

**Evaluation of Approaches for the Derivation of Defensible  
Sediment Quality Guidelines for Application at Saskatchewan Uranium Operations**

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for the Degree of Masters of Science in the  
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Charlene Carol Burnett-Seidel

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## ABSTRACT

Sediment quality guidelines (SQGs) or values (SQVs) are used to assess the potential risk of contaminants in sediment to aquatic organisms, specifically benthic invertebrates. The objectives of this research were to evaluate SQGs derived specifically for use by the uranium industry in Canada, propose alternative SQVs for use at uranium operations in northern Saskatchewan using a novel approach, and investigate the potentially bioavailable fractions of metals in sediment which could become an alternative measurement to the typically used total metal concentrations.

The screening-level concentration (SLC) approach has been used in Ontario, Canada, to derive lowest effect levels (LELs) and severe effect levels for use as SQGs. This approach was adopted by the Canadian Nuclear Safety Commission (CNSC) to set guidelines for metals (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V) and radionuclides (Ra-226, Pb-210, and Po-210) in sediment at northern Saskatchewan uranium mining and milling operations. The SLC approach is based on total metal and radionuclide concentrations in sediment, and corresponding benthic invertebrate community composition data for a specific sampling site. In this study, sediment chemistry (total metals and radionuclides) and benthic community data from northern Saskatchewan uranium operations were compiled and examined. Results indicated that the CNSC-derived SQGs had limited relationships to observed effects, or lack thereof, on benthic invertebrate communities near uranium operations in Saskatchewan. On a general basis, the correct alignment of at least one LEL exceedence at an effect site was observed 95% of the time, but on an element-specific basis many of the elements had concentrations at effect sites below their LELs. Furthermore, concentrations of the evaluated elements exceeded at least one LEL at 60% of the no-effect sites. The high number of exceedences of LELs at reference and no-effect sites (false-positives) calls to question the appropriateness of the CNSC-derived SQGs.

To address the deficiencies of the SLC approach, a new approach was developed to derive alternative metal SQVs for the uranium industry in northern Saskatchewan that was based on the use of reference and no-effect site field data. Three different sets of NE values were derived using combinations of benthic invertebrate community effect criteria (abundance, richness, evenness, Bray-Curtis index). Additionally, reference (REF) values were derived based solely on sediment metal concentrations from reference sites. In general, NE values derived using abundance, richness, and evenness (NE1 and NE2 values) were found to be higher than the

NE values derived using all four metrics (NE3 values). Derived NE values for Cr, Cu, Pb, and V did not change with the incorporation of additional effects criteria due to a lack of influence from the uranium operations on these metal concentrations (i.e., they were generally present at or near background levels). However, a gradient of exposure concentrations was apparent for As, Mo, Ni, Se, and U in sediment which allowed for acceptable exposure levels of these metals in sediment to be defined. The findings from this assessment proposed a set of new, alternate SQVs for use at the uranium industry in northern Saskatchewan.

Often, only total metals concentrations are measured in the evaluation of contaminated sediment. However, this measure may over-estimate metal exposure to benthic invertebrates. Using sediment cores collected from near the Rabbit Lake and Key Lake uranium operations, along with a series of mixed-sediments (contaminated and reference sediment), metal bioaccumulation experiments using *Chironomus dilutus* were conducted in the laboratory. Metal concentrations in extracts from single extractions with either potassium phosphate or hydrochloric acid on wet and dried sediment, pore-water, and whole-sediment were used to evaluate metal bioaccumulation in test organisms. Depending on the metal, pore-water isolated using peepers generally exhibited the best relationship with tissue metal concentrations. Based on this finding, it is suggested that pore-water sampling using peepers (an *in-situ* dialysis device) be added to environmental sampling programs at Saskatchewan uranium operations so that sediment metal availability to benthic invertebrates can be better assessed. With the eventual development of a larger pore-water metals dataset, SQGs based on pore-water metals concentrations could likely be derived as an alternative to existing SQGs based on total metal concentrations in sediment.

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## LIST OF ABBREVIATIONS

AET	apparent effects threshold
AVS	acid-volatile sulfide
BEAST	Benthic Assessment of Sediment
BIC	benthic invertebrate community
CCME	Canadian Council of Ministers of the Environment
CNSC	Canadian Nuclear Safety Commission
DET	diffusional equilibration in thin-films
DGT	diffusive gradients in thin-films
DOC	dissolved organic carbon
DVD	David Lake
EC <sub>50</sub>	median effect concentration
EDTA	ethylenediaminetetraacetic acid
EEM	environmental effects monitoring
EqP	equilibrium-partitioning
ERL	effect range low
ERM	effect range median
f-SSD	field-based species sensitivity distribution
HCl	hydrochloric acid
HC <sub>x</sub>	hazard concentration
HF	hydrofluoric acid
HP	Horseshoe Pond
ICES	International Council for the Exploration of the Sea
ISQG	interim sediment quality guideline
KH <sub>2</sub> PO <sub>4</sub>	potassium phosphate
K <sub>ow</sub>	octanol-water partitioning coefficient
LB25	lethal amount bioaccumulated to effect 25% of the population
LD	limit of detection
LEL	low-effect level
MET	minimal effect threshold

MMER	Metal Mining Effluent Regulations
MPA	maximum permissible addition
MPC	maximum permissible concentration
NC	negligible concentration
NE	no-effect
NEC	no-effect concentration
NEL	no-effect level
NOAA	National Oceanic and Atmospheric Administration
NSTP	National Status and Trends Program
NWRI	National Water Research Institute
PAH	polycyclic aromatic hydrocarbon
PEC	probable effect concentration
PEL	probable effect level
PNEC	probable no-effect concentrations
REF	reference
SD	standard deviation
SED-TOX	sediment-toxicity
SEL	severe-effect level
SEM	simultaneously extracted metals
SLC	screening-level concentration
SMOE	Saskatchewan Ministry of Environment
SSLC	species screening level concentration
SQG	sediment quality guideline
SQV	sediment quality value
TEL	threshold effect level
TEC	threshold effect concentration
TET	toxic effect threshold
TOC	total organic carbon
US EPA	United States Environmental Protection Agency
WQG	water quality guideline

## PREFACE

This thesis is organized as a series of three manuscripts related to the evaluation of sediment quality values, derivation of new sediment quality values using a novel no-effect approach, and the assessment of potential bioavailable fractions of metals in sediment to a model benthic invertebrate, *Chironomus dilutus*. Some repetition of details among chapters was therefore unavoidable.

Chapter 2 is currently published on-line in the scientific journal, *Environmental Monitoring and Assessment* (DOI: 10.1007/s10661-011-2063-1). Chapter 3 will be submitted to *Environmental Monitoring and Assessment* in the fall of 2011. Chapter 4, or a portion of it, may be submitted to a scientific journal in the future.

# **CHAPTER 1**

## **LITERATURE REVIEW**

### **1.1 Introduction**

Sediments are an important part of the aquatic ecosystem. They can act as a sink for anthropogenic inputs, such as metals, but can also act as a source for contamination to overlying water and aquatic organisms. Anthropogenic sources of metal contamination can occur from mining and milling of metals, such as uranium, with metals released into the aquatic environment through effluent and seepages. Frequent monitoring of sediments surrounding mining and milling activities generally occurs to assess the effects of potential contamination. Sediment quality guidelines (SQGs) or values (SQVs) are frequently used as tools to assess the potential of contaminants to cause adverse effects on aquatic organisms. Comparison of SQVs to measured sediment concentrations at aquatic monitoring sites downstream of seepage or effluent discharge points illustrates the potential for those sediments to cause adverse effects on aquatic organisms. Although SQGs are used frequently to assess metals in sediments, they have been criticized for being overly conservative, not taking into account the bioavailability of metals to aquatic organisms, not being site-specific, and not having a single, standard derivation method.

### **1.2 Sediment quality guidelines**

Numerous reviews of the approaches available to derive SQVs have been published, both in peer-reviewed journals (Burton 2002; Chapman 1989; McCauley et al. 2000; van Beelen 2003), by government agencies world wide (ANZECC & ARMCANZ 2000; Canadian Council of Ministers of the Environment (CCME) 2001; Florida Department of Environmental Protection 1994; ICES (International Council for the Exploration of the Sea) 2003), and, more recently, through international workshops (Wenning et al. 2005). There are numerous approaches to deriving SQVs. Differences between approaches include the amount of data required, inclusion of variables, procedures, statistics, and how the derived values are used. However, in general

sediment quality value derivation approaches can be divided into two categories; empirical and mechanistic (Vidal and Bay 2005).

## **1.2.1 Sediment quality guideline derivation approaches**

### **1.2.1.1 Empirical approaches**

Empirical SQV approaches are based on statistical analyses of large databases of sediment chemistry and toxicity data to identify relationships between chemical concentrations and biological effects (Vidal and Bay 2005). These effects-based approaches to deriving SQVs rely on laboratory tests or field data that compare concentrations of a contaminant to the effect on an organism(s). Many effect endpoints can be used (e.g., survival, growth, reproduction, species presence/absence, and biochemical markers), but cause-effect relationships are assumed rather than evaluated in empirical approaches. Chemical speciation and bioavailability are not directly addressed, but are indirectly considered because a large database with a broad cross section of data is used (Vidal and Bay 2005). A broad cross section of data incorporates many possible contaminants, sediment types, ranges of chemical concentrations, abiotic factors, biological organisms, and effects. Many of these empirical approaches are very similar, with only small differences in how values are calculated or determined (i.e. effect centiles, data criteria, etc.).

#### **1.2.1.1.1 Spiked-sediment toxicity**

The spiked-sediment toxicity test method for deriving SQVs assesses the toxicity of the contaminant(s) of interest to multiple aquatic organisms (usually benthic invertebrates) to determine a concentration-response relationship. Test sediment is spiked with increasing concentrations of a contaminant or contaminant mixture in a laboratory. The concentration-response is used to define a threshold concentration. Contaminant concentrations below the threshold are less likely to cause an effect on an organism and concentrations above the threshold are more likely to cause an effect. Multiple species of organisms are typically used because different species vary in sensitivity to a given toxicant. This approach was based on sediment toxicity testing methods developed by Gannon and Beeton (1971) and Swartz et al. (1979). A major assumption with this method is that laboratory experiments can realistically mimic real world situations (Adams et al. 1992).

#### **1.2.1.1.2 Apparent effects threshold (AET) approach**

The apparent effects threshold approach uses the chemical concentrations from field-collected sediments and laboratory test results, and at least one indicator of biological effects (e.g., benthic invertebrate structure, toxicity test endpoints) to determine the concentration that will *always* cause significant adverse biological effects (Adams et al. 1992; Chapman 1989). A relatively large database is needed (> 30 data points) with a range of concentrations. This approach assumes that using a large database accounts for the inclusion of possible interactions between contaminants and environmental factors, and that bioavailability or bioeffects can be determined based on either field or laboratory data (Chapman 1989).

#### **1.2.1.1.3 Screening-level concentration (SLC) approach**

This field-based approach estimates the concentration of a contaminant in sediment which is predicted to be tolerable to 95% of the population of benthic infauna. Persaud et al. (1993) and Thompson et al. (2005) outline this methodology, which uses co-occurring total contaminant concentrations in field-collected sediments and corresponding benthic community effect data. Screening-level concentrations for each contaminant of interest are calculated by first deriving a species screening level concentration (SSLC). This is the 90<sup>th</sup> percentile in a raw frequency distribution of contaminant concentrations at all sites where a species is present. Using all of the SSLCs for a single contaminant, another frequency distribution is constructed from which the lowest-effect level (LEL) and severe-effect level (SEL) are determined. The LEL is the 5<sup>th</sup> percentile of this distribution and is the concentration below which harmful effects on benthic invertebrates are not expected. The SEL is the 95<sup>th</sup> percentile from this distribution and is the concentration above which harmful effects on benthic invertebrates are expected. There are three assumptions to this approach, as outlined by Chapman (1989). First, it is assumed that if the database is large, then the influence of modifying factors, such as habitat variables, water depth, and sediment grain size, should not have to be considered with respect to particular contaminants (i.e., variability is taken into account due to the breadth of the database). Secondly, it is assumed that when a species is present at a site that it is due to a lack of a biological effect (i.e., the contaminants at that site are not causing an effect on that specific species). Lastly, the approach assumes that the assessment of a toxic response can be adequately measured, with appropriate sensitivity, by the presence or absence of a species (Chapman 1989).

#### **1.2.1.1.4 Effects range approach (ERL and ERM)**

This approach derives environmental risk limits and was introduced by Long and Morgan (1990) who derived values from studies performed in both saltwater and freshwater. A large database of co-occurrence data (bulk sediment chemical concentrations and related biological effects) is compiled to determine the level at which organisms are moderately (effects range median - ERM) and minimally (effect range low - ERL) effected. The ERL is the concentration below which adverse effects are not likely to occur, whereas the ERM is the concentration above which adverse effects are more likely (NOAA (National Oceanic and Atmospheric Administration) 1999). The effects-range values are derived by plotting co-occurrence data in ascending order, noting adverse effects for each corresponding sediment concentration. Because adverse effects generally increase with increasing concentration, the ERL value is defined as the 10<sup>th</sup> percentile whereas the ERM value is the 50<sup>th</sup> percentile. These values are intended as informal benchmarks to be used in the interpretation of chemical data from sediment.

#### **1.2.1.1.5 Threshold and probable effect levels (TEL and PEL)**

The threshold and probable effect levels (TELs and PELs) approach for deriving SQGs was suggested by Smith et al. (1996). Effect levels have been derived using field based sensitivity distributions as outlined in Leung et al. (2005). According to Smith et al. (1996), SQVs are derived using a weight of evidence approach by matching biological and chemical data from numerous modeling, laboratory, and field studies performed on freshwater sediment and compiled into the biological effects database for sediments. Two values, TEL and PEL, are derived from this data set. The TEL is calculated by the geometric mean of the 15<sup>th</sup> percentile of the observed effect data set and the 50<sup>th</sup> percentile of the no observed effect data set. The PEL is calculated by the geometric mean of the 50<sup>th</sup> percentile of the observed effect data set and the 85<sup>th</sup> percentile of the no observed effect data set. The TEL is the level below which adverse effects are unlikely and the PEL is the level above which adverse effects are estimated to frequently occur.

#### **1.2.1.1.6 Field-based species sensitivity distribution (f-SSD) approach**

The field-based species sensitivity distribution (f-SSD) approach was first proposed by Leung et al. (2005). This approach is similar to other effects based field approaches, such as

AET, LEL/SEL, ERL/ERM, and TEL/PEL approaches. For each separate chemical, the f-SSD values are derived by plotting the EC<sub>50</sub>s in a cumulative frequency distribution (percentiles). Various hazard concentrations (HC<sub>x</sub>; e.g., HC<sub>5</sub>) are determined by the nonparametric bootstrap method. Because only sensitive species are used to derive the HC<sub>x</sub>, the HC<sub>x</sub>s were corrected so that they would not be overprotective. This was accomplished by finding the level (*x*) of HC<sub>x</sub> that would protect 95% of the species in the community resulting in an impact of 5% of the community (HC<sub>5</sub>). The adjusted community HC<sub>5</sub> was selected as the TEL for each chemical, while the PEL was represented by the adjusted community HC<sub>10</sub> from the f-SSD (Leung et al. 2005). The construction of f-SSDs assumes that if sensitive species are protected, then other more-tolerant species are protected as well.

#### **1.2.1.1.7 Consensus approach**

Consensus based SQGs were derived for a particular contaminant from various other empirically based SQGs and have been reviewed thoroughly (MacDonald et al. 2000a; MacDonald et al. 2000b; Swartz 1999). Two levels of effect are calculated using current, previously derived SQGs. The threshold effect concentration (TEC) is the concentration of a contaminant below which an adverse effect on sediment organisms is not expected. This TEC value is based on SQGs such as TELs, ERLs, LELs, minimal effect thresholds (METs), and sediment quality advisory levels. The second effect level is the probable effect concentration (PEC) and illustrates a concentration of contaminant above which harmful effects are expected to occur frequently in sediment organisms. This PEC is based on SQGs such as PELs, ERMs, SELs, and toxic effect thresholds (TETs). These effect concentrations (TEC and PEC) are derived by calculating the geometric mean of the published values for a chemical substance (or group of substances) after three or more independent SQGs have been published (MacDonald et al. 2000b).

#### **1.2.1.1.8 Regression models**

There are two models that use the regression-type approach. The first model has been proposed as an alternative means of calculating ecotoxicological thresholds to overcome existing limitations of the available data (Field et al. 1999; Field et al. 2002). The approach estimates the percentage of samples expected to be toxic at a given contaminant concentration. It uses a large

database of co-occurring data (i.e. sediment contaminant concentrations and measurements of effects on benthic invertebrates) from field-collected samples, and applies logistic models and concentration-interval plots. The other model estimates SQVs by regressing the bioavailable contaminant concentration (amount bioaccumulated or concentration in overlying water) against concentrations in the sediment and determining the point of intersection of the regression line with critical concentrations (i.e. LB25s: lethal amount bioaccumulated to effect 25 % of the population, or water-based LC25s) (Borgmann 2003).

### **1.2.1.2 Mechanistic approaches**

Mechanistic approaches to SQV derivation incorporate factors that affect bioavailability of chemicals in sediment. Variables such as organic carbon and amorphous sulfides are considered. Because of this incorporation, mechanistically derived SQGs have a greater ability to determine which contaminant is causing toxicity in a mixture. However, more chemical data are required which results in a reduced number of published mechanistic SQVs, compared to empirical SQVs.

#### **1.2.1.2.1 Equilibrium-partitioning (EqP) approach**

The EqP approach uses a correction factor for organic carbon content in order to derive SQVs from water quality criteria/guidelines and also considers the simultaneously extracted metals/acid-volatile sulfide (SEM/AVS) ratio, which is used for predicting toxicity of sediments. Since its original development in the 1980's, this approach has been developed further by Di Toro et al. (1991). This approach makes several assumptions which include that partitioning of chemicals is based on the organic carbon content in the sediment with little consideration for other physical or chemical factors, that water column organisms are affected in a similar way as sediment dwelling benthic invertebrates, and that uptake via respiratory surfaces is the only route of exposure without considering sediment ingestion. Steady-state conditions are assumed to occur in aquatic systems and partition coefficients are assumed to be applicable to natural environments (Chapman 1989).

#### **1.2.1.2.2 Tissue residue approach**

This approach relates the maximum acceptable concentration of a chemical in the sediment to an acceptable concentration in aquatic organisms or consumers. Tissue residue concentrations, bioconcentration factors, and/or octanol-water partitioning coefficients ( $K_{ow}$ ) are used to estimate contaminant concentrations that are safe for animals or people to be in contact with or ingest (usually the latter). As well, knowledge of sediment-organism partition coefficients, equilibrium partitioning, organism preference factors, and pharmacokinetic-bioenergetic models are useful in accurately determining appropriate sediment concentrations (Adams et al. 1992).

#### **1.2.1.3 Other approaches**

##### **1.2.1.3.1 Sediment background approach**

This SQV approach uses the premise that contaminated sediments can be compared to a reference (either a nearby uncontaminated site sediment or sediment from a core prior to contamination) to reveal chemicals of concern. Reference sediments form the basis for the regulatory criteria. This approach assumes that there is no assimilative capacity or threshold for toxic effects for benthic organisms and that the toxicity of contaminants in sediments are not influenced substantially by sediment characteristics (Chapman 1989). In other words, benthic invertebrates may be able to tolerate concentrations greater than the background concentration, but that contaminant concentration difference between the toxicity threshold and the background concentration is not considered in the sediment background approach and may result in overly conservative SQVs.

##### **1.2.1.3.2 Water quality criteria approach**

This approach compares the contaminant concentrations in interstitial water (pore-water) with available water quality criteria (previously derived). The premise is that water quality criteria can be used as the backbone for sediment criteria. This approach assumes that water column organisms are as sensitive as benthic organisms, that information from aqueous bioassays are applicable to sediments, and that sediment ingestion is not a route of significant exposure (Chapman 1989). According to Chapman (1989), the advantage of this approach is that it uses a large database so that only field measurements are required. However, this approach is

limited to only those contaminants having existing water quality criteria. Without these, SQVs cannot be developed using this approach. In addition, methods for measuring interstitial water concentrations are not widely standardized or validated, criteria are only available for individual chemicals and may not be applicable to complex chemical mixtures, and this approach does not use toxicological data derived directly from the sediment of interest (Chapman 1989). This method is used in Australia and New Zealand as an alternative to their primary method of deriving SQVs (ANZECC & ARMCANZ 2000).

## **1.2.2 Uses**

### **1.2.2.1 Canada**

Canada currently has SQGs for the protection of aquatic life (CCME 1995; 2001; 2002). Some of the guideline values are “interim” (i.e., ISQG) due to limited available information, uncertainty, and information gaps regarding sediment toxicity. This may change with the availability of more data and the development of standardized methods. The CCME along with Environment Canada are responsible for these guidelines. The SQGs are used as a nation-wide sediment screening tool.

The derivation of a numerical SQG in Canada relies mainly on the National Status and Trends Program (NSTP; developed by the NOAA of the United States of America) or the TEL approach. However, an initial weight of evidence approach is completed on the contaminant of concern which includes a literature search and review of environmental chemistry and fate data, evaluation of toxicology data for acceptability, and an evaluation/establishment of background concentrations. Then, the NSTP approach is carried out whereby co-occurrence data of biological effect and chemical concentration data are used to establish an association between effects and the concentration of a contaminant in sediments. Once these two steps are complete, tables of co-occurring data are derived. If minimum data requirements are met, TEL and PEL values are derived. The SQG is then recommended by the CCME and it is determined whether or not spiked-sediment toxicity tests are needed to either complement or validate the SQG. If minimum data requirements are not met, ISQGs are adopted or default options are investigated and implemented, and the data gaps are identified. The approach can be applied to both freshwater and estuarine (saltwater) sediments and is developed on a chemical-by-chemical basis. It should be noted that SQGs for metals, refer to the “total” concentration of metal

recovered using a near-total (acid digestion) method. The CCME sediment guidelines have been criticized by Borgmann (2003) for not being based on clear cause-effect relationships. This makes it difficult to manage sediments based on these guidelines.

Sediment quality guidelines in Canada can be used in conjunction with other sediment assessment tools. No single sediment assessment tool should be used solely to predict whether adverse biological effects will occur, but should be used in conjunction with other assessment tools to make a practical and informed decision (CCME 2001). The National Water Research Institute (NWRI) and Environment Canada have developed other assessment tools, such as the Benthic Assessment of Sediment (BEAST) and the sediment-toxicity (SED-TOX) index to aid in contaminated sediment management. In the future, the spiked-sediment toxicity test approach could be used to derive SQGs in Canada, similar to the approach used in water quality guideline derivation, once methodological concerns are overcome.

Other approaches used in Canada, which are different from the CCME approach, are the EqP and SLC approaches. These derivation approaches are used in the province of Ontario (Persaud et al. 1993). In Persaud et al. (1993), three levels of effect were established – a no-effect level (NEL), a LEL, and a SEL. The derivation of a NEL used the EqP approach (Section 1.1.1.2.1) and is based on concentrations that are low enough to not be passed up the food chain. The LEL and SEL values are derived using the SLC approach (Section 1.1.1.1.3). Minimum data requirements have been established for both methods of deriving SQGs.

In Saskatchewan, uranium operations (as an example) are regulated according to several acts. Some of these include the Fisheries Act (under which the Metal Mining Effluent Regulations (MMER) are enforced), the Nuclear Safety and Control Act, and the Canadian Environmental Protection Act. These acts do not include regulation of sediments with regards to metals or radionuclides. Therefore, the CNSC recently derived SQGs for total metals and radionuclides in sediment for Canadian uranium operations using the SLC approach and data from Canadian uranium operations (Thompson et al. 2005). The SQGs derived by the CNSC and the CCME serve only as guidelines that are not enforceable under legislation, unless they are set as a license requirement for an operation. Because of the specificity of the guidelines derived by the CNSC for the uranium industry, higher regard for adherence to the guidelines may be necessary to comply with the CNSC requirements even though they are not legally enforceable.

The CNSC SQGs are frequently used in risk assessments and evaluations of sediment data at uranium operations in Saskatchewan.

#### **1.2.2.2 United States**

The United States does not have an overall sediment policy, but rather multiple sub-policies (Nord 2001). However, the United States Environmental Protection Agency (US EPA), has derived EqP benchmarks for dieldrin, endrin, PAH mixtures, and metal mixtures (Cd, Cu, Pb, Ni, Ag, Zn) in sediment. New York State has a technical guidance document for screening contaminated sediments for non-polar organic contaminants and metals. They use the EqP method to derive SQGs for non-polar organic contaminants, but have adopted the SLC approach for metal guideline derivation as established by NOAA (Long and Morgan 1990) and the Ministry of Ontario (Persaud et al. 1993). The State of Florida has sediment quality assessment guidelines for coastal waters which are applicable to freshwater. Threshold effect levels and PELs are derived for 34 contaminants using the weight of evidence approach developed by Long and Morgan (1990). In general, the approach of Long and Morgan (1990) is commonly used to derive SQVs.

#### **1.2.3 Future of sediment quality guidelines**

According to Adams et al. (1992), the future of SQVs rely on four key actions. First, SQV derivation should be incorporated into a tiered assessment approach to evaluate the significance of sediment contamination. Secondly, derived SQVs should be validated with field tests to assess the applicability to real situations. Thirdly, methods for measuring contaminants and ways of improving chronic toxicity testing capabilities should be expanded and improved. Lastly, exploring ways to delineate historical contamination from current practices would be beneficial. With the development of new statistical techniques and more sophisticated, integrated approaches for deriving SQVs, new SQVs are likely to emerge within the next decade. The start of this trend is the use of species sensitivity distributions and regression models for deriving SQVs. Derivation approaches should continue to be based on biological responses which is a very important aspect of deriving scientifically defensible SQVs (Borgmann 2003; Giesy and Hoke 1990).

### **1.3 Behavior of metals in aquatic systems**

#### **1.3.1 Sediment**

Given that sediments can act as a contaminant sink, the geochemistry of sediment is of importance downstream of effluent discharge points and in other areas of contamination around the globe. Sediments can act as a source of contaminants even after industrial or municipal discharges cease. Chemical changes that occur when an effluent is introduced to the environment can cause metals to precipitate from solution and deposit in surface sediment. Benthic organisms, especially invertebrates, may take up these metals either from solution, direct contact with the sediment, or through sediment ingestion. If sediment is disturbed by changes in overlying water conditions or through dredging, metals can be released back into solution (depending on the physiochemical properties of the water and sediment) and carried further down the drainage area, causing a spread of metal contamination in the drainage basin. Although sediments may act as a sink for contaminants and contain high concentrations of metals, especially in immediate discharge areas, only a small fraction of contaminants, such as metals adsorbed onto sediments, may be bioavailable. Metals may bind to a variety of ligands (e.g., organic matter and sediment particles) which will reduce their bioavailability (discussed further below). Binding depends on the properties and affinity to other molecules and compounds.

As alluded to above, numerous geochemical factors affect the chemistry of metals in sediment. Physical factors include grain (particle) size, surface area, surface charge, and organic carbon content. Chemical factors can be mechanistic (type of association) in nature, such as adsorption, precipitation, and cation-exchange, or they can be related to a chemical or physical phase (type and quantity of substrate), such as interstitial water, carbonates, hydrous Fe and Mn oxides, sulfides, and silicates (Horowitz 1991). As examples, adsorption is the adherence of metals to surface of sediment particles and is major mechanism for the collection of metals in sediments. Clays specifically are known to adsorb metals because they have high surface areas (relative to their volume) which allows numerous binding sites for metals and other metal “collectors”, such as organic matter and hydrous Fe and Mn oxides. In comparison, sand has much lower surface area to volume ratios (comprised of larger particles) and thus will not bind metals to the extent that clay would. Clearly, physical factors and chemical mechanisms that affect metals in sediment are strongly interrelated.

Additionally, metals in sediment can be influenced by the overlying water and pore-water chemistry. In this scenario, sediments can act as a source for contamination rather a sink, as discussed above. Dissolution and precipitation depends on the properties of the metal and the conditions of the aquatic environment (such as pH and hardness). Generally, as pH decreases the solubility of metals increases, thus releasing metals that are adsorbed to sediment into the surrounding water. High hardness concentrations in water generally decrease the availability of metals to aquatic organisms due to the competition of Ca and Mg ions with the uptake of other metal ions. The contribution of the water associated with sediments influences the dynamic chemistry of metals in sediments.

### **1.3.2 Pore-water**

Toxicants in sediment are available to benthic invertebrates via sediment pore-water. Dissolved pore-water metals make up a small fraction of the total metals measured in bulk sediment. However, only total metal concentrations in bulk sediment are typically measured and reported.

Pore-water contains water soluble and colloiddally-adsorbed contaminants. Sediment properties, such as AVS content, percent total organic carbon (TOC), particle size distribution (affects surface area and charge), and dissolved ligands (i.e., dissolved organic carbon (DOC), dissolved sulfide) govern the partitioning of divalent cationic metals between the solid and liquid phases, and hence the bioavailability of metals within pore-water (Di Toro et al. 1991; Mahony et al. 1996). Once these ligands are exhausted, excess metals occur as 'free' (uncomplexed) metal in the pore-water. This results in potential interactions of the available metal fraction and benthic invertebrates.

A variety of ligands affect the availability of metals to aquatic organisms. In oxic sediments, organic carbon, iron and manganese oxyhydroxides, and other components of geochemical processes can bind with metals to reduce their availability (Bryan and Langston 1992; Tessier and Campbell 1987; Tessier et al. 1993). In anoxic sediments, AVS and organic carbon have been found to bind with divalent, cationic metals to make them unavailable to aquatic organisms (Di Toro et al. 1991; Mahony et al. 1996).

Acid volatile sulfide can play a major role in binding metals in sediment. Acid volatile sulfides are defined as the sulfide liberated and collected from sediments during cold

hydrochloric acid treatment (Burton et al. 2007; Carbonaro et al. 2005). Acid-volatile sulfides and SEM are collected at the same time (Di Toro et al. 1991). The ratio of SEM/AVS is used as a tool to predict the bioavailable fraction of selected metals, such as Cd, Cu, Ni, Pb, and Zn (Burton et al. 2007; Hansen et al. 1996). Ratios of SEM/AVS > 1.0 indicate sediments potentially toxic to benthic invertebrates (i.e., excess metals may be present which are not bound to AVS and may illicit toxic action on benthic invertebrates via pore-water exposure), whereas an SEM/AVS ratio < 1.0 indicates that sediments are likely nontoxic (i.e., excess binding sites for metals with no bioavailable metal fraction) (Burton et al. 2007; Di Toro et al. 1990; Di Toro et al. 1991; Hansen et al. 1996).

A number of methods exist for collecting pore-water. The whole-core squeezer method involves squeezing a sediment core between two pistons with pore-water being collected via a sample tube (Bender et al. 1987). The centrifugation-filtration method, which is more widely used in research today, involves centrifuging a sediment sample and collecting the overlying solution which is filtered prior to analysis (Emerson et al. 1980). As well, peepers or *in situ* dialysis samplers have become increasingly popular. Peepers are sampling vessels filled with purified water and covered with a semi-permeable membrane (Hesslein 1976). They are placed directly into sediment and allowed to equilibrate over time via diffusive processes. Once equilibrium has been reached, the peepers are removed and the pore-water sample analyzed for dissolved metals.

Benthic invertebrates are generally in contact with pore-water, which is a potential route of contaminant exposure for many benthic and epi-benthic invertebrates. It has been shown that pore-water metal concentrations, such as copper, compared to water-only toxicity data, accurately predict toxicity (presence and extent) for the amphipod *Hyaella azteca* in test sediments (Ankley et al. 1993). Likewise, ammonia toxicity was accurately predicted from pore-water concentrations for the midge *Chironomus tentans* and the oligochaete *Lumbriculus variegatus* (Whiteman et al. 1996). Speciation of metals in pore-water has been correlated with accumulation in benthic organisms (Vink 2002). Therefore, contaminants in pore-water can be used to predict or at least correlate with effects on benthic organisms.

In light of this, SQVs based on measurements of bioavailable contaminant fractions have been developed. The criteria include pore-water and associated AVS and SEM measurements for metals (Ankley et al. 1996; Leonard et al. 1996). As well, they incorporate equilibrium

partitioning theory, which assumes that chemicals are at equilibrium and are partitioned between the solid and dissolved phases (Di Toro et al. 1991). For example, using the partitioning of organic contaminants between organic matter and pore-water, sediment quality criteria have been developed for nonionic organic chemicals (Di Toro et al. 1991). Sediment quality criteria based on bioavailable fractions of contaminants in sediment are thought to be a more reliable and scientifically defensible than those derived using total (bulk) contaminant concentrations in sediment.

### **1.3.3 Bioavailability and modifying factors of toxicity**

Bioavailability can be defined as the “proportion of total metals that is available for incorporation into biota (bioaccumulation)” (John and Leventhal 1995) or the “degree to which a chemical is able to move into or onto an organism” (Benson et al. 1994). Horowitz (2001) defines bioavailability as the “portion of dissolved, biologically-, or sediment-associated chemical constituents that are readily accessible to biota either through physical contact or by ingestion” which is a general and operationally defined term.

There are many factors which can modify the availability (and toxicity) of metals in sediment. These modifying factors include sediment organic matter, Fe and Mn oxyhydroxide and sulfide content, particle size distribution, pH, and hardness of water and pore-water. Organic matter (quantified as organic carbon) is found naturally in sediment and is derived from plants, microbes, and animals. Organic matter has varying sorption capacities depending on the composition. Generically, when a metal binds to organic matter a complex is formed. This complex has varying degrees of stability and the affinity of the metal may depend on the speciation of the metal and the type of organic matter. Iron and manganese oxyhydroxides and sulfides bind metals in a manner similar to organic matter. Sediment particles also have the ability to bind metals in a similar fashion. Smaller sediment particles (i.e., clay, silt) have large surface areas relative to their volume (more binding sites) and therefore bind more metal than the same mass of larger sediment particles (i.e., sand). The hydrogen ion content (pH) of pore-water and overlying water has an impact on bioavailability as well. Generally, the lower the pH (more  $H^+$  ions) the more metals (especially cationic metals) will be in a free form (unbound) as the result of competition between the  $H^+$  and metal ions. This competition results in the displacement (liberation) of metals from sediment. Elevated water hardness can decrease the

toxicity of some metals (i.e., Cd, Co, Cu, Zn) (Mayer et al. 1994) due to competition at metal uptake sites.

## **1.4 Chemical extractions**

Various chemicals can be added to sediment to extract various operationally-defined fractions of metals. This typically involves mixing a chemical solution with sediment, allowing for a mixing period, and then isolating the aqueous phase. This procedure may be done sequentially (numerous chemicals are added to a single sediment sample one after another following each isolation), or singly (only one chemical is added to a sediment sample). The metal concentrations extracted from a sediment sample will vary depending on the process used, the properties of the chemicals, the metals of interest, and the characteristics of the sediment.

### **1.4.1 Sequential extractions**

Sequential extraction is a means of partitioning sediment metals into operationally and chemically defined fractions, depending on speciation and binding properties of the metal and the type of particulate matter available. The procedure is based on the concept that specific fractions could be defined with appropriate chemicals and the purpose of the sequential extraction procedure was to provide “detailed information about the origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport of trace metals” (Tessier et al. 1979). Tessier et al. (1979) operationally defined the metal fractions in sediment to be (from most to least potentially bioavailable): exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter, and residual. The sequential extraction procedures developed by Tessier et al. (1979) are well cited and used. However, many subsequent studies have modified the methods of Tessier et al. (1979) in some way such that each similarly named extracted fraction is somewhat operationally different.

Each subsequent fraction in a sequential extraction sequence removes metals that are more tightly bound to sediment particles than the previous fraction. Extraction reagents are chosen based on their ability to selectively extract metals from a particular fraction. A list of potential chemicals used to extract metals from sediments and soils, along with their approximate relative mobility and extraction strength, are listed in Figure 1.1. A single sediment sample is used throughout a sequential extraction; therefore, the total sediment metal concentration is equal

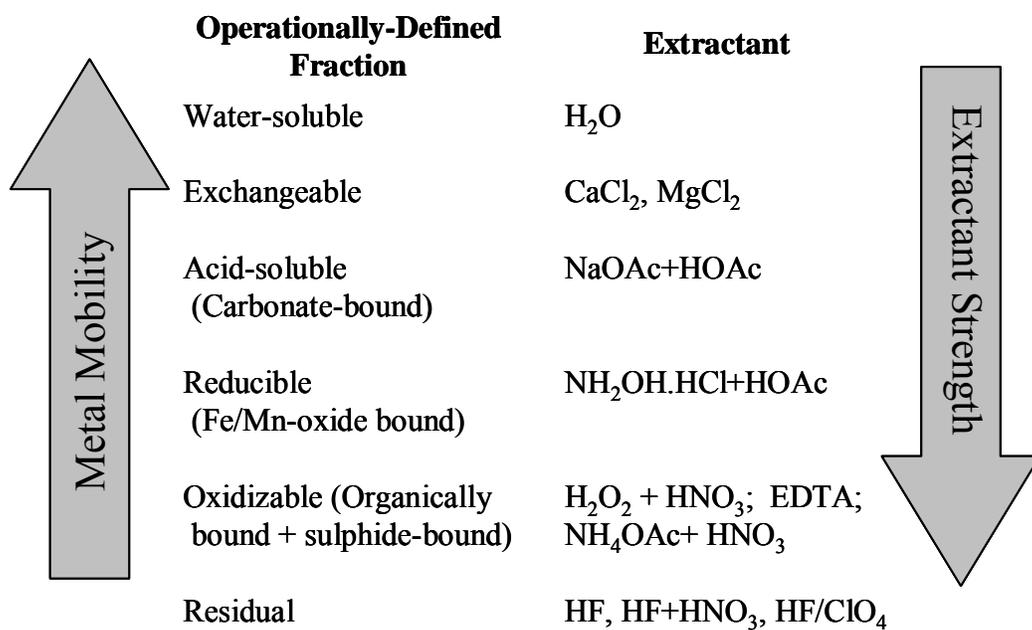


Figure 1.1. Relationship between metal mobility in the different operationally-defined phases and extractant strength of common chemical reagents used in single and sequential extractions (modified from Filgueiras et al. (2002) and Tessier et al. (1979)).

to the sum of all the fractions. In other words, all the concentrations from fractions in a sequential extraction, ending in an hydrofluoric acid (HF) extraction as an example, should add up to the concentration from digestion of sediment with HF only.

Bioavailable fractions often make up only a small percentage of the total metal concentration in sediment, depending on the metal and sediment (Lopez-Sanchez et al. 1993; Tessier et al. 1979). Bioavailable fractions are thought to include those in overlying water or pore-water (Buykx et al. 2000), the exchangeable fraction, and the fraction bound to organic matter (Carapeto and Purchase 2000). In the sequential extraction scheme shown in Figure 1.1, the most bioavailable fractions would be the water-soluble, exchangeable and acid-soluble fractions.

Many modifications of the Tessier et al. (1979) approach have been explored with extraction results varying with each unique approach. The reasoning behind such modifications are that the five-step sequential extraction technique (Tessier approach) is very time-consuming and labour-intensive (Carapeto and Purchase 2000). The European Commission, through the Standards, Measurement, and Testing Programme, has developed a three-step sequential extraction procedure known as the BCR (Community Bureau of Reference; the old name for the Standards, Measurement, and Testing Programme) approach (Lopez-Sanchez et al. 1993; Pueyo et al. 2001; Quevauviller et al. 1997; Rauret et al. 1999).

Sequential extraction data can be correlated with biological data to evaluate the relationship between extracted contaminant fractions and biological effects. Ramos et al. (1999) investigated the relationship between sequentially extracted Cu, Pb, Cd, and Zn in sediments with tissue concentrations of exposed earthworms and the best results generally correlated well with the fraction where the metal was mainly associated (i.e., fraction with the highest % of the total concentration). For instance, the exchangeable fraction represented about 53% of the total Cd concentration in sediment and the residual and organic fractions together accounting for about 80% of the total Cu concentration in sediment (Ramos et al. 1999). This area of research has not been extensively pursued, especially for freshwater sediments.

#### **1.4.2 Single extractions**

A single extraction, as the name implies, involves a single application of a chemical solution to sediment. Single extractions are similar to sequential extractions in that a chemical

solution is added to sediment or soil to extract an operationally-defined fraction of metal. A specific fraction is targeted and the procedure can be modified to extract particular metals or groups of metals depending on the chemical (extractant) used. This simpler procedure is more time and cost effective than sequential extractions when there is a particular fraction of interest rather than multiple fractions.

