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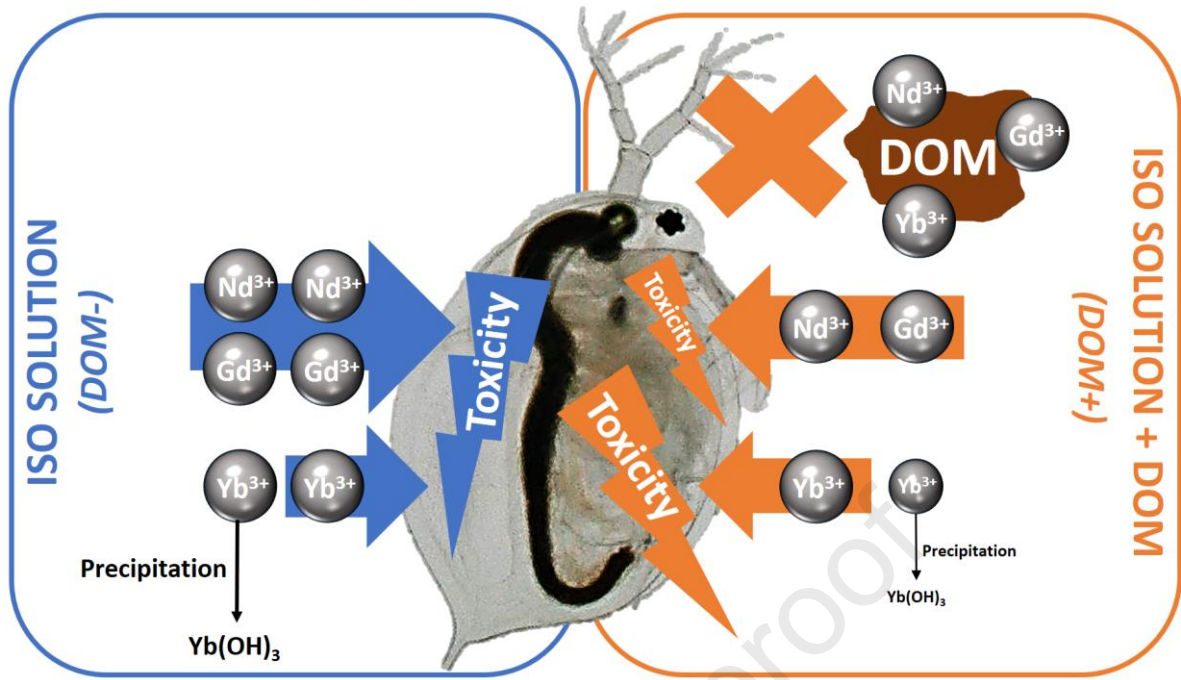
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## Implications of speciation on rare earth element toxicity: a focus on organic matter influence in *Daphnia magna* standard test

Nicolas Lachaux<sup>a,b\*</sup>, Charlotte Catrouillet<sup>c</sup>, Rémi Marsac<sup>c</sup>, Laurence Poirier<sup>d</sup>, Sandrine Pain-Devin<sup>a,b</sup>, Elisabeth Maria Gross<sup>a,b</sup>, Laure Giamberini<sup>a,b</sup>

<sup>a</sup>Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

<sup>b</sup>LTSER-Zone Atelier Moselle, F-57000 Metz, France

<sup>c</sup>Géosciences Rennes, University Rennes, CNRS, UMR 6118, 35000 Rennes, France

<sup>d</sup>Nantes University, Institut des Substances et Organismes de la Mer, ISOMer, UR 2160, F-44000 Nantes, France

E-mail contact: nicolas.lachaux@univ-lorraine.fr

### Abstract

1 Rare earth elements (REE) have become essential in high- and green-technologies. Their  
2 increasing use lead to the release of anthropogenic REE into the environment including aquatic  
3 systems. The limited data available on the aquatic ecotoxicology of REE indicate their  
4 biological effects are highly dependent on their speciation, posing challenges for a reliable  
5 environmental risk assessment (ERA). The current study assessed the influence of speciation  
6 on the toxicity of neodymium (Nd), gadolinium (Gd) and ytterbium (Yb) in the *Daphnia magna*  
7 mobility inhibition test (ISO 6341:2012). REE toxicity was assessed individually and in ternary  
8 mixture, in the absence and presence of dissolved organic matter (DOM). Speciation was  
9 predicted by modeling and REE bioaccumulation by *D. magna* was measured to better  
10 understand the relationship between REE speciation and toxicity. DOM decreased significantly  
11 the toxicity of Nd, Gd and the mixture towards this freshwater crustacean. This was explained  
12 by a lower REE bioaccumulation in the presence of DOM due to REE-DOM complexation,  
13 which reduced REE bioavailability. DOM effects on Yb toxicity and bioaccumulation were  
14 limited because of Yb precipitation. We show that the way of expressing EC50 values (based

15 on nominal, measured or predicted REE concentrations in solution) drastically changed REE  
16 toxicity assessment and that these changes were influenced by REE speciation. This study  
17 demonstrates for the first time that REE speciation, and especially REE-DOM complexation,  
18 significantly influences REE bioaccumulation and toxicity towards *D. magna*. Our results have  
19 implications for the subsequent ERA of REE.

## 20 **Keywords**

21 Rare earth elements; mixture; toxicity; bioaccumulation; speciation; dissolved organic matter

## 22 **1 Introduction**

23 Rare earth elements (REE) are a group of 17 metals including the lanthanide series, scandium  
24 (Sc) and yttrium (Y). They are naturally present in the environment, constituting a chemical  
25 group sharing similar characteristics (Feng et al., 2013). The group is divided essentially into  
26 three subgroups based on their ionic radii: LREE (Light Rare Earth Elements), MREE (Medium  
27 Rare Earth Elements), and HREE (Heavy Rare Earth Elements) but the division is sometimes  
28 limited to LREE and HREE (Zepf, 2013). They are little known to the public although they are  
29 used daily by everyone *via* smartphones, computers, cars, household appliances and more.  
30 Since around 30 years, REE have become essential in high- and green-technologies (e.g. wind  
31 turbines, solar panels, electrical vehicles) but their supply capacity is below the increasing  
32 demand. Thus, REE are now considered as critical raw materials (European Commission,  
33 2011). Despite their use in the production of clean energy, they are of growing ecotoxicological  
34 concern because of their release into the environment during mining extraction, uses in  
35 industrial and agricultural activities and because of e-waste management, among others  
36 (Gwenzi et al., 2018). The presence of anthropogenic REE in aquatic systems was first  
37 demonstrated with the release of gadolinium (Gd) contained in contrast agents used in magnetic  
38 resonance imaging (MRI), disturbing natural REE biogeochemical cycles (Bau and Dulski,

39 1996). Rare earth element applications, especially as permanent magnets, and associated  
40 releases in the environment are expected to increase in the future (Haque et al., 2014). However,  
41 still little is known about the fate and ecotoxicology of REE, which prevents the establishment  
42 of a reliable environmental risk assessment (ERA) (Blinova et al., 2020). Studies on REE  
43 toxicity and bioaccumulation in aquatic systems are scarce and focused mainly on lanthanum  
44 (La) and cerium (Ce) (Blinova et al., 2020). There are limited data on other REE, notably on  
45 neodymium (Nd), terbium (Tb) and HREE such as thulium (Tm) and ytterbium (Yb), which  
46 should thus be studied with priority, particularly LREE that are more abundant and  
47 bioaccumulated than HREE (Blinova et al., 2020). Most studies evaluated the individual effects  
48 of REE but not the combined effects of REE mixtures (Gonzalez et al., 2014) although different  
49 REE occur together in the environment. Missing data on the toxicity of some REE and their  
50 mixtures prevent the establishment of a consensus on the uniformity of different REE (Gonzalez  
51 et al., 2014). Moreover, biological effects observed with REE are heterogeneous (Blinova et  
52 al., 2020; Gonzalez et al., 2014). This can be explained partly by differences in test solution  
53 composition, which strongly influence REE speciation (i.e. the different chemical and physical  
54 forms of REE) and in consequence induces different biological effects (Barry and Meehan,  
55 2000; Borgmann et al., 2005; Gonzalez et al., 2014; Vukov et al., 2016). Dissolved organic  
56 matter (DOM) is an ubiquitous component of aquatic systems known to influence the  
57 speciation, bioavailability and the subsequent toxicity of metals (Wood et al., 2011). DOM is  
58 taken into account in the definition of environmental quality standards of some priority metals  
59 (i.e. lead and nickel) through the application of the biotic ligand model (BLM) (European  
60 Parliament, 2013) in order to allow for a more realistic ERA (Rüdel et al., 2015). Although  
61 DOM effects on REE speciation and fate in water are well known (Davranche et al., 2017;  
62 Johannesson et al., 2004; Marsac et al., 2011; Pourret et al., 2007a, 2007b; Sonke and Salters,

63 2006; Tang and Johannesson, 2003), few studies have investigated DOM effects on REE  
64 toxicity.

