Environmental Pollution 239 (2018) 359–366

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Uranium toxicity to aquatic invertebrates: A laboratory assay☆

Melissa Bergmann [a, *], Olimpia Sobral [a], João Pratas [b], Manuel A.S. Graça [a]

[a] MARE, Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[b] MARE, Marine and Environmental Sciences Centre, Department of Earth Sciences, University of Coimbra, 3030-790 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 30 September 2017
Received in revised form
24 March 2018
Accepted 2 April 2018

Keywords:
Shredders
Sublethal effects
Bioaccumulation
Mining

ABSTRACT

Uranium mining is an environmental concern because of runoff and the potential for toxic effects on the biota. To investigate uranium toxicity to freshwater invertebrates, we conducted a 96-h acute toxicity test to determine lethal concentrations (testing concentrations up to 262 mg L^{-1}) for three stream invertebrates: a shredder caddisfly, *Schizopelex festiva* Rambur (Trichoptera, Sericostomatidae); a detritivorous isopod, *Proasellus* sp. (Isopoda, Asellidae); and a scraper gastropod, *Theodoxus fluviatilis* (Gastropoda, Neritidae). Next, we ran a chronic-toxicity test with the most tolerant species (*S. festiva*) to assess if uranium concentrations found in some local streams (up to 25 μg L^{-1}) affect feeding, growth and respiration rates. Finally, we investigated whether *S. festiva* takes up uranium from the water and/or from ingested food. In the acute test, *S. festiva* survived in all uranium concentrations tested. LC_{50}-96-h for *Proasellus* sp and *T. fluviatilis* were 142 mg L^{-1} and 24 mg L^{-1}, respectively. Specimens of *S. festiva* exposed to 25 μg L^{-1} had 47% reduced growth compared with specimens under control conditions (21.5 ± 2.9 vs. 40.6 ± 4.9 μg of mass increase $animal^{-1} \cdot day^{-1}$). Respiration rates (0.40 ± 0.03 μg $O_2 \cdot h^{-1} \cdot mg\ animal^{-1}$) and consumption rates (0.54 ± 0.05 μg μg $animal^{-1} \cdot day^{-1}$; means ± SE) did not differ between treatments. Under laboratory conditions *S. festiva* accumulated uranium from both the water and the ingested food. Our results indicate that uranium can be less toxic than other metals or metalloids produced by mining activities. However, even at the low concentrations observed in streams affected by abandoned mines, uranium can impair physiological processes, is bioaccumulated, and is potentially transferred through food webs.

1. Introduction

The world energy demand is expected to increase by 48% from 2012 to 2040, with non-fossil renewable energy and nuclear power expected to increase by 2% per year during the same period (EIA, 2016). Currently, nuclear power contributes about 7% of the global energy demand (Asif and Muneer, 2007), with a tendency to increase. The nuclear energy demand involves mining of more than 100,000 tons of uranium during the next 15 years (Dittmar, 2012), with consequent production of mining wastes.

Extensive information is available on the environmental effects of several metals and metalloids, but comparatively little is known about the effects of uranium. Although uranium appears to be toxic at high concentrations, it is a non-essential metal that accumulates on aquatic biota even in low levels and concerns to protect it must be considered. For the protection of freshwater life under chronic (long-term) exposure, Canadian Water Quality Guidelines for Uranium (CWQG) recommends a maximum of 15 μg L^{-1}, and 33 μg L^{-1} for short-term exposure during transient events (CEQG, 2011).

Exposure of aquatic invertebrates to uranium may cause induction of reactive oxygen species (ROS), leading to DNA damage (Simon et al., 2011) Uranyl ions bind to nucleotides through phosphoric groups (ATP-UO_2^{2+}), compete with calcium and magnesium ions and inhibit ATPase activity and ATP production (De Stefano et al., 2005). Uranium also indirectly affects the heme group of oxyhemoglobin, interfering with O_2 binding (Kumar et al., 2016). The U-hemoglobin complex can be a pathway for uranium to enter animal organs that are not directly exposed (Bucher et al., 2016). Tagliaferro et al. (2018) found that exposure of the caddisfly shredder *Calamoceras marsupus* to 50 μg L^{-1} of uranium decreased Na^+/K^+ ATPase activity, an enzyme related to signal transduction and regulation of cell growth (Xie and Askari, 2002). Regarding to physiological effects, studies have reported that invertebrate

☆ This paper has been recommended for acceptance by Dr. Harmon Sarah Michele.