Single versus sequential extractions have been compared by Tack and Verloo (1999). They found metal concentrations in acid extractable, reducible, and residual fractions from single extractions generally agreed with those from sequential extraction fractions. Each of these various approaches to fractionation of soil or sediment metal concentrations use similar groups of chemicals to extract a particular fraction. However, there are slight variations in extraction efficiency within each fraction, depending on the sediment properties (i.e., clay, sand, and organic matter proportions) and methods employed (i.e., mixing time, use of wet or dried sediment, temperature, ratios of chemicals, amount of chemical used per amount of sediment).

## **1.5 Benthic invertebrates**

Benthic invertebrates reside in or on sediment. Given this close association, benthic invertebrates are often good indicators of sediment toxicity. Benthic invertebrates, such as *H. azteca* and *Chironomus dilutus* (formerly *C. tentans*), are routinely used in laboratory toxicity testing to evaluate sediments (Norberg-King et al. 2006). In the field, bioaccumulation of contaminants in benthic invertebrates can be used (along with other lines of evidence) to characterize the partitioning of metals in ecosystems (Frag et al. 2007). More commonly, benthic invertebrate communities are used to measure the effects of mining activities on fish habitat in environmental effects monitoring programs (Environment Canada 2002).

Summary metrics, such as family richness, abundance of organisms, the evenness index, and the similarity index (e.g., Bray-Curtis Index), are used to measure the difference between reference and exposure benthic communities. Any significant difference between reference and exposure site summary metrics results in the exposure site being considered as adversely affected. This would indicate that sediment, or potentially another component of the aquatic ecosystem, is causing an adverse effect on the benthic community which in Canada is considered fish habitat (Environment Canada 2009).

## **1.6 Uranium industry in northern Saskatchewan**

### **1.6.1 Mining process**

Methods used to mine uranium (U) ore include open pit mining, underground mining, and *in situ* recovery. Open pit mining involves excavating the overburden to access the ore body and then using explosives to break up the rock which is hauled to the surface for milling. Underground mining involves access and ventilation shafts being dug into the ground to access the ore. The ore is extracted using raise and jet boring techniques, processed underground into sand which is mixed with water to form a slurry, and transferred to the surface for milling. *In situ* recovery involves pumping solutions into the ore bodies. The solutions dissolve the U, which is then pumped back to the surface for U extraction.

### **1.6.2 Milling process**

Milling is the act of processing the extracted ore to isolate U from other minerals and rock material. The ore is first crushed (unless already in solution) and treated with acid to separate the U from other materials. The solution is then purified with chemicals to dissolve the U and chemically separated to precipitate U out of solution. The precipitate is dried with the resulting powder being U oxide concentrate,  $U_3O_8$ , more commonly referred to as yellowcake. Yellowcake is packed in drums and shipped to a refinery. Before the mined U can be used in a nuclear reactor, it must undergo refining, conversion, and enrichment.

### **1.6.3 Uranium operations**

The U operations in northern Saskatchewan were chosen as a case study to evaluate a new approach to deriving SQGs and to investigate various methods of measuring bioavailable metal fractions in sediment. Within the Athabasca Basin in northern Saskatchewan, concentrated uranium ore is found in abundance. The individual uranium operations are relatively similar to each other and are therefore satisfactory models for deriving regional SQVs. There are presently five uranium operations in Saskatchewan which are operated by either Cameco Corporation or AREVA Resources Canada (Figure 1.2). Currently, uranium ore is mined at the McClean Lake, Rabbit Lake, McArthur River, and soon, Cigar Lake, operations. The ore is milled at McClean Lake, Rabbit Lake, or Key Lake operations to extract uranium. The processed yellowcake is packaged and sent to refineries outside of the province. Four other mine/mill operations (Cluff

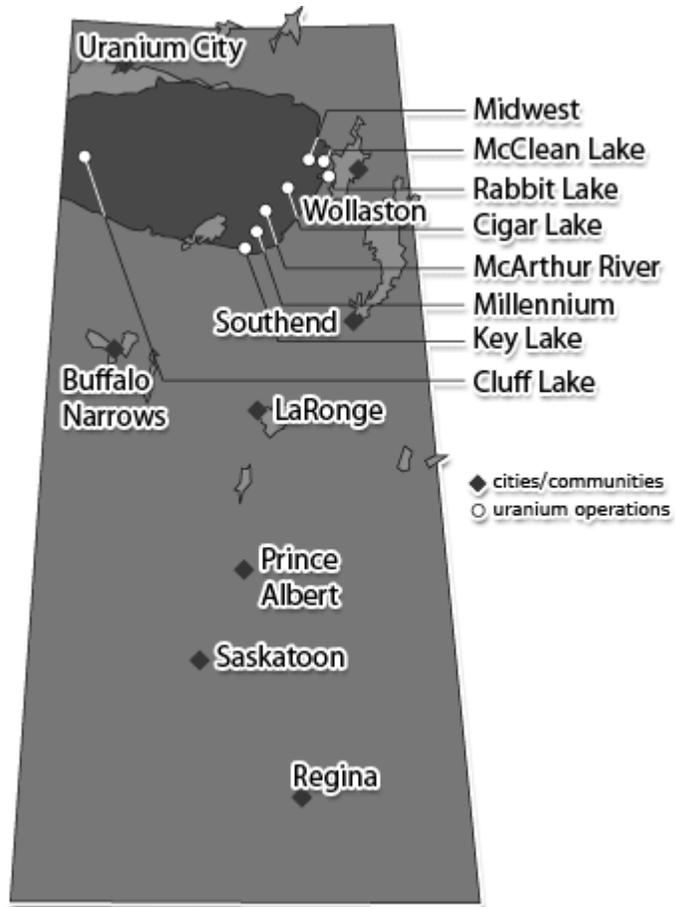


Figure 1.2. Locations of uranium mining and milling projects in Saskatchewan, Canada (from [www.cameco.com](http://www.cameco.com)).

Lake, Beaverlodge, Gunnar, Larado) in Saskatchewan are undergoing decommissioning, reclamation and remediation activities.

#### **1.6.4 Metals of potential concern**

In this case study, the metals of primary concern are uranium, selenium, arsenic, molybdenum, and nickel. These specific metals co-occur in uranium ore bodies and could potentially cause adverse environmental effects if elevated concentrations are released in the mine effluent. They are also of particular interest because they are known to be bioavailable to aquatic organism to varying degrees depending upon the environmental conditions. Other metals of interest include chromium, copper, lead, and vanadium.

#### **1.6.5 Regulatory framework**

Uranium operations in Saskatchewan are regulated according to several provincial and federal acts, and require licenses through the Saskatchewan Ministry of Environment (SMOE) and the CNSC. The specific acts include the Fisheries Act (under which the MMER are enforced), the Nuclear Safety and Control Act, the Environmental Management and Protection Act, and the Canadian Environmental Protection Act. The specifics for license requirements vary by each operation, but they generally include mine plans, environmental monitoring programs, worker safety programs, and quality management systems requirements. These acts and license requirements are strictly enforced and in place to protect the environment and mine/mill employees.

Using data from Canadian uranium operations, the CNSC recently derived LELs using the SLC approach developed by Persaud et al. (1993) based on total metal and radionuclide concentrations in sediment (Thompson et al. 2005). The SLC approach is reportedly suitable for metals and is applicable to benthic invertebrates (Persaud et al. 1993). Co-occurring total metal and radionuclide concentrations in sediments and associated benthic community effects data from uranium mine sites in northern Saskatchewan and northern Ontario originally collected between 1985 and 2001 were used to derive the guidelines (Thompson et al. 2005). The CNSC, using this approach, has derived SQGs for As, Cr, Cu, Pb, Mo, Ni, Se, U, V,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$ , and  $^{210}\text{Po}$ . These guidelines provide benchmarks not previously derived for some metals and radionuclides. However, the approach has limitations in that the full range of species tolerances

for all contaminants may not be represented by the data obtained (Thompson et al. 2005). In addition, total sediment metal concentrations, rather than bioavailable metal measurements, are correlated with species presence or absence.

The CNSC-derived SQVs are used to determine the likelihood of adverse effects on benthic invertebrate communities due to sediment contamination resulting from mine/mill effluent. They are also intended to facilitate decisions about effluent control and to determine the scale and magnitude of potential remediation efforts (Thompson et al. 2005). If a SQV is exceeded, then it is anticipated that there would be an effect on the benthic community and a concerted effort by the company to alleviate the contamination (e.g., stop or decrease effluent discharge, employ remediation efforts). These SLC SQGs derived by the CNSC are frequently used to evaluate sediments near uranium operations in Saskatchewan, even though they are not legally enforceable (unless it is specified in an operations license requirements).

## **1.7 Research goals and objectives**

The overall goal of this research was to evaluate the current methods for deriving SQVs for metals and to propose a scientifically defensible and user-friendly alternative applicable to Saskatchewan uranium operations. To achieve this, the following research objectives were completed:

- I) Conduct a critique and assessment of the predictive capabilities of the current SQVs derived for the uranium industry using data from uranium operations in northern Saskatchewan and identify possible options for alternative SQV derivation approaches;

H<sub>0</sub>: There is no difference between the predictive capabilities of currently available SQVs for the uranium industry (based on Thompson et al. (2005)) and that conducted using a more recent data set.

- II) Using a novel derivation approach, derive regionally-specific SQVs for Saskatchewan uranium mines based on reference and no observed-effect concentrations associated with benthic communities;

H<sub>0</sub>: There is no difference between regionally-specific NE SQVs and other no- or low-effect SQVs currently available for each of the metals investigated (As, Cr, Cu, Ni, Pb, Mo, Se, U, V).

- III) Identify a scientifically-defensible, user-friendly approach for measuring bioavailable fractions of metals in sediments.

H<sub>0</sub>: Pore-water or chemical extractions are no better than total sediment metal concentrations at predicting sediment metal bioavailability to the model benthic invertebrate *C. dilutus*.

**CHAPTER 2**

**EVALUATION OF SEDIMENT QUALITY GUIDELINES DERIVED  
USING THE SCREENING-LEVEL CONCENTRATION APPROACH  
FOR APPLICATION AT URANIUM OPERATIONS  
IN SASKATCHEWAN, CANADA\***

**2.1 Abstract**

Sediment quality guidelines (SQGs) can be derived using different approaches and are commonly used in environmental management, reclamation, and risk assessment. The screening-level concentration (SLC) approach has been used in Ontario, Canada, to derive lowest effect levels (LELs) and severe effect levels (SELs) for use as SQGs. This approach was adopted by the Canadian Nuclear Safety Commission (CNSC) to set guidelines for metals (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V) and radionuclides (Ra-226, Pb-210, and Po-210) in sediment at northern Saskatchewan uranium mining and milling operations. The SLC approach is based on total metal and radionuclide concentrations in sediment, and corresponding benthic community composition data for a specific sampling site. In this study, sediment chemistry (total metals and radionuclides) and benthic community data from northern Saskatchewan uranium operations were compiled and examined. Results indicate that the CNSC-derived SQGs had limited relationships to observed effects, or lack thereof, on benthic invertebrate communities near uranium operations in Saskatchewan. The LELs were found to correctly align with effects at 95% of the sites that had effects, on a general basis, but on an element-specific basis many of the elements had concentrations at effect sites below their LELs. Furthermore, concentrations of the evaluated elements exceeded at least one LEL at 60% of the no-effect sites. The high number of exceedences of LELs at reference and no-effect sites (false-positives) calls to question the appropriateness of the CNSC-derived SQGs. It is suggested that alternatives to the SLC approach be explored.

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## 2.2 Introduction

Although the manner in which they are used varies, sediment quality guidelines (SQGs) are generally used to help protect aquatic environments from adverse effects of various stressors. Typically, total concentrations of contaminants in whole sediment are compared to guideline values to determine whether there is a potential for benthic invertebrate community impairment. Sediment quality guidelines can be used in environmental assessments in combination with other measures (e.g., water quality, concentrations of contaminants in resident biota, benthic community indices, in situ experimental results) to evaluate the risk to aquatic ecosystems from anthropogenic activities (e.g., industrial development, effluent discharges). Sediment quality guidelines may also be used as target values for particular contaminants of concern during reclamation of contaminated sites.

Many approaches to SQG derivation have been used to date. These can generally be divided into two broad categories: mechanistic and empirical approaches. Mechanistic SQG derivation approaches (e.g., the equilibrium partitioning approach (Di Toro et al. 1991; US EPA 2005)) incorporate chemical and biological factors that are known to modify the bioavailability of contaminants. Empirical SQG approaches are based on statistical analyses of large databases of co-occurring sediment chemistry and sediment toxicity/biology data to identify relationships between chemical concentrations and biological effects (Vidal and Bay 2005). Various endpoints, such as survival, growth, reproduction, bioaccumulation, presence/absence of taxa, and biochemical markers, can be used to identify an effect. The datasets used to derive empirical guidelines may incorporate many possible contaminants, sediment characteristics, chemical concentrations, abiotic factors, chemical interactions, and biological effects. Examples of empirical approaches include the effects range approach (Long and Morgan 1990), the threshold and probable effect level approach (Smith et al. 1996), the consensus approach (MacDonald et al. 2000a, b), and the screening level concentration (SLC) approach (Persaud et al. 1993; Thompson et al. 2005).

The focus of this evaluation is the SLC approach. The Canadian Nuclear Safety Commission (CNSC) reported to have chosen this approach for derivation of SQGs for use at uranium mining and milling operations in Canada because of the abundance of historical and current environmental monitoring data that already existed related to that industry, and because the approach was reportedly applicable to situations involving benthic invertebrates and metals

(Thompson et al. 2005). The CNSC guidelines presented by Thompson et al. (2005) are derived according to the approach of Persaud et al. (1993). Briefly, this approach involves plotting species-specific benthic invertebrate distributions in relation to total contaminant concentrations in whole sediment. The 90th percentiles derived from each of the various species-specific distributions of contaminant concentrations are then plotted in a species sensitivity distribution. The 5th and 95th percentiles of the species sensitivity distribution represent the lowest effect level (LEL) and severe effect level (SEL) SQGs, respectively. The SQGs thus derived by Thompson et al. (2005) provide a set of regional SQGs to monitor and assess the condition of sediments near uranium operations in northern Saskatchewan.

Although currently in use, there are limitations to the SLC approach which reflect the shortcomings in the SQGs derived for the uranium industry in Canada. Specifically, the SLC-derived SQGs may not accurately reflect the true effects of trace element concentrations on benthic organisms. Trace element concentrations may be misclassified as the effect concentrations because an element is “guilty by association”. For example, some benthic organisms may be absent at a site due to the concentration of one particular trace element. However, because the SLC approach does not recognize cause–effect relationships, all trace element concentrations present at that site would be classified as effect concentrations (regardless of causality). Because the SLC approach is solely based on the presence or absence of taxa, the resulting SQGs have very limited application and are difficult to defend scientifically. Due to their reliance on statistical and mathematical relationships between invertebrate community data and total sediment contaminant concentrations, and the potential for under sampling rare species and over representing common species, authors such as von Stackelberg and Menzie (2002) and Smith and Jones (2005) have previously cautioned against the use of the SLC approach and other similar empirical approaches when deriving SQGs. Guidelines derived using the SLC approach have little relationship to toxicity thresholds and can be similar to the background concentrations associated with the dataset used in the derivation process (Smith and Jones 2005). In essence, the SQGs derived using the SLC approach are simply a correlational function of the data and are not based on cause–effect relationships.

The objective of this research was to independently evaluate the SQGs derived by the CNSC for application at Saskatchewan uranium operations. To achieve this, SQGs derived by Thompson et al. (2005) were thoroughly compared with sediment chemistry and benthic

invertebrate community effects data collected from Cameco Corporation uranium projects in northern Saskatchewan, Canada. The data in this evaluation were independent of what was used in the original assessment conducted by Thompson et al. (2005). Thompson et al. (2005) assessed their SQGs using a subset of the data that was used in the derivation of the SQGs. This could have biased the results of the evaluation of the SQGs. This case study of regionally derived SQGs will serve as a measure of how effective the empirical SLC approach is at predicting effects (or the lack thereof) of metals and radionuclides on resident benthic invertebrate communities.

### **2.3 Material and methods**

Environmental monitoring data from sites near Cameco Corporation uranium mining and milling projects located in northern Saskatchewan, Canada, were used to evaluate the SLC approach. Scientific reports containing the data used in the evaluation were obtained from Cameco Corporation, various environmental consulting firms, an academic thesis, and a field study completed by the authors (CanNorth Environmental Services 2001, 2005b, 2008; Conor Pacific Environmental Technologies Inc. 1999; Golder Associates Ltd. 2002, 2003, 2005a, b, c, 2008a, b; Robertson 2006; Terrestrial & Aquatic Environmental Managers Ltd. 1997). The data were from the Key Lake, Rabbit Lake, McArthur River, and Cigar Lake uranium operations, and collected between 1996 and 2007. A total of 15 reports were reviewed which collectively include 33 different sampling locations and 87 different datasets of co-occurring sediment chemistry (total metal and radionuclide concentrations) and benthic invertebrate community data. Of the 87 datasets, 28 were from reference sites and 59 were from locations downstream of mining and milling activities (i.e., effluent discharge, potentially contaminated seepage). As a result of temporal environmental monitoring programs, 24 of the 33 sampling locations were sampled more than once. The majority of the benthos sampling programs described in the various reports followed the procedures outlined in the Environmental Effects Monitoring (EEM) guidance document (Environment Canada 2002) for sampling benthic invertebrates (e.g., Ekman grab samples). Sediment fractions for metals analysis were obtained from 0–2 cm to approximately 0–5 cm horizons and were collected using a variety of equipment (e.g., Ekman grab sampler, Tech-Ops extruder corer, hand-corer). Generally, five replicate samples for sediment chemistry and benthic invertebrate community assessment were collected. Sediment

and benthic invertebrate sampling took place throughout the year, but occurred most frequently in the fall.

Collected data were compared to the SQGs derived by Thompson et al. (2005). First, sediment chemistry data (mean metal concentrations) from each site were screened for exceedences of these SQGs. The guideline values derived by Thompson et al. (2005) using their “weighted method” are presented in Table 2.1 and were used in all comparisons in this study. These SQGs include LELs and SELs for As, Cr, Cu, Mo, Ni, Pb, Se, U, V, Pb-210, Po-210, and Ra-226. These elements were either deemed by Thompson et al. (2005) to be of concern at uranium operations in northern Saskatchewan, routinely monitored at metal mines, or have other SQGs derived for the elements using other approaches. According to Thompson et al. (2005), if a total trace element concentration in sediment was less than the LEL, adverse effects on the benthic community were not expected, and if the concentration in sediment was greater than the SEL, harmful effects on the benthic invertebrate community were expected to occur. Thompson et al. (2005) recommended the use of their “weighted” LEL SQGs for use in ecological risk assessments as concentrations below which adverse effects on the benthic invertebrate communities are not expected but found that their SEL SQGs were not good predictors of severe impacts on benthic invertebrate communities.

The next step in the evaluation presented here was to screen the various exposure site benthic community data for evidence of adverse effects. In this study, an effect on the benthic invertebrate community was defined as a statistical difference between a reference and exposure site with respect to total abundance (one report presented only mean abundance) or taxon richness (generally at family level). If statistical comparisons were not made for abundance or richness within the report evaluated, then an effect was defined as a >20% difference in either abundance or richness as compared to the reference site. These definitions were chosen to correspond to the effect criteria defined by Thompson et al. (2005), which defined an effect between reference and exposure sites as a >20% difference in either abundance or richness, and also the Canadian EEM Program (Environment Canada 2002) which defines an effect on the benthic community as a statistical difference between reference and exposure sites.

After SQG exceedences and benthic community effects were evaluated for each dataset, the sediment chemistry data and benthic community effects data were integrated to determine the ability of SQGs to accurately predict an effect, or lack thereof, on benthic invertebrate

Table 2.1. Summary of select metal ( $\mu\text{g/g}$  dry wt) and radionuclide ( $\text{Bq/g}$  dry wt) lowest effect levels (LELs) and severe effect levels (SELs) from Thompson et al. (2005).

	As	Cr	Cu	Mo	Ni	Pb	Se	U	V	Pb-210	Po-210	Ra-226
LEL	9.8	47.6	22.2	13.8	23.4	36.7	1.9	104.4	35.2	0.9	0.8	0.6
SEL	346.4	115.4	278.8	1238.5	484.0	412.4	16.1	5874.1	160.0	20.8	12.1	14.4

Table 2.2. Summary of water depth and physical sediment characteristics for sites used in the evaluation of SLC approach-derived sediment quality guidelines.

	Water Depth (m)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Content (%)	Total Organic Carbon (%)
Minimum	0.5	0.01	0.3	1.0	1.0	19.1	0.2
Maximum	78.0	0.13	98.0	89.2	67.0	98.4	27.1
Average	5.9	0.04	28.7	50.5	21.7	84.6	8.3
Median	2.5	0.03	17.8	55.2	16.1	89.2	7.6

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Table 2.3. Number of sampling sites (with the % of total number of sites) with either an effect or no-effect on the benthic invertebrate community in relation to exceedences of the lowest effect level (LEL) and severe effect level (SEL) sediment quality guidelines (SQGs) derived by Thompson et al. (2005).

Metal and Radionuclide Concentrations	Number of sites ( <i>n</i> )	Benthic Community	
		Reference/No-Effect	Effect
No exceedence of LELs	19 (22%)	17 (37%)	2 (5%)
Exceeded at least one LEL	68 (78%)	29 (63%)	39 (95%)
Exceeded at least one SEL	33 (38%)	15 (33%)	18 (48%)
Total number of sites ( <i>n</i> )	87	46	41

communities. Predictive ability thus assessed how well SQGs predicted the correct outcome (i.e., effect or no-effect site). This measure of reliability was assessed through four possible combinations of outcomes whereby benthic invertebrate communities were or were not affected and SQGs were or were not exceeded. Reliability was assessed on a general and specific level. General predictive ability identified exceedences of any metal or radionuclide at a single site. Specific predictive ability identified exceedence of an individual metal or radionuclide (on a metal-specific basis) at a single site.

Thompson et al. (2005) also conducted an evaluation of their SQGs based on predictive ability using a subset of the data used to derive their LELs and SELs. It should be noted that the datasets used in the evaluation of the SLC approach in this study were not identical to the data used in the SQG derivation process used by the CNSC (Thompson et al. 2005). Only four sampling sites (one report from 1996) overlapped between the data used in this evaluation of the SLC guidelines and the data used by the CNSC to derive the SLC guidelines.

## **2.4 Results**

Of the 87 datasets used in this assessment, 28 were from reference locations. Benthic communities were significantly affected at 41 of the 59 exposure site locations. Eighteen of the 59 exposure sites were determined to be unaffected (no measurable effect on the benthic invertebrate community). Sediments in this region can vary widely in their physical characteristics (e.g., particle size distribution, moisture content, and total organic carbon content). Sediment characteristics for the sites used in this assessment are summarized in Table 2.2. Graphical presentation of % organic carbon and % sand in sediments from reference and exposure sites illustrated that, generally, there was an even distribution of sediment physical characteristics at reference and exposure sites (unpublished data).

### **2.4.1 General predictive ability**

It was anticipated that an exceedence of one or more SQGs would be found at effect sites. Out of all the sites evaluated (reference, no-effect, and effect), 78% had element concentrations that exceeded at least one LEL and 38% had concentrations that exceeded at least one SEL (Table 2.3). Of the sites exceeding at least one LEL value, 57% (39/68) was found to have an effect on the benthic invertebrate community and 43% (29/68) was either reference or no-effect

sites (Table 2.3). Of the sites exceeding at least one SEL value, 55% (18/33) was found to have an effect on the benthic community and 45% (15/33) where either reference or no-effect sites (Table 2.3). Looking at the data in a different way, it was found that exceedences of at least one trace element LEL were found at 95% of the effect sites (Table 2.3). Conversely, reference and no-effect sites should not have trace element concentrations exceeding either the LEL or SEL SQGs. Only 37% of the reference and no-effect sites had all trace element concentrations below their respective LELs (Table 2.3).

#### **2.4.2 Specific predictive ability**

To further investigate the predictive ability of the SQGs derived by Thompson et al. (2005), the percentage of LEL and SEL exceedences were explored for each metal and radionuclide individually. The percentage of reference, no-effect (including and excluding reference sites), or effect sites (based on benthic invertebrate community abundance and richness) that exceeded the LEL and SEL SQGs derived by Thompson et al. (2005) are provided in Table 2.4. These values reflect the ability of the SQG for each metal or radionuclide to accurately predict a potential effect, or the lack of an effect, on the benthic community at the sites investigated.

For As, Mo, Ni, Se, U, Pb-210, Po-210, and Ra-226, no-effect sites (including reference sites) were found to have concentrations of these elements in sediment that were above individual LELs, but below SELs, 15% to 33% of the time. Arsenic, Cr, Mo, Ni, Se, and Po-210 concentrations in sediment were above the SELs 2% to 27% of the time at no-effect sites (including reference sites). More than 77% of the time no-effect sites (including reference sites) had Cr, Cu, Pb, U, V, and Ra-226 sediment concentrations below their LEL.

At effect sites, sediment concentrations for all 12 elements were below LELs 18% to 89% of the time. For Cr, Cu, Pb, and V, metal concentrations at effect sites were below their LELs 67% to 89% of the time. Conversely, for As, Mo, Se, and U, concentrations in sediment were generally greater than LELs 53% to 82% of the time. Apart from the elements found above the LEL, all other elements present at effect sites would be categorized as “guilty by association”.

Table 2.4. Percent of reference, no-effect, or effect sites, based on location (reference sites) and benthic invertebrate community abundance and richness, which had metal or radionuclide concentrations below or above the lowest effect level (LEL) and severe effect level (SEL) sediment quality guidelines (SQGs) derived by Thompson et al. (2005).

	Metals and Radionuclides											
	As	Cr	Cu	Mo	Ni	Pb	Se	U	V	Pb-210	Po-210	Ra-226
<u>Percent of Reference Sites that are:</u>												
< LEL	79	96	100	85	85	100	75	85	90	67	71	88
> LEL but < SEL	11	0	0	15	11	0	25	15	10	33	21	12
> SEL	11	4	0	0	4	0	0	0	0	0	8	0
<i>n</i> =	28	24	28	26	26	25	12	27	21	24	24	24
<u>Percent of No-Effect Exposure Sites (only) that are:</u>												
< LEL	22	100	89	47	25	100	20	65	100	50	67	83
> LEL but < SEL	56	0	11	18	63	0	20	35	0	50	33	17
> SEL	22	0	0	35	12	0	60	0	0	0	0	0
<i>n</i> =	18	17	18	17	16	16	10	17	14	12	12	12
<u>Percent of No-Effect Sites (including reference sites) that are:</u>												
< LEL	57	98	96	69	62	100	50	77	94	67	69	85
> LEL but < SEL	28	0	4	17	31	0	23	23	6	33	25	15
> SEL	15	2	0	14	7	0	27	0	0	0	6	0
<i>n</i> =	46	41	46	42	42	41	22	44	35	36	36	34
<u>Percent of Effect Sites that are:</u>												
< LEL	41	84	73	18	54	89	19	47	67	57	63	63
> LEL but < SEL	39	16	27	59	44	11	39	48	26	40	34	31
> SEL	20	0	0	23	2	0	42	5	7	3	3	6
<i>n</i> =	41	37	41	39	39	37	26	40	30	35	35	35

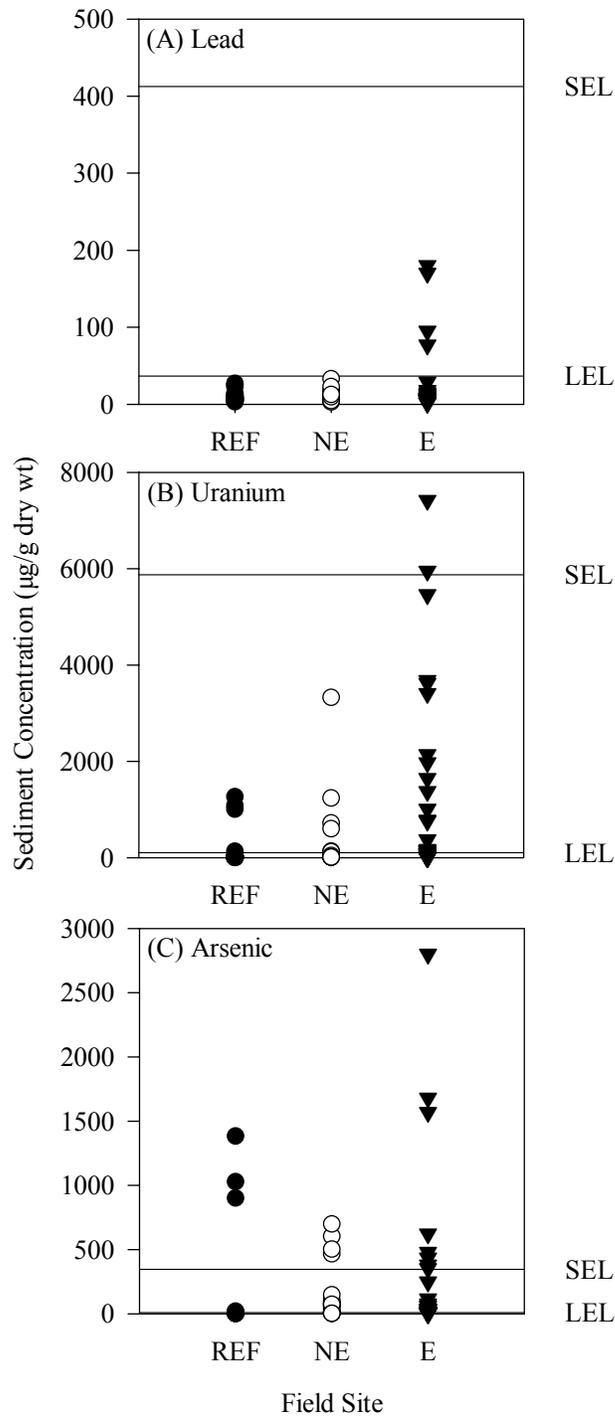


Figure 2.1. Comparison of metal (Pb, U, As) concentrations in sediment at reference (REF), no-effect (NE) and effect (E) sites relative to the lowest effect level (LEL) and severe effect level (SEL) sediment quality guidelines derived by Thompson et al. (2005).

Graphs of selected trace element concentrations at reference, no-effect, and effect sites in comparison to SLC SQGs illustrate the above mentioned exceedences (Figure 2.1). Lead (Figure 2.1a), for example, illustrates the situation where reference and no-effect sites have Pb concentrations below the LEL and effect sites concentrations both above and below the LEL. Figure 2.1b depicts numerous exceedences of the U LEL at reference and no-effect sites, and a range of U concentrations at effect sites which fall both above and below the LEL and SEL. Figure 2.1c shows the exceedence of As LEL and SEL values at all sites.

## **2.5 Discussion**

According to Apitz et al. (2007), “The goal of SQGs is to provide a screening-level indication of whether contaminants at a given level in sediments are likely to be non-toxic, possibly toxic, probably toxic, or extremely toxic”. In other words, they categorize a gradient of toxicity, from non-toxic to potentially or definitely toxic. The Canadian Council of Ministers of the Environment (2001) state “Sediment quality guidelines provide scientific benchmarks, or reference points, for evaluating the potential for observing adverse biological effects in aquatic systems.” Thus, it is generally agreed upon that SQGs should be used as a primary screening tool to assess the potential for adverse effects in aquatic systems. However, Burton (2002) points out that chemical benchmarks, such as SQGs, are often used as a “primary decision-making tool, with little or no site validation of biological effects”. Therefore, the validity of establishing SQGs as screening tools to determine true biological effects can be debated, although advances have recently been made using, for example, the consensus-based approach.

Sediment quality guidelines have been used in a variety of ways. They are used in the management of dredged materials (Casado-Martinez et al. 2006) and as sediment quality remediation targets (Crane et al. 2002; Crane and MacDonald 2003). They are solely used or integrated as a line of evidence into environmental risk assessments to evaluate the potential for adverse effects on aquatic life (Chang et al. 2004; Long et al. 2006; Thompson et al. 2005; Apitz et al. 2007). They are also a part of environmental guidance, such as the Canadian Environmental Quality Guidelines (Canadian Council of Ministers of the Environment 2001), and as such serve as national benchmarks. Furthermore, SQGs can be used in other applications to assess potentially adverse effects, protect the quality of the environment, and prevent harm to an ecosystem.

Advantages of the SLC approach largely relate to the use of large databases of chemical and biological data in the derivation of the guidelines (Florida Department of Environmental Protection 1994; McCauley et al. 2000). In addition, the approach is relatively inexpensive to implement, the dataset is easily collected (assuming it is available in the first place), the approach uses common analyses (e.g., benthic invertebrate metrics and concentrations of elements in sediment; McCauley et al. 2000), and it can be used with any contaminant and existing databases (Chapman 1989; Florida Department of Environmental Protection 1994). Chapman (1989) further mentions that the approach is based on an objective method which is similar to setting water quality criteria that do not assume any specific mechanisms of toxic action.

Despite the various advantages of the SLC approach, there are limitations to this method of SQG derivation. The major weaknesses to the SLC approach are that causality is not determined and bioavailability is not considered (Florida Department of Environmental Protection 1994; McCauley et al. 2000). The approach uses a large database, but may be incomplete if data are not available for all contaminants of interest, all benthic organisms, or the specific areas of concern (Florida Department of Environmental Protection 1994). Taxonomic identification of benthic invertebrates may be challenging and expensive depending on the level of accuracy needed and the number of samples to be analyzed (Chapman 1989). Also, it is difficult to distinguish effects of mixtures of contaminants from effects of single contaminants, and measured contaminants may be influenced, to varying degrees, by unmeasured contaminants or sediment characteristics, all of which may have an effect on benthic invertebrate communities (Chapman 1989). These limitations were the motivation to further explore the applicability of the SLC approach.

### **2.5.1 Predictive ability of the SLC approach**

Exceedences of LELs and occasionally even SELs at reference and no-effect sites were routinely observed in this study. There was only a small degree of agreement between lack of effects (reference and no-effect sites) and concentrations below LELs and SELs. One would expect that if the SLC approach generated appropriate guidelines then less false-positives should occur.

Reasons for these observed exceedences at reference and no-effect sites are twofold. First, some LELs (and possibly even some SELs) derived by Thompson et al. (2005) are too low (overly conservative), especially for those elements which appear to be “guilty by association” (e.g., one element may cause an effect due to an elevated concentration, but other elements with low concentrations are co-associated with the effect). Secondly, no-effect sites were not truly “no-effect” sites. This could result from insufficient statistical power to determine true differences in benthic community composition, (i.e., resulting from low replication), or because the indices used in the assessment of effects (i.e., abundance and richness) were not sensitive enough to identify true effects at exposure sites designated as no-effect sites. Using additional benthic invertebrate community composition metrics (e.g., evenness, diversity, or similarity indices) may help identify such effects, if they truly are there. Only abundance and richness were used here in order to be consistent with the SLC derivation methodology used by Thompson et al. (2005). When evaluating individual metals and radionuclides, it was found that some elements were either below or above their LEL and SEL values at no-effect and reference sites more so than other elements. For instance, Cr, Cu, Pb, V, and Ra-226 were found more than 80% of the time at concentrations below their LEL values at reference and no-effect sites (Table 2.4). This would be expected for appropriate SQGs. Lead is shown as an example in Figure 2.1a. On the other hand, over half of the metals and radionuclides evaluated exceeded the LELs at reference and no-effect sites at least 20% of the time. These elements include As, Mo, Ni, Se, U, Pb-210, and Po-210. Examples of these exceedences are illustrated in Figure 2.1b and 2.1c for U and As, respectively. The numerous exceedences at reference and no-effect sites are cause to question the SLC derivation approach. Not surprisingly, there was relatively good agreement between effect sites and exceedences of the LELs and SELs because these values are very conservative. At effect sites, at least one of the LELs and SELs were exceeded 95% and 48% of the time, respectively (Table 2.3).

When the data are viewed from a different perspective, by evaluating exceedences of the SQGs and how this relates to benthic invertebrate community effects, it reinforces that the SLC-derived guidelines are deficient in accurately predicting when an effect will occur. Of the sites exceeding the LEL and SEL values, only 57% (39 of 68 datasets) and 55% (18 of 33 datasets), respectively, were found to have effects on the benthic invertebrate community (Table 2.3). One would expect that if the SLC approach generated appropriate guidelines then exceedences should

occur more often at effect sites than seen here (in our opinion ideally  $\geq 80\%$  of the time for specific elements), if the effects are due to the elements of concern. Furthermore, these data suggest that the ability of SELs to predict a greater magnitude of effect on benthic communities is limited, which is consistent with the findings presented by Thompson et al. (2005), who explicitly recommended the use of the LEL SQGs and not the SEL SQGs.

When exceedences of LELs at effect sites were assessed on a per element basis it was found that elements below the LEL co-occurred with effects. For instance, Cr, Cu, Pb, V, Po-210, and Ra-226 were below the LEL at effect sites  $\geq 60\%$  of the time. These elements are therefore unlikely to be the cause of toxicity at these sites, yet they are associated with an effect on the benthic invertebrate community. This illustrates that using concentrations of all elements quantifiable at effects sites can confound the derivation of appropriate environmental guidelines. The evidence presented from this assessment demonstrates that the SLC SQGs performed poorly at predicting an absence of effect. If SQGs are set too low, the guideline will likely predict the possibility of an effect when there is no real effect (Type I error). If SQGs are set too high, adverse effects may occur even though not anticipated (Type II error). Therefore, there is a need to balance conservatism with accuracy. Despite a high percentage of LEL exceedences at effect sites, high concentrations of many elements were also found at no-effect sites. This suggests that SQGs derived using the SLC approach overestimate the presence of effects on benthic invertebrate communities near uranium operations in northern Saskatchewan. These false-positives could result in unnecessary and costly additional investigation or remediation. However, given that each species has its own tolerance level for toxicants and that the benthic invertebrate community composition (species assemblage) naturally varies from site to site, establishing a generic SQG may not be practical, especially if based on total contaminant concentrations.

One of the re-occurring problems in SQG derivation procedures is the use of total metal concentrations in bulk sediment as the measure of choice. Sediment characteristics (e.g., organic carbon content, particle size distribution, acid-volatile sulfide content) are known to influence metal bioavailability in sediment and thus strongly influence site-specific benthic community composition. Sediment quality guidelines derived using total metal concentrations in sediment are generally thought to be less effective at predicting biological effects relative to mechanistically derived SQGs. Furthermore, given that there can be great uncertainty as to

which element(s) measured in sediment at an effect site are the actual cause(s) of an effect, it may be impossible to determine the cause solely by using SQGs (Borgmann 2003; Chapman and Hollert 2006). With the SLC approach, if one contaminant, measured or unmeasured, causes an effect on the benthic community, then all contaminants measured at the site are “guilty by association”. This is not scientifically defensible.

The results presented here suggest that the SQGs developed by the CNSC for northern Saskatchewan uranium mining and milling operations should be revisited in an effort to improve their predictive capability for determining both adverse effects and the absence of effects at field sites. To properly relate an effect with a measure that is biologically relevant, alternative approaches for deriving SQGs would ideally be based on measurement of bioavailable contaminant fractions (e.g., concentrations in pore-water) rather than total concentrations of elements in bulk sediment so that SQGs are based on more biologically relevant measurements of exposure and bioaccessibility. Alternative approaches for deriving SQGs could also employ the evaluation of metal concentrations in sediment from no-effect sites to define potentially “safe”, site-specific levels. Both of these alternative approaches are currently under investigation.

## CHAPTER 3

### DERIVATION OF NO-EFFECT AND REFERENCE-LEVEL SEDIMENT QUALITY VALUES FOR APPLICATION AT SASKATCHEWAN URANIUM OPERATIONS

#### 3.1 Abstract

To-date, the majority of empirical approaches used to derive sediment quality values (SQVs) have focused on metal concentrations in sediment associated with adverse effects on benthic invertebrate communities. Here we propose the no-effect (NE) approach. This SQV derivation methodology uses metal concentrations in sediment associated with unaffected benthic communities (i.e., from reference sites and lightly contaminated no-effect sites) and accounts for local benthic invertebrate tolerance and potential chemical interactions at no-effect exposure sites. This NE approach was used to propose alternative regional SQVs for uranium operations in northern Saskatchewan. Three different sets of NE values were derived using different combinations of benthic invertebrate community effects criteria (abundance, richness, evenness, Bray-Curtis index). Additionally, reference (REF) values were derived based solely on sediment metal concentrations from reference sites. In general, NE values derived using abundance, richness, and evenness (NE1 and NE2 values) were found to be higher than the NE values derived using all four metrics (NE3 values). Derived NE values for Cr, Cu, Pb, and V did not change with the incorporation of additional effects criteria due to a lack of influence from the uranium operations on the concentrations of these metals. However, a gradient of exposure concentrations was apparent for As, Mo, Ni, Se, and U in sediment which allowed for tolerable exposure levels of these metals in sediment to be defined. The findings from this assessment have suggested a range of new, alternate SQVs for use at the uranium industry in northern Saskatchewan.

