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Ecotoxicology & Environmental Chemistry

Lanthanide atomic mass and chemical behaviour in solution influence their solubility and ecotoxicity for *Daphnia magna*: Implications for risk assessment of aquatic organisms

Patrice Couture *based on peer reviews by* **Carrie J. Rickwood** *and 1 anonymous reviewer*

Davide A.L. Vignati, Loïc Martin, Laurence Poirier, Aurore Zalouk-Vergnoux, Chantal Fouque, Clément Bojic, Christophe Hissler, Carole Cossu-Leguille (2024) Ecotoxicity of lanthanides to *Daphnia magna*: insights from elemental behavior and speciation in a standardized test medium. HAL, ver. 3, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry.
https://hal.univ-lorraine.fr/hal-04302491v3

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The demand for lanthanides (LN) has seen a steady increase and is anticipated to continue to grow. Due to their unique properties, they have become essential in key components of new technologies, such as batteries, wind turbines, electronic components and other devices needed to facilitate energy transition away from fossil fuels. These elements are also increasingly used in a range of new technologies, including medical applications and telecommunication. In this context, the concentrations of lanthanides are expected to increase in freshwater environments (Gwenzi *et al.*, 2018). Our limited knowledge about the risk that they pose to organisms limits our ability to develop guidelines for environmental protection. Research on this issue has so far been hindered by the peculiar properties of lanthanides, that tend to form insoluble precipitates when added in standard ecotoxicological test media (Blinova *et al.*, 2018). This and other challenges of studying lanthanide toxicity were addressed in this in-depth study that leaves few stones unturned.

The study by Vignati and colleagues (2024) is the first to investigate the acute toxicity of all LN, with the exception of promethium, a radioactive element, on *Daphnia magna*, a model test species, following the ISO 6341 (2012) norm. The authors designed their study to generate data useable for the development of risk assessment guidelines for the LN series and to generate data-based recommendations for future studies on LN ecotoxicity. They exposed daphnids to nine to ten dilutions of all tested LN in a medium and carried out 48-hour acute immobilization assays. Initial and final pH was measured along with concentrations of LN in the test solutions sampled at various intervals by ICP-MS. This data allowed calculation of LN speciation, performed using VisualMinteq software. Effect concentrations were also calculated using different metrics based on initial (nominal), time-averaged or modelled LN3+ exposure concentrations.

In their multi-faceted investigation, the authors reported several observations that clearly contribute to a better understanding of the ecotoxicity of LN to aquatic organisms and provide useful advice for future studies, briefly summarized here. Proper characterization of exposure concentrations is a key in any ecotoxicological study. Their project shows that even for a short, 48 h exposure, LN concentrations decrease due to a combination of precipitation and, possibly, adsorption. The concentration decrease was inversely proportional to the LN atomic mass, which may reduce the analytical requirements for future studies using the same test medium. The addition of LN to the test medium also modified pH and a detailed hypothesis is formulated to explain this phenomenon that has implications for ecotoxicological endpoints. Conclusions on LN ecotoxicity drawn in this study are based on experimental data and on extensive thermodynamic speciation modeling. The values of EC50 presented in the study varied by several order of magnitude depending on the chosen exposure metric, underscoring the urgent need for consensus-building on this issue across the research community. The authors also provide a comparison of their conclusions on EC50 values for daphnids with the limited data available in the literature, further validating their data with cautions carefully laid out about experimental design. The paper concludes with a list of seven caveats that should be considered both for regulators who will want to use the data presented in the paper for environmental LN concentrations regulations and for future studies. These caveats highlight the importance of considering LN speciation and chemical behavior during ecotoxicity assays, their influence on exposure concentrations, and their importance for risk assessment. They also reiterate that since LN concentrations in filtered water collected in the field are not directly comparable to EC50 values derived from laboratory studies using total or free LN3+ concentrations, an effort must be made to harmonize the methods of LN concentration measurements in field and laboratory studies. Overall, this paper may be one of the most rigorous studies in the current literature about LN ecotoxicity in freshwater systems. In its approach, it sets a precedent for future studies aiming at generating EC50 values or other toxicological endpoints of inorganic contaminants. The paper, carefully reviewed by Carrie Rickwood and by an anonymous reviewer, is a major contribution towards our understanding of LN ecotoxicity. **References**

Blinova, I., Lukjanova, A., Muna, M., Vija, H., & Kahru, A. (2018). Evaluation of the potential hazard of lanthanides to freshwater microcrustaceans. Sci. Tot. Environ. 642 :1100-1107. https://doi.org/10.1016/j.scitotenv.2018.06.155

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N, Dunjana, N., Sanganyado, E. (2018). Sources, behaviour, and environmental and human health risks of high technology rare earth elements as emerging contaminants. Sci. Total Environ., 636:299-313. https://doi.org/10.1016/j.scitotenv.2018.04.235

