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Effects of environmental concentrations of gadolinium on adults and juveniles of the earthworm *Lumbricus terrestris*

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ABSTRACT

Rare earth elements (REEs), including gadolinium (Gd), are increasingly released into the environment because of their widespread use in medical imaging, electronics, and renewable energy technologies. Despite growing concerns over their accumulation in soil ecosystems, the effects of Gd on terrestrial organisms are poorly understood. To address this gap, we evaluated the toxic effects of Gd on the soil organism *Lumbricus terrestris* at both the adult and juvenile stages. Adult earthworms were exposed for 28 days to 1 mg/kg or 10 mg/kg Gd to assess both acute and sublethal effects, including cellular and oxidative stress, neurotoxicity, growth and reproductive performance. The offspring were exposed to 1 mg/kg of Gd for 28 days, followed by an additional 28 days of exposure to 5 mg/kg, and the same sublethal parameters were assessed. The results revealed a low accumulation of Gd in adults and a lack of acute and sublethal effects in Gd-treated worms, except for an increase in lysosomal membrane destabilization. Juveniles were more susceptible, showing increased growth and glycogen content. Upon exposure to Gd, catalase activity was inhibited, whereas acetylcholinesterase activity increased. The effects on glycogen and catalase were exacerbated in juveniles exposed to relatively high Gd concentrations. Overall, the results indicate that *L. terrestris* is susceptible to Gd exposure, highlighting the need for further research on its long-term effects.

1. Introduction

Rare earth elements (REEs) consist of 15 lanthanides belonging to group III B of the periodic table (Hoshino et al., 2016). They are widely used across multiple applications, including agriculture, medicine, automotive manufacturing, and metallurgy. They are also essential in advanced electronics and optical devices, such as hard disk drives, display screens, and camera lenses, as well as in renewable energy technologies, including batteries, magnets for hybrid vehicles, and wind turbines (Arciszewska et al., 2022). The growing demand for REEs inevitably leads to their widespread release in the environment, where their concentration is expected to increase (Lima and Ottosen, 2021). Rare earth elements can reach the soil environment through different routes: the deposition of industrial atmospheric emissions, mining processes, solid and electronic wastes, recycling plants, road runoff, the spread of fertilizers and irrigation with contaminated wastewaters (Gwenzi et al., 2018; Lima and Ottosen, 2021). Moreover, these elements can migrate through the soil and surface waters, expanding to other areas far from their primary inputs (Aubert et al., 2002).

Among REEs, gadolinium (Gd) has various applications such as in control rods for nuclear energy plants and to make garnets for microwaves. Gadolinium is used mainly in contrast agents for clinical imaging. After being injected into the bloodstream in Gd-based pharmaceuticals, Gd is excreted mainly through the urine. It can reach the aquatic environment directly (Kulaksız and Bau, 2007), almost unaltered, because biological wastewater treatment plants are unable to remove it from the final effluent (Kümmerer and Helmers, 2000). Moreover, no *ad hoc* collection systems for Gd-based contrast agents are currently available in hospitals (Kümmerer and Helmers, 2000).

Currently, Gd can be detected in surface waters from Europe and North America at concentrations as high as hundreds of ng/L (Rogowska et al., 2018). Increasing levels of Gd have also been recorded in drinking

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water (Brünjes and Hoffman, 2020; Wysocka et al., 2023).

The levels of Gd measured in soil and sediment in different areas of the world range from 0.71 mg/kg to 16.8 mg/kg, as shown in Table S1, whereas levels up to 42.1 mg/kg have been detected in abandoned mines (Brouziotis et al., 2024). Few studies have shown that Gd can affect the growth of plant species (Tai et al., 2010; Liu et al., 2021). Its accumulation in plants can reach levels <2 μg/kg dry weight, but in the case of lichens, its concentration can increase to 500 μg/kg dry weight (Emsley, 2011). In summary, the effects of Gd on the soil ecosystem are largely unexplored. Conversely, the toxicity of Gd to several aquatic taxa has been investigated (Tai et al., 2010; González et al., 2015; Martino et al., 2017; Hanana et al., 2017; Henriques et al., 2019; Trapasso et al., 2021; Cunha et al., 2022; Cesarini et al., 2024; Piarulli et al., 2024), as summarized in Albarano et al. (2025). Furthermore, to date, most of the research conducted on REEs has focused on the short-term effects of these contaminants, whereas the impacts on different life stages and on parameters such as growth and reproduction are understudied (Egler et al., 2022).

To fill this knowledge gap, it is of primary importance to acquire information on the bioavailability and bioaccumulation of Gd in terrestrial organisms as well as on its potential acute and sublethal effects. Therefore, this study aimed to evaluate the bioaccumulation of Gd and its effects on *Lumbricus terrestris* (Linnaeus, 1758). Given their role as ecosystem engineers, earthworms are keystone organisms in terrestrial ecosystems because they contribute to several soil processes, such as soil formation, litter decomposition, nutrient cycling and soil pore creation (Blouin et al., 2013). They also provide valuable ecosystem services, such as waste recycling, water regulation and pollution control (Keith and Robinson, 2012). *Lumbricus terrestris* is widely used in ecotoxicological studies to test the effects of different types of environmental pollutants (Huerta Lwanga et al., 2016; Šrut et al., 2017; Puddepatt et al., 2022; Montalvão et al., 2024). In particular, this species is prone to accumulate metals and metalloids and is susceptible to their toxic effects (Lévèque et al., 2013).

The hypotheses beyond this study were as follows: i) adults of *L. terrestris* accumulate Gd and are susceptible to adverse effects; ii) juveniles of *L. terrestris* are more susceptible to the toxicity of Gd; iii) acclimatization to Gd might occur in juveniles; and iv) the effects in juveniles can be recovered once Gd is removed from the soil. To answer these questions, both adults and juveniles were exposed to environmental concentrations of Gd (Fig. S1). Mortality, reproduction and different sublethal effects were evaluated by means of the application of a biomarker suite related to cytotoxicity, metabolism, oxidative stress and damage, neurotoxicity and reproductive performance.

2. Materials and methods

2.1. Earthworm rearing and in vivo exposures

The adult specimens of *L. terrestris* were provided from a laboratory culture reared at the Helmotz Zentrum Münken. Organisms were kept in stable temperature conditions (21 ± 1 °C), pH (6.5–7.0) and humidity (about 40%), inside a compost bin. They were provided with food and water supplies weekly.

Adult earthworms of at least 3 months old, presenting the clitellum and a wet mass between 250 and 600, mg were used. Before the start of the experiment, the earthworms were placed inside a tank with standardized soil (LUFA SP2.2 Speyer Germany) for one week ensuring acclimation. After this period, organisms were distributed in different tanks and exposed to the following condition for 28 days: control (CTRL), Gd1 (1 mg/kg of Gd) and Gd10 (10 mg/kg Gd) being representative of real soil scenarios. The gadolinium standard (Gd_2O_3 99.95 % purity) was purchased via Scharlab (Italy). The experimental conditions were in accordance with OECD protocol 222 (OECD, 2016). Three replicates for each treatment were used containing 6 animals each. As shown in Fig. S1, in the first experiment, the organisms were exposed to 1 mg/kg. Animals were weighed every 7 days. At the end of 28 days (t28), the earthworms were collected, weighed and observed under an optical microscope and subsequently kept for 24 h, to purge their intestinal contents. During the purging phase, coprolites were collected to measure any Gd loss via casts. After the depuration phase, organisms were snap frozen in liquid nitrogen and kept at −80 °C for subsequent biomarker analysis. In addition, after the first 28 days the eggs were left in the tanks lasting the exposure for another 28 days. Juveniles were recovered heating the tank up to 60 °C according to the OECD 222 protocol, counted and weighted. Subsequently, the offspring was divided into two equal sub-groups, one aliquot from each tank was frozen for further biomarker analysis, as done for adults, while another aliquot was divided into four groups: C-C, C-Gd, Gd-C and Gd-Gd (where C stands for control and Gd stands for Gd at 5 mg/kg, and the code before - indicates the origin, while the one after indicates the destination). This phase lasted 28 days.