#### 3.2 Introduction

Sediment quality values are used primarily for comparison to sediment contaminant concentrations to determine if there is a potential for adverse effect to aquatic life. This screening process is often combined with other lines of evidence (e.g., water quality, tissue trace-

metal concentrations, benthic community indices, *in situ* experiments) to determine whether anthropogenic contamination has impacted a site (Chapman et al. 2002; McDonald et al. 2007). Environmental effects monitoring programs and environmental impact assessments use SQVs to help characterize and predict risks to aquatic ecosystems. In addition, SQVs can be used as target clean-up values for contaminants of concern in reclaiming or remediating resource extraction sites.

To date, various SQV derivation approaches have been developed. These approaches can generally be divided into two broad categories: mechanistic and empirical approaches. Mechanistic SQV derivation approaches, such as the equilibrium partitioning approach (Di Toro et al. 1991; US EPA 2005), incorporate chemical and biological factors known to modify the bioavailability of metals and metalloids (metals and metalloids will be hereafter referred to as metals). Empirical SQV approaches identify relationships between chemical concentrations and biological effect based on statistical analyses of large datasets of co-occurring sediment chemistry and toxicity data (Vidal and Bay 2005). The datasets used to derive empirical guidelines may incorporate many possible contaminants, sediment characteristics, chemical concentrations, abiotic factors, chemical interactions, and biological effects. Various endpoints, such as survival, growth, reproduction, contaminant bioaccumulation, presence/absence of taxa, and biochemical markers, can be used to determine or predict an effect. Examples of empirical approaches include the spiked-sediment toxicity approach (Gannon and Beeton 1971; Swartz et al. 1979), the effects range approach (Long and Morgan 1990), the threshold and probable effect levels approach (Smith et al. 1996), the consensus approach (MacDonald et al. 2000a; MacDonald et al. 2000b), and the screening-level concentration (SLC) approach (Persaud et al. 1993; Thompson et al. 2005). Each of these approaches can be used to calculate contaminant concentrations likely to be associated with low, medium and severe levels of biological effects, depending on the definition of an effect, the data used, and the derivation method employed.

Variations in the derivation of no-effect level (NEL) values relate to the defined effect criteria, predictability of the absence or presence of effects, and how the values will be used (e.g., in risk assessment or as reclamation objectives). Persaud et al. (1993) introduced NELs for contaminants in sediment in Ontario, Canada. They stated that the NEL in sediment was the "...level at which the chemicals in the sediment do not affect fish or the sediment-dwelling organisms" and that there was expected to be "...no transfer of chemicals through the food chain

and no effect on water quality”. Persaud et al. (1993) derived NELs for non-polar organics in sediment using a partitioning approach based on water quality guidelines and sediment-water partitioning coefficients. Ingersoll et al. (1996) defined the no-effect concentration (NEC) as the “...maximum concentration of a chemical in a sediment that did not significantly adversely affect the particular response (e.g., survival, growth, maturation) compared to the control” and derived NECs for metals, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons (PAHs) using laboratory toxicity data associated with field-collected sediment. The negligible concentration (NC) and maximum permissible concentration (MPC) for metals were derived for use in the Netherlands. The NC for a metal was defined as the background concentration plus a negligible addition (the sediment maximum permissible addition (MPA) divided by a safety factor of 100). The MPC was derived using a modified equilibrium-partitioning method based on the sediment background contaminant concentration, the partitioning co-efficient, and the calculated MPA for water (Crommentuijn et al. 2000). Altin et al. (2008) selected environmentally safe threshold concentrations for offshore drilling operations near Norway by deriving probable no-effect concentrations (PNECs). These PNECs were calculated and evaluated for metals, PAHs, and aliphatic hydrocarbons using a variety of derivation approaches (e.g., assessment factor, equilibrium partitioning, field-species sensitivity distributions). Predicted no-effect concentrations have also been derived for bisphenol A using various species sensitivity distribution methods (Staples et al. 2008). Sheppard et al. (2005) recommended a predicted no-effect concentration for uranium in sediment of 100 µg/g based on unpublished and in preparation works. They consider the limited sediment toxicity data (unpublished work) and Saskatchewan background concentrations in relation to the low-effect levels (LELs) suggested by an in preparation version of the Thompson et al. (2005) manuscript. A summary of SQVs applicable to freshwater ecosystems and for select metals of interest to this assessment are listed in Table 3.1. Considering that the Sheppard et al. (2005) PNEC for uranium in sediment was simply an adopted Thompson et al. (2005) LEL, that PNEC was not used for comparison here. Notably, the type of endpoint (e.g., bioaccumulation versus survival or growth), the application (e.g., field versus laboratory), and the breadth of organisms protected (e.g., multiple trophic levels versus individual organism) varies among threshold level derivation approaches for sediment.

Table 3.1. Comparison of select no-effect and low-effect sediment quality values from the literature (all values are in µg/g dry weight).

Element	NEC <sup>a</sup>	MPC <sup>b</sup>	NC <sup>c</sup>	SLC LEL <sup>d</sup>	Canadian ISQG <sup>e</sup>	Ontario LEL <sup>f</sup>	MEL <sup>g</sup>	NET <sup>h</sup>	MET <sup>i</sup>
As	100	190	31	9.8	5.9	6	7	-	-
Cr	95	1720	116	47.6	37.3	26	55	-	-
Cu	580	73	36	22.2	35.7	16	28	-	-
Mo	-	250	3	13.8	-	-	-	-	-
Ni	130	44	35	23.4	18	18	35	35	35
Pb	130	4800	132	36.7	35	35	23	23	42
Se	-	2.9	0.72	1.9	-	-	-	-	-
U	-	-	-	104.4	-	-	-	-	-
V	-	56	42	35.2	-	-	-	-	-

<sup>a</sup>NEC: No effect concentration based on 28-d *Hyalella azteca* tests, Ingersoll et al. (1996).

<sup>b</sup>MPC: Maximum permissible concentration for the Netherlands, Crommentuijn et al. (2000).

<sup>c</sup>NC: Negligible concentration for the Netherlands, Crommentuijn et al. (2000).

<sup>d</sup>SLC LEL: Screening-level concentration approach lowest effect level using the weighted method for Canadian uranium operations, Thompson et al. (2005).

<sup>e</sup>ISQG: Interim sediment quality guidelines for Canada, CCME (2011).

<sup>f</sup>LEL: Lowest effect level, Persaud et al. (1993).

<sup>g</sup>MEL: Minor effect level, MacDonald et al. (2000).

<sup>h</sup>NET: No effects threshold for the St. Lawrence River, CCME (1999).

<sup>i</sup>MET: Minimal effects threshold for the St. Lawrence River, CCME (1999).

Note: A CCME SQV for Ni was available in 1999, but not thereafter. Regardless, the CCME 1999 ISQG for Ni has been included in this table and discussion for comparison purposes.

Here, uranium operations in northern Saskatchewan, Canada, were chosen as a case-study for deriving regional specific no-effect (NE) SQVs. Occasionally, sediment metal concentrations at reference sites within this region, which represent natural background conditions, exceed existing guidelines derived for use in Canada (e.g., CCME 2011; Thompson et al. 2005). Regional NE values would incorporate background metal concentrations in the aquatic environment near uranium operations in northern Saskatchewan and thus better reflect the local tolerance or adaptation of benthic invertebrates to elevated sediment metal concentrations.

Due to the geology of northern Saskatchewan, uranium usually co-occurs with other metals in sediment. Many of these metals, which are also associated with uranium ore extraction and processing activities, do not have national SQVs. Using data obtained near uranium operations in Ontario and Saskatchewan, Thompson et al. (2005) were the first to derive sediment quality guidelines (SQGs) for the protection of aquatic life for specific elements associated with the Canadian uranium industry (e.g., Se, Mo, U, V). However, Burnett-Seidel and Liber (2011; Chapter 2) found that the SQGs derived by Thompson et al. (2005) resulted in numerous false-positives when compared to reference and no-effect site data which suggested that their SQGs are quite conservative. Furthermore, given that there are limited peer-reviewed sediment toxicity data available for comparison for some of these elements, these guidelines should be used with some caution until additional field and laboratory toxicity information is available to validate the derived LEL SQGs. Until such studies are conducted, an alternate or supplemental approach based on different benthic invertebrate community (BIC) effect criteria, as presented in this paper, would aid in setting SQVs for less investigated metals of concern (e.g., Mo, Se, U, V).

The NE approach derives SQVs from total metal concentrations in sediment. Metals, among other potential contaminants, are of concern downstream of effluent discharge points at Saskatchewan uranium operations (Muscatello et al. 2006; Robertson and Liber 2007). Many of these metals (e.g., Mo, Se, U, V) have not been investigated as thoroughly as many other metals (e.g., Cd, Cu, Pb, Zn). Furthermore, metals are almost exclusively measured or reported as total metal concentrations in sediment. However, metal bioavailability is an important factor when considering metal toxicity to all organisms, especially to benthic invertebrates. Basing guidelines on total sediment metal concentrations is therefore not ideal. However, until sediment

sampling and analysis protocols routinely include pore-water contaminant concentrations or other measurements more strongly related to metal bioavailability (e.g., simultaneous-extracted metals and acid-volatile sulfide measurements, sequential or single chemical extractions, diffusional equilibration in thin-film (DET) or diffusive gradients in thin-film (DGT) technologies), guidelines for contaminated site evaluations and risk assessments will likely continue to be based on total metal concentrations in sediment.

In this study, we propose a method for deriving regionally-specific NE SQVs for metals. This approach relies on co-occurring sediment chemistry data and BIC metrics from reference and no-effect sampling sites in northern Saskatchewan, Canada. Three different levels of BIC effect criteria (based on different community metrics) were used to define NE datasets. Reference SQVs were derived by following the same approach, but using only reference site data (background concentrations). Derived NE and REF values were compared to available SQVs and sediment toxicity data from the peer-reviewed literature, where appropriate. The SQVs derived in this assessment can be used as screening tools in contaminated sediment assessments at uranium operations in northern Saskatchewan, Canada.

### **3.3 Materials and methods**

#### **3.3.1 Study sites**

The data used to derive NE values were gathered from consultant and research reports, and student theses, that investigated the aquatic environments near Cameco Corporation operations in northern Saskatchewan, Canada (Appendix; CanNorth Environmental Services 2001; 2005b; 2008; Conor Pacific Environmental Technologies Inc. 1999; Golder Associates Ltd. 2002; 2003; 2004; 2005a; 2005b; 2005c; 2008a; 2008b; Robertson 2006; Terrestrial & Aquatic Environmental Managers Ltd. 1997). Data were collected from watersheds near the Key Lake, Rabbit Lake, McArthur River, and Cigar Lake uranium operations between 1996 and 2007. A total of 15 reports were reviewed which collectively include 33 different sampling locations (e.g., specific areas within a waterbody) and 87 different datasets of co-occurring sediment chemistry (total metal and radionuclide concentrations) and BIC data. Of these sites, 28 were reference sites and 59 were believed to be influenced by mining activities (e.g., effluent discharge, seepages from tailings facilities). Due to temporal environmental monitoring programs, data were available for some sites for more than one year. Sampling sites had a broad

range of water depth and sediment characteristics (Table 3.2). Upon carefully evaluating these sediment characteristics, no bias towards one type of sediment in either reference or exposure sites was found (data not shown). Although a large range of habitat characteristics were apparent, reference and exposure sites were matched as best as possible during each field investigation to minimize the potential for observing effects from habitat differences.

### **3.3.2 Classification of datasets**

Reference sites (REF) were identified and BIC data were screened in order to define no-effect/effect sites based on three different BIC effects criteria (NE1, NE2, NE3). Reference sites were defined as sample locations that were upstream of mining or milling sources, or were part of completely separate, but near by, drainage to those influenced by these activities. For the derivation of No-Effect 1 (NE1) values, effects on the BIC were defined as a statistical difference between a reference and exposure site in terms of total organism abundance or taxon richness (family level) which is consistent with the methods presented in the Canadian Environmental Effects Monitoring (EEM) guidance document (Environment Canada 2011). No-Effect 2 (NE2) values were defined as a statistical difference between a reference and exposure site in abundance, richness, or evenness (a measure of the relative abundance of each species at a site). No-Effect 3 (NE3) values were calculated using the metrics listed for NE2, but included the Bray-Curtis similarity index as an additional effect criterion. Each report evaluated outlined the specific method used to calculate each metric which were generally consistent with the Canadian EEM guidance document (Environment Canada 2011). Usually a single reference site was used for comparisons to exposure sites within a dataset, as outlined in each report. Only one study used multiple reference sites. In this case, a statistical difference in the above metrics between an exposure site and any of the reference sites was interpreted as an effect. If a statistical comparison was not reported for an index in the original data source, then an effect was defined as a > 20% difference relative to the reference site. These definitions of an effect incorporate the effects criteria defined as a > 20% difference by Thompson et al. (2005) and as a statistically significant difference by the Canadian EEM program for metal mines (Environment Canada 2002). Both reference sites and unaffected sites (exposure sites showing no difference from a reference site) were classified as no-effect sites.

Table 3.2. Water depth and sediment characteristics for the no-effect and reference sites used in the derivation of sediment quality values for the uranium industry in northern Saskatchewan, Canada.

	Water Depth (m)	Particle Size Distribution (%)			Moisture Content (%)	Total Organic Carbon (%)
		Sand (>0.062 mm)	Silt (0.0039 to 0.062 mm)	Clay (<0.0039 mm)		
Average	5.9	35.7	46.1	21.1	83.8	8.9
Median	2.5	26.8	48.9	13.9	88.0	7.5
Minimum	0.6	1.8	1.0	1.0	19.1	0.2
Maximum	22.0	98.0	88.2	67.0	98.4	27.1

The exact number of datasets used in the derivation of NE and REF SQVs varied depending upon the effects criterion employed and the metal of interest. All 28 reference sites were used in the derivation of REF values. The NE1 dataset included 18 NE sites and 28 reference sites, for a total of 46 data pairs. The NE2 dataset included 16 NE sites and 28 reference sites (44 data pairs). The NE3 dataset had eight NE sites and 28 reference sites (36 data pairs). The total number of data pairs also varied for each metal because not all of the metals of interest were reported in every field study.

### **3.3.3 No-effect value derivation approach**

Three sets of NE values and one set of REF values were derived for each of nine metals (As, Cr, Cu, Mo, Ni, Pb, Se, U, V) using the same approach, but different data sub-sets (based on the above NE1 to NE3 criteria). These metals are deemed either to be of potential concern at uranium operations in northern Saskatchewan or are routinely monitored at those operations. It is proposed that if the total concentration of a particular metal in sediment at some northern Saskatchewan field site is less than its NE value, adverse effects on the local BIC due to that metal are unlikely. Conversely, if the total metal concentration in sediment is greater than the NE value, there is increased potential for adverse BIC effects and further investigation may be warranted. In addition to NE values, REF values were also derived. The REF dataset contained sediment data from established reference areas only. These data represent background sediment metal concentrations for the region, concentrations that are considered to have no adverse effects on native benthic organisms.

For each of the nine metals evaluated, the sediment metal concentrations from the available datasets were plotted using a log-normal probability plot with concentration (x-axis) on a log scale and the cumulative frequency (centiles; y-axis) on a linear scale. A logistic regression (three parameter equation) was fit to the data and the 90<sup>th</sup> centile of the regression was used to define each NE or REF value for that particular metal. An example of a graph used to derive SQVs is depicted in Figure 3.1. The upper 90<sup>th</sup> centile of the cumulative frequency distribution was selected as the SQV because it makes use of all available data and incorporates some conservatism. Each NE and REF value was compared to corresponding LELs derived by Thompson et al. (2005) and other available LEL SQVs.

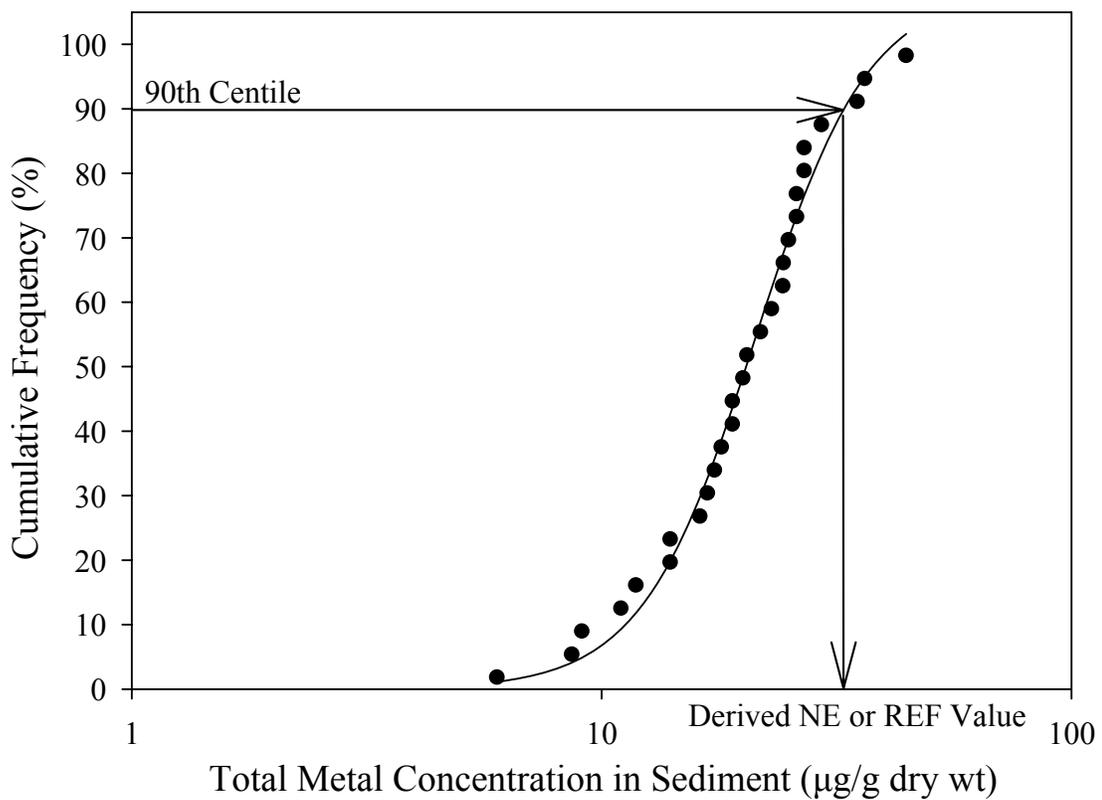


Figure 3.1. Example of a logistic regression line fit to total metal concentrations in sediment in order to derive reference (REF) and no-effect (NE) sediment quality values. Each point on the curve represents one mean metal concentration describing either a reference or no-effect site.

Two specific data issues resulted in minor deviations from the derivation approach outlined above. First, if the curve fit of the data distribution resulted in the upper plateau falling below the 90<sup>th</sup> centile, then an NE or REF value could not be interpolated from the logistic regression line. In these cases (9 of 36 derived values), the NE value was derived based on an interpolation of the 90<sup>th</sup> centile from the actual dataset (rather than fit of the logistic regression line) using the standard percentile method. The standard percentile method represents the largest integer rather than nearest integer related to rank within the dataset (SigmaPlot 11.0, NIST 2010). The second issue resulting in a deviation from the primary methodology were in cases where measured metal concentrations were below the limit of detection (LD). In these cases, a sensitivity analysis was conducted for the element of interest to determine the effect of including values less than the LD on SQV derivation when (i) the LD was used in place of the unknown (< LD) value, or (ii) when the value was excluded from the dataset. SigmaPlot 11.0 (Systat Software Inc. 2008) was used for the derivation of all NE and REF values. All SQVs are presented in µg/g dry weight.

### **3.3.4 Evaluation criteria**

Once the NE and REF values were generated, it was desirable to test how strongly these SQVs correlated with lack of *in-situ* effects on the BICs. To assess predictive ability of their SQVs, Thompson et al. (2005) used a subsample of the data used to derive their SQGs. However, given that this can result in a biased assessment, this approach was not used here. Ideally, northern Saskatchewan data not used in the SQV derivation process would be used to “validate” SQVs.

A search for additional datasets from uranium operations in northern Saskatchewan (data not used here in the derivation of REF and NE values) identified four reports containing sediment metal concentrations and benthic invertebrate indices from two different operations not included in the initial assessment: Millennium (an exploration site and potential future uranium mine site; CanNorth Environmental Services 2009) and Beaverlodge (a decommissioned, legacy uranium mine and mill site; CanNorth Environmental Services 2002; 2005a; 2007) operations. While datasets from these locations were heavily weighted with either reference or high exposure sites, sediment metal concentrations from Millennium and Beaverlodge reference and exposure sites were compared to the REF and NE values derived here in an effort to validate the

proposed SQVs. Of the exposure sites from Beaverlodge, only a single no-effect site (based on abundance and richness criteria) was observed in the reports evaluated.

### **3.4 Results**

#### **3.4.1 Reference and no-effect value trends**

As a greater number of biological effects endpoints were included and the number of data pairs decreased, the derived NE SQVs for As, Mo, and Se generally decreased (NE1 to NE3 values; Table 3.3). In other words, an increase in the number of benthic invertebrate effects criteria used to define each NE value generally resulted in a subsequent decrease in the NE value. Exceptions included uranium where NE1 and NE3 values were similar, but the NE2 value was 2 to 3 times higher. The U NE values ranged from 9- to 24-fold higher than the U REF value. Nickel NE values were similar to each other (between 320 and 383 µg/g), but were between 15- and 18-fold higher than the Ni REF value. Chromium, Cu, Pb, and V NE values remained relatively constant regardless of the effects criteria employed.

#### **3.4.2 Influence of non-quantifiable measurements on derived SQVs**

No-effect and REF SQVs were occasionally dependent on the percentage of total metal concentrations which were below the limit of detection. For Cr, between 2.4 and 4.0 % of the total data were < LD (Table 3.3), depending on the effects criteria used to define the dataset. The < LD data had little influence on the derived Cr NE and REF values. Similarly, between 2.4 and 4.5 % of the data were < LD in the Mo datasets. Overall, the inclusion of non-detect Mo values (using the LD as the <LD value) in the NE datasets decreased the REF value by 1.7 %, the NE1 value by 3.2 %, the NE2 value by 22.7 %, and did not change the NE3 value (0% change). The Mo NE2 value changed by over 22 % due to the removal of a single data point. For this curve fit, the 90<sup>th</sup> centile was near the very top plateau of the logistic curves which is sensitive to large changes in concentrations (X-axis) with small changes in centiles (Y-axis) which resulted in a substantial change in the derived value. Selenium concentrations in sediment were generally very low with approximately half of the Se datasets consisting of non-detect values. Thus, inclusion of < LD data resulted in a decrease of all Se NE and REF values.

Table 3.3. No-effect (NE) and reference (REF) sediment quality values ( $\mu\text{g/g}$  dry wt) derived for use at uranium operations in northern Saskatchewan based on various benthic invertebrate community effect criteria with consideration of the influence of metal values that were below the method detection limit (<LD).

Metal	NE1		NE2		NE3		REF	
	No <LD	With <LD (%) <sup>a</sup>	No <LD	With <LD (%) <sup>a</sup>	No <LD	With <LD (%) <sup>a</sup>	No <LD	With <LD (%) <sup>a</sup>
Cr	25.7	25.9 (2.4)	26.0	26.2 (2.5)	24.2	24.6 (3.1)	29.9	31.5 (4.0)
Cu	11.0	- <sup>b</sup>	11.3	- <sup>b</sup>	9.7	- <sup>b</sup>	9.1	- <sup>b</sup>
Pb	19.2	- <sup>b</sup>	19.7	- <sup>b</sup>	24.2	- <sup>b</sup>	16.3	- <sup>b</sup>
V	31.8	- <sup>b</sup>	31.8	- <sup>b</sup>	32.8	- <sup>b</sup>	35.1	- <sup>b</sup>
As	592	- <sup>b</sup>	522 <sup>c</sup>	- <sup>b</sup>	82.6 <sup>c</sup>	- <sup>b</sup>	20.8	- <sup>b</sup>
Mo	1366 <sup>c</sup>	1322 <sup>c</sup> (4.5)	316	245 (2.4)	34.0	34.0 (2.9)	23.0	22.6 (3.7)
Ni	320 <sup>c</sup>	- <sup>b</sup>	326 <sup>c</sup>	- <sup>b</sup>	383 <sup>c</sup>	- <sup>b</sup>	21.4	- <sup>b</sup>
Se	52.0 <sup>c</sup>	31.1 <sup>c</sup> (44.4)	52.8 <sup>c</sup>	29.7 (46.5)	16.6 <sup>c</sup>	5.2 (51.4)	4.0	3.6 (55.6)
U	1012 <sup>c</sup>	- <sup>b</sup>	2296	- <sup>b</sup>	839	- <sup>b</sup>	96.7	- <sup>b</sup>

NE1: No-effect 1 value; invertebrate abundance and richness used as measures of effect.

NE2: No-effect 2 value; invertebrate abundance, richness, and evenness index used as measures of effect.

NE3: No-effect 3 value; invertebrate abundance, richness, evenness index, and Bray-Curtis similarity index used as measures of effect.

REF: Reference sediment value derived using total metal concentrations in sediment from reference sites only.

LD: Limit of detection.

<sup>a</sup> Percentage of metal concentrations that were less than the limit of detection presented in parenthesis.

<sup>b</sup> All metal data used were above the method detection limit.

<sup>c</sup> Value derived from the 90th centile of the dataset rather than the logistic curve because the 90th centile was above the fit line.

Table 3.4. Comparison of no-effect (NE) values and reference (REF) values with select Canadian sediment quality values (all values in  $\mu\text{g/g}$  dry weight).<sup>a</sup>

Metal	NE and REF SQVs (this study)				Other Canadian SQVs		
	NE1	NE2	NE3	REF	CNSC SLC LEL	Canadian ISQG	Ontario LEL
Cr	25.9	26.2	24.6	31.5	47.6	37.3	26
Cu	11.0	11.3	9.7	9.1	22.2	35.7	16
Pb	19.2	19.7	24.2	16.3	36.7	35	35
V	31.8	31.8	32.8	35.1	35.2	-	-
As	592	522 <sup>b</sup>	82.6 <sup>b</sup>	20.8	9.8	5.9	6
Mo	1322 <sup>b</sup>	245	34.0	22.6	13.8	-	-
Ni	320 <sup>b</sup>	326 <sup>b</sup>	383 <sup>b</sup>	21.4	23.4	18	18
Se	31.1 <sup>b</sup>	29.7	5.2	3.6	1.9	-	-
U	1012 <sup>b</sup>	2296	839	96.7	104	-	-

<sup>a</sup> When NE and REF values were derived both with and without data <LD, the value calculated using <LD is presented.

<sup>b</sup> Value derived from the 90th centile of the dataset rather than the logistic curve because the 90th centile was above the fit line.

NE1: No-effect 1 value; invertebrate abundance and richness used as measures of effect.

NE2: No-effect 2 value; invertebrate abundance, richness, and evenness index used as measures of effect.

NE3: No-effect 3 value; invertebrate abundance, richness, evenness index, and Bray-Curtis similarity index used as measures of effect.

REF: Reference sediment value derived using total metal concentrations in sediment from reference sites only.

SLC LEL: Screening level concentration approach low effect level (CNSC), Thompson et al. (2005).

Canadian ISQG: Canadian interim sediment quality guideline, CCME (2011).

Ontario LEL: Ontario low effect level, Persaud et al. (1993).

Note: A CCME SQV for Ni was available in 1999 but not thereafter. Regardless, the CCME (1999) ISQG for Ni has been included in this table and discussion for comparison purposes.

### **3.4.3 Comparison of reference and no-effect values with other sediment quality values**

The derived NE and REF values were compared to existing Canadian guidelines (CCME) and to the screening values of Thompson et al. (2005) (Table 3.4). Arsenic, Mo, Ni, Se, and U NE values (NE1 through NE3 values) exceeded all Canadian guidelines. Even the REF As value exceeded all Canadian guidelines by at least 2-fold which suggests that the Canadian guidelines may not be suitable for some regions of northern Saskatchewan due to higher naturally occurring concentrations of As in sediments. The NE values for Ni were also higher than all other Ni guideline values, whereas the REF value for Ni was similar to the SLC LEL of 23.4 µg/g. The high Ni concentrations in the datasets used to derive the NE values were influenced by a single no-effect site that had > 1000 µg Ni/g dry wt and was sampled on two occasions (due to temporal sampling). This single site was thus responsible for the elevated Ni NE values. Derived Ni NE values without these two influential points did not result in substantial changes as there were less than a 10% difference between the NE1 and NE2 values and a 22% difference between the NE3 values derived with and without the two elevated Ni sediment concentrations. The SLC LEL for Se was exceeded by the REF and NE values by approximately 2 to 16-fold. The REF value for U was similar to the SLC LEL and the NE values for U ranged from approximately 8 to 23 times higher than the SLC LEL (104 µg/g). The presence of As, Mo, Ni, Se, and U at concentrations above other Canadian guideline values could be due to either natural or anthropogenic influences. However, it should be emphasized that the sediment metal concentrations represented by the derived values were not associated with measurable effects on the BIC. In contrast, Cr, Cu, Pb, and V values were either similar to or below all comparative Canadian guidelines.

### **3.4.4 Validation of reference and no-effect values**

Mean concentrations of metals in sediment from separate reference and exposure sites located at or near the Millennium and Beaverlodge operations in northern Saskatchewan (Table 3.5) were compared to REF and NE values (Table 3.3). Millennium and Beaverlodge reference site values (means) were all very similar to or lower than the REF values presented here. Only maximum concentrations of Cr, Cu, and V were above the derived REF values. Therefore, the presented REF values appear to be representative of reference sediment metal concentrations in northern Saskatchewan. The exposure site sediment metal concentrations were solely from the

Table 3.5. Reference and exposure site metal concentrations from the Millennium and Beaverlodge projects in northern Saskatchewan, Canada ( $\mu\text{g/g}$  dry weight).

Metal	Reference Sites ( $n = 11$ )			No-Effect Site ( $n = 1$ )	Effect Sites ( $n = 9$ )		
	Mean $\pm$ SD	Min	Max	Mean	Mean $\pm$ SD	Min	Max
Cr	15.6 $\pm$ 13.8	5.1	46.4	49.6	59.7 $\pm$ 30.5	20.2	106.6
Cu	11.4 $\pm$ 18.9	3.3	67.6	76.0	84.8 $\pm$ 50.6	12.0	158.0
Pb	8.4 $\pm$ 3.0	2.2	13.5	5.0	404.0 $\pm$ 507.7	9.2	1264
V	23.0 $\pm$ 14.2	6.8	50.0	42.4	323.1 $\pm$ 291.4	29.8	704.0
As	5.1 $\pm$ 4.4	0.7	17.8	5.0	9.7 $\pm$ 9.3	3.1	32.8
Mo	1.7 $\pm$ 3.0	0.4	10.8	15.0	40.9 $\pm$ 76.8	0.5	206.0
Ni	9.3 $\pm$ 4.6	5.1	20.0	30.6	43.6 $\pm$ 26.6	13.8	80.2
Se <sup>a</sup>	1.3 $\pm$ 0.3	<0.5	1.9	2.8	32.3 $\pm$ 75.8	1.8	234.0
U	3.2 $\pm$ 5.8	0.4	19.5	2218	4925 $\pm$ 12465	113.6	38060

<sup>a</sup>Two sites had concentrations of Se below the limit of detection of 0.5  $\mu\text{g/g}$  dry wt. These sites were not included in the calculation of the average, standard deviation, or median values.

decommissioned Beaverlodge uranium mining operations that are currently undergoing reclamation. Within the exposure dataset, only one no-effect dataset was identified (Table 3.5). This single no-effect site was below NE3 values for As, Mo, Ni, Pb, and Se, but above NE3 values for the remaining metals evaluated (i.e., Cr, Cu, U, and V). At effect sites (Table 3.5), the mean sediment metal concentrations were higher than derived NE3 and REF values for all metals evaluated except for As. Comparisons of effect-related sediment concentrations with the less conservative NE1 and NE2 values showed that Cr, Cu, Pb, Se, U, and V concentrations in sediment were above these values and As, Mo, and Ni concentrations were below the NE values. This suggests that generally there are expected relationships between field data at reference and exposure (no-effect and effect) sites and the derived NE and REF values (i.e., more exceedences of NE values at effect sites than at no-effect and reference sites) and that metals of potential concern can be identified using the derived NE values presented here. However, validation would have been stronger if more exposure sites were no-effect sites.

### **3.5 Discussion**

There is a need to improve upon current empirical approaches for deriving SQGs, such as the SLC approach. Current empirical approaches utilize the abundance of available data and relate co-occurring effects on BICs with concentrations of contaminants in sediment. However, the use of these approaches results in some contaminant concentrations in sediment being “guilty by association” because not all measured contaminants in sediment at an effect site are the cause of the observed effect. This can result in some guidelines being lower than necessary (over-conservative) because constituents in the sediment that are not truly causing the effect are included in the datasets used to derive the guidelines. An example of this is the SLC approach, which can result in numerous false-positives when field contaminant concentrations are above a guideline value (Chapter 2; Burnett-Seidel and Liber 2011).

Many of the assumptions underlying the SLC approach limit the derivation of defensible SQGs. For instance, if a large database is used it is assumed that factors that modify toxicity do not have to be taken into account, total metal concentrations in sediment adequately characterize exposure, all metal concentrations in sediment at a site are safe to the species if the species is present, all metal concentrations in sediment where a species is absent are the cause of the absence, contaminants act independently, the presence or absence of a species is an appropriate

assessment of a toxic response, and the species in the database are representative of species you would find elsewhere.

This study presents a new, simple approach to deriving SQGs based on no-effect field data related to local BIC tolerances. The approach utilizes data associated with reference and no-effect sites. The biological criteria used were consistent with effects criteria used in the Canadian EEM program (Environment Canada 2011) and those used in the SLC approach presented by Thompson et al. (2005). Because NE values are based on BIC metrics, the derived NE values may not always protect individual species. However, the derived NE values should be protective of aquatic habitat in general. Using data from uranium operations in northern Saskatchewan, Canada, as a case-study to evaluate the NE approach, three NE values, based on combinations of BIC metrics, and a single REF value for each metal evaluated were derived. The approach reduces the number of assumptions made relative to the SLC approach. Using only metal concentrations in sediment from no-effect and reference sites eliminates the assumption that all metal concentrations in sediment are the cause of an absence of a single species or taxon. In addition, the proposed approach for derivation of NE values does not try to estimate a value related to effects (such as the SEL), because only data related to a lack of effects are used to derive the SQV. In short, contaminants can no longer be guilty by association. Derivation of REF values further eliminates the assumptions related to biological effects since the values are based solely on sediment chemistry.

During the derivation process and the evaluation of the results, certain factors were found to influence the resulting NE and REF values. Firstly, an increase in the number and general sensitivity of the benthic community metrics used in the datasets for each NE value was found to result in a decrease of the calculated NE value. For example, NE1 value datasets were selected based on invertebrate abundance or richness and generally consisted of higher sediment metal concentrations than NE3 values that were derived using abundance, richness, evenness, and the Bray-Curtis index. Secondly, some level of contamination above reference (background) levels was necessary for a threshold concentration to be derived. Without elevated metal concentrations in sediment, the NE values derived were indistinguishable from the derived REF values, which was the case for the Cr, Cu, Pb, and V NE values. Therefore, these four metals are not influenced by the uranium operations. A tolerable metal concentration in sediment greater than background concentrations likely exist for each of these metals and could be derived if a

larger range of metal concentrations in sediment were available at field locations. Lastly, a sensitivity analysis on the influence of below detection limit metal concentrations in sediment on the NE values derived revealed that excluding below detection limit data from the dataset or using the method detection limit as a surrogate for the non-detect value can influence the derived NE value depending on the percentage of non-detects in the dataset. Generally, the higher the percentage of non-detects in the dataset, the greater the variation in derived NE or REF values. Overall, the BIC metrics used to determine biological effects, the level of contamination present in sediment, and the prevalence of below detection limit concentrations can influence the NE or REF values derived and should be considered during the threshold derivation process.

While considering the above-mentioned factors that influence SQV derivation, the results presented here yield two distinct groups of metals – those that are present at no-effect sites at concentrations similar to background, and those that represented elevated but apparently tolerable concentrations in sediment based on BIC-metrics. For instance, Cr, Cu, Pb, and V NE values were similar to REF values indicating that these metals were not influenced by the uranium operations at these exposure sites. On the contrary, As, Mo, Ni, Se, and U NE values were elevated above REF values indicating that some level of contamination was present at no-effect sites although there was no observed effect on the BIC based on the selected effect criteria (e.g., abundance, richness, evenness, Bray-Curtis index). Because of these elevated metal concentrations in sediment, a tolerable metal concentration in sediment to BICs could be derived. Based on this, the threshold values for As, Mo, Ni, Se, and U are recommended for local use in the evaluation of sediment quality. The NE values for Cr, Cu, Pb, and V provide no more useful information than if comparisons were made to reference sediment concentrations and they are therefore not recommended for the evaluation of acceptable metal concentrations in sediment.

Within these two groups of NE values, variation results from the datasets used to derive the NE values (based on the selected BIC metrics). Invertebrate abundance and richness are measures of presence and absence of certain species and are the criteria used in the derivation of NE1 values. In addition to abundance and richness, NE2 values also included the evenness metric. This resulted in some NE1 exposure no-effect site(s) being reclassified as effect sites, thereby removing some metal concentrations in sediment from each dataset (i.e., the NE2 dataset was smaller than the NE1 dataset for each metal). The NE2 values were generally lower than the NE1 values. The NE3 values used an additional effects criterion, the Bray-Curtis similarity

index, which is known to be a sensitive indicator of changes in BICs (Faith et al. 1991) and has elsewhere resulted in an erroneous conclusion that two unexposed lakes of similar habitat characteristics were different (CanNorth Environmental Services 2009). As expected, the addition of the Bray-Curtis index as an indicator of effects on BICs resulted in further reduction in the number of values in the NE3 datasets. This resulted in NE3 values that were generally the lowest derived NE values, often quite similar to REF values (background sediment metal concentrations). Therefore, the derived SQVs were found to be influenced, as expected, by the effects criteria employed.

To evaluate the SQVs derived in this study, comparisons were made to select low effect level guidelines used in Canada (Table 3.4). The group of metals which had NE values that were similar to REF values (Cr, Cu, Pb, and V NE values) were all below other comparable SQVs. This should be expected. Furthermore, NE levels for this group of metals do not represent effect thresholds for BICs. The other group of NE values were above background concentrations (As, Mo, Ni, Se, and U NE values) and therefore can be used to estimate a threshold response. These NE values were all above the comparable national and international guideline values. Even the REF values for As, Mo, and Se were above published guidelines. This calls to question the utility of the current LELs and ISQGs at northern Saskatchewan uranium operations, especially where reference areas naturally have elevated concentrations of some metals in sediment. Furthermore, it can be expected that BICs would have some level of tolerance to metals above background sediment concentrations, which is why the NE values of As, Mo, Se, Ni, and U are above REF values and other comparable guidelines values, yet still represent “safe” sediment metal concentrations to local BICs.

It is important to keep in mind that the premise of the NE approach presented here is that a BIC (as a whole) can tolerate some level of metals in sediment, and that sublethal and food chain transfer effects are not fully accounted for. Benthic invertebrate communities represent an indicator for aquatic habitat status for fish. If a single invertebrate species is lost, the ecological function of the system is likely to not be impaired. However, if multiple species or families are affected, which should be detected with the chosen BIC metrics, then the effect could be due to metals (or some other constituent) in sediment. The derived NE values do not fully take into account sublethal effects (e.g., growth, reproduction) because the criteria used in this approach are based on presence/absence of species. If sublethal effects are developing at an exposure site,

then BIC metrics would not indicate that there is an effect until the community structure was significantly altered. Furthermore, the presented NE values do not consider the effects of metals in sediment which biomagnify in higher trophic levels.

