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Acute toxicity of single and combined rare earth element exposures towards *Daphnia similis*

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ABSTRACT

The increasing use of Rare Earth Elements (REE) in emerging technologies, medicine and agriculture has led to chronic aquatic compartment contamination. In this context, this aimed to evaluate the acute toxic effects of lanthanum (La), neodymium (Nd) and samarium (Sm), as both single and binary and ternary mixtures on the survival of the microcrustacean *Daphnia similis*. A metal solution medium with (MS) and without EDTA and cyanocobalamin (MSq) as chelators was employed as the assay dilution water to assess REE bioavailability effects. In the single exposure experiments, toxicity in the MS medium decreased following the order La > Sm > Nd, while the opposite was noted for the MSq medium, which was also more toxic than the MS medium. The highest MS toxicity was observed for the binary Nd + La (1:1) mixture (EC_{50} 48 h of 11.57 ± 1.22 $mg.L^{-1}$) and the lowest, in the ternary Sm + La + Nd (2:2:1) mixture (EC_{50} 48 h 41.48 ± 1.40 $mg.L^{-1}$). The highest toxicity in the MSq medium was observed in the single assays and in the binary Sm + Nd (1:1) mixture (EC_{50} 48 h 10.60 ± 1.57 $mg.L^{-1}$), and the lowest, in the ternary Sm + La + Nd (1:2:2) mixture (EC_{50} 48 h 36.76 ± 1.54 $mg.L^{-1}$). Concerning the MS medium, 75 % of interactions were additive, 19 % antagonistic, and 6 % synergistic. In the MSq medium, 56 % of interactions were synergistic and 44 % additive. The higher toxicity observed in the MSq medium indicates that the absence of chelators can increase the concentrations of more toxic free ions, suggesting that the MS medium should be avoided in REE assays. Additive interactions were observed in greater or equivalent amounts in both media and were independent of elemental mixture ratios. These findings improve the understanding of environmental REE effects, contributing to the establishment of future guidelines and ecological risk calculations.

1. Introduction

Interest in understanding environmental Rare Earth Element (REE) effects has increased due to various REE applications, particularly in emerging technologies, green industries, agriculture, and medicine (Sousa Filho et al., 2019). This higher use of strategic elements increases the probability of REE exposure and environmental contamination, making effect assessments paramount (Blinova et al., 2018, 2020; González et al., 2014, 2015; Herrmann et al., 2014). REEs comprise 17 chemical elements, namely 15 lanthanides plus scandium and yttrium (Andrade, 2014; Barry and Meehan, 2000). These elements display similar properties, depending on their electronic distribution (González et al., 2015; Martins and Isolani, 2005), and are found associated with other elements, such as iron, phosphorus, titanium, zirconium, niobium, tin, copper, gold, thorium, radium, uranium, making them likely to be found naturally present in the environment (Blinova et al., 2020;

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González et al., 2015; Haxel et al., 2002).

Varied REE applications are possible to their chemical, spectroscopic, magnetic, and luminescent properties (Martins and Isolani, 2005), favoring the use of these elements in high-technology industries, which now present increasing demands (Andrade, 2014; Haxel et al., 2002; Peixoto et al., 2012). Because of this, REE emissions to freshwater ecosystems are estimated to increase due to wastewater inputs originated from mining activities and inadequate post-consumption disposal, industrial emissions from pre-consumption beneficiation processes, surface runoff from diffuse sources and, finally, atmospheric deposition resulting from fertilizer-contaminated particulates (Balusamy et al., 2015; Blaise et al., 2008; Blinova et al., 2020; González et al., 2015; Herrmann et al., 2014).

Lanthanides exhibits chemical and physical characteristics similar to calcium (Ca), in particular their ionic radius (González et al., 2014, 2015). This similarity causes lanthanides, especially La, Pr, Nd, Tm and Tb, to react with tissue components, displacing and replacing Ca^{2+} in different cellular functions, competing for active binding sites, and directly influencing the chemical processes of exposed organisms (Barry and Meehan, 2000; Blinova et al., 2020; Brown et al., 1990; Martins and Isolani, 2005). Thus, REE interactions with Ca-dependent biological systems interfere with their metabolism, resulting in functional impairment (Barry and Meehan, 2000; Das et al., 1988; González et al., 2015; Thomas et al., 2014). Although only some cases of lanthanide contamination have been reported, environmental and human exposures are on the rise (Gwenzi et al., 2018; Pagano et al., 2015a). Furthermore, REE bioaccumulation has been reported in soils (Gong et al., 2021a, 2021b, 2019), plants (Li et al., 2010), and human hair (González et al., 2015; Herrmann et al., 2014), and REE exposure has been reported as damaging the lungs, liver, spleen, blood, and brain (Haley, 1965; Pagano et al., 2015b; Shi et al., 2022). Therefore, REE ecotoxicity, bioaccumulation and mode of action studies are required for a better understanding and prevention of potential risks associated with their disposal (González et al., 2014, 2015).

Microcrustaceans belonging to the Cladocera order and *Daphnia* genus are widely employed as test organisms in ecotoxicology assays (Domingues and Bertoletti, 2006; Lingott et al., 2016), comprising important bioindicators, as they ingest dissolved and solid contaminants through the gills and digestive system (Lingott et al., 2016). They occur in different aquatic ecosystems niches and present several characteristics that make them suitable for use in aquatic ecotoxicity tests, such as availability, taxonomic stability, small size, sensitivity to a wide variety of substances, wide distribution, parthenogenetic reproduction, known cultivation techniques, large litters and high population density and growth (Sarma and Nandini, 2006).

Toxicity studies employing Y, La, Ce, Nd, Sm, Gd, Dy, and Er have indicated the influence of medium hardness used in test on acute toxicity in *D. carinata* and *D. magna* (Barry and Meehan, 2000; Cardon et al., 2019). Adult mortality in chronic trials was the most sensitive endpoint compared to *D. magna* growth or juvenile production (Barry and Meehan, 2000; Blinova et al., 2018; Van Hoecke et al., 2009). REE (Y, La, Ce, Nd, Sm, Gd, Dy, and Er) effects on *D. magna* reproduction, such as transient fecundity increases, delayed in maturation, production of larger litters, smaller body size, and neonatal mortality have also been reported (Barry and Meehan, 2000; Blinova et al., 2018; Galdiero et al., 2019; Lürling and Tolma, 2010). Other studies point to *D. magna* digestive tract La and Gd accumulation and damage, reduced feeding and swimming ability following La exposure, and molt inhibition and decreased growth following Ce exposure (Balusamy et al., 2015; Gaiser et al., 2011; Lingott et al., 2016), acute *D. similis* La, Nd and Sm toxicity in both single and combined exposures (Bergsten-Torralba et al., 2020), and acute *D. pulex* Gd effects (Vukov et al., 2016). Chronic Ce and Er toxicity assays have also been reported as activating detoxification processes in *D. magna*, such as antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione s-transferase (GST), and the expression of ATP-binding cassette (ABC) transporter genes (Galdiero et al., 2019).

However, although organisms are exposed to complex REE mixtures in aquatic environments (Blinova et al., 2020), most studies still employ single REE test concentrations (Blinova et al., 2020). In this regard, mixture assessments display the potential to further understanding on the combined effects of substances present in effluents from different contaminant sources, discharged into water bodies (Balusamy et al., 2015; Blaise et al., 2008; Blinova et al., 2020; González et al., 2015, 2015; Herrmann et al., 2014).

In this context, understanding toxic aquatic organism REE levels in both single and/or combined exposure conditions is extremely useful to determine guidelines in risk assessment studies, as well as in remediation assessments and in residue recycling and disposing in soils and water bodies (Blinova et al., 2018; De Sá Paye et al., 2016). Some factors that determine REE toxicity inhibition include competition with Ca^{2+} and Mg^{2+} ions, precipitation in the form of carbonates and phosphates at high pH, speciation, and complexation with organic and inorganic ligands (El-Akl et al., 2015; Romero-Freire et al., 2019; Sneller et al., 2000; Weltje et al., 2002). This clearly indicates that REE interactions in *Daphnia* spp. exposed to binary and ternary mixtures and resulting toxic effects should be further evaluated.

Thus, the aims of the present study comprised (i) assessments on toxicity following exposure to La [$La(NO_3)_3$], Nd [$Nd(NO_3)$] and Sm [$Sm(NO_3)_3$], in single, binary and ternary mixture exposure assays on the survival of *Daphnia similis* (Crustacea, Daphniidae); (ii) comparisons of the toxic effects of these REE in two media, with and without chelators; (iii) identification of the toxicity order of the investigated REEs and types of interactions and (iv) association of the observed toxicities with elemental mixture ratios.

