QUANTIFYING AND MODELING THE BIOAVAILABILITY OF SEDIMENT-ASSOCIATED URANIUM TO THE FRESHWATER MIDGE (*CHIRONOMUS DILUTUS*)

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ABSTRACT

Uranium (U) enters aquatic environments from natural and anthropogenic sources, often accumulating in sediments to concentrations that could, if bioavailable, adversely affect benthic organisms. Current assessments of U-contaminated sediments typically rely on total U concentrations measured in the sediment, which may not be representative of the concentration of U bioavailable to benthic organisms. However, the factors and mechanisms that influence U bioavailability in sediment have not been thoroughly evaluated, despite evidence that sediment properties can influence the sorption and availability of other sediment-associated metals. The lack of detailed knowledge about the factors that modify sediment-associated U bioavailability can hinder our ability to properly use sediment quality guidelines to predict adverse effects, or lack thereof, of U-contaminated sediments to benthic communities. Therefore, the overall objective of this research was to quantify and model the key physicochemical properties of sediment that modify the bioavailability of U to a model freshwater benthic invertebrate, *Chironomus dilutus*.

To assess the influence of sediment properties on the bioavailability of U, several 10-day sediment bioaccumulation experiments were performed exposing *C. dilutus* larvae to a wide range of formulated and field-collected sediments spiked with U (5, 50, 200 or 500 mg U/kg d.w.). Bioaccumulation of U in *C. dilutus* larvae differed by over one order of magnitude when exposed to different sediments spiked with the same total concentration of U, thus total U concentrations in the sediment displayed weak or insignificant relationships with U bioaccumulation. Concentrations of U in both the overlying water and sediment pore water collected just above and below the sediment surface displayed significant positive relationships with U bioaccumulation in *C. dilutus* larvae for all experiments. Significant inverse relationships were observed between the bioaccumulation of U in *C. dilutus* larvae and key binding properties of sediment (e.g., organic matter, fine fraction or clay, cation exchange capacity and Fe content). Simple regression equations based on the physicochemical properties of sediment successfully described the bioaccumulation of U within a factor of two and provided a significant improvement in predicting the bioaccumulation of U to *C. dilutus* larvae over the use of total U concentrations in the sediment.

To further assess the influence of key binding properties of sediments on U bioavailability, the sorption of U to different field sediments was quantified in 48-h batch equilibrium experiments as a function of solution pH and U concentration. The degree of U adsorption to sediment was greatest at pH 6 and 7, and was significantly reduced at pH 8. The adsorption of U had a strong positive relationship with increasing binding properties of sediment up until a threshold [i.e., sediments with greater than 12% total organic carbon, 37% fine fraction (≤ 50 µm), or 29 g/kg of iron content], which generally corresponded with the observed reductions in U bioaccumulation.

The data from the sorption and sediment bioaccumulation experiment were further assessed using the Windermere Humic Aqueous Model, version 7.0.4 (WHAM7), which produced reliable predictions of U sorption, total U solution concentrations, free ion concentrations and species distribution of U, which are all important for determining the risk and bioavailability of U to benthic invertebrates. More importantly, the use of WHAM7 led to successful predictions of the >20-fold difference in the U accumulation in *C. dilutus* larvae observed across the wide range of physicochemical characteristics of the solid and aqueous phases examined in the present studies.

Overall, the research presented in this thesis quantified the significant influence of key physicochemical properties of sediment on U sorption and bioaccumulation. The use of simple regression equations and WHAM7 were able to better predict the bioaccumulation of U in *C. dilutus* larvae over the use of total U concentrations in the sediment. These results strongly suggest that key sediment properties such as total organic carbon, particle size distribution and iron content of sediment should be incorporated into the risk assessment and sediment quality guidelines for U in order to better predict the bioavailability of U to benthic invertebrates from U-contaminated sediments.

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°C	Degrees Celsius
α	Alpha
µm	Micrometer
µM	Micromolar
µS/cm	Micro Sieverts per centimetre
Ω	Ohm
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
AVS	Acid volatile sulphide
BLM	Biotic Ligand Model
C_0	Concentration of contaminant in initial solution
C_e	Concentration of contaminant in solution at equilibrium
CCME	Canadian Council of Ministers of the Environment
CEC	Cation exchange capacity
CNSC	Canadian Nuclear Safety Commission
CWQG	Canadian water quality guidelines
d	Day
d	Diameter
DCB	Dithionite-citrate-bicarbonate extraction method
DGT	Diffusive gradients in thin films
DO	Dissolved oxygen
DOC	Dissolved organic carbon
d.w.	Dry weight
EARMP	Eastern Athabasca Regional Monitoring Program
EC	Environment Canada
EC50	Median effect concentration
EqP	Equilibrium partitioning
FA	Fulvic acid

FIAM	Free (metal) ion activity model
g	Grams
g	Gravitational force
h	Hour
HA	Humic acid
IAEA	International Atomic Energy Agency
IC	Ion chromatography
ICP-MS	Inductively couple plasma-mass spectrometry
K_d	Sediment-solution partition (or distribution) coefficient
LC50	Median lethal concentration
LEL	Lowest effect level
m	Mass
M	Molar concentration (mol/L)
Meq/100g	Milliequivalent of hydrogen per 100 g of dry sediment (same as cmol/kg)
mg/kg	Milligram per kilogram
mg/L	Milligrams per liter
min	Minute(s)
ml	Milliliter
mM	Millimolar (mmol/L)
mol	Mole
n	Sample size
NEA	Nuclear Energy Agency
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
OM	Organic matter
PNEC	Probable no-effect concentration
r	Correlation coefficient
R^2 or r^2	Correlation of determination
Rho	density (g/cm^3); ρ

RMSE	Root-mean-square error
SA	Surface area (m^2/g)
SD	Standard deviation
SE	Standard error
SEL	Severe Effect Level
SEM	Simultaneous extracted metals
SQG	Sediment Quality Guidelines
SLC	Screening Level Concentration
SSR	Solid-to-solution ratio
STIR	Sediment testing intermittent renewal
SWI	Sediment-water interface
TOC	Total organic carbon
U	Uranium
U.S. EPA	United States Environmental Protection Agency
V	Volume
WHAM	Windermere Humic Aqueous Model
w.w.	Wet weight

NOTE TO READERS

This thesis is organized and formatted to follow the University of Saskatchewan College of Graduate Studies and Research guidelines for a manuscript-style thesis. Chapter 1 is a general introduction and literature review, including the project goal and objectives, and Chapter 6 contains a general discussion and conclusions tying the chapters together. Chapters 2, 3, 4 and 5 of this thesis are organized as manuscripts for publication in peer-reviewed scientific journals. Chapter 2 has been published in Science of the Total Environment, Chapter 3 has been published in Chemosphere, Chapter 4 was submitted to Environmental Pollution, and Chapter 5 will be submitted to Environmental Toxicology and Chemistry. Full citations for the published research manuscripts are provided below. As a result of the manuscript-style format, there is some repetition of material in the introduction and materials and methods sections of the thesis. The tables, figures, supporting information and references cited in these research chapters have been reformatted here to a consistent thesis style. References cites in each chapter are combined and listed in the References section of the thesis. Supporting information associated with research chapters are presented in the Appendix section at the end of this thesis.

The content of Chapter 2 was reprinted (adapted) from Science of the Total Environment, (http://dx.doi.org/10.1016/j.scitotenv.2015.05.116). S. Crawford and K. Liber, "Effects of clay minerals and organic matter in formulated sediment on the bioavailability of sediment-associated uranium to the freshwater midge, *Chironomus dilutus*" 532: 821-830. Copyright (2015), with permission from Elsevier.

The content of Chapter 3 was reprinted (adapted) from Chemosphere, (http://dx.doi.org/10.1016/j.chemosphere.2015.12.123). S. Crawford and K. Liber, "Sediment properties influencing the bioavailability of uranium to *Chironomus dilutus* larvae in spiked field sediments" 148: 77-85. Copyright (2016), with permission from Elsevier.

The author contributions for each research chapter included:

> S.E. Crawford (University of Saskatchewan) managed and conducted all research and field sampling, performed all chemical, statistical and modeling analysis, and drafted each of the manuscripts.

Dr. Karsten Liber (University of Saskatchewan) provided scientific guidance and input, manuscript review, comments and editorial corrections, as well as funding and direction for all of the research.

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CHAPTER 1:
GENERAL INTRODUCTION

1 Introduction

The projected increase in demand for nuclear energy production is expected to result in the increased mining and milling of uranium (U) from areas with rich deposits (Environment Canada, 2003; IAEA, 2009). The mining and milling of U ore and associated processing operations, nuclear fuel production, power station discharge and waste management and treatment processing can lead to potential releases of U to the environment (CCME, 2011; Environment Canada, 2003; OECD-NEA and IAEA, 2014). In particular, aquatic environments surrounding U mine and mill activity are the most likely to receive inputs of U from U tailings and treated effluent (Environment Canada, 2003; Trenfield et al., 2011). Thus, concentrations of U in aquatic environments have the potential or have been observed to cause immediate or long-term adverse effects to aquatic organisms (Environment Canada, 2003; Liber et al., 2011; Alves et al., 2008; Antunes et al., 2007). In order to protect aquatic organisms from U contamination, it is necessary to accurately assess the fate and availability of U in the aquatic environment.

1.1 Properties, sources and occurrence of uranium in the aquatic environment

Uranium is a member of the actinide series of elements and has an atomic number of 92. It has 10 radioactive isotopes, but has been found to be more chemotoxic than radiotoxic to aquatic organisms (Alves et al., 2008; Bleise et al., 2003; CCME, 2007; Environment Canada, 2003; Lagauzère et al., 2009c; Li et al., 2006; Zeman et al., 2008). Uranium occurs in 4 oxidative states (+3, +4, +5, +6), however, the hexavalent state (i.e., UO_2^{2+}) is the most stable and most common form in oxic waters (Alves et al., 2009; CCME, 2007; Sheppard et al., 2005). Uranyl ions also readily form complexes with carbonate, phosphate, and sulphate ions, which increase solubility allowing for easy transport and possible accumulation of U in aquatic organisms. The hexavalent uranyl (UO_2^{2+}) and hydroxouranyl (UO_2OH^+) ions are the U species found to be most responsible for the harmful effects of U in aquatic organisms (Alves et al., 2009; Environment

Canada, 2003; Markich, 2002). In contrast, the tetravalent state (U^{4+}) is predominant under reducing conditions, such as in anoxic water and sediment, which results in a strong tendency for U to adsorb to sediment binding phases and precipitate, making U more immobile (Sheppard et al., 2005).

Natural sources of U can arise from the weathering and erosion of concentrated deposits of U found around the world. In contrast, anthropogenic sources of U are primarily associated with the nuclear fuel cycle and accompanying mining and milling of U, but other sources of U can include the combustion of fossil fuels, the manufacturing and application of phosphate fertilizers, and military use of U (CCME, 2011; Environment Canada, 2003; Markich, 2002). As a result, a variety of chemical forms of U are present in soil, sediment, water, and food (Bleise et al., 2003). The richest deposits of U discovered to date are located in the Athabasca region of northern Saskatchewan, Canada (CCME, 2007; Painter et al., 1994; World Nuclear Association, 2016). Northern Saskatchewan is home to several of the world's largest U mining and milling operations, producing approximately 15% of the global U supply (OECD-NEA and IAEA, 2014). Due to the rich deposits of U and geochemistry of these areas, natural enrichment of U may result in exceedances of environmental quality guidelines (where they exist), which does not necessarily mean that ecosystems are in jeopardy (CCME, 2007).

Background median aqueous concentrations of U in areas such as northern Saskatchewan are generally in the range of 0.05-0.35 µg/L upstream of U mines, while median sediment concentrations range between 3.7-29 mg/kg dry weight (d.w.) (Environment Canada, 2003). These aqueous concentrations of U are below the Canadian water quality guidelines (WQGs) for the protection of aquatic life from short-term (33 µg/L) and long-term (15 µg/L) exposures of U (CCME, 2011). However, anthropogenic activities such as mining and milling have been shown to significantly increase U concentrations in the environment, particularly in downstream receiving environments (Alves et al., 2009; Antunes et al., 2007; Baborowski and Bozau, 2006; Lagauzère et al., 2009c; Liber et al., 2011; Saari et al., 2008). Elevated U concentrations, particularly from historical legacy contamination, have been reported to range from 100 to over 500 µg/L in surface waters and to exceed 1,000 mg/kg d.w. in sediments downstream of U mining and milling operations in northern Saskatchewan (Environment Canada, 2003; Hart et al., 1986; Hynes et al., 1987; Neame et al., 1982). Such concentrations of U downstream from mines have the potential to impact aquatic organisms.

1.2 Fate and behaviour of uranium in the aquatic environment

The environmental fate of U, similar to that of other metals, is determined by chemical speciation and sorption processes, which are affected by physicochemical characteristics of the water and sediment, as well as the metal (Alves et al., 2008; Environment Canada, 2003; Lofts et al., 2015; Reiller et al., 2011; Sheppard et al., 2005). The speciation of U is likely to affect toxicity and is dependent upon pH as uranyl ions form stable hydroxide or carbonate complexes under acidic and alkaline conditions, respectively. Previous chemical speciation models used with data from northern Saskatchewan U mining areas (e.g., Beaverlodge site) predicted that 98% of U was present as uranyl-carbonate species in slightly alkaline waters with less than 0.5% of U in the form of the more harmful species, UO_2^{2+} and UO_2OH^+ (Environment Canada, 2003). In comparison, only 15% of U was complexed by carbonate species in poorly buffered and slightly acidic water bodies (e.g., at the Key Lake mine in northern Saskatchewan), with a greater proportion (50-60%) of U present in the form of uranyl sulphate (UO_2SO_4) complexes, due to the high sulphate content of the mill effluent (Environment Canada, 2003). Additionally, increasing the acidity of poorly buffered water bodies with the addition of U effluent can also result in greater amounts of harmful U species, UO_2^{2+} (17-29%) and UO_2OH^+ (4-17%), than would typically be found under well-buffered, circumneutral or alkaline conditions (Environment Canada, 2003). Acidic pH conditions of the overlying water have also been reported by Alves et al. (2008) to favour the formation of harmful U species, which corresponded with greater U toxicity in *Hyalella azteca*. However, acidic pH conditions have also been observed to reduce toxicity of U to *Chironomus crassiforceps*, which may be associated with the counteracting effects of increased competition of H^+ ion with uranyl ions for binding sites on sediment surfaces with lower pH (Fortin et al., 2004; Markich, 2002; Peck et al., 2002). Thus, it is evident that pH can influence the fate and proportion of harmful U species, but the mechanisms by which toxicity occurs for various U species to different organisms are not fully understood.

The fate of U can also be influenced by water hardness. The presence of Ca^{2+} and Mg^{2+} have been shown to reduce the toxicity of U in some cases, likely due to the competitive inhibition of U uptake at cellular surfaces (Environment Canada, 2003; Sheppard et al., 2005). A reduction in toxicity with corresponding increases in water hardness is well established for many metals (Cd, Cu, Ni, Pb, and Zn), however, there is a lack of consistent data relating hardness-reduced uptake and toxicity for U. For example, Alves et al. (2008) found that Ca^{2+} had little effect on the uptake of U, despite the influence of Ca^{2+} on U speciation. The inconsistencies reported in the literature may be the result of confounding effects associated with distinguishing between water hardness and alkalinity, measured as calcium carbonate concentrations, as well as the concurrent effects of pH and H^+ concentrations. The complexation of U with carbonate has been found to alter U speciation, reducing the concentration of harmful U species (Ames et al., 1983; Echevarria et al., 2001; Goulet et al., 2015; Sylwester et al., 2000). Although not consistent, it is evident that hardness, and alkalinity, or a combination of both, may affect U availability and toxicity to some aquatic organisms.

Many of the same water chemistry variables that affect U speciation can also affect the sorption of U to sediment solid phases in aquatic systems. Several studies have suggested that dissolved carbonate concentrations and pH are the two most important factors influencing the sorption behaviour of U in soils (Echevarria et al., 2001; Pandit et al., 2012; US EPA, 1999; Vandenhove et al., 2009a; Vandenhove et al., 2009b). The increased U solubility associated with increasing carbonate content results from the formation of negatively charged uranyl-carbonate complexes that have a lower sorption affinity to sediment (Echevarria et al., 2001; Markich, 2002; Pandit et al., 2012). The influence of pH are predominantly due to the associated changes in U speciation. In general, at pH values less than 3, sorption of U to sediment is low (U present as uranyl ions), followed by a rapid increase in sorption with increasing pH from 3 to 5. Additionally, as conditions become more acidic the number of exchange sites on variable charged surfaces to which U could bind (e.g., iron oxides and organic matter) decrease (Pandit et al., 2012). Maximum U sorption is reached at pH 6 to 7 (uranyl ions hydrolyze forming aqueous hydroxide complexes which are influenced by the absence of dissolved inorganic ligands), and sorption is observed to significantly decrease with increasing pH values above 7 due to the predominance of weak-adsorbing U species (i.e., highly soluble carbonate complexes predominate) (Langmuir, 1978; Pandit et al., 2012; US EPA, 1999; Vandenhove et al., 2009a; Vandenhove et al., 2009b). Overall, there are a number of studies available in the literature that have examined the effects of water chemistry on the fate and behaviour of U (CCME, 2011), but very few studies have assessed the influence of physicochemical characteristics of the sediment on U behaviour in aquatic environments. The role of sediment is particularly important as U generally favours adsorption to soil and sediment particles, with sediment-solution partition coefficients of up to 1×10^6 ml/g reported under various test and field conditions (Echevarria et al., 2001; Pandit et al., 2012; US EPA, 1999). Sorption of U to sediment and particulate phases can directly influence the mobility and fate of U in aquatic environments.

1.2.1 Sediment as a sink and source of uranium

The amount of U in aquatic environments surrounding U mining and milling operations can occasionally exceed ecologically safe concentrations (Hart et al., 1986; Hynes et al., 1987; Lagauzère et al., 2009c; Liber et al., 2011). Since U does not degrade, the tendency of U to accumulate in sediment can result in a long-term environmental risk, particularly for historical legacy contamination. The adsorption and accumulation of U in sediment is a result of U surface complexation processes and interactions with mineral coatings associated with sediment particles. After a metal is bound to a particle surface or sorbed into the interior matrix, biotransformation and desorption of the metal is usually slow (Burton, 2002). Therefore, sediment can often act as a sink for U-contamination, resulting in concentrations in the sediment that are often several orders of magnitude greater than those in the water column (Faria et al., 2007; Simkiss et al., 2001). The U may not always be bound irreversibly to sediment and may redistribute into the overlying water as a result of various processes, such as bioturbation or resuspension of sediment. The partitioning of U to the aqueous phase may increase the availability of U for uptake and assimilation by various aquatic organisms (Burton Jr, 1991; Faria et al., 2007; Lagauzère et al., 2009a). Furthermore, sediment-associated U may pose a threat to biota that live in or on the sediment at the sediment-water interface (SWI) through the assimilation or ingestion of U directly from the sediment phase, as well as U associated with sediment pore water (i.e., interstitial water) and the immediate overlying water. Thus, it is important to assess the toxicity of U-contaminated sediment to aquatic organisms.

1.3 Assessment of U-contaminated sediment toxicity

The assessment of U-contaminated sediments generally involves the measurement of total or near-total U concentration in the sediment from either U-spiked sediments or from U-contaminated whole field sediments. In laboratory studies sediments are often spiked with uranyl nitrate, uranyl sulphate or uranyl acetate, and aged for an appropriate period of time to allow for pseudo-equilibrium to occur between the sediment, pore water and overlying water. Sediments can consist of either field or formulated (also called reconstituted, artificial or synthetic) sediment that can vary in composition (e.g., organic matter and particle size), geochemical characteristics (e.g., mineral coatings), and U concentrations. The Organisation for Economic Co-operation and Development (OECD) guidelines recommend the use of formulated sediments over field sediments in spiked sediment toxicity tests, as they provide a reproducible "standardized matrix", are readily available (e.g., no time, seasonal, or storage constraints), free of contaminants and indigenous organisms, and reduce the cost associated with collecting field sediments (OECD, 2004). Formulated sediments have also been used as suitable alternatives for the use of field sediments in toxicity tests (ASTM, 1995; Höss et al., 2010; Kemble et al., 1999; OECD, 2004; Roman et al., 2007; Suedel and Rodgers Jr, 1994a; Suedel et al., 1996). For example, Suedel and Rodgers Jr. (1994) observed similar survival and growth of several aquatic organisms exposed to both field sediments and formulated sediments. The formulated sediments were prepared to mimic the field sediments with respect to physical and chemical compositions through the use of plant material, manure and peat moss, and varying quantities of kaolinite clay, montmorillonite clay and quartz sand. However, the use of field sediments can be equally appropriate for the assessment of U-contaminated sediments as they offer the ability to investigate site-specific or ecologically relevant conditions. Field sediments can be tested either *in-situ* (e.g., caged experiments) at contaminated sites, or as field-contaminated whole sediments in the laboratory under controlled test conditions. Field sediments collected from reference sites can be spiked with known concentrations of U for testing, or can be used as controls for additional U-contaminated field sediments collected for testing in the laboratory. However, it can sometimes be difficult to obtain appropriate uncontaminated reference sediments from areas near the target sites that have similar sediment properties to serve as controls, which can be a limiting factor in interpreting the results obtained from sediment tests (OECD, 2004). For example, the survival and growth of some aquatic organisms can be influenced solely by the particle size and organic matter content of uncontaminated formulated and field sediments (Sibley et al., 1997a; Suedel and Rodgers Jr, 1994b). Overall, the use of field and formulated spiked sediments and field-contaminated sediment each have their advantages and will depend on the study objectives and design.

1.4 Toxicity of uranium-contaminated sediment

A number of investigations and reviews are available regarding the toxicity of aqueous U to aquatic organisms (Alves et al., 2009; CCME, 2007; Environment Canada, 2003; Hynes et al., 1987; Liber et al., 2004; Markich et al., 2000; Markich, 2003; Muscatello and Liber, 2009; Muscatello and Liber, 2010; Pyle et al., 2001; Pyle et al., 2002; Robertson and Liber, 2007; Sheppard et al., 2005). However, little information is available on the toxicity of U-contaminated sediment, particularly with regard to effects on sediment-dwelling organisms. Sediment-associated U has the potential to adversely impact benthic communities, which can have adverse consequences for ecological processes and food-web dynamics. Benthic invertebrates have a high potential for exposure to U-contaminated sediment due to their close contact with sediment. Exposure of benthic invertebrates to sediment-associated U can occur via the aqueous phases at the SWI (e.g., overlying water and pore water) and/or from exposure and ingestion of U-contaminated food and sediment particles.

It is common to use benthic invertebrates as test organisms in sediment toxicity tests for contaminants that persist in the sediment, such as U. The freshwater dipteran *Chironomus* species (*C. dilutus* and *C. riparius*) are often the organism recommended for use in toxicity testing, and subsequently, standard culturing and testing protocols are readily available (ASTM, 1995; Environment Canada, 1997; OECD, 2004). The use of chironomids is beneficial as contact with sediment occurs throughout larval development, which is often the most sensitive life stage for many organisms exposed to contaminants (Giesy and Hoke, 1989). Furthermore, *C. dilutus* is a common species throughout North American freshwater environments, including U mining areas in northern Saskatchewan (Benoit et al., 1997; Muscatello and Liber, 2009; Wiramanaden et al., 2010). Thus, sediment toxicity tests, in which benthic organisms, including chironomids, are exposed to field-contaminated sediment or U-spiked sediment, act as an essential tool for evaluating the potential hazard of sediment-associated U (ASTM, 1995; Höss et al., 2010; Ingersoll et al., 1995; OECD, 2004). However, U has been poorly investigated in toxicological studies using freshwater benthic invertebrates, particularly with respect to the toxicity associated with sediment-associated U contamination.

A review of the limited literature available on the toxicity of U-contaminated sediments to benthic invertebrates is presented in Table 1.1. Early stages of benthic invertebrates have been observed to display greater sensitivity to U-spiked sediment than adults (e.g., *Hyalella azteca*; BEAK International Inc., 1998 cited in Environment Canada, 2003). Dias et al. (2008) reported significant adverse effects on mortality, development time, and growth of *C. riparius* larvae at

Table 1.1 Toxicity data for freshwater benthic invertebrates exposed to U-spiked sediments.

Authors	**Species**	**Endpoint**	**Effect concentration (mg U/kg d.w. sediment)**
BEAK International Inc. (1998) in Environment Canada (2003)	*H. azteca* (juvenile)	LC_{50} 14 d	57
	H. azteca (adult)	LC_{50} 14 d	436
Dias et al. (2008)	*C. riparius*	LC_{50} 10 d	5.3
Lagauzère et al. (2009a)	*T. tubifex*	LC_{50} 12 d	2,320
		EC_{50} 12 d growth	547
	C. riparius	LC_{50} 12 d	856
		EC_{50} 12 d growth	787
Lagauzère et al. (2009c)	*T. tubifex*	LC_{50} 12 d	2,910
		EC_{50} 12 d autotomy*	3,580
Liber et al. (2011)	*C. dilutus*	LC_{50} 10 d	10,551
		EC_{50} 10 d growth	2,695
	H. azteca	LC_{50} 10 d	2,442
		EC_{50} 10 d growth	1,918
Peck et al. (2002)	*C. crassiforeps*	LC_{50} 72 h	> 5,000

*Autotomy – An effect measured by the severing of caudal parts.

6.07, 6.07 and 2.97 mg U/kg d.w., respectively. Additionally, Liber et al. (2011) demonstrated that growth of chironomids was a sensitive endpoint appropriate for U-spiked sediment tests. A reduction in larval growth could result in reduced adult emergence for chironomid species, with implications for reproductive output (Muscatello and Liber, 2009).

For the few studies conducted with U-contaminated sediment, a wide range of effect concentrations have been reported for various benthic invertebrates (Table 1.1). A 10-d LC_{50} of 5.03 mg U/kg d.w. was observed for *C. riparius* larvae from U-spiked formulated (88% silica sand and 12% α-cellulose) sediment (Dias et al., 2008), while Liber et al. (2011) reported a 10-d LC_{50} of 10,551 mg U/kg d.w. for *C. dilutus* larvae in U-spiked field sediment (57% sand, 43% fine fraction and 7.4% TOC). Additionally, Peck et al. (2002) reported no sublethal effects for any of the U-spiked sediment concentrations tested (up to 5,000 mg U/kg d.w.) on *C. crassiforeps* in field sediment (8% TOC, 13 meq/100 g). The different responses to U observed among the studies and organisms presented in Table 1.1 may be a result of different species sensitives to U. Previous species sensitivities, *Hyalella azteca* ≥ *C. riparius* > *T. tubifex*, have been observed for sediments contaminated with Cu, Cd, and Ni (Milani et al., 2003). However, the differences could also be the result of the various experimental approaches and conditions, as well as the parameters investigated (e.g., sediment type), all of which may affect the availability and toxicity of U to benthic organisms.

The physicochemical characteristics of any sediment used in toxicity tests should be ecologically relevant, while also remaining within a range shown to support the survival and growth of test organisms. It has been previously shown that physicochemical properties themselves can affect test organisms directly and result in the misinterpretation of toxicity data (Ankley et al., 1993a; Ankley et al., 1994a; Höss et al., 2010; Sibley et al., 1997a; Suedel and Rodgers Jr, 1994b). Additionally, physicochemical properties and composition of sediment can directly influence the toxicity of metals and alter the interpretation of results. For example, in a study by Campana et al. (2013), silty sediments with greater organic carbon content resulted in a significant improvement (8-fold) of the EC_{50} for bivalve growth when compared to sandy sediments with lower organic carbon spiked with the same total Cu concentration. From the same study, bivalve survival was better predicted from Cu concentrations normalized to the organic carbon content of the < 63 µm fine fraction of sediment than by the total Cu concentration in the sediment (Campana et al., 2013). Therefore, all important parameters affecting U toxicity, including the choice of sediment (i.e., field and formulated) and the composition of sediment utilized in a test should be measured and reported to enhance the utility of the toxicity results.

1.5 Sediment Quality Guidelines (SQGs)

Benthic community health can be adversely impacted by the contamination of sediment with U. Adverse effects to benthic communities are typically assessed through the use of biological effect data and can be an indication of ecosystem health (CCME, 1999). Thus, sediment quality guidelines (SQGs) in Canada, or similar criteria elsewhere, have been developed to provide threshold values for total concentrations of individual contaminants, which if not exceeded, are expected to protect benthic invertebrate communities and associated food chains (Burton, 2002; CCME, 1999; Chapman et al., 1998). Many SQG approaches are derived using statistical methods based on empirical data that establish associations between the co-occurrence of total concentrations of a metal in sediment and adverse biological effects on benthic communities (CCME, 1999; Thompson et al., 2005). No assumptions are made about cause-and-effect and the specific mechanism of toxic action (Burnett-Seidel and Liber, 2012; Thompson et al., 2005). Sediment quality guidelines often serve as a screening tool for management decisions, reinforcing that the quality of data required for the derivation and use of SQGs are important to ensure their appropriateness and effectiveness in protecting benthic communities.

The Screening Level Concentration (SLC) approach is currently used to develop region-specific SQGs for use in regulating metals (As, Cr, Cu, Pb, Mo, Ni, Se, U, and V) and radionuclides (Ra-226, Pb-210, and Po-210) in sediment surrounding U mining and milling operations in Canada, including northern Saskatchewan. The SLC approach was implemented by the Canadian Nuclear Safety Commission (CNSC) to predict the adverse effects of U-contaminated sediment, or lack thereof, to benthic communities based on the lowest effect level (LEL; 104 mg U/kg d.w.) and the severe effect level (SEL; 5874 mg U/kg d.w.) derived for U (Thompson et al., 2005). No adverse effects on a benthic community are expected for total U concentrations in the sediment that are below the LEL value for U, whereas exceedances of the SEL indicate a high probability of an adverse effect of U on the benthic community (i.e., reduction in abundance and species richness ≥ 40 %; Thompson et al., 2005). The 56-fold gap between the LEL and SEL is often where adverse effects of U on benthic communities, or lack thereof, are most inaccurate and difficult to predict. For example, an evaluation of the SLC approach by Burnett-Seidel and Liber (2012) reported a high number of exceedances of the lowest effect levels (LELs) for U in sediments from both reference and no-effect sites, indicating an increased probability of adverse effect being predicted when there were none observed (false-positives). Furthermore, for many SQG approaches, including the SLC, it is difficult to account for factors that modify the availability of contaminants, or to distinguish between effects of co-contaminants and individual contaminants. Sediment quality guidelines can thus result in contaminants that may be classified as "guilty by association" (e.g., falsely classified as the cause of toxicity) (Burnett-Seidel and Liber, 2012). Consequently, many SQGs have been criticized for overlooking the important role of contaminant bioavailability and the modifying factors that control the behaviour of contaminants in aquatic ecosystems (Ahlf et al., 2009; Borgmann, 2000; Burton, 1995; Burton, 2002; Meyer, 2002). Sediments are not always homogenous and can vary significantly even within a specific site. The differences in types of sediments have been shown to influence the concentrations, bioavailability, and fate of metals in sediments (Bjørgesæter and Gray, 2008; Burton, 1995; Echevarria et al., 2001; Sheppard and Evenden, 1992). Thus, it is crucial that sediment characteristics that modify the bioavailability of sediment-associated U be thoroughly investigated to improve U-SQGs and the assessment of U-contaminated sediments.

1.6 Bioavailability of uranium

Bioavailability is the amount of a contaminant that is available for uptake by an organism, such that not all of a contaminant present in the environment necessarily contributes to bioaccumulation or toxicity. For this reason, it is widely recognized that bioavailability influences metal toxicity in aquatic environments, and the importance of understanding the bioavailability of sediment-associated U toxicity is becoming more apparent. However, regulatory guidelines and research often focus on water-only exposures for U. This can be unrepresentative of U exposure as there is always a continuous interaction between U in aqueous phases (overlying water, pore water, and groundwater) and solid phases (sediment, suspended matter) in a typical dynamic aquatic system (Ahlf et al., 2009; Atkinson et al., 2007; Simpson et al., 2005). Relevant exposure pathways for U, similar to other metals, include concentrations of U in the dissolved phases, U-complexes, particle bound-U, as well as U through dietary sources

(Ahlf et al., 2009; Lagauzère et al., 2009c; Muscatello and Liber, 2010; Worms et al., 2006). Reactions with different binding sites on the biological surfaces of organisms can also play a role in modifying U bioavailability.

Recently, studies have begun to describe toxicity of contaminants to aquatic organisms through the use of metal bioavailability rather than total metal concentrations from the sediment (Ahlf et al., 2009; Allen and Janssen, 2006; Ankley et al., 1996c; Batley et al., 2004; Borgmann, 2000; Burton, 2010). In some instances, the bioavailability of metals (measured through bioaccumulation or toxic responses in organisms) have been observed to correlate better with dissolved concentrations of metals in pore water than with that of total metal content in whole-sediment or particulate-bound metals (Ahlf et al., 2009; Ankley et al., 1994b; Atkinson et al., 2007; Burton, 2010; Chapman et al., 1998; Doig and Liber, 2006; Liber et al., 2011; Prica et al., 2010). As a result, a great amount of attention has been given to free ion concentrations (e.g., free ion activity model; FIAM) as a relevant exposure pathway and thus an indication of metal bioavailability. Free ion activity of metals is influenced not only by geochemical conditions, but also by metal-particle interactions and organism activity (i.e., metabolism and bioturbation) (Ahlf et al., 2009; Ankley et al., 1996a; Borgmann, 2000; Campbell, 1988; Eggleton and Thomas, 2004; Lagauzère et al., 2009a). Bioaccumulation of U has also been suggested as a more reliable indicator of U toxicity and bioavailability than U concentration in water or sediment (Alves et al., 2008; Borgmann, 2000; Muscatello and Liber, 2009).

Although much progress has been made in understanding bioavailability, the modifying effects of sediment characteristics on U bioavailability have been studied and quantified less frequently than the effects of water chemistry conditions (e.g., pH, hardness, alkalinity, and dissolved organic matter). The degree of toxicity exhibited by U-contaminated sediments is influenced by the degree of adsorption of U to sediment solid phases, which will modify the potential exposure and bioavailability of the U to biota (Di Toro et al., 1990). Quantifying the bioavailability of U is therefore important as the concentration of U that is harmful in one type of sediment might have no impact in another type of sediment. Identifying and examining relevant sediment characteristics that influence the partitioning and interaction of U between the solid and aqueous phases will help enhance our understanding of U bioavailability for use in risk assessment of U-contaminated sediments.

1.7 Influence of sediment characteristics on uranium bioavailability

Sediment characteristics, pH, and the properties of a metal are largely responsible for governing the partitioning and fate of sediment-associated metals in freshwater systems and, as a result, affect the bioavailability of metals to aquatic organisms. Sediment properties can influence sorption, precipitation, solubility, and availability of metals in aquatic systems because sediments offer many sites for metal interaction. Sediment characteristics have been investigated with respect to their role in altering the chemical fate and mobility of U in the environment, but with little consideration of the modifying effects on U bioavailability to freshwater organisms. There are a number of physicochemical characteristics of sediment that modify the sorption and bioavailability of other metals, which may be of importance to U bioavailability, as discussed below.

1.7.1 Clay minerals

Clay minerals are hydrous aluminosilicate minerals of soils, sediments, rocks and water that are < 2 µm in size (e.g., often colloids). Clays contain a number of exchangeable cations and anions on their surfaces that can be readily exchanged with metal ions through ion exchange, coordination, or ion-dipole interactions (Bhattacharyya and Gupta, 2008). Both strong and weak interactions can also result from H-bonding, van der Waals interactions, or hydrophobic bonding, depending on the type of structural or other features of the clay mineral (Bhattacharyya and Gupta, 2008). Thus, clay minerals play an integral part in the partitioning of metals between the aqueous and solid phases in sediment. Uranium has been observed to adsorb to clay mineral surfaces at both fixed-charge and variably-charge sites (Ames et al., 1983; Bostick et al., 2002; Catalano and Brown Jr, 2005; Chisholm-Brause et al., 1994; McKinley et al., 1995; Pabalan and Turner, 1996), which may decrease U solubility in the aquatic environment and in turn decrease U bioavailability.

The type and content of clay minerals in sediment can often differ across field sites, such that an investigation of one clay mineral may not be representative of clays in sediment of another site. Additionally, clay minerals used in formulated sediments may not always be representative of site-specific or natural, ecologically relevant sediments. Standard formulated sediments are typically prepared with kaolin (kaolinite) clay as recommended by OECD (2004), but montmorillonite clay is the predominant clay mineral found in sediments and soils around U mines in northern Saskatchewan, followed by illite, kaolinite and dickite, with minor amounts of chlorite (Drits et al., 1993). Differences in the distribution and occurrence of clay minerals are particularly important as different clay minerals have been observed to have different affinities for adsorbing metals. For example, Wang et al. (2011) found the following trend in U adsorption among different types of soils: 6-line-ferrihydrite > North Carolina chlorite ≈ California clinochlore > quartz ≈ Michigan chlorite > illite > montmorillonite. The differences in clay minerals are usually based on the physicochemical properties of clay (Table 1.2). The binding capacities of clay minerals are usually determined by cation exchange methods, as it is not simply the chemistry of their exposed surfaces that are the driving force for binding, but also the charge deficiencies within the solid phase from isomorphic substitution (Horowitz, 1985; Luoma and Davis, 1983). Thus, an investigation of the various binding capacities of different clay minerals is important, as the differential adsorption of U may alter the amount of U available to aquatic biota.

1.7.2 Particle size distribution

The effects of particle size on the partitioning of many metals in sediment have been well established (Eggleton and Thomas, 2004; Horowitz, 1985), but not specifically for U. Particle size is one of the most important factors controlling the capacity of both suspended- and bottom-sediments in adsorbing and retaining metals. However, it is difficult to examine influences of particle size without considering the associated effects of particle surface area and associated surface chemistry. For example fine-sized particles, such as clay minerals, offer major sites for surface adsorption and complexation of U (Catalano and Brown Jr, 2005; Chisholm-Brause et al., 1994; Wang et al., 2011), which also correspond with greater surface area (Table 1.2). Greater surface area allows for greater adsorption, catalysis, precipitation, microbial colonization, and other surface phenomena. In a study by Höss et al. (2010), it was found that the variability in responses of some organisms exposed to metals in sediment toxicity tests were associated with differences in both particle size distribution and organic matter content. Similar responses may be observed for U as extractable concentrations of U from sediment have been significantly correlated with clay content (Sheppard and Evenden, 1992), suggesting that U may be less readily bioavailable with greater contents of clay.

Table 1.2 Summary of properties of four common clay minerals; kaolinite, montmorillonite (only expanding type), illite and chlorite. Adapted from Horowitz (1987) and Bhattachryya and Gupta (2008).

Property/Mineral	**Kaolinite**	**Montmorillonite**	**Illite (Hydrous Mica)**	**Chlorite**
Layer Structure	1:1	2:1	2:1	2:1:1
Bonding	Strong	Very weak	Strong	Moderately strong
Shrink-swell capacity	Very low	High	Low	None
CEC (meq/100 g)	1-15	80-150	20-30	10-40
Specific surface area (m^2/g)	5-20	700-800	50-200	--
Basal spacing (nm)	0.72	0.98-1.8+	1.0	1.4

CEC – cation exchange capacity.

Particle size integrates most other factors controlling the adsorption of metals to sediment, including surface area, CEC, surface charge, and increasing concentrations of geochemical substrate (Horowitz, 1985). A very strong positive correlation has been observed between decreasing particle size and increasing metal concentrations due to the sorptive nature and large reactive surface area of fine-sized sediments (Eggleton and Thomas, 2004; Horowitz, 1985; Sutherland, 2003; Zhong and Wang, 2008). However, some studies have found that coarse particles can also accumulate high concentrations of metals (Stone and Marsalek, 1996; Whitney, 1975). This may be the result of the metal oxide coatings often associated with larger particles, which can act as an "adsorber" of metals (Whitney, 1975). Regardless, greater adsorption of metals to sediment suggest that the bioavailability of metals may be reduced in aquatic systems. However, aquatic ecosystems are dynamic with continuous sediment-water interactions that may result in the release or increased partitioning of U into the aqueous phase, suggesting that particle size distribution may play an important role in U bioavailability.

1.7.3 Organic matter

Organic matter (OM) in freshwater sediments consist of autochthonous primary production, microorganisms and their exudates, and allochthonous material entering from terrestrial environments (Luoma and Davis, 1983). Despite the various sources and different amounts of OM entering a system, some generalizations can be made about the binding properties of OM, present as either coatings on inorganic particles or as particulate OM (Burton, 2002; Horowitz, 1985; Zhong and Wang, 2008). Organic matter can adsorb metals by surface area, CEC, surface charge, and physical trapping via weakly acidic functional groups, primarily carboxylic and phenolic groups that are involved in the complexation of metals (Horowitz, 1985; Luoma and Davis, 1983). Humic substances, such as humic acids (HA) and fulvic acids (FA) are important organic materials in sediment that can have considerable effects on metal adsorption due to their reactive functional groups (Doig and Liber, 2007; Zhong and Wang, 2008). For example, the range of chelating functional groups (carboxyl, thiol) associated with humic substances act to reduce free ion concentrations and bioavailability of Ni to *Daphnia magna* (Cloran et al., 2010). Peat moss is an additional type of OM consisting primarily of lignin and cellulose, and due to its polar nature has been observed to adsorb metals to a great extent (Crist et al., 1996; Qin et al., 2006; Tipping, 1998; Zhirong and Shaoqi, 2010). Peat moss is a large component of the organic substrate found in wetland regions of northern Saskatchewan and is the

OM constituent recommended for use in formulated sediments by OECD (2004) (Dunn, 1981; Leventhal et al., 1987; Zielinski and Meier, 1988).