ISO. (2012). Water quality — Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) — Acute toxicity test (norm 6341). https://www.iso.org/standard/54614.html

Vignati, D.A.L., Martin, L.A., Poirier, L., Zalouk-Vergnoux, A., Fouque, C., Clément, B., Hissler, C., Cossu-Leguille, C. (2024). Ecotoxicity of lanthanides to *Daphnia magna*: insights from elemental behavior and speciation in a standardized test medium. Ver.3 peer-reviewed and recommended by Peer Community In Ecotoxicology and Environmental Chemistry. https://hal.science/LIEC-UL/hal-04302491v3

Reviews

Evaluation round #2

DOI or URL of the preprint: https://hal.univ-lorraine.fr/hal-04302491v2
Version of the preprint: 2

Authors' reply, 18 June 2024

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Decision by Patrice Couture, posted 10 June 2024, validated 10 June 2024

Minor revisions needed

Dear Davide Vignati,

Thank you for your thorough revision of your manuscript. I have reviewed your replies to the reviewer's comments and suggestions. I consider that you correctly addressed all comments and concerns addressed by the two reviewers. I also reviewed your revised manuscript. I identified a few of grammatical errors, mostly in the newly added text. I also made a suggestion for the last paragraph of the introduction for your consideration.

The supplementary files accompanying your manuscript are acceptable without modification.

Please review the corrections and resubmit a clean version of your manuscript. I attach the annotated document for your consideration.

Once again, thank you for considering PCI EcotoxEncChem for this interesting work. **Download recommender's annotations**

Evaluation round #1

DOI or URL of the preprint: https://hal.univ-lorraine.fr/hal-04302491
Version of the preprint: 1

Authors' reply, 23 May 2024

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Decision by Patrice Couture, posted 10 January 2024, validated 11 January 2024

Revisions requested

Dear Dr. Vignati,

Thank you for submitting your manuscript to PCI EcotoxEnvChem. We have now received two reviews of your manuscript. I will be looking forward to receive a revised version of your manuscript following major revisions, including a point-by-point reply to each comment and suggestion made by the reviewers.

Regards,

Patrice Couture

Recommender for PCI EcotoxEnvChem

Reviewed by Carrie J. Rickwood, 04 January 2024

General comment: This was a very well written paper and provides critical data for understanding the ecotoxicity of lanthanides. In addition, the discussion provides highly relevant commentary on the status of lanthanide research in terms of identifying trends and highlights some important data/research gaps that require investigation. A very timely paper that I would highly recommend for publication. A few comments follow:

1) Line 130 – acute immobilization test was conducted in the dark – could the authors provide justification for why the test was conducted in the dark? Other protocols (i.e. OECD, US EPA) suggest this test to be completed with 16h light: 8h dark with the option of complete darkness if test substances are unstable in light. Is it thought the lanthanides would be unstable under the 16h light:8h dark regime? Some explanation as to why this test was conducted in the dark would be welcomed here to guide future ecotoxicity testing methodology with LN.

2) Line 133, the authors provide the composition of the standardized medium, could they also provide the hardness value?

3) Line 211 – reference to the TWM formula from OECD 2008 is made but this reference is not in the reference list – please include.

Results

1) Line 213 = it states that Conct(x=1) is the measurement concentration at 24. I believe this should be Conct(x+1).

2) Calculation of time weighted mean (lines290-295) – could you provide more justification for why two different equations were used to calculate TWM for 24h (equation 2) and 48h (equation 5). The text refers to Table S11 for a detailed explanation, but there is no explanation included in this table when extracted into excel. Could the authors either provide this explanation in the text or as a separate document. Also there is reference to the 24h TWM EC50 being calculated using equation 4 but it is not clear from Table S11 whether the 24h EC50 results reported are the TWM or derived from nominal.

Discussion

Line 334 – the authors reference section 3.4, do you mean 4.4?

Line 380 – the discussion regarding LN solubility being time and concentration dependent is a critical one. It would be beneficial here to discuss the various alternative ways to calculate TWM to include that temporal change. For example, the approach used for inhalation toxicity where the hours of exposure to specific concentrations are incorporated to better understand the actual exposure. A mention of other methods and how the equations used in this paper compare would be useful.

Table S10 – a separate table comparing nominal and measured EC50 values would be appropriate here (i.e. keep the theoretical solubility limits in S10 and create a separate table for the EC50 data). Including EC50s at 24h and 48-h (nominal) , the measured EC50s and TWM EC50s will allow the reader to compare the values without having to find them in different tables.