* Corresponding author.
E-mail address: mbergmann@student.uc.pt (M. Bergmann).

https://doi.org/10.1016/j.envpol.2018.04.007
0269-7491/

exposure to uranium results in malformations, reduction on development time and survival (Dias et al., 2008; Lagauzère et al., 2009), and reduced growth and reproduction (Beaudouin et al., 2012; Mooney et al., 2016). For instance, decreased growth at 300 μg L^{-1} in water was reported in *Chironomus tentans* for 9 days (Muscatello and Liber, 2010), and at 27 μg L^{-1} in *Sericostoma vittatum* for 60 days (Gonçalves et al., 2011), while the inhibitory concentration for growth (IC_{50}, 10 days) for *Chironomus dilutus* was 0.91 mg L^{-1} of uranium (Liber et al., 2011).

Although these studies reported some physiological effects at sublethal concentrations of uranium, we still need to know whether environmentally realistic levels found in streams are high enough to affect biological processes, and whether uranium is accumulated by stream-dwelling consumers. To address these questions, we first conducted a 96-h acute toxicity assay to determine lethal uranium concentrations for three stream-dwelling invertebrates, the shredder *Schizopelex festiva* Rambur, 1842 (Trichoptera, Sericostomatidae), the detritivore *Proasellus* sp. (Isopoda, Asellidae), and the scraper *Theodoxus fluviatilis* Linnaeus, 1758 (Gastropoda, Neritidae). We then exposed the most tolerant species, *S. festiva*, for 37 days to uranium concentrations similar to those found in streams affected by abandoned uranium mines in Portugal, to test for effects on survivorship, feeding, growth and respiration rates. Finally, we investigated if *S. festiva* accumulates uranium, and if so, whether it is taken up from the water or from the ingested food. We selected the *S. festiva* species because Sericostomatidae are common leaf-shredders in Europe, playing an important role on organic matter cycling in low order streams (Feio and Graça, 2000). If the most tolerant species is affected by environmental realistic concentrations of uranium, them all other species will likely be affected as well.

2. Material and methods

2.1. Invertebrates and water

Specimens of *S. festiva* were collected from sandy substrates in a reference stream at Múceres (N 40° 32′ 01″; W 08° 09′ 15″, pH 6.9, 0.35 ± 0.10 μg L^{-1} of uranium in the water and 1.40 ± 0.50 mg kg^{-1} in the sediments). *Proasellus* sp. were collected from a stream in Póvoa de Luzianes (N 40° 30' 43″ W 07° 49' 02″, pH 7.0, conductivity: 156.50 ± 17.70 μS/cm, 2.20 ± 1.60 μg L^{-1} of uranium in the water and 24.40 ± 7.10 mg kg^{-1} in the sediments). *T. fluviatilis* were sampled at the Anços River source (N 39° 58′ 43″ W 08° 34' 23", pH 7.3, conductivity: 539 μS/cm, 0.25 ± 0.08 μg L^{-1} of uranium in the water). The specimens, leaf litter, and some stones with periphytic algae (in the case of *T. fluviatilis*) were transported in stream water to the laboratory and acclimated for 5 days with aeration, 18 ± 1 °C and photoperiod of 14:10 h L:D. On day 3, half of the stream water was replaced by standard water to be used in the test. The standard water was based on the USEPA international recommendations for moderately hard water (Lewis et al., 1994): $MgSO_4$ (120 mg L^{-1}), $NaHCO_3$ (96 mg L^{-1}), KCl (4 mg L^{-1}), and $CaSO_4.2H_2O$ (60 mg L^{-1}). During acclimation, invertebrates were fed with alder leaves (*Alnus glutinosa*) conditioned in the laboratory (as described below). At the end of day 5, the invertebrates were transferred to cups containing the test solutions. As source of uranium we used uranyl nitrate hexahydrate ($N_2O_8U.6H_2O$) for acute and sublethal tests.