A second experiment was carried out exposing worms to 10 mg/kg, considered a worst-case environmental scenario, representative of mining areas. Worms were kept for 28 days in the same conditions described above. At the end of day 28, the earthworms were processed as already described and both organisms and coprolites were collected. Experiments considering Gd1 and Gd10 were carried out in series.

At time zero (t0, start of the experiment) and t28, an aliquot of soil (50 g) was collected from each tank and stored at −20 °C for Gd chemical determination. At the end of day 28, six adults from each treatment were also collected and stored at −20 °C for Gd bioaccumulation.

2.2. Cytotoxicity

The coelomic fluid of 3 organisms for each treatment was collected in LBSS (Lumbricus Balanced Salt Solution pH 7.3) from each treatment and the Neutral Red retention time test (NRRT) was conducted following the Lowe et al. (1995) method. The slides were observed at 30 min intervals, until 50% of hemocytes are destabilized, or upon reaching 120 min.

2.3. Biochemical markers

An aliquot (about 0.5 g) of post-clitellar portion was collected from each earthworm and homogenized in 100 mM potassium phosphate buffer (added with KCl 100 mM, EDTA 1 mM, dithiothreitol 1 mM and protease inhibitors 1:100 v/v, pH 7.4). Samples were homogenized using the QIAGEN® TissueLyser II, through three cycles of 30 s and with a maximum frequency of 30/s. Then, the samples were centrifuged for 20 min at 4 °C at 15,000*g*. The following biochemical biomarkers were analyzed on the supernatant: protein and glycogen (GLY) content, content of reactive oxygen species (ROS), activity of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), activity of detoxifying enzyme glutathione-S-transferases (GSTs) and acetylcholinesterase activity (AChE). The analysis was carried out on 6 individuals for each treatment. Juveniles were analyzed in a pool (N = 6 pool for each treatment). Due to the small biological sample obtained, only the following measurements were carried out: GLY, ROS, CAT, GSTs and AChE.

All parameters were measured in triplicate and data were normalized to the protein content. The total protein content of each sample was measured using the Bradford (1976) assay. The protein concentration was quantified, using a calibration line of bovine serum albumin (BSA) (5–40 μg/mL; $r^2 > 0.98$). For the analysis of GLY content, the protocol of Dubois et al. (1956) was followed. Absorbance was measured at 492 nm by Ensight™ plate reader. For the final quantification a glucose standard curve (0–5 mg/mL) was used. Data were expressed as mg GLY x g fresh weight^{-1}. The ROS level was calculated using dichlorofluorescein-diacetate as a fluorescent substrate (DCFH-DA). The fluorescence was measured at λ_{ex} = 485 nm and λ_{em} = 530 nm. ROS

concentration was expressed as fluorescent units (FU) mg protein^{-1}. The SOD activity was calculated following the method of Magni et al. (2018). The activity was expressed in SOD unit (U SOD) x mg protein^{-1}. One U SOD is the amount of enzyme needed to inhibit the rate of reduction of cytochrome by 50 %. The activity of the CAT enzyme was analyzed monitoring the decrease in H_2O_2 at $\lambda = 240$ nm. The activities were expressed as: mmol x min^{-1} x mg protein^{-1}. GSTs activity was quantified following Habig et al. (1974) protocol. The activity was expressed as mmol min^{-1} x mg proteins^{-1}.

The analysis of AChE activity was carried out following the protocol developed by Ellman et al. (1961) using DTNB (5 mM) and acethyltiocoline (1 mM) as substrates. The absorbance was read at $\lambda = 412$ nm. Data were expressed as nmol min^{-1} x mg prot^{-1}.

The content of lipid peroxidation (LPO) was measured only in adults, according to the method described by Nigro et al. (2021). LPO content was determined by mixing Trichloroacetic acid (TCA), 20 % (w/v) and 2-thiobarbituric acid (TBA) 0.5 % (w/v). Absorbance was measured at $\lambda = 535$ nm and LPO levels were expressed in nmol x g fresh weight^{-1}.

2.4. Chemical determination of Gd

Gadolinium concentrations were measured using inductively-coupled plasma mass spectrometry (ICP-MS, Aurora M90, Bruker Daltonics Inc. Nitric acid (HNO_3) at 69 % v/v, classified as Ultratrace@ ppb-trace analysis grade, was supplied by Scharlau (Barcelona, Spain). For ICP-MS analysis, all samples were prepared in a 2% v/v HNO_3 solution and the analysis was conducted in Normal Sensitivity mode. Calibration curves for Gd determination spanned from 0.5 to 1000 μg/L and were freshly prepared before analysis using standard solutions. The internal standard used for both the calibration curve and sample analysis was ^{115}In.

2.5. Statistical analyses

Data are expressed as mean ± standard deviation (SD). The statistical analysis was carried out using the GraphPad Prism 8.0.2 program. An unpaired *t*-test was applied to biological parameters and Gd levels to verify the presence of statistically significant differences between the control and the 1 mg/kg treatment both in adults and in juvenile specimens and between control and the 10 mg/kg treatment. The two-way ANOVA statistical test was used for growth and NNRT, setting time and treatment as factors, followed by Tukey's *post-hoc* test. Finally, the one-way test ANOVA, was used for juvenile earthworms exposed to 5 mg/kg, taking treatment as a factor. The results were considered statistically significant if they had a p-value <0.05.

3. Results

3.1. Effects on adults

The onset of mortality in adult organisms exposed to 1 or 10 mg/kg Gd did not occur throughout the entire duration of the test. The microscopic examination revealed no morphological alterations in the exposed organisms. At both concentrations tested, the weights of the treated organisms did not significantly change compared with those of the controls (Fig. 1).

The onset of lysosomal membrane destabilization was observed in the haemocytes of organisms exposed to Gd for 28 days (Fig. 2). Compared with those in control organisms, a significant increase in the percentage of destabilized cells was observed at 30, 90 and 120 min in organisms exposed to Gd (treatment $p < 0.0001$, $F_{2,24} = 170.1$; time $p < 0.0001$, $F_{3,24} = 88.03$; interaction $p < 0.0001$, $F_{6,24} = 10.05$). Furthermore, treatment with 10 mg/kg Gd resulted in 50% cell destabilization at 90 min.

The measurements of biochemical biomarkers related to oxidative stress/damage, detoxification, metabolic conditions and neurotoxicity are shown in Table 1. The results did not show any modulation in the earthworms exposed to either tested concentration of Gd with respect to the controls.