65 In the present work, we aimed for a better understanding of the influence of speciation on  
66 REE toxicity. To assess whether different elements of the REE group share similar toxicity and  
67 characteristics, we tested Nd (LREE), Gd (MREE) and Yb (HREE) individually and in mixture.  
68 The selected elements are of concern because of their human use (Nd as permanent magnet and  
69 Gd in MRI), their occurrence in aquatic systems (anthropogenic Gd from MRI (Louis et al.,  
70 2020)) and missing ecotoxicological data (Yb and Nd are among the least studied REE (Blinova  
71 et al., 2020)). REE toxicity was assessed on the key freshwater zooplankton species, *Daphnia*  
72 *magna*, using the mobility inhibition test guideline (ISO, 2012). REE speciation was predicted  
73 by modeling and its influence on REE toxicity was investigated in the ISO solution modified  
74 or not by the addition of DOM. Because speciation regulates exposure, bioavailability and  
75 toxicity, the EC50 values were calculated and expressed in different ways, based on nominal,  
76 measured and predicted REE concentrations in solution. In addition, REE bioaccumulation by  
77 *D. magna* was analysed to better understand the relationship between speciation and toxicity.

## 78 **2 Materials and Methods**

### 79 **2.1 Rare Earth Elements (REE) and Dissolved Organic Matter (DOM) preparation**

80 REE stock solutions were prepared by dissolution in MilliQ water ( $5 \text{ g L}^{-1}$  REE salt;  
81 neodymium nitrate ( $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ ), gadolinium chloride ( $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ ), ytterbium chloride  
82 ( $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ ); all purity > 99%; Sigma Aldrich). Salts of chlorides and nitrates were used  
83 because the complexation of Nd, Gd and Yb with  $\text{Cl}^-$  and  $\text{NO}_3^-$  is negligible. REE  
84 concentrations were measured in the stock solutions and no precipitation was observed. For  
85 each REE concentration tested, an intermediate solution was prepared by dilution of the REE  
86 stock solution so that an equivalent volume of REE solution could be added to all test flasks.

87 The used DOM had been obtained from Wageningen University, by purification of  
88 groundwater from Netherlands. It is mainly composed of fulvic acid (>80 %) as determined by  
89 the method of van Zomeren and Comans, (2007). The stock solution was stored in the dark at  
90 5°C. We worked with a dissolved organic carbon (DOC) concentration of 8 mg C L<sup>-1</sup> because  
91 this is representative of concentrations found in freshwater systems such as rivers (2-25 mg C  
92 L<sup>-1</sup>) and lakes (2-30 mg C L<sup>-1</sup>) (Thurman, 1985). The DOM stock solution was diluted in MilliQ  
93 water to obtain the desired DOC concentration of 8 mg C L<sup>-1</sup> in the test flasks.

## 94 **2.2 *Daphnia magna* mobility inhibition test**

95 The mobility inhibition of the freshwater crustacean *D. magna* was evaluated following the  
96 protocol described in detail in the European NF EN ISO 6341:2012 guideline (ISO, 2012).  
97 Complementary information about the protocol can be found in the international equivalent  
98 OECD 202:2004 guideline (OECD, 2004). Briefly, the toxicity of Nd, Gd and Yb was first  
99 tested individually (single test) and afterwards in ternary equitoxic mixture (mixture test),  
100 which means that each element was present at the same fraction of their own individual toxicity  
101 (Minguez et al., 2018). Therefore, the REE concentrations tested in the mixture were selected  
102 according to the nominal EC<sub>x</sub> values (EC<sub>5</sub>, EC<sub>10</sub>, EC<sub>30</sub>, EC<sub>50</sub>, EC<sub>65</sub>, EC<sub>75</sub>, EC<sub>80</sub>) of each REE  
103 determined in the single tests. All tests included five REE concentrations inducing from 0 to  
104 100% inhibition and a control (no REE).

105 In addition, all tests were performed in the absence (0 mg C L<sup>-1</sup> DOC, denoted DOM-) and  
106 presence (8 mg C L<sup>-1</sup> DOC, denoted DOM+) of DOM to modify REE speciation and to  
107 assess its influence on REE toxicity. In the single tests, REE concentrations were the same in  
108 DOM- and DOM+. Prior to the test, the flasks (glass test tubes, 15 mL capacity) were exposed  
109 to 10 mL of ISO solution (294 mg L<sup>-1</sup> CaCl<sub>2</sub>, 2H<sub>2</sub>O; 123 mg L<sup>-1</sup> MgSO<sub>4</sub>, 7H<sub>2</sub>O; 65 mg L<sup>-1</sup>  
110 NaHCO<sub>3</sub>; 6 mg L<sup>-1</sup> KCl) spiked with REE during at least 12 h in order to equilibrate glass wall  
111 adsorption sites with REE and to limit REE losses during the test. After this preconditioning,



112 the flasks were emptied and the solution was renewed. Then, ISO solution, DOM (in DOM+  
113 tests) and REE were added consecutively to the flask constituting the test solution. The  
114 daphnids were immediately added after REE addition in order to enhance REE exposure before  
115 REE precipitation, which occurs relatively fast (Romero-Freire et al., 2019). We considered the  
116 beginning of the test ( $t_0$ ) after adding the daphnids to the test solution.

117 Neonates (less than 24h), originating from our laboratory culture, were exposed to REE  
118 during 48 h at 20°C (+/-2°C) in the dark. At the end of the test, after 48h exposure ( $t_1$ ), REE  
119 toxicity was assessed by evaluating *D. magna* mobility. The daphnids were considered as not  
120 mobile when they did not move during the 15 seconds following a gentle tube shaking. A test  
121 was performed with at least four replicates (1 replicate = 5 daphnids) and repeated twice.

122 Prior to the addition of DOM, REE and daphnids, the pH of the ISO solution was  
123 measured and adjusted, with a 3.2% HCl solution, at 6.5 instead of 7.8 (recommended in the  
124 ISO guideline) in order to enhance REE solubility and bioavailability. Such pH value (6.5)  
125 reflects naturally acidic freshwaters, which tend to have higher DOC concentration (Thurman,  
126 1985). The pH was measured again at  $t_1$  in the solutions of the control and extreme  
127 concentrations as recommended in the OECD guideline (OECD, 2004). The pH in the solution  
128 of intermediate concentrations was estimated from the equation of the linear correlation  
129 between pH values and total REE concentrations measured at  $t_1$  (Table S1).

130 Several tests with the reference substance, potassium dichromate, indicated that the  
131  $EC_{50_{24h}}$  of the latter was included in the range, [0.6 – 2.1] mg L<sup>-1</sup>, recommended by the ISO  
132 guideline (ISO, 2012), which validated the reliability of the test conditions.

### 133 **2.3 REE concentration measurement and analysis**

134 For one of the two tests performed, REE concentrations in the test solution and in daphnids  
135 were measured after 48 h exposure by Inductively Coupled Plasma - Mass Spectrometry (ICP-

136 MS, Nexion 350x PerkinElmer). The test solution was filtered using a mix cellulose ester filter  
137 (MF-Millipore, reference: HAWP04700) in order to separate the particle phase ( $>0.45 \mu\text{m}$ )  
138 retained by the filter and the dissolved phase ( $<0.45 \mu\text{m}$ ) constituting the filtrate. For each  
139 concentration tested, the test solution of all replicates was filtered on a unique filter and the  
140 filtrates were pooled. The REE concentration was measured in the pool of filtrates. We consider  
141 this concentration as the measured dissolved concentration at  $t_1$  (denoted  $[\text{REEdiss}]$ ). In  
142 addition, the amount of REE retained on the filter was quantified after mineralization and the  
143 concentration of particulate REE in the test solution was recalculated and added to the dissolved  
144 concentration to determine the total REE concentration at  $t_1$  (denoted  $[\text{REEtot}]$ ). Because  
145 measured in the pool of replicates, the unique value of  $[\text{REEdiss}]$  and  $[\text{REEtot}]$  obtained for  
146 each concentration represents an average value. Prior to ICP-MS analysis, the filtrate was  
147 acidified at 1% [v/v] with  $\text{HNO}_3$  69% in Milli-Q water and stored at  $4^\circ\text{C}$ ; filter membranes  
148 were digested with 2 mL  $\text{HNO}_3$  65% (Fischer Scientific) at  $80^\circ\text{C}$  until complete mineralization.

