mixture. Replication of laboratory control and FE was n=6. Replication of bentonite, Cu, and Cu+FE was n=8. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$).

sediment exposure compared to laboratory and bentonite controls (Figure 3.4).

### *Water Quality*

Dissolved organic carbon, pH, and alkalinity were observed to increase with increasing FE percent concentration both with and without the addition of Cu-spiked sediment (Table 1). Hardness decreased in exposures with Cu-spiked sediment with increasing FE percent concentrations, but this was not observed in exposures that did not

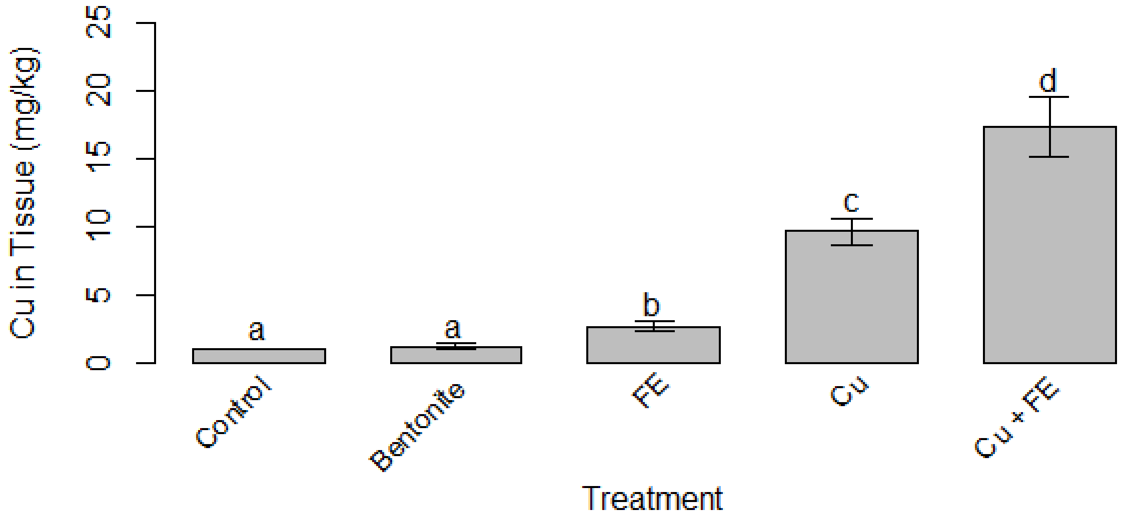

Figure 3. 3. Mean whole-body Cu concentration ± standard error for *H. azteca* following exposure to 12% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment bound Cu+12% FE mixture. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$). n = 3.

contain Cu-spiked sediment (Table 2.1). All other water quality parameters were within acceptable deviations of average values described in the Canadian Environmental Protection Service Biological Test Method (Government of Canada, 2012).

## Discussion

### *Survival, growth, and bioaccumulation*

We found that the combination of a Cu-enriched sediment with FE interact to cause more-than-additive toxic effects on *H. azteca*. The Cu+FE combination induced a severe decrease in survival following 14-day exposure to 225 mg Cu/kg and 12.5% FE.

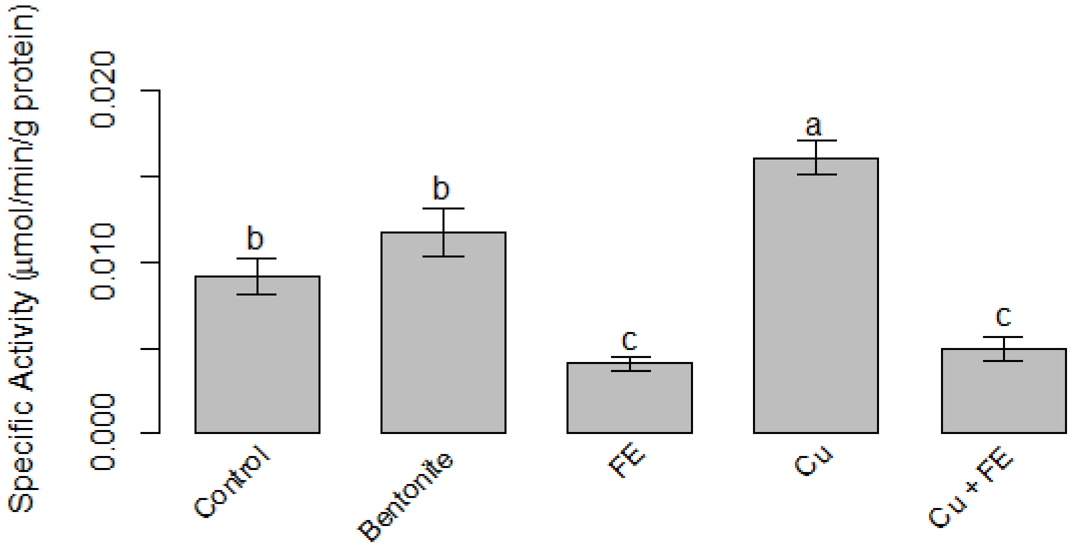

Figure 3. 4. Specific activity ± standard error for *H. azteca* following exposure to 12% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment bound Cu+12% FE mixture. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$). n=3.

