

Dear Prof. Couture,
Dear PCI Editorial Board,

Thank you very much for allowing us to revise our submission to PCI Ecotoxicology and Environmental Chemistry based on the comments from Dr. Carrie Rickwood and one anonymous reviewer. We are grateful to both colleagues for the time they spent to suggest improvements to the original submission.

The detailed responses to all comments are presented below. We hope that they will be satisfactory to all the involved parties.

Best regards,
Davide Vignati on behalf of all co-authors.

Reviewer 1

NOTE:

Line numbers in reviewer's query are those in the original submission.

Line numbers in author's response are those in the revised version (version 2) on HAL deposit.

Reviewer's query	Authors response
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.	We do not expect lanthanides to be unstable under light. Simply, the norm NF ISO 6341 states that 'exposures should be carried out in the dark or under a 16h light:8h dark regime'. Indeed, the phrasing of ISO 6341 differs from OECD guideline 202 stating "A 16-hour light and 8-hour dark cycle is recommended. Complete darkness is also acceptable especially for test substances unstable in light". Because we followed the NF ISO6341, exposures were carried out in the dark, i.e. the first option suggested by this norm. We have added a note 'as per guideline specifications' in the revised text (line 130).
2) Line 133, the authors provide the composition of the standardized medium, could they also provide the hardness value?	The calculated hardness value (250 mg/L as CaCO ₃) has been added to the text (line 133). Moles Ca in medium: approx. 2 mM Moles Mg in medium: approx. 0.5 mM Molecular weight Ca(CO ₃): 100.075 mg/mM Total hardness as Ca(CO ₃): 2.5 mM * 100.075 mg/mM = approx. 250 mg/L
3) Line 211 – reference to the TWM formula from OECD 2008 is made but	The in-text citation has been corrected to OECD (2012), which is the correct reference (Line 217).

<p>this reference is not in the reference list – please include.</p>	<p>OECD 2012 is already in the reference list and is an update of the former 2008 version that we mistakenly left in the first submission.</p>
<p>Line 213 = it states that $Conct(x=1)$ is the measurement concentration at 24. I believe this should be $Conct(x+1)$.</p>	<p>Line 219. Text has been corrected. The reviewer is right.</p>
<p>Lines 290-295. Calculation of time weighted mean (lines 290-295) – 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.</p>	<p>We modified the text at lines 317-329 of the revised version to address this comment.</p> <p>The following elements were added or revised for clarity: All TWM were estimated using equation 2. In the case of 24h exposure, experimentally measured concentrations ($t=0$ and $t=24$) could be entered directly into equation 2. In the case of 48h exposure, we used the nominal 48h EC50 values obtained from ecotoxicity tests for the concentrations at $t=0$. Equation 5 was then used to estimate the corresponding decrease in exposure concentration after 48 hours.</p> <p>In relation to Table S11, we made a mistake in the order in which we uploaded the files on the data repository. The order of the data files has been corrected. Table S11 now presents the results of TWM estimations described in section 3.4 of the text. The caption of Table S11 (in the readme file of the data depository) describes the calculation procedures. The revised text explicitly direct readers to the table caption for the detailed calculation procedure.</p>
<p>Line 334 - the authors reference section 3.4, do you mean 4.4?</p>	<p>The text has been corrected (line 376). Indeed, we wanted to refer to section 4.4.</p>
<p>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</p>	<p>This is a very interesting point. In the revised text (lines 431–445), we started by acknowledging that TWM is just of the possible methods to account for decreasing exposure concentrations. For comparison purposes, we added simple geometric mean calculations to Table S11. The advantage of geometric mean is that it requires concentration only at $t=0$ and $t=$ ‘end of the text’; i.e., it does not require an excessive</p>