While the derived NE values were not intended to be directly compared to spiked sediment toxicity data from the literature, comparisons are made to put the derived NE values into context. Sediment toxicity tests evaluate exposure of a single element under controlled conditions (which may or may not be representative of field conditions) to a specific species. For instance, the 10-d IC25s (inhibition concentrations of 25% of the organisms tested) for *Chironomus dilutus* and *Hyalella azteca* exposed to As were 174 and >462 but <724 µg/g dry weight (Liber et al. 2011). These IC25s are at or below the NE1 (592 µg/g) and NE2 (522 µg/g) values presented here, indicating that sublethal effects on these two species could potentially occur at the NE values, but that *in-situ* the NE values appear to be tolerable to the BIC. For Mo, 10-d IC25s for *C. dilutus* and *H. azteca* were both >3742 µg/g dry weight (Liber et al. 2011) indicating that some invertebrate species are able to tolerate Mo concentrations in sediment well above the derived NE values which ranged from 34.0 to 1322 µg/g. A wide range of 10-d and 28-d IC50s have been reported for Ni (40 to 1281 µg Ni/g dry weight), with *H. azteca* being the most sensitive and *C. tentans* (now known as *C. dilutus*) the most tolerant of the five species tested (Milani et al. 2003; Liber et al. 2011). The Ni NE concentrations derived here ranged from 320 to 383 µg/g dry weight which suggests that some individual species could potentially be affected at these Ni concentrations based on sublethal endpoints. A similar observation was made for uranium in sediment where 10- to 12-d IC/EC50 values for *Chironomus riparius*, *T. tubifex*, *H. azteca*, and *C. tentans* (now known as *C. dilutus*) range from 547 to 2695 µg/g dry weight (Lagauzere et al. 2009; Liber et al. 2011) and the derived NE values ranged from 839 to 1012 µg/g dry weight. Overall, these comparisons show that the proposed NE values, which are based on the assessment of entire BICs *in-situ*, are in reasonable agreement with available spiked-sediment toxicity data.

Another way that the proposed NE and REF values were validated was through a comparison with metal concentrations in sediment from northern Saskatchewan sites that were not included in the datasets used to derive the NE and REF values. The mean metal concentrations in sediment from Millennium and Beaverlodge reference sites were all below the REF values (Table 3.5) with the exception of Cu. However, the median Cu concentration of 4.6

µg/g at these reference sites (data not shown) was below the REF value of 9.1 µg/g. This suggests that the calculated REF values are representative of reference/background metal concentrations near uranium operations in northern Saskatchewan. As for the NE values, the more conservative NE3 values were compared to data from exposure sites near the Beaverlodge properties. The exposure sites consisted of only a single no-effect site, which provides limited confidence in comparing derived NE values to this exposure dataset. Nonetheless, this single no-effect site had sediment metal concentration that were below the NE3 values for As, Mo, Ni, Pb, and Se, but above values for the remaining metals evaluated (Cr, Cu, U, V; Table 3.5). This indicates that NE3 values were likely reasonable for identifying sediment metal concentrations that should not cause adverse effects on BICs. Overall, comparisons with data not included in the derivation of the proposed SQVs suggested that the REF and NE values represent reasonable SQVs for use at uranium operations in northern Saskatchewan.

The REF and multiple NE values derived in this study can be used as initial screening tools to assess the condition of aquatic habitats near northern Saskatchewan uranium operations. Although only data related to a specific industry and region were used in this case-study to highlight this new derivation approach, this flexible and easy to use approach can potentially be used with any no-effect and/or reference dataset to derive suitable, alternate SQVs. Specifically, this approach should improve risk assessment of contaminated sediments near Saskatchewan uranium operations (less false-positives).

## CHAPTER 4

### ASSESSMENT OF METHODS FOR THE ESTIMATION OF BIOAVAILABLE METALS FRACTIONS IN SEDIMENT

#### 4.1 Abstract

Two approaches were used to assess metals bioavailability across a gradient of sediment metals concentrations. The first approach involved mixing contaminated and reference sediments (both field-collected) in different ratios to create a gradient of metals exposure (“mixed-sediment experiment”). The second approach used sediment cores collected from a variety of contaminated and reference sites, spanning a range of sediment metals concentrations (“field-core experiment”). Midge larvae (*Chironomus dilutus*) were exposed to the sediments of both experiments and then analyzed for whole-body (“tissue”) metals accumulation. Metal concentrations in tissues were then correlated with various metal fractions to determine the usefulness of these fractions for assessing metal bioavailability in sediment. The sediment phases evaluated in each of the experiments included pore-water (isolated using both centrifugation and peepers) and the extracts from single chemical extractions performed on both wet and dry sediment subsamples. A broader variety of extractions were evaluated in a preliminary experiment, with potassium phosphate and hydrochloric acid being selected for further evaluation in the mixed-sediment and field-core experiments. In the mixed-sediment experiment, tissue-metals correlations with metals concentrations in each sediment phase were very strong (all correlation coefficients were greater than 0.890 and positively correlated). This was likely the result of co-correlation of both sediment characteristics and metals concentrations due to the dilution of one contaminated sediment in a step-wise fashion. Conversely, the correlation coefficients were inconsistent (range of 0.066 to 0.999) in the field-core experiment and the slopes were both positive and negative. This was likely due to the variability in sediment characteristic and metal concentrations among the different sediments. For the subset of metals evaluated (As, Mo, Ni and U), pore-water metal concentration had the best relationship with tissue metal concentration (both experiments). Based on these findings, it is suggested that single chemical extractions (e.g., HCl and KH<sub>2</sub>PO<sub>4</sub>) would be useful in focused, single-metal

sediment studies, where a particular metal (e.g., Ni and U) is targeted for an investigation but that pore-water may be useful more broadly.

## 4.2 Introduction

Metals in sediment are most commonly measured and expressed as a total (or bulk) concentration; however, it is well accepted that total sediment metal concentrations are often poorly correlated with metal bioavailability. Cause-effect relationships between metals in sediment and adverse effects on benthic invertebrates would be better described using a measure of metals (some fraction of the total concentration) that is more descriptive or predictive of metal bioavailability to these organisms. The bioavailable fraction can be defined as “the degree and rate at which a substance (as a drug) is absorbed into a living system or is made available at the site of physiological activity” (Merriam-Webster 2010). In the context of sediments, Horowitz (1991) describes the bioavailable fraction as “... that portion of dissolved, biologically-, or sediment-associated chemical constituents that are readily accessible to biota either through physical contact or by ingestion; this is an operationally defined term.” Horowitz refers to “operationally defined” as “the quantitation of a physical or chemical constituent or property which is dependent on the method used for its determination. If the method is altered the measurement changes also; many of the physical and chemical determinations performed on sediment/solid samples fall into this category.” When discussing the bioavailability of metals in sediment to benthic invertebrates in an *in situ* setting, bioavailable metals is considered to be an operationally defined fraction of the total sediment metal concentration that is available for uptake by organisms.

Bioavailable metal fractions can be estimated and quantified in numerous ways using different methods. These methods include measuring dissolved pore-water metal concentrations and quantifying the extraction of metals from sediments using various chemicals. Pore-water is the water found between sediment particles and is more commonly extracted by centrifuging sediment (Emerson et al. 1980) or by using passive-diffusion devices such as peepers (Hesslein 1976). The relationship between pore-water measurements and toxicity or bioaccumulation of contaminants in aquatic invertebrates has been previously demonstrated (Ankley et al. 1993; Vink 2002; Whiteman et al. 1996). In addition to being present in pore-water, metals in sediment can be associated with various solid phases (organic matter, iron and manganese

oxides, sulfides, clay surfaces, etc.). Chemical extractions (single chemicals or chemicals in combination) seek to subdivide the total metal concentrations in sediment into specific operationally-defined metal fractions. This process can provide insight into trace metal origin, biological and physicochemical availability, mobilization, and transport in sediment (Tessier et al. 1979). Examples of operationally-defined metal fractions extracted from dry sediments that are most likely available for uptake by benthic invertebrates include the exchangeable (Tessier et al. 1979), organic-associated (Carapeto and Purchase 2000), or mobile (Maiz et al. 1997) fractions. These fractions can be extracted in a single step and represent metals that are weakly-bound or adsorbed to sediment particles and are readily dissolved in aqueous solution. They likely represent the metals already found in pore-water of wet sediment. Although these fractions are only a portion of the total metal concentration in sediment, dissolved and weakly-bound metal fractions in sediment are thought to represent that portion of sediment metal that is most available to benthic invertebrates. Weakly-bound metals partition between the solid and dissolved phases and can therefore be taken up either indirectly by benthic invertebrates through respiration (the gill surface), particle ingestion (the gut epithelium), or directly through dermal contact with sediment. Extraction procedures do not directly estimate metal exposure, but generate metal measures that potentially co-vary with benthic invertebrate metal uptake and accumulation. For example, Ramos et al. (1999) found that the exchangeable Cu fraction in contaminated sediment was best correlated with the tissue concentrations of Cu in earthworms (*Allophora mollen*) exposed to this sediment. Although studies such as Ramos et al. (1999) have investigated the bioavailability of various metal fractions in sediment, this topic has not been broadly studied, and the data are particularly limited for freshwater sediments.

The objective of this investigation was to evaluate different methods of estimating the bioavailable fraction of metals in sediment to the benthic invertebrate *Chironomus dilutus* (formerly *C. tentans*) ( $H_0$ : Pore-water or chemical extractions are no better than total sediment metal concentrations at predicting sediment metal bioavailability to the model benthic invertebrate *C. dilutus*). Given the limitations of using total metal concentrations in sediment to evaluate metal bioavailability and toxicity, and the previous successes linking specific metal fractions in sediment with metal availability, the intent of this investigation was to identify a simple, one-step method to better evaluate the risk that a sediment may pose to the associated benthic invertebrate community (BIC). It is believed that for a relatively small amount of

additional effort (in addition to a complete sediment digestion), a single chemical extraction could provide much needed information on metal bioavailability and associated risk.

Based on a literature review, two metal fractions (metals in pore-water and metals weakly-adsorbed to sediment particles) were identified as having the greatest potential for assessing metal availability. These two fractions likely account for the majority of metals taken up by benthic invertebrates through either aqueous or dietary routes of exposure. Larvae of the benthic invertebrate *C. dilutus* were exposed under controlled laboratory conditions to a gradient of metal concentrations in sediments using field sediments (reference and contaminated) collected near uranium operations in northern Saskatchewan. The contaminants of interest included those metals and metalloids already having sediment quality guidelines (SQGs; As, Cr, Cu, Pb, Mo, Ni, Se, U, and V); however, emphasis was placed on those metals having sediment concentrations clearly elevated compared to the reference sediments. Two approaches were used to achieve a gradient of metals concentrations in sediments: i) sediments cores were collected from known contamination gradients downstream of uranium mining and milling operations in northern Saskatchewan and ii) a highly contaminated sediment was mixed with a reference sediment in various ratios. The relationships between whole-body metal concentrations in midge larvae exposed to these sediments and either pore-water metals concentrations (isolated using centrifugation and peepers) or metal concentrations in the chemical extractions were then evaluated and compared.

### **4.3 Materials and methods**

As stated above, two approaches were used to assess metal bioavailability across gradients of sediment contamination. The first approach involved mixing contaminated and reference sediments (both field-collected) in different ratios to create a gradient of metals exposure. This is referred to hereafter as the “mixed-sediment” experiment. The second approach used sediment cores collected from a variety of contaminated and reference sites (spanning a range of sediment metals concentrations) and is referred to hereafter as the “field-core” experiment. Midge larvae (*C. dilutus*, a common benthic invertebrate test organism) were exposed to the sediments from both experiments and then analyzed for whole-body metals accumulation (“whole-body” will be referred to as “tissue” hereafter). Metal concentrations in tissues were then correlated with various metal fractions to determine the usefulness of these

fractions in assessing metal bioavailability in sediment. The metal fractions evaluated in both of the experiments included pore-water (isolated using both centrifugation and peepers, two commonly used pore-water extraction methods) and the extracts from two different single chemical extractions. A variety of extraction options were evaluated in a preliminary experiment, with potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and hydrochloric acid (HCl) being selected for further evaluation in the mixed-sediment and field-core experiments (discussed further below). Extractions were performed on both wet and dry sediment subsamples. It is a common practice to dry sediment samples prior to digestion or chemical extraction; however, drying can alter the speciation of metals in sediment and thus confound our understanding of metal bioavailability in sediments *in situ*. In addition to dry sediment subsamples, wet sediment subsamples were therefore extracted to evaluate the effects of sediment drying on tissue-metal concentration and extractant-metal concentration relationships.

#### **4.3.1 Sediment collection and processing**

##### **4.3.1.1 Field sediments (grab samples)**

Bulk sediments used in this study were collected near two uranium mining and milling operations in northern Saskatchewan, Canada. Contaminated sediment was collected from Horseshoe Pond (HP) on September 18, 2007. Located near the Rabbit Lake Operation, this pond has received treated effluent since the 1970s and is known to have elevated concentrations of metals in sediment. Bulk reference sediment was collected from David Lake (DVD; August 23, 2007) located near the Key Lake Operation. This lake is upstream of any influence from mining and milling operations. Both sediments were collected using a small Ekman grab sampler and were stored in sealed plastic pails at 4°C until use. Prior to use, excess pore-water (resulting from sediment consolidation) was gently decanted and the sediment homogenized with a large plastic spoon. The two sediments were combined in different ratios to create a gradient of metals exposure for the mixed-sediment experiments (described below). Only Horseshoe Pond sediment was used in the extractant selection experiment (described in Section 4.3.2.3)

Using a plastic spoon, aliquots of wet sediment (600 g total per bottle) were added to pre-cleaned 1-L wide-mouth HDPE bottles. Each bottle contained a different mixture of DVD and HP sediment, mixed on a wet-weight basis. The treatments included a control (DVD sediment

only), 25% HP (25% HP sediment + 75% DVD sediment), 50% HP (50% HP sediment + 50% DVD sediment), 75% HP (75% HP sediment + 25% DVD sediment), and 100% HP sediment.

Each sediment treatment was homogenized with a clean, plastic spoon and shaken for 1 h at 300 oscillations per minute on a Mighty Magnum Wrist-Action<sup>®</sup> Shaker with Snap-N-Lock container clamps (Burrell Scientific, Inc., Pittsburgh, PA). All treatments were then stored in the dark at 4°C for 27 days to allow metals to re-partition. After 27 days, each treatment was again shaken for 5 minutes at 300 oscillations/minute to ensure that all sediments were well mixed.

#### **4.3.1.2 Field sediment (core samples)**

Sediment cores were collected from two uranium operations, Key Lake (August 21 to 23, 2007) and Rabbit Lake (September 18 and 19, 2007), in northern Saskatchewan, Canada.

Sample locations near the Key Lake operation included DVD (reference), Fox Lake (FOX), Unknown Lake (UNK), and Delta Lake (DTA). Rabbit Lake sampling sites included Raven Lake (RAV; reference), HP, and Parks Lake at the outflow (PLO) and near a seepage point (PLS). Contaminated waters from a seepage point from the above ground tailings management facility enter Parks Lake at the north-west end of the lake.

Sediment samples were collected in 5-cm diameter acrylic core-tubes (Wildlife Supply Company, Buffalo, NY, USA) using a custom-made hand-held corer. All sediment cores were sealed with plastic caps with minimal head space after retrieval. Sediment cores were stored upright in the dark at 4°C until used (~15 months after sampling). Although the sediment-core storage period was longer than recommended for sediments intended for contaminant analysis, the intent of core sampling was to collect a gradient of sediment metal concentrations and to assess metal bioavailability across this gradient. Given that the speciation and bioavailability of the metals in the sediment cores could have changed during storage (not evaluated), results from the core experiments presented here may not reflect exact field conditions at the time of sampling; however, the purpose here was to evaluate the relationship between bioavailable fractions of metals in sediment and *C. dilutus* tissue concentrations, not to describe the change(s) in metal speciation or bioavailability related to the aging of sediments.

## **4.3.2 Sediment metal fractions**

### **4.3.2.1 Peeper pore-water**

The peeper is an *in-situ* dialysis device used to sample dissolved metal concentrations in water and sediment. Miniature peepers (mini-peepers) modified for use in laboratory toxicity tests (Doig and Liber 2000) were used to sample dissolved metals immediately above and below the sediment-water interface. Each of the two chambers of the mini-peeper (simply called a peeper hereafter) was filled with ultrapure (Barnstead® 18.2 Ω) water and covered with a semi-permeable membrane (0.45-μm pore size, Supor® polyethersulfone filter membrane, Pall Life Sciences, East Hills, NY, USA). The membrane was held in place by a thin face plate secured by small stainless steel screws. After assembly, the peepers were placed in deionized water and bubbled with nitrogen for > 12 hours to remove any oxygen, which has the potential to alter metal speciation in anoxic sediment. The peepers were then placed vertically into test sediment such that one chamber was above and one chamber was below the sediment-water interface. Solutes (i.e., metals) pass through the membrane allowing the peeper cells to equilibrate over time with either the pore-water or overlying water. When peepers are removed from sediment, they are gently rinsed with deionized water to remove sediment particles. Each chamber is sampled by puncturing the membrane with a 1-mL pipette tip and the cell contents transferred to a pre-cleaned 8-mL Nalgene® bottle. A new pipette tip was used to sample each chamber in this study. Each sample for metals analysis was preserved with high-purity HNO<sub>3</sub> (to approximately 2%).

### **4.3.2.2 Centrifuged pore-water**

Pore-water was also isolated using centrifugation of sediment collected from both the mixed-sediment and field-core experiments. The top 2.5-cm of sediment were sampled from test beakers using a small scoop and placed into a new glass beaker. This sediment was then homogenized and a sub-sample removed and centrifuged at 3000 rpm (Eppendorf Centrifuge Model 5810, Mississauga, ON, Canada) at room temperature for 15 minutes to isolate the pore-water. Once centrifuged, pore-water (supernatant) was collected using a pipette and transferred to a plastic vial. Pore-water samples for metals analysis were filtered through a 0.45-μm pore size syringe filter (Supor® polyethersulfone filter membrane) and preserved with high-purity HNO<sub>3</sub> (to approximately 2%) in a pre-clean 8-mL Nalgene® bottle. The pore-water sample was

also analyzed for dissolved organic carbon (DOC; Shimadzu Total Organic Carbon Analyser, Model TOC-5050A, Mandel Scientific, Guelph, ON, Canada), pH (ORION<sup>®</sup> perpHect Ross Sure-Flow electrode, Model 8272BN, Thermo Scientific, Beverly, MA, USA connected to a ORION<sup>®</sup> PerpHect LogR meter, Model 370, Thermo Scientific, Beverly, MA, USA), ammonia (Thermo Orion<sup>®</sup> ammonia electrode, Model 9512 BN, Thermo Scientific, Beverly, MA, USA connected to a VWR meter, Model SB301, VWR International, Arlington Heights, IL, USA), hardness, and alkalinity (both alkalinity and hardness were measured using a Hach Digital Titrator, Model 16900, Hach Company, Loveland, CO, USA) when the volume of pore-water was sufficient to conduct these analyses (sandy sediments contains less pore-water than clay or silt-type sediments).

#### **4.3.2.3 Chemical extractions**

##### *Extraction selection experiment*

A preliminary experiment was conducted to evaluate which extractants to use in subsequent experiments. Using bulk sediment collected from Horseshoe Pond (previously described), this experiment evaluated five extractants (deionized water, 1M MgCl<sub>2</sub>, 0.1M KH<sub>2</sub>PO<sub>4</sub>, 0.05M EDTA, 1M HCl) for their efficacy in extracting metals from wet and dry sediment during a single chemical extraction. Deionized water was evaluated because it could theoretically be used as a surrogate for pore-water in a dry sediment extraction. Magnesium chloride was used to isolate the exchangeable metal fraction (Tessier et al. 1979). Ethylenediaminetetraacetic acid (EDTA), a chemical known for binding metals, was used to evaluate the carbonate or oxidizable (organic and sulfide-bound) metal fractions in sediment (Filgueiras et al. 2002). Potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) has previously shown potential for extracting Se species (oxy-anions; Sharmasarkar and Vance 1997), a metal of concern downstream of northern Saskatchewan uranium operations. Potassium phosphate also has potential to extract other oxy-anions such as Mo. Hydrochloric acid was evaluated because it is likely to represent a fraction of the total metal concentration in sediment that would not underestimate metal bioavailability and it can liberate metals from Fe and Mn oxides, organic phases, and sulfides (Snape et al. 2004). Based on the overall percentage of the total metals extracted, two chemicals were chosen for use in subsequent bioaccumulation experiments. For comparison to these chemical extractions, pore-water was isolated using peepers and sediment centrifugation

and analyzed for dissolved metals. No test organisms were used in this experiment. This static, renewal experiment was conducted using nine replicate 250-mL glass beakers. Each beaker contained ~150 mL of contaminated field sediment (depth of ~3.5 cm) and ~200 mL of overlying reconstituted hard water formulated to mimic field site water quality (pH = 7, alkalinity = 13 mg CaCO<sub>3</sub>/L, hardness = 1310 mg CaCO<sub>3</sub>/L). Peepers were added to three of the nine replicates to isolate pore-water. All replicates had an equilibration period of 12 days in the test beakers with renewal of the overlying water on day six to mimic water changes in the mixed-sediment and field-core experiments. Overlying water temperature and dissolved oxygen were monitored throughout the experiment and samples for measurement of pH, conductivity, alkalinity, total hardness, ammonia and metals were collected on day 0 and day 12 of the experiment. Both overlying-water and pore-water peeper samples, and the pore-water collected from centrifugation of sediment, were collected on day 12 and subsequently processed and analyzed for dissolved metals. Three replicates were used for wet sediment extractions and three replicates were used for dried sediment extractions. For these six replicates, the top 2.5 cm of sediment were isolated, homogenized, subsampled, and extracted using the procedures described previously.

Comparisons of the metals concentrations in the different chemical extractants ( $\mu\text{g}$  metal per g pore-water) were made based on the change in concentration of each metal (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V) relative to peeper pore-water concentrations (expressed as  $\mu\text{g}$  metal per g pore-water). The measurement units were standardized on a per gram of pore-water basis so that chemical extracts and pore-water could be directly be compared. In the extract of EDTA, a precipitate formed after acidification with nitric acid which could not be dissolved with hydrogen peroxide. Thus, EDTA extracts could not be analyzed. In general, the order of extraction efficiency was: water < MgCl<sub>2</sub> < KH<sub>2</sub>PO<sub>4</sub> < HCl. Extractions using dried sediment yielded higher concentrations of metals than extractions using wet sediment. Because KH<sub>2</sub>PO<sub>4</sub> and HCl extracted a moderate (0.04 to 46%, depending on the metal) to high proportion (0.78 to 96%, depending on the metal), respectively, of the metals in HP sediment, these reagents were selected for use in subsequent laboratory sediment bioaccumulation experiments. In addition, HCl should extract only a portion than the total metal concentration in sediment, but should not underestimate the potentially bioavailable fraction. Potassium phosphate will most likely extract metals that are anions. The intent of the extraction selection experiment was to select two

extractants for use in subsequent bioaccumulation experiments. As such, the results of the extractant selection experiment are not presented herein.

*Single chemical extractions (wet and dry sediments)*

Single chemical extractions were conducted on wet and dry sediments from the mixed-sediment and field-core bioaccumulation experiments to evaluate the effect(s) of drying sediment prior to chemical extraction and metals analysis. In these experiments, a single replicate from each sediment treatment was subsampled for chemical extraction of both wet and dry sediment. The overlying water from these replicates was first sampled for water quality analysis (described further below) and then completely removed using a pipette or siphon. If the depth of the sediments was >2.5 cm (as in the field-core experiment), the top 2.5-cm of sediment was removed from each beaker (using a plastic scoop), transferred to a new beaker, and homogenized using a plastic spoon. In the mixed-sediment experiment, where the total sediment depth was ~2.5 cm across treatments and replicates, the sediments were not transferred to a clean beaker before homogenization. Sediment samples (from both experiments) destined for sediment chemical extractions were each transferred to a 30-mL wide-mouth Nalgene<sup>®</sup> bottle. Those samples destined for wet sediment extraction were refrigerated at 4°C until chemical extraction could be completed (within 24 h). The remaining subsamples for chemical extractions and sediment characterization were dried for > 48 h at 60°C.

Two chemicals, 0.1 M potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) and 1 M hydrochloric acid (HCl), used individually, were used to extract metals from both wet and dried sediment. For chemical extraction on dry sediment, 1 g of dried sediment was added to a clean, pre-weighed 30-mL Nalgene<sup>®</sup> bottle followed by 10 mL of extractant. For chemical extraction on wet sediment, approximately 8 g of wet sediment (exact weight was recorded) was added to a clean, pre-weighed 30-mL Nalgene<sup>®</sup> bottle. The volume of extractant added to the wet sediment subsample was corrected for sediment moisture content such that for every 1 g dry weight of sediment, 10 mL of extractant was added to the wet sediment. The weights of the sediment and extractant added in each vial were recorded. Using these weights, the metal concentrations in the extractants were corrected on a per gram of dry sediment basis. Extract metal concentrations from chemical extraction of dry sediment were corrected using the metal concentration of the extract, the weight of the extract, and the dry weight of the sediment used in the extraction.

Extract metal concentrations from chemical extraction of wet sediment were corrected using the metal concentration of the extract, the weight of the extract, the theoretical weight of the pore-water based on the wet weight of the sediment and percent moisture content, and the dry weight equivalent of the wet sediment used in the extraction. All extractions were performed on a Mighty Magnum Wrist-Action<sup>®</sup> Shaker for 2 h at 300 oscillations per minute and then left for 30 minutes to allow the sediment to settle. Chemical extracts were filtered using a 5-mL syringe and a 0.45- $\mu\text{m}$  pore size syringe filter (VWR<sup>®</sup> polyethersulfone filter membrane). The  $\text{KH}_2\text{PO}_4$  extracts were preserved with high-purity  $\text{HNO}_3$  (2% of sample volume) and stored at 4°C until analyzed using by ICP-MS. Extracts using HCl were also stored at 4°C until analyzed directly (without the addition of  $\text{HNO}_3$ ) using ICP-MS.

The sediment subsamples for sediment characterization were also taken from the same replicates used for chemical extractions. These subsamples were dried for > 48 h at 60°C. They were analyzed for total organic carbon (TOC) content using a Leco C632 organic and total carbon analyzer (Leco, St. Joseph, MI, USA). In addition, they were digested using a microwave-assisted (MARS 5 Accelerated Microwave Reaction System, CEM Corporation, Matthews, NC, USA) digestion protocol (Wu et al. 1996, Wiramanaden et al. 2010) (nitric acid, hydrogen peroxide, and hydrofluoric acid digestion). The resulting solution was analyzed for metals using an ICP-MS as described in Section 4.3.4.

### **4.3.3 General sediment bioaccumulation experiment procedures**

The test organism used in this study was *C. dilutus* larvae. Organisms were cultured in-house at the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, Canada) following protocols similar to those outlined in Environment Canada (1997). Briefly, *C. dilutus* were cultured in 20-L aquaria with each tank being fed 20 mL of a 100 g/L Nutra-Fin<sup>®</sup> (Rolf Hagen, Montreal, QC) fish food slurry three times per week. The culture water was carbon-filtered municipal water (Saskatoon, SK, Canada) which was aerated for a minimum of 24 h in a 50-L Nalgene<sup>®</sup> carboy before use. For breeding, isolated *C. dilutus* adults were added from the main colony via aspiration to a 1-L glass jar with a screened lid, a small Parafilm<sup>®</sup> floating platform, ~100 mL of culture water, and a piece of rectangular plastic mesh to provide a mating surface. The breeding jar was surrounded with cardboard to reduce visual disturbances. The morning after the addition of adults, egg masses laid in the breeding jar were added to new 20-L tanks

containing aerated culture water and a 1-cm deep layer of clean silica sand (particle size of ~250 to 425 µm). Food was introduced 48 h after the egg masses were placed in the tanks. These animals were fed three times per week with 5 ml of a 100 g/L Nutra-Fin<sup>®</sup> fish food slurry. The tank water was changed after ~1 week. Twelve days from the time the egg masses were added (approximately 10 days post-hatch), larvae were transferred to a glass pan containing culture water and were gently teased out of their cases for use in experiments. Larvae were isolated this way for all bioaccumulation experiments. Endpoints evaluated after exposure to sediments for 10 days were survival, growth (as dry weight), and whole-body bioaccumulation of metals (referred to as tissue metals).

#### **4.3.3.1 Mixed-sediment bioaccumulation experiment**

The mixed-sediment experiment consisted of six treatments. A sand control, a reference sediment from DVD, and four mixed-sediment exposure treatments (25, 50, 75, and 100% HP sediment), as described previously. Each treatment consisted of six replicate beakers with ten *C. dilutus* larvae per beaker.

Each replicate consisted of approximately 100 g of sand or sediment added to a 300-mL tall-form glass beaker with approximately 175 mL of overlying water (carbon-filtered municipal water, Saskatoon, SK, Canada) added to the beaker using a funnel to minimize sediment disturbance. Each beaker was covered with Parafilm<sup>®</sup> to prevent evaporation and stored in the dark at 4°C for six days. The beakers were then placed in a controlled environmental chamber (~23°C) for 24 h. After 24 h, the overlying water was siphoned off and a peeper (described in Section 4.3.2.1) inserted into four of the six replicates per treatment. Clean carbon-filtered municipal water was then added to each beaker with minimal re-suspension of the sediment. All beakers were aerated to maintain adequate dissolved oxygen levels. After 48 h, the water was changed again prior to adding the test organisms to remove metals that had leached into overlying water.

Once the final pre-test change was complete, ten 8 to 10-d post-hatch *C. dilutus* larvae were added to five of the six replicates per treatment. The remaining replicate in each treatment was used for initial sediment characterization (total metals, pore-water isolation via centrifugation, and chemical extractions). Throughout the experiment, larvae were fed 0.1 mL of fish-food slurry (Nutra-Fin<sup>®</sup> 10 g/L of dechlorinated water) each day until excess food was

visible in the overlying water (feeding was reduced to limit ammonia build-up in the overlying water). The experiments were conducted in a light (16 h light: 8 h darkness) and temperature controlled (~23°C) environmental chamber in the Toxicology Centre (University of Saskatchewan, Saskatoon, SK, Canada). Overlying water was changed in each replicate every two days to reduce potential ammonia build-up (i.e., maintain good overlying water quality). Routine water chemistry (pH, ammonia, hardness, alkalinity, dissolved oxygen, and temperature) and metal concentrations in overlying water were monitored throughout the experiment in both old and new water when water was changed (every two days).

At the completion of this experiment (day 10), all mini-peepers were removed and processed as previously described to sample pore-water. Each of these replicates was then sieved to retrieve all surviving larvae. These larvae were gut-purged for 24 h in beakers containing clean, carbon-filtered municipal water and a sand substrate. Food was added (0.1 mL of fish-food slurry) to enhance gut clearance. Gut purging was done to reduce the influence of gut contents (e.g., sediment particles) on measured whole-body metals concentrations. After gut purging, larvae were dried at 60°C for > 48 h and then weighed and digested for metals analysis (ICP-MS; described in Section 4.3.4). Tissues were cold digested by adding increments of nitric acid (5 mL, Omnitrace Ultra; EM Science) and peroxide (1.5 mL, 30%, Super Pure; EMD Chemicals), evaporated to near-dryness on a hot plate at no more than 75°C after reagent additions, and then diluted in 5 mL of 2% nitric acid for metals analysis. To verify the tissue digestion method, Tort-2 lobster hepatopancreas (National Research Council) was used as a certified standard reference material. The remaining replicate from each treatment was used to subsample the sediment for final total metals analysis, moisture content and TOC, pore-water isolation via centrifugation, and chemical extractions. The centrifuged pore-water was analyzed for dissolved metals and routine water quality (pH, ammonia, DOC, hardness, and alkalinity). Some sediments had low pore-water sample volumes; for these sediments the full suite of analyses could not be performed.

#### **4.3.3.2 Field-core bioaccumulation experiment**

The field-core sediment experiment consisted of nine treatments. A laboratory sand control was used to verify larvae survival and growth. Sediment cores from the Key Lake and Rabbit Lake operations (described in Sections 4.3.1.1 and 4.3.1.2) made up the remaining

treatments and were described previously. Each treatment had five replicates. Cores were allowed to warm up to room temperature for about 4 h before transfer (with overlying water) into individual 300-mL tall-form beakers. The cores were extruded into the tall-form beakers in a manner that reasonably maintained the profile of each core. After transfer, the contents of the beaker were allowed to settle overnight. The overlying water was then siphoned off and replaced with approximately 175 mL of carbon-filtered municipal water. Peepers were then added to four replicates per treatment. After 48 h, the overlying water was once again siphoned off and replaced with clean water to remove metals that had diffused into overlying water. Ten *C. dilutus* larvae (8 to 10-d old) were then added to each replicate. The standard experimental procedures detailed above (Section 4.3.3) were followed. One replicate from each treatment was sampled on Day 0 of the experiment for total metals analysis, pore-water metals (by centrifugation) analysis, and to conduct chemical extractions. This experiment was maintained for 10 days with water changes every two days (~75% water replacement). Routine water chemistry (pH, ammonia, hardness, alkalinity, dissolved oxygen, and temperature) and metal concentrations in the overlying water were monitored throughout the experiment in both old and new water on water change days.

Upon termination of the experiment, the peepers were removed and processed (as described previously) and larvae were isolated from three replicates per treatment. Larvae were rinsed with dechlorinated water to remove sediment particles (rather than gut-purging) and dried at 60°C for > 48 h. Tissues were cold digested as previously described. The dried larvae were weighed, digested and analyzed to determine tissue metals concentrations. The remaining replicate was sampled to analyze sediment subsamples for total metals, percent moisture, TOC, isolate pore-water by centrifugation, and perform chemical extractions. Dissolved metals and routine water quality analyses (pH, ammonia, dissolved organic carbon, hardness, and alkalinity) were performed on the centrifuged pore-water. Some sediments had low pore-water sample volumes; for these sediments the full suite of analyses could not be performed.

#### **4.3.4 Metals analysis**

Metals of interest in this study were As, Cr, Cu, Mo, Ni, Pb, Se, U, and V. These metals were selected because there are SQGs available for these metals for uranium mines and mills in Canada, as per Thompson et al. (2005).

All metals analyses were conducted on an inductively coupled plasma mass spectrometer (ICP-MS; Thermo Fisher Scientific X-Series, Waltham, MA, USA) housed in the Toxicology Centre at the University of Saskatchewan, Canada. Blanks, standard reference material (SRM) for sediment (PACS-2 [National Research Council Canada]) and water (1640 [National Institute of Standards and Technology] and SLRS-4 [National Research Council Canada]), duplicates, and calibration standard samples were analyzed alongside test samples to verify the accuracy of all metals analyses. For the extractant samples, standards were made in each extractant to account for matrix interferences. In general, if the majority of metals of interest were within  $\pm 20\%$  of the quality assurance samples (e.g., SRM, duplicates, standards), then all data for all the metals were presented and used in the data analysis. Some metals (some As, U, and V concentrations in the HCl extractions) were consistently more than 20% higher than the SRM. Despite this, these data were presented and used to investigate general trends in sediment exposure-*C. dilutus* metal accumulation relationships. The instrument detection limits for each sample run are provided rather than method detection limits.

#### **4.3.5 Statistics and data analysis**

All treatment data were tested for normality (Kolmogorov-Smirnov Test) and equal variance (Levene Median test) prior to statistical analysis, such as Analysis of Variance (ANOVA) or a *t*-test (Systat Software Inc., 2008). If these assumptions were not met, then the data were log-transformed. If statistical assumptions were not met following transformation, then a non-parametric statistical test was used (e.g., Mann-Whitney Rank Sum Test). Post-hoc tests, such as Tukey or Dunnett's, were conducted if a statistical difference among treatments was found. Percent survival data were arcsin square-root transformed prior to statistical analysis.

Correlations between metal concentrations in tissue and each of the sediment phases were only evaluated for a subset of metals that were present in sediment above background concentrations and yielded a sufficient gradient (As, Ni, Mo, U). Each dataset was tested for normality (Kolmogorov-Smirnov Test or Shapiro-Wilk Test) prior to conducting correlation analysis on non-transformed data. Correlations were determined using the Pearson Product Moment Correlation to evaluate potential relationships between metal concentrations in chironomids and measured metal concentrations in whole-sediment, pore-water, and chemical extractions. Of all the data evaluated, there were only three sets of data from the field-core

experiment that did not meet normality: molybdenum concentrations in HCl extracts, nickel concentrations in centrifuged pore-water samples, and uranium concentrations in  $\text{KH}_2\text{PO}_4$  extracts. To keep the method of statistical analysis consistent among the metals and sediment phases evaluated, the Pearson Product Moment Correlation was still used to analyze these non-normal sets of data.

For data that were not quantifiable, half the method detection limit was used for all statistical analyses. An alpha value of 0.05 was used in all statistical comparisons. All statistical analyses were completed using SigmaPlot Version 11.0 (Systat Software Inc., 2008).

## **4.4 Results**

### **4.4.1 Mixed-sediment experiment**

With few exceptions, overlying water quality in the mixed-sediment experiment was relatively consistent during the 10-day exposure period and similar across all control and exposure treatments. The mean ranges across treatments were: DO concentration of 7.1 to 7.3 mg/L, pH of 8.0 to 8.3, alkalinity of 85 to 119 mg  $\text{CaCO}_3/\text{L}$ , total hardness of 139 to 181 mg  $\text{CaCO}_3/\text{L}$ , and DOC of 5.2 to 9.3 mg/L. Substantial differences were noted for ammonia concentrations in new (0.43 to 0.84 mg N/L) and old (2.19 to 3.37 mg N/L) water samples collected during water changes. Metal concentrations in overlying water were generally higher on Day 10 than Day 0 in all treatments, which was attributed to leaching of metals from sediment.

Sediment physical characteristics and total metal concentrations from the mixed-sediment experiment are listed in Table 4.1. Generally, whole-sediment metal concentrations were fairly constant between Day 0 and Day 10 samples (i.e., low standard deviation) with As, Mo, and U concentrations being somewhat more variable. Thus, metal concentrations were calculated as an average of Day 0 and Day 10 measurements. Sediment metal concentrations and TOC content increased with an increased percentage of contaminated HP sediment. Arsenic, Mo, Ni, and U were significantly elevated in HP sediment and as a result, produced only a good range of metals concentrations for these elements among the mixed-sediments. Chromium, Cu, and V were only slightly elevated in HP sediment and as such produced a small metals gradient. Lead and Se concentrations in HP sediment were low and did not result in a measurable gradient in the mixed-sediment experiment.

Table 4.1. Mean whole-sediment metal concentrations and physical sediment characteristics ( $\pm$  standard deviation) from the mixed-sediment experiment ( $n = 2$ ; Day 0 and Day 10).

Variable	Control	Reference Sediment	Mixed-sediment Treatments			
	Sand	100 % DVD	25% HP	50% HP	75% HP	100% HP
As	0.03 $\pm$ 0.02	1.03 $\pm$ 0.22	3.82 $\pm$ 0.46	9.30 $\pm$ 3.45	24.60 $\pm$ 1.72 <sup>a</sup>	31.76 $\pm$ 5.09 <sup>a</sup>
Cr	0.24 $\pm$ 0.33	2.89 $\pm$ 0.64	2.76 $\pm$ 0.57	4.93 $\pm$ 1.74	12.49 $\pm$ 0.65	13.71 $\pm$ 3.57
Cu	0.64 $\pm$ 0.22	1.00 $\pm$ 0.15	2.24 $\pm$ 0.46	4.52 $\pm$ 0.58	13.08 $\pm$ 1.16	15.30 $\pm$ 1.79
Mo	0.02 $\pm$ 0.02	0.23 $\pm$ 0.20	86.30 $\pm$ 2.09 <sup>a</sup>	240.29 $\pm$ 138.86 <sup>a</sup>	681.51 $\pm$ 13.11 <sup>a</sup>	877.62 $\pm$ 177.49 <sup>a</sup>
Ni	0.74 $\pm$ 0.08	0.60 $\pm$ 0.11	3.17 $\pm$ 0.25	9.07 $\pm$ 4.83	21.53 $\pm$ 0.95	27.55 $\pm$ 3.86 <sup>a</sup>
Pb	0.45 $\pm$ 0.04	1.98 $\pm$ 0.35	2.09 $\pm$ 0.02	2.68 $\pm$ 0.31	3.79 $\pm$ 0.12	3.89 $\pm$ 0.86
Se	<0.001	<0.001	<0.001	1.00 $\pm$ 0.83	4.29 $\pm$ 0.25 <sup>a</sup>	5.83 $\pm$ 0.95 <sup>a</sup>
U	0.20 $\pm$ 0.03	0.48 $\pm$ 0.10	58.89 $\pm$ 3.95	150.32 $\pm$ 72.46 <sup>a</sup>	449.38 $\pm$ 17.61 <sup>a</sup>	577.22 $\pm$ 94.32 <sup>a</sup>
V	0.36 $\pm$ 0.05	1.78 $\pm$ 0.58	2.86 $\pm$ 0.20	5.05 $\pm$ 1.67	11.05 $\pm$ 0.47	12.62 $\pm$ 2.28
Moisture	19.05 $\pm$ 1.59	48.70 $\pm$ 2.02	57.49 $\pm$ 2.1	62.45 $\pm$ 4.69	79.07 $\pm$ 0.99	82.15 $\pm$ 0.26
TOC	0.03 $\pm$ 0.01	2.28 $\pm$ 0.08	3.24 $\pm$ 0.28	4.05 $\pm$ 0.3	8.81 $\pm$ 0.64	11.7 $\pm$ 0.49

All metal concentrations in  $\mu\text{g/g}$  dry weight.