These results are in agreement with research done by Gauthier *et al.* (2015) who performed a study with *H. azteca* exposed to combinations of waterborne Cu with pyrogenic PAHs, which are constituents of FE. Amelioration of Cu toxicity by DOC present in the FE was not observed which is incongruent with decreased toxicity found for *Ceriodaphia dubia* exposed to Cu and DOC by Kim *et al.* (1999). This inability to ameliorate Cu toxicity may be due to exposure route. The benthic amphipods are very likely consuming Cu that is bound to DOC but when the molecule enters the acid environment of the digestive system, it releases Cu in its ionic form which is bioavailable to the organism.

The addition of FE did not affect growth of *H. azteca* differently than Cu-spiked sediment alone. Observing a difference in survival but not in growth could indicate that the mechanisms of combined toxicity have a threshold that, when reached, causes death in the organism before more sensitive endpoints such as growth can be affected. Before this threshold is met, amphipods would only be affected by the Cu-spiked sediment. The use of mixed-age animals could have contributed to this effect with younger, more sensitive animals reaching threshold toxicity at lower concentrations than adults. García *et al.* (2010) studied the age-related sensitivity of *Hyalella curvispina* to cadmium and found neonates to be more sensitive with an LC50 five times lower than adults. For the Cu+FE exposures that had complete mortality, it is theorized that this threshold of toxicity was met for both age classes before the end of the exposure. It could also simply be that growth is not affected by this exposure.

This study found that amphipods exposed to Cu+12% FE had a more-than-additive accumulation of Cu compared to either contaminant individually. Excess metals are not easily transported across membranes but polycyclic aromatic hydrocarbons present in the FE can disrupt membrane fluidity leading to increased ion uptake (Sikkema *et al.*, 1994). Once in the cell,  metals produce ROS leading to cell damage and enzyme alteration including cytochrome p450 1A1 (CYP) (Gauthier et al., 2014). Inactivation of CYP 1A1 reduces the ability of the cell to break down and detoxify PAHs (Gauthier et al., 2014). In this way, Cu and PAHs both facilitate each other to increase their individual toxicological effects, leading to the more-than-additive effect on mortality observed in this study.

### *AChE activity*

One possible mechanism of toxicity studied was inhibition of AChE activity. Acetylcholinesterase activity in *H. azteca* was found to be diminished when exposed to FE which is likely due to the presence of PAHs in the FE. Gauthier *et al.* (2016) found similar results for *H. azteca* exposed to PHE with AChE activity at 77 ± 7% of control. Due to their non-polar nature, PAHs enter the cell through passive diffusion across membranes. Once in the cell, PAHs can inactivate AChE when nitro-aromatic compounds bind to the α-anionic site of AChE inactivating the enzyme (Pohanka, 2011). However, other studies have reported decreased AChE activity in animals exposed to non-nitro containing PAHs which could be due to in vivo transformation of PAHs to nitro-PAHs (Gauthier *et al.*, 2016; Shailaja *et al.*, 2006). Similar toxic mechanisms could explain the results found in this study but further investigation into pyrogenic PAH toxicity is warranted.

Contrary to results found in this study, previous research has shown that aquatic invertebrates exposed to metals generally demonstrate a decrease in AChE activity (Forget, *et al.*, 1999) or no difference from controls (Gauthier et al., 2016). However, these studies all used waterborne concentrations of metals while this experiment employed sediment-bound Cu which could perhaps be contributing to the differences found through differences in exposure route leading to differences in the animal toxicant concentrations. Some studies have observed AChE activation upon exposure to toxicants similar to those used in the present study. Abou-Donia *et al.* (2002) studied the effects of uranyl acetate on the central nervous system in rats where a significant increase in AChE activity was observed in the cortex. The authors suggested that an increase in AChE could deplete acetylcholine at the synapse, leading to abnormal neural transmission. Similar effects were observed by Dethloff *et al.* (1999) and Romani *et al.* (2003) in rainbow trout and sea bream, respectively, in the brain when exposed to Cu. Romani et al., (2003) theorized that Cu ions improve the catalytic efficiency of AChE by enhancing the anionic moiety of the active site; therefore, increasing the formation of the enzyme-substrate complex with choline molecules. A similar effect could be occurring in the amphipods of this study; however, whole body AChE was used in the present study, not isolated brain tissue.

### *Conclusions*

This study revealed that the presence of Cu-enriched sediment can exacerbate the toxicity of FE leading to a more-than-additive decrease in survival and a more-than-additive increased in whole-body Cu concentrations for the freshwater amphipod *H. azteca*. Several pathways may be involved in producing these more-than-additive effects in *H. azteca*, particularly those involving Cu and PAH co-toxicity, but they require further work. The co-toxic effect of Cu-enriched sediment and wildfire runoff is a concern that warrants further investigation to determine how the combination interacts and affects aquatic ecosystems.

## General Conclusions

The objectives of the present study were two-fold: first, to determine if metals associated with the 2014 MPMC tailings breach were bioavailable to aquatic organisms at concentrations sufficient to cause toxicity by using a biotic vector; and second, to determine the extent to which forest fire runoff exacerbates or ameliorates Cu toxicity.

The present study concludes that Cu, and other metals, associated with the MPMC tailings breach of 2014 are bioavailable to aquatic organisms. For the sake of ecological risk assessment, despite the value and efficiency of bench-top testing for bioavailability, the best way to test bioavailability of contaminants is to use biota. While many studies employ geochemical assessments to determine the potential for sediment-bound metal bioavailability, the present study highlights the importance of confirming the results of geochemical studies with biological models. Exposure route to sediments, such as ingestion, can have major effects on metal bioavailability when ions are released from molecules in the acid environment of the gut. The complex biological systems and regulatory mechanisms present within organisms can make predictions of bioavailability based on geochemical assessment alone unreliable and prone to underestimating the toxic potential of sediments in contaminated water bodies.

