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Lanthanide ecotoxicity: First attempt to measure environmental risk for aquatic organisms

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ABSTRACT

The geochemical cycles of lanthanides are being disrupted by increasing global production and human use, but their ecotoxicity is not fully characterized. In this study, the sensitivity of *Aliivibrio fischeri* and *Pseudokirchneriella subcapitata* to lanthanides increased with atomic number, while *Daphnia magna*, *Heterocypris incongruens*, *Brachionus calyciflorus* and *Hydra attenuata* were equally sensitive to the tested elements. In some cases, a marked decrease in exposure concentrations was observed over test duration and duly considered in calculating effect concentrations and predicted no effect concentrations (PNEC) for hazard and risk assessment. Comparison of PNEC with measured environmental concentrations indicate that, for the present, environmental risks deriving from lanthanides should be limited to some hotspots (e.g., downstream of wastewater treatment plants). However, considering the increasing environmental concentrations of lanthanides, the associated risks could become higher in the future. Ecotoxicological and risk assessment studies, along with monitoring, are required for properly managing these emerging contaminants.

1. Introduction

The lanthanide group includes 15 elements, from lanthanum (Z = 57) to lutetium (Z = 71), which have atomic properties that are highly similar to one another (Greenwood and Earnshaw, 1997). Generally, based on the electronic configuration of each element, lanthanides can be roughly divided into two groups: light lanthanides, from lanthanum to europium (La–Eu), and heavy lanthanides, from gadolinium to lutetium (Gd–Lu). Together with yttrium (Y) and scandium (Sc), they form the rare earth elements (REEs), which have a broad spectrum of applications in agriculture, clean energy and other industrial sectors (US. EPA, 2012). Lanthanides commonly occur at low concentrations in soil and water, but their low mobility could promote their accumulation in the environment (Cao et al., 2000; Zhang and Shan, 2001) as a consequence of anthropogenic inputs. Their wide range of applications is causing lanthanide enrichment in the environment as microcontaminants in the hydrosphere (Kulasiz and Bau, 2011a) or in tap water (Kulasiz and Bau, 2011b). REE uses as fertilizers in oriental agriculture, and mining activities connected with their extraction, are being associated with their bioaccumulation in soils and vegetables (Li et al., 2013) and also in human hair (Wei et al., 2013) in China.

Although reported cases of lanthanide contamination are traditionally scarce, they have been emerging in recent literature, highlighting the need to develop a general understanding of lanthanides' ecotoxicity, bioaccumulation and mode of action in order to predict the possible risks associated with their current anthropogenically driven release into the environment (Gonzalez et al., 2014). In this study, we assessed the ecotoxicity of three lanthanides: cerium (Ce) and lutetium (Lu) representing light and heavy lanthanides, respectively, and gadolinium (Gd) in between, using a test battery including *Daphnia magna* (Crustacea: Branchiopoda), *Heterocypris incongruens* (Crustacean: Ostracoda), *Hydra attenuata* (Cnidaria:Hydrozoa), *Brachionus calyciflorus* (Rotifera: Monogononta), *Pseudokirchneriella subcapitata* (Chlorophyta: Chlorophyceae), *Aliivibrio fischeri* (Proteobacteria: Gamma proteobacteria) and LuminoTox ®. The aims of this study were: (1) to generate ecotoxicological information regarding these poorly

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studied elements, (2) to identify, if possible, an ecotoxicity order between them, and (3) to compare ecotoxicity endpoints with concentrations already found in the environment to determine the current risk associated with these elements.

2. Materials and methods

2.1. Preparation of contaminated solutions

The experiments were conducted using commercially available chemicals (purity > 99.99%): Cerium chloride ($CeCl_3$–$7H_2O$), gadolinium chloride ($GdCl_3$–$6H_2O$) and lutetium chloride ($LuCl_3$-$6H_2O$). All chemicals were purchased from Sigma Aldrich (St. Louis, Missouri U.S.A.). Stock solutions of each metal were prepared in MilliQ water, and exposure concentrations were prepared by diluting the stock solutions in the exposure medium appropriate for each organism (see Annex 1). Salts were diluted to reach the following concentrations for each lanthanide: 100, 200, 400, 800, 1600, 3200, and 6400 μg L^{-1}. Measured concentrations were determined for each exposure level in all tests at t-0 (at the start of test) and t-1 (at the end of the test). For measured concentrations, samples were filtered using sterile filter units (Millex 0.22 μm from MerckMillipore, Molsheim, France). After filtration, trace elements were measured by inductively coupled plasma-mass spectrometry (ICP-MS) using a Hewlett Packard 4500 STS instrument.

2.2. Ecotoxicity tests

2.2.1. D. magna immobilization test

A 48-h immobilization test with *D. magna* was performed according to the international guideline ISO (2012a) at 25 ± 2 °C. In glass beakers of 50 mL capacity, five individuals (less than 24 h old) were exposed to 30 mL test medium. All lanthanide concentrations were tested in triplicate and two independent tests were performed to evaluate the variability of the procedure. Potassium dichromate was used as a positive control at 0.9 mg L^{-1}. After a 48 h exposure period, immobilized individuals were checked and counted. The validity criterion of the *D. magna* assay was an immobilization percentage lower than 10% in negative controls.

