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Long-term toxicity of lanthanum to amphipods *Hyalella azteca*

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ABSTRACT: The impact of rare-earth elements (REEs) on living organisms is still unexplored. This study aimed to determine the toxicological properties of lanthanum (La) under prolonged exposure to the benthic amphipod *Hyalella azteca*, Saussure. The artificially created environmental condition models were water with La spiking in concentrations ranging from 0.16 to 160 μmol L^{-1} (mass concentrations of 0.0006 - 0.6 mg L^{-1}) as $La_2(SO_4)_3 \cdot 8H_2O$. The observation period lasted 40 days. Based on mortality rates, La concentrations of 160 and 16 μmol L^{-1} (22.4 and 2.24 mg L^{-1}) were toxic in the acute experiment. In contrast, concentrations of 1.6, 0.8 and 0.16 μmol L^{-1} (0.224, 0.112, 0.022 mg L^{-1}) exerted delayed chronic toxic effects on *H. azteca*. The linear dimensions and body mass of the amphipods increased in response to La concentrations of 0.16 and 0.8 μmol L^{-1}, but further increases of metal concentrations reduced the parameters compared to lower concentrations. In all test groups except 0.16 μmol L^{-1}, a significant increase in food consumption (biomass of *Acer platanoides* L.) was observed compared to the control. This effect is attributed to the compensation of toxic stress through increased feeding activity. *H. azteca* exhibits adverse effects of La exposure. Mortality and morphological parameters serve as the most sensitive test endpoints for these amphipods.
Keywords: bioassay; survival; feeding behavior; chronic toxicity.

Toxicidade do lantânio para anfípodes *Hyalella azteca* com exposição prolongada

RESUMO: Muitas questões sobre os efeitos dos elementos de terras raras sobre organismos vivos ainda não foram estudadas. O objetivo deste trabalho foi determinar as propriedades toxicológicas do lantânio em exposição prolongada em anfípodes bentônicos *Hyalella azteca* Saussure. Os meios-modelo foram água com aditivo La na faixa de concentração de 0,16 - 160 μmol L^{-1} (concentrações em massa de 0,0006 - 0,6 mg L^{-1}) na forma de $La_2(SO_4)_3 \cdot 8H_2O$. A duração das observações foi de 40 dias. Em termos de mortalidade, as concentrações de La de 160 e 16 μmol L^{-1} (22,4 e 2,24 mg L^{-1}) foram tóxicas no experimento agudo, e as concentrações de La de 1,6, 0,8 e 0,16 μmol L^{-1} (0,224, 0,112 e 0,022 mg L^{-1}) tiveram efeito tóxico crônico tardio na *H. azteca*. O tamanho linear e o peso dos crustáceos aumentaram em resposta às concentrações de La de 0,16 e 0,8 μmol L^{-1}, elevando ainda mais a concentração do metal em comparação com concentrações mais baixas. Em todas as variantes, exceto 0,16 μmol L^{-1}, houve um aumento significativo no consumo de alimentos (biomassa de *Acer platanoides* L.) em comparação com o controle, devido à compensação do estresse tóxico pela nutrição. Assim, o *H. azteca* experimenta os efeitos negativos do La. A morte e os parâmetros morfológicos são as funções de teste mais sensíveis nesses crustáceos.
Palavras-chave: bioensaio; sobrevivência; comportamento alimentar; toxicidade crónica.

1. INTRODUCTION

Rare earth elements (REEs) are a family of 17 chemical elements from group III of the short-form periodic table. Based on their chemical properties and co-occurrence in nature, they are divided into the yttrium group (Y, La, Gd–Lu) and the cerium group (Ce–Eu), while by atomic mass, they are classified as light (Ce–Eu) and heavy (Gd–Lu) lanthanides. Lanthanum is a silvery-white soft metal with an atomic mass of 138.9 amu, an ionic radius of 1.061 pm. It actively reacts with oxygen and water, forming an oxide film on its surface (JADHAV et al., 2025). With an average crustal abundance of 35 mg/kg, lanthanum is relatively abundant in the Earth's crust compared to many heavy metals and other REEs (TAO et al., 2020). It occurs primarily in complex minerals, such as monazite and bastnäsite, where lanthanum accounts for 25% and 38% by mass, respectively (ZHENG et al., 2022).

