Ecotoxicology and Environmental Safety 163 (2018) 486–491

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Ecotoxicity responses of the freshwater cnidarian *Hydra attenuata* to 11 rare earth elements

Christian Blaise[a], François Gagné[a,*], Manon Harwood[b], Brian Quinn[c], H. Hanana[a]

[a] Aquatic Contaminants Research Division, Environment and Climate Change Canada, 105 McGill street, 7th Floor, Montréal, QC, Canada H2Y 2E7
[b] Quebec Laboratory for Environmental Testing, Environment and Climate Change Canada, Montréal, QC, Canada
[c] University of the West of Scotland, Centre for Environmental Research, Paisley, United Kingdom

ARTICLE INFO

Keywords:
Hydra attenuata
Toxicity
Lanthanides
Rare earth elements
Freshwater

ABSTRACT

Lanthanides are the major family of rare earth elements (REEs) owing to the essential properties these metallic species provide in diverse fields of today's world economy. They are now being mined and produced as never before. This raises new environmental concerns in terms of their expected future discharges notably to aquatic systems. Interspecies studies of their ecotoxicity are sparse and effects on aquatic life are still poorly understood. Absence of such information for cnidarians, an ecologically relevant freshwater community, thus prompted the present research on REEs toxicity using *Hydra attenuata* as our animal model. Lethal and sublethal ecotoxicity data generated with the 11 REEs displayed LC50 values ranging from 0.21 to 0.77 mg $L^{-1}$and EC50 values ranging from 0.02 to 0.27 mg L^{-1}, thereby confirming the inherent sensitivity of *Hydra* to REE exposure at environmentally relevant concentrations. Additionally, two properties of REEs were shown to modulate *Hydra* (sub)lethal toxicity (LC50 and EC50) which decreases with increasing atomic number and with decreasing ionic radius. Compared to studies carried out with different taxonomic groups, *Hydra* toxicity responses to REEs proved to be among the most sensitive, along with those of other invertebrate species (i.e., *Daphnia magna*, *Ceriodaphnia dubia*, *Hyalella azteca*), suggesting that members of this community are likely more at risk to eventual REE discharges in aquatic environments. Demonstrated *Hydra* sensitivity to REE exposure strongly justifies their future use in toxicity testing battery approaches to evaluate liquid samples suspected of harbouring REEs.

1. Introduction

Lanthanides, La (Z = 57) to Lu (Z = 71), form a group of 15 metallic species that, along with yttrium (Z = 39) and scandium (Z = 21), are known as the rare earth elements (REEs). Chemical properties of lanthanides are similar within the group, all closely mirroring that of lanthanum (La), and hence their collective appellation "REEs" referring to any of these metals.

Because of their increasing use in diverse areas linked to the world economy (e.g., agronomy, medicine, industry), mining of REEs and production of REE-containing products have skyrocketed over the past decades thereby raising new environmental concerns for the biosphere. An increased presence of REEs has been shown, for example, in aquatic bodies (Kulaksiz andBau (2011), in sediments (Sneller et al., 2000) and soils (Cao et al., 2000).

More specifically linked to the issue of water quality in ecosystems, REEs have been measured in soil runoff, effluent and in the hydrosphere itself owing to varied agricultural/industrial practices (Protano and Riccobono, 2002) as well as to medical applications (Bau et al., 2006; Rabiet et al., 2009). In our digital age, the indirect release of REEs to aqueous environments via e-wastes is an additional preoccupation because of the absence of proper recycling technologies in many countries of the world, thereby posing further potential risks to environmental and human health (Dodson et al., 2012).

With reference to aquatic ecotoxicity, a recent review by a French group flagged 91 references dealing with (freshwater and saltwater) toxicity studies relating exposure of selected lanthanides to biota representative of different biological levels (Gonzalez et al., 2014). As

Abbreviations: EC50, effective concentration sub-lethally impacting 50% of the exposed population; LC50, lethal concentration killing 50% of the exposed population; REE, refers to the 15 rare earth elements also known as lanthanides or a group thereof; NOEC, a no observable effect concentration in an exposed population; REE, rare earth element