Line 548 – Hyalella Azteca should be Hyalella azteca

Reviewed by anonymous reviewer 1, 05 January 2024

Recommendation: accepted with major changes

The manuscript entitled ” Behaviour and speciation of lanthanides during standardized ecotoxicity tests with Daphnia magna: implications and recommendations for hazard and risk assessment ” represents a meaningful

and pointless work where the authors provided key recommendations for any study focused on lanthanides (LN) toxicity. Several aspects regarding the speciation, the exposure conditions including the exposure time, the pH variation, and the measured concentrations of these contaminants were carefully discussed. As well, further discussion related to the LN group behaviour in terms of solubility, stability and toxicity were adequately presented. Although the manuscript provides interesting and pertinent recommendations for LN toxic testing works, the way this topic was addressed still required some improvements. I do not recommend this article for publication in the journal unless major changes are made. Even with these modifications, more perspectives and critical limitations of the current study are needed.

General comments:

The considerations/recommendations proposed for hazard and risk assessment: From the title to the end of the manuscript, the authors stated several times the contributions of several considerations/recommendations regarding LN toxicity testing to hazard and risk assessment. I think the authors should be more sincere and limit the recommendations provided in the manuscript. In a short run, your conclusions are of great importance for any study focused on Ln toxicity (using D. magna as well as other aquatic organisms). In a long run, these pertinent recommendations will help provide, ultimately or eventually, realistic toxicological data for environmental assessment (more for hazard than for risk assessment). I think a rereading through the manuscript is needed to better express this idea concerning the implication of such recommendation for ecotoxicological studies on LN and then the potential importance of such considerations for eventual environmental assessment. This point could reinforce the contribution of the current work. A good example of what I am proposing is found on line 106 (" in future research on the ecotoxicity of LN")

Lanthanides as a contaminant group of interest: In the results and discussion sections, the authors used the terms heavy and light Ln or rare earth elements (REE) at times (e.g. line 260) without making any definition of such terms in previous sections. In addition, in the results presented in some figures (e.g., figs. 2, 4) some differences are observed between both groups but little is written about it. Why the authors are not comfortable in using such terms? And more importantly, why the differences between both groups in their chemical properties such affinity for O-containing ligands, ionic radius, other binding preferences, etc., are not used for more discussion of such results (when examining solubility, stability, toxicity)? Please, be consistent with the abbreviation REE or REY in the document. In the introduction section, you use the term critical raw material, why not using just critical elements? At several times (e.g. line 84, 92, 705), you made reference to Ln mixtures, but as you do not address this point in your results and discussion sections, I think all these passages related to REE mixture have to be removed.

Animal model used: On lines 78-87, the authors justified the animal model chosen for the present work, but as there are some solid works done about the solubility, speciation, and toxicity of REE using algae, why is still important to look at all these aspects for D. magna? Is not possible to take some lesson learn from these previous studies on algae when once decides to work for D. magna? I highly recommend mentioning that in these lines (78-87).

Methodological information: In general, I appreciate the information provided for the experiments conducted by the authors, but I found a little hard to follow some experiments as well as some graphics. For that reason, I ask for a better organization or for adding more information, which helps to make a clearer and more understanding reading of the manuscript.

-In your experiment set, you used only one "negative" control (the same condition without contaminants), but why not using another negative control to check the influence of NO3 or Cl added in the experiment media as Ln salt? On lines 469-475, you showed the EC50 for both anions are far from the concentrations used in your experiments. However, why not consider any interaction between Ln and the anion (NO3 or Cl) which would effect/perturb the toxicity you observed? Some words about this issue should be included in the discussion section.

-You prepared mother solutions or initial solutions of Ln (using salts), from which you make some dilution

to obtain difference Ln concentrations in your experiments (n = 9-10). Did you measure the concentrations of such initial solutions? Such measurements which help reduce the differences observed between nominal and measured concentrations (see Fig. 2) in your "initial" toxic testing time. An important element to be discussed in the manuscript

-On lines 143-153: you mentioned that two independent definitive tests were performed for each element. I think it could be good to explain here the difference between one and the second definitive tests used (24h versus 48 h?). What is your argument to assume homogenous initial pH (at t = 0) for all exposure concentrations? I cannot understand why you did not take any precaution decision to be sure that there is not any initial difference in terms of pH at the beginning of the exposure for each condition.

-On line 164: Did you test if the material of the filter used remove lanthanides by adsorption? Any perturbation caused by the filter material used? It could be useful to mention that as recommendations for further experiments.

-On line 184: Why was the pH not measured in all testing conditions? Or what was your criteria to select some exposure conditions?

