Bioaccumulation and Toxicity of Uranium, Arsenic, and Nickel to Juvenile and Adult *Hyalella azteca* in Spiked Sediment Bioassays

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**Abstract:** Uranium (U) mining and milling release arsenic (As), nickel (Ni) and U to receiving waters, which accumulate in sediments. The objective of the present study was to investigate if As, Ni, and U concentrations in tissue residue of *Hyalella azteca*, overlying water, sediment porewater, and solids could predict juvenile and adult survival and growth in conditions similar to lake sediments downstream of U mines and mills. We conducted 14-d static sediment toxicity tests spiked with U, As, and Ni salts. For U, we spiked uranyl nitrate with sodium bicarbonate to limit U precipitation once in contact with circumneutral sediment. The median lethal concentrations for As, Ni, and U of juveniles and adults based on measured concentrations in sediments were 134 and 165 mg/g, 370 and 787 mg/g, and 48 and 214 mg/g, respectively. Adult survival and growth linearly decreased with increasing bioaccumulation. For juveniles, metal accumulation linearly predicted survival. We calculated median lethal body concentrations for juveniles and adults of 5 and 36 mg As/g, 14 and 49 mg Ni/g, and 0.4 and 1.0 mg U/g. The concentrations of As, Ni, and U in tissue residue leading to a 20% decrease in adult growth were 32 mg As/g, 44 mg Ni/g, and 1 mg U/g. Overall, the present study showed that U was the most toxic element, followed by As and Ni; that juveniles were more sensitive to the 3 metals tested than adults; and that threshold body concentrations can support assessment of benthic invertebrate community impairment. Environ Toxicol Chem 2018;37:2340–2349. © 2018 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC.

**Keywords:** Uranium; Nickel; Arsenic; *Hyalella azteca*; Life stage; Lethal body concentration

**INTRODUCTION**

Treated effluent discharged from uranium (U) mines and mills contributes to the accumulation of metals in sediments. Past environmental effects monitoring of sediments downstream of U mine and mill sites identified local impairment to benthic communities inhabiting these sediments [1]. Gates and Lipsit [2] indicated that metals of primary concern with respect to sediment contamination were U, molybdenum (Mo), arsenic (As), and nickel (Ni). However, Liber et al. [3] ruled out Mo as a potential cause of impacts to benthic invertebrates because benthic invertebrates were tolerant to a wide range of Mo concentrations.

Determining whether As, Ni, or U caused these observed localized impacts to benthic invertebrates is challenging. First, each contaminant has specific affinities with a wide variety of particulate and dissolved ligands, which will affect partitioning to sediments and bioavailability to benthic invertebrates [4–6]. Second, each contaminant has its own specific toxicity [7,8], and the relative toxicity of contaminants to benthic invertebrates will depend on rates of uptake from pools such as the overlying water, sediment porewater, and solids [9,10]. Some studies have indicated that benthic invertebrates mainly take up metals from porewater and/or overlying water [4,9,10]. Liber et al. [3] investigated the toxicity of As, Ni, and U to *Hyalella azteca* and *Chironomus dilutus* exposed to contaminated water, sediment porewater, and solids. They expressed sediment toxicity on the basis of total sediment and sediment porewater concentrations. Comparison of median lethal concentrations (LC50s) from water-only experiments with LC50s calculated from porewater in the spiked sediment tests supported the hypothesis that porewater concentrations are useful for predicting the potential toxicity of sediments.
However, metal toxicity predictions from sediment porewater are not less challenging because the free metal ion is often the best predictor of toxicity [11–13], and other parameters affect the uptake and toxicity of metals. First, pH, calcium, and magnesium compete with metals for uptake sites [14,15] where an increase in cations and protons impedes metal uptake. Second, dissolved organic carbon [16–19] and bicarbonate [20] can bind metals, reducing their uptake by aquatic organisms. An additional challenge is that the free-ion activity model does not consider uptake of metals from food, which can be an important pathway of exposure [21]. Therefore, predicting metal exposure and toxicity to benthic invertebrates based on porewater concentration remains challenging.

Recently, investigators have explored the possibility of using metal body concentrations, including U, as predictors of toxic effects [22–28]. The approach suggests that at lower exposure concentrations organisms maintain metal excretion at or above metal uptake and detoxify the accumulating metals by sequestering them into nonbiologically active pools, such as metallothionein-like proteins or metal-rich granules. However, at high metal exposure concentrations, organisms take up metals at rates exceeding metal excretion, and/or metal levels in nonbiologically active pools reach a saturation point, with metal spilling over into biologically active pools, causing deleterious effects [29]. The advantage of deriving threshold body concentrations is that metal accumulation is a true measure of exposure and less dependent on water chemistry [30], and the individual contaminant causing the observed toxic effects can be identified [27,31]. However, these threshold body concentrations will vary with the balance between metal uptake and detoxification and efflux rates.

