Toxicity and bioaccumulation of sediment-associated metals and elements from wildfire impacted streams of southern Alberta on *Hyalella azteca*

by

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Author’s declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners

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Abstract

There is increasing global recognition of the effects of large scale land disturbance by wildfire on a wide range of water and ecosystem services. In 2003, the Lost Creek wildfire burned a contiguous area of 21,000 ha on the eastern slopes of the Rocky Mountains in southern Alberta. This disturbance had a significant and prolonged impact on the water quantity and quality of downstream river reaches and reservoirs in the Oldman watershed. Previous research in this watershed demonstrates that dissolved metal concentrations in rivers draining burned landscapes were 2 to 15 times greater than in unburned reference streams (Silins et al. 2009a). Currently there is no information on the effects of wildfire on the bioaccumulation and toxicity of sediment-associated metals in fire-impacted streams in Alberta.

This study was designed to evaluate the linear downstream disturbance effects of wildfire in the Crowsnest River located in southern Alberta. The toxicity and bioaccumulation of particulate-associated metals from wildfire impacted tributaries to the Crowsnest River on freshwater amphipod *Hyalella azteca* were evaluated. Phillips samplers were deployed to collect suspended solids in streams draining burned zone impacted by the Lost Creek wildfire and reference (unburned) zones within the area. Metal toxicity and bioaccumulation were determined in the laboratory by exposing the epi-benthic freshwater amphipod *Hyalella azteca* to particulates collected from the Crowsnest River. A metal effects addition model (MEAM) was used to assess the impact of metal mixtures and to predict chronic mortality (Norwood et al. 2013). Increased concentrations of Al, Ba, Co, Cr, Mn and Zn were found in the tissues of *H. azteca* exposed to particulates from burned watersheds in comparison to the unburned watersheds. *H. azteca* mean survival was similar when exposed to the particulates samples from both burned and unburned sites indicating that 9 years after this landscape disturbance, there was little impact due to the wildfire. However, at burned site (B1), the observed survival was lower than survival predicted by MEAM. The data suggests that factors other than the metals examined in this study were influencing the

survival of *H. azteca*. The concentrations of sediment-associated metals have decreased in the nine years since the wildfire, and minimal metal toxicity was observed in *H. azteca.* Although metal toxicity in *H. azteca w*as minimal 9 years after the Lost Creek Fire, the short term effects of wildfire on metal toxicity remain largely unknown. In addition, other factors such as burn severity, stream size and hydroclimatic conditions can influence the effects of wildfire on abundance and diversity of aquatic invertebrates (Minshall et al. 2001). Therefore, the influences of those factors on metal toxicity as a result of wildfire should be rigorously assessed in future studies.

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1. Introduction

1.1 Problem Statement

In recent years the frequency and magnitude of severe wildfire have increased in North America (Westerling et al. 2006; Kurz et al. 2008). Landscape disturbance by wildfire has been shown to increase sediment-associated contaminant fluxes to downstream water supplies (reservoirs), which can increase the operational costs of water treatment (Dudley and Stolton 2003). In addition, wildfire can significantly impact downstream ecosystem services (Stein and Butler 2004) and drinking water supplies (Emelko et al. 2011).

Wildfire-impacted forested landscapes typically experience increased erosion rates, soil hydrophobicity, runoff and sediment delivery (Silins et al. 2009b; Owens et al. 2010; Ryan et al. 2011). Sediment is the primary vector for contaminant transfer in aquatic systems (Horowitz 1985) and the health of aquatic systems is directly linked to the transfer and fate of sediment-associated nutrients and contaminants (Luoma 1989). In burned forested catchments, elevated metal concentrations can exceed environmental quality guidelines (Schwesig and Matzer 2000; Gallaher et al. 2002; Silins et al. 2009b). However, solely assessing total metal concentration content in sediment does not provide a true indication of metal toxicity (Chapman 1990). The Sediment Quality Triad developed by Chapman (1990) suggests that the assessment of degradation caused by the sediment-associated pollution should include toxicity tests apart from sediment chemistry and the structure of the aquatic invertebrate community. Sediment-associated metals generated by wildfire are known to influence the diversity and abundance of aquatic invertebrates (Rinne 1996; Minshall et al. 2001; Hall and Lombardoozi 2008). However, little is known about metal bioaccumulation and toxicity in wildfire impacted streams and how long these effects occur.

Results of this thesis may provide information on ecosystem recovery in response to wildfire generated metal fluxes to streams.

In 2003, the Lost Creek wildfire burned a contiguous area of 21, 000 hectares in the eastern slopes of the Rocky Mountains in southern Alberta. These kinds of large scale land disturbance have been shown to significantly impact the water quantity and quality of downstream river reaches and reservoirs (Beschta 1990). In particular, dissolved metal concentrations in rivers draining burned landscapes have elevated metal concentrations that are 2 to 15 times those measured in adjacent reference streams (Silins et al. 2009a). With the exception of studies on the bioaccumulation of Hg (Beganyi and Batzer 2011), there is no information on the effects of wildfire on sediment-associated metal bioaccumulation and toxicity in fire-impacted watersheds globally. This thesis is the first study of its kind to directly quantify metal bioavailability and toxicity to benthic invertebrates in a pristine river ecosystem disturbed by wildfire.

Metal toxicity and bioaccumulation tests are necessary to determine whether the aquatic life in a watershed has been impacted by sediment-associated metals. However, there is currently no information on the effects of wildfire on sediment-associated metal toxicity and bioaccumulation, despite the fact that metal concentrations in wildfire impacted rivers can exceed environmental quality guidelines (Schwesig and Matzer 2000; Gallaher et al. 2002; Silins et al. 2009b). The frequency and severity of wildfire has increased globally (Westerling et al. 2006) and there is a need to better understand the impacts of wildfire on aquatic life. Research questions addressed in this thesis are:

i. What are the long-term effects (after 9 years) of Lost Creek wildfire on concentrationsconcentrations of 13 sediment-associated metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Tl Zn) and do they exceed CCME sediment quality guidelines?

ii. Do elevated wildfire-generated sediment-associated metals bioaccumulate in aquatic life and cause mortality?

iii. Are the predicted survivals rates from the metal effects addition model (MEAM) comparable to survival observed in the toxicity tests? Can MEAM be applied to wildfire disturbed watershed to determine the survival of aquatic life?

The purpose of this study is to quantify sediment-associated metal toxicity and bioaccumulation in wildfire impacted streams and natural (unburned) streams located on the eastern slope of Rocky Mountains. Specific objectives of the study are to:

i. Characterize the metal chemistry of sediment and water from rivers in burned and unburned regions of the Crowsnest River basin

ii. Conduct chronic toxicity and bioaccumulation tests developed by Environment Canada on cultured amphipod *Hyalella azteca* with sediment and water samples collected from burned and unburned sites

iii. Compare the observed and predicted survival of the *H. azteca* using the metal effects addition model (MEAM)

It is hypothesized that:

i. There is no difference in metal concentrations between the impacted (burned) and reference (unburned) sites

ii. There is no difference in metal bioaccumulation between the impacted and reference tissues

iii. There is no difference in survival rates of *H. azteca* between the impacted and reference sites

1.2 Literature Review

1.2.1 Metals in the Environment

Metals are ubiquitous in the environment (Forstner and Wittmann 1979). They are mobilized through natural processes such as weathering of geological materials (Stumm and Morgan 1981) as well as anthropogenic disturbances. These disturbances include agriculture (Stone & Droppo 1996), mining (Gillis et al. 2006), sewer discharge (Forstner et al. 1981), landfill (Øyard et al. 2008) and urbanization (Stone and Marsalek 1996). Although mining and metal plating are the most prominent sources of metal pollution (Malueg et al. 1984; Smolders et al. 2003), wildfire is also an important contributor of metals to the environment (Table 1).

Table 1 Sediment-associated metal concentration (Range and mean) due to land disturbance type

Land disturbances	Sediment-associated metal concentrations (mg/kg)						References
	Cd	Cr	Cu	Ni	Pb	Zn	
Agricultural		147.6	31.9-1281	60.5	67-158	230-326	Stone & Droppo 1996; Quinton & Catt 2007
Mining	16.2-107		296-2400		85.5-902	3019-9058	Forstner et al. 1981; Smolders et al. 2003; Gillis et al. 2006
Sewer	45.9				178	1019	Forstner et al. 1981
Metal plating	18.2	19053	5557	5954		1350	Malueg et al. 1984
Landfill leachates	0.04-0.56	3.16-25.2	2.08-76	3.4-12.4	0.6-4.4	20-220	Øyard et al. 2008
Urbanization	<0.2-2.8	25-314	27-802	20-38	33-261	52-2728	Stone and Marsalek 1996
Wildfire	60	500	900	2000	600	30000	Gallaher et al. 2002; Gallaher et al. 2004

The transfer of metals from terrestrial to aquatic environments and subsequent mobility of these metals in aquatic environments are governed by a complex series of physical (Horowitz 1985), chemical (Stumm and Morgan 1981) and biological (Luoma 1989) processes. These processes are strongly influenced by wildfire (Rhoades et al. 2011; Ryan et al. 2011; Corbin 2012). For example, soil hydrophobicity can increase after wildfire (Certini 2005) and during rain events, the transfer of soils and associated metals to receiving streams increase (Debano 1981; Blake et al. 2010; Smith et al. 2011), which can impact downstream water quality (Blake et al. 2009). Metal concentrations downstream of land disturbances are elevated because metals bound to fine sediment are vastly mobile (Horowitz 1985).

1.2.2 Effects of Wildfire on the Ecosystem

Wildfire disturbances strongly influence vegetation cover, soil characteristics, hydrology, water quality, aquatic life and their abundance and diversity of aquatic biota (Earl and Blinn 2003; Silins et al. 2009b; Blake et al. 2010; Bodí et al. 2011). Once the vegetation cover is removed by wildfire, canopy interception is reduced (Shakesby 2006). In addition, hydrophobic burned soils often reduce water infiltration rates in wildfire-impacted areas (Certini 2005). Accordingly, sediment fluxes to receiving streams typically increase during precipitation events and are reduced once vegetation cover is regenerated (Beyers et al. 2005). Water quality is degraded by wildfire-induced contaminants, but their effects on aquatic life require further investigation. Aquatic life provides essential ecosystem service to humans, for example, fish as a source of food, and can have tremendous economic value to society. In turn, fish populations rely on aquatic invertebrates as a major food source. Aquatic invertebrates are an important ecosystem service because of their role in organic matter decomposition as well as nutrient and elemental cycling (Wallace and Webster 1996). Examining the effects of wildfire on aquatic invertebrates can illustrate implications of wildfire on some aquatic ecosystem services.

1.2.2.1 Abundance and diversity of aquatic invertebrates

Aquatic invertebrates are commonly used indicators of the magnitude and effect of various disturbances in aquatic ecosystems (Minshall 2003). The abundance and diversity of aquatic invertebrates have been used as a method to determine the relative impacts of these disturbances on aquatic environments. Table 2 presents some of the findings of studies designed to determine the impacts of wildfire on the abundance and diversity of benthic invertebrates. The magnitude of the wildfire impacts can be influenced by timing of the fire, size of catchments, severity of the burn and precipitation events (Minshall et al. 2001). These studies show that the effects of wildfire on abundance and diversity of aquatic invertebrates is not often obvious in short term studies (e.g. less than one-year studies) because such studies often show no overall changes in impacted sites compared to reference sites (Table 2). However, Corbin (2012) claims that effects of wildfire are reduced when precipitation and runoff rates are low. Crowther and Papas (2005) suggest that an insufficient number of impact and control sites were the reasons that differences between the burned and control sites were not observed in their study. Studies longer than four years indicate contradictory results and higher abundance and diversity have been found at both burned sites compared to unburned sites (Table 2). Silins et al. (2009a) reports that an increase in the abundance and diversity of macroinvertebrate in burned sites is related to an increase in algal availability resulting from high nutrient loading to streams. The magnitude of wildfire impact on animal density is related to the presence of ash and how long it is present in the water column of the stream (Earl and Blinn 2003). Table 2 shows that wildfire impacts on benthic invertebrate abundance and diversity may not be observed immediately post-fire and effects can linger up to 10 years (Minshall et al. 2001). Although the abundance and diversity of aquatic invertebrates are affected by wildfire, little is known about the bioaccumulation and toxicity of wildfire-generated metals on their survival.

Table 2 Studies assessing the impact of wildfires on benthic invertebrates

STUDY (YR)	WILDFIRE	YEAR	CONCLUSIONS	SOURCES
1	North-east Victoria	2003	No overall difference in density or species richness	Crowther and Papas (2005)
1	Tod River	2005	No obvious changes in abundance or diversity	Corbin (2012)
2	Hayman	2002	Density and biomass were 60%-80% lower in burned streams. Data show recovery in year 2.	Hall and Lombardozzi (2008)
2	Togo fire	2003	Higher density in burned than control sites. Diversity was lower in burned catchments and dominated by Chironomae	Mellon et al. (2008)
2	Okefenokee Swamp	2007	Only marginal different in overall community composition. Midges still dominated but number of water mites decreased. Biomass of midges were unaffected. Greater density of Corixids in burned sites.	Beganyi and Batzer (2011)
3	Dude Fire	1990	Density reduced to zero one month post fire. Recovered 25-30% within a year.	Rinne (1996)
4	Lost Creek Fire	2003	Benthic invertebrates abundance elevated by 1.5x in the burned streams in year 4. Higher diversity in burned sites.	Silins et al. (2009a)
5	Yellowstone fire	1988	Minimal change in density, biomass, and richness. Chironomidae abundance increased 1 to 3 years post fire. Mayfly increase 3-5 years post fire.	Minshall et al. (1997)
5	Gile River	1995	Density reduction is minimal to dramatic depending on duration of ash flow. Density recovered within 1 year.	Earl and Blinn (2003)
10	Mortar Creek Fire	1979	Richness and abundance is lower in burned catchments, but recovered with 7 years. Density of disturbance-adapted species increase after fire, other species decreased.	Minshall et al. (2001)

1.2.3 Metals in Aquatic Systems

The survival of aquatic life is related to the mobilization of metals at the sediment-water interface (Luoma 1989). In aquatic systems, sediment can act as a sink or source for metals (Figure 1). In the water column, metals bound to suspended sediments can be deposited as bed sediment. “Pore water” is defined as water (20%-50%) trapped between sediment particles (Teasdale et al. 1995). At steady state, metals are in equilibrium with sediment particulates, pore water and overlying water. Metal exposure to aquatic life can occur and in some environments when the environmental conditions are favorable for the release of metals (INAC 2002). The term “metal bioavailability” refers to the amount of metals that is directly exposed to aquatic organisms and several environmental factors influence the rate of release and uptake (Luoma 1983). One of the controlling factors of bioavailability is "metal speciation", which is defined as 'the partitioning among various metal forms in which they might exist' (Tessier et al. 1979). The following sections discuss the factors that influence the relationship between metal bioavailability and the metal speciation in aquatic systems. Specifically this literature review addresses metal mobility related to water chemistry, porewater and sediment chemistry.

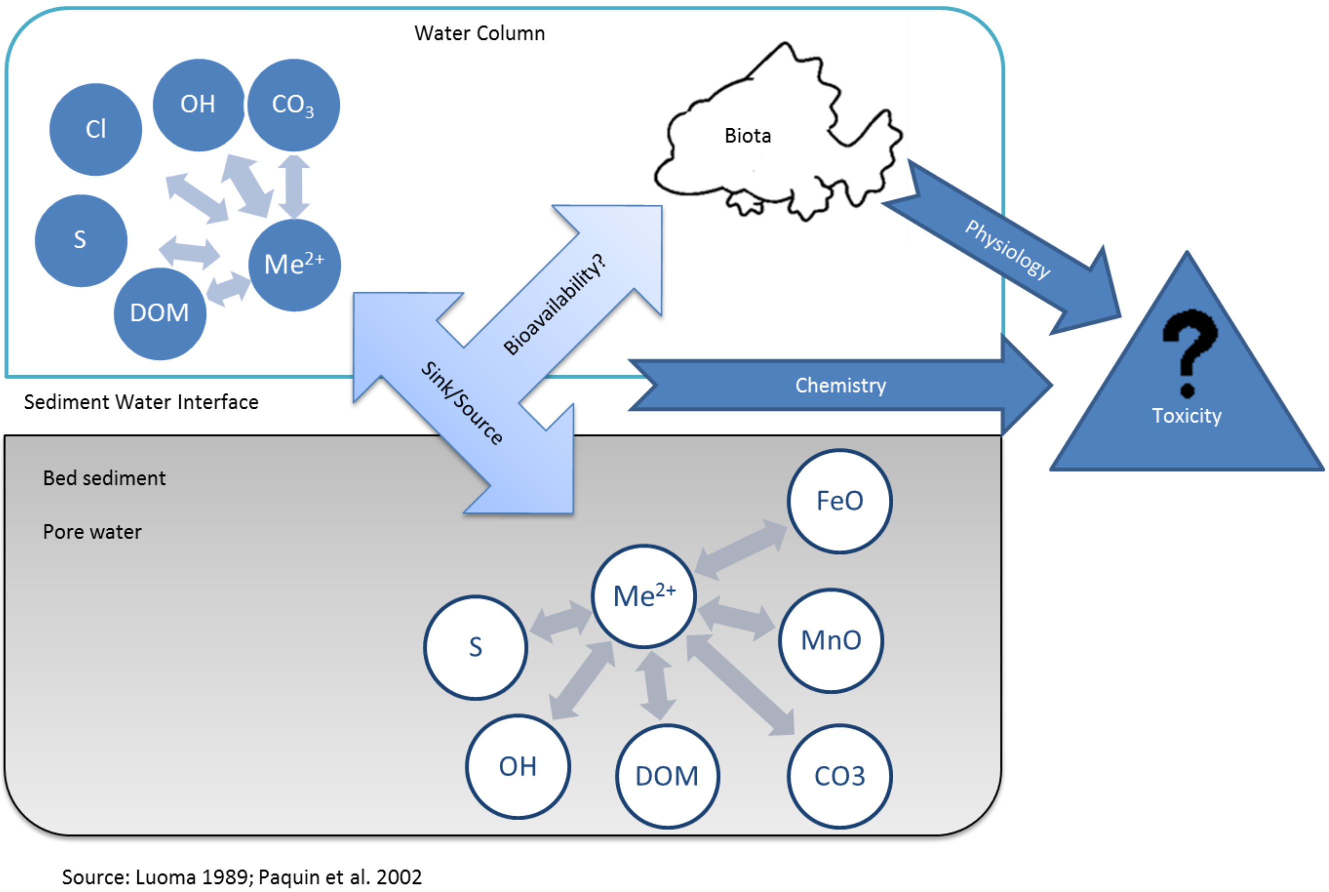

Figure 1 Conceptual diagram of the biotic ligand model, which incorporates metal chemistry (water and sediment) and the physiology of organisms to predict toxicity

1.2.3.1 *Water chemistry and metal bioavailability*

Metal speciation is one of the environmental factors controlling metal bioavailability in the water column (Stumm and Morgan 1981). Depending on the pH and water hardness, metals species can bind with ligands such as hydroxide (OH), carbonate (CO_3) and dissolved organic matter (DOM) such as humic substances (Morel et al. 1973; Mota and Santos 1995). Major ions such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) control water hardness, which may influence the metal bioavailability to aquatic life (Stephenson and Mackie 1989). The total metal concentration is composed of the bioavailable free metal fraction and complexed metal fraction (Allen 1993). The generic expression of the total concentration of a divalent metal (Me_T) such as cadmium (Cd^{2+}) including free divalent metal (Me^{2+}), hydroxide (OH^-), chloride (Cl^-), sulphate (SO_4^{2-}), carbonate species (CO_3^{2-}, HCO_3^-) and organic ligands (L) is described in equation (i) as reported by (Evans et al. 2003):

$$[M]_T = [Me^{2+}] + [MeOH^+] + [MeCO_3{}^0] + [MeHCO_3{}^+] + [MeCl^+] + [MeSO_4{}^0] + [MeL^0] \qquad \text{(i)}$$

The proportion of dissolved metal species is dependent on pH, water hardness and amount of ligands in the water column (Evans 1989). Anions such as SO_4^{2-} can reduce bioavailability of Se^{2+} (Forsythe and Klaine 1994) and Cl^- can reduce the bioavailability of Hg^{2+} and Cd^{2+} (Borgmann 1983). Mercury (Hg) is bound to Cl^- at lower pH, but as water becomes more alkaline (pH increases), more OH^- ions are available to bind with Hg (Figure 2).

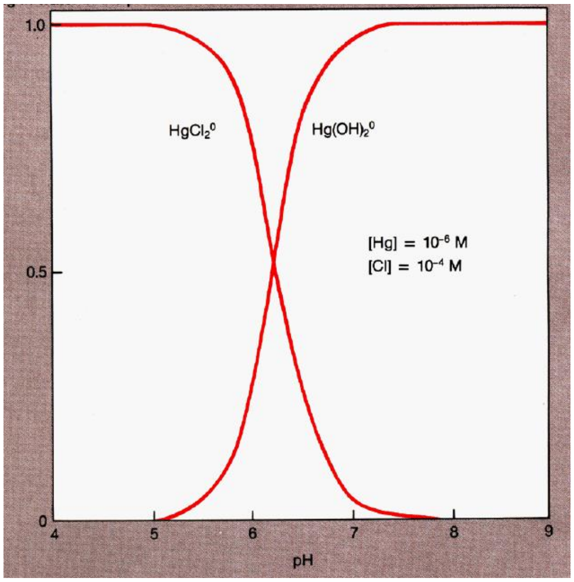

Figure 2 The proportion of mercury (Hg) speciation with chloride (Cl) and hydroxide (OH) as a function of pH in natural water (Evans 1989)

Carbonate species originate from the dissolution of mineral e.g. calcite ($CaCO_3$) and atmospheric carbon dioxide ($CO_{2\,(g)}$) that reacts with silicates, oxides and carbonates in rocks (Stumm and Morgan 1995). Carbonate species in freshwater systems, that are open to the atmosphere, depend on the partial pressure of carbon dioxide gas ($pCO_{2\,(g)}$), and alkalinity (bicarbonates). Total dissolved inorganic carbon (DIC) is described by equation (ii) (Stumm and Morgan 1995):

$$C_T = [CO_{2\,(aq)}] + [H_2CO_3] + [HCO_3^{\,-}] + [CO_3^{\,2-}] \qquad \text{(ii)}$$

Formation of metal complexes with DIC in the water column is a function of pH, which influences the availability of its species. For example, carbonates can influence dissolved metal species as described in the following chemical equations (iii to v):

$$Me^{2+} + CO_3^{\,2-} \leftrightarrow MeCO_3^{\,0} \qquad \text{(iii)}$$

$$Me^{2+} + 2(CO_3^{\,2-}) \leftrightarrow Me(CO_3)^{2-} \qquad \text{(iv)}$$

$$Me^{2+} + HCO_3^{\,-} \leftrightarrow MeHCO_3^{\,+} \qquad \text{(v)}$$

Because carbonate species act as buffers in aquatic systems, the concentration of carbonate species and the amount of metals complexed with carbonates is typically very low (Allen and Hansen 1996). Metal induced mortality of benthic invertebrates is reduced by dissolved inorganic carbon (DIC). For an instance, DIC complexation can reduce bioavailability for Cu (Stiff 1971; Andrew 1977).

Dissolved organic matter (DOM) in aquatic systems can reduce metal bioavailability and toxicity by binding with metals (Borgmann et al. 1991; Meador 1991; Kim et al. 1999). Dissolved organic matter is composed of humins, humic acids, fulvic acids and yellow organic acids (Jonasson 1977). In freshwater, 60% to 80% of DOC contains humic substances, which are mainly humic and fulvic acids (Reuter and Perdue 1977). In particular, fulvic acids play a major role in binding with metals because they have lower molecular weight and have a larger number of functional groups, such as amino ($-NH_2$), carbonyl (=O), alcohol (-OH), thioether (-S-), carboxyl (-COOH), phenolic (-OH) and thiol (-SH) groups (Jenne 1976). The freshwater cladoceran C*eriodaphnia dubia* has a lower percent survival with non-DOM-complexed copper (Cu) than DOM-complexed Cu (Kim et al. 1999) (Figure 3). Accordingly, DOM complexation reduces the bioavailability of Cu by tightly binding with it. Consequently, DIC, DOC, and major ions such as SO_4^{2-} and Cl^- should be considered when conducting metal analysis because of their influence on the free ion activity of metals in natural water.

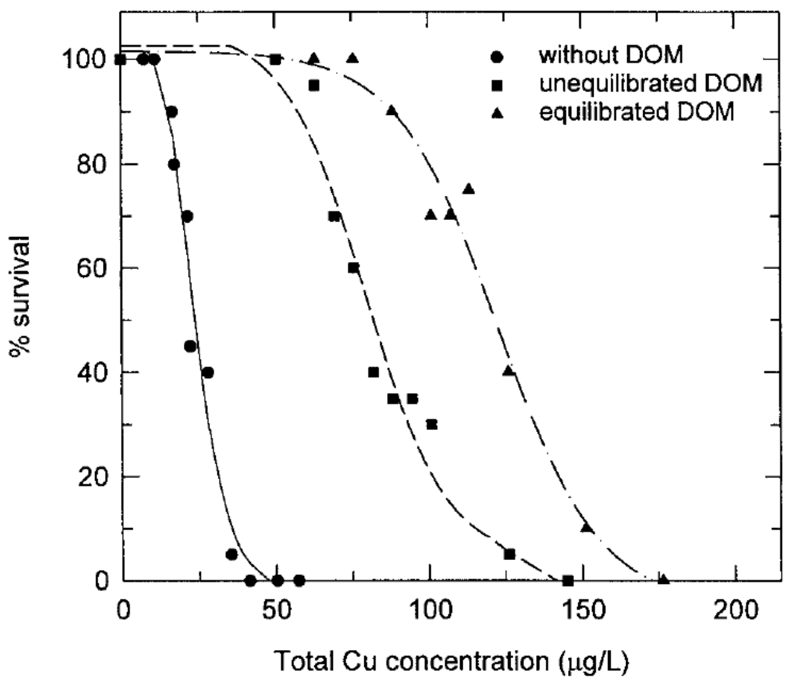

Figure 3 The effect of dissolved organic matter (DOM) on survival of *Ceriodaphnia dubia* with increasing Cu concentration (Kim et al. 1999)

1.2.3.2 Pore water chemistry and metal bioavailability

Metal bioavailability is strongly governed by pore water chemistry (INAC 2002). Figure 4 shows that as redox potential decreases with sediment depth, organic matter (OM) begins to break down and release various byproducts such as manganese (Mn^{2+}), iron (Fe^{2+}) and sulphate (SO_4^{2-}) into the water column (Zhang et al. 1995). The reduction of sulphate to hydrogen sulphide (H_2S) is an important process in anoxic sediment because under reducing conditions sulphide (S^{2-}) precipitates with various metals and metal bioavailability is reduced (Gaillard et al. 1986; Evans 1989; Santos-Echeandia et al. 2009). Conversely, metals are more bioavailable near the sediment-water interface where oxygen concentrations are higher and the rate of metal precipitation is lower (Zhang et al. 1995).

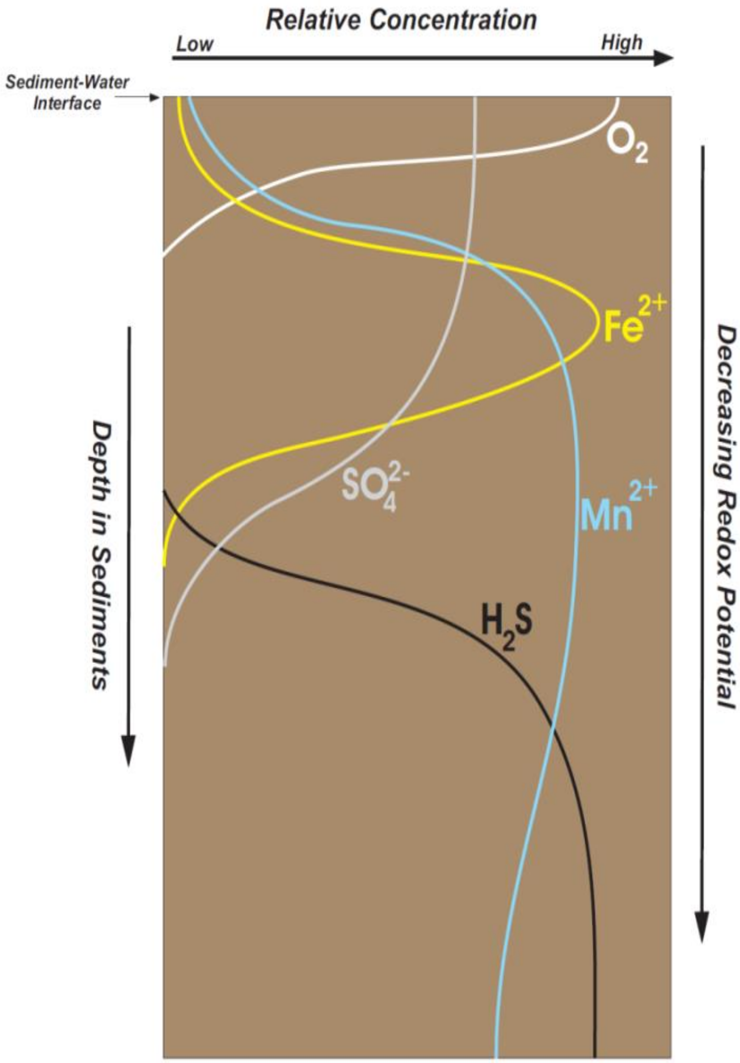

Figure 4 Porewater metal bioavailability in the sediment under varying depth, redox potential, oxygen, sulfate and hydrogen sulphide levels (INAC 2002)

1.2.3.3 Sediment chemistry and metal bioavailability

Metal bioavailability is related to changing pH conditions and metal speciation with ligands including clay minerals, organic matter (OM) and iron and manganese oxides (FeO/MnO) in bed sediment (Jenne 1977). Sediment with high clay mineral content can bind metals because of its large surface area and surfaces with metal-oxy hydroxides (OM, FeO and MnO) (Allen 1993). Clay minerals such as kaolinite and chlorite have negatively charged surfaces (due to hydroxyl ions on the surface structure) that attract metallic cations by adsorption (Hirst 1962). Some expandable clay minerals such as vermiculite and smectite adsorb metallic ions in between their inter-layer sheets as well as on their edges (Kinniburgh and Jackson 1981). Phyllosilicate clay minerals reduce metal bioavailability and metal toxicity due to their ability to adsorb metals (Singh et al. 1991; Usman et al. 2005; Zhang et al. 2011).

Organic matter (OM) surface coatings in sediment bind with metals and reduce metal bioavailability (Fu et al. 1992; Fagnani et al. 2012; Hernandez-Soriano and Jimenez-Lopez 2012). Soluble humic acids and fulvic acids can play an important role in binding aqueous phase metals. In contrast, humins and yellow organic acids absorb metals in the sediment phase since they are relatively insoluble (Jenne 1976; Jonasson 1977). Oragnic matter-bound metals are often adsorbed on the surface of clay minerals as well, which reduces metal bioavailability (Curtis 1966).

In the oxidized sediment layer, hydrous iron (Fe) and manganese (Mn) oxides are efficient metal collectors that are commonly coated on clay mineral surfaces (Forstner and Wittmann 1979). Metals can be bound to hydrous Fe and Mn oxides by precipitation and/or adsorption (Kinniburgh and Jackson 1981). Although the influence of Fe and Mn oxide on metal toxicity is not well studied, metals are highly adsorbed by oxides of Fe and Mn, which potentially reduce metal bioavailability in oxidized sediment layers (Dong et al. 2000; Li et al. 2003; Besser et al. 2008; Øygard et al. 2008).

The total metal concentration in sediment may not entirely reflect its toxicity for aquatic biota as many inorganic and organic ligands are present in the water column and sediment to bind with metals (Luoma 1989) thereby reducing metal bioavailability. Accordingly, it is necessary to consider metal speciation in the ambient aqueous and sediment system because of their combined influence on metal bioavailability. In addition to metal speciation in the aquatic system, metal bioavailability also depends on the biological interactions of the aquatic life.

1.2.4 Metals Interactions with Aquatic Invertebrates

When aquatic invertebrates are exposed to metals they undergo physiological changes to process the metals (Table 3). Although guidelines have been developed by Canadian Council of Ministers of the Environment (CCME) to protect the aquatic life (Table 3) and physiological mechanism of metal-fish interaction has been well studied (Morgan et al. 1997; Bury et al. 1999; Wood et al. 1999), the physiological effects of metals on specific aquatic invertebrates are comparatively well-studied.

Table 3 Metal physiological effects and sediment quality guidelines from CCME (1999)

Metals	**Metal physiological effects to benthic invertebrates**	**CCME ISQG (ppm)**
Aluminium (Al)	Disruption of ion regulation and loss of sodium (Otto and Svensson 1983)	N/A
Arsenic (As)	Suppressed AChE activity and considered neurotoxicant (Chakraborty et al. 2012)	5.9
Cadmium (Cd)	Inhibition of calcium influx (Wright 1980; Craig et al. 1999)	0.6
Copper (Cu)	Inhibition of Na+/K+/ATPase (Brooks and Mills 2003)	35.7
Iron (Fe)	Action inhibition and smothering due to precipitation (Vouri 1995)	N/A
Lead (Pb)	Poorly understood	35
Manganese (Mn)	Block calcium channels (Simkiss and Taylor 1995)	N/A
Mercury (Hg)	Poorly understood	0.17
Zinc (Zn)	Poorly understood	123

Generally, monovalent metals such as silver (Ag^{+}) impair sodium (Na^{+}) regulation and divalent metals such as cadmium (Cd^{2+}) and zinc (Zn^{2+}) disrupt internal calcium (Ca^{2+}) concentrations (Paquin et al. 2002). Aquatic invertebrates have a variety of mechanisms deal to deal with elevated metal concentrations (Rainbow 2002). This section summarizes knowledge regarding the general mechanisms of metal uptake, detoxification and excretion by aquatic invertebrates.

Aquatic invertebrates can take up metals by ingestion through diet, diffusion through the body surface and adsorption to the exoskeleton (Rainbow 2007). Dietary ingestion is known to be the major source of metal contribution from sediment (Lee et al. 2000; Yu and Wang 2002). After ingestion, non-essential metals such as silver and cadmium cross the endoderm of the gut (Rainbow 1988). Alternatively, invertebrates use ion channels to transport essential ions such as chloride, sodium and calcium to maintain ionic-regulation in their bodies (Paquin et al. 2002). Metals such as silver inhibit sodium and chloride pumps by disrupting the sodium/potassium dependent adensosine (Na/K ATP) as shown in *Daphnia magna (*Bianchini and Wood 2003). Metals can also be adsorbed onto the exoskeleton, but this pathway is not a major source of uptake (Rainbow 2007).

Once metals are taken up, aquatic invertebrates may respond differently depending on whether the metal is essential or non-essential (Rainbow 2002). Essential metals such as zinc and copper are necessary for metabolism of enzymes and proteins such as carbonic anhydrase and haemocyanin. Conversely, non-essential metals such as cadmium and silver do not contribute to metabolic needs (Rainbow 2002). When the concentration of essential metals exceeds metabolic requirements or non-essential metals are taken up, detoxification in aquatic invertebrates occurs by metal storage in proteins and granules, which causes metals to become metabolically unavailable (Brown 1982; Rainbow 1988). For example, *D. magna* synthesize metallothionein, which is a cysteine-rich protein (Stuhlbacher et al. 1992). This protein serves to bind and detoxify metals such as cadmium, copper, mercury, silver, and zinc (Amiard et al. 2006).