149 REE recovery at  $t_1$  was calculated as the ratio between  $[\text{REEtot}]$  and the nominal  
150 concentration at  $t_0$ . More than 90% of  $[\text{REEtot}]$  values did not remain within 80-120% of the  
151 nominal concentration (recovery  $<80\%$  or  $>120\%$ ) (Table S1), thus the toxicity results were  
152 systematically based on measured concentration as recommended in the ISO guideline (ISO,  
153 2012) and REE exposure concentrations were corrected calculating the geometric mean  
154 concentration according to Romero-Freire et al. (2019):

$$155 \quad [\text{REE}]_{\text{mean}} = \sqrt{(C_0 \times C_1)} \quad (1)$$

156 with  $C_0$  being the nominal concentration at  $t_0$  (nominal was used instead of measured because  
157 REE concentration at  $t_0$  was not measured here) and  $C_1$  being the measured (total or dissolved)  
158 concentration at  $t_1$ . When using  $[\text{REEtot}]$  to calculate the geometric mean, the latter is denoted  
159  $[\text{REEtot}]_{\text{mean}}$  and when using  $[\text{REEdiss}]$  it refers to  $[\text{REEdiss}]_{\text{mean}}$ . For substances such as REE

160 where the exposure concentration decreases significantly during the test (e.g. Blinova et al.,  
161 2018; Romero-Freire et al., 2019; Vukov et al., 2016; Weltje et al., 2002), the mean  
162 concentration represents more properly the real exposure along the test than using the unique  
163 concentration at the beginning or the end of the test (OECD, 2000).

164 For each concentration tested, the daphnids of all replicates ( $\geq 20$  daphnids) were pooled  
165 and the REE concentration was measured in this pool (denoted  $[REE]_{D.magna}$ ) in order to  
166 estimate REE bioaccumulation. The organisms were dried at 50°C during 24 h and digested  
167 with 1 mL of HNO<sub>3</sub> 65% at 80°C before analysis. The Bioconcentration factor (BCF) was  
168 calculated to compare the bioaccumulation between the different exposure conditions using the  
169 formula:

$$170 \quad BCF = \frac{[REE]_{D.magna}}{[REE]_{diss}^{mean}} \quad (2)$$

171 For ICP-MS analysis, all samples, blanks and standards were diluted to a final concentration of  
172 HNO<sub>3</sub> at 2%. The following isotopes were measured: <sup>146</sup>Nd, <sup>157</sup>Gd, <sup>173</sup>Yb. Rhenium (<sup>187</sup>Re) was  
173 used as internal standard. Limits of detection (LOD) and quantification (LOQ) were determined  
174 and ranged from 0.1 to 0.3 µg L<sup>-1</sup> for each element in aqueous samples and from 0.01 to 0.04  
175 mg g<sup>-1</sup> dry weight in solid samples (daphnids, filters). Analytical and procedural blanks were  
176 prepared and analysed using the same analytical procedure as for samples. The accuracy of the  
177 analytical protocol was validated using international certified materials (BCR-667 and BCR-  
178 668) consisting, respectively, of estuarine sediments and mussel tissues from LGC standards.  
179 The recovery of Nd, Gd and Yb were respectively 111, 113 and 100 %.

## 180 **2.4 Geochemical speciation modeling**

181 PHREEQC (version 2) (Parkhurst and Appelo, 1999) is a computer code based on an ion-  
182 association aqueous model, which was designed to perform speciation and saturation-index  
183 calculations in water. The thermodynamic database "Minteq.v4.dat" provided with PHREEQC

184 was used. This database has been updated by the incorporation of the well-accepted reactions  
185 and stability constants at 0 M ionic strength and 25°C for REE inorganic anion complexation  
186 relevant to the present work (Table 1 and references therein). Ionic strength corrections have  
187 been performed with the Davies equation. REE binding modeling to DOM was considered  
188 using the humic-ion binding model “Model VII” (Tipping et al., 2011). PHREEQC-Model VII  
189 coupling is described thoroughly elsewhere (Marsac et al., 2017, 2014). Briefly, Model VII  
190 assumes that the complexation of ions by humic acids (HA) occurs through various discrete  
191 groups (carboxylic and phenolic groups), which can form either mono-, bi- or tri-dentate  
192 binding sites. Because HA are large and negatively charged polyelectrolytes or colloids,  
193 electrostatic effects are also accounted for the quantification of metal ions-HA binding (Tipping  
194 et al., 2011). Because no parameter is available for REE complexation to fulvic acids (FA), the  
195 most recently optimized binding parameters for REE and HA were used (Marsac et al., 2021)  
196 as a proxy of REE-FA binding parameters. By using [REE<sub>tot</sub>] and pH values measured at t1  
197 and accounting for potential REE sorption to the flask, we performed simulations to determine  
198 each REE distribution between three distinct compartments: (i) a solid phase of potential  
199 REE(OH)<sub>3(s)</sub> precipitates, (ii) a colloidal phase that includes REE bound to HA and (iii) truly  
200 dissolved REE that include REE aquo-ions (REE<sup>3+</sup>) and all dissolved complexes (with OH<sup>-</sup>,  
201 CO<sub>3</sub><sup>2-</sup>, Cl<sup>-</sup>, etc.).

## 202 2.5 Data analysis

203 The dose response-curves, the EC<sub>x</sub> values and their confidence interval (95% certainty) were  
204 obtained using R (version 3.6.1), package “drc” (Ritz et al., 2015). A log-logistic model with  
205 two parameters (LL.2) was used to fit the data. Because speciation affects REE exposure and  
206 bioavailability, the EC<sub>50</sub> were calculated and expressed based on the nominal (theoretical), the  
207 geometric mean of total ([REE<sub>tot</sub>]<sub>mean</sub>) and dissolved ([REE<sub>diss</sub>]<sub>mean</sub>) concentrations, and the  
208 predicted free ion concentrations (calculated by modeling) to assess the influence on REE

209 toxicity assessment. The comparison of EC50 values was based on confidence interval overlap.  
210 The relationship between two variables was evaluated through a simple linear regression test  
211 with the function “lm” in R.

## 212 **3 Results**

### 213 **3.1 REE exposure concentrations and speciation**

214 In the absence (DOM-) and presence (DOM+) of DOM, all concentrations of Nd, Gd and Yb  
215 measured in the water at t1 were lower than the nominal ones. The recovery was slightly higher  
216 in DOM+ (62-89%) than in DOM- (34-79%) but most of the time it was below 80% (Table S1).  
217 Therefore, REE exposure concentrations were corrected calculating the geometric mean of the  
218 total ( $[REE_{tot}]_{mean}$ ) and dissolved ( $[REE_{diss}]_{mean}$ ) concentrations (cf section 2.3).

219 Nd and Gd presented a similar geochemical speciation with the absence of precipitation  
220 and an occurrence only in dissolved form (Table 2). On the contrary, Yb was calculated to be  
221 present in often precipitated form (i.e.  $Yb(OH)_{3(s)}$ ) between 21 and 38% in DOM- and between  
222 12 and 31% in DOM+ (Table S1). With increasing Yb total concentrations, the fraction of  
223 precipitated Yb first increased and then decreased (Figure S1). In the mixture tests, Yb occurred  
224 only in dissolved form (i.e. no precipitation occurred) as well as Nd and Gd (Table S2).