<p>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.</p>	<p>analytical burden during ecotoxicological investigation.</p> <p>The ATSDR guidance for inhalation toxicity provides instruction to integrate hours of exposure to different concentration. However, it seems to us that ATSDR considers fixed concentrations for variable time, which is not exactly the case in the present article. In this study, concentrations vary with time, while ATSDR assumes fixed concentrations for variable duration.</p> <p>On the other hand, the TWM approach can integrated multiple measurements over time. In these cases, it is enough to add the exposure duration for each period as specified at pages 22 and 23 of OECD (2012). This procedure is also explained and applied in the revised version and the corresponding results has also been added to Table S11.</p> <p>The revised version better supports our recommendation for the need of a community consensus on how to account for concentration decrease during ecotoxicity testing of lanthanides.</p> <p>We are ready to make explicit mention of the ATSDR procedure should the reviewer deem this necessary.</p>
<p>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.</p>	<p>Table S10 belonged to those that were erroneously labelled in the data repository containing the supporting information (see also comment to lines 290-295 of original submission).</p> <p>In the revised version, table S10 contains the information on nominal EC50s at 24h and 48h plus the corresponding TWM EC50, in line with the reviewer request.</p> <p>The caption of table S10 has been modified accordingly.</p>
<p>Hyaella Azteca should be Hyaella azteca</p>	<p>Modified as requested.</p>

Reviewer 2

We have tried to make the necessary changes to include “more perspectives and critical limitations of the current study” as recommended by the reviewer.

Considering the breadth of some comments, we organized our response in ‘general’ and ‘specific comments’

<p>General comment 1</p> <p>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.</p> <p>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”)</p>	<p>We appreciate the overall positive perception of the reviewer on the potential interest of our study.</p> <p>This overarching comment seems to call for a more critical appreciation of the possible implications and ramification of our work in relation to the actual results collected and presented in the paper.</p> <p>In the absence of more detailed request, we proceeded as follows.</p> <p>Sub-section 4.6 of the original submission was transformed into a new, independent section 5. In this way, it is clear from the beginning that what is presented does not necessarily belong to the discussion of the results obtained in the present paper.</p> <p>The title of this new section is ‘Recommendations for future studies on LN ecotoxicity’. The new title is more focused on the specific topic of the paper without claims for major contributions to the broader areas of hazard and risk assessment.</p> <p>The text in the various subsections has then been modified to highlight how the results of the present study provide suggestions for future research. We tried to be as clear as possible about the specific contribution of the present study vs. knowledge already available from previous work.</p> <p>The text of caveat #1 was re-organized to better stress the two points specific to the present study: changes in total and filterable concentrations over time and the relation between pH and formation of LN precipitates.</p>
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	<p>In caveat #2, thanks to the reviewers' comments, we can now be sincerer about the actual recommendations originating from our results. In particular, we discuss the implications of the rapid decrease in LN concentrations at the beginning of the tests and the existence of linear relationships between LN atomic masses and concentration decrease. We removed the considerations on the possible ecotoxic effects of LN precipitates (see modifications to caveat #3) and on DGT use (already mentioned in moved to caveat #7).</p> <p>Caveat #3. We start this subsection by clearly stating that our results and approaches do not allow to address the specific issues related to the possible ecotoxicity LN precipitated chemical species. We then provide a few recommendations based on current knowledge. Because of the widespread occurrence of LN precipitates in ecotoxicity media, some authors explicitly recommended using total concentrations as the best exposure metric to assess LN ecotoxicity. We explicitly mention this to acknowledge that our recommendation in caveat #1 do not represent a consensus across the research community. Mentioning of LN mixtures has been deleted to avoid overstretching of our results.</p> <p>No modifications were done to the subsection caveat #4 and 5, except minor editorial changes.</p> <p>Some text in caveat #6 has been deleted to avoid overstretching the significance of our work.</p> <p>The text of caveat#7 has been modified to include the reviewers remark at section 2.4 (see below).</p>
<p>General comment 2</p> <p>Lanthanides as a contaminant group of interest: In the results and discussion</p>	<p>This comment contains several observations that we address below.</p> <p>Light vs. heavy LN. We did not mean to refer to the distinction between light and</p>

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

heavy lanthanides (or REE) that is often used in the literature. We simply wanted to highlight differences between the behavior of the elements. Because we focus on the entire LN series, dividing LN into light and heavy would add little to the paper. We have modified the text to avoid confusion.

