Toxicokinetics and Bioaccumulation of Metals in Wood Frog Tadpoles (*Lithobates sylvaticus*) Exposed to Sediment Near Oil Sands Mining in Northern Alberta

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Abstract

Bitumen extraction in the Athabasca oil sands in Alberta releases metals to the region. In this study, I performed an uptake-elimination experiment with wood frog tadpoles (*Lithobates sylvaticus*) to determine the bioaccumulation potential of metals from exposure to MacKay River sediment, an area affected by oil sands contamination, and to uncontaminated reference sediment. Wood frog tadpoles, Gosner stages 28-32, were exposed to two sediments: (1) MacKay River sediment that is enriched in petrogenic hydrocarbons from natural and anthropogenic sources; and (2) an uncontaminated reference sediment. Tadpole exposures to sediments lasted 4 days, followed by a depuration phase for an additional 4 days where tadpoles were allowed to eliminate excess metals from their bodies. The metal concentrations at various time points during the uptake and elimination phases were determined in order to define toxicokinetic parameters, such as uptake and elimination first order rate constants, accumulation by ingestion, and assimilation efficiencies for specific metals.

It was determined that tadpoles exposed to the MacKay sediment had higher concentrations of Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn throughout the uptake phase of the study compared to tadpoles exposed to reference sediment. We also observed little to no decrease in concentrations of Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn throughout the elimination phase of the study. In addition, biota-sediment accumulation factors (BSAF) revealed that Cu, Zn, Cr, and V had among the highest bioaccumulation potential in our trials. The experiment was subsequently repeated by preventing direct contact of the tadpoles to sediment with a screen, exposing tadpoles only to metals in water. By comparing tadpole exposures to metals from 'aqueous' and 'aqueous +sediment' in separate trials, and by tracking sediment ingestion rates, I am able to show that sediment ingestion constitutes the primary source of metal bioaccumulation by tadpoles. Not only were metal concentrations higher in tadpoles that were ingesting sediment, but they also had greater metal uptake rates compared to tadpoles that were only exposed to contaminated water. It was also determined that assimilation efficiencies were higher in tadpoles exposed to reference sediment compared to ones exposed to MacKay River sediment.

Using toxicokinetic parameters defined by the uptake-elimination experiment, I developed a computational model using STELLA$^{\text{TM}}$ system dynamics software to accurately estimate first order uptake and depuration rate constants for metals in exposed aquatic animals. The model estimated metal uptake and depuration kinetics with a mean relative error of 2.25 ± 0.93 % (±SE, n=9) for the uptake study and 2.53 ± 2.61 % (±SE, n=9) for the depuration study. With increased oil-sands production anticipated, we recommend continued monitoring of contaminants from oil-sands for the purpose of understanding the potential risks they may have on northern Alberta’s ecosystems.

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List of Abbreviations

Aq	Aqueous experiment
Al	Aluminum
As	Arsenic
Ag	Silver
ANOVA	Analysis of variance
Ba	Barium
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
Be	Beryllium
Bi	Bismuth
BSAF	Biota-sediment accumulation factor
Ca	Calcium
Cd	Cadmium
Cr	Chromium
Co	Cobalt
Cu	Copper
CCME	Canadian Council of Ministers of the Environment
DW	Dry weight
Fe	Iron
Hg	Mercury
HCl	Hydrogen Chloride
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
k_1	Uptake rate constant
k_2	Elimination rate constant
k_{tmax}	Uptake rate at time of maximum concentration
K	Potassium

Li	Lithium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
P	Phosphorus
PAH	Polycyclic Aromatic Hydrocarbon
Pb	Lead
SedAq	Sediment + aqueous experiment
Seds	Sediment concentration
Sb	Antimony
Se	Selenium
Sr	Strontium
Sn	Tin
SGS	Mineral Service Canada
STELLA™	Systems thinking, experimental learning laboratory with animation
TS	Time series
Ti	Titanium
Tl	Thallium
TOC	Total organic content
U	Uranium
U.S. EPA	United States Environmental Protection Agency
V	Vanadium
WW	Wet weight
Y	Yttrium
Zn	Zinc

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1.0. General Introduction

1.1. Introduction

The Athabasca oil sands region is the third largest oil reserve in the world that occupies 141,000 km^2 of boreal forest and muskeg in northern Alberta, Canada. Along with the many economic benefits are challenges related to environmental contamination. Employment across Canada, as a result of oil sands investment in Alberta, is expected to grow from 151,000 jobs to over 225,000 jobs by 2038 and is predicted to contribute over $4 trillion to the Canadian economy (CERI 2015). However, due to intensive oil sands mining and bitumen extraction, many contaminants are being released into nearby aquatic ecosystems in the Athabasca region. These contaminants present several environmental challenges, such as negatively impacting the quantity and quality of habitats for aquatic and terrestrial organisms, accumulation of waste in large tailings ponds and increasing the emission of greenhouse gases. More specifically, metal contaminants that accumulate in sediment and water are exposed to aquatic organisms. Previous studies suggest aquatic organisms, such as mussels and snails, experience an increase in the bioaccumulation of Be, V, Ni, Pb, Al, Cr, Co and Mo when placed in the Athabasca River near Fort McMurray (Pilote *et al.* 2017, Baker *et al.* 2017, Galal *et al.* 2017, Vu *et al.* 2017). There are a variety of metals that have been deposited from anthropogenic activities that have been detected in nearby ecosystems and they have proven hazardous to regional wildlife (Headley *et al.* 2005). Studies have shown that larval amphibians exposed to metals in sediment experience an increase in mortality and a decrease in growth and survival rates (Lanctôt *et al.* 2017). Lefcort *et al.* (1998) exposed *Rana luteiventris* tadpoles to Pb, Zn and Cd separately and as mixtures in sediment. Not only were the tadpoles accumulating higher concentrations of metals when exposed to the metals as a mixture, they also experienced an increase in mortality, a delay in development and reduced antipredator behavior. Water and sediment samples collected from the Athabasca River showed concentrations of Al, Fe, Mn, Ni, Co, Cu, Zn, Ag and Pb that exceeded concentration limits set by the Canadian Council of Ministers of the Environment (CCME) for the protection of aquatic life. Hg, Cr and As are of particular concern as they can cause toxicological effects at concentrations below the CCME recommended limit (Government of Alberta 2016, Headley *et al.* 2005). Recent evidence suggests that these metals are readily accumulated by aquatic animals and then stored in their tissues and organs, which increases their hazard to aquatic organisms and human health even at low concentrations (Singh *et al.* 2016, Rice 2001). Developing amphibians are especially susceptible to metal accumulation compared to terrestrial adults as they can accumulate metals through sediment ingestion, dermal, and respiratory pathways (Milani *et al.* 2002, Yologlu *et al.* 2015). In addition to having multiple exposure pathways, frog tadpoles are also highly active during spring melt and are thus directly affected by metals retained by snow (Dayyani *et al.* 2016). Even so, there are very few toxicokinetic metal studies done with amphibians from the surface minable oil sands region in northern Alberta.

My overall objectives for this thesis are to: (1) examine the bioaccumulation potential of metal contaminants in tadpoles from sediment collected near oilsands mining areas and reference sediment to assess their accumulation and depuration kinetics and determine their bioaccumulation potential from sediment and aqueous exposures; and (2) establish key toxicokinetic parameters that can be utilized to computationally replicate and predict toxicological endpoints for metal contaminant exposures.

1.2. Sources of Metals

Metal contaminants are potentially toxic elements when exposure exceeds probable effect levels. Sources of metal contaminants in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He *et al.* 2005). Environmental pollution from metals is very well known in areas such as mining, foundries and smelters, and other metal-based industrial operations (Tchounwou *et al.* 2014). Contamination can occur through atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension, and metal evaporation from water resources to soil and groundwater (Nriagu *et al.* 1989). Natural phenomena such as volcanic eruptions, weather, and forest fires have also been reported to contribute to metal pollution (Yang *et al.* 2005). Even though metals are naturally occurring elements that are found throughout the earth's crust, environmental contamination results from anthropogenic activities, such as petroleum combustion and oil sands mining operations.

1.3. Athabasca Oil Sands

The oil sands bitumen reserves located in northern Alberta, Canada are considered to be the third largest reserves in the world, containing around 165 billion barrels of recoverable oil (CAPP 2016). The oil sands reside in three main regions, which consist of Athabasca, Cold Lake, and Peace River, and combine for a total area of over 141,000

km^2. The Athabasca oil sands region is positioned along the Athabasca River and is considered the largest of the three oil sands regions in Alberta. It is also the only region suitable for open pit extraction as opposed to extraction through *in-situ* methods.

In 2015, Alberta produced 2.37 million barrels of crude oil per day and this production is predicted to increase to 3.67 million barrels per day by the year 2030 (Summers *et al.* 2016, Government of Alberta 2016). This rapid growth in oil sands activities will have environmental effects. Mining, extraction and refining of bitumen results in alteration of air, water and soil that may damage aquatic and terrestrial areas; these include overcompensation of water from water resources and an increase in contamination from hazardous chemical substances, such as polycyclic aromatic compounds, naphthenic acids and fly ash (JOSM 2016). Bitumen extraction also releases large quantities of metal and polycyclic aromatic compounds, which are then deposited in nearby ecosystems (Wayland *et al.* 2007).

1.4. Metal Toxicology

Metal contaminants that are of concern due to oil sands activity in Alberta include Al, Fe, Mn, Cu, Zn, Ag, Pb, Hg, Cr and As (Government of Alberta 2016, Headley *et al.* 2005, Boutin *et al.* 2017). Many metals are essential to biological systems, as they are necessary in some biochemical reactions; others are nonessential and can be toxic at low concentrations (Shaw *et al.* 1987). When present in excessive amounts, metals can disrupt natural biogeochemical processes (Gobas *et al.* 2000) and at sufficiently high concentrations, all metals can be toxic (Hellou *et al.* 2012). For instance, iron is known to participate in physiological processes such as oxygen transportation, xenobiotic metabolism and oxidative phosphorylation, but when iron is absorbed excessively by an organism, it becomes highly toxic, as shown by numerous iron related diseases including cancer (Galaris 2002). Similar considerations are valid for a number of other metals, such as As, Cd and Zn (Frank *et al.* 2014, Singh 2016, Lucas *et al.* 2017).

Essential metals, such as Zn and Cu, are involved in protein, carbohydrate, and DNA metabolic processes, and can act as cofactors for several enzymes, but can become toxic if concentrations exceed physiological sufficiency (Carvalho *et al.* 2017). Nonessential metals, such as Pb and Cd, released into the environment by human activity may be carcinogenic, genotoxic, and capable of interrupting many molecular mechanisms and generating reactive oxygen species (Lanctot *et al.* 2017). They are considered highly toxic to aquatic organisms because they are known to deplete antioxidants (glutathione and sulfhydryl proteins) and increase the production of reactive oxygen species, which can lead to lipid degradation and oxidative stress (Carvalho *et al.* 2017).

Metals, like other environmental carcinogens, have the potential to damage DNA and cause mutations in human somatic cells. The metal ions of Cu, Cd, Cr, Ni, and V are capable of generating reactive oxygen species (Galaris and Evabgelso 2001). These species have the ability to induce lipid peroxidation, DNA damage, and depletion of sulfhydryl groups (Symons *et al.* 1998, Stohs *et al.* 1995). Carcinogenesis is a possible result of metal exposure because DNA damage tends to play a central role in cancer development.

1.5. Metal Contaminants in Aquatic Ecosystems

The large quantities of metal contaminants discharged into aquatic ecosystems within the Athabasca region due to oil sands mining and processing can result in various sub-lethal or lethal effects on local aquatic and terrestrial animals (Jianbo *et al.* 2017). Once accumulated in sediment of aquatic ecosystems, metals may be re-released to water through sediment re-suspension, desorption, reduction or oxidation reactions, and the degradation of organic tissues (Islam *et al.* 2018). These processes are capable of increasing the concentration of dissolved metals in natural waters.

Metals that enter river water can react with organic polymers or clay minerals, forming complexes or chelates that settle and accumulate in sediments (Fu *et al.* 2014). Metals are also found in pore water and suspended solids, and are easily transmitted to and accumulated in animals (Alonso *et al.* 2014). These metals undergo a series of physical, chemical and biological transformations, which is a concern to aquatic wildlife.

1.6. Wood Frog Tadpoles

Amphibians are commonly used as indicator species for measuring ecosystem health and are well known for accumulating metals (Ermakov *et al.* 2016, Simon *et al.* 2010, Linder *et al.* 2003). Tadpoles are ideal bioindicators because they are readily available in large numbers, are easily managed in a laboratory setting and are good representatives of freshwater ecosystems. In addition, larval amphibians are capable of accumulating metals more readily than adults due to greater skin permeability, continuous contact with water, and from their feeding habits, which can include the consumption of sediment and algae (Kelepertzis *et al.* 2012).

Wood frog tadpoles (*Lithobates sylvaticus*) were used as model organisms to study the bioaccumulation and toxicokinetics of metal contaminants in aquatic organisms because of their frequent contact with sediment substrate, their lower efficiency in metabolizing metals compared to higher trophic organisms, their sufficient biomass for analysis, and their ubiquity in the Athabasca oil sands region. These larval amphibians can also accumulate metal contaminants in water through dermal absorption and across respiratory pathways, as well as from sediment through dietary absorption since they ingest sediment while feeding. Furthermore, *L. sylvaticus* tadpoles are prey to a variety of species, such as fish, waterfowl, wading birds, and larger invertebrates, making them an essential component of freshwater ecosystems. Many studies have shown that amphibians are sensitive to metal exposure and that their growth, development, and survival can be affected by exposure to various metals (Santana *et al.* 2017).

1.7. Bioaccumulation of Metal Contaminants

Bioaccumulation of a chemical compound in an aquatic organism can be characterized as the net available amount of the chemical following a period of uptake, elimination, and metabolism (Gobas *et al.* 2000). Aquatic organisms are capable of taking up metal contaminants from a variety of sources, including prey, sediment, water, and fine suspended particles in water and air. More specifically, larval amphibians are more capable of bioaccumulating metals compared to adults due to their semi-permeable and highly vascularised skin, which allows for a high accumulation of environmental contaminants in their tissue from sediment and water. For instance, Carvalho and Utsunomiya (2017) exposed both adult and larval *L. catesbeianus* to Zn, Cu, and Cd separately and in combination for 16 days, and found that larval *L. catesbeianus* accumulated all three metals in various tissues more efficiently than adults. Amphibians, both larval and adult, are a source for biomagnification of metals because they are capable of accumulating environmental pollutants and serve as both predator and prey in many food webs.

Biota-sediment accumulation factors (BSAF) have been used in previous studies to describe the bioaccumulation potential of metals in aquatic organisms relative to the metal concentrations in sediment (Thomann *et al.* 1998, Van Hop 2017, Wu *et al.* 2017). Van Hop *et al.* (2017) prioritized metals of concern that were detected in the Tien Estuary in Vietnam using BSAF calculations to determine the bioaccumulation potential of Cd, Ni, Cr, As, Pb, Cu and Zn in hard clams, *Meretrix lyrata*. Concentrations of all 7 tested metals were higher in *M. lyrata* tissue compared to sediment concentrations, indicating that all 7 metals bioaccumulated in *M. lyrata* with Cd possessing the highest BSAF. This study on metal contaminants in benthic invertebrates indicates that further field and laboratory investigations of metal body burdens, bioaccumulation potentials, and toxicokinetics in other aquatic organisms are warranted.

Although there is a significant amount of research on the bioaccumulation of metal contaminants in the environment (Singh *et al.* 2016, Dey 2016, Santana *et al.*

2017), further investigation on the toxicokinetics and bioaccumulation potential of metals is needed. The majority of studies investigate metals individually or in mixtures containing 2-3 metals (Rowe 2017, Kelepertzis *et al.* 2012), even though metals are found in complex mixtures in the environment (Frank *et al.* 2014). Studies that included environmentally relevant metal concentrations only looked at a single exposure route: either ingestion or dermal absorption (Gagne *et al.* 2012). Accumulation-elimination experiments have successfully determined uptake and elimination kinetics of metal contaminants in many aquatic organisms, including clams, mussels, fish, and amphibians (Santana 2017, Wu *et al.* 2017, Lucas *et al.* 2017). However, a more comprehensive accumulation-elimination study comparing the toxicokinetics and bioaccumulation potential of metals found in sediment collected near oil sands activity with those in reference sediment is needed. Doing so would further our understanding on the effects of metals in the environment and provide important information on key kinetic parameters of metals, which are proven to be useful in bioaccumulation studies and biomonitoring programs.

1.8. Study Objectives and Predictions

The first objective of this study is to determine the bioaccumulation potential of metals in wood frog tadpoles through exposure to a mixture of metals in both sediment and water from the Athabasca oil sands region in order to interpret metal residues measured in animal tissue. Recent studies suggest ingestion is the more dominant route of exposure for metals compared to metal uptake via dermal absorption (Dayyani *et al.* 2016, Clark *et al.* 1998, Iqbal *et al.* 2017), and consequently, we predict that wood frog tadpoles with access to sediment will accumulate higher metal concentrations than those exposed only to contaminated water. Subsequently, toxicokinetic parameters for both the uptake and depuration phases will be established for each metal contaminant.

The second objective of this study is to compare the bioaccumulation potential of metals between contaminated sediment collected near oil sands activity and reference sediment collected outside of the oil sands formation. Previous studies have shown that metal concentrations detected in sediment and water closer to the oil sands formation are higher compared to samples collected approximately 50 km away (Headley *et al.* 2005). This study will allow us to assess the consequences of metal contamination on aquatic organisms that inhabit these affected ecosystems. I predict that wood frog tadpoles exposed to sediment collected closer to oil sands mining activity will accumulate more metals compared to frog tadpoles exposed to a reference sediment collected outside of the oil sands formation.

The final objective of this study is to develop a predictive modeling tool using system dynamics to computationally replicate the toxicokinetics and bioaccumulation of metals in wood frog tadpoles. Not only can this tool be used to predict how a change in metal concentration will impact metal uptake and elimination rates in the future, it will also be used to determine key toxicokinetic parameters.

2.0. Laboratory Study

2.1. Introduction

Since commercial production of oil from the Alberta oil sands began in 1967, oil sands mining and bitumen extraction has increased exponentially in order to meet a growing global demand for energy. Crude oil production is predicted to increase from 2.37 million barrels per day in 2015 to 3.67 million barrels per day by 2030 with tailings ponds covering more than 130 km^2 (CAPP 2016). The development of the oil sands is associated with increased mining and processing operations. These operations produce tailings that are contaminated with metals, naphthenic acids and polycyclic aromatic compounds. Unfortunately, leaks are commonly observed in tailings ponds, which have raised concerns for downstream ecosystems (Raine *et al.* 2017). Oil sands development has the potential to affect water quality on a large scale, cumulative impacts on wildlife, and human and ecosystem health (Arens *et al.* 2017, Frank 2014). Aquatic organisms are especially susceptible to xenobiotics including metals, polycyclic aromatic compounds, and naphthenic acids (Bakker *et al.* 2017, Ohiozebau *et al.* 2015). These contaminants are produced and released from the extraction and processing of unconventional oil, and accumulate in both water and sediment of nearby aquatic habitats (Headley 2005).

2.1.1. Sources of Metals

The Alberta oil sands are known to cause increased concentrations of Al, Cd, Cu, Fe, Pb, Ni, Ag, Zn, Hg, Cr, and As in nearby sediment and water (Kelly *et al.* 2010). The metals detected in nearby ponds, rivers, and sediment likely originate from petroleum coke, land disturbances, seepage of tailings ponds, volatilization, and atmospheric deposition from upgraders and tailings ponds (Frank *et al.* 2014). Accidental pipeline spills and emissions of airborne particles also contribute to the quantity of metals introduced into the environment (Gerner *et al.* 2017). These metals are a concern because of their toxicological and bioaccumulative potential in exposed aquatic organisms. Unfortunately, most studies involving toxic metals and aquatic biota only investigate a select few metals; they rarely assess the impact of mixtures (of metals or other contaminants) on organisms. Considering the anticipated increase in oil sands activity in Alberta in the near future, metal concentrations are also predicted to rise. Thus, investigating the uptake, elimination, and bioaccumulation of these metals in aquatic organisms is of vital importance.

Metal contaminants are distributed into aquatic ecosystems through aqueous pathways and bed sediment. The fraction of free metal ions that remain dissolved in water is small owing to hydrolysis, co-precipitation, and adsorption. Metal distribution and bioavailability differs between water and sediment, and consequently, it is important to understand how aquatic biota are exposed to metals. Sediments are a major sink for metals, and thus they may act as a major source of metal exposure to aquatic organisms. These organisms are also capable of taking up free metal ions that are not bound to sediment. The chemical species or form is an important factor that can dictate the fate, mobility and bioavailability of metals. Mastrengelo *et al.* 2011 exposed toads, *Rhinella arenarum*, to aqueous solutions containing different concentrations of free Cd^{2+} ions and Ca. Cd^{2+} exposure resulted in high uptake rates, but Cd^{2+} uptake decreased with increased

Ca exposure, providing some degree of protection against free Cd^{2+} ion uptake. Many metal exposure studies have been conducted on aquatic species including clams, trout, snails, and mussels (Santana 2017, Lucas *et al.* 2017, Wu *et al.* 2017). To my knowledge, only one paper explored the toxicokinetics of metals in *L. catesbeiana*, and it focused solely on the uptake and elimination of lead (Rice *et al.* 2001). No other studies have investigated the toxicokinetics of metals in exposed anuran larvae.

2.1.2. Bioaccumulation of Metals

Wood frog tadpoles were used to determine the bioaccumulation and toxicokinetics of metal contaminants for this laboratory study because they are commonly exposed to sediment in the wild, they can provide adequate biomass for metal analysis, they are involved in important ecological roles (e.g. prey, predator, nutrient cycling, and biological indicators of ecosystem health), and they are abundantly found within the Athabasca oil sands region. In addition, the larval stage can provide important information on the uptake of metals via both water and sediment exposure. Wood frog tadpoles accumulate metals not only from water through dermal absorption and across respiratory pathways, but also from sediment through dietary exposure as they are known to ingest sediment while feeding. Consequently, we designed a two-part study to examine the accumulation and elimination of metals in tadpoles. The first part of the study examines tadpole exposure to contaminated sediment and water and the second examines tadpole exposure to contaminated water only. This design allowed me to separate the relative contributions of aqueous and sediment exposure pathways for metal uptake in tadpoles.

Tadpoles are known to accumulate metals over time when exposed (Clark *et al.* 1998). Metal concentrations accumulated in exposed tadpoles will eventually reach a steady state as shown in Figure 2.1. Following bioaccumulation, tadpoles begin to eliminate metals through egestion or excretion (Carvalho *et al.* 2017). To account for these processes, I conducted an uptake and depuration experiment as shown in Figure 2.1.

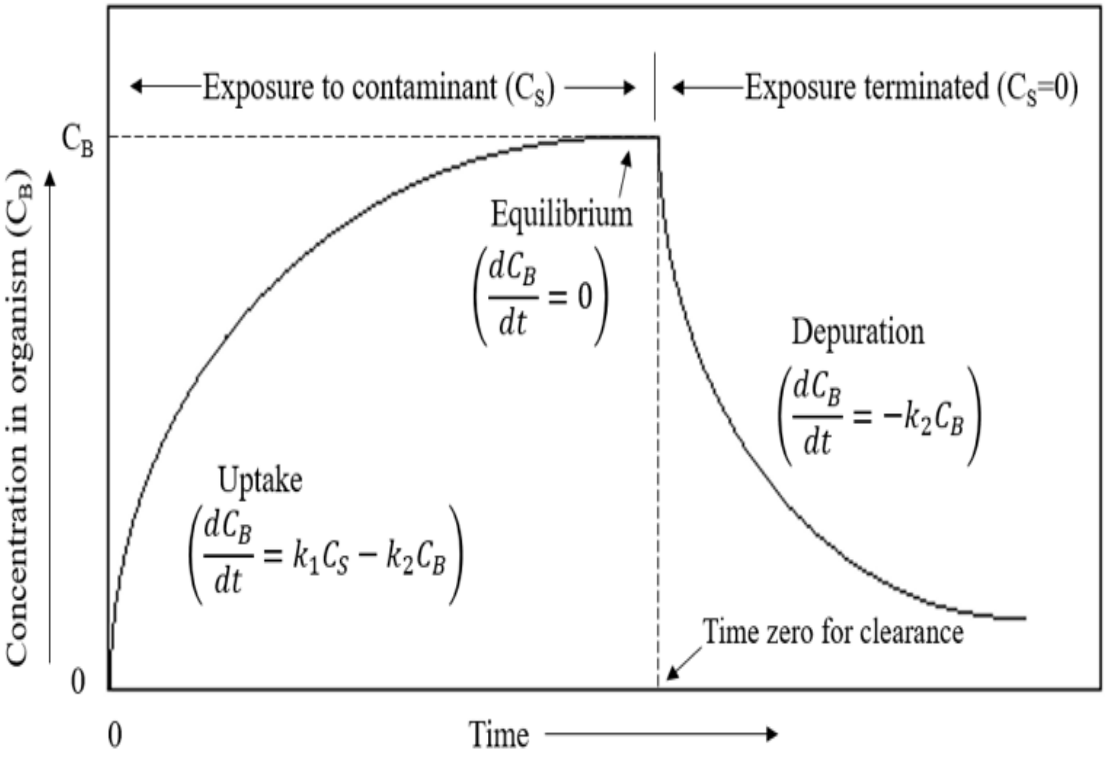


Figure 2.1. Idealized uptake and depuration curves of accumulation-elimination, where the concentration of a contaminant in an organism (C_B) increases during exposure to the source of the contaminant (C_S) until it reaches a state of equilibrium. First order uptake (k_1) and elimination (k_2) rate constants are generated with such an uptake-depuration study. During the uptake phase, kinetics are dictated by both uptake and elimination. The organisms are then allowed to depurate and kinetics in this elimination phase is solely a consequence of elimination. Subsequently, the k_2 elimination rate and k_1 uptake rate of the contaminant can be calculated (Boethling and MacKay 2000).

