

# Peer Community Journal

Section: Ecotoxicology and Environmental Chemistry

Research article

Published  
2024-07-19

Cite as

Davide A.L. Vignati, Loïc A. Martin, Laurence Poirier, Aurore Zalouk-Vergnoux, Chantal Fouque, Clément Bojic, Christophe Hissler and Carole Cossu-Leguille (2024) *Ecotoxicity of lanthanides to Daphnia magna: insights from elemental behavior and speciation in a standardized test medium*, Peer Community Journal, 4: e66.

Correspondence

david-anselmo.vignati@univ-lorraine.fr

Peer-review

Peer reviewed and recommended by PCI Ecotoxicology and Environmental Chemistry, <https://doi.org/10.24072/pci.ecotoxenvchem.100143>



This article is licensed under the Creative Commons Attribution 4.0 License.

## Ecotoxicity of lanthanides to *Daphnia magna*: insights from elemental behavior and speciation in a standardized test medium

Davide A.L. Vignati<sup>1</sup>, Loïc A. Martin<sup>1,2,3</sup>, Laurence Poirier<sup>4</sup>, Aurore Zalouk-Vergnoux<sup>4</sup>, Chantal Fouque<sup>1</sup>, Clément Bojic<sup>1</sup>, Christophe Hissler<sup>2</sup>, and Carole Cossu-Leguille<sup>1</sup>

Volume 4 (2024), article e66

<https://doi.org/10.24072/pcjournal.440>

### Abstract

Lanthanides (LNs) are a group of 15 elements with steadily increasing economical importance due to their multiple uses in technologies essential for sustainable ecological, digital and energetic transitions. Although knowledge on LN ecotoxicology has greatly improved over the last decade, uncertainty persists with regard to their actual hazard and risk in freshwater environments. In particular, only limited information is available on i) the actual relationships between LN speciation vs. ecotoxicological responses in standardized laboratory tests and ii) the existence of regular and predictable patterns in LN ecotoxicity (expressed as e.g., EC<sub>50</sub>) along the LN series. The present paper provides the first report on the ecotoxicity of all lanthanides (except Pm) for the freshwater crustacean *Daphnia magna* along with an unprecedented level of detail on LN speciation in the exposure medium. Experimental data show that exposure concentrations can decrease by up to 95 % over the test duration, with the percentage decrease being inversely related with LN atomic mass. Thermodynamic speciation calculations confirm the possible formation of insoluble species, mainly LN carbonates. However, the corresponding theoretical solubility limits do not fully agree with measured concentrations at the end of the tests. Experimental verification of exposure concentrations (as a minimum at the beginning and end of laboratory tests) remains therefore mandatory to reach proper conclusions as to the ecotoxicity of each LN. A decreasing trend in ecotoxicity can actually be observed along the LN series when temporal changes in the exposure concentrations are properly accounted for. However, this trend remains dependent on exposure time and selected exposure metrics. This and other caveats must be considered in future research to reach a community-based consensus for the proper hazard and risk assessment of LN towards daphnids and other aquatic organisms.

<sup>1</sup>Université de Lorraine, CNRS, LIEC, F-57000 Metz, France, <sup>2</sup>CAT/ENVISION, Luxembourg Institute of Science and Technology, L-4422 Belvaux, Luxembourg, <sup>3</sup>Géosciences Environnement Toulouse, CNRS/IRD/CNES/Université Toulouse III, 14 avenue Edouard Belin, 31400 Toulouse, France, <sup>4</sup>Nantes Université, Institut des Substances et Organismes de la Mer, ISOMer, UR 2160, F-44000 Nantes, France

## 1. Introduction

The transition from a global economy dominated by fossil fuels to one based on renewable energy sources and the increasing digitalization of our society will both lead to a more intensive use of several mineral resources that are collectively termed critical raw materials (Bobba et al., 2020; IEA, 2021, 2023). Some Rare Earth Elements (REY), which include lanthanides (LN), Yttrium and Scandium, are needed in a large number of strategic, interlinked technologies and sectors such as wind energy generation, electric mobility (fuel cells and traction motors), robotics, drones, information and communication technologies at large, and medical applications (Bau & Dulski, 1996; Bobba et al., 2020). The increasing demand for REY has raised a) geopolitical issues in relation to supply chains (Bobba et al., 2020; IEA, 2021, 2023), b) prospecting for new resources across the globe (Dushyantha et al., 2020; Pell et al., 2021), c) concerns about the disruption of their natural biogeochemical cycles (Hissler et al., 2016; Kulaksız & Bau, 2013; Lerat-Hardy et al., 2019; Louis et al., 2023; Martin et al., 2021), and d) concerns about the insufficient knowledge as to their potential environmental and human health risks (Blinova et al., 2020; Gonzalez et al., 2014; Malhotra et al., 2020).

Specific challenges arise when assessing REY ecotoxicity. One of the most important lessons already learned by the ecotoxicological community is that the test media and experimental conditions (notably pH values) used in (standardized) ecotoxicity testing cause lanthanides to precipitate as insoluble chemical species such as carbonates, phosphates and hydroxides (Blinova et al., 2018; Gonzalez et al., 2014; Kang et al., 2022; Lachaux et al., 2022). It is also noteworthy that these chemical speciation issues are independent of the chosen biological model because they arise from the chemical properties of LN and the composition of the test media. The formation of such precipitates leads to variable exposure conditions during ecotoxicity tests, which makes the choice of exposure metrics particularly critical when deriving concentration vs. response relationships for hazard and risk assessment. Proposed solutions include modification of standard media to avoid, in principle, the formation of precipitates (Aharchaou et al., 2020; Blinova et al., 2018; El-Akl et al., 2015; Ma et al., 2016; Revel et al., 2023; Vukov et al., 2016) or the choice of exposure metrics based on analytically measured concentrations at the end of the tests or based on geometric means between initial and final filterable concentrations (Lachaux et al., 2022; Vukov et al., 2016). However, modifications in standard procedures should be consensus-based not to sacrifice reliability, *sensu* Roth and Ciffroy (2016), for relevance (note that we mean no criticisms to the scientific validity of the approaches adopted in any of the cited studies). Furthermore, modification of test medium does not necessarily avoid the formation of colloidal-sized precipitates at circumneutral pH values (El-Akl et al., 2015; Revel et al., 2023). Meaningful and informative comparisons among studies on LN ecotoxicity must therefore give the utmost consideration to LN behavior and speciation in the different test media and experimental conditions (Gonzalez et al., 2014).

*Daphnia magna* is a well-studied model filter-feeder used in standardized ecotoxicity testing (ISO, 2012; OCDE, 2004). However, information on its responses following exposure to REY remains quite limited with a few acute ecotoxicity studies on selected LN (Blinova et al., 2018; González et al., 2015; Lachaux et al., 2022; Ma et al., 2016; Revel et al., 2023) and a handful of works on chronic ecotoxicity, transgenerational effects and life-history traits (Blinova et al., 2018; Blinova et al., 2022; Deng et al., 2022; Galdiero et al., 2019; Lürling & Tolman, 2010; Shu et al., 2023). Studies on the effects of selected LN mixtures are also available and suggest that LN mixtures could follow a concentration addition model for mixture ecotoxicity (Lachaux et al., 2022; Romero-Freire et al., 2019). However, to the best of our knowledge, there is no peer-reviewed investigation published to date that compares the effects of the full LN series (i.e., 14 elements when radioactive Pm is excluded) on *D. magna*.

Because lanthanides exhibit regular variation in their physico-chemical properties from Lanthanum (La) to Lutetium (Lu), ecotoxicologists have been looking for similar predictable patterns in ecotoxicity along the LN series or for ecotoxicological predictions based on physico-chemical properties (Le

Faucheur et al., 2021). The possible presence of ecotoxicological trends along the LN series is attractive in that it would allow to predict the effects of all LN from a reduced number of studies on selected LN. Apart from its fundamental importance, this scenario would result in considerable time and money savings without compromising reliability and relevance of ecotoxicological results; a highly desirable situation for both fundamental and applied research in this field. The few studies examining at least ten LN have identified both increasing and decreasing trends in their ecotoxicological effects along the LN series (Blaise et al., 2018; Borgmann et al., 2005; Lin et al., 2022; Manusadžianas et al., 2020; Weltje, 2002). Except for Weltje (2002), these observations require further confirmation because of missing or incomplete information on LN speciation in the exposure medium, a key factor when looking for trends along the LN series (Gonzalez et al., 2014).

The present study provides the first report on the acute ecotoxic effects of all LN to the model species *D. magna* according to the norm ISO 6341 (2012). The behavior and speciation of LN in the exposure medium are examined with an unprecedented level of detail to i) provide reliable and relevant data for hazard and risk assessment, ii) explore the existence of ecotoxicity trends along the complete LN series for this model organisms, and iii) summarize evidence-based recommendations to ensure maximum reliability and relevance in future research on the ecotoxicity of LN.

## 2. Methods

### 2.1. Chemicals

All lanthanides salts (chlorides or nitrates) were purchased from Sigma-Aldrich and had a minimal purity of 99% (Table S1). Chemicals for preparing the test medium for acute tests with *Daphnia magna* were also purchased from Sigma-Aldrich. Concentrated hydrochloric acid (HCl Suprapur 30%) and concentrated nitric acid (HNO<sub>3</sub> 67%, Prolabo) were from VWR. Ultrapure water for washing/preconditioning of plasticware and preparing test medium and LN stock/working solutions was obtained from a Veolia Purelab® Chorus apparatus.

### 2.2. Ecotoxicity testing with *Daphnia magna*

Parthenogenetic specimen of *D. magna* were reared in Lefebvre-Czarda (LC)-Volvic medium (Table S2) at 20 °C and under a 16h light : 8h dark photoperiod (light intensity 35 μmol/s/m<sup>2</sup>). The growth medium was changed three times a week and daphnids were fed an algal mixture of *Chlorella vulgaris*, *Raphidocelis subcapitata* and *Scenedesmus subspicatus* (2.5, 5, and 2.5 × 10<sup>6</sup> cells/day/daphnia, respectively) after each medium renewal.

Acute immobilization tests (48h) were performed according to international guideline NF EN ISO 6341 (2012) at 20 ± 2 °C in the dark as per guideline specifications. Daphnids less than 24h old were used in all tests and were obtained from parent organisms aged from two to six weeks. Organisms were not fed during the tests. The composition of the standardized ISO 6341 medium was: CaCl<sub>2</sub> × 2H<sub>2</sub>O (294 mg/L), MgSO<sub>4</sub> × 7H<sub>2</sub>O (123 mg/L), NaHCO<sub>3</sub> (64.7 mg/L), and KCl (5.75 mg/L). Calculated medium hardness was approx 250 mg/L as CaCO<sub>3</sub>.

In range-finding tests (data not shown), daphnids were exposed to a series of seven dilutions of all LN (except the radioactive Pm) at concentrations ranging from 0.05 to 45 mg/L of the corresponding salt (see Table S3 for details and the equivalent elemental concentrations). This range had to be extended up to 1,750 mg/L of LN salts to identify the appropriate test concentrations for the definitive tests with La, Nd, Sm, Tb, Dy, Ho, Er and Tm (Table S3). Preliminary tests were performed at an initial pH of 7.8 units, as recommended by the ISO 6341 norm, and of 6.5 units to reduce the visible precipitation of lanthanides carbonate species without compromising organisms' survival. In the preliminary tests performed at the initial pH value of 7.8, observed mortality did not span the 0–100% range required for optimal EC<sub>x</sub> estimations. Definitive tests were therefore carried out only at an initial pH of 6.5 units.

In definitive tests, minimum and maximum nominal exposure concentrations (expressed as elemental values) were 0.7 to 15 mg/L and 20 to 70 mg L<sup>-1</sup>, respectively. A dilution series of nine or ten concentrations plus one control (test medium without added LN) were used for each element (Table S4). Test tubes were preconditioned for 24h with the corresponding test solutions to minimize adsorption losses on test vials. The preconditioning solutions were discarded before starting the experiments. Four replicate exposures (10 mL total volume) with five organisms each were used for all test concentrations. Two independent definitive tests were performed for each element. During each test, immobilization of daphnids was evaluated after 24 and 48h of exposure (Table S4). The pH of exposure solutions was measured in control tubes at t = 0 and 48h and also in selected exposure concentrations at t = 48h. Initial pH was therefore assumed to be homogenous for all exposure concentrations. Although this assumption could have been violated in some cases, a proper discussion of ecotoxicological results remains possible thanks to the presence of linear relationships between pH and added LN (see sections 4.1 and 4.4). Potassium dichromate was used as a positive control with EC<sub>50</sub> 24h values between 0.6 and 2.1 mg/L of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> as recommended by the ISO 6341 norm (2012).

### 2.3. Analytical verification of exposure concentrations in the test medium

Analytical determinations were carried out by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS, Nexion 350x, Perkin Elmer). The following isotopes were measured: <sup>139</sup>La, <sup>140</sup>Ce, <sup>141</sup>Pr, <sup>146</sup>Nd, <sup>147</sup>Sm, <sup>153</sup>Eu, <sup>157</sup>Gd, <sup>159</sup>Tb, <sup>163</sup>Dy, <sup>165</sup>Ho, <sup>166</sup>Er, <sup>169</sup>Tm, <sup>173</sup>Yb, <sup>175</sup>Lu. Rhenium (<sup>187</sup>Re) was used as internal standard. Limits of detection (LOD) and quantification (LOQ) ranged from 0.1 to 0.8 µg/L (Table S5). LOD, analytical and procedural blanks were prepared and analyzed using the same analytical procedure as for samples. The accuracy of the whole analytical protocol was validated using international certified material (BCR-668- mussel tissues) from LGC standards. The recovery of LN ranged from 105 to 116% (Table S5).

Actual exposure concentrations were analytically verified at t = 0 and 48h for total (unfiltered) concentrations and at t = 0, 5, 24 and 48h for filterable concentrations. Filtrations were performed with 0.45 µm syringe filters (Millex 33 mm diameter, mixed cellulose esters – MCE; Millipore, reference SLHA033SB, lot R7HA27577). The day before use, filters were washed with 10 mL of diluted (10% v/v) HCl, rinsed three times with 10 mL of ultrapure water and stored at 4 °C in the dark in a zip-bag with a few drops of MilliQ water to prevent drying of the membranes. Filtered and unfiltered solutions were collected in 15 mL falcon tubes previously preconditioned in two baths of diluted 10% v/v HNO<sub>3</sub> followed by two baths of MilliQ water. The soaking time was 24h in each bath. Except for t = 0h, all solutions for analytical measurements were incubated in the presence of daphnids as described for ecotoxicity tests. The first 2 mL of filtered solutions were discarded and any living daphnids or visible debris were eliminated from the unfiltered samples before transfer into the falcon tubes. All samples were acidified at 1% v/v with concentrated nitric acid and stored at 4 °C in the dark pending analysis. Analyses at t = 0 and 48h were performed in duplicate for both total and filterable concentrations. Measurement precision was estimated using the formula:

$$(1) \quad Precision = \sqrt{abs\left(\frac{m_1 - m_2}{2}\right)}$$

where  $m_1$  is the first replicate measurement,  $m_2$  is the second replicate measurement and  $abs$  is the absolute value of the difference.