Threshold body concentrations remain scant in the open literature, especially for sensitive aquatic organisms. The amphipod *H. azteca* is routinely used in toxicity investigations because it is sensitive to metals [32] including *U* [3,15,33]. Among the 3 metals of interest downstream of U mines and mills, Ni has the most data on threshold body concentrations, which consider a wide range of hardness, pH, dissolved organic carbon, and metal contamination [10,26,30]. Regarding As, threshold body concentrations are more limited and often derived using city tap water [22,34]. For *U*, threshold body concentrations are also scant. Alves et al. [28] derived *U* uptake rates and critical body concentrations in juvenile *H. azteca* as a function of body size in water-only exposure. Alves et al. [35] also derived critical body concentrations in juvenile *H. azteca* when exposed to spiked sediments. However, they used city tap water instead of water with chemical characteristics similar to natural waters receiving treated effluent discharge from U mining and milling operations. Liber et al. [3] did measure toxicity of *U* to adult *H. azteca* in conditions relevant to lakes downstream of U mines and mills but only reported toxicity thresholds based on total sediment and porewater concentrations. In addition, these *U* toxicity studies used uranyl nitrate acidic solutions to spike sediments. Adding a uranyl nitrate acidic solution to sediments forms *U* precipitates under neutral pH and low carbonate concentrations typically encountered in natural sediments. However, U mills use carbonate leach to extract U from crushed ore, and the excess carbonate ends up in the circumneutral treated effluent and, once released, increases the pH and carbonate levels in the receiving environment. In such conditions, toxicity tests may underestimate the toxicity of *U*. Finally, these studies have not compared the sensitivity of juvenile and adult *H. azteca* to *U*. Therefore, current studies that derived critical body concentrations for *U*, As, and Ni do not consistently consider the sensitivity of the life stage of *H. azteca*, the pH and carbonate levels typically encountered downstream of U mines and mills, and that spiking procedures have to mimic as much as possible environmental exposure conditions.

Therefore, the objective of the present study was to derive *U*, Ni, and As toxicity thresholds for survival and growth of juvenile and adult *H. azteca* on the basis of body burden, overlying water, sediment porewater, and solids using water chemistry representative of water bodies downstream of Canadian U mines and mills [36].

**METHODOLOGY**

**Sediment collection**

Freshwater sediment was collected from Lake St. François (Coteau Landing, latitude 45°15'00.6", longitude 74°12'54.3") along the St. Lawrence River in Canada. Sampling and homogenization were carried out using the guidelines outlined in Environment Canada [37]. Two Ponar grabs were used, one with a 5-L capacity and the other with a 12-L capacity. Approximately 75 L of sediment was collected and stored the same day in a walk-in refrigerator at 4 °C. The following morning, all of the sediment was put into one large container and homogenized. All large debris, rocks, and visible gastropods were manually removed. The sediment was then separated into 4 buckets (20-L capacity each, lined with 2 polyethylene bags) and stored in the dark at 4 °C.

**Sediment preparation and spiking procedures**

Sediments were spiked in accordance with Environment Canada procedures [36]. First, the moisture content of sediments was measured by drying 3 subsamples of sediments at 100 °C for 24 h. The mean wet/dry weight ratio was then calculated and used for calculating the concentrations to be spiked.

The containers used for spiking the sediment were 1-L glass jars. A 500-mL subsample of sediment (mean dry wt/wet wt ratio 0.495) was added to each jar. Sediments were spiked with sodium m-arsenite (NaAsO₂; CAS no. 7784-46-5), nickel sulfate hexahydrate (NiSO₄ · 6H₂O; CAS no. 10101-97-0), or uranyl nitrate (UO₂(NO₃)₂ · 6H₂O; CAS no. 13520-83-7).

