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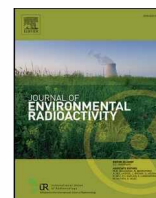
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Chronic toxicity of uranium to three benthic organisms in laboratory spiked sediment

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ABSTRACT

Due to mining activities, concentration of uranium (U) in the environment nearby former and operating sites can be higher than in other areas. The derivation of quality criteria for U in freshwater ecosystems, rivers and lakes includes the consideration of contaminated sediments and the associated risk to the benthic life. Therefore, the derivation of a quality criteria for sediment has been viewed as a logical and necessary extension of the work already done to establish water quality criteria. In order to contribute to the determination of a Quality Standard for sediment (QS_{sediment}) according to the European recommendations, this study focuses on the acquisition of a new toxicity dataset, to enrich the few rare existing data, most often unsuitable. A basic set of organisms, including three complementary benthic organisms (*Chironomus riparius*, *Hyalella azteca*, *Myriophyllum aquaticum*), was chronically exposed to U spiked to a standard laboratory-formulated sediment, according to the related bioassay guidelines (ISO/FDIS16303, OECD 218/9, ISO/DIS 16191). We looked to determine when possible both NOEC and EC₁₀ values for each organism. For *C. riparius*, a NOEC (emergence rate) value was estimated at 62 mgU, kg⁻¹, dm and the EC₁₀ value reached 188 mgU, kg⁻¹, dm (CI_{95%} 40–885 mgU kg⁻¹, dm). For *H. azteca*, a NOEC (survival rate) value of 40 mgU kg⁻¹, dm was observed while the EC₁₀ value at 296 mgU kg⁻¹, dm (CI_{95%} = 155–436 mgU kg⁻¹, dm) was slightly higher than for growth at 199 mgU kg⁻¹, dm (CI_{95%} = 107–291 mgU kg⁻¹, dm). Finally, the less sensitive organism seemed to be the plant, *M. aquaticum*, for which we determined a NOEC value of 100 mgU kg⁻¹, dm. Results obtained regarding the toxicity of U made it possible to suggest a preliminary QS_{sediment} value of 4 mgU kg⁻¹, dry mass. This value was shown conservative compared to U sediment quality criteria derived by other jurisdictions.

1. Introduction

Sediments are well known to act in freshwater ecosystems as sink or source of contamination, questioning the protection of aquatic resources from their potential toxicity (Loska and Wiechula, 2003). Contaminated sediments pose a risk to the aquatic organisms (Ciutat and Boudou,

2003; Lagauzère et al., 2014; Méndez-Fernández et al., 2014; Stesevic et al., 2007; Vandegheuchte et al., 2013). Sediment toxicity depends not only on the physico-chemical characteristics of the contaminant and the sediments (Chapman, 2007; Di Toro et al., 2005; Méndez-Fernández et al., 2014; Roman et al., 2007; Väänänen et al., 2018), but also on the biological characteristics of the exposed benthic animal and plant

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Table 1Nominal uranium sediment concentrations (mgU kg⁻¹, dry mass), number of replicates and test duration for each bioassay.

Bioassays	U concentration mg kg ⁻¹ , dry mass							Replicates for each treatment			Test duration (d)	
	0	3.9	7.8	15.6	31	62	125	250	500	1000		
<i>C. riparius</i>	0	3.9	7.8	15.6	31	62	125	250	500	1000	4	28
<i>H. azteca</i>	0	40	80	130	200	250	300	444	667	1000	5	14
<i>M. aquaticum</i>	0	12.5	25	50	400	500					4	15

species (uptake rate, assimilation efficiency, feeding behaviour). Benthic invertebrates, in particular polychaetes and amphipods, have potential for sediment toxicity tests because of their privileged exposure to sediments either through their burrowing activity or via the ingestion of particles (Dias et al., 2008; Leppänen et al., 2006; Vandegheuchte et al., 2013). As the uptake and so toxicity likely differs between epibenthic burrowing amphipods or deeper burrowing polychaetes, the use of multiple species and endpoints (survival, growth, reproduction) is necessary to estimate the sediment toxicity (Chapman, 2007; EC-TG N°27, 2011; Sheppard et al., 2005). Plants represent autotrophic life forms which also present a high potential to detect toxicity caused by particulate-bound pollutants (Stesevic et al., 2007).