Bioaccumulation is defined as the net accumulation of contaminants from all possible routes of uptake (Boethling and MacKay 2000). The bioaccumulation potential of metals in aquatic organisms is often quantified using bioconcentration factors (BCFs) and biota-sediment accumulation factors (BSAFs) (Thomann *et al.* 1995, Singh *et al.* 2014). BCFs refer to the concentration of a metal in an organism's tissue divided by its equilibrium concentration in water, whereas BSAFs are the lipid and carbon normalized ratios of metal concentrations in organisms relative to those in the sediment in which they reside (Boethling and MacKay 2000).

BSAFs are commonly used as a first level-screening tool in order to compare the bioaccumulation potentials of different contaminants in exposed organisms (Nasirian 2017, Hop 2017, Wu 2017). For instance, Hop (2017) exposed the invertebrate, *Meretrix lyrata*, to contaminated sediment collected from the Tien Estuary located in Southern Vietnam. This sediment had high concentrations of Cd, Ni, Cr, As, Pb, Cu, and Zn. Hop concluded that the *M. lyrata* that reside in the Tien Estuary are safe for human consumption based on the low BSAFs determined in the study. Knowing the bioaccumulation potential of metals would be of value for future research examining metals in aquatic organisms as well as for monitoring programs that assess the health of aquatic ecosystems located near large mining operations.

2.1.3. Study Objectives

The first objective of this laboratory study is to determine the bioaccumulation potential of metals in wood frog tadpoles through exposure to environmentally realistic mixtures of metals in both sediment and water. I hypothesize that BSAFs for individual metals will be dependent on metal concentration ratios between the exposed wood frog tadpoles and sediment used throughout the exposure experiment (C_B / C_S) such that they will increase as these ratios increase. The second objective is to determine the relative contribution of sediment and aqueous exposure on metal uptake and accumulation in amphibian larvae. I predict that wood frog tadpoles exposed to both metal-contaminated sediment and water will have higher concentrations of metal during the uptake phase relative to wood frog tadpoles that were exposed to metal-contaminated water alone. This laboratory study will ultimately provide critical information on the uptake, accumulation, and elimination of metals in amphibian larvae exposed to oil sands- contaminated media.

2.2. Methodology

2.2.1. Wood Frog Tadpole Care

L. sylvaticus tadpoles were reared at the University of Ottawa's Aquatic Facility under the BL-2202 Animal Care Protocol. Adult frogs were collected from Bishops Mills Ottawa, Ontario by Dr. Vance Trudeau's lab at the University of Ottawa and were used to produce egg masses. All eggs, and subsequent tadpoles, were kept in flow-through tanks containing 14 ^{o}C water provided by the University of Ottawa's Aquatic Facility (domestic municipal water with the chloramines removed). Following facility protocols, the photoperiod consisted of 12:12 L:D and the tadpoles were fed daily (sera® micron powdered algae prior to development of mouthparts, and crushed Nasco Frog Brittle afterwards). When aquarium tanks were used during transfers, they were cleaned using Accel® PREVention Concentrate disinfectant (1:40 dilution) to ensure optimal health of the tadpoles. Approximately 8000 tadpoles were hatched and matured to Gosner stage 21-36. 1935 tadpoles were used in the exposure study, all of similar mass, size and age (Gosner 28-30).

2.2.2. Experimental Design

An exposure experiment was performed in July 2016; *L. sylvaticus* tadpoles were exposed to sediment collected from the Athabasca oil sands region as well as a reference sediment collected from an isolated and relatively uncontaminated lake. The Athabasca oil sands sediment was collected late June of 2016 from the MacKay River in Fort MacKay, Alberta, a First Nation community situated 58 km north of Fort McMurray, Alberta. The MacKay River flows northeast through the surface mineable bituminous sands area until it reaches the Athabasca River. Five gallons of surficial sediment were collected 1 m from the water's edge, upstream of Range Rd 110A Bridge (N 57 10'1.84, W 111 38'6.10). The sediment contained a mean total carbon content of 1.6 ± 0.1% (SE, n=3). The sediment also consisted of a clastic fraction of about 50% sand, 30% silt and 20% clay (RAMP 2004). 5 gallons of sediment from an isolated lake, 335 km south of Fort MacKay (N 55 00.857, W 112 01.608), were also collected in June 2016, positioning the site well outside oil sands activity impact. This sediment contained a mean total carbon content of 0.3 ± 0.02% (SE, n=3) and was used as reference sediment throughout the exposure study. All tanks and glassware were treated and cleaned with Accel PREVention^{TM} Concentrate disinfectant (1:40 dilution) to ensure optimal health of the tadpoles. Nitric acid was used to remove any existing metals from the aquaria prior to the experiment.

2.2.3. Aqueous Only Exposure (Aq)

An eight-day exposure was conducted using a total of twenty 9.5 L glass aquarium tanks. Each tank consisted of 6 L of water and 2250 g of sediment. Each tank housed 45 wood frog tadpoles in order to conduct triplicates consisting of 15 tadpoles each. 15 tadpoles were enough to produce at least 0.5 g of tissue (dry weight), which was the minimum mass of biota sample needed for analysis. Ten tanks contained sediment from Fort McKay and the remaining 10 contained of reference sediment. Tadpoles were separated from the substrate by dividers that were placed 7 cm above the substrate surface to ensure that exposure to metals would be via aqueous exposure only (tadpoles were unable to contact or ingest the sediment throughout the study). Dividers were manufactured using aquarium-safe sealant, wooden dowels and nylon fabric netting.

Dividers were first placed in 5 metric buckets of distilled water for 3 days in order to allow any unwanted chemicals to leach out prior to being placed in the exposure tanks. Three control tanks, consisting of 6 L of water and dividers, were used to determine if the dividers introduced any additional metals during the exposure study. Before the tadpoles were placed into exposure tanks, the water was left to equilibrate with the sediment for 7 days. 900 tadpoles (Gosner 28-30) fasted for 24 hours before being placed into the exposure tanks. 450 tadpoles were placed in tanks consisting of sediment collected from Fort MacKay and the remaining 450 tadpoles were placed in tanks consisting of reference sediment.

Three sets of 15 tadpoles were analyzed for metals prior to the start of the experiment (0h). During the uptake phase of the exposure study, 225 tadpoles were removed from exposure tanks and analyzed for metal concentrations at the following time points: 6, 12, 24, 72 and 96 hours. At each time point, 3 sets of 15 tadpoles, and subsequently, a water and sediment sample, were collected. During the depuration phase, the remaining 225 tadpoles in each exposure tank were removed and placed in uncontaminated flow-through tanks. 3 sets of 15 tadpoles were removed from uncontaminated flow-through tanks and analyzed for metal concentrations at 6, 12, 24, 72 and 96 hour time points. Water samples were collected from the depuration flow-through tanks at the time of each tadpole collection.

2.2.4. Sediment + Aqueous Exposure (SedAq)

In order to have a more holistic perspective on the uptake of metals in wood frog tadpoles, I performed another eight-day exposure experiment where tadpoles were exposed to metals in both water and sediment. These tadpoles were not restricted by dividers and were allowed access to the sediment, enabling them to accumulate metals from both the water (via dermal absorption, ingestion, and respiratory pathways) and sediment ingestion.

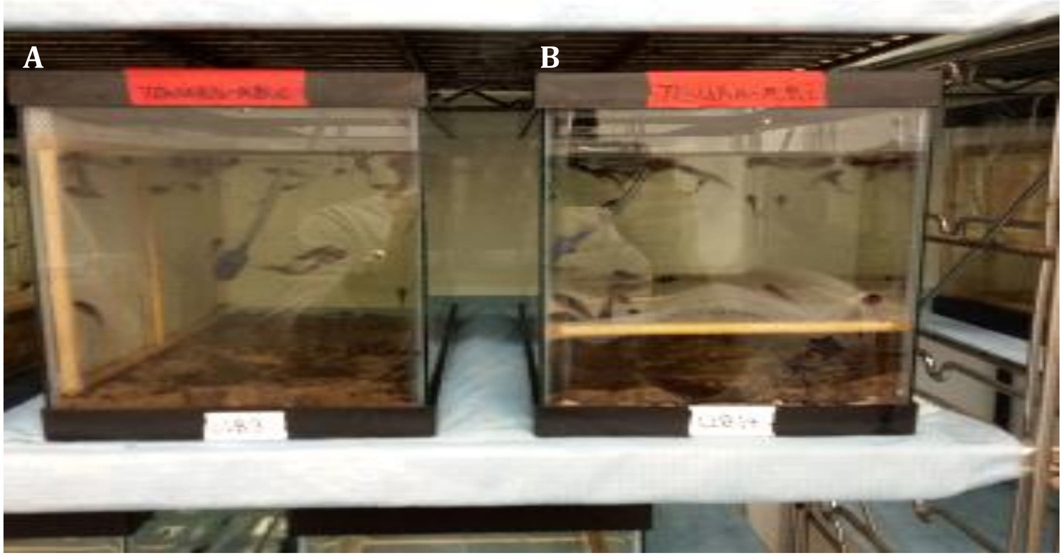


Figure 2.2. Photograph of a typical SedAq exposure tank (A) and Aq exposure tank (B). These tanks were used throughout the uptake phase of the study.

Similar to the aqueous only exposure study, twenty 9.5 L tanks containing 2250 g of sediment, 6 L of water and 45 wood frog tadpoles were used. Ten tanks contained sediment collected from Fort McKay and the remaining contained reference sediment. A Divider was placed along the inside of one of the walls of the exposure tanks to account for any effect of the dividers in the aqueous-only exposure experiment. Aside from the divider placement, all the parameters in the aqueous-only and the sediment + aqueous exposure were identical (Figure 2.2).

2.3. Metal Analysis

2.3.1. SGS Mineral Services Canada

Collected wood frog, water and sediment samples were sent to SGS Mineral Service Canada for analysis by inductively coupled plasma mass spectrometry (ICP-MS). All analyses were completed to the standards of the Canadian Analytical Laboratory Association (CALA).

2.3.2. Wood Frog Tadpoles, Sediment and Water

In accordance with Animal Care protocol BL-2202, wood frog tadpoles were euthanized prior to analysis using tricaine methanesulfonate (AquaLife TMS, Syndel Laboratories Ltd, B.C., Canada) and dissected after being stored at -20 ^{o}C for 24 hours. Gastrointestinal tracts and stomachs were removed from each tadpole to ensure that metal concentrations detected derived from tadpole bodies and not from ingested sediment remaining in the gastrointestinal tract. Tadpoles were dissected under a Leica L2 stereoscopic microscope and cut with stainless steel micro-dissecting scissors. Tadpole samples were placed in scintillation vials and freeze dried for 2 days. Biomass samples were homogenized and sent to SGS Mineral Services Canada for analysis. Based on the SGS protocol for metal analysis, samples were placed in digestion tubes and a small amount of deionized water was added to each tube along with 9 mL of concentrated trace metal HCl and 3 mL of concentrated trace metal HNO_3. Samples were then placed on hot blocks at 90 °C+/-2 °C for 40 minutes and then left to cool to room temperature. Samples were diluted to 50 mL with deionized water, shaken, and then centrifuged for 5 minutes. The solids were allowed to separate and settle before they were analyzed by ICP-MS.

Sediment and water samples were also analyzed by SGS Mineral Services Canada following the same protocol as described above. Notably, water samples were first filtered through 45 µm filter pores and then acid treated with 70% nitric acid (HNO_3) before analysis in order to analyze the dissolved metal concentrations.

2.3.3. Percent Organic Carbon and Nitrogen

Percent organic carbon and nitrogen were determined at the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa. After acid desiccation using nitric acid, samples were flash combusted in a Micro Cube elemental analyzer (Elementar, Germany) at a temperature of 1800 ^oC. They were carried by helium through reducing/oxidizing columns before being trapped within a single "trap and purge" adsorption column. Detection was accomplished by a thermal conductivity detector, with a routine analytical precision of +/-0.1%.

2.3.4. Bioaccumulation

The bioaccumulation potential of each metal was determined for the sediment + aqueous exposure experiments. Biota-sediment accumulation factors (BSAFs) were calculated at t=96 h (equation 2.1). This bioaccumulation factor describes the bioaccumulation of each metal in the wood frog tadpoles relative to the metal concentration in the MacKay River sediment:

$$BSAF = \left(\frac{C_b}{C_s}\right) \cdot \left(\frac{TOC}{L}\right) \qquad (2.1)$$

C_b is the concentration of metals in the organism (µg g^{-1} organism), C_s is the concentration of metals in the sediment (µg g^{-1} dw sediment), TOC is the total organic carbon content in the sediment (g organic carbon g^{-1} dw sediment), and L is the lipid content in the organism (g lipid g^{-1} organism) (Boethling and MacKay 2000).

The following equation was used to determine the bioconcentration factor for wood frog tadpoles exposed to contaminated water only:

$$BCF = \frac{C_b}{C_w} \qquad (2.2)$$

C_b is the concentration of metals in the organism (µg g^{-1} organism) and C_w is the concentration of metals measured in water (mg L^1).

2.3.5. Statistical Analysis

Student’s or paired T-tests and Mann-Whitney tests were performed to test for differences in metal concentrations in the two different types of sediments used in the exposure study. These tests were also used to evaluate differences in metal uptake and bioaccumulation concentrations in wood frog tadpoles that were exposed to different conditions. Shapiro-Wilk and Levene’s tests were used to verify the normality of distribution and the homogeneity of variance, with a p-value of 0.05. When required, the data were log-transformed prior to statistical analysis to meet parametric assumptions. All statistical analyses were done using SigmaPlot 12 software.

2.4. Results

2.4.1. Sediment + Aqueous (SedAq) and Aqueous Only (Aq) (192 h) Exposure and Depuration Experiment

The metal profiles for sediments from the Athabasca River and reference site are shown in Figure 2.3. When comparing the McKay sediment to the reference sediment, it is clear that the majority of metals detected in the McKay sediment are greater than those detected in the reference sediment (Figure 2.3). Not only did the MacKay sediment contain a greater number of detectable metals, it also had concentrations approximately three times greater for individual metals when compared to the reference sediment. The most abundant metals detected in the MacKay sediment that did not reach detectable limits in the reference sediment are (in descending order) Zn, Sr, V, Ni, Y, Cu, Li, Pb, As Cr, and Co.

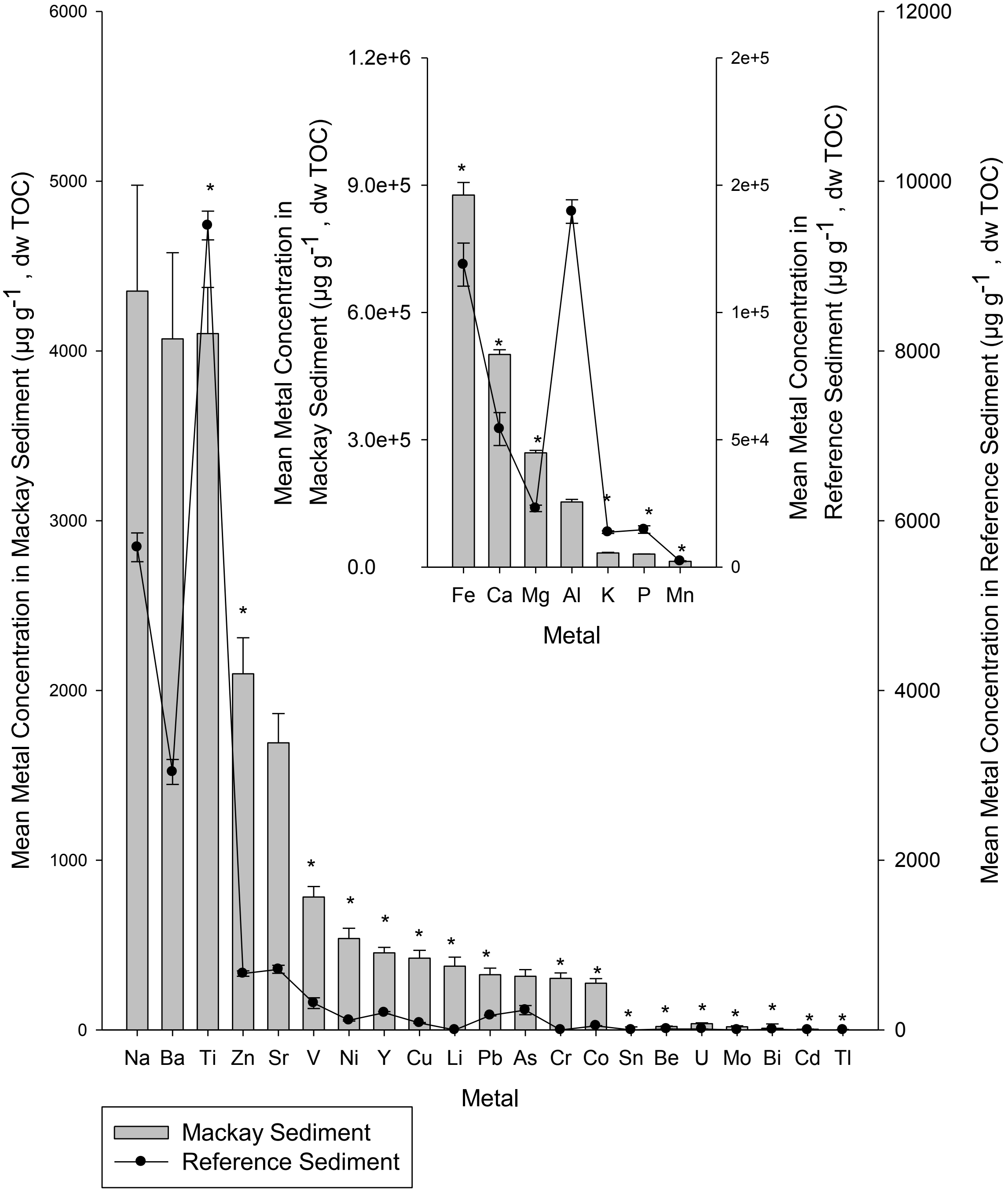


Figure 2.3. Mean (±SE) metal concentration profile (µg g^{-1}, dry weight TOC) detected in MacKay River sediment and reference sediment collected 335 km south of Fort MacKay (n=3). Both types of sediments were used in all four types of exposure conditions. When the p-value of the student t-test was lower than 0.05, the difference between metal concentrations were considered significant (*) (Table A.23).

MacKay River water and reference water samples used in wood frog tadpole SedAq and Aq exposure studies were characterized for metals (Figure 2.4). Water samples from the two sources have similar metal concentrations with the exception of greater concentrations of Ca, Mg, Fe, Sr, Al, Ba, Mn, Li, Ti, Ni, Mo, As, V, and U in MacKay water samples.

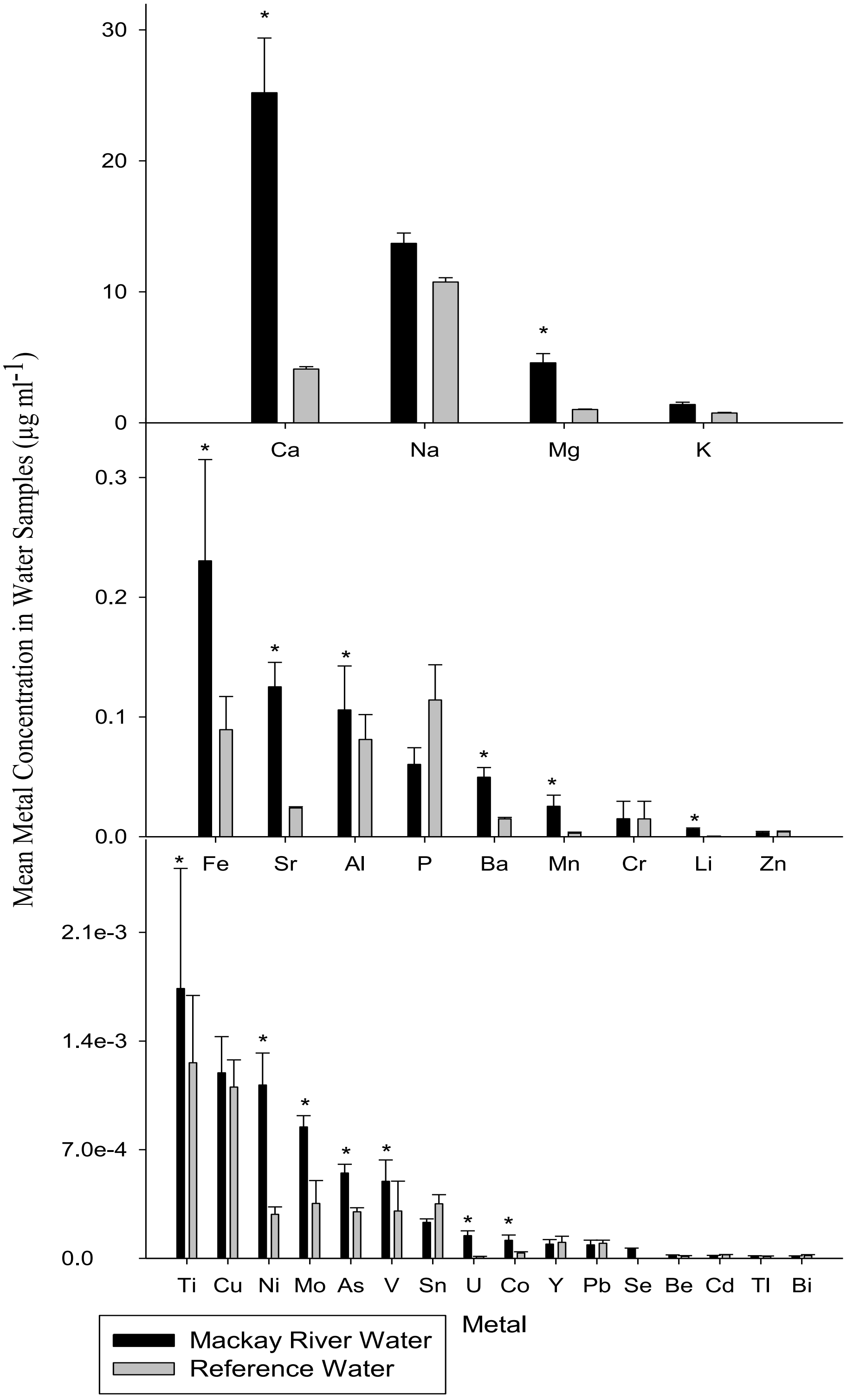


Figure 2.4. Mean (±SE) metal concentrations profile (μg mL^{-1}) detected in MacKay River water and reference water (n=3). Both types of water were used in all four types of exposure conditions.

V and Ni are highly enriched in crude oil and bitumen and are abundantly found in sediments that have been affected by oil sands activities (Lewan *et al.* 1982). V and Ni concentrations in dilbit, MacKay River sediment, and the reference sediment, were characterized and compared in Figure 2.5. Vanadium and nickel concentrations are much greater in dilbit compared to both types of sediment. MacKay sediment contains much higher vanadium and nickel concentrations compared to the reference sediment, suggesting that the MacKay sediment was affected by oil sands processes to a higher degree compared to the reference sediment used in this exposure study.