With respect to reproduction performance, the number of juveniles was 188 ± 35 individuals in the control group and 202 ± 14 individuals in the group exposed to 1 mg/kg Gd. The statistical analysis did not reveal any significant difference between the different treatments ($p = 0.6183$). Additionally, all the juveniles were weighed to observe any differences in reproduction timing and/or growth alterations. However,

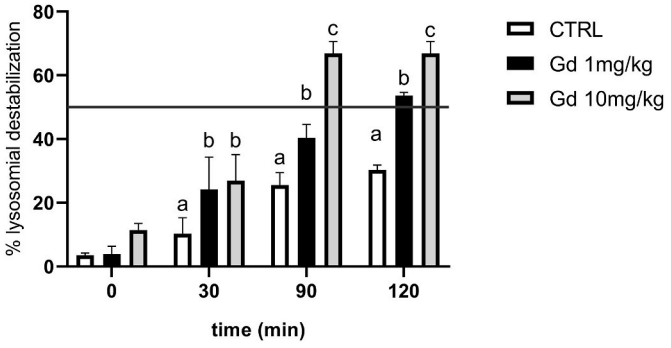

Fig. 2. Percentage of destabilized hemocytes in earthworms exposed to Gd. Values are expressed as mean ± SD (N = 3 individuals each treatment). Different letters indicate a statistically significant difference ($p < 0.05$).

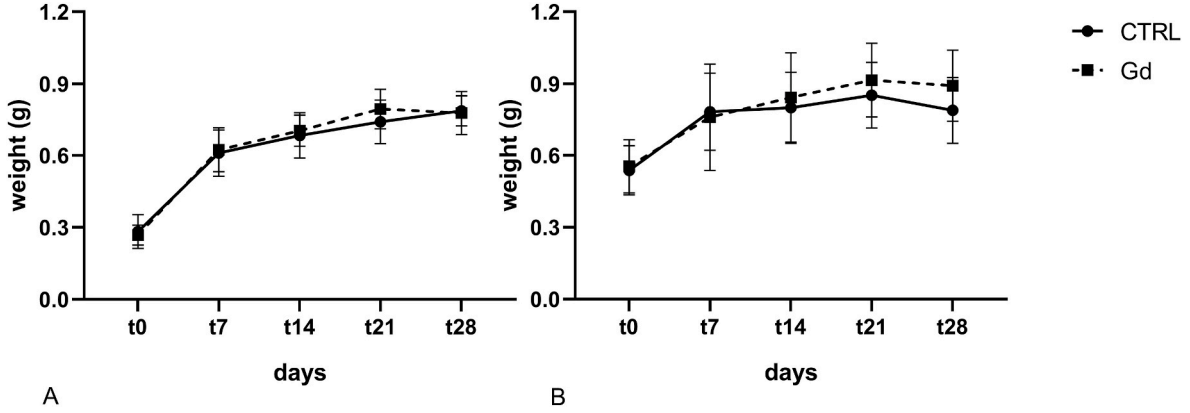

Fig. 1. Weight of earthworms exposed to Gd 1 mg/kg (A) and 10 mg/kg (B) during 28 days of experimentation. Values are expressed as mean ± SD (N = 18 individuals each treatment).

Table 1
Biomarkers in adult worms exposed to Gd 1 mg/kg and Gd 10 mg/kg. Data are expressed as mean ± standard deviation (N = 6 individuals each treatment). In brackets the p value of each endpoint.

	CTRL	Gd 1 mg/kg	CTRL	Gd 10 mg/kg
GLY mg g w. w.$^{-1}$	22.89 ± 10.50	19.23 ± 4.07 (0.4489)	35.16 ± 0.32	34.81 ± 0.16 (0.0603)
ROS AUF mg prot^{-1}	2.38E^8 ± 8.33 E^7	2.25E^8 ± 5.98 E^7 (0.7604)	5.79 ^8 ± 8.33 E^7	4.70 ^8 ± 1.24 E^8 (0.3531)
SOD U mg prot^{-1}	231.12 ± 75.01	185.44 ± 94.31 (0.7215)	508.13 ± 136.86	470.11 ± 78.82 (0.5685)
CAT mmol min^{-1} mg prot^{-1}	0.52 ± 0.17	0.46 ± 0.12 (0.4833)	0.43 ± 0.22	0.36 ± 0.24 (0.6243)
LPO MDA mM g w.w. $^{-1}$	18.36 ± 8.34	14.07 ± 4.35 (0.9380)	34.98 ± 14.28	30.12 ± 9.19 (0.5283)
GSTs mmol min^{-1} mg prot^{-1}	1.32 ± 0.35	1.32 ± 0.35 (0.4689)	0.58 ± 0.22	0.55 ± 0.21 (0.5606)
AChE µmol min^{-1} mg prot^{-1}	2.56 ± 0.938	2.55 ± 0.59 (0.9894)	3.08 ± 1.51	3.92 ± 0.92 (0.1774)

the analysis of the results did not reveal statistically significant differences between the weights of the control (2.258 ± 0.311 g) and 1 mg/kg Gd (2.454 ± 0.280 g) (p = 0.4644) groups.

3.2. Effects on juveniles

Owing to the small size of juveniles, analyses of NRRT and of SOD and LPO were not carried out. The measurement of GLY content revealed a significant increase in organisms exposed to Gd (p = 0.0004) (Fig. 3 Table S2). With respect to the oxidative stress parameters, no statistically significant differences were observed in the ROS content between the controls and the organisms exposed to 1 mg/kg Gd (p = 0.1011). Similarly, GST activity was not significantly modulated in response to exposure to Gd (p = 0.5209). Conversely, CAT activity was significantly lower in individuals exposed to Gd than in controls (p = 0.0011). The analysis of the AChE enzyme revealed a statistically significant increase in juveniles exposed to Gd compared with the controls (p = 0.0133) (Fig. 3, Table S2).

3.3. Effects of increasing Gd concentration in juveniles

A survival rate higher than 93% was observed in all the treatments. Furthermore, the weights of the treated organisms were significantly modified (treatment $p < 0.0001$, $F_{3,420} = 82.77$; time $p < 0.0001$, $F_{4,40} = 296.6$; interaction $p < 0.0001$, $F_{12,40} = 7.396$) (Fig. 4). Specifically, on Day 7 (t7) and Day 14 (t14), the C-Gd group was significantly different from the other groups, whereas at t14, the C-C group was significantly different from the Gd-Gd group. On Day 21, however, the experimental groups were all significantly different from each other, and finally, at the end of the exposure, the C-C and C-Gd groups were significantly