As explained above, we had no intention of dividing LN in two groups, but to examine the behavior of all LN along the series. Link between LN solubility and toxicity vs. LN atomic mass are examined section 4.2 (figure 5) and 4.4 (figure 6), respectively. Because atomic mass correlates with other chemical properties (e.g., ionic radius), we decided to limit our attention to atomic mass.

Chemical properties such as affinity for O-containing ligands are also accounted for during speciation calculation where hydroxide precipitation was allowed (line 193 of the original submission). There are no other O-containing ligands in our simple laboratory test medium.

When discussing our own results, we consistently used LN in the revised version. In the introduction and in caveat 6, we used the abbreviation REY which is more inclusive of those studies focusing on lanthanides plus Sc and Y.

In keywords, we changed ‘critical raw materials’ to ‘technologically critical elements’. In the first sentence of the introduction, no modification was done because reference is made to ‘mineral resources’ and not necessarily to individual elements.

Indeed, the paper does not deal with LN mixtures.

<p>discussion sections, I think all these passages related to REE mixture have to be removed.</p>	<p>At lines 88-90, we use some references to acknowledge that work exists on LN mixtures.</p> <p>At line 97, text has been modified not to convey the idea that this paper dealing with LN mixtures.</p> <p>At line 705, we are examining the possible ramification of our findings and we would like to stand to our original wording.</p>
<p>General comment 3</p> <p>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 <i>D. magna</i>? Is not possible to take some lesson learn from these previous studies on algae when once decides to work for <i>D. magna</i>? I highly recommend mentioning that in these lines (78-87).</p>	<p>The literature on REE speciation and ecotoxicity has grown steadily and important results are indeed available for model organisms other than <i>D. magna</i>. In our work, a number of examples in this sense are listed in Table 1 (which does not claim at all to be exhaustive).</p> <p>In the original submission, we quickly explained these issues at lines 61-77 (of original submission), again with specific references to <i>D. magna</i> which is the focus of our work.</p> <p>In the revised version, we added two sentences (lines 63 to 69 of revised version) to better acknowledge the similar issues have already been reported. This should better clarify the actual scope of our contribution.</p>
<p>General comment 4</p> <p>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.</p>	<p>We made efforts to address this general comment by providing answers to the detailed requests made by the reviewer on this issue.</p>

General comment 5

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.

The strong statement in the original version (... , the toxic effects ...can be ascribed exclusively to the added LN) has been revised to a milder one based on the approach described in this response to reviewers (lines 552–560).

In particular, we adopted a more quantitative approach to analyze the available data. We calculated the toxic units corresponding to the maximum added LN concentrations. For example, in the case of La, the maximum La concentration added to the test medium was 53.5 mg/L. This concentration corresponds to 5.25 Toxic Units (first series) or 2.7 TU (second series) of La. TU are obtained by dividing the maximum added concentration by the 48h EC₅₀. The 48h exposure was chosen because literature data for Cl⁻ and NO₃⁻ are available for this exposure duration.

Using the same conceptual approach, we estimated the toxic units for chlorides or nitrates. TU were estimated using the maximum added chloride or nitrate concentration divided by the lowest EC₅₀ reported in the literature. With this approach we obtain 0.15 TU for chlorides and 0.05 TU for nitrates. The worst-case scenario (i.e., the highest contribution of counteranions) is therefore in relation to the results of the first series; i.e. 2.7 TU of La. In this case, the TU for Cl would be 5% of those for La. This quantitative approach was applied to all elements and the contribution of anions was below 5% in all cases except Eu (second test series).