Analytical verifications were carried out on solutions initially amended with a concentration corresponding to the 24h EC<sub>50</sub>, hereinafter C<sub>EC50</sub>, determined in the first round of definitive test. Tubes for the analytical verification of LN concentrations were incubated in parallel with the second definite tests and pH values were verified at t = 0 and 48h for selected concentrations (see sections 3.3 and 3.4). For selected elements Lanthanum (La), Neodymium (Nd), Gadolinium (Gd) and Ytterbium (Yb), total and filterable concentrations were measured also in solutions amended with the minimum (C<sub>min</sub>) and

maximum ( $C_{MAX}$ ) test concentrations (Table S4). These additional measurements were performed in duplicate at  $t = 0, 24$  and  $48\text{h}$  using solutions where daphnids were initially added (except at  $t = 0\text{h}$ ). The pH was checked in all the  $C_{min}$  and  $C_{MAX}$  solutions immediately before filtration and/or transfer to falcon tubes for analysis. Procedures for filter cleaning and removal of debris from unfiltered solutions were as previously described.

#### 2.4. Speciation of lanthanides in the test medium

Speciation calculations were performed using VisualMinteq (VM) version 3.1. The precipitation of carbonate and hydroxide complexes was allowed as ‘possible solid phases’. The default VM database specifies values for carbonate solubility products only for La, Neodymium (Nd), Samarium (Sm), Europium (Eu), Gd, Dysprosium (Dy) and Yb. Additional calculations were therefore performed using the carbonate solubility products determined by Firsching and Mohammadzadei (1986) who provided values for all lanthanides except Ce (Table S6). The value reported by Li et al. (2004) was used for  $\text{Ce}_2(\text{CO}_3)_3$  solubility. Possible precipitation of sulfate and nitrate complexes was not considered because it requires LN concentrations well above those used in our experiments; *i.e.* above tens or hundreds of mM for  $\text{LN}_2(\text{SO}_4)_3$  precipitation (Judge et al., 2023; Moldoveanu et al., 2024) and in the molar range for  $\text{LN}(\text{NO}_3)_3$  precipitation (Siekierski & Salomon, 1985).

Input temperature was fixed at  $20^\circ\text{C}$ , medium alkalinity was specified as  $0.831\text{ meq/L CaCO}_3$  and the Debye-Hückel equation was used for activity correction. Calculations were performed in pH range 5 to 9 using increments of 0.2 units and for nominal concentrations from  $10\text{ ng/L}$  ( $5.7\text{--}7.2 \times 10^{-11}\text{ M}$ ) to  $50\text{ mg/L}$  ( $2.9\text{--}3.6 \times 10^{-4}\text{ M}$ ). The upper and lower limit of the molar concentration ranges refer to La and Lu, respectively. The nominal concentration range used for speciation calculation covered the range of tested and measured concentrations in the present study. For each element, theoretical solubility limits were estimated from speciation calculation by subtracting the percentage of precipitated concentrations from the corresponding nominal values.

Speciation was also modelled for solutions amended with concentrations corresponding to the 24h  $\text{EC}_{50}$  obtained in the first round of definitive tests for each element. In this case, the elemental concentration for VM runs was entered as the time-weighted mean (TWM) of the 24h  $\text{C}_{\text{EC}_{50}}$  to account for the concentrations decreases observed over the test duration (see the Results section for details). The TWM was calculated from the experimentally measured concentrations at 0 and 24 hours using the formula (OECD, 2012):

$$(2) \quad TWM = \frac{\text{Conc}_{t(x)} - \text{Conc}_{t(x+1)}}{\text{Ln}(\text{Conc}_{t(x)}) - \text{Ln}(\text{Conc}_{t(x+1)})}$$

where:  $\text{Conc}_{t(x)}$  is the measured concentration at  $t = 0\text{h}$ ;  $\text{Conc}_{t(x+1)}$  is the measured concentration at  $24\text{h}$ ; Ln is the Napierian logarithm of the measured concentrations at any time ‘t’.

Because TWM concentrations are mathematical estimates, the corresponding pH values to specify for VM calculations were estimated as follows. Changes in pH were monitored between 0 and  $48\text{h}$  for several concentrations of all tested elements (Table S7). Except for Praseodymium (Pr), linear relationships existed between pH at  $48\text{h}$  and LN nominal concentrations. These relationships were used to estimate the expected pH values for the calculated TWM concentrations and for the concentrations measured at  $t = 24$  and  $48\text{h}$  (Table S8). In the case of Pr, the pH value of the concentration closer to the TWM  $\text{EC}_{50}$  was used. It will be shown later that pH changes were minor between 24 and  $48\text{h}$ .

#### 2.5. Data analysis and statistics

The presence of statistically significant linear relationships between variables was verified using the standard Pearson least squares method and a robust MM estimation method that is less sensitive to outliers and non-normally distributed errors (Susanti et al., 2014; Venables & Ripley, 1999). The letters



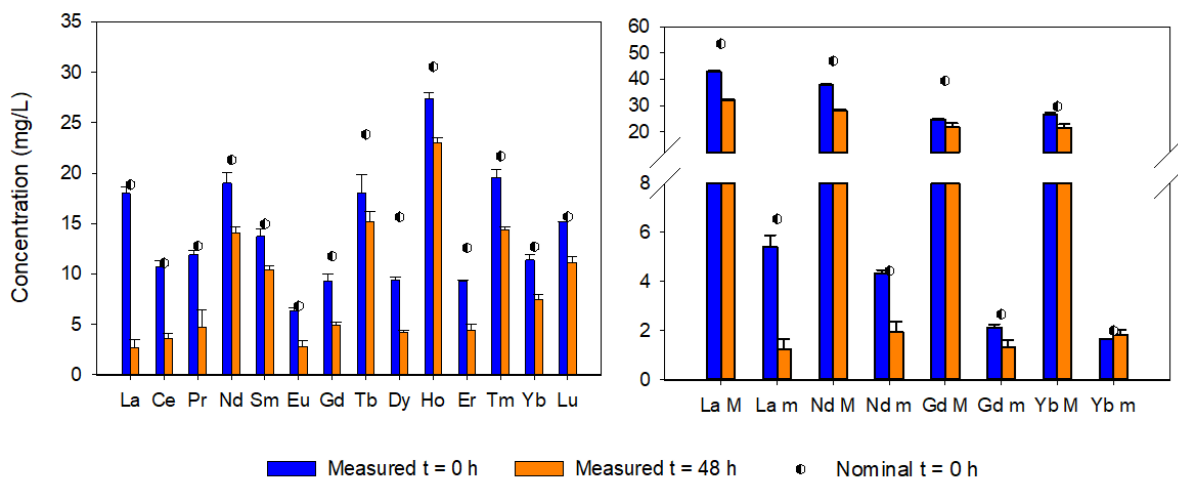
MM indicate that the estimation is of the maximum likelihood type, but do not form an acronym. Rather, they refer to the type of estimation procedure as explained in Susanti et al. (2014). Linear relationships were obtained with R version 3.6.0 (R Core Team 2019). Graphs were prepared using Sigma Plot (version 14).

Effect concentrations ( $EC_x$ ) were calculated with the Excel macro REGTOX (version EV7.0.7) using the Hill model. The macro and the related documentation are available in French at: [https://www.normalesup.org/~vindimian/fr\\_index.html](https://www.normalesup.org/~vindimian/fr_index.html).

### 3. Results

#### 3.1. Total concentrations in the test medium

Measured levels of LN in control solutions were always below the corresponding analytical detection limits (Table S5). The relative standard deviation (RSD) for replicate measurements of total LN concentrations was less than 10% at  $t = 0$  and usually within 30% at  $t = 48$  h. Total concentrations at  $t = 0$  were within 20% of the expected nominal values except for solutions amended with Dy at  $C_{EC50}$  where measured values were 60% of the expected ones (Fig. 1 and Table S9). This discrepancy, possibly due to dilution errors, does not affect the objectives of the present paper nor the data interpretation presented below. At the end of the tests (48h), total concentrations in solutions initially amended with  $C_{EC50}$  were within 25% of initial values for Nd, Tb, Ho, Tm, Sm and Lu, but had decreased by more than 40% for the other elements (Fig. 1A and Table S9). Total concentrations also decreased by more than 50% in medium aliquots spiked with  $C_{min}$  of La, Nd, and Gd (6.55, 4.44 and 2.67 mg/L, respectively), but remained relatively constant for Yb (2.0 mg/L). In solutions spiked with  $C_{MAX}$ , total concentrations (La = 53.5, Nd = 47.0, Gd = 39.4 and Yb = 29.6 mg/L) showed little variation over 48h (Fig. 1B, Table S9).



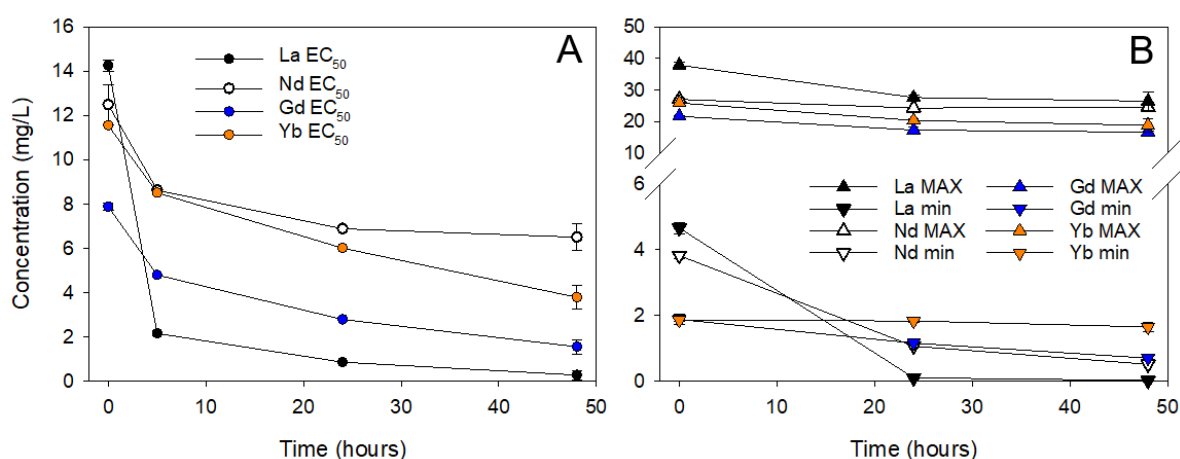
**Figure 1** - (A) Total concentrations of lanthanides (LN) measured at 0 and 48 hours in solutions initially amended with LN concentrations equal to the corresponding 24h  $EC_{50}$  for *D. magna*. (B) Same as panel A for solutions initially amended with the maximum (LN M) and the minimum (LN m) concentrations of La, Nd, Gd and Yb used in ecotoxicity tests. All measurements were performed in duplicate. Error bars were calculated using Formula 1 in the text. Nominal values expected at  $t = 0$  h are indicated for comparison purposes in both panels.

#### 3.2. Filterable concentrations in the test medium

In solutions amended with  $C_{EC50}$ , the ratio between total and filterable concentration at  $t = 0$  h was between 80 and 120% for all elements except Nd (65%), Pr (77 %) and Sm (77%) (see Table S9 for raw

results and section 4.2 for discussion). In the same solutions, filterable concentrations decreased by 50–98% over the test duration according to three temporal patterns as described in figure 2A for selected elements and in Table S9 and Figure S2 for the entire LN series. In pattern one (La and Ce), filterable concentrations decreased by more than 80% within 5h from the start of the incubation and fell below 5% of the initial values after 48 hours. In pattern two (Pr, Sm, Eu, Gd, Er, Dy, Yb), the concentration decrease was less rapid and reached 70–95% after 48 hours. In pattern three (Nd, Tb, Tm, Ho, Lu), the overall concentration decrease was less than 50% with little changes between 24 and 48h. After 48h, the differences between total and filterable concentrations were less than 2-fold for Nd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, but between 3- and 34-fold for the other elements of the series. Small differences between total and filterable concentrations suggest either a higher solubility or the formation of precipitates that were not retained by the 0.45  $\mu\text{m}$  filters.

In solutions amended with  $C_{\text{min}}$  and  $C_{\text{MAX}}$ , the ratio between total and filterable concentrations at  $t = 0\text{h}$  was between 85 and 110% except for Nd at  $C_{\text{MAX}}$  (see section 4.2 for discussion). At  $C_{\text{min}}$ , the behavior of La, Nd, Gd and Yb essentially agreed with the observations at  $C_{\text{EC50}}$ , while filterable concentrations remained within 30% of the initial values for  $C_{\text{MAX}}$  scenarios (Fig. 2B, Table S9).

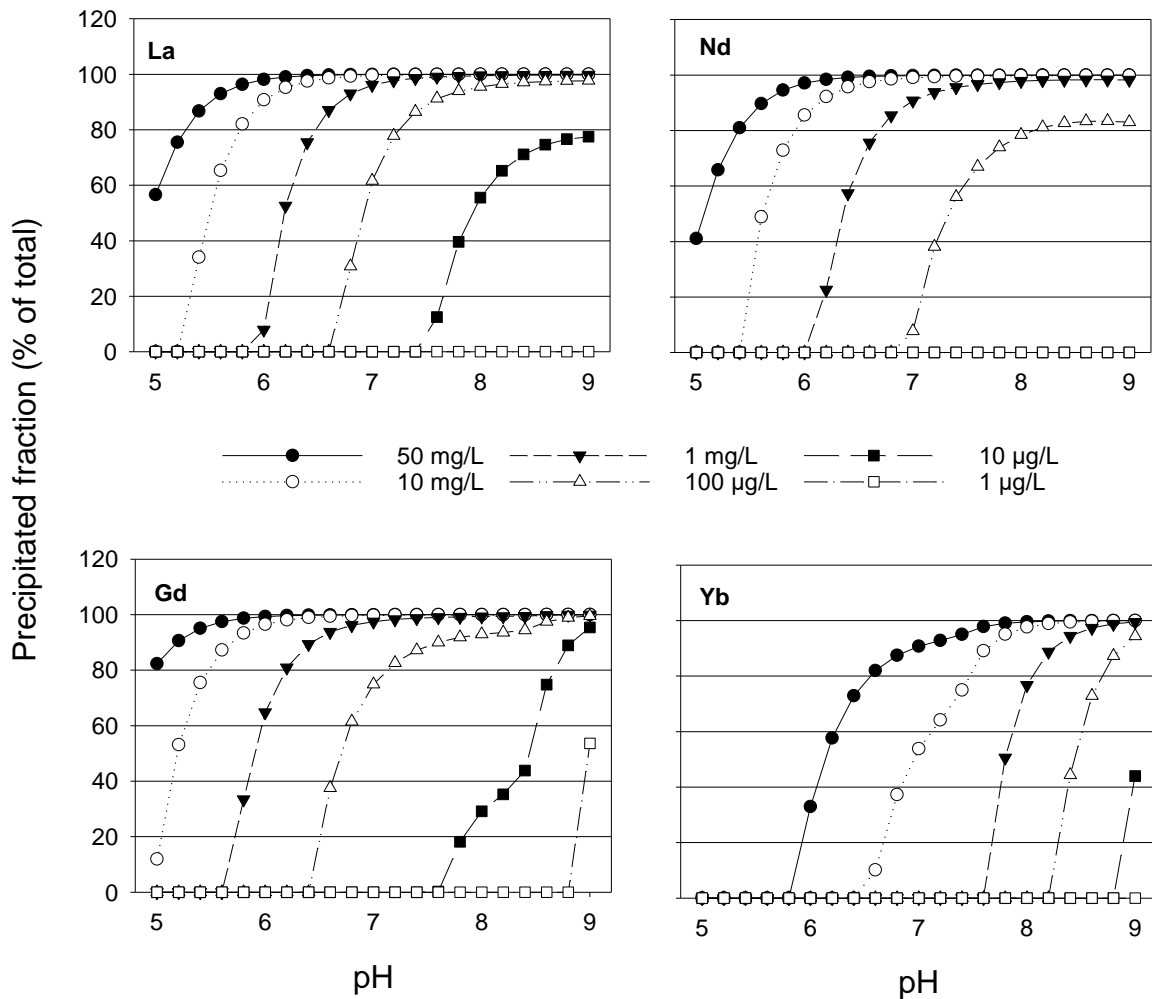


**Figure 2 -** (A) Temporal changes in the filterable concentrations ( $< 0.45 \mu\text{m}$ ) of La, Gd, Nd, Yb for solutions initially amended with lanthanides concentrations equal to the corresponding  $EC_{50}$  at 24h. The selected elements are representative of the three different behaviors across the whole lanthanides series (see text for details and Table S9 and Fig. S2 for full results). Measurements at  $t = 0$  and  $t = 48\text{h}$  were performed in duplicate. (B) Same as panel A, but for solutions initially amended with maximum (MAX) and minimum (min) tested concentrations. All measurements performed in duplicate. Error bars may be smaller than the corresponding symbols.