2. Material and methods

2.1. REE stock-solutions

The three oxides (La_2O_3, PIDC, CAS# 1312–81–8, Nd_2O_3, PIDC, CAS# 7440–00–8 and Sm_2O_3, PIDC, CAS# 7440–19–9) used in the assays were provided by the Coordination of Metallurgical and Environmental Processes (COPMA) belonging to the Brazilian Mineral Technology Center (CETEM). These REEs were chosen because they are among the four most abundant REEs in the Earth's crust (La – 19 $g.t^{-1}$, Nd – 24 $g.t^{-1}$, and Sm – 6.5 $g.t^{-1}$) (Abrão, 1994) and because they are widely applied in agricultural fertilizers and green industries (Herrmann et al., 2014; Shi et al., 2022). The synthetic REE nitrate solutions were prepared from 5 $g.L^{-1}$ stock solutions obtained by solubilizing each oxide in nitric acid (HNO_3, Qhemis, CAS# 7697–37–2) p.a. at 70 %. As the initial acid pH of the stock solutions is outside the tolerance range of the test organism (ABNT, 2016; US.EPA, 2002), all stock solution aliquots and test solutions pH values were adjusted to 6.0 ± 0.5 prior to the assays using 0.001, 0.01, 1, 2 and 4 $mol.L^{-1}$ NaOH solutions.

2.2. *Daphnia similis* assays

Fifty to 60 *Daphnia similis* specimens were maintained in a metal solution (MS) (ABNT, 2016) medium, with pH between 7 and 7.6 (DM-22 Digimed), hardness between 40 and 48 mg of $CaCO_3$. L^{-1} and dissolved oxygen (DM-4 P Digimed) over 5 mg L^{-1} at 20 ± 2 °C, a 16 h/8 h light dark photoperiod and lighting from 500 to 1000 lux in 2 L glass containers. Specimens were divided per age group into 0 – 7 day, 7 – 14 day, 14 – 21 day and 21 – 28 day classes. The cultures were fed daily with an algal suspension of *Raphidocelis subcapitata* from 1 to 5×10^5 cells per organism. Sensitivity assays were performed monthly with sodium chloride (NaCl) as the reference substance (ABNT, 2016; US. EPA, 2002).

Acute tests were performed according to ABNT (2016) and US.EPA (2002) standards. Test solutions were prepared using the MS medium as dilution water and control comprising the MS medium only. The MS medium contained ethylenediaminetracetic acid (EDTA) and cyanocobalamin as chelators, which can complex with available REEs and make them non-bioavailable to tested organisms (Tang et al., 2018). To test for chelation effects, assays were also performed with the MS medium without both chelators (MSq). Twenty juveniles born 6–24 h previously were exposed in four replicates under the same culture conditions without light and food for 48 h. pH and DO values were determined at the beginning and end of the assays. At the end of the experiments, organisms were counted, and the results expressed as EC_{50} 48 h, median effective concentration. Assay validation was achieved when the mortality in the control is less than or equal to 10 % of exposed organisms and the results of the sensitivity assay performed with NaCl are within the range of ± 2 SD (standard deviation) of the means of previous assays (ABNT, 2016). The control chart value was $EC_{50} = 2252.9 \pm 198.67$ NaCl $mg.L^{-1}$.

Assays were carried out in the MS and MSq media in single REE exposures (nominal concentrations of 2.5 mg,L^{-1}; 5.0 mg,L^{-1}; 10.0 mg, L^{-1}; 15 mg,L^{-1}; 20 mg,L^{-1}; 30 mg,L^{-1} and 40 mg,L^{-1}) and in REE mixtures at three binary ratios (v/v), as Sm + La, Sm + Nd and Nd + La at 1:1, 1:2 and 2:1 and in seven ternary ratios (v/v), as Sm + La + Nd at 1:1:1, 1:2:1, 1:1:2, 2:1:1, 1:2:2, 2:1:2 and 2:2:1. The mixture ratios were prepared with the same solute test concentrations employed in the single REE assays.

Chemical analyses of the test concentrations were performed at the Coordination of Mineral Analysis (COAMI) belonging to the Mineral Technology Center (CETEM) employing a Ultima-2 Horiba-Jobin Yvon inductively coupled plasma optical emission spectrometer (ICP OES) (Li et al., 2017). The spectral lines used for elemental quantifications and limits of detection calculations were as follows: La [ES1] (333.749 nm, 0.006 mg L^{-1}; La [ES2] (398.852 nm, 0.02 mg L^{-1}; Sm [ES1] (360.948, 0.006 mg L^{-1}; Sm [ES2] (446.734 nm, 0.04 mg L^{-1}; Nd [ES1] (395.115 nm, 0.06 mg L^{-1}; Nd [ES2] (410.946 nm, 0.03 mg L^{-1}), respectively.

2.3. Data analyses

The Shapiro-Wilks test was used to test data normality, followed by the t-Student test for independent samples for parametric data and the Mann-Whitney test for non-parametric data. The Trimmed Spearman-Karber program was used to EC_{50} calculation. The Additive Concentration (AC) model was applied to determine the type of REE interaction, which considers that all elements within a mixture exhibit similar mechanisms of action and toxicity (Blinova et al., 2018). The results are expressed as Toxic Units (Panouillères et al., 2007), according to Eq. (1):

$$TU_m = \sum(\text{Concentration of REE at } EC_{50} \text{ of mixture} / EC_{50} \text{ of REE alone}) \quad (1)$$

Where: TU_m – Toxic mixture unit; REE – Rare Earth Element; EC_{50} - median effective concentration.

When the sum is equal to 1, interactions are considered additive, and mixture compounds do not interact with each other. If the sum is less than 1, the compounds present in the mixture interact antagonistically, and the effect is less than additive, with one element partially or totally inhibiting the action of the other. If the sum is greater than 1, the mixture presents synergistic effects, with an effect greater than in the additive interaction, and one compound potentiates the effects of the other (ATSDR, 2004). Statistically significant difference between the EC_{50} results was determined by assessing the confidence limits. When an overlap of the confidence limits of the EC_{50} was noted, the standard error of the mean differences was compared through Eq. (2), (US.EPA, 1985):

$$G = \sqrt{\left(\log\left(SL_{(1)} \div EC_{50(1)}\right)\right)^2 + \left(\log\left(SL_{(2)} \div EC_{50(2)}\right)\right)^2} \quad (2)$$

Where: SL – upper limit of the confidence interval for test 1 or 2. Subsequently, $H = 10^{G}$ and Z are calculated, with $Z =$ upper $EC_{50} \div$ lower EC_{50}. If $Z > H$, a significant difference between EC_{50} values is observed.

All analyses were performed separately, for the REEs in the single exposure, in the binary mixtures and in the ternary mixtures. All replicates are identified by numbers. When similar, each replicate is preceded by a number (1, 2 and 3), and when similar to another replicate, it receives the number of the similar one (i.e., 123, 12, 13, 23).

3. Results and discussion

The ICP-OES results of the nominal concentrations employed in the single and mixture exposure assays are presented in Table 1. Variations of the determined concentrations in relation to the nominal test solution concentrations in different media at the beginning and end of toxicity assays have been reported for both *D. magna* (Blinova et al., 2018, 2020; González et al., 2015; Lürling and Tolma, 2010; Romero-Freire et al., 2019) and *D. carinata* (Barry and Meehan, 2000). These have been attributed to medium characteristics (pH, hardness, formation of inorganic and organic complexes, speciation), exposure time (acute or chronic), and nominal test solution concentrations, with decreased concentrations observed at the end of the tests in the dissolved phase (Blinova et al., 2018, 2020; González et al., 2015; Lürling and Tolma, 2010; Romero-Freire et al., 2019). However, Blinova et al. (2022) mentioned that as crustaceans are particle ingesters, they can ingest soluble REE complexes from the water column and insoluble precipitates, being not only exposed to the dissolved phase of REE from the medium.

In the present study, chemical test solution analyses were performed after preparation, with total sample solubilization. In general, values remained within the appropriate bias range (± 15 %) (Desimoni and Brunetti, 2011). The analytical results of the nominal concentrations of the three REE in the MS and MSq media were highly precise, with low coefficients of variation (CV ≤ 15 %, data not shown – Table 1). The pH values at the beginning and at the end of the assays were close to neutral, ranging from 6.25 ± 1.08 at the beginning to 6.58 ± 0.71 at the end during single REE exposures, from 6.13 ± 0.75–6.44 ± 0.58 in the binary mixture assays and from 6.23 ± 0.65–6.69 ± 0.49. in the ternary mixture assays.