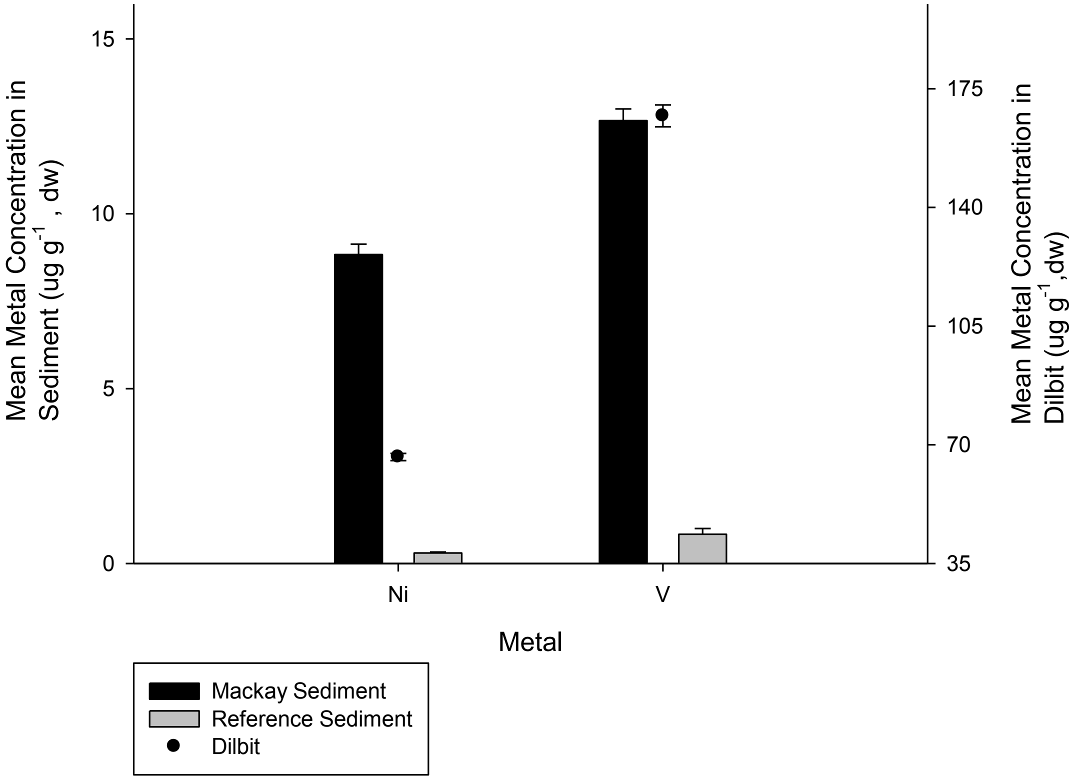


Figure 2.5. Mean (±SE) Ni and V concentrations (µg g^{-1}, dry weight) in McKay River sediment and reference sediment used in the tadpole exposure experiments (n=3). Ni and V concentrations in diluted bitumen (dilbit) are shown for comparison.

Based on guidelines set by the Canadian Council of Ministers of the Environment (CCME), Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn exceeded the guideline for the protection of aquatic life within the Athabasca region, which includes MacKay River sediment (Government of Alberta 2016, Headley *et al.* 2005). Values for these individual metal concentrations in the MacKay and reference sediments are shown in Figure 2.6, where the most abundant metals are (in descending order) Mg, Al, Zn, V, Ni, Cu, Pb, Cr, and Co. With the exception of Al, concentrations of all individual metals in the MacKay sediment are more than double those detected in the reference sediment.

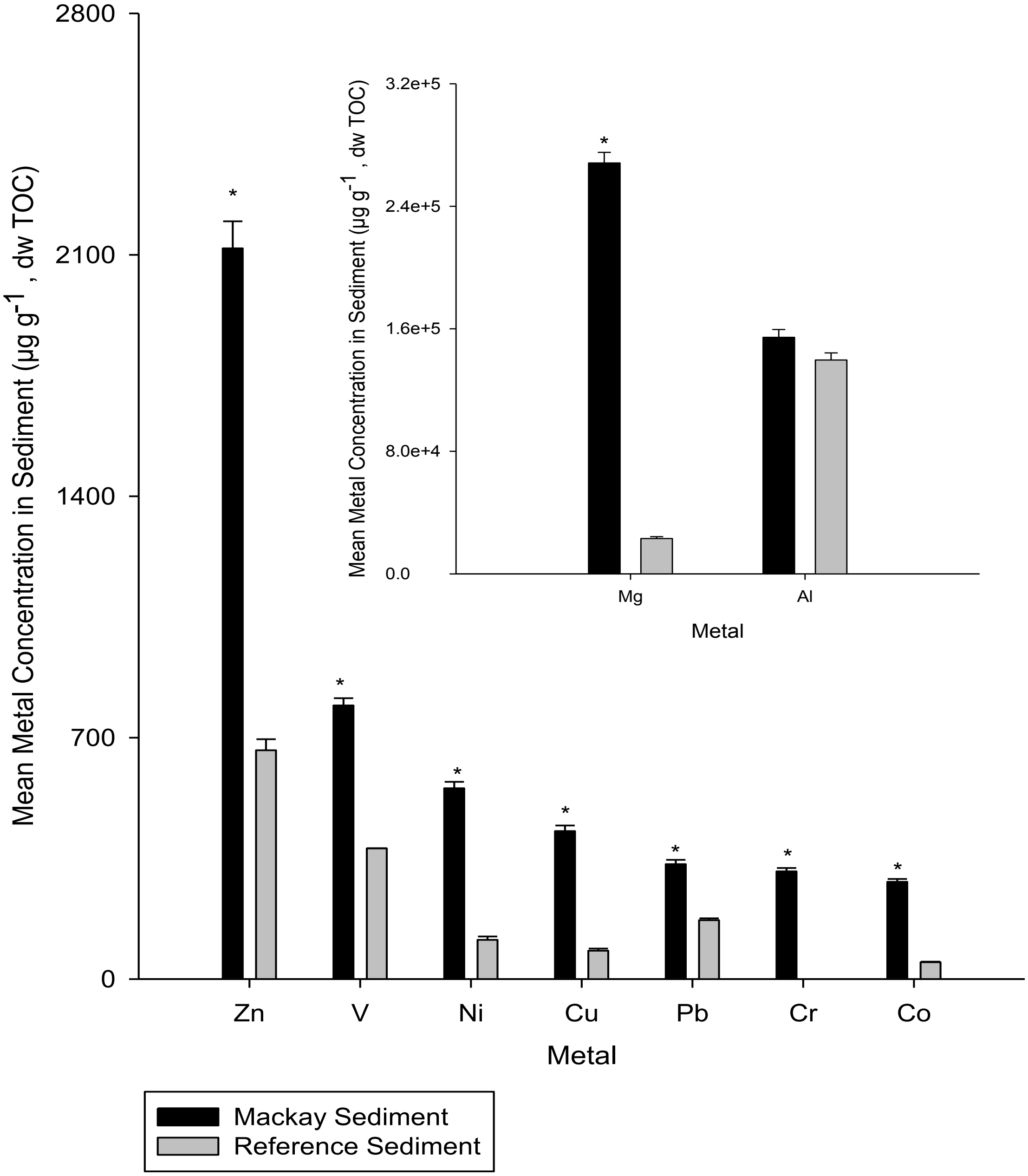


Figure 2.6. Comparison of mean (±SE) metal concentrations (µg g^{-1}, dry weight TOC) in MacKay sediment and reference sediment (n=3). Metals included in this figure exceed guidance concentrations for the protection of aquatic wildlife in water and sediment samples collected downstream of the Athabasca River (Kelly *et al.* 2010). When the p-value of the Student's t-test was lower than 0.05, the difference between metal concentrations was considered significant (*) (Table A.23).

Concentrations of Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn are also similar throughout different time points and tanks during the entire exposure experiment. Metal concentrations in both sediment and water for tanks that consisted of either the MacKay or reference sediment remained unchanged from 0 hours to 96 hours of exposure (Figure 2.7). Metal concentrations detected in water samples experienced an initial spike in concentration during the uptake phase of the study and returned to initial concentrations during the depuration phase when wood frog tadpoles were removed from contaminated conditions and placed in clean flow-through tanks (Figure 2.7). This demonstrates that wood frog tadpoles were exposed to similar water, sediment, and tank conditions for all four exposure conditions throughout the entire experiment. However, it is important to note that water within the depuration flow-through tanks contained metal concentrations, which are defined in Figure 2.7.

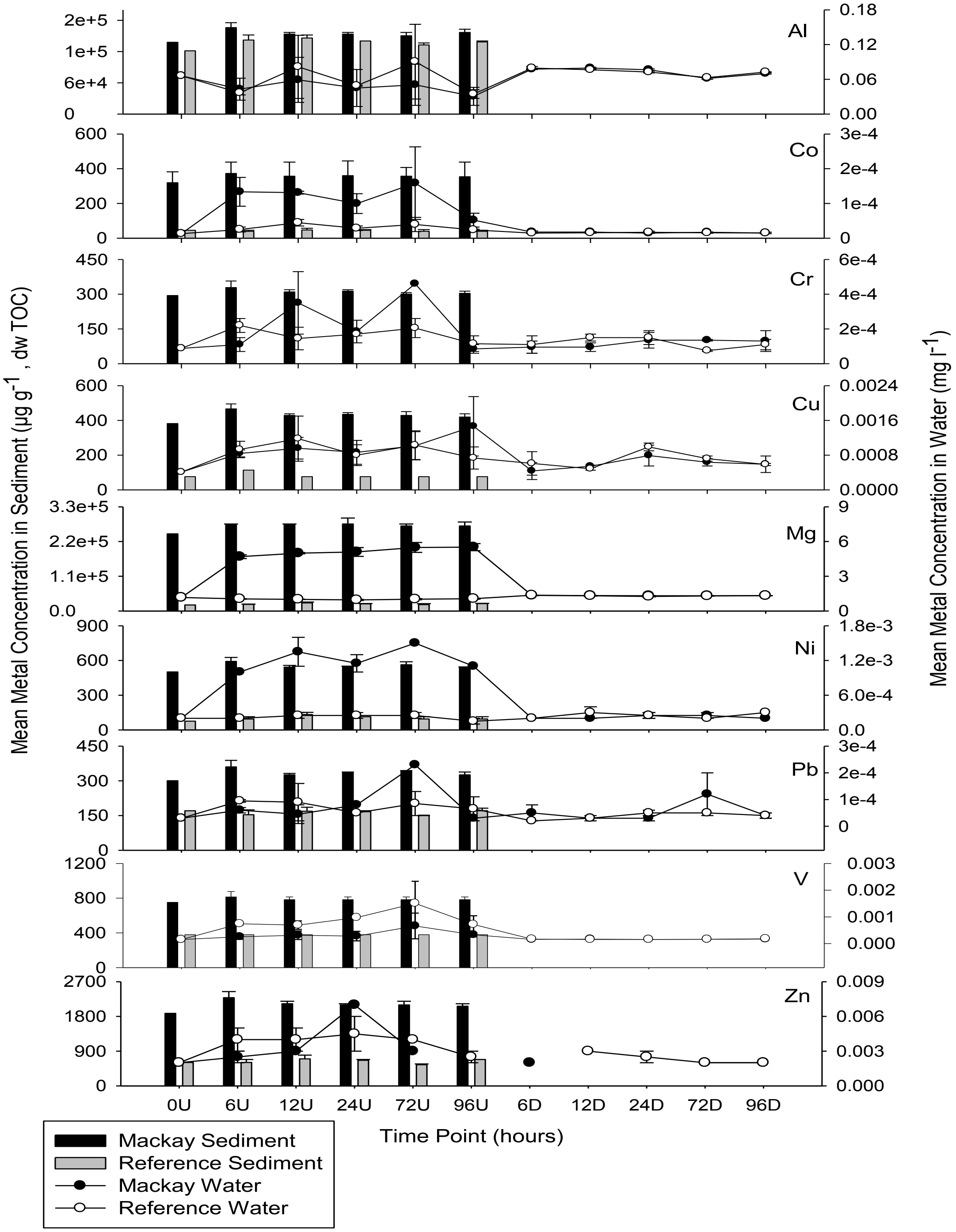


Figure 2.7. Mean (±SE) metal concentrations in sediment (µg g^{-1}, dry weight TOC) and water (mg L^{-1}) samples collected in exposure tanks for each time point (n=3). Concentrations of metals in water and sediment from tanks containing either MacKay sediment or reference sediment are presented. Metals displayed exceed concentrations for the protection of aquatic wildlife in water and sediment samples collected downstream of the Athabasca River.

Wood frog tadpoles that were used for both SedAq and Aq exposure experiments had a mean weight of 0.28 g ww and were a total length of 27.2 ± 4.1 mm long (±SE n=507). Initially, all tadpoles were between Gosner stages 29-31 and experienced no significant growth during the study; wood frog tadpoles had a mean lipid content of 1.64 ± 0.38% (±SE n=3). The mean ingestion rate of sediment during the exposure experiment (3.96 ± 0.37 mg g^{-1} h^{-1} (±SE, n=22)) was determined by dissecting GI tracts from wood frog tadpoles that were exposed to sediment at different time points. These where then dried and weighed to calculate the amount of sediment ingested per hour of exposure (Bilodeau, 2016).

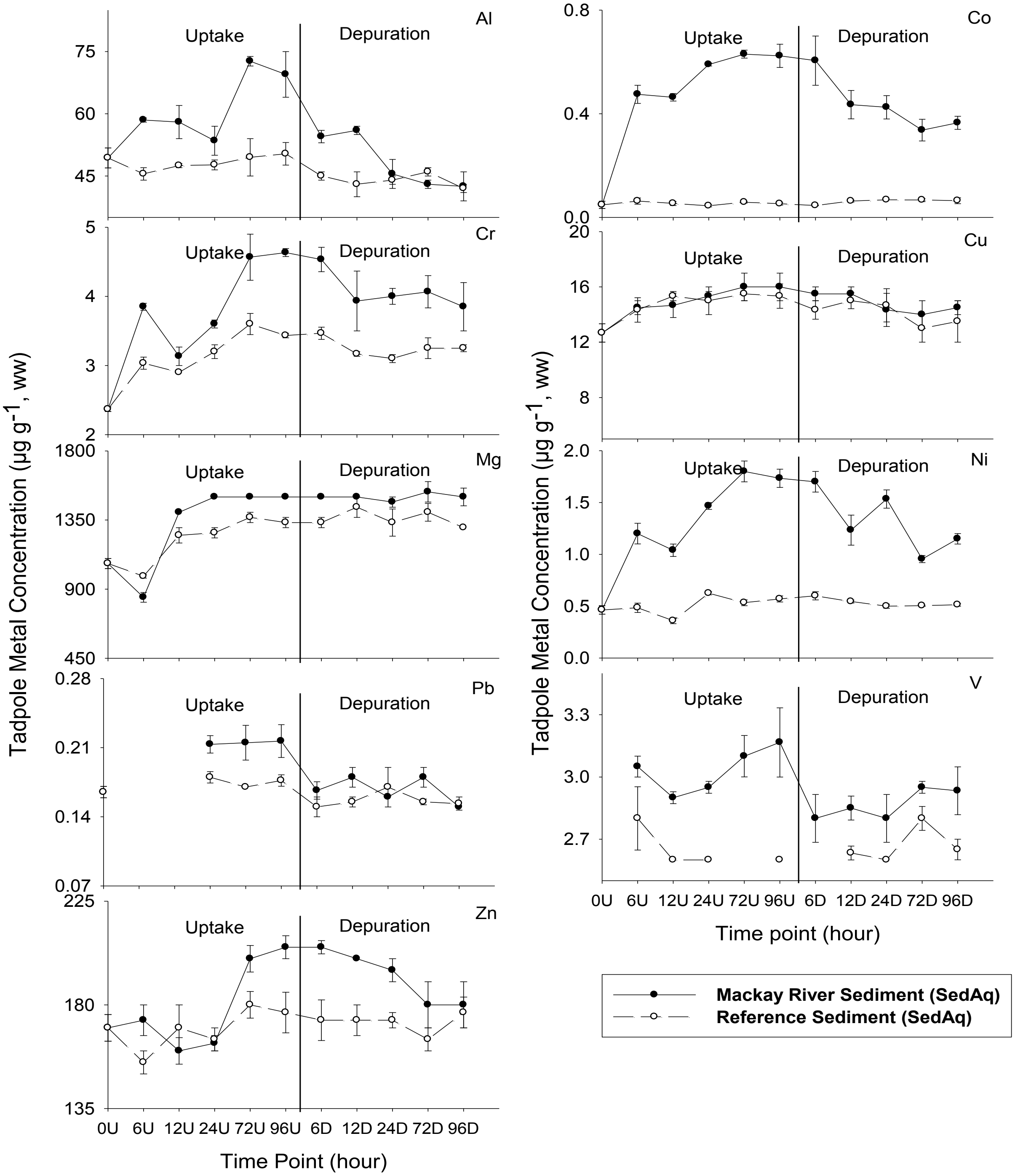


Figure 2.8. Mean (±SE, n=3) metal concentrations in wood frog tadpoles (μg g^{-1}, wet weight) in SedAq tanks. Tadpoles were exposed to MacKay River sediment and reference sediment over 96 hours. Metals included in the analyses were those that exceeded concentration of the protection of aquatic wildlife in water and sediment samples collected downstream of the Athabasca River (Kelly *et al.* 2010). U indicates the uptake phase of the exposure study and D indicates the depuration phase.

Wood frog tadpoles in the SedAq exposure with Mackay sediment accumulated the highest concentrations of all nine metals compared to all other exposure conditions (Figure 2.8, 2.9). Metal uptake concentrations for wood frog tadpoles in the SedAq exposures with MacKay River sediment are significantly greater for Al, Co, Cr, Ni, Mg, V, and Zn when compared to tadpoles in the SedAq exposure tanks with reference sediment; Cu and Pb concentrations were not significantly different between the two exposure conditions (Table 2.1). Concentrations for all nine metals detected in wood frog tadpoles in the Aq exposure with both MacKay and reference sediment and the SedAq exposure with reference sediment are similar. All nine metals that exceed the protection of aquatic life according to the CCME guidelines gradually increased in concentration as time progressed during the uptake phase and gradually decreased in concentration as time progressed during the depuration phase, with the exception of Mg, which did not decrease in concentration during the depuration phase. All nine metals reached an equilibrium or steady state between 24 to 72 hours of exposure during the uptake phase, but only Al, Pb, and Zn returned to initial concentrations after 96 hours of depuration; wood frog tadpoles were unable to fully eliminate the remaining six metals.

Table 2.1. Statistical differences between metal concentrations in wood frog tadpoles exposed to MacKay River sediment (SedAq 96 h) and reference sediment (SedAq 96 h), n=3. Data were log-transformed when normalization was possible. When the p-value of the Student's t-test was lower than 0.05, the difference between metal concentrations were considered significant (*).

	SedAq (96 h)	**SedAq (96 h)**	
Metal	**Mean MacKay (μg g^{-1})**	**Mean Reference (μg g^{-1})**	**p-value**
Al	69.5	50.3	0.01*
Co	0.62	0.05	<0.001*
Cr	4.63	3.43	<0.001*
Cu	16	15.3	0.64
Mg	1500	1330	0.007*
Ni	1.73	0.57	<0.001*
Pb	0.22	0.18	0.09
V	3.17	0.00	<0.001*
Zn	205	177	0.038*

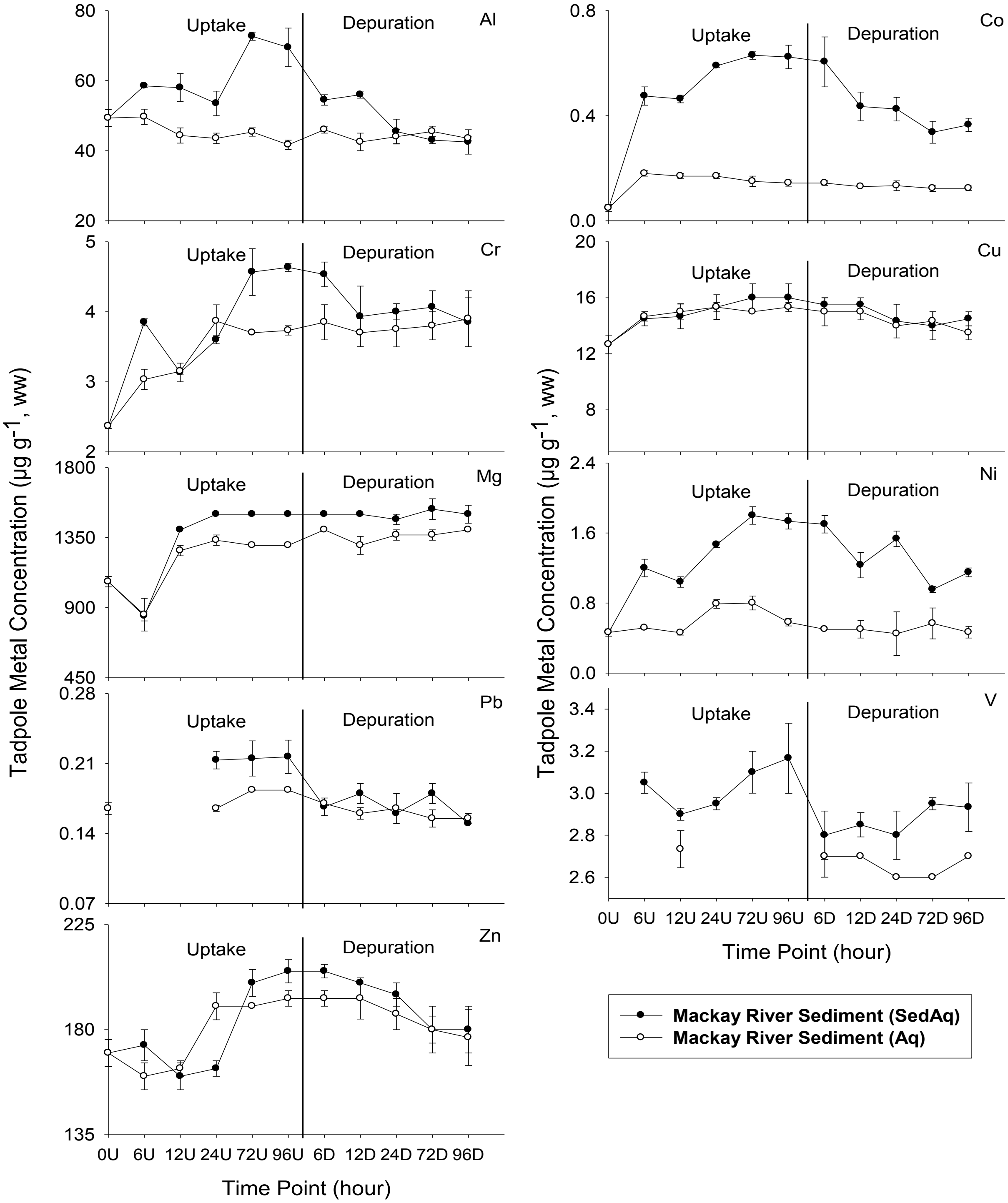


Figure 2.9 Mean (±SE) concentration of metals (exceeding the concentration for the protection of aquatic wildlife in water and sediment according to CCME guidelines) in wood frog tadpoles (μg g^{-1}, wet weight), n=3, in SedAq and Aq tanks over different time points (hours). Uptake (U) time points indicate the uptake phase of the exposure study and depuration (D) time points indicate the depuration phase.

Uptake and depuration concentrations not presented are a result of metal concentrations falling below detection limits. In addition, wood frog tadpoles in the SedAq and Aq tanks exposed to MacKay River sediment have significantly different uptake concentrations. The SedAq exposure condition resulted in significantly greater uptake concentrations for Al, Co, Cr, Ni, Zn, and V; Cu, Mg, and Pb concentrations were similar between the SedAq and Aq exposures and were not significantly different (Table 2.2). Metal concentrations below the detection limit are not shown in Figures 2.8 and 2.9.

Table. 2.2. Metal concentrations in wood frog tadpoles exposed to MacKay River sediment (SedAq 96 h) and MacKay River sediment (Aq 96 h), n=3. Data were log-transformed when normalization was necessary. When the p-value of either the Mann-Whitney (M-W) or the student t-test (T-test) was lower than 0.05, the difference between metal concentrations are considered significant (*).