A new supplementary table has been added (Table S13) to show these calculations for all elements.

<p>General comment 5</p> <p>-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</p>	<p>We thank the reviewer for the possibility to expand our reasoning on this important point. Kindly note that there are no ‘nominal’ concentrations given in Figure 2. Nominal values are given only in Figure 1. Our response is therefore based on both figures 1 and 2.</p> <p>As explained in the original submission, total concentrations at t=0 were within 20 % with the exception of Dy (original submission, lines 231-232). We acknowledge this oversight, but we maintain that this does not affect data interpretation in figure 1 as already discussed in the original submission (original submission, lines 232-234).</p> <p>In relation to figure 2, the reviewer prompted us to add a comment on the difference between total concentrations and filterable concentrations at t = 0 (lines 277-284). The ratio between total and filterable concentrations at t = 0 were between 80 and 120 % for Gd, Yb, Tb, Ho, Tm, La (79%), Eu, Ce, Lu, Dy, and Eu. Lower ratios were observed for Nd = 65%, Sm = 73% and Pr = 77 %.</p> <p>This additional information also allows to address the comment to line 164 of the original submission concerning the possible adsorption of LN on filter materials.</p>
<p>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?).</p> <p>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</p>	<p>The original text was unclear about the first point of this comment. Both the first and second definitive test provided results about LN effects at 24h and 48h. Text has been revised for improved clarity (lines 150-151).</p> <p>In relation to the second point of this comment, we have modified the text to acknowledge that the assumption of homogeneous initial pH could have been violated (lines 151-157). At the same time,</p>

<p>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.</p>	<p>we notice that the presence of linear relationships between pH and added LN concentrations (Table S8) will allow us to properly evaluate the ecotoxicological results.</p> <p>In practice, we chose to follow, given technical and practical constraints, how the exposure systems evolved over time. A posteriori, taking measures to ensure homogenous pH at the beginning of the tests could have been a better option. However, it would not have eliminated the issue of changing pH over the test duration. This will remain a limitation of the study. In the revised version, we acknowledge it in caveat #2 of section 5 (section 4.6 in the original submission).</p>
<p>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.</p>	<p>Following our reply to a previous comment on LN concentration in mother solution, we have been able to add a few caveats and recommendation in section 4.2 (lines 378-384).</p> <p>Other comments on this issue have been added to section 4.3 following the reviewer's observation at lines 410-413 of the original submission (see below).</p>
<p>On line 184: Why was the pH not measured in all testing conditions? Or what was your criteria to select some exposure conditions?</p>	<p>The initial idea was simply to check that the pH did not fell to values with potential adverse effects on <i>D. magna</i> (as explained in section 4.4, lines 464-469 of the original submission).</p> <p>In general, we measured controls, the lowest exposure concentration and the highest exposure concentrations. Intermediate concentrations were sometimes added depending on actual observation of daphnids mortality.</p> <p>Considering that we extensively discuss the linear relationships between added LN and pH (section 4.4 and Table S8), we have not modified the text.</p>
<p>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</p>	<p>Tests were performed in the standardized medium specified by the norm ISO 6341. We note that the objective of speciation calculation performed in our paper simply aimed at estimating the concentration of free LN₃₊ ions for each element and the</p>

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.

solubility of individual LN in the test medium.

Speciation calculations took into account all the inorganic components present in the medium; i.e., the cations Ca, Mg, Na and K and the anions Cl, SO₄²⁻ and HCO₃⁻. Protons (H⁺) and hydroxo (OH⁻) anions are considered by default in the speciation calculation. The counterions concentrations added with each lanthanide were also considered in the speciation calculations. On the other hand, the thermodynamic speciation calculations of the present study cannot account the competitive role of Ca and Mg (or other ions) on REE uptake as it is done, for example, in Biotic Ligand Model.