* Corresponding author.
E-mail addresses: tiamat3600@bell.net (C. Blaise), francois.gagne@canada.ca (F. Gagné), manon.harwood@canada.ca (M. Harwood), brian.Quinn@uws.ac.uk (B. Quinn), Houda.hanana@canada.ca (H. Hanana).

https://doi.org/10.1016/j.ecoenv.2018.07.033
Received 8 March 2018; Received in revised form 3 July 2018; Accepted 8 July 2018

expected, REE ecotoxicity varied across species tested, influenced in part by experimental conditions imposed by testing procedures. While arthropods, algae and fish received the most attention in the assessment of ecotoxicity on selected REEs in these investigations, cnidarians were virtually left out of any such appraisal. Another recent review focusing solely on Lanthanum demonstrated its bioaccumulation in several types of aquatic biota with varying ecotoxicity responses across levels (microorganisms, protozoans, micro-algae, macrophytes, invertebrate sp., fish), but information on La cnidarian toxicity was lacking (Hermann et al., 2016).

Inexistent ecotoxicity studies related to cnidarian responses toward RREs thus triggered our desire to acquire data in this respect. Since cnidarians (*Hydrozoa*) of the genus *Hydra*, ubiquitous and ecologically relevant in freshwater environments, have been shown to be particularly sensitive to heavy metals (Quinn et al., 2012; Ginou and Holway, 2013), generating toxicity responses with a *Hydra* species model exposed to REEs also appeared entirely justified to evaluate hazard toward this aquatic community.

Herein, we report our findings of REEs ecotoxicity responses generated with *Hydra attenuata*, also known as *Hydra vulgaris* (Pallas, 1766), an animal model used extensively since the 1980s to undertake developmental (Johnson and Gabel, 1982) and diverse toxicity (Fu et al., 1992; Pardos et al., 1999; Pascoe et al., 2002; Quinn et al., 2008) studies. Lastly, it is noteworthy to mention that ever since miniaturization of the testing procedure in microplates was developed in our laboratory, *Hydra* toxicity assays have become simple and cost-effective to perform (Blaise and Kusui, 1997; Trottier et al., 1997). The morphological aspect of the polyp can be used to assess toxicity of aqueous contaminants and can determine sub-lethal and lethal endpoints (De Jong et al., 2016).

2. Materials and methods

The 11 REEs salts tested were purchased from Sigma-Aldrich. They are listed in Table 1 along with their atomic number and ionic radii characteristics. In preparation for subsequent toxicity testing, each lanthanide salt was processed as follows. First, a 1 g/L stock solution for each was prepared in Hydra medium (made up of 0.15 g $CaCl_2$.2H2O and 0.1 g TES (N-tris [hydroxymethyl]methyl 1-2- aminoethanesulfonic acid) buffer, pH 7.0). The 1 g/L solution was well below the reported solubility limit of the chloride salts in water (Guminski et al., 2016). Then, 400 µL were withdrawn from a 40-mL solution of *Hydra* medium and 400 µL of each stock solution were re-added to the *Hydra* medium to make up REEs working solutions of 10 mg $L^{-1}$for subsequent bioanalysis. Controls consist of 400 µL of distilled water and 400 µL of Hydra medium.

The toxicity testing procedure using *Hydra attenuata* has been extensively detailed previously (Blaise and Kusui, 1997; Trottier et al., 1997). Hydra were raised and maintained in culture in 20-cm diameter crystallisation bowls at 20–22 °C in the Hydra medium as described above. They were fed daily with *Artemia salina* brine shrimps. Briefly, exposure tests were conducted in 12-well polystyrene microplates with three animals placed in each of three adjacent wells (n = 9) for each of 11 serially-diluted REEs concentrations (10 mg $L^{-1}$to 0.0098 mg L^{-1}) using *Hydra* medium as the dilution medium. Experimental animals (n = 3 in each well) bathed in 4 mL of *Hydra* medium (controls) or of REEs dilutions thereof. After a 96-h exposure at room temperature (20–25 °C), changes in *Hydra* morphology based on the Wilby scale (Wilby, 1989) from controls (normal-sized animals with long and slender tentacles) indicating sublethal (clubbed and/or shortened tentacles) and lethal (tulip and/or disintegrated stages) effects were scored with a stereomicroscope at 6 × magnification (the number of hydra with morphological alterations is the endpoint to calculate the EC50). Representative examples for each key morphological changes are included (Fig. 1). Hydra were not fed during the 96-h exposure period.