The active use of REEs since the mid-20th century, including lanthanum, has led to large-scale exploration and development of corresponding mineral resources worldwide. Due to their unique physicochemical properties, REEs are used in the production of automotive catalytic converters, ceramics, glass additives and polishing agents, battery alloys, phosphors and fluid catalytic cracking. The physicochemical properties of lanthanum have found applications in many fields, such as the production of nickel-metal hydride batteries, high-strength and conductive copper alloys, potentiometric chlorine sensors, high-temperature lanthanum zirconate-based thermal barrier coatings, petroleum cracking catalysts, electron-dense indicators in molecular biology, and even lanthanum carbonate has been proposed as a pharmaceutical agent (ZHI et al., 2020). Thus, the demands of modern industries have intensified REEs mining and global trade, which, according to experts, will only increase in the coming decades (BLINOVA et al., 2018; FIGUEIREDO et al., 2022).

The extraction and application of REEs inevitably lead to their significant release into the environment and pose threats to aquatic ecosystems. Natural waters in REEs mining areas contain their concentrations ranging from nanomolar to millimolar levels (LIU et al., 2019; WANG et al., 2022). Although knowledge in the field of REE ecotoxicology has significantly expanded over the past decade, uncertainty remains regarding their actual hazard and risk to freshwaters. However, international environmental regulations still lack established safe concentration limits for REEs. For drinking water, maximum allowable concentrations (MACs) have been officially defined only for europium (Eu, 0.3 mg L^{-1}) and samarium (Sm, 0.024 mg L^{-1}) (SANPIN 2.1.4.1074-01, 2010). Based on the analysis of available data, a standard for the maximum safe content in fresh water of 4 µg L^{-1} has been proposed for La (HERRMANN ET AL., 2016).

Data on the correlation between individual REEs and responses in standard laboratory tests, as well as predictable toxicity patterns (e.g., expressed as LC50 values), remain limited. Consequently, lanthanum compounds should be classified as substances requiring detailed investigation of their ecotoxicological properties.

This paper aims to evaluate the range of response reactions in the amphipod *Hyalella azteca* to increasing concentrations of lanthanum under chronic exposure conditions.

2. MATERIALS AND METHODS

The study used lanthanum sulfate octahydrate $La_2(SO_4)_3 \cdot 8H_2O$ (the purity of the reagent is 99.5%). Test concentrations in the range of 0.16-160 µmol L^{-1} (equivalent to 0.022-22.4 mg L^{-1}) were prepared by serial dilution of a saturated $La_2(SO_4)_3$ solution with a concentration of 160 µmol L^{-1} using tap water (pH 7.0-7.5, total hardness 4.0-4.5 meq L^{-1} Ca^{2+} and Mg^{2+}, concentration of heavy metals, natural anions and cations within the limits of Russian and international standards for natural waters). The same water was used as a control. The solubility of lanthanum in water is 2.33 g/100 cm^3 at 20 °C (Available at: http://dictionary.sensagent.com/Solubility_table/en-en/).

The concentration range was selected based on published data regarding the effects of lanthanum sulfate on survival, lifespan, growth, development, and fecundity in cladocerans (LOZHKINA; TOMILINA, 2016; SYSOLYATINA; OLKOVA, 2022).

The amphipod *Hyalella azteca*, Saussure, 1858, from laboratory culture was used as the test organism. Experiments were conducted according to standard methodology (Ingersoll; Nelson, 1990; Rybina et al., 2019), with additional parameters being evaluated. Neonatal amphipods (1-3 days old) were sieved through the Standard Sieve Series, USA No. 30 (600 µm) and No. 50 (300 µm) to separate specimens not exceeding 3 mm in size. Juveniles were individually collected using a 2 cm^3 pipette and placed in glass chemical beakers (10 individuals per 200 mL of test medium). The animals were fed with maple leaves (*Acer platanoides* L., 1753) 1.8 cm in diameter. The leaves were pre-soaked in water to remove tannins. Three leaves were provided per beaker. The experiment duration was 40 days.