Lanthanum was the most toxic REE in the single assays in the MS medium ($EC_{50} = 17.61 \pm 0.63$ $mg.L^{-1}$), followed by Sm ($EC_{50} = 22.34 \pm 0.20$ $mg.L^{-1}$) and Nd ($EC_{50} = 25.43 \pm 1.60$ $mg.L^{-1}$) (Table 2). In the MSq medium, Nd was the most toxic REE (EC_{50} 48 h = 9.19 ± 0.64 mg. L-1), followed by Sm ($EC_{50} = 10.79 \pm 1.25$ $mg.L^{-1}$) and lanthanum ($EC_{50} = 12.60 \pm 0.99$ $mg.L^{-1}$). Neodymium was significantly different only from La, with its upper confidence interval (CI = 10.99) smaller than the lower La confidence intervals (CI = 11.47). Samarium ($EC_{50} = 10.79 \pm 1.25$ $mg.L^{-1}$) was not significantly different from either La ($EC_{50} = 12.60 \pm 0.99$ $mg.L^{-1}$) or Nd ($EC_{50} = 9.19 \pm 0.64$ $mg.L^{-1}$).

Two different responses were observed comparing the EC_{50} 48 h values (Table 2) for two media, the first comprising an inverse response to the most toxic element in the media and the second, lower EC_{50} 48 h values in the MSq medium for Sm and Nd, except for La which was not significantly different between the two media. This toxicity difference may be a result of preferential REE binding by the same sorbent, in this case MS chelators. Metal complexation/adsorption related to their ionic potential (oxidation number to ionic radius ratio) (Camargo et al., 2001). Ion valence and ionic radii are paramount in ion retention (Camargo et al., 2001). Retention preference for ions of the same valence is related to ionic radii, where smaller ionic radii favor attraction to the sorbent surface (Camargo et al., 2001), and consequently lower bioavailability. The ionic radii of La^{3+}, Nd^{3+} and Sm^{3+} are, respectively, 122, 104 and 100 pm (Manusadžianas et al., 2020), resulting in greater Sm and Nd complexation compared to La. Thus, the greater affinity of Sm and Nd for the chelators present in the MS medium leads to lower toxicity. Both Nd and Sm became more available in the MSq medium, which contained no chelators, so no complexing effect took place, favoring greater exposed organism assimilation and resulting

Table 1
Chemical analysis and standard deviation of nominal concentrations determined by ICP-OES in the Metal Solution (MS) medium and in the MSq chelator-free medium used in acute *Daphnia similis* exposure assays. * outside the precision range - ± 15 %.

Concentrations (mg.L^{-1}) and media/elements and mixtures	Nominal	Determined					
		MS			Chelator-free MS		
		Sm	La	Nd	Sm	La	Nd
Sm	2.5	2.45 ± 0.02			2.78 ± 0.04		
	5	5.05 ± 0.03			5.23 ± 0.03		
	10	9.79 ± 0.12			10.54 ± 0.13		
	20	19.68 ± 0.25			19.82 ± 0.16		
	30	29.96 ± 0.45			31.23 ± 0.47		
	40	40.38 ± 0.62			41.92 ± 0.16		
La	2.5		2.21 ± 0.02			2.31 ± 0.02	
	5		4.28 ± 0.05			4.53 ± 0.09	
	10		8.65 ± 0.08			9.25 ± 0.14	
	20		16.86 ± 0.08*			17.76 ± 0.03	
	30		25.04 ± 0.02*			25.30 ± 0.30*	
	40		34.01 ± 0.19			34.61 ± 0.37	
Nd	2.5			1.83 ± 0.04*			2.24 ± 0.02
	5			3.81 ± 0.04*			4.35 ± 0.16
	10			7.84 ± 0.01*			8.78 ± 0.14
	20			15.2 ± 0.19*			17.03 ± 0.07
	30			22.76 ± 0.14*			24.84 ± 0.40*
	40			30.6 ± 0.41*			34.44 ± 0.43
Sm-La							
1:1	2.5	2.5 ± 0.02	2.22 ± 0.03		2.5 ± 0.03	2.4 ± 0.05	
	5	5.01 ± 0.08	4.48 ± 0.04		5.0 ± 0.03	4.6 ± 0.08	
	10	10.53 ± 0.12	9.18 ± 0.38		10.50 ± 0.18	9.4 ± 0.02	
	15	15.45 ± 0.51	14.3 ± 0.35		15.40 ± 0.17	13.30 ± 0.28	
	20	20.53 ± 0.52	18.8 ± 0.43		20.50 ± 0.17	18.40 ± 0.69	
	25	24.88 ± 0.25	22.67 ± 0.42		25.8 ± 0.21	18.40 ± 0.69	
	30	31.09 ± 0.39	28.5 ± 0.81		30.40 ± 0.50	26.80 ± 0.11	
	40	41.14 ± 0.50	38.3 ± 0.60		40.60 ± 0.15	35.50 ± 1.04	
1:2	2.5	2.7 ± 0.05	4.71 ± 0.14		2.5 ± 0.03	4.6 ± 0.01	
	5	5.31 ± 0.09	9.5 ± 0.37		5.0 ± 0.01	9.0 ± 0.07	
	10	10.38 ± 0.17	18.95 ± 0.37		10.30 ± 0.13	18.6 ± 0.29	
	15	16.25 ± 0.17	28.37 ± 0.67		15.60 ± 0.23	27.80 ± 0.59	
	20	20.59 ± 0.34	36.33 ± 0.15		20.30 ± 0.29	36.30 ± 0.04	
	25	25.24 ± 0.60	49.04 ± 0.64		25.50 ± 0.13	45.70 ± 0.87	
	30	30.3 ± 0.27	56.57 ± 1.92		31.30 ± 0.24	55.30 ± 0.77	
	40	40.45 ± 0.15	74.84 ± 1.84		41.10 ± 0.25	74.0 ± 1.36	
2:1	2.5	5.4 ± 0.09	2.41 ± 0.11		5.0 ± 0.04	2.4 ± 0.05	
	5	11.01 ± 0.05	4.84 ± 0.04		10.20 ± 0.12	4.6 ± 0.09	
	10	20.46 ± 0.18	9.8 ± 0.23		20.90 ± 0.15	9.50 ± 0.06	
	15	30.71 ± 0.34	14.3 ± 0.18		30.40 ± 0.29	13.80 ± 0.28	
	20	41.62 ± 0.79	19.1 ± 0.28		41.40 ± 0.30	18.20 ± 0.40	
	25	52 ± 0.53	24.61 ± 0.59		50.0 ± 0.31	22.40 ± 0.51	
	30	64.82 ± 0.36	28.82 ± 0.38		61.20 ± 0.40	27.80 ± 0.37	
	40	84.58 ± 1.38	38.5 ± 0.60		81.30 ± 0.85	35.80 ± 1.17	
Sm-Nd							
1:1	2.5	2.61 ± 0.03		2.41 ± 0.05	2.60 ± 0.15		2.10 ± 0.01*
	5	5.17 ± 0.04		4.78 ± 0.18	5.0 ± 0.06		4.50 ± 0.05
	10	9.85 ± 0.24		9.38 ± 0.18	10.50 ± 0.10		9.40 ± 0.03
	15	15.93 ± 0.18		14.69 ± 0.15	14.90 ± 0.16		13.60 ± 0.08
	20	20.28 ± 0.56		18.73 ± 0.07	20.90 ± 0.20		18.60 ± 0.22
	25	24.82 ± 0.19		23.78 ± 0.79	25.10 ± 0.25		23.10 ± 0.04
	30	30.22 ± 0.67		28.33 ± 0.88	31.60 ± 0.39		28.20 ± 0.65
	40	39.49 ± 0.31		37.41 ± 0.06	41.90 ± 0.29		37.50 ± 0.25
1:2	2.5	2.69 ± 0.03		4.49 ± 0.03	2.50 ± 0.56		4.60 ± 0.06
	5	5.27 ± 0.11		9.02 ± 0.1	5.20 ± 0.05		9.70 ± 0.29
	10	9.83 ± 0.1		18.49 ± 0.28	11.30 ± 0.04		19.40 ± 0.17
	15	15.43 ± 0.06		26.86 ± 0.32	17.10 ± 0.16		29.60 ± 0.23
	20	20.17 ± 0.06		38.04 ± 1.23	23.0 ± 0.11		40.90 ± 0.38
	25	25.37 ± 0.47		44.35 ± 1.31	30.10 ± 0.30*		52.50 ± 0.44
	30	29.69 ± 0.54		54.9 ± 0.71	37.70 ± 0.34*		65.70 ± 1.27
	40	40.01 ± 1.41		73.07 ± 0.88	50.70 ± 0.31*		91.50 ± 1.69
2:1	2.5	5.16 ± 0.09		2.57 ± 0.08	5.30 ± 0.05		2.40 ± 0.04
	5	10.04 ± 0.06		4.95 ± 0.15	10.30 ± 0.23		4.80 ± 0.05
	10	20.43 ± 0.16		10.08 ± 0.18	20.20 ± 0.24		10.20 ± 0.30
	15	30.39 ± 0.58		15.61 ± 0.43	30.10 ± 0.15		14.70 ± 0.26
	20	40.52 ± 0.46		20.01 ± 0.38	40.60 ± 0.59		20.10 ± 0.41
	25	50.26 ± 0.38		25.26 ± 0.33	50.50 ± 0.56		24.70 ± 0.70
	30	60.54 ± 0.25		29.78 ± 0.71	60.90 ± 0.91		29.40 ± 0.87
	40	81.36 ± 1.63		39.37 ± 0.71	82.30 ± 1.47		39.0 ± 0.56
Nd-La							
1:1	2.5		2.26 ± 0.06	2.27 ± 0.03		2.60 ± 0.02	2.20 ± 0.03