2.2.2. Ostracodtoxkit F® test

A 6-d mortality and growth inhibition test with *H. incongruens* was performed according to the Standard Operational Procedure (Microbiotest, 2001) with some modification. This test uses neonates of the benthic ostracod *H. incongruens* (within 24 h of hatching). Cysts were incubated for 52 h at 25 °C, and freshly hatched ostracods were pre-fed spirulina powder 4 h before the beginning of the test. 5 organisms, instead of the standard 10, were exposed to 2 mL test solution in 6-well plates. Each well also contained 1 mL calibrated reference sediment (standard sand) and 2 mL alga food suspension (*Scenedesmus* spp.). After a 6-d exposure at 25 °C in darkness, the ostracods were retrieved from the substrate, and their mortality and growth variation were determined. All lanthanide concentrations were tested in triplicate, and two independent tests were performed to evaluate the variability of the procedure. The validity criterion of the ostracod assay was a survival of at least 80% of the control test organisms.

2.2.3. H. attenuata toxicity test

A 96-h acute toxicity test with *H. attenuata* was performed as indicated by Environment Canada (1996). Each hydra was exposed to 4 mL of test solution in 12-well plates, and the organisms were not fed during the test. After 96 h at 23 ± 2 °C and under a standard 16/8 h light–dark photoperiod, scores and lethality in control and exposed organisms were recorded. Toxicity in Hydra is indicated by body and tentacle contraction as well as other morphological changes (Wilby, 1988), scored from 10 (normal) to 0 (disintegrated). Tulip phase (score 5) and disintegration (score 0) morphologies were recorded as death (Trottier et al., 1997). All concentrations were tested in triplicate, and two independent tests were performed to evaluate the variability of the procedure.

2.2.4. B. calyciflorus population growth inhibition test

A 48-h population growth inhibition test with *B. calyciflorus* was performed according to the standardized French Guideline (NF T 90-377, 2000). This test uses neonates of the female rotifer *B. calyciflorus* (less than 2 h old), which can be obtained by incubating cysts (MicroBioTest Inc.) at 25 °C for approximately 18–20 h. Each organism was exposed to 0.9 mL test solution in 24-well plates (8 replicates per concentration), with the addition of 0.1 mL algal suspension (*P. subcapitata* at a concentration of 2×10^7 cells mL^{-1}) as food. After 48 h of exposure at 25°C ± 1 °C in darkness, surviving females were counted. Using the mean number of surviving females in samples and controls, the growth inhibition was calculated by using the equation I = (Fc-Fs/Fc) *100, where I is % growth inhibition, Fc is mean number of surviving females in a control, and Fs is mean number of surviving females in each sample concentration tested. Two independent tests were performed to evaluate the variability of the procedure. The validity criterion of the rotifer assay was a reproduction rate of at least 87.5% and at least 3 female rotifers surviving in control at the end of the test.

2.2.5. P. subcapitata growth inhibition test

A 72-h growth inhibition test with the alga *P. subcapitata* (Korshikov, strain 61.81 SAG, Göttingen, Germany) was performed according to the ISO (2012b) standardized protocol. The inoculations came from the exponential phase of cell growth, and tests were conducted in microplates according to a published methodology (Radetski et al., 1995). Potassium dichromate was used as a positive control at 1.2 mg L^{-1}. The cell density of the algal suspension was adjusted to 10^4 cells mL^{-1} by dilution with ISO test medium. Algal suspensions were exposed to lanthanide concentrations in triplicate; two independent tests were performed to evaluate the variability of the procedure. After 72 h at 23 ± 2 °C with a continuous illumination of 70 μm E m^{-2} s^{-1} (from cool-white fluorescent lamps), the inhibitory effect based on algal fluorescence activity was measured with a FLUOstar microplate reader (BGM Labtechnologies).

2.2.6. LumisTox test

A bacterial luminescence inhibition test (using *Aliivibrio fisheri*, formerly known as *Vibrio fisheri*) was performed according to ISO (1998). The lyophilized bacterial reagent was obtained from Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSM N♯

Table 1
Levels of cerium (Ce), gadolinium (Gd) and lutetium (Lu) measured in each medium as percent of expected (nominal) for all test battery. The interval represents the recovery percentage for the seven nominal concentration tested (100–6400 μg L^{-1}).

	Ce		Gd		Lu	
	T0	T1	T0	T1	T0	T1
H. attenuata	[81–101]	[88–111]	[78–103]	[82–96]	[82–108]	[88–102]
P. subcapitata	[44–95]	[53–92]	[70–94]	[51–96]	[60–97]	[61–92]
B. calcyflorus	[85–101]	[53–92]	[96–106]	[53–92]	[55–115]	[33–106]
H. incongruens	[12–59]	[0]	[41–60]	[0]	[41–72]	[0–1]
D.magna	[33–60]	[0–10]	[57–66]	[0–12]	[59–72]	[0–24]
V.fisheri	[93–108]	nm	[88–103]	nm	[85–106]	nm
LuminoTox®	[96–103]	nm	[96–100]	nm	[95–102]	nm

(nm) = no measured; T0-beginning of the test; T1-end of the test (different times depending on the test. See Annex 1).