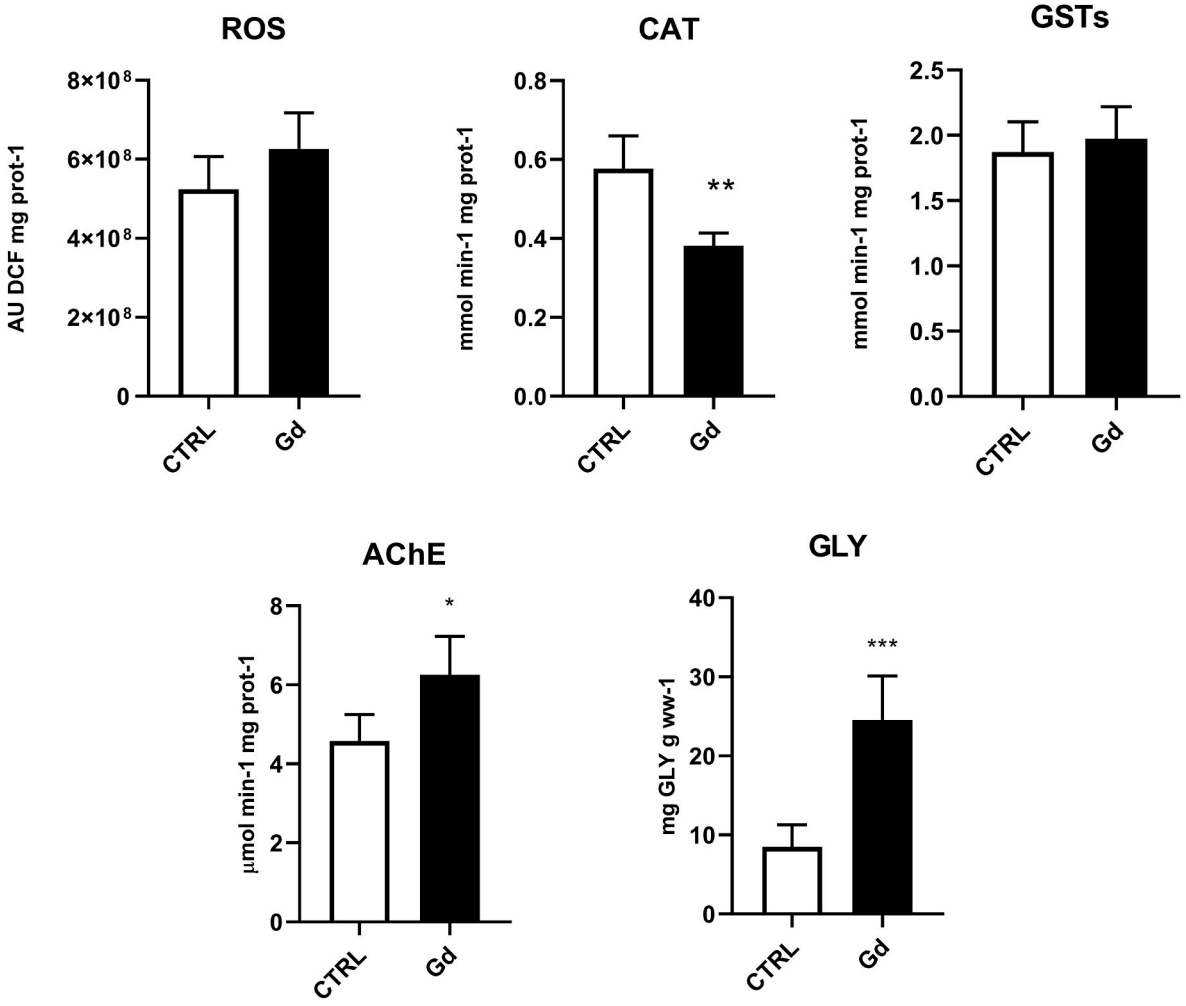

Fig. 3. Biochemical parameters in juvenile earthworms exposed to Gd. Values are expressed as mean ± SD (N = 6 replicates each treatment). Asterisk indicates significant difference with respect to control (*p < 0.05, **p < 0.001, ***p < 0.0001).

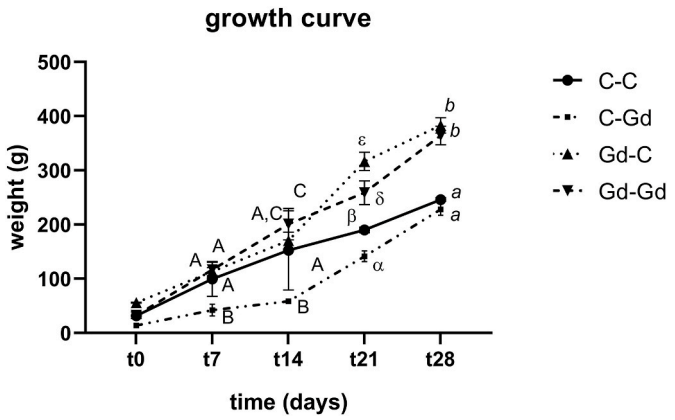

Fig. 4. Weight of juvenile earthworms exposed for 28 days to 5 mg/kg of Gd or reference soil. Values are expressed as mean ± SD (N = 3 replicates each treatment). Different letters indicate significant differences among groups ($p < 0.05$).

different from the Gd-C and Gd-Gd groups.

In terms of biochemical biomarkers, the GLY content significantly differed among the different treatments ($p < 0.0001$, $F_{3,19} = 25.53$). Specifically, an increase was observed in all the organisms exposed to Gd (C-Gd, Gd-C, and Gd-Gd) compared with the controls (C-C) (Fig. 5, Table S3).

With respect to the oxidative stress parameters (Fig. 5, Table S3), no significant differences in the content of ROS were observed among the treatments ($p = 0.0802$, $F_{3,19} = 2.625$). However, the activities of the CAT and GST enzymes were significantly altered in response to the different treatments ($p = 0.0031$, $F_{3,19} = 6.594$ for CAT; $p < 0.0001$, $F_{3,20} = 28.64$ for GSTs). Specifically, the CAT activity decreased in the Gd-Gd group compared with the other treatments, whereas the GST activity increased in the organisms exposed to Gd (Gd-C and Gd-Gd) compared with those in the C-C and C-Gd groups; moreover, the activity in the latter group was significantly greater than that in the C-C group. With respect to the AChE enzyme, no statistically significant difference was observed between the various treatments ($p = 0.2100$, $F_{3,20} = 1.649$).