Detoxified metals are generally excreted but some animals may store detoxified metals in their body (Rainbow 1998). Metal excretion is carried out through feces secretion in form of granules and lysosome broken down from metallothionein (Rainbow 2007). Some aquatic invertebrates can accumulate metallothionein-bound metals instead of excreting them and these metabolically unavailable toxic metals are stored inside the body (Rainbow 1998).

Metal toxicity is based on the amount of metal accumulated in the body of aquatic invertebrates, which is termed “bioaccumulation” (Borgmann and Norwood 1999). Metal bioaccumulation in benthic invertebrates is proportional to the bioavailable metal concentration (Stephenson and Mackie 1989; Rainbow 1995). Accordingly, the sediment quality guidelines provided by CCME (Table 1) do not accurately depict metal toxicity because they are based on total metal concentration instead of bioavailable metal concentration (MacDonald et al. 1996). Furthermore, metals such as aluminum, iron, manganese and nickel have no defined guidelines, thus, other approaches are required to regulate their toxicity.

Physiological processes in benthic invertebrates can alter metal bioaccumulation (Rainbow 2007). Intuitively, if the rate of metal uptake is greater than the rate of metal detoxification and excretion, then the total body metal concentration will be higher. Although metals adsorbed onto the exoskeleton contribute to total body metal concentration, they do not affect the animals internally since they are not bioaccmulated in the body through this pathway (Rainbow 2007). The total body burden of metals in an animal is composed of the metal in its body (bioavailable) and in its gut content (non-bioavailable) (Chapman 1985; Hare et al. 1989; Cain et al. 1995; Amyot et al. 1996). Neumann et al. (1999) demonstrate that gut contents can contribute a substantial amount of non-bioavailable cadmium in the freshwater amphipod *Hyalella azteca*. For this reason, it is vital to clear the gut before conducting metal analysis to avoid overestimation of bioavailable metal concentration in the body, and most standard toxicity test protocols include this as a recommended post-exposure step (ASTM 2003).

1.2.5 Metal Toxicity and Bioaccumulation Models

1.2.5.1 Biotic ligand model (BLM)

The biotic ligand model (BLM) was developed to quantify the toxicological responses of aquatic invertebrates by considering the ambient chemistry, as well as metal bioavailability in the aquatic system (Paquin et al. 2002). The BLM evolved from the gill surface interaction model (GSIM) following Pagenkopf (1983) and the free ion activity model (FIAM) of Morel (1983). The BLM predicts the amount of metals bound at the biotic ligand (membrane of the animal) and assumes that metals bound at the site of action causes animal mortality (Paquin 2002). Metal bioaccumulation can result through dietary uptake, but this exposure pathway is not considered in the BLM (Di Toro et al. 2005). The BLM predicts acute toxicity based on the individual metal binding coefficients at the site of action, but it does not consider effects of chronic toxicity and metal mixtures (Niyogi and Wood 2003; Norwood et al. 2003). Regardless, the BLM relates bioaccumulation and toxicological response. Other models have been specifically developed to predict chronic toxicity in aquatic invertebrates.

1.2.6 Metal Effects Addition Model (MEAM) and the Biotic Ligand Model (BLM)

The MEAM predicts toxicity based on metal accumulated in the body because metal bioaccumulation ultimately causes animal mortality despite of the exposure pathway (Norwood et al. 2013). Di Toro et al. (2001) states that the BLM predicts that metals at lower concentrations do not compete at the same site, where as MEAM predicts additive effects of metals at low concentrations. The MEAM is considered a more reliable tool to predict chronic survival than sediment quality guidelines (SQG) since SQGs do not consider the effects of mixtures of metals (Norwood et al. 2003). The MEAM is used to quantify chronic survival of freshwater amphipod *Hyalella azteca* in a mixture of 10 toxic metals (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) (Norwood et al. 2013). MEAM uses background corrected, 24-hour depurated, body concentrations of those 10 metals to predict chronic mortality (Norwood et al. 2013). This study evaluates the ability of MEAM to predict metal chronic survival in wildfire impacted sites.

2 Methodology

2.1 Study Area

The study area is located in the Crowsnest River basin in southwestern Alberta, Canada (Figure 5). The vegetation varied as elevation ranged from 1100 m to 3100 m: lower areas contained mixed conifer, mid elevations contained primarily subalpine forest, and higher elevated area consisted primarily of alpine meadow vegetation and bare rock extending above the tree line (Allin et al. 2012). At higher elevation, the mean annual precipitation was 1020 mm/yr. From 2004 to 2008, 53% of the precipitation was in the form of rainfall and 47% as snowfall (Silins et al. 2009a).

This river was recognized as a high quality fishing habitat and it supplies water for agricultural and recreational uses as it flows eastward through the Crowsnest Pass and into the Oldman Rservoir. The Oldman River flows through the Peigan Indian Reserve, the City of Lethbridge, and south where it joins the Bow River to become part of the Saskatchewan-Nelson River system (Glenn 1999).

The Crowsnest River is an internationally recognized flyfishing river and the fish species feed on a range of invertebrates including Ephemeroptera, Plecoptera, Trichopetera, Chironomidae (Kiffney & Clements 1994; Silins et al. 2009a).

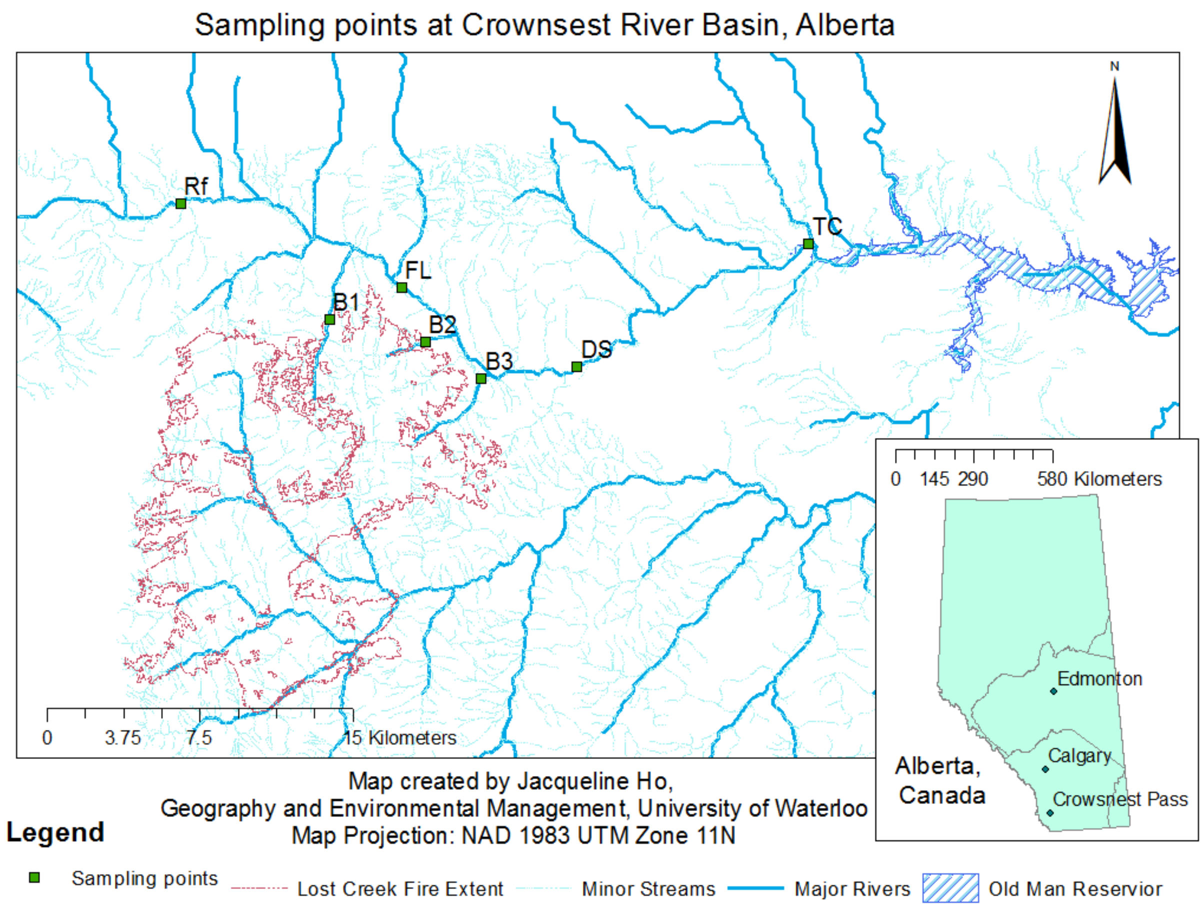

Figure 5 Study area and sampling locations

In 2003, the Lost Creek fire burned a nearly contiguous area of 21,000 hectares including the headwater area of the Crowsnest River. There are six watersheds that drain north into the Crowsnest River, including South York Creek, Drum Creek, Star Creek, North York Creek, Lyons Creek East-fork, and Lyons Creek West-fork. Rivers running parallel with Crowsnest River include the Oldman River and Castle River. The reference site (Rf) of the Crowsnest River was located northwest of the burn area, mid-river located north of the burned area (B1, B2, and B3) and the downstream sites (DS and TC) of the Crowsnest River is northeast of the burned area. The arm of Crowsnest River leads into the receiving catchment of the burned sites, the Oldman Reservoir. The characteristics of the sampling sites are summarized in Table 4.

Table 4 Characteristics of the study watersheds

Site ID	Stream	Category	Burn percent (%)
Rf	Crowsnest River	Reference	
B1	Lyons Creek	Burned and salvage- logged	82
FL	Frank Lake	After water treatment plant	N/A
B2	Drum Creek	Burned	100
B3	Byron Creek	Burned	60
DS	Downstream of Crowsnest River	Downstream	10
TC	Crowsnest River before Todd Creek	Downstream	N/A

2.2 Experimental Design

The purpose of this thesis is to determine sediment-associated metal toxicity and bioaccumulation in wildfire impacted streams and natural (unburned) streams located on the eastern slope of the Rocky Mountains in the Crowsnest River basin. Phillips samplers (Figure 6) were deployed from May to August, 2012 to collect suspended particulates from various land use type areas (burned, reference/unburned). Three samplers were deployed at the reference site (Rf), one sampler each at three burned sites (B1, B2, B3) and at a downstream site (DS) (Figure 5). Two additional grab samples were collected in acid-washed high density polyethylene (HDPE) containers at the surface of sediment where particles are fine and re-suspend easily (Figure 7). One grab sample was collected at Frank Lake (FL) to determine the effects of a sewage treatment upstream of this site and another grab sample was collected in Crowsnest River above the confluence of Todd Creek (TC). In total, nine water and sediment samples were collected.

Figure 6 Phillips samplers deployed at the upstream reference (unburned site) (Rf)

Figure 7 Fine sediment deposits where grab samples were collected in the Crowsnest River (Site TC).

2.3 Experimental Preparation

2.3.1 Culturing

The freshwater amphipod *Hyalella azteca* collected at a small lake in Valens Conservation Area was cultured in the Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, ON (Figure 8) (Borgmann et al. 2005a). *H. azteca* is a sensitive species that often borrows in the top-oxic sediment layer where metals are released from porewater (Borgmann et al. 1989; Borgmann et al.

2005a). Thus, *H. azteca* is suitable for sediment toxicity. As well, *H. azteca* is as a major food source for many fish, waterfowl and larger invertebrates (Table 5) (Dryer et al. 1965; Wojcik et al. 1986).

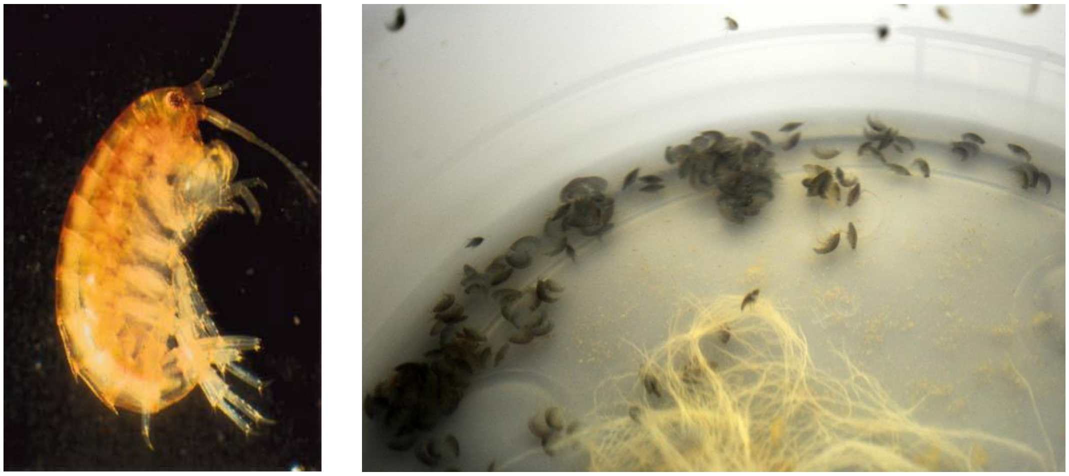

Figure 8 The freshwater amphipod *Hyalella azteca*

Table 5 Life history characteristics of *H. azteca*

Species (Order)	*Hyalella azteca* (Amphipoda)
Optimum habitat	Warm water at 23-25°C Near shore & shallow areas
Geographic distribution	North America, Central & northern South America
Burrowing activity	Burrow in fine, organic rich, upper oxic sediment. Cling on cotton gauze
Reproduction rate	1-3 young per week per adult
Required essential ions	Bromide, bicarbonate, calcium, sodium
Predator	Waterfowl, Fish, Large Invertebrates

H. azteca were cultured at CCIW since 1985 in incubators at 25°C with 16 hours of light and 8 hours of dark and light intensity of 50-80 $\mu E/m^2/s$ in de-chlorinated (deChlor) tap water with a hardness of 130 mg/L, alkalinity 90 mg/L, Ca 40mg/L, Mg 8 mg/L, Na 12 mg/L, SO_4 28 mg/L, Cl 24 mg/L, pH 7.9-8.6 and DOC 2 mg/L. Another culturing medium is SAM-5S, developed by Borgmann (1996), which contains all the essential ions that *H. azteca* requires to survive, including bromide (0.01 mM NaBr), 1 mM $CaCl_2$, 1mM NaHCO3, 0.25 mM $MgSO_4$, and 0.05 mM KCl. Twenty to 30 animals were held in 2 L polypropylene containers with cotton gauze as a substrate. Each container was fed 5 mg of ground Tetra-Min® fish food 3 times per week. Each adult produced 1-3 young per week. Adults and juveniles were separated weekly into petri dishes using sieve mesh sizes of 650 and 270 µm, respectively.

2.4 Chronic Bioaccumulation and Toxicity Test

The method developed by Borgmann et al. (2005) uses 1 L Imhoff settling cones (Figure 9). Each cone contained 15 mL of sediment and 1 L of water resulting in a water: sediment ratio of 67:1. Stoppers were added to the cones tightly to prevent leakage. To minimize disturbance to the sediment in the cone, water was added slowly and carefully to prevent re-suspension of sediment. An air tube was connected to supply air and extended to the bottom of the cone to ensure sufficient oxygenation. The advantages of using Imhoff settling cones over conventional beakers are: the higher volume of cones (1L) allows higher volume for chemical analysis; prevents deterioration of water quality for a 4-week chronic toxicity test; which increases the survival of control test organisms (Borgmann et al. 2005a).

For each site listed in Table 4, up to three replicates were setup depending on the amount of sediment that was available. For example, only one cone was setup for Byron Creek (Site B3) and 2 cones were setup for downstream site (DS) due to insufficient sediment samples collected by the time-integrating suspended sediment sampler. In addition to the sediment collected from the field, two control sediments from uncontaminated sites (Lake Erie and Lake Restoule) were used to demonstrate the good health and survival of the animals. These sediments have been tested repeatedly and have shown good survival consistently (Borgmann et al. 2001). All cones were filled with their respective site water, except for control cones where deChlor water was used.

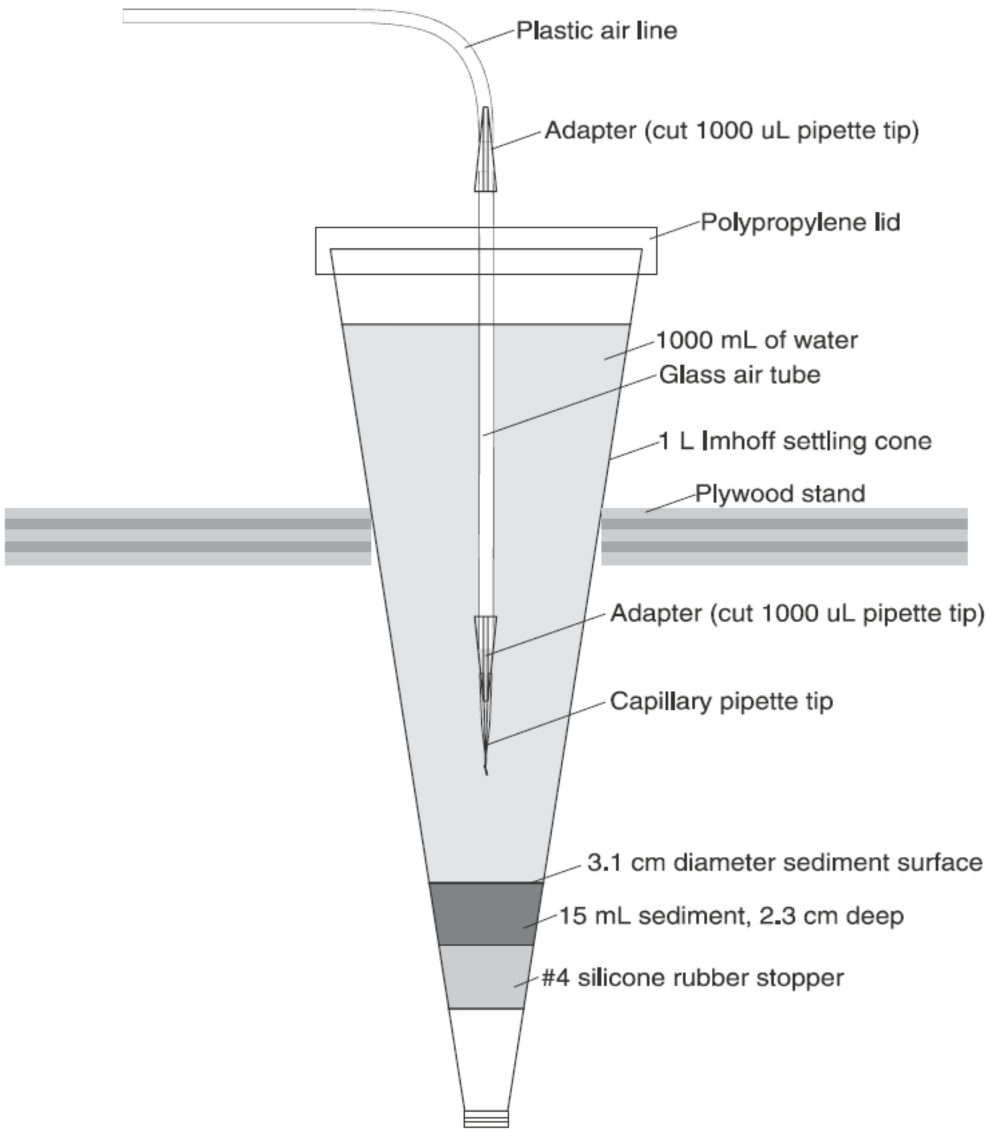

Figure 9 Imhoff settling cones setup for sediment toxicity tests (Borgmann et al. 2005a)

2.4.1 Two-week Equilibrium

The Imhoff cones were packed with sediment and site water then placed in the incubator at constant temperature for two weeks in the dark at 25°C to allow sediment and overlying water to equilibrate. To increase the accuracy of adding 15 mL of sediment in the cone, a 15 mL marker line was drawn on the cone measuring from the top of the stopper (Figure 10). Before adding the sediment to the cones, excess water from the storage container was decanted carefully with the amount of water recorded and sediment samples were mixed thoroughly. At the beginning and the end of two weeks, water quality was tested and recorded. Ten mL of water was collected from the cones for metal analysis described below.

Figure 10 The 15 mL line on Imhoff cones

After two weeks of equilibration, 15 young (0-1 week-old) *H. azteca* were added to the cones. Animals were placed into cups three at a time until 15 animals were in each cup. Young animals are used in chronic exposure so that growth, survival and bioaccumulation can be quantified (Borgmann et al. 2005a). The 15 juveniles in each sample cup were then poured into the prepared cones in random orders and rinsed with nanopure water to ensure all animals were transferred into the cone. A squirt bottle was used to spray on the water surface of the cones to ensure food and animals were not stuck due to water tension. The bubbling tube was moved down to 1 cm from the sediment surface to ensure the animals have sufficient oxygen supply, but not disturbed by the bubbling of the tube.

The chronic toxicity test duration was 28 days. In week 1 and 2, the animals in the cones were fed 2 mg of grounded fish food; 2.5 mg as fed in week 3; and 5 mg was fed in the final week. Depending on the food residue, the amount fed to the animals was reduced slightly to maintain adequate cone water quality. The air tube was checked frequently to ensure continuous air flow.

Before taking down the cones on Day 28 of the toxicity test, a 10 mL water sample was taken from each cone for metal analysis. In addition, 100 mL and 500 mL of water samples were collected for major ions, dissolved organic and inorganic carbon analysis. Detailed procedures to process water the samples for chemical analyses are described in Section 2.6.

After collecting water samples, the remaining overlying water was decanted carefully without losing any animals. When the water concentrations reached close to the sediment, the remaining water was poured through a 363 µm mesh to catch the animals. In the fume hood, the remaining cone contents were rinsed through the mesh to flush. The animals collected on the mesh were transferred into a counting bowl and the number of survivors was recorded.

2.4.2 Gut Clearance

For gut clearance, the remaining survivors were placed in 50 µM EDTA solution for 24 hours (Neumann et al. 1999). The animals were fed 2.5 mg grounded fish food and provided with cotton gauzes. After 24 hours, the animals were removed from EDTA solution. Then, tissue digestion was carried out as described in Section 2.6.3.

2.5 One-week Bioaccumulation Test (water only)

On Day 6 of the experiment, 10 adult animals (4-6-week old) were randomly placed into sampling cups filled with deChlor water two at a time, similarly to the method described in Section 2.4. Adults are used instead of young animals as bioaccumulation can be quantified in adults within 1 week and mortality is often lower than using young animals (Borgmann et al. 2001). Cages were installed on the top of the cone with clips for secure attachment (Figure 11). Animals were added to the cages in a similar manner as in Section 2.4. Small pieces of cotton gauzes were added for the animals to cling on. The caged animals were fed 2.5 mg of fish food 3 times for the one-week bioaccumulation test. The bioaccumulation test only required 1 week as the body metal concentration reaches a steady state for most metals within a week (Borgmann and Norwood 1995).

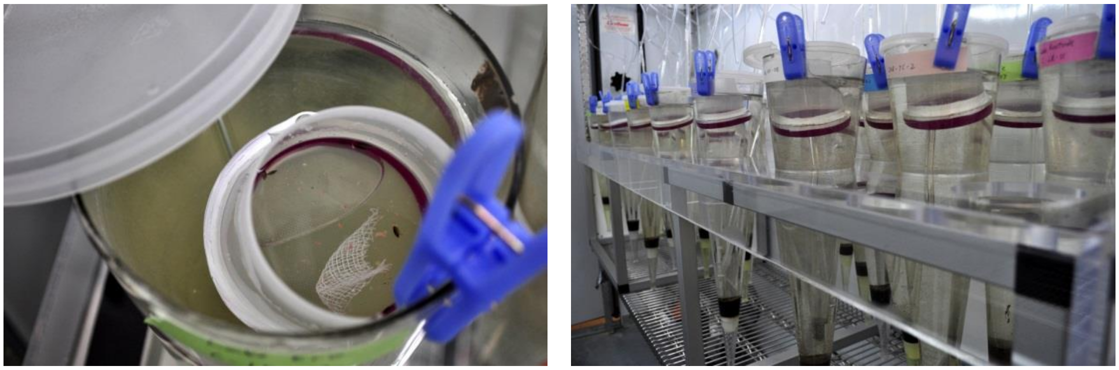

Figure 11 showing the cages attached on the cones for bioaccumulation tests

After 7 days (Day 13 of the experiment), the cages were detached from the cone. The survivors were transferred from the cages into counting bowls with 50 µM EDTA solution. After recording the number of survivors, the animals were gut-cleared as illustrated in Section 2.4.1. Tissue digestion was also carried out as described in Section 2.6.3.

2.6 Chemical Analysis

All samples including water, sediment and tissues were submitted to the National Laboratory for Environmental Testing (NLET) at Environment Canada in Burlington, ON for chemical analysis. Quality assurance/quality control (QA/QC) methodology is described in Borgmann et al. (2007). Each batch of samples submitted included four blanks in case of contamination during handling. The detection limit of each metal is calculated by multiplying the standard deviation of the four blanks of each site by 3 (Norwood et al. 2007). All raw data are corrected by their mean blank concentrations before any unit conversion or analysis. This caused some values to be negative when the raw data fell below the detection limit and these values are indicated as “<DL” in summary tables.

2.6.1 Water Chemistry Analysis: Sample Preparation

Water samples tested for major ions (calcium, chloride, manganese, potassium, sodium, and sulphate) were collected in 500 mL-containers, dissolved inorganic and organic carbon (DIC/DOC) in 100 mL-glass containers, and 48 metals (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Ge, In, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, Pb, Pt, Rb, Sb, Sc, Se, Sn, Sr, Te, Ti, Tl, U, V, W, Y, Zn and Zr) in 10 mL polypropylene containers. Although 48 metals are provided by NLET, only 13 metals (Al, As, Ba, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Tl, Zn) were discussed in this study as those metals are commonly found elevated in burned catchments (Smith et al. 2011). For DIC/DOC analysis, water samples were filtered through air pump with 0.45 µm glass microfiber filter paper. For metal analysis, additional water samples were filtered using syringes with Pall Acrodisc® Ion Chromatography (IC) syringe 0.45 µm filters (Caliper automation certified). Water samples were acidified with 100 µL of ultrapure 70% nitric acid for preservation before submission for metal analysis. Four blanks filled with nanopure water were also analyzed to determine background concentrations. Detection limits were calculated by multiplying standard deviation of the blanks by 3, while the mean blank concentrations were used to correct the raw data provided by NLET through subtraction.

2.6.1.1 Measuring water chemistry

Water quality assessments including pH, electrical conductivity (EC), dissolved oxygen (DO), alkalinity, and ammonia concentrations were conducted throughout the experiment including before cone setup (site water), after 2-week equilibrium (Day 1), and the last day of the experiment (Day 28). The measurements were recorded in a spreadsheet. Each water sample was placed on a stir plate and well-mixed during measurement. The pH meter was calibrated each time water chemistry was measured with a Thermo Scientific Orion 4 star pH meter. The calibration for pH buffer 4, 7, and 10 were recorded. All meter probes were rinsed with nanopure water and dried with a Kim wipe in between samples. In order to obtain accurate water conductivity (mS) measurements with the Amber Science Inc. EC meter (model 1056), the conductivity probe was dipped in the sample repeatedly until the meter gave a consistent reading. Before measuring dissolved oxygen (DO) (mg/L), the Thermo Scientific Orion 4 start

DO meter was calibrated. Ammonia concentrations were measured with a freshwater ammonia test kit. Using a micro-pipette, 1000 µL of water sample was transferred into a test tube. Then, 60 µL of test solution bottle #1 and bottle #2 are added to the test tubes. The test tube is vortexed with Vortex Maxi Mix in between and after adding solutions. The sample can then be compared with the freshwater ammonia color card to determine ammonia concentrations.

2.6.1.2 Filtered and Unfiltered samples

In cases where water samples were filtered for metal analysis, filtered and unfiltered metal concentrations were compared to determine the amount of particulate-associated metals removed during filtration (Norwood et al. 2006). Filtration contaminated some metal analysis thus only non-filtered results for all 13 metals (excluding Al) were to indicate the exposure concentration. Dissolved metal concentrations less than 100% of the total metal concentrations indicate a portion of the particulate associated metal was removed by filtration. Since there were not sufficient samples, statistical analysis could not be conducted to determine whether a significant amount of particulate associated metal is removed by filtered. Therefore, the mean of the filtered and unfiltered concentration was used to determine the exposure concentration. In each media (sediment, water and tissue), the reference site (Rf) was compared with other sites to determine whether there is a change in chemical composition further downstream from the unburned site. This was demonstrated with a ratio between the reference site and sites further downstream from it.

2.6.2 Sediment Digestion Method

The wet weight of sediment samples were measured in pre-zeroed cryovials on Mettler Toledo XP 205 DeltaRange Analytical Balance before drying in the oven at 60°C until a dry pellet was formed from the sediment. The dry weights of sediment samples were then measured on a Sartorius CP2P-F Micro Balance to determine percent moisture. The sediment pellets were then ground with a mortar and pestle before transferring approximately 4 mg to 15 mL falcon tubes. Four 4 mg TORT samples and four blank samples were included. The following solutions were added to the falcon tubes to digest the sediment and TORT samples: 250 µL ultrapure 70% nitric acid (digest for 6 days); 200 µL 30% hydrogen peroxide (digest for 1 day); and the falcon tube was topped up with 9550 µL nanopure water. The falcon tubes were vortexed after each solution has been added to ensure thorough mixing of the solution. When the sediment digestion process was complete, sediment samples were submitted to NLET for metal analysis. Once raw data was obtained, the blank corrected sediment metal chemistry measured in µg/L was converted to final sediment concentration (mg/kg).

2.6.3 Tissue Digestion Method

After gut clearance (Section 2.4.1), surviving animals from either the cages or cones were transferred with an eye dropper onto a large folded Kimwipe to damp dry. The number of animals was recorded again in case of mortality during gut clearance. The surviving animals were carefully brushed onto a pre-zeroed weigh boat, and the wet weights were recorded. The animals were transferred into a labeled cryovial and dried in 60°C oven for a 48 hr. Six dried animals (if available) were transferred onto a pre-zeroed weigh boat made of tinfoil, and the dry weights were recorded. The weighted tissues were then transferred into 15 mL falcon tubes. In addition, 4 TORT samples and 4 blank samples were included for reference. To digest the tissue samples, 150 µL of ultrapure 70% nitric acid was added to the falcon tube for 6 days. Then, 120 µL of 30% hydrogen peroxide was and the sample digested for 1 day and 5730 µL of nanopure water was added to fill the falcon tube. After adding each reagent, the falcon tubes were vortexed to ensure the solution was well-mixed. The digested tissues were then submitted to NLET for metal analysis with Inductively Coupled Plasma-Mass Spectrometry (ICP-MC).

MEAM was used to predict *Hyalella azteca* survival with the measured body concentration for 10 metals (Norwood et al. 2013). The raw data obtained were converted from µg/L was converted to nmol/g as shown in the equation vi. In addition, the measured background body concentration of the culture was subtracted from sample.

$$Final\ body\ burden\ (nmol/g) = \frac{Blank\ corrected\ concentration\ (\mu g/L)}{molar\ mass\ (g/mol) \times 1000} \div \frac{1000/Digestionvol\ (mL)}{Tissue\ Dry\ weight\ (mg)} - background\ concentration\ (nmol/g) \quad \text{(vi)}$$

3 Results

The metal (Al, As, Ba, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Tl, Zn) content of sediment from each study site is presented in Section 3.1. Water chemistry, including metals and major ions is described in Section 3.2. In addition, cone water chemistry data including metals and major ions, and related water quality parameters such as pH and ammonia concentrations are compared. In Section 3.3, metal concentrations measured in the tissue of *H. azteca* during the experiments are compared. In Section 3.4, the observed survival of *H. azteca* is compared to the survival predicted by MEAM.

While the detection limits of metal analyses in sediment, water and tissue samples are listed in Appendix A, raw dataset are presented in Appendices B to P.

3.1 Sediment Metal Chemistry

Total metal concentrations in sediment for each study site are summarized in Table 6. Metal concentrations varied (CV) from 8% to 27% across all sites. The CV for most metals (Al, Ba, Cd, Cr, Cu, Mn, Ni, Tl and Zn) was ≥15%. Cadmium concentrations ranged from 0.385 mg/kg to 0.862 mg/kg and varied (CV) by 27%. The highest Cd concentration was measured in sediment collected at one of the burned sites (B2). Metal concentrations for Al, Ba, Co and Cu in sediment collected in steams draining burned catchments were 1.5x greater than sediment in the unburned catchment. Aluminum concentrations ranged from 4,522 mg/kg to 7,739 mg/kg, Ba ranged from 153 mg/kg to 277 mg/kg, Co ranged from 4.25 mg/kg to 8.66 mg/kg and Cu ranged from 7.29 mg/kg to 12.1 mg/kg. The highest concentrations of these four metal concentrations were observed at burned site (B2).

Table 6 Sediment metal chemistry (N=1)

Site ID *	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Tl	Zn
	sediment metal concentration (mg/kg)												
Rf	4520	4.23	164	0.557	4.25	8.1	12.3	10000	273	17.9	10.5	0.211	76.8
B-1	6090	3.98	237	0.385	5.50	7.3	16.9	12100	310	14.6	11.0	0.147	49.9
FL	5450	3.23	153	0.427	5.73	7.4	21.5	11700	252	15.7	11.4	0.137	75.1
B-2	7740	4.00	277	0.862	8.66	12.1	24.7	15900	471	26.0	12.1	0.209	78.9
B-3	6890	3.41	239	0.615	6.93	9.9	19.5	13800	404	19.6	10.0	0.161	64.0
DS	6380	3.17	204	0.475	6.44	8.9	14.4	13200	345	16.9	9.3	0.144	76.0
TC	6570	3.40	250	0.698	6.70	9.4	20.4	13900	263	19.9	11.0	0.196	86.9
Max	7740	4.23	277	0.862	8.66	12.1	24.7	15900	471	26.0	12.1	0.211	86.9
Min	4520	3.17	153	0.385	4.25	7.3	12.3	10000	252	14.6	9.3	0.137	49.9
Mean	6230	3.63	218	0.574	6.32	9.0	18.5	12900	331	18.7	10.7	0.172	72.5
SD	960	0.40	43	0.155	1.27	1.6	4.0	1700	75	3.5	0.9	0.030	11.1
CV (%)	15	11	20	27	20	17	21	13	23	19	8	17	15

*Rf (Reference unburned site); B-1, B-2 and B-3 (Burned sites); FL (Frank Lake); DS (Downstream); TC (Tod Creek)

3.2 Site and Cone Water Chemistry

In this section, the chemistry (metals, major ions, alkalinity, DIC, DOC, pH) of both raw water and cone water is presented. Filtered (dissolved metal concentration) and unfiltered (total metal concentrations) in samples were measured to estimate the final exposure concentration to *H. azteca*. Metal data from the unburned reference site are compared to the other study sites (B-1, FL, B-2, B-3, DS, and TC).