Moisture and TOC in %.

TOC = Total organic carbon.

<sup>a</sup> Exceeds lowest effect level (LEL) sediment quality guidelines listed in the Appendix (CCME 2011).

Mixed-sediment treatments presented as percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP) sediment added to the reference sediment from David Lake (DVD).

Table 4.2. Mean centrifuged pore-water variables ( $\pm$  standard deviation;  $n = 2$ ) from the mixed-sediment experiment.

Water Quality Variable	Reference Sediment	Mixed-sediment Treatments			
	100 % DVD	25% HP	50% HP	75% HP	100% HP
Ammonia (mg N/L) Day 0 <sup>a</sup>	NA	3.21	3.69	3.17	3.59
Ammonia (mg N/L) Day 10 <sup>a</sup>	4.68	2.99	3.09	4.57	3.40
pH	6.5 $\pm$ 0.8	6.8 $\pm$ 0.9	6.9 $\pm$ 0.8	7.1 $\pm$ 0.7	7.0 $\pm$ 0.6
Hardness (mg CaCO <sub>3</sub> /L)	40 <sup>a</sup>	124 $\pm$ 45	204 $\pm$ 147	292 $\pm$ 209	363 $\pm$ 258
Alkalinity (mg CaCO <sub>3</sub> /L)	NA	75 <sup>a</sup>	66 $\pm$ 13	72 $\pm$ 3	96 $\pm$ 9
DOC (mg/L)	9.3 $\pm$ 3.7	12.6 $\pm$ 3.7	19.3 $\pm$ 2.3	25.3 $\pm$ 3.7	26.3 $\pm$ 3.4

DOC = Dissolved organic carbon.

NA = Not measured because sample volume was too small.

<sup>a</sup>  $n = 1$  for each treatment.

Sand control pore-water sample volume was too low for these analyses.

Mixed-sediment treatments presented as percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP) sediment added to the reference sediment from David Lake (DVD).

Table 4.3. Mean centrifuged ( $n = 2$ ) and peeper pore-water ( $n = 3$ ) metal concentrations ( $\pm$  standard deviation;  $\mu\text{g/L}$ ) from the mixed-sediment experiment.

Metal	Control Sand		Reference Sediment 100% DVD		Mixed-sediment Treatments 25% HP	
	CPW <sup>1</sup>	Peeper	CPW	Peeper	CPW	Peeper
	As	2.80	<1.2	2.02 $\pm$ 0.75	<1.2	20.22 $\pm$ 4.28 <sup>a</sup>
Cr	4.56	4.34 $\pm$ 0.48	2.89 $\pm$ 0.27	6.05 $\pm$ 4.69	3.90 $\pm$ 1.47	3.33 $\pm$ 0.81
Cu	14.10 <sup>a</sup>	9.06 $\pm$ 3.91 <sup>a</sup>	0.80 $\pm$ 0.13	2.75 $\pm$ 0.30 <sup>a</sup>	1.01 $\pm$ 0.02	2.38 $\pm$ 0.43 <sup>a</sup>
Mo	2.53	3.03 $\pm$ 0.04	<1.9	<2.4	430.94 $\pm$ 608.10 <sup>a</sup>	568.30 $\pm$ 45.90 <sup>a</sup>
Ni	4.36	4.54 $\pm$ 2.42	<3.4	<3.9	5.09 $\pm$ 1.92	<3.9
Pb	0.09	0.44 $\pm$ 0.07	<0.002	0.34 $\pm$ 0.00	0.01 $\pm$ 0.01	0.33 $\pm$ 0.05
Se	<0.76	<1.5 <sup>b</sup>	<0.76	<1.5 <sup>b</sup>	<0.76	<1.5 <sup>b</sup>
U	1.74	1.23 $\pm$ 0.13	0.09 $\pm$ 0.01	<0.006	14.87 $\pm$ 6.44	22.88 $\pm$ 1.27 <sup>a</sup>
V	2.39	1.61 $\pm$ 0.27	0.93 $\pm$ 0.04	1.01 $\pm$ 0.01	1.02 $\pm$ 0.06	1.06 $\pm$ 0.05

Metal	Mixed-sediment Treatments					
	50% HP		75% HP		100% HP	
	CPW	Peeper	CPW	Peeper	CPW	Peeper
As	30.18 $\pm$ 16.28 <sup>a</sup>	14.06 $\pm$ 5.79 <sup>a</sup>	41.59 $\pm$ 17.84 <sup>a</sup>	25.97 $\pm$ 4.81 <sup>a</sup>	45.83 $\pm$ 15.16 <sup>a</sup>	31.42 $\pm$ 14.28 <sup>a</sup>
Cr	4.18 $\pm$ 1.18	4.84 $\pm$ 2.78	4.88 $\pm$ 1.39	4.61 $\pm$ 1.72	10.50 $\pm$ 7.06 <sup>a</sup>	4.55 $\pm$ 0.79
Cu	1.07 $\pm$ 0.14	1.75 $\pm$ 0.33	1.39 $\pm$ 0.72	1.70 $\pm$ 0.32	2.17 $\pm$ 1.69 <sup>a</sup>	2.33 $\pm$ 0.90 <sup>a</sup>
Mo	2550 $\pm$ 1373 <sup>a</sup>	1312.7 $\pm$ 293.64 <sup>a</sup>	5196 $\pm$ 2804 <sup>a</sup>	2192.2 $\pm$ 378.59 <sup>a</sup>	7203 $\pm$ 4217 <sup>a</sup>	3115.0 $\pm$ 325.3 <sup>a</sup>
Ni	7.17 $\pm$ 2.81	4.41 $\pm$ 2.48	10.16 $\pm$ 1.74	5.65 $\pm$ 0.28	11.55 $\pm$ 1.89	8.67 $\pm$ 1.27
Pb	<0.002	0.39 $\pm$ 0.05	0.05 $\pm$ 0.07	0.35 $\pm$ 0.03	<0.003	1.10 $\pm$ 1.22 <sup>a</sup>
Se	0.86 $\pm$ 0.02	<1.5 <sup>b</sup>	1.37 $\pm$ 0.13 <sup>a</sup>	<1.5 <sup>b</sup>	1.56 $\pm$ 0.08 <sup>a</sup>	<1.5 <sup>b</sup>
U	111.37 $\pm$ 97.93 <sup>a</sup>	131.32 $\pm$ 59.35 <sup>a</sup>	598.85 $\pm$ 549.97 <sup>a</sup>	148.31 $\pm$ 16.71 <sup>a</sup>	1926 $\pm$ 2118 <sup>a</sup>	359.22 $\pm$ 47.58 <sup>a</sup>
V	1.54 $\pm$ 0.46	1.76 $\pm$ 0.42	1.95 $\pm$ 0.49	1.63 $\pm$ 0.04	2.55 $\pm$ 0.83	1.92 $\pm$ 0.53

<sup>a</sup> Exceeds water quality guidelines outlined in the Appendix.

<sup>b</sup> The method detection limit exceeds the water quality guidelines outlined in the Appendix (CCME 2011).

CPW = centrifuged pore-water.

<sup>1</sup>  $n = 1$ .

Mixed-sediment treatments presented as percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP) sediment added to the reference sediment from David Lake (DVD).

Due to the limited peeper sample volume, general pore-water chemistry (Table 4.2) was characterized only for the centrifuged pore-water samples. In addition, pore-water chemistry (Table 4.3) could not be measured in the sand treatment due to the limited pore-water volume isolated during centrifugation (only 19% moisture in sediment). Pore-water As, Mo, Ni, and U concentrations (peepers and centrifuged) were elevated and, similar to the whole-sediment concentrations of these metals, they were present in a gradient among the mixed-sediment treatments.

Metal concentrations in the  $\text{KH}_2\text{PO}_4$  and HCl extracts are presented on a sediment dry-weight basis (Table 4.4 and Table 4.5). In the  $\text{KH}_2\text{PO}_4$  extracts, Cr, Cu, and Pb were below the method detection limit in the majority of sediments. Metals that had a broad range in concentration in the mixed-sediment  $\text{KH}_2\text{PO}_4$  extracts included As and Mo. Although Ni, Se, and U were similarly present in a gradient of concentrations in the  $\text{KH}_2\text{PO}_4$  extracts, the range in the concentrations of these elements was narrower. Arsenic, Mo, Ni, and U displayed a good gradient of concentrations in the HCl extracts. For both  $\text{KH}_2\text{PO}_4$  and HCl extractions, dry sediment yielded higher concentrations of metals than wet sediment, although the difference was sometimes marginal. For example, among all sediments, As, Cu, and Mo concentrations in HCl extractions and Mo, Ni, Se, U, and V concentrations in  $\text{KH}_2\text{PO}_4$  extractions performed on dry sediment were higher than those performed on wet sediment. Of the four metals that displayed a good gradient of concentrations in the HCl extracts, Ni and U were found at much higher concentrations in HCl extracts compared to the  $\text{KH}_2\text{PO}_4$  extracts. There was no discernable difference in As and Mo concentrations between the HCl and  $\text{KH}_2\text{PO}_4$  extracts (the data were quite variable). Likely due to potential polyatomic interferences with the ClO ion, V was not quantifiable in the HCl extracts using ICP-MS. Matrix spike samples (spiked extracts) were used for quality assurance.

There was no statistical difference in survival ( $P = 0.500$ ; one-way ANOVA on Ranks) or growth ( $P = 0.642$ ; one-way ANOVA) among the mixed-sediment treatments and either the sand or field sediment controls. The tissue-metal concentrations in *C. dilutus* larvae exposed to the mixed-sediment treatments are summarized in Table 4.6. Of the metals evaluated, only V and Cr tissue concentrations did not vary statistically among treatments ( $P = 0.543$  for V;  $P = 0.162$  for Cr). Tissue metal concentrations generally increased with increasing whole-sediment, overlying

Table 4.4. Metal concentrations in potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) extractions performed on wet and dry sediment from the mixed-sediment experiment (µg metal/g dry sediment; *n* = 1).

Metal	Control				Mixed-sediment Experiment <sup>a</sup>	
	Sand		DVD Sediment		25% HP	
	Wet	Dry	Wet	Dry	Wet	Dry
As	<0.004	<0.004	0.050	0.050	1.77	1.54
Cr	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
Cu	<0.003	0.011	<0.003	<0.003	<0.003	<0.003
Mo <sup>b</sup>	<0.013	<0.013	0.115	0.209	2.31	4.11
Ni	0.004	0.006	<0.001	<0.001	0.029	0.069
Pb	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Se	<0.001	0.001	<0.001	0.006	0.021	0.044
U <sup>b</sup>	<0.001	<0.001	<0.001	<0.001	0.597	1.77
V	0.004	0.006	0.006	0.010	0.010	0.018
Metal	Mixed-sediment Experiment <sup>a</sup>					
	50% HP		75% HP		100% HP	
	Wet	Dry	Wet	Dry	Wet	Dry
As	2.82	2.43	8.14	8.34	13.8	12.5
Cr	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
Cu	<0.003	<0.003	<0.003	0.059	<0.003	0.097
Mo <sup>b</sup>	4.91	8.03	23.5	28.4	45.8	46.1
Ni	0.042	0.116	0.118	0.300	0.186	0.402
Pb	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Se	0.027	0.062	0.060	0.124	0.086	0.159
U <sup>b</sup>	0.720	2.41	1.31	3.99	1.84	4.49
V	0.011	0.021	0.022	0.042	0.031	0.054

<sup>a</sup>Mixed-sediment treatments presented as a percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP) sediment added to the reference sediment from David Lake (DVD).

<sup>b</sup>Some matrix spike quality assurance samples did not meet general acceptability criteria (within ± 20% of nominal value).

Table 4.5. Metal concentrations in hydrochloric acid (HCl) extractions performed on wet and dry sediment from the mixed-sediment experiment ( $\mu\text{g}$  metal/g dry sediment;  $n = 1$ ).

Metal	Control				Mixed-sediment Experiment <sup>a</sup>	
	Sand		Sediment (DVD)		25 % HP	
	Wet	Dry	Wet	Dry	Wet	Dry
As <sup>c</sup>	0.299	0.449	1.42	1.48	6.76	7.34
Cr	<0.024	<0.024	<0.024	<0.024	<0.024	<0.024
Cu	0.078	0.083	0.245	0.266	0.364	0.990
Mo <sup>c</sup>	0.002	<0.001	0.006	0.010	10.9	30.0
Ni	0.025	0.030	0.120	0.113	2.16	2.09
Pb	0.141	0.139	1.22	1.00	1.50	1.17
Se	<0.0013	<0.0013	<0.0013	<0.0013	<0.0013	<0.0013
U <sup>c</sup>	0.060	0.062	0.191	0.169	213	172
V	NQ	NQ	NQ	NQ	NQ	NQ

Metal	Mixed-sediment Experiment <sup>a</sup>					
	50% HP		75% HP <sup>b</sup>		100 % HP	
	Wet	Dry	Wet	Dry	Wet	Dry
As <sup>c</sup>	6.34	11.2	2.25	26.8	1.58	37.3
Cr	<0.024	<0.024	0.539	0.684	0.946	1.09
Cu	0.200	1.42	<0.0013	3.93	<0.0013	5.55
Mo <sup>c</sup>	11.2	46.8	10.4	121	5.79	164
Ni	2.27	3.15	6.39	11.1	11.9	15.1
Pb	1.04	1.14	1.58	2.27	1.23	2.09
Se	<0.0013	<0.0013	<0.0013	0.048	<0.0013	0.062
U <sup>c</sup>	232	286	419	972	804	1710
V	NQ	NQ	NQ	NQ	NQ	NQ

NQ = not quantifiable due to matrix interference.

<sup>a</sup> Mixed-sediment treatments presented as percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP) sediment added to the reference sediment from David Lake (DVD).

<sup>b</sup> Reanalyzed run due to instrumental problems during initial analysis.

<sup>c</sup> Some matrix spike quality assurance samples did not meet general acceptability criteria (within  $\pm 20\%$  of nominal value).

Table 4.6. Mean metal concentrations ( $\pm$  standard deviation;  $\mu\text{g/g}$  dry wt) in *Chironomus dilutus* larvae on initiation of the experiment (Day 0) and after 10 days of exposure to control, reference, and mixed-sediment treatments ( $n = 3$ ) and 24 h of gut-purging.

Metal	Mixed-sediment Treatments			
	25% HP	50% HP	75% HP	100% HP
As	2.24 $\pm$ 0.71 <sup>b</sup>	3.28 $\pm$ 1.18 <sup>b</sup>	4.16 $\pm$ 0.74 <sup>b</sup>	4.95 $\pm$ 0.17 <sup>b</sup>
Cr	0.85 $\pm$ 0.20	0.74 $\pm$ 0.30	0.87 $\pm$ 0.12	1.50 $\pm$ 0.82
Cu	13.92 $\pm$ 3.11	8.83 $\pm$ 3.49 <sup>c</sup>	8.94 $\pm$ 2.49 <sup>c</sup>	9.23 $\pm$ 2.18 <sup>c</sup>
Mo	50.42 $\pm$ 11.62 <sup>b</sup>	73.52 $\pm$ 39.21 <sup>b</sup>	92.88 $\pm$ 14.50 <sup>b</sup>	114.10 $\pm$ 2.57 <sup>b</sup>
Ni	1.65 $\pm$ 0.50 <sup>c</sup>	2.24 $\pm$ 1.18 <sup>b</sup>	2.35 $\pm$ 0.34 <sup>b</sup>	3.10 $\pm$ 0.36 <sup>b</sup>
Pb	0.87 $\pm$ 0.22	0.68 $\pm$ 0.33	0.68 $\pm$ 0.03	0.67 $\pm$ 0.17
Se	3.86 $\pm$ 0.38	4.62 $\pm$ 1.82	6.02 $\pm$ 0.57	7.00 $\pm$ 0.47 <sup>b</sup>
U	37.22 $\pm$ 4.11 <sup>b</sup>	40.20 $\pm$ 20.18 <sup>b</sup>	46.04 $\pm$ 10.01 <sup>b</sup>	51.52 $\pm$ 7.54 <sup>b</sup>
V	0.74 $\pm$ 0.22	0.62 $\pm$ 0.31	0.71 $\pm$ 0.03	0.72 $\pm$ 0.05

Metal	Day 0	Control	Reference Sediment
		Sand <sup>a</sup>	100% DVD
As	<0.001	0.58 $\pm$ 0.17	0.30 $\pm$ 0.04
Cr	3.52 $\pm$ 4.88	1.54 $\pm$ 0.62	0.70 $\pm$ 0.62
Cu	26.62 $\pm$ 6.09	56.22 $\pm$ 4.82	22.32 $\pm$ 0.24
Mo	0.05 $\pm$ 0.09	0.25 $\pm$ 0.03	0.25 $\pm$ 0.05
Ni	0.72 $\pm$ 1.24	0.91 $\pm$ 0.29 <sup>c</sup>	0.62 $\pm$ 0.07
Pb	1.47 $\pm$ 0.60	3.22 $\pm$ 1.20 <sup>c</sup>	0.60 $\pm$ 0.16
Se	0.93 $\pm$ 0.06	0.71 $\pm$ 0.05	0.64 $\pm$ 0.05
U	0.23 $\pm$ 0.14	1.13 $\pm$ 0.31	0.84 $\pm$ 0.12
V	0.19 $\pm$ 0.08	0.67 $\pm$ 0.28	0.44 $\pm$ 0.05

<sup>a</sup>  $n = 5$ .

<sup>b</sup> Statistically significant different ( $P < 0.05$ ) from both of the controls.

<sup>c</sup> Statistically significant different ( $P < 0.05$ ) from the sediment control.

Mixed-sediment treatments presented as percentage (mixed on a wet weight basis) of contaminated Horseshoe Pond (HP)

sediment added to the reference sediment from David Lake (DVD).

water, and pore-water metal concentrations, with the exception of Cu and Pb. Tissue metal concentrations ranged the most for As, Mo, Ni, Se, and U (at least a factor of 10).

Tissue metal concentrations were correlated (Pearson Product Moment correlations) with whole-sediment metal concentrations ( $\mu\text{g/g}$  dry weight), pore-water concentrations ( $\text{mg/L}$ ) and metal concentrations in the  $\text{KH}_2\text{PO}_4$  and HCl extracts on a sediment dry weight basis ( $\mu\text{g/g}$  dry weight) (Table 4.7). Correlations are presented for only those metals that were elevated above background metal concentrations and that had a good gradient of concentrations in both the different sediment phases and the chironomid tissues. As such, the metals evaluated included As, Mo, Ni, and U and the correlations were based on the 25% HP, 50% HP, 75% HP, and 100% HP treatment data. Despite there sometimes being a good gradient in the tissue-Se concentrations among sediments, Se was excluded from the evaluation since it is known that dietary exposure via ingestion of biofilm and detritus is the primary mode of accumulation in benthic macroinvertebrates, not exposure to pore-water and whole-sediment (Wiramanaden et al. 2010). In order to determine which relationship best describes the bioavailability of As, Mo, Ni and U in sediment to *C. dilutus*, the two highest R-values for each metal were identified (Table 4.7).

The majority of correlations for the metals evaluated were statistically significant relationships ( $R > 0.890$ ;  $P < 0.05$ ). Peeper and centrifuged pore-water metal concentrations had the strongest correlations with tissue-metal concentrations for As, Mo, and Ni. As for U, the strongest correlations were with whole-sediment and the extract from the  $\text{KH}_2\text{PO}_4$  extraction performed on wet sediment. The strong relationship among all of the sediment phases and tissue-metal concentrations was likely due to the mixed-sediment being created by diluting a single contaminated sediment. As a result, all phases were somewhat co-correlated with one another, including the fraction most likely responsible for most of the metal availability (e.g., pore-water dissolved metal concentration).

#### **4.4.2 Field-core sediment experiment**

Overlying water chemistry was relatively constant for the duration of the exposure within treatments of the field-core sediment experiment. Overlying water alkalinity, hardness, and DOC concentration varied among treatments (alkalinity = 49 to 112  $\text{mg CaCO}_3/\text{L}$ ; total hardness

Table 4.7. Relationships (Pearson Product Moment correlation) between select metal concentrations in *Chironomus dilutus* and different sediment phases from the exposure sediments (25%, 50%, 75%, and 100% Horseshoe Pond sediment) in the mixed-sediment experiment ( $n = 4$ ).

Sediment Phase	R Values			
	As	Mo	Ni	U
Sediment (dw)	0.978	0.976	0.920 <sup>a</sup>	0.988
Centrifuged PW	0.991	0.997	0.926 <sup>a</sup>	0.946
Peeper PW	0.994	0.997	0.994	0.940 <sup>a</sup>
HCl-D (dw)	0.966	0.968	0.890 <sup>a</sup>	0.974
HCl-W (dw)	NQ	NQ	0.915 <sup>a</sup>	0.958
KP-D (dw)	0.954	0.963	0.909 <sup>a</sup>	0.980
KP-W (dw)	0.949	0.948	0.923 <sup>a</sup>	0.994

Shaded boxes highlight the two strongest relationships for each metal evaluated. Only metals that were substantially elevated in Horseshoe Pond sediment were evaluated.

PW = pore-water.

dw = dry weight.

HCl-D = Hydrochloric acid extraction on dry sediment.

HCl-W = Hydrochloric acid extraction on wet sediment.

KP-D = Potassium phosphate extraction on dry sediment.

KP-W = Potassium phosphate extraction on wet sediment.

NQ = No quantifiable relationship due to unexplainable analytical results.

<sup>a</sup> Not statistically significant;  $P > 0.05$ .

= 86 to 225 mg CaCO<sub>3</sub>/L; DOC = 5.0 to 12.3 mg/L). Ammonia concentrations differed between water changes with ammonia concentrations in new water ranging from 0.31 to 0.81 mg N/L and old water ranging from 1.57 to 3.90 mg N/L. In general, metal concentrations in overlying water increased slightly from Day 0 to Day 10 with the greatest changes noted for As, Cr, Mo, and Ni.

The field-cores evaluated had a range of whole-sediment metal concentrations and different TOC concentrations (Table 4.8). Metal concentrations varied depending on the metal, sediment type, and proximity of the sampling site to the effluent discharge point (e.g., Fox Lake was nearest to the treated effluent discharge point; Delta Lake was the furthest site downstream). Metal concentrations in whole-sediment from Parks Lake (both sampling areas) were not substantially different from the reference sediment. A good gradient of whole-sediment metal concentrations existed among Delta Lake, Unknown Lake, Fox Lake, and Horseshoe Pond sediments. Metals elevated above background concentrations in these four sediments were As, Ni, Mo, and U. A comparison between field sediment cores processed soon after sampling (referred to in the Appendix) and the field sediment cores used in the bioaccumulation experiment described here indicated that despite the length of time the cores were stored (~15 months), whole-sediment metal concentrations in the cores did not change substantially.

Centrifuged pore-water total hardness, alkalinity, pH, and DOC (Table 4.9) varied among sediment field-cores from the various lakes. Similar to the overlying water results, pore-water ammonia concentrations were higher on Day 10 than Day 0. Generally, pore-water collected using centrifugation yielded similar dissolved metal concentrations between Day 0 and Day 10 (Table 4.10) with the exception of Mo, which increased in concentration over the course of the experiment. Metal concentrations in pore-water from peepers (Table 4.11) were slightly lower than those concentrations in pore-water isolated via sediment centrifugation for most metals. The metals that were elevated above background in pore-water were As, Mo, Ni, and U. Overall, pore-water metal concentrations remained relatively stable throughout the experiment, the two methods of pore-water isolation were generally comparable, and As, Mo, Ni, and U appear to be the metals of greatest concern in the pore-water of the sediments evaluated.

Metal concentrations in the KH<sub>2</sub>PO<sub>4</sub> and HCl extracts from the field-core sediments are presented on a sediment dry weight basis in Table 4.12 and Table 4.13. In the KH<sub>2</sub>PO<sub>4</sub> extracts, As, Mo, Ni, Se, and U were the metals having the largest range in concentrations among the

Table 4.8. Mean whole-sediment metal concentrations and physical sediment characteristics ( $\pm$  standard deviation) from the field-core experiment ( $n = 2$ ; Day 0 and Day 10).

Variable	Control	Key Lake Field-Cores			
	Sand	David Lake	Delta Lake	Unknown Lake	Fox Lake
As	0.63 $\pm$ 0.02	1.41 $\pm$ 0.07	10.77 $\pm$ 6.51 <sup>a</sup>	197.19 $\pm$ 46.38 <sup>a</sup>	31.81 $\pm$ 4.12 <sup>a</sup>
Cr	0.33 $\pm$ 0.36	2.41 $\pm$ 0.21	5.58 $\pm$ 1.62	18.52 $\pm$ 0.48	4.62 $\pm$ 0.01
Cu	3.15 $\pm$ 4.42	1.39 $\pm$ 0.25	2.56 $\pm$ 0.31	5.67 $\pm$ 2.29	1.54 $\pm$ 0.21
Mo	<0.001	0.05 $\pm$ 0.01	453.2 $\pm$ 265.79 <sup>a</sup>	597.19 $\pm$ 115.78 <sup>a</sup>	153.09 $\pm$ 34.81 <sup>a</sup>
Ni	<0.003	0.90 $\pm$ 0.13	12.85 $\pm$ 8.98	41.83 $\pm$ 13.87	3.82 $\pm$ 0.45
Pb	3.05 $\pm$ 0.39	2.13 $\pm$ 0.48	4.99 $\pm$ 0.93	7.25 $\pm$ 0.83	4.01 $\pm$ 0.24
Se	<0.005	<0.005	3.52 $\pm$ 2.77 <sup>a</sup>	19.56 $\pm$ 6.96 <sup>a</sup>	3.18 $\pm$ 0.50 <sup>a</sup>
U	18.64 $\pm$ 3.41	<0.008	19.61 $\pm$ 4.96	26.85 $\pm$ 3.06	16.50 $\pm$ 1.58
V	0.36 $\pm$ 0.01	2.72 $\pm$ 0.07	6.84 $\pm$ 2.96	18.50 $\pm$ 1.75	7.78 $\pm$ 3.69
Moisture <sup>b</sup>	21.0 $\pm$ 1.4	68.8 $\pm$ 1.7	75.6 $\pm$ 12.4	91.9 $\pm$ 1.5	52.3 $\pm$ 14.2
TOC	0.03 $\pm$ 0.01	3.1 $\pm$ 0.7	3.7 $\pm$ 2.3	19.9 $\pm$ 0.4	0.9 $\pm$ 0.5

Variable	Rabbit Lake Field-Cores			
	Raven Lake	Park Lake Outflow	Park Lake Seepage	Horseshoe Pond
As	2.60 $\pm$ 0.67	2.80 $\pm$ 0.05	2.54 $\pm$ 0.04	81.66 $\pm$ 31.46 <sup>a</sup>
Cr	12.27 $\pm$ 1.13	17.19 $\pm$ 0.22	17.11 $\pm$ 2.31	19.78 $\pm$ 0.20
Cu	6.52 $\pm$ 4.10	8.92 $\pm$ 0.01	3.82 $\pm$ 0.22	31.71 $\pm$ 7.81 <sup>a</sup>
Mo	1.32 $\pm$ 0.95	0.91 $\pm$ 0.11	1.00 $\pm$ 0.10	1857.73 $\pm$ 518.25 <sup>a</sup>
Ni	6.34 $\pm$ 0.05	14.74 $\pm$ 1.04	8.04 $\pm$ 0.41	57.26 $\pm$ 6.37 <sup>a</sup>
Pb	8.99 $\pm$ 0.08	13.55 $\pm$ 0.53	14.36 $\pm$ 0.20	5.46 $\pm$ 0.33
Se	<0.005	<0.005	<0.005	18.05 $\pm$ 6.07 <sup>a</sup>
U	69.90 $\pm$ 30.20	21.00 $\pm$ 1.42	20.61 $\pm$ 0.36	1335.25 $\pm$ 412.52 <sup>a</sup>
V	31.00 $\pm$ 10.49	19.37 $\pm$ 0.27	19.34 $\pm$ 0.08	16.87 $\pm$ 0.80
Moisture <sup>b</sup>	78.1 $\pm$ 10.1	71.8 $\pm$ 3.0	60.7 $\pm$ 6.6	89.6 $\pm$ 2.5
TOC	3.7 $\pm$ 2.0	4.2 $\pm$ 0.3	1.1 $\pm$ 0.02	13.1 $\pm$ 1.0

TOC = Total organic carbon.

All metal concentrations in  $\mu\text{g/g}$  dry weight.

Moisture and TOC in %.

<sup>a</sup> Exceeds Screening-Level Concentration approach sediment quality guidelines (Thompson et al. 2005; Appendix).

<sup>b</sup>  $n = 3$ .

Table 4.9. Mean centrifuged pore-water quality variables ( $\pm$  standard deviation;  $n = 2$ ) from the field-core experiment. Sediment field-cores collected near the Key Lake and Rabbit Lake uranium operations.

Water Quality Variable	Control	Key Lake Field-Cores			
	Sand	David Lake	Delta Lake	Unknown Lake	Fox Lake
Ammonia (mg N/L) Day 0 <sup>a</sup>	NA	0.20	0.10	1.02	0.48
Ammonia (mg N/L) Day 10 <sup>a</sup>	NA	4.82	5.79	5.72	4.26
pH	7.1 <sup>a</sup>	4.4 $\pm$ 0.8	5.2 $\pm$ 1.0	5.7 $\pm$ 0.2	5.7 $\pm$ 1.6
Hardness (mg CaCO <sub>3</sub> /L)	NA	37 $\pm$ 16	202 $\pm$ 99	550 <sup>a</sup>	NA
Alkalinity (mg CaCO <sub>3</sub> /L)	NA	1132 <sup>a</sup>	3 $\pm$ 4	0 <sup>a</sup>	NA
DOC (mg/L)	NA	7.9 $\pm$ 2.3	9.6 $\pm$ 3.7	15.4 $\pm$ 2.2	15.1 $\pm$ 2.8
Water Quality Variable	Rabbit Lake Field-Cores				
	Raven Lake	Park Lake Outflow	Park Lake Seepage	Horseshoe Pond	
Ammonia (mg N/L) Day 0 <sup>a</sup>	0.98	0.05	0.08	0.25	
Ammonia (mg N/L) Day 10 <sup>a</sup>	6.56	4.88	2.31	6.70	
pH	5.9 $\pm$ 0.5	5.0 $\pm$ 0.0	6.3 $\pm$ 0.4	6.8 $\pm$ 0.0	
Hardness (mg CaCO <sub>3</sub> /L)	30 <sup>a</sup>	143 $\pm$ 78	112 $\pm$ 11	480 <sup>a</sup>	
Alkalinity (mg CaCO <sub>3</sub> /L)	34 <sup>a</sup>	38 $\pm$ 3	48 $\pm$ 6	NA	
DOC (mg/L)	16.7 $\pm$ 1.5	9.8 $\pm$ 2.9	11.3 $\pm$ 1.0	15.5 $\pm$ 7.3	

DOC = Dissolved organic carbon .

NA = Not measured because sample volume was too small.

Means are an average of Day 0 and 10 data.

<sup>a</sup>  $n = 1$ .

Table 4.10. Mean centrifuged pore-water metal concentrations ( $\pm$  standard deviation;  $\mu\text{g/L}$ ) from the field-core experiment ( $n = 2$ ).

Metal	Control	Key Lake Field-Cores			
	Sand	David Lake	Delta Lake	Unknown Lake	Fox Lake
As	2.90 $\pm$ 0.14	1.41 $\pm$ 0.17	8.61 $\pm$ 0.02	138.26 $\pm$ 29.72 <sup>a</sup>	66.36 $\pm$ 53.69 <sup>a</sup>
Cr	3.18 $\pm$ 0.06	3.88 $\pm$ 0.71	4.45 $\pm$ 2.69	4.12 $\pm$ 1.23	4.32 $\pm$ 1.34
Cu	5.16 $\pm$ 3.33 <sup>a</sup>	0.89 $\pm$ 0.75	0.18 $\pm$ 0.10	0.20 $\pm$ 0.13	1.19 $\pm$ 0.55
Mo	2.46 $\pm$ 0.16	<1.9	813.33 $\pm$ 878.25 <sup>a</sup>	663.41 $\pm$ 837.76 <sup>a</sup>	2804.76 $\pm$ 1490.97 <sup>a</sup>
Ni	3.31 $\pm$ 2.28	<3.4	6.02 $\pm$ 2.37	10.69 $\pm$ 6.87	61.63 $\pm$ 75.92 <sup>a</sup>
Pb	0.02 $\pm$ 0.03	1.14 $\pm$ 1.61 <sup>a</sup>	<0.002	<0.002	0.01 $\pm$ 0.02
Se	<0.76	<0.76	<0.76	0.78 $\pm$ 0.00	4.30 $\pm$ 1.07 <sup>a</sup>
U	2.71 $\pm$ 0.78	0.10 $\pm$ 0.00	0.18 $\pm$ 0.02	0.63 $\pm$ 0.02	1.42 $\pm$ 0.02
V	4.41 $\pm$ 2.12	0.78 $\pm$ 0.14	1.20 $\pm$ 0.06	1.88 $\pm$ 0.50	6.95 $\pm$ 6.36

Metal	Rabbit Lake Field-Cores			
	Raven Lake	Park Lake Outflow	Park Lake Seepage	Horseshoe Pond
As	1.73 $\pm$ 0.08	2.86 $\pm$ 0.82	2.50 $\pm$ 1.36	52.72 $\pm$ 9.87 <sup>a</sup>
Cr	3.00 $\pm$ 0.36	4.10 $\pm$ 1.71	7.34 $\pm$ 6.86	3.57 $\pm$ 0.10
Cu	1.44 $\pm$ 0.79	1.97 $\pm$ 1.36	1.02 $\pm$ 0.26	0.67 $\pm$ 0.15
Mo	2.82 $\pm$ 2.64	<1.9	<1.9	3338.44 $\pm$ 1036.48 <sup>a</sup>
Ni	<3.4	3.64 $\pm$ 0.21	<3.4	10.26 $\pm$ 3.43
Pb	<0.002	0.24 $\pm$ 0.32	0.17 $\pm$ 0.02	<0.002
Se	<0.76	<0.76	<0.76	0.91 $\pm$ 0.05
U	4.21 $\pm$ 2.22	0.42 $\pm$ 0.28	0.44 $\pm$ 0.15	412.91 $\pm$ 454.44 <sup>a</sup>
V	5.20 $\pm$ 2.95	1.38 $\pm$ 0.59	1.71 $\pm$ 0.39	2.03 $\pm$ 0.29

<sup>a</sup> Exceeds water quality guidelines outlined in the Appendix (CCME 2011).

Table 4.11. Mean peeper pore-water metal concentrations ( $\pm$  standard deviation;  $\mu\text{g/L}$ ) in sediment pore-water samples (collected using peepers) from the field-core experiment ( $n = 3$ ).

Metal	Control	Key Lake Field-Cores			
	Sand	David Lake	Delta Lake	Unknown Lake	Fox Lake
As	1.53 $\pm$ 1.61	0.94 $\pm$ 0.59	8.24 $\pm$ 4.93	86.75 $\pm$ 24.77 <sup>a</sup>	86.19 $\pm$ 65.90 <sup>a</sup>
Cr	6.20 $\pm$ 3.25	9.03 $\pm$ 8.85 <sup>a</sup>	4.56 $\pm$ 1.40	6.19 $\pm$ 3.30	9.28 $\pm$ 3.27 <sup>a</sup>
Cu	8.44 $\pm$ 0.15 <sup>a</sup>	1.97 $\pm$ 2.10	11.77 $\pm$ 19.18 <sup>a</sup>	0.65 $\pm$ 0.24	3.15 $\pm$ 0.38 <sup>a</sup>
Mo	3.77 $\pm$ 1.55	<2.4	4401.6 $\pm$ 5590.6 <sup>a</sup>	684.60 $\pm$ 127.87	6532.1 $\pm$ 672.56 <sup>a</sup>
Ni	5.93 $\pm$ 0.60	5.69 $\pm$ 6.47	7.75 $\pm$ 3.94	5.38 $\pm$ 1.16	10.99 $\pm$ 2.09
Pb	0.32 $\pm$ 0.05	2.21 $\pm$ 3.09 <sup>a</sup>	1.31 $\pm$ 1.49 <sup>a</sup>	0.27 $\pm$ 0.02	0.56 $\pm$ 0.16
Se	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>	7.71 $\pm$ 2.56 <sup>a</sup>
U	1.52 $\pm$ 0.24	<0.006	0.23 $\pm$ 0.16	0.77 $\pm$ 0.24	4.22 $\pm$ 3.34
V	2.79 $\pm$ 0.43	1.09 $\pm$ 0.16	1.18 $\pm$ 0.25	1.58 $\pm$ 0.51	2.89 $\pm$ 1.68

Metal	Rabbit Lake Field-Cores			
	Raven Lake	Park Lake Outflow	Park Lake Seepage	Horseshoe Pond
As	<1.2	<1.2	<1.2	25.08 $\pm$ 16.74 <sup>a</sup>
Cr	4.31 $\pm$ 0.65	3.25 $\pm$ 0.28	5.51 $\pm$ 0.80	7.29 $\pm$ 1.79
Cu	1.66 $\pm$ 0.65	1.20 $\pm$ 0.19	1.79 $\pm$ 0.39	0.85 $\pm$ 0.09
Mo	6.11 $\pm$ 0.82	<2.4	<2.4	3320.6 $\pm$ 1684.7 <sup>a</sup>
Ni	<3.9	2.63 $\pm$ 1.17	4.37 $\pm$ 2.14	6.33 $\pm$ 1.62
Pb	0.37 $\pm$ 0.04	0.35 $\pm$ 0.11	0.48 $\pm$ 0.10	0.32 $\pm$ 0.00
Se	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>	<1.5 <sup>b</sup>
U	6.73 $\pm$ 3.84	0.18 $\pm$ 0.11	0.25 $\pm$ 0.15	278.72 $\pm$ 280.30 <sup>a</sup>
V	4.62 $\pm$ 0.73	1.30 $\pm$ 0.38	0.93 $\pm$ 0.27	1.79 $\pm$ 0.14

<sup>a</sup> Exceeds water quality guidelines outlined in the Appendix (CCME 2011).

<sup>b</sup> The method detection limit exceeds the water quality guideline (CCME 2011).

Table 4.12. Metal concentrations in potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) extractions performed on wet and dry sediment from the field-core experiment ( $\mu\text{g metal/g dry sediment}$ ;  $n = 1$ ).

