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ABSTRACT

Lanthanide (LNs) release into the environment is expected to greatly increase in the coming years due to a high demand for new technologies. However there is a gap in the ecological risk assessment of these metals because most of the ecotoxicological studies have been performed with only one element, although they are usually found in nature as a group. This research evaluated the effects of mixtures of three lanthanides, cerium (Ce), gadolinium (Gd), and lutetium (Lu), representative of the light, middle and heavy rare earth elements, respectively, on seven aquatic species (*A. fischeri*, *R. subcapitata*, *C. vulgaris*, *B. calyciflorus*, *H. incongruens*, *D. magna* and *D. rerio*). Lanthanide content decreased over time in all toxicity test media and it was observed that LN sedimentation starts at the beginning of the tests with a steep decline of the available LN amount. Potential toxic effects of LNs were observed only in five species of the seven studied, predominantly in the unicellular organism (*A. fischeri*) and in the organisms belonging to the lower trophic levels (*R. subcapitata* and *B. calyciflorus*). The multi-toxicity approach performed in this study showed synergistic effects in tests performed with the bacteria *A. fischeri* and the algae *R. subcapitata*, and antagonistic effects for the rotifer *B. calyciflorus*. Although predicting the response of aquatic organisms exposed to multi-elements is not an easy task and can be masked by potential interactions with other compounds or even by nutrient removal. The variation in toxic action among species observed in this study reveals that lanthanide interaction in toxicity mechanisms should not be discarded, and supports that further studies with LN mixtures are required to properly understand their toxic behaviour in nature ecosystems.

CAPSULE Mixtures of lanthanides showed toxic effects in bacteria and organisms of low trophic levels, with potential synergistic or antagonistic effects depending on the studied organism.

Keywords: ecotoxicology, bioassays, rare earth elements, toxic units, multi-element

1. INTRODUCTION

Rare earth elements (REE), more specifically lanthanides (LNs), play an essential role in modern technology. Along with a growing demand for applications from electronics and fuel additives (UNCTAD, 2014) to agriculture (Pang et al., 2002) and even environmental remediation (Stauber, 2000), lanthanide production rates are expected to increase (Alonso et al., 2012), and thus their release into the environment. The trend towards a greater environmental input of LNs indicates a pressing need to assess the potential hazard they pose (Alonso et al., 2012, Zhang et al., 2017). In overviews about the effect of lanthanides on aquatic organisms, the toxic effective concentration values have been reported in the ⌠g to mg per liter range for algae, crustaceans, nematodes and fish (González et al., 2014; Herrmann et al., 2016). Different mechanisms for LN inhibitive action, including competition with Ca, replacement of Mg or precipitation with phosphates or carbonates (Sneller et al., 2000), have been previously observed. Additionally it has been also observed that toxicity-inducing free LN concentration in some test media can be drastically reduced by pH increase (Weltje at al., 2004). Metal inhibitive effects are, therefore, not only connected to their direct toxicity, but complexation with ligands and competition with other metals could interfere in the study of their effects (El-Akl et al., 2015).

The additional input of LNs into the environment usually results in a concentration into the ng L^{-1} range (Kulaksiz and Bau, 2013, Klaver et al., 2014, Hatje et al., 2016). Concentrations due to anthropogenic input do not normally reach levels that induce ecotoxicity in standard laboratory tests, except at some point sources (Kulaksiz and Bau, 2011, González et al., 2015). Toxicity assessment is mainly done by acute tests, however Blinova et al. (2018a) stated that acute toxicity data of lanthanides are not reliable and the environmental risk assessment of lanthanides have been shown to be beneficial for plants (Pang et al., 2002) and

bacteria (Wehrmann et al., 2017); and even used as fertilizers in agriculture (Zhang et al., 2001). To date, the only regulatory levels for LN concentrations in freshwaters are available for the Netherlands, where the maximal permissible levels range from 1.8 µg L^{-1} for Nd to 22.1 µg L^{-1} for Ce (Sneller et al., 2000).

The evaluation of the toxicity of LN's has mostly been on one element, with a special emphasis on La, Ce and Gd effects (Pagano et al., 2015; González et al., 2015). To date, information about the toxicity of other LNs remains relatively scarce, and studies on LN mixture toxicity in aquatic environments are almost absent. Blinova et al. (2018a) suggested that LN should be considered as an uniform group of elements with a similar mode of action based on a study using crustaceans. Tai et al. (2010) found that the inhibitive effect of LNs remained the same for the algae *Skeletonema costatum* whether the toxicant was an individual LN or an equimolar mixture of all LNs. Tan et al. (2017) observed in a study with the algae *Chlamydomonas reinhardtii* and different LNs that they can share common transport sites and, therefore, can have competitive effects. Thus, the combined effects should not be overlooked since different LNs occur together in nature, albeit with variable relative abundances.

In the present work, the effects of mixtures of three lanthanides, cerium (Ce), gadolinium (Gd), and lutetium (Lu), representative of the light, middle and heavy rare earth elements respectively, (ranked by atomic weight), were studied on aquatic species from different trophic levels (the marine bacteria *A. fischeri*, representative of decomposers; the algae *R. subcapitata* and *C. vulgaris* as primary producers; *B. calyciflorus*, *H. incongruens* and *D. magna* as primary consumers; and the fish *D. rerio* as secondary consumer). Our aim was to establish a preliminary approach of lanthanide mixture ecotoxicology to characterize and assess their

toxic effects as well as to obtain a better understanding of lanthanide-interactions in aquatic ecosystems.

2. MATERIAL AND METHODS

2.1. ARTIFICIALLY SPIKED WATERS

The experiments were conducted using spiked aqueous solutions prepared with soluble salts of the three studied lanthanides: $CeCl_3$-7Hon$_2$O (purity 99.9%), $GdCl_3$-6H_2O (purity 99.0%) and $LuCl_3$-6H_2O (purity 99.9%) (Sigma Aldrich, St. Louis, Missouri U.S.A). Before starting the tests, exposure concentrations were prepared by diluting stock solutions, previously made by dissolving the salts in deionized water and stored refrigerated, in the corresponding exposure medium. Lanthanide concentrations used in the present study were selected based on the effective concentration causing a 10% reduction (EC_{10}) for the least sensitive species obtained previously by González et al. (2015) in a battery of tests with aquatic organisms. Lu was the exception, where a lowered value, according to its lower occurrence in the environment, was selected (855 µg L^{-1}). The mixtures were done according to three different ratios and for three different concentrations (Table 1). The first mixture (CE1) had a ~~natural~~ ratio of Ce:Gd:Lu (Ce:Gd=20:1 and Gd:Lu=17:1), in accordance with the ratio obtained in a freshwater system in a natural area with LN geological enrichment (Romero-Freire et al. 2018; own data). The second mixture (GD1) was enriched in Gd and the third mixture (LU1) was enriched in Lu. These three treatments were diluted twice by a dilution factor of 2, maintaining the established ratios (CE2, CE3, GD2, GD3, LU2, LU3). In addition, dual salt mixtures (A, B, C) were combined from the highest LN concentrations used in the triple salt mixtures, with a final number of 12 treatments (Table 1). Because the spiking was prepared using lanthanide chlorides, a control with $CaCl_2$ (final Cl^- concentration 4.8 mg L^{-1}, the

highest quantity of Cl^- added in the treatments) was used in addition of the specified control for the different exposure media.

