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PREDICTING THE BIOAVAILABILITY OF SEDIMENT-BOUND URANIUM TO THE FRESHWATER MIDGE (*CHIRONOMUS DILUTUS*) USING PHYSICOCHEMICAL PROPERTIES

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Abstract: Assessment of uranium (U)-contaminated sediment is often hindered by the inability to accurately account for the physicochemical properties of sediment that modify U bioavailability. The goal of this research was to determine whether sediment-associated U bioavailability could be predicted over a wide range of conditions and sediment properties using simple regressions and a geochemical speciation model, the Windermere Humic Aqueous Model (WHAM7). Data from U-contaminated field sediment bioaccumulation tests, along with previously published bioaccumulation studies with U-spiked field and formulated sediments were used to examine the models. Observed U concentrations in *Chironomus dilutus* larvae exposed to U-spiked and U-contaminated sediments correlated well ($r^2 > 0.74$, $p < 0.001$) with the WHAM-calculated concentration of U bound to humic acid (HA), indicating that HA may be a suitable surrogate for U binding sites (biotic ligands) in *C. dilutus* larvae. Subsequently, the concentration of U in *C. dilutus* was predicted with WHAM7 by numerically optimizing the equivalent mass of HA per gram of organism. The predicted concentrations of U in *C. dilutus* larvae exposed to U-spiked and U-contaminated field sediment compared well with the observed values, where one of the regression models provided a slightly better fit (mean absolute error [*MAE*; mg U/kg d.w.] = 18.1) than WHAM7 (*MAE* = 34.2). The regression model provides a predictive capacity with a minimal number of variables, while WHAM7 provides additional complementary insight into the chemical variables influencing the speciation, sorption and bioavailability of U in sediment. Our results indicate that physicochemical properties of sediment can be used to account for variability in U bioavailability as measured through bioaccumulation in chironomids exposed to U-contaminated sediments.

Keywords: Metal bioavailability, Uranium, Adsorption, Benthic macroinvertebrates, Bioaccumulation, Windermere Humic Aqueous Model (WHAM), Sediment assessment

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INTRODUCTION

The purpose of sediment quality guidelines (SQGs) is to set thresholds that can be used to protect benthic invertebrate communities from hazardous concentrations of contaminants in the sediment [1,2]. However, the use and derivation of SQGs for metals have been criticized for using total metal concentrations in sediment, which do not incorporate variations in metal bioavailability [3,4]. Total metal concentrations poorly reflect metal bioavailability because sediments can vary in their physicochemical properties, and therefore have different capacities to adsorb metals, often resulting in significant differences in metal uptake by organisms (i.e., bioavailability) [5-7]. As a result, 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 unreliable predictions of potential adverse effects to benthic communities. Inaccurate predictions of adverse effects on benthic communities are a particular issue with region-specific SQGs developed for use in and around uranium (U) mining areas in northern Saskatchewan, Canada [4]. Northern Saskatchewan contains some of the richest deposits of U ore in the world [8]. In freshwater environments, the hexavalent state, U(VI), is the predominant form of U under oxic conditions, found 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 [9,10]. The uranyl ion strongly interacts with solid phases, such as suspended solids, sediments and various minerals, which can result in substantial 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 [11,12]. Uranyl ions also readily form complexes with carbonate, phosphate, and sulphate ions, as well as with dissolved organic matter, all of which increase solubility allowing for easy transport and possible accumulation of U in aquatic

organisms. Thus, the tendency of U to accumulate in sediments can pose a risk to benthic communities [13,14], particularly under conditions that favour high U bioavailability.

The current approach for the assessment of U-contaminated sediments surrounding U mining operations in Canada is the Screening Level Concentration (SLC) approach [2]. The SLC approach, similar to other sediment quality criteria approaches, compares total metal concentrations measured in the sediment to derived upper (severe effect level; SEL) and lower (lowest effect level; LEL) guideline values to estimate the potential occurrence of adverse impacts on the benthic community. A site where the total U concentration in the sediment is below the LEL (104 mg U/kg d.w.) is not expected to display an adverse impact on the benthic community, whereas sites with total U above the SEL (5874 mg U/kg d.w.) indicate that an adverse effect of U to the benthic community is expected (i.e., reduction in community abundance and species richness $\geq$ 40 %; [2]). The 56-fold difference between the lower and upper guideline values of the U-SLC approach represents a large range in total U concentrations in the sediment where adverse effects on benthic communities, or lack thereof, become difficult to predict with a resulting large degree of uncertainty. Thus, U-SQGs would benefit from the development of a practical model that more accurately predicts adverse effects by incorporating U bioavailability based on the presence of modifying factors, instead of relying on total U concentrations.

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 [7,15-20]. However, the difficulty in incorporating modifying factors of bioavailability into risk assessments and SQGs for many metals is largely due to the absence of a model applicable for a wide range of sediments and site conditions. As a

result, no scientifically-acceptable approach has been adopted for use in the regulation of U-contaminated sediments in Canada that incorporate modifying factors of U bioavailability. Some success at predicting the behaviour and accumulation 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 [21-23]. 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) [24-26]. 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 [25], although a version for sediment organisms has also been developed [27]. WHAM can compute solution speciation and adsorption to solid surfaces [28] and has been used to model the variability in the uptake of metals by organisms [29]. Recent work has demonstrated support for the use of WHAM-calculated metals and protons bound to humic acid (HA) as a proxy for the binding of metals and protons by organisms at steady-state ("metabolically available" cations), with good agreement between calculated HA-bound metals and metal accumulation by aquatic organisms (i.e., extent of binding is assumed to be a measure of bioavailability) [23,30]. The WHAM toxicity function (F_{TOX}) model [23] quantifies the combined toxic effects of these metabolically available protons and metal cations toward biota through the linear combination of the products of organism-bound cations and the toxic potency coefficient for each cation. WHAM also offers an advantage in that it considers modifying factors of metal bioavailability, such as competitive binding of metals to ligands in solution and competitive uptake by organisms. Water chemistry and physicochemical characteristics of sediments (e.g., solution pH,

total organic carbon (TOC), carbonate and Fe content, and particle size) have previously been demonstrated to be significant modifiers of U bioavailability in sediment and soil [5,15,16,31-36]. Thus, the incorporation of modifying factors into a model to predict U bioavailability is essential for improving U-SQGs and the risk assessment of U-contaminated sediments.