2.2. Leaf conditioning

To feed the invertebrates in all chronic tests and for biosorption of uranium assay, senescent air-dried *A. glutinosa* leaves from Mondego River Park (Coimbra, Portugal) were microbially conditioned in the laboratory. This was done by inoculating a ~6 L aquaria with stream water and litter at different decomposition stages (from Múceres, Central Portugal) as inoculum. Leaves were enclosed into 500 μm-mesh bags (19.50 × 13 cm), ~30 g/bag and conditioned for 7 days under strong aerations. Stream water was changed every two days to prevent accumulation of litter leachates.

2.3. Acute (96-h) toxicity test

We tested 10 uranium concentrations, 4 replicates of 5 specimens each. We selected invertebrates with similar sizes in terms of case, body size and shell length for *S. festiva*, *Proasellus* sp., and *T. fluviatilis*, respectively. We used 200 specimens of each species, with biomasses of 2.30 ± 0.08 mg (mean ± SE; dry weight) in the case of *S. festiva*, 0.75 ± 0.035 mg (length 5.35 ± 0.09 mm) in the case of *Proasellus* sp., and 2.50 ± 0.46 mg (5.0 ± 0.62 mm shell length) in the case of *T. fluviatilis*. The test vessels were plastic cups, 10 cm high, 5.5 cm diameter, containing 150 mL test solution (see below). For *S. festiva,* we also added 10 g of stream sand, previously sieved through a 1-mm mesh, incinerated at 450 °C for 8 h and washed in distilled water.

Uranium treatments for *S. festiva* and *Proasellus* sp. ranged from 0 to 262 mg L^{-1}. Nominal concentrations were 0.004 mg L^{-1} and multiples of 4 up to 262.14 mg L^{-1} (9 concentrations plus control). However, at the end of the experiments the final concentrations in water were in average 0.0025, 0.014, 0.044, 0.121, 0.634, 2.67, 10.77, 38.20, and 254.50 mg L^{-1} (reduction of 32 ± 15%). Uranium concentrations in test with *T. fluviatilis* ranged from 10.0 mg L^{-1} to 75.9 mg L^{-1} (1.5 × increases) based on a preliminary assay in which no mortality occurred below 10.0 mg L^{-1} and 100% mortality occurred above 75.9 mg L^{-1}. Survivorship was measured every 24 h. Mortality (%) was calculated as the number of dead organisms divided by initial number of individuals, multiplied by 100 (n = 20 specimens/concentration). The pH, dissolved oxygen and conductivity were documented daily in a random set of cups. At the end of the test, the water from each microcosm was acidified with 65% HNO_3 to pH = 2 (v/v) and stored at 4 °C until analysis of uranium.

2.4. Sublethal tests − Growth, consumption and respiration rates

To test for sublethal uranium toxicity, we performed three assays to measure growth, food consumption and respiration rates by *S. festiva*, the most tolerant species from the previous experiment. To test the environmentally realistic concentrations, we measured the uranium concentrations in several streams affected by abandoned uranium mines in central Portugal, and obtained values from the literature for rivers in the region (Table 1; Table S1). Since the mean levels of uranium from polluted streams ranged from 1.98 to 35.45 μg L^{-1}, we exposed *S. festiva* to 25 μg L^{-1} and 0 μg L^{-1}.

For growth and consumption assays, *S. festiva* specimens were individually allocated to cups (150 mL test water, replaced every 2 days), 20 replicates for each concentration, 25 μg L^{-1} and 0 μg L^{-1} of uranium. To measure size, we regressed the case opening (CO, mm) on body dry mass (W, mg): W = (8.27 × CO) − 15.366; n = 42, $R^2 = 0.90$, $p < 0.001$. Case opening was measured in a stereoscopic microscopic (ocular with graduated scales) at 16 ×. To feed the specimens, we conditioned (see above) and soaked alder leaves for 48 h in the testing uranium concentrations. The initial body mass was estimated by regression from a set of specimens not used in the tests, and the final dry mass was obtained by weighing the tested specimens after 37 days. Growth rate (μg·mg $animal^{-1}$·day^{-1}) was determined as:

$$GR = \frac{\ln(Wf/Wi)}{t}$$