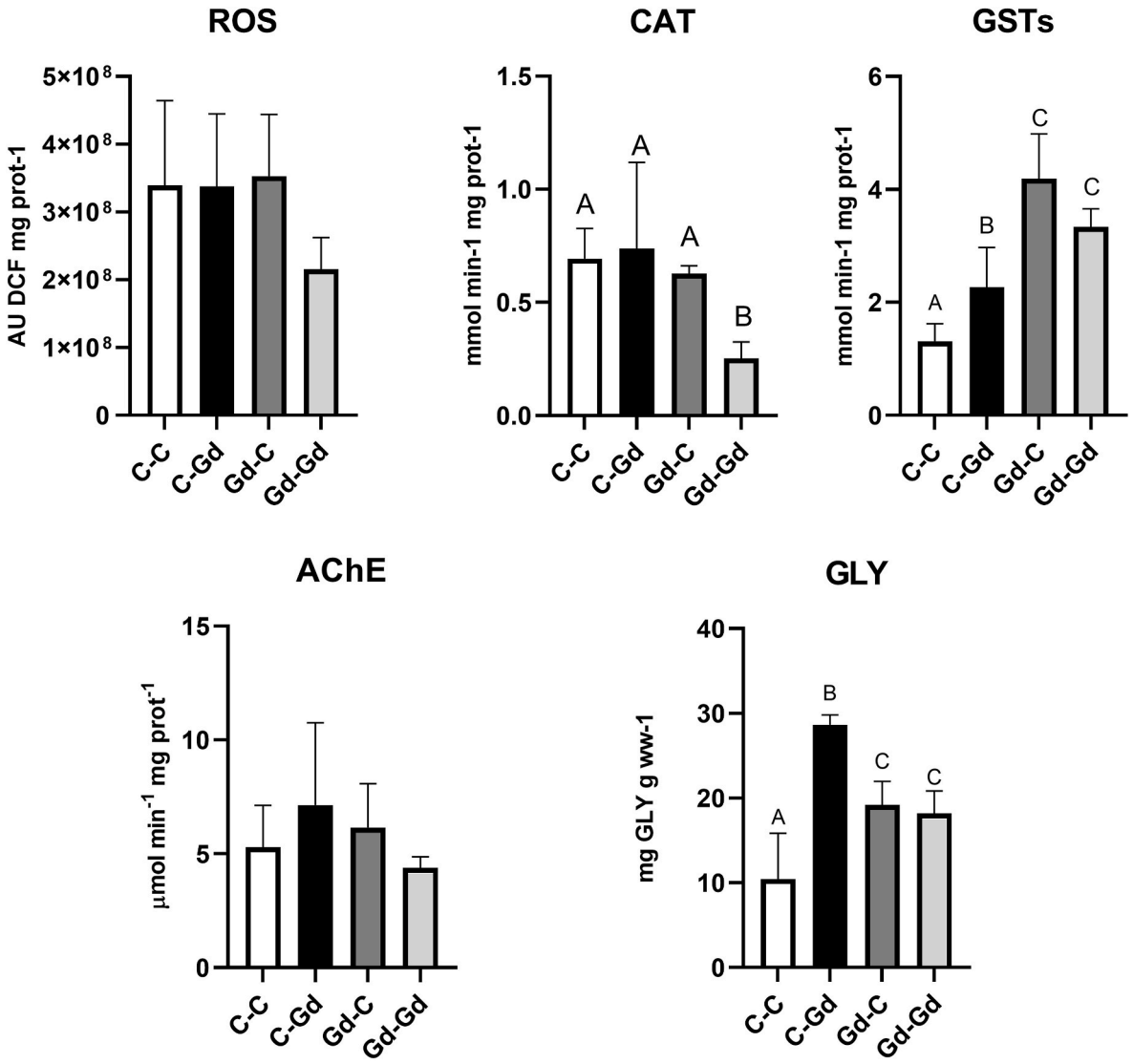

Fig. 5. Biochemical parameters juvenile earthworms exposed for 28 days to 5 mg/kg of Gd or reference soil. Values are expressed as mean ± SD (N = 5 replicates each treatment). Different letters indicate significant differences among groups ($p < 0.05$).

3.4. Accumulation of Gd

The measurement of the Gd content in the soil revealed the presence of Gd in the standard soil, ranging from 1.627 ± 0.196 mg/kg at t0 to 1.447 ± 0.335 mg/kg at t28. The Gd content in the soil spiked with 1 mg/kg Gd was 2.188 ± 0.076 mg/kg at t0 and 2.343 ± 0.329 mg/kg at t28, whereas the Gd content in the soil spiked with 10 mg/kg Gd was 10.212 ± 0.073 at t0 and 10.777 ± 0.032 at t28, confirming the stability of Gd content in the soil during the experiment.

A significantly greater content of Gd was measured in the earthworms exposed to both concentrations than in the control earthworms ($p = 0.0015$ for 1 mg/kg Gd and $p < 0.001$ for 10 mg/kg Gd) (Fig. 6). Similarly, a higher Gd content was observed in coprolites from the treated groups than in those from the control groups ($p = 0.031$ for 1 mg/kg Gd and $p = 0.0068$ for 10 mg/kg Gd) (Fig. 6). Additionally, the data indicate that Gd levels in coprolites are higher than those in tissues.

4. Discussion

Currently, there is still very limited knowledge about the potential impact of Gd on soil environments, which is restricted to two main scenarios: mine tailings and mine wastewater reuse *in situ* and *ex situ* and the reuse of biosolids in agriculture. Unfortunately, none of these scenarios have been clearly investigated from an analytical viewpoint. Moreover, the available information concerning the toxicity to terrestrial organisms is not sufficient to assess the environmental risk of this element to the soil ecosystem. Therefore, this study aimed to fill a knowledge gap by evaluating the bioaccumulation of Gd in earthworms and studying its acute and sublethal effects, as well as its impacts on reproduction, providing the first results concerning the effects of Gd not only on adult individuals but also on juveniles of *L. terrestris*.

Data obtained from adults revealed the limited toxicity of this element, even at 10 mg/kg. The only parameter that was significantly modulated compared with the controls was the NRRT. The interaction of Gd with lysosomes has already been observed in the freshwater bivalve *Corbicula fluminea* (Lachaux et al., 2023). This result aligns with the evidence of previous studies showing that earthworm exposure to metals can induce lysosomal destabilization (Maboeta et al., 2002; Boughattas et al., 2016), which could predict effects at higher biological levels (Moore et al., 2006). Nonetheless, exposure to Gd did not affect worm growth or reproduction.

With respect to oxidative stress, Xia et al. (2011) demonstrated the ability of Gd to promote neurotoxicity through oxidative stress *in vitro*. In line with this finding, in the marine mussel *Mytilus galloprovincialis*,

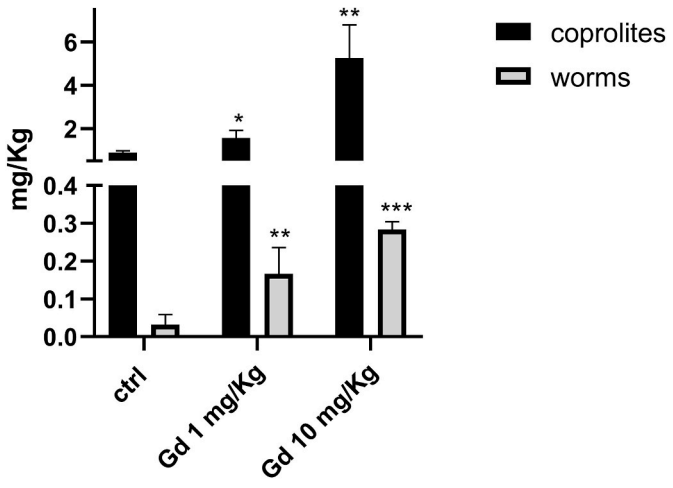

Fig. 6. Gd content in earthworms and coprolites. Values are expressed as mean ± SD (N = 3 replicates each treatment). Asterisk indicates significant difference with respect to control (*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$).

the induction of antioxidant enzymes was observed (Henriques et al., 2019; Trapasso et al., 2021). In contrast, in our study, Gd did not induce oxidative stress in adults, which was also reflected in the absence of oxidative damage (LPO). The induction of oxidative stress is a known effect triggered by metals, and several studies have shown the modulation of biomarkers related to oxidative stress and oxidative damage in earthworms exposed to metals (Wang et al., 2018; Mkhinini et al., 2019; Yan et al., 2021; Li et al., 2020; Hattab et al., 2023; Yadav et al., 2023 and citations therein). The inconsistencies found compared with previous studies could be attributed to various reasons. First, most of the studies tested mixtures of metals or provided concentrations of metals higher than those in the present research. Another explanation could be that the susceptibility of earthworms to metal toxicity is species-specific and dependent on the eco-physiology of the species (Fourie et al., 2007; Suthar et al., 2008). Finally, the difference could be attributed to the toxicokinetic properties of Gd. Further studies focused on Gd uptake and disposition in *L. terrestris* compared with other biological models are necessary to confirm the last hypothesis.

The mild toxicity observed could be related to the low accumulation of Gd in worms because its dose is not sufficient to induce relevant biological effects at both the biochemical and organismal levels. Chemical analyses, in fact, have shown that most of the Gd is excreted in casts. However, chemical analyses revealed the presence of Gd in LUFA 2.2 soil. Similarly, Bastos et al. (2014) reported that LUFA 2.2 can contain traces of priority metals, including cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), zinc (Zn), nickel (Ni), lead (Pb), arsenic (As) and mercury (Hg). This may have reduced the biological effects related to exposure to 1 mg/kg Gd.

The results concerning Gd levels in organisms and coprolites showed that earthworms can modify the distribution of Gd in the soil, potentially affecting its availability for other organisms, as already suggested for other metals (Ruiz et al., 2011).