In this paper, WHAM7 (version 7.0.4) and previously developed regression models that incorporate individual modifying factors of sediment properties [5] were evaluated and compared for their applicability in predicting the bioavailability of U to chironomid larvae in freshwater sediment. Water chemistry characteristics and sediment properties representative of a wide range of conditions typical of areas surrounding U mines in northern Saskatchewan, Canada, were used as input parameters for the models. Model predictions were compared to actual measurements of U concentrations in larvae of the freshwater midge, *Chironomus dilutus*, exposed to U-spiked and "natural" U-contaminated field sediments. Additionally, WHAM7 was used to calculate the sorption, aqueous chemical speciation, and accumulation of protons and U cations by a model freshwater benthic invertebrate compared to previously published data from U-spiked sediment experiments [5,6] and sorption tests [36].

MATERIALS AND METHODS

Laboratory exposure: uranium-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 1). Sediments were collected 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 (Umpherville River near Wollaston Lake;[5]).

The U-contaminated field sediments were used in a 10-d sediment bioaccumulation test using *C. dilutus* larvae. The 10-d test followed guidelines outlined by Environment Canada [37] and OECD [38] for testing of chironomids in sediment tests. The specific protocol used is described in detail by Crawford and Liber [5], except that field 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 freshwater environments surrounding U mines in northern Saskatchewan and because *C. dilutus* is a standard test species for sediment toxicity assessments [37]. Six biological replicates each with ten 8-d old (second instar) *C. dilutus* larvae were used for bioaccumulation assessments for the control and each of the four U-contaminated sediments. Additionally, two chemistry replicates from each sediment and the control were used for the analysis of dissolved organic carbon (DOC) from extracted pore water. The control treatment consisted of the same un-spiked silica sand (particle size of 106 to 425 µm) described in Crawford and Liber [5,6]. No field sediments were used as controls because our previous studies with reference field sediments (i.e., UR) as controls, having similar physicochemical characteristics of the U-contaminated field sediments, resulted in no adverse effects on the growth or survival of the *C. dilutus* larvae [5].

The 10-d tests were conducted in a modified sediment testing intermittent renewal (STIR) system, previously described [5,6,39], which allowed for automated renewal of overlying water (15% volume per beaker; carbon-filtered, bio-filtered municipal water) three times a day throughout the duration of the test. 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; [6,40]) inserted into each biological test beaker. Temperature, dissolved oxygen, conductivity, pH, ammonia, alkalinity, total hardness, and DOC

were analyzed in 20 mL overlying water samples collected from three test beakers per treatment throughout the test following the procedures described by Crawford and Liber [5]. Approximately 1 g d.w. of sediment was also collected from three test beakers per treatment on day 0 and 10 for analysis of total U concentrations in the sediment through microwave-assisted digestion with acids (HNO_3, H_2O_2 and HF) as outline by Crawford and Liber [5]. Organism survival, weight (tissue mass dry weight; 60°C oven for 24-h), and U accumulation (via tissue digestion) were determined after gut purging animals (12-h) following the same EDTA-rinse procedure used and described in previous U-spiked sediment experiments [5,6].

Field exposure: uranium-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 sediment sites in the field to quantify U concentrations in the native organisms. Field-collected benthic organisms were also sampled from reference areas of the Umpherville River and used as control references for the analysis of natural background U concentrations in native benthic organisms. Field organisms were removed from the sediment at each site (via Ekman grabs), initially by rinsing grab samples in a sieve bucket, subsequently removing them from sorting trays using forceps, and sorting them taxonomically into order, family or genus, if possible. All collected chironomid species 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 [5,6]. Field organisms were subsequently transported back to the laboratory, dried at 60°C for 24 h, weighed, and digested using HNO_3 and H_2O_2 for determination of U bioaccumulation. Additionally, overlying water samples were collected at reference (control) and U-contaminated field sites in the Wollaston Lake area using a Van Dorn sampler from approximately 10 to 15 cm

above the sediment surface for analysis of conductivity, pH, alkalinity, total hardness, DOC, and U concentration.

Chemical analysis

Water, digested sediment, and digested tissue samples were filtered (0.45-µm pore size, polyethersulfone membranes) and acidified (2% HNO_3) for analysis of U by inductively coupled 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. The method detection limit for U was 0.05 mg/L, with instrumental and method recoveries within 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 [41].

Model analysis

Concentrations of U in *C. dilutus* larvae were used to assess and validate regression models previously developed by Crawford and Liber [5] for predicting U bioaccumulation in chironomids from contaminated sediment. The previous laboratory tests used field sediments collected from reference sites around U mining areas in northern Saskatchewan (Wollaston Bay area) in a series of 10-d U-spiked sediment tests to determine differences in U concentrations in *C. dilutus* larvae as a function of sediment properties. The most practical and reliable regression equations were based on the significant correlations between observed concentrations of U in *C. dilutus* larvae (U_{tissue}) and fine fraction content (≤ 50 µm particle size) of field sediments spiked

with 50 mg U/kg d.w. (Eq. 1, $r^2 = 0.74$, $p < 0.05$) and 500 mg U/kg d.w. (Eq. 2, $r^2 = 0.79$, $p < 0.05$) [5].

$$\log U_{tissue} = 1.61 - 0.45 \log \text{fine fraction} \quad \text{(Eq. 1)}$$

$$\log U_{tissue} = 3.06 - 0.79 \log \text{fine fraction} \quad \text{(Eq. 2)}$$

In addition to the regression models, WHAM7, version 7.0.4 [21,22] was used to determine aqueous speciation, sorption, and accumulation of U by a model freshwater benthic invertebrate with the most current, reviewed thermodynamic stability constants for U(VI) complexes (presented in [36]). WHAM7 is fully described and extensively calibrated [22,42]. The WHAM7 model includes Humic Ion-Binding Model VII, a discrete site/electrostatic submodel of cation binding to humic substances [22,42,43] and a surface complexation model [44] for ion binding to mineral oxides. These submodels are parameterized for the binding of 46 cationic species, including U(VI), to humic and fulvic acids and amorphous Fe(III) oxide.