The results of the present study highlight the importance of studying the toxicity of multiple contaminants when assessing the toxicological potential of contaminants on freshwater organisms. The discovery of more-than-additive effects on toxicological endpoints of wildfire runoff in combination with a Cu-enriched sediment could have implication for future risk assessment. Climate change has increased the frequency and severity of wildfire worldwide with this trend expected to continue with rising global temperatures (Dale et al., 2001; Stocks et al., 1998). It is not unreasonable to predict that these wildfires will interact with metal-contaminated environments such as metal mining sites that are in forested areas. This could be the case for locations such as Polley and Quesnel Lake that have been impacted by Cu contamination and wildfire runoff. It is important to have effective risk assessment to understand how co-toxic events, such as the combination of Cu and wildfire runoff, will impact aquatic ecosystems. Results from this study strongly suggest that Cu-contaminated water bodies that receive runoff from nearby wildfires may be at risk of enriched toxicological effects.

Future study should be done on amphipod sublethal endpoints following exposure to sediment and water collected from Quesnel and Polley Lake. Endpoint such as reproduction, body length, and activity could be affected and have implications for the ecological health of the waterbodies. A similar sediment-exclusion study should be preformed with a fish analog to see if observation in the present study correlate with a vertebrate model. Further work should be done testing the toxicity of more representative wildfire runoff, such as including undergrowth foliage and soil, in combination with a Cu-enriched sediment to determine if more-than-additive toxicity is still observed. Additionally, while a PAH – Cu mechanism has been proposed by Gauthier et al. (2014), this mechanism does not account for the other constituents of wildfire runoff such as ammonia, DOC, P, and N and so requires further work to be more informative for risk assessors.

## References

Abatzoglou, J. T., & Williams, A. P. (2016). Impact of anthropogenic climate change on wildfire across western US forests. *Proceedings of the National Academy of Sciences*, *113*(42), 11770–11775. https://doi.org/10.1073/pnas.1607171113

Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*(1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Abou-Donia, M. B., Dechkovskaia, A. M., Goldstein, L. B., Shah, D. U., Bullman, S. L., & Khan, W. A. (2002). Uranyl acetate-induced sensorimotor deficit and increased nitric oxide generation in the central nervous system in rats. *Pharmacology Biochemistry and Behavior*, *72*(4), 881–890. https://doi.org/10.1016/S0091-3057(02)00772-4

Al-Reasi, H. A., Scott Smith, D., & Wood, C. M. (2012). Evaluating the ameliorative effect of natural dissolved organic matter (DOM) quality on copper toxicity to Daphnia magna: Improving the BLM. *Ecotoxicology*, *21*(2), 524–537. https://doi.org/10.1007/s10646-011-0813-z

Amiard-Triquet, C., & Berthet, B. (2015). Chapter 10 - Endobenthic invertebrates as reference species. In C. Amiard-Triquet, J.-C. Amiard, & C. Mouneyrac (Eds.), *Aquatic Ecotoxicology* (pp. 229–252). https://doi.org/10.1016/B978-0-12-800949-9.00010-3

Aparicio, A. T., & Canales, P. de J. H. (2017). Gestión de riesgos y desastres socioambientales. El caso de la mina Buenavista del cobre de Cananea. *Investigaciones Geográficas*, *93*. https://doi.org/dx.doi.org/10.14350/rig.54770

APHA. (1989). *Standard methods for the examination of water and wastewater* (17th Edition; L. S. Clesceri, A. E. Greenburg, & R. R. Trussel, Eds.). Washington, D.C.: American Public Health Association.

ATSDR. (2004). *Toxicological profile for copper*. Retrieved from Agency for Toxic Substances and Disease website: https://www.atsdr.cdc.gov/toxprofiles/tp132.pdf

Bailey, D. G. (1990). *Geology of the central Quesnel Belt, British Columbia*. British Columbia. Ministry of Energy, Mines and Petroleum Resources.

Bartlett, A. J., Struger, J., Grapentine, L. C., & Palace, V. P. (2016). Examining impacts of current-use pesticides in Southern Ontario using in situ exposures of the amphipod *Hyalella azteca*. *Environmental Toxicology and Chemistry*, *35*(5), 1224–1238. https://doi.org/10.1002/etc.3265

BC Wildfire Service. (2017). *Fire-perimeters - current*. Retrieved from https://catalogue.data.gov.bc.ca/dataset/fire-perimeters-current

BCMOE. (2017). *British Columbia working water quality guidelines: aquatic life, wildlife & agriculture*. Retrieved from https://www2.gov.bc.ca/assets/gov/environment/air-land-water/water/waterquality/wqgs-wqos/bc_env_working_water_quality_guidelines.pdf

BCMOE. (2018). *British Columbia approved water quality guidelines: aquatic life, wildlife and agriculture*. Retrieved from https://www2.gov.bc.ca/gov/content/environment/air-land-water/water/water-quality/water-quality-guidelines/approved-water-quality-guidelines

Besser, J. M., Brumbaugh, W. G., May, T. W., & Ingersoll, C. G. (2003). Effects of organic amendments on the toxicity and bioavailability of cadmium and copper in spiked formulated sediments. *Environmental Toxicology and Chemistry*, *22*(4), 805–815. https://doi.org/10.1002/etc.5620220419