Uranium (U) was recently the subject of toxicity studies in order to derive quality criteria for ecological risk assessment (Beaugelin-Seiller et al., 2015; Caetano et al., 2014; Crawford et al., 2018; Goulet et al., 2011; Sheppard et al., 2005; Thompson et al., 2005). The implementation of the Water Framework Directive in the European Union (European Commission, 2000) is notably based on the determination of Environmental Quality Standards (EQS) as threshold value below which no expected adverse effect occurs to aquatic ecosystems. The compliance with EQS aims to prevent the deterioration of ecosystems and to enhance the status of aquatic ecosystems, to promote sustainable water and to reduce pollution from priority substances listed within the European Water Framework Directive (European Commission (2008)). EQS is determined based on Quality Standards (QS), themselves derived for different protection objectives based on direct ecotoxicity to pelagic aquatic organisms, secondary poisoning of predators, human consumption of fishery products, human consumption of drinking water and to protect benthic sediment-dwelling species. When the data are sufficient, QSs are derived from short- and long-term toxicity data by probabilistic approach adopting Species Sensitivity Distribution (SSD) modelling. Otherwise, a deterministic approach is used applying appropriate assessment actors (AF) to the lowest reliable toxicity data.

High concentrations of uranium have also been found in sediment (median concentration of 2 mgU kg⁻¹ with a range from 1 to 90 mgU kg⁻¹ in Europe (IRSN, 2010)) and may lead to toxicity for benthic species, depending on the physico-chemical (redox, pH, complexes with hydroxides, carbonates, organic matter) properties of this environment and biological characteristics of organisms (filtering behaviour, burrowing activity, microbial activity, bioturbation) (Goulet et al., 2011; Lagauzère et al., 2014).

Regarding sediment ecotoxicity of U, the in-depth review of Sheppard et al. (2005) recommended a Predicted No Effect Concentration (PNEC) of 100 mgU kg⁻¹, dry mass (dm) for sediment, based on the Canadian methodology for setting sediment guidelines. The recommended value is in line with the lowest effect level determined by Thompson et al. (2005) using the same methodology (32–104 mg kg⁻¹). A complementary analyse of the most recent literature from laboratory experiments made possible to determine the range of the QS_{sediment} values, between 5.3 × 10⁻³ mgU kg⁻¹, dm (calculated from one EC₅₀ = concentration at which an effect of 50% is observed, to which an assessment factor equal to 1000 was applied) (Dias et al., 2008) and 7.4 mgU kg⁻¹, dm (calculated from one NOEC - No Observed Effect Concentration, to which an assessment factor of 100 was applied) (Liber et al., 2011). Note that the acquisition of the associated underlying ecotoxicity data did not rigorously follow OECD guidelines. *In situ* studies showed that the lowest no-effect values, based on invertebrate abundance and taxon richness, for recommended application at

Saskatchewan (Canada) uranium operations were 1 mgU kg⁻¹, dm (Burnett-Seidel and Liber, 2013). This obvious data scarcity leads to large uncertainties in determining benchmarks (ranging on three orders of magnitude), calling for the acquisition of complementary and robust information following international guidance.

The main goal of our study was to acquire the minimal ecotoxicity dataset required to derive a QS_{sediment} for uranium in freshwater sediments, from robust and consistent results of dedicated 3 ecotoxicity assays following OECD and ISO guidelines. Chronic U ecotoxicity was assessed for three taxa with an artificial U-spiked sediment. Tested species were chosen from applicable guidelines to encompass a variety of feeding and life strategies. Both lethal and sub-lethal endpoints were evaluated for the amphipod *Hyalella azteca* (mortality and growth; ISO/FDIS16303), the midge *Chironomus riparius* (emergence rate; OECD 218/9) and the macrophyte *Myriophyllum aquaticum*, (growth rate; ISO/DIS 16191). In addition to the guidelines recommendations, the distribution of U in the system was characterised to qualify the actual level of exposure by checking the concentrations of U both in pore water and sediment.

2. Materials and methods

2.1. Sediment preparation and spiking procedure

The same sediment was used for both animal assays in order to ensure identical chemical characteristics and associated U bioavailability. Our choice was to use for both animals the sediment composition proposed in the OECD protocol N°218/9 (75% quartz sand (100–300 μm), 20% kaolinite clay and 5% sphagnum moss peat (<1 mm)). The sediment composition used for the plant was slightly different with 74% quartz sand (100–300 μm), 1% calcium carbonate, 20% kaolinite clay and 5% sphagnum moss peat (<1 mm) as indicated in the guideline ISO/DIS 16191. The sediment constituents were dry-mixed in a rotary shaker (Turbula® Type 12C, Switzerland) at room temperature for 3 h. All tests were conducted in 201 cm² surface area experimental unit (EU) containing 125 g sediment dry mass (dm) mixed with 40 ml of ultrapure water. The pH in the system was manually adjusted to 7–7.5 by adding HNO₃ solution over time.