### 3.3. Theoretical solubility of lanthanides in the test medium

The present section will focus on speciation results obtained using the carbonate solubility products ( $K_{\text{SP}}$ ) established by Firsching and Mohammadzadei (1986), except for La and Ce. In the case of La, using the  $K_{\text{SP}}$  of Firsching and Mohammadzadei (1986) clearly overestimated La solubility compared with experimental results. The default  $K_{\text{SP}}$  in the VisualMinteq (VM) database was therefore preferred (Table S6). An indicative  $K_{\text{SP}}$  for  $\text{Ce}_2(\text{CO}_3)_3$  was taken from Li et al. (2004), because no value for Ce was available in Firsching and Mohammadzadei (1986).

In the pH range from 6.0 to 7.8 (covering the values measured in ecotoxicity tests, Table S7), formation of insoluble carbonate species was predicted for all LNs and for concentrations as low as 10  $\mu\text{g/L}$  (Fig. 3). Formation and precipitation of hydroxides,  $\text{LN}(\text{OH})_3$ , was also predicted for Er, Tm, Yb and Lu.



**Figure 3** - Theoretical solubility of La, Nd, Gd and Yb as a function of pH in ISO 6341 medium for acute toxicity testing with *Daphnia magna*. Model calculations were performed for theoretical concentration from 1 µg/L to 50 mg/L. No precipitation was predicted in theoretical solutions amended with 10 and 100 ng/L (plots not shown).

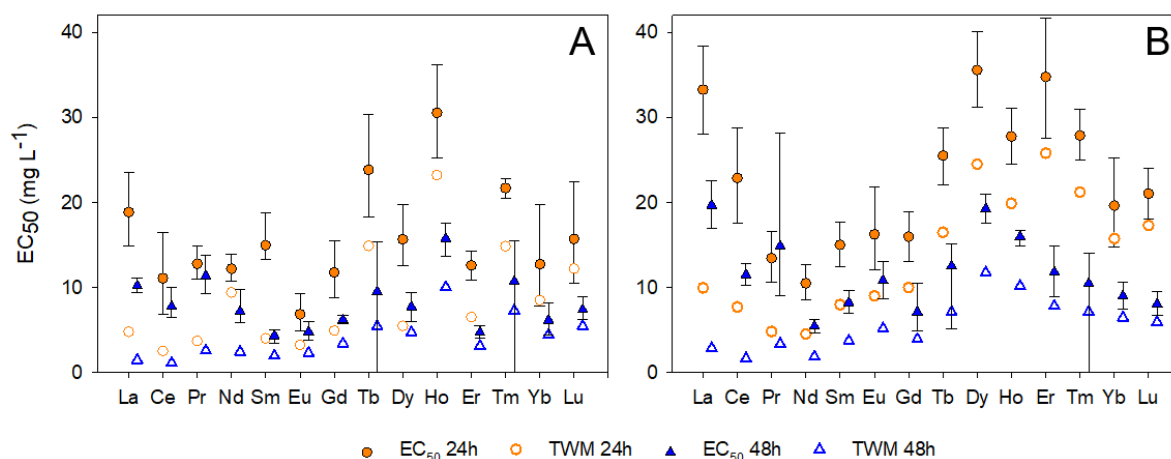
### 3.4. Lanthanides ecotoxicity

In the first round of definitive test, nominal  $EC_{50}$ s ranged from 6.84 to 30.5 mg/L and from 4.70 to 15.7 mg/L after 24 and 48h of exposure, respectively (Fig. 4a; Table S10). To account for the decrease in LN concentrations over time (section 3.1), the TWM  $EC_{50}$  were calculated using Formula 2. For the 24h TWM  $EC_{50}$ , the experimental data presented in section 3.1 (*i.e.*, the filterable concentrations at  $t = 0$  and  $t = 24$  h) were directly entered into Formula 2. For the 48h TWM  $EC_{50}$ , the nominal 48h  $EC_{50}$  values were used as input for the concentrations at  $t = 0$ h. The corresponding LN concentrations at 48h were estimated using Formula 5 (see section 4.2) and then used as input for the concentrations at 48h in Formula 2. Note that the experimental measurements at 48h (section 3.1) could not be used for this purpose, because they refer to medium aliquots initially spiked with concentrations equal to the 24h  $EC_{50}$ . Results from the calculation procedure are summarized in Table S11. A detailed explanation of the calculation procedure is provided in the caption of Table S11 in the readme file accompanying the supporting information. For comparison purposes, the same procedure was also performed for the 24h TWM  $EC_{50}$  estimated using Formula 4 (Table S11).

The TWM 24h  $EC_{50}$  calculated using experimental values differed from the nominal  $EC_{50}$  by more than 2-fold for La, Ce, Pr, Sm, Eu, Gd and Dy (Fig. 4A). The 24h TWM  $EC_{50}$  estimated using Formula 4 were within



20 % of those obtained using experimental data for La, Pr, Eu, Tb, Ho, Tm, Yb, Lu and within a factor of two for the remaining elements except Nd (Table S11). Differences between nominal and TWM 48h EC<sub>50</sub> were more than 2-fold for La, Ce, Pr, Nd, Sm and Eu (Table S11). The observations confirm that TWM correction would avoid underestimating the ecotoxicity of La, Ce, Pr, Sm, Dy and, to a lesser extent, Eu and Gd (Fig. 4A). For the second round of definitive tests, 24h and 48h EC<sub>50</sub> ranged from 10.5 to 35.6 mg/L and 5.4 to 19.7 mg/L, respectively (Table S10, Fig. 4B). The TWM corrections were performed using Formulas 6 and 7 (Table S11), leading to results and conclusions equivalent to those discussed for the first round of tests.

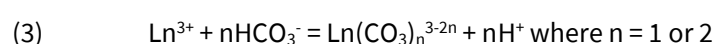


**Figure 4** - Nominal and Time-Weighted Mean (TWM) EC<sub>50</sub> values for specimen of *Daphnia magna* exposed to lanthanides for 24 or 48h in the first (panel A) and second (panel B) series of definitive tests. Bars represent 95% confidence intervals for nominal EC<sub>50</sub> (estimation not possible for TWM). Values for 24 and 48h have been slightly offset for graphical representation purposes.

## 4. Discussion

### 4.1. Temporal changes in total LN concentrations

In the present study, total LN concentrations at t = 0h were in good agreement with expected values, but showed an unexpected decrease at the end of the test for several LN (Fig. 1 and Table S9). Although LN tend to form insoluble precipitates in the test medium, we cannot exclude that the decrease in total LN concentrations could partly originate from adsorption losses onto the tubes walls despite preconditioning of the test tubes. However, daphnids can actively ingest LN precipitates forming into the test medium and contribute to the observed losses, although the extent of such ingestion is element-dependent (Revel et al., 2023). The effect of LN addition on the pH of the test medium (Tables S7, S8, S9) can also contribute to the observed temporal changes in their concentrations. The inverse relationship between added LN concentrations and pH (Table S8) reflects the formation of LN<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub> complexes in test solutions along with the release of protons according to the following equilibrium (Luo & Byrne, 2004):



As for alkaline waters in equilibrium with atmospheric CO<sub>2</sub> (Luo & Byrne, 2004), carbonate complexes are expected to dominate LN speciation regardless of the added concentrations. The formation and subsequent precipitation of such complexes consume the alkalinity of the test medium (provided by the NaHCO<sub>3</sub> component), reduce its buffering capacity, shift its pH toward more acidic values via the release

of protons (Formula 3) and eventually increase LN solubility at high concentrations (see section 3.3, Tables S7 and S9). The formation of protons following LN addition to the test medium explains why pH remained acidic in medium aliquots amended with  $C_{MAX}$  (Table S9) and why pH and LN concentration were inversely correlated (Tables S7 and S8). However, medium pH increased from the beginning to the end of the tests in control exposures and in medium aliquots amended with  $C_{EC50}$  and  $C_{min}$  (Tables S7 and S9). These observations can be explained by considering that test tubes were opened to the atmosphere; *i.e.*, the carbonate system ( $H_2CO_3 - HCO_3^- - CO_3^{2-}$ ) of the medium could equilibrate with atmospheric  $CO_2$  over time. In control exposures, the protons added to lower the initial pH to 6.5 (section 2.2) shifted the equilibrium of the carbonate system toward to formation of carbonic acid ( $H_2CO_3$ ). The newly formed  $H_2CO_3$  will dissociate into  $H_2O$  and  $CO_2$ , with  $CO_2$  evading from the solution into the atmosphere. The overall outcome of this process is a removal of protons from the medium resulting in an increase in pH. In solutions amended with  $C_{EC50}$  and  $C_{min}$ , both processes (Formula 3 and the equilibrium of the carbonate system) contributed to determine the pH of the solutions at the end of the test.

Considering that pH differences could exceed one unit across the exposure range (Table S7), LN speciation was not necessarily homogenous for all exposure concentrations. The ecotoxicological significance of such variability remains to be ascertained and will be further discussed in section 4.4.

#### 4.2. Temporal changes in filterable LN concentrations

Filtrable concentrations at  $t = 0$  agreed well with the corresponding total ones except for Pr, Nd, and Sm (section 3.2 and Table S9). Although filters were acid washed and preconditioned before use (section 2.3), we cannot completely exclude possible adsorption losses onto filter membranes. Possible (element specific) filtration artifacts may require specific investigations, especially when comparing results from different studies or combining them for regulatory purposes (see section 5).

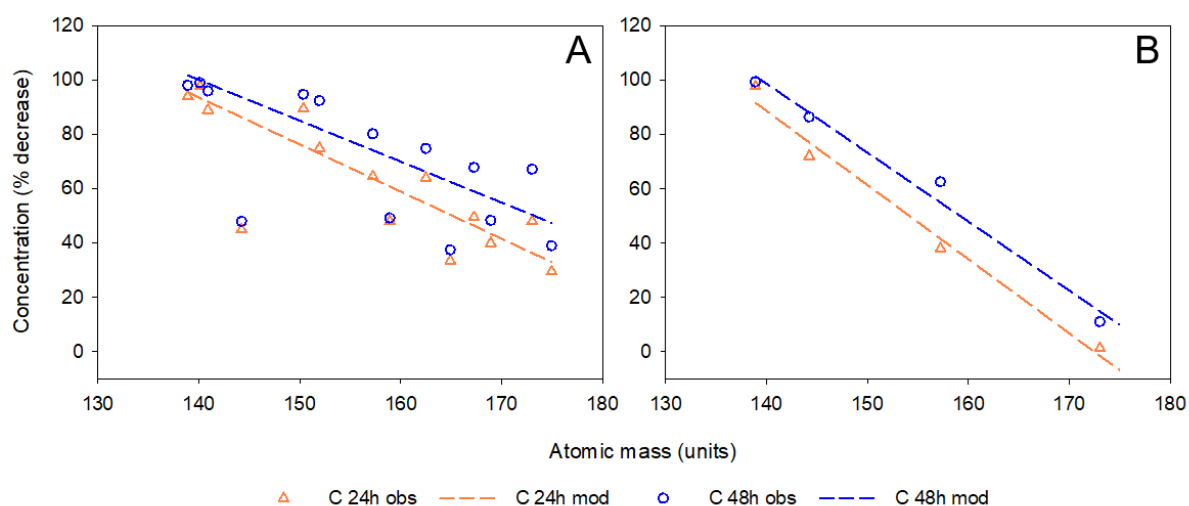
Assuming that our results are internally coherent (same filter type and batch, same procedures for all elements), the decrease in filterable concentrations was always more marked than the corresponding total ones, suggesting the presence of insoluble LN particles large enough to be retained by the pores of 0.45  $\mu m$  filters (see also section 3.3). Inverse linear correlations (Formulas 4 to 7) existed between the decrease in filterable concentrations at 24 and 48h (expressed as % of initial concentrations) and LN atomic masses in solutions amended at  $C_{EC50}$  and  $C_{min}$  (Fig. 5). Robust MM regressions showed a better fit compared with conventional partial least-squares regressions for the  $C_{EC50}$  scatterplots, while the performance of the two regressions was equivalent for  $C_{min}$  (data not shown). For the sake of clarity, only robust MM regressions will be presented and discussed.

$$(4) \quad \%Loss C_{EC50\_24hMM} = (-1.7353 \pm 0.2517) \text{ atomic mass} + (337 \pm 40) \quad R^2 = 0.70$$

$$(5) \quad \%Loss C_{EC50\_48hMM} = (-1.5065 \pm 0.3772) \text{ atomic mass} + (311 \pm 59) \quad R^2 = 0.54$$

$$(6) \quad \%Loss C_{min\_24hMM} = (-2.7296 \pm 0.2438) \text{ atomic mass} + (471 \pm 38) \quad R^2 = 0.98$$

$$(7) \quad \%Loss C_{min\_48hMM} = (-2.5407 \pm 0.2461) \text{ atomic mass} + (454 \pm 38) \quad R^2 = 0.98$$



**Figure 5** - Scatterplots of percentage decrease in lanthanides concentrations vs. atomic mass at 24 and 48h in solutions initially amended with LN concentrations equivalent to the corresponding  $C_{EC50}$  (panel A) and to the minimum tested concentration  $C_{min}$  (panel B). Triangles and circles correspond to the observed (obs) percentage concentration decreases calculated from experimental data. Dotted lines are the corresponding modeled (mod) linear fits to the observed values using robust MM regressions (Formulas 4 to 7). Note that a 100% decrease means a filterable concentration equal to zero after 24 or 48h, while a 0% decrease means that concentrations did not change over time. See text and Figure S1 for the actual meaning of modelled values above 100% or below 0.