2.2. ECOTOXICITY TESTS

Seven ecotoxicity tests with organisms representative of the main trophic levels (decomposers, primary producers and consumers and secondary consumers) were selected. The methodology followed and the specific modifications performed in the different toxicity tests are summarized in Table 2. The marine bacteria *Aliivibrio fischeri* (Microtox test, MIT), formerly known as *Vibrio fischeri*, representative of decomposers, was tested following the ISO 11348-1 standard protocol (2007), but with the standard medium diluted twice (NaCl 1%). For the primary producer algae *Raphidocelis subcapitata*, the standard toxicity test (ALT) was performed in the EPA medium (OECD, 2011). This medium was selected because it contains less carbonate (CO_3 = 10.71 mg L^{-1}, 70.0% less) and phosphates (PO_4 = 0.73 mg L^{-1}, 34.6% less) than the common used OECD 201 media, but 3 times greater EDTA content (Table SI.1). Additionally, the LuminoTox test (LUT), with the algae *Chlorella vulgaris*, was performed in deionized water (Bellemare et al., 2006). Primary consumers were represented by three organisms: *Brachionus calyciflorus*, *Heterocypris incongruens* and *Daphnia magna*. *B. calyciflorus* (ROT) and *H. incongruens* (OST) toxicity tests were performed according to the bench protocols (Microbiotest, 2001a, b) but the final test concentrations (EPA moderately hard freshwater) had media diluted twice. Toxicity test with *D. magna* (DAT) was carried out according to OECD protocol (2004), but to minimize the matrix effects shown by Lurling and Tolma (2010), the same test medium used for ROT and OST was selected, after supplementation with extra $CaSO_4 \cdot 2H_2O$ to ensure minimum required water hardness (140 mg L^{-1} as $CaCO_3$). Finally, an additional toxicity test with the fish *Danio rerio* (FET) (secondary consumer) was conducted according to the protocol FET test without

modifications (OECD, 2013), carried out respecting the policy regulation (Directive 2010/63/CE – Décret 2013-118). The eggs used were obtained from an in-house culture (agreement number: A-57-463-3).

Before performing the toxicity tests, the organisms were incubated in the modified test media to ensure the viability of the organisms in the selected media and to not mask the potential toxic effects of LNs with inhibition due to lack of nutrients. For the organisms obtained from in-house cultures, $ZnCl_2$ (for algae), $CuSO_4$ (for fish) and $K_2Cr_2O_7$ (for daphnid) were used as positive controls respectively. Responses obtained in the tests were scaled to range from 0% for samples that did not exhibit toxicity, to 100% for samples with maximum toxicity. In addition pH (edge®blu, Hanna Instruments, USA) was checked at the beginning of all tests.

2.3. EVALUATION OF LANTHANIDE CONCENTRATION AND HAZARD

At the start of each toxicity test (t0h) and at the end (tXh) of tests with a longer exposure than 24 h, LN concentrations were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Ce (Z=58), Gd (Z=64) and Lu (Z=71)). Instrument measurements were carried out in triplicate with a PE SCIEX ELAN-5000 spectrometer using Rh as the internal standard. Aliquots for measuring were obtained directly from the supernatant at t0h and after centrifuging at 300 g for 10 minutes at tXh. Aliquots were acidified with HNO_3 (65%) at 1% for preserving. The supernatants were obtained from the wells of the toxic tests, however for the algae test, due to the small quantities used (250 µL per sample), 15 ml falcon tubes with the studied artificial spiked waters and with and without algae addition (same content in algae cells) were incubated at the same time under the same conditions. For determining ICP-MS precision, surface water (SPS-SW1 Batch 120, LGC standard, UK) without specific treatment, was measured. Average recovery values for SPS-SW1 (n=5) were 120±32% (Ce), 106±37% (Gd) and 101±21% (Lu). From the LN concentrations at the start and at

the end of the tests, and after observing concentrations decline significantly over the exposure period of the tests, the geometric mean (√t0*tXh) was calculated for assessing the toxic effects obtained (OECD, 2000). Additionally, in the algae test, a temporary evolution of P content in the used EPA medium spiked with individual salts of Ce, Gd and Lu at the highest used concentrations (4000, 1400 and 855 µg L^{-1}, respectively), according to the same experimental conditions, was determined by spectrometry (Analytik Jena Specord 205).

To predict and understand multi-element toxicity, the toxic unit approach was used, calculated by dividing the total concentration of each element by their corresponding EC_{50} obtained in González et al. (2015). Finally, total toxic units (TU) were obtained as the sum of all the toxic units (TU_i) calculated from a single element (Weltje, 1998). TU calculation was applied from the nominal concentration, the measured LN content at time 0h (t0h); at the end of each test (tXh); the calculated geometric mean (√t0*tXh); and from the free LN content obtained by modelling speciation, when data was available. From the obtained TU values, the effective concentrations causing a 50% reduction for the corresponding endpoint (EC_{50}) and the 95% confidence intervals (95%CI) were obtained by fitting a log-logistic model to the data (Doelman et al., 1989) using the method of Marquardt (proc NLIN, SAS 9.1, SAS Institute, Cary, NC). Data interpretation of the obtained EC_{50} was based on ATSDR (2004) and the interactions were identified as follows: TU near the unit was identified as additive (no apparent influence). When TU>1, it was identified as antagonistic, effect less than additive (inhibition or masking); and when TU<1, it was identified as synergistic, effect greater than additive (potentiation).

Additionally, to better understand the toxic action of the mixtures, which with LN show similar physicochemical properties and occur together in nature (Walker et al., 2001), cumulative concentration of the mixtures (CCM) were calculated as the sum of each LN composing the mixture, and they corresponding EC_{50} were calculated. Obtained values were used as representative of the LN content as a whole, and they were compared to the individual toxic values previously reported by González et al. (2015).

Finally in order to show the hazard of the mixtures, the predicted no effect concentration (PNEC) was estimated using an assessment factor (1000) and the EC_{50} of the most sensitive species, according to TGD (2003). While the predicted environmental concentration (PEC), was derived from the mean natural concentrations in a freshwater system (Romero-Freire et al., 2018; own data: Ce=1.67 μg L^{-1}; Gd= 0.09 μg L^{-1}; Lu=0.01 μg L^{-1}). PEC/PNEC ratio higher than the unit indicates that unacceptable effects on organisms are likely to occur (Smit et al., 2005).

2.4. DATA ANALYSIS

Chemical equilibrium model VisualMINTEQ 3.1 (KTH Royal Institute of Technology, Stockholm, Sweden) was used to calculate LN speciation in the seven studied toxicity tests. Calculations were performed using inputs of measured pH, nominal concentrations of the lanthanides, cations and anions of the media, and temperature (set to those used in each toxicity test). Algae added as food/test organism (ROT, OST, ALT, LUT) and sand added in OST test, were not included in the model.

Data distributions were established by calculating the mean values and the standard deviations (±SD). The differences between the individual means of the treatments were compared using ANOVA and post-hoc Duncan's tests ($p<0.05$), either to compare among tests or to do

multiple comparisons inside each test. Moreover, in order to analyse the influence of LN content in ecotoxicological effects, Spearman's correlations were performed. Analyses were done with a confidence level of 95% by using SPSS v.20.0 (SPSS Inc., Chicago, USA).

3. RESULTS

3.1. EFFECT OF EXPERIMENTAL CONDITIONS ON LN RECOVERY

Lanthanide content in all test media before and after organisms' exposure is summarized as percentages of recovery from the nominal LN in Table 3. Lanthanide content after spiking (t0h) showed good recovery of the nominal content, ranging between 85 and 122%. In the zebrafish test (FET), LN at t0h was not determined, but the same procedure from stock dilution was followed as in the other tests. LN contents for microtox (MIT) and luminotox (LUT) tests were not measured at the end of the tests because of the short test duration (≤4h). In algae test (ALT), LN content was determined both with and without the addition of algae (*R. subcapitata*). Obtained recoveries show that the addition of algae has a decreasing effect of LN concentration, with a maximal recovery of 37%, whereas without algae addition, it was up to 81% (both for Lu). Additionally in this test, temporal evolution of P was determined for 3 treatments (Figure SI-1). Phosphorous content decreased during the test with greater decreases for treatments with Gd>Ce>Lu. In rotifer (ROT) and ostracod (OST) tests, where algae were added as food sources, maximal LN recoveries were 24% (Lu) and 27% (Gd) at the end of the tests. In tests involving algae (ROT, OST, ALT), agglomeration of cells was observed in the higher concentrations of LNs (Figure SI-2). In OST, standard sand was added to the media and the assay was the longest in duration (144h), which likely caused it to have one of the lowest LN recoveries (1-5% for total LN). Ambient lanthanide concentrations were followed over time in OST for the highest added concentrations (CE1, GD1 and LU1) (Figure SI-3), and it was observed that LN decreased

quickly in the first hours. In the 96h zebrafish assay, the observed recoveries, obtained from the nominal content, were also low (1-10%).