Since OM plays an important role in the adsorption and binding of metals, the amount of OM present in sediment, or the lack thereof, may greatly determine the proportion of free metal ions present in the aqueous phases (i.e., overlying and pore water). The content of sediment OM has been shown to be predictive of biological effects from metals in the absence of other sediment binding phases (Bjørgesæter and Gray, 2008; Chapman et al., 1998). In a study by Lock and Janssen (2001), an approximately 13-fold change in the toxicity of Zn and Cd to the potworm (*Enchytraues albidus*) was observed for soils in which the type of clay and OM differed. Similar conclusions have been reached for Cu, where the toxicity of Cu-spiked sediment to *Daphnia magna* and other freshwater organisms was reduced with the addition of organic carbon (as peat moss) in formulated test sediment (Ankley et al., 1993b; Malueg et al., 1986). Overliming of peat soils and other disturbances to freshwater environments have been observed to cause significant remobilization of U to the aqueous phase (Zielinski and Meier, 1988), which can increase bioavailability of U in the environment. Thus, determining the degree of U adsorption to OM would be important in enhancing our understanding of U bioavailability in sediment. Sorption of U to OM would also be particularly important for deposit feeders (e.g., some species of chironomid larvae), which feed on OM deposited on or present within sediment, since increased ingestion would likely play a role in the bioaccumulation and subsequent toxicity of sediment-associated U. Overall, the partitioning of metals between aqueous and solid sediment phases can change as a function of OM content, such that a greater content of OM can mitigate metal toxicity to aquatic organisms (Cloran et al., 2010; Doig and Liber, 2006; Doig and Liber, 2007).

1.7.4 Iron and manganese oxyhydroxides

Iron (Fe) and manganese (Mn) oxyhydroxide (oxides and hydroxides) minerals may only constitute a small percentage of bulk sediments, but are suspected to be responsible for 10-50% of the total metal sorption in some freshwater sediments (Zhong and Wang, 2008). The concentration of Fe in sediment is typically greater than Mn, but the latter is suspected to be more reactive with metals (Zhong and Wang, 2008). However, sorption of metals associated with Fe and Mn oxyhydroxides can be both metal-specific and pH-dependent. For example, Whitney

(1975) found that the adsorption of Zn and Cd to sediment most often paralleled that of Mn, while the adsorption of Pb was less consistent, being found to be related to both the content of Fe and Mn. Metals have been found to bind directly to the -OH groups of Fe and Mn oxyhydroxides in neutral waters, and to the functional groups of OM adsorbed on Fe oxyhydroxides in more acidic waters (Tessier et al., 1996). The influence of Fe and Mn oxides as strong adsorbers of metals can be complicated as they often occupy the surfaces of OM and clay minerals as coatings (Luoma and Davis, 1983). The partitioning of metals from the sediment into the aqueous phase can occur at different rates due to the various degrees of metal adsorption to hydrous oxides of Fe and Mn. This emphasizes the importance in identifying and quantifying the influence the various sedimentary components (OM, clay minerals and Fe/Mn oxides) for the assessment of the availability of sediment-associated U to aquatic organisms.

1.7.5 Acid volatile sulphides

The role of acid volatile sulphides (AVS) in binding metals has been well studied and determined to have a strong influence on the bioavailability of divalent, cationic metals (e.g., Ag, Cd, Cu, Ni, Pb, Zn) in anoxic freshwater sediment (Ankley, 1996; Ankley et al., 1996b; Berry et al., 1996; Carbonaro et al., 2005; Gonzalez, 1996; Hansen et al., 1996; Hare et al., 1994; Liber et al., 1996; Peterson et al., 1996; Prica et al., 2008). For this reason, the United States Environmental Protection Agency (U.S. EPA) has adopted the use of the simultaneously extracted metals-acid volatile sulphides (SEM-AVS) approach to derive no-effect criteria for some divalent cationic metals found in sediment (Ankley et al., 1996a; US EPA, 2005). This approach determines the amount of amorphous sulphides available to competitively bind with divalent cationic metals, which form insoluble metal sulphide complexes. If there is an excess of AVS, then such metals will not be available. In contrast, if SEM exceeds the AVS present in the sediment, then metals are potentially bioavailable. The insoluble metal sulphides formed in sediment would represent a permanent sink if left undisturbed; however, oxidation by molecular oxygen can occur in surficial sediments, subsequently solubilizing these metals and enhancing their bioavailability. Alternatively, the concentration of AVS and its influence on metal bioavailability may not be as significant in oxic sediments, or under oxic conditions at the SWI which benthic invertebrates often inhabit (Campana et al., 2013; Campbell et al., 2015; Wang and Chapman, 1999). Therefore, it is widely accepted that the amount of AVS present in sediment can have a direct impact on the bioavailability of certain cationic metals, but knowledge about the influence of AVS on U behaviour in sediments in lacking.

1.8 Sorption behaviour of uranium in sediment

Many of the physicochemical characteristics of sediment that modify U bioavailability are a result of the different binding capacities or sorption strengths associated with different sediment phases and properties. A common method for describing the sorption behaviour of metals is through the use of sediment-solution partition coefficient (K_d) values, defined as the ratio between the concentration of a metal in the sediment solid phase and the metal concentration in the aqueous solution (ml/g), under equilibrium conditions (OECD, 2000). The quantification of K_d values provide a method to assess and describe the sorption and partitioning of metals in sediment, such that high K_d values represent a greater adsorption of metals to the sediment phase and low K_d values represent low adsorption and thus greater mobility of the metal in solution. The use of K_d values can consequently help to quantify the influence of different test and field parameters that may affect the transport, accumulation, and bioavailability of metals. For example, sorption of Zn in soils has been observed to be significantly influenced by soil composition, such as soil OM content, and solution chemistry (e.g., pH and Zn concentration) (Shi et al., 2008). Additionally, changes in pH and subsequently changes in the speciation of metals can result in significant differences in binding affinities between metals and sediment phases (Echevarria et al., 2001; Peck et al., 2002; Sheppard, 2011). Thus, the quantification of K_d-U values may provide better insight into the sediment properties that influence U sorption and bioavailability.

There is a large range in the K_d values reported for U (<1 to 1×10^6 ml/g) for a number of different experimental parameters and conditions (CCME, 2011; US EPA, 1999). The quantification of K_d values has been shown to change based on the experimental methods used (e.g., *in-situ* vs. laboratory batch equilibrium experiments), water chemistry, solid-to-solution ratio (SSR), concentrations of HA and FA, and equilibration time (Ren et al., 2010; US EPA, 1999; Zhirong and Shaoqi, 2010). Work by Ren et al. (2010) concluded that sorption of U from aqueous solution onto bentonite (i.e., montmorillonite) clay is strongly dependent on pH and ionic strength of the aqueous phase. Additionally, a study by Zhirong et al. (2010) found that the adsorption of uranyl ions onto peat moss increased with increasing pH (range 2-6), with higher

HA concentrations, and with decreasing ionic strength. Additionally, sorption of U to sediment has been observed to be dependent upon the physical, chemical and mineralogical properties of sediment, including the content and type of clay minerals and OM, iron oxides and hydroxides, pH, presence of colloids, and presence of counter-ions (Echevarria et al., 2001; Pandit et al., 2012; Ren et al., 2010; Sheppard et al., 1989; Sheppard, 2011; US EPA, 1999; Vandenhove et al., 2007; Vandenhove et al., 2009a; Vandenhove et al., 2009b; Zhirong and Shaoqi, 2010). For example, both clay- and organic-rich sediment are generally observed to have greater K_d-U values in comparison to loamy and sandy sediments (Sheppard, 2011). Additionally, Vandenhove et al. (2007) found a strong dependence of K_d-U on organic carbon and amorphous Fe oxides contents in soils. Conversely, Echevarria et al. (2001) did not find a relationship between OM or clay content for U partitioning and instead concluded that pH was the most important factor driving U partitioning in soils. Therefore, the mechanisms of U sorption are not fully understood and should be further investigated in order to complement the quantification of U bioavailability.

1.9 Modeling U sorption and bioavailability

In addition to quantifying the influence of sediment characteristics on the bioaccumulation and sorption of U, predictions of U bioaccumulation and sorption would contribute to improved environmental monitoring and risk assessment of U-contaminated sediments. One model that can be used to predict metal sorption to substrates is the Windermere Humic-Aqueous Model (WHAM). This model is designed to calculate equilibrium chemical speciation in surface and ground waters, and in sediment and soil, in particular situations where chemical sorption is dominated by OM (humic substances) (Tipping, 1994). The WHAM model includes a (i) Humic Ion-Binding Model, a discrete site/electrostatic submodel of cation binding to humic substances (Tipping et al., 2011) and (ii) a surface complexation model (Lofts and Tipping, 1998) for ion binding to mineral oxides. These submodels are parameterized for the binding of 46 cations, including U(VI), to humic and fulvic acids and amorphous Fe(III) oxide. WHAM is also an integral component of the biotic ligand model (BLM), which accounts for the complexion and competition between metals and aqueous ions for biotic ligands to predict the bioavailability of metals in aquatic environments (De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2005). While the BLM relies heavily on aqueous ions and water chemistry, WHAM additionally accounts for precipitation of Al and Fe oxides, cation-exchange on an idealized clay mineral, and adsorption-desorption reactions of FA. For example, Shi et al. (2008) presented a Zn kinetics model incorporating WHAM that was able to account for different water chemistries and OM concentrations of sediment to describe the nonlinear binding of Zn to OM. Additional studies by De Schamphelaere et al. (2002, 2005) generated a model that predicted Cu and Ni bioavailability through the integration of WHAM into the BLM. Di Toro et al. (2005) outlined the use of a sediment BLM in which partitioning and toxicity were modeled through the use of WHAM and BLM, respectively. The advantage of WHAM is that it provides a user-friendly computer model that produces output through a set of best estimate parameters for the adsorption of a variety of adsorbing cations to humic substances, CO_3 and to Fe(III) oxide. Recent studies have also demonstrated that WHAM optimized parameters for predicted HA-bound metals act as a suitable surrogate for the accumulation of metals in organisms (Antunes et al., 2012; He and Van Gestel, 2015; Stockdale et al., 2010; Tipping et al., 2008), thus providing a measure of bioavailability. Therefore, if used appropriately, models may allow for successful predictions of U bioavailability for a wide range of water chemistries and physicochemical properties of sediment, which would ultimately provide a simplified tool for the management and risk assessment of U-contaminated sediments.

1.10 Project summary and rationale

Due to the potential for release of U, especially from U mining and milling into aquatic environments, and the associated accumulation of U in sediments, there is a need to investigate the bioavailability of sediment-associated U to freshwater benthic invertebrates. Few studies have assessed the influence or modifying effects of different physicochemical properties of sediment on the bioavailability of U to benthic invertebrates. Lack of such knowledge can impede the use and improvement of U-SQGs for the management and protection of aquatic environments near U mines and mill tailings.

1.10.1 Summary of research objectives and null hypotheses

The overall goal of the research presented in this thesis was to quantify (Chapter 2-4) and model (Chapter 5) the influence of sediment physicochemical properties on the bioavailability of sediment-associated U to *C. dilutus* larvae, a model freshwater benthic invertebrate. The specific research objectives were:

1. a) To quantify the bioaccumulation of U in a model freshwater benthic invertebrate exposed to U-spiked formulated sediments amended with different clay minerals to determine the role of different types and quantities of clay minerals on U bioavailability in 10-d U-spiked sediment bioaccumulation tests.

   H_0: There will be no statistically significant differences in the amount of U accumulated in *C. dilutus* larvae when exposed to formulated sediments composed of three different types of clay minerals (kaolin, montmorillonite, and illite) spiked with the same concentrations of U.

   b) To quantify the bioaccumulation of U in a model freshwater benthic invertebrate exposed to U-spiked formulated sediments with different sand size fractions or clay content to determine the role of particle size distribution on U bioavailability in 10-d U-spiked sediment bioaccumulation tests.

   H_0: There will be no statistically significant differences in the amount of U accumulated in *C. dilutus* larvae exposed to the same concentrations of U in formulated sediments amended with either varying amounts of kaolin clay content or with different sand size fractions.

   c) To quantify the bioaccumulation of U in a model freshwater benthic invertebrate exposed to U-spiked formulated sediments amended with different quantities of organic matter (OM) to determine the influence of OM type and content on U bioavailability in 10-d U-spiked sediment bioaccumulation tests.

   H_0: There will be no statistically significant differences in the accumulation of U in *C. dilutus* larvae exposed to formulated sediments composed of different amounts of either peat moss or α–cellulose as the OM source, spiked with the same concentrations of U.

2. To quantify and develop simple regression equations to predict the bioaccumulation of U in a model freshwater benthic invertebrate exposed to U-spiked field sediments with a wide range of physicochemical properties (i.e., TOC, particle size distribution, CEC, and content of Fe and Mn) to determine changes in the bioavailability of U in 10-d U-spiked sediment bioaccumulation tests.

H_0: There will be no statistically significant relationship between the accumulation of U in *C. dilutus* larvae and the different field sediments spiked with the same concentrations of U, irrespective of different physical, chemical, and mineralogical properties of the sediments.

3. To quantify and model the sorption behaviour and sediment partition coefficients (K_d) of U for various field sediments as a function of pH and U concentration to better characterize the influence of different physical, chemical and mineralogical properties of sediment on U bioavailability using batch equilibrium sorption tests.

   H_0: There will be no statistically significant differences in the K_d-U values among different sediments or under different test conditions (i.e., U concentrations, pH).

4. To model U sorption, speciation, and bioaccumulation using WHAM, an existing computer model, and to compare WHAM and simple regressions predictions to experimental observations to assess how well the models performed in predicting U bioavailability to a model freshwater benthic invertebrate.

   H_0: The use of WHAM or simple regression models will not allow for reasonable approximations of U sorption to various sediments or the prediction of U bioaccumulation in *C. dilutus* larvae exposed to different U-contaminated sediments.

CHAPTER 2:
EFFECTS OF CLAY MINERALS AND ORGANIC MATTER IN FORMULATED SEDIMENTS ON THE BIOAVAILABILITY OF SEDIMENT-ASSOCIATED URANIUM TO THE FRESHWATER MIDGE, *CHIRONOMUS DILUTUS*

2 Preface

This chapter focuses on the influence of physicochemical properties of sediment, specifically associated with formulated sediments, on the bioavailability of U to a model freshwater benthic organism, *Chironomus dilutus*. Differences in clay minerals (kaolin, montmorillonite, illite), particle size distribution (sieved sand size fractions and content of kaolin clay), and the type (peat moss vs. α-cellulose) and content of organic matter were investigated in several 10-d U-spiked formulated sediment experiments. The type of clay minerals and organic matter, as well as the range of particle size distribution used in this chapter aimed not only to support the growth of the test organism, but also to represent ecologically relevant sediment characteristics typical of northern Saskatchewan U mining areas. Results from this chapter demonstrate that increasing contents of sediment clay and organic matter play a significant role in reducing U bioavailability, which is important for informing risk assessments of U-contaminated sediments and in the development of site-specific sediment quality guidelines for U.

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2.1 Introduction

Uranium (U) is a naturally occurring element in the Earth's crust that can enter the environment from a number of sources. One of the largest occurrences of U released into the environment comes from the mining and milling of U-rich ore for energy production (CCME, 2011). Once U enters a freshwater ecosystem, it most often becomes associated with sediments due to its relatively high sediment partition coefficient (K_d; ranging from 0.36 to 4400 ml/g) (US EPA, 1999). As a result, elevated concentrations of U have been documented downstream of U mining operations around the world, occasionally exceeding 1,000 mg U/kg dry weight (d.w.) in some sediments (CCME, 2011; Liber et al., 2011). The tendency of U to accumulate in sediment makes benthic invertebrates among the most highly exposed organisms. Additionally, benthic invertebrates are an important group of organisms used for the evaluation of metal impacts in aquatic environments and for the development of sediment quality guidelines (SQGs) in Canada and equivalent criteria around the world (Thompson et al., 2005).

Despite the important role sediment has in U sequestration and associated exposure to aquatic organisms, few studies have exposed benthic invertebrates to U-spiked sediments, and results of these tests have been equivocal. Early life stages of amphipods display sensitivity to U-spiked sediments and growth of chironomids has been shown to be a sensitive endpoint for evaluating toxicity from chronic U exposure (Liber et al., 2011). Research has also been conducted on the effects and exposure of U-contaminated sediment to *Tubifex tubifex* and *Chironomus riparius*, with concentrations greater than 599 mg U/kg d.w. causing malformations and reduced survival, biomass and burrowing activity (Lagauzère et al., 2009a; Lagauzère et al., 2009c). Of greater interest, however, is the high degree of divergence in effect concentrations reported in the literature. For example, 10-d LC50 values have been reported as low as 5.3 mg U/kg d.w. in sediment for *C. riparius* larvae (Dias et al., 2008), to over 10,000 mg U/kg d.w. for *Chironomus dilutus* larvae (Liber et al., 2011). The difference in lethal concentrations among published studies is likely associated with differences in experimental parameters (i.e., water chemistry, type and composition of sediment), which can substantially alter U bioavailability and hence observed toxicity.

Different sediment characteristics, such as total organic carbon (TOC) and cation exchange capacity (CEC), will influence the partitioning of metals between the solid and aqueous phases in sediment, thus altering metal bioavailability. Furthermore, fine-sized particles, such as clay minerals, tend to demonstrate a strong positive correlation with metal partitioning due to their sorptive nature and large reactive surface area (Horowitz, 1985; Zhong and Wang, 2008). Uranium adsorption to sediment is known to vary with mineral type and total U concentration (Davis et al., 2004; Wang et al., 2011), but we know little about the influence of U adsorption in sediment on its uptake and bioaccumulation by benthic invertebrates.

The purpose of this research was to compare the influence of key sediment physicochemical properties on U bioavailability and associated bioaccumulation in a model freshwater benthic invertebrate, *C. dilutus* (Diptera, Chironomidae). *Chironomus dilutus* larvae were selected as the test organisms as they are a standard test species and a common freshwater benthic organism found in Saskatchewan with close contact to the sediment throughout larval development, and they allowed for easy determination of bioaccumulation effects from spiked sediment tests (Benoit et al., 1997). The sediment physicochemical properties examined were clay mineral type and content, particle size distribution, and total organic matter type and content. Previous studies have shown that the use of formulated (reconstituted) sediments can appropriately mimic natural field sediment and reduce the confounding factors often associated with field sediment (Kemble et al., 1999; Suedel and Rodgers Jr, 1994a). Thus, formulated sediments were utilized in this research in order to manipulate individual variables to quantify their influence on metal bioavailability. The hypothesis was that sediments with different physicochemical properties would have different capacities for adsorbing U and hence would differentially alter the bioavailability of U in these sediments. The specific objective was thus to quantify U bioaccumulation in *C. dilutus* larvae when exposed to U-spiked formulated sediment with different sediment physicochemical properties in a series of 10-d bioaccumulation tests.

2.2 Materials and methods

2.2.1 Test organism and materials

The midge, *C. dilutus*, was cultured in-house at the Toxicology Centre, University of Saskatchewan (SK, Canada) following guidelines described in Environment Canada (1997). Carbon-filtered, bio-filtered municipal water from the City of Saskatoon, SK, was used for culturing and experimental exposures. Test animals were housed in an environmental chamber maintained at an average daily temperature of 23 ± 1°C, a 16h light:8h dark photoperiod, and an illumination intensity of 500-1000 lux.

Uranium as uranium (VI) dinitrate oxide hexahydrate ($UO_2(NO_3)_2{\cdot}6H_2O$) (Strem Chemicals, Inc., Newburyport, MA, USA) was used in all experiments since the uranyl ion (UO_2^{2+}) is the predominant form of U found in oxic aquatic environments (CCME, 2011). Ultra-pure 18 MΩ-cm (Barnstead NANOpure®, Thermo Scientific, Dubuque, IA, USA) water was used for dilution of samples for analysis and to make up any stock solutions used throughout the experiments.

Formulated sediments were composed of commercially available substrates (Table 2.1). The base material was industrial, pre-sieved silica sand with a particle size range of 106 to 425 µm unless otherwise stated, which was triple rinsed with deionized and clean test water before use. Clay-based sediments included the addition of kaolin clay, montmorillonite clay, or illite clay (Table 2.1), which are all common clay minerals found in freshwater sediments. These three clay minerals are also different with regard to their CEC, surface area, and other properties that are likely to alter U adsorption and bioavailability (Table 2.1). Organic matter sources used in the formulated sediments included sphagnum peat moss and α-cellulose, which have both been used as suitable surrogates for natural organic matter in previous sediment spiking studies (Dias et al., 2008; Kemble et al., 1999; Lacey et al., 1999). Sphagnum moss peat (Table 2.1) was prepared following OECD (2004) guidelines for use with formulated sediments, but was neutralized with a 1 M solution of sodium hydroxide (EMD Chemicals, Inc., Gibbstown, NJ, USA) prior to conditioning.

2.2.2 Experimental design

The bioavailability of U associated with different formulated sediments was investigated in eight experiments examining the influence of clay type, particle size, and organic matter. Details of the different experimental designs are found in Table 2.2. The three clay minerals selected are common clays found in many environments (Horowitz, 1985). The first component of the particle size study aimed at investigating the influence of silica sand size fractions (surface area) on U bioavailability without the influence of more complex surface chemistry. The range of sand size fractions were selected to encompass United States Department of Agriculture (USDA) classification of soil particle sizes for fine to coarse sand (Pansu and Gautheyrou, 2007). Selection of sand size fractions were also based on the ranges that would be most feasible to sieve and obtain adequate quantities of desired size classes from commercially available sources of sand. Using Eq. (2.1), the theoretical, geometric surface area was calculated using an average particle diameter and assuming sphericity of particles (Horowitz, 1985),

$$SA = \frac{6x10^{-4}}{rho \cdot d} \qquad \text{(Eq. 2.1)}$$

where, SA is surface area (m^2/g), rho is average density (g/cm^2), and d is particle diameter (µm). From this equation, the sand size fractions used had average surface areas as follows: fine (median diameter of 125 µm) = 0.0313 m^2/g; medium (median diameter of 362.5 µm) = 0.0108 m^2/g; and coarse (median diameter of 725 µm) = 0.0054 m^2/g.

For the second component of the particle size study, the combination of surface area and surface chemistry was examined using different concentrations of clay. Kaolin clay was selected as the clay mineral for this experiment since no adverse effects on *C. dilutus* larval growth and survival had been obtained in earlier experiments with this material and because this is the standard clay recommended for use in formulated sediments (OECD, 2004). The influence of different types of organic matter (OM) in sediment on U bioavailability was examined in two separate experiments using either peat or α-cellulose, two common organic carbon sources (Kemble et al., 1999). The second part of the OM study examined the role of a wide range of environmentally relevant OM contents using peat (Table 2.2) because peat is recommended as the OM source in standard formulated sediments (OECD, 2004). Controls (without U) for all experiments included a sand-only treatment, as well as each formulated substrate treatment (unless otherwise stated) to ensure there were no adverse effects due to the substrate formulations alone.

2.2.3 Sediment spiking procedure and test system

Sediments were spiked with desired U concentrations and allowed to age for 20 d prior to each 10-d test (the last 3-d in the test system) following methods described by Liber et al. (2011). In brief, test batches of dry sediments (approximately 650 g d.w. for each batch) were formulated to achieve appropriate sediment compositions as outlined in the previous Experimental Design section and were thoroughly mixed in 1-L polypropylene bottles before spiking. Once homogenized, stock solutions of U and/or un-spiked ultra-pure water were added in small aliquots and hand shaken for 1 min between each addition until the sediment mixture was saturated with “pore water”. After spiking, sediment bottles were placed on a Wrist Action

Table 2.1 Description of materials used in the formulated sediments and their associated physicochemical properties.

Property	**Silica sand**	**Kaolin**	**Montmorillonite**	**Illite**	**Peat**	**Alpha-cellulose**
Source	Unimin Corp, Ottawa, MN, USA	Plainsman Clays Ltd., Medicine Hat, AB, CAN	Canadian Clay Products, Inc., Wilcox, SK, CAN	Sun Clay Therapy, Pasadena, CA, USA	Sun Gro Horticulture Inc, Seba Beach, AB, CAN	Sigma-Aldrich, St. Louis, MO, USA
Layer Structure[a]	n/a	1:1	2:1	2:1	n/a	n/a
Bonding[a]	n/a	Strong	Very weak	Strong	n/a	n/a
Shrink-swell capacity[a]	n/a	Very low	High	Low	n/a	n/a
CEC (meq/100 g)	2[a]	1-15[a]	80-150[a]	20-30[a]	n/a	n/a
		11[b]	42 [b]	3 [b]		
Specific surface area (m^2/g)[a]	0.005-2	5-45	700-800	50-200	n/a	n/a
TOC (%)[c]	0.026 ± 0.001	n/a	n/a	n/a	44.1 ± 0.8	44.4 ± 0.7
Background U concentration (mg U/kg d.w.)[d]	0.28 ± 0.10	22.06 ± 2.08	6.20 ± 0.20	4.72 ± 0.33	0.74 ± 0.23	< LOD

CEC – cation exchange capacity. TOC – total organic carbon. n/a – not applicable and/or not measured. < LOD – Less than limit of detection for U (0.005 µg/kg d.w.).

[a] Adapted from Horowitz (1985) and Pansu and Gautheyrou (2007).

[b] Measured at ALS Environmental, Saskatoon, SK.

[c] Mean (± SD) TOC determined by LECO carbonator, Department of Soil Science, University of Saskatchewan, SK (*n* = 3).

[d] Mean (±SD) concentration of U determined by ICP-MS after complete sediment digestion (*n* = 3).

Table 2.2 Summary of study parameters and experimental design for each of the eight studies performed using formulated sediments/substrates spiked with different concentration of uranium.

Study	**Experiment**	**Substrate formulation**[a]	**Concentration of U (mg U/kg d.w.)**	**Treatments (N)**[b]
Clay mineral type	Kaolin	0, 2, 20% clay	0, 50, 200	7[c]
	Montmorillonite	0, 2, 20% clay	0, 50, 200	9
	Illite	0, 2, 20% clay	0, 50, 200	9
Particle size distribution	Surface area: sand	Fine (100 – 150 µm)	0, 5, 50[d]	3
		Medium (300 – 450 µm)	0, 5, 50[d]	3
		Coarse (600 – 850 µm)	0, 5, 50[d]	3
	Surface chemistry: clay (kaolin)	0, 0.74, 20% clay	0[e]	3
		0, 0.25, 0.74, 2.2, 6.7, 20, 60% clay	50	7
Organic matter	Percent OM (peat)	0% OM	0[f]	1
		0, 0.25, 0.74, 2.2, 6.7, 20, 40% OM	50	7
	Source of OM: Peat	0, 5, 15% OM	0, 50, 200	9
	Source of OM: α-cellulose	0, 5, 15% OM	0, 50, 200	9

[a] Silica sand (106 – 425 µm, unless otherwise stated) was used as the base material for all formulated sediments (i.e., 0%).

[b] All treatments had 6 replicates (*n* = 6).

[c] The kaolin experiment did not include a 0% treatments at 50 and 200 mg U/kg d.w.

[d] Lower concentrations of U were selected due to the limited binding capacity of U in pure sand as reported in the literature (Ames et al., 1983) and as determined in a preliminary test (S. Crawford, unpublished data).

[e] Controls only included un-spiked (0 mg U/kg d.w.) for substrate formulations 0% (sand), 0.74 and 20% kaolin clay.

[f] It was concluded from earlier experiments that peat content (within the range of concentrations tested) did not have an adverse effect on the survival or growth of *C. dilutus* larvae, thus only a sand-only control was used.

Laboratory Shaker (Burrell® Corporation, Pittsburgh, PA, USA) for 1 h (~300 oscillations per min) and subsequently stored at 4°C in the dark for 10-d.

After the 10-d storage period, sediment bottles were removed from the refrigerator and shaken on the Wrist Action Laboratory Shaker for 10 min (~300 oscillations per min). Aliquots of 75 ml of homogenized sediment were then transferred into six 300-ml high-form replicate test beakers (described below) for each treatment and control. Dialysis sampling devices, modified mini-peepers (Doig and Liber, 2000), were inserted into each test beaker so that one peeper chamber was above the sediment surface (for overlying water sampling) and one chamber was below the sediment surface (for pore water sampling). Peeper chambers (each 1 ml volume) were filled with ultra-pure water and covered with a semi-permeable membrane (0.45 µm pore size; Supor®-450 polyethersulfone membrane filter, Pall Corporation, Ann Arbor, MI, USA). Clean test water (125 ml) was carefully added to each beaker with the aid of a plastic disc covering the sediment surface layer to minimize disturbance to the sediment. All beakers were then stored in the dark at 4°C for 7 d after which time the beakers were transferred to the test system.

Sediment bioaccumulation tests were performed using a previously developed sediment testing intermittent renewal (STIR) system (Benoit et al., 1993), which was modified as outlined in detail by Liber et al. (2011). In brief, the system included six replicate 300-ml high-form glass beakers (7 cm dia.) placed within each of the glass holding tanks. Each holding tank received temperature-controlled test water from a common plexiglass head-box. Water exited the holding tanks through self-starting glass siphons. Inflow and outflow rates to each tank were controlled individually with on-line plastic valves. Each beaker had two 1.5-cm diameter opposing holes in their side walls located 7 cm above the beaker bottom and were covered with stainless steel screens (25-µm mesh) to allow for water exchange between beakers and the surrounding holding tank. Automated overlying water renewal (approx. 15% of the overlying water per beaker per renewal) began 1 d after the addition of test beakers to each treatment holding tank and was allowed to run an additional 2 d prior to the addition of test organisms. The pretest renewal period was included to facilitate diffusion and flushing of "excess" unbound U from the sediments. A timer ensured that water renewals from the head-box ran for 20 min every 8 h, with renewal continuing throughout the 10-d test. Aeration was only used in experiments with OM amendments in order to ensure that dissolved oxygen levels remained above the recommended minimum of 3 mg/L (Environment Canada, 1997).

2.2.4 Experimental set-up

At each test initiation, ten second instar (8- to 10-day old) *C. dilutus* larvae were randomly added to each beaker and exposed to the U-spiked or control sediment for 10 days following recommended guidelines (Environment Canada, 1997). A separate study has shown that pseudo-steady state between U concentrations in water and in *C. dilutus* larvae is reached rapidly, allowing reasonable estimates of bioaccumulation to be obtained within a 10-day exposure period (Muscatello and Liber, 2010). Three extra replicates of ten individual organisms were also collected at test initiation (not gut purged) to determine average initial dry weight after 24 h drying at 60 °C. Mean initial dry weight of individual *C. dilutus* larvae at the start of each test was on average (± SE) 0.16 ± 0.09 mg d.w. per organism. During each test, organisms were fed 6 mg of Nutrafin® fish flakes (Rolf C. Hagen Inc., Montreal, QC, CAN) slurry per beaker daily.

Water temperature and dissolved oxygen (DO) levels were measured every second day in three replicate beakers for each control and U-spiked treatment. Three 20 ml overlying water samples were also taken on days 0, 5 and 10 from three randomly selected beakers for each treatment and control in order to measure conductivity, pH, total hardness, alkalinity, and ammonia. Water samples for U analysis were obtained from the pore water and overlying water chambers of the peeper devices retrieved from three randomly selected replicate beakers on day 0 and day 10 for each control and U-spiked treatment. Peeper samples were collected by piercing the membrane of each chamber with a pipettor to extract the 1 ml water sample. Each sample was then acidified to 2% HNO_3 (double distilled ultra-pure nitric acid 1 M; EMD Millipore Chemicals Inc., Gibbstown, NJ, USA) and stored in the dark at 4°C until analysis. In instances where fine clay colloids were visible in the 0.45 µm filtered peeper samples, the samples were centrifuged at 10,600 *g* for 10 min to remove as much of the colloids as possible. Dissolved organic carbon (DOC; TOC-V CPN model 5000, Shimanzu Co., Kyoto, Japan) was also measured when applicable (i.e., OM experiments) from diluted water samples obtained from both the pore water and overlying water peeper chambers sampled on days 0 and 10 prior to acidification. Sediment samples (approx. 1 g w.w.) were collected for total U analysis on days 0 and 10 from the top 1-cm layer of sediment using a wide-tipped transfer pipettor. Sediment samples were dried at 60°C for 24 h, homogenized, and digested with H_2O_2, HNO_3, and HF in a Microwave Accelerated Reaction System (MARS-5®, CEM Corporation, Mathews, NC, USA) in preparation for total U analysis. Total organic carbon (TOC; LECO carbonator Model C632) was also analyzed in the OM experiments from the batch stock of each sediment treatment.

After the 10-d exposure period, surviving organisms were retrieved and counted. Organisms were then placed in clean culture water for 10 min, transferred to a 1 mM ethylenediaminetetra-acetic acid (EDTA; Sigma Chemical Co., St. Louis, MO, USA) solution for 15 min to remove any U adsorbed to the surface of the larvae, and subsequently rinsed with ultra-pure water. Test organisms were then placed in 250-ml beakers with clean water, coarse sand, and 6 mg of Nutrafin fish flake slurry food for a 12-h gut clearance period (Muscatello and Liber, 2010; Sibley et al., 1997b). Subsequently, organism dry weights were measured after a 24-h drying period at 60°C. Organisms were then acid digested (H_2O_2 and HNO_3) prior to analysis of total U concentration.

All U analyses were performed at the Toxicology Centre, University of Saskatchewan using inductively coupled plasma-mass spectrometry (ICP-MS; X-series II ICP-MS spectrometer with PlasmaLab software and collision cell, Thermo Electron Ltd, Mississauga, ON, CAN) with a minimum detection limit of 0.01 ppb U and an instrumental and method recovery between 80-120%. Certified reference material (PACS-2 Marine Sediment for trace metals, National Research Council Canada, Ottawa, CAN; 1640a trace elements in natural water, National Institute of Standards and Technology, Gaithersburg, USA), duplicates, and blanks were used for digestions and analytical procedures to ensure analytical accuracy and validity.

2.2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics®, version 20 (SPSS Inc., Chicago, IL, USA) and plotted with Sigmaplot®, version 11 (San Jose, CA, USA). All tests were conducted at $\alpha = 0.05$ after checking for compliance with parametric assumptions of normal distribution (Shapiro-Wilk test) and homogeneous variance (Levene’s test). Mean survival and weight of *C. dilutus* larvae, and mean U concentrations in sediment, overlying water, pore water, and *C. dilutus* larvae were analysed using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc tests for all experiments. Data that did not meet parametric assumptions were transformed (arcsin square root (%), $\log_{10}$, or $\log_{10}(x+1)$) prior to statistical analysis. If data did not meet the normality and homogeneity of variances assumptions after transformation, then a non-parametric Kruskal-Wallis test was used, followed by multiple individual Mann-Whitney post-hoc tests. Linear regressions were conducted to determine the relationship between mean U concentrations in sediment, overlying water, or pore water, relative to mean U concentration in *C. dilutus* larvae, where appropriate.

2.3 Results

2.3.1 Water quality

Water quality variables were generally similar in all treatments for all 10-d test (Table 2.3). Lower DO levels were measured in the peat experiment, which resulted in the use of aeration two days into the test and for all other OM tests. However, DO always remained above the recommended threshold of 3 mg/L (> 40% of saturation; Environment Canada, 1997) for *C. dilutus* larvae in all of the treatments throughout all experiments. Conductivity was greatest in the 20% montmorillonite clay treatments, which caused a greater overall conductivity measurement for the montmorillonite experiment. Ammonia generally increased slightly over each 10-d test, but mean ammonia concentrations across all tests ranged from 0.0 to 0.8 mg/L, with greater concentrations observed in the montmorillonite clay test.

2.3.2 Growth and survival of test organisms

Mean (±SE) survival of *C. dilutus* larvae across all 10-d experiments was 90 ± 8%, which met the minimum acceptable requirement of ≥ 70% survival (Environment Canada, 1997), with the exception of one control treatment containing 20% kaolin clay and all treatments containing 20% montmorillonite clay. The 20% kaolin control had a mean (±SD) survival of 63 ± 22%, compared to the four other treatments containing 20% kaolin clay, whether controls or U-spiked, which had mean survivals over 80%, suggesting it was not the kaolin clay substrate that caused poor control survival. In contrast, all sediments containing 20% montmorillonite clay exhibited low mean (± SD) survival of 38 ± 19%, 37 ± 22%, and 22 ± 10% in the control, 50 and 200 mg U/kg d.w. treatments, respectively. The low survival suggests that the 20% montmorillonite clay substrate was not a suitable substrate for *C. dilutus* larvae.

Across all experiments, mean (± SE) dry weights of *C. dilutus* larvae, 1.2 ± 0.3 mg d.w. per organism, were substantially greater than the recommended minimum dry weight at the end of a 10-d test of 0.6 mg per individual organism (Environment Canada, 1997). The only exception was in treatments containing 20% montmorillonite clay, which had a mean (± SE)

Table 2.3 Measured water quality variables (mean ± SE) for each 10-d experiment.

Experiments	**Temperature (°C)**	**Dissolved oxygen (mg/L)**	**Conductivity (μS/cm)**	**pH**	**Ammonia (mg/L)[a]**	**Total hardness (mg/L)[b]**	**Alkalinity (mg/L)[b]**
Test (source) water[c]	21.6 ± 1.3	9.0 ± 0.7	426.1 ± 20.4	7.9 ± 0.2	0.02 ± 0.04	133.2 ± 11.3	102.9 ± 12.1
Kaolin	21.5 ± 0.2	6.6 ± 0.5	443.0 ± 8.1	8.0 ± 0.1	0.4 ± 0.1	135.8 ± 3.2	82.8 ± 4.7
Montmorillonite	19.7 ± 0.5	6.3 ± 0.4	620.6 ± 187.6	7.8 ± 0.1	0.8 ± 0.2	161.8 ± 16.6	131.6 ± 13.0
Illite	20.6 ± 0.3	6.3 ± 0.3	486.4 ± 30.5	7.9 ± 0.0	0.1 ± 0.1	158.1 ± 10.2	123.5 ± 8.1
Surface area – sand	19.8 ± 0.3	7.7 ± 0.2	449.7 ± 17.8	8.1 ± 0.1	0.3 ± 0.1	144.2 ± 3.1	102.6 ± 1.9
Surface chemistry – (kaolin) clay	20.7 ± 0.2	6.6 ± 0.2	446.0 ± 11.6	7.6 ± 0.3	0.1 ± 0.0	156.8 ± 4.5	111.8 ± 4.4
Percent OM (peat)	21.6 ± 0.2	6.2 ± 0.9	458.2 ± 29.6	8.0 ± 0.1	0.3 ± 0.1	147.6 ± 13.3	120.2 ± 12.3
Source of OM – peat	21.2 ± 0.4	4.9 ± 1.2	530.4 ± 30.0	7.6 ± 0.1	0.2 ± 0.2	158.0 ± 8.2	139.6 ± 16.5
Source of OM – α-cellulose	20.4 ± 0.2	6.4 ± 0.4	455.3 ± 11.0	7.9 ± 0.1	0.04 ± 0.03	164.1 ± 9.8	116.5 ± 15.4

Means (± SE) represent the overall mean for each experiment (i.e. an average of three replicates sampled on each respective sampling day, averaged for all treatments within each experiment; *n* = 7-10 depending on the number of treatments tested in each experiment).

[a] Measured as ammonia nitrogen.

[b] Measured as calcium carbonate.

[c] Test (source) water represents water sampled from the head-tank before it was delivered to individual treatment tanks, averaged across all eight experiments.

final weight of 0.5 ± 0.1 mg d.w. per organism. The low survival and smaller mass of *C. dilutus* larvae in montmorillonite treatments made it difficult to interpret the bioaccumulation data; thus, caution should be taken when drawing conclusion on the bioavailability of U to *C. dilutus* larvae in the montmorillonite clay experiment.

2.3.3 Background concentrations of uranium in controls

Mean background U concentrations in all control sediments were less than 1.2 mg U/kg d.w. across all eight experiments, with the exception of the control treatments containing 20% kaolin clay, which contained 4.7 ± 2.0 mg U/kg d.w., due to the natural background concentration of U found in the kaolin clay (Table 2.1). Mean (± SE) concentrations of dissolved U in pore water and overlying water from controls across all eight experiments were 0.019 (± 0.003) and 0.0034 (± 0.0016) mg U/L, respectively. The mean concentration of U accumulated by *C. dilutus* larvae in control treatments was below 1.0 mg U/kg d.w. in all experiments.