The better goodness-of-fit at 24h reflects the presence of elements showing little concentration decrease between 24 and 48h (*i.e.*, pattern three in Figs. 2 and S2). Regression slopes were higher for solutions amended with  $C_{min}$  (Formulas 6 and 7) than with  $C_{EC50}$  (Formulas 4 and 5). These results indicate that i) the concentrations of lanthanides decreased faster in solutions amended with  $C_{EC50}$  than with  $C_{min}$  and ii) the differences in concentration decrease between  $C_{min}$  and  $C_{EC50}$  progressively became more important from La to Lu (see Fig. S1 for details and examples). In solutions amended with  $C_{EC50}$ , the observed percentage decrease in Nd concentrations (atomic mass = 144.24) was markedly lower than expected at both 24 and 48h (Fig. 5). We do not have a definite explanation for Nd behavior, but we notice that El-Akl et al. (2015) reported the formation of Nd-colloidal particles with an approximate size of 60 nm in a non-complexing test medium for freshwater algae. Particles of such sizes can probably pass through the pores of standard 0.45  $\mu\text{m}$  filters. However, it remains unclear while only Nd would form such small-sized particles compared with other LN. Some modelled % losses fall outside the 0–100 % range; *i.e.* the actual boundaries for experimental observations. These model artifacts do not invalidate the clear trend in increasing LN solubility from La to Lu in the ISO 6341 test medium for acute ecotoxicity testing with *D. magna*. The proposed linear models can be used to optimize analytical efforts to check for exposure concentrations in ISO test medium for *D. magna* over the test duration (at least for concentration up to  $C_{EC50}$  and with the possible exception of Nd).

Although decreasing total or filterable LN concentrations have been previously reported in standardized test media for *D. magna* or *Daphnia* spp. (Barry & Meehan, 2000; Blinova et al., 2018; González et al., 2015; Lachaux et al., 2022; Ma et al., 2016; Vukov et al., 2016), the present study provides the first data of the different temporal trends in concentration decrease across the whole LN series. Overall, LN solubility appears to be time and concentration dependent. Correct interpretation of LN ecotoxicity data therefore needs a detailed follow up of changes in exposure concentrations over test duration.

We acknowledge that TWM (Formula 2) is just one of the available approaches to account for decreasing exposure concentrations during ecotoxicity tests. For example, using geometric means instead of TWM concentrations would result in lower, average exposure concentrations over the test duration in the case of 24h EC<sub>50</sub> for the first round of definitive tests (Table S11). More refined calculations of the TWM are also possible when exposure concentrations are verified at multiple time points during ecotoxicity tests. The data available for t = 0, 5 and 24h (Figure 2 and Table S9) provide a useful example in this sense. First, the average exposure concentrations between 0–5h (0.21 days) and 5–24h (0.79 days) can be calculated using a slightly modified version of Formula 2 that was presented earlier:

$$(8) \quad \text{Average concentration}_{\text{period}} = \frac{\text{Conc}_{t(x)} - \text{Conc}_{t(x+1)}}{\text{Ln}(\text{Conc}_{t(x)}) - \text{Ln}(\text{Conc}_{t(x+1)})} * \text{Days}$$

As already seen for Formula 2, t(x) and t(x+1) refer to concentration at the beginning and at the end of each exposure period. The calculated average concentration during each period is then multiplied by the duration of the exposure period itself (in days). Finally, the overall TWM is obtained by summing the average concentration for each period and dividing by the total duration in days; *i.e.*, one day in the present case. We notice that Formula 8 simplifies to Formula 2 when measurements are available only at 0 and 24h.

The TWM obtained with Formula 8 are lower than those obtained using Formula 2, except for Nd, and in good agreement with the corresponding geometric means (Table S11). Considering the variety of possible approaches, the research community should reach a consensus about the preferred calculation method(s) to estimate average exposure concentrations during ecotoxicity tests with LN when their concentrations are not stable over the entire test duration. This consensus is especially necessary to facilitate comparisons across studies used for regulatory decisions.

Caution is also required to understand the impact of adding large quantities of LN to the test medium. Indeed, LN concentrations remained within  $\pm 30\%$  of the initial values in solutions amended with C<sub>MAX</sub> for La, Nd, Gd and Yb; contrary to what observed for C<sub>EC50</sub> and C<sub>min</sub> (Figure 2A, B). Furthermore, the pH in solutions amended with C<sub>MAX</sub> remained around the initial value of 6.5 units or slightly decreased over time, again in contrast with observations of C<sub>EC50</sub> and C<sub>min</sub> where pH increased to about 7.5 units over the test duration (Table S7 and S9). To the best of our knowledge, these are the first results considering the changes in pH during standardized ecotoxicity test with *D. magna* following protocol ISO 6341 (2012). These findings could also bear relevance for correct interpretation of chronic ecotoxicity tests such as the *Daphnia magna* reproduction test (OCDE, 2012). Depending on the tested LN, exposure concentration can decrease by up to 80% of initial values in five hours; a span much shorter than the 48 h among medium renewal usually adopted in chronic tests with *D. magna*.

### 4.3. Theoretical solubility of LN

At the initial pH for the definitive ecotoxicity tests (range from 6.4 to 6.6, Table S7), all LN concentrations were above the corresponding theoretical solubility except for the C<sub>min</sub> of Er, Tm, Yb and Lu (Table S12). The decrease in total and filterable LN concentrations over time (Fig. 1, Table S9) therefore agreed with the modelling results. However, the agreement between modelled and observed LN solubility was far from perfect, reflecting the limitations of thermodynamic speciation models for our experimental matrix and conditions.

In solutions spiked with C<sub>EC50</sub>, measured filterable concentrations at t = 24 and 48h were consistently higher than the corresponding theoretical solubility values in the pH range from 6.6 to 7.4 (Table S12). The pH range 6.6–7.4 covers the corresponding values estimated from concentration–pH relationships in solutions initially spiked with C<sub>EC50</sub> (Table S8). The discrepancies between theoretical and measured concentrations could simply indicate that the system had not yet reached equilibrium at the end of the experiments. This explanation holds for La, Ce, Pr, Sm, Eu, Gd, Dy, Er, Tm and Yb. The filterable

concentrations of these elements continuously decreased between 0 and 48h (Fig. 2) and the trend could have continued beyond 48h. On the other hand, the non equilibrium explanation does not apply to Nd, Tb, Ho, Tm and Lu whose filterable concentrations plateaued after 24h while remaining above the corresponding theoretical solubility limits (Fig. 2). One possibility is that the measured filterable concentrations of these elements included colloidal-sized precipitates small enough to pass through the filter pores. The presence of colloids could have increased the apparent solubility of these elements compared with VM calculations that do not consider the particle size of the precipitates. The formation of such colloidal-sized particles (60 nm in the specific case) has indeed been reported in a simple ecotoxicological medium spiked with Ce (El-Akl et al., 2015). Furthermore, the filterable elemental fraction may vary depending on the filter material as well as the characteristics and quantity of filtered waters (Hall et al., 2002; Horowitz, 2013). While we expect our filtration results to be internally coherent (same medium and same filter type for all samples), the true percent filterable metal contained in medium ISO6341 may well vary depending on methodological choices for filtration. Finally, the conditional nature of VM modelling parameters could also contribute to the observed differences for all LNs (Rodríguez de San Miguel et al., 2023).

The reasoning developed for solutions amended with  $C_{EC50}$  also applies to experiments using  $C_{min}$  for La, Nd, Gd and Yb. In solutions amended with  $C_{MAX}$  for the same elements, all daphnids died within 24 h and pH values at  $t = 48h$  had dropped below the initial value of 6.5 (Table S9). Low pH values are expected to increase LN solubility and likely contributed to the stability of filterable concentrations in these solutions (Fig. 2) even if the added LN levels initially exceeded the corresponding theoretical solubilities and precipitation could be expected (Fig. 3 and Table S12). Furthermore, the death of daphnids likely released different organic molecules into test solutions. These molecules may also increase LN solubility as observed for humic acids (Pourret et al., 2007), soil organic matter (Lachaux et al., 2022) and organic ligands such as oxalate and citrate (Wen et al., 2024), but this hypothesis remains to be verified.

#### 4.4. Comparison of different exposure metrics for lanthanides ecotoxicity

The two rounds of tests gave equivalent results (*i.e.*, overlapping 95% confidence intervals of nominal  $EC_{50}$ ) for Pr, Nd, Gd, Tb, Ho, Tm, Yb, Lu and, at 24 h only, Sm (Table S10). In all other cases (La, Ce, Eu, Dy, Er, and, at 48 h only, Sm),  $EC_{50}$  were higher in the second round of definitive tests than in the first one (Table S10, Fig. 4). Except for Eu (see below), we surmise that these differences were caused by the higher pH values of the exposure solutions during the second round of tests (Table S7).

In both experiences, pH values were measured at  $t = 0h$  in control solutions (assumed to be representative of all exposure solutions at the beginning of the tests) and at 48h for selected concentrations (Table S7). Measurements of pH after 24h of exposure were not systematically performed during definitive tests to avoid additional stress to daphnids. However, available data showed limited pH variability between 24 and 48h (Table S9).

Statistically significant inverse linear relationships were found between the measured pH values at 48 h and the initial LN concentration in the test medium (Table S8). These linear relationships were used to estimate the pH value in hypothetical aliquots of test media spiked with the corresponding nominal  $EC_{50}$  (Table S8). Because pH controls the solubility of  $Ln_2(CO_3)_3$ , a higher pH value at the end of the test (data in Table S7) implies i) a larger and probably faster decrease in the actual exposure concentrations over the test duration (see sections 3.2 and 3.3) and hence ii) higher  $EC_{50}$  values in the second round of definitive tests. The pH explanation does not hold for Eu whose exposure solutions showed lower pH values for the higher  $EC_{50}$  in the second test series (Table S7 and S8). We acknowledge that the proposed simple explanation does not account for kinetics processes and for confounding processes linked to the presence of daphnids in the exposure solutions.

Besides the formation of insoluble precipitates, expressing the  $EC_{50}$  in terms of free  $Ln^{3+}$  concentrations in the dissolved fraction can provide further insight into the ecotoxicity of LNs and

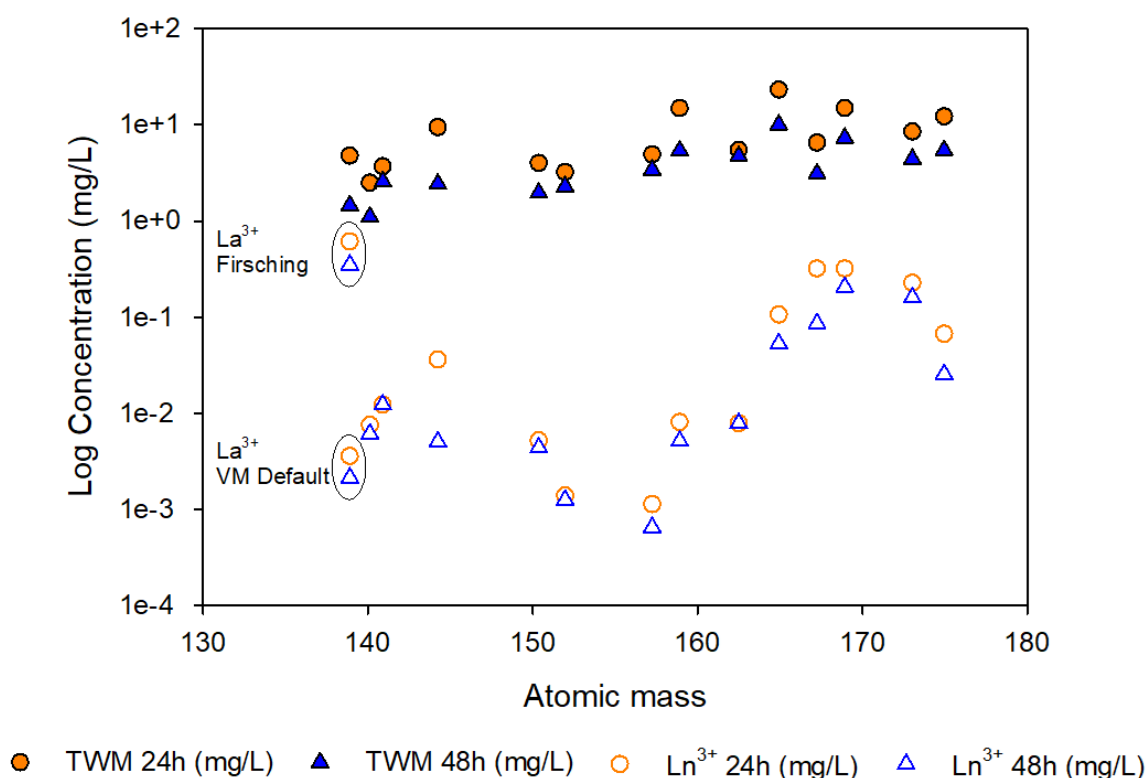


increase the reliability and relevance of concentration–response relationships. Estimation of  $\text{Ln}^{3+}$  concentrations was done for TWM  $\text{EC}_{50}$  values using the same assumptions described in section 3.3 for the choice of stability constants for  $\text{Ln}_2(\text{CO}_3)_3$  precipitation. These estimations will be presented only for the first series of definitive tests.

In absolute terms,  $\text{Ln}^{3+}$  based  $\text{EC}_{50}$  were between 1 to 10  $\mu\text{g/L}$  for La, Ce, Sm, Eu, Gd, Tb and Dy and in the tens to hundreds of  $\mu\text{g/L}$  for the remaining elements. Differences between  $\text{Ln}^{3+}$  and TWM-based  $\text{EC}_{50}$  were between 1 and 3 orders of magnitude at both 24 and 48h of exposure (Fig. 6). The actual ecotoxicological significance of such estimations depends on how well the constants used in the thermodynamic models are representative of the true LN speciation in the test medium. For example, estimates of free  $\text{La}^{3+}$  concentrations were three orders of magnitude higher when using the  $\text{La}_2(\text{CO}_3)_3$  solubility product reported by Firshing & Mohammadzadei (1986) compared with the default value in the VisualMinteq database (Fig. 6). Further work is needed to experimentally measure the free  $\text{Ln}^{3+}$  concentration in exposure medium and to determine confidence intervals to  $\text{EC}_{50}$  values based on TWM or free  $\text{Ln}^{3+}$ .