3.2.1 Site Water Metal Chemistry

Total metal concentrations in site water are presented in Table 7. Due to financial constraints, only water at sites Rf, FL and TC were filtered and analyzed for metals. For Al, Co, Cr,Ni, Pb, and Tl in filtered water, metal concentrations exceeded that measured in unfiltered samples (>100%) (Appendix C). These data suggest that despite following standard analytical protocols that some samples were may have been contaminated during the filtration process thus the unfiltered concentration is used as exposure concentration. For sites with unfiltered metal concentrations < total metal concentrations, the mean filtered and unfiltered data are reported herein. Concentrations of Cd, Cr, Cu and Pb in river water were near or below detection limit (<DL) at various sites (Table 7; Appendix D). CV of total metal concentrations varied from -162% to 317% (Table 7). Concentrations of Cr were < DL and some of these values were negative. Metals including Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, and Pb were at least 2x higher in the burned sites compared to the reference site. Aluminum concentrations were highly variable and at site B1 (4.52 µg/L) and B2 (5.21 µg/L) and were >2x that of the reference site (2.10 µg/L). Concentrations of Ba were less variable but at sites B1 (106 µg/L), B2 (115 µg/L) and B3 (93.2 µg/L) were approximately 2x > the reference site (46.9 µg/L). The concentration of Co at site B2 (0.0422 µg/L) was 4x > the reference site (0.00978 µg/L). Across the study sites, Cu concentrations were highly variable (CV = 70%). While most Cu concentrations were <DL, its concentration at site B1 (0.350 µg/L) was 20x > the reference site (0.0144 µg/L). Fe concentrations with CV of 86% was highly variable and its concentration at burned site B2 (16.9 µg/L) was 2x > its reference site (5.74 µg/L). Mn concentrations with CV of 93% is highly variable and its concentration at burned site B2 (1.80 µg/L) was 2x > its reference site (0.761 µg/L). Lead concentrations were highly variable (CV = 317%) and its concentration (0.0397 µg/L) at burned site B3 was 8x > its reference site.

Table 7 Total metal concentration in site water (µg/L)

Me	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Tl	Zn
Site	Metal concentration in site water (µg/L)												
Rf	2.1	0.234	47	*<DL*	0.0098	*<DL*	*<DL*	5.7	0.76	0.442	*<DL*	0.0166	1.00
B1	4.5	0.295	106	*<DL*	0.0173	*<DL*	0.350	9.7	*0.28*	0.192	*<DL*	*0.0026*	*<DL*
FL	3.5	0.197	58	*<DL*	0.0134	*<DL*	*<DL*	11.6	0.74	0.399	*<DL*	0.0164	*<DL*
B2	5.2	0.150	115	*<DL*	0.0422	*<DL*	*<DL*	16.9	1.80	0.596	*<DL*	0.0053	*<DL*
B3	2.2	0.152	93	*<DL*	0.0185	*<DL*	*<DL*	3.8	*0.05*	0.198	*<DL*	0.0042	*<DL*
DS	2.6	0.227	67	*<DL*	0.0203	*<DL*	*<DL*	7.8	*0.36*	0.210	*<DL*	0.0147	*<DL*
TC	19.2	0.340	73	*<DL*	0.0421	*<DL*	*<DL*	41.5	2.75	0.452	*<DL*	0.0112	*<DL*
Max	5.2	0.295	115	0.00807	0.0422	0.0671	0.350	16.9	1.80	0.596	0.0397	0.0166	1.00
Min	2.1	0.150	47	0.00223	0.0098	-0.0990	0.014	3.8	0.05	0.192	-0.0167	0.0026	0.06
Mean	5.6	0.228	80	0.00455	0.0234	-0.0304	0.154	13.9	0.96	0.355	0.0060	0.0101	0.46
SD	5.6	0.065	24	0.00173	0.0123	0.0493	0.108	12.0	0.90	0.146	0.0190	0.0056	0.34
CV	101%	29%	30%	38%	53%	-162%	70%	86%	93%	41%	317%	55%	73%

<DL: below detection limit (Appendix A)

3.2.2 Site Water Characteristics: Major Ions and Water Quality

This section describes a range of water quality parameters and major ions measured in site water prior to conducting the toxicity experiments (Table 8). The mean and standard deviation pH of the site water was 8.57±0.07. The DOC concentrations ranged from 1.20 mg/L to 3.10 mg/L and the highest DOC concentrations were observed at burned site (B1), which was 2x > the reference site (1.30 mg/L). Concentrations of SO_4^{2-} ranged from 5.6 mg/L to 37.8 mg/L and the lowest concentration was observed at burned site B1. The Cl^- concentrations at the three burned sites B1 (0.0900 mg/L), B2 (0.220 mg/L) and B3 (0.270 mg/L) are < a quarter of that measured at the reference site (1.21 mg/L). Concentrations of Na^+ at burn sites B1 (4.76 mg/L), B2 (8.64 mg/L) and B3 (4.11 mg/L) were at least 2x > its reference sit (1.69 mg/L). Concentration of Mg^{2+} ranged from 6.79 mg/L to 16.5 mg/L. The lowest Mg^{2+} concentration was observed at burn site (B1). Concentration of K^+ ranged from 0.350 mg/L to 0.730 mg/L and its concentration in the burned site B1 (0.720 mg/L), B2 (0.730 mg/L) and B3 (0.690 mg/L) were approximately 2x > the reference site (0.350 mg/L). Other parameters such electrical conductivity (EC) (mean=350uS ±42.7), DIC (36.0 mg/L ±5.28), Ca^{2+} (50.6 mg/L ±4.31), hardness (156 mg/L ±22.5) and alkalinity (3.00 mol/L ±0.440) were relatively similar across sites.

Table 8 Chemical characteristics of site water at each study site

Parameters	Units	Sites						
		Rf	B1	FL	B2	B3	DS	TC
pH	-	8.62	8.52	8.43	8.65	8.64	8.59	8.56
COND.	uS	319	261	363	401	372	363	371
NH_3*	mM	0	0	0	0	0	0	0
DOC	mg/L	1.30	3.10	1.40	1.30	1.40	1.20	1.50
DIC	mg/L	30.0	30.0	34.4	45.7	41.0	34.8	36.0
SO_4^{2-}	mg/L	31.2	5.6	37.8	20.8	23.7	37.3	36.9
Cl^-	mg/L	1.21	0.09	2.03	0.22	0.27	2.24	2.48
Ca^{2+}	mg/L	48.8	40.8	52.8	53.8	53.7	52.3	52.3
Mg^{2+}	mg/L	10.1	6.8	11.8	16.5	14.4	12.3	12.6
Na^+	mg/L	1.69	4.76	4.36	8.64	4.11	5.07	5.61
K^+	mg/L	0.350	0.720	0.530	0.730	0.690	0.530	0.600
Hardness as $CaCO_3$	mg/L	132	132	148	199	177	151	155
Alkalinity	CO_3 mol/L	2.50	2.50	2.86	3.80	3.41	2.90	3.00

*Ammonia is not present in the site water

3.3 Cone Water Metal Chemistry

This section reports water chemistry (metals, major ions and a range of water quality parameters) measured in cone water on Day 1 and Day 28 of the toxicity experiment. The mean total concentrations of 13 metals measured on Day 1 and Day 28 are summarized in Table 9 and data for all 48 metals are presented in Appendix F (Day 1) and Appendix G (Day 28).

Variation (CV) in total metal concentrations for Day 1 and Day 28 is presented in Table 10. Variability in total metal concentration was high across all groups on both days: CVs ranged from 63% to 374% on Day 1 and 87% to 285% on Day 28. The highest concentrations of metals including Al, As, Cr, Cu, and Ni were observed in the cone containing water overlying the burned site sediment (Table 9). Concentrations of Al ranged from 0.482 µg/L to 130 µg/L on Day 1 and 0.354 µg/L to 47.1 µg/L on Day 28. Although the highest Al mean concentration was observed in the water of a cone containing sediment from burn site (B1) on Day 1, its concentration had decreased by 83% on Day 28. The CV of As was similar on both days, its concentration ranging from 0.0165 µg/L to 1.56 µg/L on Day 1 and 0.0239 µg/L to 2.91 µg/L on Day 28. The highest As concentrations were observed in water overlying sediment from site (B2) on both days, with the As concentration increasing by 86% on Day 28. The CV of Cr concentrations were similar across both days, and its cone water concentration ranged from 0.0260 µg/L to 0.143 µg/L on Day 1 and 0.0186 µg/L to 0.0947 µg/L on Day 28. The highest Cu concentration was observed in burned cone water (B3) on Day 1, but decreased by 76% on Day 28. Cu concentration ranged from 1.09 µg/L to 3.09 µg/L on Day 1 and from 0.755 µg/L to 2.14 µg/L on Day 28. The highest Ni concentration was observed in burned cone water (B2) on Day 1, but decreased by 61% on Day 28. Cone water concentration of Ni ranged from 0.533 µg/L to 3.94 µg/L on Day 1 and 0.828 µg/L to 1.73 µg/L on Day 28.

Table 9 Total metal concentrations (µg/L) (Mean and standard error) in cone water on Day 1 and Day 28

Me	Day	Rf		LR		LE		B1		FL		B2		B3		DS		TC	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Al	D1	5	1	28	2	47	6	130	35	3	0	14	3	9		42	7	4	1
	D28	8	1	47	15	33	7	22	5	7	2	14	2	16	0	9	2	9	2
As	D1	0.93	0.05	0.66	0.02	0.79	0.03	0.55	0.03	1.20	0.10	1.56	0.11	0.31		0.51	0.03	0.86	0.02
	D28	2.24	0.10	0.74	0.03	1.11	0.02	2.18	0.21	1.32	0.11	2.91	0.04	1.83	0.03	1.69	0.07	1.39	0.20
Ba	D1	53	1	33	0	27	0	124	1	73	0	101	3	85		68	1	78	0
	D28	49	1	30	1	27	0	103	1	72	3	98	4	62	0	61	1	77	2
Cd	D1	0.0091	0.0018	0.0531	0.0251	0.0164	0.0018	0.0614	0.0429	0.0057	0.0018	0.0185	0.0091	0.0247		0.0141	0.0058	0.0359	0.0310
	D28	0.0092	0.0010	0.0263	0.0047	0.0154	0.0023	0.0179	0.0036	0.0072	0.0018	0.0129	0.0015	0.0098		0.0224	0.0026	0.0071	0.0017
Co	D1	0.033	0.012	0.201	0.036	0.013	0.003	0.102	0.004	0.026	0.004	0.096	0.036	0.064		0.071	0.021	0.041	0.006
	D28	0.047	0.003	0.191	0.029	0.050	0.008	0.111	0.009	0.069	0.005	0.105	0.009	0.077		0.091	0.005	0.128	0.016
Cr	D1	0.076	0.003	0.046	0.002	0.043	0.004	0.133	0.020	0.051	0.003	0.079	0.008	0.104		0.143	0.023	0.026	0.004
	D28	0.039	0.002	0.095	0.012	0.054	0.010	0.036	0.006	0.037	0.016	0.031	0.005	0.023		0.056	0.003	0.019	0.003
Cu	D1	1.43	0.32	2.68	0.88	2.12	0.92	2.32	0.69	1.09	0.18	2.43	0.76	3.09		2.30	0.41	1.90	0.71
	D28	1.11	0.10	1.27	0.05	2.14	0.11	1.63	0.13	1.16	0.07	1.31	0.05	0.75		1.34	0.03	2.02	0.72
Fe	D1	8	3	13	1	6	3	132	36	6	3	19	6	5		66	16	7	4
	D28	6	1	86	37	22	14	13	5	8	4	8	3	9		8	4	11	4
Mn	D1	0	0	917	48	0	0	2	1	0	0	2	1	0		3	1	1	0
	D28	2	1	269	89	2	1	4	2	5	3	6	3	3	2	1	0	4	2
Ni	D1	0.77	0.13	1.28	0.63	0.53	0.08	1.87	1.18	0.72	0.24	3.94	2.31	1.91		0.67	0.18	0.79	0.18
	D28	1.07	0.05	0.92	0.04	1.07	0.14	1.00	0.07	1.08	0.10	1.54	0.08	0.83		1.20	0.01	1.73	0.25
Pb	D1	0.069	0.026	0.176	0.103	0.123	0.074	0.253	0.032	0.012	0.006	0.113	0.056	0.214		0.132	0.004	0.094	0.048
	D28	0.059	0.009	0.241	0.109	0.083	0.016	0.103	0.044	0.022	0.005	0.042	0.006	0.023		0.035	0.003	0.050	0.013
Tl	D1	0.0186	0.0008	0.0208	0.0001	0.0201	0.0009	0.0086	0.0007	0.0169	0.0004	0.0088	0.0006	0.0064		0.0171	0.0009	0.0140	0.0005
	D28	0.0156	0.0010	0.0438	0.0060	0.0312	0.0041	0.0094	0.0011	0.0144	0.0014	0.0093	0.0007	0.0095	0.0014	0.0160	0.0016	0.0122	0.0015
Zn	D1	0.4	0.2	2.0	0.6	3.4	0.9	1.9	0.9	0.0	0.1	10.8	10.7	1.0		0.7	0.3	2.1	1.7
	D28	0.9	0.2	1.7	0.4	2.1	0.3	0.6	0.2	0.2	0.3	0.2	0.3	0.4		1.3	0.4	0.3	0.2

Table 10 Total metal concentrations (µg/L) in cone water on Day 1 and Day 28

Metals	Day	Max	Min	Mean	SD	CV
Al	D1	130	3	31	38	121%
	D28	47.1	7.1	18.4	12.9	70%
As	D1	1.56	0.31	0.82	0.36	44%
	D28	2.94	0.74	1.72	0.63	37%
Ba	D1	124	27	71	29	41%
	D28	103	27	64	25	39%
Cd	D1	0.0721	0.0091	0.0339	0.0217	64%
	D28	0.0263	0.0071	0.0142	0.0065	45%
Co	D1	0.201	0.013	0.072	0.054	75%
	D28	0.191	0.047	0.097	0.042	44%
Cr	D1	0.143	0.028	0.078	0.039	49%
	D28	0.0947	0.0312	0.0471	0.0187	40%
Cu	D1	3.09	1.09	2.14	0.59	28%
	D28	2.48	1.31	1.79	0.35	19%
Fe	D1	132	5	29	41	140%
	D28	86.2	6.4	19.2	24.1	126%
Mn	D1	917	0	103	288	279%
	D28	269	1	33	83	255%
Ni	D1	3.94	0.53	1.38	1.03	74%
	D28	1.83	0.98	1.31	0.26	19%
Pb	D1	0.253	0.012	0.131	0.070	53%
	D28	0.241	0.044	0.086	0.057	66%
Tl	D1	0.0208	0.0064	0.0146	0.0051	35%
	D28	0.0438	0.0093	0.0179	0.0112	62%
Zn	D1	10.8	0.0	2.5	3.1	125%
	D28	2.71	0.59	1.34	0.64	48%

3.3.1 Major Ions and Water Quality in Cones

This section presents the water chemistry measured in the toxicity experiments on Day 1 and of Day 28. The raw data (major ions and water quality parameters, namely pH, ammonia concentrations, dissolved oxygen concentration and electric conductivity) in each cone are listed in Appendix H. The mean concentrations of these data are summarized in Tables 11 and 12. The pH of cone water on Day 1 (mean= 8.49±0.101) and Day 28 (mean=8.53±0.243) were similar (Table 11). Other parameters including dissolved oxygen (DO) (8.88 mg/L ±0.0851 on Day 1 and 9.05 mg/L ±0.481 on Day 28), electrical conductivity (309 µS ±23.5 on D1 and 306 µS ±35 on D28) and ammonia (0.00250mM ±0.00680 on D1 and 0.0130 mM ±0.0051 on D28) were similar for both days.

DOC ranged from 2.5 mg/L to 28.1 mg/L (Appendix G). The highest DOC mean concentration of 12.0 mg/L was observed in cone water at burned site B1, which was 3x > in the reference cone water (3.66 mg/L) (Table 12). The mean concentration of SO_4^{2-} was 27.8 mg/L ± 10.0, and was approximately one quarter of the value in cone water from the burned site B1 (6.42 mg/L), but comparable to reference cone water (25.4 mg/L). Chloride (Cl^-) ranged from 1.31 mg/L to 29.7 mg/L, while only half the reference site concentration (2.41 mg/L) was measured at burned cone water (B3) (1.33 mg/L). The concentration of Na^+ ranged from 2.23 mg/L to 16.4 mg/L. Cones including B1 (6.10 mg/L), B2 (10.0 mg/L) and B3 (5 mg/L) were double the Na^+ concentration than in the reference cone water (2.47 mg/L). Other major ions were similar across all sites: DIC (25.7 mg/L ±6.03), Ca^{2+} (37.6 mg/L ±8.10), Mg^{2+} (10.8 mg/L ±2.64), K^+ (1.29 mg/L ±0.379), hardness as $CaCO_3$ (115 mg/L ±27.2), and alkalinity (2.14 CO_3 mol/L ±0.502).

Table 11 Chemistry of cone water (mean and standard deviation) on Day 1 and Day 28

WQ	units	D	sites								
			Rf	LR	LE	B1	FL	B2	B3	DS	TC
pH	-	1	8.50 (0.02)	8.26 (0.01)	8.37 (0.03)	8.55 (0.04)	8.53 (0.02)	8.63 (0.02)	8.58	8.55(0.01)	8.50 (0.01)
		28	8.50 (0.28)	8.29 (0.29)	8.45(0.14)	8.91 (0.17)	8.50 (0.08)	8.50 (0.03)	8.94	8.39 (0.04)	8.57 (0.04)
O_2	mg/L	1	8.88 (0.11)	8.90 (0.04)	8.84 (0.03)	8.95 (0.10)	8.88 (0.06)	8.90 (0.15)	8.89	8.82 (0.04)	8.84 (0.07)
		28	9.13 (0.57)	9.32 (0.42)	9.03 (0.29)	9.73 (0.22)	8.66 (0.08)	8.63 (0.05)	9.65	8.63 (0.13)	8.72 (0.03)
COND.	µS	1	293 (12)	308 (7)	336 (12)	265 (5)	326 (4)	335 (24)	311	326 (1)	317 (6)
		28	284 (19)	293 (10)	356 (20)	253 (7)	338 (3)	336 (22)	260	317 (6)	338 (7)
NH_3	mM	1	0.008 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0	0(0)	0(0)
		28	0.0150(0)	0.0150(0)	0.0150(0)	0.0150(0)	0.01 (0.01)	0.005 (0.1)	0	0.0150(0)	0.0150(0)

Table 12 Major ion chemistry in cone water on Day 28

Major Ions	Units	DeChlor Water	Rf	LR	LE	B1	FL	B2	B3	DS	TC
DOC	mg/L	2	3.7	3.7	3.4	12.0	3.5	4.4	4.8	3.5	12.0
DIC	mg/L		25.0	12.4	20.8	27.3	30.3	35.3	23.6	27.0	29.6
SO42-	mg/L	28	25.4	38.1	37.1	6.4	36.0	17.2	21.0	35.4	36.2
Cl-	mg/L	24	2.4	28.1	28.5	2.2	3.2	1.6	1.3	3.5	4.0
Ca2+	mg/L	40	40.0	26.9	37.5	27.2	47.3	39.2	26.7	42.3	41.1
Mg2+	mg/L	8	10.1	7.7	9.1	7.0	12.2	16.1	14.3	12.0	12.9
Na+	mg/L	12	2.5	15.4	15.8	6.1	5.2	10.0	5.0	5.8	6.7
K+	mg/L		0.97	1.90	2.07	1.09	1.17	1.33	1.37	1.12	1.24
Hardness (CaCO3)	mg/L	130	112	54	94	127	135	158	111	118	132
Alkalinity	CO3 mol/L	90	2.08	1.04	1.73	2.27	2.52	2.94	1.96	2.24	2.46

3.3.2 Tissue Metal Concentrations

This section presents metal concentration data measured in tissue of *H. azteca* from the cages (1-week bioaccumulation test) and the cones (28-day chronic toxicity test). The final body burdens of 48 metals are shown in Appendix J (cages) and Appendix K (cones) while 13 metal tissue concentrations are summarized in Tables 13 and 14. Variation (CV) in metal body burden in cages ranged from -17% to 556%, indicating that metal concentrations were highly variable across all sites. The CV of As (-17%) and Cu (-7%) were negative because their concentration was typically below the background, which caused metal concentrations in some samples to be negative (Table 13). The CV of some metals (Cr, Mn, Ni, Pb and Zn) in tissues sampled from caged *H. azteca* was > 100%.

The highest tissue concentrations for Ni, Pb, and Zn were measured in *H. azteca* from burned treatments. Mean Ni concentration in caged tissues was 4.07 nmol/g ± 5.10 and the highest mean body concentration of 59.2 nmol/g ±44.5 was observed in a cage from burn site (B1). The highest Pb tissue concentration (15.3 nmol/g ± 15.4) was observed in a cage from burn site (B1) while its mean body concentration in caged tissues was 1.49 nmol/g ± 1.54. Mean body concentration of Zn in caged tissues was 174 nmol/g ± 106 and the highest tissue concentration of 1,116 nmol/g ± 1067 was observed in burned cage (B1). Accordingly, highest metal concentrations were observed in the caged tissues at burned sites (B1).

The concentration of 13 main metals measured in the *H. azteca* tissues are summarized in Table 14. Because there were no survivors in cone Rf-2 and Rf-8, no tissue was available for metal analysis. These samples may be outliers because there is no direct evidence indicating that metal concentrations should result in complete mortality, which means factors other than metals (e.g. bad handling of animals) are causing the mortality in those cones. The CV of body burden in *H. azteca* in these cones ranged from -196% to 510%, which indicates highly variable concentrations across all sites. The CV of Cr (-519%), Cu (-

48%) and Ni (-296%) were below zero as majority of *H. azteca* in the cones have body concentrations below the background body concentration. Tissue metals with higher variability (CV > 100%) were As (106%), Cd (510%), Mn (172%), Pb (127%) and Zn (180%). Cd had the highest CV and the highest tissue concentration in the burned cone B3. Cd tissue concentrations in most cones were below the background body concentration (3.64 nmol/g) except for cone B3, which had the highest tissue concentration of 96.1 nmol/g (Table 14). The burned/unburned ratios showed that Al, Cd, Co, Cr and Mn in the tissue from the burned cone were at least 2x > the reference cone excluding control cones. The Al concentration (mean= 2276 nmol/g ± 283) in burned cone B1 (2978 nmol/g ±1070), B2 (2978 nmol/g ±871) and B3 (4891 nmol/g) were 2x to 3x > the reference site (1329 nmol/g ± 119). Mean of Cd tissue concentration was 3.59 nmol/g ± 3.34 and its tissue concentration at burned cone B1 (2.76 nmol/g ± 1.58) and B3 (96.11 nmol/g) were 3x and 70x > its reference cones (-1.28 nmol/g ± 0.40) respectively. Body concentration of Co (mean= 5.17 nmol/g ± 0.82) at burned cones B1 (9.31 nmol/g± 1.00) and B3 (18.34 nmol/g) were 2x to 4x > the reference cones (3.08 nmol/g± 0.34). Most Cr concentrations were below background concentration except for cone B2, where its mean concentration (3.61 nmol/g ±2.15) was 2x > the reference cone (-2.62 nmo/g ±1.99). The mean concentration of Mn was 837 nmol/g ± 262 and tissue concentrations in cone B1 (1054 nmol/g ± 405) and B3 (1325 nmol/g) were 2x and 3x > the reference cones (318 nmol/g ± 151), respectively.

Table 13 Metal body burden (mean and standard error) in cages (7-Day bioaccumulation test)

Me	Back-ground	Rf (N=9)		LR (N=3)		LE (N=3)		B1 (N=3)		FL (N=3)		B2 (N=3)		B3(N=1)	DS (N=3)		TC (N=3)	
		Cage mean body burden and standard error (nmol/g)																
Al		1060	190	700	100	1700	300	1200	260	780	150	990	150	1350	810	370	1570	280
As	13.8	<BG		<BG		<BG		<BG		<BG		<BG		<BG	<BG		<BG	
Ba	2.25	164	7	199	18	114	4	453	65	230	10	258	31	264	196	39	208	8
Cd	3.64	3.84	0.44	3.51	0.31	4.33	0.48	6.23	1.87	2.82	0.16	3.55	0.07	3.22	3.25	0.74	2.27	0.28
Co		1.24	0.14	1.82	0.15	1.18	0.15	3.15	1.15	1.39	0.10	1.19	0.13	0.82	1.97	0.25	1.12	0.18
Cr	-0.1	1.4	0.6	14.0	7.6	1.7	0.4	8.2	2.9	8.7	2.8	1.9	0.9	4.2	10.9	2.8	1.8	0.9
Cu	1539	<BG		<BG		<BG		<BG		<BG		<BG		<BG	<BG		<BG	
Fe		900	50	760	30	1020	80	1180	210	890	50	950	50	1180	1020	90	850	20
Mn	107	<BG		4710	810	<BG		17	36	71	44	<BG		<BG	<BG		5	7
Ni	16	<BG		<BG		<BG		59.2	44.5	<BG		0.4	3.0	8.1	2.3	3.0	1.4	5.3
Pb	0.199	<BG		0.0	0.1	0.0	0.0	15.3	15.4	-0.1	0.0	<BG		0.0	0.1	0.1	0.0	0.1
Tl	0.124	0.393	0.042	0.867	0.312	0.389	0.126	0.336	0.054	0.381	0.052	0.306	0.045	0.751	0.699	0.059	0.541	0.093
Zn	924	73	20	66	24	95	17	1116	1067	77	23	33	13	77	106	16	42	8

<BG: values are below body background concentrations

Table 14 Metal body burden (mean and standard error) in cones (28-Day toxicity test)

Metals	Back-ground	Rf (N=7)		LR (N=3)		LE (N=3)		B1 (N=3)		FL (N=3)		B2 (N=3)		B3(N=1)	DS (N=3)		TC (N=3)	
		Cone body burden and standard error (nmol/g)																
Al		1330	120	1350	110	1260	280	2980	1070	2130	1010	2870	870	4890	4920	1530	2660	980
As	13.8	4.7	1.0	<BG		9.2	3.8	1.9	3.2	6.7	1.5	4.3	2.5	1.9	1.3	1.6	13.9	1.7
Ba	2.25	600	90	470	20	260	30	1380	350	830	50	1230	150	1380	920	40	640	110
Cd	3.64	<BG		3.7	0.5	1.5	1.6	2.8	1.6	<BG		<BG		96.1	1.9	0.6	<BG	
Co		3.1	0.3	4.6	0.9	1.7	0.3	9.3	1.0	2.1	0.7	4.5	0.9	18.3	13.4	5.4	3.9	0.2
Cr	-0.1	<BG		0.1	0.5	<BG		2.5	8.1	<BG		3.6	2.2	<BG	<BG		<BG	
Cu	1539	<BG		<BG		<BG		<BG		<BG		<BG		<BG	<BG		<BG	
Fe		1460	130	1290	80	800	40	2180	630	2710	700	2160	410	970	3160	790	2040	490
Mn	107	320	50	4250	1470	210	70	1050	410	280	30	300	40	1330	490	190	220	60
Ni	16	<BG		<BG		4.6	13.8	3.8	13.1	<BG		0.8	4.2	<BG	<BG		<BG	
Pb	0.199	0.206	0.060	0.810	0.074	0.267	0.183	0.366	0.260	0.344		0.340	0.114	<BG	0.529	0.306	0.196	0.046
Tl	0.124	0.317	0.053	0.496	0.031	0.179	0.034	0.016	0.079	0.332	0.053	0.195	0.013	0.093	0.517	0.069	0.090	0.081
Zn	924	109	28	80	27	129	89	52	40	26	76	44	25	<BG	37	51	<BG	

<BG: values are below body background concentrations

3.4 MEAM Predicted Survival vs. Observed Survival

This section presents the observed survival data in the cone and the MEAM predicted survival in the cage and cone. All survival data are shown in Appendix N, while predicted survival in the cones and cages, and observed cone survival are summarized in Figure 12. Some sites including Rf, LE, B1, and B3 had relatively high descrepacy between predicted and observed survivals.

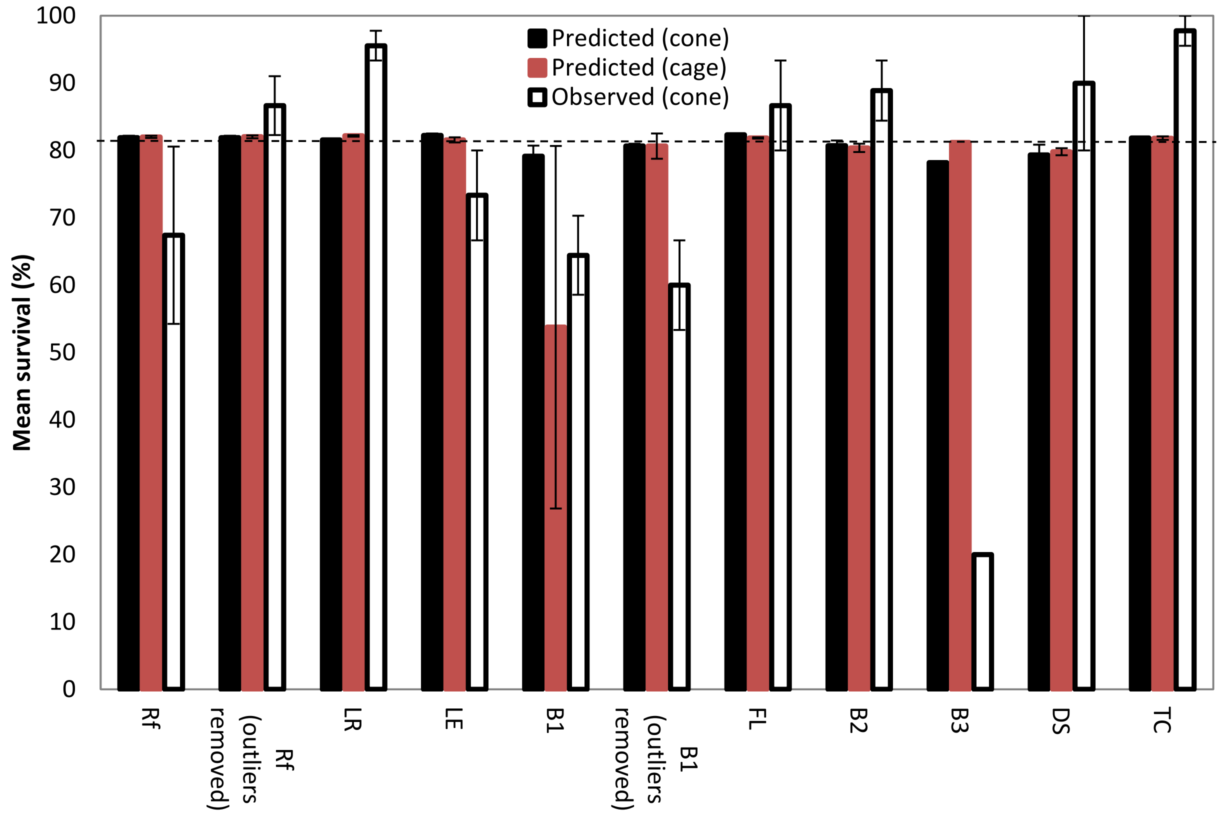

Figure 12 Predicted MEAM survival and observed mean survival with standard error

The mean observed survival in the reference cones ranged from 20% to 97.8%. The mean observed survival (67.4% ±12.4) was nearly 15% < the predicted survival (81.6% ±0.2). The discrepancy between the observed and predicted survival were related to the zero observed survival in cone Rf-2 and Rf-8 (Figure 12). Rf-2 and Rf-8 data points are removed as outliers as they are inconsistent with its replicates which are above 80% survival. The zero survival rates in Rf-2 and Rf-8 may be caused by poor handling of the test animals. When outliers (Rf-2 and Rf-8) were removed, the observed and predicted survivals matched (Figure 12).

Survival in the cones containing burned site sediments ranged from 20% to 93% (Figure 13). For site B1-R2, the predicted survival in the cage (54%) was much lower than the predicted and observed survival in the cones and it had a relatively high standard error of 27%. The predicted survival in cage B1-R3 was zero due to extremely high metal (Ni, Pb and Zn) concentration in tissues, which was inconsistent with its replicates (B1-R1 and B1-R2) (Figure 13). Therefore, data point B1-R3 is removed as outlier due to contamination of Ni, Pb and Zn in the tissue.

The observed survival in site B3-R1 is 20%, and is approximately 60% lower than both predicted survival, which means survival in site B3 is reduced by other factors that are not considered in MEAM (Figure 13). Since there were no replicates for site B3 due to lack of sediment samples, standard errors could not be calculated. Data point B3-R1 is removed as its survival are caused by factors not considered in MEAM, which may be bad handling of animals, other contaminants not measured (organic contaminants, other metals, lack of oxygen) and biological agents (parasites and predators). With these two data points (B1-R3 and B3-R1) removed, the discrepancy were reduced and predicted survival in the cage increased to 81%, observed survival in the cone increased to 77% and predicted survival in the cone increased to 81% (Figure 12). In addition, the standard error of survival in the cage decreased from 12% to <1%.

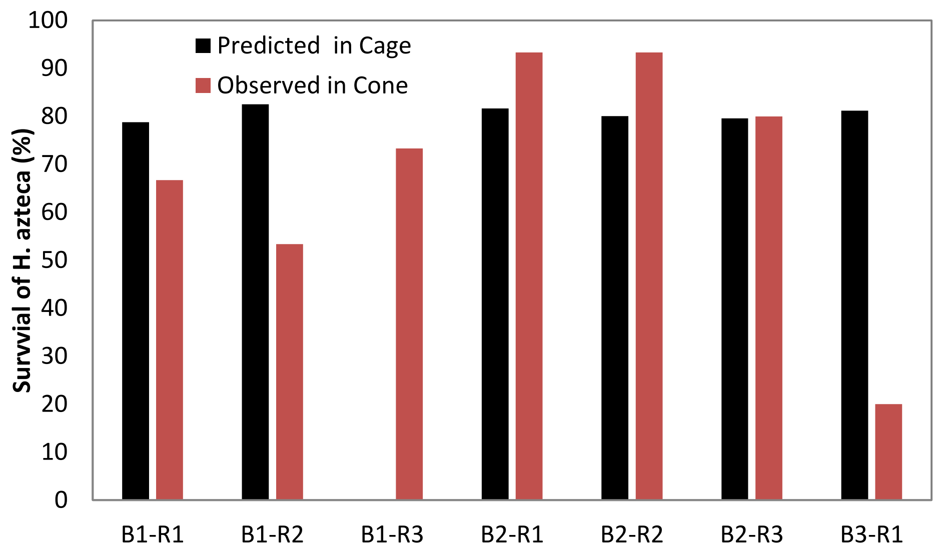

Figure 13 Survival in the burned treatments

4 Discussion

There is abundant literature related to the impacts of wildfire on the quality and quantity of water (Smith et al. 2011) and the abundance and diversity of aquatic invertebrates (Minshall et al. 2001) in many regions of the world. Many studies report elevated metal concentrations in the sediment and water for a range of wildfire-impacted sites (Beyers et al. 2005; Owens et al. 2010). However, very few studies have investigated whether wildfire-generated metals are a threat (bioaccumulation and toxicity) to aquatic life and there are no peer reviewed studies that have evaluated the effects of wildfire on metal toxicity in aquatic invertebrates.