225 In the dissolved phase, Nd, Gd and Yb, individually and in mixture, were present in the  
226 same forms, mainly as  $REE^{3+}$  (7-74%),  $REECO_3^+$  (1-53%) and  $REESO_4^+$  (1-25%) (Table 2). In  
227 DOM+, the proportion of free ion REE decreased relatively to DOM-, by 12%, 20%, 7%, 13%  
228 for Nd, Gd, Yb and the mixture, respectively (Table S1).

229 The presence of DOM modified the geochemical speciation of all REE through the  
230 formation of REE-organic (REE-DOM) complexes. The speciation model predicted between 6  
231 and 38% of total Nd (Gd 6-73%, Yb 6-58%, mixture 11-43%) were complexed to DOM (Table  
232 2), with declining values at higher REE concentration in solution (Figure S1). The proportions

233 of Nd, Gd and Yb bound to DOM between single (Table S1) and mixture tests (Table S2) were  
234 relatively close.

### 235 3.2 REE bioaccumulation and toxicity

236 REE concentrations measured in the daphnids ranged from 2 to 137 mg g<sup>-1</sup> (in DOM-) and  
237 from 3 to 109 mg g<sup>-1</sup> (in DOM+) (Table 2) and were significantly related to the REE  
238 concentrations measured in solution (linear regressions: p-values < 0.05, Figure 1; Table S3).  
239 Bioaccumulated concentrations tended to follow the order Nd > Gd > Yb (Table 2).  
240 Bioaccumulation decreased in DOM+ compared with DOM- for Nd, Gd, the mixture and, in a  
241 lesser extent, for Yb (Table 2). The respective mean BCF values were 2.8, 2.6, 2.1 and 1.3-fold  
242 lower in DOM+ than in DOM- for Nd, Gd, the mixture and Yb. The lower REE  
243 bioaccumulation in DOM+ is also visible by the regression line for DOM+ (in orange) being  
244 below the regression line obtained for DOM- (in blue) (Figure 1).

245 Mobility inhibition was positively related to REE concentration measured in the  
246 daphnids (linear regression: R<sup>2</sup>=0.84 and 0.76 in DOM- and DOM+ respectively, p-values <  
247 0.05, Table S4) and to REE concentration in solution, which is visible by the dose response-  
248 curves (Figure 1). The EC50 values and their confidence interval based on nominal,  
249 [REEtot]<sub>mean</sub>, [REEdiss]<sub>mean</sub> and free ion concentrations are reported in the Table S5. In DOM-  
250 , the EC50 values of Nd, Gd and Yb in single and mixture tests ranged between 8.3 and 8.8 mg  
251 L<sup>-1</sup> (nominal concentration), 4.7 and 6.2 mg L<sup>-1</sup> ([REEtot]<sub>mean</sub>), 2.5 and 4.9 mg L<sup>-1</sup>  
252 ([REEdiss]<sub>mean</sub>) and between 0.6 and 1.9 mg L<sup>-1</sup> (free ion concentration) (Figure 2). The EC50  
253 confidence interval of the three REE overlapped excluding those of Yb when considering  
254 [REEdiss]<sub>mean</sub> and free ion concentration (Figure 2). The EC50 values were 2.8, 1.9 and 1.3 fold  
255 higher in DOM+ than in DOM- for Nd, Gd and the mixture respectively (based on dissolved  
256 concentrations) (Figure 2). Their EC50 values were close and ranged from 10.4 to 12.8 mg L<sup>-1</sup>  
257 (nominal concentration), 8.5 to 10.1 mg L<sup>-1</sup> ([REEtot]<sub>mean</sub>), 4.7 to 6.3 mg L<sup>-1</sup> ([REEdiss]<sub>mean</sub>)

258 and 2.1 to 2.9 mg L<sup>-1</sup> (free ion concentration) (Figure 2). The lower toxicity in DOM+ is also  
259 visible by the dose-response curves for DOM+ (in orange) being below the curves obtained for  
260 DOM- (in blue) (Figure 1). On the contrary, Yb EC50 values were similar in DOM- and DOM+  
261 and their confidence intervals and dose-response curves overlapped (Figures 1, 2). In DOM+,  
262 the toxicity (i.e. EC50 values) of Yb was higher than Nd, Gd and the mixture if considering  
263 nominal and free ion concentrations (Figure 2). In DOM- and DOM+, the EC50 values of the  
264 mixture were close to the mean of the EC50 values of the three single REE (Figure 2).

## 265 **4 Discussion**

266 In this work, we investigated the influence of REE speciation on the toxicity to a key freshwater  
267 zooplankton species, *D. magna*. To tackle this issue, we added DOM to the test solution and  
268 expressed REE EC50 values in different ways. REE bioaccumulation was analysed to better  
269 understand the relationship between speciation and toxicity. Nd (LREE), Gd (MREE) and Yb  
270 (HREE) were tested individually and in mixture to assess the uniformity of their effects.

### 271 **4.1 REE toxicity individually and in mixture**

272 Nd, Gd and Yb, individually and in mixture, showed a similar toxicity with EC50 values  
273 ranging in a relatively narrow interval (Figure 2). If considering nominal, [REE<sub>tot</sub>]<sub>mean</sub> and  
274 [REE<sub>diss</sub>]<sub>mean</sub> concentrations, the discrepancy between Nd, Gd and Yb EC50 values (in single  
275 and mixture tests) never exceeded a factor of 2 (Table S5). Our results corroborate the similar  
276 toxicity of La, Ce, Pr, Nd and Gd observed with *D. magna* (Blinova et al., 2018). The  
277 homogenous toxicity of REE is probably linked to their comparable physicochemical  
278 properties. Based on our EC50 values (expressed as [REE<sub>tot</sub>]<sub>mean</sub> and [REE<sub>diss</sub>]<sub>mean</sub>), Nd, Gd  
279 and Yb could be included in the same category (category 2: EC50 > 1 to ≤ 10 mg L<sup>-1</sup>) of the  
280 classification of hazardous substances to the aquatic environment (United Nations, 2021). Such  
281 a range of REE concentrations (1-10 mg L<sup>-1</sup>) can be found in stream water located in REE

282 mining areas (Liang et al., 2014; Liu et al., 2019). However, REE concentrations usually found  
283 in natural freshwaters are ranged between 30 to 3000 ng L<sup>-1</sup> with median values around 150 and  
284 370 ng L<sup>-1</sup> for rivers and lakes respectively (Noack et al., 2014). According to the toxic values  
285 determined in the current study, the environmental risk caused by REE is mostly located in  
286 REE mining areas and to a lesser extent in wastewater treatment plant influent or downstream  
287 for hospital areas (González et al., 2015). However, additional toxicity data on other species  
288 belonging to different trophic levels (e.g. algae, fish) are necessary to realize a proper ERA of  
289 REE.

290 The toxicity of the mixture was similar to the toxicity of the individual REE. This can be  
291 explained by the similar chemical and physical behavior of REE when present individually or  
292 in mixture. Indeed, the speciation and bioaccumulation of Nd, Gd between single (Table S1)  
293 and mixture tests (Table S2) were relatively close. These results suggest no interactions between  
294 REE in mixture. Empirically, the absence of interactions lead to additive toxic effects in  
295 mixture, which is mostly the case for substances having similar toxicity (Kortenkamp et al.,  
296 2009), such as REE.