Data analyzed with WHAM7 included the current laboratory-exposed and field-collected data for U-contaminated field sediments. Additional data included 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 [36], as well as previous bioaccumulation tests with U-spiked formulated sediments [6] and field sediments [5]. The previous U bioaccumulation tests were conducted with 25 field sediments and 48 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

these previous studies included solution pH, temperature, particulate humic acid, fulvic acid (FA) and Fe oxide, DOC as colloidal FA, alkalinity, and concentrations of major ions (Table 1 and data from [5,6,36]). 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 [45]. 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 was comprised of 50% carbon and that the measured TOC had ion-binding properties of 50% HA and 50% FA, reflecting the average "binding activity" of TOC based on previous studies [22,30]. Concentrations of DOC were used to calculate colloidal FA for input into WHAM7 by assuming that dissolved organic matter (DOM) was 50% carbon (i.e. doubling the measured DOC) and that 65% of the DOM behave as active FA with respect to cation binding [30]. 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. [46], with additional thermodynamic stability constants presented in Crawford et al. [36]. Alkalinity measurements were used to define the carbonate contents of the systems. Key outputs from WHAM7 are discussed below, but include modeled contributions of U bound to particulate and colloidal phases of sediment, percent distribution of dissolved U species, and HA-bound U (as a potential surrogate for bioaccumulation in *C. dilutus*).

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, weight and concentration of U in *C. dilutus* larvae, and mean concentrations of U in sediment and water among treatments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. 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 variance assumptions, then a non-parametric Kruskal-Wallis one-way ANOVA on ranks was used, followed by a Tukey's pairwise multiple comparison post-hoc test. Correlations were performed using the Pearson product-moment correlation. Mean absolute values (*MAE*) and root-mean-square error (*RMSE*) values were calculated to describe the average model-performance error, where lower *MAE* and *RMSE* values indicated a better fit of the model to the observed data (presented in Table S1). Uranium binding data were fitted by numerically optimizing the equivalent mass of HA per gram of organism dry weight (E_{HA}, g/g) using the Solver function in Excel (i.e., minimizing the sum of weighted absolute deviations between the observed and calculated values of the squared differences between the observed and calculated values of log m, where m is the moles of metal bound per gram of humic matter). The E_{HA} values are also presented in the Supplemental data (Table S1).

RESULTS AND DISCUSSION

Uranium-contaminated field sediments

Test conditions. The physicochemical properties of the U-contaminated field sediments are presented in Table 1 and the associated water chemistry from the 10-d laboratory bioaccumulation sediment tests and field conditions are presented in Table S2 of the Supplemental Data. Mean (± SD) DOC concentration of the overlying water from the Umpherville River reference (control) 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). Concentrations of DOC were greater in the overlying water of the U-contaminated field sediment sites (5.5 ± 0.2 mg/L, n = 8) and the laboratory U-contaminated sediment treatments (6.0 ± 0.4 mg/L, n = 24). Concentration of DOC can be important in the assessment of U bioavailability as DOC was observed to ameliorate U toxicity to algae (*Chlorella* sp. and *Euglena gracilis*), green hydra (*Hydra viridissima*), bivalve (*Velesunio angasi*), and northern trout gudgeoun (*Mogurnda mogurnda*) by 6 to 9% with each 1 mg/L addition of DOC, up to 30 mg/L [35].
Other water chemistry variables such as pH, hardness, and alkalinity can also influence the sorption and bioavailability of U [33,47,48]. The sorption of U is significantly pH-dependent with peak sorption occurring at circumneutral pH values [15,36], which can influence the availability of U. For example, the 72-h LC50 of *C. crassiforceps* to U was shown to decrease from 36 mg/L at pH 6 to 58 mg/L at pH 4 [13]. 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 and are important input for models such as WHAM7.

Biological endpoints and uranium bioaccumulation. 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 [37], with no significant difference between the control and U-contaminated field sediment treatments. 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 = 30). Concentrations of U in *C. dilutus* larvae from the controls of the 10-d sediment test and in the chironomids collected directly from Umpherville River reference (control) areas were negligible (≤ 0.3 mg U/kg d.w.). In contrast, exposure to the U-contaminated field sediment resulted in concentrations of U that ranged from 5 to 35 mg U/kg d.w. in the laboratory-exposed *C. dilutus* larvae and from 6 to 68 mg U/kg d.w. in the field-collected chironomid species (Fig. 1). There was a 2- to 3-fold difference in concentration of U in the whole-organisms between the laboratory-exposed and field-collected chironomids for each of the respective U-contaminated field sediments. The differences in accumulation of U between the laboratory-exposed and field-collected organisms could be a result of differences in the sample size, life stage, and species of chironomids, as well as exposure conditions (i.e., length of exposure: 10-d vs resident biota). Organisms in the laboratory were exposed to constant and homogenous U concentrations (i.e., sediments were thoroughly mixed prior to testing and water chemistry and conditions were stable), which was likely not the case for the field-collected chironomid species exposed to field sediments and conditions that are variable (i.e., stratified sediment and various solution associations). Due to the potentially complex exposure conditions of the field-collected chironomids, the concentrations of U in the field-collected organisms were used only for visual comparisons and not statistical conclusions (Fig. 1).

The concentrations of U in *C. dilutus* larvae had a significant positive relationship with dissolved concentrations of U in the overlying water ([U_{OW}]; 0.022 to 0.16 mg U/L) in the laboratory test ($\log [U_{tissue}] = 0.97 \log [U_{OW}] + 2.38$, $r^2 = 0.95$, $p < 0.001$, $n = 4$). Similar relationships have been observed previously for U-spiked formulated sediment ($\log [U_{tissue}] = 0.98 \log [U_{OW}] + 2.15$, $r^2 = 0.77$, $p < 0.001$, $n = 47$; [6]) and U-spiked field sediment ($\log [U_{tissue}] = 0.68 \log [U_{OW}] + 2.37$, $r^2 = 0.61$, $p < 0.001$, $n = 28$; [5]), even with total U concentrations in the sediment remaining the same. The relationships between the concentration of U in *C. dilutus* larvae and the concentrations of U in the overlying water do not fully explain the variance, indicating that additional factors are likely involved in modifying the partitioning and availability of U from sediments. In particular, the influence of physicochemical characteristics of sediment have previously been demonstrated to significantly modify U bioavailability due to the various binding phases that influence the adsorption and partitioning of U between the aqueous and solid sediment phases [5].

Application of regression equations

To investigate the ability of predicting U bioavailability from sediment, previous regressions developed for modeling U concentrations in *C. dilutus* larvae using a single, easily-measured sediment physicochemical property, fine fraction (≤ 50 μm particle size), were further examined. The regression equations were developed by Crawford and Liber [5] using reference field sediments collected from areas near Saskatchewan U mines that were spiked with 50 mg U/kg d.w. (Eq. 1) or 500 mg U/kg d.w. in the laboratory (Eq. 2). Equations 1 and 2 were evaluated in the present study (Fig. 1) for use in predicting concentrations of U in *C. dilutus* larvae exposed to U-contaminated field sediment (Table 1). Predictions of U concentrations in

the whole-organisms of *C. dilutus* larvae were generally within a factor of 3 of the observed U concentrations in the laboratory-exposed organisms.