	SedAq (96 h)	**Aq (96 h)**		
Metal	**Mean MacKay (μg g^{-1})**	**Mean MacKay (μg g^{-1})**	**p -value**	**Statistical Test**
Al	69.50	41.7	0.001*	T-test
Co	0.62	0.14	<0.001*	T-test
Cr	4.63	3.73	<0.001*	T-test
Cu	16.00	15.3	0.56	T-test
Mg	1500	1300	0.10	M-W
Ni	1.73	0.58	<0.001*	T-test
Pb	0.22	0.18	0.12	T-test
V	3.17	2.60	0.027*	T-test
Zn	205	193	0.06	T-test

The biota-sediment accumulation factors (BSAFs) and bioconcentration factors (BCFs) between types of metals were examined (Table 2.3). There are several differences between BSAFs and BCFs of tadpoles exposed to MacKay River sediment and reference sediment after 96 h of exposure. BSAFs for tadpoles exposed to MacKay River sediment in the SedAq exposure are greater for all nine metals that were above water quality

Table 2.3. Differences between biota-sediment accumulation factors (BSAF) and bioconcentration factors (BCF) of metal concentrations above water quality guidelines for the protection of aquatic organisms for four exposure conditions conducted on wood frog tadpoles exposed to MacKay River sediment and a reference sediment.

	MacKay		**Reference**	
	SedAq	**Aq**	**SedAq**	**Aq**
Metal	**Mean BSAF** (±SE) **(t=96 h)**	**Mean BCF** (±SE) **(t=96 h)**	**Mean BSAF** (±SE) **(t=96 h)**	**Mean BCF** (±SE) **(t=96 h)**
Al	0.03 (2.2E-03)	585 (0.66)	0.02 (4.4E-04)	722 (2.3)
Co	0.13 (0.01)	1670 (3.4)	0.07 (0.01)	1500 (5.6)
Cr	0.65 (0.33)	248 (0.10)	-	-
Cu	2.33 (0.12)	10700 (2.1)	1.1 (0.16)	10200 (7.2)
Mg	0.33 (0.01)	340 (0.18)	0.32 (0.02)	422 (0.79)
Ni	0.19 (0.02)	457 (0.64)	0.11 (0.01)	861 (5.9)
Pb	0.13 (3.6E-03)	3110 (15.5)	0.04 (0.01)	2440 (9.5)
V	0.28 (0.01)	4850 (12.1)	-	-
Zn	5.87 (0.08)	40200 (10.7)	5.58 (0.28)	61700 (1.9)

guidelines for the protection of aquatic organisms. Cr and V are only detectable in tadpoles that participated in the SedAq tanks and were exposed to MacKay River sediment (Table 2.3). In addition, BCFs were similar, if not greater, for wood frog tadpoles in the aqueous only exposure to reference water compared to tadpoles exposed to MacKay River water (Table 2.3).

2.5. Discussion

2.5.1. Sediment + Aqueous (SedAq) and Aqueous Only (Aq) Exposure

The majority of metals that accumulate in aquatic environments reside in sediment and are more likely to be exposed to and taken up by benthic and epibenthic organisms (Figure 2.7). One of the main objectives of this study is to assess the importance of including both sediment and aqueous exposures to *L. sylvaticus* tadpoles through two accumulation-elimination experiments (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone). Wood frog tadpoles involved in the SedAq (96 h) experiment resulted in greater overall metal bioaccumulation concentrations (51.17 ± 42 mg g^{-1} at t=96 h) than those from the Aq (96 h) experiment (24.54 ± 42 mg g^{-1} at t=96 h). Therefore, accumulation of metals was greater in wood frog tadpoles that were exposed to MacKay River sediment compared to wood frog tadpoles that were only exposed to water contaminated by MacKay River sediment. Evidently, when tadpoles have contact with sediments, the dominant route of metal exposure is from sediment. It is important to note that tadpoles in control tanks, that only contained uncontaminated water and dividers, did not accumulate additional metals from the materials used to construct the dividers and that differences in metal uptake between exposure conditions are due to differences in sediment and sediment accessibility.

In aquatic ecosystems, sediment acts as a main sink for metal contaminants that are introduced into the environment from natural and anthropogenic sources and can therefore be a source for metal exposure to aquatic organisms (Alonso *et al.* 2013, Milani *et al.* 2002, Amato 2007). Through two exposure experiments, using spiked sediment and freshwater, Campana (2013) exposed snails, *Hydrobia ulvae*, to both sediment and water that were spiked with Cu, Cd, and Zn and determined that sediment ingestion, specifically, was the most significant route of metal assimilation. In addition, Metian and Warnau (2009) performed a 13-day study exposing scallops, *Pecten maximus*, to Co, Mn, and Zn; they explored three different routes of uptake, which included uptake via water, sediment, and diet. It was determined that uptake and bioaccumulation was highest for sediment, which accounted for 88% of Zn and 89% of Co uptake and bioaccumulation. It was also determined that more than 85% of metals taken up from sediment were strongly retained in scallop tissue despite a lower transfer efficiency for metals bound to sediment than when dissolved in water. Conversely, we observed lower metal transfer efficiency in sediment relative to water. This is evidenced by similar metal concentrations in wood frog tadpoles exposed to MacKay River sediment in both the SedAq and Aq exposures where Cu, Cd, and Zn uptake and elimination concentrations are similar. Furthermore, previous studies have reported greater metal concentrations in sediment compared to water (Singh *et al*. 2016, Oyoo-Okoth *et al. 2012*). Sediment can therefore contribute significantly to the total metal uptake in aquatic organisms, which is evident when comparing the concentrations of Al, Co, Cr, Ni, Pb, and V in wood frog tadpoles; these concentrations were greater in tadpoles exposed to MacKay River sediment in the SedAq exposure versus the tadpoles in the Aq exposure. Evidently, it is important to consider sediment exposure when assessing the uptake and bioaccumulation of metals in aquatic organisms.

2.5.2. MacKay River Sediment and Reference Sediment

This study was able to determine (1) that sediment exposure for Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn is more important than aqueous exposure in amphibian larvae and (2) that sediment collected closer to oil sands activity (MacKay River sediment) results in greater metal uptake concentrations and bioaccumulation compared to sediment collected from an isolated lake located 335 km south of the oil sands mining area (reference sediment). Wood frog tadpoles exposed to the MacKay River sediment accumulated greater amounts of metals, exceeding water quality guidelines for the protection of aquatic organisms compared to tadpoles exposed to reference sediment (Kelly *et al.* 2010). Correspondingly, Co, Cr, Ni, and V are more concentrated in wood frog tadpoles exposed to MacKay River sediment at various time points throughout the depuration phase. It was predicted that wood frog tadpoles exposed to the contaminated MacKay River sediments would accumulate higher metal concentrations and result in larger biota-sediment accumulation factors (BSAF) compared to tadpoles exposed to reference sediment. BSAFs were greater for metals in tadpoles exposed to MacKay sediment (SedAq exposure) than for tadpoles exposed to reference sediment (SedAq exposure).

Ziyaandin *et al.* (2016) collected mollusks from different contaminated sites in Iran and found BSAFs for Ni, Cu, As, Pb, Hg, and Cd are higher when there are higher concentrations of these metals detected in the sediment from different wetlands. In addition, Thomann *et al.* (1995) measured BSAF values along a gradient of metal contamination and found BSAFs are three orders of magnitude greater for Zn, Cd, Ni, Pb, Cr, and Hg in eastern oysters and blue mussels in the more contaminated sites. Similarly, our study suggests that aquatic organisms located in habitats within a 50 km radius of oil sands operations are susceptible to greater bioaccumulation of metals than aquatic organisms located 3350 km south. Aquatic organisms closer to oil sands activity are also capable of bioaccumulating a larger selection of metals, including Cr and V.

Background levels of metals in the wood frog tadpoles in this study (at t=0 h) varied considerably between exposures and were higher than expected. The tadpoles used in this laboratory study may have had higher metal concentrations since they were raised in water with a similar metal profile and housed in aquaria tanks that could not be aggressively acid washed in order to maintain the structural integrity of the tanks. In addition, the food provided as well as wood frog tadpole feces could have introduced additional sources of metal exposure. Nonetheless, metal concentrations at t=0 h were low enough in the wood frog tadpoles to observe the clear uptake and accumulation of metals once they were placed in the exposure tanks.

2.6. Conclusion

This study compared accumulation of Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn in *L. sylvaticus* tadpoles exposed to a contaminated sediment (MacKay River) collected from the Athabasca oil sands region and a reference sediment located far from contamination sources. It is clear that wood frog tadpoles, exposed to sediment collected within the oil sands mining area significantly accumulated more Al, Co, Cr, Ni, Mg, V, and Zn compared to wood frog tadpoles exposed to sediment collected from an uncontaminated and isolated lake situated 335 km south of the oil sands mining area. Al, Co, Cr, Ni, and V uptake and accumulation are also significantly greater in tadpoles exposed to both sediment and water compared to tadpoles exposed to water alone, indicating that the dominant route of exposure for wood frog tadpoles is sediment. Evidently, this laboratory study highlights the possible impact of oil sands mining with regards to metal uptake and bioaccumulation and how important it is to include sediment exposures in metal toxicokinetic studies for aquatic organisms.

3.0. System Dynamics Modeling

3.1. Introduction

The use of animals in the field of chemical and environmental toxicology provides important information on the effects of anthropogenic contaminants on physiological pathways and ecosystem health (Duarte *et al.* 2017, Ferrante *et al.* 2017, Khidkhan *et al.* 2017). Animal testing is commonly used to assess contaminated sites and to determine the risk to humans (Gao 2017, Tiecher *et al.* 2017). The most impactful and reliable data generated from animal testing include those with replication of experimental methods. As a result, these studies use and sacrifice a large number of test organisms.

Toxicological and chemical databases have expanded substantially during recent years as computational methods and programs have been fine-tuned, so that data produced by *in silico* approaches to toxicity assessments are more reliable and can be incorporated to biomonitoring programs (Knudsen 2013, Kongsbak *et al.* 2014). The U.S. EPA and the National Research Council have recommended that *in silico* approaches should be included in future assessments of toxicology with the aim to reduce animal testing and refine existing methods (Taboureau 2016, NRC, 2007). While computational simulations cannot completely substitute for biological testing, they can help focus on particular substances and targets, thus giving priority to contaminants of greater concern and allowing for more efficient testing. *In silico* methods can also be used as predictive tools in ecotoxicology risk assessments (Faust *et al.* 2003, Kim *et al.* 2012). The discovery of parameters that are not definable through experimental testing due to limitations can be calculated or estimated using computational toxicology methods. The main functions of computational toxicology are to effectively assess hazards posed by chemicals in the environment, determine the risk of chemicals at low levels of exposure, more accurately predict the effects of chemicals across species, and to reduce experimental cost (US EPA 2007). Ultimately, computational approaches can reduce the number of animals used in experiments and can be used as tools that exercise the 3Rs (replacement, reduction, refinement). In this study, the bioaccumulation data collected in the exposure study will be modeled using STELLA^{TM} system dynamics software and equations 3.1 – 3.6. Not only will this allow us to define toxicokinetic parameters and variables, such as assimilation efficiencies, uptake rate coefficients and elimination rate coefficients, it can also be used as a predictive tool for biomonitoring programs and risk assessments related to oil sands activities.

3.1.1. STELLA^{TM} System Dynamics Software

Systems dynamics is a methodology for studying and managing complex systems that change over time. The method uses computer modeling to focus on the information feedback loops that give rise to dynamic behavior. Systems thinking, experimental learning laboratory with animation (STELLA^{TM}) is a systems dynamic modeling program that allows users to run mathematical models created as graphical representations of a system using four fundamental building blocks known as stocks, flows, converters, and connectors. Stocks are represented as the squares (Figure 3.1) and can be defined as a reservoir that collects calculated data over a period of time; they represent any entity that accumulates or depletes over time. Flows are represented as the circle and arrow figures placed on either side of a stock (Figure 3.1) and are defined as inputs and outputs that influence the rate of change of a connected stock. Converters are the individual circles displayed in Figure 3.1 and are defined as variables that influence a flow, either increasing or decreasing the influence a flow has on a stock. The arrows connecting the converters to the flows are defined as connectors and are responsible for delegating which set of converters will influence a flow.

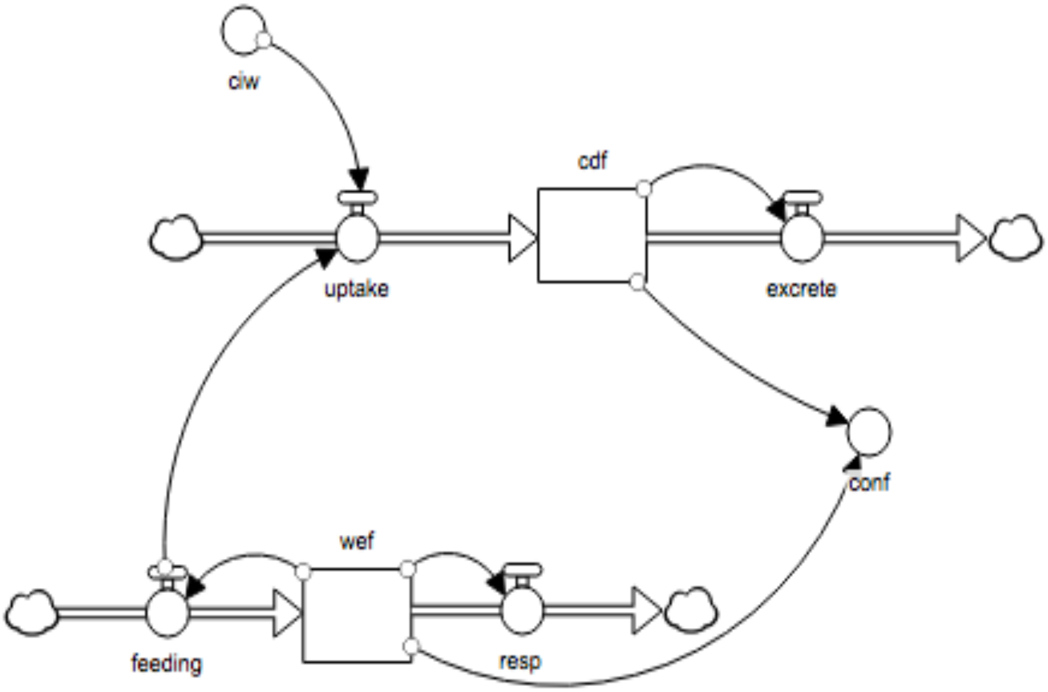


Figure 3.1. Example of a STELLA^{TM} generated model representing the system dynamics of Cd uptake in fish. Variables include the weight of the fish (wef), metal concentration in the water (ciw), concentration in fish (conf), feeding rate, uptake efficiency and respiration rate of fish (resp) (Azanu *et al.* 2016).

STELLA^{TM} has been used in many studies to assess the mass transfer of a variety of chemical substances including polycyclic aromatic hydrocarbons (PAHs) and atrazine (Kim *er al.* 2011, Ouyang *et al.* 2010). Kim (2011) used STELLA^{TM} to generate a mass balance model to estimate the transport of PAHs to a coastal environment in Masan Bay, Korea, which receives large amounts of industrial and sewage discharges and waste from shipping activity. STELLA^{TM} generated PAH concentrations that were similar to laboratory measured PAH concentrations in sediment and water samples collected from Masan Bay with a relative error of 5.89%.

3.1.2. Metal Bioaccumulation

An ideal uptake and elimination pattern is demonstrated in Figure 3.2. During the exposure phase, there should be a short, gradual increase in the concentration of contaminants in test animals; this concentration should eventually reach a steady state. A steady state generally indicates that the exposed organism has reached its accumulation maximum. During the depuration phase (when the animals are removed from the contaminated medium), there should be a steady, gradual decrease in the concentration of contaminants. System dynamic model programs, such as STELLA^{TM}, Powersim and

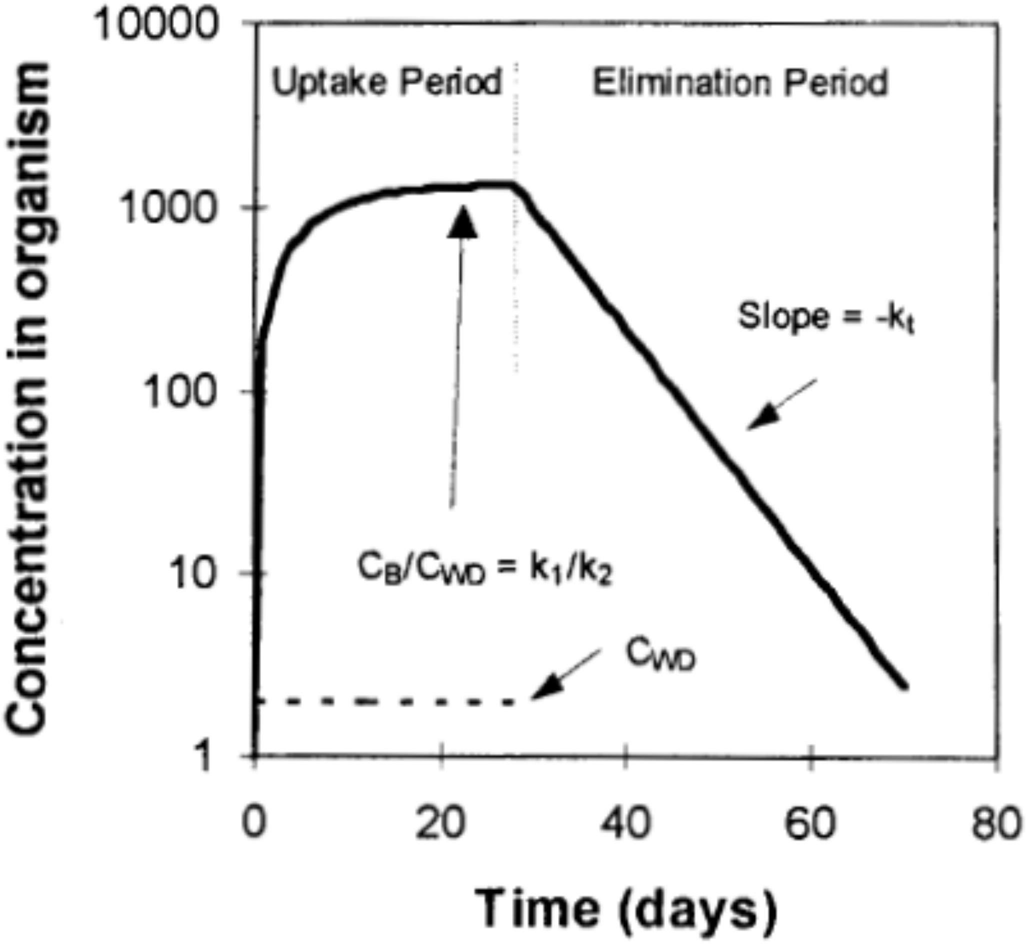


Figure 3.2. Illustrated example of uptake and elimination period modeled though time. This type of data is generated with an uptake-elimination study. During the uptake phase, kinetics are dictated by both uptake and elimination. The organisms are then allowed to depurate and kinetics in this elimination phase is solely a consequence of elimination (Newman 2010).

Vensim, are capable of modeling nonlinear curves as seen in Figure 3.2 through stock and flow models. Previous studies suggest that aquatic organisms that accumulate metals from different environmental media over time reach a steady state within 7 days of exposure and experience lower first order elimination rate constants than uptake rate constants. For example, Wadige *et al.* (2017) exposed mussels, *Hyridella australis*, to mining-impacted sediment collected from the Molonglo River in New South Wales for

28 days. It was reported that laboratory exposed *H. australis* reached a maximum uptake concentration for Zn, Cu, Pb, and Cd after 3 days of exposure; *H. australis* caged within nearby wetlands in the field experienced similar uptake rates, but resulted in higher total metal concentrations. System dynamic models can be used to predict maximum uptake concentrations and bioaccumulation potential for exposed metals in aquatic organisms based on kinetic parameters and metal concentrations found within different environmental media.

3.1.3. Study Objectives

The objective of this study is to define toxicokinetic parameters for metal uptake and elimination by wood frog tadpoles exposed to MacKay River sediment and reference sediment. These parameters will be determined using data collected from the uptake-depuration exposure experiment and results of these parameters will be modeled using system dynamics modeling. It is predicted that stock and flow models created using STELLA^{TM} will be capable of generating uptake and elimination curves similar to those defined in the uptake-depuration experiment. Variables, such as metal concentrations in sediment (C_s, µg g^{-1}), metal concentrations in water (C_w µg mL^{-1}), sediment ingestion rate (R, g h^{-1}), and metal concentrations in tadpoles at various time points (C_t, µg g^{-1}), are known to influence the uptake and elimination of metals in aquatic organisms, and will be defined by the laboratory uptake-depuration exposure experiment performed in June 2016. Other variables, such as metal absorption efficiency (α), metal uptake rate (k_1, h^{-1}) and elimination rate (k_2, h^{-1}), were solved for using equations 3.2 to 3.6. Not only will this allow us to mathematically estimate metal concentrations for time points that were not included in the uptake-depuration laboratory experiment, stock and flow models developed by STELLA^{TM} can also be used as a predictive tool when assessing the bioaccumulation of metals in wood frog tadpoles that are located in different wetlands within the oil sands formation.

3.2. Methodology

3.2.1. Toxicokinetics

Exposure conditions were modeled computationally using variables that were determined during the experimental exposure study performed in 2016. A STELLA^{TM} systems dynamic diagram was generated to model equations that are used to predict the uptake of metals over a 96-hour exposure study. The uptake rate coefficient was determined by fitting the experimental data to the following equation:

$$k_1 = \frac{m}{C_w} \tag{3.1}$$

Where the slope (m) is defined by the metal concentrations detected in wood frog tadpoles (µg g^{-1}) over time (h), k_1 (h^{-1}) is the uptake rate coefficient and C_W (µg mL^{-1}) is the metal concentration detected in the water of the exposure tank.

Aqueous only (Equation 3.2), sediment only (Equation 3.3), and sediment+aqueous (Equation 3.4) uptake from water and dietary exposure experiments were determined using the following equations (Newman 2009):

$$C_t = C_W \cdot \left(\frac{k_1}{k_2}\right) . (1 - e^{-k_2 t}) \tag{3.2}$$

$$C_t = \frac{\alpha R C_S}{k_2} . (1 - e^{-k_2 t}) \tag{3.3}$$

$$C_t = \frac{C_W * k_1 + \alpha R C_S}{k_2} . (1 - e^{-k_2 t}) \tag{3.4}$$

Where k_2 (h^{-1}) is the elimination rate coefficient, t is the time of the uptake phase (h), C_t ($\mu g\ g^{-1}$) is the metal concentration in wood frog tadpoles at 96 h, and C_W ($\mu g\ mL^{-1}$) is the metal concentration detected in the water of the exposure and depuration tanks. Equation 3.2 modeled the uptake of metals from water only, Equation 3.3 modeled the uptake of metal contaminates from sediment ingestion only, and Equation 3.4 modeled the uptake of metal contaminants from both water absorption and sediment ingestion. The absorption efficiency is represented as α, R ($g\ h^{-1}$) is the rate of sediment ingestion, and

C_S (µg g^{-1}) is the measured concentration of metals within the sediment the tadpoles were ingesting.

$$SS = \sum(C_{modeled} - C_{measured})^2 \tag{3.5}$$

The sum of squares (SS) between experimentally measured data ($C_{measured}$, µg g^{-1}) and modeled data ($C_{modeled}$, µg g^{-1}) was used to define optimum assimilation efficiencies (α) and uptake and first order elimination rate coefficients (k_1 and k_2, h^{-1}).

$$\frac{C_B}{C_W} = \frac{k_1}{k_2} \tag{3.6}$$

To determine the elimination rate coefficients of individual metals, equation 3.6 was used where C_B is the metal concentration detected in wood frog tadpoles at steady state in µg g^{-1} and k_1 and k_2 are uptake and elimination rate constants in h^{-1} units.