#### 297 **4.2 DOM influence on REE speciation, bioaccumulation and toxicity**

298 The presence of DOM in solution changed REE speciation, which significantly  
299 influenced REE bioaccumulation and toxicity, especially for Nd, Gd and the mixture. DOM  
300 modified REE speciation by the formation of REE-DOM complexes (Table 2). The decreasing  
301 proportions of REE-DOM complexes with increasing total REE concentrations (Figure S1) can  
302 be explained by both (i) the concomitant decrease in pH (Table S1) (ii) metal loading of DOM  
303 (saturation of the complexation sites) (Marsac et al., 2010). The complexation between REE  
304 and DOM reduced REE bioavailability and bioaccumulation because of a decreased proportion  
305 of free ion REE in DOM<sup>+</sup> solution (Table 2), which is largely accepted to reflect metal  
306 bioavailability (Di Toro et al., 2001). Our results corroborate those of MacMillan et al. (2019),



307 who suggested that DOM reduces REE bioavailability. They demonstrated in Canadian lakes  
308 that zooplankton accumulated less REE with decreasing REE/DOC ratio. Bioaccumulated  
309 metals are distributed at subcellular level between sensitive/ toxic fractions (biologically active  
310 metals) and detoxified fractions (biologically inactive metals) (Wallace et al., 2003). Cardon et  
311 al. (2019) demonstrated that *D. magna* accumulated most yttrium (Y) (75%) in a detoxified  
312 form (as metal-rich granules), but Y concentrations measured ( $0.00002\text{-}0.055\text{ mg g}^{-1}\text{ w.w.}$ )  
313 were much lower than the REE concentrations we measured ( $2\text{-}137\text{ mg g}^{-1}\text{ d.w.}$ ). This can be  
314 explained by the lower concentrations they tested ( $0.2\text{-}1.2\text{ mg Y L}^{-1}$ ) compared to our  
315 concentrations ( $0.4\text{-}28.4\text{ mg REE L}^{-1}$ ). Indeed, we demonstrated that the higher REE  
316 concentration was in solution, the higher REE concentration was in *D. magna* (Figure 1). Our  
317 results are in agreement with the positive correlation found between REE accumulation by  
318 zooplankton and REE concentration measured in natural waters (MacMillan et al., 2019). It is  
319 likely that we underestimated bioaccumulation because mortality occurred, especially at higher  
320 tested REE concentrations. Died daphnids will have a lower bioaccumulation period compared  
321 to alive daphnids. The consideration of alive daphnids only would have enhanced the  
322 relationship between REE bioaccumulation and REE concentration in solution.

323 REE bioaccumulation was related to REE toxicity (Table S4). Indeed, lower toxicity  
324 was observed with lower REE bioaccumulation. The presence of DOM divided by 2.1-2.8 the  
325 bioaccumulation and by 1.3-2.8 the toxicity of Nd, Gd and the mixture towards *D. magna*. Our  
326 results are in accordance with previous works studying DOM influence on the toxicity of Tm  
327 (Loveridge et al., 2021) and Dy (Vukov et al., 2016) towards the crustacean *Hyalella azteca*.  
328  $9.3\text{ mg C L}^{-1}$  from Suwannee River divided by 2 Dy toxicity (Vukov et al. 2016). Compared to  
329 these works, our approach is specific in combining speciation, bioaccumulation and toxicity  
330 towards *D. magna*. To our knowledge, only Aharchaou et al., (2020) also conducted the  
331 simultaneous investigation of DOM effects on speciation, bioaccumulation and resulting

332 toxicity of REE towards a microalga species. They showed that malic acid, nitrilotriacetic acid  
333 (NTA) and iminodiacetic (IDA) decreased Ce and La uptake and toxicity towards *Chlorella*  
334 *fusca* because of REE complexation with the organic ligands. A reduction of REE (Sm, Nd, La,  
335 Ce, Sc, Y, Eu, Tm) bioavailability and uptake in the microalga *Chlamydomonas reinhardtii* has  
336 been demonstrated in the presence of different organic ligands such as FA (El-Akl et al., 2015;  
337 Rowell et al., 2018), HA (Rowell et al., 2018), malic and citric acid (Tan et al., 2017; Yang et  
338 al., 2014; Yang and Wilkinson, 2018; Zhao and Wilkinson, 2015), diglycolic acid (Tan et al.,  
339 2017) and NTA (Nitrilotriacetic acid) (Zhao and Wilkinson, 2015). The attenuating effects of  
340 DOM on metal bioavailability and toxicity in aqueous media are well documented (Wood et  
341 al., 2011). Examples are reported for silver on microalgae (Paquet, 2007), for copper on  
342 crustaceans (Kramer et al., 2004), for zinc on bivalves (Vercauteren and Blust, 1996), and for  
343 lead and cadmium on fish (Schwartz et al., 2004). Our work demonstrates for the first time that  
344 DOM could also mitigate Nd and Gd (individually and in mixture) bioaccumulation and toxicity  
345 towards *D. magna*.

346 Contrary to Nd and Gd, DOM did not influence Yb toxicity and bioaccumulation. The  
347 calculated concentration of  $\text{Yb}^{3+}$  in DOM+ and DOM- are similar (Table S1). In fact, contrary  
348 to Nd and Gd, Yb partly occurred as  $\text{Yb}(\text{OH})_{3(s)}$  solid form (Table 2), which controls the  
349 concentration of  $\text{Yb}^{3+}$  in solution. On the contrary,  $\text{Nd}^{3+}$  and  $\text{Gd}^{3+}$  concentrations are controlled  
350 by the complexation with the DOM. The lower bioaccumulation of Yb relative to Nd and Gd  
351 would be explained also by  $\text{Yb}(\text{OH})_{3(s)}$  precipitation in single tests.

352 The described effects of DOM on REE bioaccumulation and toxicity are likely even  
353 stronger in natural waters where REE concentrations are much lower ( $\text{ng L}^{-1}$ ) than those we  
354 tested ( $\text{mg L}^{-1}$ ). The relative amount of REE bound to DOM strongly increases with decreasing  
355 REE/C ratio (Marsac et al., 2010), showing that the REE concentration in solution influences  
356 the binding of REE to DOM. At environmental REE/C ratios, we can expect a higher

357 complexation of Yb to DOM because the few strong binding sites are preferentially complexing  
358 HREE (Marsac et al., 2010).

### 359 **4.3 Implications of speciation on REE toxicity assessment**

360 By adding DOM, we demonstrated that test solution composition significantly  
361 influenced REE toxicity because it determines REE speciation. Similarly, water hardness  
362 reduces REE toxicity (Barry and Meehan, 2000; Borgmann et al., 2005; Cardon et al., 2019).  
363 Solution composition also influences REE uptake mechanisms, and thus REE toxicity. The  
364 toxicity of Dy towards *H. azteca* was reduced with increased Ca and Na concentrations and  
365 lower pH, explained by competition between  $\text{Ca}^{2+}$ ,  $\text{Na}^{2+}$ ,  $\text{H}^{+}$  and  $\text{Dy}^{3+}$  for the binding to the  
366 biotic ligand (Vukov et al., 2016). Test solution variability can partly explain the heterogeneity  
367 of REE EC50 values reported in acute toxicity studies on crustaceans, which range from 9.4 to  
368  $122.4 \text{ mg L}^{-1}$  (nominal concentration), from 0.043 to  $5 \text{ mg L}^{-1}$  (measured total concentration)  
369 and from 0.3 to  $1.5 \text{ mg L}^{-1}$  (measured dissolved concentration) (Table S5). Therefore, solution  
370 composition and speciation should be considered in the assessment of REE toxicity. Based on  
371 the same test solution composition, our results corroborate those of Gonzalez et al. (2015) and  
372 Ma et al. (2016) (Table S5). By contrast, the EC50 values (based on measured concentrations)  
373 determined in other studies (Blinova et al., 2018; Galdiero et al., 2019) using the same solution  
374 are much lower (by more than 10 fold) than those we found in the current study (Table S5).  
375 This may be explained by a significant REE precipitation reported in the study of Blinova et al.  
376 (2018), which might have caused high variations in REE concentration measurements, making  
377 an exact identification of EC50 values based on measured concentrations difficult.

378 We demonstrate that solution composition and the subsequent speciation may significantly  
379 affect REE exposure concentrations and bioavailability. Subsequently, the EC50 values were  
380 calculated according to nominal,  $[\text{REE}_{\text{tot}}]_{\text{mean}}$ ,  $[\text{REE}_{\text{diss}}]_{\text{mean}}$  and predicted free ion  
381 concentrations in order to evaluate the influence on toxicity assessment. Nd, Gd and Yb tested

382 individually or in mixture, with or without DOM exhibited EC50 values in the following order:  
383 nominal >  $[\text{REE}_{\text{tot}}]_{\text{mean}} > [\text{REE}_{\text{diss}}]_{\text{mean}} >$  predicted free ion (Figure 2). This expected pattern  
384 has been observed also in other studies (Table S5). In the current work, the predicted free ion  
385 EC50 are on average 8-fold lower than nominal EC50, and up to 16-fold lower for Yb in DOM+  
386 (Figure 2). This gap between EC50 values is highly relevant because it can strongly influence  
387 the subsequent ERA. The way of expressing EC50 should thus be selected with caution.  
388 Nominal concentration is not suitable because the concentrations of Nd, Gd and Yb measured  
389 in the water at the end of the test were drastically lower than nominal ones (Table 2). This can  
390 be explained by REE adsorption on glass material and/or by REE precipitation (Blinova et al.,  
391 2018; González et al., 2015; Romero-Freire et al., 2019). The preconditioning of the test flask  
392 may have reduced but not completely avoided REE adsorption on flasks. According to the  
393 classification of hazardous substances to the aquatic environment (United Nations, 2021), Nd  
394 and Gd in DOM+ would be classified in the category 3 ( $\text{EC}_{50} > 10$  to  $\leq 100 \text{ mg L}^{-1}$ ) if  
395 considering nominal concentrations and in the category 2 ( $\text{EC}_{50} > 1$  to  $\leq 10 \text{ mg L}^{-1}$ ) if  
396 considering  $[\text{REE}_{\text{tot}}]_{\text{mean}}$  and  $[\text{REE}_{\text{diss}}]_{\text{mean}}$  (Figure 2). Consequently, the use of nominal  
397 concentrations can lead to underestimation of REE toxicity (Gonzalez et al., 2014; Vukov et  
398 al., 2016).