Mortality was assessed daily by the complete absence of swimming. Control group mortality (7%) met methodological requirements. Test solutions and consumed maple leaves were replaced with fresh ones every 10 days. During each water change, the linear dimensions of surviving specimens and the post-consumption mass of maple leaves were measured. Trophic activity was evaluated as a percentage reduction in maple leaf mass relative to initial mass. Laboratory scales VSL-60 (Russia) with an accuracy of 0.001 g were used. Amphipod body dimensions were measured under a binocular microscope Micromed MC-2-ZOOM (Russia) on days 14, 28 and 40. Body length was measured from the base of the first antenna to the end of the last abdominal segment's appendage. Individual mass of surviving amphipods was determined from linear measurements using the formula (INGERSOLL et al., 2008):

$$M = (0.177 \times L - 0.0292)^3 \quad (01)$$

where: M is mass, in mg; and L is length, in mm.

The experiments were conducted in triplicate. Optimal environmental conditions were maintained: water temperature 22 ± 1°C, pH 7.5-8.0, dissolved oxygen at saturation level through forced aeration, and light regime (920 lux) with daylight lamps (16 h light and 8 h dark). The control group of test organisms was kept under identical conditions in tap water without La addition.

Results are presented as mean X ± SD, where X is the mean value for the entire experimental period and SD is the standard deviation. Statistical significance of differences was assessed by analysis of variance (ANOVA, LSD test) at p = 0.05 (SOKAL; ROHLF, 1995). Correlation analysis between studied parameters that did not show normal distribution (Shapiro-Wilk test) was performed using the non-parametric Spearman's coefficient (rs, p=0.05).

3. RESULTS

In the acute experiment, the concentration causing 50% mortality of amphipods after 48-hour exposure was determined as LC_{50} = 100 µmol L^{-1}. Mortality of *H. azteca* in La-containing solutions consistently increased with rising concentration. Acute toxic effects of La were recorded at 160 µmol L^{-1} on day 2 of the experiment. By day 14, 100% mortality of amphipods was observed at concentrations of 16 and 160 µmol L^{-1}. At the experiment's conclusion, minimal mortality values (close to control levels) were recorded in the 0.16 µmol L^{-1} solution.

The minimal amphipod sizes on days 14, 28 and 40 of the experiment were recorded in the control group. In all experimental solutions, this parameter was higher (Figure 2). This pattern was first observed on day 14 at high concentrations of 160 and 16 µmol L^{-1}, which subsequently demonstrated maximal toxic effects. This phenomenon is likely attributable to the competitive and trophic advantages of surviving amphipods. The maximal amphipod sizes compared to controls were recorded in solutions with La 0.8

µmol L^{-1} concentration. At day 14, specimens in the lowest concentration solutions showed no size differences from controls, though a growth trend was noticed. By experiment's end, these differences became statistically significant.

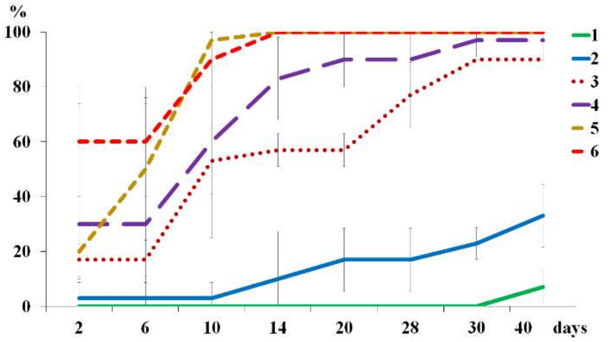

Figure 1. Effect of La exposure on mortality of *H. azteca* under chronic experiment conditions (1: control; 2: 0.16 µmol L^{-1}; 3: 0.8 µmol L^{-1}; 4: 1.6 µmol L^{-1}; 5: 6 µmol L^{-1}; 6: 16 µmol L^{-1}; 7: 160 µmol L^{-1}).
Figura 1. Efeito da exposição a La na mortalidade de *H. azteca* em condições crônicas de experimento (1: controle; 2: 0,16 µmol L^{-1}; 3: 0,8 µmol L^{-1}; 4: 1,6 µmol L^{-1}; 5: 6 µmol L^{-1}; 6: 16 µmol L^{-1}; 7: 160 µmol L^{-1}).