CETIS software (version 1.8.7.7) was used to determine 96 h-LC50s (Spearman-Karber method; Finney, 1964) and 96-h EC50s (Log-Logit method) values. Pearson-moment correlation analyses were undertaken using Statistica software (version 13.). Significance was set at $p < 0.05$. Correlations with REE ionic radius were performed by using ionic radii values provided in Emsley (1989).

3. Results and discussion

Lethal and sublethal ecotoxicity data generated with the 11 REEs are shown in Table 1. LC50 values are strikingly similar and range from 0.21 to 0.77 mg L^{-1} (3.7-fold difference). EC50 values display somewhat more variability and range from 0.02 to 0.27 mg L^{-1}, i.e. by slightly over one order of magnitude (13.5-fold difference). The toxicity data plots from which LC50 and EC50 values were derived are shown in Fig. 2. The lighter REEs Y, La, Ce and Pr were generally more toxic than the heavier ones (Nd, Sm, Gd, Tb, Dy, Er) with the exception of the heaviest Lu which was more toxic. With one exception, LC50/EC50 ratios demonstrate that sublethal effects appear at exposure concentrations that are not far from those eliciting lethality. Indeed, 10 of the 11 REEs have ratios ranging from 2.6 to 7.3. In contrast, Pr stands out with an LC50/EC50 ratio of 28. Pr also produced the lowest EC50 value (i.e., 0.020 mg L^{-1}) intimating it would likely be more harmful to *Hydra* if present in aquatic environments. A further estimate of REEs hazard potential is provided by reporting our data according to the EU-Directive 93/67/EEC classification scheme (CEC Commission of the European Communities, 1996). Their span of toxicity responses ranges from "extremely toxic (effects < 0.1 mg L^{-1}), "very toxic (effects between 0.1 and 1 mg L^{-1})", "toxic (effects between 1 and 10 mg L^{-1})",

Table 1
Lanthanides tested, chemical characteristics and corresponding lethal and sublethal ecotoxicity data (expressed in mg. L^{-1}).

Lanthanide	Symbol	Atomic number	Crystalline Ionic radius (III)	96 h-LC50 (CI)[a]	96 h-EC50 (CI)[b]	LC50/EC50 ratio
Yttrium (III) chloride hexahydrate	Y	39	106	0.22 (0.18–0.28)	0.03 (0.02–0.04)	7.3
Lanthanum (III) chloride heptahydrate	La	57	122	0.21 (0.19–0.23)	0.07 (0.05–0.09)	3
Cerium (III) chloride heptahydrate	Ce	58	107	0.33 (0.24–0.45)	0.05 (0.03–0.07)	6.6
Praseodymium (III) chloride	Pr	59	106	0.56 (0.41–0.76)	0.020 (0.016–0.025)	28
Neodynium (III) chloride hexahydrate	Nd	60	104	0.31 (0.25–0.39)	0.09 (0.06–0.13)	3.4
Samarium (III) chloride hexahydrate	Sm	62	100	0.77 (0.65–0.92)	0.18 (0.13–0.25)	4.3
Gadolinium (III) chloride hexahydrate	Gd	64	97	0.52 (0.43–0.63)	0.10 (0.07–0.15)	5.2
Terbium (III) chloride hexahydrate	Tb	65	93	0.70 (0.57–0.88)	0.10 (0.07–0.14)	7
Dysprosium (III) chloride hexahydrate	Dy	66	91	0.69 (0.56–0.84)	0.27 (0.22–0.32)	2.6
Erbium (III) chloride hexahydrate	Er	68	89	0.40 (0.32–0.57)	0.10 (0.07–0.14)	4
Lutetium (III) chloride hexahydrate	Lu	71	85	0.29 (0.22–0.36)	0.10 (0.07–0.15)	2.9

a) Concentration of REEs salts producing a 50% lethality effect (Lethal Concentration or LC50) in exposed *Hydra* after a 96h exposure period, along with their 95% confidence intervals (CI).
b) Concentration of REEs salts producing a 50% sub-lethality effect (Effective Concentration or EC50) in exposed *Hydra* after a 96h exposure period, along with their 95% confidence intervals (CI).