To ensure that U would remain in solution during the spiking process and thereby mix evenly with the sediment, chemical speciation modeling was conducted using PHREEQC. Modeling results showed that it was necessary to extract porewater from the sediments, spike the porewater with uranyl nitrate, and raise the carbonate levels and the pH from 2.93 to 6.74 with sodium bicarbonate prior to remixing of spiked porewater with sediment. Adding sodium bicarbonate ensured that all solid U species remained undersaturated and would not
precipitate from the low-pH stock solution immediately on contact with sediments. Specifically, a 200-mL volume of porewater was removed from 500 mL of sediment by centrifugation at 3000 rpm for 10 min at 4°C. The following day the porewater was mixed with an appropriate volume of stock solution and dilution water (for a total of 50 mL of diluted stock solution per 500 mL of sediment, as with the spiking of As and Ni), and the spiked porewater was adjusted to pH 6.7 or above with sodium bicarbonate.

All sediments were mixed by the wet sediment-rolling technique using an adapted version of the jar-rolling apparatus developed by Ditsworth et al. [38]. All 3 metals were mixed at 23°C for a minimum of 2 h on the first day, left upright overnight, and then mixed again the following morning for another 2 h.

To assess whether equilibrium had been reached, subsamples of overlying water were taken after 2 and 4 wk in the highest concentration treatment. Arsenic levels were 250 μg/L after 2 wk and 200 μg/L after 4 wk. Nickel levels were 120 mg/L in overlying water after 2 wk and 110 mg/L after 4 wk. Sediments appeared to have reached equilibrium after 4 wk, and the total equilibration time for the sediment was 8 and 10 wk for As and Ni, respectively. We did not measure when U reached equilibrium. Liber et al. [3] allowed spiked sediment to equilibrate for 3 wk, whereas Alves et al. [28,35] waited 4 wk before starting their U toxicity experiments. In the present study, sediments were allowed to equilibrate for 14 wk.

**Toxicity tests**

*Hyalella azteca* has been successfully cultured in our contracted laboratory for several years. The 14-d toxicity tests were conducted per the Environment Canada protocol [36] with the exception that we used 1-L glass jars with 200 mL of spiked sediment and 350 mL of overlying water instead of 100 mL of spiked sediment and 175 mL of overlying water. More overlying water was added to limit the transfer of recently spiked metals in sediments to the overlying water [39]. The tests were static with aeration, and the test vessels were checked daily to ensure that the aeration was adequate. Ten adults (>21 d) and 10 juveniles (2–9 d) were added to each test vessel. During the experiments, adult amphipods were fed a mixture of yeast, Cerophyll, and trout chow 3 times per week. We added twice the amount of food recommended for 10 juveniles.

The number of treatments and their concentrations of metals were determined based on the results of range-finding toxicity tests. For As, juvenile and adult amphipods were exposed to 6 treatments (control, 20, 40, 77, 145, and 324 mg As/kg dry wt) with 5 replicates per treatment (n = 60 test vessels). For Ni, juvenile and adult amphipods were exposed to 7 treatments (control, 130, 320, 580, 1100, 2100, and 4200 mg Ni/kg dry wt) with 5 replicates per treatment (n = 70 test vessels). For U, juvenile amphipods were exposed to 8 treatments (control, 5, 10, 30, 250, 710, 1400, and 1900 mg U/kg dry wt) with 5 replicates per treatment, and adult amphipods were exposed to 8 treatments (control, 5, 10,
of approximately 95 °C for 30 min. Extracted samples were transferred into a 100-mL amber glass and stored at 4 °C until analysis.

At the end of each test, all organisms alive were collected by sieving the sediments, purged of their gut content for a period of 4 h [41], counted, and dried at 60 °C for 24 h. Dried amphipods were pooled (i.e., replicates 1 and 2 and replicates 3–5 were combined together) to have 2 replicates per concentration. Dried amphipod replicates were transferred into a weighing cup and weighed on a microbalance to 0.01 mg. If there was insufficient survival of juveniles or adults (i.e., insufficient tissue weight, i.e., fewer than 5 surviving amphipods per replicate) to analyze 2 replicates, only one was analyzed. If there were fewer than 5 surviving individual juveniles or adults when replicates were combined, there was insufficient tissue weight to proceed with the analysis. Samples were placed in glass jars (previously rinsed with 10% nitric acid). Amphipods were digested in 70% ultrapure nitric acid for 7 d, then 30% hydrogen peroxide was added. Digestion was allowed to continue for another 24 h [42,43]. Distilled water was then added to each sample to make up a volume of 0.5 mL per sample in a 20-g glass jar and kept at 4 °C until analysis.

All samples were shipped to Philip Analytical Services and analyzed within 6 mo as required by USEPA SW486 method 6020 and USEPA series method 200.8. Metal levels in digested sediment solid extracts, extracted porewater, surface water, and digested amphipods were quantified by inductively coupled plasma–mass spectrometry.