(*continued on next page*)

Table 1 (*continued*)

Concentrations (mg.L^{-1}) and media/elements and mixtures	Nominal	Determined					
		MS			Chelator-free MS		
		Sm	La	Nd	Sm	La	Nd
	5		4.32 ± 0.04	4.61 ± 0.02		4.70 ± 0.03	4.10 ± 0.03*
	10		8.95 ± 0.19	9.81 ± 0.12		9.90 ± 0.20	9.20 ± 0.29
	15		13.24 ± 0.16	14.29 ± 0.18		14.10 ± 0.28	13.50 ± 0.24
	20		17.3 ± 0.26	19.48 ± 0.40		19.60 ± 0.18	17.80 ± 0.60
	25		23.11 ± 0.50	24.43 ± 0.45		24.40 ± 0.47	22.40 ± 0.30
	30		27.33 ± 0.08	28.41 ± 0.45		27.80 ± 0.87	26.80 ± 0.69
	40		36.07 ± 0.84	37.92 ± 0.65		37.50 ± 0.44	32.20 ± 6.84*
1:2	2.5		4.67 ± 0.17	2.44 ± 0.01		4.60 ± 0.11	2.0 ± 0.03*
	5		6.84 ± 0.31*	4.82 ± 0.08		9.10 ± 0.11	4.10 ± 0.03*
	10		18.06 ± 0.58	10.21 ± 0.26		17.50 ± 0.46	8.70 ± 0.12
	15		27.99 ± 0.51	15.53 ± 0.44		26.50 ± 1.07	12.80 ± 0.64
	20		36.7 ± 0.41	19.98 ± 0.26		36.10 ± 1.08	17.0 ± 0.19
	25		47.2 ± 1.17	23.97 ± 0.10		45.0 ± 0.68	21.70 ± 0.24
	30		56.1 ± 0.42	29.57 ± 0.65		53.40 ± 0.93	25.40 ± 0.61*
	40		72.8 ± 0.42	38.73 ± 0.54		73.30 ± 0.87	34.20 ± 0.33
2:1	2.5		2.42 ± 0.02	4.82 ± 0.20		2.40 ± 0.02	3.90 ± 0.09*
	5		4.38 ± 0.05	9.49 ± 0.13		4.60 ± 0.03	8.60 ± 0.10
	10		8.95 ± 0.21	18.73 ± 0.14		9.70 ± 0.23	17.0 ± 0.38
	15		13.31 ± 0.19	28.33 ± 0.28		13.30 ± 0.19	25.30 ± 0.79*
	20		17.64 ± 0.11	39.43 ± 0.60		18.20 ± 0.43	34.90 ± 0.81
	25		22.78 ± 0.92	48.8 ± 0.35		21.90 ± 0.52	41.40 ± 0.70*
	30		27.17 ± 0.38	61.79 ± 0.19		25.80 ± 0.45	49.90 ± 0.42*
	40		35.63 ± 0.46	78.13 ± 1.22		36.50 ± 0.87	66.20 ± 0.33*
Sm-La-Nd							
1:1:1	2.5	2.63 ± 0.04	2.48 ± 0.02	2.78 ± 0.09	2.50 ± 0.05	2.40 ± 0.01	2.20 ± 0.04
	5	5.25 ± 0.07	4.53 ± 0.12	4.81 ± 0.05	5.0 ± 0.02	4.50 ± 0.01	4.40 ± 0.03
	10	10.43 ± 0.21	8.82 ± 0.14	9.67 ± 0.08	9.90 ± 0.15	8.70 ± 0.04	10.0 ± 0.12
	15	15.95 ± 0.29	13.38 ± 0.17	13.87 ± 0.25	14.50 ± 0.23	12.60 ± 0.04	13.60 ± 0.13
	20	19.73 ± 0.06	16.91 ± 0.13*	18.1 ± 0.37	19.40 ± 0.08	17.30 0.22	17.90 ± 0.43
	25	24.85 ± 0.59	21.11 ± 0.67*	22.78 ± 0.24	24.20 ± 0.51	21.50 ± 0.20	22.30 ± 0.40
	30	30 ± 0.35	25.88 ± 0.93	27.15 ± 0.59	29.70 ± 0.15	26.0 ± 0.36	27.80 ± 0.29
	40	40.27 ± 0.76	34.43 ± 0.80	35.01 ± 0.33	38.20 ± 0.38	33.90 ± 0.55*	34.80 ± 0.13
1:2:1	2.5	2.76 ± 0.04	4.83 ± 0.09	2.59 ± 0.06	2.50 ± 0.01	4.50 ± 0.08	2.10 ± 0.05
	5	5.13 ± 0.08	9.01 ± 0.01	4.82 ± 0.08	5.10 ± 0.06	9.0 ± 0.25	4.60 ± 0.16
	10	11.06 ± 0.23	17.21 ± 0.45	9.68 ± 0.13	9.90 ± 0.08	18.0 ± 0.45	9.20 ± 0.11
	15	15.44 ± 0.27	27.06 ± 0.76	14.3 ± 0.34	15.30 ± 0.09	26.40 ± 0.17	13.80 ± 0.16
	20	19.67 ± 0.28	36.61 ± 1.06	17.82 ± 0.44	19.10 ± 0.20	34.30 ± 0.80	17.90 ± 0.54
	25	25.01 ± 0.11	45.28 ± 0.68	23.8 ± 0.67	24.60 ± 0.18	43.50 ± 1.17	22.40 ± 0.22
	30	29.61 ± 0.56	52.55 ± 1.04	26.15 ± 0.43	29.50 ± 0.50	51.9 ± 0.87	27.10 ± 0.41
	40	40.08 ± 0.43	68.54 ± 1.82	36.85 ± 0.87	37.90 ± 0.34	67.80 ± 0.95	35.0 ± 0.51
1:1:2	2.5	2.69 ± 0.04	2.66 ± 0.05	4.6 ± 0.11	2.50 ± 0.03	2.30 ± 0.02	4.30 ± 0.11
	5	5.23 ± 0.05	4.71 ± 0.05	9.12 ± 0.08	4.90 ± 0.09	4.40 ± 0.08	8.30 ± 0.09*
	10	10.51 ± 0.30	9.05 ± 0.08	17.75 ± 0.17	9.90 ± 0.02	8.90 ± 0.20	17.20 ± 0.55
	15	15.76 ± 0.22	14.07 ± 0.05	27.14 ± 0.75	14.30 ± 0.12	12.90 ± 0.24	25.70 ± 0.38
	20	21.02 ± 0.13	17.37 ± 0.19	36.4 ± 0.77	19.0 ± 0.08	17.0 ± 0.63	33.90 ± 0.32*
	25	24.98 ± 0.50	21.55 ± 0.42	45.76 ± 0.79	24.40 ± 0.13	20.70 ± 0.40*	43.20 ± 0.61
	30	31.32 ± 0.27	26.6 ± 0.21	54.4 ± 2.23	29.10 ± 0.30	25.80 ± 0.35	51.30 ± 0.63
	40	39.94 ± 0.03	34.65 ± 1.20	74.16 ± 2.32	37.20 ± 0.27	33.80 ± 0.40*	68.30 ± 0.27
2:1:1	2.5	5.14 ± 0.05	2.49 ± 0.03	2.65 ± 0.04	4.80 ± 0.07	2.40 ± 0.05	2.30 ± 0.05
	5	10.59 ± 0.21	4.78 ± 0.11	5.32 ± 0.17	9.40 ± 0.06	4.30 ± 0.07	4.50 ± 0.03
	10	20.44 ± 0.29	9.56 ± 0.19	9.97 ± 0.03	19.10 ± 0.30	8.80 ± 0.07	9.60 ± 0.08
	15	31.74 ± 0.10	13.9 ± 0.29	14.96 ± 0.14	29.0 ± 0.22	12.80 ± 0.23	14.60 ± 0.08
	20	40 ± 0.67	18.13 ± 0.23	18.84 ± 0.23	38.80 ± 0.30	17.20 ± 0.17	19.0 ± 0.27
	25	50.67 ± 0.23	21.58 ± 0.40	23.42 ± 0.79	48.70 ± 0.60	21.60 ± 0.12	24.40 ± 0.48
	30	62.16 ± 0.59	23.12 ± 0.11	29.28 ± 0.29	57.20 ± 0.64	25.40 ± 0.11*	27.80 ± 0.54
	40	80.64 ± 1.30	35.44 ± 0.21	37.25 ± 0.74	76.10 ± 1.40	33.70 ± 0.21*	37.0 ± 1.00
1:2:2	2.5	2.83 ± 0.01	4.83 ± 0.09	4.78 ± 0.12	2.50 ± 0.05	4.40 ± 0.01	4.0 ± 0.07*
	5	5.33 ± 0.09	9.41 ± 0.23	9.49 ± 0.10	5.0 ± 0.02	8.90 ± 0.10	8.60 ± 0.08
	10	10.91 ± 0.16	17.78 ± 0.11	18.43 ± 0.02	10.0 ± 0.10	16.90 ± 0.17*	17.0 ± 0.40
	15	15.73 ± 0.63	27.59 ± 0.68	27.29 ± 0.99	14.30 ± 0.18	25.40 ± 0.46*	25.10 ± 0.43*
	20	20.44 ± 0.14	37.01 ± 1.28	38.72 ± 0.30	19.10 ± 0.15	34.20 ± 1.09	33.50 ± 0.65*
	25	26.1 ± 0.17	45.54 ± 2.02	45.07 ± 1.02	23.60 ± 0.05	41.20 ± 0.47*	41.20 ± 1.36*
	30	29.48 ± 0.28	52.42 ± 0.61	54.21 ± 1.18	28.30 ± 0.25	49.80 ± 0.82*	50.60 ± 1.31*
	40	39.7 ± 0.55	72.16 ± 0.76	74.36 ± 1.68	37.30 ± 0.62	67.30 ± 0.92*	65.30 ± 1.51*
2:1:2	2.5	4.87 ± 0.08	2.39 ± 0.01	4.51 ± 0.09	4.70 ± 0.07	2.30 ± 0.02	4.20 ± 0.05*
	5	10.06 ± 0.15	4.31 ± 0.06	9.11 ± 0.28	9.40 ± 0.17	4.30 ± 0.07	8.60 ± 0.16
	10	19.6 ± 0.11	8.62 ± 0.20	17.67 ± 0.58	19.10 ± 0.13	8.90 ± 0.14	17.30 ± 0.24
	15	29.92 ± 0.45	12.89 ± 0.21	27.17 ± 0.47	27.50 ± 0.31	12.40 ± 0.18*	25.90 ± 0.14
	20	40.94 ± 0.94	17.62 ± 0.49	37.28 ± 0.87	37.10 ± 0.43	16.70 ± 0.33*	34.70 ± 0.45
	25	51.24 ± 0.15	21.02 ± 0.75*	46.84 ± 1.70	45.80 ± 0.22	20.20 ± 0.29*	42.0 ± 0.58 *
	30	61.18 ± 1.59	25.66 ± 0.28	55.49 ± 1.78	55.80 ± 0.68	24.30 ± 0.54*	51.0 ± 1.94
	40	80.24 ± 1.81	32.6 ± 0.28	73.96 ± 1.21	74.0 ± 1.16	32.50 ± 0.45*	66.30 ± 0.80 *