The main objectives of this thesis were to measure and compare the metal content in sediment, from reference (unburned) and impacted (burned) sites in the Crowsnest River and determine whether metals in these samples were bioavailable and toxic to the freshwater amphipod *H. azteca*. In this chapter, the chemistry of all analyzed medium (sediment, water, and tissue) and the survival are categorized and discussed by treatment: (1) unburned reference (Rf), (2) burned (B1, B2 and B3), (3) Frank Lake (FL) immediately downstream of the sewage treatment plant and (4) downstream composite sites (DS and TC). Metal concentrations in sediment and water samples are compared with their respective CCME (1999) guidelines to determine the level of risk to aquatic life. Tissue metal concentrations were evaluated to determine whether metals from the impacted zones were bioavailable to *H. azteca.* Finally*,* metal concentrations in tissue samples were also compared with lethal body concentration at 25% mortality (LBC25) to determine the level of risk on survival of *H. azteca*. Predicted survivals using the MEAM were compared with the observed survival and the utility of MEAM to wildfire disturbed watersheds is discussed. In the following sections, results of this study are compared and discussed in the context of the literature.

4.1 Sediment Metal Chemistry

Large scale land disturbance by wildfire can cause increased sediment loads and elevated particulate-associated metal concentrations in streams (Burke et al. 2010; Owens et al. 2010). For example, Gallaher et al. (2002) report that maximum concentrations of Fe (510 - 42000 mg/kg), Mn (37- 16900 mg/kg) and Tl (0.003- 18 mg/kg) exceeded the EPA screening concentrations as a result of the Cerro Grande Fire in New Mexico, United States. Five years after the Lost Creek Fire in southern Alberta, Stone (unpublished data) found that metal (Cd, Cu, Ni and Zn) concentrations in burned catchments were greater than the threshold effect concentration (TEC) of the CCME Sediment Quality Guideline (SQG) (CCME 1999). In this section, sediment metal concentration from 2012 samples were compared with the CCME Interim Sediment Quality guideline (ISQG) to determine whether metal concentrations, nine years post-fire were of concern (Table 15).

Table 15 Sediment metal concentrations (Mean and standard error) compared with CCME ISQG (CCME 1999)

	CCME ISQG	Unburned (N=1)	Burned (N=3)		Frank Lake (N=1)	Downstream (N=2)	
		Mean	Mean	SE	Mean	Mean	SE
Me	mg/kg	Metal concentration in sediment (mg/kg)					
Al	**N/A**	2520	6910	390	5450	6470	70
As	**5.9**	4.23	3.80	0.16	3.23	3.28	0.08
Ba	**N/A**	164	251	11	153	227	16
Cd	**0.6**	0.557	**0.620**	0.112	0.427	0.587	0.079
Co	**N/A**	4.25	7.03	0.75	5.73	6.57	0.09
Cr	**37.3**	8.05	9.78	1.14	7.36	9.16	0.16
Cu	**35.7**	12.3	20.4	1.9	21.5	17.4	2.1
Fe	**N/A**	10000	13900	900	11700	13600	200
Mn	**N/A**	273	395	38	252	304	29
Ni	**N/A**	17.9	20.1	2.7	15.7	18.4	1.1
Pb	**35**	10.5	11.0	0.5	11.4	10.1	0.6
Tl	**N/A**	0.211	0.173	0.015	0.137	0.170	0.019
Zn	**123**	76.8	64.3	6.8	75.1	81.5	3.9

Bolded values exceed the CCME (1999) ISQG

Although Cd (0.620±0.195 mg/kg) in the sediment from the burned site exceeded the CCME (1999) sediment quality guideline (0.6 mg/kg) (Table 15), the standard error for Cd in the burned site also indicated similarity in Cd concentration between the burned and unburned sites, thus there may not be a difference in Cd concentrations. After nine years post Lost Creek fire, total metal concentrations (Al, Ba, Co, and Cu) remained slightly elevated compared to the unburned sediment. While concentrations of Cu were below the respective ISQGs, there is no CCME SQG for Al, Ba and Co. Concentrations of Al in this study (4500-7700 mg/kg) were within the range of concentrations reported by Gallaher et al. (2002) (200- 61700 mg/kg), which was also below the EPA screening level. Ba concentrations in the Crowsnest watershed (153-277 mg/kg) were lower than in Gallaher et al. (2002) (25-2000 mg/kg). Shuhaimi-Othman (2008) measured Ba of 87.8 mg/kg in the sediment from Richard Lake, Sudbury and did not report any toxicity associated with Ba. Although there are no CCME SQGs for Al, Ba and Co, the bioavailability in sediment can be investigated in the tissue (Section 4.3).

In 1903, Turtle Mountain beside Frank Lake had undergone a rock slide (Frank Slide) and the fragmented deposits from Turtle Mountain were still apparent in 2012 (Figure 15). It is possible that the weathered deposits of Turtle Mountain increased the sediment flux into Frank Lake (Korup 2005). Accordingly, the increased sediment load could have diluted the impacted sediment from the burned site and lowered concentrations of Al, Ba, Cd and Co in the sediment in Frank Lake.

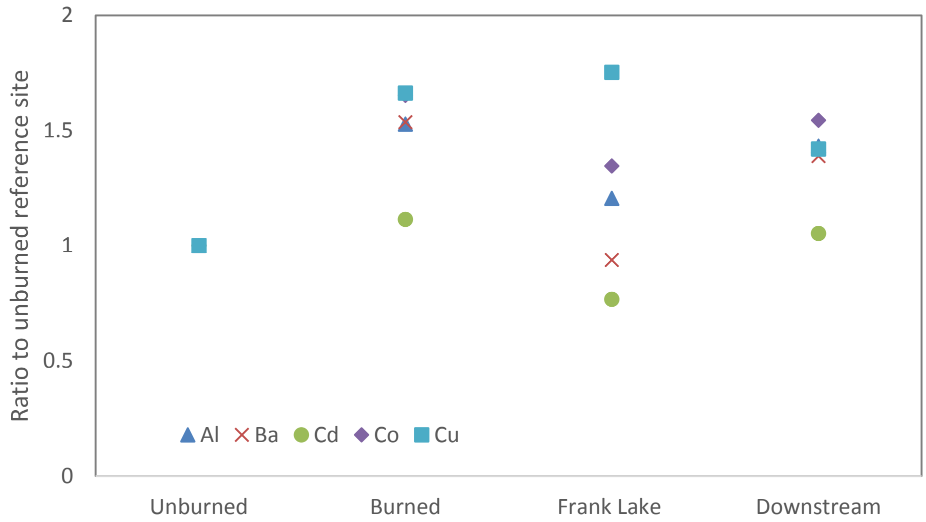

Figure 14 Ratio of selected sediment-associated metals (Al, Ba, Cd, Co, and Cu) in the unburned site to other sites (Burned/Frank Lake/Downstream)

Figure 15 Frank Lake (right indicated by an arrow) at the base of Turtle Mountain (left) in 2012

4.2 Water Chemistry

After large scale land disturbance such as wildfire, sediment-associated metals can be released into the water column (Luoma 1989). Many studies report increased total and dissolved metal concentrations in burned watersheds (Gallaher et al. 2002; Leak et al. 2003; Townsend and Douglas 2004). Townsend and Douglas (2004) reported mean concentrations of Fe (330 µg/L) and Mn (6 µg/L) post fire at Kajadu National Park. Gallaher and Koch (2004) measured Al (73- 1,500,000 µg/L), Ba (0-190 µg/L), Cd (0.1- 57.3 µg/L), Co (5-1,100 µg/L), Ni (2-826 µg/L), Pb (0-1180 µg/L), Zn (3-3610 µg/L) after the Cerro Grande Fire.

From 2004 to 2008, Silins et al. (2009a) showed that metals including Al, Co, Pb, Mn, and Mo were consistently elevated in the Crowsnest River, which they attributed to the effects of large scale land disturbance by the Lost Creek Fire. In this section, the 2012 chemistry of site water at the Crowsnest River and cone water (metal, major ions and water quality) on Day 1 and Day 28 of the toxicity test are compared to the literature. In addition to comparing the burned with the reference (unburned) sites, temporal changes in water chemistry in cone water from Day 1 to Day 28 are described. Metal concentrations in the water are compared with CCME freshwater water quality guidelines for the protection of aquatic life to evaluate the risk to aquatic life in the Crowsnest River. Several studies have documented the role of major ions on metal toxicity (Stephenson and Mackie 1989; Wurts and Perschbacher 1994; Borgmann et al. 2005b). In Section 4.2.3, water characteristics such as pH, conductivity and major ions are evaluated regarding their potential influence on metal bioavailability and toxicity in the present research.

4.2.1 Site Water Metal Chemistry

This section discusses total metal concentrations of the site water in the burned and reference (unburned) sites and the potential impacts these metals may have on downstream reaches of the Crowsnest River. For the site water in the burned zone, metals concentrations (Ba and Co) were at least 2x > the reference site (Figure 16). Other metals were not elevated in the burned site and the concentrations of some metal were below the detection limit (<*DL* in Table 16). Both Ba and Co concentrations in Frank Lake were lower than the burned sites and similar to the reference (unburned) site (Figure 16). The rock deposits as a result of the Frank Slide are composed of limestone; it is possible that Ba (Shahwan et al. 2002) and Co (Komnitsas et al. 2004) sorbed onto those deposits that entered Frank Lake, and thus lowering the Ba and Co concentrations in the lake. Co in the downstream zone was 3.5x > the unburned zone (Figure 16), therefore Co concentrations generated in the burned sites may have mobilized downstream in the Crowsnest River by binding to suspended sediment (Gibbs 1994). The mean Ba concentration (105 µg/L ± 6) measured in this study was within the range measured in Gallaher and Koch (2004). Mean Co (0.0260 µg/L ± 0.0081) in this study was lower than the range reported by Gallaher and Koch (2004). According to the site water metal chemistry, the aquatic life in the Crowsnest River should not be threatened by the increased concentration of Ba and Co.

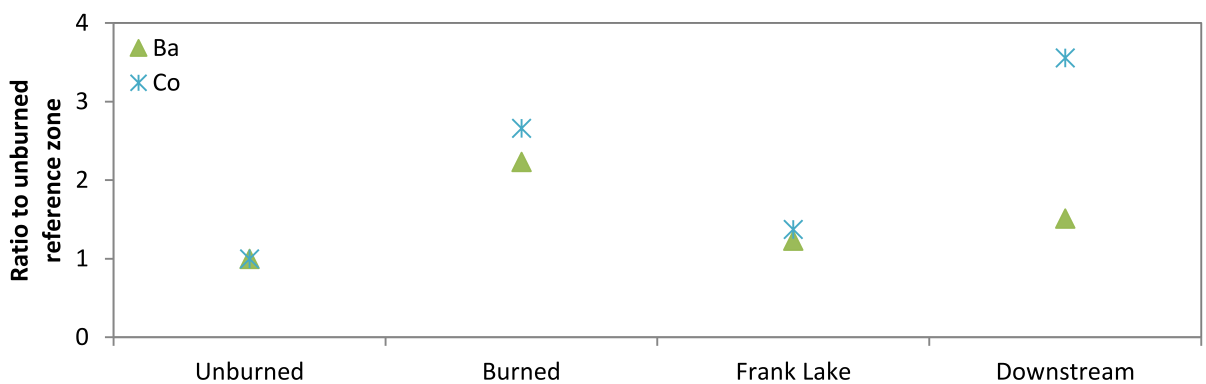

Figure 16 The total metal concentration ratio of the unburned reference and other zones (Burned/Frank Lake/Downstream)

Table 16 Site water total metal concentration in the Crowsnest River (Mean and Standard Error)

Treatment		Unburned		Burned		Treated Water		Composite	
	CCME WQG (µg/L)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Me		Mean Concentration in Site Water (µg/L)							
Al	100	2.1	2.1	4.0	0.9	3.5	3.0	13.7	12.6
As	5	0.234	0.009	0.199	0.048	0.303	0.020	0.303	0.038
Ba		47	0	105	6	71	2	71	3
Cd		<DL	<DL	<DL	0.00102	<DL	0.00134	<DL	0.00258
Co		0.0098	N/A	0.0260	0.0081	0.0348	0.0019	0.0348	0.0134
Cr		*0.0036*	0.0088	<DL	0.0493	<DL	0.0106	<DL	0.0313
Cu	2	*0.014*		*0.237*	0.068	*0.133*		*0.133*	0.048
Fe	300	5.7	4.8	10.2	3.8	30.3	8.7	30.3	26.6
Mn		0.76	0.78	0.71	0.55	1.95	0.71	1.95	1.73
Ni		0.442	0.102	0.328	0.134	0.372	0.113	0.372	0.091
Pb	1	<DL	N/A	<DL	0.0169	<DL	0.0106	<DL	0.0286
Tl	0.8	0.0166	0.0004	0.0040	0.0008	0.0123	0.0002	0.0123	0.0035
Zn	30	1.00	0.46	0.24	0.10	0.55	0.17	0.55	0.57

4.2.2 Cone Water Metal

This section discusses changes in total metal concentrations in the cone water from Day 1 to Day 28. Mean total metal concentration and its standard error of two zones (burned and downstream) are summarized and compared with the literature and CCME water quality guidelines (CCME 1999). Metals including Al, Ba, Cd, Co, Fe, Ni, and Zn were elevated in the cone water at the impacted (burned) sites when compared to the unburned site (Table 17).

Table 17 Percentage of total metal concentration elevated compared to the unburned zone on Day 1

	Zone	
Metal	Burned	Downstream
Al	1202%	315%
Ba	203%	139%
Cd	417%	95%
Co	287%	157%
Fe	798%	323%
Mn	423%	305%
Ni	357%	97%
Pb	271%	154%
Zn	309%	150%

The Al concentration on Day 1 (63.0 µg/L ± 27) increased by 1500% compared to site water after field collection and in the burned zone it was 12x > the unburned zone (5.24 µg/L ± 1.26) (Table 18). Al bound to particulate matter from the fire may have been released to the overlaying cone water during the 2-week equilibration. Although the mean concentration of Al on Day 1 is below the CCME guideline, the Al concentration of site B1-R2 (166 µg/L) and B1-R3 (162 µg/L) exceeded the CCME guideline of 100 µg/L (Figure 17). The mean Al concentration in the burned zone was lower than that reported in Gallaher and Koch (2004) and the individual measurements in B1-R2 and B1-R3 were within the lower range of that study (73-1,500,000 µg/L). The overlay water of B1-R2 and B1-R3 could contribute to toxicity of aquatic life as their elevated Al concentrations exceeded the CCME guideline. On Day 28 in the burned treatment (17.9 µg/L ± 2.5), Al decreased by approximately 70%. One of the explanations of lowered Al concentrations on Day 28 is that Al is rebound to residual fraction of the sediment (Khan et al. 2013). Boudot et al. (1994) studied Al speciation in soil and claims Al bind to inorganic and organic anionic ligands by forming soluble complexes, which lowers toxicity to plants. Al concentration in tissues in this study showed that Al was bioaccumulated in *H. azteca* (Ingersoll et al. 1994), which could have resulted in the lower survival in burned cones (B1) of 64% ± 8 (Section 3.4). Accordingly, tissue samples of *H. azteca* in cone B1 were examined closely for Al bioaccumulation in Section 4.3. Al concentrations in cone water containing Frank Lake sediment (2.7 µg/L ±0.48) on Day 1 were not elevated, hence it is possible that runoff of rock deposits (limestone) from Turtle Mountain reduced the dissolved Al from the overlaying water (Cravotta and Trahan 1999). However, Al (16.5 µg/L ± 8.4) concentrations in the cone water from the downstream sites increased by 1400% on Day 1 and they were 3x > the total Al concentrations in the unburned zone. This observation suggests that Al bound to particulate may have been released to overlaying water during the 2-week equilibration. Therefore, particulates with elevated Al from the burned zones may have transferred downstream in the Crowsnest River. Al concentrations in the downstream sites decreased by approximately 50% on Day 28, thus Al may have been absorbed by *H. azteca* (Ingersoll et al. 1994).

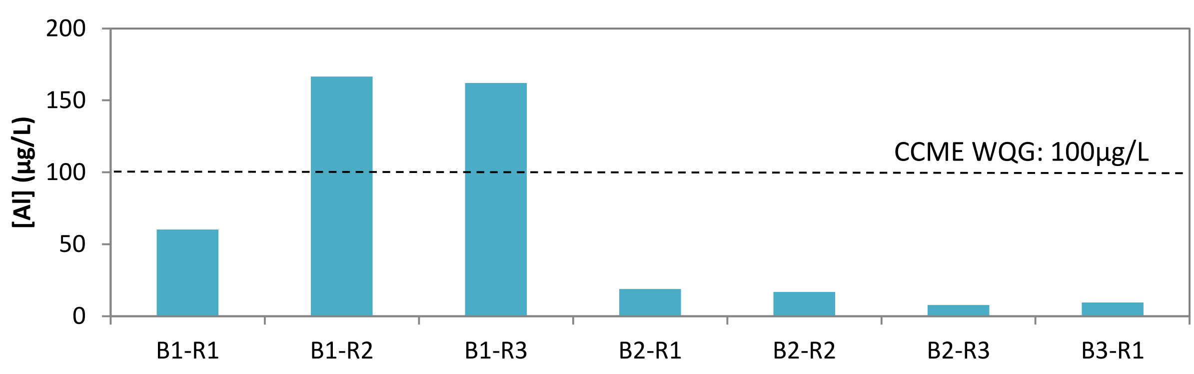

Figure 17 Level of Al in Day 1 of burned cone water

No CCME WQG has been established for Ba, but its concentration in the water was consistently 2x > in the unburned treatments: after field collection (105 µg/L ± 6), Day 1 (108 µg/L ± 4) and Day 28 (95.2

μg/L ± 6.4) of the toxicity test (Table 17 & 18). The Ba concentrations measured in this study were on the lower end of the range reported (18-29800 μg/L) by Gallaher and Koch (2004). Similarly to the site water, Ba concentrations in the cone water of Frank Lake and downstream Crowsnest River site were comparatively less elevated than in the burned sites on both days (Table 17), thus elevated Ba concentrations in the burned sites may not have mobilized downstream.

Increased Cd concentrations were observed in cone water of burned zones (Table 17). The Cd concentration measured in this study was below the range reported in Gallaher and Koch (2004) of 0.05-3.92 μg/L. Cd concentrations in the burned zone (0.0377 μg/L ± 0.0019) on Day 1 increased by 500% after field collection (<DL) (Table 18), which indicates particulate-bound Cd may have been released to the overlay cone water. Cd concentrations in the burned zone were 4x > in the unburned zone on Day 1. However, Cd concentrations in the burned zone on Day 28 were reduced by 60%, which implies that Cd maybe bioaccumlated by *H. azteca* (Borgmann et al. 1991) or rebound to Fe and Mn oxides and hydroxides of the sediment (Linnik and Zubenko 2000). Cd concentration in the Frank Lake and downstream sites were less elevated than the burned sites, thus elevated Cd from the burned zones may have precipitated as Cd carbonate with rock deposit (limestone) (Wang and Reardon 2001) from Turtle Mountain in Frank Lake, or it may not have mobilized downstream in the Crowsnest River.

Table 18 Total metal concentrations (µg/L) in overlying cone water on Day 1 and Day 28

Me	Day	CCME WQG (µg/L)	Unburned		Control (LE and LR)		Burned		Frank Lake		Downstream	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Al	1	100	5.2	1.3	37.6	5.1	63.0	27.0	2.7	0.5	16.5	8.4
	28		8.0	1.3	40.1	8.0	17.9	2.5	7.1	1.5	8.9	1.5
As	1	5	0.93	0.05	0.73	0.03	0.95	0.22	1.20	0.10	0.72	0.09
	28		2.24	0.10	0.92	0.06	2.31	0.16	1.32	0.11	1.47	0.16
Ba	1	N/A	53	1	30	1	108	6	73	0	74	3
	28		49.0	1.0	28.5	0.8	95.2	4.2	72.3	2.7	70.4	2.8
Cd	1	N/A	0.0091	0.0018	0.0348	0.0139	0.0377	0.0186	0.0057	0.0018	0.0086	0.0029
	28		0.0092	0.0010	0.0208	0.0030	0.0150	0.0019	0.0072	0.0013	0.0132	0.0029
Co	1	N/A	0.0327	0.0122	0.1072	0.0451	0.0939	0.0146	0.0257	0.0044	0.0513	0.0092
	28		0.047	0.003	0.120	0.026	0.105	0.006	0.069	0.005	0.113	0.011
Cr	1	N/A	0.076	0.003	0.044	0.002	0.106	0.013	0.051	0.003	0.073	0.030
	28		0.0393	0.0019	0.0743	0.0097	0.0330	0.0036	0.0367	0.0163	0.0351	0.0068
Cu	1	2	1.43	0.32	2.40	0.58	2.48	0.40	1.09	0.18	2.03	0.47
	28		1.11	0.10	1.71	0.20	1.40	0.12	1.16	0.07	1.85	0.54
Fe	1	300	8.2	2.8	9.4	2.2	65.1	27.4	5.6	2.7	26.4	13.4
	28		6.4	1.5	54.2	21.1	10.2	2.7	7.8	3.8	9.7	2.6
Mn	1	N/A	0	0	459	206	2	1	0	0	1	1
	28		2	1	123	59	5	2	5	3	3	2
Ni	1	N/A	0.77	0.13	0.90	0.33	2.76	1.06	0.72	0.24	0.75	0.13
	28		1.07	0.05	1.00	0.07	1.18	0.11	1.08	0.10	1.55	0.19
Pb	1	1	0.069	0.026	0.149	0.058	0.187	0.036	0.012	0.006	0.107	0.032
	28		0.059	0.009	0.178	0.058	0.073	0.025	0.022	0.005	0.045	0.010
Tl	1	0.8	0.0186	0.0008	0.0204	0.0005	0.0083	0.0005	0.0169	0.0004	0.0153	0.0008
	28		0.0156	0.0010	0.0375	0.0040	0.0093	0.0006	0.0144	0.0014	0.0137	0.0012
Zn	1	30	*0.37*	0.22	2.71	0.58	1.13	0.54	*0.03*	0.11	1.66	1.12
	28		0.91	0.17	1.88	0.26	0.43	0.16	*0.18*	0.27	0.78	0.29

CCME WQG is not available for Co, but elevated concentrations of Co were observed in cone water from the burned sites (Table 17). The Co concentrations measured in this study were well below the ranged reported by Gallaher and Koch (2004) of 5 - 1,100 µg/L. Cone water concentrations of Co of the burned zone (0.0939 µg/L ± 0.0145) increased by 260% on Day 1 from site water (0.0260 µg/L) (Table 18). Particulate-bound Co may have been released to the overlaying cone water during the 2-week equilibration and as they were nearly 3x > those in the unburned cone water sample on Day 1 (Table 17). Concentrations of Co in the burned zone (0.105 µg/L ± 0.008) on Day 28 did not vary much from Day 1.

Concentrations of Fe in the burned zone (65.1 µg/L ± 27.4) increased by 540% on Day 1 compared to site water, and it was approximately 8x > in the unburned zone (8.2 µg/L ± 2.8) (Table 17). When compared to Townsend and Douglas (2004), the Fe concentration measured in this study was well below their reported mean concentration (330 µg/L). In the Crowsnest River, Fe concentrations were below the CCME WQG (300 µg/L) (Table 18). Moreover, Fe is highly ubiquitous in freshwater ecosystem (Forstner and Wittmann 1979).

Increased concentrations of Ni were observed in the burned zones (Table 17). The Ni concentrations measured this study were below the range detected in Gallaher and Koch (2004) of 5.17-1,300 µg/L. On Day 1, concentration of Ni (2.76 µg/L ± 1.06) increased by 700% compared to site water and it was at least 3.5x > in the unburned zone (0.77 µg/L ± 0.13), thus particulate-bound Ni may have been released to the overlay cone water during the 2-week equilibration. On Day 28, Ni concentrations decreased by almost 60% compared to Day 1, thus some Ni may have been absorbed by *H. azteca* (Borgmann et al. 2001), or rebound to residual fraction of sediment (Fan et al. 2002). Ni concentrations in Frank Lake were lower, which could possibly be precipitated by limestone deposits (Aziz et al. 2008) from Turtle Mountain as a result of Frank Slide. Ni concentrations downstream zone were also lower, which indicates that elevated Ni in the burned zone may not have mobilized downstream in the Crowsnest River.

On Day 1, Zn concentrations increased by 350% in the burned treatment (2.71 µg/L ± 0.58) and it was 3x > the unburned treatment (0.37 µg/L ± 0.22) (Table 18). On Day 28, Zn concentrations in the burned treatment (1.88 µg/L± 0.26) decreased by 60%, which show that Zn may have been absorbed by *H. azteca* (Borgmann and Norwood 1995) or bound to organic matter and residual fraction of the sediment (Tessier et al. 1979). Zn concentrations in the Frank Lake and downstream zone were not elevated, which indicates that elevated Zn concentrations from the burned sites may have precipitated with limestone from Turtle Mountain at Frank Lake and Zn may not have mobilized downstream in the Crowsnest River. When compared to Gallaher and Koch (2004), the Zn concentrations measured in this study was just below their detected range (2.94- 47,000 µg/L). In addition, Zn concentrations in none the samples exceeded the CCME WQG (Table 18).

To summarize, dissolved metals including Al, Ba, Cd, Co, Fe, Ni, and Zn were elevated in the sites impacted by the wildfire. All total metal concentrations were below the CCME WQG while no such guidelines are available for Ba and Co. Concentrations of Ba and Co were monitored in the tissues samples and these data are discussed in Section 4.3.

4.2.3 Water Characteristics (site and cone)

Major ions and other water parameters such as pH are known to influence the bioavailability and toxicity of metals (Schamphelaere et al. 2002; Borgmann et al. 2005b). This section discusses water quality (Table 19) and major ions (Table 20) of the site water and cone water (Day 28). Major ion concentrations are compared with the DeChlor water used to culture *H. azteca.* Major ions were not analyzed on Day 1 of the experiment because of limited sample size. The pH of all sites was slightly basic (7.9-8.6) on all three days and was similar to DeChlor water. Ammonia concentrations were all ≤

0.015 mM and the oxygen concentrations on Day 1 and Day 28 of the cone water ranged from 8.83 mg/L to 9.24 mg/L. Other parameters including DIC, Ca^{2+} and hardness were similar or greater than the concentrations of DeChlor water (Table 20). Various patterns were observed for other major ions including DOC, SO_4^{2-} , Cl, Na, and alkalinity (Figure 18).

Table 19 Characteristics of site water and cone water (Day 1 and Day 28)

Parameters	Units	Days	Culture DeChlor Water	**Unburned**	**Control**	**Burned**	**Frank Lake**	**Downstream**
pH	-	Site	7.9-8.6	8.62		8.60	8.43	8.58
	-	1	-	8.50	8.31	8.59	8.53	8.52
	-	28	-	8.50	8.37	8.74	8.50	8.50
COND.	us	Site	-	319		345	363	367
	us	1	-	293	322	302	326	320
	us	28	-	284	325	289	338	329
NH_3	mM	Site	-	0		0	0	0
	mM	1	-	0.00833	0	0	0	0
	mM	28	-	0.015	0.015	0	0.01	0.015
O_2	mg/L	1	-	8.88	8.87	8.92	8.88	8.83
	mg/L	28	-	9.13	9.17	9.25	8.66	8.68

Table 20 Major ion concentrations and water characteristics of the study sites

Major Ions	Units	DeChlor Water		Unburned	Control	Burned	Frank Lake	Downstream
DOC	mg/L	2	Site	1.30	-	1.93	1.40	1.35
			28	3.66	3.53	7.70	3.53	8.60
DIC	mg/L	N/A	Site	30.0	-	38.9	34.4	35.4
			28	25.0	16.6	30.2	30.3	28.5
SO_4^{2-}	mg/L	28	Site	31.2	-	16.7	37.8	37.1
			28	25.4	37.6	13.1	36.0	35.9
Cl^-	mg/L	24	Site	1.21	-	0.19	2.03	2.36
			28	2.41	28.3	1.84	3.23	3.81
Ca^{2+}	mg/L	40	Site	48.8	-	49.4	52.8	52.3
			28	40.0	32.2	32.3	47.3	41.6
Mg^{2+}	mg/L	8	Site	10.1	-	12.6	11.8	12.5
			28	10.1	8.4	11.9	12.2	12.5
Na^+	mg/L	12	Site	1.69	-	5.84	4.36	5.34
			28	2.5	15.6	7.6	5.2	6.3
K^+	mg/L	N/A	Site	0.350	-	0.713	0.530	0.565
			28	0.97	1.98	1.23	1.17	1.19
Hardness ($CaCO_3$)	mg/L	130	Site	132	-	169	148	153
			28	112	74	138	135	126
Alkalinity	CO3 mol/L	90	Site	2.50	-	3.24	2.86	2.95
			28	2.08	1.38	2.51	2.52	2.38

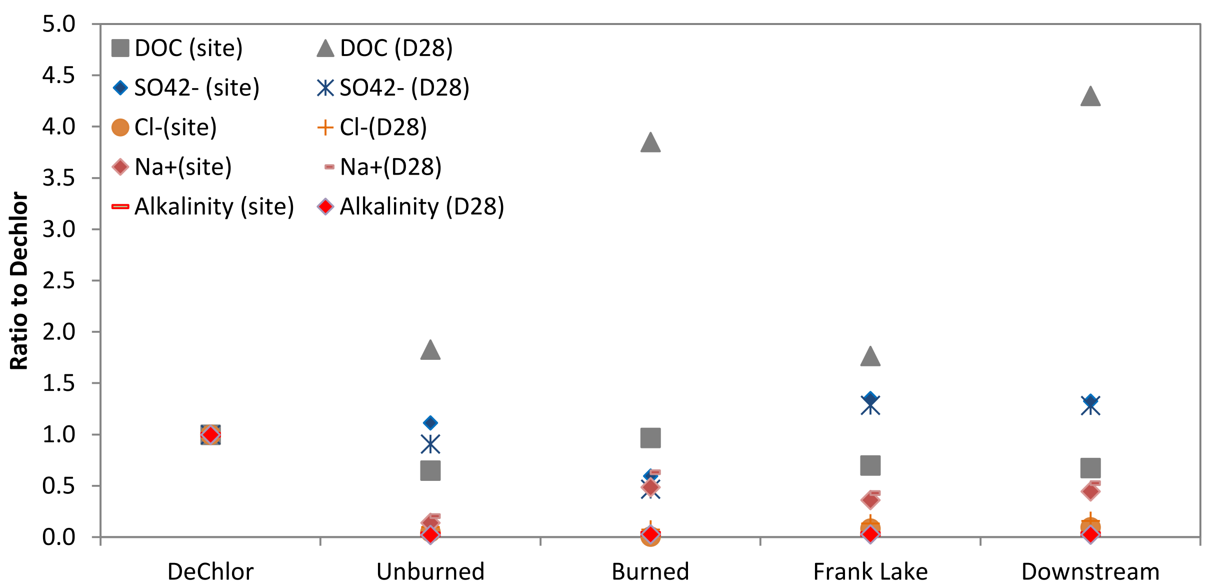

Figure 18 Ratio of major ions in each zone (Unburned/Burned/Frank Lake/Downstream) to Dechlor culture water

DOC concentrations in the burned and downstream site water on Day 28 increased by 300% and 500% when compared to site water, respectively and were well above the DeChlor water DOC concentrations (2 mg/L) (Figure 18). Higher concentrations of DOC can increase the metal complexing capacity and reduce metal bioavailability in the burned and downstream zones (Borgmann et al. 1991). Concentrations of SO_4^{2-} in the burned site water (13.1 mg/L) was 40% lower than DeChlor water (28 mg/L) (Figure 18), which may lower the ability to complex with metals compared to DeChlor water such as Cd (Benjamin and Leckle 1982). Concentrations of Cl^- in all treatments were at least 90% lower than DeChlor water (Figure 18), thus the ability of the water to complex metals (such as Cd) with Cl^- ions may be much lower than the DeChlor water (Borgmann 1983). The concentrations of Na^+ in the cone water on Day 28 were 30% to 80% < the concentrations in DeChlor water (12 mg/L) (Figure 18), especially the concentration at the cone water from the unburned site (2.47 mg/L) (Table 20). In this study, the survival of *H. azteca* was > 80% when Na^+ concentrations were approximately 10 mg/L in burned cone water (B2) (Figure 19). When the level of Na^+ lowered to 5 mg/L in burned cone water B3, it may have reduced survival of *H. azteca* (20%) as Na^+ is an essential ion to *H. azteca* (Figure 19) (Borgmann 1996).

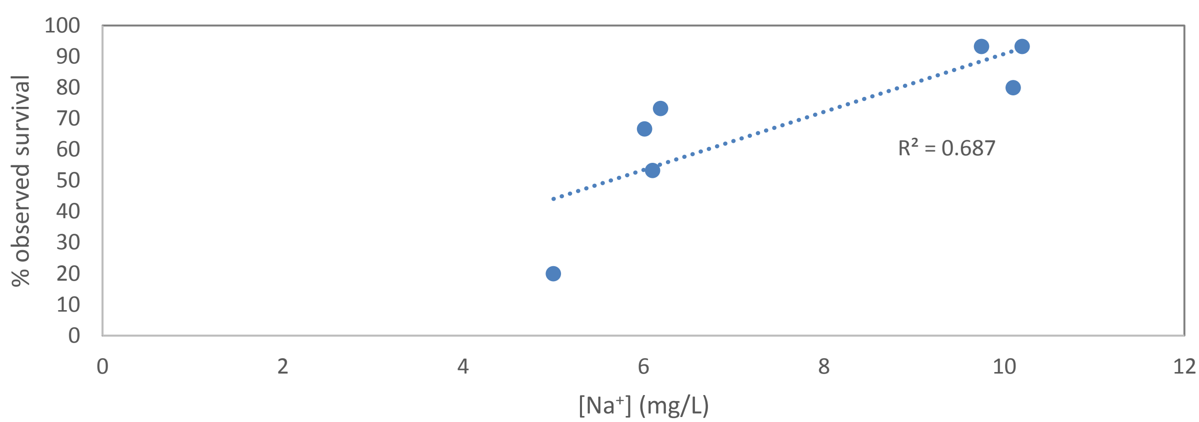

Figure 19 Relationship between Na^+ concentrations and observed survival in burned cone on Day 28

Alkalinity of site water and cone water was at least 95% < alkalinity of DeChlor water (Figure 20). Although the effect of alkalinity on metal toxicity of *H. azteca* is not well known, studies have found alkalinity often reduce metal toxicity (Stiff 1971; Andrew 1977). For example, Wurts and Perschbacher (1994) found 100% morality of catfish with 20 mg/L alkalinity and lower mortality (63%) with higher alkalinity (250 mg/L) at lethal copper concentrations. Accordingly, site water with lower alkalinity may have lower survival of *H. azteca* from metal toxicity (Figure 20).