Experimental units were completed with overlying water whose chemical composition was that recommended by guidelines (ISO/FDIS16303, OECD 218/9, ISO/DIS 16191). Volumes of 400 ml and 20 ml of overlying water were added to the EUs, for animals and plants respectively. For the toxicity bioassays, U-spiked sediment was equilibrated with overlying water for 10 days in order to achieve near-equilibrium conditions for U distribution before introducing organisms.

U-spiked sediment was prepared by mixing thoroughly by a 10 min hand-shaking a solution of U (UO₂(NO₃)₂, 6H₂O, Sigma-Aldrich, 99.65%²³⁸U, dissolved in ultrapure water (40 ml)) with the sediment. Quantities of U were adjusted in 40 ml to obtain the desired range of concentrations from 0 to 10,000 mgU kg⁻¹, dm (Table 1), embracing the previously proposed PNEC of 100 mgU kg⁻¹, dm. During a preliminary test, U concentrations in sediment were checked before introducing overlying water and organisms. U concentrations were measured in four UE (replicates) per concentration. Three analyses were also performed in one UE per concentration (intra-replicate). Variability of U concentration in sediment was evaluated between the 4 replicates and between the 3 intra replicates inside one EU.

Before starting toxicity bioassays, a second preliminary test was

performed to characterize the time needed to achieve an apparent equilibrium of uranium distribution between sediment solid phases and water. Uranium concentrations in overlying water were measured over time (1 d, 5 d) at two depth locations (water/sediment interface and mid-water column) in EUs without organisms, for the 3 lowest concentrations of uranium (1, 10, 100 mgU kg⁻¹, dm).

2.2. U analysis

After filtration at 0.45 µm (syringe, PC filter), water samples (10 ml) were acidified to 2% HNO₃ (Merck, 65%) prior analysis. The U concentration in water samples was followed only during the preliminary experiment. Sediment samples (0.5–2 g wet weight) were dried, mineralised in a glass tube with HNO₃ (3 ml, Merck, 65%) and evaporated, and then evaporated again after addition of perchloric acid (2 ml, Merck, 33%). The residue was finally diluted in 1–5 ml of acidified (2% v/v, nitric acid) ultrapure water. All samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES Optima 4300 V, PerkinElmer, Wellesley, MA, USA, Detection limit: 10 µg L⁻¹). All samples were spiked with a known quantity of yttrium (as an internal standard, PerkinElmer, 1000 µg ml⁻¹, 2% HNO₃) for analysis using a Multi-component Spectral fitting correction to decrease the matrix effect of biological samples. Protocol was adapted from U measurement in tissues (Simon et al., 2013b; Simon and Garnier-Laplace, 2004, 2005).

2.3. Endpoint bioassay procedure

Test animals were obtained from stock cultures maintained at our laboratory according to the related guideline recommendations. These cultures were initiated from eggs of *C. riparius* and adults of *H. azteca* provided by the Institut National de l'Environnement Industriel et des Risques (INERIS, France) and Institut national de Recherche en Sciences et Technologies pour l'Environnement et l'Agriculture (IRSTEA, France) respectively. *C. riparius* was maintained at 22.7 ± 0.6 °C with a photoperiod of 16 h light and 8 h dark, according to culturing conditions described in OECD N°218/9; the breeding substrate was a ca. 3 cm layer of quartz sand (<500 µm) with culturing medium added. The pH was manually adjusted at 7.5 twice a day. *H. azteca* was cultured at 23.4 ± 0.6 °C in a temperature-controlled chamber with a photoperiod of 16 h light and 8 h dark. Culturing conditions, in particular a method for obtaining known-age test organisms and culturing medium are described in ISO/FDIS 16303:2013. *M. aquaticum* was provided by the Institut für Gewässerschutz (Mesocosm GmbH, Germany). Culturing conditions are described in ISO 16191:2013.

The bioassay on *C. riparius* was conducted on first instar larvae (24 h old, 20 individuals per EU) obtained from egg sacs hatched in culturing

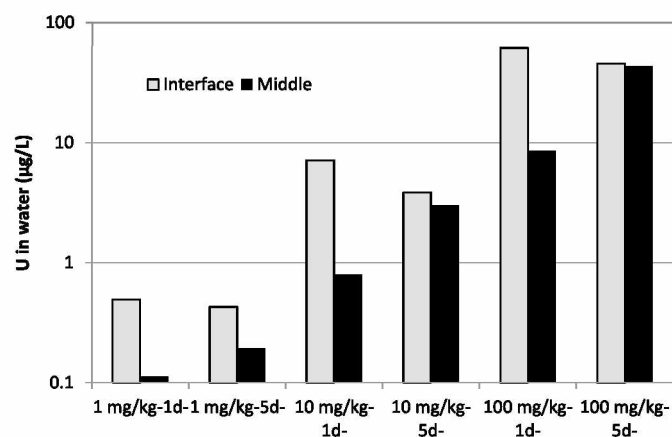


Fig. 1. Uranium concentration (µg L⁻¹, n = 1) in water at various depths (interface and mid-water column) for 3 spiked-sediment after 1 and 5 days.

medium. Larvae were fed with food solution (2–5 ml/day/EU of 1 g of TetraMin® flakes prepared as slurry by mixing with 20 ml of clean water). Emergence was followed from the 11th day of exposure to the 28th day. The adults were sexed. Cumulative emergence rate at 28 days and emergence time were assessed for all U treatments.