Quality assurance and quality control of chemical analyses in water, sediment, and amphipod tissues consisted of mass calibration and resolution checks, internal standard intensity, interference check solution, calibration blank analysis, initial and continuous calibration verification, method blank, blank spikes, matrix duplicates, matrix spikes, matrix spike duplicates, dilution tests, and postdigestion spikes. Detection limits for metals in water, sediment, and amphipods are presented in Table 1.

Statistical analysis

Statistical analysis for both growth and survival was performed using TOXSTAT™. The results obtained with each measured concentration of metal in sediments, porewater, overlying water, and amphipod tissue were compared with the results obtained with the control amphipods to test whether there was a significant difference (p < 0.05). The statistical analyses used to determine if growth and survival for each metal were different from the control were the nonparametric Tukey test and the nonparametric Steel’s many-one rank test, respectively. Lethal concentration (LC) and lethal body concentration (LBC) for each metal was calculated using the LC50 Calculation Statistical Program [44]. For effect (i.e., growth inhibition) concentration and effect (i.e., growth inhibition) body concentration, the inhibition concentration method [45] was used to calculate the median inhibition concentrations for each metal.

We analyzed the relationship between metal concentrations in tissue residue and metal concentration in overlying water, sediment porewater, and solids as well as survival and growth with metal body burden using the linear regression function in Sigma Plot 8.0. All data were log-transformed to address the normality and homoscedasticity of residuals in our data sets. Survival and growth endpoints were left untransformed because the normality and homoscedasticity assumptions were met. We also compared the regression slopes of metal tissue residue with survival between adults and juveniles by an analysis of covariance (ANCOVA) using Systat, Ver 10. We also verified the assumptions of the ANCOVA by identifying any outliers, verifying the normality distribution of the data and the homogeneity of slopes.

Metal speciation calculations

Speciation of As, Ni, and U in water was calculated using the Windemere Humic Acid Model, Ver 7.0 [46]. The database of thermodynamic constants was verified against Guillaumont et al. [47] and the National Institute of Standards and Technology’s metal complex database [48].

RESULTS

Sediment toxicity to juveniles and adults

Because Lake St. François is exposed to anthropogenic sources of metals, we determined if these sediments were toxic

| Table 1: Arsenic, nickel, and uranium concentrations (dry wt) in adult and juvenile Hyalella azteca body and Lake St. François water and sediment |
|------------------------------------------------|
| **Variable** | **n** | **Value** | **Detection limit** | **Guideline** |
| Sediment     |      |          |                    |              |
| As mg/kg     | 3    | <5–6     | 5                  | 10*          |
| Ni mg/kg     | 3    | 23–61    | 5                  | 23*          |
| U mg/kg      | 3    | <0.1–1.2 | 0.1                | 104*         |
| Water        |      |          |                    |              |
| As μg/L      | 4    | <20      | 20                 | 5 [49]       |
| Ni μg/L      | 3    | <10–20   | 10                 | 25 [50]      |
| U μg/L       | 2    | 1–2      | 1                  | 15 [51]      |
| Background juvenile H. azteca body concentration |      |          |                    |              |
| As mg/kg     | 2    | 0.7–0.8  | 0.2                | —            |
| Ni mg/kg     | 2    | 1.9–2.6  | 0.1                | —            |
| U mg/kg      | 2    | <0.1     | 0.1                | —            |
| Background adult H. azteca body concentration |      |          |                    |              |
| As mg/kg     | 2    | 0.8–0.9  | 0.2                | —            |
| Ni mg/kg     | 2    | 0.9–1.2  | 0.1                | —            |
| U mg/kg      | 2    | <0.1–0.1 | 0.1                | —            |