To assess the effect of test duration on LN content, the values of LN recoveries at the end of the test in respect to the start (tXh-t0h) were compared to test duration using Spearman correlation. For the zebrafish test (FET), the comparison was based on nominal instead of t0h concentrations. Percentages of LN recoveries after performing the 7 toxicity tests were strongly negatively correlated with time of exposition ($R=-0.899$, $p<0.01$, $n=216$). The same analysis done only from tests with a longer duration than 24h (without MIT and LUT) also showed negative correlation ($R=-0.671$, $p<0.01$, $n=144$).

3.2. EFFECT OF MEDIA COMPOSITION ON LN SPECIATION

The speciation of the lanthanides in the studied media was calculated with VisualMINTEQ for the treatments CE1, GD1 and LU1 from the nominal contents. The precipitation percentage and the % of formed species from the remaining dissolved media can be seen in Table 4. Considerable precipitation occurred in all tests with the exception of microtox (MIT) and luminotox (LUT) test where almost all available forms were present as free LNs. For MIT done in NaCl (1%), LNs also formed species of $LNCl^{+2}$. In LUT test, done in deionized water, only $LNOH^{+2}$ species were reported, and the formation of these complexes decreased with the increase in the ionic radii, with more than 1% only for Lu.

In the *R. subcapitata* (ALT) exposure media, soluble species with EDTA and insoluble species with phosphates formed in addition to the carbonates, hydroxides and sulfates mainly present in the other test media. Cerium precipitated as phosphate, Gd as hydroxide, while Lu precipitation was not observed. Available LNs appeared as free ions and forming species with CO_3^+ in great percentages for Ce and Gd, with higher percentage of free species for the treatments with higher nominal content, unless uneven precipitation occurred. On the

contrary, Lu distribution was mainly forming species with $EDTA^-$ for treatments at lower nominal values of Lu (CE1>GD1>LU1). In the case of higher content of Lu (LU1), Lu appeared also as a free ion and mainly formed species with CO_3^+. Other species formed in high percentages (values up to 9.3%) were with SO_4^+, which showed a trend of decreasing with the decrease in the ionic radii (this trend appeared also for HCO_3^{2+}).

For the test done with the primary and secondary consumers (ROT, OST, DAT and FET), LN appeared mainly in complexes with SO_4^+ and CO_3^+ and as free forms (LN^{+3}). The percentage of free forms and sulphate complexes decreased with the decrease in the ionic radii (Ce>Gd>Lu), and, to the detriment of these complexes, species with CO_3^+ increased for these four tests with the decrease in the ionic radii. In all tests, the formed species with OH^{+2} and $(CO_3)_{2-}$, also showed an increase with the decrease in the ionic radii, although in lower percentages.

3.3. TOXICITY RESPONSE OF THE ORGANISMS

Organisms in experimental controls were not adversely affected by media dilution (*A. fischeri*, *B. calyciflorus*, *H. incongruens* and *D. magna*); and controls with Cl^- were similar to negative controls in the tests. In all tests, negative controls met all the validation criteria suggested in the corresponding protocols. Positive controls for house cultures (*R. subcapitata*, *D. magna* and *D. rerio*) showed, in the case of algae, effective concentration (EC) values similar to those reported in literature (Franklin et al., 2007); for daphnids $K_2Cr_2O_7$ EC_{50} values were more restrictive (3-13 times lower) compared to the frequently used medium (OECD 202); and in the zebrafish test, the positive control done with $CuSO_4$, systematically used in our laboratory, validated the sensitivity of the organisms (EC_{50}≈60µg Cu L^{-1}).

Toxic effects are reported for the different studied organisms in Figure 1. From a Duncan test performed with all treatments, effect was ranged from higher to lower toxicity in the

following order: ALT, MIT, ROT>LUT>OST, DAT, FET. Significant inhibition ($p<0.05$) by the mixtures compared to the controls, obtained by multiple comparisons, was found in the bacterium (MIT) for all treatments except CE3. In the test with primary producers, the luminotox test showed significant inhibitions only for treatments GD1-GD3 and in all treatments for *R. subcapitata* (ALT). In the primary consumers, significant differences were obtained for almost all treatments of the rotifer test (ROT), while for *D. magna* (DAT) significant inhibitions were reported only in CE3 and GD3. From the 7 studied tests, only the ostracod *H. incongruens* (OST) and *D. rerio* (FET) were not relevantly inhibited by any treatment. Comparing toxicity test results, only MIT and ALT showed a direct correlation for the inhibition reported (R=0.892, $p<0.01$). Toxicity observed in the dual salt mixtures (A, B, C) showed in general values similar to the obtained for the triple-mixtures, with a trend to have less inhibition in microtox and rotifer tests in treatment B (without Ce addition, and lower total LN content than A and C).

The inhibitory effects were consistently concentration-dependent (according to the nominal values) only for *A. fischeri* (MIT) (Spearman: R=0.882, $p<0.01$, n=36), *R. subcapitata* (ALT) (R=0.450, $p<0.01$, n=72) and *B. calyciflorus* (ROT) (R=0.883, $p<0.01$, n=24). This dependence was also studied with the free LN ion and with the dissolved LN obtained in the modelling calculation from the averages (n=12), with correlation again only for MIT, ALT and ROT. Thus, these three tests, both for showing inhibition values higher than 50% and for the observed correlations with LN content, were selected for further multi-toxicity studies.

3.4. LANTHANIDE MULTI-TOXICITY APPROACH

In order to predict multi-element toxicity, a correlation study between the inhibitions obtained for microtox (MIT), algae (ALT) and rotifer (ROT) tests and the multi-LNs and the individual content for all studied treatments was done (Table 4). A direct correlation between inhibition

and nominal LN content, as well as with LN content at the start of the tests (t0h), was observed in the three studied tests. At the end of the test for MIT, due to the shortness of test time duration, LN content was not determined. In the case of ALT an inverse correlation between inhibition and recovered LN content was found at 72h, while for ROT a positive correlation was obtained at 48h. From the study of the individual LN content (Ce, Gd and Lu), positive correlations were obtained for all cases (nominal and t0h) in MIT test. The ALT test showed positive correlation between inhibition and individual LN content only for the nominal value and t0h. In the ROT test inhibition showed only correlation with Ce, at all studied times and with Gd for nominal and t0h. In addition, toxic units (TU) for the nominal and the LN content at t0h were calculated for the 3 tests using the effective concentration values (EC_{50}) obtained by González et al. (2015). For TU direct correlations with inhibition for all cases were observed.

For interpreting the potential interaction of LN in the observed toxicity, EC_{50} values were calculated both from the toxic unit approach (TU) and from the cumulative concentration of the mixtures (CCM). Effective concentrations obtained by CCM approach were compared to the individual EC_{50} reported by González et al. (2015) (Table 5). For microtox (MIT) EC_{50} values were obtained only from the nominal and the measured content at the beginning of the test. Additionally calculated free lanthanide ($\sum LN^{3+}$) by modeling with VisualMINTEQ was also obtained for CCM. Obtained EC_{50} values did not show significant differences (no superposition of confidence intervals) in both approaches (TU, CCM). EC_{50} values obtained from TU were lower than the unit, which implies potential synergism. Whereas the comparison of the EC_{50} values obtained from CCM were lower than the individual nominal EC_{50} values reported by González et al. (2015), which support the idea of potential synergism effects. For algae test (ALT) EC_{50}

values were obtained additionally for the LN content at the end of the test (t72h). Obtained EC_{50} values did not show either significant difference in both approaches. TU approach showed EC_{50} values lower than the unit, potential synergism, and CCM approach showed EC_{50} values lower than the individual ones in almost all cases, which helps to corroborate the synergistic effect. In the rotifer test (ROT) it was also possible to calculate EC values also from the geometric mean. For the TU approach EC_{50} obtained were in all cases higher than the unit (potential antagonistic effect). Whereas the comparison of the EC_{50} values obtained by CCM were higher than the individual nominal EC_{50} values reported by González et al. (2015), which underscores the idea of potential antagonistic effects. In this test obtained EC showed significant differences, when comparing the confidence intervals. Differences were mainly with the EC calculated from the nominal and the measured at the beginning of the test, and the obtained at the end of the test or by modeling and calculation approaches.