3.2.2. Stock and Flow Model Development

A stock and flow skeleton was created in order to be able to model the toxicokinetics of metal contaminants in wood frog tadpoles exposed to sediment collected near oil sands activity. The stock and flow model presented in Figure 3.3 is a projection of equation 3.4, which is used to model the metal concentrations being taken up by frog tadpoles involved in the sediment + aqueous exposure tanks. All variables were defined by the exposure experiment, which include initial and final metal concentrations (C_f, C_i), uptake and elimination rate constants (k_1, k_2) and sediment ingestion rates (R). Assimilation efficiencies (α) were defined by best fit in the SedAq accumulation trials, using k_1 and k_2 values determined from the Aq accumulation study. Best fit was achieved by minimizing the sum of squares between modeled and measured metal concentrations in the SedAq exposures (Equation 3.5).

$$C_t = \frac{C_W k_1 + \alpha R C_S}{k_2} . (1 - e^{-k_2 t})$$

Sediment + Aqueous

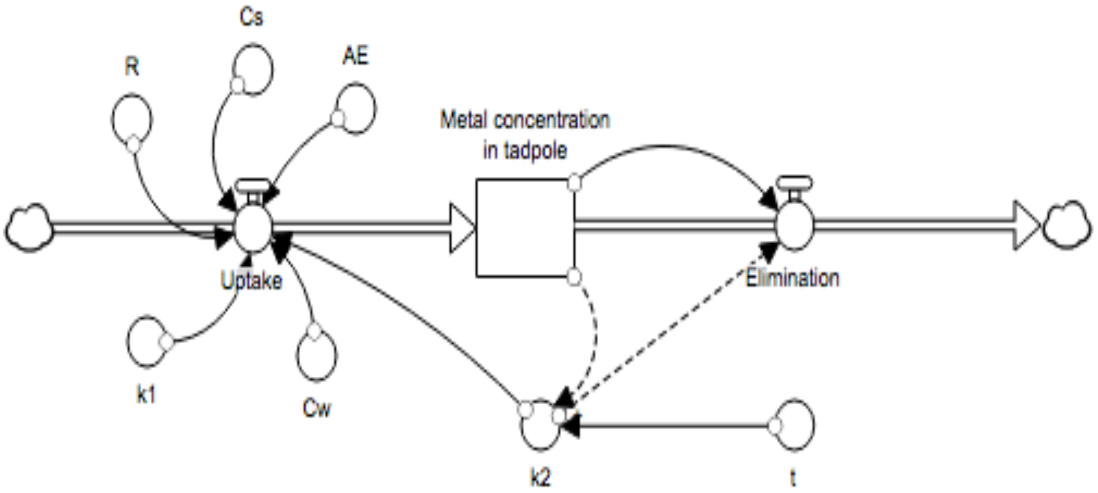


Figure 3.3. STELLA^{TM} generated stock and flow model used to calculate the toxicokinetics of metal uptake in wood frog tadpoles exposed to sediment collected near oil sands mining activity. The variables involved include uptake rate (k_1), assimilation efficiency (AE, α), ingestion rate (R), metal concentration in sediment (C_s), metal concentration in water (C_w), depuration rate (k_2) and time (t).

As seen in Figure 3.3, the stock in the model is defined as the metal concentration that accumulates in the tissue of the exposed wood frog tadpoles and the two flows are defined as the metal contaminants being taken up and eliminated by the exposed wood frog tadpoles. In addition, all converters are variables that influence the uptake and elimination of metals as shown in Equation 3.3. The “metal absorbed” flow is influenced by both the metal uptake rate and elimination rate. This is because both factors are simultaneously active during the exposure experiment.

$$\boxed{\begin{array}{l} lnC_F(t) = lnC_O - k_2t \\ \textbf{First Order Elimination Rate} \end{array}}$$

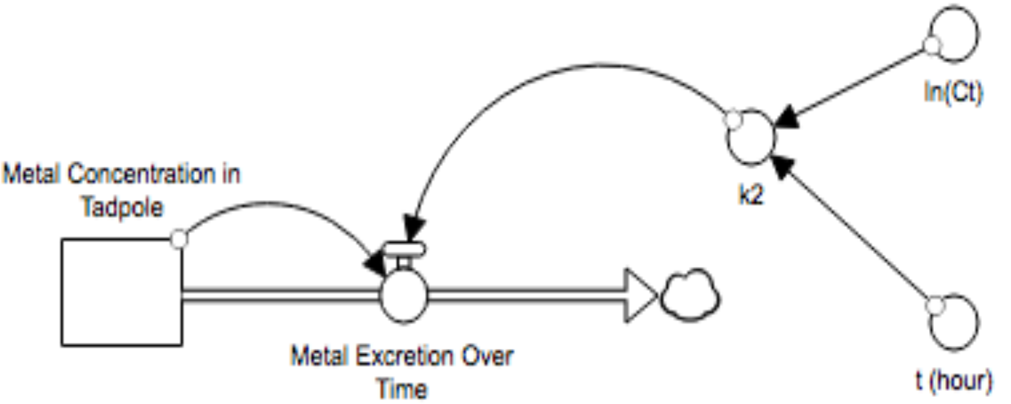


Figure 3.4. STELLA^{TM} generated stock and flow model used to calculate the toxicokinetics of metal elimination in wood frog tadpoles exposed to sediment collected near oil sands mining activity and then placed in flow-through depuration tanks where elimination rates (k_2) are in h^{-1} units, ($\ln(C_t)$) is the natural log of metal concentrations of tadpoles and t if time in h units.

The stock and flow model in Figure 3.4 was used to calculate the rate of metal elimination in tadpoles during a theoretical period of depuration. Metal concentrations were analyzed at different time points to properly model the elimination of metals over time. The stock in Figure 3.4 is defined as the tadpole and represents the concentration of metals within the wood frog tadpole. The flow is defined as the amount of metals being eliminated by the wood frog tadpoles as time progresses. The converters, k_2, *ln*C_0(t), and time, are factors influencing the flow as described by equation 3.2. Not only were all input parameters collected from the uptake-elimination exposure experiment for tadpoles that were exposed to MacKay River sediment (Table 3.2A), they were also collected from the uptake-elimination exposure study for tadpoles exposed to reference sediment as well (Table 3.2B). In order to account for the metal concentrations detected in the water of the flow-through tanks, k_2 values were calculated using Equation 3.4. In addition, different uptake and elimination rate coefficients were defined using both experimental data and computational modeling (Table 3.1). All uptake and elimination rate coefficients were used to optimize the STELLA^{TM} generated systems dynamic models in order to determine the k_1 and k_2 values that produce the best fit corresponding to the experimentally measured metal concentrations over time. Modeled uptake and elimination coefficients were determined and optimized using STELLA^{TM} (Figure 3.3). Measured uptake and elimination coefficients were determined by the slope of the concentration of metals detected in wood frog tadpoles over time as defined by the uptake-elimination experiment. Uptake and elimination values were also calculated using Equation 3.4 and by estimating metal concentrations for time intervals using the difference between metal concentrations defined by measured time points (Table A.22).

Table 3.1. Uptake and elimination rate constants generated through computational modeling (±SE). Modeled rates were generated using a combination of systems dynamic modeling and equation 3.4, 3.5 and 3.6.

	MacKay River Sediment Exposure		**Reference Sediment Exposure**	
Metal	**k_1** (±SE) **h^{-1}**	**k_2** (±SE) **h^{-1}**	**k_1** (±SE) **h^{-1}**	**k_2** (±SE) **h^{-1}**
Al	1.54 (0.05)	0.04 (0.01)	1.21 (0.6)	0.01 (8.2E-03)
Co	0.26 (0.03)	0.1 (0.01)	9.0E-03 (1.5E-03)	0.05 (7.1E-03)
Cr	1.44 (0.44)	0.02 (3.1E-03)	0.21 (0.07)	8.0E-03 (2.7E-03)
Cu	0.2 (0.04)	0.1 (6.4E-03)	0.05 (0.02)	9.0E-04 (7.2E-05)
Pb	1.73 (0.58)	0.22 (0.02)	0.26 (0.08)	0.07 (1.9E-03)
Mg	4.5 (0.12)	0.07 (0.02)	1.56 (0.3)	0.05 (0.01)
Ni	0.54 (0.04)	0.05 (0.1.3E-03)	0.28 (0.02)	9.1E-03 (7.4E-03)
V	0.76 (0.03)	0.14 (0.08)	0.35 (0.11)	0.07 (0.02)
Zn	2.71 (0.87)	0.05 (0.01)	1.38 (0.77)	0.02 (2.6E-03)

3.3. Results

3.3.1. Metal Uptake and Depuration Modeling

Numerous variables were defined during the uptake-elimination laboratory study, such as time (t), sediment ingestion rate (R), metal uptake concentration in wood frog tadpoles (C_t), metal concentration in sediment to which tadpoles (C_s) were exposed and metal concentration in the water (C_w) for individual metals. Variables were determined for tadpoles exposed to MacKay River sediment and tadpoles exposed to reference sediment (Table 3.2).

Table 3.2. Variables defined by the experimental uptake-elimination exposure experiment used in order to predict metal bioaccumulation and uptake and elimination kinetics for wood frog tadpoles exposed to MacKay River sediment (A) and reference sediment (B) using STELLA^{TM} software. Time is defined as t, assimilation efficiency is defined as α, R is the ingestion rate, C_t is the concentration of metals in wood frog tadpoles during steady state, C_i is the initial concentration of metals detected in wood frog tadpoles, C_s is the metal concentration in sediment, and C_w is the metal concentration in water.

MacKay River Sediment Exposure

A

Metal	**t** **h**	**α**	**R** **g h^{-1}**	**C_t** **µg g^{-1}**	**C_i** **µg g^{-1}**	**C_s** **µg g^{-1}**	**C_w** **µg mL^{-1}**
Al	96	0.08	3.96E-03	69.5	47.0	2470	0.11
Co	96	0.45	3.96E-03	0.62	0.03	4.50	1.16E-04
Cr	96	0.53	3.96E-03	4.56	2.40	15.0	0.01
Cu	96	0.32	3.96E-03	15.4	12.7	68.5	1.20E-03
Pb	96	0.20	3.96E-03	0.22	0.17	5.32	8.67E-05
Mg	96	0.51	3.96E-03	1500	1070	6280	4.57
Ni	96	0.64	3.96E-03	1.73	0.46	8.83	1.12E-03
V	96	0.41	3.96E-03	3.40	0.00	12.7	0.50
Zn	96	0.15	3.96E-03	200	175	738	3.40E-03

Reference Sediment Exposure

B

Metal	**t** **h**	**α**	**R** **g h^{-1}**	**C_t** **µg g^{-1}**	**C_i** **µg g^{-1}**	**C_s** **µg g^{-1}**	**C_w** **µg mL^{-1}**
Al	96	0.23	3.96E-03	51.5	47.0	353	0.05
Co	96	0.73	3.96E-03	3.50	0.03	0.50	0.01
Cr	96	0.83	3.96E-03	0.06	2.40	0.10	1.95E-05
Cu	96	0.65	3.96E-03	15.2	12.7	0.20	1.00E-03
Pb	96	0.55	3.96E-03	0.17	0.17	0.41	4.10E-05
Mg	96	0.76	3.96E-03	1330	1070	55.5	1.15
Ni	96	0.83	3.96E-03	0.45	0.46	0.23	2.70E-04
V	96	0.68	3.96E-03	2.80	0.00	1.01	5.00E-04
Zn	96	0.31	3.96E-03	178	175	1.60	3.00E-03

The most accurate method for defining uptake and elimination rate coefficients was to calculate k_2 values for individual metals using Equation 3.6 and Equation 3.2. This allows one to replicate measured metal concentrations in wood frog tadpoles, predict steady state concentrations, and predict the time at which steady state is reached. The k_1 values (Table 3.1) were used in Equation 3.6 to solve for k_2 values. Both k_1 and k_2 values were then optimized using Equation 3.2 in order to generate metal concentrations that best fit the measured metal concentrations determined by the aqueous only (Aq) exposure experiment (Figure 3.5). Assimilation efficiencies were then optimized to generate metal concentrations using the uptake and elimination coefficients, (optimized from the previous step and Equation 3.4), that best fit the measured metal concentrations determined by the sediment+aqueous (SedAq) exposure experiment. Not only were these parameters optimized for tadpoles exposed to MacKay River sediment (Figure 3.5), they were also optimized using the same method for tadpoles exposed to reference sediment (Figure 3.6).

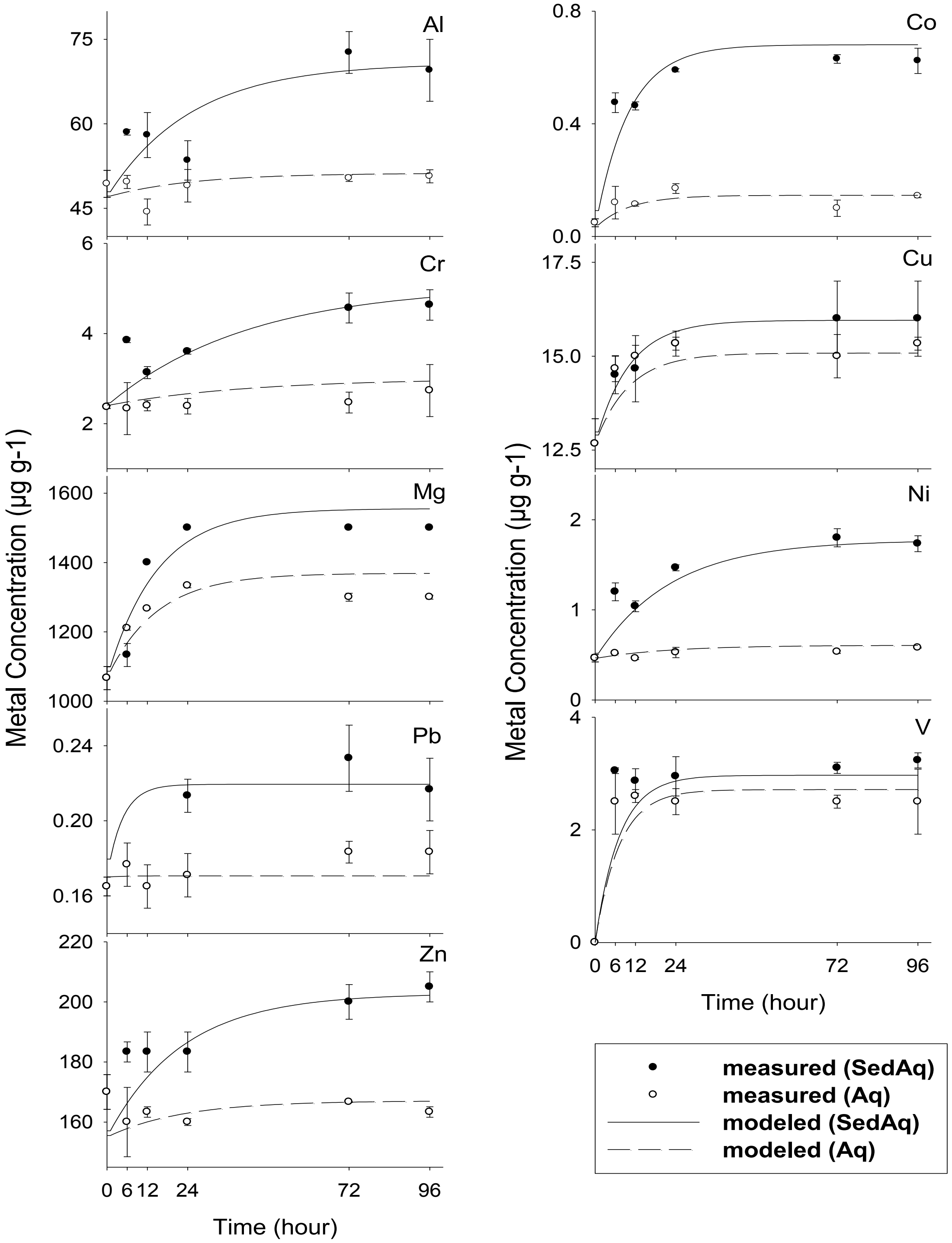


Figure 3.5. Mean measured Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn concentrations (µg g^{-1}) in wood frog tadpoles (±SE, n=3) exposed to MacKay River sediment through both the SedAq and Aq exposure and compared modeled concentrations (µg g^{-1}) generated by calculated k_1 and k_2 rate coefficients (h^{-1}) and assimilation efficiencies using equations 3.2, 3.4, and 3.6 and STELLA^{TM} modeling software (Table 3.2A).

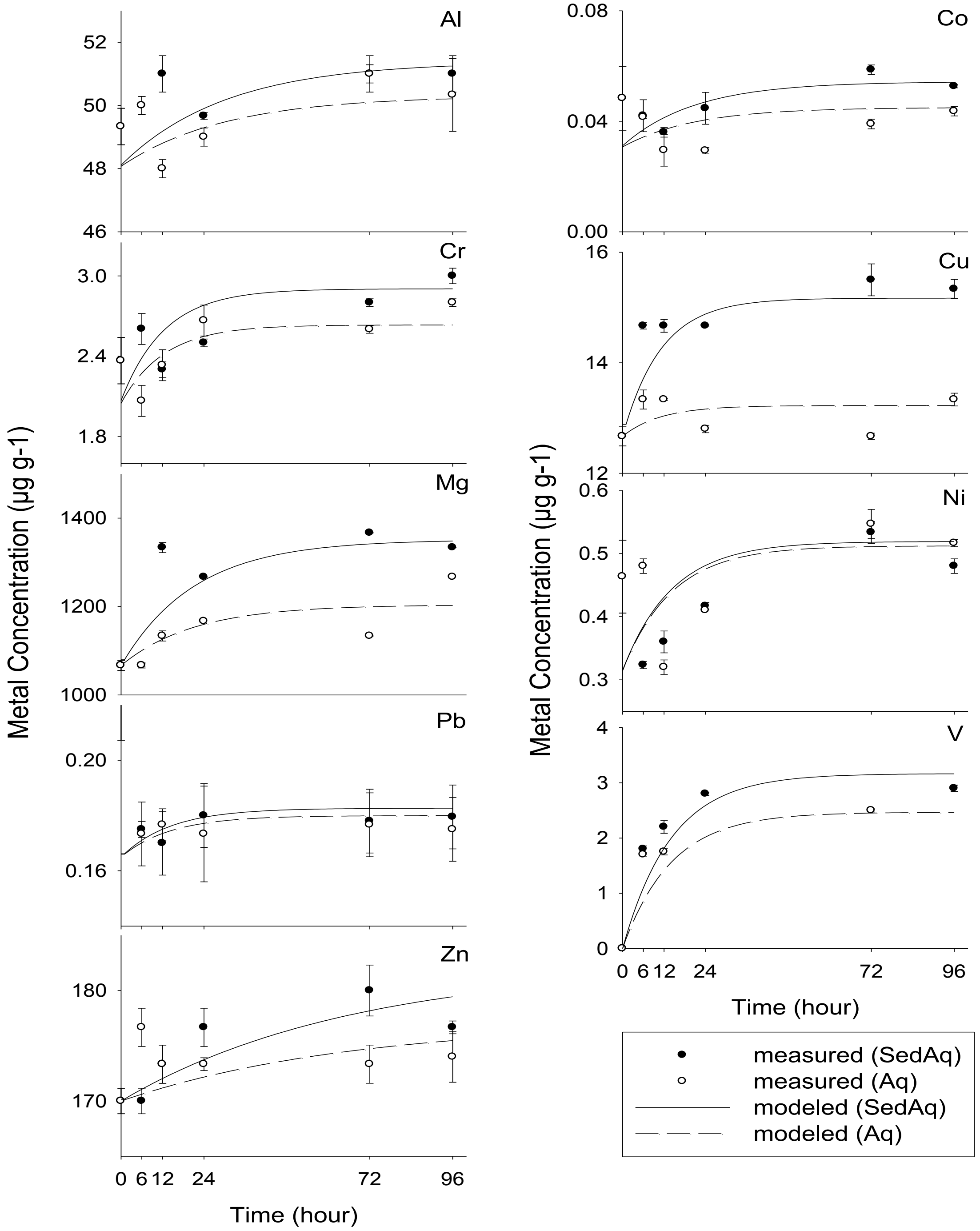


Figure 3.6. Mean measured Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn concentrations (µg g^{-1}) in wood frog tadpoles (±SE, n=3) exposed to reference sediment that participated in both the SedAq and Aq exposure and compared modeled concentrations (µg g^{-1}) generated by calculated k_1 and k_2 rate coefficients (h^{-1}) and assimilation efficiencies using equations 3.2, 3.4, and 3.6 and STELLA™ modeling software (Table 3.2B).

Using the stock and flow models generated by STELLA^{TM}, we were able to calculate uptake and depuration rate constants generated from the uptake-depuration experimental study for detected metals. Kinetic parameters from the uptake-depuration experiments provided in Equations 3.2-3.4 have been used to model the uptake and depuration of Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn from water and sediment ingestion (Figure 3.7 and 3.8). Both the uptake and depuration STELLA^{TM} models were able to accurately reproduce concentrations for Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn in wood frog tadpoles throughout various time points with a relative error of <7.27% (Table 3.3). Not only were concentrations accurately predicted, but also ideal uptake and depuration curves (Figure 3.7 and 3.8) were estimated for all nine metals that exceeded water quality guidelines for the protection of aquatic organisms. In addition, STELLA^{TM} models predicted steady states to be reached between 24 h and 72h for all nine metals during the uptake portion of the study, which corroborated the empirical data collected.

Table 3.3. Relative error (%) for STELLA™ generated metal concentrations when compared to metal concentrations defined by a laboratory uptake-depuration exposure experiment. Conditions that were modeled in a stock and flow STELLA™ model were of tadpoles that were exposed to MacKay River sediment and allowed access to the sediment during the exposure. Depuration conditions that were modeled were of tadpoles that were placed in uncontaminated flow-through tanks after being exposed to MacKay River sediment (96 h).

	MacKay (SedAq)	
Metal	**Uptake Relative Error (%)**	**Depuration Relative Error (%)**
Al	2.33	2.23
Co	1.06	6.48
Cu	2.68	2.04
Cr	1.30	0.41
Mg	2.95	0.17
Ni	2.61	7.27
Pb	2.05	1.76
V	3.93	0.27
Zn	1.32	2.16

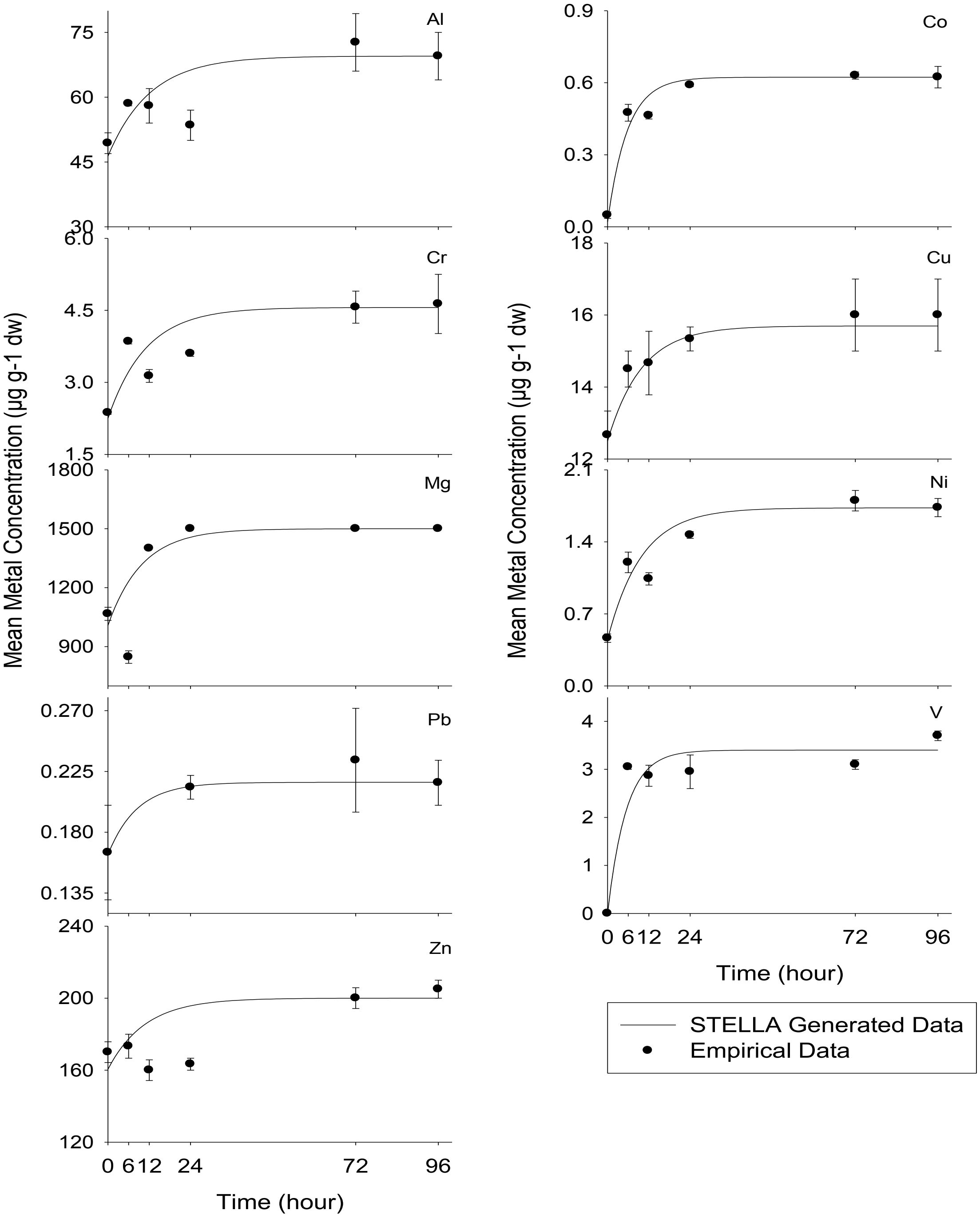


Figure 3.7. Mean (±SE, n=3) metal concentrations detected in wood frog tadpoles involved in the SedAq experiment that were exposed to MacKay River sediment during the uptake phase of the study. STELLA^{TM} generated data compared to data collected from the laboratory exposure study for metals that exceed the concentration of the protection of aquatic wildlife in water and sediment samples collected downstream of the Athabasca River in wood frog tadpoles.