*Lowest effect level published by Thompson et al. [7].
to amphipods. We first measured natural levels of As, Ni, and U in sediments of Lake St. François (Table 1). Arsenic and U were below or slightly above detection limits and below their respective lowest effect level [7]. Nickel in sediments was above the detection limit and above the lowest effect level value of 23 mg/kg (Table 1) [7]. We also measured As and Ni in overlying water of control tests on days 0 and 14 of range-finding tests and measured As, Ni, and U in controls on day 14 of the definitive toxicity tests (Table 1). In control experiments, As was below the detection limit (20 μg/L), which was, however, above the Canadian water quality guideline for the protection of aquatic life value of 5 μg/L [49]. Nickel ranged from below the detection limit to 2 times above it (detection limit = 10 μg/L) and remained below the Canadian water quality guideline for the protection of aquatic life value of 25 μg/L [50]. Uranium concentrations in overlying water were slightly above the detection limit (1 μg/L) but 10 times lower than the Canadian water quality guideline for the protection of aquatic life value of 15 μg/L [51]. Because Ni was above the lowest effect level and our As detection limit was above the Canadian water quality guideline for the protection of aquatic life value for As, we conducted toxicity tests on the natural sediments with H. azteca to confirm that the natural sediments were not toxic to the amphipod. There was 100% survival in all tests, indicating that Lake St. François sediments were not toxic to juvenile and adult amphipods.

We then verified that the salts added when spiking the sediments in the highest treatment would not contribute to any observed toxic effects in the definitive tests. Adult survival when exposed to sodium nitrate, sodium sulfate, and sodium bicarbonate was 80, 85, and 90%, respectively. In contrast, juvenile survival was 75% when exposed to all 3 salts at the highest concentration, which were slightly below the acceptable range of 80%.

Table 2 presents the range in water chemistry in the overlying water as well as the range in As, Ni, and U concentrations in amphipod tissue, overlying water, sediment porewater, and solids in all treatments. The pH generally ranged from 7.14 to 8.91, whereas temperature remained between 19.4 and 24°C in all treatments. Hardness ranged from 105 to 366 mg CaCO3/L. In the sediment solids, As ranged from 5 to 324 mg/kg dry weight, whereas its concentrations in sediment porewater ranged from 23 to 25,990 μg/kg, higher than in the overlying water where it ranged from 23 to 2996 μg/L. Nickel in sediments ranged from 59 to 4226 mg/kg dry weight, whereas its concentration in sediment porewater ranged from 18 to 58,994 μg/L, lower than in overlying water where Ni ranged from 12 to 88,050 μg/L. Uranium in sediments ranged from 1 to 1900 mg/kg dry weight, whereas its equilibrium concentration in sediment porewater was 1 to 60,928 μg/L, similar to concentrations in the overlying water where U ranged from 2 to 47,600 μg/L.

The toxicity results of As, Ni, and U based on concentrations in sediment, sediment porewater, and overlying water are presented in Table 3. Metals were less toxic to adult H. azteca than to juveniles, with LC50 values of 165 mg As/kg dry weight, 787 mg Ni/kg dry weight, and 214 mg U/kg dry weight for adults in comparison with LC50 values of 135 mg As/kg dry weight, 370 mg Ni/kg dry weight, and 48 mg U/kg dry weight for juveniles. The LC20 values exhibited a similar pattern, and the LC50 and LC20 values based on sediment porewater and overlying water also exhibited similar patterns (Table 3).

### Bioaccumulation of As, Ni, and U in juvenile and adult H. azteca

When investigating the link between bioaccumulation and toxicity, it is important to first account for the background accumulation of metals in amphipods exposed to natural (i.e., control) sediment. If metal accumulation in control experiments is appreciable, lethal and growth inhibition body concentrations may need to be corrected accordingly [22]. Under these background exposure conditions, juvenile H. azteca did accumulate 0.7 to 0.8 mg/kg dry weight, whereas adults accumulated 0.8 to 0.9 mg/kg dry weight (Table 1). For Ni, juveniles accumulated Ni 2 times more (1.9–2.5 mg/kg dry wt) than adults (0.9–1.2 mg/kg dry wt; Table 1). In amphipod tissues, U was below the limit of detection (Table 1). Bioaccumulation in tissue reported in Table 2 was therefore corrected for As and Ni background concentrations.

Bioaccumulation of As, Ni, and U by juvenile and adult H. azteca is presented in Figure 1. For juveniles, bioaccumulation of U was linearly related to U concentrations in sediment ($r^2 = 0.96$), in porewater ($r^2 = 0.98$), and in overlying water