2.3.4 Clay studies

Uranium bioaccumulation in *C. dilutus* larvae exposed to U-spiked sediment, and corresponding concentrations of U in overlying water and pore water, are presented in Fig. 2.1 for all three clay experiments combined. The sediment containing 2% montmorillonite clay spiked with 200 mg U/kg d.w. was compromised (spiking error) and thus was not presented in Fig. 2.1b, or included in any statistical analysis. Different clay types resulted in significantly different concentrations of U in the spiked sediment despite receiving the same amount of U (Fig. 2.1a and b). Kaolin clay amended sediments had the greatest retention of U among the three clays examined and most closely represented the nominal concentrations. However, the retention of U in sediment generally did not differ significantly between clay contents (0% vs 2% vs. 20%) within each individual clay type (Fig. 2.1). The exception to this was in the 20% montmorillonite sediment, which retained significantly more U than the 0% or 2% montmorillonite sediment treatments at 50 mg U/kg d.w. (Fig. 2.1a) and less U at 200 mg U/kg d.w. than 0% (Fig. 2.1b). Uranium concentrations generally decreased significantly in the overlying water, pore water, and U-exposed *C. dilutus* larvae when clay content increased from 2% to 20% for all clay types examined, except for illite clay at 200 mg U/kg d.w. (Fig. 2.1b). Among the three types of clay, kaolin had significantly lower concentrations of U in the dissolved phases than montmorillonite and illite (Fig. 2.1). However, it should be noted that the montmorillonite and illite treatments required centrifugation due to the visible presence of fine clay colloids in the pore water samples, but this process may not have been effective at removing all fine colloids which could explain the greater concentrations of U in the dissolved fractions of these treatments. There was on average a 2-fold decrease of U bioaccumulation in *C. dilutus* for all clay types as clay content increased from 2% to 20% (Fig. 2.1), except for illite clay spiked with 50 mg U/kg d.w. that resulted in a 5-fold decrease in U bioaccumulation from 2% to 20% clay.

2.3.5 Particle size distribution

2.3.5.1 Surface area - Sand

Sand size fraction did not have a significant influence on U bioavailability at nominal U treatments of 5 and 50 mg U/kg d.w. (Fig. 2.2). Total U concentrations measured in the spiked sand were consistently lower by approximately 2- to 4-fold than nominal concentrations (5 and 50 mg U/kg d.w), suggesting minimal adsorption of U to the sand. Similarly, concentrations of U in pore water were generally comparable on a ppm basis to those measured in the sand, further suggesting that there was minimal binding to the sand. On average, there was a 4-fold decrease from day 0 to 10 in the concentrations of U in both the sediment and pore water. Concentrations of U were statistically similar among the three sand size fractions at each U-spiked concentration (Fig. 2.2). Overall, U concentrations in the sand, dissolved phases, and *C. dilutus* larvae at the three different sand size fractions followed similar trends at both nominal U concentrations.

2.3.5.2 Surface chemistry – Clay

Formulated sediments containing greater than 6.7% kaolin clay retained U more closely to the nominal U-spiked concentration (50 mg U/kg d.w.; Fig. 2.3) than sediments with ≤ 2.2% clay. The U concentrations in whole sediment and pore water were similar in the sand (0%) and 0.25% clay treatments, suggesting that those substrates had a limited capacity for binding U (Fig. 2.3). In contrast, treatments containing ≥ 2.2% clay displayed a strong inverse relationship between increasing clay concentrations and decreasing U concentrations in pore water (Fig. 2.3). This trend corresponded with a strong positive relationship between concentrations of U in the pore water and increasing concentrations of U in *C. dilutus* larvae (Fig. A2.1; $\log(y) = 0.7\log(x) + 1.7$, $r^2 = 0.84$, $p = 0.004$). Organisms exposed to U-spiked sediments had approximately a 100-fold reduction in bioaccumulation of U as clay content increased from 0 to 60% clay, clearly

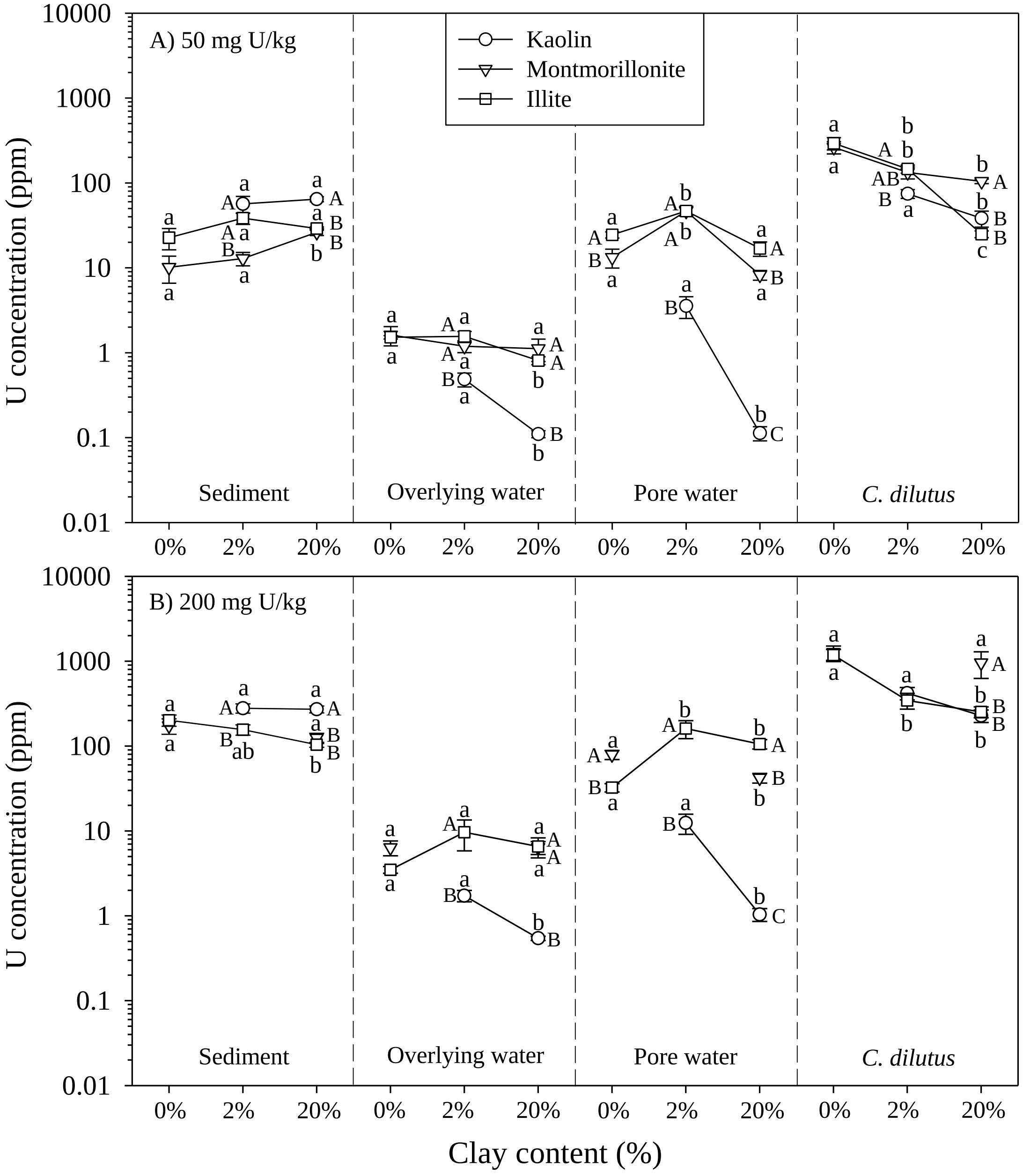

Fig. 2.1 Mean concentrations of uranium (± SE) measured in whole sediment (mg U/kg d.w.), overlying water (mg U/L), pore water (mg U/L), and whole organisms (*C. dilutus*; mg U/kg d.w.) when formulated sediments amended with 0, 2, and 20% clay d.w. (kaolin, montmorillonite, and illite) were spiked with nominal U concentrations of (A) 50 mg/kg d.w. and (B) 200 mg/kg d.w. Data points that do not share a letter are significantly different (one-way ANOVA, $p < 0.05$), with lowercase letters indicating a difference between concentrations (0% vs 2% vs 20%) for each respective clay mineral, and uppercase letters representing a difference among the three types of clay mineral at either 0, 2, or 20% clay content.

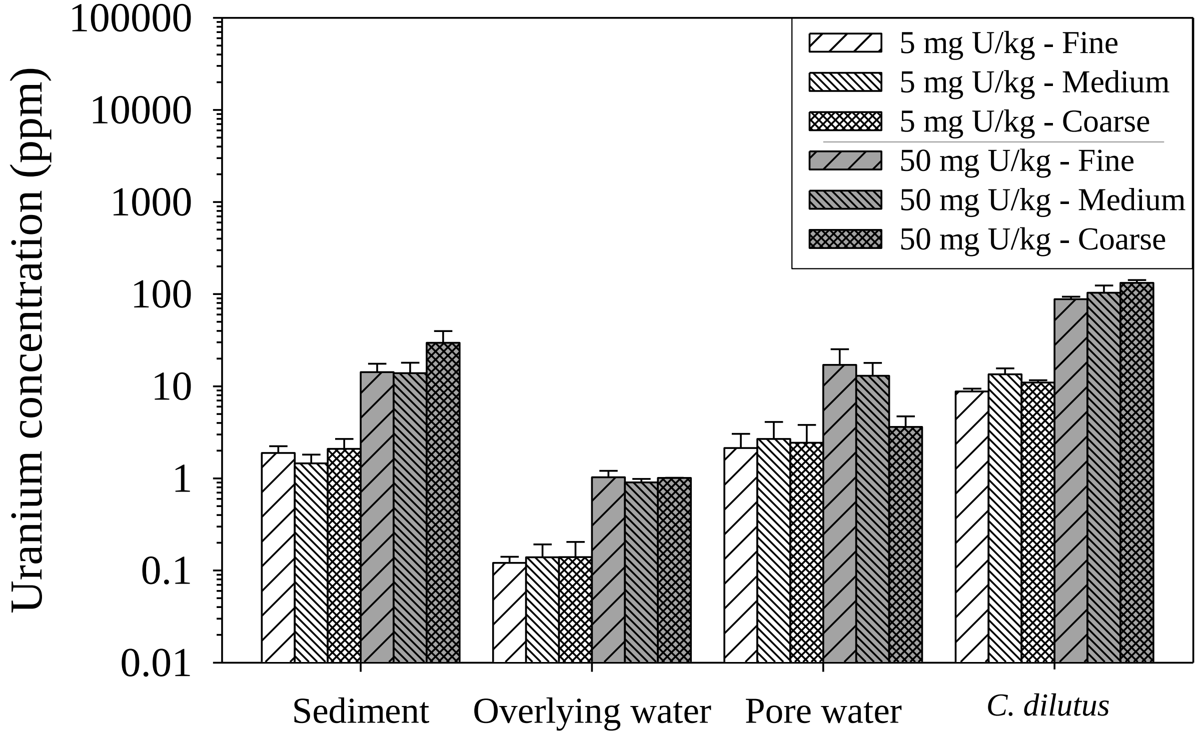

Fig. 2.2 Mean concentrations of uranium (± SE) measured in whole sediment (mg U/kg d.w.), overlying water (mg U/L), pore water (mg U/L), and whole organisms (*C. dilutus*; mg U/kg d.w.) when three sand size fractions (fine 125 µm, medium 362 µm, and coarse 725 µm) were spiked with nominal concentrations of 5 and 50 mg U/kg d.w. Uranium concentrations within any matrix were not significantly different among particle size treatments within either U treatment.

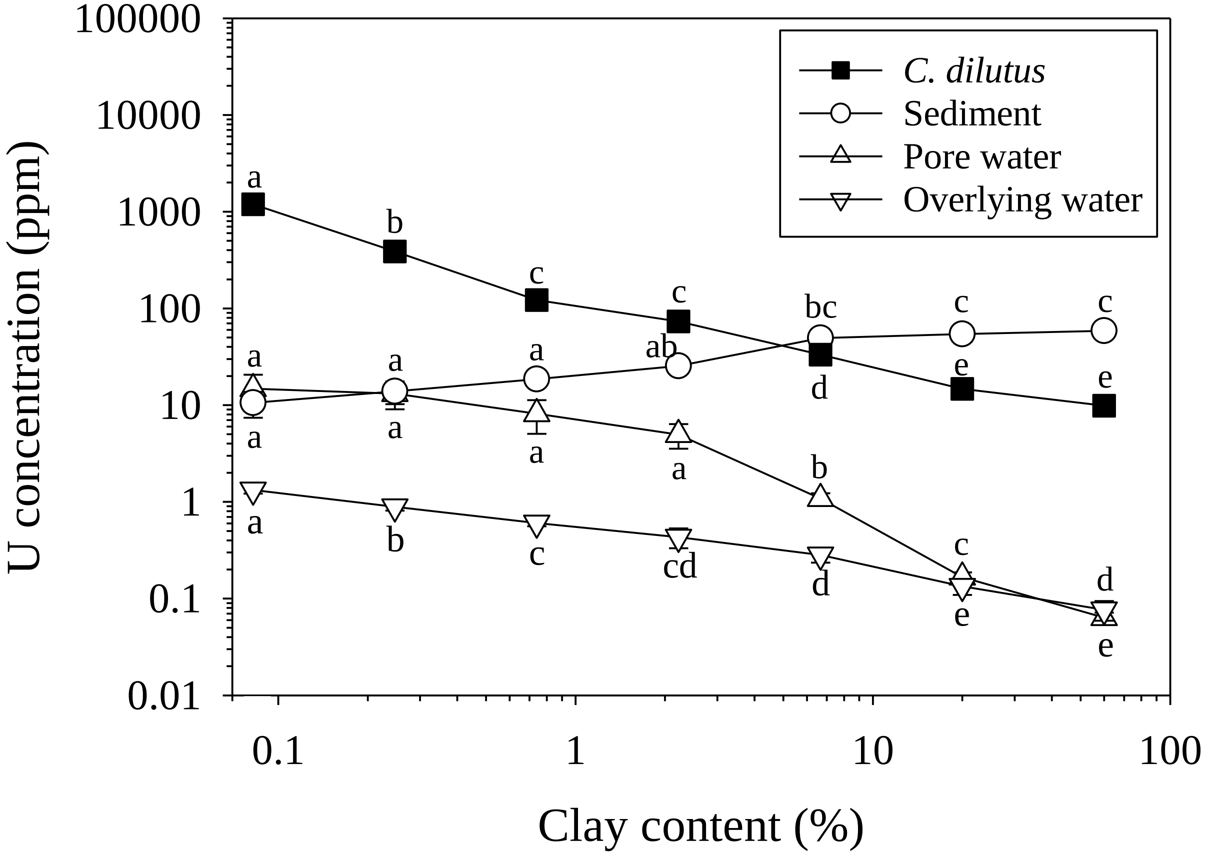

Fig. 2.3 Mean uranium concentrations (± SE) in whole organisms (*C. dilutus*; mg U/kg d.w.), whole sediment (mg U/kg d.w.), pore water (mg U/L), and overlying water (mg U/L) relative to nominal sediment kaolin clay content (% d.w.) in a 10-d test where sediments were spiked with a nominal concentration of 50 mg U/kg d.w. The first data point on each line represents a nominal zero percent clay content. Data that do not share a letter are significantly different (One-way ANOVA, $p < 0.05$).

demonstrating that sediment clay content reduced U bioavailability to *C. dilutus* larvae in a predictable fashion.

2.3.6 Organic Matter

2.3.6.1 Percent organic matter

In contrast to the percent clay experiment, all OM treatments (i.e., 0.25% to 40% OM) showed slightly higher than nominal U concentrations in the whole sediment across all treatments (50 mg U/kg d.w.; Fig. 2.4). Concentrations of U in pore water demonstrated a strong inverse relationship with sediment OM content resulting in a 31-fold decrease in mean U concentrations in pore water when OM content increased from 0% to 40% OM (Fig. 2.4). Concentrations of U in pore water also had a significant positive relationship with U concentration in the whole organism (Fig. A2.2; $\log(y) = 1.0\log(x) + 1.1$, $r^2 = 0.97$, $p < 0.001$). Bioaccumulation of U had a significant 30-fold decrease as OM concentrations increased from 0% to 40% OM d.w. (Fig. 2.4), demonstrating that U bioavailability decreased with higher concentrations of sediment OM.

2.3.6.2 Source of organic matter

In general, U concentrations were greater in peat amended sediments than α-cellulose amended sediments, but there were no significant differences in U concentration between the 5% and 15% OM treatments in either the peat or the α-cellulose experiments (Fig. 2.5). There were also no significant differences in the U concentrations of the pore water or U bioaccumulation between the 5% and 15% α-cellulose treatments. In contrast, U concentrations in pore water decreased significantly as peat content increased from 0 to 15% at both U concentrations, which corresponded with significant decreases in U bioaccumulation in *C. dilutus* at both U concentrations. Overall, the addition of 5% OM as peat or α-cellulose significantly decreased U bioaccumulation in *C. dilutus* larvae.

2.3.7 Bioaccumulation versus uranium exposure pathways

The exposure of *C. dilutus* larvae to U based on whole sediment, pore water, and overlying water pathways from all treatments in all eight experiments combined is presented in Fig. 2.6. Overall, whole sediment was a poor predictor of U bioavailability (expressed as concentration of U in the whole organism; $r^2 = 0.01$, $p = 0.028$). In contrast, the dissolved

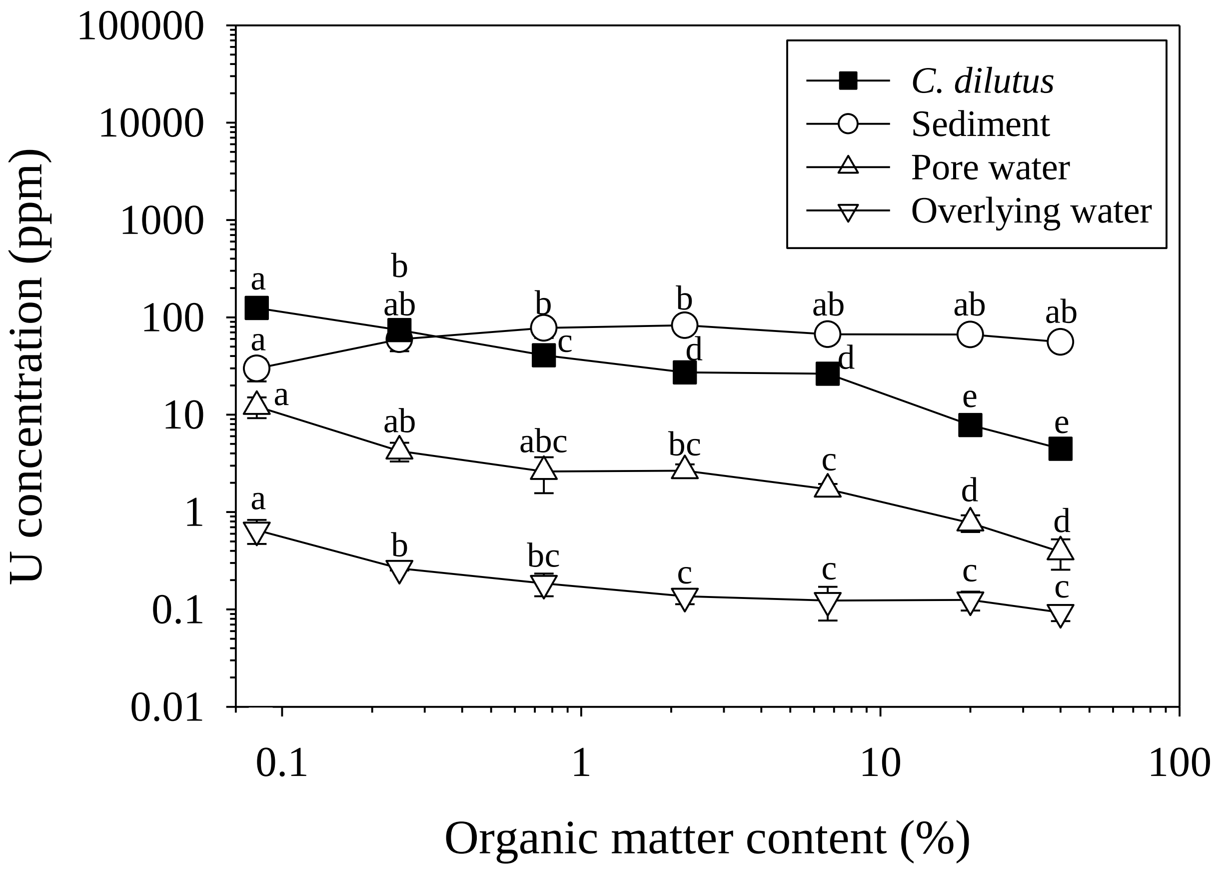

Fig. 2.4 Mean uranium concentrations (± SE) in whole organisms (*C. dilutus*; mg U/kg d.w.), whole sediment (mg U/kg d.w.), pore water (mg U/L), and overlying water (mg U/L) relative to nominal sediment organic matter content (% d.w.) in a 10-d test where sediments were spiked with a nominal concentration of 50 mg U/kg d.w. The first data point on each line represents a nominal zero percent organic matter content. Data that do not share a letter are significantly different (One-way ANOVA, $p < 0.05$).

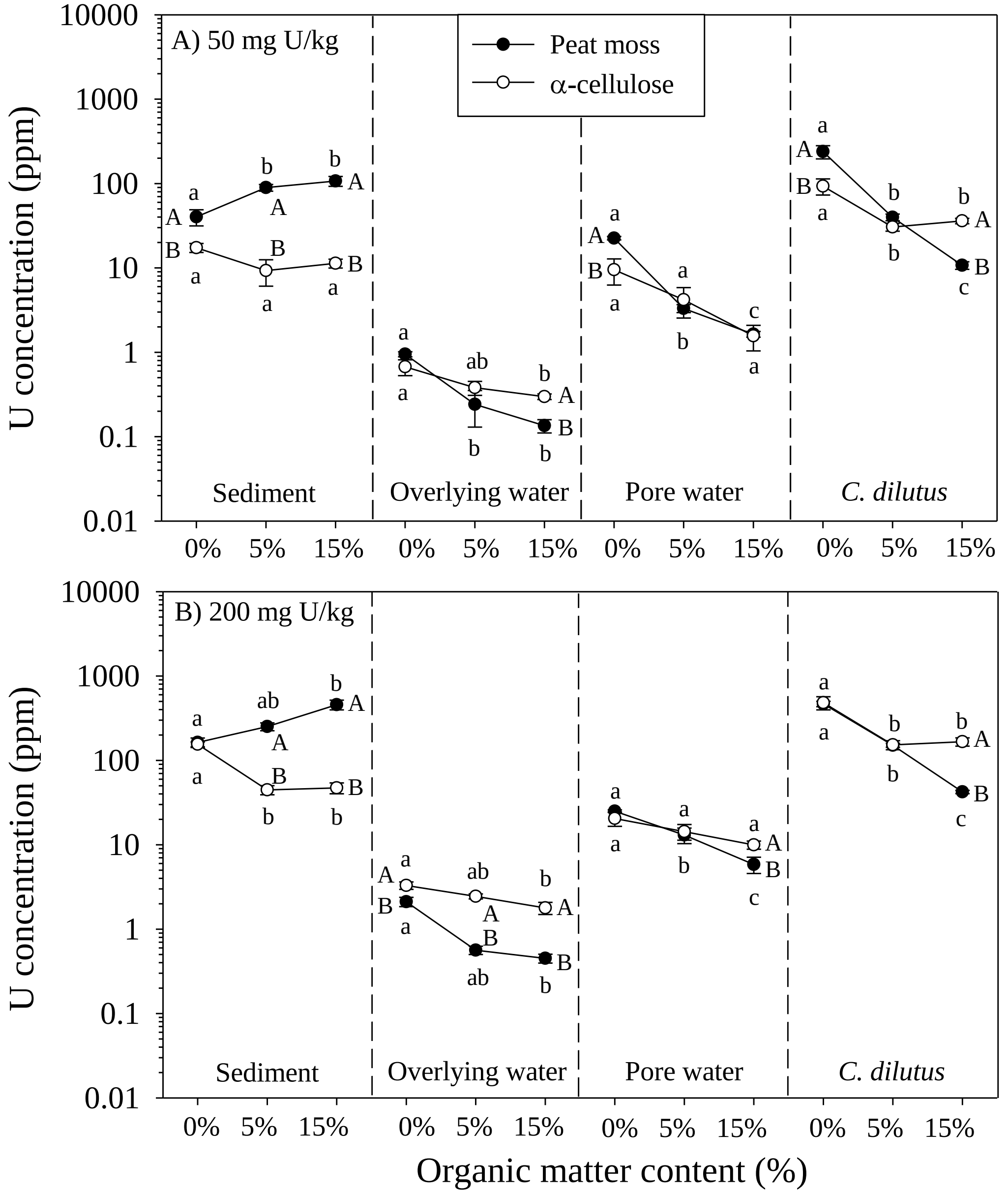

Fig. 2.5 Mean concentrations of uranium (± SE) measured in whole sediment (mg U/kg d.w.), overlying water (mg U/L), pore water (mg U/L), and whole organisms (*C. dilutus*; mg U/kg d.w.) when peat or α-cellulose amended sediments (nominal 0, 5 and 15% organic matter content d.w. basis) were spiked with nominal U concentrations of (A) 50 mg/kg d.w. and (B) 200 mg/kg d.w. Data points that do not share a letter are significantly different (one-way ANOVA, $p < 0.05$), with lowercase letters indicating a difference between concentrations (0% vs 5% vs 15%) for each respective source of organic matter, and uppercase letters representing a difference between peat and α-cellulose at each 0, 5, or 15% organic matter content.

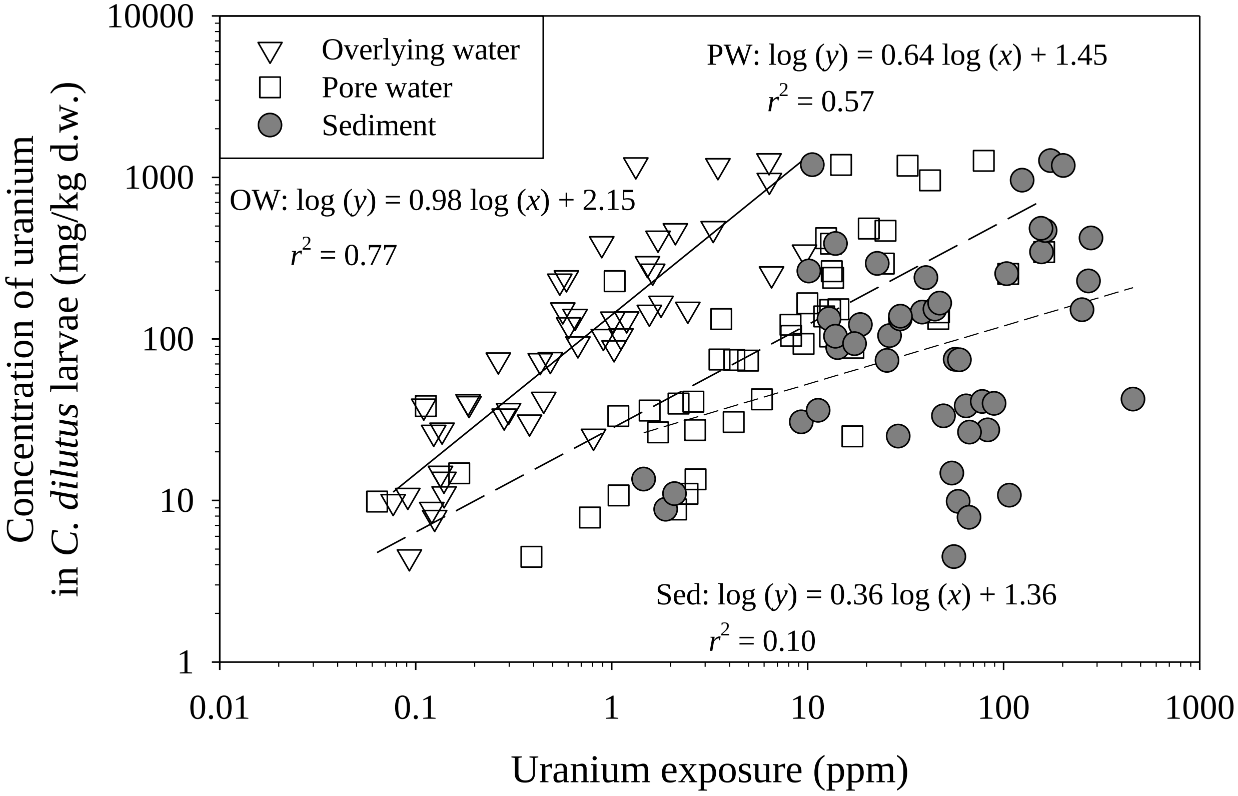

Fig. 2.6 Mean concentration of uranium in the whole organism (*C. dilutus* larvae; mg/kg d.w.) exposed to uranium in overlying water (OW; mg/L), pore water (PW; mg/L), and whole sediment (Sed; mg/kg d.w.) from all eight experiments. Simple linear regression lines are shown for each exposure matrix ($p < 0.05$).

fractions of U were better predictors of U bioaccumulation, with overlying water showing a slightly stronger relationship ($r^2 = 0.77$, $p < 0.001$) than pore water ($r^2 = 0.57$, $p < 0.001$).

2.4 Discussion

2.4.1 Effects of clay minerals on U bioavailability

The three clay experiments demonstrated a general reduction in U bioavailability to *C. dilutus* larvae as sediment clay content increased, with a better correlation observed between U bioaccumulation and aqueous U fractions than with whole sediment U. Differences in U bioaccumulation among clay types were more apparent at the higher (20%) clay amendment, which demonstrated that bioaccumulation of U in *C. dilutus* larvae was significantly reduced with kaolin and illite clay compared to montmorillonite clay (Fig. 2.1). Similar bioaccumulation was observed for both kaolin and illite treatments, despite having different U concentrations in the pore water and overlying water in these two experiments. The higher U concentrations observed in the pore water of the illite clay experiment could be the result of U adsorption to clay colloids, and thus may not represent the truly dissolved and bioavailable U concentration. The properties of the three clays examined (Table 2.1) suggest that strong adsorption of metals should occur to montmorillonite clay, followed by illite and then kaolin clay based on CEC, surface area, and clay structure (Bhattacharyya and Gupta, 2008; Catalano and Brown Jr, 2005). However, our results found the opposite with U adsorption being consistently greatest in kaolin clay, followed by illite clay and then montmorillonite (Fig. 2.1). Similar to our result, Wang et al. (2011) found that U adsorption among different types of surface area normalized minerals was greater in illite clay than montmorillonite clay.

The greater accumulation of U in *C. dilutus* larvae in the montmorillonite treatments is complicated further by the low survival. The montmorillonite results are, however, important in demonstrating that *C. dilutus* larvae may not thrive in habitats containing large deposits of montmorillonite clay. Sub-optimal conditions may be due to the high swelling capacity that is characteristic of montmorillonite clay (Table 2.1). The U retention capacity of montmorillonite might not have been as great as expected under our conditions of high ionic strength and a pH around 8, as these conditions have been shown to restrict sorption of the uranyl ion to only the edge sites of montmorillonite clays (Catalano and Brown Jr, 2005; McKinley et al., 1995; Pabalan and Turner, 1996). Additionally, measured CECs (Table 2.1), which generally reduce U bioavailability, were below the expected ranges for montmorillonite and illite clay, which may explain the under-predicted affinities for adsorption of U and associated bioavailability. However, adsorption of U onto clay minerals is complex and involves multiple mechanisms, including exchange and edge coordination sites, which can vary with pH, ionic strength, iron content, and other factors (Barnett et al., 2000; Chisholm-Brause et al., 1994; McKinley et al., 1995; Morrison et al., 1995; US EPA, 1999). Overall clay content and type influenced the bioaccumulation of U, suggesting that clay is a modifying factor of U bioavailability in sediment and thus an important factor for assessing risk of U contamination in sediment.

2.4.2 Effect of particle size distribution on U bioavailability

The presence of oxide coatings or other reactive surfaces on particles of sand have often been associated with greater adsorption of metals (Stone and Marsalek, 1996; Whitney, 1975). The commercial silica sand used in these experiments appears to have had little reactive surface chemistry, thus resulting in the negligible retention of U and the insignificant effect on U bioaccumulation among the different sand size fractions (Fig. 2.1). As a result, test organisms in this experiment were likely exposed largely to a water-only exposure matrix rather than a conventional spiked-sediment matrix. Bioaccumulation factors (BAFs) based on overlying water exposures ranged from 73 to 131, whereas pore water BAFs generally ranged from 4 to 8. In comparison, an overlying water BAF of 56 was previously reported for a 10-d water-only U experiment with *C. dilutus* larvae under similar laboratory conditions (Muscatello and Liber, 2010), further suggesting that our spiked-sand experiments largely represented a water-only exposure. Furthermore, published U sorption values for silica sand are small ranging from 0 to 10 ml/g (Ames et al., 1983; US EPA, 1999), suggesting high mobility and bioavailability, which is consistent with the low adsorption observed in this study. These observations further suggest that the presence of sand in the other formulated sediments used here should not have acted as a significant adsorber of U, and thus should not by itself reduce U bioavailability. In short, surface chemistry matters, not particle size alone.

The effect of particle surface chemistry on U bioavailability was investigated in a separate experiment with kaolin clay content, which showed a strong inverse relationship between clay content and U bioaccumulation (Fig. 2.3). This indicates that U bioavailability is reduced in sediments with higher amounts of clay, which is not routinely considered when contaminant concentrations are compared to SQGs. Similarly, work by Sheppard et al. (1992) found that $NaHCO_3$-extractable fractions of U were significantly correlated with clay content, also indicating that U was less readily available with greater clay content. The reduction in U bioaccumulation in *C. dilutus* as clay content increased mirrored the trend of decreasing concentrations of U in the dissolved phases (Fig. A2.1; pore water, overlying water), indicating that U bioavailability is more closely related to the dissolved phases than the sediment-associated phase. The same conclusion was obtained for overlying water in a study comparing U toxicity and accumulation by *Hyalella azteca* exposed to U-contaminated sediments (Alves et al., 2008).

2.4.3 Effects of organic matter on U bioavailability

Similar to the trend observed in the clay experiments, U bioaccumulation in *C. dilutus* larvae decreased as the content of OM in sediment increased (Fig. 2.4), demonstrating the importance of the peat fraction in the sorption of U. This is further supported by the greater retention of U in the sediment, along with lower concentrations of U in the dissolved phases at higher OM contents. These results are consistent with previous studies that have shown OM to act as a modifier of metal bioavailability (Ankley et al., 1993b; Cloran et al., 2010). In addition to the sorptive properties of peat, greater contents of OM often lead to higher concentrations of DOC that can also reduce metal bioavailability. Studies have shown that actinides, such as U, can form strong complexes with DOC, reducing U bioavailability (Jackson et al., 2005; Van Dam et al., 2012). Unfortunately, DOC data for both overlying water and pore water were difficult to interpret in our studies due to reduced accuracy resulting from the limited amount of sample available from the peepers. Dilution of peeper samples with ultra-pure water was necessary to reach the volume required for DOC quantification, resulting in low DOC concentrations and increased analytical variability. However, there was a clear increasing trend in DOC as sediment OM content increased from 0 to 40%, with greater DOC concentrations in the pore water (86-fold increase) than in the overlying water (19-fold increase). Additionally, there was an inverse relationship between U bioaccumulation in *C. dilutus* larvae and pore water DOC concentrations.

A comparison of the two OM types clearly showed that the addition of either type of OM to sand significantly reduced U bioaccumulation relative to sand alone. However, only the peat amendment was able to reduce U bioavailability further with an increase in OM from 5% to

15%. This might imply that there were excess binding sites on α-cellulose at 5%, thus the further addition to 15% did not offer additional U adsorption and hence no change in bioaccumulation (Fig. 2.5). However, the reduction in U bioaccumulation observed in the 15% peat treatment was more likely associated with the greater DOC concentrations found with 15% peat than with 15% cellulose. DOC concentrations did not differ between the low and high U concentrations (data not presented). The overlying water DOC concentration in 5% and 15% cellulose did not differ from the sand treatment (0% cellulose), and pore water DOC was only 2-3-fold greater than in the sand control. In comparison, overlying water DOC increased 2.5- and 6-fold with the addition of 5% and 15% peat relative to the sand treatments, while pore water DOC increased 41- and 71-fold with the addition of 5% and 15% peat, respectively. Thus, the greater concentrations of DOC associated with increasing peat content may explain the further reduction in U bioaccumulation observed with peat treatments. Thus, both OM amount and OM type are important for the assessment of U contaminated sediment, and DOC concentrations associated with OM sources may further alter the bioavailability of U in sediment.

2.4.4 Influence of sediment physicochemical properties on U exposure

Possible exposure routes of *C. dilutus* larvae to U in these experiments include the overlying water and pore water from respiration, filtering, and burrow irrigation behaviour, and from sediment due to burrowing activity (Dias et al., 2008; Thomas and Liber, 2001). We observed different burrowing behaviour in the various surface sediments. In the sand controls and treatments, the midge larvae typically lived in or on the sand surface, whereas in the clay and OM formulated sediments midge larvae exhibited better burrowing and case building behaviour. Such differences can alter, or at least influence, the importance of different contaminant exposure pathways, and thus change what pathway is most appropriately used in calculating BAFs. For example, BAFs based on U in overlying water in the clay and OM experiments ranged from 65 to 214, while the BAF based on pore water U exposure ranged from 3 to 25. These values are above and below the previously calculated water-only BAF of 56 for *C. dilutus* larvae (Muscatello and Liber, 2010), suggesting that both dissolved phases were important exposure routes in these formulated sediment experiments. However, the different burrowing behaviour observed in the sand treatments may influence the importance of U exposure routes. Thus, sandier sediments where animals are not burrowing well might exhibit more of an overlying water exposure pathway than sediments higher in OM and clay where animals burrow more, thus, resulting in more U exposure from pore water, as reflected by the BAFs in this study.

Dietary exposure of U may also be important as deposit feeders, such as *C. dilutus*, feed on OM present within sediment and make tubes from and within these substrates (Faria et al., 2007). The OM sources used in the present study (i.e., peat and α-cellulose) typically have low nutritional value compared to the Nutrafin food slurry provided (Lacey et al., 1999). The similar *C. dilutus* larvae biomasses observed among the sand, α-cellulose, and peat treatments support the notion of low nutritional value of the OM used in our experiments. Furthermore, Muscatello and Liber (2010) found no difference in U accumulation in fed and non-fed *C. dilutus* larvae in a 48-h water-only test. However, early in a 10-d test when midges are smaller, excess food can be present, thus leading to adsorption of U to the excess food particles. Food spiked with 1500 mg U/kg d.w. resulted in significant U bioaccumulation in *C. dilutus* larvae compared to the associated aqueous U exposure resulting from desorption of U from the spiked food (J. Hunt and K. Liber, unpublished data). However, in a 10-d test with formulated sediment, the addition of food is necessary for adequate survival and growth of chironomids (Environment Canada, 1997). In the present study, we did not determine if U would preferentially bind to OM sources over food, further altering the significance of U exposure pathways. This is particularly important in natural sediments that may have different sources and contents of OM present, or if supplemental feeding is not provided when investigating the effects of U on natural benthic communities. Future work will determine the partition coefficients of U to different sediments compared to food particles.

In most of the experiments described here, the total U concentration in sediment remained reasonably constant, or increased with increasing concentrations of clay or OM, while corresponding U bioaccumulation in *C. dilutus* decreased (Fig. 2.3 and Fig. 2.4). These results confirm what has been shown for other metals, that the total metal concentration in whole sediment is a poor indicator of bioavailability, especially when organisms are exposed to metals in sediments with a wide range of physicochemical properties (Ankley, 1996; Liber et al., 2011; Lock and Janssen, 2001). For example, previous studies have concluded that Ni concentrations in whole sediment were not reliable indicators of Ni bioavailability or toxicity to *H. azteca*, and instead Ni bioaccumulation or concentrations of Ni in overlying water better predicted Ni toxicity (Borgmann et al., 2001b). Additionally, toxicity to *C. riparius* in a 10-d whole-sediment test with contaminated sediment from a mine pit pond was attributed mainly to the water column, suggesting that much of the sediment-bound U was not bioavailable (Antunes et al., 2007). Uranium concentrations in the dissolved fractions (pore water and overlying water) of our experiments showed significant positive relationships with U bioaccumulation in *C. dilutus* larvae (Fig. 2.6). This conforms to previous work (Alves et al., 2008; Ankley et al., 1996b; Luoma, 1989) that has suggested that dissolved concentrations of metals correlate better with the bioavailable fraction to aquatic organisms compared to whole sediment metal concentrations.

2.5 Conclusions

The results from this research show that the sediment physicochemical factors, clay and organic matter, alter the adsorption of U in sediment, the partitioning of U into the dissolved phases, and thus U bioavailability to benthic organisms. This knowledge may be used to help improve current SQGs for U, which rely on total U concentrations in the sediment to predict probable effect levels on benthic communities (Thompson et al., 2005). Our data clearly support that whole sediment U concentrations in formulated sediments are poor predictors of U bioavailability, and hence toxicity, and that aqueous U concentrations would be more appropriate predictors of U bioavailability to benthic invertebrates from sediment-associated U. Future work will focus on quantifying the role of sediment physicochemical characteristics of field sediment to determine if results from these formulated sediment experiments are environmentally realistic, and whether a simple SQG equation can be developed that adjusts for U bioavailability. Improving our understanding of the sorption behaviour of U in different types of sediment is important as it dictates mobility and bioavailability of U in the environment and hence the risk of U to aquatic biota in U-contaminated environments.