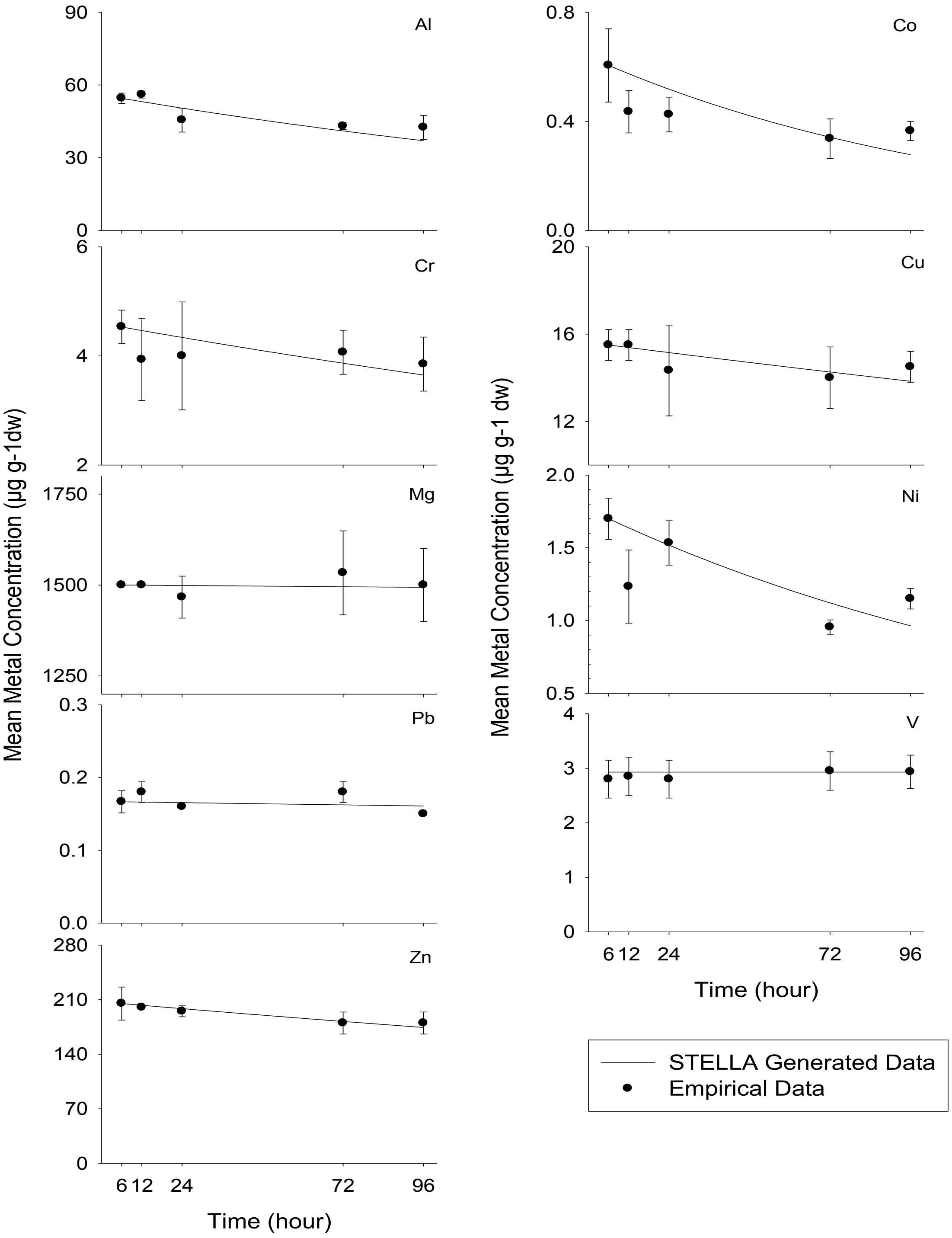


Figure 3.8. Mean (±SE, n=3) metal concentrations detected in wood frog tadpoles involved in the SedAq experiment that were exposed to sediment collected near oil sands activity during the depuration phase of the study. STELLA^{TM} generated data, relative to data collected from the laboratory exposure study for metals, that exceeded the concentration of the protection of aquatic wildlife in water and sediment samples collected downstream of the Athabasca River in wood frog tadpoles.

Metal concentrations that were undefined during the uptake-depuration laboratory exposure study, such as Pb concentrations at 6 and 12 h uptake, were estimated using STELLA^{TM} (Figure 3.7). Pb concentrations at 6 and 12 h for wood frog tadpoles that were exposed to MacKay sediment and allowed access to the sediment was estimated to be 0.19 μg g^{-1}, ww and 0.2 μg g^{-1}, ww respectively. The uptake models developed by STELLA^{TM} for all nine metals resulted in a mean relative error of 2.25 ± 0.93 % (±SE n=9), while the depuration STELLA^{TM} model resulted in a mean relative error of 2.53 ± 2.61 % (±SE, n=9) for all nine metals tested, making the uptake model the more accurate and reliable of the two.

3.4. Discussion

3.4.1. STELLA^{TM} Model Accuracy

The significant increase in toxicological and chemical data during recent years has resulted in more accurate and reliable *in silico* approaches to assessing toxicity and the health of ecosystems, as well as decreasing the amount of animal testing. One of the main objectives of this study was to develop a dynamic model to accurately replicate and predict uptake and depuration metal concentrations in wood frog tadpoles exposed to sediment collected near oil sands activity. It was found that uptake and elimination models developed by STELLA^{TM} resulted in mean relative errors of 2.25 ± 0.93 % (±SE, n=9) and 2.53 ± 2.61 % (±SE n=9) respectively. The model predicted that maximal Al, Co, Cu, Cr, Mg, Ni, Pb, V, and Zn tissue concentrations would reach 69.49, 0.62, 4.56, 15.7, 1499.96, 1.73, 0.22, 3.4, and 199.99 $\mu g\ g^{-1}$ respectively after 96 h of exposure to MacKay River sediment for wood frog tadpoles involved in the SedAq exposure tanks. Notably, a similar, but slightly higher maximum concentration was measured at 72.67, 0.63, 4.63, 16, 1500, 1.8, 0.23, 3.7, and 205 $\mu g\ g^{-1}$ after 96 h. Therefore, STELLA^{TM} generated mass balance dynamic models were able to accurately predict uptake and elimination concentrations and kinetics for all nine metals that exceeded water quality guidelines for the protection of aquatic organisms, as well as produce a good fit between the modeled and the measured data obtained for all data points. Toxicokinetic parameters were also defined for Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn uptake and elimination in wood frog tadpoles. Toxicokinetic parameters that were generated by the laboratory uptake-elimination experiment were defined using water that contained metals during the depuration phase of the study, resulting in non-realistic parameters. This study allowed us to determine uptake rate constants (k_1, h^{-1}), elimination rate constants (k_2, h^{-1}) and assimilation efficiencies (α) computationally, and account for metals that were present in the water used during the depuration portion of the uptake-elimination laboratory experiment, resulting in more accurate parameter estimations. Diet, climate, and water current are factors that may further influence the uptake and bioaccumulation of metal contaminants in wood frog tadpoles and as such, they should be incorporated into future STELLA^{TM} models. Currently, there are no published data on metal concentrations detected in ponds or wetlands within the oil sands Athabasca region that analyzed biota, water, and sediment data.

It was also determined that uptake rate coefficients were greater for Al, Co, Cr, Cu, Mg, Ni, Pb, V and Zn in wood frog tadpoles that were exposed to MacKay River sediment compared to tadpoles exposed to reference sediment. Metal concentrations were much higher in MacKay River sediment, which provides greater metal bioavailability for exposed wood frog tadpoles compared to tadpoles exposed to reference sediment. In addition, assimilation efficiencies were higher in tadpoles that were exposed to the reference sediment compared to assimilation efficiencies for tadpoles exposed to MacKay River sediment. This difference in assimilation efficiency may have resulted in an increase in metal bioaccumulation for tadpoles exposed to reference sediment, and therefore allowed these tadpoles to accumulate metal concentrations that rival bioaccumulation concentrations of tadpoles that were exposed to MacKay River sediment, despite the large difference in metal concentrations detected in the two sediments. Development and feeding habits of larval amphibians may be altered by exposure to metals at high concentrations. Unrine *et al.* (2003) exposed larvae *Rana sphenocephala* to water that was spiked with different concentrations of mercury and concluded that exposed larvae experienced an increase in metamorphosis time in order to flee contaminated conditions, which led to an increase in deformities and mortality.

To my knowledge, there are currently few studies that have used system dynamics modeling to predict metal uptake and elimination kinetics in aquatic organisms. Al-Ansari (2013) developed a mass balance dynamic model for 17α-ethinylestradiol (EE2) uptake and elimination in gold fish using STELLA^{TM}. This model was able to predict EE2 total body burden in environmentally exposed fish by using kinetic parameters obtained from uptake and depuration experiments. In addition, Azanu *et al.* 2016 developed a systems dynamic model using STELLA^{TM} in order to predict Cd, Pb, Cr, Cu, and Hg uptake concentrations in fish found within sewage treatment ponds in Southern Ghana. The model was successful in accurately predicting measured metal concentrations detected in collected fish with a percentage error of 23.2 – 41.1%.

3.5. Conclusion

This study suggests that incorporating calculated assimilation efficiencies, uptake rate coefficients and elimination rate coefficients in STELLA^{TM} models can accurately replicate and predict uptake and depuration metal concentrations in wood frog tadpoles for controlled laboratory exposure studies. Comparison of measured and predicted metal concentrations resulted in a mean relative error of 2.25 ± 0.93 % (±SE, n=9) for the uptake study and 2.53 ± 2.61 % (±SE, n=9) for the depuration study. Notably, the developed model was un-tested using *in-situ* conditions and parameters due to the limited data available on metal concentrations found within ponds located in active oil sands mining areas. Therefore, the model requires further refinement and validation in order to predict metal uptake concentrations for aquatic organisms in wetlands and ponds located within the Alberta oil sands formation.

4.0. General Conclusion and Perspectives on Future Research

Many previous studies have performed uptake and elimination experiments on aquatic organisms in order to determine the toxicokinetics and bioaccumulation of metals. This thesis is the first to examine the accumulation and elimination of metals in contaminated sediment using amphibian larvae. The results of this study are the largest and most comprehensive set of toxicokinetic and bioaccumulation information of metals in the amphibian larvae *L. sylvaticus* conducted to date. As predicted, the laboratory study determined that the concentrations of metals in wood frog tadpoles exposed to sediment collected near oil sands activity were much higher compared to wood frog tadpoles exposed to a reference sediment collect 335km south of the Athabasca oil sands formation. In addition, wood frog tadpoles exposed to metal contaminated sediment (MacKay River sediment) seem to have higher metal bioaccumulation potentials as well. The higher BSAF values for metals could be a result of higher bioavailability of metals present in the sediment.

This study is one of the few that uses complex mixtures of metals rather than individual metals. This study also evaluated the exposure of metals to amphibian larvae from both water and sediment together, and water only. By performing two accumulation-elimination experiments (one evaluating exposure to contaminated sediment and water and the other to contaminated water alone), this study determined the dominant route of exposure for wood frog tadpoles. Metal concentrations and uptake rates in wood frog tadpoles were highest when tadpoles were exposed to metal-contaminated sediment compared to reference sediment. Consequently, it was determined that the dominant route of exposure of wood frog tadpoles to metals is sediment rather than water. Similar results have been observed in other studies where dietary uptake was more effective than aqueous uptake for metals in benthic organisms (Metian *et al.* 2009, Campana *et al.* 2013).

The mass balance dynamic model of metal exposure developed using STELLA^{TM} software yielded similar metal uptake and elimination concentrations in wood frog tadpoles compared to measured concentrations from an uptake-depuration experiment. When predicting uptake and elimination concentrations for Al, Co, Cr, Cu, Mg, Ni, Pb, V and Zn, the model produced a mean relative error of 2.25 ± 0.93 % (±SE, n=9) for the uptake study and 2.53 ± 2.61 % (±SE, n=9) for the depuration study. Not only was the model able to predict metal concentrations that were undetectable in some samples due to high detection limits, it was also able to model uptake and depuration trends. Steady state concentrations and times were also predicted and more defined in the metal concentration data generated by the STELLA^{TM} model. Although this approach requires further refinement and validation in order to be a reliable tool for predicting the toxicokinetics and bioaccumulation of metals in aquatic organisms, systems dynamics modeling is capable of providing valuable information when assessing the health of aquatic ecosystems in areas within large mining operations.

As indicated by previous studies regarding the Athabasca oil sands region, this laboratory exposure study showed that aquatic organisms living closer to oil sands mining activity and exposed to larger quantities of metals are more likely to bioaccumulate metals based on the increase in bioavailability (Boutin *et al.* 2017, Huang *et al.* 2016, Kelly *et al.* 2010). Therefore, it is likely that oil sands mining and upgrading facilities within the region are contributing to metal contamination to nearby aquatic ecosystems in the Athabasca oil sands region (Pilote *et al.* 2018). These laboratory studies showed that metals bioaccumulate in tissue and that sediment is an important source of metal exposure to amphibian larvae, especially in wetlands located closer to oil sands mining activities and upgrading facilities. Determining metal concentrations in wood frog tadpoles, sediment and water within wetlands located in the oil sands formation is necessary to further assess the health of nearby aquatic ecosystems and to further validate the effectiveness of using computational modeling as a reliable tool in monitoring programs. This thesis highlights how important it is to include sediment exposure and complex mixtures of contaminants in toxicological studies for aquatic organisms.

Appendix A. List of all Analyzed Metals

Table A. 1. List of all metals analyzed using ICP-MS and their chemical symbols.

Types of Metal	**Metal**	**Symbol**
Alkali Metal	Lithium	Li
	Sodium	Na
	Potassium	K
Alkaline Metal	Beryllium	Be
	Magnesium	Mg
	Calcium	Ca
	Strontium	Sr
	Barium	Ba
Post-transition Metal	Aluminum	Al
	Thallium	Tl
	Tin	Sn
	Lead	Pb
	Bismuth	Bi
Transition Metal	Titanium	Ti
	Vanadium	V
	Chromium	Cr
	Manganese	Mn
	Iron	Fe
	Cobalt	Co
	Nickel	Ni
	Copper	Cu
	Zinc	Zn
	Yttrium	Y
	Molybdenum	Mo
	Cadmium	Cd

Metalloid	Arsenic	As
	Antimony	Sb
Non-metal	Phosphorus	P
	Selenium	Se
Actinoid	Uranium	U

Appendix B. SGS Method Detection Limits of Metals for Sediment, Water and Biota

Table A. 2. Method detection limits of metals as defined within the SGS protocol for metal analysis in biota samples using ICP-MS for tadpole, sediment and water samples.

Metal	Unit	MDL
Aluminum	µg/g	1
Aluminum	µg/g	1
Arsenic	µg/g	0.5
Barium	µg/g	0.01
Beryllium	µg/g	0.02
Bismuth	µg/g	0.09
Calcium	µg/g	1
Cadmium	µg/g	0.02
Cobalt	µg/g	0.01
Chromium	µg/g	0.5
Copper	µg/g	0.1
Iron	µg/g	0.3
Potassium	µg/g	0.3
Lithium	µg/g	2
Magnesium	µg/g	0.1
Manganese	µg/g	0.1
Molybdenum	µg/g	0.1
Sodium	µg/g	1
Nickel	µg/g	0.1

Phosphorus	µg/g	3
Lead	µg/g	0.05
Antimony	µg/g	0.8
Selenium	µg/g	0.7
Tin	µg/g	0.5
Strontium	µg/g	0.02
Titanium	µg/g	0.1
Thallium	µg/g	0.02
Uranium	µg/g	0.002
Vanadium	µg/g	1
Yttrium	µg/g	0.004
Zinc	µg/g	0.7

Appendix C. Metal Concentrations in MacKay River Sediment

Table A. 3. Mean and standard error of metal concentrations (ng g^{-1} dw, TOC) in MacKay River sediment used in exposure experiments (n=6). TOC normalized concentrations were calculated based on individual TOC values (mean=0.016 g dw sediment/g TOC).

Metal	**Mean** ng g^{-1}, dw	**±SE**	**Mean** ng g^{-1}, dw TOC	**±SE**
Aluminum	154.48	5.03	7853.52	255.59
Arsenic	0.32	0.01	16.29	0.73
Barium	4.19	0.16	212.79	8.16
Beryllium	0.02	0.00	1.03	0.05
Bismuth	0.02	0.01	0.89	0.38
Calcium	502.06	10.15	25523.93	516.12
Cadmium	0.00	0.00	0.20	0.01
Cobalt	0.28	0.01	14.33	0.43
Chromium	0.31	0.01	15.87	0.50
Copper	0.43	0.02	21.81	0.83
Iron	887.21	19.25	45104.66	978.46
Potassium	33.92	0.98	1724.59	49.61
Lithium	0.40	0.01	20.16	0.67
Magnesium	268.25	6.94	13637.53	352.79
Manganese	13.57	0.31	689.84	15.74
Molybdenum	0.02	0.00	1.06	0.07
Sodium	4.51	0.19	229.24	9.69
Nickel	0.55	0.02	28.12	0.95
Phosphorus	30.17	0.82	1533.56	41.44
Lead	0.33	0.01	16.93	0.64
Antimony	-	-	-	-
Selenium	-	-	-	-
Tin	0.01	0.01	0.27	0.27

Strontium	1.72	0.06	87.56	2.94
Titanium	4.11	0.11	209.07	5.43
Thallium	0.00	0.00	0.12	0.01
Uranium	0.04	0.00	1.94	0.08
Vanadium	0.79	0.02	40.33	1.06
Yttrium	0.45	0.01	23.03	0.70
Zinc	2.12	0.08	107.72	3.98

Appendix D. Metal Concentrations in MacKay River Water

Table A. 4. Mean and standard error of metal concentrations (mg L^{-1}) in tanks that contained MacKay River sediment used in exposure experiments (n=6).

Metal	Mean mg L^{-1}	±SE
Aluminum	1.14E-01	4.39E-02
Arsenic	6.00E-04	3.16E-05
Barium	5.75E-02	2.28E-03
Beryllium	1.30E-05	-
Bismuth	9.00E-06	7.75E-07
Calcium	2.92E+01	1.33E+00
Cadmium	1.50E-05	1.82E-06
Cobalt	1.36E-04	3.34E-05
Chromium	3.00E-04	8.35E-05
Copper	1.35E-03	2.11E-04
Iron	2.30E-01	8.45E-02
Potassium	1.56E+00	5.48E-02
Lithium	7.28E-03	2.40E-04
Magnesium	5.25E+00	2.55E-01
Manganese	3.03E-02	9.74E-03
Molybdenum	8.00E-04	6.79E-05
Sodium	1.44E+01	3.25E-01
Nickel	1.30E-03	1.14E-04
Phosphorus	7.04E-02	1.18E-02
Lead	9.80E-05	3.43E-05
Antimony	-	-
Selenium	6.20E-05	3.74E-06
Tin	2.52E-04	1.24E-05

Strontium	1.45E-01	6.79E-03
Titanium	2.07E-03	8.59E-04
Thallium	9.20E-06	9.70E-07
Uranium	1.73E-04	1.86E-05
Vanadium	5.66E-04	1.45E-04
Yttrium	1.02E-04	3.43E-05
Zinc	3.75E-03	9.92E-04

Appendix E. Metal Concentration in Reference Sediment

Table A. 5. Mean and standard error of metal concentrations (ng g^{-1} dw, TOC) in Reference sediment used in exposure experiments (n=6). TOC normalized concentrations were calculated based on individual TOC values (mean=0.026 g dw sediment/g TOC).

Metal	**Mean** **ng g^{-1}, dw**	**±SE**	**Mean** **ng g^{-1}, dw TOC**	**±SE**
Aluminum	139.63	4.63	5295.10	175.74
Arsenic	0.23	0.05	8.87	2.05
Barium	3.04	0.15	115.25	5.57
Beryllium	0.01	0.00	0.41	0.09
Bismuth	0.01	0.01	0.24	0.24
Calcium	54.21	6.46	2055.75	244.95
Cadmium	-	-	-	-
Cobalt	0.05	0.00	1.84	0.07
Chromium	-	-	-	-
Copper	0.08	0.01	3.11	0.24
Iron	118.78	8.46	4504.43	320.74
Potassium	13.71	0.51	519.93	19.44
Lithium	-	-	-	-
Magnesium	23.06	1.27	874.53	48.03
Manganese	2.31	0.18	87.45	6.93
Molybdenum	-	-	-	-
Sodium	5.69	0.17	215.64	6.43
Nickel	0.11	0.01	4.31	0.37
Phosphorus	14.72	1.48	558.26	56.22
Lead	0.17	0.01	6.47	0.21
Antimony	-	-	-	-
Selenium	-	-	-	-
Tin	-	-	-	-

Strontium	0.71	0.05	27.07	1.80
Titanium	9.48	0.17	359.40	6.43
Thallium	-	-	-	-
Uranium	0.01	0.00	0.40	0.02
Vanadium	0.32	0.06	11.98	2.40
Yttrium	0.20	0.01	7.67	0.54
Zinc	0.66	0.03	25.16	1.22

Appendix F. Metal Concentration in Reference Water

Table A. 6. Mean and standard error of metal concentrations (mg L^{-1}) in tanks that contained Reference sediment used in exposure experiments (n=6). Reference sediment was collected 335 km south of Fort MacKay.

Metal	**Mean mg L^{-1}**	**±SE**
Aluminum	8.42E-02	2.52E-02
Arsenic	3.00E-04	3.16E-05
Barium	1.58E-02	8.17E-04
Beryllium	8.50E-06	9.49E-07
Bismuth	1.65E-05	5.38E-06
Calcium	3.92E+00	6.30E-02
Cadmium	2.22E-05	2.27E-06
Cobalt	3.88E-05	7.66E-06
Chromium	2.10E-04	2.24E-05
Copper	1.24E-03	1.31E-04
Iron	8.94E-02	2.77E-02
Potassium	7.76E-01	4.77E-02
Lithium	4.00E-04	3.16E-05
Magnesium	9.83E-01	2.15E-02
Manganese	3.29E-03	1.03E-03
Molybdenum	2.08E-04	3.26E-05
Sodium	1.09E+01	3.64E-01
Nickel	3.00E-04	5.48E-05
Phosphorus	1.35E-01	2.54E-02
Lead	1.10E-04	1.82E-05
Antimony	-	-
Selenium	-	-
Tin	3.96E-04	4.70E-05

Strontium	2.34E-02	8.30E-04
Titanium	1.49E-03	4.49E-04
Thallium	6.60E-06	5.10E-07
Uranium	1.08E-05	2.33E-06
Vanadium	1.20E-03	2.87E-04
Yttrium	1.16E-04	4.46E-05
Zinc	4.40E-03	6.78E-04

Appendix G. Metal Concentrations in Wood Frog Tadpoles (Control)

Table A. 7. Mean and standard error of metal concentrations (µg g^{-1}, dw) detected in wood frog tadpoles placed in tanks containing uncontaminated water and dividers after 96 hours. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles placed in clean flow through tanks after 96 hours of depuration.