The bioassay on *H. azteca* was conducted on two-day-old amphipods (10 individuals per EU). Larvae were fed with food solution (0.05–0.1 ml/day/EU of 1 g TetraMin® in 20 ml), quantity superior to those recommended (2.7 mg) to promote control growth to meet the requirements of the guidelines. The toxicity endpoints were mortality (%) and growth inhibition (expressed by the mass decrease, assessed relatively to control data). At the end of the test (14 d), the content of each EU was sieved through at 300 µm. The mean dry mass (SE2 ultramicrobalance, precision of 0.1 µg, Sartorius, Gottingen, Germany) per surviving amphipod was calculated from the total mass of each replicate group of survivors for each treatment.

For animal bioassays, temperature was monitored every day and pH (Multi 350i/SET, WTW, Germany) was measured at the start and at the end of the test as recommended by the bioassay's guidelines. In line with the guidelines of the bioassays, which do not require the measurement of hardness, dissolved organic carbon, carbonate, we did not measure the data. Maximum dissolved oxygen levels (Multi 350i/SET, WTW, Germany) in EUs for both *C. riparius* and *H. azteca* were ensured by constant air pumping. Both tests were performed in EUs with a ratio of sediment to water being equal to 1:4. As the tests were static, the volume of overlying water (400 ml) was maintained constant every day by adding aerated, reconstituted overlying water according to guideline. This addition of water prevented us from taking any samples from the water column during the bioassays. Therefore, concentration of U in the water column was derived from the preliminary experiments.

For plant bioassay, apical part of *M. aquaticum* plants were cut into the number of whorls needed for testing (2–4 whorls maximum with five leaves per shoot). Each whorl was drained on a tissue paper, weighted and transferred to an EU. Each EU held 3 whorls. Plant whorls were maintained at 24.4 ± 1.1 °C in a temperature-controlled chamber, under continuous lighting (75 µmol m⁻² s⁻¹). The endpoints concerned (i) physiological adverse effects (necrosis, chlorosis and morphological changes) over the whole test duration, (ii) growth inhibition (%) after measuring the final fresh mass of the test plant (whole plant, including new organs: roots and shoot). Sediment was daily irrigated with culturing solution to maintain a thin layer of liquid at the sediment surface. Plants were not submerged. The test duration for *M. aquaticum* was increased from the recommended 10 days–15 days to promote the growth performance of the plants in the control samples.

Ecotoxicity tests were conducted for a control (no added uranium) and 5 (plant bioassays) to 9 (animal bioassays) uranium concentrations in sediment with 4–5 replicates for each treatment (Table 1). We tried to systematically express ecotoxic effects of uranium through the estimation of the EC₁₀ and NOEC values for each test.

2.4. Statistical analysis

The NOEC was determined as the tested concentration immediately lower to the lowest providing a significant effect compared to the control. Statistical analysis for EC₁₀ determination followed the guideline recommendations. Cochran-Armitage trend test (p < 0.05) was used to compare emergence rate of *C. riparius* between control and treatments (20 organisms per EU, 4 EU per treatment). Pair-wise comparisons of survival and growth of *H. azteca* data (10 organisms per EU, 5 EU per treatment) were made for each test treatment against same data derived for control sediment. All data were tested for normality using the Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test or other suitable tests. When the mortality data did not meet the requirement for normality and homogeneity of variance, the Welch test was used to compare survival rate between experimental conditions. The level of significance for effect on growth in *M. aquaticum* bioassay (3

Table 2Nominal and measured (mean and associated variability, n = 4 for replicate, n = 3 for intra replicate) U sediment concentrations (mgU kg⁻¹, dry mass) in bioassays.