All that considered, we surely agree that major elements such as Ca and Mg can influence REE uptake and toxicity. Because our results do not provide information to directly discuss such issues, we mentioned this potential problem in section 5 (formerly 4.6) at caveat #7 of the revised version.

The rationale behind equation 2 is explained in OCDE (2012) and is applicable to any contaminant (inorganic and organic) whose concentration does not remain stable over a given ecotoxicological tests.

We did not consider possible precipitation by sulfates or nitrates because these inorganic complexes of LN are usually soluble in water. According to two recent publications, LN sulfate solubilities at 25 °C range from 19 g/L (for Sm sulfate being the least soluble) to over 300 g/L for Lu sulfates. These solubility values for sulfates correspond to added concentrations of 4 g/L of Sm, which is at least two orders of magnitude higher than concentrations used in the present study.

The solubility of nitrate salts is in the molar range; again much higher than the added concentrations.

(continues on next page)

	<p>As for hydroxides, they were considered in our speciation calculations as written at line 193 of the original submission.</p> <p>We have added a sentence and the necessary references (see below) in the revised text (lines 200-203).</p> <p>The specified alkalinity value is for the standard ISO 6341 medium. We agree that natural waters can have different alkalinity. The modifications made to section 5 (see above) should be enough to cover this issue as well.</p> <p>References added to text in response to this comment:</p> <p>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 H2SO4 solutions (neodymium sulfate). <i>Hydrometallurgy</i>, 223, 106194. https://doi.org/https://doi.org/10.1016/j.hydromet.2023.106194</p> <p>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 H2SO4 solutions (dysprosium). <i>Hydrometallurgy</i>, 218, 106054. https://doi.org/https://doi.org/10.1016/j.hydromet.2023.106054</p> <p>Siekierski, S., & Salomon, M. (1985, 1985/07/01). Thermodynamics of saturated lanthanide nitrate-water systems. <i>Journal of Solution Chemistry</i>, 14(7), 473-484. https://doi.org/10.1007/BF00646979</p>
<p>Section 2.5: The statistical analyses required more information. It could be</p>	<p>The number of measurements has been added in Figures 1 and 2. Other figures present elaboration of raw data.</p>

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 ?)

Considering that measurements were performed in duplicate at t=0 and 48 h (and only 1 measurement was performed at t = 5 and 24 h) a statistical analysis of the differences was not attempted in figures 1 and 2.

Because figure 3 shows the theoretical solubility of LN, we will assume that the reviewer wants to refer to figure 4. Panel A and B of figure 4 give the EC₅₀ at 24h and 48h for the first (panel A) or second (panel B) round of test. Confidence intervals are given for each EC₅₀.

In relation to the differences between REE groups, the reviewer probably refers to the widely accepted distinction between light (LREE) and heavy (HREE) rare earth elements.

The patterns observed in figure 2 (and given in full in Figure S2) do not seem to conform to this grouping. Figure S2 shows that La and Ce stand out from other elements (pattern 1). However, patterns in figures 2 and S2 do not show any clear distinction conforming to the accepted grouping between LREE and HREE. For this reason, no attempt to group REE has been done for these results.

On the other hand, we have performed a statistical analysis of the EC₅₀ values presented in figure 4 and 6 based on the LREE vs. HREE grouping. The results of this new elaboration are discussed at lines 647-657 of the revised version and a new supplementary figure has been added (Figure S4). The statistics used for this elaboration are specified in the caption of figure S4, rather than in section 2.5, for the sake of clarity.

In figure 5B, it is technically correct to derive linear relationship based on 4 observations because drawing the regression requires a minimum of two degrees of freedom (which are available).