(*continued on next page*)

Table 1 (*continued*)

Concentrations (mg.L^{-1}) and media/elements and mixtures	Nominal	Determined					
		MS			Chelator-free MS		
		Sm	La	Nd	Sm	La	Nd
2:2:1	2.5	5.08 ± 0.13	4.53 ± 0.04	2.49 ± 0.04	4.70 ± 0.04	4.30 ± 0.06	2.30 ± 0.02
	5	10.16 ± 0.16	8.71 ± 0.27	4.85 ± 0.18	9.40 ± 0.11	8.0 ± 0.13*	4.50 ± 0.02
	10	20.26 ± 0.38	17.04 ± 0.52	10.07 ± 0.25	18.80 ± 0.28	16.80 ± 0.21*	9.70 ± 0.20
	15	29.41 ± 0.94	25.14 ± 0.26	14.86 ± 0.27	29.0 ± 0.34	25.80 ± 0.37	14.60 ± 0.25
	20	40.52 ± 0.69	34.19 ± 0.37	36.14 ± 0.71	38.0 ± 0.30	34.0 ± 0.22	18.70 ± 0.47
	25	50.23 ± 0.75	44.19 ± 0.95	43.65 ± 0.90*	48.80 ± 0.74	43.70 ± 0.93	24.30 ± 0.33
	30	60.28 ± 0.89	52.37 ± 0.18	51.48 ± 0.80*	57.90 ± 0.45	51.90 ± 0.47	28.10 ± 0.61
	40	84.55 ± 1.44	68.91 ± 2.56	73.88 ± 0.20*	76.90 ± 1.26	67.10 ± 1.42*	38.70 ± 0.59

in higher toxicity.

La toxicity was not different between the MS and the Msq media, potentially due to its higher ionic radius. The complexation phenomenon was evident for Sm and Nd, with EC_{50} values significantly reduced from 22.34 to 12.25 and from 25.43 to 9.19 in MSq, respectively (Table 2). As Sm and Nd have similar ionic radii (100 and 104 pm, respectively), in relation to La (122 pm), the higher Nd complexation in the MS medium, as well as its higher toxicity in the MSq medium, may have been influenced by another factor as, although not assessed herein, Sm can present configurations with different numbers of valences (Sousa Filho and Serra, 2014) that also influence the complexation process.

This complexation behavior corroborates the results of other studies employing EDTA in REE separation by titration, where the stability constants of the REE-EDTA complexes were log k 15.10, 16.4 and 17.00, for La, Nd and Sm, respectively (Abrão, 1994). The higher the K value, the greater the stability of the metal complex (Abrão, 1994). EDTA also removes most of the REE bound to cell membrane, without extracting internalized REE (El-Akl et al., 2015). The presence of organic and inorganic ligands, competition with Ca^{+2} and Mg^{+2} ions, speciation and free ion concentrations can all modify the REE flux balance through the plasma membrane through binding sites (Galdiero et al., 2019; Gong et al., 2019). Decreased toxicity in the presence of REE+EDTA complexes have been reported in studies with the cnidarian *Hydra attenuatta* (Cnidaria: Hydrozoa) employing Ce, Gd and Lu, where REE-EDTA complexes were the most abundant (> 99 %) (González et al., 2015). Furthermore, in La, Gd and Y bioconcentration assays conducted with the *Chlorella vulgaris* algae, lower bioavailability was observed for when REE-EDTA complexes were present in the media, presenting the following Bioconcentration Factors: La – 0.26, Gd – 0.38 and Y – 0.11 (Hao et al., 1997). Decreased growth-inhibiting effects have also been observed for the cyanobacterium *Microcystis aeruginosa* exposed to La (72 µmol L^{-1}) with the addition of EDTA ranging from 2.69 to 13.4 µmol L-1 (Jin et al., 2009).