U concentration mg kg ⁻¹ , dm								
Nominal	Average of 4 Replicates	SD	Average of 3 intra replicates	SD	Variability			
					min	max	RSD ^a (%)	
							Replicate	Intra replicate
0	< DL ^b	No meaning						
39	< DL							
78	74	41	89	22	56	108	41	24
156	204	25	185	129	141	259	25	70
313	234	38	165	27	162	350	38	17
625	526	17	586	307	432	613	17	52
1250	1214	35	1141	73	879	1837	35	6
2500	2950	46	2628	894	2030	4949	46	34
5000	5091	9	5183	516	4589	5702	9	10
10000	10437	25	10814	1662	7690	12807	25	15

^a RSD: Relative Standard Deviation (SDx100/mean).^b LD: Detection Limit.**Table 3**Toxicity of uranium (mgU kg⁻¹, dry mass) to *C. riparius* in a 28d spiked-sediment bioassay (n = 20 organisms per replicate, 4 replicates per treatment).

Nominal [U] (mg kg ⁻¹ , dm) in sediment	Cumulative emergence at 28d (%)	
	Mean	SD
0	95	6
3.9	96	5
7.8	93	5
15.6	86	13
31	91	6
62	91	18
125	76*	17
250	78*	10
500	23*	10
1000	2.5*	5

* significantly different from control (calculated by Cochran-Armitage trend test, p < 0.05).

organisms per EU, 4 EU per treatment) was calculated by Mann-Whitney test (p < 0.05). Logistic models (given in supplementary data) were used to fit concentration-effect relationships for the estimation of EC₁₀ value and to estimate its Confidence Interval at 95% (CI_{95%} (Ritz, 2010)). Statistical analyses were made using the R language and environment for statistical computing (R Core Team, 2013).

3. Results

3.1. Exposure conditions

The U concentration in water samples was followed only during the preliminary experiment. Uranium concentrations in water (interface and middle of the overlying) are given for 3 sediment U concentrations in Fig. 1. The U concentration in water was always higher at the sediment surface than in the mid water column. It did not evolve between the first and the fifth day, indicating an apparent equilibrium between sediment and its porous water within less than 24 h. U concentration in the mid water column reached the one at the sediment interface after 5 days, except for the lowest U concentration. The distribution of U between water at the sediment surface and the sediment itself was consistent for the three lowest concentrations, leading to an average sediment-water partition coefficient (K_d), equal to 2384 ± 202 L kg⁻¹, n = 3. As expected for a sediment bioassay, the majority of U (>99%) stayed localized in the sediment + porewater compartment and could allow to evaluate the U toxicity.

The correspondence between nominal and measured uranium concentration in sediment during toxicity bioassays is given in Table 2. A significant variability was observed for intra- (6–70%) and inter-

Table 4Toxicity of uranium (mgU kg⁻¹, dry mass) to *H. azteca* in a 14d spiked-sediment bioassay (n = 10 organisms per replicate, 5 replicates per treatment).

Nominal [U] (mg kg ⁻¹ , dm) in sediment	Survival (%) at 14d		Growth at 14d (mg, dm)	
	Mean	SD	Mean	SD
40	68	16	1.50	0.29
80	64*	11	1.50	0.29
130	78	13	1.61	0.60
200	80	12	1.54	0.37
250	58*	35	0.89	0.67
300	58*	38	1.12*	0.32
444	44*	26	0.57*	0.17
667	12*	13	0.16*	0.08
1000	0*	0	0*	0

* significantly different from control (calculated by Welch test; p < 0.05).

replicates (9–46%). The two highest U concentrations in sediment exhibited the lowest variability. For the whole range of U concentrations in sediment (from 78 to 10,000 mg kg⁻¹, dm), measurements were on average within 75–131% of the nominal concentration, the mean shift between measured and nominal concentrations being less than 5% (U_{nominal} = 1.04xU_{measured}, n = 32, R² = 0.94). Based on previous results of U solid/liquid distribution kinetics, these concentration of U within the sediment were assumed to remain stable over time. Therefore, nominal concentrations were used to analyse toxic effects and derive thresholds values. Finally, the water samples were not taken during the bioassay experiments since it is not required by the guidelines. Dissolved oxygen levels, water pH values and temperature values complied with the guideline recommendations.

3.2. Toxic effects

For *C. riparius*, 95 ± 6% of individuals initially placed in control EUs emerged (Table 3). The cumulative adult emergence rate began to decrease significantly from 125 mgU kg⁻¹, dm. For the two highest concentrations, only 23 and 2.5% adults of the 80 exposed individuals emerged respectively. No effect on the sex ratio (48–57% of adults were male, consistently with the control conditions) and no effect on the development time were observed (data not shown). The EC₁₀ value was estimated at 188 mgU kg⁻¹, dm (CI_{95%} 40–885 mgU kg⁻¹, dm) for a NOEC set at 62 mgU kg⁻¹, dm (Table 6, Figure SD1).