	<p>We have added information on how we obtained EC₅₀ in section 2.5 (lines 237-239). Thank you for pointing out this oversight.</p>
<p>-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.</p>	<p>These data are elaboration of the raw results obtained from analytical measurements and ecotoxicity testing. Following PCI requirements for separate 'Results' and 'Discussion' sections, we would stand to the presentation used in the original submission.</p>
<p>-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 <i>D. magna</i> during cultivation and releasing during Ln exposure? I think these possibilities deserve to be also discussed.</p>	<p>In the original text, precipitation issues are discussed at lines 327-334. In the revision, we have however mentioned this at the beginning of section 4.1 for clarity.</p> <p>On the other hand, no food was added during ecotoxicity testing as per the procedures described in norm ISO 6341. We mention 'no food has been added' at line 131 of the revised text.</p>
<p>-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?</p>	<p>These are two different issues.</p> <p>In systems opened to the atmosphere, the carbonate system ($\text{H}_2\text{CO}_3 - \text{HCO}_3^- - \text{CO}_3^{2-}$) will reach an equilibrium between the carbonates contained in the aqueous solutions and the atmospheric CO₂. In particular, when adding protons to lower pH, the available medium alkalinity (provided by the NaHCO₃ component) will tend to buffer the system by shifting the equilibrium toward increased formation of H₂CO₃. The H₂CO₃ will dissociate into H₂O and CO₂, with CO₂ evading from the solution into the atmosphere. Otherwise stated, H₂CO₃ will be removed from the carbonate system and excess protons will continue to react with HCO₃⁻ to form H₂CO₃ until an equilibrium between H₂CO₃ and atmospheric CO₂ is reached. This equilibrium is determined by the medium composition. According to the norm ISO6341, the pH of the standard medium should be 7.8 ± 0.5 units, which explains why pH tends to increase provided that medium alkalinity is not exhausted.</p>

	<p>On the other hand, part of the added LN would precipitate with the carbonates present in the medium. As a consequence, HCO_3^- will dissociate into H^+ and CO_3^{2-} to re-establish the equilibrium of the carbonate system (this is equation 3 of the original and revised submissions). The release of H^+ will tend to decrease pH and the pH will actually start decreasing when the buffering capacity of the system is exhausted. This is explained at lines 327-344 of the original submission.</p> <p>However, the original text was not clear enough on the fact that pH changes are determined by the combination of the two processes. In the revised submission, the entire text of section 4.1 has been re-organized and expanded to explain all these phenomena. The effect of LN addition (lowering on pH) is discussed first. Basic information on the carbonate buffering system is then mentioned to explain why, in some cases, pH increased rather than decreased during the tests. Reference to supplementary tables has also been revised.</p>
<p>-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.</p> <p>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 <i>D. magna</i> versus other aquatic organisms (mussels)?</p>	<p>We thank the reviewer for expressing his/her appreciation on this specific point.</p> <p>Indeed, some recent work would point to a role of LN-containing particles in <i>D. magna</i>. This is discussed in the revised caveat #3 and a new reference on the subject has also been added and commented in the revised version.</p>
<p>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</p>	<p>We understand and agree with the reviewer's opinion, but we feel uncomfortable to provide explicit recommendations on bioaccumulation considering that we do not provide any results on bioaccumulation. Our paper is on the importance of speciation in the exposure</p>