Unlike what was observed for adults, the biomarkers analyzed in juveniles highlighted some significant alterations between exposed organisms and controls. These results confirmed that the early stages of development are more sensitive to contaminants than adults are (Balbi et al., 2016; Capolupo et al., 2018). In particular, GLY was significantly greater in the organisms exposed to Gd than in the controls. The allocation of energy reserves can have significant effects on the health and survival of organisms (Cunha et al., 2022), whereas energy-related parameters can be used to assess the energy costs involved in the stress response (Smolders et al., 2004). A possible explanation for the increased growth of earthworms exposed to Gd could be related to the energy trade-off (Garland et al., 2022) when energy is devoted to growth if it is sufficient for sexual maturation (Aira et al., 2007). Similar resource allocation strategies were observed in an experiment conducted on the earthworm *Eisenia andrei*, reared in artificial soil under environmental stress (exposed to different pH, temperature and humidity regimes), which preferentially maintained growth, resulting in the production of fewer eggs (Van Gestel et al., 1992). Further experiments aimed at evaluating the attainment of sexual maturity and reproductive success of offspring are necessary to confirm this hypothesis.

Concerning the effects on biochemical parameters, the decline in CAT might be due to oxidative stress pressure, as already postulated for invertebrate species, including earthworms, exposed to metals (Saint-Denis et al., 2001; Vlachogianni and Valavanidis, 2007; Regoli et al., 2011; Ray et al., 2019). Nevertheless, the other biomarkers related to oxidative stress (ROS and SOD) were unaltered, and no oxidative damage occurred in the exposed organisms. Some metal ions can inhibit CAT activity through binding with SH groups (Atli et al., 2006; Regoli and Giuliani, 2014). Therefore, we hypothesize that CAT, like other metals, is a target of the toxic action of Gd, even though further investigations are needed to confirm this hypothesis. Since CAT is a key enzyme involved in the protective response against oxidative stress, the inhibition of CAT could increase the susceptibility of organisms to

further external stress.

The increase in AChE observed in juveniles exposed to Gd contrasts with other studies reporting the inhibition of AChE activity in adult earthworms exposed to metals (Mkhinini et al., 2019; Hattab et al., 2023). The opposite trend observed in juveniles confirms that adults and juveniles can behave differently in response to pollutants. Several studies have reported an increase in AChE activity in response to environmental contaminants in different species, although there is a gap in knowledge concerning the mechanism behind this effect. For example, in the earthworm *E. andrei*, AChE increased because of the hormetic effects elicited by temephos and formalin (Hackenberger et al., 2008, 2012). In fish species, an increase in AChE activity was observed in eel (*Electrophorus electricus*) exposed to low doses of monoterpenoids. The authors hypothesized that the activation could be due to a structural change of AChE towards a more favourable conformation for catalysis in response to these molecules (López et al., 2015). In *Sparus aurata*, Cu induced significant activation of AChE in the brain and white muscle (Romani et al., 2003). Other studies suggest that the induction of AChE activity might be explained by the fact that some invertebrates have several AChE isoforms that play a role in the stress response and detoxification of xenobiotics (Kang et al., 2011; Kim and Lee, 2018). A further hypothesis to explain the AChE increase is linked to potential overproduction of the neurotransmitter acetylcholine, a substrate of the acetylcholinesterase enzyme. In mammalian models, this neurotransmitter is induced under stress conditions, but the synthesis mechanisms in invertebrates are not known (Horiuchi et al., 2003). Given the central role of AChE in neurotransmission, further studies are warranted to identify the potential mechanism determining the induction of AChE in response to Gd.

The further exposure of juveniles to increasing concentrations of Gd revealed that individuals reared in Gd-contaminated soil grew more than juveniles kept in reference soil. Moreover, the GLY content increased in the same individuals. These observations are consistent with the increase in GLY observed in juveniles treated with 1 mg/kg Gd, confirming that this element can affect the allocation of energy reserves in juveniles.

For the biochemical markers, a decrease in CAT activity was observed in the Gd-Gd group, whereas the ROS content was not affected, as observed in the organisms treated with 1 mg/kg Gd. This result seems to support the hypothesis of a specific interaction between CAT and Gd. A similar result was reported by Šrut et al. (2017) in *L. terrestris* exposed to Cd. Specifically, in earthworms pre-exposed to Cd and further exposed to a relatively high concentration of the metal (corresponding to Gd-Gd), the CAT activity was significantly reduced. The authors reported that the decrease in CAT activity was accompanied by an increase in the expression of the metallothionein gene, which could act as a radical scavenger. Conversely, the individuals transferred from Gd-spiked soil to uncontaminated soil (Gd-C) presented CAT activities similar to those of the control. This trend suggested a transient effect on CAT activity that could be recovered once Gd was removed from the soil.

The GSTs were significantly different between the treatments: the earthworms pre-exposed to Gd (Gd-Gd and Gd-C) presented higher values of GSTs than did those from the control soil. In addition, organisms that were exposed to Gd for 28 days (C-Gd) presented higher values than did the control. This result aligns with those of previous studies showing the protective role of GSTs against metals in earthworms (Mkhinini et al., 2019; Hattab et al., 2023). Moreover, an increase in GST activity has already been observed in aquatic species exposed to Gd (Perrat et al., 2017; Henriques et al., 2019), supporting the hypothesis that these enzymes are involved in the detoxification mechanisms induced by worms to counteract the effects of Gd.

Finally, no statistically significant difference was observed in AChE activity, suggesting that the effect observed in juveniles exposed to Gd for 28 days was not exacerbated by a further increase in the concentration of the contaminant.

This evidence suggested that Gd is able to modify the oxidative status of earthworms, even though it is not possible to provide a mechanism-based understanding of the observed effects, and further research is needed to identify the mechanism of action of this potential contaminant, both in adults and in juveniles.

5. Conclusions

The present study provides new information on the effects of Gd on key species in the soil ecosystem. Results indicated that Gd has a cytotoxic effect on adult earthworms; however, this effect is not reflected in an increase in oxidative stress or oxidative damage. Furthermore, no effect on reproduction was observed. This is probably because Gd tends to bioaccumulate in small quantities in tissues, considering the high Gd content measured in coprolites. However, Gd affected the offspring, resulting in an increase in GLY content, a reduction in CAT enzyme activity, and an increase in AChE enzyme activity. In addition, by further increasing the concentration of Gd, some of these effects were exacerbated. Our results highlight that the presence of Gd in soils could compromise the functionality of the soil ecosystem because of the fundamental role played by this earthworm species. Therefore, more studies that focus on the effects related to long-term exposure scenarios are necessary to understand the vulnerability of soil ecosystems to Gd and other REEs.

CRediT authorship contribution statement

Giovanni Libralato: Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Martina Inversini:** Writing – review & editing, Methodology, Formal analysis. **Silvia Giorgia Signorini:** Writing – review & editing, Formal analysis. **Stefano Magni:** Writing – review & editing, Resources. **Silvia Angelillo:** Writing – review & editing, Formal analysis. **Marco Trifuoggi:** Writing – review & editing, Formal analysis. **Andrea Binelli:** Writing – review & editing, Resources. **Camilla Della Torre:** Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121621.

Data availability

Data will be made available on request.

References

Aira, M., Dominguez, J., Monroy, F., Velando, A., 2007. Stress promotes changes in resource allocation to growth and reproduction in a simultaneous hermaphrodite with indeterminate growth. Biol. J. Linn. Soc. 91, 593–600. https://doi.org/10.1111/j.1095-8312.2007.00822.x.