Considering the important quantities of LN salts added to the test medium (Table S3) and the tendency of test medium to get more acidic at high levels of added LN salts, it is important to verify the possible contribution of these two factors (pH and counterions added along with LNs) onto the survival of *D. magna*. Available reports (Chen et al., 2012) and online article abstracts (El-Deeb et al., 2011; Zhuang, 1994) suggest that effects of low pH become visible at values below 5.5 units, lower than those observed in the present study. In the case of the  $\text{NO}_3^-$  and  $\text{Cl}^-$  ions, the maximum concentration added along with LN were 76 mg/L and 46 mg/L, respectively (information on the specific counterion for each LN salt can be found in section 2.1). In the case of nitrates,  $\text{LC}_{50}$  reported in the literature for *D. magna* are in the range 323–611 mg/L  $\text{N-NO}_3^-$ , i.e., 1,430–2,705 mg/L  $\text{NO}_3^-$  (Scott & Crunkilton, 2000). In the case of chlorides,  $\text{LC}_{50}$  depends on the specific salt added to the test medium. Potassium chloride (KCl) has the lowest reported 48h  $\text{LC}_{50}$  of 660 mg/L, which corresponds to 314 mg/L of  $\text{Cl}^-$  (Mount et al., 1997). Based on this information, we estimated the toxic units ( $\text{TU} = \text{exposure concentration} / \text{LC}_{50}$ ) for each LN and the appropriate counteranion at the maximum concentrations added to the test medium. A worst-case scenario was used for the counteranion contribution; i.e., the maximum added  $\text{Cl}^-$  or  $\text{NO}_3^-$  concentration used in the present study was divided the lowest  $\text{LC}_{50}$  reported in the literature. The TU approach allows to compare the potential contribution of LN and counteranions to the overall ecotoxic effect for the maximum added LN levels. The TU values for counteranions were below 5% of those for LN in all cases except for Eu in one of the test series (Table S13). Overall, the contribution of counteranions to the observed ecotoxicity should therefore be secondary compared with that of the added LN. Depending on the added LN concentrations, future studies may consider including experimental verification of counterion ecotoxicity.

The  $\text{EC}_{50}$  values based on nominal values (Fig. 4 and Table S10) agree with those observed in previous studies with daphnids and other crustaceans (see the recent data compilation reported in Table S5 of Lachaux et al. (2022)). Comparison based on measured LN concentrations or on modelled free ion concentrations are less straightforward because of the methodological differences among the studies containing such information. In the case of Nd, Gd, and Yb, our TWM  $\text{EC}_{50}$  at 48h (Table S11) are within the 95% confidence intervals of the values reported by Lachaux et al. (2022) who used the same test medium and analogous methodologies. However, our estimations for  $\text{EC}_{50}$  based on modelled free-ion concentrations (Fig. 6) are three orders of magnitude lower than those of Lachaux et al. (2022) because of different assumptions in model calculations. In particular, we allowed the precipitation of  $\text{Ln}_2(\text{CO}_3)_3$  solids in the test medium and subtracted the precipitated concentrations from the total ones before calculating the free-ion concentrations. Both approaches can be acceptable depending on the experimenter's choice as to the complexation and solubility constants in the speciation model (see sections 3.3, 4.3 and 5 for further discussion).



**Figure 6** - Comparison between 24h and 48h Time Weighed Mean (TWM)  $EC_{50}$  expressed as total filterable concentrations and free  $Ln^{3+}$  concentrations. Free  $Ln^{3+}$  concentrations were estimated using VisualMinteq (see section 3.3 for details). Calculations for free lanthanum ( $La^{3+}$ ) are presented using two different  $La_2(CO_3)_3$  solubility products to invite caution when making ecotoxicological inference using modelled  $La^{3+}$  values (see text for details).

Blinova et al. (2018) did observe a marked decrease in La, Ce, Pr, Nd, and Gd concentrations in standard artificial freshwater (OCDE, 2004), but did not attempt to calculate 48h  $EC_{50}$  based on measured concentrations. Vukov et al. (2016) reported a 48h  $EC_{50}$  of 0.49 mg/L Dy for *D. pulex* based on the concentration measured at the end of the test. This value is 10- to 20-fold lower than the 48h TWM  $EC_{50}$  of the present study (Table S11). However, it compares favorably with the filterable Dy concentration of 2 mg/L that remained in our exposure medium after 48 hours (Table S9). Shu et al. (2023) reported 24h and 48h  $EC_{50}$  of 16.2 and 2.3 mg/L, respectively, for *D. magna* exposed to La according to OCDE guideline 202 (OCDE, 2004). These values referred to analytically measured filterable La concentrations at  $t = 0$  (after 2h of equilibration following the initial La spike). They are higher than our  $EC_{50}$  based on TWM (Table S11), but difficult to compare with nominal values considering the tendency of La concentration to rapidly decrease upon addition to the test medium (Fig. 1). Finally, Ma et al. (2016) substituted  $NaHCO_3$  with NaOH in the test medium for *D. magna* and successfully avoided carbonate precipitation when testing the ecotoxicity of Ce. As a result, the filterable (0.22  $\mu m$ ) Ce concentration showed little variation between the beginning and the end of the test. Their 48h  $EC_{50}$  of 1.5 mg/L (based on measured concentration) is in excellent agreement with our 48h TWM  $EC_{50}$  of 1.11 mg/L (1<sup>st</sup> series) and 1.64 mg/L (2<sup>nd</sup> series) (Table S11), showing that correction for concentration decrease can improve data comparability across studies. We are not aware of other studies providing  $EC_{50}$  based on free ion concentrations for *D. magna* or other daphnids, apart from the mentioned comparison with Lachaux et al. (2022).

#### 4.5. Trends in EC<sub>50</sub> along the lanthanides series

Ecotoxicologists are constantly seeking for the presence of regular, predictable variations in EC<sub>50</sub> along the LN series, in analogy with the predictable changes in physico-chemical properties such as ionic radius and ionization potential. Regular ecotoxicological patterns would allow to focus research efforts on a few representative lanthanides from which relevant information could then be derived for the whole series.

In the present study, nominal EC<sub>50</sub> values did not show any clear trend along the LN series after 24 or 48h of exposure (Fig. 4). On the other hand, linear relationships could be derived between the atomic mass of lanthanides and the EC<sub>50</sub> values expressed as TWM (Formulas 9 to 13, see Table S14 for the statistical significance of regression coefficients and calculation details). For 24h exposure in the first series of experiments, two relationships were derived; one using TWM values calculated from Formula 2 with analytically measured concentrations at 0 and 24h as inputs (Formula 9) and one using TWM estimated from Formula 4 (Formula 10). On average, the predicted values differed by 30–40 % from the input TWM values, with differences larger than 50% for many elements (Table S15).

$$(9) \quad TWM_{24h(1stSeries\_Exp)} = (0.2582 \pm 0.115) \text{ atomic mass} + (-32.0 \pm 18.1) \quad R^2 = 0.29$$

$$(10) \quad TWM_{24h(1stSeries\_Mod)} = (0.3010 \pm 0.0898) \text{ atomic mass} + (-37.5 \pm 14.1) \quad R^2 = 0.48$$

$$(11) \quad TWM_{48h(1stSeries)} = (0.136 \pm 0.0413) \text{ atomic mass} + (-17.4 \pm 6.5) \quad R^2 = 0.48$$

$$(12) \quad TWM_{24h(2ndSeries)} = (0.4516 \pm 0.1013) \text{ atomic mass} + (-56.9 \pm 15.9) \quad R^2 = 0.62$$

$$(13) \quad TWM_{48h(2ndSeries)} = (0.1720 \pm 0.0489) \text{ atomic mass} + (-21.3 \pm 7.7) \quad R^2 = 0.51$$

Expressing TWM EC<sub>50</sub> in terms of free ion concentrations resulted a clear M-shaped pattern at both 24 and 48h (Fig. 6). This pattern resembles the *tetrad effect* linked to the progressive filling of *f* orbitals along the lanthanides series (McLennan, 1994) and is also similar to the changes in the energy of electronic configuration from La to Lu (Schinzel et al., 2006). In particular, La, Gd and Lu have the largest differences between the energy of the highest occupied and lowest occupied molecular orbital and form the most stable complexes, which agrees with their higher ecotoxic potential (lower Ln<sup>3+</sup> EC<sub>50</sub>) compared with adjacent elements (Fig. 6). However, the solubility products used in the present study (Fig. S3) also showed a M-shaped trend when plotted against the atomic mass of the corresponding elements. Otherwise stated, the pattern observed in Ln<sup>3+</sup> EC<sub>50</sub> may simply be a model artifact linked to speciation calculation. Methodological developments are clearly necessary to obtain experimental measurements of LN speciation in ecotoxicological test media and verify the true meaning of this M-shaped pattern in Ln<sup>3+</sup> EC<sub>50</sub>.

Few studies examined the ecotoxic effects of the whole LN series and they reached contrasting results (Table 1). Data from Blinova et al. (2018) provide the most relevant comparison for the present study and suggest a linear decreasing trend for 48h LC<sub>50</sub> to *D. magna* based on nominal values (La > Ce > Pr > Nd ≈ Gd). However, a strong decrease in LN concentration was reported over the test duration and the trend based on measured concentration could not be validated based on the information provided in the paper. Similarly, 21 d chronic LC<sub>50</sub> did not show any trend (La ≈ Gd > Ce ≈ Pr ≈ Nd). Decreasing ecotoxicity along the LN series has been reported for *Hyaella azteca*, *Hydra attenuata* and *Danio rerio* based on nominal concentrations (Table 1). However, the heaviest lanthanides did not conform to the observed trends for *H. azteca* and *H. attenuata* (see footnotes in Table 1). These findings (*i.e.*, a decrease in ecotoxicity along the LN series) agree with the observations of the present study, but the comparisons cannot be considered fully conclusive due to the missing analytical verification of exposure concentrations in these previous works (Table 1).

**Table 1** - Summary of studies examining trends in ecotoxicity along the lanthanides series to aquatic organisms. ↓ or ↑ indicate a decreasing or increasing trend in ecotoxicity along the lanthanides series, respectively. [LN] measured refers to analytical verification of exposure concentration; Y, yes; N, no.

Elements	Model organism	Trend (Y/N)	[LN] measured	Medium composition	Reference
La, Ce, Pr, Nd, Gd	<i>Daphnia magna</i>	Y <sup>§</sup> (acute, nominal), ↓ N (chronic)	Y	Acute test: OECD (2004) Chronic test: Lake Water (pH 7.5 ± 0.2, DOC 10.6 mg/L C)	Blinova et al. (2018)
All LN	<i>Danio rerio</i>	Y, ↓	N	According to OECD 203 (2019)	Lin et al. 2022
La, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Er, Lu (and Y)	<i>Hydra attenuata</i>	Y <sup>#</sup> , ↓	N	Hydra medium CaCl <sub>2</sub> , TES, pH 7	Blaise et al. (2018)
All LN	<i>Hyalella azteca</i>	Y, ↓ <sup>§</sup>	Y	Soft water	Borgmann et al. (2005)
All LN except Pr, Ho, Yb	<i>Vibrio fischeri</i>	Y, ↑	N	0.355 M NaNO <sub>3</sub> , pH 5.5	Weltje (2002)
All LN except Ho, Tm, Yb	<i>Nitellopsis obtusa</i>	Y, ↑ <sup>  </sup>	N	Artificial Pond Water KCl, NaCl, CaCl <sub>2</sub> and HEPES (pH 7.5)	Manusadžianas et al. (2020)
All LN except Ho, Tm, Yb	<i>Thamnocephalus platyurus</i>	N	N	Standard freshwater NaHCO <sub>3</sub> , CaSO <sub>4</sub> , MgSO <sub>4</sub> and KCl	Manusadžianas et al. (2020)
All LN	Cell lines of <i>Danio rerio</i> and <i>Onchorynchus mykiss</i>	N	Y	Mixed medium	Fleurbaix et al. (2022)
La, Ce, Gd, Ho	<i>Danio rerio</i>	N <sup>§</sup>	Y	Standard dilution water CaCl <sub>2</sub> , MgSO <sub>4</sub> , NaHCO <sub>3</sub> , KCl	Kang et al. 2022
All LN	<i>Hyalella azteca</i>	N	N	Tap water	Borgmann et al. (2005)
All LN	<i>Chlorella vulgaris</i>	N	N	K <sub>2</sub> HPO <sub>4</sub> , KH <sub>2</sub> PO <sub>4</sub> , (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , KNO <sub>3</sub> (pH just below 7)	Den Dooren de Jong (1965)
All LN	<i>Skeletonema costatum</i>	N	N	f/2 medium without EDTA: NaNO <sub>3</sub> , NaH <sub>2</sub> PO <sub>4</sub> , trace elements and vitamins (pH 8.0)	Tai et al. (2010)

§, The existence of trends has been examined by the authors of the present study, but was not mentioned in the original publication. ||, only for 24 d LC<sub>50</sub> (not for shorter exposure time); #, if Er and Lu excluded from the regression; §, if Tm, Yb and Lu excluded.

At the opposite, increasing ecotoxicity along the LN series was reported for the charophyte alga *Nitellopsis obtusa* (Manusadžianas et al., 2020) and the bacterium *Aliivibrio fischeri* (Weltje, 2002) (Table 1). In the very simple medium (0.355 M NaNO<sub>3</sub>) used for testing with *A. fischeri*, free Ln<sup>3+</sup> were predicted to be the dominant species and showed the same trend as total dissolved concentrations (nominal values). However, total dissolved and free Ln<sup>3+</sup> concentration will likely have different patterns in more complexing media, especially those containing phosphates or carbonates. Gonzalez et al. (2014) warned about establishing trends in the absence of detailed and complete information on LN speciation in the various test media used for different organisms. Until minimum analytical requirements are adopted by the whole ecotoxicological community, it will remain elusive to establish meaningful and generally valid ecotoxicity trends along the LN series.

In the same vein, it is instructive to examine if differences exist in the ecotoxicity of LN after grouping them into light rare earth elements (LREE – from La to Gd) and heavy rare earth elements (HREE – from Tb to Lu); an approach that is widely used by the scientific community (Rétif et al., 2024). We are specifically interested in differences between LREE and HREE at any given time (24 or 48h) and for each

exposure metric (nominal  $EC_{50}$ , TWM  $EC_{50}$  and  $EC_{50}$  based on free ion concentrations; Figs. 4 and 6). On average, LREE appear more toxic than HREE ( $p < 0.05$ ) except when  $EC_{50}$  are expressed as nominal concentrations after 48h of exposure (Fig. S4). The use of TWM increases the probability of LREE being more toxic than HREE in all cases; in agreement with the larger decrease in the exposure concentrations for many LREE (see sections 4.1 and 4.2; Fig. S4). Although similar results have been reported (Lin et al., 2022), these observations confirm the need to reach a consensus on which metric to use for LN exposure in order to correctly interpret their ecotoxicological effects.

## 5. Recommendations for future studies on LN ecotoxicity

The results of the present study highlight a number of caveats that should be addressed to improve the reliability and relevance of laboratory studies on LN ecotoxicity. The proposed list of caveats (Table 2 and text below) considers only issues directly linked to the results and approaches used in the present study and does not claim to be exhaustive.

### *Caveat # 1. Formation of insoluble LN species during ecotoxicity testing*

The ecotoxicological community is already well aware of the tendency of LN to form insoluble precipitates in ecotoxicological test media, especially in the presence of carbonates or phosphates (Blinova et al., 2018; Lachaux et al., 2022; Ma et al., 2016). Formation of Ce-bearing colloidal particles has been reported even in a non-complexing medium (0.01 M NaHEPES +  $10^{-5}$  M  $Ca(NO_3)_2$ ) spiked with approx. 5  $\mu\text{g/L}$  of Ce at pH between 6 and 8, with 40 to 70% of the total concentration being retained by a 3 kDa centrifugal ultrafiltration device (El-Akl et al., 2015). It is therefore advisable to consider that insoluble LN species will form in any medium and take the necessary analytical steps to verify this hypothesis (Table 2, point #1).