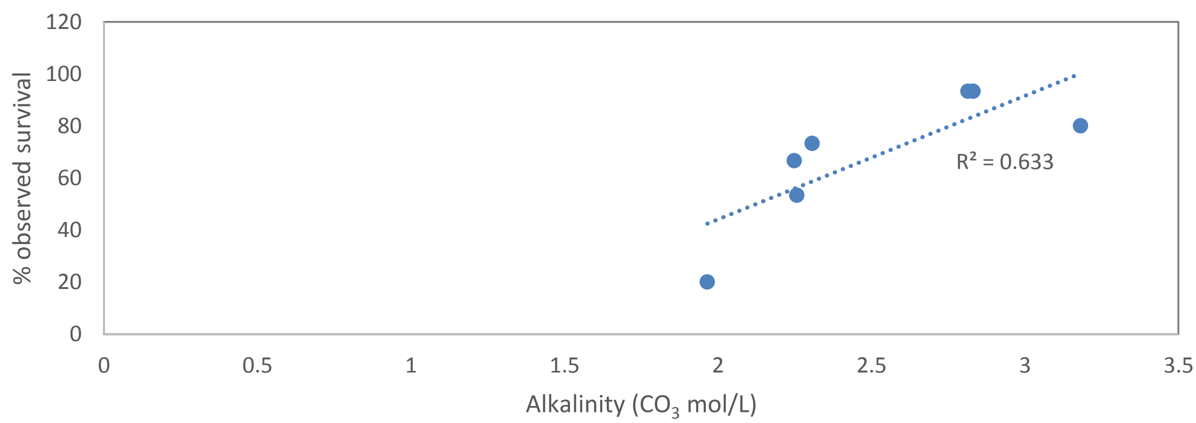

Figure 20 The relationship between alkalinity and percent observed survival in burned cones

4.3 Tissue Metal Chemistry

Very few studies have examined the impacts of wildfire on metal bioaccumulation and toxicity to aquatic life. One exception is mercury, whose bioaccumulation has been measured in aquatic life in wildfire-impacted watersheds in a number of studies (Garcia and Carignan 1999; Kelly et al. 2006; Beganyi and Batzer 2011). Measuring the total metal body concentration (BC) in aquatic invertebrates such as *H. azteca* can be used to quantify the toxicological effects of wildfire-generated metals (Borgmann et al. 1991; Norwood et al. 2007). In this section, the metal BCs of *H. azteca* from the bioaccumulation and toxicity tests are examined. The metal BC is compared with the lethal body concentration at 25% mortality (LBC25).

4.3.1 Caged Tissues

The tissues of *H. azteca* from the cages in cones containing sediment from the burned site were elevated Ba, Cr, and Zn (Figure 21). Concentrations of Ba in the tissue from the burned cages (343 nmol/g ± 48) were 2x > the unburned cages (165 nmol/g ± 7). LBC25 has not been developed for Ba, but Shuhaimi-Othman (2008) reported Ba tissue body concentration of 167 µg/g ± 32 (2980 nmol/g ± 570) in adult in *H. azteca* and toxicity potentially related to Ba was not reported. Concentrations of Cr in the tissue from the burned cages (4.90 nmol/g ± 1.65) were 3x > in unburned cages (1.40 nmol/g ± 0.65) (Table 21). The cages containing water from Frank Lake and downstream zones showed similar or higher Cr concentrations in the *H. azteca* tissue. All Cr body concentrations are below the LBC25, and Cr survival of *H. azteca* is not reduced. Concentrations of Zn (504 nmol/g ± 458) in the tissue of the burned cages were nearly 7x > unburned cages (72.5 nmol/g ± 19.8) (Table 21). Zn tissue concentrations do not exceeded the LBC25. In conclusion, *H. azteca* bioaccumulated higher concentrations of metals (Cr and Zn) in the burned cones, but the total metal concentrations are below the LBC25.

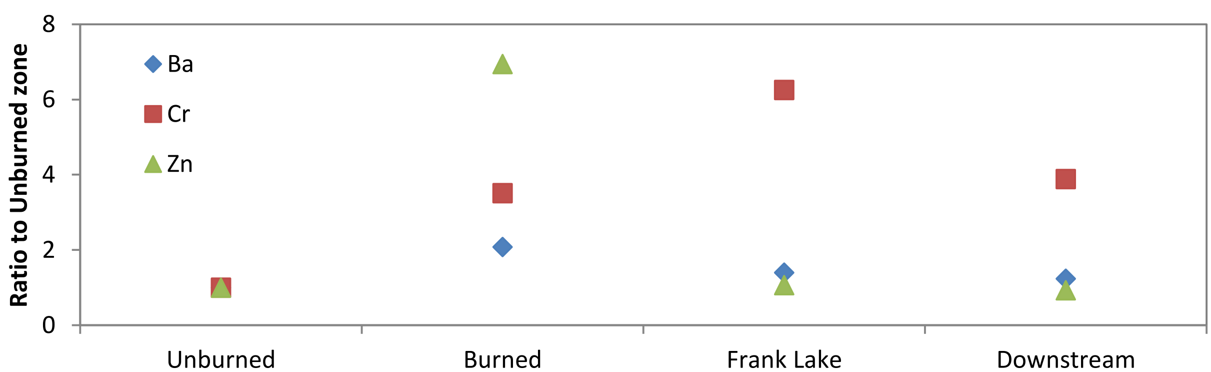

Figure 21 Ratio of caged BC of Ba, Cr, and Zn in the unburned zone and other zones (burned/Frank Lake/downstream)

Table 21 Metal body concentration of *H. azteca* in the cages (nmol/g)

			Unburned		Control		Burned		Frank Lake		Downstream	
Metals	LBC25	Background	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Al	N/A	N/A	1060	190	1200	260	1130	120	780	150	1270	270
As	83	13.8	<BG		<BG		<BG		<BG		<BG	
Ba	N/A	N/A	164	7	157	21	343	48	230	10	203	13
Cd	585	3.64	3.84	0.44	3.92	0.31	4.65	0.90	2.82	0.16	2.66	0.37
Co	90		1.24	0.14	1.50	0.17	1.98	0.61	1.39	0.10	1.46	0.24
Cr	146	-0.1	1.40	0.65	7.83	4.37	4.90	1.65	8.73	2.76	5.43	2.44
Cu	1850	1539	<BG		<BG		<BG		<BG		<BG	
Fe	N/A	N/A	900	50	890	70	1080	94	890	50	920	50
Mn	44400	107	<BG		2346	1119	<BG		70.7	43.9	1.5	4.4
Ni	169	16	<BG		<BG		26.7	20.4	<BG		1.8	3.1
Pb	38	0.199	<BG		<BG		6.54	6.60	<BG		0.04	0.05
Tl	364	0.124	0.393	0.042	0.628	0.185	0.382	0.067	0.381	0.052	0.604	0.067
Zn	938	924	73	20	81	15	504	458	77	23	68	17

<BG: values below background concentration in the culture of *H. azteca*

4.3.2 Cone Tissues

The tissues of *H. azteca* from the cones showed increased concentrations of metals (Al, Ba, Co, and Mn) in the burned zones after the 28-day toxicity test (Figure 25). The BC of Al in the burned cone (3206 nmol/g ± 409) was 2x > in the unburned cone (1329 nmol/g ± 122) and elevated BC of Al was also observed in the composite cones (3561 nmol/g ± 589) (Table 22). LBC25 has not been developed for Al in *H. azteca*. There are no studies that directly examined the relationship between Al bioaccumulation and toxicity in *H. azteca*, Havas (1985) reported bioaccumulation of Al in *Daphnia magna* ranging from 13 µmol/g (13,000 nmol/g) at pH 6.5 when dosed with 20µg/L of Al. At 50 hours, the survival of *Daphnia magna* was approximately 80% under acidic conditions (Havas 1985). Although Mackie (1989) did not report Al bioaccumulation, he found that 325 µg/L Al is not acutely toxic to *H. azteca*, which is higher than Al measured in the cone water of this study. Similar to the caged tissues, the BC of Ba from the burned cones (1316 nmol/g ± 154) was 2x > in the unburned cones (604 nmol/g ± 69). Since LBC25 has not been developed for Ba in *H. azteca*, but when compared to Shuhaimi-Othman (2008), the body concentrations of Ba measured in the cone was still lower than their reported value (1980 nmol/g ± 570).

Tissue concentrations of Co from the burned cones (8.52 nmol/g ± 1.33) were 2x > unburned cones (3.08 nmol/g ± 0.30). Tissue level of Co from the downstream cones (7.67 nmol/g ± 1.71) was also 2x > unburned cones. However, the increased tissue concentration of Co was well below the LCB25 (90 nmol/g). Mn BC in the burned cones was 2x > the unburned cones (Figure 22), but it is below the LBC25, thus it should not cause mortality of *H. azteca*.

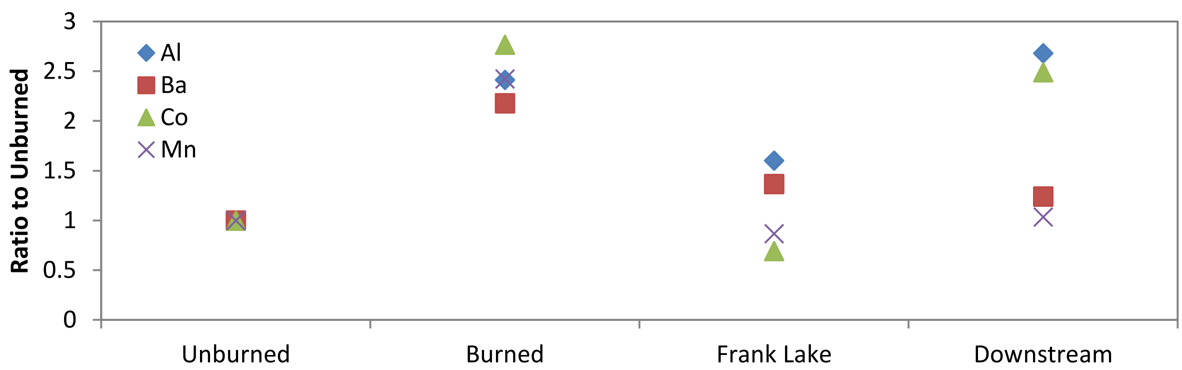

Figure 22 Ratio of cone BC of Al, Ba, Co and Mn in unburned zone and other zones (burned/Frank Lake/downstream)

Table 22 Metal body concentration of *H. azteca* in the cones in nmol/g

	LBC25*	Back-	Unburned		Control		Burned		Frank Lake		Downstream	
Me	(nmol/g)	ground	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Al	N/A	N/A	1330	120	1300	140	3210	410	2130	550	3560	590
As	83	13.8	4.68	1.16	3.91	1.79	2.96	1.24	6.65	2.40	8.86	2.61
Ba	N/A	N/A	600	70	370	40	1320	150	830	140	750	100
Cd	585	3.64	<BG		2.6	0.5	14.3	6.7	<BG		<BG	
Co	90	2.25	3.08	0.30	3.15	0.45	8.52	1.33	2.14	0.36	7.67	1.71
Cr	146	-0.1	<BG		0.00	2.41	<BG	3.80	<BG		<BG	
Cu	1850	1539	<BG		<BG	87	<BG	72	<BG		<BG	
Fe	N/A	N/A	1460	100	1050	70	2000	210	2710	510	2490	340
Mn	44400	107	320	50	2230	750	770	150	280	50	330	70
Ni	169	16	<BG		<BG		<BG		<BG		<BG	
Pb	38	0.199	0.21	0.05	0.54	0.11	0.17	3.29	0.34	0.15	0.33	0.08
Tl	364	0.124	0.317	0.035	0.337	0.104	0.104	0.055	0.332	0.035	0.261	0.085
Zn	938	924	109	18	104	22	14	231	26	37	<BG	

*LBC25 data from Norwood et al. (2003)
<BG: values below background in culture of *H. azteca*

4.4 MEAM Predicted Survival vs. Observed Survival

Wildfire is known to influence the abundance and diversity of aquatic life (Rhine 1996; Mellon et al. 2008; Silins et al. 2009a), but little is known about the toxicity of sediment-associated metals to aquatic life in wildfire impacted streams. This thesis is the first study of its kind to directly investigate the toxicological effects of sediment-associated metals on freshwater amphipod *H. azteca*. In this section, the results of the study are compared studies on *H. azteca* using sediment from other contaminated sites and the results of MEAM-predicted and observed survival of *H. azteca* are compared for the revised dataset after the outlier replicates were removed (Figure 23).

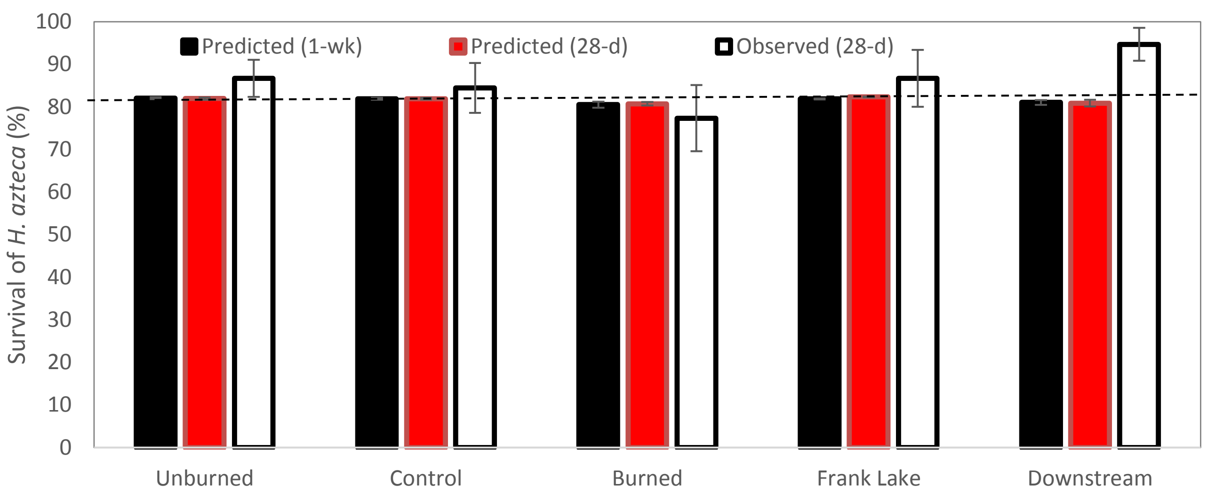

Figure 23 MEAM predicted (1-week and 28-day) and observed survival (28-day) of *H. azteca.* Control survival was 82% (----)

Previous studies have conducted sediment-associated toxicity tests to determine the level of environmental degradation. For example, Borgmann et al. (2007) found survival less than 70% in two rivers affected by metal mining. In this study, after all outliers (Rf-2, Rf-8, B1-R3 and B3) were removed eliminated, all of the treatments are comparable to the acceptable control survival (82%) (Figure 23), but with the exception for observed survival in the burned site. In The observed survival of the 28-day toxicity test was 77% ± 17, which has a relatively high standard error that is contributed from site B1 (Figure 24). The Al concentrations cone water of Day 1 in site B1 were above the CCME water quality guideline and could have had a toxic effect to the test animals. The bioaccumulation and possible toxicological effect of Al are not considered in MEAM because LCB25 for Al has not yet been developed for *H. azteca*. Accordingly, the elevated observed increased in Al concentrations could be responsible for the inconsistency identified between the between the MEAM-predicted and the observed survival (Figure 25). Further, in previous studies on wildfire generated metals, Gallaher et al. (2002) reported elevated Ba concentrations. In the present study, elevated concentrations of Ba were also found in the sediment, water and tissue samples. Currently there are no CCME guidelines nor has a LBC25 been established for Ba, it is possible that increased concentrations of Ba could have impacted the results of the present study because the relationship between the observed survival and concentration of Ba in the water shown to be correlated (Figure 25).

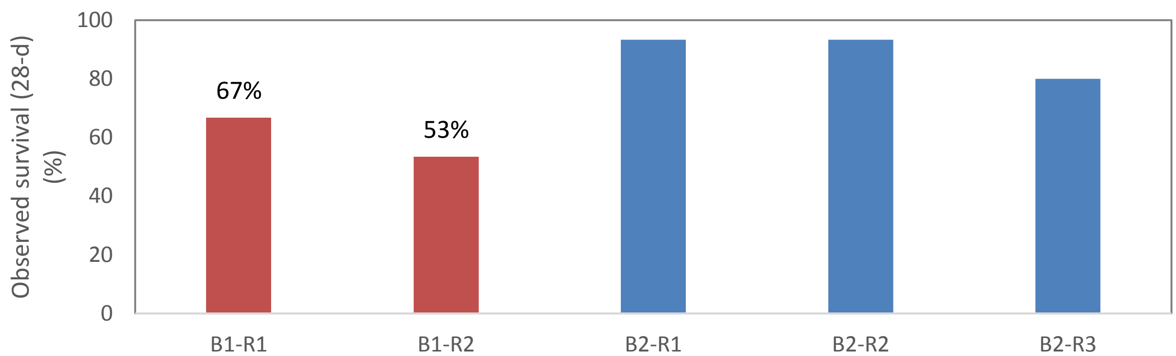

Figure 24 Individual observed survival in the cones representing the burned

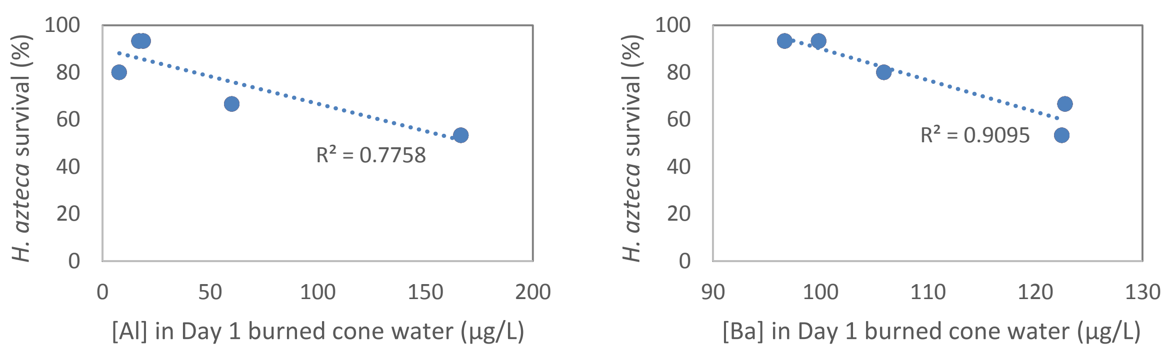

Figure 25 The relationship between the survival (28-d) of *H. azteca* and the concentration of Al and Ba in the burned cone water on Day 1

Despite the lower survival in the burned zone (Figure 23), the differences between the survival in the unburned zone and burned zone were minimal. Nine year after Lost Creek fire, increased metal concentrations in sediment, water and tissue samples were observed in all burned zones, but only slight toxicity was reflected in one of the burned zones (B1). This observation is supported by Minshall et al. (2001) who reported recovery of aquatic invertebrate population 10 years after a fire.

5 Conclusions

The conclusions of this study are:

1. Metal (Al, Ba, Cd, Co, Cu) concentrations were elevated in river sediment nine years after the Lost Creek Fire. Cd exceeded the PEL of the CCME SQG (CCME 1999).
2. After a two-week equilibration, total metal concentrations (Al, Ba, Cd, Co, Fe, Mn, Ni, Pb, and Zn) were elevated in overlaying water of cones containing sediment from the burned sites compared to reference (unburned) sediment. Al was the only metal in the overlaying water that exceeded the CCME WQGs (CCME 1999).
3. Metal concentrations in tissues from both the cage and cones were elevated in *H. azteca* exposed to sediment and water from streams draining burned landscapes compared to the upstream reference condition. No metals exceeded the lethal body concentration at 25% mortality (LCB25). Metals, including Ba, Cr and Zn, were elevated in caged tissues, while Al, Ba, Co and Mn were elevated in cone tissues. Wildfire-generated sediment-associated metals did bioaccumulate above background in tissues of *H. azteca*, but were below the LBC25. The LBC25 for Al and Ba have not been developed, thus the MEAM does not utilize their tissue concentration to predict survival. The relationship between the metal (Al and Ba) concentration and *H. azteca* survival may account for the observed survival that was lower than MEAM-predicted survival in the burned zone.
4. Nine years post Lost Creek Fire, the survival difference between the burned and unburned sites are minimal.
5. The toxicity test indicated that sediment-associated metals in the Crowsnest river basin were elevated but not toxic to *H. azteca* years after the wildfire. MEAM and LBC25 comparisons did not identify any problem metals.

Appendix

Appendix A The detection limit of each media (sediment, water, and tissues)

	Site Sediment	Site water	Cone water Day 1	Cone water Day 28	Body burden (cage)	Body burden (cone)
Units	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Ag	0.000837	0.0786	0.00431	0.00119	0.0193	0.00453
Al	0.0420	0.830	0.117	0.698	0.956	0.751
As	0.00255	0.0902	0.00348	0.00198	0.00262	0.00125
B	0.482	0.596	0.627	1.94	0.272	1.83
Ba	0.00751	0.403	0.0300	0.0333	0.112	0.0765
Be	0.000447	0.00450	0.000313	0.000472	0.000487	0.000784
Bi	0.000374	0.00257	0.00018554	0.00720	0.0233	0.00498
Ca	0.0123	0.0467	0.00845	0.0373	0.0230	0.0177
Cd	0.00268	0.00686	0.000300	0.00605	0.00225	0.000750
Ce	0.00534	0.00666	0.00019486	0.000581	0.0306	0.00148
Co	0.00201	0.00857	0.000310	0.00985	0.00479	0.00413
Cr	0.0562	0.637	0.105	0.0311	0.163	0.0336
Cs	0.000121	0.00453	0.0000746	0.0000474	0.0000776	0.000222
Cu	0.240	0.334	0.131	0.146	0.408	0.147
Fe]	1.16	1.15	0.769	0.988	1.34	2.01
Ga	0.000215	0.00717	0.0000654	0.000279	0.000384	0.000130
Ge	0.00186	0.0839	0.00218	0.00159	0.00127	0.00167
In	0.000508	0.00697	0.0000450	0.000111	0.000210	0.000133
K	0.0173	0.0629	0.00515	0.0152	0.00367	0.0171
La	0.00104	0.00672	0.000403	0.000431	0.00149	0.00117
Li	0.00911	0.179	0.00285	0.00335	0.00181	0.00388
Mg	0.00176	0.00839	0.000368	0.00204	0.00224	0.00286
Mn	0.0346	0.394	0.00789	0.0247	0.0544	0.0694
Mo	0.0117	0.0330	0.00162	0.00691	0.00699	0.0112
Na	0.00273	0.0427	0.00237	0.0344	0.0206	0.0209
Nb	0.0213	0.00253	0.0000424	0.000110	0.00404	0.0273
Ni	0.867	0.129	0.00663	0.0603	0.236	0.0399
Pb	0.0141	0.0343	0.00673	0.0355	0.0432	0.0206
Pd	0.00257	0.00424	0.000562	0.00167	0.00239	0.00460
Pt	0.000423	0.00341	0.000348	0.000723	0.000440	0.000780
Rb	0.0000735	0.0461	0.000850	0.00778	0.00209	0.0190
Sb]	0.00200	0.00878	0.000502	0.00102	0.00623	0.0189
Sc	0.00158	0.0125	0.00591	0.00172	0.00271	0.00303
Se	0.00436	0.107	0.00185	0.00488	0.00229	0.00477
Sn	0.0482	0.00696	0.00130	0.00781	0.0968	0.105
Sr	0.00884	0.783	0.00527	0.912	0.0258	0.0365
Te	0.0113	0.0204	0.0102	0.0122	0.00730	0.00718
Ti	0.0771	0.428	0.0242	0.0402	0.299	0.0546
Tl	0.00662	0.00377	0.000502	0.000745	0.00173	0.00125
U	0.000265	0.00727	0.00012794	0.000106	0.000533	0.000223
V	0.0199	0.0346	0.0127	0.0154	0.0138	0.00904
W	0.00176	0.00285	0.00192	0.000809	0.00285	0.0112
Y	0.000325	0.00693	0.00027608	0.0000808	0.00280	0.00161
Zn	0.219	0.832	0.424	1.32	2.09	1.51
Zr	0.0906	0.00417	0.00131	0.0114	0.0133	0.0165

Appendix B1 The final sediment metal concentration (nmol/g dry weight)

Site ID	Rf	B-1	FL	B-2	B-3	DS	TC
Metal [Molar mass (g/mol)]	Final Sediment concetnrations (nmol/g dry wt)						
Ag [107]	1.38	1.90	1.44	2.85	1.68	1.41	1.69
Al [27]	167,486	225,590	202,005	286,624	255,078	236,197	243,321
As [75]	56.4	53.0	43.1	53.3	45.5	42.3	45.3
B [11]	434	300	282	562	296	221	538
Ba [135]	1,211	1,754	1,135	2,052	1,774	1,510	1,853
Be [9]	48.3	64.5	55.1	70.5	58.0	58.2	63.4
Bi [209]	0.575	0.639	0.378	0.641	0.571	0.485	0.659
Ca [43]	1468	341	807	414	542	758	1209
Cd [111]	5.02	3.47	3.85	7.76	5.54	4.28	6.29
Ce [140]	136	133	103	129	123	129	116
Co [59]	72.1	93.2	97.1	147	117	109	113
Cr [52]	155	140	141	233	191	172	181
Cs [133]	6.86	7.69	6.47	7.29	6.39	7.19	7.54
Cu [63]	194	268	341	392	309	228	324
Fe [56]	179,445	216,244	208,709	283,272	246,748	236,170	247,944
Ga [71]	22.9	29.5	27.7	35.5	31.3	32.0	31.3
Ge [74]	2.87	1.95	2.02	2.44	2.30	2.02	2.31
In [115]	0.132	0.133	0.119	0.176	0.142	0.127	0.150
K [39]	18.2	22.6	18.9	22.9	26.1	24.4	25.5
La [139]	60.5	69.0	54.8	64.0	57.7	66.9	58.8
Li [7]	890	885	1,005	1,348	1,220	1,098	1,178
Mg [26]	462	207	369	244	265	339	376
Mn [55]	4,972	5,633	4,588	8,573	7,348	6,275	4,779
Mo [98]	4.27	7.32	3.81	9.79	7.70	4.14	4.79

Rf (reference site); B-1, B-2, B-3 (burned sites); FL (Frank Lake); DS (downstream); TC (Todd Creek)

Appendix B2 The final sediment metal concentration (nmol/g dry weight)

Site ID	CNR-Rf	CNR-B-1	CNR-FL	CNR-B-2	CNR-B-3	CNR-DS	CNR-TC
Metal [Molar mass (g/mol)]	Final Sediment concetnrations (nmol/g dry wt)						
Na [23]	6.37	9.07	9.93	10.3	9.66	12.4	10.3
Nb [93]	2.85	12.7	6.97	6.52	5.36	10.4	8.00
Ni [60]	298	243	261	434	326	281	332
Pb [208]	50.4	52.7	54.6	58.4	48.1	44.5	52.7
Pd [108]	-0.084	-0.134	-0.200	-0.162	*-0.226*	-0.188	-0.088
Pt [195]	0.0126	0.0115	0.0131	0.0150	0.00879	0.00523	0.0143
Rb [85]	87.6	103	78.6	109	97.5	96.8	102
Sb [121]	1.83	1.97	1.67	2.50	1.84	1.73	1.84
Sc [45]	42.4	57.7	42.3	83.0	66.6	50.1	60.8
Se [78]	38.1	7.49	9.78	22.9	13.6	14.5	16.9
Sn [120]	-4.77	-5.55	-5.28	-5.73	-5.95	-5.53	-5.47
Sr [88]	900	573	713	849	997	834	1,246
Te [128]	*0.308*	0.458	*0.304*	0.505	0.594	*0.403*	*0.341*
Ti [47]	2,178	4,421	3,880	4,858	4,894	4,539	3,823
Tl [205]	1.03	0.719	0.666	1.02	0.786	0.702	0.958
U [238]	2.54	2.80	2.41	3.80	2.50	1.83	2.11
V [51]	234	319	289	382	363	347	325
W [184]	0.520	0.436	0.483	0.427	0.532	0.477	0.598
Y [89]	146	121	95.3	136	101	88.1	103
Zn [66]	1,164	756	1,138	1,196	969	1,151	1,317
Zr [90]	61.4	64.6	38.2	71.0	43.0	41.2	62.1

Appendix C The Percentage of dissolved metal concentration in total metal concentration in the site water

Metal	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Tl	Zn
Site	% of dissolved concentration in total metal concentration												
Rf	92.36%	-0.24%	98.47%	47.24%	103.27%	-42.39%	138.09%	9.29%	-1.11%	159.61%	345.99%	104.44%	37.17%
FL	81.89%	7.10%	92.50%	38.14%	75.74%	241.25%	117.11%	14.18%	2.14%	179.43%	-31.17%	97.77%	57.23%
TC	101.80%	-0.91%	92.32%	-4.78%	36.71%	1536.14%	73.56%	-0.24%	1.50%	138.25%	-22.37%	32.27%	-6.54%

Appendix D1 The metal concentration (µg/L) in site water

Site ID	Rf	Rf-F	B1	FL	FL-F	B2	B3	DS	TC	TC-F
Metal [Molar Mass g/mol]	Metal concentration in site water (µg/L)									
Ag [107]	*-0.0189*	*-0.0193*	*-0.0210*	*-0.0203*	*-0.0210*	*-0.0179*	*-0.0200*	*-0.0206*	*-0.0192*	*-0.0210*
Al [27]	4.20	*-0.0101*	4.52	6.50	0.46	5.21	2.16	2.61	38.7	*-0.352*
As [75]	0.243	0.225	0.295	0.217	0.177	0.150	0.152	0.227	0.337	0.344
B [11]	1.40	1.43	11.0	5.89	5.42	16.5	11.8	8.62	8.50	12.8
Ba [135]	47.3	46.6	105.7	60.0	55.5	115	93.2	66.7	76.1	70.3
Be [9]	*0.000118*	*-0.000463*	*0.000988*	*0.000838*	*-0.000092*	*0.00125*	*-0.000062*	*0.000718*	0.00444	*-0.000353*
Bi [209]	*-0.00209*	*-0.00217*	*-0.00151*	*-0.00162*	*-0.00149*	*-0.00217*	0.00237	*-0.00223*	*-0.000770*	*-0.00234*
Ca [43]	45.9	44.5	38.0	48.3	44.1	48.4	48.5	46.0	45.8	42.5
Cd [111]	*0.00303*	*0.00143*	0.00491	0.00433	*0.00165*	0.00807	0.00509	0.00449	0.00852	*-0.000408*
Ce [140]	0.00540	*-0.0016225*	0.00530	0.0130	*-0.000773*	0.00641	*0.00319*	*0.00160*	0.0727	*-0.0023125*
Co [59]	0.00978	0.0101	0.0173	0.0153	0.0116	0.0422	0.0185	0.0203	0.0615	0.0226
Cr [52]	*0.0124*	*-0.0053*	*-0.0990*	*-0.0150*	*-0.0363*	*-0.0509*	*0.0671*	*-0.0471*	*-0.0075*	*-0.115*
Cs [133]	*0.000620*	*-0.000250*	*0.000490*	*0.00201*	*0.000730*	*0.00118*	*0.000620*	*0.000390*	0.00715	*-0.000710*
Cu [63]	*0.0144*	*0.0199*	0.350	*0.129*	*0.152*	*0.116*	0.246	*0.0432*	0.205	*0.151*
Fe [56]	10.5	*0.976*	9.72	20.3	2.88	16.9	3.83	7.80	83.3	*-0.196*
Ga [71]	*0.00679*	*0.00556*	*0.00416*	0.00636	*0.00390*	*0.00474*	*0.00290*	*0.00554*	0.0185	*-0.000193*
Ge [74]	*-0.00592*	*-0.00629*	*-0.0121*	*-0.00764*	*-0.00719*	*0.00705*	*-0.00934*	*-0.0104*	*-0.00721*	*-0.00696*
In [115]	*-0.0012275*	*-0.0012575*	*-0.0012375*	*-0.0011875*	*-0.0011575*	*-0.0012075*	*-0.0012275*	*-0.0012175*	*-0.000928*	*-0.0012575*
K [39]	0.368	0.331	0.731	0.497	0.455	0.670	0.653	0.511	0.590	0.549
La [139]	*0.00285*	*-0.000580*	0.00979	0.00744	*0.0000900*	0.00484	*0.00275*	*0.000780*	0.0347	*-0.00111*
Li [7]	2.67	2.65	1.34	4.70	4.36	5.40	4.03	4.66	4.99	4.61
Mg [26]	10.5	10.1	6.83	12.0	11.0	16.0	13.6	11.9	12.4	11.8
Mn [55]	1.54	*-0.0171*	0.283	1.46	*0.0312*	1.80	*0.0542*	0.355	5.42	*0.0813*
Mo [98]	0.486	*0.463*	0.649	0.542	*0.495*	1.25	*1.30*	0.600	0.647	*0.634*

Appendix D2 The metal concentration (μg/L) in site water

Site ID	Rf	Rf-F	B1	FL	FL-F	B2	B3	DS	TC	TC-F
Metal [Molar Mass g/mol]	Metal concentration in site water (μg/L)									
Na [23]	1.75	1.71	4.99	4.44	4.15	8.73	4.04	5.02	5.56	5.25
Nb [93]	*-0.000310*	*-0.000150*	*0.000240*	*0.000100*	*-0.000240*	*-0.000310*	*-0.0000200*	*-0.000210*	*0.000460*	0.0189
Ni [60]	0.341	0.544	0.192	0.285	0.512	0.596	0.198	0.210	0.380	0.525
Pb [208]	*-0.004675*	*-0.0162*	*-0.0167*	0.0162	*-0.005045*	*-0.001985*	0.0397	*-0.008285*	0.0733	*-0.0164*
Pd [108]	*-0.0003275*	*-0.0003775*	*-0.0010775*	*-0.0004675*	*-0.0003275*	*-0.0004675*	*-0.0003375*	*-0.000988*	*-0.0006975*	0.0160
Pt [195]	*-0.000305*	*-0.000275*	*-0.000375*	*-0.000545*	*-0.000125*	*-0.000605*	*-0.000375*	*-0.000425*	*-0.0000250*	*0.000415*
Rb [85]	0.205	0.187	0.224	0.337	0.301	0.493	0.278	0.296	0.338	0.294
Sb [121]	0.0394	0.0373	0.0618	0.0468	0.0399	0.0383	0.0433	0.0451	0.0572	0.0867
Sc [45]	0.0403	0.0434	0.0578	0.0401	0.0466	0.0641	0.0772	*0.0160*	0.0502	0.0416
Se [78]	1.79	1.74	0.156	1.30	1.25	0.943	0.804	1.27	1.18	1.46
Sn [120]	*-0.00241*	*-0.00210*	*-0.00282*	*-0.00261*	0.00040	*-0.00272*	0.00347	*-0.00390*	*-0.00271*	*-0.0042375*
Sr [88]	186	180	258	265	248	411	521	291	299	280
Te [128]	*0.00356*	*-0.00390*	*-0.000490*	*-0.000710*	*-0.00547*	*-0.0153*	*-0.0141*	*-0.0221*	*-0.00736*	*-0.0187*
Ti [47]	*0.0796*	*-0.00444*	*0.0104*	*0.104*	*0.0000200*	*0.00239*	*0.0798*	*-0.0601*	0.605	*0.0501*
Tl [205]	0.0162	0.0170	0.00257	0.0166	0.0162	0.00532	0.00415	0.0147	0.0169	0.00545
U [238]	0.315	0.318	0.356	0.387	0.358	0.646	0.597	0.405	0.426	0.265
V [51]	0.247	0.209	0.270	0.215	0.188	0.200	0.184	0.207	0.324	0.195
W [184]	0.00693	0.00533	*-0.00101*	0.00499	0.00460	*-0.00135*	*-0.000408*	0.00423	0.00494	0.166
Y [89]	0.0137	0.00793	0.0558	0.0226	0.00948	0.0313	0.0237	0.0134	0.0638	*0.00292*
Zn [66]	1.46	0.543	*0.0618*	0.788	0.451	0.407	*0.242*	*0.0731*	1.69	*-0.110*
Zr [90]	0.00516	0.00489	0.0511	0.00784	0.00594	0.00945	0.00863	0.00248	0.00976	0.0243