Albarano, L., Guida, M., Tommasi, F., Lofrano, G., Padilla Suarez, E.G., Gjata, I., Brouziotis, A.A., Trifuoggi, M., Giarra, A., Libralato, G., 2025. Species sensitivity

distribution of rare Earth elements: a full overview. Sci. Total Environ. 958, 178079. https://doi.org/10.1016/j.scitotenv.2024.178079.
Arciszewska, Z., Gama, S., Leśniewska, B., Malejko, J., Nalewajko-Sieliwoniuk, E., Zambrzycka-Szelewa, E., Godlewska-Żyłkiewicz, B., 2022. The translocation pathways of rare earth elements from the environment to the food chain and their impact on human health.". Process Saf. Environ. Prot. 168, 205–223. https://doi.org/10.1016/j.psep.2022.09.056.
Atli, G., Alptekin, O., Tükel, S., Canli, M., 2006. Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish Oreochromis niloticus. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 143, 218–224. https://doi.org/10.1016/j.cbpc.2006.02.003.
Aubert, D., Stille, P., Probst, A., Gauthier-lafaye, F., Pourcelot, L., Del nero, M., 2002. Characterization and migration of atmospheric REE in soils and surface waters. Geochim. Cosmochim. Acta 66, 3339–3350. https://doi.org/10.1016/S0016-7037(02)00913-4.
Balbi, T., Franzellitti, S., Fabbri, R., Montagna, M., Fabbri, E., Canesi, L., 2016. Impact of bisphenol A (BPA) on early embryo development in the marine mussel *Mytilus galloprovincialis*: effects on gene transcription. Environ. Pollut. 218, 996–1004. https://doi.org/10.1016/j.envpol.2016.08.050.
Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussand, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 161–182. https://doi.org/10.1111/ejss.12025.
Boughattas, I., Hattab, S., Boussetta, H., Sappin-Didier, V., Viarengo, A., Banni, M., Sforzini, S., 2016. Biomarker responses of *Eisenia andrei* to a polymetallic gradient near a lead mining site in North Tunisia. Environ. Pollut. 218, 530–541. https://doi.org/10.1016/j.envpol.2016.07.033.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
Bastos, A.C., Prodana, M., Oliveira, J.M., Calhôa, C.F., Santos, M.J., Soares, A.M., Loureiro, S., 2014. Water-extractable priority contaminants in LUFA 2.2 soil: back to basics, contextualisation and implications for use as natural standard soil. Ecotoxicology 23, 1814–1822. https://doi.org/10.1007/s10646-014-1335-2.
Brouziotis, A.A., Heise, S., Saviano, L., Zhang, K., Giarra, A., Bau, M., Tommasi, F., Guida, M., Libralato, G., Trifuoggi, M., 2024. Levels of rare earth elements on three abandoned mining sites of bauxite in southern Italy: a comparison between TXRF and ICP-MS. Talanta 275, 126093. https://doi.org/10.1016/j.talanta.2024.126093.
Brünjes, R., Hofmann, T., 2020. Anthropogenic gadolinium in freshwater and drinking water systems. Wat. Res. 182. https://doi.org/10.1016/j.watres.2020.115966.
Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C.S., Fabbri, E., 2018. Uptake and transcriptional effects of polystyrene microplastics in larval stages of the mediterranean mussel *Mytilus galloprovincialis*. Environ. Pollut. 241, 1038–1047. https://doi.org/10.1016/j.envpol.2018.06.035.
Cesarini, G., Spani, F., Patricelli, R., Quattrocchi, C.C., Colasanti, M., Scalici, M., 2024. Assessing teratogenic risks of gadolinium in freshwater environments: implications for environmental health. Ecotoxicol. Environ. Saf. 278, 116442. https://doi.org/10.1016/j.ecoenv.2024.116442.
Cunha, M., Louro, P., Silva, M., Soares, A.M.V.M., Pereira, E., Freitas, R., 2022. Biochemical alterations caused by lanthanum and gadolinium in *Mytilus galloprovincialis* after exposure and recovery periods. Environ. Pollut. 307, 119387. https://doi.org/10.1016/j.envpol.2022.119387.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.
Egler, S.G., Niemeyer, J.C., Correia, F.V., Saggioro, E.M., 2022. Effects of rare earth elements (REE) on terrestrial organisms: current status and future directions. Ecotoxicology 31, 689–699. https://doi.org/10.1007/s10646-022-02542-6.
Ellmann, G.L., Courtney, K.D., Andres, V Jr, Feather-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9. PMID: 13726518.
Emsley, J., 2011. Nature's Building Blocks: an AZ Guide to the Elements. Oxford University Press, USA.
Fourie, F., Reinecke, S.A., Reinecke, A.J., 2007. The determination of earthworm species sensitivity differences to cadmium genotoxicity using the comet assay. Ecotoxicol. Environ. Saf. 67, 361–368. https://doi.org/10.1016/j.ecoenv.2006.10.005.
Garland, T Jr, Downs, C.J., Ives, A.R., 2022. Trade-offs (and constraints) in organismal biology. Physiol. Biochem. Zool. 95, 82–112. https://doi.org/10.1086/717897.
González, V., Vignati, D.A., Pons, M.N., Montarges-Pelletier, E., Bojic, C., Giamberini, L., 2015. Lanthanide ecotoxicity: first attempt to measure environmental risk for aquatic organisms. Environ. Pollut. 199, 139–147. https://doi.org/10.1016/j.envpol.2015.01.020.
Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., Sanganyado, E., 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ. 636, 299–313. https://doi.org/10.1016/j.scitotenv.2018.04.235.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-Transferases. J. Biol. Chem. 249, 7130–7139.
Hackenberger, B.K., Jarić-Perkusić, D., Stepić, S., 2008. Effect of temephos on cholinesterase activity in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae). Ecotoxicol. Environ. Saf. 71, 583–589. https://doi.org/10.1016/j.ecoenv.2007.11.008.
Hackenberger, B.K., Velki, M., Stepić, S., Hackenberger, D.K., 2012. The effect of formalin on acetylcholinesterase and catalase activities, and on the concentration of oximes, in the earthworm species *Eisenia andrei*. Eur. J. Soil Biol. 50, 137–143, 0.1016/j.ejsobi.2012.02.002.
Hanana, H., Turcotte, P., Pilote, M., Auclair, J., Gagnon, C., Gagné, F., 2017. Biomarker assessment of lanthanum on a freshwater invertebrate, *Dreissena polymorpha*. SOJ Biochem. 3, 1–9. https://doi.org/10.15226/2376-4589/2/3/00108.
Hattab, S., Boughattas, I., Cappello, T., Zitouni, N., Touil, G., Romdhani, I., Livet, A., Bousserrhine, N., Banni, M., 2023. Heavy metal accumulation, biochemical and transcriptomic biomarkers in earthworms *Eisenia andrei* exposed to industrially contaminated soils from south-eastern Tunisia (gabes governorate). Sci. Tot. Environ. 887, 163950. https://doi.org/10.1016/j.scitotenv.2023.163950.
Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., Pereira, E., 2019. Toxicological assessment of anthropogenic gadolinium in seawater: biochemical effects in mussels *Mytilus galloprovincialis*. Sci. Tot. Environ. 664, 626–634. https://doi.org/10.1016/j.scitotenv.2019.01.341.
Horiuchi, Y., Kimura, R., Kato, N., Fujii, T., Seki, M., Endo, T., Kato, T., Kawashima, K., 2003. Evolutional study on acetylcholine expression. Life Sci. 72, 1745–1756. https://doi.org/10.1016/s0024-3205(02)02478-5.
Hoshino, M., Sanematsu, K., Watanabe, Y., 2016. REE mineralogy and resources. Handb. Phys. Chem. Rare Earths 49, 129–291.
Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environ. Sci. Technol. 50, 2685–2691. https://doi.org/10.1021/acs.est.5b05478.
Kang, J.S., Lee, D.W., Koh, Y.H., Lee, S.H., 2011. A soluble acetylcholinesterase provides chemical defense against xenobiotics in the pinewood nematode. PLoS One 6, e19063. https://doi.org/10.1371/journal.pone.0019063.
Keith, A.H., Robinson, D.A., 2012. Earthworms as natural capital: ecosystem service providers in agricultural soils. Economol. J. 2, 91–99.
Kim, Y.H., Lee, S.H., 2018. Invertebrate acetylcholinesterases: insights into their evolution and nonclassical functions. J Asian-Pac. Entomol. 21, 186–195. https://doi.org/10.1016/j.aspen.2017.11.017.
Kulaksız, S., Bau, M., 2007. Contrasting behaviour of anthropogenic gadolinium and natural rare earth elements in estuaries and the gadolinium input into the North Sea. Earth Planet Sci. Lett. 260, 361–371. https://doi.org/10.1016/j.epsl.2007.06.016.
Kümmerer, K., Helmers, E., 2000. Hospital effluents as a source of gadolinium in the aquatic environment. Environ. Sci. Technol. 34, 573–577. https://doi.org/10.1021/es990633h.
Lachaux, N., Otero-Fariña, A., Minguez, L., Sohm, B., Rétif, J., Châtel, A., Poirier, L., Devin, S., Pain-Devin, S., Gross, E.M., Giamberini, L., 2023. Fate, subcellular distribution and biological effects of rare earth elements in a freshwater bivalve under complex exposure. Sci. Total Environ. 905, 167302. https://doi.org/10.1016/j.scitotenv.2023.
Lévèque, T., Capowiez, Y., Schreck, E., Mazzia, C., Auffan, M., Foucault, Y., Austruy, A., Dumat, C., 2013. Assessing ecotoxicity and uptake of metals and metalloids in relation to two different earthworm species (*Eiseina hortensis* and *Lumbricus terrestris*). Environ. Pollut. 179, 232–241. https://doi.org/10.1016/j.envpol.2013.03.066.
Li, X., Wang, M., Jiang, R., Zheng, L., Chen, W., 2020. Evaluation of joint toxicity of heavy metals and herbicide mixtures in soils to earthworms (*Eisenia fetida*). J. Environ. Sci. 94, 137–146. https://doi.org/10.1016/j.jes.2020.03.055.
Lima, A.T., Ottosen, L., 2021. Recovering rare earth elements from contaminated soils: critical overview of current remediation technologies. Chemosphere 265, 129163. https://doi.org/10.1016/j.chemosphere.2020.129163.
Liu, Z., Guo, C., Tai, P., Sun, L., Chen, Z., 2021. The exposure of gadolinium at environmental relevant levels induced genotoxic effects in Arabidopsis thaliana (L.). Ecotoxicol. Environ. Saf. 215, 112138. https://doi.org/10.1016/j.ecoenv.2021.112138. Epub 2021 Mar 16. PMID: 33740487.
López, M.D., Campoy, F.J., Pascual-Villalobos, M.J., Muñoz-Delgado, E., Vidal, C.J., 2015. Acetylcholinesterase activity of electric eel is increased or decreased by selected monoterpenoids and phenylpropanoids in a concentration-dependent manner. Chem. Biol. Interact. 229, 36–43. https://doi.org/10.1016/j.cbi.2015.01.006.
Lowe, D.M., Soverchia, C., Moore, M.N., 1995. Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. Aquat. Toxicol. 33. https://doi.org/10.1016/0166-445X(95)00015-V.
Maboeta, M.S., Reinecke, S.A., Reinecke, A.J., 2002. The relation between lysosomal biomarker and population responses in a field population of Microchaetus sp. (Oligochaeta) exposed to the fungicide copper oxychloride. Ecotoxicol. Environ. Saf. 52, 280–287. https://doi.org/10.1006/eesa.2002.2180.
Magni, S., Gagné, F., André, C., Della Torre, C., Auclair, J., Hanana, H., Parenti, C.C., Bonasoro, F., Binelli, A., 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: bivalvia). Sci. Tot. Environ. 631–632, 778–788. https://doi.org/10.1016/j.scitotenv.2018.03.075.
Martino, C., Bonaventura, R., Byrne, M., Roccheri, M., Matranga, V., 2017. Effects of exposure to gadolinium on the development of geographically and phylogenetically distant sea urchins species. Mar. Environ. Res. 128, 98–106. https://doi.org/10.1016/j.marenvres.2016.06.001.
Mkhinini, M., Boughattas, I., Bousserrhine, N., Banni, M., 2019. Biochemical and transcriptomic response of earthworms *Eisenia andrei* exposed to soils irrigated with treated wastewater. Environ. Sci. Pollut. Res. 26, 2851–2863. https://doi.org/10.1007/s11356-018-3794-0.
Montalvão, M.F., Gomes, A.R., Guimarães, A.T.B., Rodrigues, A.S.L., Matos, L.P., Mendonça, J.D.S., da Luz, T.M., Matos, S.G.D.S., Rahman, M.S., Ragavendran, C., Senthil-Nathan, S., Guru, A., Rakib, M.R.J., Mubarak, N.M., Rahman, M.M., Rocha, T.L., Islam, A.R.M.T., Malafaia, G., 2024. Toxicity of carbon nanofibers in earthworms (*Lumbricus terrestris*) naturally infected with Monocystis sp. Sci. Tot. Environ. 906, 167712. https://doi.org/10.1016/j.scitotenv.2023.167712.