The present study suggests that the use analytical verifications based on total concentrations (*i.e.*, unfiltered samples) should be discouraged because they do not distinguish between soluble and (nano)particulate LN species (Vukov et al., 2016). Indeed, soluble species and precipitates do not necessarily contribute to ecotoxicity in the same way (Table 2, point # 3). The norm ISO14442 (ISO, 2006) provides useful recommendations that are applicable to the hazard assessment of elements tending to form insoluble precipitates such as LN. Further research should also better characterize the particle size distribution of LN precipitates, because filtration at 0.45 or 0.22  $\mu\text{m}$  will not detect the presence of colloidal precipitates smaller than the filter cut-off. Based on Single-Particle ICP-MS, El-Akl et al. (2015) estimated an average size of 60 nm for Ce particles formed in an exposure medium for short-term uptake experiments with *Chlamydomonas reinhardtii*. It is highly likely that part of the filterable concentrations reported in the present and previous studies actually include a certain proportion of particles having passed through the filter pores.

The formation of LN precipitates seems to strongly depend on the pH of the medium. Besides the theoretical solubility calculations supporting this hypothesis (sections 3.3 and 4.3), the decrease in filterable LN concentrations was almost suppressed when the quantity of added LN salt was high enough to keep the pH at values below 6.5 units over the duration of the test (Figures 1 and 2). Measurements of pH values at the beginning and at the end of ecotoxicity tests therefore provide valuable additional information to i) verify that LN speciation is similar across the chosen range of exposure concentrations in individual studies and ii) facilitate comparisons across studies.

Finally, we note that modifications of exposure media are a possible alternative to avoid the formation of LN precipitates and even increasing the environmental relevance of the results (Blinova et al., 2018; Ma et al., 2016). These options would not eliminate the need for a thorough preliminary verification of LN precipitation in the chosen matrix across the range of exposure concentrations. Indeed, substituting NaCl for  $NaHCO_3$  in ISO medium 6341 (*i.e.*, the medium used in the present study without modification) did not prevent La and Gd filterable concentrations from decreasing by 50–98%



(La) and 40–70% (Gd) over the exposure period of 48h (Revel et al., 2023). On the other hand, Ma et al. (2016) apparently avoided the formation of Ce precipitates larger than 0.22  $\mu\text{m}$  by substituting NaOH for NaHCO<sub>3</sub> in the same medium. Medium modifications should therefore be standardized among research groups to ensure that data across studies are comparable. A similar reasoning should ideally apply also to the procedures to determine filterable LN fractions (Horowitz, 2013; Vignati et al., 2006; Weyman et al., 2012).

**Table 2** - Caveats, recommendations and open issues for properly testing LN ecotoxicity to *Daphnia magna* and aquatic organisms in general. The recommendations are applicable to other ecotoxicological media where LN precipitation may occur. Open issues indicate possible priorities for future research and consensus-building (also see Table S16 for a more detailed presentation of each caveat). TWM, Time-Weighted Mean; LN, lanthanides.

#	Caveats	Recommendations / Possible approaches	Open issues
1	Formation of insoluble chemical species in the test medium	Provide appropriate analytical verification of filterable exposure concentrations. Verification of pH values very useful.	Presence of colloidal particles undetected by filtration. Speciation differences across the range of exposure concentration. Definition of standard non-complexing media.
2	Changeable exposure conditions for test organisms	Derive EC <sub>x</sub> using experimentally measured filterable concentrations and TWM or geometric means.	Relative contribution of soluble and particulate LN species to observed ecotoxic responses. Technical developments to measure LN bioavailable concentrations in test medium. Differences in filter type/performance can affect comparisons across studies.
3	Unknown and contrasting ecotoxicological roles of LN precipitates	Modify test medium to avoid formation of precipitates. Verify the absence of precipitates by <i>e.g.</i> , ultrafiltration or single-particle ICP-MS.	Contribution of precipitated species to ecotoxicity. Accumulation of precipitates in the gut of filter-feeding organisms during tests.
4	Use of free ionic LN concentrations as an improved exposure metric	Remember assumptions and limitations of thermodynamic speciation models.	Untested assumption that only Ln <sup>3+</sup> contribute to toxicity. Dynamic aspects of LN speciation.
5	Conditional character of stability and solubility constants in thermodynamic speciation models	Strive to combine speciation calculation and experimental verification of model predictions.	Developing techniques for direct determination of LN speciation in test media.
6	Ecotoxicity trends along the LN series may be artefacts related to methodological limitations and experimental choices	Do not establish trends in the absence of minimum verification of exposure concentrations (see # 1 and 2) and Table 1.	True trends require determination of the actual LN bioavailable fraction.
7	Inappropriate comparison between exposure metrics from ecotoxicity testing and environmentally measured levels	Clearly report what is being compared. Consider the implications of the different exposure metrics from ecotoxicity testing in terms of possible underestimation or overestimation of the risk.	Matrix effects on LN speciation remain to be fully understood. Field-based studies using DGTs may provide hints on LN bioavailability in natural conditions. Reporting conventions to be defined.

#### *Caveat # 2. Variable exposure conditions for test organisms over the test duration*

Although marked changes in LN exposure concentrations have been previously reported (see caveat #1), the present study shows that concentration decreases can be especially fast at the beginning of

ecotoxicity tests (Figure 2). It is therefore necessary to reach a consensus as to the appropriate exposure metric to calculate ecotoxicological endpoints for LN. In principle, evaluation of LN ecotoxicity should not be based on i) nominal or measured concentrations at the beginning of the test, or ii) total concentrations that may contain a mixture of soluble and precipitated LN species (Vukov et al., 2016; present study). Both choices can lead to a marked underestimation of ecotoxic effects (Fig. 4). Measured concentrations at the end of the test would be a conservative alternative when exposure concentrations decrease over time. However, we recommend either TWM or geometric mean values as the best options to estimate the average exposure concentration during the whole test duration.

In the specific case of the test medium ISO 6341 (2012) for *D. magna*, the temporal decrease in filterable LN concentrations at 24 and 48h shows a clearly linear trend along the LN series (with the possible exception of Nd) (Figure 5). Future studies using this medium may therefore rely on Formulas 4 to 7 to estimate LN concentrations at the end of the tests and provide EC<sub>50</sub> estimates as TWM (present study) or geometric mean concentrations (Lachaux et al., 2022). It would be beneficial to verify if analogous relationships exist between LN atomic mass vs. concentration decrease in other standardized ecotoxicological test media. The existence of such relationship would indeed reduce the analytical requirements necessary to establish a consensus about the best exposure metrics to evaluate LN ecotoxicity. Care should also be taken as to the possible effects of adding large quantities of LN on medium pH. In the present study, we assumed homogenous pH at the beginning of the tests for all exposure concentrations, but experimental results suggest that this assumption could have been violated in some cases. Future studies may consider either experimental verification of pH at multiple time points for all exposure concentrations or pH adjustments at the beginning of the tests. The second option does not necessarily eliminate the possibility of pH changes during the tests and hence the need for pH monitoring. Linear relationships between pH and LN concentrations may exist, as for the present study, and be used to estimate pH values across the exposure range.

### *Caveat #3. Unknown ecotoxicological role of precipitates*

The results of the present study do not allow to establish if the observed biological responses in *D. magna* were caused solely by the free ionic LN species, whose concentrations may have varied during the tests and were only a tiny fraction of filterable ones (Fig. 6), or by combined effects of soluble and particulate LN species. Considering that LN precipitation is probably ubiquitous in standard ecotoxicological media, we provide a few suggestions on how to tackle this important issue based on current knowledge.

Revel et al. (2023) recently observed that particle formation was accompanied by different effects and tissue distributions in daphnids exposed for 48h in medium aliquots initially amended with soluble La and Gd in the same concentration range. As discussed in *caveat #1*, both elements formed insoluble precipitates albeit La did so to a greater extent. Micro- and nano-XRF analysis showed that La concentrated almost totally in the digestive tract, while Gd was apparently taken up and distributed across different tissues. Only Gd caused a clear ecotoxicological response in the exposed organisms with 48h EC<sub>50</sub> of 13.9 mg/L (nominal value) or 8.22 mg/L (filterable concentration at the end of the test); comparable with the present study. The absence of La ecotoxicity suggests that precipitates were not available for uptake by internal organs, while the observed Gd effects cannot be univocally ascribed to soluble or precipitated forms. These results were confirmed by Revel et al. (2024) who suggested using total or nominal concentrations as exposure metrics for LN ecotoxicity, in contrast with our recommendations at *caveat #1*.

Identifying the possible role of precipitates in LN ecotoxicity is related, but not equivalent, to understanding the ecotoxicity of nanoparticulate LN forms. LN-bearing nanoparticles are engineered materials of known composition and primary particle-size distribution. This contrasts with the formation of precipitates following addition of soluble LN salts into the test medium. LN precipitates have variable composition, *i.e.*, different types of precipitates can be present at the same time, and unknown particle

size distribution. Their concentration may also change over the test duration depending on the stability of exposure conditions and on the solubility of the precipitates. Specific experimental approaches are required to identify the presence of precipitates and their possible contribution to LN ecotoxicity in standard laboratory tests (Table 2).

Alternatively, exposure media could be aged after spiking with soluble LN forms and the precipitates removed by filtration, ultrafiltration or centrifugation before ecotoxicity testing in analogy to approaches proposed in the ecotoxicity of engineered nanoparticles (Sørensen & Baun, 2015). Results from the present study suggest that formation of particles larger than 0.45  $\mu\text{m}$  (nominal cut-off) becomes progressively less important along the LN series (Figs. 1 and 2). However, the time to reach stable filterable concentrations varies among LN (Fig. 2). The choice of the appropriate filter cut-off also requires further information on the particle size distribution of the precipitates. Such considerations may equally apply to chronic ecotoxicity testing because theoretical calculations suggest that the solubility limits could be as low as a few tens of micrograms per liter depending on specific elements and medium pH (Fig. 3 and Table S12). These solubility limits are comparable to, or lower than, the lowest chronic ecotoxicity values reported by Shu et al. (2023) and Lüring and Tolman (2010) for La (59  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$ , respectively in artificial media), but higher than Ce and Er concentrations of 1  $\mu\text{g/L}$  used by Galdiero et al. (2019) to study multigenerational effects. Comparison with the chronic values determined by Blinova et al. (2018) for La, Ce, Pr, Nd and Gd (range 300–490  $\mu\text{g/L}$ ) is hindered by the use of a natural lake water at pH 7.5 and containing 10.6 mg/L of dissolved organic carbon.

#### *Caveats # 4 and 5. Using modelled free ion concentrations as an improved exposure metric*

According to the scientific underpinnings of the biotic ligand model (BLM), ecotoxicological responses are controlled by the bioavailable elemental fraction that binds to specific biological ligands. The bioavailable fraction is mostly controlled by the free ion concentration although contributions from other elemental species are possible (Campbell & Fortin, 2013). While suitable analytical techniques are being explored (Janot et al., 2021; Leguay et al., 2016; Nduwayezu et al., 2016), free ion concentrations for LN are usually estimated using thermodynamic speciation models. This approach facilitates comparisons across studies using different exposure media and provides ecotoxicity estimation more relevant for risk assessment. However, it has some conceptual limitations that researchers should understand to avoid overstressing the environmental significance and implications of experimental results (Table 2, points # 4 and 5).

The choice of  $\text{Ln}^{3+}$  as a metric of exposure assumes that only free ions contribute to the observed ecotoxic effects. In support of this assumption, MacMillan et al. (2018) found good overall relationships between the modelled  $\Sigma\text{REY}$  free ion concentrations and REY accumulation by indigenous zooplankton in Canadian lakes. On the other hand, studies with a variety of model organisms provide contrasting evidence as to the bioavailability of LN-containing particles formed in ecotoxicological media following the addition of soluble LN forms (Aharchaou et al., 2020; El-Akl et al., 2015; Revel et al., 2023; Vukov et al., 2016). The problem is exacerbated when comparing  $\text{Ln}^{3+}$  concentrations in ecotoxicological media and in natural waters.

Although insoluble precipitates can dominate LN speciation in the laboratory, LN in natural waters can be strongly associated with colloids and occurring as complexes with dissolved natural organic matter (Janot et al., 2021; Marsac et al., 2021; Martin et al., 2021; Pinheiro & Rotureau, 2023). Thermodynamic speciation calculations in the presence of natural organic matter still suffer from conceptual shortcomings that could lead to erroneous estimations of  $\text{Ln}^{3+}$  in natural waters (Tesfa et al., 2022; Town et al., 2019). Similarly, speciation calculation in a given standard ecotoxicological medium (or across ecotoxicological media) will yield different results depending on the stability constants chosen for modelling by the experimenters as in the case of Lachaux et al. (2022) vs. the present study. The choice to allow or not for the precipitation of insoluble species during speciation modelling (provided that the necessary solubility products are available) can also affect the comparability of free ion based

metrics across different studies. Theoretical solubility limits can also vary markedly depending on the choice of solubility values for the possible precipitate species. In the present study, solubility limits could differ by over 10-fold when comparing calculations using the 'Firsching' and the 'Default VM' databases for carbonate solubility (Table S12), notably in the case of La. Finally, estimation of free ion concentrations based on nominal or total concentrations and filterable concentrations at the beginning of the test should be avoided for the same reasons discussed in caveat #1.

#### *Caveat # 6. Identifying meaningful trends along the LN series*

The previous caveats show that defining a generally valid ecotoxicological trend along the LN series remains elusive at the current state of knowledge, but also provide some hints to ensure that future research provides valid results for verifying the actual existence of such trends. In particular, results from studies not providing minimum analytical information on exposure concentrations (Table 1) should be considered of limited reliability for hazard and risk assessment purposes until further verification. Trends based on average, measured exposure concentrations (or free ion concentrations derived from such measurements) may provide better information, but are also subject to further screening as more knowledge on LN speciation and bioavailability becomes available in both standardized test media and natural waters. The results of the present study do not end the quest for the presence or absence of regular, generally valid ecotoxicological patterns along the LN series in relation to their acute ecotoxicity toward *Daphnia magna*. They do however show a clear trend in the concentration decrease (Fig. 5), suggesting the best approaches to avoid underestimating LN ecotoxicity.

#### *Caveat # 7. Comparing laboratory-derived exposure metrics with natural concentrations*

Risk assessment is based on the comparison between the EC<sub>50</sub> derived from laboratory studies (hazard assessment) with the measured ambient concentrations in the case of site-specific investigations or probable environmental concentrations to establish environmental quality standard on a wider scale (European Commission, 2019). At the current state of knowledge, the utmost care is required to avoid both overestimation and underestimation of the potential risks arising from (increasing) concentrations of LN following anthropogenic impacts (Table 2). According to the present study, laboratory exposure metrics derived from nominal concentrations (or concentrations measured at the beginning of the tests) would clearly underestimate risk. At the opposite extreme, comparing properly derived EC<sub>50</sub> with total elemental concentrations in natural water would likely overestimate the actual risk because LN bound to environmental particles will be considered equivalent to the dissolved (bioavailable) fraction. Studies attempting risk assessment should therefore be germane as to the analytical details of the concentrations being compared. An intriguing possibility would be to base risk assessment on comparisons of time-averaged, DGT-labile concentrations measured in both laboratory media and natural freshwater by extending approaches proposed for in situ ecological risk in sediments (Gu et al., 2020; Gu et al., 2022; Paller et al., 2019). Extrapolation of laboratory-based results to real-field situations also needs more information on how the variable composition of natural aqueous matrices in terms of major ions (e.g., Ca, Mg) or organic matter content affects LN uptake by and ecotoxicity to biota. Protective effects of Ca and organic matter against LN ecotoxicity have already been reported (Aharchaou et al., 2020; El-Akl et al., 2015; Lachaux et al., 2022; Vukov et al., 2016), but generally accepted models to predict such effects still have to be developed.