Bladon, K. D., Silins, U., Wagner, M. J., Stone, M., Emelko, M. B., Mendoza, C. A., Devito, K. J., & Boon, S. (2008). Wildfire impacts on nitrogen concentration and production from headwater streams in southern Alberta’s Rocky Mountains. *Canadian Journal of Forest Research*, *38*(9), 2359–2371. https://doi.org/10.1139/X08-071

Borgmann, U, & Norwood, W. P. (1997). Toxicity and accumulation of zinc and copper in *Hyalella azteca* exposed to metal-spiked sediments. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(5), 1046–1054. https://doi.org/10.1139/f97-020

Borgmann, U., Norwood, W. P., & Babirad, I. M. (1991). Relationship between Chronic Toxicity and Bioaccumulation of Cadmium in *Hyalella azteca*. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(6), 1055–1060. https://doi.org/10.1139/f91-124

Borgmann, U., Norwood, W. P., & Clarke, C. (1993). Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. *Hydrobiologia*, *259*(2), 79–89. https://doi.org/10.1007/BF00008374

Borgmann, Uwe, Couillard, Y., Doyle, P., & Dixon, D. G. (2005). Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness. *Environmental Toxicology and Chemistry*, *24*(3), 641–652. https://doi.org/10.1897/04-177R.1

Bowker, L. N., & Chambers, D. M. (2015). The risk, public liability, & economics of tailings storage facility failures. *Earthwork Act*, 1–56.

Bowman, D. M. J. S., Balch, J., Artaxo, P., Bond, W. J., Cochrane, M. A., D’Antonio, C. M., DeFries, R., Johnston, F. H., Keeley, J. E., Krawchuk, M. A., Kull, C. A., Mack, M., Moritz, M. A., Pyne, S., Roos, C. I., Scott, A. C., Sodhi, N. S., & Swetnam, T. W. (2011). The human dimension of fire regimes on Earth. *Journal* *of Biogeography*, *38*(12), 2223–2236. https://doi.org/10.1111/j.1365-2699.2011.02595.x

Bremner, I. (1998). Manifestations of copper excess. *The American Journal of Clinical Nutrition*, *67*(5), 1069S-1073S. https://doi.org/10.1093/ajcn/67.5.1069S

British Columbia Wildfire Services. (2019, January 25). *Fire Incident Locations - Historical*. Retrieved from https://catalogue.data.gov.bc.ca/dataset/fire-incident-locations-historical

Britton, J. (2016). Exploration and mining in the Thompson-Okanagan-Cariboo region, British Columbia. In: Provincial overview of exploration and mining in British Columbia, 2015. *British Columbia Ministry of Energy and Mines, British Columbia Geological Survey, Information Circular*, 89–105.

Brown, R. J., Galloway, T. S., Lowe, D., Browne, M. A., Dissanayake, A., Jones, M. B., & Depledge, M. H. (2004). Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. *Aquatic Toxicology*, *66*(3), 267–278. https://doi.org/10.1016/j.aquatox.2003.10.001

Byrne, P., Hudson-Edwards, K. A., Bird, G., Macklin, M. G., Brewer, P. A., Williams, R. D., & Jamieson, H. E. (2018). Water quality impacts and river system recovery following the 2014 Mount Polley mine tailings dam spill, British Columbia, Canada. *Applied Geochemistry*, *91*, 64–74. https://doi.org/10.1016/j.apgeochem.2018.01.012

Chakoumakos, C., Russo, R. C., & Thurston, R. V. (1979). Toxicity of copper to cutthroat trout (Salmo clarki) under different conditions of alkalinity, pH, and hardness. *Environmental Science & Technology*, *13*(2), 213–219.

Croteau, M.-N., Luoma, S. N., & Stewart, A. R. (2005). Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. *Limnology and Oceanography*, *50*(5), 1511–1519. https://doi.org/10.4319/lo.2005.50.5.1511

Cusack, C. C., & Mihelcic, J. R. (1999). Sediment toxicity from copper in the Torch Lake (MI) Great Lakes area of concern. *Journal of Great Lakes Research*, *25*(4), 735–743. https://doi.org/10.1016/S0380-1330(99)70773-2

Dai, A. (2013). Increasing drought under global warming in observations and models. *Nature Climate Change*, *3*(1), 52–58. https://doi.org/10.1038/nclimate1633

Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., Hanson, P. J., Irland, L. C., Lugo, A. E., Peterson, C. J., Simberloff, D., Swanson, F. J., Stocks, B. J., & Wotton, B. M. (2001). Climate change and forest disturbances: Climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. *BioScience*, *51*(9), 723–734. https://doi.org/10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2

Dethloff, G. M., Schlenk, D., Hamm, J. T., & Bailey, H. C. (1999). Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. *Ecotoxicology and Environmental Safety*, *42*(3), 253–264. https://doi.org/10.1006/eesa.1998.1757

Ellenberger, S. A., Baumann, P. C., & May, T. W. (1994). Evaluation of Effects Caused by High Copper Concentrations in Torch Lake, Michigan, on Reproduction of Yellow Perch. *Journal of Great Lakes Research*, *20*(3), 531–536. https://doi.org/10.1016/S0380-1330(94)71169-2

Erickson, R. J., Benoit, D. A., Mattson, V. R., Leonard, E. N., & Nelson, H. P. (1996). The effects of water chemistry on the toxicity of copper to fathead minnows. *Environmental Toxicology and Chemistry*, *15*(2), 181–193. https://doi.org/10.1002/etc.5620150217