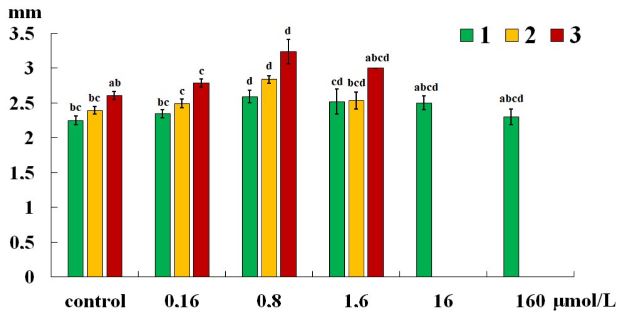

Figure 2. Effect of La exposure on the dynamics of linear dimensions of *H. azteca*. (1: 14 days; 2: 28 days; 3: 40 days).
Figura 2. Efeito da exposição a La na dinâmica das dimensões lineares de *H. azteca*. (1: 14 dias; 2: 28 dias; 3: 40 dias).

The maximum total biomass of surviving specimens was recorded in the control group. The biomass gradually decreased with increasing La concentration (Table 1).

Table 1. Weight of surviving individuals of *H. azteca* under La exposure at the end of the experiment*.
Tabela 1. Peso dos indivíduos sobreviventes de *H. azteca* expostos a La no final do experimento*.

La concentration (µmol L^{-1})	Quantity (pcs)	Weight (mg)	Weight of one individual (mg)
0.16	7	2.04	0.10 ± 0.02^{a}
0.8	3	0.49	0.16 ± 0.05^{b}
1.6	1	0.13	0.13 ± 0^{ab}
16	0	–	–
160	0	–	–
Control	27	2.61	0.09 ± 0.03^{a}

Note. "*" data are given at the end of the experiment, «-» – 100% 100% mortality of individuals was observed in the solutions. Here and in Table 2, "a, b, c" are letter indices of statistically significant differences at p<0.05.

When calculating mass per individual, the maximum statistically significant values were recorded at the 0.8 µmol L^{-1} concentration. The lowest mass values per individual were observed both in the control group and at the 0.16 µmol L^{-1} concentration (Figure 3).

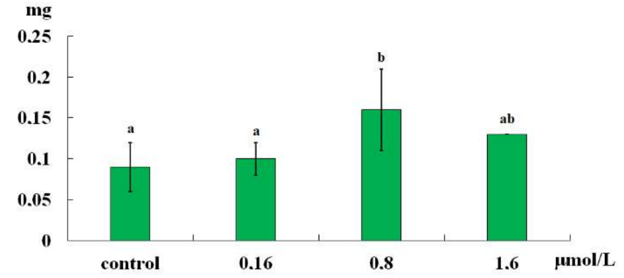

Figure 3. Mass of one surviving individual of *H. azteca* under La exposure at the end of the experiment. *Different letters mean statistically significant differences in the linear sizes of amphipods in experimental groups from control values at p <0.05.
Figura 3. Massa de um indivíduo sobrevivente de *H. azteca* exposto a La no final do experimento. *Letras diferentes indicam diferenças estatisticamente significativas nos tamanhos lineares dos anfípodes entre os grupos experimentais e os de controle, com p < 0,05.

The amphipods showed gradually increasing trophic activity from day 10 to day 20 of the experiment (Table 2). On day 10, statistically significantly higher leaf consumption was observed at the higher concentrations (16 and 160 µmol L^{-1}) compared to the control. By day 20, increased feeding activity was recorded for all concentrations except 0.16 µmol L^{-1}. No statistically significant differences in trophic activity were found between groups on day 30. On day 40, significantly elevated trophic activity was noted at 0.8 and 1.6 µmol L^{-1} concentrations. The lowest tested concentration (0.16 µmol L^{-1}) showed no effects on average trophic activity throughout the experiment. Stepwise increases in La concentration consistently led to progressively greater leaf consumption.