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**Table 2**: Measured concentrations of arsenic, nickel, and uranium at the end of the 14-d experiments in all tested concentrations in surface and porewater, sediments, and body of Hyalella azteca and measured pH, hardness, and alkalinity levels at days 0, 7, and 14 in overlying water

| Metals | Stage | pH       | Temperature (°C) | Hardness (mg CaCO3/L) | Water (μg/L) | Porewater (μg/L) | Sediment (mg/kg) | Body content (mg/kg) |
|--------|-------|----------|------------------|-----------------------|-------------|------------------|------------------|---------------------|
| As     | Juvenile | 7.99–8.71 | 20.8–24.0 | 144–270 | 23–2996 | 23–25,990 | 5–324 | 0.7–5.9 |
|        | Adult  | 7.14–8.91 | 19.8–23.2 | 105–366 | 23–2996 | 23–25,990 | 5–324 | 1.6–71 |
| Ni     | Juvenile | 7.42–8.58 | 19.4–23.5 | 124–330 | 12–88,050 | 18–58,994 | 59–4226 | 1.9–19 |
|        | Adult  | 7.98–8.72 | 21.0–23.5 | 105–366 | 12–88,050 | 18–58,994 | 59–4226 | 0.9–95 |
| U      | Juvenile | 7.48–8.74 | 20.8–22.8 | 184–255 | 2–47,600 | 1–60,928 | 1–1900 | 0.1–1.8 |
|        | Adult  | 7.12–8.77 | 20.4–24.9 | 189–344 | 2–47,600 | 1–60,928 | 1–1900 | 0.1–28 |
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(\( r^2 = 0.96 \)). Bioaccumulation of Ni was also strongly related to Ni concentrations in water (\( r^2 = 0.84 \)), sediment porewater (\( r^2 = 0.84 \)), and solids (\( r^2 = 0.87 \); Figure 1). For As, the relationship between tissue concentrations and sediment concentrations was weaker (\( r^2 = 0.67 \)) than for either Ni (\( r^2 = 0.87 \)) or U (\( r^2 = 0.96 \)). Contrary to Ni and U, As bioaccumulation was only weakly related to overlying water As concentrations (\( r^2 = 0.38 \)). In porewater As could not be linked to accumulation in juveniles because As was below the limit of detection.

In adult *H. azteca*, a similar pattern of bioaccumulation was observed, with U body concentrations being lower than both As and Ni tissue concentrations (Figure 1). Bioaccumulation of U and Ni was again linearly related to U and Ni concentrations in sediment (U, \( r^2 = 0.98 \); Ni, \( r^2 = 0.91 \)), porewater (U, \( r^2 = 0.98 \); Ni, \( r^2 = 0.90 \)), and overlying water (U, \( r^2 = 0.98 \); Ni, \( r^2 = 0.93 \)). Similar to juveniles, the regression coefficients for the relationship between As bioaccumulation in adult *H. azteca* to concentrations in test media were lower than for either U or Ni.

We noted that for all metals juveniles had higher tissue concentrations than at the lower adult exposure concentrations. However, as metal concentrations in the exposure media increased, accumulation of metals became higher in adults than in juveniles (Figure 1), mostly because adults survived to higher exposure concentrations. For instance, at the lowest 3 treatments, As concentrations in juveniles ranged from 0.7 to 2.2 mg/kg dry weight, similar to concentrations in adults, which ranged from 0.5 to 1.8 mg/kg dry weight (Figure 1). At the next 2 higher exposure treatment concentrations, As body concentration in juveniles increased from 2.2 to 5.9 mg/kg dry weight before death in the sixth treatment, whereas adult body concentrations reached 71 mg/kg dry weight in the sixth treatment. Similarly, in the 3 lowest treatments, Ni concentrations in juveniles ranged from 2.2 to 15.0 mg/kg dry weight, whereas concentrations in adults ranged from 1.0 to 4.4 mg/kg dry weight (Figure 1). In contrast, Ni body concentration in juveniles decreased to 12 mg/kg dry weight in the fourth treatment and then all individuals died in the fifth treatment, whereas adult body concentrations reached 75 mg/kg dry weight in the fifth treatment before all individuals died in the sixth treatment. As for U, U concentrations in juvenile ranged from 0.1 to 1.8 mg/kg dry weight, similar to the U concentrations in adults, which ranged from 0.1 to 1.3 mg/kg dry weight in the first 3 treatments (Figure 1). Then, at higher U concentrations in the exposure media, no juveniles survived; therefore, U was only measured in adults and increased from 3.8 to 21.9 mg/kg dry weight in the next 3 treatments. Finally, in both juvenile and adult *H. azteca*, bioaccumulation of U was lower than As and Ni bioaccumulation.