One limitation to the use of regression Eq. 1 and 2 is that they are based on specific U-spiked field sediment concentrations (i.e., 50 and 500 mg U/kg d.w., respectively). In order to derive concentration-independent equations, biota-sediment accumulation factors (BSAFs; concentration of U in *C. dilutus* larvae divided by total concentration of U in sediment) were utilized. A significant negative relationship was observed between the BSAFs and sediment fine fraction content (log BSAF = 0.12 – 0.64 log fine fraction, $r^2 = 0.78$, $p < 0.001$, $n = 25$; Fig. 2) for the U-spiked field sediments. The BSAF relationship for U-spiked field sediments was used to calculate the predicted concentration of U in *C. dilutus* larvae (i.e., $[U_{tissue}]$) in different sediment, while also incorporating total U concentration in the sediment (i.e., $[U_{sed}]$) through the following equation:

$$[U_{tissue}] = (0.12 - 0.64 \log \text{fine fraction}) \times [U_{sed}] \quad \text{(Eq. 3)}$$

Concentrations of U in whole-organisms predicted with Eq. 3 corresponded significantly (within a factor of 1) with the observed concentrations of U in *C. dilutus* larvae exposed to U-contaminated field sediments ($r^2 = 0.99$, $p < 0.01$, *MAE* = 4.2 mg U/kg d.w, Fig. 1). The use of Eq. 3 also improved the predictions of U concentrations in *C. dilutus* larvae exposed to both concentrations of U-spiked field sediments ($r^2 = 0.89$, $p < 0.001$, *MAE* = 20.4 mg U/kg d.w., Fig. 3) compared to insignificant relationships observed with Eq. 1 and Eq. 2. Use of Eq. 3 in predicting concentrations of U in *C. dilutus* larvae previously exposed to U-spiked formulated sediments were also investigated, but did not significantly correspond with observed values (p =

0.16, not shown in Fig. 3). The use of Eq. 3 may not have been applicable to U-spiked formulated sediments as the fine fraction content of the sediment (≤ 50 μm particle size) consisted of only one individual clay component rather than the complex mixture and coatings associated with different clay minerals and silt found in field sediments. However, the advantage of empirical models such as Eq. 3, are that they provide a simple yet predictive capacity of the concentration of U accumulated in the whole organism (within their limits) with a minimal number of variables required. In the present case, the total sediment concentration and fine fraction content of the sediment are the only variables that are required for use in the estimation and are routinely measured during a site assessment. Furthermore, the inclusion of fine fraction as a variable in the prediction of the concentration of U in *C. dilutus* larvae intrinsically considers the bioavailability of U since fine fraction is a significant modifier of U bioavailability. While the Eq. 3 model is useful, a mechanistic model that incorporates multiple parameters that can affect U bioavailability (via sorption and speciation of U) may better elucidate the influence of modifying factors of U bioavailability and provide additional complementary insight into the risk of U-contaminated sediments to benthic invertebrates.

Application of WHAM

WHAM7 incorporates a number of physicochemical parameters of both the water and sediment chemistry, including the partitioning of metals to sediment organic carbon and hydrous iron oxide to investigate the behaviour of metals in aquatic and terrestrial environments. Reliable predictions of sediment-solution partition coefficients (K_d) for U were calculated with WHAM7 in a previous study [36] that investigated the influence of sediment properties and pH on the sorption of U to field-collected reference sediments. Significant sorption of U to sediment organic matter and hydrous iron oxide was also observed [36]. Due to the previous success in

predicting U sorption with WHAM7, this model was used in the present study to investigate the modeled concentrations of U in whole organisms (also referred to as bioaccumulation in this paper), additional sorptive behaviour of U, and U speciation for a number of different test conditions to provide further insight into the bioavailability of U from U-contaminated field sediment.

Modeling uranium bioaccumulation. The use of WHAM7 to predict U bioaccumulation was investigated and validated with U bioaccumulation data from our previous and present bioaccumulation tests. Previous work has shown a significant relationship between measured bioaccumulation of metals in invertebrates and WHAM-calculated metals and protons bound to HA [23]. In the present study, HA-bound U was calculated in WHAM7 (Fig. 4A) and positively correlated with the concentration of U in *C. dilutus* larvae exposed to U-contaminated field sediments (Table 1), U-spiked field sediments [36], and U-spiked formulated sediments [6]. The correlation between the HA-bound U and the corresponding concentration of U accumulated by *C. dilutus* larvae from the U-spiked formulated sediments (slope = 1.03, $r^2 = 0.74$, $p < 0.001$, $n = 48$), the U-spiked field sediments (slope = 1.02, $r^2 = 0.75$, $p < 0.001$, $n = 25$), the U-contaminated field sediments (slope = 1.09, $r^2 = 0.96$, $p < 0.001$, $n = 4$), and all combined sediment datasets (slope = 1.17, $r^2 = 0.75$, $p < 0.001$, $n = 77$; Fig. 4A) generally followed a 1:1 relationship within a factor of 4. Similarly, He and Van Gestel [49] demonstrated a significant correlation ($r^2 = 0.79$ to 0.93) between the observed body concentrations of Ni and Co in *Enchytraeus crypticus* and the WHAM-calculated Ni and Co bound to HA. The similarities between HA-bound metals and observed concentration of U in *C. dilutus* larvae suggests that chironomids accumulate metals, or at least U, in a fashion comparable to that of U-binding to HA (i.e., competitive binding to weak-acid sites).