Fernandes, G. W., Goulart, F. F., Ranieri, B. D., Coelho, M. S., Dales, K., Boesche, N., Bustamante, M., Carvalho, F. A., Carvalho, D. C., Dirzo, R., Fernandes, S., Galetti, P. M., Millan, V. E. G., Mielke, C., Ramirez, J. L., Neves, A., Rogass, C., Ribeiro, S. P., Scariot, A., & Soares-Filho, B. (2016). Deep into the mud: Ecological and socio-economic impacts of the dam breach in Mariana, Brazil. *Natureza & Conservação*, *14*(2), 35–45. https://doi.org/10.1016/j.ncon.2016.10.003

Forget, J., Pavillon, J.-F., Beliaeff, B., & Bocquené, G. (1999). Joint action of pollutant combinations (pesticides and metals) on survival (LC50 values) and acetylcholinesterase activity of *Tigriopus brevicornis* (Copepoda, Harpacticoida). *Environmental Toxicology and Chemistry*, *18*(5), 912–918. https://doi.org/10.1002/etc.5620180514

Frasco, M. F., Fournier, D., Carvalho, F., & Guilhermino, L. (2005). Do metals inhibit acetylcholinesterase (AChE)? Implementation of assay conditions for the use of AChE activity as a biomarker of metal toxicity. *Biomarkers*, *10*(5), 360–375. https://doi.org/10.1080/13547500500264660

Gaetke, L. M., & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, *189*(1), 147–163. https://doi.org/10.1016/S0300-483X(03)00159-8

Gaetke, L. M., Chow-Johnson, H. S., & Chow, C. K. (2014). Copper: Toxicological relevance and mechanisms. *Archives of Toxicology*, *88*(11), 1929–1938. https://doi.org/10.1007/s00204-014-1355-y

García, M. E., Rodrígues Capítulo, A., & Ferrari, L. (2010). Age-related differential sensitivity to cadmium in *Hyalella curvispina* (Amphipoda) and implications in ecotoxicity studies. *Ecotoxicology and Environmental Safety*, *73*(5), 771–778. https://doi.org/10.1016/j.ecoenv.2009.12.022

Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2014). Metal-PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes. *Aquatic Toxicology (Amsterdam, Netherlands), 154*, 253–269. https://doi.org/10.1016/j.aquatox.2014.05.026

Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2015). Metal-polycyclic aromatic hydrocarbon mixture toxicity in *Hyalella azteca*. 2. Metal accumulation and oxidative stress as interactive co-toxic mechanisms. *Environmental Science & Technology*, *49*(19), 11780–11788. https://doi.org/10.1021/acs.est.5b03233

Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2016). Behavioural alterations from exposure to Cu, phenanthrene, and Cu-phenanthrene mixtures: Linking behaviour to acute toxic mechanisms in the aquatic amphipod, *Hyalella azteca*. *Aquatic Toxicology*, *170*, 377–383. https://doi.org/10.1016/j.aquatox.2015.10.019

Gheorghiu, C., Smith, D. S., Al-Reasi, H. A., McGeer, J. C., & Wilkie, M. P. (2010). Influence of natural organic matter (NOM) quality on Cu–gill binding in the rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, *97*(4), 343–352. https://doi.org/10.1016/j.aquatox.2010.01.003

Giacomin, M., Gillis, P. L., Bianchini, A., & Wood, C. M. (2013). Interactive effects of copper and dissolved organic matter on sodium uptake, copper bioaccumulation, and oxidative stress in juvenile freshwater mussels (*Lampsilis siliquoidea*). *Aquatic Toxicology*, *144–145*, 105–115. https://doi.org/10.1016/j.aquatox.2013.09.028

Giesy, J., Hertzberg, R., Klaassen, C., Kodell, R., Paustenbach, D., & Yang, R. (2002). Support of science-based decisions concerning the evaluation of the toxicology of mixtures: a new beginning. *Regulatory Toxicology and Pharmacology*, *36*, 34–39.

Gillett, N. P., Weaver, A. J., Zwiers, F. W., & Flannigan, M. D. (2004). Detecting the effect of climate change on Canadian forest fires. *Geophysical Research Letters, 31*(18). https://doi.org/10.1029/2004GL020876

Goldammer, J. G. (2015). Vegetation Fires and Global Change - Challenges for Concerted International Action: A White Paper directed to the United Nations and International Organizations. *Planet@Risk*, *3*(1). Retrieved from https://planet-risk.org/index.php/pr/article/view/214

Golder Associates. (2016). *Update Report: Post-Event Environmental Impact Assessment Report*.

Google. (n.d.). *Google Maps satellite images of the MPMC tailing pond breach, before and after.*

Government of Canada, P. S. and P. C. (2012). Biological test method - test for survival and growth in sediment and water using the freshwater amphipod *Hyalella azteca*.

Retrieved February 7, 2019, from http://publications.gc.ca/site/eng/9.575704/publication.html

Graves, K., Laval, B., Hockin, A., Vagle, S., Petticrew, S., & Albers, S. (2016). Quesnel Lake's response to the catastrophic Mount Polley Mine tailings impoundment failure – 1 year post-spill. *Proceedings of the 19th International Workshop on Physical Processes in Natural Waters: Bath, UK, 12-15 July 2016.*

Grimalt, J. O., Ferrer, M., & Macpherson, E. (1999). The mine tailing accident in Aznalcollar. *Science of The Total Environment*, *242*(1), 3–11. https://doi.org/10.1016/S0048-9697(99)00372-1

Hansen, J., Ruedy, R., Sato, M., & Lo, K. (2010). Global Surface Temperature Change. *Reviews of Geophysics*, *48*(4). https://doi.org/10.1029/2010RG000345