## **6. Concluding remarks and way forward**

Reliable exposure vs. response relationships are the foundation of hazard and risk assessment. Understanding the dynamic and complex speciation of LN is therefore a missing cornerstone in standardized ecotoxicity tests for these elements. In the absence of such information, it is not possible

to choose the appropriate metric to link exposure to LN with the measured biological responses even in controlled laboratory conditions.

The experimental results from the present study show that a statistically significant linear relationship exists between the atomic mass and the solubility of LN in the standard ISO 6341 test medium for *D. magna*, implying that reliable ecotoxicological endpoints must explicitly account for changes in LN concentration over the test duration. On the other hand, ecotoxicological trends along the LN series appear dependent on exposure time and the selected exposure metrics, highlighting the need for the definition of shared experimental and reporting procedures across the research community on this aspect. Given the difficulties in obtaining stable exposure concentrations of LN in many test media, one may even question the ability of standardized methods to provide reliable results on the ecotoxicity of LN to aquatic organisms. However, similar issues would equally apply to New Approach Methodologies (NAM), such as in vitro screening, and to any test method unless they adopt test media allowing a thorough control of LN speciation.

In our opinion, addressing the caveats discussed in section 5 will provide stronger basis for mutual acceptance of data across studies and help to reconcile standardized tests with the much more complex environmental reality (Vignati, 2021). Similarly, ecotoxicity trends along the LN series will remain conditional (*i.e.*, dependent on the chosen exposure metric and exposure time, among the other variables) until precise information becomes available as to the LN form(s) capable of interacting with test organisms. Alternatively, approaches based on LN bioaccumulation may be pursued to determine the critical body residues (CBR) capable of inducing adverse effects in *D. magna*. Such approaches are becoming increasingly attractive thanks to new techniques allowing a detailed mapping of element distribution across *D. magna* tissues. In this case, measurements of LN accumulation on a mass base (*i.e.*,  $\mu\text{g LN per gram tissue}$ ) should be completed by more complex approaches allowing to map and, ideally, quantifying the LN distribution across *D. magna* tissues.

## Acknowledgments

This work was partly done with resources from the Pôle de compétences en biologie environnementale ANATELo, LIEC laboratory, UMR 7360 CNRS – Université de Lorraine. We are indebted to Bénédicte Sohm (LIEC) for supervising routine laboratory functioning. We thank Armand Maul (LIEC) for statistical advice. We acknowledge the assistance of Danièle Pauly in maintenance of daphnids, reagent preparation and test organization. Preprint version 3 of this article has been peer-reviewed and recommended by Peer Community In Ecotoxicology and Environmental Chemistry (<https://doi.org/10.24072/pci.ecotoxenvchem.100143>; Couture P., 2024).

This work is licensed under a CC BY 4.0 license (2024).

## Data, scripts, code, and supplementary information availability

Data and supplementary information are available online: <https://doi.org/10.57745/NW0GF9> (Vignati et al., 2023)

## Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article. Davide A.L. Vignati is a recommender for PCI Ecotoxicology and Environmental Chemistry.



## Funding

This work is part of the French National Research Agency (ANR) ECOTREE project (contract no. ANR-16-CE34-0012) and was partly supported by the Luxembourg National Research Fund (FNR) and the in the framework of the FNR/INTER/ANR research program (contract no. INTER/ANR/15/11209808/ECOTREE).

## References

- Aharchaou, I., Beaubien, C., Campbell, P. G. C., & Fortin, C. (2020). Lanthanum and Cerium Toxicity to the Freshwater Green Alga *Chlorella fusca*: Applicability of the Biotic Ligand Model. *Environmental Toxicology and Chemistry*, 39(5), 996-1005. <https://doi.org/10.1002/etc.4707>
- Barry, M. J., & Meehan, B. J. (2000). The acute and chronic toxicity of lanthanum to *Daphnia carinata*. *Chemosphere*, 41(10), 1669-1674. [https://doi.org/10.1016/S0045-6535\(00\)00091-6](https://doi.org/10.1016/S0045-6535(00)00091-6)
- Bau, M., & Dulski, P. (1996). Anthropogenic origin of positive gadolinium anomalies in river waters. *Earth and Planetary Science Letters*, 143(1), 245-255. [https://doi.org/10.1016/0012-821X\(96\)00127-6](https://doi.org/10.1016/0012-821X(96)00127-6)
- Blaise, C., Gagné, F., Harwood, M., Quinn, B., & Hanana, H. (2018). Ecotoxicity responses of the freshwater cnidarian *Hydra attenuata* to 11 rare earth elements. *Ecotoxicology and Environmental Safety*, 163, 486-491. <https://doi.org/10.1016/j.ecoenv.2018.07.033>
- Blinova, I., Lukjanova, A., Muna, M., Vija, H., & Kahru, A. (2018). Evaluation of the potential hazard of lanthanides to freshwater microcrustaceans. *Science of The Total Environment*, 642, 1100-1107. <https://doi.org/10.1016/j.scitotenv.2018.06.155>
- Blinova, I., Lukjanova, A., Vija, H., & Kahru, A. (2022). Long-Term Toxicity of Gadolinium to the Freshwater Crustacean *Daphnia magna*. *Bulletin of Environmental Contamination and Toxicology*, 108(5), 848-853. <https://doi.org/10.1007/s00128-021-03388-0>
- Blinova, I., Muna, M., Heinlaan, M., Lukjanova, A., & Kahru, A. (2020). Potential Hazard of Lanthanides and Lanthanide-Based Nanoparticles to Aquatic Ecosystems: Data Gaps, Challenges and Future Research Needs Derived from Bibliometric Analysis. *Nanomaterials*, 10(2), 328. <https://doi.org/10.3390/nano10020328>
- Bobba, S., Carrara, S., Huisman, J., Mathieux, F., & Pavel, C. (2020). *Critical raw materials for strategic technologies and sectors in the EU : a foresight study, 2020*. <https://doi.org/10.2873/58081>
- Borgmann, U., Couillard, Y., Doyle, P., & Dixon, D. G. (2005). Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry*, 24(3), 641-652. <https://doi.org/10.1897/04-177r.1>
- Campbell, P. G. C., & Fortin, C. (2013). Biotic Ligand Model. In J.-F. Féraud & C. Blaise (Eds.), *Encyclopedia of Aquatic Ecotoxicology* (pp. 237-246). Springer Netherlands. [https://doi.org/10.1007/978-94-007-5704-2\\_23](https://doi.org/10.1007/978-94-007-5704-2_23)
- Chen, L., Fu, X. e., Zhang, G., Zeng, Y., & Ren, Z. (2012). Influences of Temperature, pH and Turbidity on the Behavioral Responses of *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) in the Biomonitor. *Procedia Environmental Sciences*, 13, 80-86. <https://doi.org/10.1016/j.proenv.2012.01.007>
- Couture, P. (2024) Lanthanide atomic mass and chemical behaviour in solution influence their solubility and ecotoxicity for *Daphnia magna*: Implications for risk assessment of aquatic organisms. *Peer Community in Ecotoxicology and Environmental Chemistry*, 100143. <https://doi.org/10.24072/pci.ecotoxenvchem.100143>
- Den Doore De Jong LE, Roman WB (1965) Tolerance of *Chlorella vulgaris* for metallic and non-metallic ions. *Antonie van Leeuwenhoek*, 31, 301-313. <https://doi.org/10.1007/BF02045910>

- Deng, L., Shu, J., & Wang, Z. (2022). Toxic Effects of Rare Earth Element Ytterbium and Neodymium on *Daphnia magna*. *Asian J. Ecotoxicology*, 17(3), 445-453. <https://doi.org/10.7524/AJE.1673-5897.20210604003>
- Dushyantha, N., Batapola, N., Ilankoon, I. M. S. K., Rohitha, S., Premasiri, R., Abeysinghe, B., Ratnayake, N., & Dissanayake, K. (2020). The story of rare earth elements (REEs): Occurrences, global distribution, genesis, geology, mineralogy and global production. *Ore Geology Reviews*, 122, 103521. <https://doi.org/10.1016/j.oregeorev.2020.103521>
- El-Akl, P., Smith, S., & Wilkinson, K. J. (2015). Linking the chemical speciation of cerium to its bioavailability in water for a freshwater alga. *Environmental Toxicology and Chemistry*, 34(8), 1711-1719. <https://doi.org/10.1002/etc.2991>
- El-Deeb, M., Ghazy, Habashy, M., & Mohammady, E. (2011). Effects of pH on Survival, Growth and Reproduction Rates of The Crustacean, *Daphnia Magna*. *Australian Journal of Basic and Applied Sciences*, 5(11), 1-10. <https://ajbasweb.com/old/ajbas/2011/November-2011/1-10.pdf>
- European Commission. (2019). *WFD-CIS Guidance document No. 38. Technical Guidance for implementing Environmental Quality Standards (EQS) for metals*.
- Firsching, F. H., & Mohammadzadei, J. (1986). Solubility products of the rare-earth carbonates. *Journal of Chemical & Engineering Data*, 31(1), 40-42. <https://doi.org/10.1021/je00043a013>
- Fleurbaix E, Parant M, Maul A, Cossu-Leguille C (2022) Toxicity of lanthanides on various fish cell lines. *Ecotoxicology*, 31, 1147–1157. <https://doi.org/10.1007/s10646-022-02574-y>
- Galdiero, E., Carotenuto, R., Siciliano, A., Libralato, G., Race, M., Lofrano, G., Fabbri, M., & Guida, M. (2019, 2019/11/01/). Cerium and erbium effects on *Daphnia magna* generations: A multiple endpoints approach. *Environmental Pollution*, 254, 112985. <https://doi.org/10.1016/j.envpol.2019.112985>
- Gonzalez, V., Vignati, D. A. L., Leyval, C., & Giamberini, L. (2014). Environmental fate and ecotoxicity of lanthanides: Are they a uniform group beyond chemistry? *Environment International*, 71(0), 148-157. <https://doi.org/10.1016/j.envint.2014.06.019>
- González, V., Vignati, D. A. L., Pons, M.-N., Montarges-Pelletier, E., Bojic, C., & Giamberini, L. (2015). Lanthanide ecotoxicity: First attempt to measure environmental risk for aquatic organisms. *Environmental Pollution*, 199(0), 139-147. <https://doi.org/10.1016/j.envpol.2015.01.020>
- Gu, Y.-G., Gao, Y.-P., Huang, H.-H., & Wu, F.-X. (2020). First attempt to assess ecotoxicological risk of fifteen rare earth elements and their mixtures in sediments with diffusive gradients in thin films. *Water Research*, 185, 116254. <https://doi.org/10.1016/j.watres.2020.116254>
- Gu, Y.-G., Huang, H.-H., Gong, X.-Y., Liao, X.-L., Dai, M., & Yang, Y.-F. (2022). Application of diffusive gradients in thin films to determine rare earth elements in surface sediments of Daya Bay, China: Occurrence, distribution and ecotoxicological risks. *Marine Pollution Bulletin*, 181, 113891. <https://doi.org/10.1016/j.marpolbul.2022.113891>
- Hall, G. E. M., Pelchat, J. C., Pelchat, P., & Vaive, J. E. (2002). Sample collection, filtration and preservation protocols for the determination of 'total dissolved' mercury in waters. *Analyst*, 127(5), 674-680. <https://doi.org/10.1039/B110491H>
- Hissler, C., Stille, P., Iffly, J. F., Guignard, C., Chabaux, F., & Pfister, L. (2016). Origin and Dynamics of Rare Earth Elements during Flood Events in Contaminated River Basins: Sr-Nd-Pb Isotopic Evidence. *Environmental Science & Technology*, 50(9), 4624-4631. <https://doi.org/10.1021/acs.est.5b03660>
- Horowitz, A. J. (2013). A Review of Selected Inorganic Surface Water Quality-Monitoring Practices: Are We Really Measuring What We Think, and If So, Are We Doing It Right? *Environmental Science & Technology*, 47(6), 2471-2486. <https://doi.org/10.1021/es304058q>
- IEA. (2021). *The Role of Critical Minerals in Clean Energy Transitions*. IEA. <https://doi.org/10.1787/f262b91c-en>
- IEA. (2023). *Critical Minerals Market Review 2023*. IEA. <https://doi.org/10.1787/9cdf8f39-en>