**Bioaccumulation and survival**

Because As, Ni, and U increased in amphipods with increasing exposure concentrations, we proceeded with linear regression analyses between metal body concentration and survival of juveniles and adults. An increase in metal body burden resulted in a statistically significant decrease in survival of juveniles and adults exposed to As, Ni, and U, with regression coefficients values ranging from 0.55 (Ni, adult) to 0.92 (U, adult; Figure 2). We then derived a LBC50 of 5 mg As/kg dry weight for juveniles and an LBC50 of 36 mg As/kg dry weight for adults (Table 3). For Ni, our LBC50 values for juveniles and adults were 14 mg/kg and 49 mg/kg dry weight.
Statistically different (slope of regressions between adults and juveniles were 32 mg/kg, 44 mg/kg, and 1.2 mg/kg. Figure 3). The calculated IBC20 values for As, Ni, and U were in metal body concentrations resulted in a statistically significant weight. In contrast, there was no relationship between As causing a 20% growth inhibition (i.e., IBC20) was 7 mg/kg dry weight, respectively. For U, we derived LBC50 values of 0.4 mg/kg and 1.0 mg/kg dry weight for juveniles and adults, respectively (Table 3). Both the LBC20 and LBC50 values indicated that juveniles were more sensitive to metals than adults. Mean survival of juveniles was significantly lower than that of adults, as confirmed by ANCOVA, suggesting that adults. Mean survival of juveniles was significantly lower than that of adults, as confirmed by ANCOVA, suggesting that juveniles were more sensitive to As, Ni, and U than adults.

The LBC50 indicates that U is more toxic than As, which is more toxic than Ni to H. azteca. An ANCOVA using metals as covariate indicated that the intercepts of the slopes of survival as a function of U body concentrations in adult and juveniles were significantly different from the slopes for As and Ni, confirming that the amphipod was more sensitive to U than As and Ni. An additional ANCOVA using only Ni and As as covariates indicated that amphipods were significantly more sensitive to As than Ni (p = 0.001). The same order of toxicity of these metals was observed when toxicity was expressed on the basis of overlying water, sediment porewater, and total metal concentration in sediment (Table 3).

**Bioaccumulation and growth**

In addition to survival, we investigated the effects of metal tissue residue on growth of juvenile and adult amphipods. For juveniles, only Ni body burden was linked to growth with a regression coefficient (r²) of 0.83. Hence, the body concentration causing a 20% growth inhibition (i.e., IBC20) was 7 mg/kg dry weight. In contrast, there was no relationship between As and U body concentrations and growth of juveniles. An increase in metal body concentrations resulted in a statistically significant decrease in growth of adults exposed to As, Ni, and U (p < 0.05; Figure 3). The calculated IBC20 values for As, Ni, and U were 32 mg/kg, 44 mg/kg, and 1.2 mg/kg.

An ANCOVA using life stage as covariate indicated that the slope of regressions between adults and juveniles were statistically different (p = 0.004). Juvenile growth decreased more markedly than that of adults, despite accumulating similar Ni levels in their tissues. An ANCOVA using metals as covariate also indicated that slopes and intercepts were statistically different (p < 0.05). Adult growth was more affected by Ni than As or U as indicated by a statistically significantly steeper slope (Figure 3). However, control individuals in the U experiment were lighter than those in the Ni and As experiment, which may explain the decrease in weight gain with increasing metal exposure.

**DISCUSSION**

**Bioaccumulation and toxicity to juveniles and adults**

The objective of the present study was to derive U, Ni, and As sediment toxicity thresholds for survival and growth of juvenile and adult H. azteca based on metal concentrations in tissue residues, overlying water, sediment porewater, and solids in water with chemical characteristics representative of water bodies downstream of Canadian U mines and mills.

The present results show that bioaccumulation of U, As, and Ni in both juvenile and adult H. azteca is equally well predicted by water, porewater, and sediment metal concentrations. These results are expected because it is the dissolved metal that accumulates in aquatic organisms, with metal accumulation being mediated first by desorption of the metal from sediment into porewater and overlying water, where it is taken up by amphipods. We noted that juveniles accumulated more metals than adults at low metal exposure concentrations. However, when metal exposure increased further, juveniles accumulated less metal and appeared to be adversely affected at lower concentrations than adults, as indicated in Figures 2 and 3. Juveniles accumulate less metal at higher concentrations, likely because at a certain exposure threshold their metal uptake
rate overwhelms their detoxification and efflux mechanisms, which leads to metal spillover. In such conditions, excess metal binds to proteins essential for metabolism and metal uptake rate begins to decrease, which then results in cascading effects that can reduce growth, leading to exhaustion and eventual death. Figures 2 and 3 indicate that adults are tolerant to a higher range of metal exposure than juveniles before experiencing reduction in growth or death.