Harris, E. D. (2001). Copper Homeostasis: The Role of Cellular Transporters. *Nutrition Reviews*, *59*(9), 281–285. https://doi.org/10.1111/j.1753-4887.2001.tb07017.x

Hashmi, S., Ward, B. C., Plouffe, A., Leybourne, M. I., & Ferbey, T. (2015). Geochemical and mineralogical dispersal in till from the Mount Polley Cu-Au porphyry deposit, central British Columbia, Canada. *Geochemistry: Exploration, Environment, Analysis*, *15*(2–3), 234–249. https://doi.org/10.1144/geochem2014-310

Haverroth, G. M. B., Welang, C., Mocelin, R. N., Postay, D., Bertoncello, K. T., Franscescon, F., Rosemberg, D. B., Dal Magro, J., & Dalla Corte, C. L. (2015). Copper acutely impairs behavioral function and muscle acetylcholinesterase activity in zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, *122*, 440–447. https://doi.org/10.1016/j.ecoenv.2015.09.012

Howarth, R. S., & Sprague, J. B. (1978). Copper lethality to rainbow trout in waters of various hardness and pH. *Water Research*, *12*(7), 455–462. https://doi.org/10.1016/0043-1354(78)90152-5

Imperial Metals. (2015). *Mount Polley Mining Corporation post-event environmental impact assessment report - key findings report*. Retrieved from https://www2.gov.bc.ca/gov/content/environment/air-land-water/spills-environmental-emergencies/spill-incidents/past-spill-incidents/mt-polley/mount-polley-key-information

Ingersoll, C. G., Brumbaugh, W. G., Dwyer, F. J., & Kemble, N. E. (1994). Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the Upper Clark Fork River, Montana. *Environmental Toxicology and Chemistry*, *13*(12), 2013–2020. https://doi.org/10.1002/etc.5620131214

IPCC. (2013). *Climate Change 2013 The Physical Science Basis*. Cambridge, UK: Cambridge University.

Jolly, W. M., Cochrane, M. A., Freeborn, P. H., Holden, Z. A., Brown, T. J., Williamson, G. J., & Bowman, D. M. J. S. (2015). Climate-induced variations in global wildfire danger from 1979 to 2013. *Nature Communications*, *6*, 7537. https://doi.org/10.1038/ncomms8537

Kamunde, C. N., Pyle, G. G., McDonald, D. G., & Wood, C. M. (2003). Influence of dietary sodium on waterborne copper toxicity in rainbow trout, *Oncorhynchus mykiss*. *Environmental Toxicology and Chemistry*, *22*(2), 342–350. https://doi.org/10.1002/etc.5620220215

Kang, J.-J., & Fang, H.-W. (1997). Polycyclic aromatic hydrocarbons Inhibit the activity of acetylcholinesterase purified from electric eel. *Biochemical and Biophysical Research Communications*, *238*(2), 367–369. https://doi.org/10.1006/bbrc.1997.7293

Karathanasis, A. D. (1999). Subsurface migration of copper and zinc mediated by soil colloids. *Soil Science Society of America Journal*, *63*(4), 830–838. https://doi.org/10.2136/sssaj1999.634830x

Kemble, N. E., Brumbaugh, W. G., Brunson, E. L., Dwyer, F. J., Ingersoll, C. G., Monda, D. P., & Woodward, D. F. (1994). Toxicity of metal-contaminated sediments from the Upper Clark Fork River, Montana, to aquatic invertebrates and fish in laboratory exposures. *Environmental Toxicology and Chemistry*, *13*(12), 1985–1997. https://doi.org/10.1002/etc.5620131212

Kerr, R. A. (2014). The Coming Copper Peak. *Science*, *343*(6172), 722–724. https://doi.org/10.1126/science.343.6172.722

Kim, S. D., Ma, H., Allen, H. E., & Cha, D. K. (1999). Influence of dissolved organic matter on the toxicity of copper to *Ceriodaphnia dubia*: Effect of complexation kinetics. *Environmental Toxicology and Chemistry*, *18*(11), 2433–2437. https://doi.org/10.1002/etc.5620181108

Landrum, P. F., Lotufo, G. R., Gossiaux, D. C., Gedeon, M. L., & Lee, J.-H. (2003). Bioaccumulation and critical body residue of PAHs in the amphipod, *Diporeia* spp.: additional evidence to support toxicity additivity for PAH mixtures. *Chemosphere*, *51*(6), 481–489. https://doi.org/10.1016/S0045-6535(02)00863-9

Lindh, S., Razmara, P., Bogart, S., & Pyle, G. (2019). Comparative tissue distribution and depuration characteristics of copper nanoparticles and soluble copper in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*, *38*(1), 80–89. https://doi.org/10.1002/etc.4282

Lippard, S. J. (1999). Free Copper Ions in the Cell? *Science*, *284*(5415), 748–749. https://doi.org/10.1126/science.284.5415.748

McEachern, P., Prepas, E. E., Gibson, J. J., & Dinsmore, W. P. (2000). Forest fire induced impacts on phosphorus, nitrogen, and chlorophyll concentrations in boreal subarctic lakes of northern Alberta. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(S2), 73–81. https://doi.org/10.1139/f00-124

Milani, D., Reynoldson, T. B., Borgmann, U., & Kolasa, J. (2003). The relative sensitivity of four benthic invertebrates to metals in spiked-sediment exposures and application to contaminated field sediment. *Environmental Toxicology and Chemistry*, *22*(4), 845–854. https://doi.org/10.1002/etc.5620220424

Milly, P. C., Dunne, K. A., & Vecchia, A. V. (2005). Global pattern of trends in streamflow and water availability in a changing climate. *Nature*, *438*(7066), 347.