CHAPTER 3:

SEDIMENT PROPERTIES INFLUENCING THE BIOAVAILABILITY OF URANIUM TO *CHIRONOMUS DILUTUS* LARVAE IN SPIKED FIELD SEDIMENTS

3 Preface

The research described in this chapter is a further investigation of the key physicochemical properties of sediment identified in Chapter 2 that influenced U bioavailability but with a focus on the physicochemical properties associated with field sediments. Eighteen field sediments with a wide range of properties were collected from reference areas surrounding northern Saskatchewan U mining operations to quantify and compare the bioaccumulation of U by *Chironomus dilutus* larvae in several 10-d U-spiked field sediment experiments. Simple regression models were developed to predict the bioaccumulation of U based on different sediment properties, with fine fraction ($\leq 50\ \mu m$ particle size) being the single most consistent and practical variable. This research strongly supports that risk assessments of U-contaminated sediments should not ignore the influence of sediment properties that can result in substantial differences in the bioaccumulation of U in benthic invertebrates.

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Crawford, S.E., Liber, K., 2016. Sediment properties influencing the bioavailability of uranium to *Chironomus dilutus* larvae in spiked field sediments. Chemosphere. 148, 77-85.

3.1 Introduction

Uranium (U) is a naturally occurring element that can be found in rich deposits around the world. Areas such as northern Saskatchewan (Canada) have some of the richest deposits of U ore globally, and thus are susceptible to U contamination from both natural and anthropogenic sources (CCME, 2011). The mining and milling of U for nuclear energy production can result in increased concentrations of U downstream of U mining activity (Liber et al., 2011). In such situations, the interactions between dissolved U and solid phases can lead to sediment U concentrations that are often several orders of magnitude higher than those in the water column, reaching concentrations of over 1000 mg U/kg dry weight (d.w.) in some sediments (Environment Canada, 2003; Liber et al., 2011). Consequently, sediments can act as a long-term sink, and subsequent source, of U and thus have the potential to cause adverse effects on freshwater aquatic organisms that live in and on sediment in areas with U contamination (Liber et al., 2011).

Despite the potential for U to accumulate to toxic levels in the sediment, there are currently no national U Sediment Quality Guidelines (SQGs) available in Canada or elsewhere. SQGs aim to protect aquatic environments from hazardous levels of contaminants in sediment, which could adversely affect aquatic life (Burton et al., 2002; Chapman et al., 1998). The Screening Level Concentration (SLC) approach (Thompson et al., 2005) is one of the few region-specific SQGs established anywhere and is used by the Canadian Nuclear Safety Commission to regulate U mines in northern Saskatchewan. However, the SLC guidelines have been shown to poorly predict impacts of U, or lack thereof, on benthic communities (Burnett-Seidel and Liber, 2012). The SLC approach, and many other SQG approaches, are based on co-occurring empirical data that are used to establish associations between total metal concentrations in sediments and adverse biological effects on benthic communities (CCME, 1999; Thompson et al., 2005). Inherent to the SLC approach, the co-occurrence of multiple contaminants and/or differences in metal bioavailability can often result in metals being falsely classified as the cause of toxicity. Recommendations for the improvement of generic SQGs include the incorporation of metal bioavailability and modifying factors that control the behaviour of metals into the assessment approach. An enhanced understanding of the modifying factors that influence contaminant bioavailability will thus allow for better identification of metal concentrations that are biologically relevant to benthic organisms.

Data on the toxicity and bioaccumulation of sediment-associated U to benthic freshwater organisms is sparse in the primary literature, limiting our understanding and quantification of the factors that influence U bioavailability. In particular, few studies have examined how sediment characteristics influence the bioavailability of U to benthic invertebrates. Crawford and Liber (2015) demonstrated the importance of organic matter (peat) and clay (kaolin) content in altering U bioavailability in formulated sediments to larvae of the freshwater midge, *Chironomus dilutus*.

However, the bioavailable fraction of metals in sediment is typically thought to be governed by a number of metal binding properties of sediment, such as cation exchange capacity (CEC), acid volatile sulphides (AVS), total organic carbon (TOC), as well as iron (Fe) and manganese (Mn) oxides, that are more complex in field sediment than in formulated sediment (Campana et al., 2013; Simpson and Batley, 2007). Furthermore, the importance of these properties and phases have not been demonstrated for U-contaminated sediment. Consideration of AVS and organic carbon (OC) content of sediment has led to improved predictions of the toxicity of divalent cationic metals and polycyclic aromatic hydrocarbons, respectively to benthic invertebrates, compared to the traditional use of total contaminant concentrations in the sediment (Berry et al., 1996; Liber et al., 1996; US EPA, 2003; US EPA, 2005). Additionally, dissolved metals present in pore water have been found to better correlate with toxicity than total metal concentrations in whole-sediment (Allen and Janssen, 2006; Ankley et al., 1996a; Batley et al., 2004; Chapman et al., 1998; Crawford and Liber, 2015). Thus, to gain an accurate understanding of the bioavailability of U in sediments, mechanistic knowledge is required on the influence of physicochemical characteristics of sediment on the partitioning of metals between the pore water and solid sediment particles, as well as on the exposure pathways for benthic organisms.

The goal of the present study was to examine the role of several sediment physicochemical characteristics in altering the bioavailability and bioaccumulation of U in *Chironomus dilutus* larvae. The hypothesis was that field-collected sediments with a wide range of physicochemical properties spiked with the same total concentration of U (50 or 500 mg/kg d.w.) would differentially influence the bioavailability and hence bioaccumulation of U by *C. dilutus* larvae exposed to the spiked sediment. The specific objectives of this study were to (i) investigate and model the effects of key physicochemical properties of 18 field sediments, specifically TOC content, particle size distribution, CEC, and the content of Fe and Mn on U bioavailability, (ii) evaluate the exposure routes of U to *C. dilutus* larvae and determine if total U concentration is an adequate predictor of U bioaccumulation, and (iii) compare U bioaccumulation from field sediments to that observed from previous U-spiked formulated sediments.

3.2 Materials and methods

3.2.1 Sediment collection, storage and handling

Sediments were collected from approximately the top 10 cm layer of surficial sediments using a small Ekman grab from reference areas surrounding U mines in northern Saskatchewan (Table 3.1). Sediment samples were placed into separate polyethylene buckets, excess overlying water decanted, and buckets were sealed (some natural headspace remaining), transported to the laboratory in Saskatoon, and stored in the dark at 4°C until use. Sediment buckets were homogenized before use, large debris removed with forceps, and aliquots were collected for analysis of total metals, TOC, particle size, Fe and Mn content, and water content prior to spiking and experimental use (Table 3.1). Any natural AVS present would have been diminished under the oxic conditions of sediment sampling, mixing and spiking, and consequently was not measured.

3.2.2 Sediment spiking and test system

Sediments were spiked to desired U concentrations (50 and/or 500 mg U/kg d.w.) using uranyl nitrate hexahydrate ($UO_2(NO_3)_2 \cdot 6H_2O$) (Strem Chemicals, Inc., Newburyport, MA, USA). A U concentration of 50 mg U/kg d.w. was selected for comparison to previously conducted U-spiked formulated sediment tests that used the same concentration (Crawford and Liber, 2015). Since field sediments were expected to have greater binding capacities than the previously used formulated sediments, an additional 10-fold greater concentration of 500 mg U/kg d.w. was also evaluated. Overall, 18 field sediments were spiked with 50 and/or 500 mg U/kg d.w. for a total of 25 different U-spiked treatments (Table 3.1). All sediment treatments were aged for 20 days prior to each 10-d bioaccumulation test following methods described by Crawford and Liber (2015). The only amendment to the spiking method was that field sediments were spiked on a wet weight basis (approx. 1000 g w.w. sediment) with additions of 5 ml of U stock solutions (in ultra-pure water, 18 MΩ/cm) to ensure minimal changes to the natural water content of the sediment. The biological controls consisted of silica sand minimally saturated with water, which received the same handling but with the addition of only un-spiked, ultra-pure water. Additionally, three uncontaminated field sediments (SL2, MC3, SL1), covering a wide range of physicochemical properties, were used as un-spiked controls to ensure that possible effects on test organisms were not due to the substrate alone.

Table 3.1 Physicochemical characteristics, water content and background uranium concentrations of field-collected reference sediments from northern Saskatchewan, Canada.

Sediment ID[a]	**TOC (%)[b]**	**% Sand (> 50 μm)[c]**	**% Silt (2 - 50 μm)[c]**	**% Clay (< 2 μm)[c]**	**% Fine fraction (silt + clay)[c]**	**CEC (meq/100 g)[d]**	**Fe (g/kg)[e]**	**Mn (mg/kg)[e]**	**Water content (%)**	**Background U (mg/kg d.w.)[f]**
UR1	0.5	97	3	0	3	2	5	1.9	26	1
UR8	1.0	95	5	0	5	4	10	11.9	34	1
SL2$^-$	1.1	99	1	0	1	5	2	0.2	80	1
UR5$^+$	1.6	75	24	1	25	7	13	1.4	35	2
UR7	2.9	86	13	1	14	9	15	4.4	48	2
UR2	3.8	29	70	1	71	12	9	0.5	50	2
MC4$^-$	4.0	90	9	1	10	10	14	4.6	86	n/a
UR6-B	5.0	90	9	1	10	9	17	0.6	53	3
UR6	5.3	77	21	2	23	12	24	0.8	54	2
WB$^+$	5.5	78	21	1	22	25	13	2.0	66	3
MC3$^-$	8.8	84	14	2	16	n/a	n/a	n/a	91	1
ML$^+$	12.4	63	35	2	37	33	29	16.4	86	2
MWB$^+$	13.6	7	80	3	83	54	41	2.3	87	3
KL$^+$	15.9	1	71	28	99	64	34	2.6	92	2
UR3	17.3	29	66	5	71	66	20	2.5	76	12
UM$^+$	18.6	2	88	10	98	66	7	1.7	87	2

Sediment ID[a]	TOC (%)[b]	% Sand (> 50 µm)[c]	% Silt (2 - 50 µm)[c]	% Clay (< 2 µm)[c]	% Fine fraction (silt + clay)[c]	CEC (meq/100 g)[d]	Fe (g/kg)[e]	Mn (mg/kg)[e]	Water content (%)	Background U (mg/kg d.w.)[f]
SL1^{-}	19.5	42	48	10	58	61	17	1.5	98	3
HL^{+}	22.1	25	68	7	75	48	8	0.7	95	1

TOC = total organic carbon; CEC = cation exchange capacity; n/a = not analyzed.

[a] Field sediments collected from the Wollaston Lake area in northern Saskatchewan, Canada; SL – Shallow Lake; MC – McClean Lake; HL – Henday Lake; KL – Konner Lake; ML – Mallen Lake; MWB – McClean West Basin; UM – Umperville Marsh; UR – Umperville River; WB – Wollaston Bay. Superscripts indicate that sediments were spiked with (-) only 50 mg U/kg d.w., (+) only 500 mg U/kg d.w., or (blank) spiked with both U-concentrations.

[b] Determined by LECO Carbonator Model C632, Department of Soil Science, University of Saskatchewan, Saskatoon, SK.

[c] Determined by mini-pipette method with removal of organic matter and carbonates, ALS Environmental, Saskatoon, SK.

[d] Determined by ammonium acetate extraction, ALS Environmental, Saskatoon, SK.

[e] Determined by dithionite-citrate-bicarbonate (DCB) extraction for total Fe/Mn (Pansu and Gautheyrou, 2007; Ryan and Gschwend, 1991).

[f] Determined by ICP-MS after complete sediment digestion, Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Test beakers consisted of 300-ml tall-form glass beakers (7 cm diameter), with two 1.5-cm diameter opposing holes in their side walls located 7 cm above the beaker bottom and covered with 25-µm stainless steel screen to allow for water exchange. After 10 days of aging, 75 ml aliquots of homogenized, spiked-sediment and 125 ml of clean culture water (Table 3.2) were transferred to each of eight replicate test beakers (six biological and two chemical analysis beakers). Previously described *in situ* dialysis devices, mini-peepers (Crawford and Liber, 2015; Doig and Liber, 2000), were used to collect approximately 1 ml of overlying water and 1 ml pore water within the biological test beakers (from just above and below the sediment surface). Mini-peepers were filled with ultra-pure water and covered with semi-permeable polyethersulfone membranes (0.45-µm pore size) and deoxygenated with nitrogen overnight prior to use to minimize oxidation reactions that can cause the pore water pH to decrease in spiked sediments. An additional aging period of 10 days was used for each test to allow the spiked U to sufficiently equilibrate between the solid sediment and the pore water, and between the pore water and the mini-peeper water prior to the addition of beakers to the test system. The modified sediment testing intermittent renewal (STIR; Benoit et al., 1993) test system and the associated automated overlying water renewal procedure (using carbon-filtered, bio-filtered municipal water) were described in detail by Crawford and Liber (2015).

3.2.3 Bioaccumulation tests

Culturing and 10-d spiked sediment bioaccumulation tests with *C. dilutus* larvae were carried out according to Environment Canada (1997) guidelines and previously described methods (Crawford and Liber, 2015). *Chironomus dilutus* larvae (Diptera, Chironomidae) were selected as the test organism as they are a standard, easily cultured sediment test species, ubiquitous in the environment and representative of Saskatchewan, have close contact with sediment throughout larval development, and are useful for the determination of contaminant bioaccumulation from spiked sediments.

At each test initiation, 10 second instar (8 to 10-d old) *C. dilutus* larvae were randomly added to 6 replicate beakers and exposed to the U-spiked or control sediment treatments for 10 days. Three extra replicates of 10 individual organisms each were also collected at test initiation (not gut purged) to determine average initial dry weight after 24-h drying at 60 °C. Mean initial dry weight of individual *C. dilutus* larvae at the start of all tests averaged (± SD, *n* = 15) 0.17 ±

0.05 mg d.w. During each test, organisms were fed 6 mg of Nutrafin® fish flake (Rolf C. Hagen Inc., Montreal, QC, CAN) slurry per beaker daily. On day 10, surviving organisms were enumerated and underwent an EDTA rinse to remove any U bound to the surface of the organisms and subsequently a 12-h gut clearance period using clean sand substrate and food as described by Crawford and Liber (2015). The gut purging period was included to remove U-contaminated stomach content associated with the different U-spiked field sediments prior to determination of U bioaccumulation. Organism dry weights were measured after a 24-h drying period at 60°C (post purge) and then stored for subsequent analysis of total U concentration in the whole organism.

On days 0, 5 and 10 of all tests, 20 ml of overlying water were collected from three randomly selected replicate beakers per treatment for routine water chemistry analysis (temperature, dissolved oxygen, conductivity, pH, ammonia, alkalinity and total hardness). On days 0 and 10, mini-peepers were removed from three randomly selected beakers, rinsed with ultra-pure water, and mini-peeper chamber membranes pierced with an Eppendorf pipette tip for collection of overlying and pore water samples that were then acidified (2% HNO_3) for subsequent dissolved U analysis. Approximately 1 g d.w. of sediment was collected from the top 1 cm layer from the same three beakers the mini-peepers were retrieved from on days 0 and 10, and dried (60° C) for later analysis of total U in the whole-sediment. Dissolved organic carbon (DOC) was analyzed on 12 ml samples of 0.45-µm filtered, overlying water from three replicate beakers per treatment on days 0 and 10 (TOC-V CPN model 5000, Shimanzu, Kyoto, Japan). Additionally, DOC was analyzed for pore water samples collected through centrifugation and vacuum filtration (0.45 µm) of whole-sediment collected on days 0 and 10 from chemical analysis beakers.

3.2.4 Chemical analysis

Uranium analyses in whole-sediment, whole organism, and water samples were performed at the Toxicology Centre, University of Saskatchewan, SK, using inductively coupled plasma mass spectrometry (ICP-MS; X-series II, Thermo Electron Ltd, Mississauga, ON, CAN) after acidification of the samples (2% HNO_3). The minimum method detection limit for U was 0.05 ppb, with instrumental and method recoveries within ± 5% and ± 15%, respectively. Total metal concentrations in sediment were determined after microwave-assisted, concentrated acid digestions using H_2O_2, HNO_3, and HF. Whole organisms (post 12-h gut clearance) were fully digested using HNO_3, H_2O_2, and heat. Certified reference materials (PACS-2 marine sediment for trace metals, NRC Canada, Ottawa, CAN; 1640a trace elements in natural water, NIST, Gaithersburg, MD, USA), duplicates, and blanks were used in the QA/QC procedures to ensure analytical accuracy in the quantification of U.

3.2.5 Statistical analysis

Statistical analyses were performed and plotted with Sigmaplot®, version 11 (San Jose, CA, USA). All tests were conducted at $\alpha = 0.05$ after checking for compliance with parametric assumptions of normal distribution (Shapiro-Wilk test) and homogeneous variance (Levene's test). Mean survival and weight of *C. dilutus* larvae, and mean U concentrations in sediment, overlying water, pore water, and *C. dilutus* larvae were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests for all experiments. Data that did not meet parametric assumptions were transformed (arcsin square root (%), $\log_{10}$, or $\log_{10}(x+1)$) prior to statistical analysis. If data did not meet the normality and homogeneity of variances assumptions after transformation, then non-parametric Kruskal-Wallis tests were used, followed by Dunn's method pairwise multiple comparison post-hoc tests. Regression analyses (linear, step-wise, and multiple) were conducted to determine the relationships between mean bioaccumulation of U by *C. dilutus* larvae and (i) U exposure concentrations in sediment, overlying water, and pore water, and (ii) sediment properties (TOC, fine fraction, CEC, Fe and Mn content). Additionally, correlations among the independent sediment properties were examined with Pearson's product-moment tests.

3.3 Results

3.3.1 Test parameters

The field sediments used had a wide range of physicochemical properties (Table 3.1). The fine fraction component (≤ 50 µm particle size) in this paper is defined as the combined clay (< 2 µm) and silt (2 – 50 µm) fractions. Overlying water quality variables for all 10-d tests were similar to the source water (Table 3.2). No significant decreases in survival or weight of *C. dilutus* larvae were observed relative to the controls in any of the experiments. Mean (± SE, *n* = 33) final weight and survival of *C. dilutus* larvae across all treatments and controls were 1.5 ± 0.1 mg d.w. per surviving individual and 89% ± 1%, respectively. Test organisms in all

Table 3.2 General water quality variables for the source water and test water for all 10-d experiments.

Variable	**Source water (*n* = 5)**	**Test water (*n* = 25)[a]**
Temperature (°C)	21.5 ± 1.6	21.1 ± 0.6
Dissolved oxygen (mg/L)	8.8 ± 0.5	8.3 ± 0.2
Conductivity (µS/cm)	406 ± 11	417 ± 17
pH	8.0 ± 0.1	8.1 ± 0.1
Ammonia (mg/L)[b]	0.0 ± 0.0	0.2 ± 0.1
Total hardness (mg/L)[c]	148 ± 20	151 ± 14
Alkalinity (mg/L)[c]	110 ± 18	112 ± 15
Dissolved organic carbon (mg/L)	3.5 ± 0.8	3.7 ± 0.5

[a] Values are means (± SE) of all experimental means (i.e., an average of three replicates sampled on each respective sampling day, averaged for all treatments within each experiment and then averaged across all experiments). Source water was carbon-filtered, bio-filtered municipal water.

[b] Measured as ammonia nitrogen.

[c] Measured as $CaCO_3$.

treatments surpassed the recommended minimum acceptable weight (0.6 mg d.w.) and survival (70%) of *C. dilutus* larvae for controls (Environment Canada, 1997). No adverse effects of U on growth or survival of *C. dilutus* were noted in any of the experiments.

3.3.2 Bioaccumulation tests

All control treatments (un-spiked lab sand and field sediment) had negligible U concentrations (average ± SE, $n = 7$) in the whole-sediment (0.89 ± 0.43 mg/kg d.w.), pore water (0.0015 ± 0.0005 mg/L), overlying water (0.0011 ± 0.0002 mg/L), and whole organisms (0.35 ± 0.10 mg/kg d.w.). The low background U concentrations in the field sediments are typical of the range found in northern Saskatchewan (≤ 12 mg/kg d.w.; Table 3.1; Environment Canada, 2003). Mean U concentrations in the spiked sediments were generally within 16% of the nominal concentrations of 50 and 500 mg U/kg d.w. (Fig. 3.1), except for UR1 (37.4 ± 12.5 mg U/kg d.w., $n = 6$) and UR3 (63.5 ± 4.8 mg U/kg d.w., $n = 6$). Despite sediments being amended with the same total concentration of 50 and/or 500 mg U/kg d.w., concentrations of U varied by orders of magnitude in both the pore water (0.004 to 2.8 mg/L and 0.016 to 7.1 mg/L) and overlying water (0.004 to 0.2 mg/L and 0.013 to 1.1 mg/L), respectively. Concentrations of U accumulated in *C. dilutus* larvae varied from 5 to 69 mg/kg d.w. for sediments spiked with 50 mg U/kg d.w. and from 20 to 452 mg/kg d.w. for sediments spiked with 500 mg U/kg d.w. (Fig. 3.1, white symbols). Bioaccumulation of U in *C. dilutus* larvae showed a significant positive relationship with concentrations of U in both the overlying water ($r^2 = 0.61$, $p < 0.001$, grey symbols) and the pore water ($r^2 = 0.47$, $p < 0.001$, black symbols).

3.3.3 Modifying factors of U bioavailability

Univariate linear regressions were used to investigate the relationships between U bioaccumulation and different sediment properties to ascertain which properties were the most important modifiers of U bioavailability and bioaccumulation (Fig. 3.2). Negative relationships were observed between bioaccumulation of U and all sediment physicochemical properties investigated (Fig. 3.2), but only fine fraction content ($r^2 = 0.74$, $p < 0.001$) and Fe content ($r^2 = 0.65$, $p < 0.05$) significantly described the variation in U bioaccumulation for the 50 mg U/kg d.w. U-spiked sediment treatments. Similar negative relationships between bioaccumulation of U in *C. dilutus* larvae from sediment treatments spiked with 500 mg U/kg d.w. were evident ($p < 0.001$) for TOC ($r^2 = 0.84$), fine fraction ($r^2 = 0.79$), and CEC ($r^2 = 0.91$), all having similar

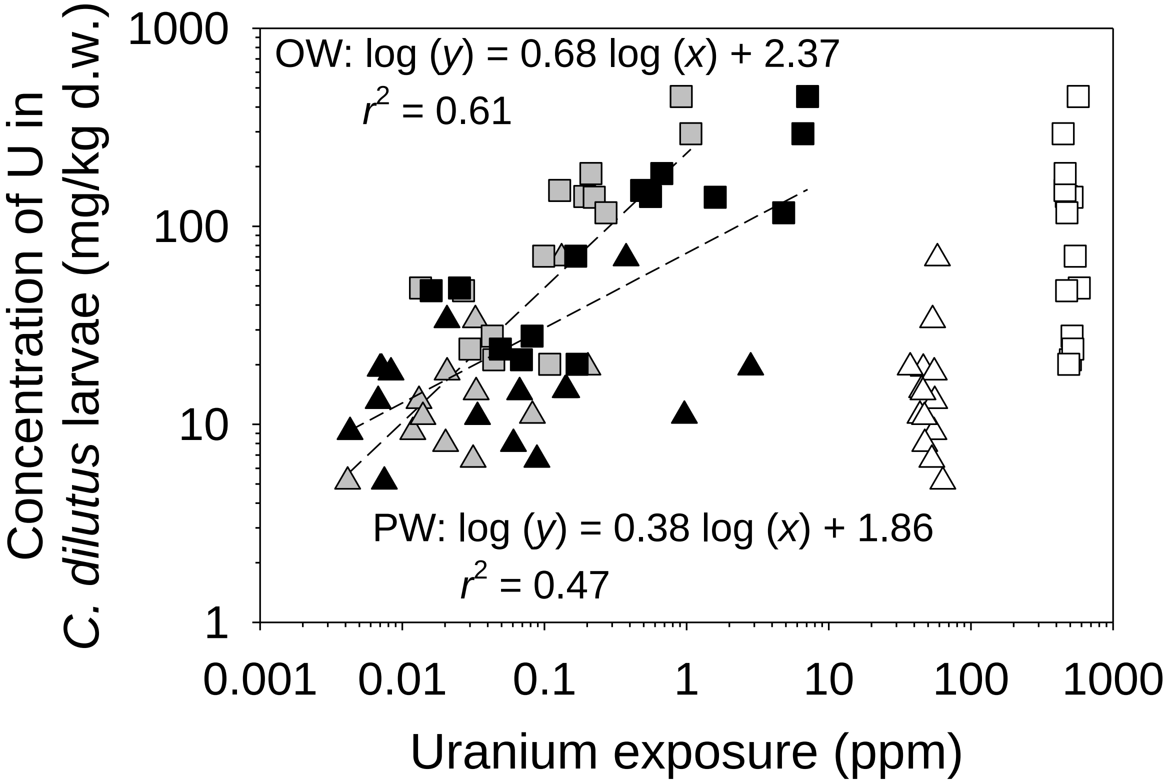

Fig. 3.1 Mean concentration of U in whole organisms (*C. dilutus* larvae; mg U/kg d.w.) as a function of mean U concentration in the overlying water (OW; grey symbols; mg U/L), pore water (PW; black symbols mg U/L), and whole-sediment (white symbols; mg U/kg d.w) for all field sediments spiked with either 50 mg U/kg d.w. (Δ symbols) or 500 mg U/kg d.w. (□ symbols). Simple linear regressions are shown for significant exposure pathways ($p < 0.001$).

slopes of -0.79, -0.79, -0.83, respectively (Fig. 3.2). The relationships between U bioaccumulation and Fe (Fig. 3.2d) or Mn content (not shown) were not significant. However, outlier analysis (standard residual values greater than two standard deviations of residual) indicated that Fe content of UM and HL sediments had a tendency to bias the regression analyses (Fig. 3.2d), and if excluded led to a stronger significant relationship (slope = -1.37, $r^2 = 0.71$, $p < 0.001$) for the 500 mg/kg d.w. treatment.

Many sediment properties co-varied and the co-correlations between different properties for the 18 sediments used in the present study are summarized in Table 3.3. Significant correlations were found among CEC, fine fraction and TOC ($r = 0.84$ to 0.97, $p < 0.001$). The content of Fe in sediment also correlated with TOC, fine fraction, and CEC, in addition to Mn ($r = 0.49$ to 0.62, $p < 0.05$). To determine if the combined effects of the sediment properties better explained U bioavailability, a multiple stepwise regression model was used to examine the relationship between U bioaccumulation in *C. dilutus* larvae and the combined sediment properties (TOC, fine fraction, CEC, and Fe content). For 50 mg U/kg d.w. spiked sediment, fine fraction and Fe content were incorporated into a model that lead to a slightly improved relationship for U bioaccumulation compared to the individual univariate equations (Eq. 3.1; $r^2 = 0.80$, $p = 0.002$).

$$\log U_{\text{tissue}} = 1.77 - (0.31 \log \text{fine fraction}) - (0.31 \log \text{Fe content}) \qquad \text{(Eq. 3.1)}$$

This model allowed for bioaccumulation of U to be predicted within a factor of two of the observed bioaccumulation values (Fig. 3.3a). In comparison, no sediment property, in addition to CEC alone, significantly contributed to explaining the variation in U bioaccumulation by *C. dilutus* larvae for the 500 mg U/kg d.w. spiked sediments (Eq. 3.2; $r^2 = 0.91$, $p < 0.001$). The univariate equation predicted U bioaccumulation within a factor of two of the observed values (Fig. 3.3b).

$$\log U_{\text{tissue}} = 2.93 - 0.83 \log \text{CEC} \qquad \text{(Eq. 3.2)}$$

3.4 Discussion

3.4.1 Exposure and bioaccumulation of U

An investigation using 18 different field sediments spiked with similar concentrations of U revealed large variations in U bioaccumulation by *C. dilutus* larvae. The 10-d bioaccumulation

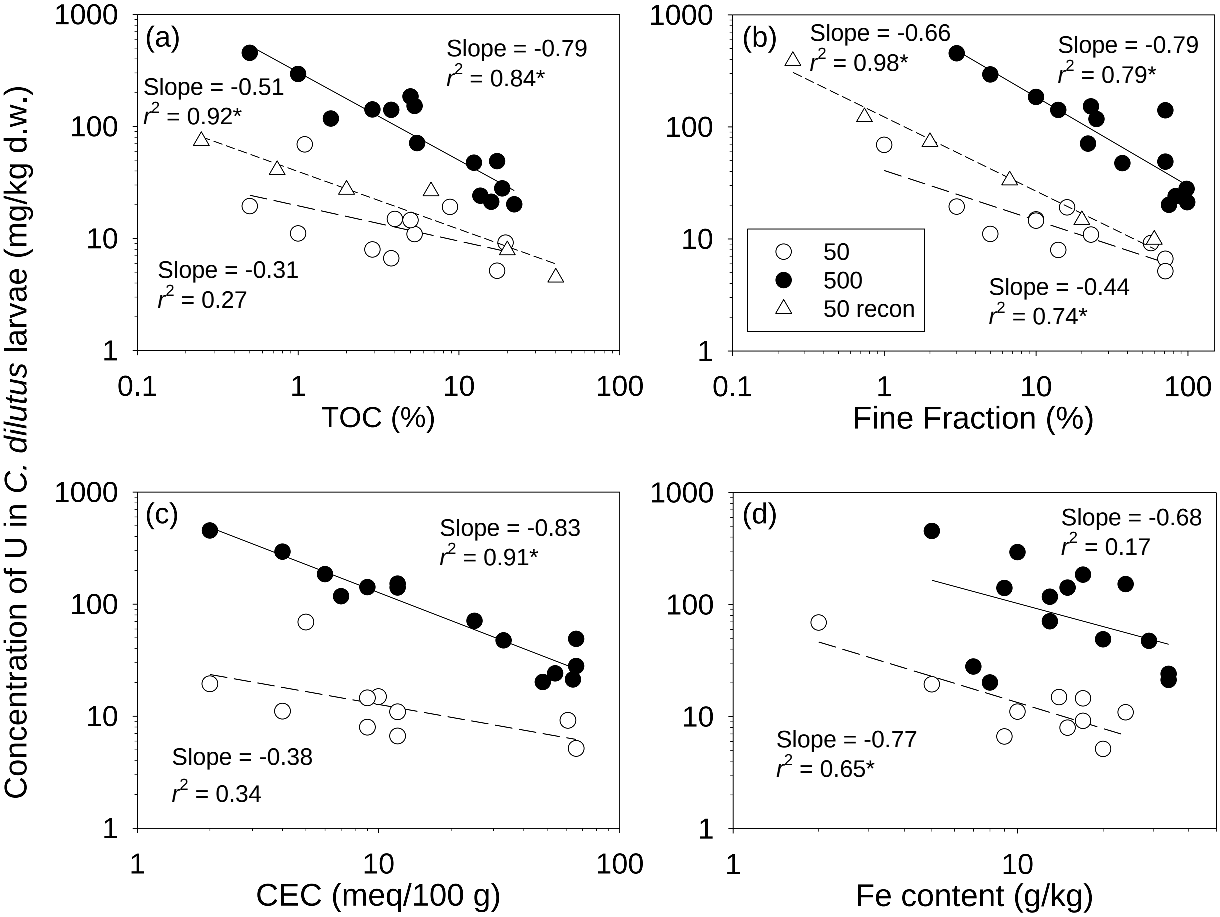

Fig. 3.2 Mean concentration of U in the whole organism (*C. dilutus* larvae; mg/kg d.w.) as a function of sediment (a) total organic carbon content (TOC; %), (b) fine fraction content (%), (c) cation exchange capacity (CEC; meq/100 g), and (d) iron content (Fe; g/kg) at U-spiked treatments of 50 mg/kg d.w. (white circle symbols, ○) and 500 mg/kg d.w. (black circle symbols, ●) in field sediment (present study), and in 50 mg U/kg d.w. formulated (recon) sediments (white triangle symbols, Δ; from Crawford and Liber, 2015). Lines represent linear regressions with an asterisk (*) indicating a significant relationship ($p < 0.05$).

Table 3.3 Correlations between sediment properties for the 18 field sediments used in the present study.[a]

Sediment property	**Correlation coefficients (*r*)**				
	TOC	**Fine fraction**	**CEC**	**Fe**	**Mn**
TOC	1.00	0.84**	0.97**	0.56*	0.20
Fine fraction		1.00	0.86**	0.62*	0.20
CEC			1.00	0.52*	0.22
Fe				1.00	0.49*
Mn					1.00

TOC = total organic carbon; Fine fraction = ≤ 50 µm particle size; CEC = cation exchange capacity.

** $p < 0.001$. * $p < 0.05$.[a] Sediment properties listed in Table 3.1 (n = 16-18).

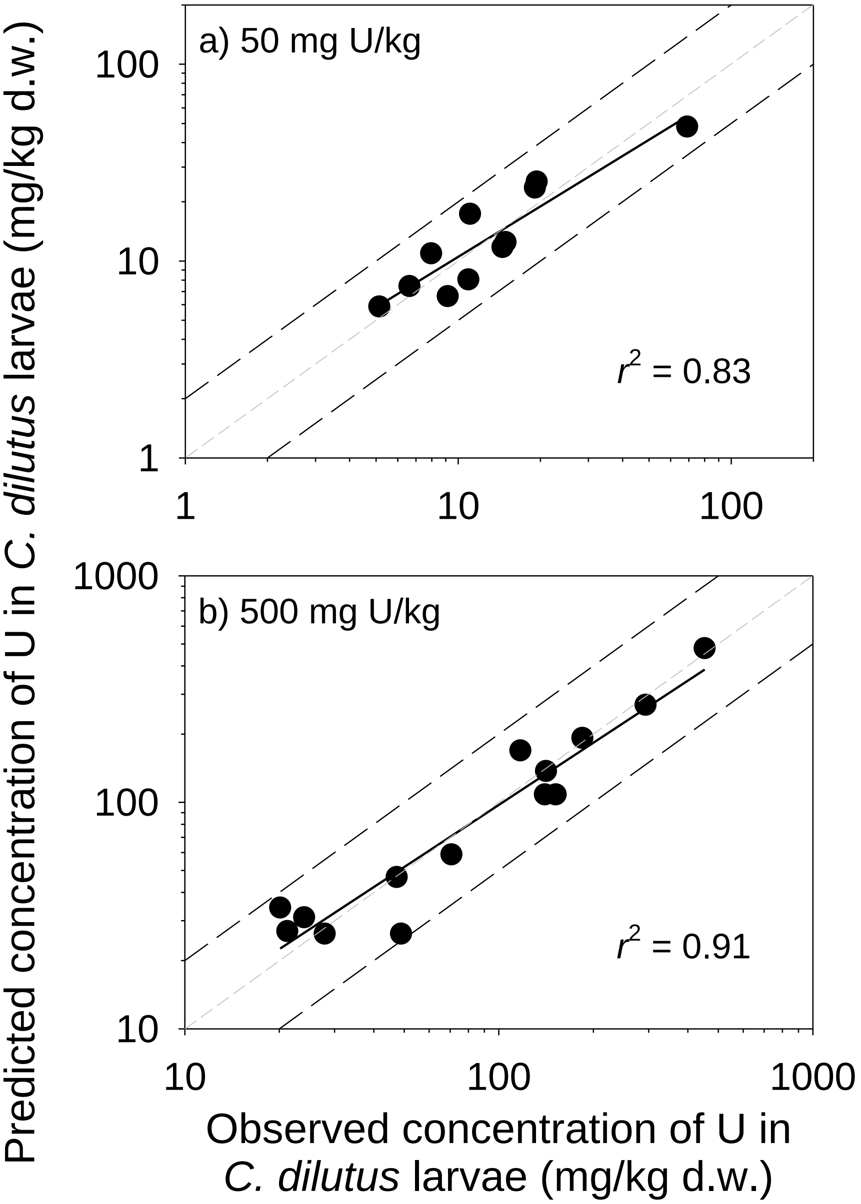

Fig. 3.3 Predicted versus observed bioaccumulation of U by *C. dilutus* larvae (mg/kg d.w.) in (a) 50 mg U/kg d.w. spiked field sediment based on bivariate regression analysis with fine fraction and Fe content as modifying factors (Eq. 3.1), and (b) 500 mg U/kg d.w. spiked field sediment based on univariate regression analysis with cation exchange capacity as the sole modifying factor (Eq. 3.2). The 1:1 line (dashed grey line) is bracketed by two-fold dashed lines (dashed black line) and presented with the linear regression (solid line).

levels differed by a factor of 14 for sediments spiked with 50 mg U/kg d.w. and by a factor of 23 for sediments spiked with 500 mg U/kg d.w. (Fig. 3.1). This large variation is in agreement with our previous study that demonstrated a > 20-fold change in U bioaccumulation from formulated sediments composed of different amounts of clay or organic matter spiked with 50 or 200 mg U/kg d.w. (Crawford and Liber, 2015). Bioaccumulation is a good indicator of bioavailability and has been shown to be a better estimator of chemical risk to benthic organisms than total metal concentrations in sediment (Alves et al., 2008; Borgmann et al., 2001a). However, U bioaccumulation data for benthic invertebrates from U-contaminated sediments are scarce in the scientific literature, with only a few published studies available (Alves et al., 2008; Crawford and Liber, 2015; Lagauzère et al., 2014). Lagauzère et al. (2014) reported an average bioaccumulation of 37.4 ± 8.3 mg U/kg d.w. by *Tubifex tubifex* in field sediment (2.4% OC, 57% fine fraction, 7.1 g Fe/kg and 0.13 g Mn/kg content) spiked with 600 mg U/kg d.w. That study demonstrated that exposure did not arise from U bound to sediment (i.e., biota-sediment accumulation factors (BSAFs) <<1 for whole-sediment), but instead from dissolved U concentrations present at the sediment-water interface (SWI) or in the pore water (Lagauzère et al., 2014). The degree of variation in U bioaccumulation from the present study, as well as our BSAFs (< 1 for whole-sediment), provide further evidence that total U concentrations in whole-sediment are poor predictors of the bioavailable fraction of U to *C. dilutus* larvae. A similar conclusion has been drawn by other authors for different metals (Ankley, 1996; Campana et al., 2013; Lock and Janssen, 2001). For example, different sediments (silty to sand) spiked with the same total Cu concentration were shown to result in approximately a 15-fold change in EC50s for amphipods and bivalves (Simpson and King, 2005). Despite the fact that total metal concentration is recognized to be a poor predictor of sediment toxicity, the present study is one of very few that provides data quantifying U bioaccumulation and the influence of sediment modifying factors on U bioavailability to benthic invertebrates.

Metal accumulation by benthic organisms often occurs from the pore water or water at the SWI via mechanisms such as respiration, filtering and burrow irrigation behaviour. The concentrations of U measured in the overlying water (using mini-peepers) in the present study are more representative of water at the SWI than what would be measured in the middle of the water column under field conditions. Therefore, the concentrations of U in the overlying water at the SWI and in the pore water both display significant correlations with U bioaccumulation in *C.*

dilutus larvae (Fig. 3.1). However, bioaccumulation of U based on pore water U concentrations more closely overlapped with bioaccumulation results from a similarly conducted 10-d water-only U test (Crawford and Liber, unpublished), suggesting that the pore water was the main route of exposure rather than the whole-sediment or the SWI overlying water. This compares well with previous publications which have suggested that concentrations of metals in the pore water are the main route of exposure for many benthic organisms, thus often proving useful for predicting the toxicity of metal-contaminated sediment (Liber et al., 1996; Liber et al., 2011; Lock, K., Janssen, C. R., 2001b). Overall, our results suggest that the uptake of U by *C. dilutus* larvae is either directly from pore water or through an exposure route closely related to or in equilibrium with pore water (e.g., SWI), and that U in the pore water is useful for estimating the bioavailable and hazardous fraction of U.

3.4.2 Relationships between sediment properties and U bioavailability

There are many factors that can influence the bioavailability of metals (Luoma, 1989; Simpson and Batley, 2007). Bioavailability is most often related to the concentration of specific chemical forms of a metal and controlled by the amount of metal bound strongly to sediment (i.e., speciation and sorption). The large variation in U bioaccumulation in *C. dilutus* larvae observed in this study after exposure to different sediments spiked with the same total U concentrations indicated that the bioavailability of U was modified by the different physicochemical properties of the 18 sediments. Sediment physicochemical factors exert varying degrees of metal-binding capacity that alter the partitioning of metals between the solid and aqueous phases, and hence influence the bioavailable fraction. For example, increasing amounts of organic matter in sediment have resulted in the increased partitioning of metals, such as U, Cd, and Cu to the solid sediment phase, resulting in lower metal concentrations in the pore water, and lower bioaccumulation and toxicity of metal-contaminated sediments to chironomids and amphipods (Besser et al., 2003; Campana et al., 2013; Crawford and Liber, 2015). Additionally, Romero-Freire et al. (2015) found that dissolved Pb concentrations varied significantly in relation to differences among soils, with higher dissolved Pb found in sandy soils than in OC-rich soils. Other studies have demonstrated the importance of silt, Fe oxides, CEC, and sulphides in altering metal sorption and influencing dissolved metal concentrations (Campana et al., 2013; Liber et al., 1996; Simpson and Batley, 2007). These results are also consistent with the equilibrium partitioning theory (EqP; Ankley et al., 1996b; Di Toro et al., 1991), which proposes that the bioavailability of metals is influenced by sorption to key binding phases, such as AVS, OC, or clay minerals, which subsequently determines the dissolved, more bioavailable metal concentrations in the aqueous phases.