Metal	Key Lake Sediment							
	David Lake		Delta Lake		Unknown Lake		Fox Lake	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
As	0.045	0.058	6.83	4.02	81.9	50.7	8.40	6.00
Cr	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
Cu	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Mo <sup>a</sup>	0.140	0.253	31.2	27.0	19.5	16.5	8.62	6.39
Ni	<0.001	<0.001	0.172	0.535	0.378	0.621	0.129	0.138
Pb	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Se	<0.001	<0.001	0.039	0.133	0.151	0.355	0.261	0.274
U <sup>a</sup>	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	0.037	0.086	0.087
V	0.006	0.011	0.016	0.039	0.048	0.055	0.052	0.037

Metal	Rabbit Lake Sediment							
	Raven Lake		Parks Lake Outflow		Parks Lake Seepage		Horseshoe Pond	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
As	<0.004	<0.004	0.115	0.123	0.045	0.061	32.1	21.5
Cr	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.086
Cu	<0.003	0.032	<0.003	0.036	0.069	0.105	<0.003	0.209
Mo <sup>a</sup>	<0.013	0.256	0.184	0.267	0.264	0.310	107	101
Ni	0.012	0.019	0.050	0.094	0.087	0.116	0.416	0.961
Pb	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Se	<0.001	0.012	<0.001	0.015	0.014	0.019	0.165	0.250
U <sup>a</sup>	0.494	1.114	<0.0009	0.031	<0.0009	0.011	3.59	7.41
V	0.101	0.147	0.029	0.047	0.047	0.045	0.050	0.088

Metal	Control Sand	
	Wet	Dry
	As	<0.004
Cr	0.028	<0.004
Cu	<0.003	0.006
Mo <sup>a</sup>	0.027	0.023
Ni	0.006	0.006
Pb	<0.0002	<0.0002
Se	<0.001	<0.001
U <sup>a</sup>	<0.0009	<0.0009
V	0.003	0.005

<sup>a</sup> Some matrix spike quality assurance samples did not meet general acceptability criteria (within  $\pm 20\%$  of nominal value).

Table 4.13. Metal concentrations in hydrochloric acid (HCl) extractions performed on wet and dry sediment from the field-core experiment ( $\mu\text{g}$  metal/g dry sediment;  $n = 1$ ).

Metal	Key Lake Sediment							
	David Lake		Delta Lake		Unknown Lake		Fox Lake	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
As <sup>a</sup>	2.36	2.01	5.76	20.4	17.5		25.3	25.9
Cr	<0.024	<0.024	0.415	0.626	<0.024		0.332	0.374
Cu	0.337	0.289	<0.001	0.858	<0.001		0.533	0.559
Mo <sup>a</sup>	0.010	0.012	9.82	225	3.38		77.6	77.5
Ni	0.187	0.150	9.39	12.9	21.9	NA	2.46	2.44
Pb	0.758	0.554	1.55	1.71	2.25		0.413	0.456
Se	<0.001	<0.001	<0.001	<0.001	<0.001		0.132	0.162
U <sup>a</sup>	0.253	0.202	2.86	9.69	8.75		4.26	4.16
V	NQ	NQ	NQ	NQ	NQ		NQ	NQ

Metal	Rabbit Lake Sediment							
	Raven Lake		Parks Lake Outflow		Parks Lake Seepage		Horseshoe Pond	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
As <sup>a</sup>	1.97	2.31	4.23	4.96	2.52	2.55	2.16	70.3
Cr	0.225	<0.024	0.397	0.600	0.42	0.615	1.29	1.74
Cu	2.91	2.79	3.29	4.28	1.68	1.57	<0.001	7.67
Mo <sup>a</sup>	0.033	0.056	0.042	0.065	0.064	0.092	15.2	283
Ni	0.509	0.555	3.22	4.21	1.11	1.16	19.8	33.8
Pb	0.670	0.600	1.86	1.88	1.44	1.40	1.19	1.95
Se	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.023	0.117
U <sup>a</sup>	78.6	65.4	5.15	6.10	2.97	3.22	1347	3802
V	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

Metal	Control Sand	
	Wet	Dry
	As <sup>a</sup>	0.371
Cr	<0.024	<0.024
Cu	0.048	0.063
Mo <sup>a</sup>	0.001	0.003
Ni	0.029	0.035
Pb	0.099	0.122
Se	<0.001	-0.003
U <sup>a</sup>	0.060	0.064
V	NQ	NQ

NA = Not available.

NQ = Not quantifiable.

<sup>a</sup> Some matrix spike quality assurance samples did not meet general acceptability criteria (within  $\pm 20\%$  of nominal value).

field-collected sediments. In the HCl extracts, As, Mo, Ni, and U had the largest range in concentrations. The other metals analyzed in each of the chemical extractions evaluated were either not quantifiable or had concentrations very similar to the metal concentrations measured in the reference sediment extracts. Metal concentrations were usually marginally higher in extractions performed on dry sediment compared to wet sediment, across all metals and both chemical extractants evaluated. Metal concentrations in HCl extracts were generally higher than those found in the  $\text{KH}_2\text{PO}_4$  extracts. Similar to the mixed-sediment experiment, some analytical problems were encountered in the metals analyses of extract samples (discussed in Section 4.3.4) and, as such, the metals data are intended to evaluate general trends and correlations rather than absolute metals concentrations.

*Chironomus dilutus* survival was only significantly reduced in the Parks Lake Seepage sediment, compared to the Raven Lake reference treatment ( $P < 0.50$ ; ANOVA with Dunnett's post-hoc). Final weight of *C. dilutus* larvae, measured as dry weight, showed no statistical difference among any of the sediment treatments ( $P = 0.16$ ; ANOVA on Ranks across all treatments). Tissue metal concentrations (Table 4.14) were generally highest in larvae exposed to Fox Lake and Horseshoe Pond sediment. Tissue metal concentrations from both Parks Lake sediments were similar to the tissue concentrations from the reference sediment. For other sediments, tissue concentrations generally increased with whole-sediment metal concentrations. The metals that showed the greatest degree of accumulation (a factor of at least 10) were As, Mo, Ni, Se, and U.

The relationships between larval tissue metal concentrations and metal concentrations measured in the sediment phases evaluated (whole-sediment, pore-water, and chemical extractions) are described using correlations (Pearson Product Moment Correlations; R-values) and are listed in Table 4.15. Similar to the mixed-sediment experiment, As, Mo, Ni, and U, had the greatest range in metal concentrations in the various sediments and phases tested and were the metals most often present in the sediment phases at concentrations above those of the reference sediments. As such, only correlations for As, Mo, Ni, and U are presented. Data from Delta Lake, Unknown Lake, Fox Lake and Horseshoe Pond treatments were chosen for inclusion in the correlations analysis because these sediments have trace metals concentrations that cover a broad range of contamination; however, U concentrations in the various sediment phases of

Table 4.14. Mean metal concentrations ( $\pm$  standard deviation;  $\mu\text{g/g}$  dry wt) in *Chironomus dilutus* larvae on initiation of the experiment (Day 0) and after 10 days of exposure to a sand control and sediments collected near the Key Lake and Rabbit Lake uranium operations ( $n = 3$ ; not gut-purged).

Metals	Control - Day 0 <sup>a</sup>	Key Lake Field-Cores - Day 10			
		David Lake <sup>b</sup>	Delta Lake <sup>b</sup>	Unknown Lake <sup>c</sup>	Fox Lake <sup>c</sup>
As	0.01 $\pm$ 0.02	1.02 $\pm$ 0.04	13.21 $\pm$ 7.45	50.27 $\pm$ 8.13	190.63 $\pm$ 55.32 <sup>‡</sup>
Cr	0.63 $\pm$ 1.24	1.02 $\pm$ 0.07	2.15 $\pm$ 0.08 <sup>*†</sup>	2.24 $\pm$ 0.50 <sup>*†</sup>	9.73 $\pm$ 0.91 <sup>*†</sup>
Cu	71.86 $\pm$ 11.02	16.60 $\pm$ 0.67 <sup>*</sup>	12.39 $\pm$ 3.10 <sup>*</sup>	17.05 $\pm$ 2.62 <sup>*</sup>	18.18 $\pm$ 2.20 <sup>*</sup>
Mo	<0.001	0.84 $\pm$ 0.87	919.72 $\pm$ 575.83 <sup>*†</sup>	133.82 $\pm$ 20.90 <sup>*†</sup>	954.92 $\pm$ 469.77 <sup>*†</sup>
Ni	0.85 $\pm$ 0.79	0.99 $\pm$ 0.10	9.98 $\pm$ 1.48 <sup>*†</sup>	6.75 $\pm$ 1.64 <sup>*†</sup>	4.79 $\pm$ 1.05 <sup>*†</sup>
Pb	2.21 $\pm$ 0.26	0.98 $\pm$ 0.03 <sup>‡</sup>	1.22 $\pm$ 0.11	1.32 $\pm$ 0.48	1.49 $\pm$ 0.21
Se	1.13 $\pm$ 0.23	0.59 $\pm$ 0.03	9.01 $\pm$ 2.45 <sup>*†</sup>	11.51 $\pm$ 2.98 <sup>*†</sup>	44.77 $\pm$ 4.77 <sup>*†</sup>
U	0.46 $\pm$ 0.11	0.44 $\pm$ 0.04 <sup>*</sup>	2.62 $\pm$ 0.15 <sup>*†</sup>	4.62 $\pm$ 1.07 <sup>*†</sup>	13.14 $\pm$ 0.93 <sup>*†</sup>
V	0.23 $\pm$ 0.05	1.96 $\pm$ 0.33 <sup>*</sup>	2.63 $\pm$ 0.41 <sup>*</sup>	2.95 $\pm$ 0.55 <sup>*</sup>	12.45 $\pm$ 2.14 <sup>*†</sup>

Metals	Control - Day 10		Rabbit Lake Field-Cores - Day 10		
	Sand <sup>c</sup>	Raven Lake <sup>c</sup>	Park Lake Outflow <sup>b</sup>	Park Lake Seepage <sup>c</sup>	Horseshoe Pond <sup>b</sup>
As	0.41 $\pm$ 0.11	0.90 $\pm$ 0.03 <sup>*</sup>	0.91 $\pm$ 0.14 <sup>*</sup>	0.80 $\pm$ 0.18 <sup>*</sup>	28.95 $\pm$ 11.03 <sup>*†</sup>
Cr	1.36 $\pm$ 0.34	3.61 $\pm$ 2.30 <sup>*</sup>	2.88 $\pm$ 0.63	2.45 $\pm$ 0.44	3.09 $\pm$ 0.83 <sup>*</sup>
Cu	50.14 $\pm$ 13.25	20.72 $\pm$ 2.08 <sup>*</sup>	19.48 $\pm$ 1.95 <sup>*</sup>	19.93 $\pm$ 3.48 <sup>*</sup>	21.51 $\pm$ 1.85 <sup>*</sup>
Mo	0.36 $\pm$ 0.44	0.92 $\pm$ 0.15	0.65 $\pm$ 0.90	0.57 $\pm$ 0.13	616.76 $\pm$ 229.81 <sup>‡</sup>
Ni	1.14 $\pm$ 0.75	1.93 $\pm$ 0.24	4.41 $\pm$ 0.78 <sup>*</sup>	3.22 $\pm$ 1.08 <sup>*</sup>	12.88 $\pm$ 4.00 <sup>*†</sup>
Pb	2.92 $\pm$ 1.23	1.21 $\pm$ 0.41 <sup>*</sup>	1.26 $\pm$ 0.26 <sup>*</sup>	1.17 $\pm$ 0.31 <sup>*</sup>	0.55 $\pm$ 0.03 <sup>*†</sup>
Se	0.72 $\pm$ 0.14	1.20 $\pm$ 0.22 <sup>*</sup>	0.92 $\pm$ 0.07	1.10 $\pm$ 0.04 <sup>*</sup>	21.35 $\pm$ 2.66 <sup>*†</sup>
U	0.77 $\pm$ 0.20	40.17 $\pm$ 5.99 <sup>*†</sup>	2.56 $\pm$ 0.36 <sup>*†</sup>	1.94 $\pm$ 0.49 <sup>*†</sup>	287.38 $\pm$ 28.29 <sup>*†</sup>
V	0.59 $\pm$ 0.15	8.73 $\pm$ 1.08 <sup>*</sup>	3.24 $\pm$ 0.48 <sup>*†</sup>	2.82 $\pm$ 0.67 <sup>*†</sup>	2.70 $\pm$ 0.66 <sup>*†</sup>

Comparisons to the Control - Day 0 tissue concentrations were not made and are listed for qualitative comparisons only.

<sup>a</sup>  $n = 5$ .

<sup>b</sup>  $n = 3$ .

<sup>c</sup>  $n = 4$ .

\* = Statistically significant difference between the Sand (Day 10) treatment ( $P < 0.05$ ; One-way ANOVA; Tukey post-hoc).

† = Statistically significant difference between the reference sediment (Raven Lake for Rabbit Lake and David Lake for Key Lake treatments ( $P < 0.05$ ; One-way ANOVA; Tukey post-hoc).

‡ = Statistically significant difference between the Sand (Day 10) treatment ( $P < 0.05$ ; One-way ANOVA on Ranks; Dunn's post-hoc).

Table 4.15. Relationships (Pearson Product Moment correlation) between select metal concentrations in *Chironomus dilutus* and different sediment phases from the exposure sediments (Horseshoe Pond, Delta Lake, Unknown Lake, and Fox Lake) in the field-core experiment ( $n = 4$ ).

Sediment Phase	R Values			
	As	Mo	Ni	U <sup>cd</sup>
Sediment (dw)	-0.210	-0.257	0.641	-0.598
Centrifuged PW	0.184	0.368	-0.716	0.983
Peeper PW	0.705	0.945 <sup>a</sup>	-0.536	0.999 <sup>a</sup>
HCl-D (dw) <sup>c</sup>	-0.336	-0.778	0.939	<sup>b</sup>
HCl-W (dw)	0.866	0.596	0.491	-0.132
KP-D (dw)	-0.276	-0.073	0.878	0.967
KP-W (dw)	-0.282	-0.066	0.534	0.984

Shaded boxes highlight the two strongest relationships for each metal evaluated. Only metals that were substantially elevated among the field-core sediments were evaluated.

PW = pore-water.

dw = dry weight.

HCl-D = Hydrochloric acid extraction on dry sediment.

HCl-W = Hydrochloric acid extraction on wet sediment.

KP-D = Potassium phosphate extraction on dry sediment.

KP-W = Potassium phosphate extraction on wet sediment.

<sup>a</sup> Statistically significant;  $P < 0.05$ .

<sup>b</sup>  $n = 2$ ; no sample for Unknown Lake in addition to the exclusion of Horseshoe Pond for these relationships.

<sup>c</sup>  $n = 3$ ; no sample for Unknown Lake.

<sup>d</sup> Correlations conducted using only Delta Lake, Unknown Lake, and Fox Lake sediment .

Horseshoe Pond sediment were more than two orders of magnitude higher than the corresponding phases from other contaminated sediments. Given that this would overly influence the outcome of the analyses, Horseshoe Pond U data were excluded from the correlations.

Compared to the mixed-sediment experiment, the correlations in the field-core experiment were weaker and inconsistent. Correlation coefficients among those metals and sediment phases evaluate ranged from 0.066 (no relationship) to 0.999 (almost perfect relationship). Tissue U concentrations were the best correlated and tissue Mo concentrations were the worst correlated with the various sediment phases. The various correlations had a mix of positive slopes (increase in tissue metal concentration with increase in the sediment phase concentration) and negative slopes (decrease in tissue concentration with increase in the sediment phase concentration). Furthermore, the majority of the correlations were not statistically significant ( $P > 0.05$ ) and the only statistically significant relationships were related to peeper pore-water relationships. Taken together, the correlations of metal concentrations in tissues with sediment phases measured in field-sediment were highly variable and inconsistent across the metals and sediment phases evaluated.

The highest two correlation coefficients for each metal evaluated are highlighted in Table 4.15. The best overall relationship with tissue metal concentrations, among all metals evaluated, was the peeper pore-water sediment phase. This finding was consistent with the results from the mixed-sediment experiment. Peeper pore-water metal concentration best correlated with tissue metal concentrations for As, Mo, and U. Nickel concentrations in chironomid tissues best correlated with the metal concentrations in dry sediment HCl extracts. Overall, there is no evidence to conclude that the two chemical extractions evaluated were better correlated with chironomid tissue concentrations than currently used alternatives such as pore-water.

## **4.5 Discussion**

Total metal concentration in whole-sediment is typically used to evaluate the risk that sediment poses to the associated benthic organisms. Pore-water dissolved metals concentrations are used similarly, but are analyzed less often. In addition to these traditional analyses, this study evaluated the usefulness of single-step chemical extractions in estimating metal bioavailability in sediments. The two chemicals evaluated, HCl and  $\text{KH}_2\text{PO}_4$ , were each

intended to isolate an operationally-defined fraction of sediment metals. For instance, HCl was used to extract metals associated with Fe and Mn oxides and sulfides in sediment. Weaker  $\text{KH}_2\text{PO}_4$  extractions (expected to extract a smaller fraction of the total metals in sediment) were used to extract metals present in anionic form, such as As, Se, Mo, and V.

Although the primary intent of this study was to evaluate the relationships between metal concentrations in different sediment phases and metal accumulation in *C. dilutus*, the influence of two different sample preparation approaches (dried versus wet sediment) was also investigated. Drying is commonly performed in sediment sequential extraction procedures (Tessier et al. 1979; Rauret et al. 1999). Although drying sediment does not affect the total metal concentration, drying can change the speciation and bioavailability of the metals within sediment. For example, Zhang et al. (2001) found that drying sediment, regardless of the method, altered the chemical speciation of metals in the sediment tested and that use of a fresh wet sample best preserved the original speciation distribution of the metals. Artefacts due to drying might therefore obscure those relationships of interest between benthic organisms and sediment metals *in situ*. Among those metals evaluated here (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V), slightly more metal was extracted from dry sediment compared to wet sediment. In addition to the possible influence of sediment drying on metal speciation, another reason for this observation could be a difference in molarity of the extractant. The amount of chemical added to both wet and dry sediment was standardized on a sediment dry weight basis rather than standardizing on the molarity of the extractant after addition to the sediment sample. Therefore, the pore-water present in the wet sediment would have diluted the extractant resulting in a lower molarity. It is possible that if molarity had been standardized, more metals would have been extracted from the wet sediment. Regardless, these small differences did not result in a noticeable change in the relationships between sediment phase (pore-water, whole-sediment, and chemical extracts) and tissue metals concentrations.

Of the complete list of metals having CNSC SQGs (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V), only As, Ni, Mo, Se and U were evaluated in this study. These metals were found to be both elevated above background (useful in creating a gradient in the mixed-sediment experiment) and present at a range of concentrations among the various field-collected cores (those used in field-core experiment). These metals were also previously identified (Chapter 3) as having a concentration gradient in sediments downstream of uranium operations in northern

Saskatchewan. The concentrations of the remaining metals were similar to those of the reference sediments or below limits of quantification and as such not useful in this study. However, Se was excluded from the evaluation since it is known that dietary exposure via ingestion of biofilm and detritus is the primary mode of accumulation in benthic macroinvertebrates, not exposure to pore-water and whole-sediment (Wiramanaden et al. 2010). In addition, Se gradients in some sediment phases were not as great as for other metals.

Although the concentrations of most metals in each of the sediment phases remained relatively constant over the duration of the exposure period (Day 0 versus Day 10 data), the overlying water metal concentrations increased between Day 0 and Day 10. This was likely due to the dissociation of metal from sediment particles into the aqueous phase and diffusion of pore-water metals into the overlying water. This phenomenon is common in laboratory sediment experiments (Van Geest et al. 2010).

#### **4.5.1 Mixed-sediment experiment**

Despite metal concentrations in all sediment phases having strong relationships with metal concentrations in chironomid tissue, metal concentration in pore-water had the best correlations overall when all four metals (As, Mo, Ni and U) were considered. However, it should be noted that the relationships between HCl-extract metal concentrations and tissue metal concentration were high, with pore-water metal concentrations being only slightly better at estimating tissue metal concentration for As, Mo, and Ni.

Although metal concentration in pore-water had the best overall correlation with tissue metal concentration, all sediment phases investigated were strongly correlated with tissue metal in the mixed-sediment experiment. Based on this, all of the measures evaluated (pore-water, whole-sediment or chemical-extraction metals concentrations) are similarly predictive of metal bioaccumulation in *C. dilutus*. Never the less, the strong positive correlations in this experiment are likely related to the nature of the sediment exposure gradient, which was created by diluting a single contaminated sediment with a reference sediment. All sediment characteristics and metal concentrations therefore varied similarly among treatments, and this likely resulted in co-correlation of the metal concentrations in tissue with the metals concentration in all of the sediment phases investigated. This ultimately limits the usefulness of these results in assessing the various approaches used to evaluate sediment metal availability.

#### 4.5.2 Field-core sediment exposure

Contrary to the mixed-sediment experiment, the relationships between tissue metal concentrations and the metal concentrations in the sediment phases investigated were weaker and inconsistent in the field-core experiment (Table 4.15). Of the sediment phases evaluated (pore-water, whole-sediment, chemical extractions of wet and dry sediment), peeper pore-water metal concentrations were again most strongly correlated with *C. dilutus* tissue concentrations for the four metals evaluated (As, Mo, Ni and U).

Although chemical extractants (HCl or  $\text{KH}_2\text{PO}_4$ ) were chosen to target the fraction of the total sediment metal that is most likely available to benthic organisms (either directly or indirectly), there was no clear and consistent relationship between metal concentration in the extractant and tissue metal concentration for each sediment phase evaluated. Despite eliminating variability in the correlations due to field-cores that represented either very low levels (Raven Lake, Parks Lake sediments, and David Lake) or, in the case of U, very high levels (Horseshoe Pond) of metals contamination, neither of the chemical extractions appeared useful for evaluating bioavailability of the four metals to *C. dilutus*. However, some extraction combinations showed promise in the evaluation of individual metal bioavailability. For example, despite the good relationship between pore-water and tissue-metal concentrations among the majority of metals evaluated, the HCl extraction yielded the best relationship for Ni. Similarly, the U concentration in  $\text{KH}_2\text{PO}_4$  extracts (both wet and dry sediments) yielded a better relationship with tissue U concentration than did total sediment U concentration. Based on this, HCl and  $\text{KH}_2\text{PO}_4$  extractions could be useful in studies targeting Ni or U, respectively, but are not likely useful to evaluate larger suites of metals. It is unlikely that any single chemical extractant or any single sediment phase would be sufficient to evaluate the bioavailability of a complex mixture of metals in field-collected sediments.

In place of chemical extractions, pore-water dissolved metal concentrations are likely a better measure for estimating the bioavailable metal fraction in sediment across a broad range of sediment characteristics and metals. However, this measure of metal bioavailability does not directly take into account the dietary route of exposure. To evaluate the metal bioavailability related to the dietary route, a solvent that simulates uptake from the animal gut could be used to extract metals from sediment (Peng et al. 2004).

#### 4.6 Conclusions

Using field-core and mixed-sediments in metal bioaccumulation experiments, it was found that chemical extractions (HCl and  $\text{KH}_2\text{PO}_4$ ) of sediment were not superior to other methods for estimating the bioavailability of a suite of metals related to the Saskatchewan uranium industry (As, Mo, Ni and U). Pore-water dissolved-metals concentrations generally had the best relationship with midge tissue metals concentrations among those metals and sediments evaluated. Correlations for metals concentrations in tissue and sediment phases were stronger in the mixed-sediment experiment, likely due to the experimental methods used. In the field-core experiment, correlations were highly variable and inconsistent due to the variability in sediment metal concentrations and sediment characteristics. Based on the findings from this study, it is suggested that single chemical extractions (e.g., HCl and  $\text{KH}_2\text{PO}_4$ ) could be useful in focused, single-metal sediment studies, where a particular metal is targeted for an investigation, but overall, pore-water is a better choice for assessing bioavailability of metals in sediment to benthic invertebrates, such as *C. dilutus*.

## CHAPTER 5

### GENERAL DISCUSSION

#### 5.1 Project rationale and goals

The rationale for this research was the lack of scientifically defensible sediment quality guidelines (SQGs) for application at uranium operations in Saskatchewan. Using the screening-level concentration (SLC) approach, specific SQGs for metals and radionuclides relevant to the uranium mining and milling industry in Canada were previously derived by Thompson et al. (2005). The SLC approach is based on the presence and absence of benthic invertebrate taxa which are associated with individual element concentrations. This approach does not take into account that not all elements in the sediment at an effect site are the cause of an observed effect or absence of a species. The SLC approach therefore results in SQGs that are often overly conservative and difficult to defend. As a result, a new approach to derive SQGs based on total sediment metal concentrations from no-effect and reference sites was evaluated. In addition, the majority of existing SQGs are based on total element concentrations in sediment rather than bioavailable metal fractions. Therefore, we also investigated the relationship between metal concentrations in sediment phases (including whole sediment) and metals in *Chironomus dilutus* tissues exposed to contaminated sediment to identify the best correlation. Using Cameco Corporation uranium operations in northern Saskatchewan as a case study, the goals of this research were to evaluate a new approach to derive SQGs for use at uranium operations in northern Saskatchewan and to investigate alternate methods to quantifying metal bioavailability in sediment to benthic invertebrates. The specific research objectives were as follows:

- i) Conduct a literature review of existing sediment quality value (SQV) derivation approaches and, based on these findings, identify possible alternative SQV derivation methods that can make use of pre-existing data;

- ii) Using field-collected benthic invertebrate community data from reference and mildly contaminated areas, derive regionally-specific SQVs based on no observed-effect concentrations, and compare these SQVs to those derived by the Canadian Nuclear Safety Commission (CNSC) for use at Saskatchewan uranium mining and milling operations, and;
- iii) Using chemical extractions and pore-water isolation techniques, investigate the possibility of identifying a simple, scientifically-defensible approach for quantifying metal exposure in sediments that can be better linked to metal bioaccumulation in benthic invertebrates than total, whole-sediment metal concentrations.

## **5.2 Synthesis and integration of project results**

Upon review of various published methods for SQG derivation, it was noted that there are many similarities among current derivation approaches, as well as numerous common deficiencies. Utilizing sediment metal concentrations associated with benthic invertebrate community effects is standard practice for many empirical approaches. An over-arching uncertainty of such SQGs is that an appropriate threshold of no-effect has been defined; one that is protective without being overly conservative. This uncertainty can result from various factors, including the range of contaminant sediment concentrations, interactions between metals in sediment, and modifying factors in sediment (such as sediment particle size, organic carbon content, pH, and redox potential) that can alter metal availability and toxicity to benthic invertebrates. The majority of existing SQG derivation techniques lack consideration and incorporation of such factors. One exception to this is the equilibrium-partitioning approach, which incorporates metal partitioning between the solid and aqueous phases to derive SQGs. Additionally, the outcome of empirical SQG derivation processes is affected by slight differences in the percentile used to derive the SQG from the dataset (e.g., 90<sup>th</sup> or 95<sup>th</sup> percentile), the criteria used to select data (e.g., effect or no-effect sites), and the actual sets of data used to derive the SQG (addition or exclusion of data will change the derived value).

Using sediment metal concentrations and benthic invertebrate community data from uranium operations in northern Saskatchewan, it was concluded that the LELs derived by Thompson et al. (2005) more accurately predicted effect sites than no-effect sites. For example,

on a general basis, the correct alignment of at least one LEL exceedence at an effect site was observed 95% of the time, but on an element-specific basis many of the elements had concentrations at effect sites below their LELs (Chapter 2; Burnett-Seidel and Liber 2011). Observed no-effect sites were often incorrectly predicted to have effects based on exceedences of the LELs. Concentrations of the evaluated elements exceeded at least one LEL at 60% of the no-effect sites (Chapter 2; Burnett-Seidel and Liber 2011). When an effect on the benthic invertebrate community is detected it is often difficult to determine the exact cause associated with that effect. Various metals typically co-occur in sediments, many of which are not likely responsible for the observed effect. Because empirical methods attribute toxicity (effects) to all metals present, many of the SQGs derived using these approaches are often overly conservative. Such false-positives could prove costly to a company required to investigate sites having SQG exceedences.

As an alternative to the SLC approach, this project evaluated the derivation of new SQGs based on sediment metal concentrations associated with benthic invertebrate communities at reference and no-effect field sites. Using data from uranium operations in northern Saskatchewan, the no-effect level derivation approach used a variety of benthic invertebrate community metrics to define an effect (and lack thereof). Overall, it was found that suitable no-effect levels could only be derived when the dataset contained an appropriate range of sediment metal concentrations (i.e., a gradient of contamination) which ideally approached benthic invertebrate community effect threshold levels. For instance, derived NE values for Cr, Cu, Pb, and V did not change with the incorporation of additional effects criteria (evenness and the Bray-Curtis index) due to a lack of influence from the uranium operations on the concentrations of these metals (i.e., they were only present at or near background levels). However, a gradient of exposure concentrations was apparent for As, Mo, Ni, Se, and U in sediment which allowed for acceptable exposure levels of these metals in sediment to be defined. In general, NE values derived using abundance, richness, and evenness (NE1 and NE2 values) were found to be higher than the NE values derived using these three metrics plus the Bray-Curtis index (NE3 values). No-effect values for As, Mo, Ni, Se, and U were between three and 95-times higher than the SQGs presented by Thompson et al. (2005), depending on the no-effect level and metal. The findings from this assessment have suggested a set of new, alternate SQVs for use at the uranium industry in northern Saskatchewan.

A set of laboratory experiments revealed that of sediment pore-water and single-chemical extractions of whole-sediment in relation to metal concentrations in the test organism, *Chironomus dilutus*, metal concentrations in peeper pore-water generally had the best overall correlation with tissue metal concentrations. In an experiment where a contaminated sediment was diluted to different degrees with a reference sediment, no single sediment phase evaluated was substantially better correlated with tissue metal concentrations than the other phases evaluated, although pore-water had the best correlation with tissue concentrations. While there was substantially more variability in the correlations from an exposure where sediment cores were collected from several contaminated lakes, pore-water metal concentrations were again found to have the strongest correlation with tissue metal concentrations, for the subset of metals evaluated. Metal concentrations in chemical extractions did not have strong, consistent correlations with tissue metal concentrations, and are therefore not recommended for use in a broad assessment of metal bioavailability in sediment at this time. No single chemical is likely to represent the bioavailable fraction for all metals. However, chemical extractions may be useful in focused, element-specific studies where previous knowledge of sediment geochemistry can be used to guide the correct chemical for use in extractions (operationally-defined fraction). Based on the results of the two extraction experiments, it is recommended that sediment pore-water sampling be added to environmental sampling programs at Saskatchewan uranium operations so that a better correlant of sediment metal availability is available for interpretation of benthic invertebrate community effects. More focus on bioavailable fractions (pore-water and weakly adsorbed metal concentrations) rather than total metal concentrations in whole-sediment will allow for better estimation of metal bioaccumulation and toxicity, and for trophic transfer modeling in ecological risk assessment.

### **5.3 Application of findings**

Overall, this research begins to address the deficiencies inherent in many current approaches used to derive SQGs. The new approach presented here using sediment total metal concentrations associated with no-effects on the benthic invertebrate community avoids the problems associated with confounded effects data. Although this approach complements existing SQG derivation approaches, metal bioavailability should also be incorporated into future SQGs, for example, by creating a database containing pore-water metals concentrations

along with measurements of factors that modify toxicity at no-effect and effect sites (based on benthic invertebrate community metrics). Such a database would better characterize benthic invertebrate exposure to a better estimator of bioavailable metals in sediment and would aid in the development of pore-water based SQGs, rather than SQGs based on total metal concentrations in sediment.

Results presented herein demonstrate that, where appropriate data exist, regional SQGs can be derived using a no-effect approach. However, in order to derive accurate SQGs the set of data must contain a range of exposure concentrations related to a suite of potential effects. Use of data from additional uranium operations in Saskatchewan, such as the McClean Lake operation, and use of data generated since this assessment was conducted, would enhance the data set and strengthen the validity of the derived SQVs derived herein.

Effects on benthic invertebrate communities were evaluated based on endpoints used in Canadian Environmental Effects Monitoring (EEM) programs. It was assumed that the endpoints used (abundance, richness, evenness, and Bray-Curtis similarity index) and the criteria of a statistical or 20% difference from a reference site together adequately defined an adversely affected benthic invertebrate community. However, Green and Chapman (2011) recently criticized the use of indices, suggesting that simpler measurements, such as abundance and richness, provide a better description of the response of benthic invertebrate communities to pollution and that if indices must be used (e.g., regulatory requirement) that they are used alongside statistical methods that retain information about the biological dataset (e.g., multivariate approaches). As well, the use of critical effect sizes (25% difference or two standard deviations) between reference and exposure endpoints has been suggested for use in monitoring programs (Munkittrik et al. 2009) in place of a statistical difference. Thus, there are simple options for defining benthic invertebrate community effects, any of which, if adopted, would redefine the no-effect dataset used here and alter the derived SQG.

Of the three sets of NE values presented here, the NE values most applicable to the uranium industry in Saskatchewan would be the set of NE2 values for As, Mo, Ni, Se, and U. The NE1 values (based on abundance and richness) could be used, but the addition of the evenness metric in the derivation of the NE2 values provides some conservatism to the NE values and a greater link to the Canadian EEM program endpoints. The NE3 values were not seen as the most suitable for broad use because the Bray-Curtis similarity index has been shown

to be very sensitive to differences in benthic invertebrate community habitat (Faith et al. 1991; Robertson 2006) and even when habitats were quite similar between two reference sites, differences based on the Bray-Curtis index, among other indices, have been observed (CanNorth Environmental Services 2009). Based on the sensitivity of the Bray-Curtis index, along with the observation that the NE3 values were often similar to or approached the derived reference values, the NE3 values can not be recommended for use by the uranium industry in Saskatchewan. Derived NE values for Cr, Cu, Pb, and V did not change with the incorporation of additional effects criteria due to a lack of influence from the uranium operations on the concentrations of these metals in sediment. Thus, NE value for these four metals can not be recommended for use by the uranium industry.

The derived REF values provide a good indicator of the natural background metal concentrations in sediment in the region and are recommended as an additional tool for assessing whether sediments contain elevated concentrations of metals. They serve to negate LEL values for As, Mo, and Se as the REF values for these metals were greater than the LEL values. Based on this, if the NE2 values are not used, a minimum, the REF values for these three metals should be used.

The derived NE values are higher (potentially less conservative) than the SQGs derived by Thompson et al. (2005) and are more site-specific. The use of the NE values in ecological risk assessments for uranium operations in northern Saskatchewan would result in a more realistic assessment of risk of elevated metal concentrations in sediment to aquatic life, especially benthic invertebrates. Overall, it is anticipated that hazard quotients, which are primarily used as an indicator in environmental risk assessments, would be lower and more appropriate if the NE values presented herein were used in place of the SQGs derived by Thompson et al. (2005). Along with this reduction in hazard quotients could be a reduction in the potential costs associated with mitigation measures needed to reduce metal concentrations in the environment to reach a hazard quotient of less than or equal to one. Thus, there is great value in using these NE values as a supplement to or alternative to the Thompson et al. (2005) SQGs.

The NE values presented herein are not guidelines or objectives that are supported by a federal or provincial regulatory agency. However, through Environment Canada (EC), Canadian Nuclear Safety Commission (CNSC), and the Saskatchewan Ministry of Environment (SMOE), the regionally-specific NE values presented herein could be adopted as suitable objectives for the

uranium industry in Saskatchewan and for assessing potential effects of contaminated sediments on benthic invertebrate communities. In the interim, the presented NE values can be used to evaluate field sediments near uranium operations in Saskatchewan in a similar manner as how the Thompson et al. (2005) values are currently used in environmental monitoring programs and site-specific risk assessments.

#### **5.4 Areas for further research**

Overall, sediment is a very complex medium to evaluate. General knowledge of factors modifying metal bioavailability and toxicity, and of factors influencing benthic invertebrate presence or absence are necessary to evaluate the potential for adverse effects. Scientists should focus on further describing the influence of factors modifying metal bioavailability in sediment (e.g., pH, organic carbon, particle size distribution) and specific relationships so that more accurate predictions of biological effects (or lack thereof) can be made. To fill in knowledge gaps, the toxicity of less researched metals (from an ecotoxicological perspective), such as uranium, molybdenum, and arsenic, to name a few, should be investigated on multiple species and over a range of sediment types. This research would inform risk assessments and potentially result in the development of new SQGs or derivation techniques.

Along with gaining more knowledge of lesser studied elements, no-effect values for other elements in sediment could be derived and evaluated. For example, no-effect values could be derived for different radionuclides using the same approach as presented here and comparisons could be made to the radionuclide SQGs derived by Thompson et al. (2005), although the total radiation dose (cumulative effects) should also be considered. Ultimately, no-effect values could be developed for any element with sufficient data. This could fill gaps where no benchmarks or guidelines exist.

Ultimately, future SQGs should be based only on those metal fractions that are bioavailable to benthic invertebrates. Guidelines based on such measures would account for the main factors modifying metal bioavailability and toxicity in sediment (e.g., organic carbon content, particle size, mineral composition, pH, redox potential); however, the importance of these factors would likely vary among elements. Toxicity testing with relevant Canadian species followed by field validation of both individual elements and metal mixtures in sediment, over a range of conditions (e.g., pH, DOC, particle size distributions), need to be further evaluated prior

to the development of SQGs based on factors modifying metal bioavailability and toxicity. Although this approach would require much more effort, it should result in the derivation of more scientifically-defensible SQGs.

The applicability of using existing water quality guidelines to evaluate the risk associated with pore-water metal concentrations should be further investigated. The procedures for deriving water quality guidelines in Canada are standardized (CCME 2007); however, the methods for pore-water collection and analysis are not. Pore-water isolated from sediment is operationally defined and differences in pore-water collection and processing can result in differences in measured pore-water metal concentrations. It is critical, therefore, that before water quality guidelines are routinely compared to pore-water metal concentrations as a method of evaluating metal bioavailability in sediment, that the various pore-water isolation methods are critically evaluated under a range of conditions. Pore-water-specific SQGs could also be developed and potentially be related to factors that modify the toxicity of dissolved metals (e.g., hardness, dissolved organic carbon, pH).

In both natural and contaminated areas, multiple metals in sediments occur together. However, single metal guidelines are compared to metal concentrations in sediment containing a mixture of metals. There is little consideration for how metals interact with each other and this is not taken into account when SQGs are used to assess risk. As such, more research is needed on the interactions and associated biological effects of metal mixtures in sediment, and mixtures of metals and other constituents such as radionuclides or organic compounds. This is an important area of field-relevant research with limited studies conducted to date; additional research could provide valuable information for predictive modeling of the interaction of metals in sediment.

## **5.5 Summary**

Overall, this research outlines deficiencies with one current approach used to derive SQGs for use at Canadian uranium operations and provides an alternative derivation approach, along with a new set of SQVs which could be used in local risk assessments and the evaluation of contaminated sediments. Furthermore, to address an underlying issue with the majority of empirically-derived SQGs (the use of total metal concentrations in sediment as a measure of exposure), the bioavailability of metals in sediment were explored by looking at the relationship between metal accumulation in a model benthic invertebrate and potentially bioavailable

fractions of metals in sediment (pore-water isolation, chemical extractions, whole-sediment). Together, this research provides alternatives to previously suggested SQGs, derivation approaches, and the measurement of metals in sediment. Ultimately, use of these alternatives could result in more defensible assessment of field sediments at northern Saskatchewan near uranium operations.

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**APPENDIX**  
**FIELD INVESTIGATION OF BENTHIC INVERTEBRATE COMMUNITY**  
**IMPAIRMENT AT TWO SASKATCHEWAN URANIUM OPERATIONS**

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**APPENDIX**

**FIELD INVESTIGATION OF BENTHIC INVERTEBRATE COMMUNITY  
IMPAIRMENT AT TWO SASKATCHEWAN URANIUM OPERATIONS**

**A.1 Abstract**

Uranium is currently mined and milled at several operations in northern Saskatchewan Canada. These activities can pose a risk to aquatic ecosystems due to either planned effluent discharge or seepage from tailings facilities. This study investigated benthic community impairment at two Saskatchewan uranium operations, Key Lake and Rabbit Lake, and the potential for metals in overlying water, porewater, and bulk sediment to be the cause of any observed effects. The Key Lake operation discharges effluent into Wolf Lake which flows into Fox Lake, then Unknown Lake, and into Delta Lake, all of which are in the David Creek drainage system. David Lake, located upstream of the discharge point, served as a reference site. At the Rabbit Lake operation, Horseshoe Pond receives effluent via Horseshoe Creek and Unknown Pond. The reference lake, Raven Lake, was located away from the effluent discharge area. A known source of seepage from an above ground tailings management facility into Parks Lake, near the Rabbit Lake operation, had previously resulted in deviation in water chemistry from baseline values so samples were also collected near the suspected seepage point (Parks Lake Seepage) and at the lake outflow (Parks Lake Outflow).

At the Key Lake operation, the benthic community at Fox Lake, the high exposure site, was significantly affected. This was possibly due to low pH levels and elevated concentrations of ammonia, Mo and Se in overlying water, and elevated concentrations of As, Mo, and Se in sediment and porewater. Although the overlying water, porewater and sediment were elevated in some trace metals, the benthic community was only mildly affected in Unknown Lake and was unaffected in Delta Lake. At the Rabbit Lake operation, likely due to effluent discharge, there were high concentrations of As, Mo, U, and Se in Horseshoe Pond sediment and porewater, and significant effects were observed on the associated benthic community (relative to the reference site). At Parks Lake, significant benthic community differences were found between the Parks

Lake Outflow site and the reference site, but no significant differences were found between the two Parks Lake sites. Although Parks Lake sediment was elevated in Ni and Pb relative to the reference site, the concentrations of both metals were consistent with historical concentrations (collected in 1993). Overall, this assessment showed that there were differences in the benthic invertebrate communities downstream of both operations.