4. DISCUSSION

4.1. LANTHANIDE CONTENT AND PREDICTED SPECIATION IN STUDIED MEDIA

LNs content in media will be influenced by the exposure method; in the present study toxicity tests were conducted under static conditions and with different exposure times. The observed decrease in LN concentration was directly correlated with time exposure, and LN precipitation seems to take place at the start of the tests with a high decline of the LN amount remaining in the solution (Figure SI-3). Differences between measured concentrations at the beginning and at the end of the tests (Table 3) were previously reported (Hermann et al., 2016). Thus in chronic tests without medium renewal, forms of LN available might not be present for all the test duration, which supports, for a more reliable risk assessment, the need of measuring LN concentration both at the beginning and at the end of the experiment. The

majority of these metals lost are possibly in colloidal forms, and the trivalent lanthanides (as most of them are present) are poorly soluble in the natural environment (El-Akl et al., 2015), therefore free ions often account for a small portion in natural water, although they have generally been found as the main predictors of LN toxicity (Tan et al., 2017; Lingott et al., 2016).

The toxicity tests performed in this study with the shortest duration, microtox and luminotox (MIT, LUT), done in deionized water and in NaCl 1% respectively, showed that LNs remained mainly as free ions (>90%). While in the test of longer duration, the addition of more chemical ligands in the media promoted the formation of additional soluble complexes mainly with SO_4^+, CO_3^+ and OH^{+2}. In spite of the expected high precipitation of LN in carbonates (Evans et al., 1990), carbonate ions ($LNCO_3$+) were also found, in general, as the main dissolved species for all LNs in our study, which was also found in González et al. (2015). Additionally, results obtained from the data modelling showed high percentages of $LNSO_4^+$. Saito and Ueno (1979) observed that the LN concentrations in the supernatant increased with increasing sulphate contents and the solubility of these forms decreased regularity with the increase in the atomic number, which was in accordance with our results.

The selection of the EPA media in the algae test (ALT) over the OECD one, based on the lower content in the anions -PO_4 and -CO was highlighted in order to obtain higher LN availability during the test. Total precipitation of these -PO_4 complexes was suggested by VisualMINTEQ modelling. A clear decrease in P during the test for solutions spiked with individual salts was observed (Figure SI-1). Phosphorous remained in the solution only in the treatment with lutetium due to the Lu concentration not exceeding the P presented in the media. The opposite occurred with Ce and Gd spiked solutions where P precipitated (Table 3). Also, modelling showed no precipitation of Lu with PO_4. Furthermore, this medium had 3

times higher content of EDTA, and LN-EDTA-complexes increased with the decrease in ionic radii, according to their binding affinity. Additionally, the comparison of Ln content in the ALT test with and without the addition of algae (Table 3), as well as the observed agglomeration by microscopy (Figure SI-2), reflected a possible effect for the presence of algae in the decrease of LN concentration in media. Tests with algae addition were LUT, ALT, ROT and OST, the two first as studied organisms and the two last ones as food addition. In ALT, ROT and OST tests (>24h), the media contained Ca and Mg (among other elements), which were observed to have an effect in competition in algae membrane transport sites with the lanthanide Sm (Tan et al., 2017). Tan et al. (2017) also reported potential competition for transport sites with other lanthanides. Whilst El-Akl et al. (2015) suggested that colloidal Ce could also accumulate by algae under high pH.

Lanthanides are known to possess low solubility and easily precipitate or bind to complexing ions (Ng et al., 2011), therefore the distribution and (bio)availability of these elements in waters, and other environments, will be strongly dependent on their speciation, which will be determined for the physicochemical properties of the media (Herrmann et al., 2016). Thus, lanthanide chemical speciation will be key for understanding their potential toxic effects on living organisms (El-Akl et al., 2015). In this work, we used Visual MINTEQ for simulating the chemical processes involved the LN in the different studied media. Lanthanides modelling are still in progress and assumptions need to be made for modelling them, therefore values have to be considered carefully according to model limitations.

4.2. TOXICITY TESTS

Toxic effects were observed only in 5 of 7 toxicity tests done. The two organisms that did not show toxicity, ostracod and zebrafish (OST and FET test) had the longest duration (>96h) and the lowest LN content at the end of the exposure period, as well as organisms used

corresponding with the highest part of the trophic chain. Results showed toxic effects in the bacteria and in the organisms belonging to the bottom of the trophic chain: algae and rotifer, but discarding the luminotox test (LUT) for having a high variability among replicates (although it rose until inhibition of 69% in some treatments). Toxicity for bacteria *A. fischeri*, algae *R. subcapitata* and rotifer *B. calyciflorus* rose until 93, 100 and 100 % of inhibition respectively in some treatments, and the recorded toxicity only showed a clear LN concentration-dependence for these three tests, although effects caused by nutrient removal should not be discarded. To highlight that, dual LN mixtures did not show marked differences compared to triple mixtures.

The highest toxic effects observed were in the algae test (ALT); in addition of the dissolved concentrations of LNs observed in this test (mainly ionic or in EDTA complexes), which could be mainly responsible for adverse effects (Weltje et al., 2002), the formation of insoluble forms of lanthanides could also be tied to the removal of other compounds (Petersen et al., 1974), which could promote indirect toxic effects due to lack of nutrients, for example the observed case of P (Figure SI-1). Also Fe could have been less available due to LNs competing with Fe for binding with EDTA. Joonas et al. (2017) observed in an assay with *R. subcapitata* that inhibitive effects could be related to the removal of essential nutrients besides LN direct toxic effects; while El-Akl et al. (2015) reported competition with calcium for the uptake sites with Ce in *C. reinhardtii*. In the microtox test (MIT), toxicity results agreed with the trends obtained for the study of individual LNs. González et al. (2015) and Weltje et al. (2004) identified that LN toxicity increases with the increase of the atomic number (Lu>Gd>Ce), which was in accordance with our results. Moreover, speciation results (Table SI-2) supported the stronger affinity of Ce to join to chloride species than Gd>Lu. Primary consumers, i.e. crustaceans and rotifers, are taxonomic groups usually used for assessing toxicity using standardized exposure media (Herrmann et al., 2016). In daphnid (DAT) and

ostracod (OST) test, toxic effects were not related with the LN content in the studied media. In these tests a high content of Ca was added for DAT; while sand, used as reference sediment, and algae, as food, were added in OST, which probably could have also influenced the direct effect of LN for the organisms. Blinova et al. (2018) also observed considerable decrease of toxicity in the OST test if sand was added. However in the speciation analysis, algae and sand additions were not taken into account, thus the absence of toxic effects could not be related to their introduction in the media. It should be noted that the use of sediment and algae does not make this test consistently less sensitive to metal toxicity compared to tests without sediments and algae (e.g. *D. magna* and *A. fischeri*) (Kudłak et al., 2011). In the rotifer test (ROT), where algae was added as food (but with a content 4 times lower than in the OST test) LN toxic effects were identified. In this test, the observed toxic effects could be directly related to the algae addition. Algae could be an additional route of LN uptake for zooplankton besides the medium, inducing a higher than expected LN uptake and toxicity, because algae concentrate LNs and form agglomerates that zooplankton feed on. Blinova et al. (2018) remarked that crustaceans, as non-selective filter-feeder organisms, can ingest both soluble and insoluble fractions, and toxicity could be attributed to both forms. For the secondary consumer, Herrmann et al. (2016), assessing La toxicity, observed that zebrafish (FET) had the lowest sensitivity; also the impact of water hardness affected the toxicity towards fish and their early development stages, which could support the absence of toxic effects observed in the FET test for this study.