Free ion concentrations seem to represent the best interaction between contaminants and organisms (Yang and Wilkinson, 2018). Results obtained with free ions have indicated that REEs are more toxic when presented in free form (González et al., 2015). For example, increased *Tritium aestivu*m root growth toxicity was noted in assays employing Y, La and Ce, (Gong et al., 2019). Higher bioconcentrations of free La, Gd and Y ions have also been reported in *Chlorella vulgaris* assays employing different chelators, such as EDTA, nitrilotriacetic acid (ANT) and citrate, resulting in a decreasing bioconcentration sequence REE^{3+} > REE-citrate > REE-ANT > REE- EDTA (Hao et al., 1997).

Concerning binary 1:1 mixtures in the MS medium, the Nd+La mixtures presented similar results to the Sm+Nd mixture, with Sm+La being the least toxic (EC_{50} 48 h 20.80 mg.L^{-1}) (Table 3). The most toxic mixture could not be determined in the MSq medium due to the 95 % CI overlap for all mixtures. The Nd+La mixture displayed the same level of toxicity in both media, while the Sm+Nd and Sm+La mixtures presented increased toxicity in the MSq medium. These results corroborate the effects observed in the single exposure conditions (Table 2). Concerning the Nd+La 1:1 mixture, La may have exerted greater toxicity in the MS medium, while the same effect was noted for Nd in the MSq medium, similarly to the single exposure of these elements. The 50 % decrease in EC_{50} values concerning the MS and MSq media for the Sm+La mixture (20.84 ± 0.44 mg.L^{-1} in the MS medium to 11.76 ± 2.10 mg.L^{-1} in the MSq medium) was greater than that observed for the 1:1 Sm+Nd mixture (14.69 ± 1.46 mg.L^{-1} in the MS medium to 9.72 ± 1.45 mg.L^{-1} in the MSq medium). Sm may have exerted a greater influence in this regard in the 1:1 Sm+La mixture when comparing the effects of these elements in the single exposure assays. Regarding the Sm+Nd 1:1 mixture, the interaction between elements and the scarcity of data in the literature on the effect of different REE ratios make it difficult to interpret the results. Tan et al. (2017) observed a 50 % decreased Sm absorption by the *Chlamydomonas reinhardtii* algae when assessing Sm bioconcentration in mixtures containing La, Ce and Eu at pH 6. Yang and Wilkinson (2018), on the other hand, when evaluating equimolar mixtures of Nd, Sm and Eu, observed that Sm and Eu decreased the uptake of Nd by *Chlamydomonas reinhardtii* cells. These authors suggest that REE competition for the same membrane binding site may have led to decreased uptakes.

The Nd+La mixture exhibited the following toxicity trend: the 1:1 ratio exhibited higher and statistically different toxicity values than the 1:2 and 2:1 ratios (1:1 > 1:2 ≈ 2:1) in the MS medium. In the MSq medium, the 1:1 ratio was similar to the 2:1 ratio but more toxic than the 1:2 ratio (1:1 ≈ 2:1 >1:2) (Table 3), with no significant difference between the two media ($t = 1.5710$, $p = 0.069$). In the MSq medium, the similar toxicity values observed for the 1:1 and 2:1 ratios may have been influenced by the greater availability of Nd in the chelator-free medium. The Sm+Nd mixtures also displayed similar toxicities in both media, of

Table 2

EC_{50} 48 h acute toxicity test results with synthetic lanthanum, neodymium and samarium solutions individually in the Metal Solution (MS) medium and chelator-free MSq medium. 95 % CI = 95 % Confidence Interval. Equal letters = non-significant statistical difference; replicate number (1, 2 and 3) = EC_{50} similar to each other; two or more numbers (123. 12. 13. 23) = similar to another replica.

Media/REE	MS			Chelator-free MS		
	EC_{50} (mg L^{-1})	CI 95 %		EC_{50} (mg L^{-1})	CI 95 %	
		Lower	Higher		Lower	Higher
Samarium	22.36^{a1}	21.13	23.67	11.28^{e1}	9.39	13.54
	22.13^{a2}	21.27	23.02	12.53^{e2}	11.18	14.03
	22.54^{a3}	21.11	24.07	12.96^{e3}	11.79	14.25
Lanthanum	16.89^{b1}	15.85	18.00	$13.26^{e123.f1}$	11.47	15.33
	18.08^{b2}	16.34	20.01	$15.37^{e3.f2}$	12.98	18.20
	$17.85^{a3.b3}$	15.89	20.06	16.05^{f3}	13.35	19.29
Neodymium	$23.59^{a123.c1}$	21.35	26.07	$9.84^{e1.g1}$	8.81	10.99
	26.45^{d1}	25.75	27.17	$9.19^{e1.g2}$	7.96	10.61
	26.25^{d2}	25.58	26.93	8.55^{g3}	7.30	10.02

Table 3

EC_{50} 48 h results and the Concentration Addition (CA) model in Toxic Units (TU) of acute toxicity tests with synthetic lanthanum, neodymium and samarium solutions in binary mixtures in the Metal Solution (MS) medium and chelator-free MSq medium.). 95 % CI = 95 % Confidence Interval. Equal letters = non-significant statistical difference; replicate number (1, 2 and 3) = EC_{50} similar to each other; two or more numbers (123. 12. 13. 23) = similar to another replica. TU – Toxic Unit. Additive. UT = 1. Synergistic, UT > 1 and Antagonistic, TU < 11 (Panouillères et al., 2007).

Type of medium/REE and mixtures	MS					Chelator-free MS				
	EC_{50} (mg L^{-1})	CI 95 %		TU	Interaction	EC_{50} (mg L^{-1})	CI 95 %		TU	Interaction
		Lower	Higher				Lower	Higher		
Sm + La 1:1	20.43^{A1}	19.38	21.53	0.976		10.14^{J1}	8.86	11.60	0.892	
	21.31^{A2}	19.20	23.65	1.019		11.02^{J2}	9.41	12.90	0.969	
	20.80^{A3}	18.33	23.60	0.994		14.13^{K1}	13.11	15.23	1.243	
			Means	0.996	Additive			Means	1.035	Additive
Sm + La 1:2	30.16^{B1}	26.69	34.07	0.967		$20.71^{A123.\ B23.\ L13}$	18.17	23.59	1.823	
	24.86^{B2}	20.34	28.94	0.797		$24.59^{A23.\ B12.3M1}$	22.19	27.25	2.164	
	26.71^{B3}	22.09	32.30	0.856		$18.19^{A123.\ L13}$	16.08	20.57	1.601	
			Means	0.873	Additive			Means	1.863	Synergistic
Sm + La 2:1	$20.32^{A123.\ C1}$	18.91	21.83	0.389		$9.57^{J12.\ N1}$	8.37	10.94	0.560	
	$19.11^{A123.\ C12}$	18.24	20.02	0.366		$10.56^{J12.\ N123}$	8.77	12.72	0.618	
	$22.68^{A23.\ B2.C13}$	20.87	24.66	0.434		$11.58^{J12.\ N23}$	10.59	12.67	0.678	
			Means	0.396	Antagonist			Means	0.619	Additive
Sm + Nd 1:1	14.96^{D1}	13.51	16.56	0.632		$12.37^{D12.\ O1}$	11.31	13.52	1.063	
	13.12^{D12}	11.84	14.55	0.554		10.03^{P1}	8.46	11.88	0.862	
	16.00^{D13}	14.75	17.36	0.678		9.39^{P2}	7.96	11.09	0.807	
			Media	0.621	Additive			Media	0.911	Additive
Sm + Nd 1:2	28.18^{E1}	21.99	36.11	0.806		$27.68^{E123.\ Q1}$	23.50	32.61	2.363	
	30.60^{E2}	27.06	34.60	0.876		$27.45^{E123.\ Q2}$	23.61	31.92	2.343	
	27.92^{E3}	24.78	31.46	0.799		$25.67^{E123.\ Q3}$	21.45	30.72	2.191	
			Means	0.827	Additive			Means	2.299	Synergystic
Sm + Nd 2:1	$25.02^{E123.\ F1}$	21.09	29.69	0.488		$24.98^{E123.\ F123.\ Q123.\ R1}$	22.39	27.86	1.462	
	$24.64^{E123.\ F2}$	20.51	29.59	0.480		$20.81^{E13.\ Q123.\ R12}$	17.03	25.42	1.218	
	$28.81^{E123.\ F3}$	24.91	33.31	0.562		$26.44^{E123.\ Q123.\ R13}$	24.56	28.46	1.548	
			Means	0.510	Additive			Means	1.409	Additive
Nd + La 1:1	11.81^{G1}	10.50	13.29	0.537		$11.18^{G123.\ S1}$	9.52	13.13	0.963	
	12.65^{G12}	11.79	13.58	0.575		$11.19^{G123.\ S2}$	9.65	12.98	0.964	
	10.25^{G13}	8.68	12.11	0.466		$11.50^{G123.\ S3}$	9.69	13.63	0.990	
			Means	0.526	Additive			Means	0.972	Additive
Nd + La 1:2	24.15^{H1}	21.59	27.01	0.827		$22.92^{H123.\ T1}$	19.61	26.78	1.989	
	25.41^{H12}	21.80	29.62	0.871		$19.14^{H12.\ T2}$	16.18	22.64	1.661	
	28.73^{H23}	25.28	32.64	0.984		$22.32^{H123.\ T3}$	19.32	25.80	1.937	
			Means	0.894	Additive				1.862	Synergystic
Nd + La 2:1	$19.59^{H12.\ I1}$	14.41	26.56	0.361		$16.17^{I123.\ S23.\ T2.U1}$	13.53	19.33	0.907	
	$23.51^{H12.\ I2}$	22.40	24.68	0.433		$12.05^{G123.\ I1.\ S23.\ U2}$	9.12	15.92	0.676	
	$23.09^{H12.\ I3}$	21.79	24.46	0.426		$13.86^{G123.\ H1.\ S23.\ T2.U3}$	9.45	20.34	0.777	
			Media	0.407	Antagonistic			Media	0.787	Additive