## **A.2 Introduction**

Uranium is currently mined and milled at multiple operations in northern Saskatchewan, Canada. Mining occurs as either underground (e.g., McArthur River) or open pit mines (e.g., Rabbit Lake). The extracted ore is milled at nearby facilities such as the Key Lake and Rabbit Lake operations. During the milling process, the ore is treated with acid to separate the uranium from the ground-up rock. The resulting solution is chemically treated to separate the uranium from other constituents and to precipitate out the uranium. The isolated uranium is then dried to form the final mining product, uranium oxide concentrate,  $U_3O_8$ , also known as yellowcake ([www.cameco.com](http://www.cameco.com)). The yellowcake is then sent to be refined (at a separate refinery) and the liquid waste produced during the milling process is treated to meet specific discharge criteria. Watersheds receiving effluent discharge are routinely monitored for chemical changes and biological effects.

Benthic communities are often used as an indicator of aquatic health. The Canadian Environmental Effects Monitoring (EEM) Program uses benthic invertebrate community density, taxa richness, an evenness index (Simpson's evenness), and the Bray-Curtis similarity index to aid in the assessment and protection of aquatic ecosystems potentially affected by metal mining (Environment Canada 2009). In Canada, regulated mines must carry out an EEM program under the Metal Mining and Effluent Regulations (MMER), which are part of the federal Fisheries Act (Environment Canada 2009). Using EEM recommended methods, many studies near the Key Lake and Rabbit Lake uranium operations have used benthic community indicators to assess effects of milling operations on aquatic habitat (Golder Associates Ltd. 2008; 2005a; 2005b; 2003; 2002; Robertson 2006).

Potential metals of concern downstream of uranium milling operations include As, Mo, Ni, Se, and U. Pyle et al. (2001; 2002) stated that As, Mo, Ni, and Se are contaminants of concern associated with uranium operations in northern Saskatchewan. Muscatello et al. (2006)

found that elevated Se levels downstream of the Key Lake operation likely caused an increased frequency in deformities and edema in northern pike fry. Robertson (2006) found that porewater total ammonia and As were potential stressors on the benthic invertebrate communities at Key Lake. Robertson (2006) also concluded that porewater total ammonia, Mn, Fe, As, and U were potential stressors at the Rabbit Lake operation. However, it was unclear whether any overlying water constituents contributed to benthic community impairment. Further investigation by Robertson (Robertson 2006; Robertson and Liber 2007) with caged *Hyaella azteca* in an *in-situ* study revealed that the observed toxicity at Key Lake was likely due to a pulse of organic mill-process chemicals (kerosene, amine, and isodecanol) in the overlying water during the time of the study, and that the toxicity at Rabbit Lake was likely due to elevated uranium concentrations in overlying water, sediment, and porewater. Thus, benthic community impairment may be due to a variety of factors in the receiving environments at each of the uranium operations.

The objective of this study was to identify benthic invertebrate communities potentially affected by uranium mining effluent or seepages at the Key Lake and Rabbit Lake operations, and relate observed effects to metal concentrations in overlying water, bulk sediment, and sediment porewater. This field investigation would provide valuable information for the assessment of the sediment quality guidelines (SQGs) published by Thompson et al. (2005) and for the development of alternate SQGs for the uranium industry in northern Saskatchewan using a new approach.

### **A.3 Materials and methods**

#### **A.3.1 Study sites**

All study sites were located in northern Saskatchewan, Canada, near two uranium mining and milling operations, Key Lake and Rabbit Lake. Open pit mining was conducted at Key Lake until the reserves were exhausted in 2002. Key Lake currently mills only ore from the McArthur River mine. The Rabbit Lake operation has an operable underground mine and mill facilities and has been in operation since 1975. The locations of the different uranium operations are shown in Figure A.1. Water, sediment, benthos, and porewater were collected at both operations from reference and potential exposure lakes. Lakes were considered to be exposure lakes if they were either downstream of an effluent discharge point or near a seepage point.

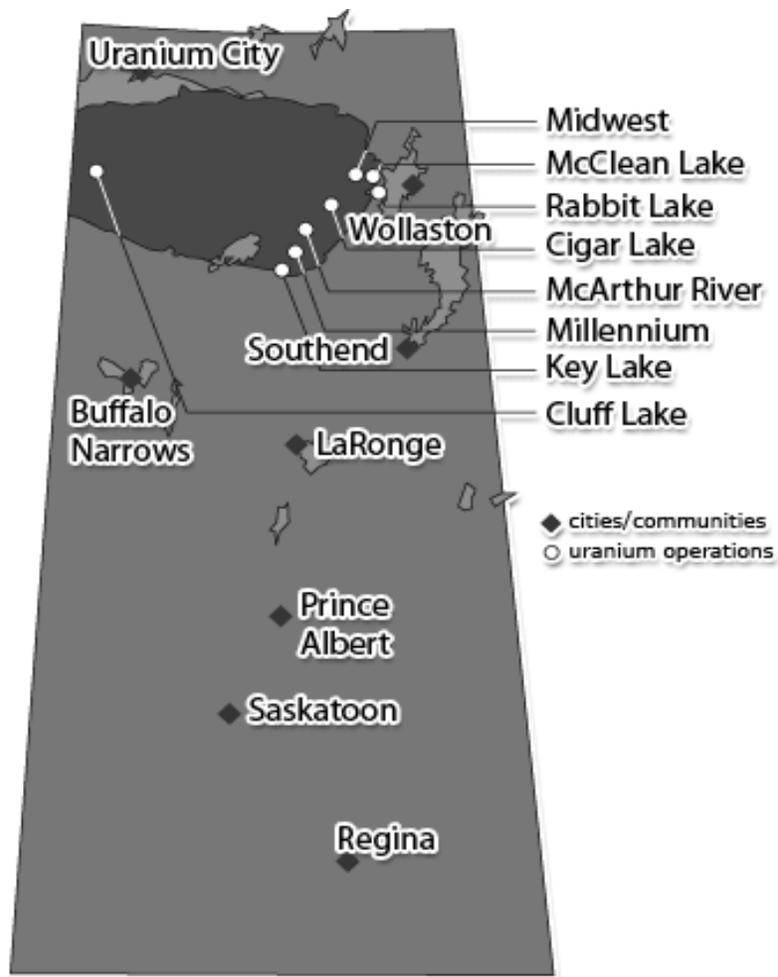


Figure A.1. Locations of uranium mining and milling operations in Saskatchewan, Canada, 2011 (From [www.cameco.com](http://www.cameco.com)).

### **A.3.1.1 Key Lake**

Samples were collected near the Key Lake operation, within the David Creek drainage area, between August 21 and 23, 2007. The reference site, David Lake, was located upstream of the effluent discharge point (Figure A.2). The exposure sites, Fox Lake, Unknown Lake, and Delta Lake, are located in a gradient of exposures downstream of the effluent discharge (Figure A.2). Effluent is discharged directly into Wolf Lake (not sampled) which flows into Fox Lake, through a boggy area into Unknown Lake, and continues downstream into Delta Lake.

### **A.3.1.2 Rabbit Lake**

Samples were collected near the Rabbit Lake operation on September 18 and 19, 2007. The reference site, Raven Lake, was located away from the effluent discharge area (Figure A.3). At the time of sampling, an exploration camp was set up next to Raven Lake with drilling going on near but not adjacent to the lake. The high exposure site was Horseshoe Pond, which is the second water body downstream from the effluent discharge point (Figure A.3). Two potential exposure sites were also located downstream of a seepage point in Parks Lake (Figure A.4). The seepage is thought to originate from a tailings pond located adjacent to the lake, the suspected source of elevated metal concentrations in sediment (Ni and Pb) and elevated major ion concentrations in surface water (Terrestrial & Aquatic Environmental Managers Ltd. 1994). In this study, the Parks Lake Seepage site was near the seepage point and the Parks Lake Outflow sample site was near the outflow of Parks Lake, downstream of the seepage point (Figure A.4).

## **A.3.2 Field characterization**

### **A.3.2.1 Overlying water**

Surface/overlying water was sampled using a Wildco<sup>®</sup> 3.2-L Van Dorn horizontal, acrylic beta water sampler (Wildlife Supply Company, Buffalo, NY, USA) from ~20 cm above the sediment surface and filtered through a 53- $\mu$ m sieve ( $n = 3$ ). Dissolved oxygen and temperature were measured in the field ( $n = 3$ ) using a Thermo Orion<sup>®</sup> dissolved oxygen meter (Model 835, Thermo Scientific, Beverly, MA, USA). Water samples were analyzed within two weeks of surface water collection. pH was measured using an ORION<sup>®</sup> perpHect Ross Sure-Flow electrode (Model 8272BN, Thermo Scientific, Beverly, MA, USA) and ORION<sup>®</sup> PerpHect LogR meter (Model 370, Thermo Scientific, Beverly, MA, USA). Conductivity was measured

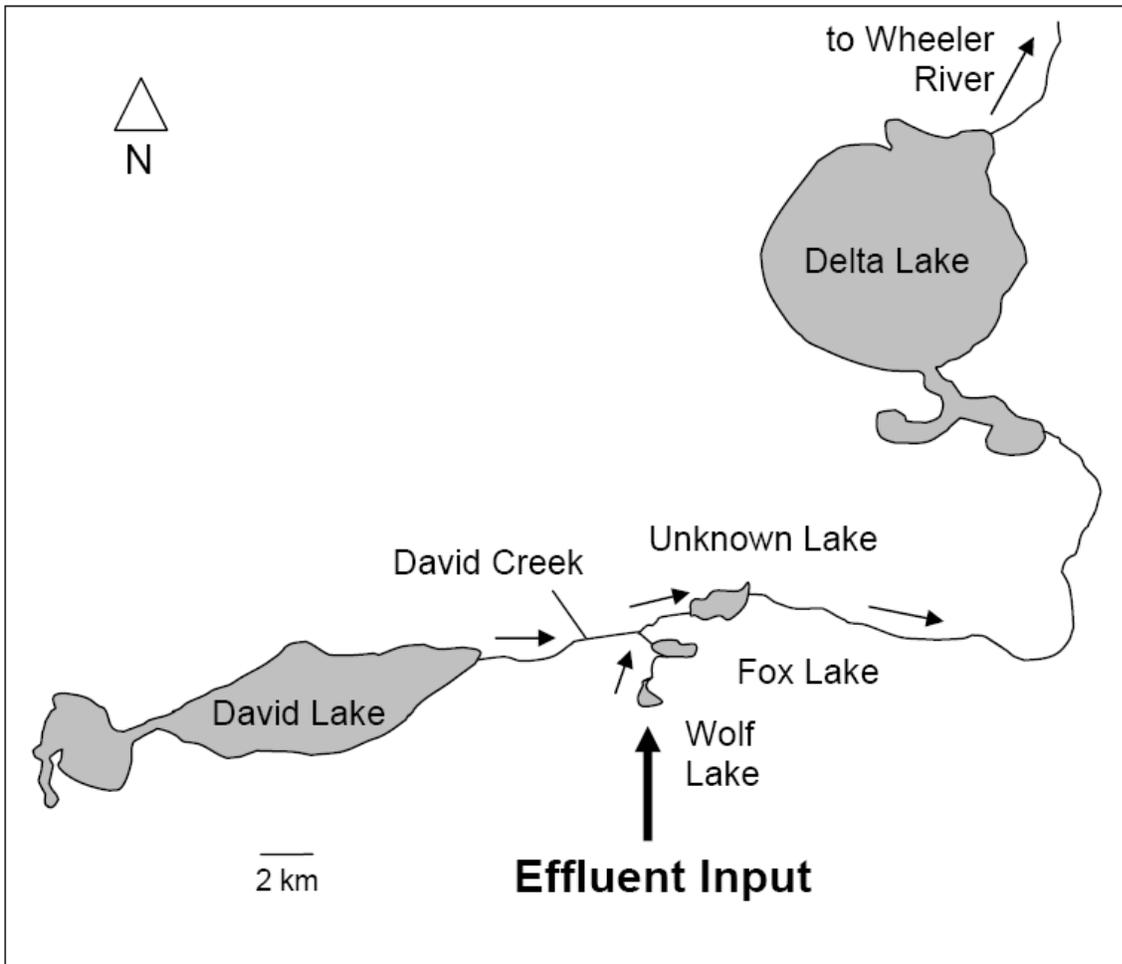


Figure A.2. Lakes sampled at the Key Lake operation, Saskatchewan, Canada. Map from Robertson (2006).

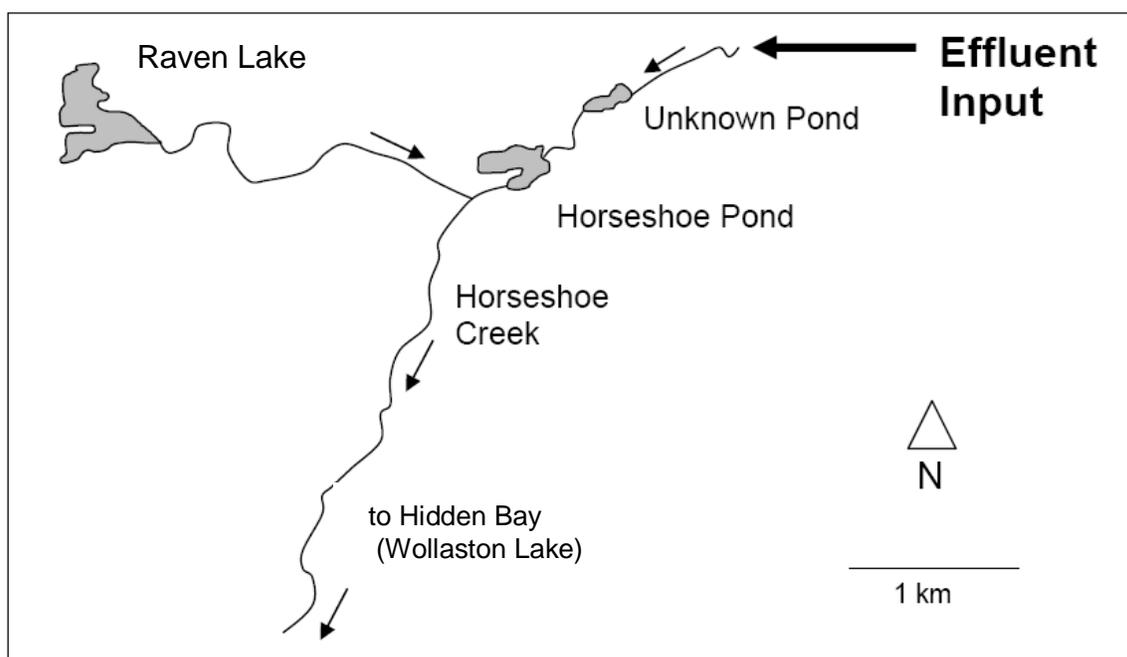


Figure A.3. Reference (Raven Lake) and high exposure (Horseshoe Pond) sample lakes at the Rabbit Lake operation, Saskatchewan, Canada. Map adapted from Robertson (2006).

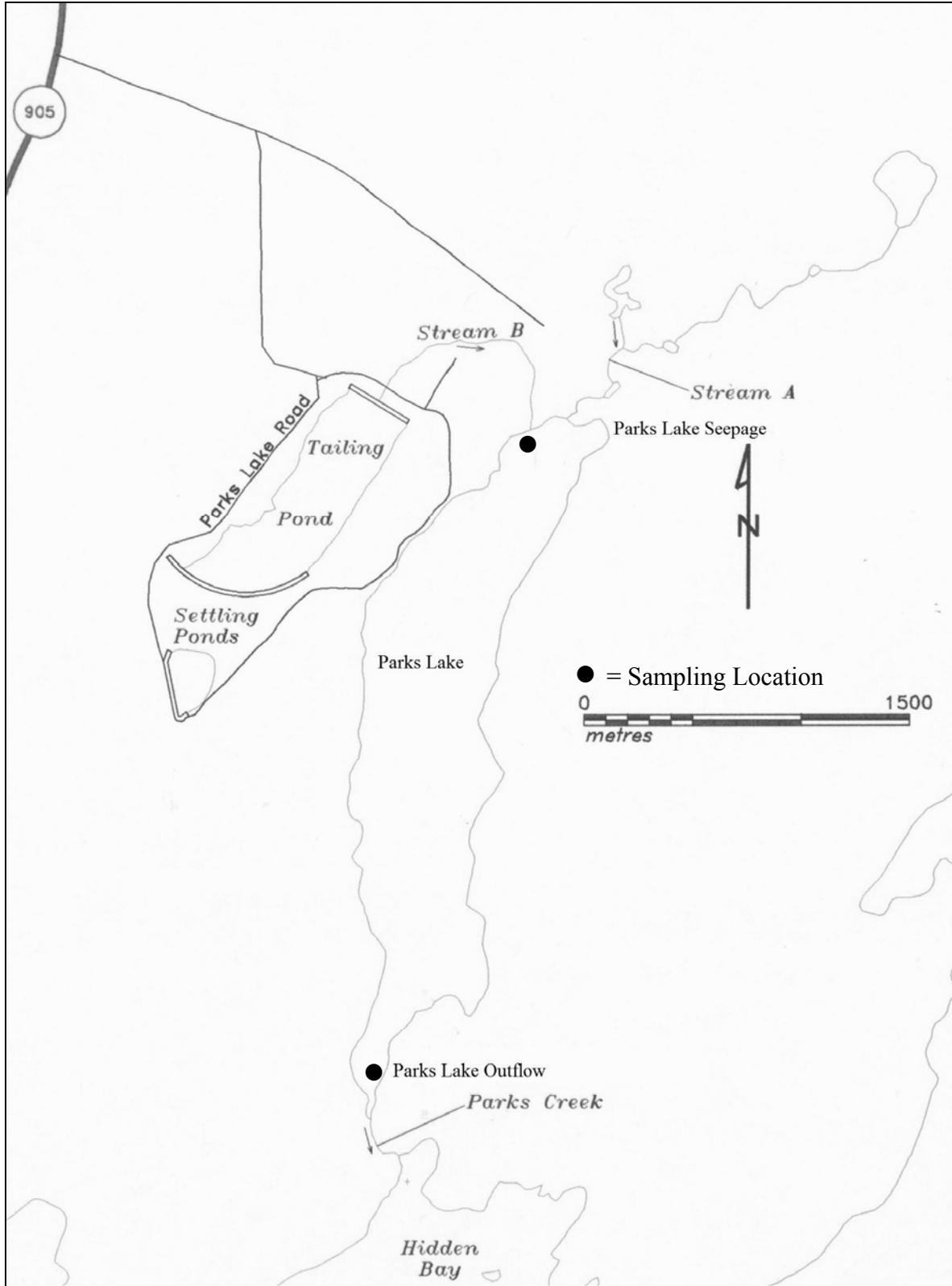


Figure A.4. Parks Lake sampling locations (Parks Lake Seepage and Parks Lake Outflow) at the Rabbit Lake operation. Map adapted from TAEM (1994).

using an ORION<sup>®</sup> ATI conductivity cell (Model 017010, Thermo Scientific, Beverly, MA, USA) and ORION<sup>®</sup> ATI meter (Model 170, Thermo Scientific, Beverly, MA, USA). Ammonia was measured using a Thermo Orion<sup>®</sup> ammonia electrode (Model 9512 BN, Thermo Scientific, Beverly, MA, USA) connected to a VWR meter (Model SB301, VWR International, Arlington Heights, IL, USA). Hardness and alkalinity were measured using a Hach Digital Titrator (Model 16900, Hach Company, Loveland, CO, USA). Dissolved organic carbon was measured using a Shimadzu Total Organic Carbon Analyser (Model TOC-5050A, Mandel Scientific, Guelph, ON, Canada). Overlying water samples collected for metals analysis were filtered through a 0.45- $\mu$ m membrane (Supor<sup>®</sup> polyethersulfone syringe filter; Pall Life Sciences, East Hills, NY, USA), acidified with high purity nitric acid, and stored at 4°C until analysis.

#### **A.3.2.2 Sediment**

Six sediment samples for porewater extraction and total metals analysis were collected in 5-cm diameter acrylic core-tubes (Wildlife Supply Company, Buffalo, NY, USA) using a hand-held corer and sealed with plastic caps at each sample area. Sediment cores were stored with no headspace (site water was added to the top of the core if necessary) and stored in the dark at 4°C until they were processed (within 2 weeks). Processing included isolating the top 2.5-cm horizon of the sediment cores, homogenizing the sample (combining two cores together for each replicate), and removing sub-samples for porewater isolation and for analysis of total metals, total organic carbon (TOC; Leco C632 organic and total carbon analyzer, Leco, St. Joseph, MI, USA), particle size (performed by ALS, Saskatoon, SK, Canada), and water content. Three replicates were analyzed for each lake. Sediment samples for total metals and TOC were oven-dried at 60°C for >48 hrs. Dried sediment samples for total metals analysis were digested using nitric acid, peroxide, hydrofluoric acid, and boric acid in a microwave digestion system (MARS 5 Accelerated Microwave Reaction System, CEM Corporation, Matthews, NC, USA) following the protocol described by Wu et al. (1996) and Wiramanaden et al. (2010). PACS-2 (Natural Resources Canada, Ottawa, ON) was used as a standard reference material during the digestion process and analysis. After sediment digestion, the solution was analysed for total metals at the Toxicology Centre (University of Saskatchewan, Canada) using ICP-MS (discussed in Section A.3.2.4).

### **A.3.2.3 Centrifuged porewater**

Porewater was isolated from sub-samples of surficial (2.5-cm horizon) sediment ( $n = 3$ ) via centrifugation (International Centrifuge Universal Model UV, International Equipment Co., Needham Heights, MA, USA) at 3000 rpm at room temperature for 15 min. Porewater was filtered through a 0.45- $\mu\text{m}$  membrane Supor® polyethersulfone syringe filter and sub-samples isolated for analysis of total metals, dissolved organic carbon, and porewater chemistry. Porewater chemistry included redox potential (Micro Eh probe, Lazar Research Laboratories, Los Angeles, CA, USA and Beckman 250 meter, Beckman-Coulter Mississauga, ON, Canada), pH, ammonia, and total hardness. Dissolved metals samples were acidified with high purity nitric acid (2.5% v/v) and stored at 4°C until analysis.

### **A.3.2.4 *In-situ* dialysis porewater**

*In-situ* dialysis devices (peepers) were used to sample overlying water and sediment porewater profiles vertically. Acrylic peepers with seven rectangular sample compartments (1-cm x 8-cm) separated vertically by 1 cm were filled with ultra-pure (Barnstead NANOpure®, Thermo Scientific, Waltham, MA, USA) water and covered with a 0.45- $\mu\text{m}$  Supor® polyethersulfone membrane. Prior to field deployment, assembled peepers were stored in a plastic pail in ultra-pure water that was bubbled with nitrogen gas (>24 hrs). Three or four peepers were placed vertically in the sediment in each lake (3 = David Lake and Delta Lake; 4 = Unknown Lake, Fox Lake, all Rabbit Lake sample sites) so that two compartments were above the sediment surface and five compartments were below the sediment surface. Peepers were allowed to equilibrate *in-situ* for 12 days. After 12 days, the peeper were retrieved and the compartment membranes pierced with a 1-mL pipette and the water samples carefully withdrawn. Sub-samples from each compartment were measured for Eh and pH while in the field. Dissolved organic carbon subsamples were analyzed in the lab. Samples for metals analysis were acidified to approximately 2.5% high purity nitric acid and stored at 4°C until analysis.

### **A.3.3 Benthic community assessment**

Benthic invertebrate community samples were collected with a standard Ekman grab sampler (6" x 6" x 6", Wildlife Supply Company, Buffalo, NY, USA) and sieved through a 500-

µm sieve bucket (Wildlife Supply Company, Buffalo, NY, USA). Three replicate samples from an approximately 3 m<sup>2</sup> area were collected at each sampling site (one sample site per lake). Each sample was preserved with 10% neutral buffered formalin in separate 1-L polyethylene containers. Benthos samples were sorted, identified, and enumerated in the laboratory of Dr. Jan Ciborowski (University of Windsor, ON, Canada) to at least the family level using standard protocols and keys.

#### **A.3.4 Metals analysis**

All water, porewater, and sediment metals samples were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS; Thermo Fisher Scientific X-Series, Waltham, MA, USA) at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK). Quality assurance and quality control (QA/QC) standards were maintained throughout all analysis (e.g., measurements within 20% of the specified standard reference material concentrations for the majority of metals analyzed, blanks less than the method detection limit). Standard reference materials used were PACS-2 (National Research Council Canada) for sediment and 1640 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and SLRS-4 (National Research Council Canada) for water. Standard reference materials, duplicates, blanks, and standards were analyzed with each batch of samples. At least one of these quality control samples were analyzed approximately every 10 samples.

#### **A.3.5 Data analysis and statistics**

Benthic community metrics were calculated to describe the benthic invertebrate community composition. Density (organisms/m<sup>2</sup>), taxon richness, Simpson's diversity, evenness, and the Bray-Curtis similarity index were calculated using the formulas found in the Canadian Metal Mining Guidance document for Aquatic Environmental Effects Monitoring (Environment Canada, 2002). Metrics were calculated based on taxonomy to the family level.

All statistical analyses were completed using SigmaPlot Version 11.0 software (Systat Software Inc.). If normality and equal variance assumptions were met, statistical significance between reference and exposure sites was tested using a one-way ANOVA. If assumptions were not met, a one-way ANOVA on ranks was used. If a significant difference between sites was found, a Dunnett's post-hoc test was used to determine which exposure sites differed from the

reference site. This approach was used for both the Key Lake and Rabbit Lake benthic community data. A *t*-test was used to assess the difference in benthic community metrics between Parks Lake Outflow and Parks Lake Seepage.

Water quality guidelines (WQGs) and SQGs were used to screen the water, porewater, and sediment concentrations of constituents (primarily metals) that may be of concern. These guidelines are presented in Table A.1.

## **A.4 Results**

### **A.4.1 Key Lake**

#### **A.4.1.1 Physicochemical characterization**

Results of the water quality analyses are listed in Table A.2. The reference sampling site depth (69 cm) was shallower than that of the exposure sites (102 to 136 cm). The pH was lower at the exposure sites (4.0 to 5.3) relative to the reference site (7.2). Ammonia, conductivity, and total hardness measurements were elevated at the exposure sites relative to the reference site and increased with proximity to the effluent discharge point (Fox Lake > Unknown Lake > Delta Lake). Dissolved organic carbon content was relative low across all sampling sites, although David Lake had the highest concentrations.

Overlying water metal concentrations from the various Key Lake locations are listed in Table A.3. Chromium, Pb, and Cu concentrations were relatively constant among sites. Arsenic, Mo, Ni, U, and V concentrations generally increased with proximity to the effluent discharge point. Metals that had concentrations below available WQGs included As, Cr, Cu, Ni, Pb, U and V. Unknown and Fox Lakes exceeded the WQG for Mo and Al. Selenium concentrations in overlying water were difficult to interpret because the limit of detection was higher than the WQG; however, Fox Lake had an overlying water concentration that was quantifiable and well above the WQG. Boron, Al, Sr, Sb, and Ba were elevated (twice that of other exposure or reference concentrations) in Fox Lake.

Sediment quality characteristics and sediment metal concentrations are listed in Table A.4 (only metals that had a SQG were evaluated). David Lake and Fox Lake sediments were sandier than Delta Lake and Unknown Lake sediments, which were classified as clay loam and clay, respectively. Unknown Lake had the highest total organic carbon content ( $19.1 \pm 1.5$  %) followed by Delta Lake ( $9.2 \pm 4.2$  %), David Lake ( $6.7 \pm 3.0$  %) and Fox Lake ( $1.0 \pm 0.1$  %).

Table A.1. Water quality guidelines (WQGs; µg/L) and sediment quality guidelines (SQGs; µg/g dry wt) used in this study for comparison to measured variables in overlying water, porewater, and sediment from the Key Lake and Rabbit Lake sites.

Variable	WQGs	SQGs	
		LEL	ISQG
Ag	0.1	-	-
Al	5 if pH < 6.5; 100 if pH ≥ 6.5	-	-
As	5	9.8	5.9
B	1500	-	-
Cd	Equation <sup>a</sup>	-	-
Co	0.9 <sup>b</sup>	-	-
Cr	8.9 <sup>c</sup> , 1.0 <sup>d</sup>	47.6	37.3
Cu	2 <sup>e</sup>	22.2	35.7
Fe	300	-	-
Hg	0.026 <sup>f</sup>	-	-
Mo	73	13.8	-
NH <sub>3</sub>	0.021 - 231 <sup>g</sup>	-	-
Ni	25 <sup>h</sup>	23.4	18
Pb	1 <sup>i</sup>	36.7	35
pH	6.5 - 9.0	-	-
Se	1	1.9	-
Tl	0.8	-	-
U	15	104.4	-
V	6 <sup>bj</sup>	35.2	-
Zn	30	-	-

WQGs = Water quality guidelines for the protection of aquatic life derived by the CCME (2011)

SQGs = Sediment quality guidelines

LEL = Lowest effect level derived by the Canadian Nuclear Safety Commission, Thompson et al. (2005)

ISQG = Interim sediment quality guideline derived by the CCME (2002)

<sup>a</sup> Cadmium WQG =  $10^{0.86[\log_{10}(\text{hardness})]-3.2}$  µg/L

<sup>b</sup> Derived by the Ontario Ministry of Environment (1994)

<sup>c</sup> Chromium III

<sup>d</sup> Chromium VI

<sup>e</sup> Copper WQG =  $e^{0.8545[\ln(\text{hardness})]-1.465} * 0.2$  µg/L; minimum of 2 µg/L regardless of water hardness

<sup>f</sup> WQG for inorganic mercury

<sup>g</sup> Temperature and pH dependent; Total ammonia in units of mg NH<sub>3</sub>/L.

<sup>h</sup> Nickel concentration =  $e^{0.76[\ln(\text{hardness})]+1.06}$  µg/L; minimum of 25 µg/L regardless of water hardness

<sup>i</sup> Lead WQG =  $e^{1.273[\ln(\text{hardness})]-4.705}$  µg/L; minimum of 1 µg/L regardless of water hardness

<sup>j</sup> Interim guideline

Table A.2. Mean ( $\pm$  SD;  $n = 3$ ) water quality variables measured in overlying water sampled near the Key Lake uranium operation in 2007.

Variable	Units	Key Lake Sampling Sites			
		David Lake	Delta Lake	Unknown Lake	Fox Lake
Depth	cm	69 $\pm$ 1	102 $\pm$ 3	136 $\pm$ 5	109 $\pm$ 4
DO <sup>a</sup>	mg/L	7.88	9.06	7.79	7.96
Temperature <sup>a</sup>	°C	13.6	16.8	15.8	15.5
pH	-	7.2 $\pm$ 0.0	4.0 $\pm$ 0.0 <sup>b</sup>	5.3 $\pm$ 0.1 <sup>b</sup>	4.8 $\pm$ 0.0 <sup>b</sup>
Conductivity	$\mu$ S/cm	24 $\pm$ 0	1236 $\pm$ 11	1411 $\pm$ 14	2723 $\pm$ 12
Ammonia	mg/L as N	0.09 $\pm$ 0.15	1.37 $\pm$ 0.13	3.82 $\pm$ 0.08	9.53 $\pm$ 1.25
Alkalinity	mg/L as CaCO <sub>3</sub>	8 $\pm$ 1	< 1	4 $\pm$ 1	4 $\pm$ 1
Hardness	mg/L as CaCO <sub>3</sub>	7 $\pm$ 2	362 $\pm$ 4	444 $\pm$ 1	977 $\pm$ 6
DOC	mg/L	5.4 $\pm$ 0.8	< 1.34 <sup>a</sup>	4.1 $\pm$ 0.2	< 2.68

DO = Dissolved oxygen

NA = Sample not available

DOC = Dissolved organic carbon

<sup>a</sup>  $n = 1$

<sup>b</sup> Not within water quality guidelines as summarized in Table A.1

Table A.3. Summary (mean  $\pm$  SD;  $n = 3$ ) of metal concentrations ( $\mu\text{g/L}$ ) in overlying water sampled near the Key Lake uranium operation in 2007.

Metal	Key Lake Sampling Sites			
	David Lake	Delta Lake	Unknown Lake	Fox Lake
Ag	0.104 $\pm$ 0.012	0.094 $\pm$ 0.006	0.089 $\pm$ 0.001	0.094 $\pm$ 0.007
Al	6.8 $\pm$ 3.0	68.0 $\pm$ 3.8 <sup>c</sup>	79.4 $\pm$ 0.37 <sup>c</sup>	188 $\pm$ 2.7 <sup>c</sup>
As	<0.23	1.2 $\pm$ 0.084	2.4 $\pm$ 0.22	6.2 $\pm$ 0.44 <sup>c</sup>
B	<11	315 $\pm$ 3.1	433 $\pm$ 2.4	939 $\pm$ 16.6
Ba	3.6 $\pm$ 0.03	23 $\pm$ 0.13	17 $\pm$ 0.11	40 $\pm$ 0.21
Cr	0.22 $\pm$ 0.14	0.37 $\pm$ 0.12	0.27 $\pm$ 0.070	0.49 $\pm$ 0.17
Cu	2.5 $\pm$ 0.081 <sup>c</sup>	3.2 $\pm$ 0.35	2.5 $\pm$ 0.079	2.6 $\pm$ 0.040
Hg	0.10 $\pm$ 0.0095	0.11 $\pm$ 0.0089	0.11 $\pm$ 0.0042	0.13 $\pm$ 0.011
Mo	<0.19	10 $\pm$ 0.21	148 $\pm$ 1.4 <sup>c</sup>	403 $\pm$ 2.8 <sup>c</sup>
Mn	4.9 $\pm$ 0.14	131 $\pm$ 2.3	69 $\pm$ 1.0	84 $\pm$ 1.4
Ni	0.39 $\pm$ 0.16	11 $\pm$ 0.24	11 $\pm$ 0.18	25 $\pm$ 0.29
Pb	0.018 $\pm$ 0.016	0.30 $\pm$ 0.059	0.049 $\pm$ 0.010	0.18 $\pm$ 0.018
Sb	<0.012 <sup>b</sup>	0.060 $\pm$ 0.0078	0.136 $\pm$ 0.0017	0.401 $\pm$ 0.0092
Se	<5	<5	<5	8.7 $\pm$ 0.5 <sup>ac</sup>
Sn	0.09 $\pm$ 0.0079	0.07 $\pm$ 0.010	0.14 $\pm$ 0.090	0.09 $\pm$ 0.018
Sr	12 $\pm$ 0.17	221 $\pm$ 1.8	255 $\pm$ 1.5	561 $\pm$ 5.8
U	0.019 $\pm$ 0.0020	0.44 $\pm$ 0.0015	0.33 $\pm$ 0.0021	2.290 $\pm$ 0.012
V	0.23 $\pm$ 0.020	0.24 $\pm$ 0.10	0.23 $\pm$ 0.084	0.44 $\pm$ 0.10

<sup>a</sup> One replicate < limit of detection (LD); used half the LD in calculating the mean and standard deviation

<sup>b</sup> Two out of three replicates < LD; therefore, no mean and standard deviation could be calculated, so stated that concentrations reported as < LD

<sup>c</sup> Exceedence of water quality guidelines summarized in Table A.1

Table A.4. Summary (mean  $\pm$  SD;  $n = 3$ ) of sediment quality characteristics and sediment metal concentrations ( $\mu\text{g/g}$  dry wt) near the Key Lake uranium operations in 2007.

Variable	Key Lake Sampling Sites							
	David Lake		Delta Lake		Unknown Lake		Fox Lake	
Sand <sup>a</sup> (%)	81		43		4		96	
Silt <sup>a</sup> (%)	9		29		29		1	
Clay <sup>a</sup> (%)	10		28		67		2	
Texture	Loamy sand		Clay loam		Clay		Sand	
TOC (%)	6.7	$\pm$ 3.0	9.2	$\pm$ 4.2	19.1	$\pm$ 1.5	1.0	$\pm$ 0.1
Moisture (%)	76.1	$\pm$ 5.8	86.6	$\pm$ 3.7	NA		48.2	$\pm$ 4.2
As	2.90	$\pm$ 1.83	60.60	$\pm$ 19.80 <sup>bc</sup>	466.02	$\pm$ 153.36 <sup>bc</sup>	61.79	$\pm$ 13.98 <sup>bc</sup>
Cr	2.38	$\pm$ 0.81	11.47	$\pm$ 3.01	26.78	$\pm$ 5.98	3.85	$\pm$ 0.78
Cu	2.89	$\pm$ 1.54	3.24	$\pm$ 0.55	7.01	$\pm$ 1.09	1.56	$\pm$ 0.79
Mo	0.22	$\pm$ 0.08	4293.70	$\pm$ 1049.5 <sup>b</sup>	1655.1	$\pm$ 602.3 <sup>b</sup>	377.40	$\pm$ 36.41 <sup>b</sup>
Ni	2.08	$\pm$ 0.95	60.88	$\pm$ 22.44 <sup>bc</sup>	100.84	$\pm$ 32.61 <sup>bc</sup>	3.07	$\pm$ 1.47
Pb	4.27	$\pm$ 1.42	5.51	$\pm$ 1.39	7.13	$\pm$ 1.16	1.85	$\pm$ 0.08
Se	<0.0067		19.25	$\pm$ 7.70 <sup>b</sup>	42.39	$\pm$ 15.59 <sup>b</sup>	3.76	$\pm$ 0.63 <sup>b</sup>
U	1.11	$\pm$ 0.38	11.41	$\pm$ 3.84	47.15	$\pm$ 13.74	5.04	$\pm$ 0.80
V	5.99	$\pm$ 1.77	11.84	$\pm$ 2.52	26.54	$\pm$ 5.42	8.60	$\pm$ 2.86

NA = Sample not available

TOC = Total organic carbon

<sup>a</sup> Only one composite sample analyzed

<sup>b</sup> Exceeds the lowest effect level (LEL) as derived by Thompson et al. (2005)

<sup>c</sup> Exceeds interim sediment quality guideline (ISQG) as derived by CCME (2011)

Sediment metal concentrations exceeded the LELs and ISQGs (Table A.1) for As, Mo, and Se at all three of the exposure sites. Delta and Unknown Lakes exceeded the Ni LEL and ISQG. Sediment metal concentrations at David Lake (reference site) were all below the SQGs. Sediment metal concentrations in Fox Lake were lower than in the two other exposure lakes which are located further downstream from the treated effluent discharge point.

Porewater characteristics and metal concentrations in porewater from centrifuged whole-sediment are listed in Table A.5. Ammonia and hardness generally increased with proximity to the effluent release point (Fox Lake > Unknown Lake  $\geq$  Delta Lake > David Lake). Metal concentrations in porewater were compared to available WQGs for the protection of aquatic life. Arsenic porewater concentrations were highest in Unknown Lake and all exposure sites were above the WQG of 5  $\mu\text{g/L}$ . Chromium porewater concentrations were highest in Unknown Lake and all sampling sites exceeded the WQG for hexavalent chromium, but were below the WQG for trivalent chromium (only total chromium concentrations were measured). Copper porewater concentrations exceeded the WQG (2  $\mu\text{g/L}$ ) at all sites with David Lake yielding the highest Cu concentrations of  $14.7 \pm 6.1 \mu\text{g/L}$ . All exposure sites had porewater Mo concentrations that exceeded the WQG and that were substantially elevated relative to the reference site, David Lake. Porewater Se concentrations were generally difficult to interpret since the limit of detection was greater than the WQG of 1  $\mu\text{g/L}$ . However, Fox Lake had a porewater Se concentration of  $8.9 \pm 0.6 \mu\text{g/L}$  which was above the WQG. The WQG for U was exceeded only in Unknown Lake porewater, although both Fox Lake and Unknown Lake showed elevated concentrations of U in the porewater relative to David Lake and Delta Lake. Other elements that exceeded available WQGs were Al, Co, Fe, and Hg.

Porewater characteristics, including metal concentrations, collected using peepers are listed in Table A.6. The number of sample replicates varied among sites since two peepers had been pulled out of the sediment in Unknown Lake by unknown causes. David Lake and Delta Lake had only three replicates deployed and retrieved due to the limited number of peepers available at the time of deployment. Chromium concentrations were above the WQG for hexavalent Cr, but less than the WQG for trivalent Cr (only total chromium concentrations were measured). Both As and Mo had elevated concentrations at all exposure sites compared to the reference lake (David Lake) and were well above their respective WQG. Again, Se porewater concentrations were difficult to interpret due to the high limit of detection (above the WQG).