Table 2. Effect of La on the dynamics of trophic activity of *H. azteca* under chronic experimental conditions.
Tabela 2. Efeito do La na dinâmica da atividade trófica de *H. azteca* em condições experimentais crônicas.

La concentration (µmol L^{-1})	Eaten leaf weight (%)			
	10 сут	20 сут	30 сут	40 сут
0.16	42.7 ± 4.6^{ab}	40.3 ± 4.3^{ab}	57.0 ± 0.6^{ab}	66.0 ± 5.1^{a}
0.8	38.0 ± 9.5^{ab}	67.0 ± 18.5^{c}	63.3 ± 2.4^{ab}	86.7 ± 3.3^{b}
1.6	41.3 ± 5.6^{ab}	66.7 ± 1.2^{c}	64.3 ± 3.8^{ab}	87.0 ± 5.0^{b}
16	61.7 ± 0.3^{cd}	72.3 ± 4.7^{c}	–	–
160	72.7 ± 2.8^{d}	69.3 ± 4.6^{c}	–	–
Control	40.0 ± 6.0^{ab}	34.7 ± 3.7^{a}	63.0 ± 3.6^{ab}	71.7 ± 2.2^{ab}

Throughout the experiment, the lowest trophic activity was observed in both the control group and at the minimally effective La concentration. In contrast, peak activity occurred at 0.8 µmol L^{-1} (Figure 4). Although *H. azteca* exhibited high feeding rates at 16 and 160 µmol L^{-1}* - comparable to those at 0.8 and 1.6 µmol L^{-1} - the daily leaf consumption percentage remained low in these higher concentrations due to high mortality of test organisms.

4. DISCUSSION

Our study used La concentrations that are currently found in natural and man-made environments. The highest concentrations of La are found in reservoirs located near REE mining areas. In particular, water samples from former mine lakes and a river in the Kinta Valley, Perak State, Malaysia, measured 17.77 and 11.51 mg L^{-1} (KHAN et al., 2016). Near the production of catalysts, the La content is even higher – 52 mg L^{-1} (KLAVER et al., 2014). The La content in urban wastewater has been reported at the level of 4.1 μg L^{-1} (ATINKPAHOUN et al., 2020). In the surface waters of urbanized territories, the La content is recorded in the range of 0.001 - 0.05 μg L^{-1} (AMORIM et al., 2019; STRAKHOVENKO et al., 2023).

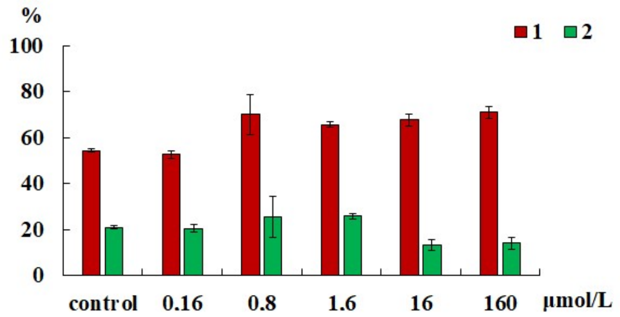

Figure 4. Effect of La on the trophic activity of *H. azteca*. (1: 40 days; 2: 1 day).
Figura 4. Efeito do La na atividade trófica de *H. azteca*. (1: 40 dias; 2: 1 dia).

The study results confirm the concentration-dependent mortality of *H. azteca* in response to increasing lanthanum concentrations. Although lanthanum sulfate undergoes weak hydrolysis in water, forming sparingly soluble lanthanum hydroxide, the Spearman correlation coefficient ($rs=0.94$, $p=0.005$) indicates high La bioavailability. This effect is likely attributable to their lifestyle. In natural habitat, *H. azteca* is primarily epibenthic, derives little nutrition from the sediments, and responds primarily to contaminants in the overlying water column (including water and food) (WANG et al., 2004). As detritivores, they consume organic matter known to accumulate contaminants (SADCHIKOV; OSTROUMOV, 2017).