Appendix E Comparison of filtered and unfiltered metal sample in cone water

Site	Rf		LR	LE	B1	FL		B2	B3	DS	TC	
N	1	9	3	3	3	1	3	3	1	2	1	3
	% of dissolved metal concentration (filtered) of the total metal concentration (unfiltered)											
Day	1	28	28	28	28	1	28	28	28	28	1	28
Al	44%	36%	25%	51%	39%	59%	50%	57%	96%	46%	56%	43%
As	117%	100%	92%	98%	100%	108%	106%	102%	97%	104%	99%	98%
Ba	100%	99%	97%	100%	100%	101%	97%	99%	99%	100%	100%	99%
Cd	38%	71%	48%	59%	41%	5%	57%	72%	101%	76%	10%	59%
Co	32%	96%	89%	73%	91%	74%	96%	94%	102%	98%	75%	91%
Cr	110%	87%	61%	44%	56%	112%	24%	63%	272%	89%	127%	205%
Cu	34%	125%	140%	131%	155%	51%	135%	202%	344%	148%	32%	44%
Fe	0%	20%	4%	3%	10%	92%	21%	14%	140%	14%	8%	17%
Mn	8%	11%	161%	10%	9%	39%	7%	5%	13%	15%	16%	5%
Ni	66%	105%	112%	134%	149%	69%	115%	121%	198%	112%	65%	110%
Pb	41%	68%	11%	114%	61%	33%	209%	168%	524%	207%	17%	112%
Tl	116%	68%	53%	55%	60%	100%	77%	75%	74%	71%	106%	73%
Zn	184%	137%	45%	110%	118%	-89%	131%	840%	779%	46%	0%	364%

Percentages in red are concentrations with filtered value greater than unfiltered value hence those are not used for exposure concentration

Appendix F1 The Day 1 cone water metal concentration in µg/L

Site	RF-1	RF-1F	RF-2	RF-3	RF-4	RF-5	RF-6	RF-7
	Day 1 Cone water metal concentration (µg/L)							
Ag	*-0.000973*	0.00868	*-0.000903*	*0.000778*	*-0.00178*	*-0.00190*	*-0.00170*	*-0.000953*
Al	5.67	2.47	12.6	5.13	2.20	1.97	5.04	3.16
As	1.09	1.09	0.811	0.741	0.942	1.10	1.08	0.811
B	3.39	3.77	3.58	4.22	3.27	3.66	4.54	3.00
Ba	52.6	53.1	50.8	52.1	48.9	55.1	55.3	54.6
Be	0.00203	0.0005125	0.0117	0.00684	0.00212	0.00590	0.00524	0.00608
Bi	0.0000450	0.0000750	*-0.0000050*	0.000145	0.000625	*-0.000105*	0.0000850	0.000745
Ca	38.4	37.6	35.2	35.8	33.3	38.8	40.4	36.0
Cd	0.00877	0.00366	0.00566	0.0174	0.00267	0.00986	0.0111	0.0188
Ce	0.0141	0.00367	0.0331	0.0118	0.00492	0.00560	0.0110	0.00793
Co	0.0237	0.0113	0.0235	0.0166	0.0139	0.0125	0.0407	0.140
Cr	0.0828	0.0836	0.0817	0.0771	0.0634	0.0781	0.0796	0.0876
Cs	0.00393	0.00358	0.00473	0.00174	0.00329	0.00241	0.00314	0.00120
Cu	0.419	0.479	1.43	1.13	0.353	1.07	1.22	1.32
Fe]	5.48	0.0339	24.4	6.22	14.8	0.310	6.33	2.65
Ga	0.0163	0.0150	0.0161	0.0145	0.0143	0.0151	0.0168	0.0153
Ge	0.0346	0.0302	0.0721	0.0265	0.0246	0.0362	0.0316	0.0291
In	0.00000500	-0.0000450	0.00982	-0.0000150	*-0.000125*	-0.00000500	-0.0000450	0.0000250
K	0.500	0.457	0.462	0.473	0.474	0.513	0.486	0.478
La	0.0126	0.00177	0.0197	0.0123	0.0112	0.0105	0.0140	0.00770
Li	2.65	2.64	2.45	2.57	2.39	2.68	2.57	2.68
Mg	9.83	9.37	9.07	9.47	8.86	9.60	9.50	9.71
Mn	0.445	0.0412	1.37	0.524	0.264	0.0645	0.596	0.225
Mo	0.629	0.627	0.617	0.656	0.615	0.644	0.653	0.672
Na	1.65	1.58	1.52	1.61	1.51	1.62	1.59	1.66
Nb	0.00119	0.000470	0.000600	0.000140	*-0.000210*	*-0.000380*	0.000110	*-0.000390*
Ni	0.507	0.509	0.515	0.783	0.398	0.599	0.854	1.43
Pb	0.0265	0.0286	0.0657	0.0241	0.00271	0.00934	0.0469	0.0470
Pd	0.000898	*-0.000213*	*-0.0000725*	*0.000468*	*-0.000483*	*-0.000663*	*-0.00139*	*-0.000863*
Pt	*0.0000250*	0.000335	*-0.000055*	*-0.000055*	0.000225	*-0.000155*	*0.000115*	0.000145
Rb	0.453	0.452	0.342	0.296	0.442	0.440	0.430	0.286
Sb]	0.0823	0.0793	0.0753	0.0766	0.0800	0.0840	0.0911	0.0827
Sc	0.0348	0.0519	0.0806	0.0415	0.0482	0.0496	0.0571	0.0140
Se	1.74	1.69	1.62	1.73	1.62	1.72	1.73	1.83
Sn	0.0116	0.0208	0.0157	0.0194	0.0181	0.0250	0.0193	0.0232
Sr	176	174	162	170	157	177	176	175
Te	*-0.000323*	*0.00249*	0.0114	*0.000568*	*0.00391*	*-0.0102*	*-0.00602*	*-0.00544*
Ti	0.119	0.132	0.249	0.152	0.0735	0.0743	0.0759	0.0755
Tl	0.0219	0.0213	0.0174	0.0167	0.0221	0.0176	0.0213	0.0168
U	0.312	0.321	0.293	0.310	0.290	0.314	0.316	0.323
V	0.419	0.389	0.520	0.519	0.496	0.425	0.534	0.587
W	0.0160	0.0136	0.0167	0.0191	0.0143	0.0170	0.0200	0.0367
Y	0.0127	0.00613	0.0323	0.0101	0.00705	0.00603	0.0162	0.00779
Zn	0.281	0.676	1.21	0.386	*-0.368*	*-0.273*	0.727	1.46
Zr	0.0110	0.0128	0.0181	0.0166	0.00626	0.0119	0.00808	0.00763

<DL

Appendix F2 The Day 1 cone water metal concentration in µg/L

Site	RF-8	RF-9	LR-1	LR-2	LR-3	LE-1	LE-2
Ag	*-0.00148*	0.0434	*0.000528*	*-0.00160*	*-0.00120*	*-0.00239*	*-0.00180*
Al	12.1	2.13	31.5	27.5	25.4	50.3	35.8
As	0.968	0.863	0.653	0.643	0.697	0.791	0.737
B	3.61	3.00	16.4	17.6	17.1	17.9	19.9
Ba	55.7	54.4	33.2	32.2	33.5	26.6	26.1
Be	0.00296	0.00661	0.00639	0.0119	0.00834	0.00689	0.00525
Bi	*-0.0000850*	*-0.000395*	*-0.0000650*	0.000185	0.000195	*-0.0000250*	*-0.000115*
Ca	39.4	37.2	27.7	26.5	28.3	33.9	31.1
Cd	0.00414	0.00851	0.0989	0.0125	0.0478	0.0198	0.0139
Ce	0.0390	0.0119	0.0179	0.0151	0.0142	0.00235	0.00851
Co	0.0243	0.0206	0.272	0.153	0.180	0.0103	0.00923
Cr	0.0767	*0.0581*	*0.0415*	*0.0497*	*0.0461*	*0.0447*	*0.0356*
Cs	0.00344	0.000593	0.00787	0.0102	0.0113	0.00339	0.00288
Cu	2.52	3.37	3.74	3.36	0.929	1.56	0.866
Fe]	20.5	0.847	14.2	11.3	12.4	0.593	5.45
Ga	0.0190	0.0149	0.0349	0.0312	0.0325	0.0286	0.0266
Ge	0.0247	0.0271	0.00811	0.00499	0.00610	0.00994	0.00967
In	*-0.0000950*	-0.00000500	*-0.000125*	*-0.0000650*	-0.0000250	*-0.000115*	*-0.000135*
K	0.492	0.479	1.54	1.57	1.56	1.75	1.70
La	0.0300	0.0128	0.0171	0.0165	0.0158	0.00145	0.00504
Li	2.60	2.51	1.92	1.90	1.94	2.30	2.16
Mg	9.51	9.37	8.08	7.93	8.22	9.18	8.65
Mn	1.24	0.0779	993	829	930	0.120	0.201
Mo	0.626	0.640	1.72	1.67	1.72	2.06	1.95
Na	1.61	1.60	14.1	14.1	14.5	15.2	14.5
Nb	0.000120	*-0.000230*	*-0.000110*	*-0.000360*	*-0.000330*	*-0.000430*	*-0.0000400*
Ni	0.487	1.39	2.51	0.470	0.851	0.668	0.391
Pb	0.193	0.207	0.381	0.0678	0.0783	0.0519	0.0464
Pd	*-0.000783*	*-0.000203*	*-0.00113*	*-0.000273*	*-0.000223*	*0.000128*	*-0.000873*
Pt	0.000215	*-0.000255*	*-0.000175*	*-0.000255*	*-0.000545*	*-0.000195*	*-0.000345*
Rb	0.319	0.298	1.50	1.47	1.46	1.08	1.04
Sb]	0.0813	0.0806	0.178	0.172	0.178	0.184	0.177
Sc	0.0663	0.0544	*0.00101*	0.0240	0.0236	0.0214	*0.00960*
Se	1.74	1.78	0.185	0.178	0.186	0.192	0.168
Sn	0.0112	0.0153	0.0486	0.0252	0.0349	0.0293	0.0266
Sr	175	171	177	168	176	197	183
Te	*-0.00491*	*-0.00654*	*0.000198*	*-0.00555*	*0.000338*	*-0.00995*	*-0.00806*
Ti	0.143	0.110	0.0799	0.145	0.114	0.0815	0.214
Tl	0.0152	0.0162	0.0205	0.0209	0.0209	0.0212	0.0209
U	0.316	0.318	0.103	0.0959	0.0985	0.151	0.155
V	0.571	0.573	0.139	0.147	0.148	0.586	0.446
W	0.0146	0.0145	0.0217	0.00926	0.0101	0.0376	0.0366
Y	0.0299	0.00807	0.00627	0.00658	0.00574	0.0101	0.00882
Zn	0.021	-0.151	3.10	1.61	1.32	4.20	1.52
Zr	0.0110	0.0105	0.0220	0.00208	0.00244	0.00826	0.00228

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Appendix F3 The Day 1 cone water metal concentration in µg/L

Site	LE-3	B1-R1	B1-R2	B1-R3	FL-1	FL-1F	FL-2
Ag	*-0.00177*	0.00336	0.00356	0.00649	*-0.000823*	0.00196	*-0.00175*
Al	55.4	60.1	167	162	4.02	1.77	2.75
As	0.853	0.517	0.528	0.614	1.25	1.29	1.34
B	19.4	11.5	9.27	11.3	10.1	10.9	9.51
Ba	27.4	123	122	125	74.4	74.1	72.8
Be	0.00854	0.0144	0.0205	0.0271	0.00810	0.00193	0.00583
Bi	0.0000050	0.000805	0.00164	0.00407	*-0.000285*	0.000235	*-0.000385*
Ca	34.1	38.5	38.0	39.8	40.2	42.6	41.0
Cd	0.0156	0.147	0.0174	0.0196	0.00921	0.00443	0.00349
Ce	0.0159	0.110	0.317	0.260	0.00921	0.00329	0.00552
Co	0.0195	0.102	0.108	0.0949	0.0389	0.0203	0.0228
Cr	*0.0481*	0.0943	0.148	0.158	*0.0465*	*0.0569*	*0.0494*
Cs	0.00434	0.0125	0.0309	0.0282	0.00221	0.00127	0.00174
Cu	3.92	1.91	1.37	3.67	1.50	0.631	1.04
Fe]	12.6	59.0	173	162	13.1	5.26	3.42
Ga	0.0294	0.0233	0.0501	0.0547	0.0126	0.0134	0.0132
Ge	0.00676	0.0119	0.00812	0.0719	0.0276	0.0238	0.0293
In	*-0.000135*	-0.00000500	0.000285	0.00731	*-0.000245*	*-0.0000650*	*-0.0000750*
K	1.79	0.815	0.799	0.837	0.682	0.720	0.691
La	0.00906	0.0761	0.222	0.202	0.00889	0.00202	0.00741
Li	2.35	1.37	1.41	1.53	4.64	4.93	4.84
Mg	9.26	6.99	6.83	7.06	12.2	13.0	12.6
Mn	0.505	1.49	3.90	2.00	0.744	0.208	0.358
Mo	2.11	1.06	0.868	0.918	0.687	0.703	0.681
Na	15.3	5.15	4.82	4.98	4.51	4.80	4.68
Nb	*0.0000500*	0.00171	0.00487	0.00759	*-0.000420*	*-0.000390*	*-0.000280*
Ni	0.539	4.22	0.685	0.709	1.42	0.537	0.451
Pb	0.272	0.247	0.200	0.311	0.0285	0.00485	0.00350
Pd	*-0.000923*	*-0.00173*	*-0.00226*	*-0.000673*	*-0.00183*	*-0.000613*	*-0.00102*
Pt	*-0.000175*	*0.00000500*	*-0.000295*	0.00319	*-0.000185*	0.000255	0.000225
Rb	1.11	0.326	0.435	0.456	0.527	0.532	0.492
Sb]	0.180	0.101	0.0892	0.0965	0.0866	0.0880	0.0858
Sc	0.0211	0.0494	0.0622	0.0929	0.0133	0.0916	0.0430
Se	0.187	0.179	0.158	0.250	1.11	1.14	1.19
Sn	0.0276	0.0292	0.0230	0.0118	0.0162	0.0523	0.00878
Sr	200	271	263	271	269	271	265
Te	*-0.0180*	*0.000888*	*-0.0151*	*-0.0160*	*-0.0102*	*-0.0172*	*-0.00452*
Ti	0.400	0.567	1.71	1.78	0.167	0.120	0.155
Tl	0.0182	0.00752	0.00821	0.00992	0.0181	0.0170	0.0161
U	0.158	0.448	0.430	0.450	0.360	0.369	0.362
V	0.534	0.598	0.766	0.787	0.605	0.631	0.598
W	0.0415	0.0128	0.00450	0.00560	0.0121	0.0138	0.0132
Y	0.0147	0.145	0.300	0.274	0.0123	0.00863	0.00950
Zn	4.48	3.67	0.898	1.09	0.339	-0.047	-0.150
Zr	0.0131	0.0912	0.102	0.126	0.00571	0.0213	0.0112

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Appendix F4 The Day 1 cone water metal concentration in µg/L

Site	FL-3	B2-R1	B2-R2	B2-R3	B3-R1
Ag	*-0.000133*	0.0473	*-0.000923*	*0.000428*	*-0.000603*
Al	2.27	18.8	16.8	7.67	9.39
As	1.00	1.67	1.35	1.66	0.313
B	10.2	17.7	17.8	17.8	12.2
Ba	72.4	99.8	96.7	106	84.9
Be	0.00673	0.00673	0.00822	0.0100	0.00592
Bi	0.000365	*-0.000115*	*-0.000375*	0.0000550	*-0.000225*
Ca	39.2	35.7	33.4	41.8	36.5
Cd	0.271	0.0101	0.00873	0.0367	0.0247
Ce	0.00522	0.0365	0.0304	0.0161	0.0144
Co	0.0206	0.0772	0.0451	0.165	0.0641
Cr	*0.0567*	0.0762	0.0673	0.0936	0.104
Cs	0.00182	0.00367	0.00388	0.00241	0.00304
Cu	1.21	3.85	1.23	2.21	3.09
Fe]	0.567	30.3	17.7	8.29	4.82
Ga	0.0132	0.0172	0.0166	0.0177	0.0101
Ge	0.0305	0.0429	0.0434	0.0459	0.00668
In	*-0.000115*	*-0.000155*	-0.0000250	*-0.000185*	*-0.000255*
K	0.713	0.913	0.873	0.901	0.856
La	0.00630	0.0285	0.0219	0.0187	0.0109
Li	4.87	5.41	5.38	5.34	4.02
Mg	12.7	16.4	16.3	16.9	14.8
Mn	0.511	3.80	1.63	1.23	0.301
Mo	0.654	1.86	1.85	1.89	1.67
Na	4.71	9.06	9.04	9.30	4.51
Nb	*-0.000470*	0.000180	*-0.000180*	*-0.000360*	*-0.000510*
Ni	0.458	8.38	0.615	2.82	1.91
Pb	0.0126	0.216	0.0228	0.101	0.214
Pd	*-0.00215*	*-0.00112*	*-0.00211*	*-0.00117*	*-0.00207*
Pt	*-0.000525*	*-0.000125*	*-0.000235*	*-0.000165*	*-0.000055*
Rb	0.504	0.794	0.753	0.767	0.389
Sb]	0.0879	0.125	0.0960	0.138	0.0799
Sc	0.0326	0.0525	0.0538	0.0680	0.0460
Se	1.13	1.01	0.965	1.06	0.867
Sn	0.0245	0.0210	0.0134	0.0399	0.0229
Sr	269	378	361	394	486
Te	*-0.00960*	*-0.0196*	*-0.0250*	*-0.0215*	*-0.0237*
Ti	0.172	0.374	0.324	0.155	0.157
Tl	0.0166	0.00881	0.00778	0.00980	0.00637
U	0.377	0.683	0.642	0.741	0.592
V	0.602	0.609	0.596	0.782	0.347
W	0.0116	0.00817	0.00857	0.0168	0.0123
Y	0.00734	0.0310	0.0244	0.0164	0.0138
Zn	-0.029	0.120	0.032	32.1	0.980
Zr	0.00841	0.0254	0.0112	0.0157	0.0123

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Appendix F5 The Day 1 cone water metal concentration in µg/L

Site	DS-1	DS-2	TC-1	TC-3
Ag	*-0.00201*	*-0.000113*	*-0.00257*	*-0.00103*
Al	35.1	49.8	2.32	2.35
As	0.471	0.541	0.847	0.840
B	8.80	6.88	12.3	14.3
Ba	68.3	67.0	77.7	78.7
Be	0.0122	0.0132	0.00710	0.00623
Bi	0.0000450	0.000575	0.000255	0.000195
Ca	41.3	39.7	35.5	33.9
Cd	0.00829	0.0198	0.00654	0.00383
Ce	0.0674	0.116	0.00372	0.00369
Co	0.0502	0.0923	0.0347	0.0375
Cr	0.120	0.165	*0.0216*	*0.0222*
Cs	0.00931	0.0126	0.00147	0.00138
Cu	2.71	1.89	1.56	1.36
Fe]	49.6	82.0	3.16	5.58
Ga	0.0234	0.0326	0.00890	0.0100
Ge	0.0107	0.0173	0.0202	0.0161
In	*-0.000155*	*-0.000165*	*-0.000135*	*-0.000165*
K	0.659	0.636	0.822	0.845
La	0.0400	0.0633	0.00521	0.00495
Li	4.67	4.55	5.15	5.22
Mg	12.6	12.3	13.5	13.5
Mn	2.41	4.25	0.251	0.465
Mo	0.787	0.774	0.710	0.708
Na	5.39	5.23	5.92	5.91
Nb	0.000330	0.00119	*-0.000590*	*-0.000710*
Ni	0.487	0.854	0.560	0.701
Pb	0.136	0.129	0.0550	0.0657
Pd	*-0.00326*	*-0.00279*	*-0.00205*	*-0.00302*
Pt	*-0.000375*	*-0.000465*	*-0.000175*	*-0.000035*
Rb	0.468	0.435	0.427	0.473
Sb]	0.0806	0.0804	0.0783	0.0852
Sc	0.0163	0.0293	0.0575	0.0174
Se	1.38	1.38	0.945	0.957
Sn	0.0242	0.0365	0.0281	0.0238
Sr	282	272	301	303
Te	*-0.0230*	*-0.0280*	*-0.0260*	*-0.0330*
Ti	0.622	0.767	0.0712	0.105
Tl	0.0161	0.0180	0.0134	0.0138
U	0.411	0.416	0.388	0.384
V	0.537	0.628	0.362	0.359
W	0.00944	0.0147	0.00734	0.0240
Y	0.0483	0.0792	0.00593	0.00584
Zn	0.415	0.973	0.400	7.21
Zr	0.00982	0.0121	0.00491	0.00492

Appendix G1 The Day 28 cone water total metal concentration in µg/L

Site	RF-1	RF-1F	RF-2	RF-2F	RF-3	RF-3F
Ag	0.00159	*0.000343*	0.0977	0.00579	0.00705	0.00120
Al	11.5	6.58	9.25	6.25	13.2	*0.402*
As	2.57	2.48	1.91	1.84	2.94	2.85
B	6.72	7.82	6.56	5.71	5.80	5.41
Ba	43.3	42.5	47.6	44.6	50.3	49.4
Be	0.00134	0.00166	0.00182	0.000965	0.00296	0.00135
Bi	*-0.00218*	*-0.00162*	*-0.00195*	*0.00400*	*-0.00226*	*-0.00101*
Ca	31.0	29.8	36.8	35.4	36.7	34.2
Cd	0.0111	*0.00307*	0.00872	0.00722	0.00844	*0.00408*
Ce	0.00947	0.00439	0.0128	0.00823	0.0168	0.00350
Co	0.0641	0.0526	0.0327	0.0342	0.0644	0.0606
Cr	0.0463	0.0350	0.0435	0.0343	0.0416	0.0356
Cs	0.00255	0.00188	0.00165	0.00158	0.00363	0.00259
Cu	1.47	0.68	1.00	1.38	0.89	1.27
Fe]	13.7	3.14	3.12	2.56	13.5	2.76
Ga	0.0128	0.0101	0.0117	0.0111	0.0156	0.00927
Ge	0.0646	0.0842	0.0564	0.0593	0.0767	0.0656
In	*0.0000475*	*0.0000375*	*-0.0000525*	*-0.0000425*	*-0.000113*	*-0.000133*
K	1.10	0.806	0.981	0.959	0.955	0.950
La	0.00981	0.00371	0.0148	0.00941	0.0276	0.00998
Li	2.73	2.59	2.49	2.24	2.52	2.32
Mg	10.4	10.0	9.61	9.14	9.69	9.27
Mn	10.1	0.784	1.12	0.0815	6.20	0.729
Mo	0.569	0.573	0.553	0.545	0.651	0.662
Na	2.34	2.27	2.15	2.06	2.16	2.05
Nb	*-0.000030*	*-0.000110*	*-0.000050*	0.000430	0.000150	0.000380
Ni	0.947	0.795	0.978	0.902	1.22	1.26
Pb	0.0819	*0.0337*	*0.0206*	0.0641	*0.0284*	*0.0336*
Pd	*0.00104*	*-0.00340*	*-0.00494*	*-0.00118*	*-0.00256*	*-0.000775*
Pt	*0.000383*	*-0.000198*	*-0.0000175*	*-0.000328*	*0.0000225*	*-0.000308*
Rb	0.782	0.769	0.761	0.743	0.696	0.678
Sb]	0.0849	0.0837	0.0735	0.0775	0.0847	0.0845
Sc	0.0237	0.0178	0.0338	0.0312	0.0382	0.0257
Se	0.547	0.612	0.458	0.453	0.665	0.670
Sn	0.0459	0.0475	0.0283	0.108	0.0309	0.0442
Sr	162	159	162	159	168	168
Te	0.0140	*0.00498*	*0.00717*	0.0275	0.0179	*0.0116*
Ti	0.125	0.0720	0.0714	0.0890	0.279	0.0763
Tl	0.0130	0.0146	0.0163	0.00991	0.0204	0.0125
U	0.212	0.221	0.183	0.185	0.219	0.225
V	0.395	0.357	0.399	0.393	0.650	0.619
W	0.00778	0.00702	0.00876	0.00885	0.0137	0.0140
Y	0.00709	0.00517	0.00893	0.00755	0.0214	0.0113
Zn	2.18	*0.095*	*0.700*	1.98	*0.636*	*0.497*
Zr	0.0478	0.0221	*0.00394*	0.0922	0.0218	0.0489

<DL

Appendix G2 The Day 28 cone water total metal concentration in µg/L

Site	RF-4	RF-4F	RF-5	RF-5F	RF-6	RF-6F
Ag	0.00153	*-0.000258*	0.00693	*0.000533*	0.00204	*0.00111*
Al	9.44	5.37	22.4	*0.620*	8.01	5.46
As	2.43	2.28	2.30	2.44	1.70	1.78
B	5.35	4.34	6.62	7.10	8.64	9.69
Ba	49.2	49.0	55.6	54.3	48.4	49.0
Be	0.00157	0.00174	0.00196	0.00555	0.00180	0.00499
Bi	*-0.00190*	*-0.00246*	*-0.00152*	*-0.00177*	*-0.00222*	*-0.00170*
Ca	36.7	34.9	39.1	41.9	35.0	38.6
Cd	0.00886	*0.00539*	0.00867	*0.00483*	0.0121	0.00665
Ce	0.00942	0.00522	0.0240	0.00318	0.0394	0.0132
Co	0.0355	0.0364	0.0485	0.0443	0.0374	0.0389
Cr	*0.0302*	0.0323	0.0592	0.0480	0.0390	0.0423
Cs	0.00180	0.00193	0.00382	0.00154	0.00311	0.00171
Cu	0.74	1.05	1.71	1.72	1.21	1.25
Fe]	4.30	3.02	23.7	1.44	10.4	2.51
Ga	0.0145	0.0102	0.0162	0.0114	0.0149	0.0148
Ge	0.0582	0.0581	0.0483	0.0572	0.0380	0.0530
In	*-0.0000725*	*-0.0000525*	*-0.0000925*	*0.0000175*	*0.0000675*	*0.0000175*
K	0.953	0.895	1.02	1.12	1.00	1.05
La	0.0175	0.00826	0.0218	0.00439	0.0394	0.0130
Li	2.43	2.26	2.70	2.95	2.39	2.64
Mg	9.37	8.87	10.2	11.0	9.55	9.92
Mn	1.40	0.175	4.30	0.178	0.545	0.122
Mo	0.646	0.651	0.668	0.690	0.650	0.671
Na	2.15	1.98	2.29	2.50	2.14	2.23
Nb	0.000120	0.000180	0.000300	0.000340	0.000230	0.000140
Ni	1.02	1.06	1.31	1.49	0.988	1.17
Pb	0.0480	0.0549	0.171	*0.0228*	0.0903	*0.0325*
Pd	*-0.00413*	*-0.00191*	*-0.00473*	*0.000365*	*-0.00381*	*0.000975*
Pt	*-0.000138*	*-0.000268*	*-0.000348*	*-0.000318*	*-0.0000175*	*-0.000708*
Rb	0.695	0.667	0.696	0.675	0.666	0.670
Sb]	0.0761	0.0787	0.0865	0.0823	0.0828	0.0768
Sc	0.0307	0.0388	0.0259	0.0287	0.0368	0.0360
Se	0.562	0.553	0.858	0.889	0.828	0.809
Sn	0.0231	0.0367	0.0353	0.0405	0.0748	0.0475
Sr	164	164	178	178	160	162
Te	*0.0113*	*-0.00181*	0.0204	*0.00968*	*0.00735*	*0.0104*
Ti	0.0853	0.105	0.308	0.141	0.155	0.251
Tl	0.0152	0.0112	0.0191	0.0131	0.0183	0.0131
U	0.205	0.214	0.237	0.233	0.216	0.220
V	0.505	0.374	0.644	0.674	0.638	0.687
W	0.0135	0.0144	0.0127	0.0125	0.0137	0.0140
Y	0.0105	0.00656	0.0286	0.0130	0.0174	0.0128
Zn	*0.535*	*0.369*	*1.07*	*0.984*	*0.671*	*0.779*
Zr	*0.00669*	0.0313	0.0128	0.0419	*0.00522*	0.0180

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Appendix G3 The Day 28 cone water total metal concentration in µg/L

Site	RF-7	RF-7F	RF-8	RF-8F	RF-9	RF-9F
Ag	0.00347	0.00209	0.00146	*-0.00122*	*0.000523*	*-0.000408*
Al	14.2	1.06	7.78	6.93	10.1	5.84
As	1.56	1.65	2.66	2.79	2.09	2.10
B	6.95	8.49	5.28	7.37	4.78	7.77
Ba	51.7	51.7	53.1	54.8	44.1	42.4
Be	0.00248	0.00545	0.00183	0.00574	0.00198	0.00594
Bi	*-0.00205*	*-0.00190*	*-0.00228*	*-0.00256*	*-0.00135*	*-0.00190*
Ca	36.1	38.1	40.8	42.7	25.2	26.1
Cd	0.0180	0.0105	0.0112	0.00763	0.00942	0.0192
Ce	0.0236	0.00818	0.00304	0.00167	0.00853	0.00254
Co	0.0586	0.0570	0.0374	0.0407	0.0523	0.0486
Cr	0.0440	0.0442	0.0340	*0.0231*	0.0404	0.0348
Cs	0.00246	0.00208	0.00179	0.00211	0.00240	0.00168
Cu	1.12	1.46	0.98	1.42	1.31	2.84
Fe	9.66	2.08	3.92	*0.907*	13.6	*0.917*
Ga	0.0141	0.0112	0.0135	0.0143	0.0157	0.0131
Ge	0.0460	0.0441	0.0439	0.0584	0.0603	0.0644
In	*0.0000475*	*-0.0000725*	*0.0000475*	0.000118	*-0.000113*	*0.0000775*
K	1.05	1.08	1.11	1.10	1.03	0.762
La	0.0292	0.0125	0.00663	0.00341	0.00941	0.00218
Li	2.52	2.85	2.54	2.90	2.41	2.68
Mg	9.77	10.6	10.1	10.6	9.42	9.74
Mn	2.23	0.686	0.899	0.113	3.67	0.407
Mo	0.707	0.729	0.627	0.642	0.678	0.694
Na	2.77	3.03	2.25	2.42	2.53	2.67
Nb	*0.000070*	0.000420	*0.000050*	0.000170	*-0.000020*	0.000280
Ni	1.04	1.18	1.21	1.16	1.27	1.47
Pb	0.0611	0.0419	*0.0166*	0.0405	0.111	0.107
Pd	*-0.00310*	*0.00138*	*-0.00506*	0.00349	*-0.000155*	0.00399
Pt	*-0.0000875*	*-0.000538*	*-0.0000675*	*-0.0000275*	*0.0000025*	*-0.000438*
Rb	0.727	0.755	0.785	0.798	0.689	0.658
Sb]	0.0847	0.0772	0.0924	0.0901	0.102	0.0840
Sc	0.0354	0.0385	0.0356	0.0596	0.0247	0.0484
Se	0.835	0.833	0.541	0.554	0.476	0.525
Sn	0.0273	0.0324	0.0317	0.0327	0.0269	0.0628
Sr	171	173	182	183	158	154
Te	*0.00555*	0.0161	*0.0121*	*-0.00293*	*-0.00221*	0.0125
Ti	*0.0322*	0.0744	0.112	0.108	0.234	0.0763
Tl	0.0259	0.0163	0.0180	0.0108	0.0201	0.0122
U	0.221	0.216	0.234	0.236	0.238	0.234
V	0.676	0.700	0.518	0.559	0.603	0.619
W	0.0179	0.0191	0.0115	0.0125	0.0163	0.0182
Y	0.0220	0.0139	0.0120	0.0134	0.00934	0.00808
Zn	2.00	2.74	*0.931*	*0.344*	1.73	6.52
Zr	*0.00957*	*0.00918*	*0.0108*	0.0209	0.0334	0.0460

Appendix G4 The Day 28 cone water total metal concentration in µg/L

Site	LR-1	LR-1F	LR-2	LR-2F	LR-3	LR-3F
Ag	0.00493	*0.0000325*	0.00536	*-0.000558*	0.00333	*-0.000308*
Al	59.3	15.0	60.1	13.2	106	28.6
As	0.812	0.778	0.801	0.759	0.712	0.592
B	13.5	16.2	14.8	17.1	15.1	16.0
Ba	31.6	30.7	33.4	32.4	26.6	25.8
Be	0.00344	0.00709	0.00416	0.00751	0.00827	0.00860
Bi	*0.000675*	*0.000395*	*0.000725*	*-0.00139*	*0.00126*	*-0.00249*
Ca	25.2	26.7	23.7	24.7	26.2	27.2
Cd	0.0266	0.0140	0.0373	0.0192	0.0429	0.0177
Ce	0.361	0.0188	0.441	0.0232	0.592	0.0269
Co	0.153	0.138	0.155	0.139	0.298	0.261
Cr	0.115	0.0760	0.0974	0.0725	0.141	0.0661
Cs	0.00948	0.00778	0.0128	0.0106	0.0117	0.00878
Cu	1.19	1.86	1.25	1.59	1.36	1.88
Fe]	137	3.83	160	9.38	202	5.16
Ga	0.0249	0.0126	0.0345	0.0190	0.0631	0.0342
Ge	0.0133	0.0165	0.0179	0.0153	0.0168	0.0142
In	*0.0000775*	*-0.0000725*	*0.0000975*	*-0.0000325*	0.000248	*-0.0000125*
K	1.96	1.95	1.95	2.02	1.97	1.73
La	0.200	0.0159	0.304	0.0233	0.289	0.0168
Li	1.49	1.63	1.46	1.58	1.57	1.69
Mg	7.44	8.00	7.46	8.04	7.88	8.37
Mn	87.2	62.7	306	281	Depleted	607
Mo	1.50	1.55	1.52	1.57	1.75	1.85
Na	13.4	14.7	13.6	15.0	14.2	15.5
Nb	0.00284	*-0.000100*	0.00290	*0.000030*	0.00468	*0.000040*
Ni	0.911	0.954	1.00	1.27	0.855	0.867
Pb	0.369	0.0446	0.401	0.0558	0.530	0.0435
Pd	*-0.00247*	*0.000405*	*-0.00299*	*0.000555*	*-0.00113*	0.00222
Pt	*-0.000308*	*-0.000498*	*0.0000625*	*-0.0000775*	*-0.000518*	*-0.000668*
Rb	1.86	1.84	1.86	1.86	1.79	1.76
Sb]	0.128	0.133	0.130	0.124	0.159	0.164
Sc	0.00822	0.0162	0.00282	0.0158	0.0241	0.0151
Se	0.240	0.229	0.218	0.219	0.218	0.221
Sn	0.0360	0.0372	0.0313	0.0450	0.0361	0.0714
Sr	172	170	162	162	174	174
Te	*0.00547*	0.0232	*0.00699*	*0.0118*	0.0133	0.0153
Ti	1.24	*0.0273*	1.10	*0.0269*	1.51	0.0783
Tl	0.0596	0.0297	0.0564	0.0307	0.0553	0.0312
U	0.0372	0.0334	0.0334	0.0303	0.0838	0.0803
V	0.592	0.458	0.623	0.477	0.484	0.246
W	0.00289	0.00177	0.00193	0.00143	0.00190	0.00290
Y	0.0749	0.0108	0.0845	0.0120	0.122	0.0147
Zn	1.34	*0.450*	2.29	*1.24*	3.35	1.48
Zr	*0.0000925*	*0.00800*	*-0.00206*	*0.0111*	*0.00500*	0.0507