In the present study, the bioaccumulation of U by *C. dilutus* larvae from U-spiked field sediment decreased as key sediment properties (TOC, fine fraction, CEC and Fe content) increased (Fig. 3.2). For example, a 10-fold decrease in U bioaccumulation was observed as the fine fraction increased from 3 to 37% in 500 mg/kg U-spiked sediment. Both fine fraction and Fe content were significant in the regression analysis for U bioaccumulation in the 50 mg U/kg d.w. treatments (Eq. 3.1), suggesting that these two factors are good predictors of reduced U bioavailability. This conclusion is in agreement with a study by Janssen et al. (1997) that concluded that these same two factors were significant in explaining Pb bioaccumulation in earthworms from 20 Pb-contaminated soils. Fine fraction and OC content of sediment have also been shown to reduce the effects of Cu on the benthic bivalve, *Tellina deltodalis,* with OC-normalized Cu concentrations in the fine fraction (< 63 µm) proposed for use in Cu-SQGs, particularly in sediments with insufficient amounts of AVS (Campana et al., 2013). In contrast, CEC was generally the single best sediment characteristic for explaining U bioaccumulation in *C. dilutus* larvae for the 500 mg U/kg d.w. spiked sediments in this study (Eq. 3.2). CEC is a measure of the amount of available cation sorption sites, and thus often incorporates metal binding to sediment organic matter, clay particles, and Fe oxides (Bradham et al., 2006). Not surprisingly then, CEC, organic matter, and fine fraction have been reported in other studies to be important sediment/soil properties modifying a number of metal effects on organisms (Bradham et al., 2006; Criel et al., 2008; Lock, K., Janssen, C. R., 2001b; Lock and Janssen, 2001; Peijnenburg et al., 1999; Romero-Freire et al., 2015; Simpson et al., 2004). Although sediment TOC did not significantly improve the models presented here for explaining U bioaccumulation at either U concentration, it may still provide an important metal-binding phase that is masked by the concurrent presence of other sediment binding phases. With the use of formulated sediments, Crawford and Liber (2015) were able to provide better insight into the significant quantitative role of individually manipulated contents of organic matter or clay without the influence of other sediment properties. Similar trends in bioaccumulation of U for the field and formulated sediments as a function of the TOC (Fig. 3.2a) and fine fraction (Fig. 3.2b) suggests that these two factors in the field sediment are still important modifiers of U bioavailability. Furthermore, the comparison of results from the field and formulated sediment experiments confirm that formulated sediments provide a reasonable surrogate for natural sediments in metal bioavailability studies, particularly for U. This is not surprising as typical types of OC and clay minerals in sediments from northern Saskatchewan include plant-based peat and kaolin clay minerals (Dunn, 1981; Fayek and Kyser, 1997), which were the materials used in creating the formulated sediments.

A review of soil/sediment properties affecting metal bioavailability and toxicity indicates that different combinations of parameters often govern the bioavailability of different metals to different organisms (Criel et al., 2008; Janssen et al., 1997; Son et al., 2009; Van Gestel, 2008; Vandenhove et al., 2007). As such, most sediments have more than one binding phase for metals as was demonstrated in our study which had significant correlations among TOC, CEC, fine fraction, and Fe content (Table 3.3). The co-correlation of sediment characteristics makes it difficult to determine the significance of individual sediment properties, thus univariate and bivariate empirical models are generally preferable to multivariate models. However, for simplicity and practicality it is often better to have one model that describes the variation in bioaccumulation consistently among different U-contaminated sediments.

$$\log U_{tissue} = 1.61 - 0.45 \log \text{fine fraction} \qquad \text{(Eq. 3.3)}$$

$$\log U_{tissue} = 3.06 - 0.79 \log \text{fine fraction} \qquad \text{(Eq. 3.4)}$$

For this reason, and because it is easily and almost always measured, fine fraction, which consistently and significantly explained the greatest variation (>74%) in U bioaccumulation for both the 50 and 500 mg U/kg d.w. sediments (Fig. 3.2b; Eq. 3.3 and 3.4, respectively), is recommended as the most important sediment property for modeling the reduction in U bioavailability in sediment.

3.4.3 SQGs for sediments with varying physicochemical properties

The ability to predict adverse effects, or the lack thereof, of metal-contaminated sediments is important not only for the assessment of contaminated sites, but also for the development of SQGs. Sediments can display strong binding affinities for metals, which means that a total metal concentration in the sediment cannot be correlated with toxicity to aquatic organisms. Conversely, sediments with low metal binding affinity will allow for greater bioavailability of toxic metals, even at low contaminant concentrations, as demonstrated in the present study. Variability of sediment binding affinities makes it difficult to develop SQGs for predicting toxic effects, or lack thereof, based solely on total metal concentrations in sediment. Relying on total metal concentration often leads to the overestimation of the true risk of metal-contaminated sediments (Burnett-Seidel and Liber, 2012). Therefore, additional data quantifying the influence of binding phases on U bioavailability in sediment, as presented in the present study, should be incorporated into U-SQGs to improve the accuracy and application of science-based guidelines.

Previously established guideline values for U toxicity to freshwater benthos include 100 mg U/kg d.w. for the predicted no-effect concentration (PNEC) (Sheppard et al., 2005) and 104 mg U/kg d.w. for the site-specific SLC approach lowest effect level (LEL) for northern Saskatchewan (Thompson et al., 2005). However, these guidelines are not mechanistically defensible and often over predict the impact of U-contaminated sediments to benthic communities because they lack the incorporation of factors that modify U bioavailability in sediment (Burnett-Seidel and Liber, 2012). Additionally, areas that require assessment of U-contaminated sediments often contain naturally higher background concentrations of U, such as in some areas of northern Saskatchewan where the background concentration of U (95th centile) in sediment is 30 mg U/kg d.w. (Environment Canada, 2003; Sheppard et al., 2005). Therefore, the present study was important in not only investigating U-contaminated sediments above guideline values (500 mg U/kg d.w.), but also those that are near background sediment U concentrations and below guidelines (50 mg U/kg d.w.).

Results from this research suggest that the incorporation of factors such as particle size, CEC, and Fe content in future assessments of U-contaminated sediments should improve the prediction of U bioavailability to benthic organisms. In the United States, EqP-based sediment quality criteria for divalent cationic metals incorporate AVS as a factor that ameliorates metal bioavailability (US EPA, 2005). However, AVS “normalization” does not apply to all metals (Ankley et al., 1996a; Chapman et al., 1999). In particular, AVS does not play a major role in moderating U bioavailability to *C. dilutus* larvae as they live close to the SWI and build tubes that they oxidize, thus reducing AVS concentrations (Campana et al., 2013). This is further corroborated by data from Campbell et al. (2015), who reported that additions of H_2S at a mining site did not decrease aqueous concentrations of U. Nevertheless, CEC, Fe content, and fine fraction were concluded to be the most important modifiers of U bioavailability among the 18 different sediments investigated here, and those correlations yielded easy to apply univariate and bivariate regression models that predicted bioaccumulation of U within a two-fold difference of the observed concentrations (Eq. 3.1 and 3.2). These three sediment physicochemical properties are routinely measured in site assessments for U and other contaminants, but are generally not utilized in calculating or modifying existing Canadian SQGs, or in quantitatively assessing the risk of existing U contamination. We recognize that SQGs for different metals will have to be modified according to metal-specific binding capacities for different sediment. However, for the wide range of sediment properties and environmentally relevant concentrations of U investigated in this study, we propose that sediment fine fraction content (Eq. 3.3 and 3.4) should be incorporated into U-SQGs.

3.5 Conclusion

Our results demonstrate that total U concentrations are generally not representative of the bioavailable fraction of U in sediment and that considerations of the interactions of U with the physicochemical matrix of the sediment are required. One of the main limitations to the incorporation of sediment properties in risk assessments and the development of SQGs is the absence of model(s) applicable to a wide range of sediments. Results from our study demonstrate that physicochemical properties of sediment, such as CEC, fine fraction, and Fe content mitigate U bioavailability to *C. dilutus* larvae, and that fine fraction may be the most easily and universally applied modifier of U bioavailability. These results contribute to our mechanistic understanding of U bioavailability in sediment, which may lend itself to the derivation of improved SQGs for the U mining industry. However, additional investigations with other benthic species would be required to propose more robust U-SQGs and further validate the incorporation of sediment properties representing different conditions into such guidelines. Uranium-contaminated sediments are a concern in many places beyond Saskatchewan and future work should aim to advance integrated models that can account for multiple modifiers of U bioavailability to benthic invertebrates, thus better evaluating the ecotoxicological significance of sediment U contamination.

CHAPTER 4:
THE ROLE OF SEDIMENT PROPERTIES AND SOLUTION pH IN THE ADSORPTION OF URANIUM(VI) TO FRESHWATER SEDIMENTS

4 Preface

Key physicochemical properties of sediment were observed to significantly influence U bioaccumulation and bioavailability in Chapter 2 and 3. The reduction in U bioavailability observed for certain sediments was likely associated with greater adsorption of U to sediments with greater binding capacity, which was investigated in this chapter. Sorption tests were employed to quantify the sorption behavior of U as a function of solution pH and U concentrations for nine uncontaminated freshwater sediments with a wide range of physicochemical characteristics. There were significant differences in the pH-dependent sorption among sediments with different physicochemical properties, with sorption increasing up until thresholds of 12% total organic carbon, 37% fine fraction (≤ 50 μm), and 29 g/kg of iron content. Experimental data were also entered into a geochemical speciation model (Windermere Humic Aqueous Model, version 7.0.4; WHAM7), which successfully predicted the sorption of U to the different sediments under various conditions of aqueous U concentrations and water chemistry. The research in this chapter provided quantitative insight into the degree of adsorption of U to different sediments, confirmed the use of WHAM7 in predicting U sorption under conditions of northern Saskatchewan U mines, and allowed for additional interpretation of the bioaccumulation data observed for the U-spiked sediments in the previous chapters.

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4.1 Introduction

Uranium (U) is an actinide element important in the nuclear fuel cycle, where it is both an initial fuel source and final waste component. Northern Saskatchewan, Canada, is home to some of the richest deposits of U in the world, making Canada the second largest producer (approx. 15%) of U globally (OECD-NEA and IAEA, 2014). Introduction of U into aquatic environments can occur in areas with U mining and nuclear processing, as well as in areas with high geological background concentrations of U. In freshwater ecosystems, under oxic conditions, U is predominantly found in the U(VI) state, either complexed to ligands or present as the aqueous hexavalent uranyl ion (UO_2^{2+}), the latter of which has been suggested to be the major species responsible for U toxicity in aquatic organisms (CCME, 2011; Markich, 2002; Sylwester et al., 2000). However, the uranyl ion strongly interacts with solid phases, such as suspended solids, sediments and various minerals, which tends to lead to large accumulation of U in depositional sediments downstream of U mine and mill sites, in some cases exceeding 1000 mg U/kg d.w in the sediment (Hart et al., 1986; Neame et al., 1982). Such accumulation of U in sediments can have adverse effects on benthic invertebrates (Alves et al., 2008; Dias et al., 2008; Lagauzère et al., 2009a; Liber et al., 2011; Thompson et al., 2005). The degree of sorption of contaminants to sediments can influence the fraction of contaminant available for uptake by aquatic organisms (i.e., bioavailability) has and subsequently the toxicity of a contaminant (Alves et al., 2008; Smit and Van Gestel, 1998; Van Gestel and Ma, 1990). Thus, quantifying U sorption behaviour is important in understanding the mobility and bioavailability of U in freshwater environments.

Uranium, in comparison to most cationic metals, has a relatively complex solid-aqueous chemistry, which depends on a number of factors that influence the partitioning of U between the aqueous and solid phases (Ames et al., 1983; Catalano and Brown Jr, 2005; CCME, 2011; Langmuir, 1978; Markich, 2002; US EPA, 1999b). Many ligands, such as phosphate, carbonate, and humic and fulvic substances can form complexes that are readily soluble and mobile in aquatic systems (Cheng et al., 2006; Franke et al., 2000; Schaller et al., 2008). However, transport of U can be limited by strong adsorption onto solid phases. Specifically, a number of studies have demonstrated the importance of single mineral or solid phases such as montmorillonite and kaolin clay minerals, organic carbon, goethite, and Fe oxides in the sorption of U (Bhattacharyya and Gupta, 2008; Catalano and Brown Jr, 2005; Cheng et al., 2004; Mibus et al., 2007; Morrison et al., 1995; Ren et al., 2010; Sachs and Bernhard, 2008; Zhirong and Shaoqi, 2010). However, the use of pure mineral/solid phases may not appropriately represent more chemically complex sediments. The number of complexing agents and/or specific types of solid phases present in a system will influence the degree of adsorption. Thus, there is often large variation in the sediment-solution partition coefficients (K_d values) for U reported in the literature, which typically range from <1 to $1x10^6$ ml/g under various solid-aqueous conditions (CCME, 2011; US EPA, 1999b). A limited number of studies have investigated the adsorption of U(VI) to field sediment with variable total organic carbon (TOC), fine fraction (clay and silt particle size), iron (Fe) and manganese (Mn) oxides contents, as well for sediments with varying cation exchange capacity (CEC) (Barnett et al., 2000; Dong et al., 2012). Additionally, sorption of U is strongly influenced by pH (Markich, 2002), but few studies have investigated the importance of pH in conjunction with physicochemical properties of sediment.

In the present study, we investigated the sorption of U(VI) onto nine field sediments in several sorption experiments, as a function of U concentration and solution pH. The sediments were collected from reference sites around U mines in northern Saskatchewan, Canada, and selected to provide a wide range of common sediment physicochemical properties, such as TOC, particle size, CEC, and Fe contents, on the sorption behaviour of U. Sorption experiments were conducted at three U concentrations and three pHs that are environmentally relevant to areas surround U mining and milling activity. The specific objectives were to: (i) measure the effects of U concentration and pH on the adsorption of U to sediment, (ii) quantify the adsorption of U onto nine field sediments from northern Saskatchewan representing different physical, chemical, and mineralogical properties, and (iii) model U sorption to field sediments using a computer model (i.e., Windermere Humic Aqueous Model) and compare model predictions to experimental observations. Our overall goal was to develop an improved understanding and predictive capability of U sorption behaviour under conditions typical of U mine sites in northern Saskatchewan, in order to better characterize U bioavailability and improve risk assessment for U-contaminated sediments.

4.2 Materials and methods

4.2.1 Characterization of field sediment

The field-collected sediments used in this study were previously characterized and used in 10-d U-spiked sediment bioaccumulation tests (Crawford and Liber, 2016). In brief, sediment was collected from the top 10-cm layer of natural sediments at 18 uncontaminated reference sites surrounding U mines in northern Saskatchewan, Canada. Nine of these 18 sediments were selected for use in the present study, chosen to represent a wide range in common physicochemical properties (Table A4.1). Each of the sediment samples were air-dried, sieved through a 2-mm sieve, and thoroughly homogenized prior to use and analysis.

4.2.2 Test solutions

Stock solutions of U(VI) were prepared from uranyl nitrate hexahydrate ($UO_2(NO_3)_2 \cdot 6H_2O$) (Strem Chemicals, Inc., Newburyport, MA, USA) and diluted with ultra-pure water (18 MΩ-cm). Test solutions were reconstituted from ultra-pure water with 0.01 M $NaNO_3$-0.005 M $CaSO_4$ to mimic water quality conditions (i.e., conductivity, total hardness, alkalinity, and major ions; Table A4.2) found downstream of Saskatchewan U mining and milling operations (EARMP, 2014; Goulet et al., 2015).

4.2.3 Adsorption batch equilibrium experiments

Sorption tests were conducted using a batch equilibrium approach following OECD 106 guidelines (OECD, 2000). Tests were conducted at room temperature (~22 °C) in 50-ml polypropylene centrifuge tubes, in duplicate. The tubes were prepared by adding 0.75 g d.w. of sediment and 30 ml of test solution (plus an extra 5 ml for water chemistry sampling prior to the test), producing a fixed solid-to-solution ratio (SSR) of 25 g/L. The pH of the suspensions were adjusted daily with 0.1 M HNO_3 and/or NaOH (less than 0.05% of the total solution volume) to the desired pH values of 6, 7 and 8 until a stable pH was achieved (maximum up to 10 days). These pH values represent the range of overlying water pH typically found downstream of Saskatchewan U mines (EARMP, 2014; Goulet et al., 2015).

Immediately prior to spiking, 5-ml aliquots of centrifuged (4000 *g,* 5 min), pH-steady suspensions were removed to determine initial conductivity, pH, total hardness, and alkalinity. At test initiation, 100-µl aliquots of U stock solutions were added to test tubes to achieve initial overlying water concentrations of 0.023, 0.23, and 2.3 mg U/L. These U concentrations are environmentally relevant to areas surrounding U mines and mills and/or within the range of sub-lethal U concentrations found in the overlying water of previous U-spiked sediment tests (Crawford and Liber, 2016; Crawford and Liber, 2015; Environment Canada, 2003; Hynes et al., 1987; Muscatello and Liber, 2010). Tests included additional duplicates of (i) *negative controls* with no added U or sediment to ensure there was no contamination, (ii) *sediment controls* with each sediment and no added U to determine if natural background U desorbed from sediment, and (iii) *U controls* with added U but no sediment to ensure spiking accuracy. The sorption experiments were conducted with all nine sediments at three U concentrations for all three pH conditions; except pH 6 treatments, which were only tested at 0.23 mg U/L based on insignificant differences among U concentrations obtained from pH 7 and 8 tests (see Results and Discussion section). Immediately after spiking, dissolved U concentrations were determined from 1-ml aliquots of centrifuged (4000 *g*, 5 min) and 0.45-μm filtered (polyethersulfone membrane) supernatant. All treatments were rapidly prepared and immediately capped to minimize exchange of CO_2 with the atmosphere. Samples were then gently agitated on a rocking platform shaker for 48 h, which had been established as an adequate time to achieve pseudo-equilibration under our experimental conditions and in previous research (Barnett et al., 2000; Cheng et al., 2004; Payne et al., 2004). The samples were removed from the shaker, centrifuged, and 1-ml aliquots of supernatant filtered to measure dissolved U concentrations. Additional aliquots of 10 ml were filtered from the supernatant to quantify dissolved organic carbon (DOC; Shimanzu TOC-V CPN model 5000). The remaining suspensions were analyzed immediately for conductivity, pH, total hardness, alkalinity, and major ions.

4.2.4 Chemical analysis and calculations

Samples for analysis of Fe, Mn, and U were acidified to a pH of ~2 (HNO_3) and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS; X-series II spectrometer with PlasmaLab software and collision cell technology, Thermo Electron Ltd., Mississauga, ON, CAN). Certified reference materials (SLRS-5; National Research Council of Canada and 1640e; National Institute of Standards and Technology), blanks, and duplicates were included with all analyses to ensure analytical accuracy and validity. The minimum method detection limits for Fe, Mn, and U were 1.34, 0.68, 0.05 mg/L, respectively, with instrumental and method recoveries of 80-120%. All major ions in solution (i.e., K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^-, PO_4^{3-}, and NO_3^-) were analyzed by Ion Chromatography (Dionex ICS-3000 dual Ion Chromatography System, Sunnyvale, CA, USA) following U.S. Environmental Protection Agency Method 300.1 (US EPA, 1997).

The sediment-solution partition (or distribution) coefficient (K_d, ml/g) was calculated for U in each sediment, as the ratio of the total concentration of U in the sediment (μg/g) and in the solution (μg/ml) at equilibrium (after 48 h) using the equation:

$$K_d = [(C_0 - C_e)/C_e]\ V/m \qquad \text{(Eq. 4.1)}$$

where C_0 is the concentration of U in the initial solution (μg/ml), C_e is the concentration of U in solution at equilibrium (μg/ml), V is the volume of the solution (ml), and m is the mass of the sediment (g) (CCME, 2011; OECD, 2000; Pandit et al., 2012).

4.2.5 Statistical analysis and modeling

Statistical analyses were performed and plotted with Sigmaplot®, version 11 (San Jose, CA, USA). All tests were conducted at $\alpha = 0.05$ after checking for compliance with parametric assumptions of normal distribution (Shapiro-Wilk test) and homogeneous variance (Levene's test). Since K_d values were log-normally distributed, all K_d-U values were $\log_{10}$ transformed and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. If data did not meet the normality and homogeneity of variances assumptions, then a non-parametric Kruskal-Wallis one-way ANOVA on ranks was used, followed by the Dunn's method pairwise multiple comparison post-hoc test. The effects of sediment physicochemical characteristics and pH on K_d-U were analyzed by linear and nonlinear (quadratic) regression. Pearson's product-moment tests were also used to identify correlations among sediment properties. The root mean square error (RMSE) was calculated as a measure of the absolute errors between the model estimates and the observed values.

The Windermere Humic Aqueous Model (WHAM7), version 7.0.4 (Lofts and Tipping, 2011; Tipping, 1994) was used to estimate the chemical speciation of U in the experimental aqueous media and predict K_d-U values under our experimental conditions. Speciation of U(VI) for the different field sediment treatments and test conditions was calculated using WHAM7 with the most current, critically reviewed thermodynamic stability constants for U(VI) complexes presented in Table A4.3. The WHAM7 model includes Humic Ion-Binding Model VII, a discrete site/electrostatic submodel of cation binding to humic substances (Tipping et al., 2011) and a surface complexation model (Lofts and Tipping, 1998) for ion binding to mineral oxides. These submodels are parameterized for the binding of 46 cations, including U(VI), to humic and fulvic acids and amorphous Fe(III) oxide, optimized using the same value of protonation-dissociation constants and surface site density. The solution complexation and particulate hydrous Fe oxide binding parameters used were those of Lofts et al. (2015), specifically adjusted for U (Table A4.3 of the Supplementary material). Free Fe(III) and Al activities were computed assuming them to be in equilibrium with $Fe(III)(OH)_3$ and $Al(OH)_3$, respectively. Precipitation of $Fe(III)(OH)_3$, UO2 and $CaUO_4$ was allowed to occur if saturation products were exceeded; however, no precipitation of either UO2 or $CaUO_4$ solid is predicted in the present sorption tests.

The input variables required for WHAM7 were based on measured physicochemical properties of the test sediments and solutions (Tables A4.1 and A4.2; pH, TOC, Fe content, DOC, alkalinity, total hardness, dissolved major ion concentrations, total U concentration in overlying water). Iron(III) oxide contents of sediments were determined from measured Fe content by assuming that all measured sediment Fe was contained in amorphous Fe(III) oxide and that 1 mole of Fe (55.85 g) corresponded to 90 g of oxide (Dzombak and Morel, 1990). Fe oxide contents were further converted to solution concentrations for input into WHAM7 as particulate hydrous Fe oxide by accounting for the sediment concentration (i.e., 25 g/L SSR). Concentrations of TOC were converted to solution concentrations for input into WHAM7 by accounting for the sediment concentration, with the additional assumption that organic matter was comprised of 50% carbon, and consisted of 50% particulate HA and FA. For measured DOC concentrations, complexation was accounted for by assuming that dissolved organic matter (DOM) was comprised of 50% carbon, with 65% of the DOM active with respect to cation binding (represented by FA), and input into WHAM7 as colloidal FA (Tipping, 1998; Tipping and Lofts, 2015). The carbonate system was simulated from measured alkalinity.

4.3 Results and discussion

4.3.1 U(VI) adsorption batch equilibrium experiments

4.3.1.1 Physicochemical parameters of test sediment and water

Sediments used in the sorption tests were collected from northern Saskatchewan and represented a wide range of TOC (1 to 22%), fine fraction (≤ 50 μm; 1 to 99%), CEC (4 to 64 meq/100 g), and Fe content (5 to 34 g/kg) as presented in Table A4.1. All sediments had low background concentrations of U (≤ 3 mg U/kg d.w.) which were similar to or below the median sediment U concentrations of 3.7 mg U/kg d.w. reported for northern Saskatchewan (Environment Canada, 2003). Furthermore, the sediments resulted in negligible concentrations of U in the test solutions (≤ 0.002 mg U/L) as measured in the sediment controls (sediment with no added U). Concentrations of U in the negative controls (no added sediment or U) were below the limit of detection of U in solution (i.e., U-LoD < 0.05 μg/L). Additionally, spiking efficiencies were within ± 4% of nominal concentrations in the U controls (U with no added sediment), with negligible loss of U to the container walls over the 48-h equilibration period, which is consistent with other U sorption investigations (Barnett et al., 2000; Cheng et al., 2004; Chisholm-Brause et al., 1994).

Water chemistry parameters for the controls and each sediment-pH treatment are summarized in Table A4.2 and were used as input parameters for WHAM7. These values are within the range of water quality characteristics representative of Saskatchewan waters receiving U mine and mill effluent (Goulet et al., 2015). Uranium speciation and sorption are thought to be affected by water hardness because the competitive binding of Ca and Mg to carbonate ions decreases the amount of carbonate species available for binding U (Goulet et al., 2015). However, contrasting literature exists regarding the importance of hardness in altering the sorption and bioavailability of U. Previous accounts of increased hardness reducing toxicity is often confounded by co-varying changes in alkalinity, or has been species-specific (Borgmann et al., 2005; Riethmuller et al., 2001; Van Dam et al., 2012). A review by Sheppard et al. (2005) suggested that sensitivity of fish to U under varying conditions of alkalinity and hardness was not due to the influence of hardness but instead to low alkalinity. This conclusion is in agreement with a recent study by Goulet et al. (2015) that concluded hardness was not the main driver for changes in toxicity as free uranyl ion concentrations remained constant despite increasing hardness, and instead that alkalinity and pH were the stronger influences. Low alkalinity results in greater free uranyl ion concentrations, whereas greater alkalinity leads to uranyl complexation with carbonate and bicarbonate ions (i.e., Section 4.3.2). In our study, alkalinity tended to increase with pH with simultaneous decreases in hardness, particularly for sediments with more silt, loam and TOC (Table A4.2). The goal of this study was not to investigate the specific influence of hardness and alkalinity on U speciation and mobility, as the test water was reconstituted, as closely as was feasible, to reflect the high hardness and low alkalinity conditions found downstream of U mines in Saskatchewan. Thus, no conclusions from the study can be made on the role of alkalinity and hardness.

Final pH measurements after 48-h showed little change (< 0.05 pH drift) from the starting solution pH for each pH treatment. Mean measured pH values (Table A4.2) were mostly within 0.1 pH units of the nominal value, except for pH 6 treatments which were consistently 0.2 to 0.3 pH units greater than the nominal. Major ions measured in the reconstituted overlying water consisted of NO_3^-, SO_4^{2-}, Ca^{2+}, and Na^{2+}, ions that are common in treated effluent from U mining and milling activities in northern Saskatchewan as a result of the U ore extraction and effluent treatment processes (i.e., lime, barium chloride, and sulfuric acid; EARMP, 2014; Goulet et al. 2015). Concentrations of DOC ranged from 5.7 to 67.0 mg/L and increased with sediment TOC content (slope = 1.83, R^2 = 0.68, n = 27, $p < 0.001$). Additionally, DOC increased as pH increased for most sediments and coincided with a decrease in K_d-U values. This is consistent with a study by Ren et al. (2010), which demonstrated that for solutions with pH > 6.5, negatively charged FA and HA resisted adsorption to clays due to electrostatic repulsion and remained in solution as pH increased. Thus, greater pH may allow for more complexation between DOC and U, which would decrease sorption to sediment (i.e., more U in solution present as U-DOC complexes). This is further supported by a study of Van Dam et al. (2012), which concluded that U toxicity was reduced to a variety of freshwater organisms by 6 to 9% with each addition of 1 mg/L of DOC (up to 30 mg/L DOC). Thus, DOC and water chemistry are important modifiers of U sorption behaviour and should be documented in all U-sorption studies.

4.3.1.2 U(VI) adsorption at different U concentrations

The K_d values for the three U concentrations investigated at each pH treatment are presented for all nine sediments in Fig. A4.1. Although K_d-U values differed among sediment types at each pH, the three U concentrations did not have a significant effect on K_d-U values for each sediment treatment at pH 7 and 8 ($p > 0.05$; one-way ANOVA). Due to the negligible effect of U concentration on K_d-U, these values for the remainder of the paper are presented as an average K_d-U for all three U concentration treatments for pH 7 and 8 (mean ± SD, $n = 6$); only one U concentration was used to determine K_d at pH 6 ($n =2$). It is worth restating that these U concentrations are environmentally relevant to areas surrounding U mining and milling activity as the medium U concentration (230 μg/L) is within the range of U concentrations reported for surface water at decommissioned mines in Beaverlodge Lake, Saskatchewan (200 to 400 μg/L; CCME, 2011). Additionally, the lowest concentration (23 μg/L) falls within the range of the long-term and short-term Canadian Water Quality Guidelines (CWQG) for U of 15 μg/L and 33 μg/L, respectively (CCME, 2011). The highest concentration (2.3 mg/L) was included as it represents a very high, but possible, pore water U concentration.

4.3.1.3 U(VI) adsorption as a function of pH

The degree of U adsorption for each sediment was significantly dependent on aqueous pH, with greater K_d-U values observed at pH 6 and 7, followed by a sharp, significant decrease at pH 8 for all sediment treatments (Fig. 4.1; $p < 0.001$). The maximum adsorption ($K_d > 1000$ ml/g), observed at all pH values between 6.2 and 7.4, was likely the result of a greater number of negatively charged binding sites (compared to pH 8) available on mineral surfaces due to the release of protons (CCME, 2011). Alternatively, the formation of uranyl-carbonate complexes at pH 8 may also decrease adsorption of U (i.e., low K_d-U values; Fig. 4.1) via negligible interactions with solid phases. The formation of soluble uranyl-carbonate complexes and low sorption at alkaline pH conditions is in agreement with previous literature for a number of other substrates, including pure Fe-minerals and amorphous Fe hydroxides (Barnett et al., 2000; Langmuir, 1978; McKinley et al., 1995; Payne et al., 2004; Vandenhove et al., 2007; Waite et al., 1994). Speciation of U, dissolution and precipitation processes, and surface charge or availability of binding sites on organic matter and oxide clay minerals are known to change as a function of pH (CCME, 2011; Maity et al., 2013). These characteristics and processes alter the availability of cationic metals and influence complexation to solid phases (Barnett et al., 2002; Ren et al., 2010). Although not investigated in our study, adsorption is also reported to be low at pH < 6 due to competition for binding sites between H^+ ions and the free UO_2^{2+} ion that

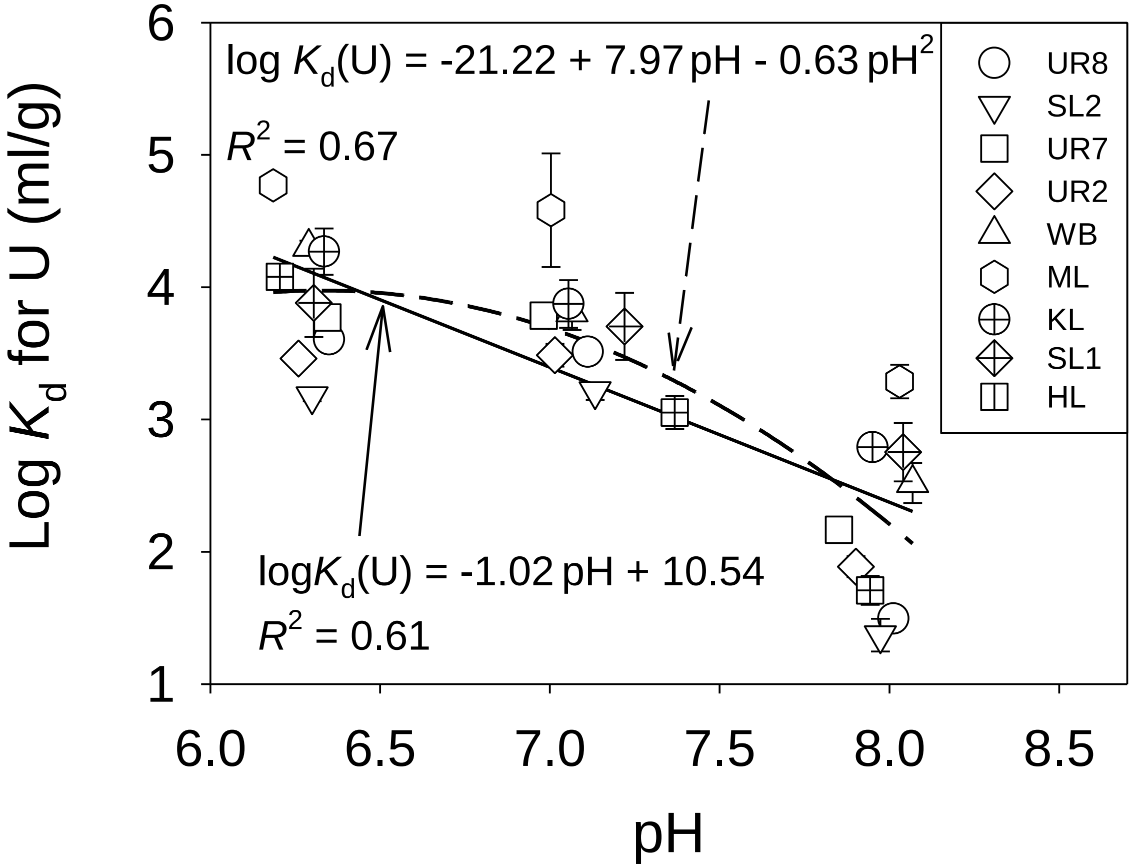

Fig. 4.1 Mean (± SD, n = 2-6) K_d values for U (log ml/g) for all nine sediments as a function of pH for nominal pH treatments of 6, 7 and 8. The solid black line represents the negative linear regression and the dashed line represents the quadratic relationship between K_d-U and pH for all sediment treatments ($p < 0.001$).

predominates at lower pH conditions (CCME, 2011; Markich et al., 2000; Riethmuller et al., 2001). Thus, sorption of U within the environmentally relevant pH range is unlike many other cationic metals; changes in pH lead to a Gaussian-type relationship with sorption increasing as pH increases, to a maximum sorption at circumneutral pH conditions, followed by a decrease in sorption as pH increases further.

A negative relationship, which could be represented by a linear equation, was observed between K_d-U and pH [$\log K_d(U) = -1.02\ \mathrm{pH} + 10.54$, $R^2 = 0.61$, $n = 27$, $p < 0.001$; Fig. 4.1, solid black line]. This trend is in general agreement with previous literature examining the relationship between pH (6-9) and U sorption to sediments and soils [$\log K_d(U) = -0.77\mathrm{pH} + 7.73$, $R^2 = 0.30$, Vandenhove et al., 2009b; $\log K_d(U) = -1.18\mathrm{pH} + 10.8$, $R^2 = 0.65$, Vandenhove et al., 2007; $\log K_d(U) = -1.07\mathrm{pH} + 9.80$, $R^2 = 0.41$, Sheppard et al., 2006; $\log K_d(U) = -1.29\mathrm{pH} + 11.0$, $R^2 = 0.76$, Echevarria et al., 2001]. General similarities in the observed relationship to other published findings confirm that pH is a significant modifier of U sorption despite differences in experimental conditions among the different studies (e.g., SSRs, U concentrations, soil vs. sediment substrate, incubation time, water chemistry). However, the K_d-U values reported here for the pH 6 and 7 treatments were not significantly different, except for sediments WB, KL and HL which had ~2- to 10-fold greater K_d-U values at a nominal of pH 6 than pH 7 ($p < 0.05$). As a result, the linear regression shown does not predict the K_d-U values at pH 7 accurately. This trend may have also been present in other published research, but masked by the variability in sediments/soils and conditions examined. One advantage of the present research is that U sorption was investigated under different pH conditions but for the same sediments, allowing for a less confounded investigation of the effects of pH on U sorption. For the present data, a quadratic function [$\log K_d(U) = -21.22 + 7.97\mathrm{pH} - 0.63\mathrm{pH}^2$, $R^2 = 0.67$, $n = 27$, $p < 0.001$; Fig. 4.1, dashed line] provided a better fit than the linear relationship, at least within the pH range investigated. But it is evident from Fig. 1 that pH alone does not influence K_d-U for the number of sediments investigated.

Generally, K_d values are an indication of the adsorption of a compound to the solid phase (via ion exchange processes and surface complexation formation) and subsequent mobility of a compound in an aqueous system. The mobility of contaminants is generally small for compounds with K_d values > 1000 ml/g as sorption to sediments is great. In contrast, mobility can be considered high when K_d values are ≤ 10 ml/g due to the negligible partitioning to sediment

(Sheppard, 2011). The K_d-U values calculated in the present study ranged over three orders of magnitude from ~20 to > 20,000 ml/g depending on the pH and sediment type (Fig. 4.1). Adsorption of U to sediments was ≥ 97% at pH 6 and 7 treatments, while adsorption ranged from 43 to 97% for the pH 8 treatment depending on the sediment used. The greater K_d-U values (> 1000 ml/g), and the extent of adsorption at pH 6 and 7, suggest that mobility of U may be of limited interest in some environmental assessments, as most U under such conditions would have a tendency to bind to sediment. In contrast, greater concentrations of U in solution at pH 8, as indicated by K_d-U values < 500 ml/g, suggest that the potential for U transport may be greater at higher pH values. Greater concentrations of dissolved U may also increase the potential uptake of U by organisms depending on the water chemistry, organism, exposure route, and type of sediment (Crawford and Liber, 2016; Liber et al., 2011). The results from the present study demonstrated the strong influence of pH on U adsorption at pH conditions (6 to 8) representative of northern Saskatchewan U mining areas, suggesting that increased U mobility and transport are more likely the higher the pH of the system.

4.3.1.4 U(VI) adsorption and sediment physicochemical properties

The effect of sediment properties on U sorption is demonstrated in Fig. 4.2a-d for four common sediment properties (TOC, fine fraction, CEC, and Fe content). The K_d-U values varied by orders of magnitude at a specific pH as a function of sediment type, with greater fine fraction and Fe content sediments (ML, KL, SL1) having greater K_d-U values than sandier sediments (SL2 and UR8). In particular, there appeared to be an interaction between pH and sediment type, as an 80-fold change in K_d-U values occurred at pH 8 while only approximately a 14-fold change in K_d-U values was observed at pH 6 and 7 for the same sediments. Sheppard (2011) reported a similar trend with a 250-fold higher K_d-U for soils containing 35% clay compared to 5% clay at pH 5.5, while at pH 6.5 only a 3-fold change in K_d occurred for the same range in clay content. Additionally, K_d-U values have previously been proposed for sand (40 ml/g), loam and clay (200 ml/g), and organic soils (2000 ml/g) (Sheppard et al., 2006), but K_d values for U can typically range from <1 to >500,000 ml/g (Campana et al., 2013; Echevarria et al., 2001; Maity et al., 2013; Pandit et al., 2012; Van Dam et al., 2012; Van Gestel, 2008; Vandenhove et al., 2007). Conversely, some studies have reported no effect of sediment properties such as CEC or TOC on U sorption (Echevarria et al., 2001; Vandenhove et al., 2007), likely due to differing pH

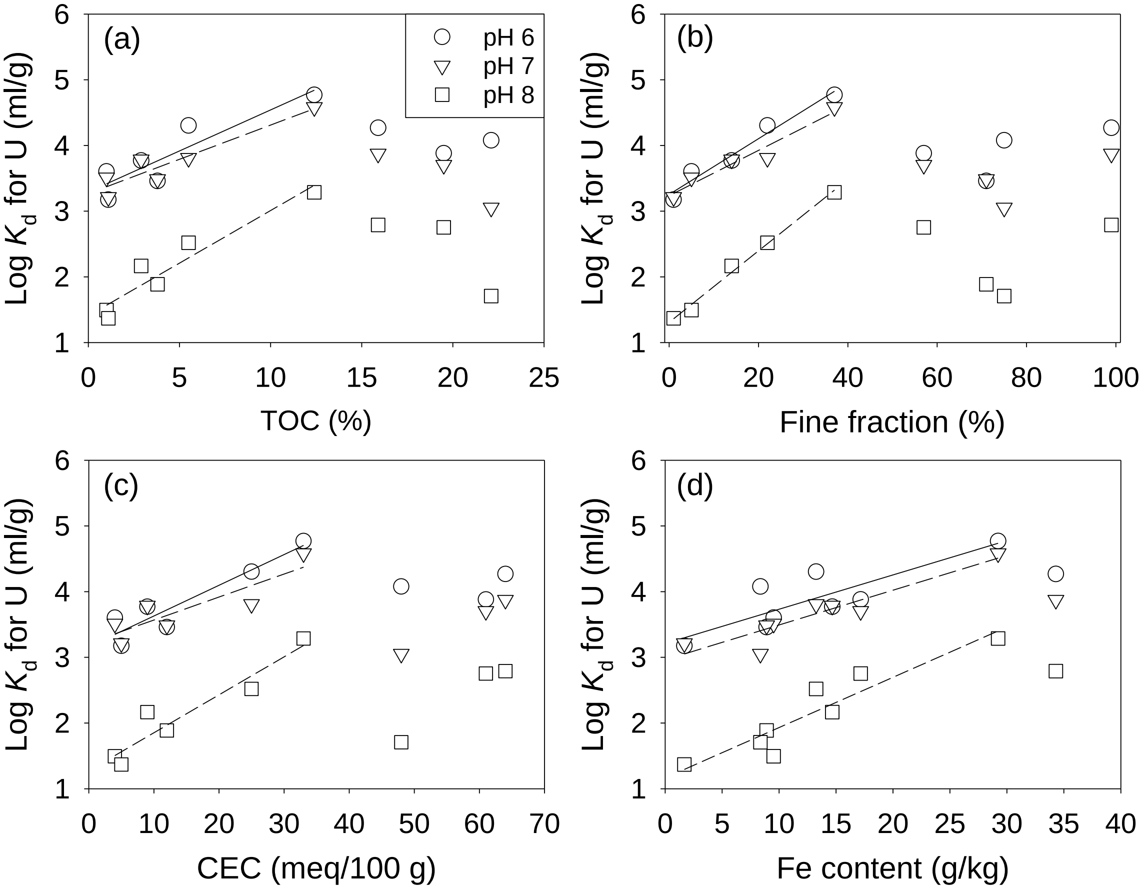

Fig. 4.2 Mean K_d for U (log ml/g) as a function of solution pH (nominal of 6, 7, and 8) and sediment (a) total organic carbon content (TOC, %), (b) fine fraction content (≤ 50 μm, %), (c) cation exchange capacity (CEC, meq/100 g), and (d) iron content (Fe, g/kg). Each symbol within a series represents one of nine different sediments. Lines represent significant linear regressions up until a threshold (Linear equations are presented in Table A4.4 of the Appendix).

conditions and the limited range in sediment/soil properties investigated (i.e., only examined with 1 to 5% organic matter).