399 Measured total concentration does not seem appropriate, neither, because REE tend to  
400 complex, precipitate and form insoluble species supposed to be not or little bioavailable and  
401 thus not toxic (Blinova et al., 2020; Gonzalez et al., 2014). Therefore, we consider that the use  
402 of measured dissolved concentration (referring in the current study to  $[\text{REE}_{\text{diss}}]_{\text{mean}}$ , which  
403 includes the measured dissolved concentration at t1) is probably the most suitable way to  
404 express REE EC50. This was also suggested by Vukov et al. (2016). The water framework  
405 directive 2013/39/EC (European Parliament, 2013) recommended the use of measured  
406 dissolved concentration ( $<0.45 \mu\text{m}$ ) to express the environmental quality standards of the

407 priority metals Pb, Cd, nickel and mercury. This is more reliable and conservative because it  
408 represents real concentration (measured), with higher bioavailability (dissolved) even if not all  
409 metal dissolved species are bioavailable. Accumulation and toxicity of metals can usually be  
410 predicted by the free ion concentration (Allen et al., 1980). The latter can be determined either  
411 experimentally, or by modeling. The experimental determination of the free metal  
412 concentrations is obviously the most reliable approach (Janot et al., 2021; Khan et al., 2017;  
413 Rowell et al., 2018). Yet, it has several drawbacks: (i) the measurements are rather expensive  
414 and time consuming, and (ii) data obtained are highly conditional because they are only valid  
415 for the investigated system and do not apply to other environmental conditions. By contrast,  
416 geochemical speciation modeling is a solution, but the data are subjected to several sources of  
417 uncertainty or mistakes made by the user of speciation codes:

- 418 (i) A complete set of the relevant reactions and thermodynamic constants must be used to  
419 perform the calculations (see e.g. Table 1 for the present work). Available default  
420 databases with speciation codes do not necessarily include all of them for all metal ions.  
421 For example, a closer look to the database provided in Visual Minteq revealed that only  
422 one hydrolysis constant and one complex with carbonate for the REE are considered.
- 423 (ii) Besides experimental uncertainties associated to any aqueous reaction constants, the  
424 value of a solubility product, used to predict mineral formation/precipitation, is supposed  
425 to vary with the size of the (nano)particles (Auffan et al., 2009; Schindler, 1967), which  
426 is difficult to determine when formed in ecotoxicological tests.
- 427 (iii) The input data are of high importance. For example, the use of nominal or measured total  
428 REE concentrations might affect the saturation index with respect to REE-mineral phase  
429 or REE-DOM loading and, hence, REE calculated speciation. Another crucial input  
430 parameter is the pH. Our calculations were based on pH values at t1 but did not take into

431 account pH evolution between  $t_0$  and  $t_1$ . This evolution might impact REE speciation  
432 because pH was in the crucial range (6.5-7.5) for REE precipitation;

433 (iv) The REE-DOM model used in the present study is the most recently updated model  
434 considering REE-DOM interactions. However, because of the use of generic DOM  
435 parameters, Model VII does not consider the variability in DOM reactivity with respect  
436 e.g. to its origin.

437 Despite these challenges the recently optimized humic-ion binding Model VII (Marsac et al.,  
438 2021) enabled the determination of relatively close EC50 values (based on free ion  
439 concentration) between DOM<sup>-</sup> and DOM<sup>+</sup> (Figure 2). This result is consistent if considering  
440 the free ion as the only metal form able to react with the biotic ligands, being taken up, and  
441 inducing toxicity, as suggested by the BLM (Di Toro et al., 2001). With all uncertainties related  
442 to the speciation presented above, it is clear that modeling the toxicity of REE using BLM  
443 remains challenging. In fact, most studies that attempted to apply the BLM to REE in the  
444 presence of DOM faced obstacles. The BLM largely underestimated REE uptake (Rowell et al.,  
445 2018; Tan et al., 2017; Yang et al., 2014; Yang and Wilkinson, 2018; Zhao and Wilkinson,  
446 2015) and/or predicted an EC50 significantly lower in the presence of DOM compared to in the  
447 absence (Aharchaou et al., 2020; Vukov et al., 2016; Yang and Wilkinson, 2018). However,  
448 speciation modeling results might have been subjected to the uncertainties listed above.  
449 Otherwise, BLM faces new challenges related to (i) the consideration of REE-DOM complex  
450 toxicity, as well as of the nanoparticles formed in the presence of DOM, and (ii) the  
451 consideration of mixtures (mixture of REE and mixture of single REE species).

## 452 **5 Conclusion**

453 To assess the uniformity of the REE group, Nd (LREE), Gd (MREE) and Yb (HREE) were  
454 tested individually and in mixture in *D. magna* mobility inhibition test (ISO, 2012). Yb showed  
455 some differences in terms of speciation and bioaccumulation relative to Nd and Gd. However,

456 the three REE, individually and in mixture, exhibited a homogeneous toxicity with EC50 values  
457 falling within in a close range. Our results are in accordance with the review of Blinova et al.  
458 (2020) on REE aquatic toxicity, which concludes that REE could be considered as a uniform  
459 group in the context of ERA despite some slight differences between light and heavy REE. The  
460 differences of electron configuration and atomic radius size between LREE, MREE and HREE  
461 did not seem to influence much REE toxicity.

462 The influence of speciation on REE toxicity was evaluated by adding DOM in solution and  
463 by calculating EC50 values in different ways (using nominal, measured and predicted  
464 concentrations). The geochemical speciation model predicted that DOM complexed a relevant  
465 proportion (6-73%) of total REE. The complexation between DOM and REE reduced the  
466 bioavailability and divided by 2.1-2.8 fold the subsequent bioaccumulation of Nd, Gd and the  
467 mixture, which explained their reduced toxicity by 1.3-2.8 times in DOM+. However, Yb  
468 toxicity was unchanged in DOM+, because similar calculated  $\text{Yb}^{3+}$  concentrations were  
469 determined in DOM+ and DOM-. Contrary to Nd and Gd, Yb was predicted to precipitate in  
470 the single tests (DOM+ and DOM-) in the form of  $\text{Yb}(\text{OH})_{3(s)}$ , controlling  $\text{Yb}^{3+}$  concentrations.

471 We demonstrated that REE speciation significantly influences the related toxicity.  
472 However, speciation is often not considered in studies on REE ecotoxicity probably because its  
473 measurement/estimation is challenging and because of few developed collaborations between  
474 ecotoxicologists and geochemists. In this work, the speciation of Nd, Gd and Yb, individually  
475 and in mixture, was predicted in the absence and presence of DOM using the most updated  
476 model available. Despite some remaining uncertainties about REE speciation mainly due to  
477 missing data and model limitations, its predictions allowed explaining observed REE  
478 bioaccumulation and toxicity. The current work provides new data allowing a better  
479 understanding of DOM influence on REE speciation, bioaccumulation and toxicity towards a  
480 model animal. Relevant environmental risk assessment of REE should include DOM and

481 speciation, as both significantly influence REE toxicity. This work highlights the relevance of  
482 a combined approach from ecotoxicology and geochemistry in order to improve the ERA of  
483 (emerging) contaminants such as REE.