<p>want to add more development on that issue, what are the precautions to take into account (deuration, desorption) when reporting total metal concentrations? I think bioaccumulation is the missing word here</p>	<p>medium along with its possible implications for hazard assessment. We opted to add a sentence in the concluding remarks (lines 887-892) to remind that bioaccumulation approaches may be useful (or even better) to understand LN ecotoxicity to <i>D. magna</i>.</p>
<p>-At several times, the name of the animal model (<i>D. magna</i>) is not in italics. Please be consistent with that through all the document.</p>	<p>All instances were checked and corrected for consistency. Apparently, some format was lost during the docx to pdf conversion in the submission.</p>
<p>-Title requires improvement. for hazard and risk assessment? Or for future REE toxicity testing?</p>	<p>Following the answers to the reviewers' comments, we proposed a revised title that better reflects the scope of the research: Ecotoxicity of lanthanides to <i>Daphnia magna</i>: insights from elemental behavior and speciation in a standardized test medium.</p>
<p>-Line 21: 14 or 15 members of Ln group? If corrected, see line 87 too.</p>	<p>We did not consider Pm. We have added this information</p>
<p>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)</p>	<p>Thank you for the suggestion. Text has been modified.</p>
<p>-Line 30: why ecotoxicology and not just toxicology?</p>	<p>The on-line Merriam Webster dictionary provides the following definitions:</p> <p>Toxicology: a science that deals with poisons and their effect and with the problems involved (such as clinical, industrial, or legal problems)</p> <p>Ecotoxicology: a scientific discipline combining the methods of ecology and toxicology in studying the effects of toxic substances and especially pollutants on the environment</p> <p>Ecotoxicology seems more appropriate to the research presented in this paper.</p>
<p>-Lines 45-46. Some keywords are included in the title.</p>	<p>Keywords have been checked to avoid redundancy with title.</p>

<p>-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...</p>	<p>Sentence at line 51 modified as requested 'Some Rare Earth Elements...' We left REY in the introduction.</p> <p>In general, we changed REE to LN considering that we studied only lanthanides.</p> <p>We changed REE to REY at line 818 because MacMillan et al. (2018) also included Sc and Y in their study.</p>
<p>-Line 73: (note that we mean no criticisms to the scientific validity of the studies having adopted this approach). Is that necessary?</p>	<p>Albeit unusual, our answer would be a qualified yes.</p> <p>The studies cited at lines 68-71 of the original submission stand scientific scrutiny as to the observed ecotoxic effects. However, in our opinion, they need improvement in the characterization of LN and speciation to establish the best 'exposure concentration' vs. 'biological response' relationship. In this sense, we want to be germane about not having any objection on the quality of the cited studies, while suggesting possible improvements based on the results (and limitations) of our contribution.</p> <p>We have slightly modified the original text to better stress our point (line 72).</p>
<p>-Line 91... series implies or series imply?</p>	<p>Implies. The subject of the sentence is 'The presence (singular)', not series (plural).</p>
<p>-Line 104: verify or explore?</p>	<p>Text was modified to 'explore'.</p>
<p>-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.</p>	<p>We performed two tests lasting 48 h each. During each test, the number of immobile daphnids was evaluated at 24h and at 48 h. The raw results are reported in Table S4. We have re-arranged the text for clarity (lines 150-157 in the revised text)</p>
<p>-Fig. 1 Why not including "n" for each measurement? Why not present these results in terms of % (which should be more illustrated)? Any \pm sd estimated in your measurements to be added?</p>	<p>The number of measurement (also given at lines 169-173 of the original submission) has been added to the figure caption. All measurements presented in figure 1 were performed in duplicate.</p>

	<p>We opted for concentration values (mg/L) in order to compare both ‘measured concentration at t=0 vs. t=48’ and ‘nominal vs. measured concentrations at t=0’ on the same figure. Information on percentage losses is given in the text and can be obtained from the available raw data.</p> <p>Error bars have been added for all measurements (all of them were available in duplicate). Formula 1 was used to calculate measurement precision.</p>
-Fig. 2. Any statistical test to be applied?	<p>As explained in the Material and methods section, some measurements were performed (at most) in duplicate to verify measurement precision (lines 169-173). There are not enough replicate measurements for reliable statistical testing.</p>
-Lines 285-295. Please, check if the equation numbers are adequately cited.	<p>This issue is addressed in response to the comment of reviewer 1 at lines 290-295 of the original text.</p>
-Line 288. the TWM EC50 were calculated as follows. I think all this information should be located in section 2. Methods	<p>Technically speaking the reviewer is right. However, estimations of TWM EC₅₀ also require the derivation of equations 4 to 5 that do not belong to the materials and methods section, but are ‘results’ from the study.</p> <p>Following the other modifications in this section of text (lines 317-329 in the revised document), we propose to leave this text in section 3.4.</p>
-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.	<p>The reply to comment at line 147 (this reviewer) should have clarified this issue. We slightly modified the caption for further improvement.</p>
-Line 312. ..expected	<p>We corrected the typo in the revised version.</p>
-Fig. 5 why did you use the term mod?	<p>We forgot to include the information in the caption of figure 5. With ‘mod’ we wanted to indicate the ‘modelled’ concentration decrease predicted using equation 4 to 7, while ‘obs’ indicates the ‘observed’ percentage concentration decrease calculated from experimental measurements.</p>