4.3. LANTHANIDE MULTI-TOXICITY

Toxicity studies using a battery of different species offer a more complete and realistic view of potential toxic effects on natural communities. However it is noted that the obtained information is especially diversified if the selected test organisms represent different trophic

levels (Maciorowski and Clarke, 1980). The need to evaluate interactions of potential toxic compounds within a mixture is well supported (ATSDR, 2004); and predicting the response of aquatic organisms exposed to multiple elements is one of the most difficult tasks in environmental toxicology and risk assessment (Norwood et al., 2003). Due to the difficulty to characterizing the modes of joint action of the toxicants, dose additivity is usually assumed for public health protection (ATSDR, 2004). However, understanding if biological responses induced by mixtures are greater (synergism), the same (additive) or lower (antagonism) than expected, based on the toxicity of individual compounds in the mixtures, could be key in risk assessment. To date, there are only three ecotoxicological studies assessing lanthanide mixture effects conducted with aquatic organisms, two dealt with algae (Tai et al., 2010, Tan et al., 2017) and one with crustaceans (Blinova et al., 2018). From the three tests, which showed toxicity related to LNs in this experiment, it is remarkable that LN content at the end of the test with algae (ALT) was not correlated with the obtained inhibition (Table 4). Sneller et al. (2000) reported that the mechanisms of toxic action of REE are in essence the competition and/or replacement with Ca/Mg as well as a phosphate deficiency to precipitations of phosphate-REEs. The decrease in algal macronutrients could lead to an indirect toxic effect through nutrient limitation (OECD, 2011). Therefore LN toxicity could be masked or multiple effects could occur at the same time (Lies et al., 2016).

The analysis of multi-toxicity performed in this study showed potential synergism for microtox and algae tests (Table 5), which is considered an undesirable event (Borgert, 2004). The synergistic effect observed in the algae test can be related with the potential combination of stressor factors (Lies et al. 2016). The observed decrease in phosphate by precipitation (Figure SI-1), and, the competition of LN with Ca in transport sites for algae (El-Akl et al., 2015), could contribute to the observed toxicity. However, the same synergistic effect obtained in the microtox test, with only the addition of NaCl in the medium and less contact

time, strongly shows that combined effects of LNs should not be discarded. Instead, in the rotifer test, toxicity appeared to be more strongly correlated with LNs with lower atomic number (Ce>Gd) (Table 4) and the multi-toxicity approaches performed support the idea by observing lower inhibition from the mixtures compared to the individual elements (antagonism) (Table 5). An assumption could be that the observed toxic effect in rotifer tests was mainly due to Ce and Gd according to the correlations observed (Table 4). Tan et al. (2017) observed that an antagonist effect could be assumed because heavy REEs (e.g. Lu) were first joined to the algae added as food, and therefore light REEs could have greater toxic effect by direct uptake from the media. On the other hand, Lingott et al. (2016), in an experiment with daphnids exposed to Gd, suggested a greater Gd content related by algae ingestion added as food. Although LN-uptake routes for the rotifer should be further studied for a better interpretation of the obtained effect, these previous results indicated that the addition of algae as food could modify the availability of LN for the organisms and therefore results should be interpreted carefully.

Toxicity was also studied from the free lanthanide content (LN^{3+}) obtained by modelling and the geometric mean calculated according to the observed decrease in Ln content over time. In general these calculated EC_{50} showed a decrease compared to the nominal and measured LN content. However only in the OST test were some significant differences between EC_{50} observed (IC not overlapping) (Table 5). This observation in the studied effect re-emphasizes the need to measure LN concentration or study their speciation in the used media during toxicity experiments for a more reliable risk assessment.

Finally, predicted no effect concentration (PNEC) of 1.30 μg L^{-1} was derived based on the EC_{10} value of the algae test, the most sensitive species (TGD, 2003). PEC value was estimated for a natural area as the addition of the concentration of the 3 studied lanthanides

with a mean PEC value of 1.77 μg L^{-1}. PEC/PNEC ratio was 1.36, which indicates the potential hazard of the mixture and therefore toxic effects due to lanthanide mixtures on organisms are likely to occur.

CONCLUSIONS

In this study, regarding the ecotoxicological assessment of mixtures of lanthanides (Ce, Gd and Lu) with seven toxicity tests, lanthanide toxic effects were reported only in bacteria and in the organisms belonging to the lower trophic levels (algae and rotifer). The strong decrease in lanthanide concentration during toxicity tests should be taken into consideration for assessing their toxicity. This decline was dependent on test media (forming mainly complexes with sulphurs and carbonates) and took place usually at the start of the tests; thus, it is directly correlated with time exposure. The multi-toxicity approaches used in this study indicated potential synergism for tests performed with the bacteria *A. fischeri* and with the algae *R. subcapitata*, and antagonism for the rotifer *B. calyciflorus*. Obtained results highlight that it should not yet be assumed that multi-LN toxicity is additive, and predicting the response of aquatic organisms exposed to multi-LNs needs further research, taking in consideration LN speciation in the media and understanding their interactions for the assessment of their risk in the environment.

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Table 1. Concentrations of REE in the tested mixtures. Bold text is used for concentrations similar to the effective concentrations inducing 10% of inhibition (EC_{10}) of the least sensitive organism tested by González et al. (2015) (Lu concentration being twice lower than least restrictive EC_{10} reported).

	Ce	**Gd**	**Lu**
		($\mu g\ L^{-1}$)	
CE1	**4000**	200	12
CE2	2000	100	6
CE3	1000	50	3
GD1	5000	**1400**	82
GD2	2500	700	41
GD3	1250	350	21
LU1	3420	1710	**855**
LU2	1710	855	428
LU3	855	428	214
A	5000	1710	
B		1710	855
C	5000		855

Table 2. Toxicity test description for seven test organisms representative of the main trophic levels: decomposer (the bacteria *Allivibrio fischeri*), primary producers (the algae *Chlorella vulgaris* and *Raphidocelis subcapitata*), primary consumers (the rotifer *Brachionus calyciflorus* and the crustacean *Heterocrypris incongruens* and *Daphnia magna*) and secondary consumer (the fish *Danio rerio*).

TEST	ORGANISMS	RATIO	REPLICATES	EXPOUSRE MEDIA	EXPOSURE CONDITIONS	ENDPOINTS	BIBLIOGRAPHY
Microtox Test **(MIT)**	*Aliivibrio fischeri (strain NRRL B-11177)*	1 ml with 10 µL of bacteria (10^6 cells mL^{-1})	3	NaCl = 10 g L^{-1}	30 min, 15 ºC, darkness	Luminiscence inhibition (%)	ISO guideline 11348-1 (2007)
Luminotox Test **(LUT)**	*Chlorella vulgaris*	2 ml with 100 µL of alga (10^6 cells mL^{-1})	3	Deionized water	4 h, room Tª, darkness	Fluorescence inhibition (%)	Bellemare et al. (2006)
Algal growth inhibition test **(ALT)**	*Raphidocelis subcapitata*	250 µl with 125 µl of algae (10^4 cells mL^{-1})	6	AAP media (U.S. EPA) Entire composition in Table SI-1.	72 h, 23 ºC, continuous ilumination (6000 lux)	Growth inhibition (%)	OECD guideline 201 (OECD, 2011) Eisentraeger et al. (2003) González et al. (2015)
Rotifer Toxicity Test **(ROT)**	*Brachionus calyciflorus* (Incubation of cysts 20 h. Organisms < 2 h old)	1 org: 1 mL	8	$NaHCO_3$ = 48 mg L^{-1}, $CaSO_4{\cdot}2H_2O$ = 60 mgL^{-1}, $MgSO_4{\cdot}7H_2O$ = 62 mg L^{-1}, KCl = 2 mg L^{-1}	48 h, 25 ºC, darkness *2	Population growth inhibition (%)	Microbiotest (2001a) French guideline NF T 90-377 (2000) ISO guideline 20666 (2008)
Ostracodtox Test **(OST)**	*Heterocrypris incongruens* (Incubation of cysts 52 h, neonates)	10 org: 4 mL*1	3	$NaHCO_3$ = 48 mg L^{-1}, $CaSO_4{\cdot}2H_2O$ = 60 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ = 62 mg L^{-1}, KCl = 2 mg L^{-1}	144 h, 25 ºC, darkness *2	Growth inhibition (%)	Microbiotest (2001b) ISO guideline 14371 (2012)
Daphnia sp. Acute Immobilisation Test **(DAT)**	*Daphnia magna* (from a culture raised as described by Toumi et al. (2013))	5 org: 10 mL	4	$NaHCO_3$ = 48 mg L^{-1}, $CaSO_4{\cdot}2H_2O$ = 107.4 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ = 62 mg L^{-1}, KCl = 2 mg L^{-1}	48 h, 20 ºC	Immobilisation (%)	OECD guideline 202 (2004)
FET Test **(FET)**	*Danio rerio < 24 h old*	1 org: 2 mL	6	$NaHCO_3$ = 63 mg L^{-1}, $CaCl_2{\cdot}2H_2O$ = 294 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ = 123.3 mg L^{-1}, KCl = 5.5mg L^{-1}	96 h, 26 ºC, LD 14:10 h (700 lux) *2	Alteration (%)	OECD guideline 236 (2013)

*1 In addition, 0.5 mL of standard sand were added according to the protocol.