Our studies demonstrated that La concentrations of 160 and 16 μmol L^{-1} (22.4 and 2.24 mg L^{-1}) were the most toxic. In contrast, concentrations of 1.6, 0.8 and 0.16 μmol L^{-1} (0.224, 0.112 and 0.022 mg L^{-1}) exerted delayed chronic toxic effects on *H. azteca* mortality. The significantly higher sensitivity of crustaceans compared to nematodes, annelids, fish, and protozoans to La exposure was confirmed (HERRMANN et al., 2016). For comparison, LC50 values ranged from 0.04 mg L^{-1} for the crustacean *Daphnia carinata* King, 1852 (most sensitive) to 278 mg L^{-1} for the ciliate Tetrahymena shanghaiensis Feng et al., 1988 (most resistant). The lethal concentrations we established are comparable to values reported in (Borgmann et al., 2005; Herrmann et al., 2016), where the 168-hour (7-day) LC50 for *H. azteca* was 0.018 mg L^{-1}. Consequently, amphipods exhibit lower resistance to lanthanum exposure compared to daphnids, though falling within similar concentration ranges.

The study results demonstrate that mortality-based bioassays show limited ecological relevance (FILENKO; TEREKHOVA, 2016). Chronic toxicity experiments prove far more informative, as they reveal sublethal biological responses at environmentally realistic contamination levels (JOHNSON et al., 2000; OLKOVA; MAHANOVA, 2018).

The results of *H. azteca* size measurements initially appeared counterintuitive. Most experimental groups showed progressive growth trends, with some exhibiting statistically significant increases compared to controls. This phenomenon may be partially explained by reduced food competition. A study with *Daphnia magna* Straus, 1820 demonstrated that increased per-capita habitat space enhances multiple biological parameters (OLKOVA et al., 2018). As established in the seminal work (Odum, 1986), abiotic and biotic factors always interact synergistically: mitigation of one stressor (population density) can increase organismal resilience to another (chemical exposure).

The Spearman correlation coefficients between concentration and linear dimensions were statistically non-significant at 0.23, 0.80, and 0.80 for days 14, 28, and 40 of the experiment, respectively. Therefore, the reduced population density cannot fully compensate for the toxic effects of La. The growth of amphipods was partially constrained by La presence in water, as evidenced by comparing the effects of 0.8 and 1.6 μmol L^{-1} solutions. The maximum body sizes, significantly different from controls, were recorded at the lower concentration (0.8 μmol L^{-1}), despite more optimal spatial and trophic conditions at 1.6 μmol L^{-1}. Similar growth inhibition patterns for *H. azteca* were reported for other metals (As, Co, and Mn) after 4-week exposures (NORWOOD et al., 2007).

The calculated amphipod mass values carry important implications for predicting the ecological effects of La contamination in aquatic ecosystems. While individual mass changes in response to La exposure were minor, the total biomass assessment of *H. azteca* at day 40 revealed that benthic biomass reduction may represent a likely ecological consequence of La exposure in natural environments. Similar effects were observed with dysprosium (another REE) exposure, showing decreased average weight of *H. azteca* by day 28 (LU, 2016). These findings may ultimately trigger cascading effects on ecosystems, as demonstrated for the herbicide simazine (WANG et al., 2021).

Based on the obtained correlation coefficients, body length and mass of *H. azteca* emerge as highly informative endpoints for La toxicity assessment, alongside mortality.

Trophic activity serves as a holistic indicator of organismal viability, while adequate food availability and consumption capacity may play a compensatory role against toxic stress (GAD, 2016). This phenomenon was evident in our assessment of La effects on amphipods: all concentrations except 0.16 μmol L^{-1} triggered statistically significant increases in food consumption relative to controls. Notably, in 160 and 16 μmol L^{-1} treatments – where population numbers were lowest due to high mortality – these trophic responses were particularly pronounced, underscoring their biological significance.

5. CONCLUSIONS

The study demonstrates that lanthanum exhibits lower toxicity compared to many metals (particularly heavy metals). However, aquatic organisms respond to its presence through morphophysiological alterations, as evidenced in our *H. azteca* experiments. Certain responses – including mortality and population biomass reduction – may initiate cascading ecological effects, ultimately impacting aquatic community structure and biodiversity of water bodies.

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Conflict of interest: The authors declare that they have no conflict of interest.

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