	We have revised the figure caption to include this information.
-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.	Agree. The work ‘kinetics’ has been modified to ‘temporal trends.’
-Lines 410-413. I think this is the place to discuss something about the impact of filter material during this step.	Indeed, filtration is much more complex than commonly assumed. Horowitz et al (1992; 1996) highlighted possible pitfalls of membrane filtration long ago. The value of filterable concentration does depend on filter type (for the same nominal filter cut-off) as well as on the total volume of filtered water. Furthermore, filter behavior may not be constant across water types. We have added these considerations at lines 482-488 of the revised version along with a couple of relevant references.
-Lines 420-421. Are you sure that the presence of more biomolecules (coming from <i>D. magna</i> death) in the exposure media will increase the Ln solubility? Why not the Ln precipitation?	<p>We are not sure that the molecules released following death of <i>D. magna</i> actually increased LN solubility. It is simply a working hypothesis to explain the trends in analytical concentration data. Previous studies would indeed suggest that LN solubility is increased in the presence of humic acids (Pourret et al. 2007). Lachaux et al. (2022) also observed increased LN solubility in the presence of soil organic matter added to standard test medium for <i>D. magna</i>. However, humic acids or soil organic matter certainly differ from the type of organic molecules released by dead daphnids in a medium otherwise containing only inorganic salts.</p> <p>An ‘as soon as published’ article in ACS Earth Space Chem (Wen et al., 2024) just showed how the interaction between simple organic molecules (oxalate, citrate and DFOB) and pH values can control the solubility of LN carbonates and phosphates (the latter being absent in our medium).</p> <p>Our original text was clearly too assertive and has been corrected to acknowledge the presence of a much more complex situation</p>

	<p>and the need for further investigations (lines 496-499 of the revised text).</p> <p>References in response to this comment: Pourret, O., Davranche, M., Gruau, G., & Dia, A. (2007, 2007/08/30/). Rare earth elements complexation with humic acid. <i>Chemical Geology</i>, 243(1), 128-141. https://doi.org/https://doi.org/10.1016/j.chemgeo.2007.05.018</p> <p>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 <i>Daphnia magna</i> standard test. <i>Environmental Pollution</i>, 307, 119554. https://doi.org/https://doi.org/10.1016/j.envpol.2022.119554</p> <p>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. <i>ACS Earth and Space Chemistry</i>. https://doi.org/10.1021/acsearthspacechem.4c00009</p>
<p>-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.</p>	<p>We have improved our reasoning in response to general comment 5. We agree with the reviewer request and added a cautionary note in this sense. However, we have not performed new experiments considering the low expected toxicity of counterions (see lines 552-561 or the revised text).</p>
<p>-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 \pm sd estimated in your measurements to be added (for 24hrs)?</p>	<p>See response to section 2.5 for the first part of this comment on the difference between light and heavy REE.</p> <p>For the second part of the comment, it is not possible to consistently provide error measurements to the reported values. Both TWm and free ion EC₅₀ are metrics derived from experimental data using calculation</p>

	<p>(TWM) or thermodynamic modelling (free ion). The error on these metrics would therefore reflect the errors on the initial analytical measurements plus or minus a constant. Because some analyses were not performed in duplicate (see section 2.3), we cannot confidently estimate error measurements from experimental data.</p>
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