Section 2.4. Speciation was estimated for each REE member in your testing conditions. But in such estimations, did you consider the presence of other metals in the medium like Ca, Mg, Fe, and Mg, which have the potential to be a competitor of REE uptake and then affecting their toxicity on D. magna? If not, that should be mentioned in the discussion section. The equation 2 used is based-on logarithmic relationships. It is validated for all REEs? It is always the case? Are you not considering Ln precipitation by sulfate or nitrate and only by carbonate as ligands? A value of 0.831 meq/L of CaCO3 is pertinent for the environment? When discussed these points in section 4.6, some lines regarding that is necessary.

Section 2.5: The statistical analyses required more information. It could be pertinent to add the number of replications (n) for each measurement you presented in the graphs and tables. Apart from the lineal relationships, I did not see any statistical analyses to be applied to observe some differences. For example, why not applying that in fig. 1 to explore differences between REE members (or REE groups) for a same measurement (measured at t = 0; 24h). Or in fig. 2 between initial measurements after 24 h or 50 h. Or in fig. 3, among REE members in terms of EC50? In fig. 5 (panel B), it is OK to represent a linear relationship with only 4 or 5 points? How did you obtain the EC50 values? (Which package ? Which model ? Log-Logistic ? How many parameters ?)

Results and Discussion

-I found that some results that have been shown in the discussion section should be located in the Results section. For example, the results discussed from Figs. 5-6, from equations 8-12; they required to be incorporated as results.

-Lines 314-316. Here, you are talking about the decrease in Ln concentrations. You mentioned some losses by Ln adsorption to tube cells, but what about i) Ln the precipitation, ii) and Ln interaction with food contained in D. magna during cultivation and releasing during Ln exposure? I think these possibilities deserve to be also discussed.

-Lines 327-334: The atmosphere here decreases the pH (towards more acid waters), but in the previous sentence (line 320) you mentioned the atmosphere increased the pH. Why this contradictory idea here? Was that really happened?

-I really appreciated the section 4.6 with all the efforts to gather some recommendations/caveats to be considered when testing Ln toxicity, but I found two points requiring special attention.

i)The Ln precipitation and their potential toxic effects. Is there any previous evidence of Ln-containing particles to be uptaken and causing toxicity in any animal model? If yes, please include and discuss this work. More development is still required to reinforce these propositions of Ln toxicity. Or is that an important issue for D. magna versus other aquatic organisms (mussels)?

ii) I was expected some lines discussing the importance of measuring bioaccumulation during LN testing

studies in any aquatic model. If you want to provide some recommendations what is your feeling about this point? If you want to add more development on that issue, what are the precautions to take into account (depuration, desorption) when reporting total metal concentrations? I think bioaccumulation is the missing word here.

-At several times, the name of the animal model (D. magna) is not in italics. Please be consistent with that through all the document.

Minor corrections:

-Title requires improvement. for hazard and risk assessment? Or for future REE toxicity testing?

-Line 21: 14 or 15 members of Ln group? If corrected, see line 87 too.

-Line 28: predictable patterns in LN ecotoxicity.. are you mentioned in terms of EC50? If yes, I recommend putting that in parentheses (if the abstract words number is allowed)

-Line 30: why ecotoxicology and not just toxicology?

-Lines 45-46. Some keywords are included in the title.

-Lines 51: please be consistent with the abbreviation REE or REY? Is all the members of REE needed for these applications? I would start by Some rare earth elements…

-Line 73: (note that we mean no criticisms to the scientific validity of the studies having adopted this approach). Is that necessary?

-Line 91… series implies or series imply?

-Line 104: verify or explore?

-Line 147: Two independent definitive tests were performed for each element. Do you have a test for 24h and 48h for each element? Or do you have a duplicate for each test 24h and then 48h. To indicate this information.

-Fig. 1 Why not including "n" for each measurement? Why not present these results in terms of % (which should be more illustrated)? Any ± sd estimated in your measurements to be added?

-Fig. 2. Any statistical test to be applied?

-Lines 285-295. Please, check if the equation numbers are adequately cited.

-Line 288. the TWM EC50 were calculated as follows. I think all this information should be located in section 2. Methods

-Fig. 4. Please a better identification of first definite series and second definite series mentioned in section 2 Methods should be needed. It is somewhat confusing.

-Line 312. ..expected

-Fig. 5 why did you use the term mod?

-Line 379. I think the word kinetics is a strong word to be used here. What about temporal changes? These are good results to going deeper about the link between chemical properties of Ln and observed behaviours.

-Lines 410-413. I think this is the place to discuss something about the impact of filter material during this step.

-Lines 420-421. Are you sure that the presence of more biomolecules (coming from D. magna death) in the exposure media will increase the Ln solubility? Why not the Ln precipitation?

-Lines 469-475. I understand your points but still a negative control should be recommended to check the influence of these anions on the toxicity observed.

-Fig. 6. Other results showing differences between light and heavy REE and more explanation according to the properties of such REE members should be done. Any ± sd estimated in your measurements to be added (for 24hrs)?