Since significant correlation was observed between calculated HA-bound U and observed concentration of U in *C. dilutus* larvae, WHAM HA-bound U was numerically optimized with the equivalent HA (E_{HA}) value to predict the concentration of U in the whole organism (Fig. 4B). The E_{HA} value is defined by Tipping and Lofts [29] as the equivalent amount of HA per gram of organism d.w. (g/g). Based on the assumption that organisms possess binding sites that have properties similar to those of HA, the use of E_{HA} optimizes a value for the binding site density on the organism relative to the binding site density of HA [29]. In the present study, the E_{HA} was 0.78 for U-spiked formulated sediments, 1.89 for U-spiked field sediments, and 5.69 for U-contaminated field sediments. The WHAM7-predicted U bioaccumulation (optimized with the respective E_{HA} values; Table S1) corresponded well with the observed concentrations of U in *C. dilutus* larvae exposed to U-spiked formulated sediments (slope = 1.03, $r^2 = 0.74$, $p < 0.001$, *MAE* = 148.7 mg U/kg d.w.), U-spiked field sediments (slope = 1.03, $r^2 = 0.75$, $p < 0.001$, *MAE* = 31.9 mg U/kg d.w.), and U-contaminated field sediments (slope = 1.09, $r^2 = 0.96$, $p = 0.021$, *MAE* = 3.6 mg U/kg d.w.), following a 1:1 relationship generally within a factor of 4 (Fig. 4B). For simplicity, all three sediment datasets were also combined and optimized with a pooled E_{HA} value of 1.15 (slope = 1.17, $r^2 = 0.76$, $p < 0.001$, *MAE* = 152.3 mg U/kg d.w.; Fig. 4C). A slightly negative bias when U bioaccumulation is low is observed in Fig. 4C (i.e., characterized by the concentration of U in *C. dilutus* exposed to U-contaminated field sediment) when fitting a smaller pooled E_{HA} value rather than the larger separate E_{HA} value applied to the U-contaminated sediment. However, regardless of whether the sediment data were pooled or separate, a similar coherent relationship was evident between the WHAM-predicted concentration of U in a model organism and the observed concentration of U in *C. dilutus* larvae exposed to all field and formulated sediments. Tipping and Lofts [29] reported a similarly good correlation (within a

factor of 2.75, r^2 =0.89, n = 467, *RMSE* = 0.44 log mol/g) between WHAM-calculated HA-bound metals and observed bioaccumulation of a number of metals in *Hyalella azteca* in laboratory and caged field animals after optimization of E_{HA} values to 0.044 and 0.11, respectively. Additional studies have also demonstrated good fits of WHAM–F_{TOX}-computed HA-bound metal with bioaccumulation, following optimization, for bryophytes [30], aquatic plants [50], oligochaetes [49], and stream macroinvertebrates [23].

The use of HA-bound U as a surrogate for bioaccumulation of U follows the general assumptions of the WHAM–F_{TOX} approach, in which the accumulation of 'metabolically active' metals occurs via their reversible binding within or on the organism, under the modifying influences of metal complexation in solution and adsorption to solid phases, and in competition for binding to sites on the organism [29]. Although it is recognized that accumulation of metals by organisms can be quite complex (e.g., regulation via uptake, excretion, incorporation and/or storage mechanisms; [29,51-53]), in the present study the use of water chemistry and sediment binding phases alone in WHAM7 predicted the steady-state accumulation of U by *C. dilutus* larvae surprisingly well (Fig. 4B). The assumption that the accumulation of metals follows the quasi-equilibrium chemical reactions of WHAM7 may be an over-simplification, but similar success in the use of WHAM7 has been previously observed for a number of metals [49,54]. Additionally, the assumptions of WHAM7 also follow the basis of the BLM in which competitive binding of cations to an active site (the biotic ligand) is the foundation of metal toxicity [24,55]. Therefore, evidence from the present study and other recent publications indicate that WHAM-calculated HA-bound metals are a suitable surrogate for metal binding sites on macroinvertebrates, at least for U. This has not previously been demonstrated for the bioaccumulation of U in chironomids and provides further support for the use of HA as a

surrogate for bioaccumulation of U, and therefore support for using WHAM7 in quantifying U bioavailability.

Modeling uranium sorption. To better understand the mechanisms behind the changes in U bioavailability among the different sediments and test conditions, 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 (UO_2^{2+}) and aqueous complexes (see *Modeling aqueous speciation of uranium* section for more detail). Examples demonstrating the distribution of different U bound fractions for four field sediments with very different compositions and characteristics are presented in Fig. 5. A description of the field sediments used in the sorption and U-spiked sediment experiments can be found in Crawford et al. [36] and Crawford and Liber [5].

The sediment phase had the greatest predicted fraction of bound U for all U experiments examined in this paper. There was a ≥ 78% association of U with the organic carbon 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. 5A). The fraction of U bound to the Fe oxide 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 (Fig. 5A). Binding of U to hydroxides under conditions of low TOC and high Fe content is consistent with the behaviour of other metals [20,31]. The present U-contaminated field sediment and previous U-spiked field sediment bioaccumulation experiments generally had a much larger (≥ 70%) fraction of U bound to the Fe oxide phase than to the TOC phase (Fig. 5B). Although some of the same sediments were used in both the sorption and bioaccumulation studies, the sediment concentrations (i.e., SSRs) were different based on the design of the tests. Particulate parameters

were calculated using SSRs to convert parameters such as TOC to solution concentrations for input into WHAM7, and different SSRs can thus influence the predicted amount of U bound to different fractions. Additionally, all Fe in the sediment was assumed to be hydrous Fe oxide (i.e., amorphous) for modeling, which may overestimate the effective amount of Fe(III) oxide surface present in the sediment since the DCB extraction method used in the present study extracts both amorphous (large reactive surface area) and crystalline (smaller reactive surface area) Fe. Consequently, running the model without the inclusion of hydrous Fe oxide for the bioaccumulation studies resulted in lower concentrations of U bound to the particulate phase, mostly affecting sediments with both low TOC and high Fe content. However, results from the inclusion of Fe in WHAM7 for 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 [10,18,56]; of which the latter was very low in our sediments ($\leq$ 0.8% $CaCO_3$). Future studies should investigate and compare the alternative use of an extraction method for amorphous Fe in sediment (e.g., ammonium oxalate-oxalic acid extraction) and their influence on U sorption.

In comparison to the sediment phases, the contribution of DOC ($\leq$1.4%) as a binding phase for U was not significant for any of the sediments from the different experiments. DOC is often more important for the binding of metals at slightly acidic to intermediate pH (6 to 7) and becomes less important at high pH as the higher CO_3 content allows for greater formation of carbonate complexes in competition with DOC. The low contribution of DOC for metal adsorption is consistent with previous predictions by WHAM (< 4.8%) for Cu, Cd, Zn, Ni, Pb [20] and U [43]. For sediments with relatively low amounts of binding phases, $\leq$ 3.8% TOC and/or $\leq$ 10 g/kg of Fe (i.e., UR8 and UR7; Fig. 5), 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%). Therefore, the distribution of U among solid and aqueous phases was demonstrated to vary as a function of both pH and the physicochemical properties of sediment, providing further support for the important role of binding phases associated with TOC and Fe content of sediment in influencing U sorption and bioavailability.