*2 In these experiments food was added: algae *Scenedesmus sp.* for *H. incongruens*, algae *R. subcapitata* for *B. calyciflorus* and shrimp *Artemia sp.* for *D. rerio*.

Table 3. Minimal and maximal recovery values (%) for total LN availability at time zero (t0h) comparing to the nominal added (nominal-t0h) and at the end of the each test (tXh) comparing to the time 0 h (tXh-t0h) for the individual LN studied (Ce, Gd and Lu) and for the total. In test with duration lower than 24 h (microtox (MIT) and luminotox (LUT) test) lanthanide content at the end of the test was not determined.

	Test duration	**Recovery of the nominal**	**Ce**	**Gd**	**Lu**	***Total***
		(nominal-t0h)		(tXh-t0h)		
	(h)	(%)		(%)		
MIT	0.5	105-122	-	-	-	-
LUT	4	85-106	-	-	-	-
ALT (without algae addition)	72	85-103	3-71	4-74	5-81	*3-72*
ALT (with algae addition)	72		<1-21	<1-24	<1-37	*1-22*
ROT	48	86-109	5-20	6-20	11-24	*5-22*
OST	144	79-109	1-4	<1-27	<1-14	*1-5*
DAT	48	97-107	1-29	2-33	5-46	*1-31*
FET	96	n.d.	<1-8[*1]	2-9[*1]	3-12[*1]	*1-10*[*1]

n.d. no data. Data at time 0 for zebrafish was not measured.

[*1] Recovery values calculated from the nominal content.

Table 4. Spearman correlation with the obtained inhibition in the tests MIT, ALT and ROT with the total content: nominal, at the start (t0h) and at the end of the tests (tXh); with the toxic units (TU) calculated from the nominal, and t0h; and with the individual LN content (Ce, Gd and Lu) for the nominal t0h and tXh. TU were calculated using the effective concentrations values (EC_{50}) obtained by González et al. (2015) (consult EC_{50} values in Table 5).

% Inhibition	MIT	ALT	ROT
∑LN-Nominal	0.883**	0.450**	0.882**
∑LN-t0h	0.883**	0.438**	0.882**
∑LN-tXh	n.c.	-0.442**	0.674**
Ce-Nominal	0.795**	0.366**	0.843**
Ce-t0h	0.781**	0.369**	0.835**
Ce-tXh	n.c.		0.596**
Gd-Nominal	0.896**	0.793**	0.555**
Gd-t0h	0.864**	0.786**	0.508*
Gd-tXh	n.c.		
Lu-Nominal	0.801**	0.852**	
Lu-t0h	0.818**	0.830**	
Lu-tXh	n.c.		
∑TU-Nominal	0.923**	0.566**	0.870**
∑TU-t0h	0.922**	0.634**	0.871**
∑TU-tXh	n.d.	n.d.	n.d.

Test duration (tXh) for ALT=72h and for ROT= 48h.
n.c. calculation not possible for test duration lower than 24h.
n.d. no EC50 data available for performing the calculations.

Table 5. Effective concentrations inducing 50% of inhibition (EC_{50}) and their confidence intervals at 95% (95%CI) in three different organisms: *A. fischeri* (MIT), *R. subcapitata* (ALT) *and B. calyciflorus* (ROT); calculated from the toxic units approach (TU) based on individual EC_{50} values obtained by González et al. (2015). TU were obtained for the nominal and for the measured LN concentration for time 0h (t0h) in MIT, ALT and ROT, and for the corresponding final time (tXh) in ALT and ROT from all studied treatments. M was also calculated from the free lanthanide (**∑LN^{3+}**) obtained by modelling speciation (using Visual MINTEQ 3.1). Geometric mean of the LN concentrations at the start and at the end of the tests (√t0*tXh) ~~(OECD, 2000)~~ was only obtained for ROT test.

		EC_{50}						
		This study				**González et al. (2015)**		
		From mixture treatments				**Ce**	**Gd**	**Lu**
		TU		**CMM (µg L^{-1})**				
MIT	**Nominal**	<0.36	*(0.31-0.43)*	2070	*(1674-2558)*	>6400[*1]	>6400[*1]	>3200[*1]
	Measured (t0h)	<0.44[*2]	*(0.38-0.52)*	2286	*(1816-2877)*			2041
	Modelled (∑LN^{3+})		n.c.	1909	*(1556-2343)*			
ALT	**Nominal**	<0.29	*(0.24-0.34)*	1302	*(1031-1643)*	>6400[*1]	3111	2062
	Measured (t0h)	0.28	*(0.23-0.35)*	1149	*(849-1553)*	6317	2219	2079
	Measured (t72h)		n.c.		n.c.			
	Modelled (∑LN^{3+})		n.c.	875	*(652-1175)*	4421	1257	809
ROT	**Nominal**	3.38	*(2.52-4.52)*	3034	*(2496-3687)*	775	1120	760
	Measured (t0h)	3.68	*(3.27-4.13)*	2532	*(2329-2752)*	620	914	575
	Measured (t48h)		n.c.	265	*(154-456)*			
	Modelled (∑LN^{3+})		n.c.	362	*(182-719)*	80.6	63.9	17.2
	Geometric mean (√t0*tXh)	1.16	n.c.	780	*(640-951)*			

[*1]For EC values with symbol higher than (>), the expressed value was used for TU calculation.
[*2]In this case, due to the lack of some EC values at t0h, nominal values were used for TU calculation.
n.c. calculation not possible.