Significant positive relationships were evident between sediment properties and K_d-U values ($R^2 > 0.71$, $p < 0.05$, Fig. 4.2 and Table A4.4) until either a threshold was reached or negative relationships were observed. These inflection points occurred for sediments with greater than 12% TOC, 37% fine fraction, 33 meq/100 g CEC, and 29 g/kg of Fe content. Vandenhove et al. (2007) demonstrated a strikingly similar positive relationship between increasing K_d-U values and greater soil organic matter ($R^2 = 0.70$) and Fe content ($R^2 = 0.63$). However, the latter study did not document a threshold after which a negative relationship was observed, as the maximum ranges examined were only approximately 15% organic matter and 20 g/kg of Fe content (Vandenhove et al., 2007), which are near or below the thresholds at which negative relationships appeared in our study. Exceptions to the positive relationships between sediment characteristics and K_d-U values in our study were observed for sediments with the greatest TOC, fine fraction, and CEC (i.e., SL1, HL, and KL). The decreasing K_d-U values associates with these sediments contradicts information from the literature that suggest that these sediments should offer the greatest number of binding sites for contaminants (Davis et al., 2004; Pabalan and Turner, 1996; Simpson et al., 2011). However, the lower K_d-U values observed for these organic carbon- and mineral-rich sediments (Fig. 4.2) may be in part due to desorption of complexing agent such as DOC, colloids, and carbonates from the sediment. Such desorption would increase dissolved U and lower K_d-U values, but without necessarily increasing U bioavailability. This is supported by results from previous experiments with the same organic- and mineral-rich sediments (SL1, HL, KL) that resulted in the lowest bioaccumulation of U by *C. dilutus* larvae (Crawford and Liber, 2016), demonstrating that much of the U was not bioavailable. These results suggest that U may be in solution and mobile even for sediments with large concentrations of some binding phases, but that the variability in dissolved U does not necessarily correlate with U bioavailability to aquatic organisms. Therefore, bioaccumulation tests complement investigations of U sorption, and information on the presence of colloids, DOC and other ligands that could be released from sediment should be provided.

The sorption tests demonstrated that all sediment properties evaluated here were important modifying factors of U sorption (Fig. 4.2); however, the use of a combination of multiple factors may have limited practicality for modeling U bioavailability in sediment. Fe content (Fig. 4.2d) was the factor that explained the greatest variation in K_d-U across the entire range of parameters examined here (at least up until ~29 g/kg), excluding the additional influence of pH. Barnett et al. (2000) also suggested that Fe oxide content exerted significant control over sorption of U across three bulk substrates with similar Fe oxide contents, as K_d values were similar despite having significant differences in fine fraction and organic matter content. Uranium species can adsorb to the surface sites of Fe oxides, and can become structurally incorporated into the oxide structure through repeated dissolution-precipitation cycling of amorphous or poorly crystalline Fe oxides (Vandenhove et al., 2007). Thus, Fe oxides in sediment can be an important adsorption-controlling phase for U, and may overcome the challenges of predicting U bioavailability associated with the presence of dissolved U-complexes from TOC, fine fraction and CEC. Additionally, Fe content of sediment has previously been observed to correlate with fine fraction, CEC, and TOC for the sediments used in this study (r = 0.84 to 0.97, $p < 0.001$; Crawford and Liber, 2016), as well as for other sediments and soils (Bradham et al., 2006; Payne et al., 2011; Vandenhove et al., 2007; Waite et al., 1994). The binding of U and other metals to sediments is not only influenced by the presence of Fe/Al oxides, but also by pH and TOC, which control the binding properties (surface area, charge and binding sites). Properties such as CEC are intrinsically accounted for through correlation with parameters such as sediment TOC and fine fraction, which are routinely measured in conjunction with field-collected sediment samples (Bradham et al., 2006; Criel et al., 2008). Recent studies have also supported the use of K_d values normalized for specific surface area (Davis et al., 2004; Pabalan and Turner, 1996; Wang et al., 2011). However, sorption can be controlled by interactions with specific surface sites, the abundance of which are not reflected by the specific surface area (Payne et al., 2011).

An exception to the positive linear relationship between Fe content and K_d-U was observed with the KL sediment, which had a slightly lower K_d-U value, despite having the greatest Fe content (34 g/kg). However, the KL sediment also had the only detectable $CaCO_3$ content (1.13%), which Pandit et al. (2012) have demonstrated to have a strong negative correlation with K_d for soils collected around U mines due to the formation of uranyl-carbonate complexes. Sheppard (2011) also suggested that carbonates were more likely to persist in fine-textured soils and KL had the highest content of clay (28%) among the sediments tested. The lower sorption in the KL sediment may therefore be a result of the formation of soluble uranyl-carbonate complexes. Overall, sediment properties, particularly Fe content, were significant modifiers of U sorption, resulting in up to an 80-fold change in K_d-U values for sediments collected from the same general area of northern Saskatchewan.

4.3.2 Modeling aqueous U(VI) speciation

The abundance of aqueous U species was estimated as a function of pH using WHAM7 for selected sediments that covered a wide range of physicochemical properties. The major aqueous U species were dominated by the (calcium)-uranyl-carbonate complexes, with $Ca_2UO_2(CO_3)_3$ and $CaUO_2(CO_3)_3^{2-}$ being greatest at pH 7 and 8, and UO_2CO_3 and $UO_2(CO_3)_2^{2-}$ being greatest at pH 6. These dominant aqueous U(VI) species are in agreement with previous modeling efforts in the literature (Lofts et al., 2015; Wang et al., 2011). Thus, these speciation calculations support the observed reduction in K_d values at pH 8, which were likely due to the formation of uranyl-carbonate complexes that dominate under alkaline conditions and inhibit adsorption to the sediment solid phase.

4.3.3 Modeling of U(VI) adsorption

While using the relationship between Fe content of sediment and K_d-U is useful for estimating changes in U bioavailability, a more universally applicable tool is desirable for the prediction and modeling of U sorption behaviour in aquatic systems. A number of studies have supported the use of surface complexation models (SCMs) for the modeling of U sorption (Barnett et al., 2000; Davis et al., 2004; Dong et al., 2012; Pabalan and Turner, 1996; Payne et al., 2004; Romero-González et al., 2007; Waite et al., 1994). Adsorption parameters may be derived directly by fitting to the solid material (the generalized composite approach), which provides optimal site-specific model performance at the expense of general applicability. A more flexible method is the assemblage approach, where adsorption is modeled on the basis of the solid phase composition using a collection of adsorption models parameterized on synthesized or isolated components (substrates) of the solid such as organic matter, sesquioxides and clays (Dong et al., 2012; Groenenberg and Lofts, 2014; Waite et al., 1994). In this study WHAM7 was applied to predict sediment K_d values using the assemblage approach, using adsorption models for particulate organic matter and Fe(III) oxide. This allowed for prediction of the variability of U sorption across the field sediments and water conditions characteristic of U mining areas in Saskatchewan, incorporating multiple solid-adsorbing parameters. The phase models used in the present study for ion binding to HA, FA, and hydrous Fe oxide allow both specific (inner-sphere) and diffuse layer binding. These binding mechanisms are supported by recent studies that have demonstrated that adsorption of U species onto clay mineral surfaces occurs via the formation of bidentate inner-sphere surface complexes at near-circumneutral pH conditions (Dong et al., 2012; McKinley et al., 1995; Pabalan and Turner, 1996; Sylwester et al., 2000). However, some of the previous studies were conducted under CO_2 free or low CO_2 conditions which means the role of uranyl-carbonate ternary surface complexes was not investigated (McKinley et al., 1995; Pabalan and Turner, 1996; Sylwester et al., 2000). Carbonate complexes with uranyl can be very important for systems containing CO_2 and have been reported as important sorption processes for U onto montmorillonite clay (Catalano and Brown Jr, 2005; Dong et al., 2012).

Modeling yielded a strong 1:1 relationship between the predicted and observed U concentrations in solution (at equilibrium) in the various sorption tests (Fig. 4.3a; slope = 1.02, R^2 = 0.961, $p < 0.001$, RMSE = 42 µg/L). This demonstrated that the model can predict, generally within a factor of two, the concentration of U in solution based on the expected binding/partitioning of U to the solid phases (i.e., sediments). Previous studies have reported issues with greater variation between the observed and predicted U concentrations near adsorption edges, where concentrations change sharply with pH (i.e., pH 8) (Barnett et al., 2002). A model examined by Barnett et al. (2002) overestimated the K_d-U by up to two orders of magnitude in the pH range of maximum adsorption (pH 6 to 7). Model predictions would be most sensitive to uncertainties in the measured pH near adsorption edges. However, aqueous U concentrations were well predicted by WHAM7 under our test conditions and parameters. The WHAM-predicted K_d-U values were generally within a factor of three of the observations for all the sediment-pH treatments (Fig. 4.3b; slope = 1.07, R^2 = 0.94, $p < 0.001$, RMSE = 0.47 ml/g log unit), with only slight deviations from the 1:1 line at the low K_d values. One possible reason for the slight overestimation of U solubility at pH 8 may be due to an underestimation of the total number or binding affinity of reactive surface sites, and/or overestimation of complexation in solution. Regardless, the similarity between the predicted and observed K_d-U values validates the trends presented in Fig. 4.2 (including the inflection points) for U sorption as a function of different sediment properties. This further demonstrates the influence of TOC, DOC, and Fe oxides, common input parameters for WHAM7, on U binding and complexation. Thus, WHAM7

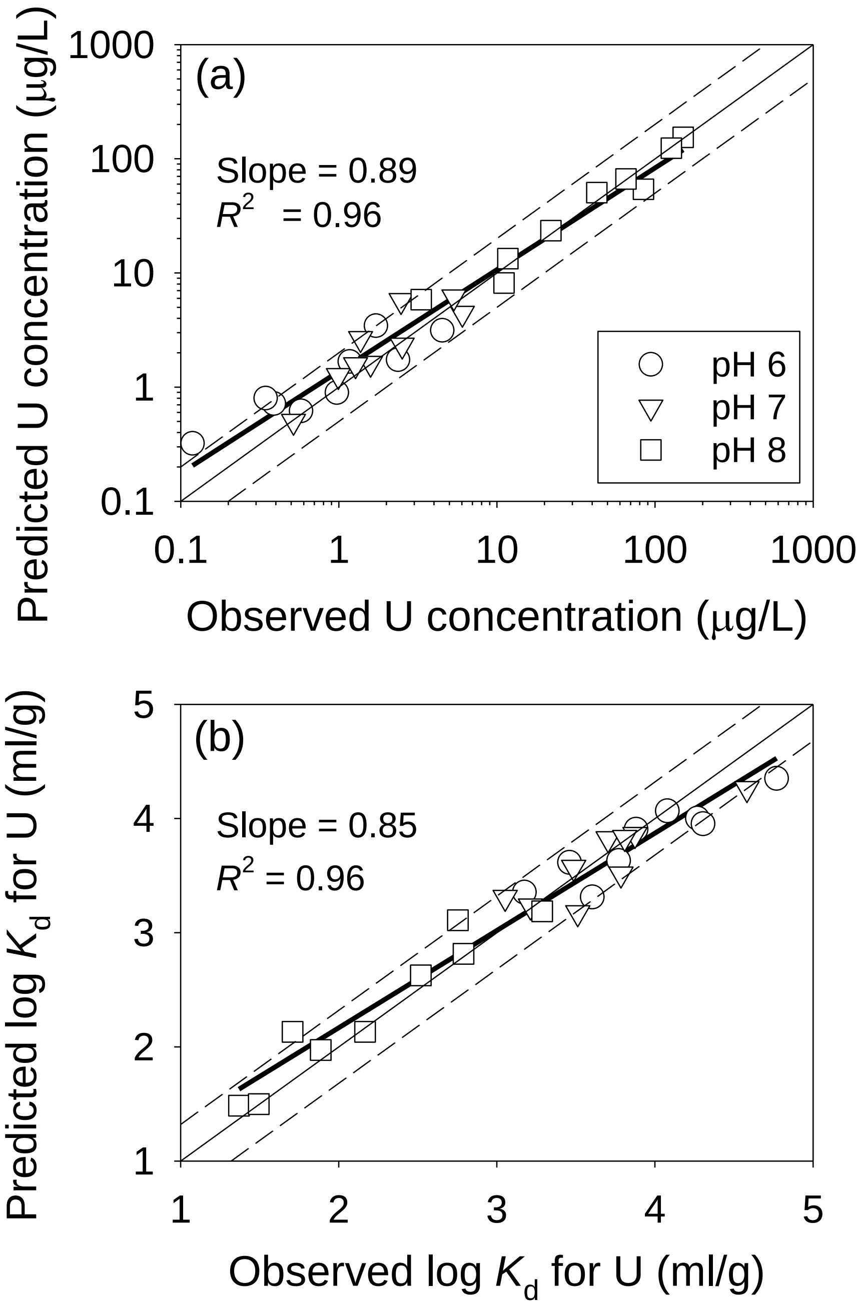

Fig. 4.3 WHAM-predicted versus observed (a) U concentration (μg/L) in the overlying water at equilibrium, and (b) log K_d for U (ml/g) for all nine sediments at pH 6, 7, and 8. Thick solid line represents the linear regression for all data points ($p < 0.05$); the 1:1 line is bracketed by dashed lines representing a factor of two.

is able to account for the complex interactions between uranyl binding to ligands on the solid sediment and in the solution in freshwater systems with a wide range of sediments and conditions characteristics of northern Saskatchewan.

4.3.4 Implications for site characterization

The present research confirms that K_d-U values are influenced by a number of sediment components including TOC, fine fraction, CEC, and Fe content, water chemistry including pH, and metal-complexing ligands such as carbonates. The extent of U adsorption was significantly pH-dependent. Due to the complex nature of U sorption with multiple and correlating sediment characteristics, it is difficult to recommend a simple relationship between the U concentrations in solution and a single sediment parameter. However, the Fe content in sediment (within the range of ~1 to 29 g Fe/kg sediment) was a consistent and strong modifier of U sorption behaviour with a relationship modeled by the equations presented in Table A4.4 of the Appendix. Fe content, along with other common physicochemical parameters of sediments, provided useful information for the application of an internationally recognized and user-friendly model, WHAM7, to predict U sorption behaviour. It is therefore recommended that Fe content should be reported, along with all other routinely measured sediment parameters, in site assessments where U-contamination is a concern. Additionally, this research highlighted the conditions that enhance the mobility of U in sediment, which will allow for further examination of these conditions to improve the risk assessment of U-contaminated sites. Adsorption of U to sediment and other particulate phases strongly influences the proportion of U available for transport and uptake by removing U from the aqueous phase or by forming complexes that render U immobile and/or not bioavailable. From this study, sediments in alkaline aquatic systems with low Fe content would favour greater U concentrations in solution and thus likely greater U mobility and possible uptake in aquatic organisms. Future work should focus on additional quantification and further validation of field and/or site-specific physicochemical properties that could improve the use of WHAM7, which may lead to more accurate risk assessments for U-contaminated sites and for development of bioavailability influenced U-SQGs.

CHAPTER 5:
PREDICTING THE BIOAVAILABILITY OF URANIUM TO CHIRONOMIDS IN FRESHWATER SEDIMENTS

5 Preface

This chapter includes additional exposure and bioaccumulation data associated with U-contaminated field sediments collected downstream of U mining operations in northern Saskatchewan. Bioaccumulation of U was compared between *Chironomus dilutus* larvae exposed to U-contaminated field sediment in a 10-d laboratory experiment and chironomid species collected directly from the field. Based on the success with the Windermere Humic Aqueous Model, version 7.0.4 (WHAM7), in predicting U sorption from Chapter 4, the ability to predict U bioaccumulation, and hence bioavailability, was evaluated through the use of either simple regressions or WHAM7. Bioaccumulation data from the U-spiked formulated sediment (Chapter 2), the U-spiked field sediment (Chapter 3) and from the U-contaminated field sediment were used to examine and validate the two models. WHAM7 was additionally used to examine the modeled speciation and fraction of U bound or present as different phases (i.e., particulate, colloidal or aqueous) in the test systems for all of the experiments presented in this thesis. Observed bioaccumulation of U in *C. dilutus* larvae correlated well with the WHAM-calculated concentration of U bound to humic acid (HA), suggesting that HA may be a suitable surrogate for U binding sites (biotic ligands) in *C. dilutus* larvae. Overall, WHAM7 produced reliable results of U sorption and bioaccumulation when applied to both laboratory and field conditions relevant to areas surrounding northern Saskatchewan U mines, and thus, may be useful for accounting for U bioavailability in regulatory assessments of U-contaminated sediments.

This chapter will be reformatted and submitted to Environmental Toxicology and Chemistry under joint authorship with Karsten Liber (University of Saskatchewan) and Stephen Lofts (NERC Centre for Ecology and Hydrology).

5.1 Introduction

The purpose of sediment quality guidelines (SQGs) is to set thresholds for contaminants that can be used to protect benthic invertebrate communities from hazardous concentrations of those contaminants in the sediment (CCME, 1999; Thompson et al., 2005). However, the use and derivation of SQGs for metals have been criticized for using total metal concentrations in the sediment because they do not incorporate metal bioavailability (Ankley et al., 1996a; Burnett-Seidel and Liber, 2012). The amount of a metal available for uptake by an organism, and thus the amount that has potential for causing adverse effects to the benthos can be significantly different from the total metal concentration due to the different binding phases of sediment that modify metal bioavailability (Campana et al., 2013; Crawford and Liber, 2016; Crawford and Liber, 2015). Thus, the use of SQGs is often hindered by the limited quantification and/or lack of incorporation of metal bioavailability and associated modifying factors, often leading to the unreliable predictions of potential adverse effects to benthic communities. Inaccurate predictions is a particular issue with regional-specific SQGs developed for use in and around uranium (U) mining areas in northern Saskatchewan, Canada (Burnett-Seidel and Liber, 2012), an area that contains some of the richest deposits of U ore in the world (OECD-NEA and IAEA, 2014). The tendency of U to accumulate in sediments can pose a risk to the benthic communities that inhabit U-contaminated sediments (Liber et al., 2011; Peck et al., 2002), particularly under conditions that favour high U bioavailability. Some of the identified modifiers of U bioavailability include solution pH and the physicochemical properties of the sediment, such as total organic carbon (TOC), Fe content, and particle size, which directly influence the partitioning of U between aqueous and solid phases (Barnett et al., 2002; Cheng et al., 2004; Crawford and Liber, 2016; Maity et al., 2013; Sheppard, 2011; Van Dam et al., 2012; Vandenhove et al., 2007; Vandenhove et al., 2009a). Therefore, there is a need to incorporate bioavailability and the associated modifying factors into SQGs to improve the accuracy of the predicted adverse effects of sediment-associated U to the benthic community.

The quantification of modifying factors and the prediction of metal bioavailability have been proposed through a number of regression models, pore water extractions, speciation models, and surface complexation models (Barnett et al., 2002; Campana et al., 2013; Cheng et al., 2004; Davis et al., 2004; Logue et al., 2004; Waite et al., 1994; Weng et al., 2002). However, the difficulty in incorporating modifying factors of metal bioavailability into risk assessments and SQGs for metals is mainly due to the absence of a model applicable for a wide range of sediments and site conditions. Additionally, there is a lack of data available to validate the models that do exist. As a result, no scientifically-acceptable approach has been adopted for use in the regulation of U-contaminated sediments that incorporate modifying factors of U bioavailability. Some success at predicting the behaviour of metals in aqueous systems has been achieved using the Windermere Humic Aqueous Model (WHAM), which incorporates a set of submodels for solution and solid phase components of aqueous systems including natural organic matter (humic substances) and mineral oxides. For example, Shi et al. (2008) presented a Zn kinetics model through use of WHAM that was able to account for multiple water chemistry parameters and Zn concentrations in describing the nonlinear binding of Zn to organic matter. Additionally, previous studies have generated models for predicting Cu and Ni bioavailability to aquatic organisms through the use of WHAM as an integral component of the biotic ligand model (BLM) (De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2005). Both WHAM and the BLM are internationally-recognized, user-friendly models based on equilibrium partitioning relationships. The BLM computes bioavailability on the basis of dissolved speciation only (De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2005), although a version for sediment organisms has been developed (Di Toro et al., 2005). WHAM can compute speciation across the solution and solid phases (Lofts and Tipping, 2000) and has also been used to model the variability in the uptake of metals by organisms (Tipping and Lofts, 2013). Since previous studies have demonstrated the significant influence of sediment properties on U behaviour in sediment and soil (Barnett et al., 2002; Crawford and Liber, 2016; Vandenhove et al., 2007), the incorporation of physicochemical properties of sediment into a model to predict U bioavailability is essential for improving SQGs. Thus, a model such as WHAM, which incorporates a number of modifying factors of U bioavailability, including those that influence the sorption, speciation and bioaccumulation of U, may be able to improve risk assessment of U-contaminated sediments and possibly U-SQGs.

In this paper, WHAM and previously developed regression models were evaluated and compared for their applicability in predicting U bioavailability under a wide range of conditions relevant to areas surrounding U mines in northern Saskatchewan, Canada. Water chemistry characteristics and sediment properties were used as input parameters for the models. The goal was to determine if a model could be used to estimate the bioavailability of U to chironomids in freshwater sediments. Both the regression models and WHAM7 were used to predict the U bioaccumulation from U-contaminated sediments for both laboratory and field exposed benthic invertebrates. Additional data from previously published U-spiked sediment experiments (Crawford and Liber, 2016; Crawford and Liber, 2015) and sorption tests (Crawford et al., 2016) were used to assess the applicability of WHAM in modeling U sorption, speciation, and bioaccumulation. The specific objectives were to (i) quantify and compare the bioaccumulation of U from U-contaminated sediment in both laboratory-exposed and field-collected organisms; (ii) evaluate the use of previous regression models in predicting U bioaccumulation to laboratory and field organisms exposed to U-contaminated sediments; (iii) examine and/or validate the WHAM-predicted sorption and speciation of U among U sorption tests, as well as U-spiked and U-contaminated sediment experiments; and (iv) investigate the applicability of WHAM in modeled U bioaccumulation in a model benthic organism.

5.2 Material and methods

5.2.1 Laboratory exposure with U-contaminated field sediments

Uranium-contaminated sediments were collected from areas downstream of a U mining operation in northern Saskatchewan, Canada (Horseshoe Creek [HC] and Hidden Bay [HB] near Wollaston Lake; Table 5.1). Sediments were collected in July 2013 from the top 10-cm layer of surficial sediment using an Ekman grab sampler. The collection, transport, and analysis of the U-contaminated field sediment followed the same protocols previously described for the collection of reference sediments from a nearby area (Umperville River near Wollaston Lake; Crawford and Liber, 2016). The use of these U-contaminated field sediments in a 10-d sediment bioaccumulation test using *Chironomus dilutus* larvae also followed an identical protocol to that described by Crawford and Liber (2016), except that sediments from the present study were not spiked with U, but tested at their “natural” field contaminated U concentrations.

Chironomus dilutus larvae were selected as the test species due to their common occurrence in areas surrounding U mines in northern Saskatchewan and because *C. dilutus* are a standard test species for sediment toxicity assessments (Environment Canada, 1997). Six biological replicates with ten 8 to 10-d old (second instar) *C. dilutus* larvae each were used for bioaccumulation assessments for the control and each of the U-contaminated sediment treatments. Additionally, two chemical replicates were used for the analysis of dissolved organic

Table 5.1 Summary of physicochemical characteristics and background uranium concentrations of the U-contaminated field sediments.

Sediment Properties/ID[a]	**HC1**	**HC2**	**HC3**	**HB1**
TOC (%)[b]	0.2	8.0	9.6	11.6
Sand (% > 50 µm)[c]	95	49	21	34
Silt (% 2 - 50 µm)[c]	5	44	71	59
Clay (% < 2 µm)[c]	0	6	8	7
Fine fraction (% silt + clay)[c]	5	50	79	66
Fe (g/kg)[d]	0.8	6.0	11.9	9.2
Water content (%)	17	58	71	73
Background U (mg/kg d.w.)[e]	7.2	213.6	401.1	444.3

TOC = total organic carbon; CEC = cation exchange capacity; Fe = iron content.

[a] Field sediments collected from the Wollaston Lake area in northern Saskatchewan, Canada; HC – Horseshoe Creek, HB – Hidden Bay.

[b] TOC determined by LECO Carbonator Model C632, Department of Soil Science, University of Saskatchewan, Saskatoon, SK.

[c] Particle size distribution determined by mini-pipette method with removal of organic matter and carbonates, ALS Environmental, Saskatoon, SK.

[d] Determined by dithionite-citrate-bicarbonate (DCB) extraction method for total Fe (Pansu and Gautheyrou, 2007; Ryan and Gschwend, 1991).

[e] Determined by ICP-MS after complete sediment digestion, Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

carbon (DOC) from extracted pore water sample for all treatments and the control. The control treatment consisted of the same un-spiked silica sand (particle size of 106 to 425 µm) as described in Crawford and Liber (2016 and 2015). Reference field sediment (i.e., Umperville R.) with similar physicochemical characteristics to the U-contaminated sediments examined here were not used as additional controls because no adverse effects on the growth or survival of the *C. dilutus* larvae had previously been observed for the reference field sediments (Crawford and Liber, 2016).

The 10-d test was conducted in a sediment testing intermittent renewal (STIR) system previously described (Benoit et al., 1993; Crawford and Liber, 2016; Crawford and Liber, 2015). Dissolved U concentrations were measured in the overlying water and pore water collected immediately above and below the sediment surface through the use of dialysis sampling devices (mini-peepers; Crawford and Liber, 2015; Doig and Liber, 2000) inserted into each biological test beaker. Temperature, dissolved oxygen, conductivity, pH, ammonia, alkalinity, total hardness, and DOC were analyzed in overlying water samples following the procedures described by Crawford and Liber (2016). Approximately 1 g d.w. of sediment was also collected from test beakers for analysis of total U concentrations in the sediment through microwave-assisted digestion with acids as outline by Crawford and Liber (2016). Organism survival, weight, and U accumulation were determined after gut purging animals (12-h) following the same EDTA-rinse procedure used and described in previous U-spiked sediment experiments (Crawford and Liber, 2016; Crawford and Liber, 2015). Water and digested sediment and tissue samples were filtered (0.45-µm pore size, polyethersulfone membranes) and acidified (2% HNO_3) for analysis of U by inductively couple plasma-mass spectrometry (ICP-MS; Thermo Scientific X-series II spectrometer with PlasmaLab software and collision cell technology, Thermo Electron Ltd., Mississauga, ON, Canada). Certified reference materials (SLRS-5; National Research Council of Canada and 1640e; National Institute of Standards and Technology), blanks and duplicates were included with all analyses to ensure analytical accuracy and validity.

5.2.2 Field exposure with U-contaminated field sediments

In addition to the exposure of laboratory-reared *C. dilutus* larvae to the U-contaminated field sediments, benthic invertebrates were collected from the same U-contaminated sediments in the field to quantify U bioaccumulation. Field-collected benthic organisms were also sampled from reference areas of the Umperville R. and used as controls for the bioaccumulation analysis. Field organisms were removed from the sediment at each site, initially by rinsing samples in a sieve bucket and subsequently removing them from sorting trays using forceps into taxonomic order, family or genus, if possible. All collected organisms were thoroughly rinsed and gut-purged in the field following a similar EDTA-rinse and 12-h gut purging procedure as that used in the laboratory experiments (Crawford and Liber, 2016; Crawford and Liber, 2015). Field organisms were subsequently transported back to the laboratory, dried at 60°C for 24 h, and digested using HNO_3 and H_2O_2 for determination of U bioaccumulation. Additionally, overlying water samples were collected at reference and U-contaminated field sites using a Van Dorn sampler approximately 10 to 15 cm above the sediment surface for analysis of conductivity, pH, alkalinity, total hardness, DOC, and U concentrations.

5.2.3 Model analysis

Bioaccumulation data from the laboratory-exposed and field-collected organisms were used to assess and validate regression models previously developed by Crawford and Liber (2016) for predicting U bioaccumulation. The previous laboratory-exposed tests used sediments collected from uncontaminated sites around U mining areas in northern Saskatchewan (Wollaston Bay area) in a series of 10-d U-spiked bioaccumulation tests to determine differences in U bioaccumulation as a function of sediment properties. The most practical and reliable regression equations were based on the significant correlations between observed U bioaccumulation in *C. dilutus* larvae and fine fraction content (≤ 50 μm particle size) of field sediments spiked with 50 mg U/kg d.w. (Eq. 5.1) and 500 mg U/kg d.w. (Eq. 5.2).

$$\log U_{\mathrm{tissue}} = 1.61 - 0.45 \log \text{fine fraction} \qquad \text{(Eq. 5.1)}$$

$$\log U_{\mathrm{tissue}} = 3.06 - 0.79 \log \text{fine fraction} \qquad \text{(Eq. 5.2)}$$

In addition to the regression model, WHAM7, version 7.0.4 (Lofts and Tipping, 2011; Tipping, 1994) was used to determine if U bioaccumulation could be predicted through the incorporation of more than physicochemical properties of sediment alone. Relevant output from WHAM7 is discussed below, but includes predicted sediment-solution partition coefficients (K_d), fractions of U bound to particulate and colloidal phases, percent distribution of relevant U species, and HA-bound U as a potential surrogate for bioaccumulation. Input for WHAM7 included data from previous U sorption tests with nine field sediments conducted at pH 6, 7 and 8 for U concentrations of 0.023, 0.23 and 2.38 mg/L under water chemistry (hardness, alkalinity, DOC and major ions) conditions typical for northern Saskatchewan (Crawford et al., 2016). Additional WHAM7 input included data from the U-contaminated field sediments and previous bioaccumulation tests with U-spiked formulated sediment (Crawford and Liber, 2015) and field sediments (Crawford and Liber, 2016). The previous U bioaccumulation tests were conducted with 25 different field sediments and over 30 formulated sediments spiked with either 5, 50, 200 or 500 mg U/kg d.w. in 10-d tests with *C. dilutus* larvae. The input parameters for WHAM7 obtained from all of these studies included solution pH, temperature, particulate humic acid (HA), fulvic acid (FA) and Fe oxide, DOC as colloidal FA, and concentrations of major solutes (Table 5.1 and obtained from Crawford and Liber, 2016 and 2015; Crawford et al., 2016). The Fe oxide content of sediments was determined from measured Fe content by assuming that 1 mole of Fe (55.85 g) corresponded to 90 g of oxide (Dzombak and Morel, 1990). Additionally, all measured sediment Fe was assumed to be hydrous Fe oxide for modeling purposes. Total organic carbon concentrations were converted to particulate HA/FA for input into WHAM7 by accounting for the sediment concentration (i.e., solid-to-solution ratio; SSR). Assumptions included that organic matter comprised 50% carbon and that the measured TOC had ion-binding properties of 50% HA and 50% FA (Lofts and Tipping, 2011). Concentrations of DOC were used to calculate colloidal FA for input into WHAM7 by assuming that FA was 50% carbon and that 65% of the DOC behaved as active FA, as previously reported by others (De Schamphelaere et al., 2005; Tipping, 1998; Tipping and Lofts, 2015; Weng et al., 2002; Zhang and Davison, 2000). Solution concentrations of Al and Fe(III) were assumed to be controlled by the solubility of their respective hydroxides. The solution complexation and particulate hydrous Fe oxide binding parameters used were those of Lofts et al. (2015), with additional thermodynamic stability constants presented in Crawford et al. (2016). Alkalinity values were input to define the carbonate content for the systems.

5.3 Results and discussion

5.3.1 U-contaminated field sediments

5.3.1.1 Test conditions

The physicochemical properties of the U-contaminated field sediments are presented in Table 5.1 and the associated water chemistry from the 10-d bioaccumulation sediment test and field conditions are presented in Table A5.1 of the Appendix. The mean (± SD) DOC concentration of the overlying water from the Umperville R. reference field sites (*n* = 3) was 3.6 ± 0.1 mg/L, with a similar mean DOC of 3.7 ± 0.4 mg/L in the overlying water of the laboratory test control treatment (*n* = 6). Slightly greater DOC concentrations were observed in the overlying water of the U-contaminated sediments (5.5 ± 0.2 mg/L, *n* = 8) and the laboratory U-contaminated sediment treatments (5 to 7 mg/L, increasing as TOC in sediments increased). The concentration of DOC can be important in the assessment of U bioavailability as DOC has been reported to ameliorate U toxicity to various aquatic organisms by 6 to 9% with each 1 mg/L addition of DOC up to 30 mg/L (Van Dam et al., 2012). Other water chemistry parameters can also influence the sorption and bioavailability of U (Goulet et al., 2015; Riethmuller et al., 2001; Sheppard, 2011). The sorption of U is significantly pH-dependent (Barnett et al., 2002; Crawford et al., 2016), which can influence the availability of U. For example, the toxicity of U to *C. crassiforceps* was observed to decrease from 36 mg/L at pH 6 to 58 mg/L at pH 4 (Peck et al., 2002). Therefore, it is important to carefully document the water chemistry characteristics of test and field conditions as they can be significant modifiers of U behaviour. Further modifications of U bioavailability by solution and sediment properties from the laboratory and *in-situ* field data are discussed below where they are incorporated into WHAM7 to predict U speciation, sorption, and bioaccumulation.

5.3.1.2 Biological endpoints and U bioaccumulation

Mean (± SD) survival of the laboratory test organisms was 84 ± 4% and final weight per surviving individual was 1.9 ± 0.2 mg d.w. for all U-contaminated and control treatments (*n* = 24). Test organisms in all treatments surpassed the recommended minimum acceptable weight (0.6 mg d.w.) and survival (70%) of *C. dilutus* larvae for controls (Environment Canada, 1997). Uranium accumulated in the *C. dilutus* larvae from the 10-d sediment test control and in the chironomids collected directly from reference areas in the Umperville R. was negligible (≤ 0.3

mg U/kg d.w.). In contrast, the bioaccumulation of U from the U-contaminated field sediment ranged from 5 to 35 mg U/kg d.w. for the laboratory-exposed *C. dilutus* larvae and from 6 to 68 mg U/kg d.w. for the field-collected chironomid species (Fig. 5.1). The differences in U accumulation between the laboratory-exposed and field-collected chironomids could be a result of the differences in the sample size, the life stage, and the species of chironomids, as well as exposure conditions. Organisms in the laboratory are exposed to constant and homogenous U concentrations, which was likely not the case for the field-exposed chironomid species, and may introduce variability into the exposure estimate. Additionally, differences in bioaccumulation of U between the field and laboratory chironomids could have arisen from the different water chemistry conditions (Table A5.1), which are important modifiers of U sorption and bioavailability (Barnett et al., 2000; Crawford et al., 2016; Crawford and Liber, 2016; Crawford and Liber, 2015; Markich, 2002). These differences are evaluated further below.

Additional investigation into the bioaccumulation of U in *C. dilutus* larvae revealed a significant positive correlation with dissolved concentrations of U in the overlying water ($[U_{OW}]$; 0.022 to 0.16 mg U/L) in the laboratory test ($\log[U_{tissue}] = 0.97 \log[U_{OW}] + 2.38$, $R^2 = 0.95$, $n = 4$, $p < 0.001$). Similar correlations between the bioaccumulation of U in *C. dilutus* larvae and the concentrations of U in the overlying water have been observed previously for U-spiked formulated sediment ($\log[U_{tissue}] = 0.98 \log[U_{OW}] + 2.15$, $R^2 = 0.77$, $n = 47$, $p < 0.001$; Crawford and Liber, 2015) and U-spiked field sediment ($\log[U_{tissue}] = 0.68 \log[U_{OW}] + 2.37$, $R^2 = 0.61$, $n = 28$, $p < 0.001$; Crawford and Liber, 2016). However, no correlation was observed between the bioaccumulation of U in the field-collected organisms (6 to 68 mg U/kg d.w., Fig. 5.1) and total U concentration in the sediment or the concentrations of U in the overlying water (0.014 to 0.015 mg U/L). This lack of correlation may be a result of the method by which overlying water was sampled. As reported with the previous use of mini-peepers in laboratory tests (Crawford and Liber, 2016), the overlying water collected with the mini-peepers is more representative of water at the sediment-water interface (SWI) than water collected mid-water column with conventional samplers in the field. Thus, U concentrations in overlying water collected 10 to 15 cm above the SWI with the Van Dorn sampler in the field may not accurately represent the concentration of U that chironomids would actually be exposed to at the SWI (which could have been different). The influence of sampling method on contaminant exposure concentrations have previously been reported comparing results for the van Dorn sampler and peepers (Robinson and Liber, 2009).

5.3.2 Application of regression equations

Differences in the bioaccumulation of U occurred between the laboratory-exposed and field-collected organisms among the different U-contaminated sediments, but could not be fully or consistently explained by total U concentrations in the sediment or by dissolved U concentrations. Subsequently, the influence of sediment physicochemical characteristics were examined as they have been previously observed to significantly modify U bioaccumulation due to the various binding phases that influence the adsorption and partitioning of U between the aqueous and solid sediment phases (Crawford and Liber, 2016). In particular, previous regressions were developed for modeling U bioaccumulation in *C. dilutus* larvae using a single, easily-measured sediment physicochemical property, fine fraction (≤ 50 μm particle size). The regression equations were developed by Crawford and Liber (2016) using reference field sediments collected from around Saskatchewan U mines that were spiked with 50 mg U/kg d.w. (Eq. 5.1) or 500 mg U/kg d.w. (Eq. 5.2). These regressions were evaluated here for use in predicting U bioaccumulation from U-contaminated field sediment for the field-collected and laboratory-exposed organisms. Equation 5.1 was used for HC1 and HC2 and Eq. 5.2 was used for HC3 and HB1, based on sediment total U concentrations (7 to 444 mg U/kg d.w.; Table 5.1). The regressions resulted in accurate predictions of U bioaccumulation for HC2 and HB1 for the field-collected and laboratory-exposed organisms, respectively (Fig. 5.1). Overall, predictions of U bioaccumulation were generally within a factor of 3 of the observed bioaccumulation, with better predictions for the field-collected organisms using Eq. 5.1, and using Eq. 5.2 for laboratory-exposed organisms.

Although the fine fraction content of the U-contaminated sediments fell within the range of fine fraction used to develop the regression models, data that fall outside the specific parameters and test conditions used to develop simple regression models is a common limitation for the use of such models. The regression models used here are limited to the modifying effect of fine fraction alone, when in reality bioavailability of U is more complex and is likely to be influenced by multiple physicochemical characteristics. Thus, a model that incorporates multiple parameters that could affect U bioavailability (via sorption and speciation of U) would better elucidate the influence of modifying factors and provide better insight into the risk of U-contaminated sediments to benthic organisms.

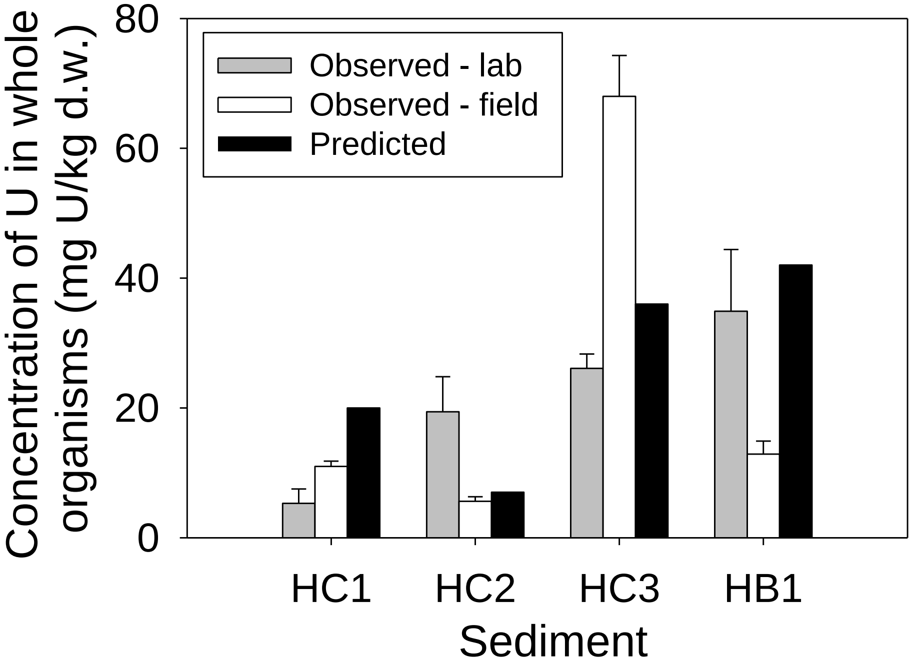

Fig. 5.1 Mean (± SD) concentration of uranium (mg U/kg d.w.) observed in laboratory test organisms (*C. dilutus*; grey bars, $n = 6$), and field-collected organisms (chironomid species; white bars, $n = 2$) relative to predicted concentration of U in benthic organisms (black bars) using Eq.5.1 (HC1 and HC2) and Eq. 5.2 (HC3 and HB1) for U-contaminated field sediments (HC, Horseshoe Creek; HB, Hidden Bay).