Nebeker, A. V., Cairns, M. A., Gakstatter, J. H., Malueg, K. W., Schuytema, G. S., & Krawczyk, D. F. (1984). Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. *Environmental Toxicology and Chemistry*, *3*(4), 617–630. https://doi.org/10.1002/etc.5620030412

Niyogi, S., & Wood, C. M. (2004). Biotic Ligand Model, a flexible tool for developing site-specific water quality guidelines for metals. *Environmental Science & Technology*, *38*(23), 6177–6192. https://doi.org/10.1021/es0496524

Norwood, W. P., Borgmann, U., & Dixon, D. G. (2007). Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. *Environmental Pollution*, *147*(1), 262–272. https://doi.org/10.1016/j.envpol.2006.07.017

Norwood, W. P., Borgmann, U., Dixon, D. G., & Wallace, A. (2003). Effects of metal mixtures on aquatic biota: A review of observations and methods. *Human and Ecological Risk Assessment: An International Journal*, *9*(4), 795–811. https://doi.org/10.1080/713610010

Nunes, B., Silva, V., Campos, I., Pereira, J. L., Pereira, P., Keizer, J. J., Gonçalves, F., & Abrantes, N. (2017). Off-site impacts of wildfires on aquatic systems — Biomarker responses of the mosquitofish *Gambusia holbrooki*. *Science of The Total Environment*, *581–582*, 305–313. https://doi.org/10.1016/j.scitotenv.2016.12.129

Olivella, M. A., Ribalta, T. G., de Febrer, A. R., Mollet, J. M., & de las Heras, F. X. C. (2006). Distribution of polycyclic aromatic hydrocarbons in riverine waters after Mediterranean forest fires. *Science of The Total Environment*, *355*(1), 156–166. https://doi.org/10.1016/j.scitotenv.2005.02.033

Osredkar, J. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology*, *s3*(01). https://doi.org/10.4172/2161-0495.S3-001

Pagenkopf, G. K., Russo, R. C., & Thurston, R. V. (1974). Effect of complexation on toxicity of copper to fishes. *Journal of the Fisheries Research Board of Canada*, *31*(4), 462–465. https://doi.org/10.1139/f74-077

Papagiannis, I., Kagalou, I., Leonardos, J., Petridis, D., & Kalfakakou, V. (2004). Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece).

*Environment International*, *30*(3), 357–362. https://doi.org/10.1016/j.envint.2003.08.002

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 & Pharmacology*, *133*(1), 3–35. https://doi.org/10.1016/S1532-0456(02)00112-6

Petticrew, E. L., Albers, S. J., Baldwin, S. A., Carmack, E. C., Déry, S. J., Gantner, N., Graves, K. E., Laval, B., Morrison, J., Owens, P. N., Selbie, D. T., & Vagle, S. (2015). The impact of a catastrophic mine tailings impoundment spill into one of North America's largest fjord lakes: Quesnel Lake, British Columbia, Canada. *Geophysical Research Letters*, *42*(9), 3347–3355. https://doi.org/10.1002/2015GL063345

Pohanka, M. (2011). Cholinesterases, a target of pharmacology and toxicology. *Biomedical Papers of the Medical Faculty of Palacky University in Olomouc*, *155*(3).

Prohaska, J. R., & Lukasewycz, O. A. (1989). Copper deficiency during perinatal development: Effects on the immune response of mice. *The Journal of Nutrition*, *119*(6), 922–931. https://doi.org/10.1093/jn/119.6.922

R Core Team. (2018). *R: A language and environment for statistical computing*. Retrieved from https://www.R-project.org/

Rainbow, P. S., Poirier, L., Smith, B. D., Brix, K. V., & Luoma, S. N. (2006). Trophic transfer of trace metals from the polychaete worm *Nereis diversicolor* to the polychaete *N. virens* and the decapod crustacean *Palaemonetes varians*. *Marine Ecology Progress Series*, *321*, 167–181. https://doi.org/10.3354/meps321167

Ranalli, A. J. (2004). *A Summary of the Scientific Literature on the Effects of Fire on the Concentration of Nutrients in Surface Waters* (No. USGS-2004-1296). Retrieved from Geological Survey, Reston, VA, website: http://www.dtic.mil/docs/citations/ADA439942

Richards, J. G., Curtis, P. J., Burnison, B. K., & Playle, R. C. (2001). Effects of natural organic matter source on reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. *Environmental Toxicology and Chemistry*, *20*(6), 1159–1166. https://doi.org/10.1002/etc.5620200604

Roesijadi, G. (1996). Metallothionein and its role in toxic metal regulation. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *113*(2), 117–123. https://doi.org/10.1016/0742-8413(95)02077-2

Romani, R., Antognelli, C., Baldracchini, F., De Santis, A., Isani, G., Giovannini, E., & Rosi, G. (2003). Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. *Chemico-Biological Interactions*, *145*(3), 321–329. https://doi.org/10.1016/S0009-2797(03)00058-9

Santore, R. C., Toro, D. M. D., 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*(10), 2397–2402. https://doi.org/10.1002/etc.5620201035

Shailaja, M. S., Rajamanickam, R., & Wahidulla, S. (2006). Formation of genotoxic nitro-PAH compounds in fish exposed to ambient nitrite and PAH. *Toxicological Sciences*, *91*(2), 440–447.