484

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493

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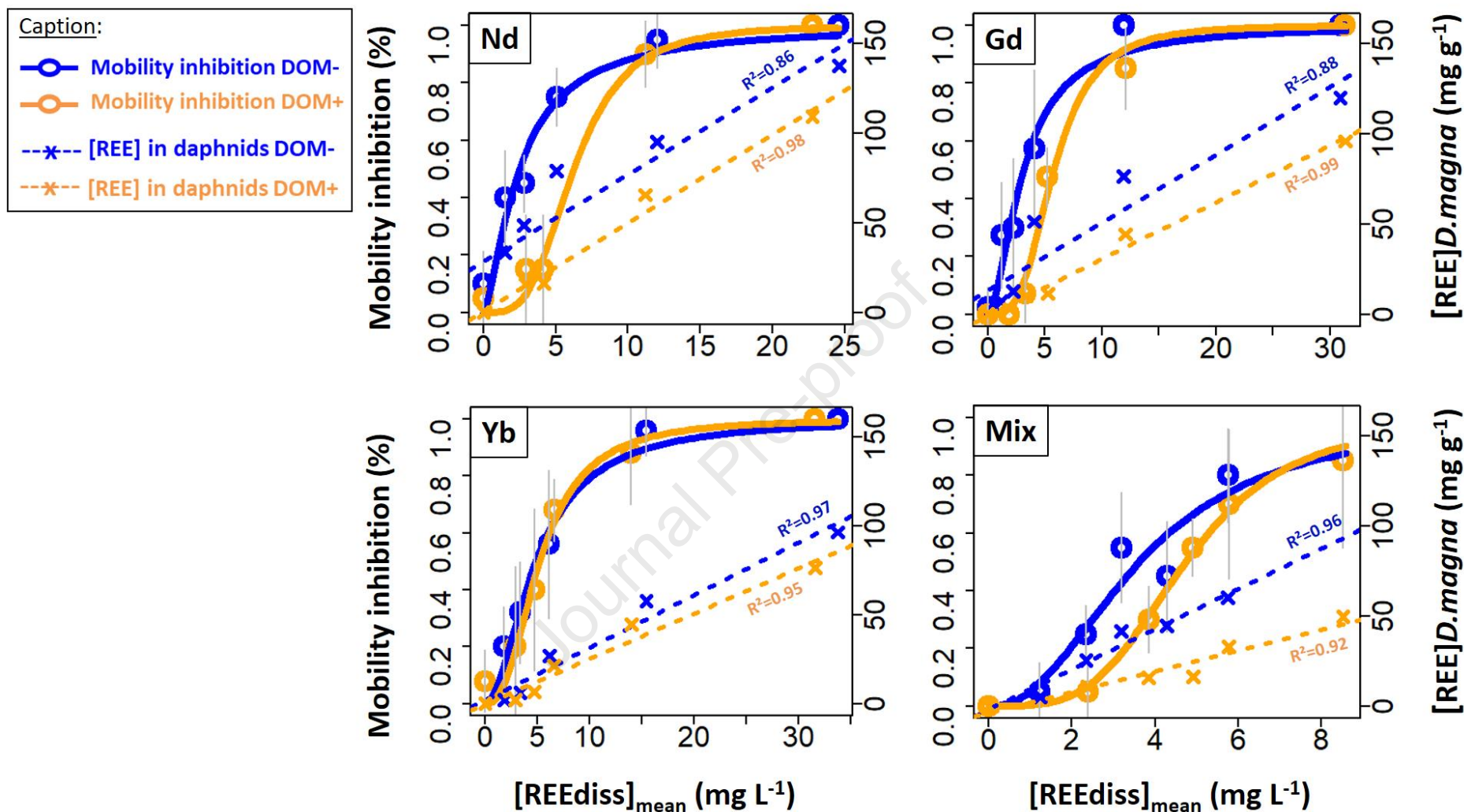
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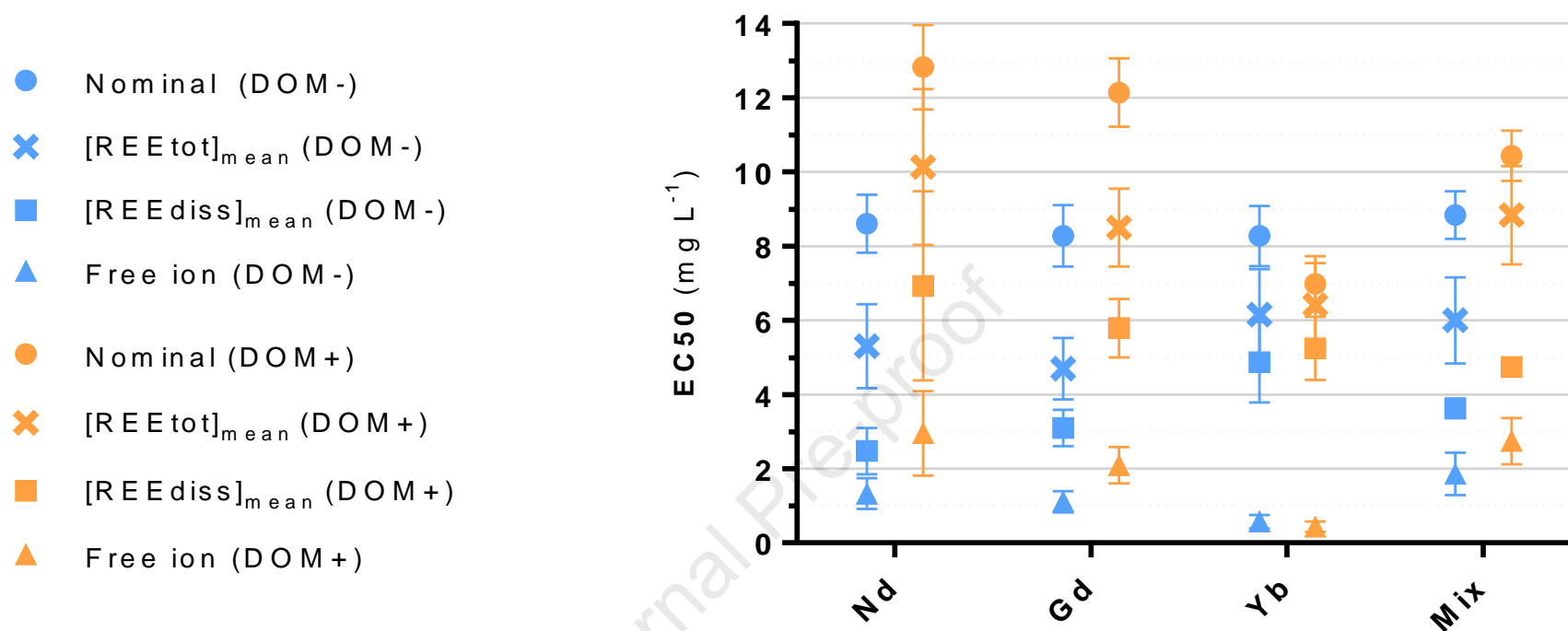
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**Figure 1: Dose-response curves and bioaccumulation of Nd, Gd and Yb, individually and in mixture.**  $[\text{REEdiss}]_{\text{mean}} = \sqrt{C_0 \times C_1}$  with  $C_0$  being the nominal concentration in solution at the test beginning ( $t_0$ ) and  $C_1$  being the dissolved concentration measured at the end ( $t_1$ ).  $[\text{REE}]D.magna$  = REE concentration measured in *D. magna* at  $t_1$ . DOM- = 0 mg C L<sup>-1</sup>; DOM+ = 8 mg C L<sup>-1</sup>. Each dot corresponds to the mean of mobility inhibition of at least 4 replicates and the associated standard deviation is represented by the vertical bar. Each cross corresponds to the REE concentration measured at  $t_1$  in a pool of daphnids ( $\geq 20$  daphnids). The dashed line represents the regression line between  $[\text{REE}]D.magna$

and  $[\text{REEdiss}]_{\text{mean}}$ .  $R^2$  = determination coefficient of the linear regression. Additional information about the regression analyses can be found in Table S3.