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. Uranium(VI), as UO_2 is generally the most predominant U species present in oxic freshwater and is considered more bioavailable than U(IV) to aquatic organisms [10,57,58]. The conditions reported to favour the formation of free UO_2^{2+} ions are generally low pH, low concentrations of organic matter, and likely low alkalinity [9,10,48,59], which were not typical of the conditions examined in our studies. Thus, WHAM7 predicted small concentrations of UO_2^{2+} (< 1% of the distributed species) under our test conditions that represented field conditions surrounding U mines in northern Saskatchewan.

Speciation of U is complex and can be influenced by a number of factors, including pH conditions and the presence of ligands, such as carbonates, sulphate ions, and DOC [13]. Speciation of U is significantly pH-dependent as previously observed in the modeled species abundance for our selected field sediments that covered a wide range of properties for the sorption test [36]. Fortin et al. [60] demonstrated a complex interaction of pH on U speciation, with increases in pH from 5 to 7 resulting in the formation of carbonate and hydroxide U complexes that reduced the free uranyl ion activity and thus reduced bioavailability. In contrast, the decrease in competing protons with increasing pH can increase uranyl bioavailability [60].

Uranyl-carbonate complexes were the predominant aqueous species modeled by WHAM7 in our studies at neutral pH conditions and increased in abundance with increasing alkalinity. Uranyl-carbonate complexes were also predominant in the bioaccumulation experiments, which is not surprising since the tests were conducted at a pH of approximately 8. The speciation in the bioaccumulation experiments typically followed a species distribution of $Ca_2UO_2(CO_3)_3$ > $CaUO_2(CO_3)_3^{2-}$ >> $UO_2(CO_3)_3^{4-}$ ≈ $MgUO_2(CO_3)_3^{2-}$ > $UO_2(CO_3)_3^{2-}$, which is in general agreement with previous literature [9,10,46]. Many of the pH-dependent uranyl-carbonate complexes are only weakly sorbed to sediment binding phases such as Fe (hydr)oxides [61,62]. The weak sorption at high pH is often a result of the formation of weakly sorbing uranyl-carbonate complexes in the presence of carbonates and subsequent increase in total concentrations of U(VI) in solution, which is consistent with the lower sorption observed in the current and previous publications for more alkaline conditions [36,56].

Concentrations of chloride, nitrate, silicate, sulfate, phosphate, and fluoride are typically low (<3 mg/L) in northern Saskatchewan U mining areas that are not impacted by effluent discharges and/or are relatively weak complexing agents of uranyl [10,63]. These ions were predicted by WHAM7 to form negligible U complexes (i.e., <1% of total U) for the sorption and bioaccumulation studies examined in this paper. Overall, WHAM7 accurately 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.

CONCLUSIONS

Both WHAM7 and Eq. 3 were able to predict changes in U bioavailability (inferred from concentrations of U in *C. dilutus* larvae) from U-spiked and U-contaminated field sediment under conditions similar to areas surrounding northern Saskatchewan U mines. The predicted concentrations of U in *C. dilutus* larvae using Eq. 3 (r^2 = 0.89, *MAE* = 18.1 mg U/kg d.w.) provided a better prediction of the concentration of U in *C. dilutus* larvae exposed to both U-spiked and U-contaminated field sediment than WHAM7 (r^2 = 0.75, *MAE* = 34.2 mg U/kg d.w.). However, the predicted concentrations of U in *C. dilutus* larvae exposed to U-spiked formulated sediment did not significantly correspond to observed values using Eq. 3, but were significant when using WHAM7 (r^2 = 0.74, *MAE* = 148.7 mg U/kg d.w.).

An empirical model such as Eq. 3, which is based solely on the total U concentration in the sediment and the fine fraction content (≤ 50 μm particle size) of sediment, can be useful for regulators that might prioritize and favour the simplicity and ease of application that a single equation model provides (i.e., low cost monitoring). In contrast, some may prioritize and favour a more mechanistic model in which the underlying mechanism behind the behaviour of the systems is understood (i.e., that all sediment binding phases and water chemistries are incorporated to fully consider the metal partitioning, speciation, and bioavailability). The benefit of using a mechanistic model such as WHAM7 is that it comprises of existing speciation models and parameters for bioavailability modeling with minimal extra work required. For example, WHAM7 allowed for the incorporation of some key physicochemical properties measured at U-contaminated field sites, such as DOC, TOC, pH, major solution ions, and particulate Fe oxide content and provided insight into the sorption and speciation of U, which improves our understanding of the controls on U bioavailability. Additionally, the benefit of using a

mechanistic model like WHAM7 is that it can complement and help explain why certain parameters are significant in the empirical models and further justify the measurement of those parameters. Many key physicochemical characteristics of the sediment and associated water chemistry input into WHAM7 are commonly measured at U-contaminated sites such as in northern Saskatchewan (i.e., EARMP [63]), and may allow for the assessment and further validation of WHAM-predicted bioaccumulation through the utilization of historical monitoring data. Our data also provide support for the use of WHAM HA-bound U as a suitable surrogate to predict bioaccumulation of U in chironomids. Overall, WHAM7 proved useful for predicting U bioavailability from U-contaminated field and formulated sediments and offers a readily-available tool to incorporate modifying.

The use of mechanistic models such as WHAM7, in combination with empirical models such as Eq. 3, to predict U bioaccumulation across different sediments has the potential to improve the risk assessment of U to benthic invertebrates. Since SQGs are expressed as total metal concentrations in the sediment, there is a need to correct for variability in bioaccumulation factors across different sediments. Our results indicate that key physicochemical properties of sediment can be used to account for variability in U bioavailability, as measured through bioaccumulation of U in chironomids exposed to U-contaminated sediments. Thus, the models presented in the present paper have the potential to improve SQGs for U by incorporating site-specific physicochemical properties of water and sediment as modifiers of bioavailability. Future research should focus on further quantifying the relationships between U bioavailability at different total U concentrations and additional combinations of physicochemical properties and conditions to improve the empirical and mechanistic understanding of U bioavailability. Overall,

this research provides a first step toward a universal model that describes U bioavailability through the incorporation of sediment and water physicochemical properties.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data, associated metadata, and calculation tools are available upon request (sarah.crawford@usask.ca, karsten.liber@usask.ca).