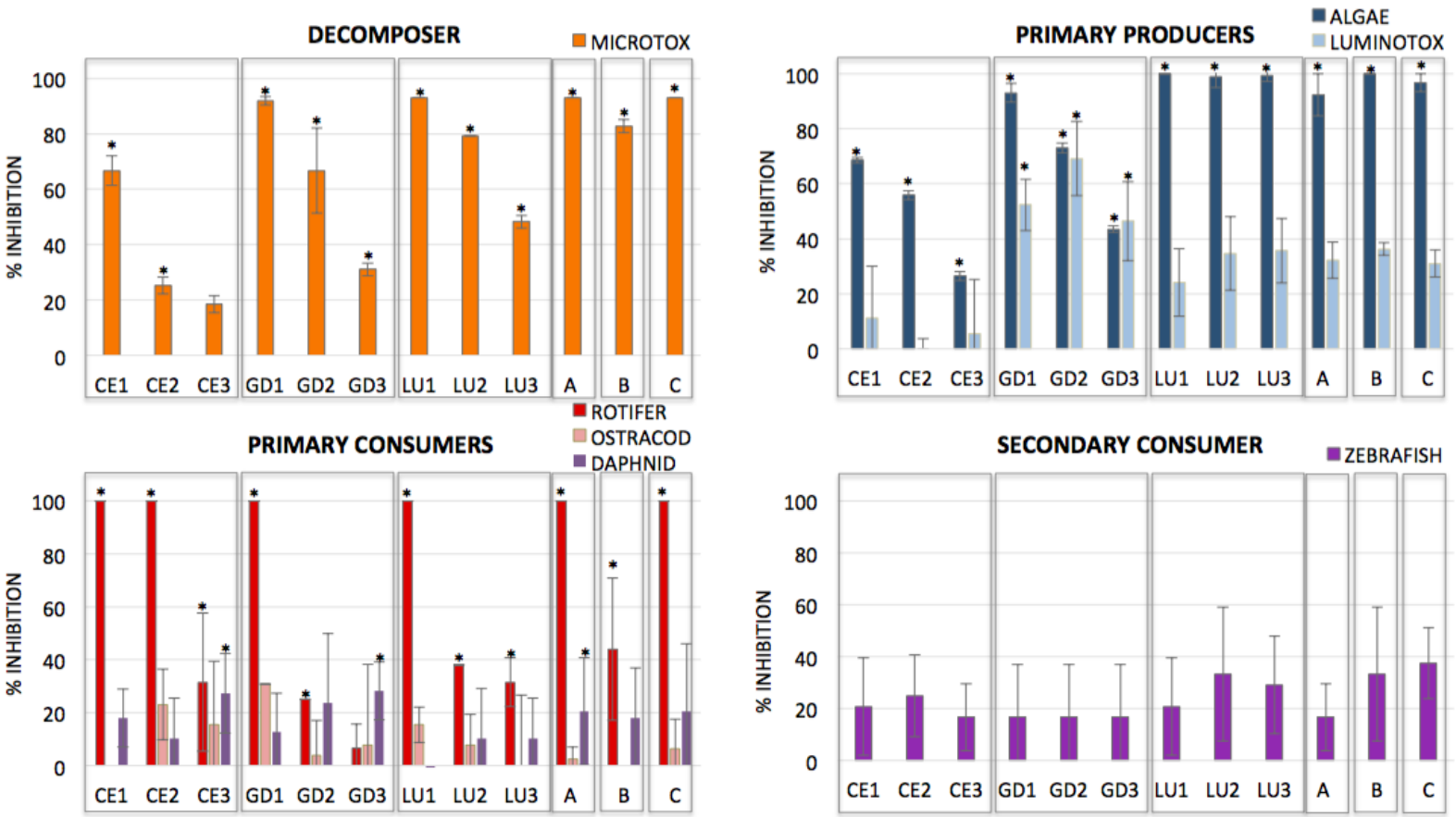

Figure 1. Inhibition (%) induced by different lanthanide mixtures towards bacteria and organisms on different trophic levels. Corresponding concentrations for the 12 treatments used are given in Table 1. Samples with * showed significant inhibition respect to the control obtained by multiple comparison (Pearson, $p<0.05$).

Assessment of the toxic effects of mixtures of three lanthanides (Ce, Gd, Lu) to aquatic biota

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Table SI-1. Composition of the media used in the ALT test (US EPA algal media) and the usual media used for algae toxicity test (OECD algal media) (OECD 201, 2011). For easily comparison, ratios of the main compounds are calculated in the last column.

Substance	**EPA final concentration (mg L^{-1})**	**OECD final concentration (mg L^{-1})**	**Ratios EPA/OECD**
NH_4Cl	-	15	
$NaNO_3$	25.5	-	
$MgCl_2 \cdot 6H_2O$	12.16	12	1.01
$CaCl_2 \cdot 2H_2O$	4.41	18	0.25
$MgSO_4 \cdot 7H2O$	14.6	15	0.97
KH_2PO_4	1.044	1.6	0.65
$FeCl_3 \cdot 6H_2O$	0.16	0.064	2.50
$Na_2EDTA \cdot 2H_2O$	0.3	0.1	3.00
H_3BO_3	0.186	0.185	1.01
$MnCl_2 \cdot 4H_2O$	0.415	0.415	1.00
$ZnCl_2$	0.00327	0.003	1.09
$CoCl_2 \cdot 6H_2O$	0.00143	0.0015	0.95
$CuCl_2 \cdot 2H2O$	0.000012	0.00001	1.20
$Na_2MoO_4 \cdot 2H_2O$	0.00726	0.007	1.04
$NaHCO_3$	15	50	0.30

Table SI-2. Percentage of precipitation and major Ce, Gd and Lu dissolved species percentage distribution (on a molar basis) among the dissolved species formed in the studied test media. Data only show for the treatments with mixtures with the highest LN contents (CE1, GD1 and LU1). Species were calculated with VisualMINTEQ 3.1. Shown are values where some specie had at least a distribution higher than 0.3%. In ALT, ROT and OST the addition of algae was not having into consideration for modeling, and, for OST test, the sand added in the final media was neither used.

		Ce			**Gd**			**Lu**		
	Nominal content (ug L^{-1})	**4000**	**5000**	**3420**	**200**	**1400**	**1710**	**12**	**82**	**855**
	%Species	**CE1**	**GD1**	**LU1**	**CE1**	**GD1**	**LU1**	**CE1**	**GD1**	**LU1**
MIT	***%Precipitation***	0	0	0	0	0	0	0	0	0
	LN^{+3}	90	90	90	94	94	94	95	95	95
	$LNCl^{+2}$	10	10	10	5.8	5.8	5.8	4.8	4.8	4.8
LUT	***%Precipitation***	0	0	0	0	0	0	0	0	0
	LN^{+3}	100	100	100	100	99	100	98	98	99
	$LNOH^{+2}$							1.4	1.5	1.1
ALT	***%Precipitation***	21	17	25	0	0	51	0	0	0
	Precipitated species		$CePO_4$			$Gd(OH)_3$				
	LN^{+3}	58	65	56	35	49	40	0.69	5.0	19
	$LNEDTA^{-}$	1.3	0.17	0	20	3.3	0.14	97	85	16
	$LNSO_4^{+}$	8.6	9.3	8.3	5.4	7.3	6.2	0.078	0.54	2.2
	$LNCO_3^{+}$	29	22	32	36	36	48	1.7	8.3	53
	$LNOH^{+2}$	1.5	1.2	1.7	2.9	2.9	4.0	0.21	1.1	7.1
	$LN(CO3)_{2-}$	0.082	0.044	0.11	0.26	0.18	0.39	0.040	0.14	1.5
	$LNHCO_3^{2+}$	1.6	1.7	1.5	1.0	1.4	1.2	0.028	0.20	0.75
ROT/OST	***%Precipitation***	0	0	0	0	60	73	0	0	0
	Precipitated species					$Gd(OH)_3$				
	LN^{+3}	21	17	16	13	9.9	9.1	6.1	4.5	4.0
	$LNSO_4^{+}$	24	20	19	16	12	11	5.5	4.0	3.6
	$LNCO_3^{+}$	51	58	60	66	71	72	74	73	73
	$LNOH^{+2}$	0.93	1.1	1.1	1.8	2.0	2.0	3.2	3.3	3.2
	$LN(SO_4)_2^{-}$	0.31	0.26	0.24	0.23	0.18	0.17	0.11	0.083	0.076
	$LN(CO_3)_2^{-}$	0.89	1.4	1.6	2.8	4.1	4.6	11	14	16
	$LNHCO_3^{2+}$	1.8	1.5	1.4	1.2	0.93	0.86	0.79	0.57	0.51
DAT	***%Precipitation***	0	0	0	4.3	83	77	0	0	48
	Precipitated species					$Gd(OH)_3$			$Lu(OH)_3$	
	LN^{+3}	32	33	36	25	26	30	16	18	23
	$LNSO_4^{+}$	44	46	49	35	37	42	17	19	24
	$LNCO_3^{+}$	22	19	13	35	32	24	54	50	42
	$LNOH^{+2}$	14	1.3	1.2	3.6	3.5	3.2	9.3	9.5	9.7
	$LN(SO_4)_2^{-}$	0.69	0.72	0.77	0.69	0.73	0.83	0.47	0.53	0.66
	$LN(CO_3)_2^{-}$	0.11	0.077	0.036	0.43	0.32	0.16	2.2	1.7	0.94
FET	***%Precipitation***	0	0	0	44	92	94	0	0	79
	Precipitated species					$Gd(OH)_3$			$Lu(OH)_3$	
	LN^{+3}	38	39	38	26	26	26	14	14	14
	$LNSO_4^{+}$	22	22	22	15	15	15	6.0	6.0	6.0
	$LNCO_3^{+}$	35	35	35	51	51	51	63	63	63
	$LNOH^{+2}$	3.0	3.0	3.0	6.4	6.4	6.4	12	12	12
	$LN(CO_3)_2^{-}$	0.30	0.30	0.30	1.1	1.1	1.1	4.5	4.5	4.5