	MacKay River Sediment			
	Post 96 h Uptake		**Post 96 h Depuration**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	47.33	8.41	51.75	3.84
Arsenic	1.45	0.04	2.75	1.18
Barium	4.63	0.20	6.93	0.12
Beryllium	-	-	-	-
Bismuth	-	-	-	-
Calcium	8333.33	176.38	9766.67	145.30
Cadmium	0.06	0.00	0.06	0.00
Cobalt	0.06	0.00	0.09	0.01

Chromium	4.20	0.06	4.17	0.03
Copper	16.33	0.33	19.67	0.33
Iron	133.33	14.53	135.00	6.67
Potassium	11666.67	333.33	11666.67	333.33
Lithium	-	-	-	-
Magnesium	1266.67	33.33	1300.00	0.00
Manganese	4.60	0.32	11.23	-
Molybdenum	-	-	-	-
Sodium	11666.67	333.33	14666.67	1452.97
Nickel	0.66	0.02	0.77	0.08
Phosphorus	10333.33	333.33	10475.00	351.19
Lead	0.37	0.06	0.48	0.09
Antimony	-	-	-	-
Selenium	2.30	0.00	1.90	0.00
Tin	-	-	-	-
Strontium	6.03	0.13	7.23	0.33
Titanium	28.67	0.88	61.67	17.17
Thallium	0.10	0.00	0.12	0.00
Uranium	0.01	0.00	0.01	0.00
Vanadium	2.83	0.15	3.20	0.17
Yttrium	0.02	0.01	0.12	0.13
Zinc	200.00	10.00	263.33	8.82

Appendix H. Metal Concentrations in Exposed Wood Frog Tadpoles (Uptake)

Table A. 8. Mean and standard error of metal concentrations (μg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 0 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment **0 h Uptake**				**Reference Sediment** **0 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**
Aluminum	55.00	2.80	55.00	2.80	55.00	2.80	55.00	2.80
Arsenic	-	-	-	-	-	-	-	-
Barium	4.93	0.34	4.93	0.34	4.93	0.34	4.93	0.34
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	-	-
Calcium	16216.67	7757.42	16216.67	7757.42	16216.67	7757.42	16216.67	7757.42
Cadmium	0.05	-	0.05	-	0.05	-	0.05	-
Cobalt	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
Chromium	2.60	0.17	2.60	0.17	2.60	0.17	2.60	0.17
Copper	14.00	0.68	14.00	0.68	14.00	0.68	14.00	0.68
Iron	113.33	3.33	113.33	3.33	113.33	3.33	113.33	3.33
Potassium	10733.33	1756.26	10733.33	1756.26	10733.33	1756.26	10733.33	1756.26
Lithium	-	-	-	-	-	-	-	-
Magnesium	1066.67	23.57	1066.67	23.57	1066.67	23.57	1066.67	23.57
Manganese	2.85	0.22	2.85	0.22	2.85	0.22	2.85	0.22
Molybdenum	-	-	-	-	-	-	-	-
Sodium	15500.00	1231.53	15500.00	1231.53	15500.00	1231.53	15500.00	1231.53
Nickel	0.64	0.08	0.64	0.08	0.64	0.08	0.64	0.08
Phosphorus	10433.33	256.47	10433.33	256.47	10433.33	256.47	10433.33	256.47
Lead	0.30	0.07	0.30	0.07	0.30	0.07	0.30	0.07

Antimony	-	-	-	-	-	-	-	-
Selenium	2.20	0.18	2.20	0.18	2.20	0.18	2.20	0.18
Tin	-	-	-	-	#DIV/0!	-	-	-
Strontium	6.43	0.19	6.43	0.19	6.43	0.19	6.43	0.19
Titanium	52.00	9.32	52.00	9.32	52.00	9.32	52.00	9.32
Thallium	-	-	-	-	-	-	-	-
Uranium	-	-	-	-	-	-	-	-
Vanadium	3.10	0.30	3.10	0.30	3.70	0.17	3.10	0.30
Yttrium	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00
Zinc	196.67	8.03	196.67	8.03	196.67	8.03	196.67	8.03

Table A. 9. Mean and standard error of metal concentrations (μg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 6 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment 6 h Uptake				**Reference Sediment 6 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**
Aluminum	71.00	2.52	49.67	2.19	18.12	1.22	62.00	5.72
Arsenic	-	-	-	-	-	-	-	-
Barium	5.33	0.20	3.35	0.20	1.25	0.32	3.83	0.27
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	1.27	0.76
Calcium	8233.33	176.38	7850.00	40.82	2689.07	163.30	8000.00	200.00
Cadmium	-	-	-	-	-	-	0.06	-
Cobalt	0.68	0.03	0.18	0.01	0.07	0.01	0.04	0.01
Chromium	3.73	0.55	3.03	0.15	1.24	0.04	3.10	0.00
Copper	16.00	0.82	15.67	0.33	5.61	1.00	15.33	0.33

Iron	180.00	5.77	186.67	17.64	70.03	14.53	130.00	20.82
Potassium	4066.67	218.58	5800.00	1250.33	2422.97	581.19	10666.67	333.33
Lithium	-	-	-	-	-	-	-	-
Magnesium	846.67	31.80	800.00	81.85	304.55	12.02	1066.67	33.33
Manganese	253.33	23.33	41.67	0.33	21.78	1.45	14.67	0.88
Molybdenum	-	-	-	-	-	-	#DIV/0!	-
Sodium	3233.33	120.19	4700.00	1357.69	2059.29	956.27	12000.00	577.35
Nickel	1.43	0.09	0.52	0.01	0.20	0.06	0.48	0.02
Phosphorus	9333.33	88.19	9366.67	88.19	3181.02	100.00	9966.67	33.33
Lead	-	-	0.13	-	0.13	0.12	0.29	0.10
Antimony	-	-	-	-	-	-	-	-
Selenium	-	-	1.80	-	1.80	-	2.30	-
Tin	-	-	-	-	-	-	-	-
Strontium	8.53	0.35	5.53	0.28	2.06	0.40	5.93	0.09
Titanium	40.00	1.73	19.67	1.20	7.53	2.08	20.00	0.58
Thallium	-	-	-	-	-	-	0.07	-
Uranium	-	-	-	-	-	-	-	-
Vanadium	3.43	0.38	-	-	0.38	0.15	2.70	-
Yttrium	0.05	0.01	0.01	-	0.01	0.01	0.04	0.01
Zinc	173.33	6.67	160.00	5.77	57.48	12.02	156.67	3.33

Table A. 10. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 12 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment 12 h Uptake				**Reference Sediment 12 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**

Aluminum	62.00	-	37.67	1.20	52.33	4.84	51.50	2.04
Arsenic	-	-	-	-	-	-	-	-
Barium	4.87	0.33	3.90	0.08	5.05	0.78	4.77	0.27
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	-	-
Calcium	8300.00	152.75	7500.00	81.65	9050.00	775.67	7333.33	202.76
Cadmium	-	-	0.06	-	-	-	-	-
Cobalt	0.46	0.01	0.17	0.01	0.06	0.01	0.10	-
Chromium	3.13	0.13	3.43	0.28	2.90	0.00	3.50	0.24
Copper	14.67	0.88	15.00	0.58	15.67	0.67	15.67	0.33
Iron	196.67	17.64	156.67	3.33	146.67	12.02	105.33	4.67
Potassium	14666.67	333.33	15000.00	1000.00	16333.33	1452.97	13333.33	881.92
Lithium	-	-	-	-	-	-	-	-
Magnesium	1400.00	0.00	1266.67	33.33	1433.33	185.59	1333.33	33.33
Manganese	62.00	3.46	38.67	7.67	10.47	2.05	17.20	4.75
Molybdenum	-	-	-	-	-	-	-	-
Sodium	20000.00	0.00	17666.67	881.92	20333.33	1855.92	16666.67	2848.00
Nickel	1.04	0.06	0.46	0.03	0.36	0.03	0.58	0.12
Phosphorus	11000.00	0.00	10666.67	333.33	12666.67	1201.85	11333.33	333.33
Lead	-	-	-	-	-	-	0.42	0.07
Antimony	-	-	-	-	-	-	-	-
Selenium	2.20	-	2.10	0.16	2.17	0.09	1.90	-
Tin	-	-	-	-	-	-	-	-
Strontium	6.97	0.20	5.37	0.22	6.93	0.56	5.17	0.23
Titanium	16.00	2.65	15.33	0.88	14.33	1.45	44.00	12.00
Thallium	-	-	-	-	-	-	0.10	0.00
Uranium	-	-	-	-	-	-	0.01	0.00
Vanadium	2.83	0.24	2.70	0.12	3.20	-	2.75	0.04
Yttrium	0.04	0.01	-	-	0.02	0.00	0.13	0.05
Zinc	160.00	5.77	163.33	3.33	196.67	27.28	173.33	8.82

Table A. 11. Mean and standard error of metal concentrations (μg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 24 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment 24 h Uptake				**Reference Sediment 24 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**
Aluminum	57.00	-	39.67	3.93	47.67	1.20	59.50	11.02
Arsenic	1.40	-	-	-	-	-	2.00	-
Barium	4.97	0.13	5.00	0.29	5.67	0.12	4.57	0.41
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	1.80	-
Calcium	8966.67	589.73	8150.00	449.07	8800.00	81.65	8366.67	548.74
Cadmium	-	-	-	-	-	-	-	-
Cobalt	0.59	0.01	0.21	0.01	0.04	0.00	0.07	0.01
Chromium	3.60	0.06	3.87	0.23	4.20	0.08	3.55	0.12
Copper	15.33	0.33	16.00	1.00	17.00	0.00	15.67	0.33
Iron	173.33	18.56	153.33	23.33	103.33	3.33	113.33	3.33
Potassium	10666.67	333.33	11666.67	333.33	10566.67	433.33	12000.00	-
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	-	1333.33	33.33	1266.67	33.33	1366.67	33.33
Manganese	65.00	1.53	87.00	16.80	12.33	0.67	8.03	1.27
Molybdenum	-	-	-	-	-	-	0.31	-
Sodium	14000.00	2516.61	13000.00	2000.00	12666.67	333.33	17333.33	333.33
Nickel	1.47	0.03	0.86	0.07	0.64	0.02	0.88	0.10
Phosphorus	10000.00	-	11000.00	0.00	10666.67	333.33	11333.33	333.33
Lead	0.21	0.01	0.19	0.03	0.18	0.01	0.24	0.03
Antimony	-	-	-	-	-	-	-	-

Selenium	-	-	-	-	-	-	1.90	-
Tin	-	-	-	-	-	-	-	-
Strontium	7.93	0.18	5.73	0.29	7.20	0.26	5.80	0.62
Titanium	33.33	3.38	28.00	3.79	32.67	3.28	17.33	1.20
Thallium	0.06	-	0.07	0.00	0.07	0.00	0.08	0.01
Uranium	0.02	0.00	0.01	0.00	0.02	0.00	0.02	0.01
Vanadium	2.90	0.33	-	-	2.80	0.16	-	-
Yttrium	0.05	0.01	-	-	0.03	0.01	0.02	0.01
Zinc	163.33	3.33	206.67	6.67	190.00	5.77	190.00	-

Table A. 12. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 72 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment 72 h Uptake				**Reference Sediment 72 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	72.67	13.68	38.67	2.19	61.33	12.12	60.50	3.67
Arsenic	-	-	1.50	-	-	-	-	-
Barium	7.40	0.60	4.93	0.28	7.33	0.20	5.23	0.38
Beryllium	-	-	-	-	-	-	-	-
Bismuth	0.34	-	-	-	-	-	-	-
Calcium	10633.33	683.94	9233.33	366.67	9850.00	122.47	8366.67	384.42
Cadmium	-	-	-	-	-	-	-	-
Cobalt	0.77	0.02	0.13	0.04	0.06	0.00	0.04	0.00
Chromium	4.40	0.41	3.83	0.13	3.75	0.04	3.90	0.16
Copper	16.00	0.82	15.00	-	16.50	0.41	17.00	-
Iron	343.33	73.56	213.33	8.82	123.33	8.82	120.00	-

Potassium	11000.00	0.00	11333.33	333.33	11333.33	666.67	7800.00	378.59
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	0.00	1300.00	-	1366.67	33.33	1133.33	33.33
Manganese	79.00	5.77	15.33	2.40	6.57	0.30	9.30	0.85
Molybdenum	0.26	0.00	-	-	-	-	-	-
Sodium	17333.33	1666.67	15333.33	1763.83	19000.00	1527.53	7733.33	600.93
Nickel	1.93	0.15	0.85	0.07	0.53	0.03	0.55	0.06
Phosphorus	9866.67	88.19	10000.00	-	11000.00	-	9733.33	266.67
Lead	0.23	0.04	0.24	0.01	0.17	-	0.18	0.01
Antimony	-	-	-	-	-	-	-	-
Selenium	1.80	-	2.00	0.12	-	-	-	-
Tin	-	-	-	-	-	-	-	-
Strontium	9.83	0.62	5.97	0.07	8.13	0.23	5.60	0.32
Titanium	25.67	3.18	21.00	1.53	22.00	2.08	20.67	2.91
Thallium	0.07	0.00	0.09	0.00	0.07	0.01	0.06	0.00
Uranium	0.03	0.00	0.01	-	0.04	0.00	0.01	-
Vanadium	3.00	-	-	-	-	-	2.50	-
Yttrium	0.12	0.04	0.01	-	0.07	0.01	0.03	0.01
Zinc	200.00	5.77	206.67	16.67	216.67	3.33	226.67	24.04

Table A. 13. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) at 96 h of exposure during the uptake phase of the experiment.

	MacKay River Sediment 96 h Uptake				**Reference Sediment 96 h Uptake**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	69.50	4.49	35.00	2.00	50.33	2.73	42.33	0.33

Arsenic	-	-	-	-	-	-	-	-
Barium	8.70	0.15	4.10	0.20	7.07	0.23	4.97	0.27
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	1.67	1.01	-	-
Calcium	10966.67	606.45	8900.00	0.00	11500.00	408.25	8866.67	328.30
Cadmium	-	-	0.05	-	0.06	-	-	-
Cobalt	0.62	0.04	0.16	0.00	0.05	0.01	0.04	0.01
Chromium	5.25	0.04	3.73	0.07	4.43	0.03	3.80	0.16
Copper	17.00	0.00	15.33	0.33	16.00	0.82	15.67	0.33
Iron	233.33	12.02	186.67	8.82	113.33	3.33	133.33	3.33
Potassium	10666.67	333.33	11666.67	333.33	11333.33	333.33	12333.33	333.33
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	0.00	1300.00	0.00	1333.33	33.33	1366.67	33.33
Manganese	58.33	2.96	21.67	1.20	11.93	1.59	12.00	4.06
Molybdenum	-	-	-	-	0.36	-	-	-
Sodium	16000.00	577.35	15666.67	2848.00	13666.67	333.33	15000.00	2516.61
Nickel	1.73	0.09	0.58	0.04	0.58	0.02	0.52	0.06
Phosphorus	10000.00	0.00	10333.33	333.33	10333.33	333.33	11333.33	333.33
Lead	0.19	0.01	0.18	0.01	0.18	0.02	0.16	0.02
Antimony	-	-	-	-	-	-	-	-
Selenium	2.53	0.29	-	-	2.20	-	-	-
Tin	-	-	-	-	1.40	-	-	-
Strontium	10.63	0.37	5.40	0.15	9.40	0.40	5.47	0.37
Titanium	40.33	0.33	19.67	0.33	32.00	1.53	24.00	2.08
Thallium	0.08	0.00	0.09	0.00	0.09	0.02	0.09	0.01
Uranium	0.03	0.00	0.01	-	0.04	0.01	0.01	0.00
Vanadium	3.70	0.10	-	-	3.10	-	-	-
Yttrium	0.08	0.00	-	-	0.04	0.01	0.01	-
Zinc	213.33	8.82	180.00	10.00	260.00	5.77	203.33	3.33

Appendix I. Metal Concentrations in Exposed Wood Frog Tadpoles (Depuration)

Table A. 14. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles that were exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) and then placed in clean flow-through tanks for 6 h during the depuration phase of the experiment.

	MacKay River Sediment 6 h Depuration				**Reference Sediment 6 h Depuration**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	55.67	1.45	66.00	8.16	55.00	0.82	40.50	2.04
Arsenic	-	-	1.45	0.04	-	-	-	-
Barium	8.70	0.33	7.75	0.78	7.90	0.42	6.15	0.20
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	-	-
Calcium	12333.33	666.67	9100.00	0.00	10900.00	898.15	8466.67	202.76
Cadmium	-	-	-	-	-	-	-	-
Cobalt	0.61	0.08	0.15	0.01	0.05	0.00	0.06	0.00
Chromium	4.53	0.18	4.15	0.04	3.80	0.16	4.55	0.12
Copper	17.00	0.00	16.00	-	15.67	0.33	17.50	0.41
Iron	193.33	12.02	216.67	14.53	140.00	5.77	130.00	11.55
Potassium	10333.33	333.33	12000.00	0.00	11666.67	333.33	13000.00	0.00
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	0.00	1400.00	0.00	1333.33	33.33	1400.00	0.00
Manganese	48.00	5.13	14.67	1.86	8.43	0.29	10.60	1.25
Molybdenum	-	-	-	-	-	-	-	-
Sodium	13333.33	1201.85	12333.33	333.33	20333.33	333.33	14000.00	577.35
Nickel	1.80	0.12	2.13	0.83	0.73	0.04	1.90	0.06

Phosphorus	10333.33	333.33	11333.33	333.33	11000.00	0.00	11333.33	333.33
Lead	0.17	0.01	0.35	0.05	0.17	0.02	0.20	0.03
Antimony	-	-	-	-	-	-	-	-
Selenium	1.90	-	2.30	-	-	-	2.70	-
Tin	-	-	#DIV/0!	-	-	-	-	-
Strontium	10.60	0.40	6.93	0.19	8.50	0.36	6.33	0.27
Titanium	32.67	2.96	27.33	2.40	23.00	1.53	33.00	4.04
Thallium	0.06	0.00	0.09	0.00	0.05	0.00	0.09	0.00
Uranium	0.03	0.00	0.01	0.00	0.02	0.00	-	-
Vanadium	2.80	0.20	3.20	0.33	-	-	2.90	0.12
Yttrium	0.07	0.02	0.05	0.00	0.03	0.01	0.02	-
Zinc	263.33	23.33	243.33	6.67	226.67	3.33	246.67	8.82

Table A. 15. Mean and standard error of metal concentrations (μg g^{-1}, dw) in wood frog tadpoles that were exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) and then placed in clean flow-through tanks for 12 h during the depuration phase of the experiment.

	MacKay River Sediment 12 h Depuration				**Reference Sediment 12 h Depuration**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**	**Mean μg g^{-1}**	**±SE**
Aluminum	60.33	4.37	72.50	6.12	63.00	2.45	74.50	4.49
Arsenic	1.60	0.24	1.90	0.12	2.05	0.29	1.90	0.26
Barium	8.15	0.20	7.40	0.08	8.00	0.41	6.75	0.29
Beryllium	-	-	-	-	-	-	-	-
Bismuth	0.32	-	-	-	-	-	-	-
Calcium	11333.33	333.33	9850.00	40.82	10450.00	449.07	9700.00	81.65

Cadmium	-	-	0.05	0.00	-	-	0.05	-
Cobalt	0.54	0.11	0.13	-	0.06	0.00	0.12	0.00
Chromium	3.93	0.43	3.70	0.16	4.17	0.03	3.90	0.24
Copper	16.50	0.41	15.67	0.33	17.33	0.33	15.00	0.58
Iron	260.00	5.77	230.00	26.46	130.00	5.77	163.33	12.02
Potassium	11666.67	333.33	10733.33	819.21	12666.67	333.33	12333.33	333.33
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	0.00	1300.00	57.74	1433.33	66.67	1333.33	33.33
Manganese	35.33	4.33	20.67	4.37	8.13	1.47	15.00	1.53
Molybdenum	-	-	-	-	-	-	-	-
Sodium	15666.67	1452.97	11933.33	1212.89	13000.00	0.00	15000.00	2000.00
Nickel	1.43	0.34	1.83	0.34	0.68	0.09	1.87	0.03
Phosphorus	11000.00	0.00	10500.00	500.00	11000.00	577.35	10666.67	333.33
Lead	0.28	0.06	0.48	0.09	0.17	0.01	0.42	0.05
Antimony	-	-	-	-	-	-	-	-
Selenium	1.80	-	2.00	0.12	-	-	-	-
Tin	-	-	-	-	-	-	-	-
Strontium	9.10	0.52	7.40	0.25	8.87	0.54	7.07	0.26
Titanium	42.33	4.41	43.33	3.71	39.33	2.73	29.00	5.13
Thallium	0.07	0.01	0.08	0.00	0.06	0.00	0.08	0.00
Uranium	0.03	0.01	0.01	0.00	0.02	0.00	0.01	0.00
Vanadium	2.85	0.20	2.70	-	2.63	0.03	2.90	-
Yttrium	0.16	0.05	0.07	0.01	0.03	0.00	0.04	0.01
Zinc	250.00	20.00	236.67	6.67	240.00	5.77	230.00	5.77

Table A. 16. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles that were exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) and then placed in clean flow-through tanks for 24 h during the depuration phase of the experiment.

	MacKay River Sediment 24 h Depuration				**Reference Sediment 24 h Depuration**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	80.50	1.22	42.67	2.19	44.00	0.82	74.50	2.86
Arsenic	3.25	0.69	1.40	0.00	2.10	-	-	-
Barium	9.20	0.24	5.25	0.04	7.35	0.37	6.90	0.49
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	2.40	-	0.30	-
Calcium	12000.00	0.00	9150.00	122.47	12000.00	0.00	8800.00	244.95
Cadmium	-	-	-	-	0.05	-	0.05	-
Cobalt	0.88	0.00	0.15	0.02	0.07	0.00	0.13	0.01
Chromium	5.00	0.24	3.75	0.20	4.05	0.04	4.40	0.16
Copper	16.00	0.58	16.00	0.00	17.00	0.58	15.67	0.33
Iron	430.00	76.38	146.67	8.82	123.33	12.02	180.00	5.77
Potassium	10633.33	366.67	12333.33	333.33	12333.33	333.33	13000.00	0.00
Lithium	-	-	-	-	-	-	-	-
Magnesium	1466.67	33.33	1366.67	33.33	1333.33	88.19	1400.00	0.00
Manganese	57.67	2.03	11.00	0.58	8.70	1.20	11.57	0.98
Molybdenum	-	-	-	-	0.31	-	-	-
Sodium	15000.00	1154.70	13833.33	3609.40	16000.00	3511.88	12666.67	881.92
Nickel	2.53	0.09	1.09	0.18	0.76	0.08	1.40	0.00
Phosphorus	9600.00	208.17	11000.00	0.00	11000.00	577.35	11666.67	333.33

Lead	0.32	0.04	0.22	0.03	0.20	0.03	0.39	0.04
Antimony	-	-	-	-	-	-	-	-
Selenium	2.25	0.04	1.80	-	-	-	2.20	-
Tin	-	-	-	-	-	-	-	-
Strontium	11.00	0.58	6.17	0.33	9.00	0.31	6.70	0.38
Titanium	41.67	2.33	22.67	2.85	27.67	0.88	30.67	2.96
Thallium	0.06	-	0.08	0.00	0.07	0.02	0.11	0.00
Uranium	0.03	0.00	0.01	0.00	0.03	0.01	0.02	0.00
Vanadium	3.83	0.30	2.60	0.00	2.95	0.04	3.30	0.16
Yttrium	0.17	0.02	0.02	0.00	0.03	0.01	0.04	0.00
Zinc	253.33	12.02	240.00	5.77	233.33	3.33	246.67	17.64

Table A. 17. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles that were exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) and then placed in clean flow-through tanks for 72 h during the depuration phase of the experiment.

	MacKay River Sediment 72 h Depuration				**Reference Sediment 72 h Depuration**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	68.00	3.27	65.50	1.22	61.00	3.27	73.50	3.67
Arsenic	1.77	0.23	1.80	0.33	2.10	0.00	2.30	-
Barium	9.30	0.57	7.00	0.33	9.35	0.04	7.35	0.53
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	-	-	-	-	-	-

Calcium	13333.33	333.33	9833.33	166.67	11666.67	333.33	10500.00	408.25
Cadmium	-	-	-	-	-	-	0.06	0.00
Cobalt	0.34	0.04	0.15	0.01	0.08	-	0.14	0.01
Chromium	4.25	0.20	4.00	0.00	4.35	0.04	5.00	0.08
Copper	17.00	0.82	17.00	0.00	16.50	1.22	16.00	1.00
Iron	313.33	56.08	243.33	8.82	126.67	6.67	183.33	28.48
Potassium	12666.67	333.33	12000.00	0.00	12666.67	333.33	11400.00	1137.25
Lithium	-	-	-	-	-	-	-	-
Magnesium	1600.00	0.00	1366.67	33.33	1400.00	57.74	1300.00	57.74
Manganese	16.00	2.52	13.67	1.67	8.97	0.24	22.33	3.84
Molybdenum	-	-	-	-	-	-	-	-
Sodium	16000.00	2516.61	16666.67	2333.33	18000.00	2645.75	13333.33	333.33
Nickel	1.07	0.12	1.04	0.06	0.81	0.03	2.03	0.37
Phosphorus	12000.00	0.00	11333.33	333.33	11000.00	0.00	11000.00	577.35
Lead	0.21	0.03	0.41	0.05	0.24	0.02	0.59	0.12
Antimony	-	-	-	-	-	-	-	-
Selenium	1.85	0.04	-	-	2.15	0.04	-	-
Tin	-	-	-	-	-	-	-	-
Strontium	10.10	0.49	6.97	0.20	8.80	0.21	8.33	0.35
Titanium	30.67	2.85	24.67	2.33	35.33	5.90	36.33	2.85
Thallium	0.08	0.00	0.09	0.00	0.07	0.00	0.09	0.01
Uranium	0.03	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Vanadium	3.25	0.04	2.60	0.00	3.60	0.49	3.67	0.22
Yttrium	0.11	0.03	0.04	0.00	0.03	0.00	0.05	0.03
Zinc	270.00	5.77	256.67	8.82	296.67	20.28	283.33	12.02

Table A. 18. Mean and standard error of metal concentrations (µg g^{-1}, dw) in wood frog tadpoles that were exposed to MacKay River sediment and reference sediment for both exposure conditions (SedAq and Aq) and then placed in clean flow-through tanks for 96 h during the depuration phase of the experiment.