5.3.3 Application of WHAM

While the regression models reasonably predicted U bioaccumulation, they do not provide additional complementary information about the mechanisms behind the modified U bioavailability, such as the sorption and speciation of U. In a previous study (Crawford et al., 2016), we investigated the influence of sediment properties and pH on the sorption of U to field-collected reference sediments and found that WHAM7 produced reliable predictions of sediment-solution partition coefficients (K_d) for U. Adsorption of U was significantly pH-dependent with ≥ 97% adsorption of U to the sediment phase occurring at pH 6 to 7, but only 43 to 97% at pH 8, depending on the type of sediment. Due to the previous success with WHAM7 in predicting sorption, WHAM7 was used to investigate additional sorptive behaviour of U, along with U speciation for different test conditions and parameters to provide further insight into the bioavailability of U.

5.3.3.1 Modeling uranium sorption

In the present study, WHAM7 was used to predict the fraction of U bound to different sediment phases (TOC and Fe oxide content) and DOC (i.e., colloidal FA), as well as U present as free ion and aqueous complexes. Several examples showing the distribution of different U bound fractions for a range of sediments with very different compositions and characteristics are presented in Fig. 5.2. A description of these sediments used in the sorption and U-spiked sediment experiments can be found in Crawford et al. (2016) and Crawford and Liber (2015, 2016).

Among all of the experiments, the sediment phase had the greatest predicted fraction of bound U. There was a ≥ 78% association of U with the TOC content in the sorption study, except for sediments containing ≤ 3.8% TOC, which had as low as 32% of U bound to TOC (lowest for pH 8 treatments; i.e. UR8, Fig. 5.2a). In contrast, the present U-contaminated field sediment test and bioaccumulation experiments had a much larger (≥ 70%) fraction of U bound to the Fe oxide phase than to the TOC phase (i.e., UR8, Fig. 5.2b). Although some of the same sediments were used in both the sorption and bioaccumulation studies, the SSRs were different based on the design of the tests. Particulate parameters were calculated using sediment concentration (i.e., SSRs) to convert parameters like TOC to solution concentrations for input into WHAM7, and different SSRs can thus influence the predicted amount of U bound to different fractions. The

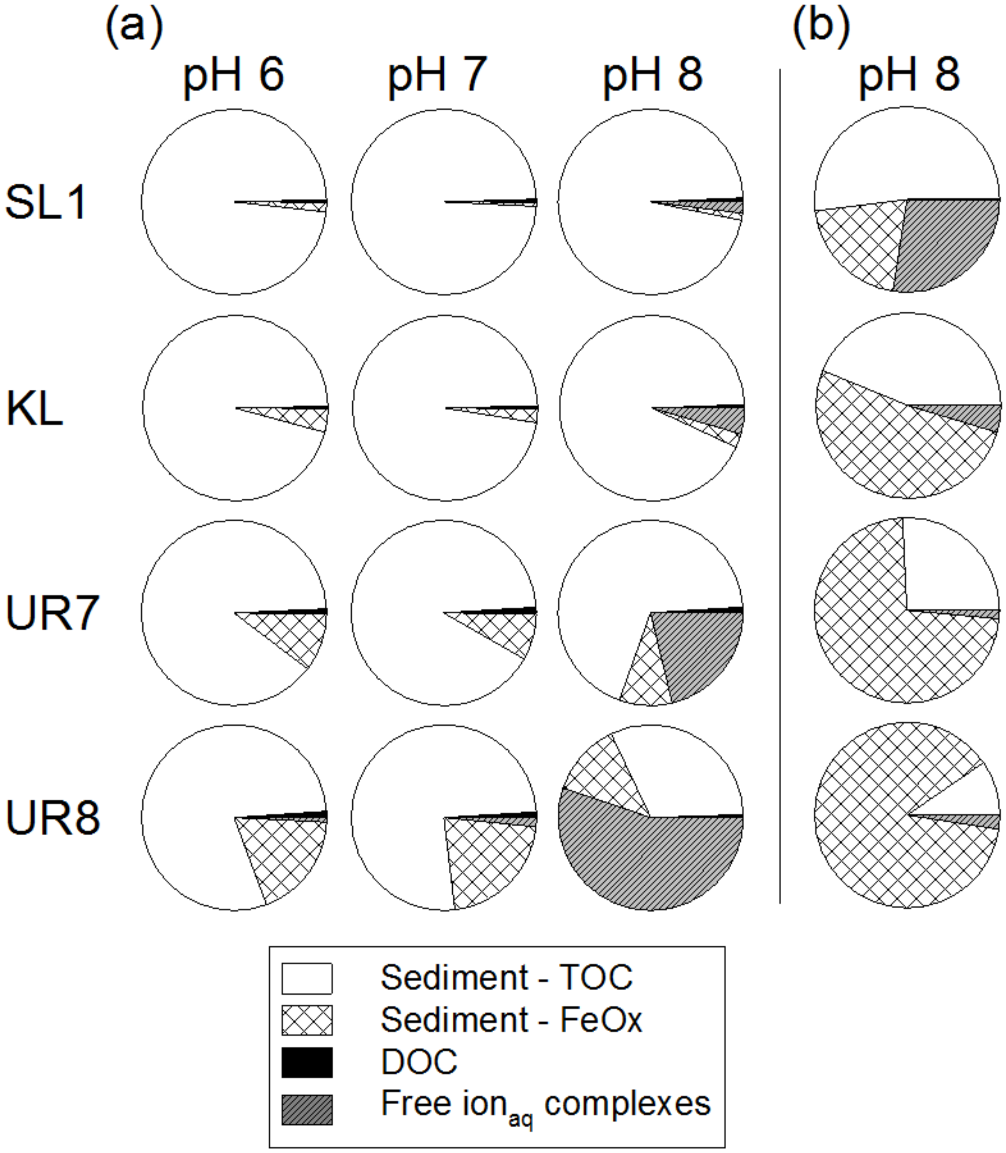

Fig. 5.2 Examples of the WHAM-modeled fraction of total uranium bound to sediment organic matter (TOC; open fill) and Fe oxide content (FeOx; open cross-hatch fill), to dissolved organic carbon (DOC; solid fill), and present as the free ion small aqueous complexes in solution (grey line fill) (a) as a function of pH for sediments described in a previous U sorption study (Crawford et al., 2016), and (b) for the U-spiked sediment bioaccumulation tests (Crawford and Liber, 2016) at a pH of 8. Sediments include Shallow Lake (SL), Konner Lake (KL), and Umperville River (UR) collected from around Wollaston Bay, Saskatchewan, Canada.

results from the bioaccumulation experiments are in agreement with other studies that have reported that Fe oxide content was the primary solid phase for the adsorption of U, followed by U bound to the carbonate sediment phase (Markich, 2002; Waite et al., 1994; Zheng et al., 2003); the latter was very low in our sediments (≤ 1.13% $CaCO_3$). Additionally, the fraction of U bound to the Fe oxide content in the sorption study, although generally low (≤ 6%), was more pronounced (8 to 22%) in sediments with low TOC (≤ 2.9%) and high Fe (≥ 10 g/kg), such as UR8 sediment (Fig. 5.2a). Binding of U to hydroxides under conditions of low TOC and high Fe content is consistent with the behaviour of other metals (Weng et al., 2002).

In comparison to the sediment phases, the contribution of DOC (≤1.4%) as a binding phase for U was not significant for any of the sediments among the different experiments. The low contribution of DOC for metal adsorption is consistent with previous predictions by WHAM (< 4.8%) for Cu, Cd, Zn, Ni, Pb (Weng et al., 2002). For sediments with low binding phases,≤ 3.8% TOC and/or ≤ 10 g/kg of Fe (i.e., UR8 and UR7; Fig. 5.2), greater proportions of U were predicted to be present as free ion aqueous complexes at pH 8 than at pH 6 and 7 in the sorption study (20 to 56%) and the bioaccumulation experiments (0.6 to 30%). Additionally, for sediments with low TOC and Fe content, U was present more as free ion aqueous complexes (0.6 to 30%) in the bioaccumulation experiments (Fig. 5.2b). Therefore, the distribution of U among solid and aqueous phases were demonstrated to vary as a function of both pH and the physicochemical properties of sediment, providing further quantification of the important role of binding phases associated with TOC and Fe content of sediment in U sorption and bioavailability.

5.3.3.2 Modeling aqueous speciation of uranium

The fraction of U that is bound or in solution does not alone determine the fate and bioavailability of U, as the aqueous speciation of U also plays an important role. In particular, the principles of the free (metal) ion activity model (FIAM) suggests that the free metal ion (i.e., U^{4+} or UO_2^{2+}) is typically the metal species that commonly accumulates and causes toxicity in aquatic organisms, with a few noted exceptions (Campbell, 1988; CCME, 2011; Markich et al., 2000). Uranium(VI) is generally the most predominant U species present in oxic freshwater and is considered more bioavailable than U(IV) to aquatic organisms (Echevarria et al., 2001; Langmuir, 1978; Markich, 2002). The conditions reported to favour the formation of UO_2^{2+} ions is generally low pH, low concentrations of organic matter, and likely low alkalinity (CCME, 2011; Markich et al., 2000; Markich, 2002; Riethmuller et al., 2001), which were not typical of the conditions examined in the U sorption test (Crawford et al., 2016) or current and previous U bioaccumulation tests (Crawford and Liber, 2016; Crawford and Liber, 2015). Thus, WHAM7 predicted that insignificant concentrations of UO_2^{2+} (< 1% of the distributed species) would have been present. Sorption and speciation of U were significantly pH-dependent (Crawford et al., 2016), as observed in the modeled species abundance presented in Fig. 5.3. Increases in pH can alter U availability and toxicity through the formation of carbonate and hydroxide U complexes that reduce the free uranyl activity (Fortin et al., 2007). Thus, the insignificant presence of the free UO_2^{2+} ion under the test conditions examined here, relevant to Saskatchewan U mining areas, is a result of the formation of the uranyl ion complexes and the U sorption that increase at pH > 6.

The abundance of U species modeled by WHAM7 as a function of pH for selected sediments that covered a wide range of properties are presented in Fig. 5.3. Uranyl-carbonate complexes were the predominant aqueous species predicted by WHAM7 at neutral pH conditions and increased in abundance with increasing alkalinity. Many of the pH-dependent uranyl-carbonate complexes are only weakly sorbed to sediment binding phases. Thus, the presence of U complexes, such as $Ca_2UO_2(CO_3)_3$, inhibit U sorption to sediment, which is consistent with the lower sorption in the current and in previous publications for more alkaline conditions (Crawford et al., 2016; Zheng et al., 2003). Speciation of U was also dependent on the type of sediment, as sandier sediments with low binding capacity (i.e., low TOC and Fe content) had greater concentrations of aqueous U species. These low binding sediments (i.e., SL2, UR8, UR7, UR2, WB) resulted in the predominance of $Ca_2UO_2(CO_3)_3$ complexes, particularly at pH 7 and 8, followed by $CaUO_2(CO_3)_3^{2-}$ species (Fig. 5.3). The latter U species surpassed the proportion of all other U species at pH 8 in high binding capacity sediments (i.e., ML, SL1, KL). The relative abundances of UO_2CO_3 and $UO_2(CO_3)_2^{2-}$ were generally the greatest, approximately ≥ 20%, at pH 6 for all sediments.

Speciation of U is complex and can be influenced by a number of other factors, including pH conditions and the presence of ligands, such as carbonates, sulphate ions, and DOC (Peck et al., 2002). The predominance of uranyl-carbonate complexes for both the sorption and

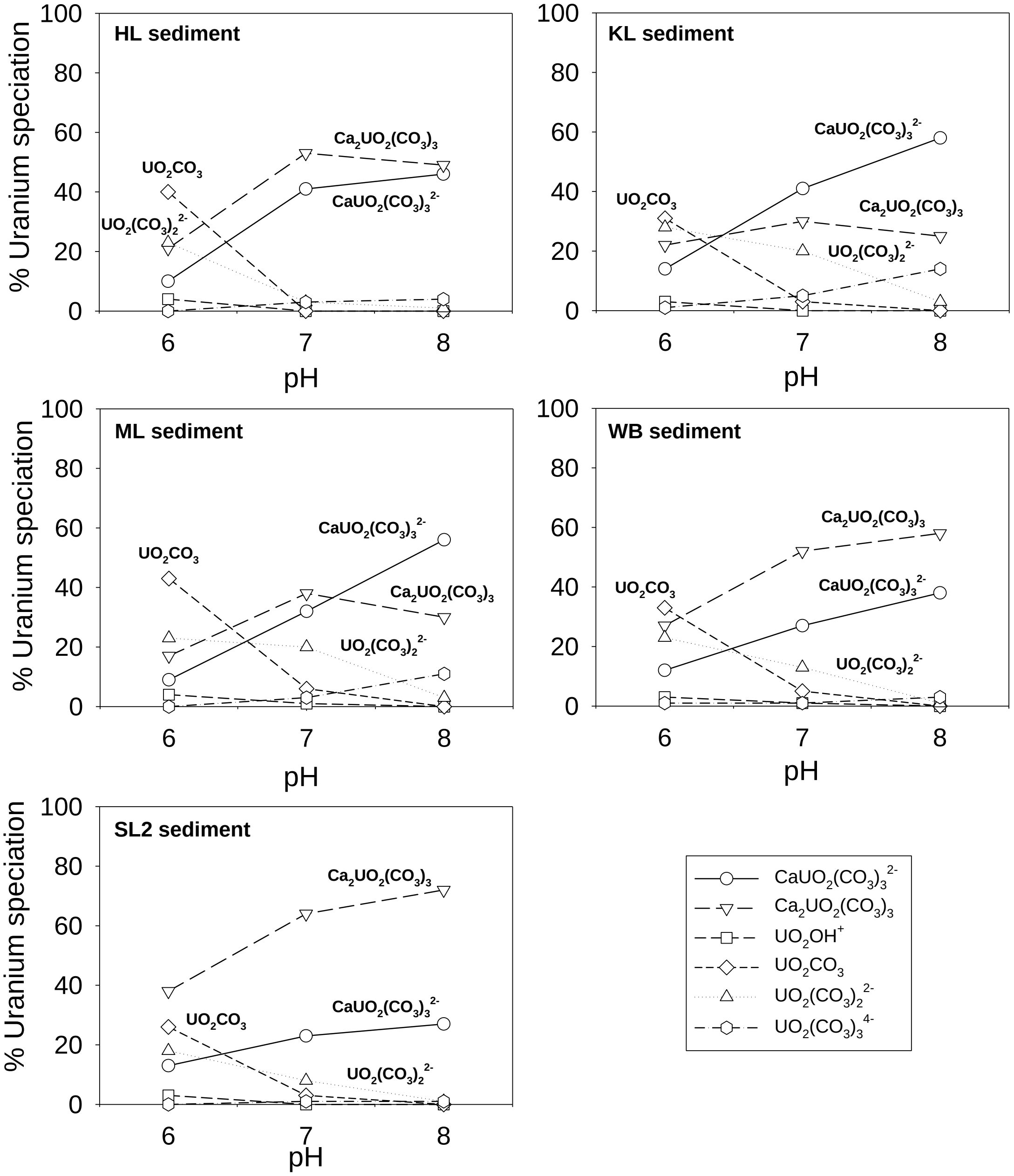

Fig. 5.3 Examples of the WHAM-predicted speciation (% distribution) of uranium (0.23 µg U/L) as a function of pH 6, 7 and 8 for a number of freshwater sediments. Uranium species <1% are excluded for clarity. Speciation of U was calculated using WHAM7 based on the sediment and water chemistry characteristics presented in Crawford et al. (2016). Sediments include Henday Lake (HL), Konner Lake (KL), Mallen Lake (ML), Wollaston Bay (WB) and Shallow Lake (SL) from around northern Saskatchewan U mining areas northern Saskatchewan U mining areas (Wollaston Lake, Saskatchewan, Canada).

bioaccumulation studies discussed here ($Ca_2UO_2(CO_3)_3$ > $CaUO_2$ $(CO_3)_3^{2-}$ >> $UO_2(CO_3)_3^{4-}$ ≈ $MgUO_2(CO_3)_3^{2-}$ > $UO_2(CO_3)_3^{2-}$) is generally in agreement with previous literature (CCME, 2011; Lofts et al., 2015; Markich, 2002). Concentrations of DOC are generally less important as complexing agents at alkaline pH conditions, as observed in our experiments, as higher CO_3 allows for greater formation of carbonate complexes in competition with DOC. Concentrations of chloride, nitrate, silicate, sulfate, phosphate, and fluoride are typically low (<3 mg/L) in northern Saskatchewan U mining areas and/or are relatively weak complexing agents of uranyl (EARMP, 2014; Markich, 2002). These ions were predicted by WHAM7 to form negligible U complexes (i.e., <1% of total U) for the present sorption and bioaccumulation studies. Overall, WHAM7 demonstrated the influence of pH, ligands such as carbonates, and the presence of different binding phases of sediment on the sorption and speciation of U under conditions typical of freshwater systems surrounding U mines in northern Saskatchewan. Knowledge about the WHAM-modeled speciation of U is important as it provides greater insight into the bioavailability of U than the use of regression equations that simply correlate sediment properties with U bioaccumulation.

5.3.3.3 Modeling uranium bioaccumulation

The use of WHAM7 in predicting U bioavailability was further investigated and validated with U bioaccumulation data from previous and present bioaccumulation tests. The accumulation of U by *C. dilutus* larvae exposed to U-contaminated field sediments (Table 5.1), U-spiked field sediments (Crawford et al., 2016), and U-spiked formulated sediments (Crawford and Liber, 2015) correlated significantly with WHAM-calculated HA-bound U (Fig. 5.4a). The correlation between the HA-bound U and bioaccumulation of U by *C. dilutus* larvae from both U-spiked formulated sediments (slope = 1.27, $R^2 = 0.87$, $n = 19$, $p < 0.001$) and U-spiked and U-contaminated field sediments (slope = 1.01, $R^2 = 0.75$, $n = 29$, $p < 0.001$) generally followed a 1:1 relationship, within a factor of 2.5 (Fig. 5.4a). Similarly, He and Van Gestel (2015) demonstrated a significant correlation between the observed body concentrations of Ni and Co in *Enchytraeus crypticus* and the WHAM-calculated amounts of metals bound to HA.

Bioaccumulation data from the U-spiked field sediments (open triangles; Fig. 5.4) overlapped with the field U-contaminated sediments (grey triangles) suggesting that the aging processes used for the U-spiked sediments allowed for adequate pseudo-equilibrium processes to occur between the chemical interactions and the exposed *C. dilutus* larvae. This conclusion is further supported in a study by Muscatello and Liber (2010) that demonstrated that a similar 10-d exposure period was an adequate period of time to allow *C. dilutus* larvae to reach steady-state conditions with the U in the surrounding aqueous phase. Aging processes can vary for different metal-spiked or metal-contaminated sediments, and times to reach equilibrium can vary for different benthic organism, depending on sediment contact, sediment composition, and metal partitioning to sediment components. For example, He and Van Gestel (2015) reported that the linear relationship between predicted Ni and Co bound to HA and observed body concentration in *E. crypticus* improved with time up until 14 d (and explained 79-93% of the variation). Thus, when conditions of equilibrium are satisfied, our data, as well as other data from the literature (He and Van Gestel, 2015; Stockdale et al., 2010; Tipping and Lofts, 2013), suggest that the predicted competitive binding of U to HA may correlate with the observed bioaccumulation of U in chironomids.

Since significant correlation was observed between predicted HA-bound U and observed U bioaccumulation in *C. dilutus* larvae, WHAM HA-bound U was fitted with the observed U accumulation using an optimized parameter, the equivalent HA (E_{HA}) value (Fig. 5.4b). The E_{HA} value is defined by Tipping and Lofts (2013) as the amount of HA per gram of organism d.w. (g/g), so that if the E_{HA} is 1 then the amount of accumulated metal follows the amount of HA-bound metal on average. Based on the assumption that organisms possess binding sites that have properties similar to those of HA, optimization with an E_{HA} value provides an ability to compare metal body burdens among different organisms (Tipping and Lofts, 2013). The E_{HA} was 0.62 for U-spiked formulated sediments and 1.89 for all U-spiked and U-contaminated field sediments. Modeled U bioaccumulation from WHAM7 resulted in a strong correlation with the observed accumulation of U in *C. dilutus* larvae from U-contaminated field sediments (slope = 1.13, R^2 = 0.97), U-spiked field sediments (slope = 1.03, R^2 = 0.75), and U-spiked formulated sediments (slope = 1.27, R^2 = 0.87). All sediments combined followed a 1:1 relationship within a factor of 2.5 (slope = 1.11, R^2 = 0.80, n = 48, $p < 0.001$; root-mean square error [RMSE] of 0.02 mol/g log units; Fig. 5.4b). Tipping and Lofts (2013) reported a similarly good correlation (R^2 =0.89, n = 467, RMSE = 0.44) between WHAM HA-bound metals and observed bioaccumulation of a number of metals in *Hyalella azteca* in the laboratory and in caged field animals after correction with an E_{HA} value of 0.044 and 0.11, respectively. Additional studies have demonstrated

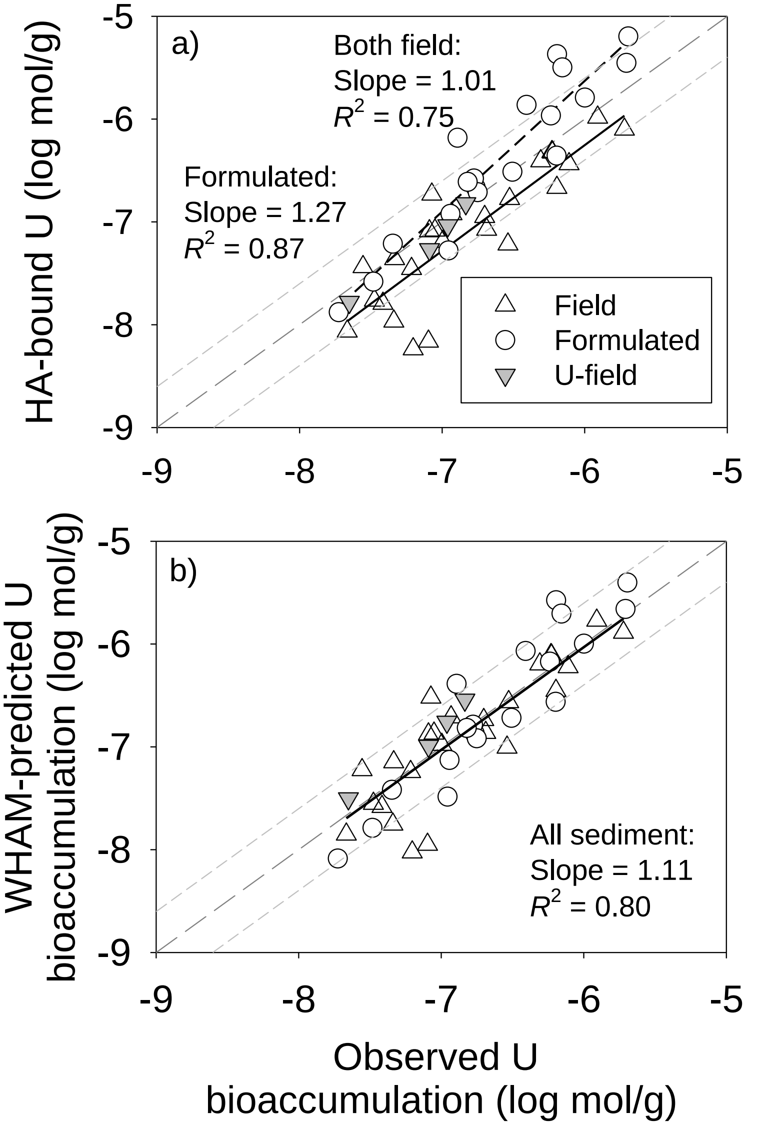

Fig. 5.4 Comparison of the (a) WHAM-calculated concentration of U bound to the humic acid (HA), or (b) WHAM-predicted U bioaccumulation via E_{HA} correction (0.62 and 1.89) to the observed bioaccumulation of U in *C. dilutus* larvae from U-spiked field sediment (open triangles; Crawford and Liber, 2016), U-spiked formulated sediment (open circles; Crawford and Liber, 2015), and U-contaminated field sediment (grey triangles). The black lines represent linear regressions ($p < 0.01$) and the long dashed grey lines represent the 1:1 line with lighter dashed lines representing a factor of 2.5.

correlations between WHAM HA-bound metals and bioaccumulation of metals under different water chemistries for bryophytes (Tipping et al., 2008), aquatic plants (Antunes et al., 2012), oligochaetes (He and Van Gestel, 2015), and stream macroinvertebrates (Stockdale et al., 2010).

The use of HA-bound U as a surrogate for bioaccumulation of U follows the general assumptions of WHAM, which are based on the chemistry of the exposure solution and chemical reactions with the solid phase. In reality, the accumulation of metals by organisms can be quite complex as organisms have the ability to regulate many metals by altering metal uptake, excretion, and/or storage mechanisms to minimize adverse effects (Goulet et al., 2011). Incorporation of biodynamic models with WHAM has been previously recommended to account for more mechanistically accurate processes such as uptake and excretion of metals by organisms (Luoma and Rainbow, 2005; Tipping and Lofts, 2013; Veltman et al., 2010). This would be particularly important for essential metals; however, U is not known to be an essential element for aquatic organisms (Goulet et al., 2011). It is evident from the present study (Fig. 5.4b) that water chemistry and sediment binding phases used in WHAM7 are strong modifiers of steady-state accumulation of U by *C. dilutus* larvae. Thus, regardless of the complex processes involved in the bioaccumulation of metals in an organism, the similarity between the observed and WHAM7 modeled bioaccumulation of U suggests that bioaccumulation of U is associated with the interactions of uranyl cations and complexes at external surfaces of chironomids exposed to the surrounding solution (chemistry) and colloids. It may be an over-simplification to conclude that the accumulation of metals follows the quasi-equilibrium chemical reactions of WHAM7, but similar success has been previously observed (He and Van Gestel, 2015; Tipping and Lofts, 2015). Additionally, the assumptions of WHAM7 are not unreasonable as they are the general basis of the BLM, in which competitive binding of metal/cations to an active site (the biotic ligand) is the basis of metal toxicity (Di Toro et al., 2001; Paquin et al., 2002). Therefore, evidence from the present study and other recent publications suggests that WHAM-calculated HA-bound metal is a suitable surrogate for metal binding sites on macroinvertebrates, at least for some metals. This has not previously been demonstrated for U accumulation by *C. dilutus* and provides further support for the use of HA as a surrogate for bioaccumulation of U, and therefore for WHAM7 in quantifying U bioavailability.

5.3.4 Incorporation of bioavailability into uranium SQGs

Uranium bioaccumulation predictions using WHAM7 (Fig. 5.4b) were an improvement over the use of the predictions of bioaccumulation using simple linear regression models or total U concentrations (Fig. 5.1). Various physicochemical characteristics relevant to site conditions, such as DOC, TOC, pH, major solution ions, and particulate Fe oxide content were utilized by WHAM7 to provide insight into the sorption and speciation of U in order to improve the ability to accurately estimate bioaccumulation of U. These key physicochemical characteristics of the sediment and associated water chemistry are commonly measured at field sites under investigation, especially for U-contaminated areas in northern Saskatchewan (i.e., EARMP, 2014). Thus, using a model like WHAM7 has the potential to improve risk assessment for U to aquatic organisms and perhaps improve environmental quality guidelines for U through the incorporation of modifying effects of site-specific water and sediment characteristics in predicting U bioaccumulation and bioavailability.

Adoption of new approaches for deriving environmental quality guidelines are often limited by the availability of databases large enough to establish and validate the proposed guidelines. In some instances, the BLM (i.e., for Cu) or similar tools have been adopted by regulatory agencies for use in risk assessment and/or for establishing environmental quality guidelines (Ankley et al., 1996a; Di Toro et al., 1991; Markich, 2013; Paquin et al., 2002). However, tools for the improvement or use in regulatory frameworks are more likely to be adopted if they can incorporate already established guideline values instead of proposing new guidelines that may require extensive data and validation. For example, the normalization of acid volatile sulphide (AVS) content of sediment have improved predictions of no adverse effects (i.e., no-effect benchmarks) for some divalent cationic metals from contaminated sediments over the traditional use of total contaminant concentrations (Ankley et al., 1996a; US EPA, 2005). Thus, WHAM7 may offer an additional benefit in that it does not necessarily require new data for use and validation, and can instead use historical data that is already available.

The current approach for the assessment of U-contaminated sediments surrounding U mining operations in Canada is the Screening level concentration (SLC) approach. The SLC approach, similar to other sediment quality criteria approaches, entails the comparison of total metal concentration measured in the sediment to an upper and lower guideline value, the lowest effect level (LEL) and the severe effect level (SEL). Specific to the SLC, a site at which the total U concentration in the sediment is below the lowest effect level (LEL; 104 mg U/kg d.w.) is not expected to have an adverse impact on the benthic community, whereas sites with total U above the severe effect level (SEL; 5874 mg U/kg d.w.) indicate that an adverse effect of U to the benthic community is expected (i.e., reduction in abundance and species richness ≥ 40 %; Thompson et al., 2005). The substantial 56-fold difference between the lower and upper guideline values of the U-SLC is often where predictions of adverse effects on benthic communities, or lack thereof, become difficult. Thus, U-SQGs would benefit from the use of a practical model that estimates U bioavailability and modifying factors, instead of relying on total U concentrations. It has been demonstrated in the present study that WHAM7 can serve that function and was able to account for the >20-fold variation in bioaccumulation that occurred in different sediments with the same total U concentration by accounting for the influence of water chemistry and sediment characteristics on U bioavailability. However, in order to utilize WHAM7 for evaluating the risk of sediment-associated U, a bioavailability-adjusted total U concentration in the sediment is required for the purpose of comparison to established SQG values. Fortunately, WHAM7 produces an output of the "particle-bound metal" concentration that is the predicted total concentration of U in the sediment. Similar to the normalization of bioavailable metal content in sediment based on AVS contents, WHAM7 could be used to calculate a correction factor for the total U concentration in sediment through the use of WHAM-predicted bioaccumulation of U and particle-bound U at a U-contaminated site. The total U concentration in sediment corrected for U bioavailability could then be used for comparison to the established regulatory SQG values for U.

This research demonstrates that it is not only the effects of water chemistry that drives the toxicity and bioavailability of U. Additional modifying factors of U bioavailability include the physicochemical properties of sediment, such as TOC and Fe oxide content. Overall, WHAM7 was able to incorporate and model the modifying effects on U bioavailability and thus may be useful for regulatory purposes. Future research should focus on further quantifying the relationships between U bioavailability and additional combinations of physicochemical properties and conditions, to improve the mechanistic understanding of U bioavailability that will lead to more accurate risk assessment procedures for U and U-SQGs.

CHAPTER 6:

GENERAL DISCUSSION

6 Discussion

6.1 Project rationale and goals

The presence of uranium (U) in the aquatic environment can arise from both natural and anthropogenic sources, including weathering processes, the use of phosphate fertilizers, and military applications, but the largest occurrences of U typically arises from the mining and milling of U-rich ore for nuclear energy production (Bleise et al., 2003; CCME, 2011). As a result, U contamination can be a concern downstream of U mining and milling operations, such as those in northern Saskatchewan, Canada. The tendency of U to accumulate in sediment downstream from mining and milling activity can lead to significant effects on local benthic communities (Dias et al., 2008; Liber et al., 2011). The risk associated with U-contaminated sediment to benthic communities is often evaluated by comparing the total concentration of U in the sediment to established sediment quality guideline (SQG) values for U, where such are available (Liber et al., 2011; Markich, 2002; Thompson et al., 2005). However, the specific U guidelines in U mining and milling regions of Canada that exist, Screening Level Concentration (SLC) approach, have been reported to frequently predict a greater likelihood of adverse effects to the benthic community at reference and no-effect sites (false positives) (Burnett-Seidel and Liber, 2012). Thus, SQGs, such as the SLC approach, have been criticized for not considering the bioavailability of metals, which can often differ from total metal concentrations in the sediment due to the modifying factors of sediment and water chemistry.

Information in the published literature about the effects and bioavailability of U-contaminated sediments to freshwater organisms is sparse, particularly for benthic invertebrates that would likely have the greatest exposure. Thus, the rationale for the present research was to increase knowledge about the key sediment properties that serve as modifying factors of U bioavailability to benthic invertebrates. Such knowledge would improve the risk assessment of

U-contaminated sediments and support the incorporation of bioavailability into future U-SQGs. For this purpose, a number of different types of formulated and field-collected sediments were spiked with U to examine the differential bioaccumulation of U in exposed *C. dilutus* larvae. Bioaccumulation is an important measurement in the assessment of the risk of U to organisms living in or on the sediment, as well as for their predators, as it quantifies the amount of U taken up in the body which ultimately can influence toxicity (Alves et al., 2008; Borgmann et al., 2001b; Timmermans et al., 1992). The quantification of U bioaccumulation also provided a direct indication of the bioavailability of U and a method for assessing the associated modifying effects of sediments with different physical, chemical, and mineralogical properties.

In addition to, and to aid in the interpretation of the U bioaccumulation data, the sorptive capacities of different field-collected sediments were quantified under a wide range of field-representative conditions. Sorption and bioaccumulation data were subsequently used to evaluate and assess the utility of the Windermere Humic Aqueous Model (WHAM, version 7.0.4; (Lofts and Tipping, 2011; Tipping, 1994) for predicting U bioavailability in sediment. The applicability of simple regression models based on relationships between bioaccumulation and physicochemical characteristics of sediment were also used evaluated for their applicability in predicting bioaccumulation of U in benthic invertebrates. To date, models such as WHAM7 have had limited application to sediment-associated U, and/or have only been used to examine relatively small datasets with little variation in the physicochemical properties of sediment. Thus, the performance of each model were compared to assess the accuracy of modeled values relative to observed bioaccumulation.

The overall goal of this research was to quantify and model the influence of sediment physicochemical characteristics on the bioavailability of sediment-associated U to a model freshwater benthic invertebrate, *Chironomus dilutus*. Specific research objectives were to:

(1) Quantify the bioaccumulation of U in *C. dilutus* larvae exposed to U-spiked formulated sediments to determine the role of different types and quantities of clay minerals, sand fractions, and organic matter on U bioavailability.

(2) Quantify and model the relationship between bioaccumulation of U in *C. dilutus* larvae exposed to U-spiked field-collected sediments to determine the influence of

physicochemical properties, specifically TOC content, particle size distribution, CEC, and the content of Fe and Mn, on U bioavailability.

(3) Quantify and model the adsorption of U to several field sediments to determine the effects of U concentration, pH, and different physical, chemical and mineralogical properties of the sediment on U sorption and solubility.

(4) Model U sorption, speciation, and bioaccumulation using a computer model (i.e., WHAM) as well as simple regression models, and to compare model predictions to experimental observations to assess the applicability and performance of the models in predicting U bioavailability.

6.2 Project summary

6.2.1 Total versus dissolved concentrations of uranium

A reoccurring conclusion among all of the studies presented in this thesis is that sediments with different physical, chemical and mineralogical properties will exert different influences on the behaviour of U, such that total U concentrations are not an appropriate indicator of the bioavailability of U. More specifically, bioaccumulation tests with both U-spiked formulated sediments (Chapter 2) and field sediments (Chapter 3) clearly demonstrated that total U concentrations were not significantly correlated with U bioaccumulation in *C. dilutus* larvae exposed to different sediments spiked with the same total U concentrations. Similar conclusions have also been made for other metals (Ankley, 1996; Campana et al., 2013; Liber et al., 2011). In contrast, dissolved concentrations of U in the U-spiked sediment tests (in both the overlying water and the pore water collected at the sediment water interface) correlated positively with the uptake of U by *C. dilutus* larvae. Previous studies have suggested that concentrations of different metals in the dissolved phase, particularly in the pore water, also correlate better with metal bioavailability to benthic invertebrates than total metal concentrations in the whole-sediment (Ahlf et al., 2009; Ankley, 1996; Burton, 2010; Liber et al., 2011). The results from this thesis, along with previous published data on different metal-contaminated sediments, demonstrate that total U concentrations in the sediment should not be used to assess the true risk of U-contaminated sediments to benthic communities.

6.2.2 Modifying effects of sediment properties on uranium bioaccumulation

Concentrations of dissolved U in the overlying water and pore water significantly correlated with U bioaccumulation, providing an indication of U bioavailability in specific sediments. Furthermore, significant relationships were observed for both the dissolved U and bioaccumulation of U as a function of different physicochemical properties of the formulated and field sediments (Chapter 2 and 3, respectively). Specific sediment properties observed to influence concentrations of dissolved U and bioaccumulation of U in *C. dilutus* larvae included the type and content of clay minerals and organic matter, individually manipulated in formulated sediment (Chapter 2(Crawford and Liber, 2015)(Crawford and Liber, 2015)(Crawford and Liber, 2015)). Bioaccumulation of U was also observed to decrease as a function of increasing TOC, fine fraction (clay + silt, ≤ 50 μm particle size), CEC and Fe content of U-spiked field sediments (Chapter 3). These result are in agreement with other studies that observed similar reductions in the bioavailability of different metals to various organisms as a result of sediment and soil properties such as organic matter, clay minerals, and reactive Fe, Al and Mn oxides (Criel et al., 2008; Lock and Janssen, 2001; Vandenhove et al., 2007).

The results presented in this thesis suggest that the physicochemical characteristics of sediment modify U bioavailability through the sorption and partitioning of U between the aqueous and solid phases. Sediments with low binding capacity (i.e., sandier sediments with low TOC and Fe content) typically resulted in greater partitioning of U to the aqueous phases, and subsequently greater aqueous concentrations of U, than sediments with high binding capacity (i.e., organic- and mineral-rich sediments with high CEC). Similar influences of sediment binding properties on different metals have been observed by others, where metals adsorbed more readily to OC-rich sediments, resulting in lower concentrations of metals in the dissolved phase, lower bioaccumulation, and lower toxicity to benthic invertebrates (Besser et al., 2003; Campana et al., 2013; Liber et al., 2011). The modifying effects of different sediment binding phases on the partitioning of U are in agreement with the principles of metal partitioning described by the equilibrium partitioning theory (EqP; Ankley et al., 1996b; Di Toro et al., 1991). Overall, U bioaccumulation significantly differed among our studies as a function of sediment properties, with increasing contents of TOC, fine fraction and Fe in sediment resulting in reduced bioaccumulation of U in *C. dilutus* larvae. The content of fine fraction provided the most practical and reliable regression equations for modeling U bioaccumulation (Eq. 3.3 and

3.4). Thus, it is recommended that the modifying effects of sediment properties be carefully considered when assessing the risk of U-contaminated sediments to benthic invertebrates.

6.2.3 Influence of sediment properties on uranium sorption

Additional insight into the U bioaccumulation data obtained in chapters 2 and 3 was gained through the quantification of sediment-solution partition coefficients (K_d) for U, which varied over two orders of magnitude as a function of field sediment composition (Chapter 4). Sorption of U, as suggested by the EqP theory, was directly related to the binding properties of sediment, thus influencing the bioavailability of U. The K_d-U values calculated in Chapter 4 generally corresponded well with the observed bioaccumulation, such that linear relationships were observed with increasing contents of TOC, fine fraction and Fe content (Chapter 4). However, this trend was only observed up until a threshold was reached and/or until a negative relationship was observed for sediments with greater than 12% TOC, 37% fine fraction, 33 meq/100 g of CEC, or 29g/kg of Fe content. Adsorption of U to sediment was expected to continually increase (i.e., greater K_d-U values) as the quantity of key binding phases of sediments increased, mirroring the decreases in dissolved U and bioaccumulation observed for similar decreases in the sediment binding properties of our bioaccumulation studies. The observed thresholds may be a result of the concentrations of DOC, colloids, and other ligands associated with high binding sediments, which likely increased the solubility of U, resulting in decreased K_d-U values, but would not necessarily increase the availability of the dissolved U-complexes. This assumption was supported by the similar trends of U sorption, free ion activity, and U complexation modeled by WHAM7 for the full range of sediment properties examined in our sorption tests (Chapter 4). Thus, sorption of U, which influences U bioavailability, was significantly modified by sediment properties and was successfully predicted through the use of WHAM7.

In addition to the influence of sediment properties on U sorption and bioavailability, the effects of pH and different U concentrations were examined (Chapter 4). Sorption of U was not significantly affected by the different U concentrations examined (0.023 to 2.3 mg U/L), but was observed to be strongly pH-dependent across the 6 to 8 pH range of our sorption experiments. As well, there was an interactive effect between pH and sediment type, which resulted in an 80-fold change in K_d-U values at pH 8, but only a 14-fold change at pH 6 and 7 for the same sediments.