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Figure 1. Mean (± SE) concentration of uranium (mg U/kg d.w.) observed in laboratory test organisms (Obs. – Lab, *C. dilutus*; black bars, $n = 6$), and field-collected organisms (Obs. – Field, chironomid species; grey solid bars, $n = 2$) relative to predicted concentration of U in a model benthic organisms using Eq.1 (Pred. – Eq 1; grey left stripe bars), Eq. 2 (Pred. – Eq 2; grey right stripe bar), Eq. 3 (Pred. – Eq 3; dark grey crosshatch bars), and WHAM7 with fitting of an E_{HA} value (Pred. – WHAM7; white crosshatch bars) for U-contaminated field sediments (HC, Horseshoe Creek; HB, Hidden Bay).

Figure 2. The biota-sediment accumulation factor (BSAF) as a function of sediment fine fraction (<2 – 50 µm; %) for field sediments spiked with 50 or 500 mg U/kg d.w. (open and filled circles, respectively). The solid line represents the linear regression for all U-spiked field sediments ($p < 0.001$). Grey triangles represent data for the *C. dilutus* larvae exposed to U-contaminated field sediments.

Figure 3. Predicted (using Eq. 3) versus observed concentrations of uranium in whole organisms (*C. dilutus* larvae) exposed to field sediments spiked with 50 or 500 mg U/kg d.w. (open and filled circles, respectively). Grey triangles represent data for the *C. dilutus* larvae exposed to U-contaminated field sediments. Data for U-spiked formulated sediment were not significant (Table S1) and thus are not presented. The long-dashed line represents the 1:1 relationship bracketed by short dashed lines representing a factor of 2.

Figure 4. Comparison of the (A) WHAM-calculated concentration of uranium (U) bound to humic acid (HA) relative to the observed concentration of U in *C. dilutus* larvae

(bioaccumulation) from U-spiked formulated sediment (open squares; combined U-spiked concentrations of 50 and 200 mg U/kg d.w., Crawford and Liber [6]), U-spiked field sediment (open circles; combined U-spiked concentrations of 50 and 500 mg U/kg d.w. Crawford and Liber [5]), and U-contaminated field sediment (grey triangles). Subsequent comparison of the WHAM-predicted concentration of U in the whole organism (bioaccumulation), via optimization with respective E_{HA} values [B] or a pooled E_{HA} value [C], relative to the observed concentration of U in *C. dilutus* larvae exposed to the respective sediments. The thick black line in (A) represents the linear regression for all combined sediments ($p < 0.001$) and the long dashed grey lines represent the 1:1 line with a factor of 4 indicated by the lighter dashed lines.

Figure 5. 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 free ion and small aqueous complexes in solution (grey line fill) (A) as a function of pH for sediments described in a previous U sorption study [36], and (B) at a pH of approximately 8 for the U-spiked sediment bioaccumulation tests [5]. Field sediments include Shallow Lake (SL), Konner Lake (KL), and Umpherville River (UR) collected from around U mines in the area of Wollaston Bay, Saskatchewan, Canada.

Table 1. Summary of physicochemical characteristics and background U 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	214	401	444

[a] Field sediments collected from the Wollaston Lake area in northern Saskatchewan, Canada; HC – Horseshoe Creek, HB – Hidden Bay. Total carbonate content of sediment was below detection limit (<0.80%), determined by the gravimetric method for loss of carbon dioxide, ALS Environmental, Saskatoon, SK.

[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 [64,65].