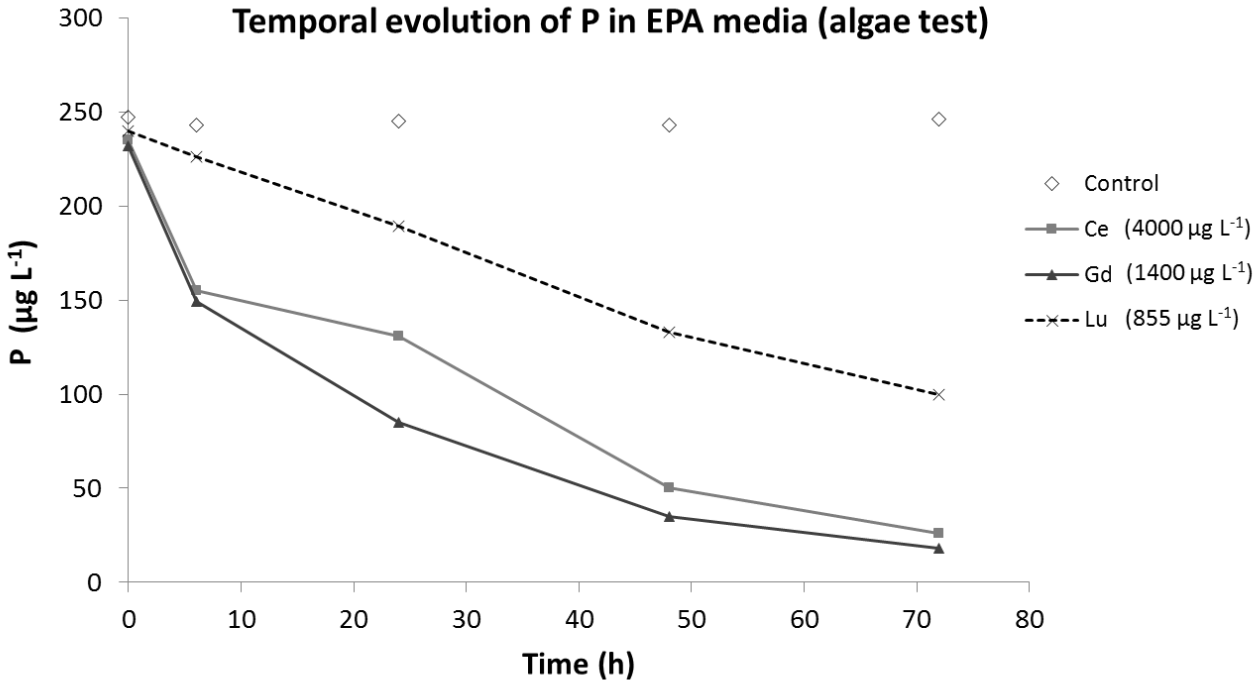

Figure SI-1. Evolution of the availability of phosphorous (P) in EPA media used in ALT test spiked with the highest concentrations used of the studied lanthanides (Ce, Gd and Lu) in individual form, without algae addition. Additional information is presented in Table SI-1 for the EPA media composition, and in Table 2 for the test procedure.

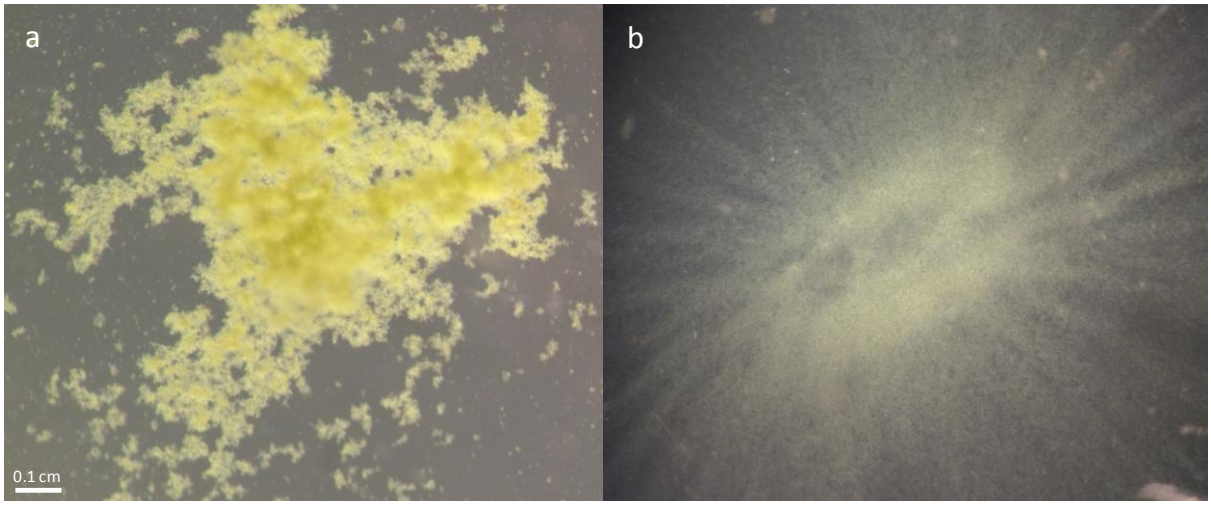

Figure SI-2. Comparison of algae distribution added as food in the rotifertox test (ROT) after 48h of exposition. a) Treatments with high LN content (CE1-2, GD1-2, LU1-2, A and C), b) controls and treatments with low LN content (CE3, GD3, LU3 and B).

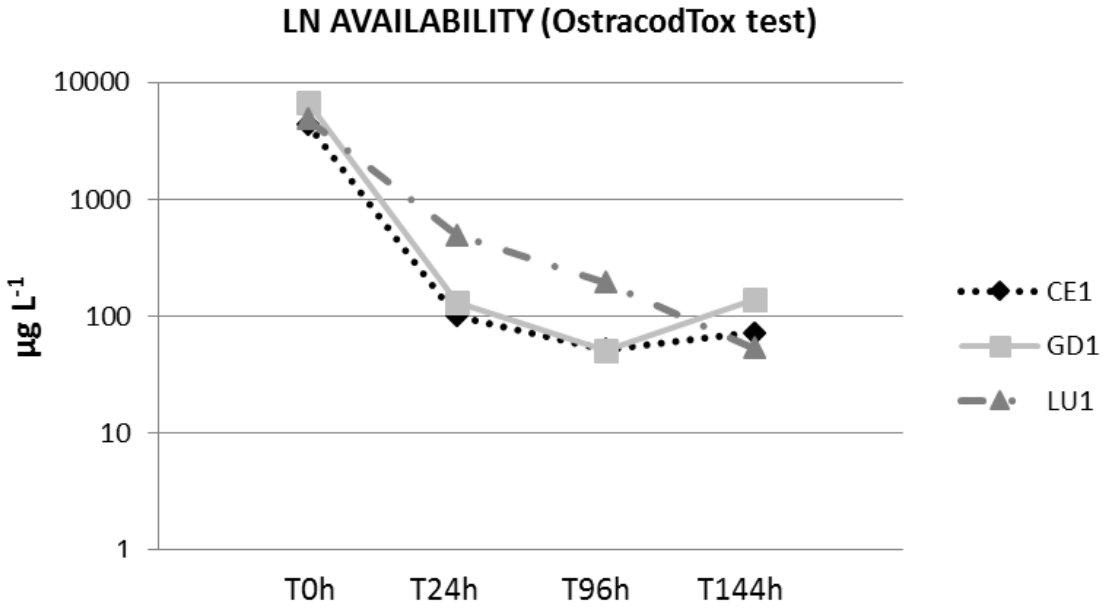

Figure SI-3. LN availability in logarithmic scale for the longest toxicity test performed (OST) at different periods of the test (time 0, 24, 96 and 144 h). Lanthanide content for each treatment is given in Table 1; while additional information for the test procedure is given in Table 2.