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**Figure 2: EC50 values of Nd, Gd and Yb, individually and in mixture, in the absence (blue) and presence (orange) of dissolved organic matter (DOM).** The EC50 values are shown for four different conditions: nominal concentrations, the geometric means of total ([REEtot]<sub>mean</sub>) or dissolved ([REEdiss]<sub>mean</sub>) concentrations and the predicted free ion concentrations. Vertical bars represent 95% confidence intervals. DOM- = 0 mg C L<sup>-1</sup>; DOM+ = 8 mg C L<sup>-1</sup>



**Table 1: Reaction and constant database for Nd, Gd and Yb.** Chemical species refer to dissolved aqueous forms or solid form (denoted (s)). \*Average value between fresh and aged solids. *References: 1 = (Klungness and Byrne, 2000); 2 = (Lee and Byrne, 1992); 3 = (Luo and Byrne, 2004); 4 = (Luo and Byrne, 2001); 5 = (Spahiu and Bruno, 1995); 6 = (Diakonov et al., 1998)*

Reaction	Nd	Gd	Yb	Reference
$\text{REE}^{3+} + \text{H}_2\text{O} \rightleftharpoons \text{REEOH}^{2+} + \text{H}^+$	-8.18	-7.83	-7.24	1,2
$\text{REE}^{3+} + 2 \text{H}_2\text{O} \rightleftharpoons \text{REE}(\text{OH})_2^+ + 2\text{H}^+$	-17.04	-16.37	-15.74	1,2
$\text{REE}^{3+} + 3 \text{H}_2\text{O} \rightleftharpoons \text{REE}(\text{OH})_3 + 3\text{H}^+$	-26.40	-25.28	-23.85	1,2
$\text{REE}^{3+} + \text{HCO}_3^- \rightleftharpoons \text{REEHCO}_3^{2+}$	2.28	2.36	2.53	3
$\text{REE}^{3+} + \text{CO}_3^{2-} \rightleftharpoons \text{REECO}_3^+$	7.28	7.39	7.81	3
$\text{REE}^{3+} + 2 \text{CO}_3^{2-} \rightleftharpoons \text{REE}(\text{CO}_3)_2^-$	12.17	12.48	13.3	3
$\text{REE}^{3+} + \text{Cl}^- \rightleftharpoons \text{REECI}^{2+}$	0.65	0.65	0.65	4
$\text{REE}^{3+} + \text{SO}_4^{2-} \rightleftharpoons \text{REESO}_4^+$	3.5	3.4	3.3	5
$\text{REE}^{3+} + 2 \text{SO}_4^{2-} \rightleftharpoons \text{REE}(\text{SO}_4)_2^-$	5.1	5.1	5.1	5
$\text{REE}(\text{OH})_{3(s)} + 3\text{H}^+ \rightleftharpoons \text{REE}^{3+} + 3\text{H}_2\text{O}$	18.28*	17.58*	16.14*	6

**Table 2: REE concentrations measured and predicted in test solution and in *Daphnia magna*.** All REE concentrations were measured at the end of the test (48h = t1). DOM- = 0 mg C L<sup>-1</sup>; DOM+ = 8 mg C L<sup>-1</sup>. REE recovery corresponds to the ratio between total concentration measured at t1 and nominal concentration at t0. REE distribution (%) among the truly dissolved, colloidal (denoted (DOM)) and solid (denoted (s)) phases were calculated by modeling. [REE<sub>tot</sub>]<sub>mean</sub> and [REE<sub>diss</sub>]<sub>mean</sub> correspond to the geometric mean ( $=\sqrt{(C_0 \cdot C_1)}$ ) where C<sub>0</sub> refers to the nominal concentration at t0 and C<sub>1</sub> refers to the total or dissolved concentration measured at t1. [REE]<sub>D.magna</sub> corresponds to the REE concentration measured at t1 in a pool of daphnids ( $\geq 20$  daphnids). BCF = Bioconcentration factor = [REE]<sub>D.magna</sub> / [REE<sub>diss</sub>]<sub>mean</sub>. Data represent the interval (min-max) of five values.

REE	DOM	Nominal [REE] (mg L <sup>-1</sup> )	REE recovery at t1 (%)	[REE <sub>tot</sub> ] <sub>mean</sub> (mg L <sup>-1</sup> )	[REE <sub>diss</sub> ] <sub>mean</sub> (mg L <sup>-1</sup> )	Predicted free ion [REE] (mg L <sup>-1</sup> )	Predicted main REE truly dissolved species (% tot)	Predicted REE colloidal and solid <sub>(s)</sub> species (% tot)	Measured [REE] <i>D.magna</i> (mg g <sup>-1</sup> dry weight)	BCF (L kg <sup>-1</sup> )
Nd	DOM-	5.2-33.9	47-68	3.5-27.9	1.5-24.6	0.7-16.3	Nd <sup>3+</sup> 32-71 NdCO <sub>3</sub> <sup>+</sup> 1-50 NdSO <sub>4</sub> <sup>+</sup> 13-25		34-137	5593-22377
	DOM+	5.2-33.9	67-76	4.3-29.6	2.9-22.7	0.7-17.3	Nd <sup>3+</sup> 20-67 NdCO <sub>3</sub> <sup>+</sup> 1-34 NdSO <sub>4</sub> <sup>+</sup> 8-24	Nd(DOM) 6-38	16-109	3067-5851
Gd	DOM-	2.5-40.6	40-65	1.6-32.8	1.2-30.9	0.3-19.6	Gd <sup>3+</sup> 27-74 GdCO <sub>3</sub> <sup>+</sup> 2-55 GdSO <sub>4</sub> <sup>+</sup> 9-21		7-120	3874-12698
	DOM+	2.5-40.6	62-72	2.1-34.4	1.9-31.3	0.1-20.3	Gd <sup>3+</sup> 7-69 GdCO <sub>3</sub> <sup>+</sup> 2-29 GdSO <sub>4</sub> <sup>+</sup> 2-20	Gd(DOM) 6-73	3-96	1569-3684
Yb	DOM-	3.6-40.1	34-79	2.1-35.6	1.8-33.7	0.3-22.0	Yb <sup>3+</sup> 11-70 YbCO <sub>3</sub> <sup>+</sup> 5-67 YbSO <sub>4</sub> <sup>+</sup> 3-16	Yb(OH) <sub>3(s)</sub> 0-38	2-96	1191-4404
	DOM+	3.6-40.1	63-80	3.0-35.9	2.9-31.5	0.3-21.0	Yb <sup>3+</sup> 5-66 YbCO <sub>3</sub> <sup>+</sup> 5-28 YbSO <sub>4</sub> <sup>+</sup> 1-15	Yb(DOM) 6-58 Yb(OH) <sub>3(s)</sub> 0-31	2-76	727-3247
Mix	DOM-	2.4-15.1	44-58	1.7-9.8	1.2-5.7	0.8-4.6	REE <sup>3+</sup> 27-63 REECO <sub>3</sub> <sup>+</sup> 9-53 REESO <sub>4</sub> <sup>+</sup> 10-20		5-60	4419-13067
	DOM+	3.5-18.1	76-89	3.1-15.0	2.4-8.5	1.8-6.9	REE <sup>3+</sup> 18-50 REECO <sub>3</sub> <sup>+</sup> 15-26 REESO <sub>4</sub> <sup>+</sup> 6-16	REE(DOM) 11-43	11 49	3294-5804

**Highlights:**

- DOM modified speciation of REE (Gd, Nd, Yb) by the formation of REE-DOM complexes
- DOM decreased REE bioaccumulation probably by reducing REE bioavailability
- DOM significantly reduced the toxicity of Nd, Gd and mixture towards *Daphnia magna*
- Yb toxicity was not influenced by DOM probably because of Yb precipitation
- Speciation influences REE bioaccumulation and the subsequent toxicity

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CRedit roles: **Nicolas Lachaux**: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Validation; Visualization; Writing - original draft. **Charlotte Catrouillet**: Data curation; Formal analysis; Resources; Writing - review & editing. **Rémi Marsac**: Data curation; Formal analysis; Resources; Writing - review & editing. **Laurence Poirier**: Data curation; Formal analysis; Resources; Writing - review & editing. **Sandrine Pain-Devin**: Conceptualization; Supervision; Validation; Writing - review & editing. **Elisabeth Maria Gross**: Conceptualization; Supervision; Validation; Writing - review & editing. **Laure Giamberini**: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Validation; Writing - review & editing.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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