For *H. azteca*, the survival rate in the control conditions reached 78 ± 21% (Table 4) and was close to the recommended value (80%). Both the survival rate (%) and the growth (dry mass of adults at 14d) were significantly different from the controls, at 80 mgU kg⁻¹, dm and 300

Table 5Toxicity of uranium (mgU kg⁻¹, dry mass) to *M. aquaticum* in a 15d spiked-sediment bioassay (n = 3 whorls per replicate, 4 replicates per treatment).

Nominal [U] (mg kg ⁻¹ , dm) in sediment	Growth rate r				Stimulation (%)	Inhibition (%)	Test Mann-Whitney	t-test
	Mean	SD	CV %	n				
0	0.09	0.02	24	4				
12.5	0.10	0.02	19	4	13.7		P = 0.127	P = 0.13
25	0.10	0.02	19	4	19.8		P = 0.078	P = 0.06
50	0.10	0.03	28	4	14.2		P = 0.219	P = 0.21
100	0.09	0.03	27	4		1.2	P = 0.755	P = 0.91
500	0.04 ^a	0.03	51	4		53.4	P < 0.001	P < 0.001

$$r = (\ln m_{t_{15d}} - \ln m_{t_{0d}}) / (t_{15d} - t_{0d})$$

m: fresh mass.

m_{t_i}: fresh mass of a single plant at the time of measurement (day), t_i.

SD: standard deviation.

CV%: coefficient of variation (SD/Mean^a100).S%: (r_{control sample} - r_{test sample}) / r_{control sample} ^a100 > 1.I%: (r_{control sample} - r_{test sample}) / r_{control sample} ^a100 < 1.^a Calculated by Mann-Whitney test.

mgU kg⁻¹, dm respectively. At 130 and 200 mgU kg⁻¹, dm, these two endpoints presented no significant difference with those measured in control conditions. The difference was regularly significant only from 250 mgU kg⁻¹, dm. For growth (Table 6, Figure SD1), the NOEC value was 250 mgU kg⁻¹, dm when the EC₁₀ was estimated at 199 mgU kg⁻¹, dm (CI_{95%} = 107–291 mg kg⁻¹, dm). Note that the NOEC is included in the confidence interval of the EC₁₀. For survival rate, a much lower NOEC of 40 mgU kg⁻¹, dm was observed while the EC₁₀ was slightly higher than for growth, at 296 mgU kg⁻¹, dm (CI_{95%} = 155–436 mg kg⁻¹, dm).

For *M. aquaticum*, the growth rate per day in the control conditions was equal to 0.09 ± 0.02 d⁻¹ (Table 5). At 500 mgU kg⁻¹, dm only, the growth rate of whorls decreased significantly compared to control. No logistic model fitted the observed data, preventing the estimation of an EC₁₀ value. We determined the NOEC value of 100 mgU kg⁻¹, dm.

4. Discussion

The whole sediment approach performed in this study combined a complex U distribution in solid phase and pore water. This approach is recommended by the European guideline to test toxicity for benthic animal and is considered as the first conservative step to acquire new data of U sediment toxicity.

4.1. Assessing U exposure

The U distribution in the experimental system was partially assessed. Even if average U concentrations measured in sediment during preliminary test were close to the nominal concentrations, difference in the U distribution was observed both between intra- and inter-replicates and could explain variability in U toxicity. Few studies have looked at the measurement of the variability of metal distribution, including uranium, in enriched sediments. Liber et al. (2011) showed a low inter-replicate variation for U concentration measured in a spiked sediment (RSD = 3.1%, n = 3), counterbalanced by a high variability of U concentrations in pore water (RSD = 120%, n = 3). Anyway, feeding activities of *C. riparius* and *H. azteca* led them to move over the entire sediment, as observed at the end of exposure. This was supposed to ensure their exposure to the total U burden introduced in EU. Measuring U concentrations in whole sediment and in pore water, as proposed par Liber et al. (2011) at the end of exposure duration should provide information on U distribution in sediment after organism activities in order to better evaluate U exposure.

U concentrations measured in the different compartments and in the absence of organisms showed that (i) an apparent equilibrium at the sediment/water interface was achieved very quickly (1 d) and (ii) equilibrium between sediment and the middle of the water column was

meanwhile reached more slowly (<5 d). In our tests, organisms were introduced at least 10 days after submersion of U-spiked sediment in order to achieve steady state conditions. This duration is comparable to that used by Roman et al. (2007), Dias et al. (2008), Liber et al. (2011) and recommended by European ERA methodology (EC-TG N°27) for artificial sediment. The duration used between U spike and introduction of organisms could be longer, several weeks for natural sediment, but in these studies the minimum time to reach steady was not really investigated (Alves et al., 2008; Goulet and Thompson, 2018; Liber et al., 2011).