1:1 > 1:2 ≈ 2:1. No significant differences between the three ratios in the two media was observed ($U = 27$, $p = 0.129$). The investigated ratios presented similar toxicities concerning the binary Sm+La mixtures in both studied media, of 1:1 ≈ 2:1 > 1:2. Comparing the results observed for both media and corroborating the results of the single exposures, the MSq medium displayed greater toxicity ($t = 3.8874$, $p = 0.008$). The 1:1 ratio, the most toxic in the MS medium, was not significantly different from the 1:2 ratio in the MSq medium, the least toxic in this medium (Table 3).

No significant difference was noted for 80 % of the ternary mixtures, (Table 4). The most toxic ratios could not be determined in the MS medium, due to the 95 % CI overlap of, but the less toxic ratios were Sm+La+Nd at 1:2:2 and 2:2:1 ratios, both containing 2-fold La concentrations. The most toxic ratios could not be determined in the MSq medium as well, also due to the 95 % CI overlap. No significant difference between the two means was observed ($t = 1.4258$, $p = 0.081$).

The types of interaction between the studied elements under mixture conditions in the MS medium were 75 % additive (12), 19 % antagonistic (3) and 6 % synergistic (1) (Tables 3 and 4). In the MSq medium, 56 % were synergistic (9) and 44 %, additive (7). In MS medium, the binary mixtures displayed most of the additive interactions (Table 3), except for the Sm+La and Nd+La 2:1 ratios, both antagonistic (Table 3). Antagonism was also observed in the ternary Sm+La+Nd mixtures at a 1:1:2 ratio (Table 4). Lanthanum, the most toxic element in the single exposure assays, is apparently partially or totally inhibited by the other REE. A synergistic effect was observed in the Sm+La+Nd mixture at a 2:2:1 ratio, the only ratio with Sm and La present at higher concentrations, in which these REE seemingly potentiate the toxic effect of the mixture. Most of the binary mixtures in the MSq medium displayed additive interactions, except for the 1:2 Sm+La, Sm+Nd and Nd+La ratios, which displayed synergistic interactions. In this medium, the most toxic element in the single exposure was Nd, while La and Nd are present at 2-fold concentrations in the first and third, and in the two latest mixtures, respectively. Apparently, these 2-fold elements potentiate mixture toxicity (Tables 3 and 4). In ternary mixtures, only one mixture with all elements in single ratios presented additive interactions, while all the others were synergistic.

The type of biological response induced by mixtures, whether greater (synergism), displaying no interaction (additive) or smaller (antagonistic) than the expected REE mixture, is still a significant knowledge gap (Gong et al., 2019; Warne, 2010). The interpretation of bioaccumulation or toxicity results is complex due to binding site interactions and/or interactions between physiological processes and potential chemical interactions with medium constituents, affecting chemical speciation (free ions, ligand complexes) and bioavailability (Gong et al., 2019; González et al., 2014; Romero-Freire et al., 2019). Gong et al. (2019) consider that complex interactions that may take place in REE mixtures can be explained and modeled by bioavailability-based toxicity models, and that antagonistic interactions can be explained as a competition effect for contaminant absorption, translocation, distribution, and detoxification processes (Gong et al., 2019).

Table 4

EC_{50} 48 h results and the Concentration Addition (CA) model in Toxic Units (TU) of acute toxicity tests with synthetic lanthanum, neodymium and samarium solutions in ternary mixtures in the Metal Solution (MS) medium and chelator-free MSq medium.). 95 % CI = 95 % Confidence Interval. Equal letters = non-significant statistical difference; replicate number (1, 2 and 3) = EC_{50} similar to each other; two or more numbers (123. 12. 13. 23) = similar to another replica. TU – Toxic Unit. Additive. UT = 1. Synergistic, UT > 1 and Antagonistic, TU < 11 (Panouillères et al., 2007).

Type of medium/REE and mixtures	MS					Chelator-free MS				
	EC_{50} (mg L^{-1})	CI 95 %		TU	Interaction	EC_{50} (mg L^{-1})	CI 95 %		TU	Interaction
		Lower	Higher				Lower	Higher		
Sm + La + Nd 1:1:1	22.04^{I13}	19.92	24.38	0.862		$15.72^{\mathrm{I2.\ V23.\ XI1}}$	14.13	17.49	1.409	
	16.79^{I23}	14.36	19.62	0.853		$16.32^{\mathrm{I23.\ V123.\ XI2}}$	14.97	17.79	1.426	
	19.31^{I123}	16.67	22.37	0.847		$17.29^{\mathrm{I23.\ V123.\ VI1.\ XI3}}$	16.35	18.28	1.441	
			Means	0.854	Additive			Means	1.425	Additive
Sm + La + Nd 1:2:1	28.31^{II1}	26.42	30.33	1.066		$18.33^{\mathrm{I23.\ V123.\ VI13.\ XI23.\ XII1}}$	16.47	20.40	1.550	
	$24.66^{\mathrm{I1.\ III1}}$	21.94	27.73	1.056		$19.65^{\mathrm{I123.\ V123.\ VI13.\ XI3.\ XII2}}$	17.16	22.51	1.562	
	38.10^{IV1}	34.61	41.95	1.089		$15.88^{\mathrm{I23.\ V123.\ XI23.\ XII3}}$	13.41	18.82	1.576	
			Means	1.071	Additive			Means	1.563	Synergystic
Sm + La + Nd 1:1:2	$19.22^{\mathrm{I123.\ V1}}$	16.43	22.48	0.496		$19.84^{\mathrm{I1.\ III1.\ V123.\ VI123.\ XI1.\ XII1XIII1}}$	16.08	24.47	2.052	
	$18.35^{\mathrm{I123.\ V2}}$	14.95	22.52	0.478		$27.45^{\mathrm{I1.\ II1.\ III1.\ VI2.\ VII1.\ XI1.\ XII1.\ XIV1}}$	26.04	28.94	2.087	
	$17.32^{\mathrm{I23.\ V3}}$	14.56	20.60	0.428		$25.48^{\mathrm{I1.\ II1.\ III1.\ VI12.\ VII1.\ XI1.\ XII1.\ XIV2}}$	23.28	27.88	2.122	
			Means	0.467	Antagonistic			Means	2.087	Synergystic
Sm + La + Nd 2:1:1	$20.69^{\mathrm{I123.\ V13.\ VI1}}$	17.05	25.10	0.719		$19.19^{\mathrm{I123.\ V123.\ VI123.\ XI23.\ XII123.\ XIII1.\ XV1}}$	16.58	22.21	1.750	
	$24.59^{\mathrm{I13.\ II1.\ III1.\ V13.\ VI2}}$	20.82	29.05	0.701		$20.26^{\mathrm{I123.\ V123.\ VI123.\ XII12.\ XIII1.\ XV2}}$	17.76	23.12	1.765	
	$20.32^{\mathrm{I13.\ V13}}$	17.95	22.99	0.705		$21.44^{\mathrm{I123.\ III1.\ V123.\ VI123.\ XI3.\ XII12.\ XIII1.\ XIV2.\ XV3}}$	17.08	26.90	1.780	
			Means	0.708	Additive			Means	1.765	Synergystic
Sm + La + Nd 1:2:2	$30.11^{\mathrm{II1.\ VII12}}$	25.41	35.68	0.884		$36.27^{\mathrm{IV1.\ VII23.\ IX13.\ XVI1}}$	34.38	38.27	3.136	
	$33.86^{\mathrm{III1.\ VI2.\ VII12}}$	31.20	36.75	0.882		$38.49^{\mathrm{IV1.\ VII3.\ VIII1.\ X13.\ XVII13}}$	37.64	39.36	3.178	
	$36.59^{\mathrm{III1.\ IV1.\ VII23}}$	34.21	39.13	0.910		$35.52^{\mathrm{IV1.\ VII123.\ IX13.\ XVI12}}$	33.47	37.71	3.221	
			Means	0.892	Additive			Means	3.178	Synergystic
Sm + La + Nd 2:1:2	$39.45^{\mathrm{VII123.\ VIII1.\ IX1}}$	37.31	41.71	1.018		$23.46^{\mathrm{I1.\ III1.\ V1.\ VI12.\ XII2.\ XIII1.\ XIV2.\ XV23.\ XVII2.\ XVIII13}}$	20.53	26.81	2.188	
	$34.82^{\mathrm{VII123.\ IX23}}$	32.06	37.82	0.977		$28.24^{\mathrm{III1.\ III1.\ VI12.\ VII1.\ IX2.\ XIV12.\ XVII2.\ XVIII2}}$	24.88	32.05	2.218	
	$33.46^{\mathrm{II1.\ III1.\ IV1.\ V1.\ VII123.\ IX23}}$	29.87	37.49	0.963		$25.32^{\mathrm{I1.\ III1.\ VI12.\ VII1.\ XIV2.\ XV3.\ XVII2.\ XVIII123}}$	24.29	26.39	2.248	
			Means	0.986	Additive			Means	2.218	Synergystic
Sm + La + Nd 2:2:1	43.07^{X1}	40.65	45.63	1.559		$31.67^{\mathrm{VII12.\ IX12.\ XVIII2.\ XIX1}}$	28.29	35.44	2.823	
	$40.40^{\mathrm{VII23.\ VIII1.\ X2}}$	36.95	44.18	1.653		$32.66^{\mathrm{VII12.\ IX12.\ XVIII2.\ XIX2}}$	30.76	34.68	2.842	
	$40.98^{\mathrm{III1.\ IV1.\ VII23.\ VIII1.\ X3}}$	36.79	45.65	1.648		$33.45^{\mathrm{VII12.\ XVI1.\ IX12.\ XVII12.\ XIX3}}$	32.35	34.70	2.861	
			Means	1.620	Synergystic			Means	2.842	Synergystic