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

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

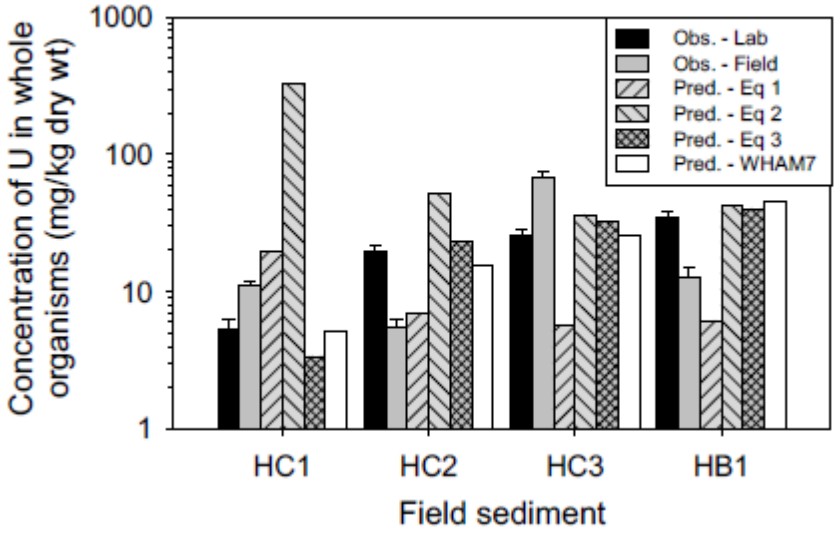

Figure 1

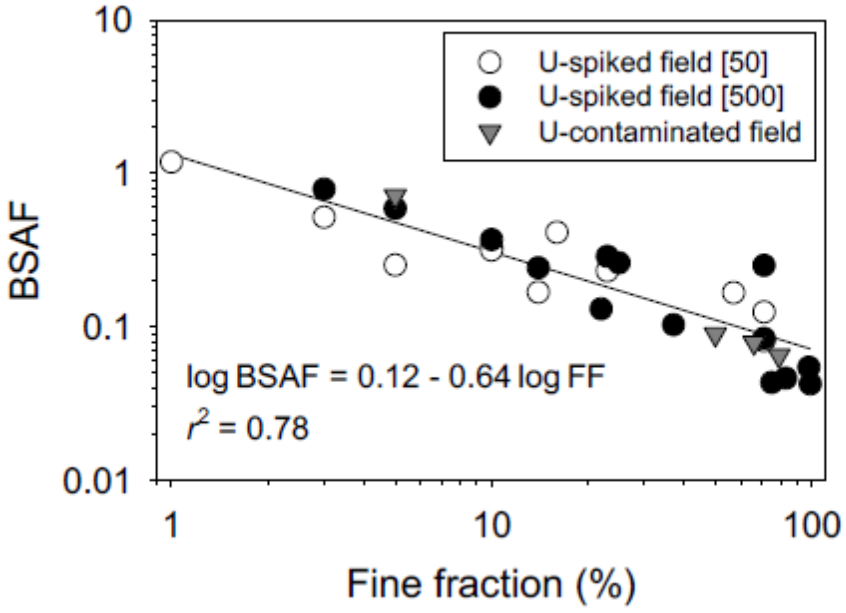

Figure 2

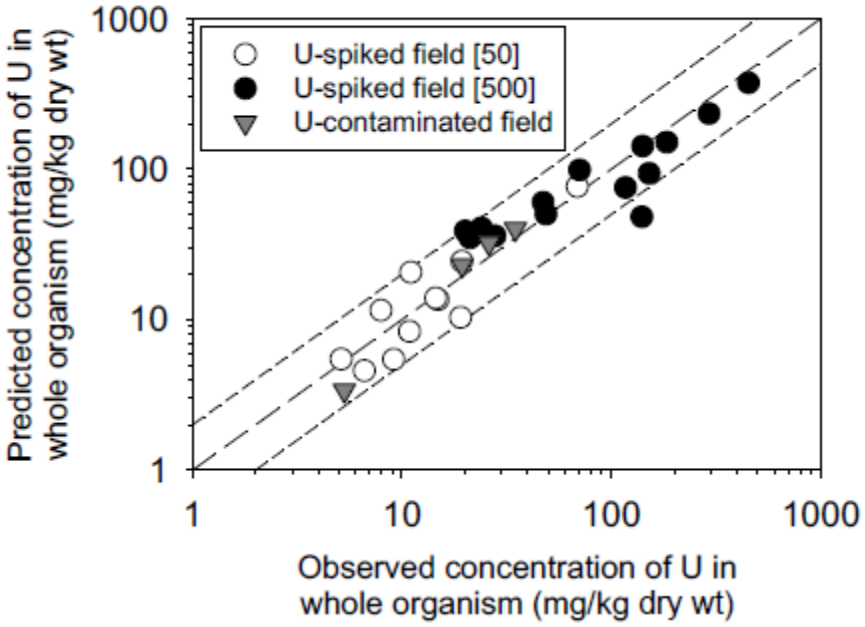

Figure 3

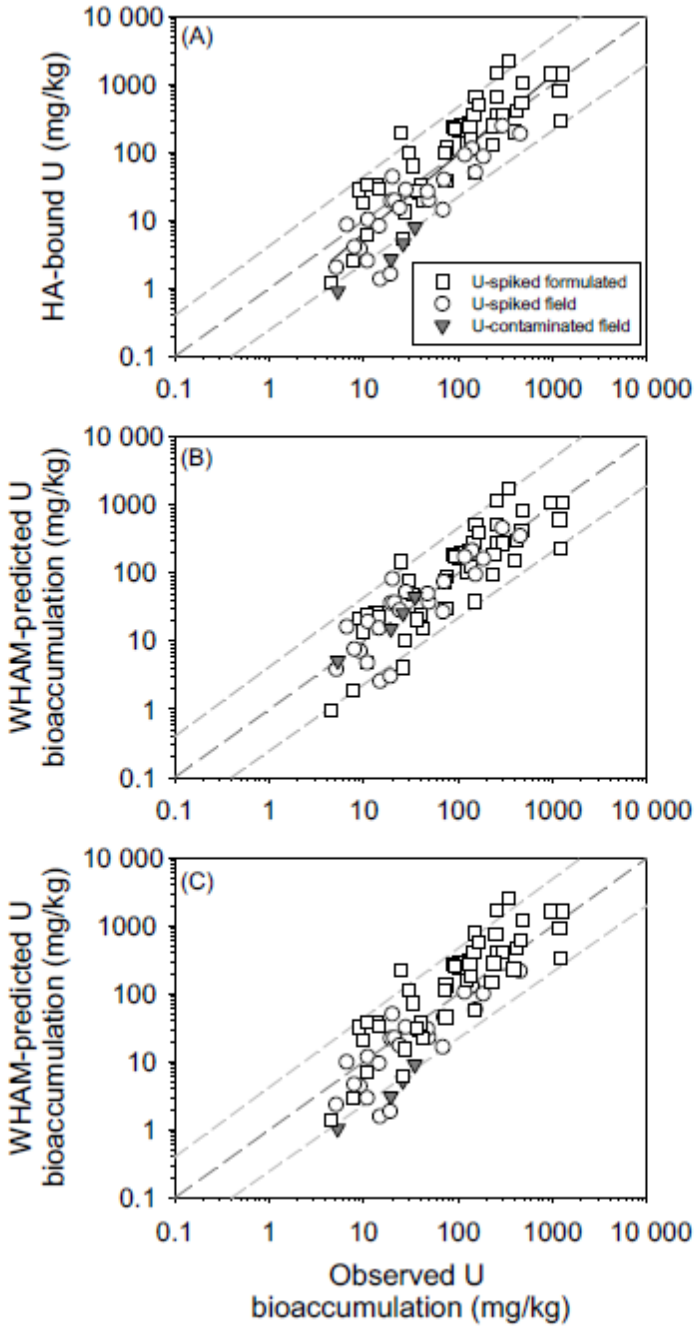

Figure 4

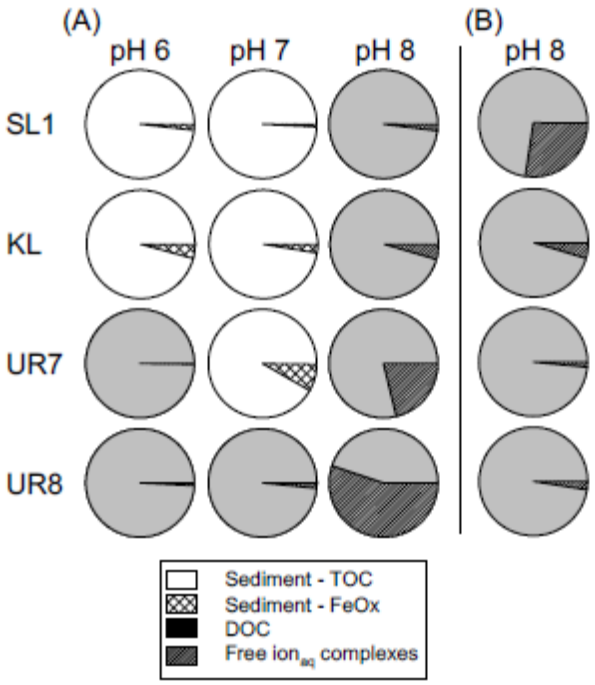

Figure 5