A study by Sheppard (2011) reported a similar interactive effect between pH and clay content of soils for K_d-U values. Maximum adsorption of U was observed in our study, as well as in previous studies (Barnett et al., 2000; Cheng et al., 2004; Davis et al., 2004), at circumneutral pH values, which may explain the lower variation in K_d-U values among the different sediment types for the pH 6 to 7 treatments. In contrast, the low K_d-U values observed for the pH 8 treatments may result in a greater influence of sediment composition on U sorption and/or the influence of the uranyl-carbonate complexes that predominate at pH 8 (Catalano and Brown Jr, 2005; Peck et al., 2002; Zheng et al., 2003), explaining the 80-fold change observed in the K_d-U values. Overall, the sorption results from Chapter 4 provided further support and quantification of the strong influence of pH and physicochemical properties of sediment on the U sorption to and bioavailability from U-contaminated sediments.

6.2.4 Modeling U bioavailability

Results from this thesis quantified the effects of physicochemical properties of sediment on the sorption and bioavailability of U. Simple regression models using individual (Eq. 3.3 and 3.4) or a limited number of sediment properties (Eq. 3.1 and 3.2) could be used to predict U bioaccumulation fairly well, within the range of sediment properties examined. However, the use of regression models that incorporate multiple parameters can be complex and they cannot be accurately used when sediment conditions fall outside the ranges of the parameters used to develop the models. Thus, a model, such as WHAM7, that incorporates a much wider range of variables that can modify U bioavailability was evaluated in Chapter 5 to determine if it could accurately predict U bioavailability. Successful predictions of U sorption by WHAM7 had already been observed in Chapter 4, so WHAM7 was evaluated further to investigate its ability to predict the fraction of U associated with the particulate, colloidal and aqueous phases in complex sediments. As shown by the sorption experiments, U was generally associated with the sediment phase, with both TOC and Fe oxide content providing major phases for U binding. Aqueous complexes of U tended to correlate with the binding capacity of sediment and with aqueous pH, such that sediments with low TOC and Fe content in alkaline solutions generally had greater predominance of aqueous complexes. The fraction of U predicted to bind to DOC was not significant, which is in agreement with the binding contribution of DOC for other metals such as Cu, Pb, Cd, Zn and Ni (Weng et al., 2002). However, the study by Weng et al. (2002), concluded that even a small amount of metal complexation with DOC resulted in an increase in the concentration of dissolved metals by more than two orders of magnitude, with greater occurrence of metal-DOC complexation for lower free metal activity and higher pH conditions. Thus, complexes with DOC associated with higher binding sediments (i.e., TOC content) can lead to enhanced "solubility" and mobility of metals, which are presumably not bioavailable based on the observations from this research.

In addition to the influence of sorption, speciation of U in the aqueous phase(s) is a key factor in the assessment of U bioavailability, as the presence of certain U species can directly influence the ability of organisms to accumulate U (Fortin et al., 2004). The type and abundance of U species for different sediments and test conditions were modeled in WHAM7, incorporating both sediment and solution parameters. The concentration of UO_2^{2+}, which is assumed to be the most bioavailable form of U, and thus most toxic, was predicted to be insignificant in abundance. Instead, the major species of U modeled in WHAM7 were shown to be strongly dependent on a number of factors such as pH and the presence of ligands, particularly carbonates. The observed predominance of uranyl-carbonate complexes are consistent with previous literature that suggests carbonates are significant ligands responsible for controlling U speciation (Catalano and Brown Jr, 2005; Peck et al., 2002; Zheng et al., 2003). Speciation and solubility of U were also influenced by complexation and sorption processes, which are dependent on sediment properties such as clay minerals, oxides and organic matter (OM). Thus, U was most soluble and bioavailable in the present studies under conditions of alkaline pH and sediments with low OM, Fe, and fine fraction content. Focusing on conditions under which U sorption and speciation result in the greatest bioavailability of U may help reduce the uncertainty in risk assessment of U-contaminated sediments.

The last step in evaluating the use of WHAM7 for predicting U bioavailability was to determine if bioaccumulation of U could be accurately modeled for a wide range of sediment and water characteristics that modify bioavailability. WHAM7 successfully modeled accumulation of U in *C. dilutus* larvae (via optimization with HA-bound U) across a wide range of sediments and test conditions (e.g., pH, particulate TOC and Fe, basic water chemistry). This was somewhat unexpected since bioaccumulation of metals is controlled by more than just chemical reactions because organisms can directly influence the exposure, uptake, storage and excretion of metals via species-specific strategies (Goulet et al., 2011; Luoma and Rainbow, 2005; Simpson and Batley, 2007). However, similar success in predicting metal bioaccumulation in various organisms was observed in previous studies using WHAM-modeled HA-bound metals (Antunes et al., 2012; He and Van Gestel, 2015; Stockdale et al., 2010; Tipping and Lofts, 2015). The present results support the use of WHAM HA-bound U as a suitable surrogate for predicting bioaccumulation of U in chironomids. Overall, WHAM7 was able to incorporate a wide range of sediment and water characteristics to successfully predict sorption, speciation and bioaccumulation of U, suggesting that WHAM7 may be a useful tool for the assessments of U bioavailability in aquatic ecosystems and the risk of U-contaminated sediments to benthic invertebrates.

6.3 Significance to uranium risk assessment and SQGs

The research presented in this thesis provided quantitative cause-effect relationships for the bioaccumulation of U by *C. dilutus* larvae exposed to different U-spiked sediments. Bioaccumulation of U varied significantly (approx. > 20-fold) as a function of sediment properties for sediments spiked with the same total U concentrations. As a result of the variability in sediment properties and their associated binding affinities, it is difficult to develop and apply SQGs based solely on total U concentrations in the sediment. False positives for toxicity can arise if SQGs, based on total concentrations in whole-sediment, are erroneously applied to U in high binding sediments. The results from this thesis emphasize the need for accounting for the bioavailability associated with different sediment properties, such as particle size and Fe content. Quantification of a wide range of sediment properties and conditions that influence U partitioning between sediment and water, and associated U speciation, are required to improve U-SQGs.

The application of WHAM7 in modeling U sorption, speciation and bioaccumulation demonstrates the usefulness and utility of potentially applying such models in the assessment of U-contaminated sediment sites. Routinely measured site characterization parameters, including water chemistry and sediment physicochemical characteristics are incorporated into WHAM7. Thus, data from previous site assessments may be entered into WHAM7 to further validate the use and prediction of U bioavailability under varying conditions and for different organisms. Additional research is required before the practical use of WHAM7 in SQG derivation and risk assessment of U-contaminated sediments can be fully assessed. Future studies should investigate lethal body burdens in *C. dilutus* larvae, and other benthic invertebrates, in order to gain a better understanding of the adverse effects and subsequent “bioavailability-corrected” SQG limits required for the protection of aquatic life.

6.4 Future research considerations

The research presented in this thesis helps quantify and demonstrate the need for the incorporation of sediment physicochemical properties as modifiers of U bioavailability into U risk assessment and in the development of appropriate U-SQGs. However, several recommendations for future work include, but are not limited to, the topics discussed below that may help improve our understanding and use of U bioavailability in risk assessments.

6.4.1 Evaluation of dissolved uranium

Alternative methods for evaluating bioavailable concentrations of U, in comparison to the methods used in the present work, could be investigated through the use of traditional extraction methodologies or alternative passive sampling techniques for different U-contaminated sediments. The mini-peepers used in the present studies sampled dissolved U concentrations on a diffusion gradient basis, allowing the sample chambers to passively equilibrate with the surrounding solution as metal solutes pass through the pores in the membrane (Doig and Liber, 2000). The limitation to mini-peepers, however, is that they rely on the filtration of water to obtain the dissolved phase, operationally defined as solutes that are < 0.45 µm in size. As observed in the present work and in other literature, the operationally defined “dissolved” metals can lead to the overestimation of labile metals, as the membrane filters do not distinguish between soluble metals and those associated with dissolved complexes and colloidal phases (Crawford et al., 2016; Zhang and Davison, 2000). An alternative method for sampling dissolved metal species include diffuse-gradients in thin-film (DGTs) devices, which are passive samplers composed of a porous hydrogel covered by a membrane filter with a Chelex resin (Costello et al., 2012). The advantage of DGTs are that they measure the mean flux and labile species of metals in sediments and soils regardless of the binding ligand, metal sulfide, or Fe oxide sorbed metals. These soluble species of metals are generally more representative of the bioavailable concentration of metals than the operationally defined dissolved metal concentrations (Zhang et al., 2014). The results from DGT deployment also automatically account for sediment and soil properties, including pH (Zhang et al., 2014). Thus, the use of DGTs has been proposed and successfully used as a surrogate for a sentinel organism in assessing metal bioavailability in sediments and soils (Amato et al., 2014; Costello et al., 2012; Zhang et al., 2014). However, the use of DGTs for metals are fairly recent, can be complex, and only a limited number of studies have compared DGT-metals to measured bioaccumulation in benthic organisms (Costello et al., 2012). The use of DGTs should be compared with measured bioaccumulation of U in benthic invertebrates to assess DGT use in predicting U bioavailability, and perhaps in validating modeled predictions of U bioaccumulation by WHAM7. Additional research into DGTs would be needed before they can be used as an alternative method to mini-peepers in sampling dissolved metal concentrations for the assessment of U-contaminated sediment for regulatory purposes. Regardless, the use of DGTs for sampling dissolved U concentrations may offer further insight in predicting and modeling U bioavailability.

6.4.2 Additional modifying properties of U bioavailability

Although a wide range of physicochemical properties of sediment were examined in this research, it would be useful to examine additional site and test conditions outside those relevant to northern Saskatchewan. For example, $CaCO_3$ content of sediment, which can significantly influence U behaviour (Echevarria et al., 2001; Pandit et al., 2012), was typically below detection in the sediments from northern Saskatchewan used here. Some environments with sediment associated U-contamination may also have pH conditions outside the range examined here (i.e., pH 6 to 8). As demonstrated in this work and other literature (Fortin et al., 2004; Franklin et al., 2000; Vandenhove et al., 2007), pH is a significant modifier of U bioavailability. Therefore, additional investigations could be conducted on the influence of pH as a modifier of U bioavailability, as sediment bioaccumulation experiments from the present research were only conducted at the pH of the overlying water used (i.e., 7.6 to 8.2). The influence of pH in the bioaccumulation tests was not investigated in the current research due to the scope of the project and the logistical challenge of amending the pH for the large volumes of water used by the STIR system (i.e., minimum 1800 L of water require per 10-d test). Nevertheless, it would be beneficial to evaluate the applicability of WHAM7 and the modifying effects of sediment parameters and pH in predicting U bioavailability for different geographic areas that have similar geological background concentrations of U (i.e., Kazakhstan and Australia). Additional research conducted on field sediments from downstream of U mining and milling operations may also help elucidate the effects of co-occurring contaminants and/or different concentrations of U that may alter predictions of U bioavailability differently than modeled in the present work. For instance the range of total U concentrations examined in this research only ranged from 5 to 500 mg U/kg d.w., but the investigation of U concentrations closer to the upper effect level of U-SQGs (i.e., SEL of 5874 mg U/kg d.w. for the SLC approach) may provide additional insight into the speciation, sorption and bioaccumulation of U, which ultimately influence U bioavailability.

6.4.3 Bioavailability of U to different organisms

The ability to accurately assess the risk of U-contaminated sediment would be improved with investigations using different organisms to evaluate the modifying effects of sediment physicochemical properties on U bioavailability. Little work has been conducted with benthic invertebrates exposed to U-contaminated sediment, and although the results from this research suggest that WHAM7 can accurately model the accumulation of U in *C. dilutus* larvae, validation for alternative organisms are needed. Chironomids live in and on the sediment surface, building tubes, burrowing in and feeding on the sediment, which significantly influences the particular exposure chironomids receive, along with the influence chironomids have on their surrounding environment (i.e., bioturbation activity; Dias et al., 2008; Lagauzère et al., 2009a and b; Thomas and Liber, 2001). For example, chironomids are responsible for creating oxic microenvironments, via irrigation of their tubes within even anoxic sediments, which may significantly alter their exposure to metals compared to other organisms (Campana et al., 2013; Lagauzère et al., 2009a; Lagauzère et al., 2009b). Additional species representative of different habitat preferences, feeding and burrowing behaviours, and geographic locations (e.g., tropical *Chironomus* spp.) should also be investigated with regard to their predicted bioaccumulation of U from freshwater sediments. Quantification of the bioaccumulation of U in chironomids and other benthic invertebrates are not only important for demonstrating the modifying effects of sediment properties on U bioavailability, but may indicate the potential impact of U accumulation in benthic organisms on upper trophic levels. For example, freshwater predatory invertebrates, such as some caddisfly larvae, have been reported to bioaccumulate Cd via ingestion of contaminated *C. riparius* larvae (Timmermans et al., 1992). Organisms can also develop adaptation and the ability to tolerate greater concentrations of contaminants, which can obscure the relationship between exposure and biological effects (CCME, 2011; Smolders et al., 2009). Adaptation of local benthic communities to U was not investigated in this research, but can be an important consideration for site-specific guidelines. Therefore, it may be prudent to quantify bioaccumulation differences between laboratory and field populations in a more extensive and controlled manner.

6.4.4 Uranium exposure routes for benthic organisms

To better elucidate the relative significance of U exposure routes in U bioaccumulation, it would be beneficial to expose both benthic and epibenthic species to similar U-contaminated sediments and determine the importance of sediment physicochemical parameters on U bioaccumulation. A common assumption for many benthic organisms is that exposure primarily occurs via pore water, but that has not always been observed (Ahlf et al., 2009; Kraaij et al., 2002; Lagauzère et al., 2014). In some instances, the ingestion of sediment particles by bivalves and other deposit feeders is considered the main route of exposure to sediment-associated contaminants (Simpson and King, 2005). Dietary exposure should also be examined in future research, as OM was observed in our research to preferentially adsorb U within the sediment phase and may be important as many chironomids are deposit feeders that feed on OM and its associated microbial community (Faria et al., 2007). The dietary exposure of U associated with the OM sources (peat moss or α-cellulose) discussed in Chapter 2 was likely negligible as the reconstituted OM components offer low nutritional value compared to the Nutrafin food slurry provided (Lacey et al., 1999). The insignificant difference in biomass between organisms from OM-amended and sand treatments corroborates the assumption that the chironomids did not feed on the extra food provided by the addition of OM. Similar biomass was also observed in the organisms exposed to the field-collected sediments, with reduced bioaccumulation of U in *C. dilutus* larvae from OM-rich sediments, further suggesting the adsorption of U to OM sources does not act as a significant dietary route of uptake. Alternatively, the lower bioaccumulation of U associated with exposure to OM-rich sediments appears to be the result of greater sorption to the sediment and thus less U in the dissolved phase, which also decrease the likelihood of adsorption of U to the supplemented food source. A previous 48-h water-only U tests had observed no difference in bioaccumulation of U between fed and non-fed *C. dilutus* larvae, suggesting adsorption of U to the Nutrafin food source was not a significant route of bioavailable dietary exposure. However, further investigation would be required to determine the partition coefficient of U to the Nutrafin food source (compared to other OM sources), the equilibration time of U sorption, and the amount of the food chironomids are consuming daily throughout the 10-d test to better quantify the importance of the dietary route of exposure to chironomids.

6.5 Conclusions

The results from the research presented in this thesis clearly demonstrate that physicochemical properties of sediment can significantly modify U availability to *C. dilutus* larvae under conditions typical of aquatic environments near northern Saskatchewan U mines. The use of a geochemical speciation model, WHAM7, was shown to be a promising method for the prediction of U bioavailability through the incorporation of different sediment-aqueous interactions that modify U sorption, speciation and bioaccumulation. Overall, the conclusions from this work strongly suggest that WHAM7, and possibly other models of U bioavailability, should explicitly consider physicochemical properties of sediment, such as TOC, fine fraction and Fe content as phases that can significantly reduce bioaccumulation, depending on the pH of the system. These results are not only relevant to environments receiving U in northern Saskatchewan, but also to other areas around the world with U mining and milling operations. The quantification and incorporation of bioavailability in assessments of U-contaminated sediment will ideally lead to regulations that are scientifically-based, cost-effective and environmentally relevant.

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APPENDIX [a]

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[a] Supplement material and information for this thesis are provided in this Appendix (5 tables and 3 figures in total). Data include the regressions between different exposure routes and the associated bioaccumulation in *C. dilutus* larvae from both the percent kaolin clay and percent peat experiments (Chapter 2). For Chapter 4, the sediment physicochemical properties and water chemistry for each sorption test, and the subsequent WHAM7 input parameters are provided. A table of solubility and binding constants for U(VI) species is also provided. Additionally, the linear regression equations between sorption of U and sediment properties are also presented, as well as the sorption data as a function of U concentration (Chapter 4). Water chemistry data for the U-contaminated field sediment are provided for Chapter 5.

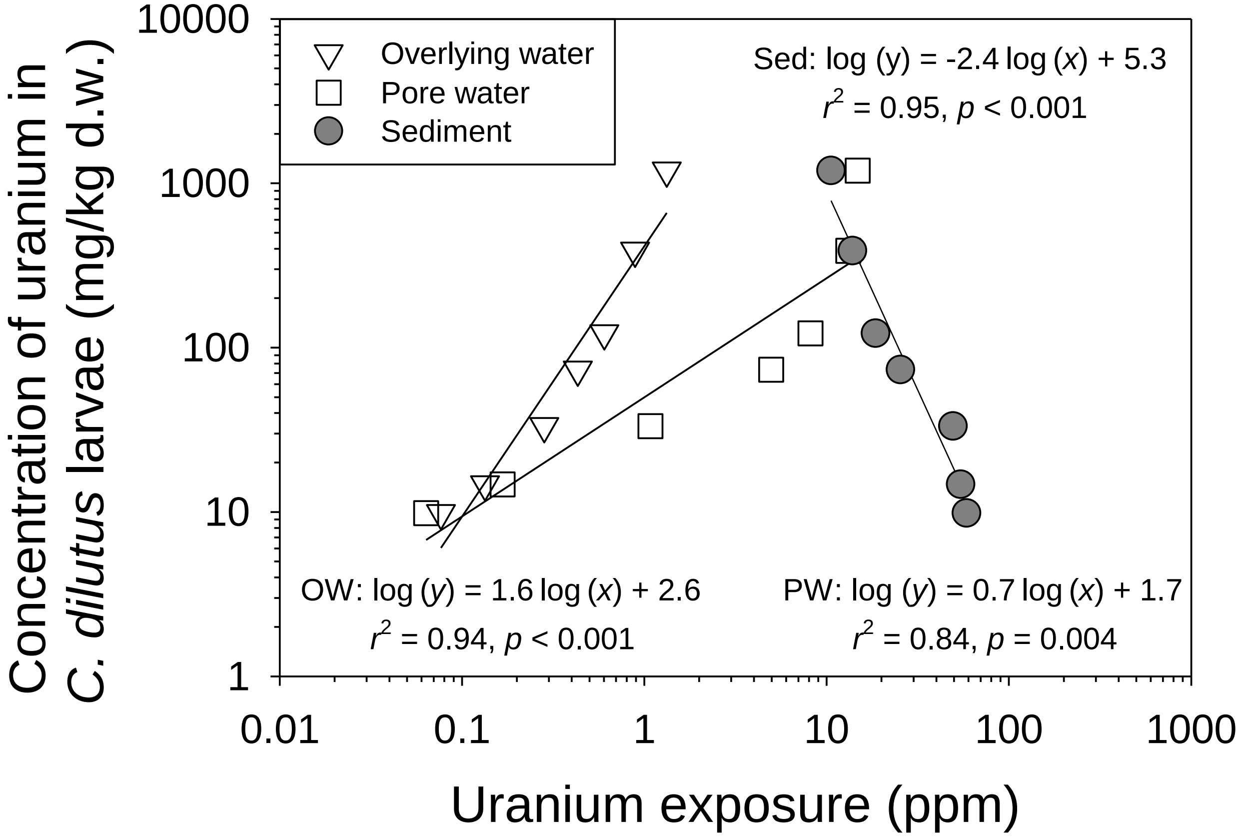

Fig. A2.1 Mean concentration of uranium in the whole organism (*C. dilutus* larvae; mg/kg d.w.) relative to U exposure from overlying water (OW; mg/L), pore water (PW; mg/L), and whole sediment (Sed; mg/kg d.w.) in the percent kaolin clay experiment. Simple linear regression lines are shown for the significant matrixes.

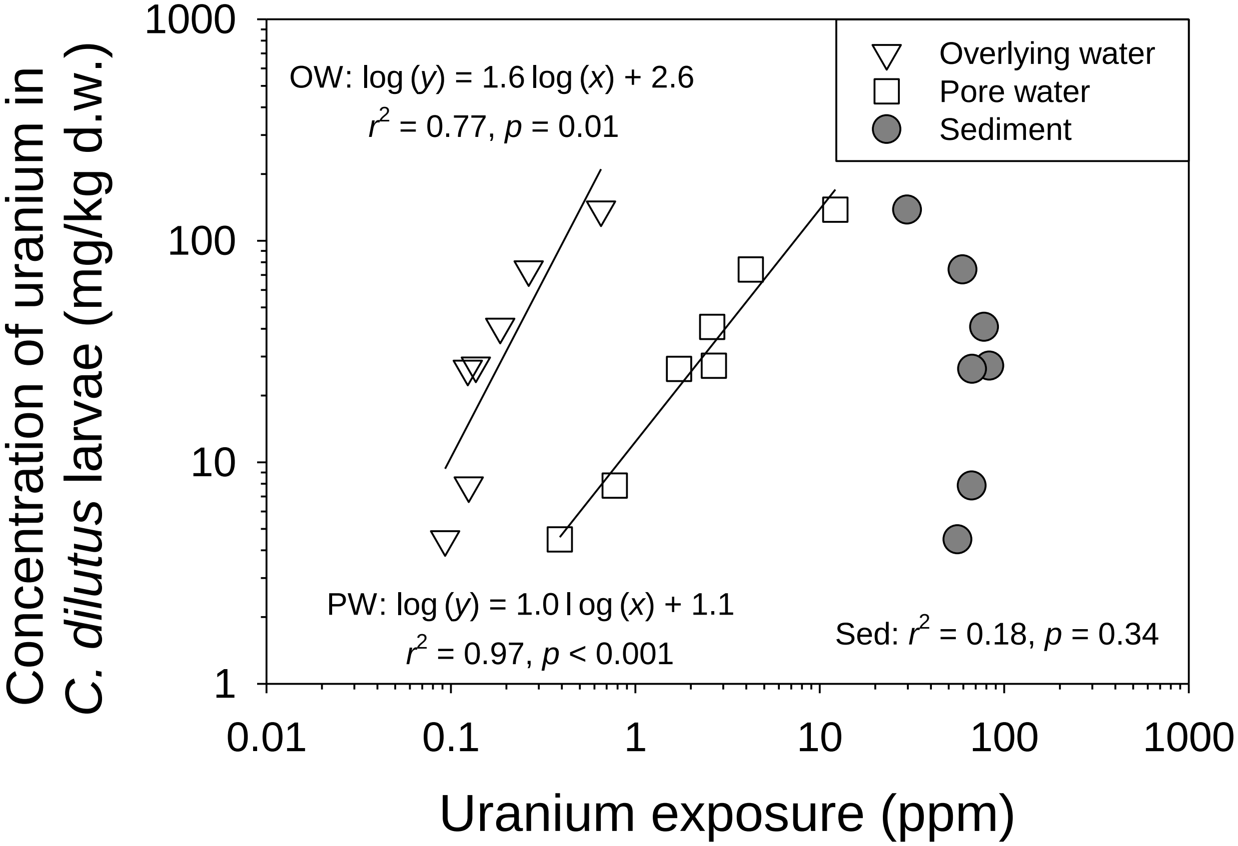

Fig. A2.2 Mean concentration of uranium in the whole organism (*C. dilutus* larvae; mg/kg d.w.) relative to U exposure from overlying water (OW; mg/L), pore water (PW; mg/L), and whole sediment (Sed; mg/kg d.w.) in the percent peat experiment. Simple linear regression lines are shown for the significant matrixes.

Table A4.1. Summary of sediment physicochemical characteristics, background uranium concentrations, and pH of field collected reference sediments. Modified from Crawford and Liber (2016).

Sediment ID[a]	**Sediment type**	**TOC (%)[b]**	**Fe (g/kg)[c]**	**Fine fraction (% ≤ 50 μm)[d]**	**CEC (meq/100 g)[e]**	**Water content (%)**	**Background U (mg/kg d.w.)[f]**	**pH (-log[H^+])**
UR8	Sand	1.0	10	5	4	34	1.1	7.2
SL2	Sand	1.1	2	1	5	80	0.8	6.7
UR7	Sand/Loamy sand	2.9	15	14	9	48	1.7	6.7
UR2	Silt loam	3.8	9	71	12	50	2.3	6.8
WB	Loamy sand	5.5	13	22	25	66	2.7	6.8
ML	Sandy loam	12.4	29	37	33	86	1.6	6.4
KL	Silty clay loam	15.9	34	99	64	92	1.6	6.5
SL1	Loam	19.5	17	58	61	98	3.0	5.5
HL	Silt loam	22.1	8	75	48	95	1.2	5.7

TOC – total organic carbon. CEC – cation exchange capacity.

[a] Field sediments collected from the Wollaston Lake area in northern Saskatchewan, Canada: SL – Shallow Lake; HL = Henday Lake; KL = Konner Lake; ML = Mallen Lake; UR = Umperville River; WB = Wollaston Bay.

[b] Determined by LECO Carbonator Model C632, Department of Soil Science, University of Saskatchewan, Saskatoon, SK.

[c] Determined by dithionite-citrate-bicarbonate (DCB) extraction method for total Fe/Mn (Pansu and Gautheyrou, 2007; Ryan and Gschwend, 1991).

[d] Determined by mini-pipette method with removal of organic matter and carbonates, ALS Environmental, Saskatoon, SK.

[e] Determined by ammonium acetate extraction, ALS Environmental, Saskatoon, SK.

[f] Determined by ICP-MS after complete sediment digestion, Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Table A4.2. Measured overlying water quality variables for each sorption experiment and input variables for WHAM7.[a]

Treatment		Conductivity (μS/cm)[a]	pH (-log$[H^+]$)[a]	Hardness (mg/L)[a,b]	Alkalinity (mg/L)[a,b]	Major ion (mg/L)[c]							DOC (mg/L)[d]
Sediment	pH					SO_4^{2-}	Cl^-	NO_3^-	K^+	Na^+	Ca^{2+}	Mg^{2+}	
Test solution		1055 (4)	5.6 (0.1)	230 (1)	8 (1)	223	–	303	–	113	94	n.a.	–
Control	6	1134 (37)	6.3 (0.06)	223 (7)	35 (6)	221	–	299	–	118	94	–	2.4 (0.3)
Control	7	1120 (18)	7.1 (0.06)	220 (3)	34 (5)	224	–	303	–	126	95	–	1.7 (0.5)
Control	8	1301 (39)	8.0 (0.1)	194 (4)	36 (3)	230	–	310	–	143	98	–	2.1 (0.2)
UR8	6	1205 (12)	6.4 (0.1)	235 (0)	35 (10)	200	42	270	32	120	120	0.31	6.0 (0.3)
	7	1074 (6)	7.1 (0.01)	216 (8)	25 (3)	209	n.a.	275	n.a.	123	117	n.a.	5.7 (0.1)
	8	1134 (10)	8.0 (0.1)	195 (4)	42 (3)	209	n.a.	269	n.a.	155	110	0.25	6.8 (1.6)
SL2	6	1100 (1)	6.3 (0.01)	218 (13)	30 (5)	208	15	266	11	122	114	0.37	6.0 (0.2)
	7	1190 (36)	7.1 (0.02)	209 (5)	19 (3)	206	20	266	19	126	111	0.36	7.3 (0.2)
	8	1286 (85)	8.0 (0.04)	201 (6)	40 (4)	207	20	265	23	150	111	0.05	8.4 (0.9)
UR7	6	1160 (1)	6.3 (0.08)	210 (5)	33 (3)	206	18	261	20	128	106	0.42	10.3 (1.1)
	7	1108 (5)	7.0 (0.1)	197 (6)	24 (3)	213	n.a.	271	n.a.	143	101	0.57	14.9 (0.2)
	8	1187 (7)	7.9 (0.05)	167 (4)	52 (2)	213	6	262	n.a.	184	90	0.47	18.2 (2.3)
UR2	6	1146 (6)	6.3 (0.07)	153 (18)	33 (8)	209	18	262	20	134	100	0.45	13.9 (0.4)
	7	1119 (6)	7.0 (0.02)	167 (3)	25 (3)	200	9	249	n.a.	146	84	0.40	17.0 (0.3)
	8	1216 (7)	7.9 (0.04)	136 (3)	69 (2)	210	n.a.	248	n.a.	196	77	0.37	22.8 (0.1)
WB	6	1171 (1)	6.3 (0.1)	163 (2)	30 (0)	209	18	260	21	139	87	0.66	9.2 (0.7)
	7	1268 (46)	7.1 (0.03)	159 (8)	21 (2)	219	48	268	50	159	81	0.56	14.0 (0.9)
	8	1257 (28)	8.1 (0.08)	117 (6)	42 (2)	212	10	257	7	200	63	0.48	20.6 (0.2)

Treatment		**Conductivity (μS/cm)**[a]	**pH (-log[H^+])**[a]	**Hardness (mg/L)**[a,b]	**Alkalinity (mg/L)**[a,b]	**Major ion (mg/L)**[c]							**DOC (mg/L)**[d]
Sediment	**pH**					**SO_4^{2-}**	**Cl^-**	**NO_3^-**	**K^+**	**Na^+**	**Ca^{2+}**	**Mg^{2+}**	
ML	6	1186 (2)	6.2 (0.1)	145 (5)	30 (5)	220	21	267	26	160	79	1.10	7.9 (0.5)
	7	1154 (4)	7.0 (0.02)	96 (3)	31 (3)	231	4	257	n.a.	198	50	0.74	11.6 (0.7)
	8	1273 (10)	8.0 (0.04)	25 (3)	78 (5)	230	5	239	n.a.	275	24	0.36	18.0 (1.0)
KL	6	1190 (1)	6.3 (0.1)	125 (5)	35 (0)	215	20	249	20	161	65	0.98	26.6 (0.5)
	7	1185 (3)	7.1 (0.02)	69 (4)	57 (3)	213	n.a.	163	n.a.	194	33	0.51	38.0 (0.2)
	8	1361 (9)	8.0 (0.03)	24 (3)	144 (4)	217	13	106	n.a.	291	22	0.30	48.7 (1.8)
SL1	6	1174 (1)	6.3 (0.1)	175 (35)	35 (0)	221	15	259	14	160	71	1.70	40.6 (0.4)
	7	1266 (34)	7.2 (0.09)	105 (7)	38 (5)	230	30	238	26	198	42	1.10	53.2 (0.2)
	8	1412 (26)	8.0 (0.07)	53 (15)	97 (3)	232	19	222	16	285	26	0.62	67.0 (0.4)
HL	6	1202 (6)	6.2 (0.01)	160 (10)	30 (0)	219	31	236	25	133	87	2.45	30.6 (0.4)
	7	1128 (5)	7.4 (0.05)	124 (7)	106 (5)	208	4	113	n.a.	159	56	1.69	41.8 (0.9)
	8	1310 (7)	7.9 (0.03)	118 (19)	162 (23)	220	n.a.	82	n.a.	244	50	1.59	48.3 (2.8)

DOC = dissolved organic carbon. (–) = not measured. n.a. = not available; no peak detected, thus below detection limit (LoD) of 0.91, 19.6, 5.8, 4.0, 3.6, 9.8, and 3.7 μg/L for the seven major ions, respectively.

[a] Data represent overall mean values and standard error between time 0 h and 48 h (SE, *n* = 6). Conductivity measured with ORION ATI conductivity meter (model 170; Thermo Scientific); pH measured with ORION PerpHecT LogR meter (model 370; Thermo Scientific); hardness and alkalinity measured with Hach 16900 Digital Titrator (Hach).

[b] Measured as $CaCO_3$.

[c] Major ion concentration measured at time 48 h (*n* = 1) by Ion Chromatography (Dionex ICS-3000 dual Ion Chromatography System). No detectable PO_4^{3-} in system (< LoD; 52.6 μg/L).

[d] Data represent mean values and standard deviation (SD, *n* = 3) at time 48 h by total organic carbon analyzer (TOC-V CPN model 5000; Shimanzu).

Table A4.3. Thermodynamic data for solution and solid complexes of U(VI) simulated by WHAM7.

Complex	**log K, 25 °C**	**ΔH° (kJ/mol)**	**Reference**
Solution [a]			
UO_2OH^+	-5.2	(-2.6)	Read and Broyd (1991)
$UO_2(OH)_2^0$	-11.9	–	Read and Broyd (1991)
$UO_2(OH)_3^-$	-21	–	Read and Broyd (1991)
$UO_2(OH)_4^{2-}$	-32.4	(-16)	Duro et al. (2006)
$(UO_2)_2(OH)_2^{2+}$	-5.6	(-17.1)	Read and Broyd (1991)
$(UO_2)_3(OH)_5^+$	-15.7	(-42.7)	Read and Broyd (1991)
$UO_2CO_3^0$	9.4	(1.1)	Read and Broyd (1991)
$UO_2(CO_3)_2^{2-}$	16.4	(3.5)	Read and Broyd (1991)
$UO_2(CO_3)_3^{4-}$	21.6	(-9.3)	Hummel et al. (2002)
$Ca_2UO_2(CO_3)_3^0$	29.82	–	Dong and Brooks (2007)[b]
$CaUO_2(CO_3)_3^{2-}$	26.37	–	Dong and Brooks (2007)[b]
$MgUO_2(CO_3)_3^{2-}$	25.3	–	Dong and Brooks (2007)[b]
$(UO_2)_2CO_3(OH)_3^-$	-0.84	–	Guillaumont et al. (2003)
$(UO_2)_3CO_3(OH)_3^+$	0.6	–	Guillaumont et al. (2003)
$(UO_2)_{11}(CO_3)_6(OH)_{12}^{2-}$	-47.5	–	Guillaumont et al. (2003)
$UO_2SO_4^0$	3	–	Read and Broyd (1991)
$UO_2(SO_4)_2^{2-}$	4.14	–	Guillaumont et al. (2003)
$UO_2(SO_4)_3^{4-}$	3.02	–	Guillaumont et al. (2003)
UO_2Cl^+	0.17	(1.9)	Hummel et al. (2002)
$UO_2Cl_2^0$	-1.1	(3.6)	Hummel et al. (2002)
Solid			
$UO_{3\,(s)}$[c]	5.39	-49.79	log K: Smith et al. (2003); ΔH°: NIST (1992)
$CaUO_{4\,(s)}$[d]	15.942	-131.3	Grenthe et al. (1992)

[a] Uranyl hydrolysis product and mixed hydroxyl-carbonate complex formation constants have been converted to refer to the generic reaction schema $nUO_2^{2+} + mH_2O + qCO_3^{2-} \Leftrightarrow (UO_2)_m(OH)_n(CO_3)_q^{2m-n-2q} + mH^+$. Conversion used the log *K*, 25ºC and ΔH values in the same database.

[b] Converted to WHAM–compatible schema using the $UO_2CO_3^0$ formation constant of Hummel et al. (2002).

[c] Schema: $UO_2^{2+} + 2H_2O \Leftrightarrow UO_{3\,(s)} + 2H^+$

[d] Schema: $UO_2^{2+} + Ca^{2+} + 2H_2O \Leftrightarrow CaUO_{4\,(s)} + 4H^+$

Table A4.4. Linear regression analyses for significant positive relationships up until an inflection point or threshold (Fig. 4.2) between mean K_d for U (log ml/g) and sediment properties (a-d).

Sediment properties (inclusive data range)	**Nominal pH**	**Equation**	$\boldsymbol{R^2}$	$\boldsymbol{p}$
(a) TOC (1.0-12.4 %)	6	Log K_d(U) = 0.12 TOC + 3.29	0.82	0.014
	7	Log K_d(U) = 0.10 TOC + 3.27	0.88	0.005
	8	Log K_d(U) = 0.16 TOC + 1.41	0.91	0.003
(b) FF (1-37 %, ≤ 50 μm)	6	Log K_d(U) = 0.04 FF + 3.25	0.96	0.003
	7	Log K_d(U) = 0.03 FF + 3.24	0.94	0.006
	8	Log K_d(U) = 0.05 FF + 1.31	0.99	<0.001
(c) CEC (4-33 meq/ 100g)	6	Log K_d(U) = 0.05 CEC + 3.16	0.87	0.007
	7	Log K_d(U) = 0.04 CEC + 3.22	0.76	0.024
	8	Log K_d(U) = 0.06 CEC + 1.27	0.91	0.003
(d) Fe content (2-29 g/kg)	6	Log K_d(U) = 0.05 Fe + 3.21	0.71	0.009
	7	Log K_d(U) = 0.05 Fe + 2.96	0.86	<0.001
	8	Log K_d(U) = 0.08 Fe + 1.17	0.87	<0.001

TOC = total organic carbon. FF = fine fraction. CEC = cation exchange capacity. Fe = iron content.

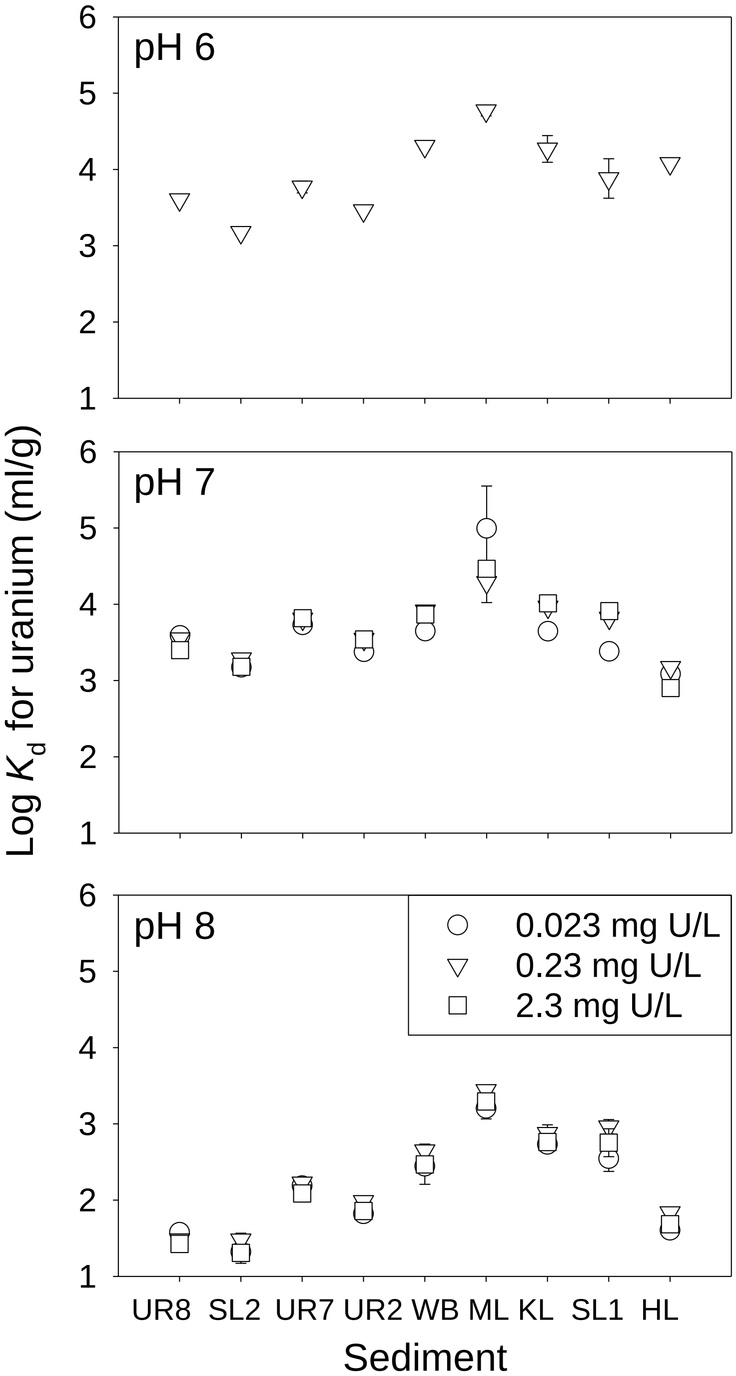

Fig. A4.1 Sediment partition coefficients (log K_d; ml/g) for each sediment at three U concentrations (0.023, 0.23, and 2.3 mg/L) and at nominal pH levels of 6, 7 and 8. Data points represent mean values (± SD, *n* = 2).

Table A5.1. Mean (± SD) water quality variables for the overlying water of the 10-d sediment bioaccumulation experiment and field conditions associated with the four U-contaminated field sediment.

Variable	**Laboratory conditions (*n* = 45)**	**Field conditions (*n* = 12)**
Temperature (°C)	21 ± 0.2	n/a
Dissolved oxygen (mg/L)	8.3 ± 0.4	n/a
Conductivity (µS/cm)	415 ± 18	1129 ± 74
pH	8.1 ± 0.1	7.3 ± 0.3
Ammonia (mg/L)[a]	0.16 ± 0.1	n/a
Total hardness (mg/L)[b]	168 ± 6	268 ± 32
Alkalinity (mg/L)[b]	125 ± 9	34 ± 1

n/a –not analyzed.

[a] Measured as ammonia nitrogen.

[b] Measured as calcium carbonate.

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