The present higher toxicity values for adults based on body burden, overlying water, sediment porewater, and solids compared with juveniles further indicate that juveniles are more sensitive to metals than adults (Table 2). The present results are well aligned with findings reported elsewhere suggesting that early life stages are the most sensitive to metals [15,35,52,53]. Juveniles are likely more sensitive to metals because they are smaller, and their detoxification mechanisms are sometimes immature and less able to cope with sudden increases in metal uptake. As a result, juveniles will experience toxic effects sooner than adults at a given exposure and at lower exposure concentrations.

The significant relationship between bioaccumulation and toxicity in juvenile and adult amphipods suggests that bioaccumulation leads to toxic effects. As a result, measuring metal concentrations in organisms to assess sediment toxicity can be another line of evidence to support the identification of the contaminant potentially responsible for benthic invertebrate community impairment. However, as Rainbow [54] explained, metal accumulation by organisms and internalization to their organs do not necessarily translate readily into a toxic response. A toxic response is proportional to the rate of uptake and how detoxification rates can cope with sudden changes in uptake rates [29,54]. Consequently, a combination of toxicity benchmarks, field bioaccumulation data, and uptake and detoxification rates along with information on metal partitioning in sediments and on chemistry of surface water and porewater would support more robust assessments of the cause of benthic invertebrate community impairment than relying mainly on toxicity based on total metal concentration in sediments.

Relative toxicity of U, As, and Ni

A secondary objective of the present study was to provide data on the relative toxicity of As, Ni, and U to strengthen our understanding of which metal may be responsible for the localized impacts to the benthic invertebrate communities downstream of U mines and mills [1]. Results from the present study on the toxicity of As, Ni, and U based on tissue residues, overlying water, sediment porewater, and solids indicate that U is more toxic than As and Ni (Table 3). Even if total U concentrations in overlying water, sediment porewater, and solids were similar to concentrations of As and Ni, both juvenile and adult amphipods consistently accumulated less U than either As or Ni (Figure 1). Amphipods accumulated less U because the U carbonate complex was 10 000 times more abundant than the more bioavailable free uranyl ion [12], whereas free As and Ni ions were dominant species in water. These differences in bioavailability are supported by lower water/amphipod bioaccumulation factor values for U than for either As or Ni.
Despite U being less bioavailable, resulting in lower accumulation by adult and juvenile amphipods, U remained approximately 100 times more toxic to amphipods than either As and Ni. In fact, juvenile mortality was complete at the 2 highest U concentrations in our survival experiment (Figure 3). This contrasts with the finding of Liber et al. [3], who reported that U was less toxic to amphipods than As and Ni. We can explain the difference in U toxicity in the present study and the study of Liber et al. [3] by the spiking procedures. Although both studies used uranyl nitrate salts to spike the sediments, we spiked the poraware with sodium carbonate, which increased carbonate and pH. Otherwise, U would have precipitated immediately on contact with the natural sediments. Liber et al. [3] directly spiked their sediments with U nitrate salts, and considering that porewater pH was 6.3 and that carbonate species availability was more limited, an appreciable proportion of U precipitated and was likely not available for uptake [14], thus explaining the apparent higher tolerance of H. azteca to U in their study. It has been shown that U accumulation and toxicity are linked to the dissolved metal [12–14] rather than to the solids. Hence, we modified our spiking method by adding sodium bicarbonate to ensure that partitioning of U was not being controlled by the pH of the spiking solution and the low availability of carbonate resulting in the formation of solids during the sediment spiking procedure.

CONCLUSION

In conclusion, sediment toxicity calculated based on metal concentrations in tissue residues, overlying water, sediment porewater, and solids consistently indicated that juveniles were more sensitive to the 3 tested metals than adults. In addition, under our experimental conditions, U was found to be more toxic than As, which was more toxic than Ni. Uranium was more toxic than As and Ni because our U spiking procedures minimized precipitation of U, which raises the issue of how to best spike sediments when assessing the toxicity of U.

The assessment of sediment toxicity is often based on total metal concentration. The present results advance our understanding of As, Ni, and U toxicity in sediments. Further assessments of the cause of benthic invertebrate community impairment should include the derivation of toxicity benchmarks for juveniles based on metal concentrations in tissue residues, water, sediment porewater, and solids with appropriate sediment spiking procedures to natural sediments. In addition, it may be desirable to further investigate uptake and detoxification rates by juveniles along with information on metal partitioning in sediments and on chemistry of surface water and porewater.

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