<DL

Appendix G5 The Day 28 cone water total metal concentration in µg/L

Site	LE-1	LE-1F	LE-2	LE-2F	LE-3	LE-3f
Ag	*0.0000225*	*-0.0000675*	0.00217	*0.000833*	0.0323	*-0.000548*
Al	66.6	24.3	37.5	15.3	28.2	27.4
As	1.09	1.00	1.16	1.16	1.10	1.12
B	16.8	19.5	18.1	21.2	17.8	19.4
Ba	26.7	25.8	26.8	27.4	27.0	27.4
Be	0.00436	0.00703	0.00259	0.00811	0.00152	0.00721
Bi	*-0.00158*	*-0.00125*	*0.00371*	*-0.00230*	*-0.00155*	*-0.00214*
Ca	32.7	33.3	39.5	41.5	42.8	42.5
Cd	0.0136	0.00902	0.0240	0.0123	0.0204	0.0131
Ce	0.0795	0.00513	0.0608	0.00316	0.00499	0.00734
Co	0.0860	0.0532	0.0531	0.0383	0.0328	0.0339
Cr	0.0902	*0.0288*	0.0733	0.0328	0.0606	0.0373
Cs	0.00886	0.00421	0.00760	0.00513	0.00588	0.00492
Cu	2.23	3.16	2.27	3.12	1.93	2.16
Fe]	84.7	3.85	41.5	*0.497*	2.86	*0.150*
Ga	0.0339	0.0216	0.0265	0.0202	0.0239	0.0234
Ge	0.00711	0.00672	0.00603	0.00550	0.00487	0.00675
In	*0.0000675*	*0.0000175*	*-0.0000125*	*0.0000875*	*0.000108*	*-0.0000725*
K	2.28	2.03	2.15	2.33	2.39	2.29
La	0.0362	0.00339	0.0428	0.00279	0.00669	0.00680
Li	2.27	2.39	2.14	2.40	2.36	2.49
Mg	9.52	9.35	8.93	9.67	9.81	9.93
Mn	6.66	0.689	1.37	0.0594	0.416	0.0741
Mo	2.06	2.07	2.00	2.17	2.11	2.19
Na	15.4	15.6	14.4	15.9	15.8	16.3
Nb	0.00185	0.000140	0.00114	0.000200	0.000260	0.000130
Ni	1.36	2.39	0.941	0.970	0.916	0.931
Pb	0.116	0.109	0.0774	0.0828	*0.0297*	0.0628
Pd	*0.000425*	*-0.000425*	*-0.00235*	*0.000045*	*0.000095*	*-0.000435*
Pt	*0.0000925*	*-0.000458*	*-0.000538*	*0.000183*	*-0.000338*	*-0.000408*
Rb	1.54	1.46	1.50	1.54	1.59	1.59
Sb]	0.154	0.149	0.152	0.158	0.158	0.154
Sc	0.0140	0.00702	0.00450	0.00975	0.0166	0.00942
Se	0.123	0.117	0.181	0.186	0.109	0.115
Sn	0.0365	0.0481	0.0500	0.0716	0.0423	0.0612
Sr	205	202	202	204	215	216
Te	*-0.0000650*	*0.00703*	*0.00484*	0.0147	*-0.00622*	*0.00125*
Ti	1.19	*0.0110*	0.726	0.125	*0.0336*	0.0749
Tl	0.0416	0.0207	0.0423	0.0225	0.0368	0.0232
U	0.117	0.112	0.113	0.114	0.0842	0.114
V	0.466	0.370	0.646	0.572	0.598	0.591
W	0.0322	0.0269	0.0330	0.0368	0.0320	0.0357
Y	0.0347	0.0103	0.0322	0.00972	0.00477	0.0107
Zn	2.42	5.65	2.79	*1.16*	2.54	1.68
Zr	0.0168	0.0332	*0.0000625*	0.0125	0.0392	0.0124

<DL

Appendix G6 The Day 28 cone water total metal concentration in µg/L

Site	B1-R1	B1-R1F	B1-R2	B1-R2F	B1-R3	B1-R3F
Ag	*0.000673*	0.00233	0.00247	0.00493	0.00187	0.00295
Al	20.4	9.33	35.3	14.5	39.5	13.0
As	1.60	1.59	2.18	2.22	2.75	2.75
B	9.20	12.0	9.49	11.9	9.48	10.1
Ba	100	103	104	102	107	104
Be	0.00257	0.00592	0.00422	0.00753	0.00400	0.00721
Bi	*0.00000500*	*-0.00215*	0.0255	*-0.00216*	*-0.000705*	*-0.000705*
Ca	35.8	35.3	36.8	36.4	36.5	36.2
Cd	0.0198	0.00762	0.0273	0.0107	0.0288	0.0130
Ce	0.0188	0.00885	0.0306	0.00935	0.0308	0.00970
Co	0.0888	0.0804	0.128	0.117	0.130	0.119
Cr	0.0403	*0.0291*	0.0610	*0.0176*	0.0385	0.0316
Cs	0.00394	0.00226	0.00526	0.00212	0.00490	0.00198
Cu	1.27	3.21	1.88	1.73	1.62	2.46
Fe]	15.0	1.09	29.6	3.00	26.4	2.77
Ga	0.0108	0.00935	0.0154	0.00937	0.0155	0.00986
Ge	0.0113	0.00969	0.0138	0.0155	0.0126	0.0153
In	*-0.0000425*	*-0.0000825*	*0.0000175*	*-0.000193*	*0.0000275*	*-0.0000825*
K	1.41	1.16	1.44	1.15	1.47	1.15
La	0.0299	0.0105	0.0242	0.00832	0.0406	0.0113
Li	1.32	1.31	1.33	1.31	1.37	1.36
Mg	7.34	7.45	7.65	7.67	7.78	8.15
Mn	3.59	0.513	7.47	0.608	11.4	0.812
Mo	1.73	1.79	1.89	1.93	1.93	2.02
Na	6.19	6.43	6.28	6.39	6.31	6.63
Nb	0.000190	0.000690	0.000610	0.000680	0.000500	0.000590
Ni	0.923	2.46	1.21	0.957	0.917	1.13
Pb	*0.0204*	0.101	0.314	*0.0350*	0.0483	0.0997
Pd	0.00232	*-0.00122*	0.00415	*-0.00102*	0.00238	*-0.00104*
Pt	*-0.000388*	*-0.000408*	*-0.0000475*	*-0.000408*	*-0.000208*	*-0.000108*
Rb	0.652	0.652	0.751	0.721	0.763	0.747
Sb]	0.101	0.110	0.139	0.137	0.137	0.142
Sc	0.0498	0.0332	0.0467	0.0372	0.0498	0.0409
Se	0.153	0.179	0.165	0.243	0.189	0.208
Sn	0.0514	0.102	0.0394	0.0701	0.0198	0.0383
Sr	246	244	260	262	271	273
Te	*-0.00264*	*0.0116*	*-0.0000250*	0.0138	*-0.00226*	*-0.000645*
Ti	0.344	*0.0257*	0.247	0.0937	0.418	0.142
Tl	0.0130	0.00621	0.0117	0.00855	0.0103	0.00644
U	0.311	0.326	0.364	0.369	0.391	0.396
V	0.580	0.583	0.651	0.629	0.645	0.658
W	0.00438	0.00438	0.00621	0.00760	0.00725	0.00689
Y	0.0480	0.0402	0.0619	0.0421	0.0586	0.0379
Zn	*0.067*	2.03	*1.28*	*0.342*	*0.868*	*0.234*
Zr	0.0593	0.174	0.0864	0.161	0.0518	0.0949

<DL

Appendix G7 The Day 28 cone water total metal concentration in µg/L

Site	FL-1	FL-1F	FL-2	FL-2F	FL-3	FL-3F
Ag	0.00241	*0.00114*	0.00177	0.00362	0.00316	0.00172
Al	13.5	6.27	7.32	1.95	7.39	5.92
As	1.40	1.34	1.51	1.79	1.01	1.05
B	15.7	14.1	14.0	20.5	15.3	16.3
Ba	77.9	76.1	76.5	75.3	65.7	62.3
Be	0.00420	0.00189	0.00294	0.0129	0.00205	0.00726
Bi	*-0.00114*	*-0.00271*	*-0.00239*	*-0.00216*	*-0.00234*	*-0.00134*
Ca	46.6	47.6	48.1	48.1	46.1	44.6
Cd	0.00825	*0.00428*	0.0109	*0.00233*	0.00813	0.00900
Ce	0.0173	0.00450	0.0156	0.00397	0.00800	0.00242
Co	0.0879	0.0756	0.0553	0.0528	0.0672	0.0725
Cr	*0.0278*	*0.0209*	0.0377	*-0.005715*	0.112	*0.0274*
Cs	0.00545	0.00475	0.00341	0.00326	0.00312	0.00298
Cu	1.08	1.00	1.32	1.58	1.22	2.31
Fe]	26.2	6.03	5.52	*0.703*	6.69	1.52
Ga	0.0151	0.0139	0.0159	0.00195	0.0128	0.0133
Ge	*0.00115*	0.00240	0.00322	0.00238	0.00287	*0.00115*
In	*0.0000375*	*-0.000133*	*0.0000375*	*-0.0000625*	*-0.0000425*	*-0.0000925*
K	1.28	1.24	1.24	1.28	1.20	1.18
La	0.0184	0.00587	0.0188	0.00688	0.0108	0.00461
Li	5.47	5.54	5.59	5.95	5.48	5.54
Mg	13.8	14.3	14.4	14.3	13.8	13.2
Mn	17.4	1.04	7.31	0.534	1.97	0.371
Mo	0.731	0.723	0.764	0.835	0.694	0.629
Na	5.51	5.65	5.78	5.90	5.58	5.43
Nb	*0.000070*	0.000240	0.000150	0.0168	0.000150	0.000540
Ni	1.33	1.30	0.881	0.871	1.04	1.57
Pb	0.0364	*0.00520*	*0.0218*	*0.0201*	*0.0272*	0.153
Pd	*-0.00256*	*-0.00246*	*-0.00179*	0.214	*-0.00244*	*-0.000705*
Pt	*0.0000725*	*0.0000325*	*-0.000438*	0.00438	*-0.000128*	*-0.000358*
Rb	0.965	0.932	0.877	0.879	0.793	0.755
Sb]	0.0906	0.0856	0.0896	0.0923	0.0722	0.0729
Sc	0.00350	0.00205	0.00774	0.0128	0.00397	0.00326
Se	0.447	0.436	0.419	0.582	0.653	0.667
Sn	0.0281	0.0489	0.0158	0.141	0.0241	0.113
Sr	279	277	283	285	269	260
Te	*0.000495*	*-0.000765*	*0.00272*	*0.00424*	*0.00356*	*0.00421*
Ti	0.0907	*0.0361*	*0.0368*	0.178	0.153	0.140
Tl	0.0172	0.0171	0.0152	0.00912	0.0164	0.0113
U	0.244	0.242	0.269	0.0384	0.253	0.235
V	0.356	0.342	0.475	0.466	0.450	0.418
W	0.00863	0.00927	0.0106	0.0778	0.00986	0.00884
Y	0.0225	0.0137	0.0132	0.0112	0.0141	0.0124
Zn	*0.967*	*0.077*	*0.092*	*-0.699*	*0.475*	2.64
Zr	0.0122	0.0118	0.0171	0.462	*0.00741*	0.423

<DL

Appendix G8 The Day 28 cone water total metal concentration in µg/L

Site	B2-R1	B2-R1F	B2-R2	B2-R2F	B2-R3	B2-R3F
Ag	0.00242	*0.000673*	0.00325	0.00284	0.00447	0.00266
Al	17.3	10.9	14.6	10.7	23.0	9.60
As	2.91	3.00	2.97	3.04	2.84	2.86
B	16.4	18.2	13.7	16.8	14.8	15.9
Ba	93.6	92.5	91.8	90.1	110	111
Be	0.00306	0.00951	0.00242	0.00978	0.00331	0.00946
Bi	*-0.000395*	*-0.00248*	*-0.00218*	*-0.00285*	*-0.00240*	*-0.00272*
Ca	33.6	34.3	31.8	34.1	39.1	40.4
Cd	0.0118	0.00932	0.0147	0.0141	0.0187	0.00901
Ce	0.0293	0.00824	0.0205	0.00415	0.0454	0.00923
Co	0.0976	0.0869	0.0891	0.0924	0.139	0.125
Cr	0.0376	*0.0209*	*0.0259*	*0.0180*	0.0515	0.0334
Cs	0.00405	0.00342	0.00424	0.00395	0.00568	0.00401
Cu	1.30	3.48	1.23	2.80	1.39	1.63
Fe]	14.1	1.52	7.49	2.06	18.3	2.20
Ga	0.0229	0.0202	0.0185	0.0178	0.0230	0.0200
Ge	0.0525	0.0593	0.0595	0.0588	0.0674	0.0610
In	*-0.0000525*	*-0.0000425*	*0.0000075*	*-0.0000825*	*-0.000133*	*0.0000275*
K	1.38	1.39	1.37	1.40	1.36	1.45
La	0.0342	0.0142	0.0224	0.00446	0.0531	0.0122
Li	4.97	5.28	4.90	5.12	4.97	5.41
Mg	16.4	16.6	15.7	15.9	16.2	17.4
Mn	5.70	0.341	5.93	0.356	20.9	1.05
Mo	1.96	1.98	1.90	1.90	1.96	2.05
Na	10.0	10.3	9.31	9.72	9.28	10.2
Nb	0.000400	*0.000090*	0.000280	0.000160	0.000550	0.000450
Ni	1.68	2.29	1.43	1.70	1.49	1.56
Pb	0.0482	0.0907	*0.0320*	0.102	0.0543	*0.0328*
Pd	*-0.000495*	*-0.00243*	*-0.000195*	*-0.00169*	*0.00107*	*-0.00170*
Pt	*-0.000448*	*-0.000268*	*-0.000318*	*-0.0000475*	*-0.000198*	*-0.000338*
Rb	1.08	1.07	1.16	1.15	1.22	1.25
Sb]	0.119	0.124	0.117	0.114	0.144	0.143
Sc	0.0246	0.0255	0.0252	0.0235	0.0421	0.0397
Se	0.747	0.747	0.419	0.419	0.817	0.798
Sn	0.0256	0.0430	0.0205	0.0338	0.0266	0.0366
Sr	340	334	341	337	376	371
Te	*0.00143*	*0.00512*	*0.00395*	*0.0118*	*-0.00397*	*0.00984*
Ti	0.185	0.110	0.160	0.241	0.219	0.142
Tl	0.0120	0.00706	0.00959	0.00795	0.0101	0.00888
U	0.513	0.505	0.489	0.477	0.611	0.620
V	0.968	0.981	0.780	0.796	0.962	0.960
W	0.0197	0.0184	0.00702	0.00604	0.00923	0.00570
Y	0.0314	0.0281	0.0227	0.0222	0.0481	0.0352
Zn	*-0.160*	3.23	*0.831*	1.72	*-0.061*	*0.159*
Zr	0.0206	0.0594	0.0175	0.0347	0.0264	0.0509

<DL

Appendix G9 The Day 28 cone water total metal concentration in µg/L

Site	B3-R1	B3-R1F	DS-1	DS-1F	DS-2	DS-2F
Ag	*-0.000238*	*-0.000618*	0.00169	0.00126	0.00296	*-0.000338*
Al	16.5	15.8	12.6	3.68	13.2	8.17
As	1.86	1.80	1.75	1.85	1.62	1.67
B	8.82	10.9	7.44	7.75	5.03	7.25
Ba	62.1	61.2	62.9	62.7	59.6	59.6
Be	0.00146	0.00590	0.00304	0.00562	0.00220	0.00465
Bi	*-0.00164*	*-0.00259*	*-0.00174*	*-0.00233*	*-0.00160*	*-0.00253*
Ca	23.7	24.1	36.1	39.0	34.9	36.3
Cd	0.00976	0.00988	0.0297	0.0212	0.0213	0.0174
Ce	0.00132	0.00172	0.0209	0.00383	0.0289	0.00555
Co	0.0772	0.0788	0.0825	0.0841	0.102	0.0970
Cr	*0.0235*	0.0639	0.0551	0.0566	0.0630	0.0482
Cs	0.00313	0.00332	0.00454	0.00266	0.00377	0.00199
Cu	0.75	2.60	1.37	1.98	1.30	1.98
Fe]	8.83	12.4	15.6	2.63	13.7	1.53
Ga	0.00933	0.00913	0.0133	0.0120	0.0151	0.0125
Ge	0.0278	0.0284	0.0281	0.0202	0.0156	0.0258
In	*-0.0000925*	*-0.000183*	*-0.0000325*	*-0.0000725*	0.000148	*-0.0000925*
K	1.33	1.10	1.07	1.14	0.979	1.04
La	0.00287	0.00180	0.0226	0.00371	0.0314	0.00610
Li	3.49	3.68	4.01	4.33	3.80	4.03
Mg	13.7	14.1	11.5	12.4	10.8	11.6
Mn	4.85	0.633	1.97	0.157	1.25	0.314
Mo	2.27	2.31	1.05	1.07	0.920	0.959
Na	4.69	4.90	5.37	5.78	5.04	5.53
Nb	*0.000030*	0.000250	0.000650	0.000390	0.000410	0.000170
Ni	0.828	1.64	1.20	1.37	1.19	1.30
Pb	*0.0229*	0.120	0.0378	0.0528	*0.0319*	0.0913
Pd	*0.000145*	0.00293	*-0.00261*	*0.000815*	*-0.000745*	*-0.00134*
Pt	*-0.000168*	*-0.000218*	*-0.000608*	*-0.000508*	*-0.000438*	*-0.000358*
Rb	1.07	1.07	0.842	0.867	0.783	0.769
Sb]	0.141	0.137	0.0821	0.0837	0.0957	0.0846
Sc	0.0224	0.0290	0.0323	0.0395	0.0285	0.0471
Se	0.317	0.331	0.867	0.903	0.925	0.918
Sn	0.0235	0.0479	0.0380	0.0663	0.0346	0.0473
Sr	406	389	257	264	245	241
Te	*-0.00504*	*0.00881*	*-0.000115*	*0.00765*	*-0.00607*	*0.0106*
Ti	0.0557	0.0770	0.127	0.128	0.263	0.0594
Tl	0.0109	0.00809	0.0186	0.0134	0.0188	0.0131
U	0.455	0.445	0.311	0.304	0.292	0.286
V	0.293	0.304	0.640	0.674	0.616	0.624
W	0.00336	0.00321	0.0117	0.0107	0.0132	0.0114
Y	0.00932	0.0120	0.0287	0.0208	0.0274	0.0199
Zn	*0.429*	3.34	2.51	*1.08*	*1.05*	*0.561*
Zr	0.0226	0.0534	0.0135	0.0720	*0.00733*	0.0455

<DL

Appendix G10 The Day 28 cone water total metal concentration in µg/L

Site	TC-2	TC-2F	TC-3	TC-3F
Ag	0.00146	*-0.000268*	0.00871	*-0.000188*
Al	18.1	4.36	9.75	5.09
As	1.94	1.38	1.40	1.95
B	18.5	16.0	15.8	18.0
Ba	83.6	73.7	73.1	83.2
Be	0.00419	0.00514	0.00238	0.00849
Bi	*-0.000785*	*-0.00259*	*-0.00105*	*-0.00235*
Ca	42.0	41.4	41.8	44.3
Cd	0.00781	*0.00201*	*0.00456*	0.00610
Ce	0.0256	0.00251	0.0119	0.00288
Co	0.181	0.104	0.120	0.173
Cr	*0.0271*	*0.0186*	*0.0188*	*0.0113*
Cs	0.00332	0.00265	0.00327	0.00176
Cu	1.02	1.32	5.55	1.36
Fe]	26.5	2.64	16.3	4.36
Ga	0.0253	0.0113	0.0142	0.0231
Ge	0.00349	*0.00157*	0.00267	0.00431
In	*-0.0000725*	*-0.0000925*	*-0.0000025*	*-0.000103*
K	1.27	1.33	1.40	1.32
La	0.0259	0.00505	0.0150	0.00537
Li	5.13	5.58	5.48	5.51
Mg	13.2	14.0	14.4	13.7
Mn	4.75	0.198	3.47	0.230
Mo	1.40	0.824	0.819	1.44
Na	6.49	7.21	7.31	6.77
Nb	0.000670	*-0.000020*	0.000320	*0.000060*
Ni	2.39	1.70	1.67	2.72
Pb	0.0812	*0.0182*	*0.0211*	0.133
Pd	*-0.00206*	*-0.00181*	*-0.00228*	*-0.00179*
Pt	*-0.000288*	*-0.000258*	*-0.000318*	*-0.000578*
Rb	0.778	0.840	0.839	0.786
Sb]	0.213	0.103	0.0997	0.214
Sc	0.00413	0.00898	0.00662	0.00511
Se	0.895	0.409	0.410	0.916
Sn	0.0224	0.0315	0.0137	0.0304
Sr	282	301	305	285
Te	0.0135	*0.00941*	*0.00509*	0.0134
Ti	0.167	0.0740	0.194	0.0562
Tl	0.0129	0.0103	0.0183	0.00740
U	0.393	0.292	0.301	0.391
V	1.19	0.607	0.622	1.17
W	0.0337	0.0117	0.0127	0.0331
Y	0.0315	0.0179	0.0188	0.0233
Zn	*0.514*	*0.570*	*-0.141*	2.70
Zr	0.0213	0.0521	0.0176	0.0664

<DL

Appendix H The raw data for major ions and water quality of cone water on Day 28

Major Ions	DOC	DIC	SO42-	Cl-	Ca2+	Mg2+	Na+	K+	Hardness as CaCO3	Alkalinity
Units	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	CO3 mol/L
RF-1	5.3	22.2	23.5	2.1	28.8	9.8	2.3	0.8	101	1.85
RF-2	3.1	26.7	24.1	2.1	42.5	9.9	2.2	1.0	116	2.22
RF-3	3.2	26.5	25	2.11	42.3	9.94	2.31	1	115	2.21
RF-4	3.2	25.1	24.1	2.12	43	9.68	2.32	1	114	2.09
RF-5	3.3	27.7	26.5	2.18	45.4	10.6	2.45	1.07	121	2.31
RF-6	3.2	24.5	26.3	2.22	42.8	9.84	2.35	1.05	110	2.04
RF-7	3.5	24.9	27.6	3.43	42.4	10.5	3.01	1.05	110	2.07
RF-8	3.3	28.1	25.8	2.3	47.8	10.4	2.45	1.05	129	2.34
RF-9	4.8	19.5	25.9	3.06	25.4	9.8	2.83	0.76	89.8	1.62
LR-1	2.8	11.6	39	28	29.3	7.78	15.2	1.96	51.1	0.97
LR-2	3	11.7	37.1	27.8	27.7	7.49	15.3	2.03	50.4	0.97
LR-3	5.3	14	38.1	28.6	23.7	7.83	15.6	1.7	61	1.17
LE-1	5	19.4	37.2	29.7	31.8	9.29	16.4	2	84.9	1.62
LE-2	2.5	21.3	36.5	27.1	43.9	8.83	15.2	2.11	94.7	1.77
LE-3	2.6	21.6	37.5	28.6	36.9	9.1	15.8	2.09	101	1.80
B1-R1	22.3	27	6.45	2.2	32.1	6.74	6.01	1.19	125	2.25
B1-R2	6.8	27.1	6.35	2.31	18	6.86	6.1	1.04	126	2.26
B1-R3	6.9	27.7	6.46	2.18	31.5	7.29	6.19	1.03	129	2.31
FL-1	3.7	30.2	34.9	3.3	47.1	12.4	5.11	1.2	137	2.51
FL-2	3.8	31.3	36.7	3.22	47.9	11.8	5.27	1.18	140	2.61
FL-3	3.1	29.4	36.4	3.16	46.9	12.4	5.12	1.12	129	2.45
B2-R1	4.2	33.8	17.6	2.14	36.9	15.8	10.2	1.22	153	2.81
B2-R2	4.3	34	16.6	1.39	36.1	16	9.75	1.36	150	2.83
B2-R3	4.6	38.2	17.3	1.31	44.5	16.6	10.1	1.42	170	3.18
B3-R1	4.8	23.6	21	1.33	26.7	14.3	5	1.37	111	1.96
DS-1	3.8	27.2	36	3.33	43.5	12.5	5.97	1.18	120	2.26
DS-2	3.1	26.7	34.7	3.61	41	11.5	5.64	1.05	115	2.22
TC-1	4	29.9	32.3	3.51	41	12.6	6.39	1.2	133	2.49
TC-2	4	30.5	35	4.92	37.3	13.4	7.17	1.25	137	2.54
TC-3	28.1	28.4	41.4	3.68	45	12.6	6.41	1.26	127	2.36
Max	28.1	38.2	41.4	29.7	47.9	16.6	16.4	2.11	170	3.18
Min	2.50	11.6	6.35	1.31	18.0	6.74	2.23	0.760	50.4	0.966
Mean	5.39	25.7	27.8	7.77	37.6	10.8	7.21	1.29	115	2.14
SD	5.46	6.03	10.0	10.3	8.10	2.64	4.74	0.379	27.2	0.502
CV	101%	24%	36%	133%	22%	24%	66%	29%	24%	24%

Appendix I The raw data for water quality of cone water on Day 1 and Day 28

WQ parameters	pH	pH	pH	pH	O_2	O_2	COND.	COND.	NH_3	NH_3
units			mV	mV	(mg/L)	(mg/L)	(us)	(us)	(mM)	(mM)
sites	D1	D28	D1	D28	D1	D28	D1	D28	D1	D28
RF-1	8.47	8.95	-86.1	-114	8.83	9.28	301	263	0.0300	0.0150
RF-2	8.48	8.38	-86.4	-81.1	8.97	9.01	279	284	0	0.0150
RF-3	8.50	8.29	-87.6	-75.5	8.93	8.77	281	285	0	0.0150
RF-4	8.47	8.31	-85.9	-77.0	9.04	8.80	276	285	0.0150	0.0150
RF-5	8.50	8.35	-87.8	-79.4	8.96	8.93	306	296	0.0150	0.0150
RF-6	8.48	8.38	-86.5	-81.1	8.80	8.96	304	287	0.0150	0.0150
RF-7	8.51	8.35	-88.2	-79.3	8.83	8.83	291	294	0	0.0150
RF-8	8.53	8.49	-89.4	-87.1	8.68	9.00	304	313	0	0.0150
RF-9	8.52	9.04	-88.8	-119	8.89	10.61	294	249	0	0.0150
LR-1	8.27	8.18	-74.5	-69.3	8.88	9.16	314	303	0	0.0150
LR-2	8.25	8.08	-73.5	-64.0	8.94	9.00	300	284	0	0.0150
LR-3	8.25	8.62	-73.5	-95.1	8.87	9.79	311	293	0	0.0150
LE-1	8.36	8.58	-79.5	-92.7	8.86	9.35	342	334	0	0.0150
LE-2	8.35	8.30	-79.1	-76.6	8.81	8.80	322	361	0	0.0150
LE-3	8.40	8.48	-81.8	-87.1	8.86	8.93	344	373	0	0.0150
B1-R1	8.50	8.72	-87.4	-101	8.99	9.48	269	245	0	0.0150
B1-R2	8.57	9.03	-91.9	-119	8.84	9.80	260	254	0	0.0150
B1-R3	8.58	8.98	-92.3	-116	9.03	9.90	267	259	0	0.0150
FL-1	8.53	8.47	-89.3	-86.3	8.88	8.73	329	339	0	0.0150
FL-2	8.55	8.59	-90.3	-93.6	8.93	8.57	327	341	0	0
FL-3	8.52	8.43	-89.0	-84.1	8.82	8.68	322	335	0	0.0150
B2-R1	8.61	8.49	-94.0	-87.8	8.77	8.64	331	325	0	0.0000
B2-R2	8.62	8.48	-94.4	-86.9	9.07	8.68	313	321	0	0.0150
B2-R3	8.65	8.54	-96.3	-90.6	8.87	8.58	360	361	0	0
B3-R1	8.58	8.94	-92.0	-113	8.89	9.65	311	260	0	0
DS-1	8.54	8.36	-90.1	-80.2	8.84	8.54	326	321	0	0.0150
DS-2	8.55	8.41	-90.7	-83.0	8.79	8.72	325	312	0	0.0150
TC-1	8.50	8.55	-87.7	-90.9	8.83	8.70	313	332	0	0.0150
TC-2	8.51	8.54	-87.9	-90.5	8.78	8.75	324	335	0	0.0150
TC-3	8.50	8.62	-87.8	-95.0	8.91	8.71	314	346	0	0.0150
Max	8.65	9.04	-73.5	-64.0	9.07	10.6	360	373	0.0300	0.0150
Min	8.25	8.08	-96.3	-119	8.68	8.54	260	245	0	0
Mean	8.49	8.53	-87.0	-89.9	8.88	9.05	309	306	0.00250	0.0130
SD	0.101	0.243	5.75	14.1	0.0851	0.481	23.5	35.1	0.00680	0.00510
CV	1%	3%	-7%	-16%	1%	5%	8%	11%	272%	39%

Appendix J1 The body burden of 1-week bioaccumulation test in the cage (*<DL)*

Site	Rf-1	Rf-2	Rf-3	Rf-4	Rf-5	Rf-6	Rf-7	Rf-8
Ag	0.976	0.964	1.02	0.913	1.33	0.736	0.677	1.02
Al	777	2,418	1,179	599	818	1,170	490	1,026
As	9.14	11.1	10.0	9.85	10.4	11.3	10.4	10.1
B	177	165	116	186	97.1	123	142	265
Ba	191	162	189	129	175	136	173	174
Be	0.168	0.163	0.202	0.103	0.116	0.0710	0.144	0.0973
Bi	*-0.0158*	*-0.0292*	*-0.0160*	*0.0117*	*0.0063*	*-0.0123*	*0.0630*	*-0.0002*
Ca	2,354	2,421	2,513	2,703	2,420	1,932	2,147	2,713
Cd	10.0	6.44	7.44	6.99	8.39	5.34	7.75	8.01
Ce	*0.0355*	*0.0503*	*0.0344*	*-0.0321*	0.242	*0.0598*	*0.0679*	*0.0456*
Co	3.12	3.50	3.90	3.12	3.77	2.71	3.85	3.83
Cr	-0.113	1.44	0.465	0.921	5.38	0.266	1.82	0.878
Cs	0.182	0.196	0.165	0.156	0.183	0.123	0.177	0.186
Cu	771	816	740	736	778	632	807	746
Fe]	719	1,026	871	777	847	739	1,137	1,093
Ga	0.330	0.330	0.316	0.286	0.294	0.268	0.302	0.311
Ge	0.123	0.0971	0.111	0.0894	0.154	0.0708	0.100	0.108
In	*0.000228*	*0*	*0.00119*	*0.00162*	*0.00147*	*-0.00186*	*0.00087*	*-0.00163*
K	197	215	209	214	208	169	206	213
La	0.0698	0.114	0.0455	0.0192	0.0734	0.106	0.0889	0.0607
Li	3.02	4.08	3.49	2.95	3.72	3.09	2.95	3.46
Mg	59.3	54.7	61.4	50.1	60.7	44.1	61.9	53.0
Mn	108	111	95.5	70.6	104	77.8	89.7	85.4
Mo	5.91	5.82	6.00	5.17	5.76	4.37	5.48	5.44
Na	272	310	290	328	298	245	276	363
Nb	*-0.0133*	0.0120	0.0137	0.00457	-0.00148	0.00311	0.00337	-0.00300
Ni	9.03	8.92	13.2	9.13	14.4	10.5	13.7	11.3
Pb	0.192	0.0793	0.214	*-0.0192*	0.327	0.0438	0.280	0.0235
Pd	-0.0919	-0.120	-0.114	*-0.148*	*-0.172*	*-0.152*	-0.110	*-0.138*
Pt	0.00548	*0.000250*	*-0.000513*	*0.00134*	*0.000548*	*0.000232*	*0.000651*	*0.00194*
Rb	108	112	98.9	102	110	82.1	101	110
Sb]	0.186	0.0984	0.134	0.136	0.442	0.266	0.300	0.179
Sc	0.382	0.332	0.256	0.354	0.191	0.146	0.266	0.298
Se	14.4	15.0	12.6	12.6	12.0	11.3	14.0	14.1
Sn	0.654	1.27	1.74	1.47	2.28	2.87	1.78	1.59
Sr	2,410	2,343	2,628	2,267	2,463	1,734	2,209	2,410
Te	*-0.0616*	*0.0725*	*0.0259*	*0.0761*	*0.00641*	*-0.0362*	*0.0720*	*0.0864*
Ti	26.2	27.8	26.5	29.5	28.9	24.2	23.3	33.1
Tl	0.534	0.538	0.594	0.561	0.596	0.249	0.479	0.416
U	0.0322	0.0596	0.0807	0.0615	0.0434	0.0568	0.0598	0.0588
V	1.10	1.62	2.63	2.13	1.49	1.56	1.73	2.01
W	0.0625	0.212	0.135	0.0826	0.161	0.561	0.0842	0.167
Y	0.0870	0.254	0.152	0.106	0.149	0.186	0.133	0.169
Zn	940	1,048	1,063	988	1,007	874	1,005	1,049
Zr	-0.151	-0.565	-0.503	*-0.558*	*-0.667*	*-0.592*	*-0.525*	*-0.596*