The K_d value estimated at 5 days (2384 ± 202 L kg⁻¹) in our experimental conditions indicated that most of U remained fixed in the sediment (>99.9%) for the 3 lowest U concentrations. This value is in the range of the environmental U-K_d values (20–10,000 L kg⁻¹) reported in the literature for various environmental conditions (Crawford et al., 2017; IAEA, 2010; Kaplan and Serkiz, 2001; Salminen et al., 1998). Results from spiked-sediment bioassays did not seem to accentuate the partitioning of metals disproportionately to the dissolved phase. Therefore, the exposure modalities of the organisms were considered sufficiently robust for an evaluation of the toxicity of U in this experimental system.

4.2. Assessing U toxicity

Aquatic plants are known to accumulate toxic metals (as U) and are regularly used as bioindicator/biomonitoring tool (Charles et al., 2006; Hogan et al., 2010; Pratas et al., 2017). However, to our knowledge there are no data on the effect of U on *Myriophyllum*. In this study, the effects on *M. aquaticum* were observed only for the highest tested concentrations (500 mgU kg⁻¹, dm), and confirmed that plants are comparatively less sensitive to U than benthic animals. The results obtained on the toxicity of U to benthic invertebrates contributed to enrich the relative limited information previously reported: the toxicity observed on emergence, survival and growth are representative endpoints with respect to population dynamics, the targeted level that should ensure the maintenance of the structure and functioning of ecosystems.

The obtained toxicity values differ slightly to those reported in the literature. For *H. azteca*, the EC₁₀ value on both survival rate and growth endpoints (296 (CI_{95%} = 155–436) and 199 (CI_{95%} = 107–291) mgU kg⁻¹, dm, respectively) are c.a. 1 order of magnitude lower than EC₁₀ values obtained by Liber et al. (2011) (NOEC survival = 2551 ± 268, NOEC growth = 807 ± 133 mgU kg⁻¹, dm) after a 10-day laboratory bioassay. EC₁₀ on survival rate (296 mgU kg⁻¹, dm) was surprisingly higher than LC₅₀ values for juveniles and adults (48 and 214 mgU kg⁻¹, dm, respectively) exposed to U-spiked sediments (with limiting the U precipitation) respectively (Goulet and Thompson, 2018). To a lesser

extent, the EC₁₀ value on emergence for *C. riparius* (188 mgU kg⁻¹ dm, (CI_{95%} = 40–885 mg kg⁻¹, dm)) was surprisingly much higher than the lethal concentration (LC₅₀ = 5.3 mgU kg⁻¹, dm, (CI_{95%} = 3.9–7.2 mg kg⁻¹, dm)) reported by Dias et al. (2008) after a 10-d static bioassay.

The differences observed between the present study and previously reported data may potentially be attributed to the influence of sediment composition on U toxicity and to the consecutive variation in U bioavailability. Indeed, the results were obtained with three different chemical compositions of sediments (composition recommended by the OECD guideline for this study; artificial sediment made of 88% silica with particle size 150–300 μm and 12% of alpha-cellulose for Dias et al. (2008); natural sediment for Liber et al. (2011)) and Goulet and Thompson (2018). These results confirm the need of new tools to better integrate the bioavailability into ecological risk assessment (Chapman, 2007; Goulet and Thompson, 2018; Peijnenburg and Jager, 2003; Peijnenburg et al., 1997; Vandegehuchte et al., 2013).

Toxicity in this study could be due to uranium bound to the solid phase of the artificial sediment as well as to U dissolved in pore water. The presence of dissolved complexing agents in the pore water phase may have counteracted adsorption to the solid matrix and led to the formation of soluble U species (Crawford et al., 2017, 2018; Krachler et al., 2018). However, the measurements indicated that the most of U remained fixed in the sediment (>99.9%). The neutral pH in the water column (between 7 and 7.5) does not favor U bioavailability (Fortin et al., 2004, 2007; Goulet et al., 2015; Markich, 2002, 2013) and consequently results in a low U toxicity of pore water in equilibrium with water column. Chironomids live exclusively buried in sediment, so effects observed on these animals are unlikely attributable to the U dissolved in the overlying water. *H. azteca* is an epibenthic amphipod and as such could be exposed to waterborne U. However, the physico-chemical conditions of our experiment did not favor the presence of bioavailable chemical species thereby reduced accumulation by direct route. This was consistent with our objective by using the standardized test to evaluate the contamination by the sediment. The toxicity was most likely due to the presence of U on the solid phase of the sediment and its transfer across trophic route to organism. Facing this complex pore water/solid system, measurements of U concentrations in particles, in pore water and in water column could contribute to explain the toxicity as proposed by several authors (Burnett-Seidel and Liber, 2012, 2013; Crawford et al., 2018; Goulet and Thompson, 2018; Liber et al., 2011). Nevertheless, it appears necessary to better characterize the different exposure routes in these bioassays. The transfer from the water column with and without sediment in *H. azteca* should be determined. One research perspective would consist in determining the internalization of pollutants, as previously carried out for the characterization of the direct route (Simon and Boudou, 2001; Simon et al., 2013a, 2013b, 2018, 2019).