Shuhaimi-Othman, M. (2008). Metals concentrations in the sediments of Richard lake, Sudbury, Canada and sediment toxicity in an amphipod *Hyalella azteca*. *Journal of Environmental Science and Technology*, *1*(1), 34–41.

Sikkema, J., de Bont, J. A., & Poolman, B. (1994). Interactions of cyclic hydrocarbons with biological membranes. *Journal of Biological Chemistry*, *269*(11), 8022–8028.

Silins, U., Bladon, K. D., Kelly, E. N., Esch, E., Spence, J. R., Stone, M., Emelko, M. B., Boon, S., Wagner, M. J., Williams, C. H. S., & Tichkowsky, I. (2014). Five-year legacy of wildfire and salvage logging impacts on nutrient runoff and aquatic plant, invertebrate, and fish productivity. *Ecohydrology*, *7*(6), 1508–1523. https://doi.org/10.1002/eco.1474

Silins, U., Stone, M., Emelko, M. B., & Bladon, K. D. (2009). Sediment production following severe wildfire and post-fire salvage logging in the Rocky Mountain headwaters of the Oldman River Basin, Alberta. *CATENA*, *79*(3), 189–197. https://doi.org/10.1016/j.catena.2009.04.001

Silva, V., Abrantes, N., Costa, R., Keizer, J. J., Gonçalves, F., & Pereira, J. L. (2016). Effects of ash-loaded post-fire runoff on the freshwater clam *Corbicula fluminea*. *Ecological Engineering*, *90*, 180–189. https://doi.org/10.1016/j.ecoleng.2016.01.043

Silva, V., Pereira, J. L., Campos, I., Keizer, J. J., Gonçalves, F., & Abrantes, N. (2015). Toxicity assessment of aqueous extracts of ash from forest fires. *CATENA*, *135*, 401–408. https://doi.org/10.1016/j.catena.2014.06.021

Sinani, D., Adle, D. J., Kim, H., & Lee, J. (2007). Distinct mechanisms for Ctr1-mediated copper and cisplatin transport. *Journal of Biological Chemistry*, *282*(37), 26775–26785. https://doi.org/10.1074/jbc.M703973200

Singer, D. A. (2017). Future copper resources. *Ore Geology Reviews*, *86*, 271–279. https://doi.org/10.1016/j.oregeorev.2017.02.022

SNC-Lavalin. (2014). *Comprehensive environmental impact assessment and action plan*. Retrieved from https://www2.gov.bc.ca/gov/content/environment/air-land-water/spills-environmental-emergencies/spill-incidents/past-spill-incidents/mt-polley/mount-polley-key-information

SRK. (2015). *Mount Polley Mining Corporation Post-event Environmental Impact Assessment Report*. Retrieved from https://www2.gov.bc.ca/assets/gov/environment/air-land-water/spills-and-environmental-emergencies/docs/mt-polley/p-o-r/2016-06-03_peeiar_summary_report_v2.pdf

Stocks, B. J., Fosberg, M. A., Lynham, T. J., Mearns, L., Wotton, B. M., Yang, Q., Jin, J.-Z., Lawrence, K., Hartley, G. R., Mason, J. A., & McKENNEY, D. W. (1998). Climate change and forest fire potential in Russian and Canadian boreal forests. *Climatic Change*, *38*(1), 1–13. https://doi.org/10.1023/A:1005306001055

Swan, C., Epps, D., & Miller, L. (2014). Impact assessment monitoring in the Quesnel Lake Watershed after the Mount Polley Mining Company tailings dam breach. *Poster Sessions Presented at the Society of Environmental Toxicology and Chemistry, Vancouver, B.C.*

Toro, D. M. D., 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*(10), 2383–2396. https://doi.org/10.1002/etc.5620201034

US EPA. (2018, January 1). Method 3051A (SW-846): Microwave assisted acid digestion of sediments, sludges, and oils [Data and Tools]. Retrieved July 1, 2019, from US EPA website: https://www.epa.gov/homeland-security-research/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges

Verrhiest, G., ClÉment, B., & Blake, G. (2001). Single and combined effects of sediment-associated PAHs on three species of freshwater macroinvertebrates. *Ecotoxicology*, *10*(6), 363–372. https://doi.org/10.1023/A:1012223014534

Wang, Z., Fingas, M., Shu, Y. Y., Sigouin, L., Landriault, M., Lambert, P., Turpin, R., Campagna, P., & Mullin, J. (1999, August 12). Quantitative characterization of PAHs in burn residue and soot samples and differentiation of pyrogenic PAHs from petrogenic PAHs−The 1994 Mobile Burn Study [Research-article]. https://doi.org/10.1021/es990031y

Webb, B. W., Hannah, D. M., Moore, R. D., Brown, L. E., & Nobilis, F. (2008). Recent advances in stream and river temperature research. *Hydrological Processes*, *22*(7), 902–918. https://doi.org/10.1002/hyp.6994

West, C. W., Mattson, V. R., Leonard, E. N., Phipps, G. L., & Ankley, G. T. (1993). Comparison of the relative sensitivity of three benthic invertebrates to copper-contaminated sediments from the Keweenaw Waterway. *Hydrobiologia*, *262*(1), 57–63. https://doi.org/10.1007/BF00010989

Westerling, A. L., Hidalgo, H. G., Cayan, D. R., & Swetnam, T. W. (2006). Warming and earlier spring increase western U.S. forest wildfire activity. *Science*, *313*(5789), 940–943. https://doi.org/10.1126/science.1128834