	MacKay River Sediment 96 h Depuration				**Reference Sediment 96 h Depuration**			
	SedAq		**Aq**		**SedAq**		**Aq**	
Metal	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**	**Mean µg g^{-1}**	**±SE**
Aluminum	87.50	9.39	58.50	3.67	57.00	3.27	55.00	1.00
Arsenic	2.30	-	2.55	0.45	3.85	2.00	1.85	0.04
Barium	11.50	0.41	7.60	0.33	11.00	0.00	7.40	0.41
Beryllium	-	-	-	-	-	-	-	-
Bismuth	-	-	1.95	1.10	-	-	-	-
Calcium	14333.33	333.33	9650.00	1102.27	12500.00	408.25	10250.00	612.37
Cadmium	0.05	0.00	0.05	0.00	0.06	0.00	0.06	0.00
Cobalt	0.37	0.02	0.13	0.01	0.08	0.00	0.11	0.00
Chromium	5.35	0.69	4.40	0.08	4.75	0.04	4.35	0.12
Copper	15.00	0.82	16.00	0.00	17.50	0.41	18.50	0.41
Iron	350.00	55.68	200.00	5.77	113.00	7.00	133.33	3.33
Potassium	10966.67	1033.33	12333.33	333.33	10600.00	400.00	12333.33	666.67
Lithium	-	-	-	-	-	-	-	-
Magnesium	1500.00	57.74	1400.00	0.00	1300.00	0.00	1433.33	33.33
Manganese	22.67	4.41	10.80	0.76	14.67	0.88	10.83	1.30
Molybdenum	-	-	0.45	#DIV/0!	-	-	-	-
Sodium	16333.33	2403.70	14000.00	1527.53	16000.00	1154.70	16333.33	3527.67
Nickel	1.63	0.32	1.00	0.11	1.50	0.45	1.33	0.13
Phosphorus	10466.67	533.33	11000.00	0.00	10166.67	440.96	11333.33	333.33

Lead	0.39	0.08	0.45	0.12	0.32	0.01	0.26	0.02
Antimony	-	-	-	-	-	-	-	-
Selenium	2.60	-	2.70	0.24	2.20	-	1.95	0.12
Tin	-	-	1.90	-	-	-	-	-
Strontium	11.67	0.88	7.13	0.47	10.67	0.33	7.73	0.58
Titanium	53.00	9.61	36.67	5.67	57.00	15.01	32.00	5.29
Thallium	0.07	0.00	0.13	0.02	0.08	0.00	0.10	0.00
Uranium	0.03	0.00	0.04	0.01	0.03	0.00	0.01	0.00
Vanadium	4.53	0.77	3.30	0.35	4.40	1.15	3.30	0.16
Yttrium	0.13	0.03	0.06	0.02	0.03	0.01	0.02	0.00
Zinc	303.33	16.67	246.67	6.67	300.00	0.00	306.67	21.86

Appendix J. Metal Uptake and Elimination Rates

Table A. 19. Uptake (k_1) and elimination (k_2) rates determined for metals measured in wood frog tadpoles during the experimental exposure experiment (96 h) that consisted with the MacKay River sediment. Uptake rates were determined using the slope between metal concentrations detected in tadpoles and the amount of time of exposure for both the SedAq and Aq exposures. Elimination rates were determined using the slope between the natural log of metal concentrations detected in wood frog tadpoles during the depuration phase of the study and the amount of time spent in clean flow-through tanks.

	MacKay River Sediment Exposure			
	SedAq		**Aq**	
Metal	**k1** $\mu g\ g^{-1}\ h^{-1}$	**k2** h^{-1}	**k1** $\mu g\ g^{-1}\ h^{-1}$	**k2** h^{-1}
Copper	0.62	0.03	0.42	0.02
Chromium	0.40	0.04	0.27	0.01
Nickel	0.25	0.10	0.05	0.02
Cobalt	0.10	0.13	0.01	0.03
Vanadium	0.10	0.02	0.02	0.02
Lead	0.03	0.05	0.01	0.03
Magnesium	120.76	0.00	73.38	0.01
Zinc	6.67	0.03	5.95	0.02
Aluminum	4.30	0.11	1.27	0.01

Table A. 20. Uptake (k_1) and elimination (k_2) rates determined for metals measured in wood frog tadpoles during the experimental exposure experiment (96 h) that consisted with the reference sediment. Uptake rates were determined using the slope between metal concentrations detected in tadpoles and the amount of time of exposure for both the SedAq and Aq exposures. Elimination rates were determined using the slope between the natural log of metal concentrations detected in wood frog tadpoles during the depuration phase of the study and the amount of time spent in clean flow-through tanks.

	Reference Sediment Exposure			
	SedAq		**Aq**	
Metal	**k1** **µg g^{-1} h^{-1}**	**k2** **h^{-1}**	**k1** **µg g^{-1} h^{-1}**	**k2** **h^{-1}**
Copper	0.47	0.03	0.41	0.02
Chromium	0.20	0.01	0.29	0.02
Nickel	0.03	0.03	0.02	0.04
Cobalt	0.00	0.06	0.00	0.05
Vanadium	0.02	0.02	-	-
Lead	0.01	0.01	0.01	0.01
Magnesium	71.43	0.00	49.52	0.00
Zinc	2.24	0.00	1.29	0.00
Aluminum	0.82	0.02	0.85	0.01

Appendix K. Biota-Sediment Accumulation Factors and Bioaccumulation Factors of Metals

Table A. 21. Biota-sediment accumulation factors (BSAFs) and bioconcentration factors (BCFs) of metals measured in wood frog tadpoles after 96 hours of sediment + aqueous and aqueous only exposure experiments for both MacKay River sediment and reference sediment exposed tadpoles.

	MacKay		**Reference**	
	SedAq	**Aq**	**SedAq**	**Aq**
Metal	**BSAF (t=96h)**	**BCF (t=96h)**	**BSAF (t=96h)**	**BCF (t=96h)**
Aluminum	0.03	612.60	0.02	521.52
Arsenic	0.36	3954.55	-	-
Barium	0.16	153.69	0.14	333.93
Beryllium	-	-	-	-
Bismuth	-	159183.67	-	-
Calcium	1.68	395.50	12.90	2163.48
Cadmium	0.84	3030.30	-	-
Cobalt	0.08	1251.80	0.06	1159.42
Chromium	0.99	299.11	-	255.75
Copper	2.41	14086.47	11.35	13894.26
Iron	0.02	962.09	0.06	1491.39
Potassium	21.19	8728.93	50.26	16618.01
Lithium	-	-	-	-
Magnesium	0.35	302.59	3.52	1347.58
Manganese	0.09	482.93	0.31	4205.61
Molybdenum	-	531.50	-	-
Sodium	218.09	1119.22	146.14	1396.00
Nickel	0.15	914.93	0.30	1835.29

Phosphorus	22.65	185082.87	42.68	99270.04
Lead	0.05	4961.54	0.06	1655.17
Antimony	-	-	-	-
Selenium	-	43548.39	-	-
Tin	-	8201.44	-	-
Strontium	0.38	56.31	0.47	227.92
Titanium	0.62	17641.42	0.21	19047.62
Thallium	1.96	8461.54	-	-
Uranium	0.05	175.20	-	-
Vanadium	0.31	6080.54	-	-
Yttrium	0.02	566.73	0.01	96.77
Zinc	8.23	74019.61	16.19	42500.00

Appendix L. Map of Sediment Collection Sites

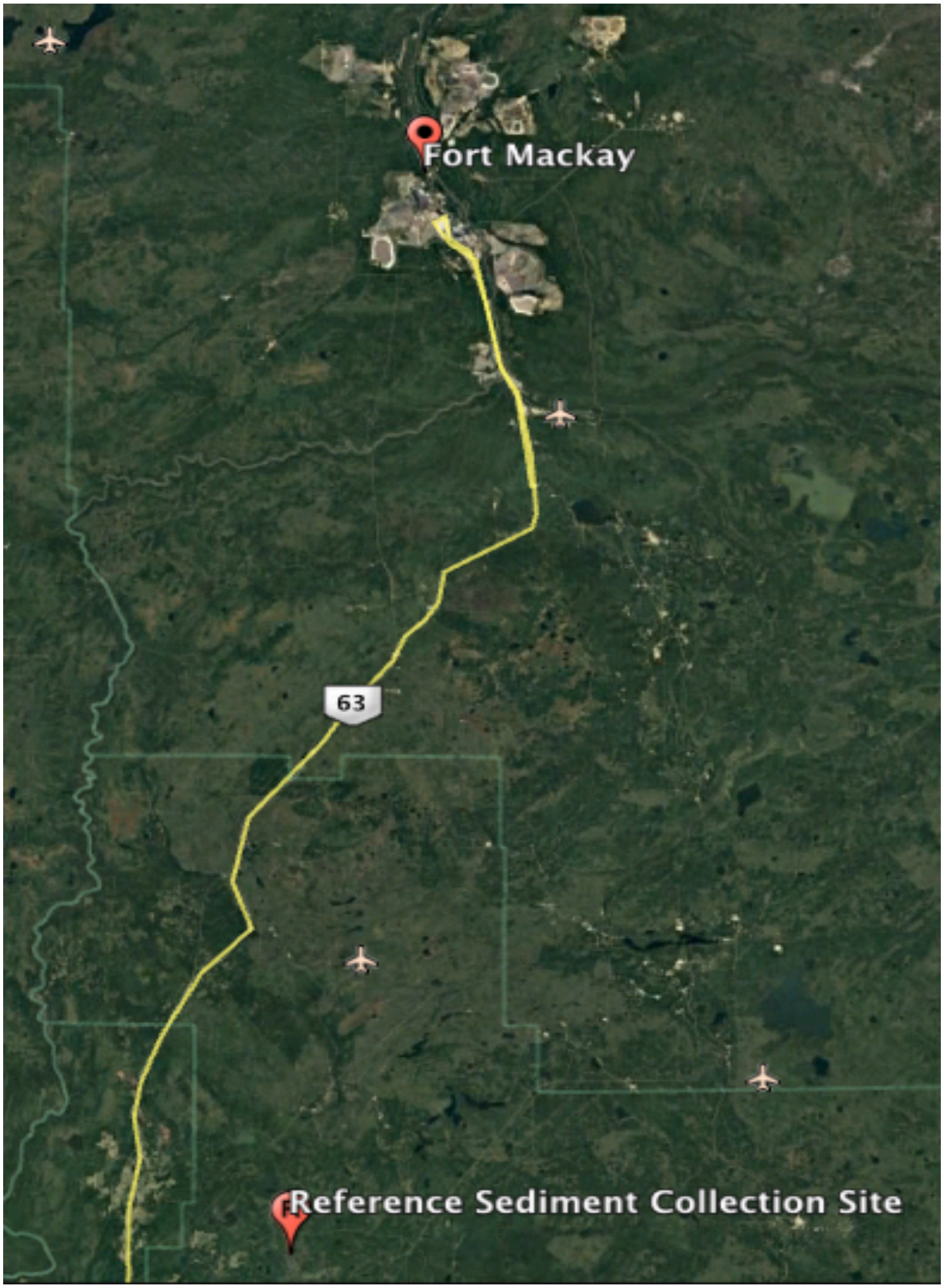
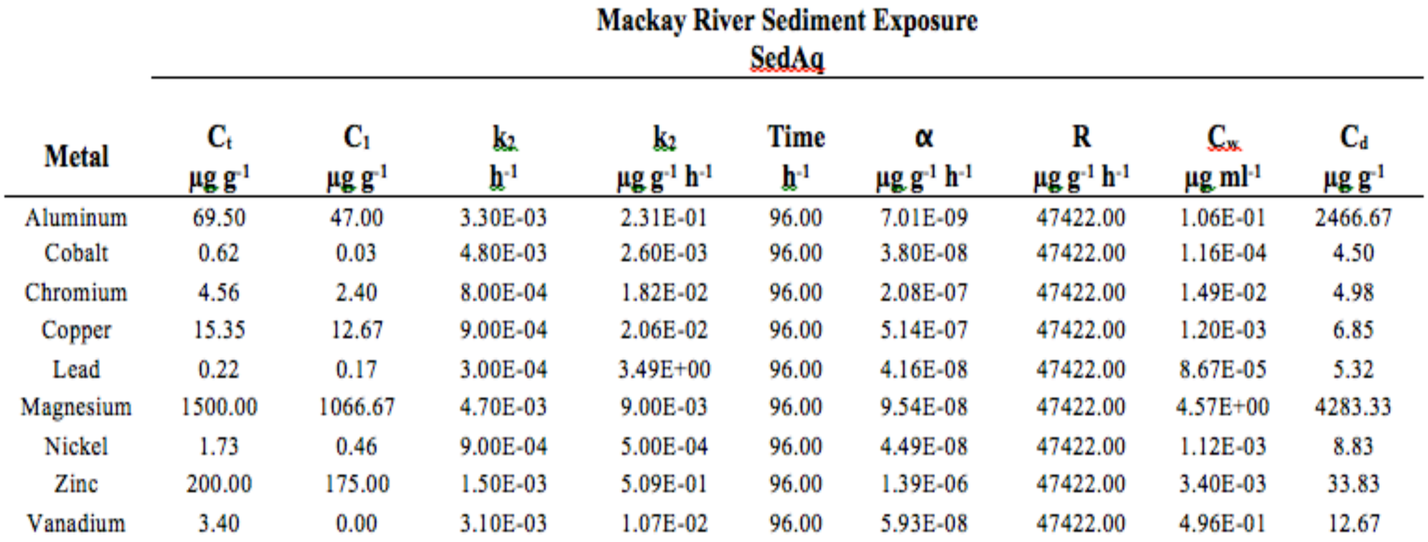


Figure A. 1. Map of sample collection sites. Reference sediment was collected 335 km south from the collected MacKay River sediment.

Appendix M. STELLA^{TM} Variables for Metals

Table A. 22. Variables defined by the experimental uptake-elimination exposure experiment used in order to predict metal bioaccumulation and uptake and elimination kinetics for wood frog tadpoles using STELLA^{TM} software.



Mackay River Sediment Exposure
SedAq

Metal	C_t µg g^{-1}	C_1 µg g^{-1}	k_2 h^{-1}	k_2 µg g^{-1} h^{-1}	Time h^{-1}	α µg g^{-1} h^{-1}	R µg g^{-1} h^{-1}	C_w µg ml^{-1}	C_d µg g^{-1}
Aluminum	69.50	47.00	3.30E-03	2.31E-01	96.00	7.01E-09	47422.00	1.06E-01	2466.67
Cobalt	0.62	0.03	4.80E-03	2.60E-03	96.00	3.80E-08	47422.00	1.16E-04	4.50
Chromium	4.56	2.40	8.00E-04	1.82E-02	96.00	2.08E-07	47422.00	1.49E-02	4.98
Copper	15.35	12.67	9.00E-04	2.06E-02	96.00	5.14E-07	47422.00	1.20E-03	6.85
Lead	0.22	0.17	3.00E-04	3.49E+00	96.00	4.16E-08	47422.00	8.67E-05	5.32
Magnesium	1500.00	1066.67	4.70E-03	9.00E-03	96.00	9.54E-08	47422.00	4.57E+00	4283.33
Nickel	1.73	0.46	9.00E-04	5.00E-04	96.00	4.49E-08	47422.00	1.12E-03	8.83
Zinc	200.00	175.00	1.50E-03	5.09E-01	96.00	1.39E-06	47422.00	3.40E-03	33.83
Vanadium	3.40	0.00	3.10E-03	1.07E-02	96.00	5.93E-08	47422.00	4.96E-01	12.67

Appendix N. Measured, Calculated and Modeled k_1 and k_2 Values

Table A.23. Uptake and elimination rate constants generated through an uptake-elimination laboratory study and computational modeling (±SE). Measured and time series (TS) rate coefficients were generated using data from the laboratory exposure, modeled rates were generated using systems dynamic modeling and calculated rates were generated using equation 3.4.

Metal	**k_1 (modeled) h^{-1}**	**k_1 (measured) h^{-1}**	**k_1 (calculated) h^{-1}**	**k_1 (TS) h^{-1}**	**k_2 (modeled) h^{-1}**	**k_2 (measured) h^{-1}**	**k_2 (calculated) h^{-1}**	**k_2 (TS) h^{-1}**
Aluminum	1.54	4.30	1.86	1.54	0.04	1.2E-03	4.52E-03	0.03
±SE	5.09	6.27	8.14	0.86	9.10	6.00	0.82	6.00
Cobalt	0.26	0.10	0.28	0.70	0.1	0.01	2.27E-03	0.09
±SE	0.32	5.83	0.67	3.83	0.99	7.25	7.12	6.08
Chromium	1.44	0.40	0.01	0.44	0.02	0.11	1.11E-03	7.38E-04
±SE	4.42	0.78	0.13	0.91	8.33	0.72	0.27	0.31
Copper	0.2	0.62	0.01	0.36	0.1	0.13	4.51E-03	9.10E-03
±SE	4.08	7.25	0.18	5.92	8.33	6.42	7.12	4.17
Lead	1.73	0.03	2.88	4.73	0.216	0.04	1.36E-03	3.16E-04
±SE	4.94	0.07	0.18	0.73	0.99	0.11	0.18	1.95
Magnesium	4.5	12.1	0.02	0.04	0.068	0.03	7.69E-02	1.08E-03
±SE	0.12	0.65	0.02	0.08	0.22	0.27	0.30	0.09
Nickel	0.54	0.25	1.61E-02	4.64E-03	0.045	0.11	3.10E-03	2.95E-04
±SE	0.49	9.77	3.39	0.75	0.98	8.16	2.10	0.74
Zinc	2.71	6.67	1.88	0.21	0.045	0.03	3.37E-03	7.61E-03
±SE	1.53	0.44	0.16	2.59	2.94	0.89	0.87	2.79
Vanadium	0.76	0.04	3.02E-03	0.76	0.14	0.02	6.00E-03	3.93E-04
±SE	0.30	0.06	0.75	0.35	0.11	0.12	1.10	0.76

Appendix O. Sensitivity Test Using Different k_1 and k_2 Values

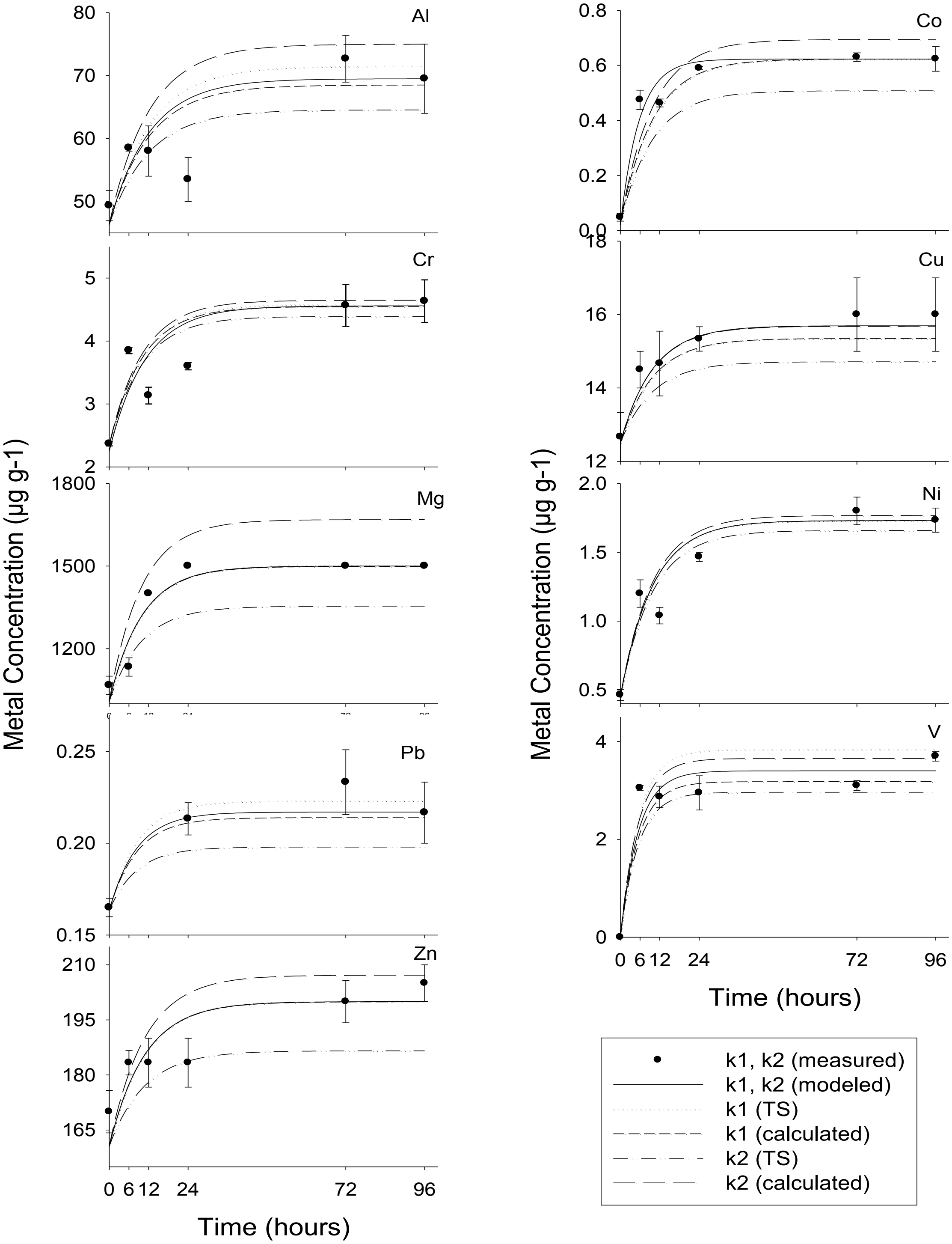


Figure A.2. Mean measured Al, Co, Cr, Cu, Mg, Ni, Pb, V, and Zn concentrations (µg g^{-1}) in wood frog tadpoles (±SE, n=3) compared to a hypothetical extrapolation of the modeled concentrations generated by different uptake and elimination rate constants (h^{-1}).

Appendix P. Statistical Analysis of All Metals Detected in MacKay River Sediment and Reference Sediment

Table A.24. Statistical differences between metal concentrations in MacKay River sediment and reference sediment. Data were log-transformed when normalization was possible. When the p-value of either the Mann-Whitney (M-W) or the student t-test (T-test) was lower than 0.05, the difference between metal concentrations were considered significant (*).

Metal	MacKay River Sediment (µg g^{-1})	Reference Sediment (µg g^{-1})	P-value	n	Statistical Test
Na	4509.11	5686.37	0.09	6	T-test
Ba	4185.54	3039.05	0.06	6	T-test
Ti	4112.47	9477.28	<0.001*	6	T-test
Zn	2118.86	663.41	0.001*	6	T-test
Sr	1722.23	713.95	0.10	6	M-W
V	793.27	315.91	<0.001*	6	T-test
Ni	553.20	113.73	<0.001*	6	T-test
Y	453.00	202.18	0.002*	6	T-test
Cu	428.99	82.14	<0.001*	6	T-test
Li	396.63	0.00	<0.001*	6	T-test
Pb	332.96	170.59	0.004*	6	T-test
Ar	320.44	233.77	0.40	6	T-test
Cr	312.09	0.00	<0.001*	6	T-test
Co	281.82	48.65	<0.001*	6	T-test
Sn	5.22	0.00	1.00	6	M-W
Be	20.35	10.74	0.029*	6	T-test
U	38.20	10.49	<0.001*	6	T-test
Mo	20.88	0.00	<0.001*	6	T-test
Bi	17.43	6.32	0.007*	6	T-test
Cd	3.86	0.00	0.002*	6	T-test
Tl	2.30	0.00	<0.001*	6	T-test
Fe	887208.60	118781.86	<0.001*	6	T-test
Ca	502055.69	54210.02	<0.001*	6	T-test
Mg	268250.13	23061.37	<0.001*	6	T-test
Al	154478.67	139631.87	0.436	6	T-test
K	33922.68	13710.46	<0.001*	6	T-test
P	30165.09	14721.37	0.004*	6	T-test
Mn	13569.07	2306.14	<0.001*	6	T-test

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