- ISO. (2006). *Water quality - Guidelines for algal growth inhibition tests with poorly soluble material, volatile compounds, metals and waste water (norm 14442)*.
- ISO. (2012). *Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) – Acute toxicity test (norm 6341)*. <https://doi.org/10.3403/30213505>
- Janot, N., Groenenberg, J. E., Otero-Fariña, A., & Pinheiro, J. P. (2021). Free Eu(III) Determination by Donnan Membrane Technique with Electrochemical Detection: Implementation and Evaluation. *Aquatic Geochemistry*. <https://doi.org/10.1007/s10498-021-09392-4>
- Judge, W. D., Ng, K. L., Moldoveanu, G. A., Kolliopoulos, G., Papangelakis, V. G., & Azimi, G. (2023, 2023/04/01/). Solubilities of heavy rare earth sulfates in water (gadolinium to lutetium) and H<sub>2</sub>SO<sub>4</sub> solutions (dysprosium). *Hydrometallurgy*, 218, 106054. <https://doi.org/10.1016/j.hydromet.2023.106054>
- Kang, S., Guo, C., Ma, C., Mu, H., Liu, Z., & Sun, L. (2022). Assessment of the biotoxicity of lanthanides (La, Ce, Gd, and Ho) on zebrafish (*Danio rerio*) in different water environments. *Ecotoxicology and Environmental Safety*, 246, 114169. <https://doi.org/10.1016/j.ecoenv.2022.114169>
- Kulaksız, S., & Bau, M. (2013). Anthropogenic dissolved and colloid/nanoparticle-bound samarium, lanthanum and gadolinium in the Rhine River and the impending destruction of the natural rare earth element distribution in rivers. *Earth and Planetary Science Letters*, 362(0), 43-50. <https://doi.org/10.1016/j.epsl.2012.11.033>
- Lachaux, N., Catrouillet, C., Marsac, R., Poirier, L., Pain-Devin, S., Gross, E. M., & Giamberini, L. (2022). Implications of speciation on rare earth element toxicity: A focus on organic matter influence in *Daphnia magna* standard test. *Environmental Pollution*, 307, 119554. <https://doi.org/10.1016/j.envpol.2022.119554>
- Le Faucheur, S., Mertens, J., Van Genderen, E., Boulemant, A., Fortin, C., & Campbell, P. G. C. (2021). Development of Quantitative Ion Character–Activity Relationship Models to Address the Lack of Toxicological Data for Technology-Critical Elements. *Environmental Toxicology and Chemistry*, 40(4), 1139-1148. <https://doi.org/10.1002/etc.4960>
- Leguay, S., Campbell, P. G. C., & Fortin, C. (2016). Determination of the free-ion concentration of rare earth elements by an ion-exchange technique: implementation, evaluation and limits. *Environmental Chemistry*, 13(3), 478-488. <https://doi.org/10.1071/EN15136>
- Lerat-Hardy, A., Coynel, A., Dutruch, L., Pereto, C., Bossy, C., Gil-Diaz, T., Capdeville, M.-J., Blanc, G., & Schäfer, J. (2019). Rare Earth Element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW France): Hospital effluents as a source of increasing gadolinium anomalies. *Science of The Total Environment*, 656, 409-420. <https://doi.org/10.1016/j.scitotenv.2018.11.343>
- Li, Y. X., Zhou, X. Z., Wang, Y., & You, X. Z. (2004). Preparation of nano-sized CeO<sub>2</sub> by mechanochemical reaction of cerium carbonate with sodium hydroxide. *Materials Letters*, 58(1), 245-249. [https://doi.org/10.1016/S0167-577X\(03\)00454-3](https://doi.org/10.1016/S0167-577X(03)00454-3)
- Lin, Y.-T., Liu, R.-X., Audira, G., Suryanto, M. E., Roldan, M. J. M., Lee, J.-S., Ger, T.-R., & Hsiao, C.-D. (2022). Lanthanides Toxicity in Zebrafish Embryos Are Correlated to Their Atomic Number. *Toxics*, 10(6), 336. <https://doi.org/10.3390/toxics10060336>
- Louis, P., Vignati, D. A. L., Pontvianne, S., & Pons, M.-N. (2023). Spatial distribution of Rare Earth Elements in a transnational watershed: The case of the Danube River. *Science of The Total Environment*, 164368. <https://doi.org/10.1016/j.scitotenv.2023.164368>
- Luo, Y.-R., & Byrne, R. H. (2004). Carbonate complexation of yttrium and the rare earth elements in natural waters. *Geochimica et Cosmochimica Acta*, 68(4), 691-699. [https://doi.org/10.1016/S0016-7037\(03\)00495-2](https://doi.org/10.1016/S0016-7037(03)00495-2)
- Lüring, M., & Tolman, Y. (2010). Effects of lanthanum and lanthanum-modified clay on growth, survival and reproduction of *Daphnia magna*. *Water Research*, 44(1), 309-319. <https://doi.org/10.1016/j.watres.2009.09.034>

- Ma, Y., Wang, J., Peng, C., Ding, Y., He, X., Zhang, P., Li, N., Lan, T., Wang, D., Zhang, Z., Sun, F., Liao, H., & Zhang, Z. (2016). Toxicity of cerium and thorium on *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 134, 226-232. <https://doi.org/10.1016/j.ecoenv.2016.09.006>
- MacMillan, G. A., Clayden, M. G., Chételat, J., Richardson, M. C., Ponton, D. E., Perron, T., & Amyot, M. (2018). Environmental Drivers of Rare Earth Element Bioaccumulation in Freshwater Zooplankton. *Environmental Science & Technology*. <https://doi.org/10.1021/acs.est.8b05547>
- Malhotra, N., Hsu, H.-S., Liang, S.-T., Roldan, M. J. M., Lee, J.-S., Ger, T.-R., & Hsiao, C.-D. (2020). An Updated Review of Toxicity Effect of the Rare Earth Elements (REEs) on Aquatic Organisms. *Animals*, 10(9), 1663. <https://doi.org/10.3390/ani10091663>
- Manusadžianas, L., Vitkus, R., Gylytė, B., Cimmperman, R., Džiugelis, M., Karitonas, R., & Sadauskas, K. (2020). Ecotoxicity Responses of the Macrophyte Algae *Nitellopsis obtusa* and Freshwater Crustacean *Thamnocephalus platyurus* to 12 Rare Earth Elements. *Sustainability*, 12(17), 7130. <https://doi.org/10.3390/su12177130>
- Marsac, R., Catrouillet, C., Davranche, M., Bouhnik-Le Coz, M., Briant, N., Janot, N., Otero-Fariña, A., Groenenberg, J. E., Pédrot, M., & Dia, A. (2021). Modeling rare earth elements binding to humic acids with model VII. *Chemical Geology*, 567, 120099. <https://doi.org/10.1016/j.chemgeo.2021.120099>
- Martin, L. A., Vignati, D. A. L., & Hissler, C. (2021). Contrasting distribution of REE and yttrium among particulate, colloidal and dissolved fractions during low and high flows in peri-urban and agricultural river systems. *Science of The Total Environment*, 790, 148207. <https://doi.org/10.1016/j.scitotenv.2021.148207>
- McLennan, S. M. (1994). Rare earth element geochemistry and the “tetrad” effect. *Geochimica et Cosmochimica Acta*, 58(9), 2025-2033. [https://doi.org/10.1016/0016-7037\(94\)90282-8](https://doi.org/10.1016/0016-7037(94)90282-8)
- Moldoveanu, G. A., Kolliopoulos, G., Judge, W. D., Ng, K. L., Azimi, G., & Papangelakis, V. G. (2024, 2024/01/01/). Solubilities of individual light rare earth sulfates (lanthanum to europium) in water and H<sub>2</sub>SO<sub>4</sub> solutions (neodymium sulfate). *Hydrometallurgy*, 223, 106194. <https://doi.org/10.1016/j.hydromet.2023.106194>
- Mount, D. R., Gulley, D. D., Hockett, J. R., Garrison, T. D., & Evans, J. M. (1997). Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). *Environmental Toxicology and Chemistry*, 16(10), 2009-2019. <https://doi.org/10.1002/etc.5620161005>
- Nduwayezu, I., Mostafavirad, F., Hadioui, M., & Wilkinson, K. J. (2016). Speciation of a lanthanide (Sm) using an ion exchange resin [10.1039/C6AY02018F]. *Analytical Methods*, 8(37), 6774-6781. <https://doi.org/10.1039/C6AY02018F>
- OECD. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test*. <https://doi.org/10.1787/9789264069947-en>
- OECD. (2012). *Test No. 211: Daphnia magna Reproduction Test*. <https://doi.org/10.1787/9789264185203-en>
- Paller, M. H., Harmon, S. M., Knox, A. S., Kuhne, W. W., & Halverson, N. V. (2019). Assessing effects of dissolved organic carbon and water hardness on metal toxicity to *Ceriodaphnia dubia* using diffusive gradients in thin films (DGT). *Science of The Total Environment*, 697, 134107. <https://doi.org/10.1016/j.scitotenv.2019.134107>
- Pell, R., Tijsseling, L., Goodenough, K., Wall, F., Dehaine, Q., Grant, A., Deak, D., Yan, X., & Whattoff, P. (2021). Towards sustainable extraction of technology materials through integrated approaches. *Nature Reviews Earth & Environment*, 2(10), 665-679. <https://doi.org/10.1038/s43017-021-00211-6>
- Pinheiro, J. P., & Rotureau, E. (2023). Electroanalytical Trace Metal Cations Quantification and Speciation in Freshwaters: Historical Overview, Critical Review of the Last Five Years and Road Map for Developing Dynamic Speciation Field Measurements. *Molecules (Basel, Switzerland)*, 28(6), 2831. <https://doi.org/10.3390/molecules28062831>

- Pourret, O., Davranche, M., Gruau, G., & Dia, A. (2007). Rare earth elements complexation with humic acid. *Chemical Geology*, 243(1), 128-141. <https://doi.org/10.1016/j.chemgeo.2007.05.018>
- R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for statistical computing, Vienna. <https://www.r-project.org/>
- Rétif, J., Briant, N., Zalouk-Vergnoux, A., Le Monier, P., Sireau, T., & Poirier, L. (2024). Distribution of rare earth elements and assessment of anthropogenic gadolinium in estuarine habitats: The case of Loire and Seine estuaries in France. *Science of The Total Environment*, 922, 171385. <https://doi.org/10.1016/j.scitotenv.2024.171385>
- Revel, M., Medjoubi, K., Charles, S., Hursthouse, A., & Heise, S. (2024). Mechanistic analysis of the sub chronic toxicity of La and Gd in *Daphnia magna* based on TKTD modelling and synchrotron X-ray fluorescence imaging. *Chemosphere*, 353, 141509. <https://doi.org/10.1016/j.chemosphere.2024.141509>
- Revel, M., Medjoubi, K., Rivard, C., Vantelon, D., Hursthouse, A., & Heise, S. (2023). Determination of the distribution of rare earth elements La and Gd in *Daphnia magna* via micro and nano-SXRF imaging. *Environmental Science: Processes & Impacts*. <https://doi.org/10.1039/D3EM00133D>
- Rodríguez de San Miguel, E., González-Albarrán, R., & Rojas-Challa, Y. (2023). Conditional Equilibrium Constants Reviewed. *Critical Reviews in Analytical Chemistry*, 53(4), 775-797. <https://doi.org/10.1080/10408347.2021.1977609>
- Romero-Freire, A., Joonas, E., Muna, M., Cossu-Leguille, C., Vignati, D. A. L., & Giamberini, L. (2019). Assessment of the toxic effects of mixtures of three lanthanides (Ce, Gd, Lu) to aquatic biota. *Science of The Total Environment*, 661, 276-284. <https://doi.org/10.1016/j.scitotenv.2019.01.155>
- Roth, N., & Ciffroy, P. (2016). A critical review of frameworks used for evaluating reliability and relevance of (eco)toxicity data: Perspectives for an integrated eco-human decision-making framework. *Environment International*, 95, 16-29. <https://doi.org/10.1016/j.envint.2016.07.011>
- Schinzel, S., Bindl, M., Visseaux, M., & Chermette, H. (2006). Structural and Electronic Analysis of Lanthanide Complexes: Reactivity May Not Necessarily Be Independent of the Identity of the Lanthanide Atom – A DFT Study. *The Journal of Physical Chemistry A*, 110(39), 11324-11331. <https://doi.org/10.1021/jp060876d>
- Scott, G., & Crunkilton, R. L. (2000). Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia*, and *Daphnia magna*. *Environmental Toxicology and Chemistry*, 19(12), 2918-2922. <https://doi.org/10.1002/etc.5620191211>
- Shu, J., Chen, W., Wang, Z., Jiang, D., Xiao, Y., & Li, Z. (2023). Two-phase effects of environmentally relevant lanthanum on life-history traits of *Daphnia magna* and transgenerational bioenergetic profiles: Implications for nutritional and environmental consequences. *Aquatic Toxicology*, 255, 106380. <https://doi.org/10.1016/j.aquatox.2022.106380>
- Siekierski, S., & Salomon, M. (1985, 1985/07/01). Thermodynamics of saturated lanthanide nitrate-water systems. *Journal of Solution Chemistry*, 14(7), 473-484. <https://doi.org/10.1007/BF00646979>
- Sørensen, S. N., & Baun, A. (2015). Controlling silver nanoparticle exposure in algal toxicity testing – A matter of timing. *Nanotoxicology*, 9(2), 201-209. <https://doi.org/10.3109/17435390.2014.913728>
- Susanti, Y., Pratiwi, H., Sulistijowati, S., & Twenty, L. (2014). M estimation, S estimation, and MM estimation in robust regression. *International Journal of Pure and Applied Mathematics*, 91, 349-360. <https://doi.org/10.12732/ijpam.v91i3.7>
- Tai P, Zhao Q, Su D, Li P, Stagnitti F (2010) Biological toxicity of lanthanide elements on algae. *Chemosphere*, 80, 1031–1035. <https://doi.org/10.1016/j.chemosphere.2010.05.030>
- Tesfa, M., Duval, J. F. L., Marsac, R., Dia, A., & Pinheiro, J.-P. (2022). Absolute and Relative Positioning of Natural Organic Matter Acid–Base Potentiometric Titration Curves: Implications for the Evaluation of the Density of Charged Reactive Sites. *Environmental Science & Technology*, 56(14), 10494-10503. <https://doi.org/10.1021/acs.est.2c00828>



- Town, R. M., van Leeuwen, H. P., & Duval, J. F. L. (2019). Rigorous Physicochemical Framework for Metal Ion Binding by Aqueous Nanoparticulate Humic Substances: Implications for Speciation Modeling by the NICA-Donnan and WHAM Codes. *Environmental Science & Technology*, 53(15), 8516-8532. <https://doi.org/10.1021/acs.est.9b00624>
- Venables, W. N., & Ripley, B. D. (1999). *Modern Applied Statistics with S-plus*, 3<sup>rd</sup> ed. Springer-Verlag. <https://doi.org/10.1007/978-1-4757-3121-7>
- Vignati, D., Martin, L., Poirier, L., Zalouk-Vergnoux, A., Fouque, C., Bojic, C., Hissler, C., & Cossu-Leguille, C. (2023). *Supporting information for: Behavior and speciation of lanthanides during standardized ecotoxicity tests with Daphnia magna: implications and recommendations for hazard and risk assessment* Version V1) [Dataset Image Other]. Recherche Data Gov. <https://doi.org/10.57745/NW0GF9>
- Vignati, D. A. L. (2021). Reconciling standard procedures and environmental realism in ecotoxicology: conceptual and practical challenges. Habilitation à Diriger des Recherches. Université de Lorraine. <https://hal.science/tel-03484547/>
- Vignati, D. A. L., Loizeau, J.-L., Rossé, P., & Dominik, J. (2006). Comparative performance of membrane filters vs. high-surface area filtration cartridges for the determination of element concentrations in freshwater systems. *Water Research*, 40, 917-924. <https://doi.org/10.1016/j.watres.2005.12.024>
- Vukov, O., Smith, D. S., & McGeer, J. C. (2016). Acute dysprosium toxicity to *Daphnia pulex* and *Hyaella azteca* and development of the biotic ligand approach. *Aquatic Toxicology*, 170, 142-151. <https://doi.org/10.1016/j.aquatox.2015.10.016>
- Weltje, L. (2002). *Bioavailability of lanthanides to freshwater organisms. Speciation, accumulation and toxicity*. Delft University of Technology]. Delft (The Netherlands).
- Wen, Y., Liu, P., Wang, Q., Zhao, S., & Tang, Y. (2024, 2024/04/25). Organic Ligand-Mediated Dissolution and Fractionation of Rare-Earth Elements (REEs) from Carbonate and Phosphate Minerals. *ACS Earth and Space Chemistry*. <https://doi.org/10.1021/acsearthspacechem.4c00009>
- Weyman, G. S., Ruffli, H., Weltje, L., Salinas, E. R., & Hamitou, M. (2012). Aquatic toxicity tests with substances that are poorly soluble in water and consequences for environmental risk assessment. *Environmental Toxicology and Chemistry*, 31(7), 1662-1669. <https://doi.org/10.1002/etc.1856>
- Zhuang, D. (1994). The effects of low pH value on survival, growth and reproduction of *Daphnia magna* (Crustacea: Cladocera). *Zhongguo Huanjing Kexue*, 14(2), 107-111. <http://europepmc.org/abstract/CBA/273135>