Our findings are similar to those reported in an acute toxicity study performed by Bergsten-Torralba et al. (2020) on *D. similis*. In single exposures, although toxicity decreased in the order Nd $>$ La $>$ Sm, the EC_{50} 48 h results for La (12.92 mg.L^{-1} – 95 % CI = 10.26–16.28) and Nd (9.41 mg.L^{-1} – 95 % CI = 8.94–9.91) were similar to those obtained for the MSq medium in the present study (Table 3). Similar findings were also observed for the binary Nd+La (12.56 mg.L^{-1} – 95 % CI = 11.43–12.55) mixtures in the MS medium and the Sm+Nd mixtures (10.09 mg.L^{-1} - 95 % CI = 8.86–11.50) in the MSq medium. The ternary mixture results for La+Nd+Sm (17.26 mg.L^{-1} – 95 % CI = 15.75–18.91) were also similar to the MSq medium findings reported herein (Table 4). However, the additive, antagonistic or synergistic interaction results were different from those reported herein.

Sneller et al. (2000) reported that toxic REE mechanisms of action consist of mainly Ca/Mg competitions and/or substitutions, as well as a phosphate deficiency for phosphate-REE precipitations. On the other hand, excess Ca^{2+} ions in a certain medium can directly compete with La^{3+} ions for binding sites and, thus, reduce La toxicity towards *Daphnia* spp (Barry and Meehan, 2000). When assessing La, Gd and Y bioconcentrations by *Chlorella vulgaris*, Hao et al. (1997) observed that REE adhesion to superficial cell membrane sites (comprising functional groups of ligand proteins with high affinity) would be a first step prior to cell entry. Thus, the REE bioconcentration values in the algae would be proportional to the amount of REE adhered to the wall, which in turn would be directly associated with the amount of free ions in the external environment (Hao et al., 1997).

A toxicity hardness effect was reported by Barry and Meehan (2000) in their study on acute and chronic La toxicities towards *D. carinata*, where La was more toxic in dechlorinated tap water (hardness 22 mg $CaCO_3$. L^{-1}, pH 7.8) with a 48 h acute EC_{50} of 43 μg La.L^{-1}, in a growth medium (DW) containing sea salt (hardness 98 mg $CaCO_3$. L^{-1}, pH 7.8), with an EC_{50} 48 h of 49 μg La.L^{-1}, and less toxic in an ASTM medium (hardness 160 mg $CaCO_3$. L^{-1}, pH 7.5) with an EC_{50} 48 h of 1180 μg La. L^{-1}. In the present study, the hardness of the MS and MSq media used as dilution water in the test solutions were adjusted to between 40 and 48 mg $CaCO_3$. L^{-1} (ABNT, 2016) and pH values were adjusted to 6.0 ± 0.5, lower than in the aforementioned study. Lower EC_{50} toxicity values were also verified, of 16.89, 17.85 and 18.08 mg.L^{-1} in the MS medium and of 13.26, 15.37 and 16.05 $mg.L^{-1}$ in the MSq medium, probably due to differences in the applied media, species and parameters.

In their study on the acute and chronic toxic effects of La and Nd on *Daphnia magna* in a synthetic medium of artificial freshwater and in natural samples obtained from two lakes, Blinova et al. (2018) reported higher toxicity in the synthetic medium compared to the natural samples, with nominal acute EC_{50} 48 h values of 31.1 ± 9.1 mg $La.L^{-1}$ and 20.8 ± 3.8 mg $Nd.L^{-1}$ in the synthetic medium. Our results indicated higher La toxicity values, of 16.89, 17.85 and 18.08 $mg.L^{-1}$ in the MS medium and of 13.26, 15.37 and 16.05 $mg.L^{-1}$ in the MSq medium, as well as for Nd, of 23.59, 26.25 and 26.45 $mg.L^{-1}$ in the MS medium and 8.55, 9.19 and 9.84 $mg.L^{-1}$ in the MSq medium, also probably due to differences in the applied media, species and parameters.

4. Conclusion

Acute toxicity tests were carried out herein with three lanthanides, La, Nd and Sm, in single and in binary and ternary mixture exposure assays at different ratios, a still understudied field. Acute toxic effects were observed in both MS and MSq media, the latter without chelators EDTA and cyanocobalamin. Differences were observed between the elements in both the single exposure assays and in mixtures, and observed interactions were additive, antagonistic, and synergistic in nature.

Higher toxicity values were observed in the MSq medium compared to the MS medium in the individual assays and in the three binary Sm+La mixture ratios, probably due to the lower presence of inorganic complexes in the presence of chelators, resulting in higher free REE ion concentrations. This indicates that the use of dilution water with chelators should be avoided in toxicity tests, as it may influence REE bioavailability.

The toxicity order of the studied REEs seems to be determined by the media. In the MS medium, La was the most toxic, while in the MSq medium, Nd was the most toxic. Sm toxicity values were intermediate in both media. Higher Nd toxicity was also observed in natural dilution water. Most interactions were additive, followed by synergistic and antagonistic. Regarding antagonistic interactions, La toxicity in the binary mixtures in the MS medium seems to have been inhibited by 2-fold Sm and Nd concentrations, while Sm toxicity seems potentiated in the synergistic interaction in ternary mixtures. Concerning the synergistic binary mixtures, the toxicity of Nd and La when present at 2-fold concentrations seems to be potentiated. These findings can improve the understanding of environmental REE effects, contributing to the establishment of future guidance values and ecological risk calculations.

CRediT authorship contribution statement

Silvia G. Egler: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft and Writing – review & editing, Visualization, Supervision, Project administration. **Tamine M. Roldão:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation. **Gabriel O. Santos:** Validation, Investigation, Data curation. **Gisele P. Heidelmann:** Validation, Investigation, Data curation. **Ellen Cristine Giese:** Conceptualization, Methodology, Writing – review & editing. **Fabio V. Correia, Enrico M. Saggioro:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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