U toxicity was evaluated from a set of three standardised sediment bioassays. Experimental conditions (T°C, pH, dissolved oxygen, food) in the control experiment led to endpoint values (95 ± 6% for insect emergence rate, 78 ± 21% of average survival for amphipods and 0.09 ± 0.02% for plant growth rate) acceptable with regard to applicable guidelines. The mean value of the survival rate was slightly inferior to that recommended in the ISO/FDIS16303 (at least 80%), which is included in the confidence interval we determined. Thus, we considered our bioassays as valid.

According to the European technical guidance for deriving a QS_{sediment} (EC-TG N°27, 2011), a small dataset as the one we provided in the present study is meets the minimum requirement for the application of the Assessment Factor method. However, the data, presented here in particular for the amphipod *Hyalella azteca* will have to be completed by other complementary tests to strengthen our conclusion. We determined 5 long term data (EC₁₀ for both tested animals, and NOEC for three species representing different living and feeding strategies) from exposure to artificial sediment, as recommended by The Technical Guidance document on risk assessment (EC-TG N°27, 2011). In order to illustrate

Table 6

Toxicity thresholds for uranium to *C. riparius*, *H. azteca* and *M. aquaticum* in spiked sediment bioassays.

Bioassays	Endpoints	NOEC	EC ₁₀ (CI95%)
		(mg kg ⁻¹ , dry mass)	(mg kg ⁻¹ , dry mass)
<i>C. riparius</i>	emergence	62	188 (40–885)
<i>H. azteca</i>	survival	40	296 (155–436)
	growth	250	199 (107–291)
<i>M. aquaticum</i>	growth	100	nd
	nd: not determined		

the order of magnitude of what could be the QS_{sediment} for uranium in freshwaters, we sought to implement the recommendations of the assessment factor method. An assessment factor (AF) of 50 should be applied to the lowest of the EC₁₀ values we determined (188 mgU kg⁻¹, dm, Table 6). It would result in a QS_{sediment} value close to 4 mgU kg⁻¹, dm. Note that QS_{sediment} value derived from the 3 NOEC values we had from our set of tested concentrations (AF = 10 on the lowest NOEC = 40 mgU kg⁻¹, dm) would also led to this value of 4 mgU kg⁻¹, dm.

The QS_{sediment} value we proposed based on our U sediment toxicity experiments data is included in the range of geochemical background concentrations of U in sediments (2–10 mg kg⁻¹, with an average of 8 mg kg⁻¹ around facilities upstream of the nuclear fuel cycle, in France (Picat et al., 2002)). Consistently with this observation, the QS_{sediment} we suggested here is about two orders of magnitude lower than the value recommended by Sheppard et al. (2005) (100 mgU kg⁻¹, dm) and proposed by Thompson et al. (2005) based on natural sediment data. Such differences were already observed for other trace metals for which bioassays performed on artificial sediments led to lower toxicity thresholds compared to those obtained with natural sediments (Chapman, 2007; Roman et al., 2007).

5. Conclusion

Toxicity of U contaminated sediment was characterised for two benthic animal species, exhibiting different lifestyle and feeding behaviour, and an aquatic plant. Sensitivity to U was higher for benthic animal species than for the plant we tested, potentially in link with U uptake modalities. Several endpoints commonly tested (emergence, survival rate and growth) were altered by the exposure of organisms to U in sediment. EC₁₀ values assessed for both benthic species were similar (ca 200 mgU kg⁻¹, dm) despite their differences in terms of feeding behaviour and tested endpoints. This study based on artificial spiked sediment bioassays was a first step in the proposition of a QS_{sediment} value for uranium in freshwater ecosystem. We illustrated the process by deriving a preliminary QS_{sediment} of 4 mgU kg⁻¹, dm as a conservative value. Sediments with U concentration below this value are assumed to be not harmful for benthic life. Because of this low U-QS_{sediment} value compared to natural geochemical background level, and the potentially variation on U bioavailability depending on site specific conditions, further experiments are necessary to identify the main factors controlling U toxicity in sediment. In a second approach, it will be necessary to better characterize the dose/effect relationship for the determination of robust basic ecotoxicity data require to determine QS. Finally, sediment quality guidelines for standardized bioassay could be improved by using laboratory bioassays with field-collected sediments and environmental site-specific physicochemical properties that will allow to consider factors governing bioavailability for both direct and trophic pathways.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvrad.2021.106776>.

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