Appendix J2 The body burden of 1-week bioaccumulation test in the cage (*<DL)*

Site	Rf-9	LR-1	LR-2	LR-3	LE-1	LE-2	LE-3	B1-R1	B1-R2
Ag	0.943	0.903	0.767	1.01	0.814	1.02	0.980	0.892	1.06
Al	1,087	867	718	524	1,130	1,818	2,148	997	889
As	10.1	10.9	11.2	9.73	10.5	10.2	10.3	10.3	10.5
B	158	171	293	289	282	321	261	201	226
Ba	152	236	184	178	120	115	106	415	364
Be	0.162	0.127	0.134	0.108	0.0942	0.120	0.178	0.225	0.135
Bi	*0.0884*	*-0.0129*	*-0.0140*	*-0.0133*	*0.0100*	*-0.0049*	*-0.0148*	*-0.0160*	*-0.0219*
Ca	2,422	2,325	2,203	2,451	2,362	2,364	2,587	2,531	2,460
Cd	6.94	7.59	6.55	7.31	7.20	8.85	7.85	8.64	7.43
Ce	*-0.0399*	0.189	*0.0638*	*0.0655*	*0.0328*	*0.119*	0.191	*0.0423*	*0.0271*
Co	3.57	3.99	4.36	3.85	3.13	3.60	3.57	4.48	4.03
Cr	-1.14	1.23	0.235	0.283	0.884	1.55	5.94	16.5	-0.388
Cs	0.176	0.193	0.218	0.216	0.169	0.177	0.194	0.217	0.152
Cu	765	682	767	733	756	818	739	813	807
Fe]	854	695	792	783	868	1,088	1,107	1,035	911
Ga	0.304	0.552	0.414	0.463	0.302	0.326	0.361	0.325	0.329
Ge	0.0734	0.0433	0.0597	0.0561	0.0654	0.0626	0.0558	0.0680	0.0829
In	*0*	*-0.00155*	*0.00165*	0.00455	*-0.00124*	*0.00314*	*0.00228*	*0.00414*	*0.00044*
K	203	210	206	211	214	199	210	208	208
La	0.0292	0.116	0.0868	0.0643	0.0434	0.0829	0.153	0.0898	0.0858
Li	4.26	1.80	1.93	1.83	2.28	2.74	3.24	2.44	2.41
Mg	53.7	58.5	58.9	58.5	60.9	54.3	51.2	54.1	54.7
Mn	93.4	6,359	3,593	4,503	79.7	95.2	89.0	100	76.5
Mo	5.82	5.36	4.95	5.44	6.59	6.03	6.30	7.01	7.05
Na	291	253	271	300	289	298	338	300	278
Nb	0.000995	0.0224	-0.000668	-0.00160	0.0123	0.0152	0.0323	-0.00286	-0.00142
Ni	13.6	12.6	10.2	9.06	12.8	10.4	18.6	16.0	47.1
Pb	*-0.0322*	0.360	0.183	0.0485	0.181	0.187	0.0907	0.179	0.0859
Pd	*-0.176*	-0.0949	-0.124	-0.103	*-0.134*	-0.0699	*-0.128*	*-0.147*	*-0.177*
Pt	*0.00202*	*0.00141*	*0.000303*	*0.00113*	0.00172	*0.000822*	*0.00104*	*0.000874*	*-0.000447*
Rb	100	117	119	121	102	98.8	106	101	82.0
Sb]	0.354	0.147	0.121	0.166	0.154	0.483	0.335	0.179	0.227
Sc	0.355	0.342	0.213	0.299	0.246	0.304	0.281	0.370	0.262
Se	16.3	8.82	10.2	9.13	10.2	10.6	10.2	9.83	10.1
Sn	1.85	1.65	1.08	0.407	0.704	1.27	0.234	0.731	1.81
Sr	2,154	2,913	2,757	2,824	2,692	2,669	2,793	3,266	2,975
Te	*0.000568*	*0.0165*	*0.0250*	*0.0259*	*0.0676*	*0.0923*	*0.0340*	*-0.0221*	*-0.00484*
Ti	23.0	34.7	31.9	26.4	31.1	31.6	28.0	28.6	31.6
Tl	0.682	1.48	0.408	1.09	0.663	0.613	0.262	0.541	0.359
U	0.0621	0.0565	0.0401	0.0746	0.0576	0.0691	0.0707	0.0449	0.0629
V	2.13	1.75	1.67	1.31	1.67	2.01	3.18	1.70	2.17
W	0.441	0.0899	0.0848	0.100	0.204	0.341	0.276	0.125	0.0549
Y	0.128	0.162	0.118	0.110	0.127	0.209	0.180	0.223	0.208
Zn	996	1,032	989	950	1,022	989	1,047	982	965
Zr	*-0.648*	0.268	-0.353	-0.409	-0.358	-0.482	-0.504	*-0.573*	-0.450

Appendix J3 The body burden of 1-week bioaccumulation test in the cage (*<DL)*

Site	B1-R3	FL-1	FL-2	FL-3	B2-R1	B2-R2
Ag	1.18	0.809	0.865	0.761	0.724	0.784
Al	1,706	477	885	969	1,186	707
As	9.53	9.88	9.96	10.7	9.78	9.96
B	1,976	309	166	249	130	233
Ba	580	247	211	232	277	198
Be	0.544	0.141	0.127	0.0603	0.156	0.124
Bi	0.128	0.108	*-0.0105*	*0.0058*	*-0.0178*	*-0.0156*
Ca	2,321	2,350	2,260	1,954	2,277	2,281
Cd	13.5	6.36	6.77	6.26	7.05	7.27
Ce	0.420	*0.0236*	*-0.0221*	*-0.0126*	*-0.00189*	*-0.0263*
Co	7.69	3.82	3.61	3.48	3.71	3.36
Cr	25.5	2.02	0.844	1.90	2.41	9.75
Cs	0.289	0.165	0.184	0.229	0.163	0.164
Cu	814	787	706	813	699	744
Fe]	1,592	789	937	936	893	895
Ga	0.417	0.302	0.319	0.328	0.291	0.318
Ge	0.0275	0.129	0.145	0.0996	0.0835	0.0846
In	*-0.00142*	*-0.00416*	*-0.00019*	*0.00233*	*-0.000473*	*-0.00173*
K	280	200	206	197	191	214
La	1.12	0.0395	0.0329	0.0258	0.0689	0.0315
Li	30.0	3.72	3.21	6.30	2.82	1.91
Mg	70.1	58.2	61.2	61.2	63.8	59.9
Mn	196	264	150	120	93.3	69.5
Mo	7.99	5.66	5.38	5.94	6.10	6.46
Na	322	273	285	275	236	289
Nb	0.0222	-0.00333	0.00661	-0.00427	0.00560	0.000309
Ni	162	15.0	12.0	10.0	13.8	13.1
Pb	**46.3**	0.0843	0.0573	0.0474	0.133	0.0650
Pd	*-0.122*	*-0.130*	*-0.196*	*-0.181*	*-0.147*	*-0.141*
Pt	0.00457	*0.00140*	*0.000173*	*0.000933*	*0.000861*	0.00259
Rb	85.0	101	108	109	112	123
Sb]	1.34	0.327	0.119	0.228	0.185	0.316
Sc	0.258	0.185	0.262	0.109	0.331	0.392
Se	9.79	20.7	19.1	11.6	11.2	12.3
Sn	6.48	0.828	1.30	0.863	1.07	0.428
Sr	3,187	2,875	2,814	2,562	3,946	3,494
Te	*0.0207*	0.140	*0.0467*	0.156	*-0.00189*	*0.0485*
Ti	33.2	30.6	30.8	26.2	29.1	33.0
Tl	0.480	0.513	0.591	0.411	0.342	0.459
U	0.114	0.0739	0.0639	0.0613	0.0806	0.0884
V	5.54	1.67	2.16	2.20	1.17	1.50
W	0.175	0.0533	0.124	0.228	0.0976	0.0698
Y	0.316	0.203	0.182	0.148	0.176	0.127
Zn	4,174	981	1,048	975	931	976
Zr	-0.454	*-0.565*	*-0.636*	*-0.712*	*-0.478*	*-0.507*

Appendix J4 The body burden of 1-week bioaccumulation test in the cage (*<DL)*

Site	B2-R3	B3-R1	DS-1	DS-2	TC-1	TC-2	TC-3
Ag	0.921	0.630	0.962	1.00	0.837	0.991	1.02
Al	1,075	1,350	443	1,180	1,130	1,485	2,104
As	9.03	9.32	9.15	11.3	9.42	10.3	10.5
B	177	233	295	254	235	201	320
Ba	300	264	235	157	194	207	222
Be	0.0766	0.137	0.203	0.103	0.171	0.118	0.184
Bi	*-0.0165*	*-0.0173*	*-0.0217*	*0.00104*	*-0.00396*	*-0.0233*	*-0.00632*
Ca	2,297	2,223	2,121	2,216	2,144	2,117	2,257
Cd	7.25	6.86	6.15	7.63	6.13	5.35	6.24
Ce	*0.0973*	*0.0512*	*-0.0312*	*0.0176*	*0.0432*	*-0.0358*	*0.0319*
Co	3.26	3.07	3.96	4.47	3.31	3.71	3.09
Cr	12.0	4.15	13.7	8.07	2.80	0.0354	2.55
Cs	0.167	0.196	0.193	0.182	0.166	0.148	0.158
Cu	641	655	684	749	736	720	681
Fe]	1,056	1,179	933	1,113	839	809	892
Ga	0.346	0.343	0.298	0.318	0.310	0.337	0.347
Ge	0.101	0.0435	0.0591	0.0589	0.0656	0.0601	0.0745
In	*0.00276*	*0.00134*	*-0.000268*	*0.00121*	*-0.00169*	*0.00319*	*0.00539*
K	210	215	209	214	200	201	202
La	0.0567	0.0613	0.0312	0.0580	0.0698	0.0241	0.0517
Li	3.92	2.76	3.10	2.98	3.21	5.94	5.02
Mg	71.0	68.7	64.3	55.5	54.8	53.7	59.8
Mn	79.4	102	100	107	101	125	109
Mo	7.20	6.80	6.12	5.75	5.09	5.50	5.08
Na	258	300	264	297	276	269	278
Nb	0.0107	*-0.00664*	-0.000964	0.00633	0.00279	*-0.00472*	0.0156
Ni	22.4	24.1	21.3	15.3	13.3	11.0	28.0
Pb	0.175	0.197	0.166	0.362	0.196	0.100	0.371
Pd	*-0.142*	*-0.133*	-0.107	*-0.148*	*-0.119*	*-0.128*	*-0.147*
Pt	*0.000247*	0.00267	*0.000273*	*0.00132*	*0.000839*	*0.00108*	0.00282
Rb	125	110	115	106	93.7	95.9	92.4
Sb]	0.180	0.0908	0.0693	0.182	0.398	0.140	0.229
Sc	0.289	0.265	0.135	*0.0563*	0.230	0.218	0.464
Se	10.7	12.0	11.6	17.2	11.5	13.9	14.0
Sn	0.195	0.155	2.64	0.931	1.87	1.81	1.08
Sr	3,913	4,528	2,811	2,628	2,912	2,656	3,195
Te	*0.0714*	*-0.0611*	*0.108*	*0.0327*	*0.0151*	*0.111*	*0.0419*
Ti	31.0	25.6	29.9	28.9	27.9	29.9	36.0
Tl	0.489	0.875	0.882	0.764	0.482	0.729	0.784
U	0.0628	0.0768	0.0661	0.0730	0.0535	0.0946	0.112
V	2.25	1.54	1.64	1.81	2.27	1.51	2.00
W	0.125	0.314	0.0867	0.219	0.145	0.0914	0.219
Y	0.176	0.214	0.118	0.158	0.125	0.157	0.219
Zn	965	1,001	1,047	1,014	955	961	981
Zr	*-0.529*	*-0.599*	*-0.539*	*-0.620*	*-0.550*	*-0.601*	0.231

Appendix K1 The body burden of 28-day toxicity test in the cones*(<DL)*

Site	RF-1	Rf-2	Rf-3	Rf-4	Rf-5	Rf-6	Rf-7	Rf-8	Rf-9
Ag	2.62	NA	3.48	2.02	3.11	2.58	1.83	NA	4.21
Al	1,851	NA	1,273	1,331	1,626	1,093	744	NA	1,381
As	21.7	NA	16.9	15.7	21.8	20.3	13.9	NA	19.0
B	590	NA	210	44.2	320	345	323	NA	546
Ba	465	NA	488	1,041	572	387	400	NA	876
Be	1.20	NA	1.03	2.40	1.09	1.19	0.852	NA	1.12
Bi	*-0.106*	NA	*-0.0734*	0.0039	-0.0241	0.0546	*-0.0353*	NA	0.0014
Ca	2,349	NA	2,312	2,973	2,083	2,078	2,222	NA	2,558
Cd	1.27	NA	1.31	1.77	2.54	2.24	2.64	NA	4.78
Ce	0.111	NA	0.0470	-0.0681	0.130	0.105	0.0568	NA	0.0516
Co	4.67	NA	6.15	5.38	4.85	3.70	6.82	NA	5.72
Cr	-8.44	NA	-1.22	-10.9	7.52	-0.28	-2.32	NA	-3.40
Cs	0.156	NA	0.127	0.173	0.161	0.144	0.130	NA	0.177
Cu	1,033	NA	1,295	1,040	1,233	1,293	1,096	NA	1,248
Fe]	1,282	NA	1,214	2,280	1,387	1,637	1,166	NA	1,258
Ga	0.557	NA	0.485	0.679	0.556	0.501	0.453	NA	0.580
Ge	0.286	NA	0.209	0.210	0.245	0.175	0.121	NA	0.214
In	*-0.0478*	NA	*-0.00525*	*-0.0202*	*0.00149*	0.0149	*-0.00762*	NA	*-0.00272*
K	233	NA	230	213	251	244	242	NA	239
La	0.232	NA	0.124	0.175	0.139	0.106	0.0764	NA	0.144
Li	8.52	NA	5.71	9.64	8.59	6.40	3.85	NA	6.87
Mg	65.4	NA	72.3	93.7	72.3	62.0	59.3	NA	86.8
Mn	476	NA	573	532	296	212	306	NA	581
Mo	5.30	NA	6.01	4.52	7.26	5.98	6.26	NA	6.71
Na	319	NA	342	426	299	325	304	NA	399
Nb	*0.344*	NA	*0.0300*	*-0.0165*	*0.0206*	*-0.0042*	*0.00456*	NA	*-0.0604*
Ni	4.52	NA	6.25	-2.00	12.0	9.61	10.1	NA	16.7
Pb	0.440	NA	0.419	0.502	0.578	0.260	0.074	NA	0.563
Pd	*-0.557*	NA	*-0.360*	*-0.830*	*-0.357*	*-0.237*	*-0.182*	NA	*-0.454*
Pt	*-0.00557*	NA	*-0.00221*	*-0.00860*	*-0.00258*	*-0.000631*	*-0.00123*	NA	*-0.00267*
Rb	98.6	NA	72.6	91.8	77.7	70.7	68.3	NA	70.9
Sb]	*0.275*	NA	*0.202*	*0.107*	*0.177*	*0.178*	*0.000130*	NA	*0.0880*
Sc	*-0.173*	NA	*0.0830*	*-0.0318*	*-0.0159*	*0.118*	0.110	NA	*-0.225*
Se	27.1	NA	29.9	31.1	29.1	30.9	26.2	NA	32.2
Sn	71.5	NA	28.9	83.4	24.5	23.3	18.3	NA	35.7
Sr	2,139	NA	2,048	2,799	2,014	1,882	1,929	NA	2,621
Te	*0.202*	NA	*0.243*	*-0.214*	*0.222*	*0.084*	*0.087*	NA	*-0.106*
Ti	31.5	NA	25.1	41.0	32.8	45.5	32.2	NA	64.9
Tl	0.324	NA	0.525	0.460	0.522	0.470	0.639	NA	0.152
U	0.174	NA	0.0788	0.0597	0.0904	0.0848	0.112	NA	0.0780
V	2.61	NA	2.56	3.89	3.64	3.07	3.66	NA	3.28
W	*0.115*	NA	*0.0450*	0.535	*0.0805*	*0.0466*	*0.0547*	NA	*0.0725*
Y	1.66	NA	0.423	0.312	0.559	0.486	0.477	NA	0.832
Zn	936	NA	941	1,176	1,040	1,048	1,013	NA	1,079
Zr	-0.825	NA	-0.892	-1.97	-0.807	-0.619	-0.195	NA	-0.738

Appendix K2 The body burden (nmol/g) from the 28-Day toxicity test (*<DL)*

Site	LR-1	LR-2	LR-3	LE-1	LE-2	LE-3
Ag	2.25	1.24	2.09	0.947	0.712	0.456
Al	1,169	1,314	1,555	1,247	768	1,749
As	11.0	14.0	12.1	27.2	15.3	26.5
B	245	151	321	238	248	158
Ba	442	492	485	253	316	201
Be	0.932	0.947	1.00	0.913	0.987	1.05
Bi	0.100	0.0438	*-0.0164*	*-0.0200*	-0.0109	*-0.0500*
Ca	2,408	2,491	2,271	1,931	2,437	2,452
Cd	7.99	7.61	6.43	2.22	5.58	7.63
Ce	0.834	1.24	1.39	0.0735	0.0113	-0.000364
Co	5.71	6.27	8.55	4.12	4.45	3.30
Cr	0.69	0.26	-1.02	-1.33	-1.31	2.13
Cs	0.440	0.529	0.512	0.210	0.186	0.228
Cu	1,288	1,326	1,254	1,288	1,516	1,161
Fe]	1,243	1,180	1,455	852	728	814
Ga	0.564	0.663	0.777	0.541	0.518	0.596
Ge	*0.023*	0.047	0.096	0.052	*0.005*	*-0.001*
In	*-0.00751*	*-0.00909*	*-0.00683*	*-0.0107*	*-0.00363*	0.0171
K	245	251	220	231	221	243
La	0.565	0.756	0.788	0.0846	0.0572	0.0500
Li	4.06	3.62	2.90	4.49	2.68	5.54
Mg	68.6	68.4	63.4	72.5	65.2	68.0
Mn	1,768	4,425	6,867	397	165	377
Mo	6.77	6.34	5.16	5.65	5.64	4.52
Na	330	313	290	251	268	314
Nb	*0.00110*	*0.00191*	*0.0118*	*-0.0091*	*-0.0181*	*-0.0250*
Ni	6.82	8.19	7.43	6.52	6.95	48.2
Pb	1.15	0.984	0.896	0.276	0.832	0.289
Pd	*-0.211*	*-0.225*	*-0.195*	*-0.195*	*-0.203*	*-0.396*
Pt	*-0.00238*	*-0.000103*	*-0.00334*	*0.000772*	*0.00140*	*-0.00356*
Rb	131	137	116	86.3	78.4	100
Sb]	*0.0779*	*0.0303*	*0.0452*	0.301	*0.0434*	*-0.000422*
Sc	0.237	0.190	0.258	*0.0085*	*0.0691*	0.231
Se	13.3	13.4	14.0	12.4	12.8	13.2
Sn	31.0	22.0	24.1	20.1	17.7	28.4
Sr	3,021	3,127	2,868	2,219	2,422	2,298
Te	*0.119*	0.327	*0.109*	0.224	*0.089*	*0.184*
Ti	44.4	37.6	52.9	45.7	39.4	41.1
Tl	0.559	0.655	0.645	0.240	0.315	0.355
U	0.0744	0.0858	0.105	0.0850	0.0660	0.115
V	2.54	1.94	2.21	2.08	1.35	3.51
W	0.0819	*0.0664*	0.0820	0.160	0.135	0.179
Y	0.577	0.623	0.667	0.230	0.192	0.442
Zn	1,009	1,047	956	959	969	1,231
Zr	-0.627	-0.645	-0.600	-0.694	-0.626	-0.965

Appendix K3 The body burden (nmol/g) from the 28-Day toxicity test (*<DL)*

Site	B1-R1	B1-R2	B1-R3	FL-1	FL-2	FL-3
Ag	0.769	2.46	2.47	2.74	3.51	1.35
Al	2,263	1,587	5,082	764	4,103	1,518
As	9.57	20.5	17.1	20.4	23.1	17.8
B	266	124	327	361	194	193
Ba	695	1,779	1,677	850	902	727
Be	1.20	1.57	2.58	1.09	3.86	1.21
Bi	0.0351	0.0028	-0.0605	*-0.0190*	0.204	0.0033
Ca	2,600	2,001	2,302	2,537	2,961	2,281
Cd	3.52	6.71	8.97	1.41	4.00	1.76
Ce	0.373	-0.0811	0.864	0.0605	1.61	0.420
Co	9.66	12.0	13.0	3.05	5.40	4.71
Cr	-3.63	-7.55	18.5	0.31	-1.69	-1.01
Cs	0.157	0.192	0.279	0.190	0.312	0.169
Cu	964	1,200	1,217	1,161	1,427	976
Fe]	1,872	1,281	3,395	1,598	4,003	2,526
Ga	0.589	0.566	0.829	0.515	0.752	0.560
Ge	*-0.015*	*-0.039*	*-0.057*	0.106	0.256	0.073
In	*0.00119*	*-0.0211*	*0.00118*	*-0.00486*	*-0.00512*	*-0.000100*
K	252	249	245	228	221	222
La	0.263	0.119	0.680	0.102	0.995	0.300
Li	7.82	4.41	17.3	9.12	15.0	8.10
Mg	59.9	72.1	73.8	71.0	74.4	70.5
Mn	448	1,851	1,184	332	432	384
Mo	10.4	9.42	7.28	5.09	4.17	5.47
Na	369	244	361	299	463	280
Nb	*-0.0381*	*-0.0973*	*-0.0868*	*-0.000841*	*0.0101*	*-0.0081*
Ni	45.5	2.85	11.1	8.23	15.5	11.6
Pb	1.07	0.192	0.439	0.222	0.992	0.415
Pd	*-0.340*	*-0.671*	*-0.888*	*-0.152*	*-0.513*	*-0.192*
Pt	*-0.00471*	*-0.0123*	*-0.00780*	*0.000461*	*-0.00422*	*0.00132*
Rb	68.2	49.9	62.0	76.4	96.4	75.0
Sb]	*0.227*	*-0.0132*	*-0.0573*	*0.00194*	*0.463*	*0.0415*
Sc	*0.0329*	*-0.461*	*-0.761*	0.364	0.377	0.281
Se	9.97	12.2	12.8	25.1	26.6	21.5
Sn	28.2	43.5	62.7	19.7	36.1	11.3
Sr	3,081	2,920	3,406	3,098	3,560	2,828
Te	0.320	*0.386*	*0.346*	0.244	*0.456*	0.290
Ti	39.4	36.8	36.7	29.9	65.1	38.6
Tl	0.297	0.0711	0.0525	0.402	0.562	0.405
U	0.209	0.112	0.178	0.183	0.117	0.211
V	2.63	3.05	5.92	1.45	7.08	3.16
W	*0.0386*	*0.0525*	*0.184*	*0.0468*	*0.0341*	*0.0614*
Y	1.24	0.493	1.58	0.351	2.95	0.561
Zn	1,038	902	989	1,096	842	910
Zr	-0.916	-2.24	-3.06	0.371	-1.33	-0.265

Appendix K4 The body burden (nmol/g) from the 28-Day toxicity test (*<DL)*

Site	B2-R1	B2-R2	B2-R3	B3-R1	DS-1	DS-2
Ag	2.10	2.89	1.61	0.807	2.28	2.25
Al	4,583	2,307	1,730	4,892	3,383	6,448
As	17.3	22.8	14.3	15.7	16.7	13.4
B	172	383	17.7	-500	263	318
Ba	1,408	924	1,351	1,376	967	879
Be	1.47	1.67	1.17	6.14	1.65	2.30
Bi	0.0270	*-0.0520*	0.0212	*-0.583*	*-0.0262*	0.0679
Ca	2,182	2,220	2,193	2,598	1,895	2,233
Cd	1.69	2.95	1.97	99.8	4.93	6.17
Ce	0.567	0.388	0.290	-0.405	0.494	1.69
Co	5.51	8.48	6.14	20.6	10.2	21.0
Cr	1.84	7.78	0.92	-43.4	-1.19	0.47
Cs	0.218	0.208	0.171	0.218	0.228	0.300
Cu	1,416	1,375	1,227	918	1,429	1,344
Fe]	2,864	2,157	1,446	973	2,369	3,948
Ga	0.802	0.628	0.589	0.750	0.742	1.00
Ge	0.250	0.258	0.439	*-0.315*	0.106	0.269
In	0.00493	*-0.00914*	*-0.00270*	*-0.126*	*0.0000331*	0.0106
K	245	252	238	231	224	227
La	0.356	0.287	0.220	0.781	0.326	0.966
Li	15.5	11.8	7.24	8.54	11.6	22.9
Mg	86.6	83.6	80.1	90.5	79.4	78.3
Mn	325	438	468	1,432	408	783
Mo	7.45	6.83	6.14	3.91	5.50	5.98
Na	292	287	264	337	225	281
Nb	*0.00101*	*-0.0408*	*-0.0284*	*-0.748*	*-0.0051*	*0.0436*
Ni	25.1	12.3	13.1	-25.9	9.37	14.7
Pb	0.573	0.326	0.718	-0.758	0.422	1.03
Pd	*-0.140*	*-0.334*	*-0.255*	*-3.45*	*-0.242*	*-0.252*
Pt	*-0.000292*	*-0.00323*	*0.00131*	*0.00740*	*-0.000723*	*########*
Rb	73.8	83.2	85.0	98.5	68.6	73.7
Sb]	*0.131*	*0.0341*	*0.200*	*1.02*	*0.0441*	*0.0854*
Sc	0.266	*0.0906*	0.194	*-4.59*	*-0.0390*	0.293
Se	14.7	21.1	15.1	17.7	27.0	22.8
Sn	8.37	24.3	15.7	321	8.61	4.81
Sr	4,299	4,017	3,895	6,207	2,746	2,944
Te	*0.078*	0.283	0.281	*1.72*	0.234	0.232
Ti	50.4	35.7	60.0	39.9	38.4	72.4
Tl	0.298	0.317	0.341	0.217	0.572	0.709
U	0.256	0.107	0.0928	0.0208	0.140	0.296
V	3.85	3.97	2.48	3.22	3.78	7.40
W	0.106	0.123	*0.0673*	*0.218*	0.0973	0.274
Y	1.05	0.866	0.685	0.441	0.924	2.39
Zn	982	919	1,002	733	910	1,013
Zr	-0.287	-0.961	-0.606	-13.3	-0.549	-0.738

Appendix K5 showing the body burden (nmol/g) from the 28-Day toxicity test (<*DL)*

Site	TC-1	TC-2	TC-3
Ag	2.38	1.73	1.02
Al	1,082	4,457	2,436
As	28.9	30.0	24.3
B	381	138	260
Ba	467	831	609
Be	0.975	1.51	1.49
Bi	-0.0116	0.0134	0.0647
Ca	2,562	2,501	2,405
Cd	2.75	0.842	1.00
Ce	0.0177	0.711	0.329
Co	6.26	6.30	5.82
Cr	-2.66	0.59	-2.04
Cs	0.161	0.189	0.187
Cu	1,233	1,114	1,016
Fe]	1,067	2,688	2,352
Ga	0.510	0.811	0.595
Ge	0.102	0.162	0.150
In	*-0.00679*	*0.00321*	*-0.0150*
K	222	235	235
La	0.0823	0.415	0.255
Li	5.20	16.1	7.77
Mg	66.7	78.2	75.9
Mn	409	206	375
Mo	5.63	6.57	5.68
Na	330	292	293
Nb	*-0.0393*	*0.101*	*-0.0475*
Ni	11.4	15.5	13.0
Pb	0.378	0.481	0.324
Pd	*-0.231*	*-0.205*	*-0.326*
Pt	*0.00392*	*NA*	*-0.000030*
Rb	68.3	65.7	82.0
Sb]	*0.0343*	*0.0395*	*0.0381*
Sc	*-0.187*	0.236	*-0.0257*
Se	21.0	22.0	19.1
Sn	19.1	9.19	17.8
Sr	3,202	3,300	3,225
Te	0.193	0.245	*0.205*
Ti	30.6	67.4	33.1
Tl	0.0569	0.262	0.323
U	0.138	0.190	0.167
V	1.70	6.04	3.83
W	0.159	0.162	0.159
Y	0.381	1.04	0.681
Zn	884	917	889
Zr	-0.663	-0.563	-1.09

Appendix L The measure body concentration (nmol/g) in cages

Background (nmol/g)	13.8	3.64	2.25	-0.100	1539	107.0	16.0	0.199	0.124	924
LBC25 $_{X24hr}$ (nmol/g)	**83**	**585**	**90**	**146**	**1850**	**44400**	**169**	**38**	**364**	**938**
TISSUE	**Cage Measured Body Concentrations (nmol/g)**									
Sample	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Tl**	**Zn**
RF-1	-4.66	6.38	0.869	-0.013	-768	1.25	-12.6	-0.00705	0.410	16.0
Rf-2	-2.75	2.80	1.25	1.54	-723	3.78	-13.3	-0.120	0.414	124
Rf-3	-3.77	3.80	1.65	0.565	-799	-11.5	-8.62	0.0148	0.470	139
Rf-4	-3.95	3.35	0.868	1.02	-803	-36.4	-12.4	-0.218	0.437	63.9
Rf-5	-3.44	4.75	1.52	5.48	-761	-3.09	-8.22	0.128	0.472	82.7
Rf-6	-2.48	1.70	0.461	0.366	-907	-29.2	-11.3	-0.155	0.125	-50.3
Rf-7	-3.43	4.11	1.60	1.92	-732	-17.3	-7.51	0.0808	0.355	80.6
Rf-8	-3.68	4.37	1.58	0.978	-793	-21.6	-10.8	-0.176	0.292	125
Rf-9	-3.69	3.30	1.32	-1.04	-774	-13.6	-8.76	-0.231	0.558	72.1
LR-1	-2.85	3.95	1.74	1.33	-857	6252	-7.99	0.161	1.35	108
LR-2	-2.59	2.91	2.11	0.335	-772	3486	-11.8	-0.0160	0.284	65.1
LR-3	-4.07	3.67	1.60	0.383	-806	4396	-12.1	-0.150	0.963	25.8
LE-1	-3.25	3.56	0.877	0.984	-783	-27.3	-7.85	-0.0178	0.539	97.7
LE-2	-3.64	5.21	1.35	1.65	-721	-11.8	-10.8	-0.0119	0.489	65.1
LE-3	-3.53	4.21	1.32	6.04	-800	-18.0	-2.65	-0.108	0.138	123
B1-R1	-3.46	5.00	2.23	16.6	-726	-6.69	-5.57	-0.0198	0.417	58.0
B1-R2	-3.26	3.79	1.78	-0.288	-732	-30.5	25.3	-0.113	0.235	41.0
B1-R3	-4.27	9.90	5.44	25.6	-725	88.8	142	46.1	0.356	3250
FL-1	-3.92	2.72	1.57	2.12	-752	157	-6.49	-0.115	0.389	57.0
FL-2	-3.84	3.13	1.36	0.944	-833	42.8	-9.93	-0.142	0.467	124
FL-3	-3.14	2.62	1.23	2.00	-726	12.6	-12.3	-0.152	0.287	50.6
B2-R1	-4.02	3.41	1.46	2.51	-840	-13.7	-6.81	-0.0656	0.218	7.30
B2-R2	-3.84	3.63	1.11	9.85	-795	-37.5	-7.53	-0.134	0.335	51.6
B2-R3	-4.77	3.61	1.01	12.1	-898	-27.6	1.49	-0.0236	0.365	41.2
B3-R1	-4.48	3.22	0.822	4.25	-884	-4.76	2.53	-0.00212	0.751	76.6
DS-1	-4.65	2.51	1.71	13.8	-855	-6.58	-0.12	-0.0328	0.758	123
DS-2	-2.52	3.99	2.22	8.17	-790	0.24	-6.10	0.163	0.640	90.0
TC-1	-4.38	2.49	1.06	2.90	-803	-5.88	-7.71	-0.00255	0.358	30.8
TC-2	-3.53	1.71	1.46	0.135	-819	18.0	-10.2	-0.0986	0.605	36.9
TC-3	-3.35	2.60	0.836	2.65	-858	1.79	5.80	0.172	0.660	57.0

Appendix M The measure body concentration (nmol/g) in cones

Background (nmol/g)	13.8	3.64	2.25	-0.100	1539	107.0	16.0	0.199	0.124	924
$LBC25_{x24hr}$ (nmol/g)	**83**	**585**	**90**	**146**	**1850**	**44400**	**169**	**38**	**364**	**938**
TISSUE	**Cone Measnured Body Concentrations (nmol/g)**									
Smpl	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Tl**	**Zn**
RF-1	7.94	-2.37	2.42	-8.34	-506	369	-11.5	0.241	0.200	11.9
Rf-2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rf-3	3.06	-2.33	3.90	-1.12	-244	466	-9.75	0.220	0.401	17.1
Rf-4	1.94	-1.87	3.13	-10.8	-499	425	-18.0	0.303	0.336	252
Rf-5	8.05	-1.10	2.60	7.62	-306	189	-3.96	0.379	0.398	116
Rf-6	6.49	-1.40	1.45	-0.176	-246	105	-6.39	0.0614	0.346	124
Rf-7	0.145	-1.00	4.57	-2.22	-443	199	-5.91	-0.125	0.515	89.4
Rf-8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rf-9	5.17	1.14	3.47	-3.30	-291	474	0.691	0.364	0.0277	155
LR-1	-2.76	4.35	3.46	0.785	-251	1661	-9.18	0.948	0.435	84.8
LR-2	0.198	3.97	4.02	0.363	-213	4318	-7.81	0.785	0.531	123
LR-3	-1.66	2.79	6.30	-0.920	-285	6760	-8.57	0.697	0.521	31.7
LE-1	13.4	-1.42	1.87	-1.23	-251	290	-9.48	0.0769	0.116	34.8
LE-2	1.55	1.94	2.20	-1.21	-23.2	57.7	-9.05	0.633	0.191	44.9
LE-3	12.7	3.99	1.05	2.23	-378	270	32.2	0.0904	0.231	307
B1-R1	-4.23	-0.118	7.41	-3.53	-575	341	29.5	0.866	0.173	114
B1-R2	6.72	3.07	9.73	-7.45	-339	1744	-13.2	-0.007	-0.0529	-21.6
B1-R3	3.34	5.33	10.8	18.6	-322	1077	-4.93	0.240	-0.0715	64.8
FL-1	6.63	-2.23	0.801	0.406	-378	225	-7.77	0.023	0.278	172
FL-2	9.28	0.364	3.15	-1.59	-112	325	-0.536	0.793	0.438	-82.1
FL-3	4.04	-1.88	2.46	-0.909	-563	277	-4.45	0.216	0.281	-13.6
B2-R1	3.51	-1.95	3.26	1.94	-123	218	9.14	0.374	0.174	58.3
B2-R2	9.00	-0.693	6.23	7.88	-164	331	-3.73	0.127	0.193	-4.60
B2-R3	0.452	-1.67	3.89	1.02	-312	361	-2.91	0.519	0.217	77.8
B3-R1	1.95	96.1	18.3	-43.3	-621	1325	-41.9	-0.957	0.0934	-191
DS-1	2.88	1.29	7.95	-1.09	-110	301	-6.63	0.223	0.448	-14.2
DS-2	-0.370	2.53	18.8	0.572	-195	676	-1.34	0.834	0.585	88.5
TC-1	15.1	-0.891	4.01	-2.56	-306	302	-4.59	0.179	-0.0671	-39.6
TC-2	16.2	-2.80	4.05	0.686	-425	99.4	-0.517	0.282	0.138	-6.68
TC-3	10.5	-2.64	3.57	-1.94	-523	268	-3.05	0.125	0.199	-35.5

Appendix N The predicted survival from MEAM and observed survival from bioaccumulation and toxicity tests

Test	1-week bioaccumulation		28-Day toxicity	
Type	Predicted	Observed	Predicted	Observed
Cones	Survival (%)			
RF-1	82.2	90.0	82.5	80.0
Rf-2	81.5	100	N/A	0
Rf-3	81.7	100	82.2	100
Rf-4	81.8	90.0	82.1	80.0
Rf-5	80.4	100	80.9	93.3
Rf-6	82.2	100	82.3	93.3
Rf-7	81.3	100	81.8	66.7
Rf-8	81.6	100	N/A	0
Rf-9	82.1	100	82.5	93.3
LR-1	81.4	90.0	81.9	93.3
LR-2	81.7	100	82.3	100
LR-3	81.8	100	82.3	93.3
LE-1	81.8	100	82.1	80.0
LE-2	81.4	90.0	81.9	80.0
LE-3	80.4	100	80.8	60.0
B1-R1	78.1	100	78.8	66.7
B1-R2	81.8	90.0	82.5	53.3
B1-R3	0.0	80.0	0.0	73.3
FL-1	81.2	100	81.7	93.3
FL-2	81.7	100	82.1	93.3
FL-3	81.4	100	81.8	73.3
B2-R1	81.2	100	81.6	93.3
B2-R2	79.7	80.0	80.0	93.3
B2-R3	79.3	100	79.6	80.0
B3-R1	80.9	100	81.2	20.0
DS-1	78.8	100	79.3	80.0
DS-2	79.7	100	80.3	100
TC-1	81.2	100	81.5	93.3
TC-2	82.0	100	82.4	100
TC-3	81.3	100	81.6	100

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