Table A.5. Summary (mean  $\pm$  SD;  $n = 3$ ) of porewater characteristics and metal concentrations measured in centrifuged porewater from sediment (top 2.5 cm) sampled near the Key Lake uranium operations in 2007.

Variable	Units	Key Lake Sampling Sites									
		David Lake		Delta Lake		Unknown Lake		Fox Lake			
pH	-	7.7 $\pm$ 0.1	5.7 $\pm$ 0.2 <sup>a</sup>	7.0 $\pm$ 0.1	5.4 $\pm$ 0.1 <sup>a</sup>						
Ammonia	mg N/L	0.26 $\pm$ 0.29	1.51 $\pm$ 0.17	6.85 $\pm$ 0.26	8.88 $\pm$ 0.26						
Hardness	mg CaCO <sub>3</sub> /L	17 $\pm$ 2	379 $\pm$ 24	393 $\pm$ 18	962 $\pm$ 20						
DOC	mg/L	5.2 $\pm$ 1.4	6.3 $\pm$ 1.5	17.3 $\pm$ 1.3	7.4 $\pm$ 1.2						
Ag	$\mu$ g/L	0.12 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>a</sup>						
Al	$\mu$ g/L	89.42 $\pm$ 40.17	12.37 $\pm$ 5.57 <sup>a</sup>	9.72 $\pm$ 0.40	641.27 $\pm$ 130.64 <sup>a</sup>						
As	$\mu$ g/L	0.86 $\pm$ 0.10	10.93 $\pm$ 2.87 <sup>a</sup>	183.33 $\pm$ 35.78 <sup>a</sup>	21.32 $\pm$ 7.03 <sup>a</sup>						
B	$\mu$ g/L	14.7 $\pm$ 3.2	372.6 $\pm$ 21.1	541.2 $\pm$ 30.2	1098.3 $\pm$ 15.2						
Ba	$\mu$ g/L	4.12 $\pm$ 1.20	46.94 $\pm$ 6.40	28.82 $\pm$ 8.51	43.38 $\pm$ 0.55						
Be	$\mu$ g/L	<1.6	<1.6	<1.6	<1.6						
Cd	$\mu$ g/L	<0.014 <sup>c</sup>	0.04 $\pm$ 0.06 <sup>d</sup>	0.05 $\pm$ 0.03	0.13 $\pm$ 0.02						
Co	$\mu$ g/L	0.09 $\pm$ 0.04	0.36 $\pm$ 0.06	0.21 $\pm$ 0.02	3.41 $\pm$ 0.96						
Cr <sup>b</sup>	$\mu$ g/L	1.71 $\pm$ 0.15	2.03 $\pm$ 1.16	3.61 $\pm$ 1.27	2.69 $\pm$ 2.82						
Cu	$\mu$ g/L	14.66 $\pm$ 6.06 <sup>a</sup>	6.79 $\pm$ 0.49	5.87 $\pm$ 0.11	6.70 $\pm$ 0.26						
Fe	$\mu$ g/L	384 $\pm$ 179	19800 $\pm$ 7751	1996 $\pm$ 73	208 $\pm$ 199						
Hg	$\mu$ g/L	0.03 $\pm$ 0.00 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>a</sup>	0.47 $\pm$ 0.09 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>						
Mn	$\mu$ g/L	24.2 $\pm$ 8.5	248.5 $\pm$ 85.5	126.7 $\pm$ 45.2	87.4 $\pm$ 6.4						
Mo	$\mu$ g/L	0.3 $\pm$ 0.0	987.1 $\pm$ 651.2 <sup>a</sup>	1366.0 $\pm$ 605.0 <sup>a</sup>	472.9 $\pm$ 71.0 <sup>a</sup>						
Ni	$\mu$ g/L	2.19 $\pm$ 0.86	16.98 $\pm$ 2.15	6.22 $\pm$ 0.81	53.73 $\pm$ 23.82 <sup>a</sup>						
Pb	$\mu$ g/L	0.19 $\pm$ 0.05	0.02 $\pm$ 0.01	0.07 $\pm$ 0.04	0.27 $\pm$ 0.21						
Sb	$\mu$ g/L	0.04 $\pm$ 0.01	1.02 $\pm$ 0.48	2.42 $\pm$ 0.67	0.78 $\pm$ 0.18						
Se	$\mu$ g/L	<3.2	<3.2	<3.2	8.93 $\pm$ 0.63 <sup>a</sup>						
Sn	$\mu$ g/L	0.17 $\pm$ 0.02	0.13 $\pm$ 0.02	0.12 $\pm$ 0.01	0.15 $\pm$ 0.02						
Sr	$\mu$ g/L	6.96 $\pm$ 1.80	219.50 $\pm$ 17.34	217.87 $\pm$ 6.67	501.50 $\pm$ 3.13						
Tl	$\mu$ g/L	0.01 $\pm$ 0.00	0.05 $\pm$ 0.03	0.01 $\pm$ 0.00	1.84 $\pm$ 0.70						
U	$\mu$ g/L	0.03 $\pm$ 0.02	0.09 $\pm$ 0.02	8.56 $\pm$ 1.75	2.91 $\pm$ 0.60						
V	$\mu$ g/L	0.71 $\pm$ 0.65	0.19 $\pm$ 0.06	0.88 $\pm$ 0.12	0.85 $\pm$ 0.34						
Zn	$\mu$ g/L	1.75 $\pm$ 0.79	4.14 $\pm$ 0.82	1.88 $\pm$ 0.41	15.83 $\pm$ 3.03						

DOC = Dissolved organic carbon

<sup>a</sup> Not within water quality guidelines as summarized in Table A.1

<sup>b</sup> Speciation analysis was not measured in samples so can not determine if samples exceed water quality guidelines

<sup>c</sup> Two out of three replicates < limit of detection (LD); therefore, no mean and standard deviation could be calculated, so concentrations listed as < LD

<sup>d</sup> One replicate <LD; used half the LD in calculating the mean and standard deviation

Table A.6. Water quality characteristics and concentrations of select variables (mean  $\pm$  SD) from sediment porewater collected from peepers sampling to the 0 - 2 cm fraction of sediment at sites near the Key Lake uranium operation in 2007.

Variable	Units	Key Lake Sampling Sites			
		David Lake	Delta Lake	Unknown Lake	Fox Lake
pH	-	6.3 $\pm$ 0.1 <sup>a</sup>	6.5 $\pm$ 0.3	7.0 $\pm$ 0.1	5.2 $\pm$ 0.4 <sup>a</sup>
DOC	mg/L	8.3 $\pm$ 1.9	7.1 $\pm$ 3.2	37.3 $\pm$ 5.3	5.2 $\pm$ 0.6
As	$\mu$ g/L	0.64 $\pm$ 0.48	6.11 $\pm$ 1.97	126.55 $\pm$ 34.72 <sup>a</sup>	133.50 $\pm$ 253.87 <sup>a</sup>
Cr <sup>b</sup>	$\mu$ g/L	1.01 $\pm$ 0.27	1.10 $\pm$ 0.14	2.56 $\pm$ 1.15	1.83 $\pm$ 1.06
Cu	$\mu$ g/L	0.23 $\pm$ 0.14	0.20 $\pm$ 0.13	1.17 $\pm$ 1.49	0.53 $\pm$ 0.37
Mo	$\mu$ g/L	0.26 $\pm$ 0.07	3101.30 $\pm$ 1701.38 <sup>a</sup>	424.35 $\pm$ 401.99 <sup>a</sup>	4249.38 $\pm$ 7708.24 <sup>a</sup>
Ni	$\mu$ g/L	0.12 $\pm$ 0.06	3.38 $\pm$ 3.03	2.97 $\pm$ 0.99	20.78 $\pm$ 4.31
Pb	$\mu$ g/L	0.06 $\pm$ 0.05	0.48 $\pm$ 0.97	0.08 $\pm$ 0.03	0.09 $\pm$ 0.06
Se	$\mu$ g/L	<6	<6	<6	8.98 $\pm$ 2.14 <sup>a</sup>
U	$\mu$ g/L	0.02 $\pm$ 0.01	0.66 $\pm$ 0.78	3.62 $\pm$ 0.33	3.54 $\pm$ 1.24
V	$\mu$ g/L	0.29 $\pm$ 0.18	0.20 $\pm$ 0.07	4.40 $\pm$ 1.12	1.17 $\pm$ 1.83
<i>n</i>	-	3	3	2	4

<sup>a</sup> Exceeds water quality guidelines as summarized in Table A.1

<sup>b</sup> Speciation analysis was not measured so can not determine if samples exceed a water quality guideline

Table A.7. Mean ( $\pm$  SD, *n* = 3) benthic invertebrate community metrics for samples collected near the Key Lake uranium operation in 2007.

Metric	Key Lake Sampling Sites			
	David Lake	Fox Lake	Unknown Lake	Delta Lake
Family Richness	11 $\pm$ 2	4 $\pm$ 1*	9 $\pm$ 1	9 $\pm$ 1
Density (individuals/m <sup>2</sup> )	9609 $\pm$ 4917	8478 $\pm$ 4570	6449 $\pm$ 3871	9087 $\pm$ 2711
Simpson's Diversity	0.54 $\pm$ 0.08	0.25 $\pm$ 0.16*	0.54 $\pm$ 0.09	0.46 $\pm$ 0.12
Bray-Curtis Index	0.19 $\pm$ 0.16	0.41 $\pm$ 0.09	0.68 $\pm$ 0.11*	0.37 $\pm$ 0.10
Evenness	0.21 $\pm$ 0.01	0.38 $\pm$ 0.06*	0.26 $\pm$ 0.05	0.22 $\pm$ 0.03

\* Statistically different (ANOVA, Dunnett's post-hoc test,  $p \leq 0.05$ ) from the reference site (David Lake)

However, the Se concentrations in Fox Lake were greater than at the other sample sites and exceeded the WQG.

#### **A.4.1.2 Benthic invertebrate communities**

The benthic invertebrate community generally varied with proximity to the effluent discharge point based on the metrics used (Table A.7). Fox Lake was statistically different from David Lake in family richness (lower), Simpson's diversity (lower), and evenness (higher). There was no significant difference between exposure and reference sites in benthic invertebrate density. The Unknown Lake Bray-Curtis index was statistically different (elevated) from that of the reference lake.

#### **A.4.2 Rabbit Lake**

##### **A.4.2.1 Physicochemical characterization**

Results of the water quality analyses are listed in Table A.8. Water sampling depths were between 87 and 108 cm over all sites at Rabbit Lake. Dissolved oxygen and temperature were similar across all sites, averaging  $12.0 \pm 1.6$  mg/L and  $6.0 \pm 1.7$  °C, respectively. Overlying water pH was fairly consistent among sampling sites with only Horseshoe Pond having a low pH of 6.2. Conductivity and water hardness were lowest at the reference site, Raven Lake, slightly elevated at both of the Parks Lake sample sites, and highest at Horseshoe Pond. All sites had low buffering capacity (alkalinity below 20 mg/L). Ammonia concentrations at all sampling sites were below 0.2 mg N/L. Overlying water metal concentrations are listed in Table A.9; concentrations were below WQGs at all sites for As, Cr, Ni, Pb, and V. Copper was above available WQGs at Raven Lake and at both Parks Lake sampling sites. Horseshoe Pond did not exceed the WQG for Cu because of its high water hardness. Horseshoe Pond exceeded the WQGs for Al, Mo and U. Parks Lake Seepage and Outflow sites were very similar in overlying water metal concentrations. Most elements increased with proximity to the seepage or effluent discharge point. Horseshoe Pond had the highest metal concentrations in the overlying water.

Sediment characteristics and whole-sediment metal concentrations are listed in Table A.10. The sediment types ranged from sandy loams (Raven Lake, Parks Lake Seepage), and silt loam (Parks Lake Outflow), to clay (Horseshoe Pond). The sediment total organic carbon content was highest in Horseshoe Pond ( $11.5 \pm 1.2\%$ ), with the other sites having approximately

Table A.8. Mean ( $\pm$  SD;  $n = 3$ ) water quality variables measured in overlying water sampled near the Rabbit Lake uranium operation in 2007.

Variable	Units	Rabbit Lake Sampling Sites			
		Raven Lake	Horseshoe Pond	Parks Lake Seepage	Parks Lake Outflow
Depth	cm	108 $\pm$ 3	87 $\pm$ 3	101 $\pm$ 1	94 $\pm$ 1
DO <sup>a</sup>	mg/L	13.8	11.3	NA	10.8
Temperature <sup>a</sup>	°C	5.3	6.7	NA	5.9
pH	-	7.1 $\pm$ 0.1	6.2 $\pm$ 0.0 <sup>b</sup>	6.9 $\pm$ 0.0	6.9 $\pm$ 0.1
Conductivity	$\mu$ S/cm	58 $\pm$ 0	3400 $\pm$ 46	245 $\pm$ 0	235 $\pm$ 2
Ammonia	mg/L as N	0.09 $\pm$ 0.02	0.17 $\pm$ 0.00	0.12 $\pm$ 0.03	0.03 $\pm$ 0.02
Alkalinity	mg/L as CaCO <sub>3</sub>	18 $\pm$ 1	7 $\pm$ 1	6 $\pm$ 1	7 $\pm$ 1
Hardness	mg/L as CaCO <sub>3</sub>	18 $\pm$ 3	1317 $\pm$ 12	66 $\pm$ 1	65 $\pm$ 1
DOC	mg/L	7.8 $\pm$ 1.8	4.8 $\pm$ 0.1	7.7 $\pm$ 0.2	8.2 $\pm$ 0.6

DO = Dissolved oxygen

NA = Sample not available

DOC = Dissolved organic carbon

<sup>a</sup> $n = 1$

<sup>b</sup>Not within WQGs as summarized in Table A.1

Table A.9. Metal concentrations ( $\mu\text{g/L}$ ) in overlying water sampled near the Rabbit Lake uranium operation in 2007 (mean  $\pm$  SD;  $n = 3$ ).

Metal	Rabbit Lake Sampling Sites							
	Raven Lake		Horseshoe Pond		Parks Lake Seepage		Parks Lake Outflow	
Ag	0.094	$\pm$ 0.003	0.089	$\pm$ 0.001	0.090	$\pm$ 0.003	0.092	$\pm$ 0.002
Al	12.29	$\pm$ 0.07	85.41	$\pm$ 5.95 <sup>a</sup>	24.29	$\pm$ 1.01	20.95	$\pm$ 0.87
As	<0.23		2.91	$\pm$ 0.11	<0.23		<0.23	
B	<11		182.37	$\pm$ 13.70	<11 <sup>c</sup>		< 11	
Ba	3.92	$\pm$ 0.08	23.86	$\pm$ 0.35	6.25	$\pm$ 0.02	6.13	$\pm$ 0.11
Cr	0.18	$\pm$ 0.01	0.65	$\pm$ 0.13	0.19	$\pm$ 0.05	0.20	$\pm$ 0.02
Cu	2.42	$\pm$ 0.02 <sup>a</sup>	2.68	$\pm$ 0.15	2.58	$\pm$ 0.14 <sup>a</sup>	2.82	$\pm$ 0.18 <sup>a</sup>
Hg	0.09	$\pm$ 0.00	0.11	$\pm$ 0.00	0.09	$\pm$ 0.01	0.09	$\pm$ 0.01
Mn	1.76	$\pm$ 0.08	172.13	$\pm$ 7.54	36.92	$\pm$ 0.15	31.29	$\pm$ 0.89
Mo	0.28	$\pm$ 0.01	1932.0	$\pm$ 36.1 <sup>a</sup>	0.46	$\pm$ 0.34	0.49	$\pm$ 0.03
Ni	0.35	$\pm$ 0.07	16.29	$\pm$ 0.53	1.19	$\pm$ 0.01	1.05	$\pm$ 0.03
Pb	0.13	$\pm$ 0.11	0.03	$\pm$ 0.01	<0.006		0.02	$\pm$ 0.00
Sb	<0.012 <sup>c</sup>		0.214	$\pm$ 0.023	0.012	$\pm$ 0.005 <sup>b</sup>	0.011	$\pm$ 0.004 <sup>b</sup>
Se	<5		<5 <sup>c</sup>		<5		<5	
Sn	0.09	$\pm$ 0.00	0.12	$\pm$ 0.02	0.05	$\pm$ 0.00	0.06	$\pm$ 0.01
Sr	17.83	$\pm$ 0.24	485.43	$\pm$ 13.83	25.09	$\pm$ 0.41	24.43	$\pm$ 0.19
U	1.41	$\pm$ 0.02	89.10	$\pm$ 1.23 <sup>a</sup>	0.24	$\pm$ 0.00	0.26	$\pm$ 0.00
V	0.22	$\pm$ 0.04	0.36	$\pm$ 0.12	0.27	$\pm$ 0.08	0.19	$\pm$ 0.05

<sup>a</sup> Exceeds water quality guidelines as summarized in Table A.1

<sup>b</sup> One replicate < limit of detection (LD); used half the LD in calculating the mean and standard deviation

<sup>c</sup> Two out of three replicates < LD; therefore, no mean and standard deviation could be calculated, so concentrations listed as < LD

Table A.10. Sediment characteristics and metal concentrations ( $\mu\text{g/g}$  dry wt) sampled near the Rabbit Lake uranium operations in 2007 (mean  $\pm$  SD;  $n = 3$ ).

Variable	Rabbit Lake Sampling Sites			
	Raven Lake	Parks Lake Outflow	Parks Lake Seepage	Horseshoe Pond
Sand <sup>a</sup> (%)	75	37	60	20
Silt <sup>a</sup> (%)	11	50	32	38
Clay <sup>a</sup> (%)	15	13	9	42
Texture	Sandy loam	Silt loam	Sandy loam	Clay
TOC (%)	2.9 $\pm$ 0.7	2.7 $\pm$ 0.6	2.0 $\pm$ 0.5	11.5 $\pm$ 1.2
Moisture (%)	82.9 $\pm$ 8.3	73.6 $\pm$ 1.6	61.3 $\pm$ 4.2	85.6 $\pm$ 2.5
As	1.62 $\pm$ 0.66	1.84 $\pm$ 0.53	2.11 $\pm$ 1.84	72.34 $\pm$ 42.41 <sup>bc</sup>
Cr	12.58 $\pm$ 1.59	17.41 $\pm$ 0.35	14.29 $\pm$ 4.11	19.58 $\pm$ 2.17
Cu	7.19 $\pm$ 0.65	7.36 $\pm$ 0.30	3.60 $\pm$ 0.36	27.20 $\pm$ 6.30 <sup>b</sup>
Mo	2.13 $\pm$ 0.26	2.01 $\pm$ 0.28	1.25 $\pm$ 0.35	1520.70 $\pm$ 517.99 <sup>b</sup>
Ni	6.04 $\pm$ 0.27	15.24 $\pm$ 0.36	9.37 $\pm$ 1.32	53.33 $\pm$ 22.48 <sup>bc</sup>
Pb	8.22 $\pm$ 0.61	12.64 $\pm$ 0.31	9.73 $\pm$ 6.43	4.60 $\pm$ 0.08
Se	<0.0067	<0.0067	<0.0067	14.59 $\pm$ 6.17 <sup>b</sup>
U	77.69 $\pm$ 8.78	6.84 $\pm$ 0.68	3.94 $\pm$ 0.65	1234.28 $\pm$ 425.44 <sup>b</sup>
V	44.48 $\pm$ 8.86 <sup>b</sup>	19.84 $\pm$ 0.34	18.33 $\pm$ 3.40	17.81 $\pm$ 1.47

TOC = Total organic carbon

<sup>a</sup> Only one composite sample analyzed

<sup>b</sup> Exceeds the lowest effect level (LEL) as derived by Thompson et al. (2005)

<sup>c</sup> Exceeds interim sediment quality guideline (ISQG) as derived by CCME (1999)

2 to 3 % organic carbon. Horseshoe Pond yielded the highest metal concentrations as compared to the other sites and exceeded the As, Cu, Mo, Ni, Se, and U LELs and ISQGs. Raven Lake was the only site to exceed the V LEL. Generally, sediment metal concentrations at Parks Lake sample sites were similar to each other and to Raven Lake.

Porewater characteristics and metal concentrations collected from centrifuging whole sediment are found in Table A.11. Ammonia, hardness, and DOC increased with proximity to anthropogenic inputs (Raven Lake < Parks Lake Outflow < Parks Lake Seepage < Horseshoe Pond). All sites were either at or below the WQGs for Cr, Ni, Pb, and V. Horseshoe Pond generally had the highest concentrations of metals in centrifuged porewater and exceeded the WQG for As, Mo, U, B, Fe, and Zn. Raven Lake marginally exceeded the WQG for Al. Parks Lake Seepage and Outflow sample sites exceed the Al, Fe, and Co WQGs. All sites exceeded the WQG for Cu.

Porewater characteristics and metal concentrations collected using peepers are listed in Table A.12. There are uneven replicates at sampling sites because one peeper had been pulled out of the sediment by unknown causes in the Parks Lake Outflow sampling site. Peeper porewater ranged from a pH of 6.2 to 6.8 and DOC ranged from 5.0 to 9.8 mg/L. Horseshoe Pond generally had the highest metal concentrations in peeper porewater and exceeded the WQGs for As, Mo, and U. All sites nearly or just exceeded the available WQG for hexavalent Cr, but not for the trivalent form of Cr. Only total metal concentrations of Cr were measured but comparisons to the speciation based Cr WQG were made as it is the only available WQG.

#### **A.4.2.2 Benthic invertebrate communities**

Metrics used to evaluate the benthic invertebrate community generally varied with proximity to the influence of mine waters (Table A.13). There was no significant difference between Raven Lake (reference) and the other sampling locations in family richness, density, and evenness. Horseshoe Pond and Parks Lake Outflow were statistically different from Raven Lake based on the Simpson's diversity metric. The Bray-Curtis similarity indices were not statistically different among all potential exposure sites and the reference site, Raven Lake (an ANOVA on Ranks was conducted since assumptions of normality and equal variance could not be met even when the data was log-transformed). When Parks Lake Seepage and Parks Lake

Table A.11. Summary (mean  $\pm$  SD; n = 3) of porewater characteristics and metal concentrations measured in centrifuged porewater from sediment (top 2.5 cm) sampled near the Rabbit Lake uranium operations in 2007.

Variable	Units	Rabbit Lake Sampling Sites			
		Raven Lake	Parks Lake Outflow	Parks Lake Seepage	Horseshoe Pond
pH	-	6.9 $\pm$ 0.2	6.6 $\pm$ 0.0	6.6 $\pm$ 0.0	7.2 $\pm$ 0.3
Ammonia	mg N/L	0.61 $\pm$ 0.15	0.58 $\pm$ 0.24	1.94 $\pm$ 0.46	5.87 $\pm$ 1.42
Hardness	mg CaCO <sub>3</sub> /L	20 $\pm$ 3	53 $\pm$ 1	99 $\pm$ 2	1127 $\pm$ 28
DOC	mg/L	5.0 $\pm$ 0.5	8.7 $\pm$ 2.3	15.1 $\pm$ 3.3	16.0 $\pm$ 1.2
Ag	$\mu$ g/L	0.11 $\pm$ 0.01 <sup>b</sup>	0.12 $\pm$ 0.01 <sup>b</sup>	0.11 $\pm$ 0.00 <sup>b</sup>	0.13 $\pm$ 0.04 <sup>b</sup>
Al	$\mu$ g/L	100.94 $\pm$ 52.98 <sup>b</sup>	448.40 $\pm$ 102.49 <sup>b</sup>	679.73 $\pm$ 163.95 <sup>b</sup>	13.57 $\pm$ 0.54
As	$\mu$ g/L	0.53 $\pm$ 0.25	1.43 $\pm$ 0.37	2.79 $\pm$ 0.91	31.18 $\pm$ 1.08 <sup>b</sup>
B	$\mu$ g/L	24.9 $\pm$ 9.9	<11 <sup>c</sup>	<11 <sup>c</sup>	285.4 $\pm$ 21.6
Ba	$\mu$ g/L	8.97 $\pm$ 1.94	25.10 $\pm$ 1.75	19.21 $\pm$ 2.54	33.36 $\pm$ 5.38
Be	$\mu$ g/L	<1.6	<1.6	<1.6	<1.6
Cd	$\mu$ g/L	<0.014 <sup>c</sup>	0.08 $\pm$ 0.02	0.04 $\pm$ 0.02	0.13 $\pm$ 0.04
Co	$\mu$ g/L	0.10 $\pm$ 0.05	1.25 $\pm$ 0.33 <sup>b</sup>	2.44 $\pm$ 0.22 <sup>b</sup>	0.69 $\pm$ 0.13
Cr <sup>a</sup>	$\mu$ g/L	0.99 $\pm$ 0.48	1.59 $\pm$ 1.02	1.62 $\pm$ 0.13	1.13 $\pm$ 0.54
Cu	$\mu$ g/L	15.58 $\pm$ 6.24 <sup>b</sup>	10.32 $\pm$ 0.72 <sup>b</sup>	9.40 $\pm$ 0.74 <sup>b</sup>	5.90 $\pm$ 0.08
Fe	$\mu$ g/L	197 $\pm$ 111	949 $\pm$ 99 <sup>b</sup>	2665 $\pm$ 521 <sup>b</sup>	13814 $\pm$ 5072 <sup>b</sup>
Hg	$\mu$ g/L	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00 <sup>b</sup>	0.03 $\pm$ 0.00 <sup>b</sup>	0.24 $\pm$ 0.07 <sup>b</sup>
Mn	$\mu$ g/L	25.6 $\pm$ 6.0	1374.0 $\pm$ 365.2	1373.0 $\pm$ 227.3	468.6 $\pm$ 120.3
Mo	$\mu$ g/L	0.6 $\pm$ 0.1	0.6 $\pm$ 0.0	3.0 $\pm$ 2.2	4285.3 $\pm$ 779.1 <sup>b</sup>
Ni	$\mu$ g/L	2.06 $\pm$ 0.86	3.61 $\pm$ 1.01	2.83 $\pm$ 0.31	12.22 $\pm$ 1.36
Pb	$\mu$ g/L	0.29 $\pm$ 0.09	0.95 $\pm$ 0.17	1.35 $\pm$ 0.29 <sup>b</sup>	0.05 $\pm$ 0.02
Sb	$\mu$ g/L	0.05 $\pm$ 0.00	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01	0.69 $\pm$ 0.22
Se	$\mu$ g/L	<3.2	<3.2	<3.2	<3.2
Sn	$\mu$ g/L	0.15 $\pm$ 0.04	0.13 $\pm$ 0.00	0.12 $\pm$ 0.01	0.18 $\pm$ 0.01
Sr	$\mu$ g/L	10.03 $\pm$ 1.50	22.92 $\pm$ 0.77	34.51 $\pm$ 1.58	469.20 $\pm$ 6.96
Tl	$\mu$ g/L	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00
U	$\mu$ g/L	4.5 $\pm$ 2.4	0.8 $\pm$ 0.2	1.6 $\pm$ 0.7	2483.3 $\pm$ 230.6 <sup>b</sup>
V	$\mu$ g/L	1.07 $\pm$ 0.18	1.19 $\pm$ 0.19	3.13 $\pm$ 0.82	0.31 $\pm$ 0.04
Zn	$\mu$ g/L	2.73 $\pm$ 0.80	5.12 $\pm$ 1.06	5.05 $\pm$ 1.45	24.99 $\pm$ 38.68 <sup>b</sup>

DOC = Dissolved organic carbon

<sup>a</sup> Speciation analysis was not measured in samples so can not determine if samples exceed water quality guidelines

<sup>b</sup> Exceeded water quality guidelines as summarized in Table A.1

<sup>c</sup> Two out of three replicates < Limit of Detection (LD); therefore, no mean and standard deviation could be calculated, so concentrations listed as < LD

Table A.12. Water quality characteristics and concentrations of select elements (mean  $\pm$  SD) from sediment porewater collected from peepers exposed to the 0 - 2 cm fraction of sediment at sites near the Rabbit Lake uranium operation in 2007.

Variable	Units	Rabbit Lake Sampling Sites			
		Raven Lake	Parks Lake Outflow	Parks Lake Seepage	Horseshoe Pond
pH	-	6.4 $\pm$ 0.1 <sup>a</sup>	6.3 $\pm$ 0.1 <sup>a</sup>	6.2 $\pm$ 0.4 <sup>a</sup>	6.8 $\pm$ 0.1
DOC	mg/L	5.0 $\pm$ 1.6	8.2 $\pm$ 1.5	9.8 $\pm$ 2.6	7.5 $\pm$ 2.8
As	$\mu$ g/L	<0.18	0.14 $\pm$ 0.07	0.29 $\pm$ 0.17	5.46 $\pm$ 4.78 <sup>a</sup>
Cr <sup>b</sup>	$\mu$ g/L	1.03 $\pm$ 0.31	1.48 $\pm$ 1.47	0.77 $\pm$ 0.15	0.97 $\pm$ 0.22
Cu	$\mu$ g/L	0.17 $\pm$ 0.03	0.28 $\pm$ 0.14	0.25 $\pm$ 0.13	0.35 $\pm$ 0.17
Mo	$\mu$ g/L	0.52 $\pm$ 0.16	0.71 $\pm$ 0.45	0.44 $\pm$ 0.19	1700.75 $\pm$ 233.76 <sup>a</sup>
Ni	$\mu$ g/L	0.12 $\pm$ 0.08	1.01 $\pm$ 0.34	2.25 $\pm$ 0.27	14.73 $\pm$ 5.54
Pb	$\mu$ g/L	0.08 $\pm$ 0.02	0.04 $\pm$ 0.02	0.08 $\pm$ 0.07	0.12 $\pm$ 0.06
Se	$\mu$ g/L	<6	<6	<6	<3.6
U	$\mu$ g/L	1.72 $\pm$ 1.45	0.10 $\pm$ 0.07	0.53 $\pm$ 0.68	424.96 $\pm$ 641.53 <sup>a</sup>
V	$\mu$ g/L	0.26 $\pm$ 0.12	0.25 $\pm$ 0.04	0.36 $\pm$ 0.16	0.23 $\pm$ 0.22
<i>n</i>	-	4	3	4	4

<sup>a</sup> Exceeds WQGs as summarized in Table A.1

<sup>b</sup> Speciation analysis was not measured so can not determine if samples exceed a water quality guideline

Table A.13. Mean ( $\pm$  SD, *n* = 3) benthic invertebrate community metrics for samples collected near the Rabbit Lake uranium operation in 2007.

Metric	Rabbit Lake Sampling Sites			
	Raven Lake	Horseshoe Pond	Parks Lake Seepage	Parks Lake Outflow
Family Richness	11 $\pm$ 2	7 $\pm$ 4	7 $\pm$ 3	9 $\pm$ 2
Density (individuals/m <sup>2</sup> )	5246 $\pm$ 1750	4986 $\pm$ 2985	4580 $\pm$ 3112	7188 $\pm$ 3240
Simpson's Diversity	0.73 $\pm$ 0.03	0.33 $\pm$ 0.24*	0.57 $\pm$ 0.05	0.38 $\pm$ 0.15*
Bray-Curtis Index	0.20 $\pm$ 0.19	0.60 $\pm$ 0.06	0.56 $\pm$ 0.09	0.60 $\pm$ 0.08
Evenness	0.35 $\pm$ 0.08	0.26 $\pm$ 0.10	0.40 $\pm$ 0.24	0.19 $\pm$ 0.06

\* Statistically different (ANOVA, Dunnett's post-hoc test,  $p \leq 0.05$ ) from the reference site (Raven Lake)

Outflow were compared, there was no significant difference between the two sites for family richness, density, diversity, evenness, and Bray-Curtis indices.

## **A.5 Discussion**

Environmental quality guidelines are used as primary screening tools to evaluate the potential for an element or substance to have harmful effects on organisms and their habitat. Water and sediment quality guidelines are intended to protect aquatic life. Such guidelines are among the management tools used to monitor and prevent environmental damage to aquatic ecosystems due to anthropogenic activities, such as effluent discharge or seepages from tailings facilities. Examples of other tools used to monitor aquatic ecosystems include benthic community composition, fish tissue contaminant concentrations, fish health, *in-situ* studies, and laboratory toxicity testing.

The purpose of this study was to identify benthic invertebrate communities that were both affected and not affected by treated effluent or tailings facility seepage at two Saskatchewan uranium operations. The results from this field study would then be used in the assessment of SQGs derived by Thompson et al. (2005) (Chapter 2) and in the derivation of proposed no-effect SQGs (Chapter 3). Linking reference and no-effect benthic invertebrate communities (based on EEM metrics) with co-occurring whole-sediment metal concentrations should identify total metal concentrations that are tolerable for benthic invertebrate communities at these locations in northern Saskatchewan. As well, co-occurring overlying water and porewater samples were collected alongside benthic invertebrate community measures for additional characterization of metal exposure.

### **A.5.1 Key Lake**

Benthic invertebrate community structure can be influenced by habitat (i.e., sediment type) (Hartwell and Claflin 2005; Hartwell and Hameedi 2006). David Lake, the reference lake, had a loamy sand sediment type and was most similar to Fox Lake, whereas the other two sites had clay type sediment. Although particle size distribution can influence benthic invertebrate community composition, the differences in community composition between reference and exposure sites were most likely due to overlying water and/or sediment contamination (and the associated porewater contaminant concentrations). This conclusion is supported by the

observation that half of the invertebrate metrics calculated for Fox Lake were statistically different from the reference site, even though these sediments were physically similar. This, along with the elevated concentrations of metals in overlying water, sediment, and porewater, suggests that the benthic invertebrate community in Fox Lake was influenced by effluent from the Key Lake milling operation rather than by habitat differences. However, the observed difference in the benthic invertebrate community in Unknown Lake could have been influenced by the different sediment particle size and total organic carbon content. Based on the benthic invertebrate community metrics, Delta Lake does not appear to be adversely affected and could be classified as a no-effect site. Overall, there is a gradient of effects downstream of the effluent discharge point in the David Creek drainage which receives treated mill effluent from the Key Lake operation.

The gradient of effects does not necessarily align with exposure concentrations or the derived SQGs. Although Delta Lake was the furthest downstream, sediment concentrations of As, Mo, Ni, and Se exceeded SQGs and this lake had the highest concentration of Mo of all sites sampled. Additionally, Delta Lake peeper porewater exceeded As and Mo WQGs. This could indicate that either benthic invertebrates are able to tolerate high concentrations of Mo, or that the SQG for Mo could be overly conservative. One may therefore question the appropriateness of the WQGs and SQGs for these elements, at least when applied in this region, considering that there where no biological effects observed with these exceedences.

Potential stressors in the Key Lake drainage area varied depending on the matrix evaluated. In overlying water, pH, conductivity, ammonia, Cu, Mo, Se, and Al were notably different at exposure sites relative to the reference lake. Concentrations of As, Mo, Ni, and Se in sediment exceeded available SQGs. The pH in centrifuged porewater at all exposure sites were lower than the recommended range for the protection of aquatic life (Canadian Council of Ministers of the Environment 2011). Porewater ammonia was elevated at all exposures sites, as compared to the reference site, but was at or below available LC50s for *Hyalella azteca* (9.2 – 18 mg as N/L; Besser et al. 2009; Whiteman et al. 1996; Borgmann 1994). Centrifuged porewater exceeded WQGs for As, Cu, Mo, Ni, Se and U, whereas peeper porewater exceeded the WQGs for only As, Mo, and Se. Previous studies have also determined that sediment centrifugation tends to result in higher porewater metal concentrations relative to porewater isolated by peepers (Robertson 2006). Based on this information, metals that exceed available guideline values in all

compartments were pH, ammonia, As, Mo, and Se. These elements can be identified as the constituents of most concern in the Key Lake drainage area. This is consistent with findings from previous studies (Robertson 2006; Pyle et al. 2001; Muscatello et al. 2006; Robertson and Liber 2007).

#### **A.5.2 Rabbit Lake**

Habitat differences among sites, as mentioned above, can influence benthic community structure. Raven Lake, the Rabbit Lake reference site, had a loamy sand sediment. The sediments in Parks Lake were similar between the two sampling locations and similar to the reference site, although Parks Lake Outflow had more silt and less sand than Parks Lake Seepage and Raven Lake. Horseshoe Pond had a clay sediment type, which was a different sediment type than Raven Lake. The higher percentage of TOC, and clay and silt sized particles, could have resulted from the treated effluent which was also noted by Robertson (2006). Despite the differences in sediment composition, the influence of treated effluent (containing high concentrations of metals) may have had the greatest effect on benthic invertebrate community composition at the Horseshoe Pond sample site.

As expected, Horseshoe Pond usually had the highest metal concentrations in the various compartments analyzed and the benthic community composition reflected this. Effects on the benthic invertebrate community in Horseshoe Pond were identified based on a statistical difference in Simpson's diversity relative to the reference site. Metals of concern, based on exceedences of environmental quality guidelines, were Al, Mo, and U in overlying water; As, Cu, Mo, Ni, Pb, Se, U, and V in sediment; and As, Mo, and U in porewater (parameters that were exceeded in both methods of isolating porewater). The common metals among the different phases were Mo and U. These two metals were therefore considered to be of most concern and could be contributing to or be the cause of the observed differences in the benthic invertebrate community.

Despite the similar contaminant profiles between sampling sites in Parks Lake, and in comparison to the reference site, Parks Lake Outflow was statistically different from Raven Lake (reference) in Simpson's diversity. In a statistical analysis between the two Parks Lake sampling sites, it was found that there was no difference between the two sites in any of the metrics calculated in this study. This shows that within Parks Lake, the benthic invertebrate community

is not notably different. The conflicting evidence provided regarding the Parks Lake sites makes it difficult to draw definitive conclusions based on EEM benthic invertebrate community endpoints and contaminant exposure concentrations.

Elevated concentrations of two metals, as compared to SQGs, were found at the reference site, Raven Lake (U and V, 78 µg/g dry wt and 45 µg/g dry wt, respectively), but only V exceeded the SQG LEL. The concentrations of these metals were likely due to their natural abundance in the sediment and rock in the region. It should be noted that a uranium exploration camp was setup adjacent to Raven Lake with drilling taking place a few kilometers away. The location of the camp demonstrates that naturally elevated concentrations of uranium in the area are very likely. Despite the elevated concentrations, Raven Lake served as a suitable reference site because it is not influenced by anthropogenic activities (e.g., seepages, treated effluent) and should be reasonably representative of the benthic invertebrate communities found in this region of northern Saskatchewan.

At the sampling sites near the Rabbit Lake uranium operation, potential stressors on the benthic invertebrate community were similar to those at the Key Lake operation, but were unique in some respects. Overlying water concentrations of Al, Cu, Mo, and U exceeded WQGs at one or more of the sampling sites, with Cu the most frequently exceeding the WQG. Sediment quality guidelines for As, Cu, Mo, Ni, Se, and U were exceeded at Horseshoe Pond, whereas Raven Lake exceeded the SQG for V. Park Lake Outflow and Seepage sites had fairly similar concentrations of metals in sediment which did not exceed SQGs. Metals in centrifuged porewater which exceeded WQGs at one or more sample sites were Al, As, B, Co, Cu, Fe Mo, U, and Zn, whereas peeper porewater only exceeded As, Mo, and U. Selenium may be an issue in porewater, but due to high limits of detection, which were above the WQG, conclusions cannot be drawn regarding the possible influence of Se. Parks Lake Seepage and Horseshoe Pond had elevated levels of ammonia in porewater, but these were below concentrations likely to cause effects on benthic invertebrates (Whiteman et al. 1996). These elements and parameters are similar to the potential stressors identified for Key Lake sampling sites.

## **A.6 Conclusions**

In conclusion, effects observed on the benthic invertebrate communities near the Key Lake were likely due to, or substantially influence by, elevated sediment and porewater

concentrations of pH, ammonia, As, Mo, and Se whereas observed effects near the Rabbit Lake uranium operations were likely due to Al, As, Cu, Mo, U, and Se. This is consistent with findings from previous studies evaluating the environmental effects of these operations (Robertson 2006; Terrestrial & Aquatic Environmental Managers Ltd. 1994). In addition, concentrations of some metals in sediment and porewater at a reference or no-effect site were elevated, which was somewhat unexpected. Overall, the number of guidelines exceeded or not exceeded did not necessarily correlate with statistical differences in benthic invertebrate community structure. This somewhat calls to question the adequacy of the environmental quality guidelines used for evaluating aquatic environments.

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