Moore, M.N., Allen, I.J., McVeigh, A., 2006. Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Mar. Environ. Res. 61, 278–304. https://doi.org/10.1016/j.marenvres.2005.10.005.

Nigro, L., Freitas, R., Maggioni, D., Hamza, H., Coppola, F., Protano, G., Della Torre, C., 2021. Coating with polysaccharides influences the surface charge of cerium oxide NPs and their effects to *Mytilus galloprovincialis*. Nanoimpact 24, 100362. https://doi.org/10.1016/j.impact.2021.100362.

OECD, 2016. Test No. 222: Earthworm Reproduction Test (Eisenia Fetida/eisenia Andrei), OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264264496-en.

Perrat, E., Parant, M., Py, J.S., Rosin, C., Cossu-Leguille, C., 2017. Bioaccumulation of gadolinium in freshwater bivalves. Environ. Sci. Pollut. Res. Int. 24, 12405–12415. https://doi.org/10.1007/s11356-017-8869-9.

Piarulli, S., Riedel, J.A., Fossum, F.N., Kermen, F., Hansen, B.H., Kvæstad, B., Olsvik, P. A., Farkas, J., 2024. Effects of gadolinium (Gd) and a Gd-based contrast agent (GBCA) on early life stages of zebrafish (*Danio rerio*). Chemosphere 350, 140950. https://doi.org/10.1016/j.chemosphere.2023.140950.

Puddepatt, K.J., McCarthy, L.H., Serre, B.M., 2022. Assessing the potential chronic, sublethal and lethal ecotoxicity of land-applying biosolids on *Folsomia candida* and *Lumbricus terrestris*. Ecotoxicology 31, 1520–1535. https://doi.org/10.1007/s10646-022-02606-7.

Ray, S., Gautam, A., Ray, A., Das, S., Ray, M., 2019. Analysis of oxidative stress and cellular aggregation in the coelomocytes of earthworms collected from metal contaminated sites of industrial and agricultural soils of West Bengal, India. Environ. Sci. Pollut. Res. Int. 26, 22625–22640. https://doi.org/10.1007/s11356-019-05438-x.

Regoli, F., Benedetti, M., Giuliani, M.E., 2011. Antioxidant defenses and acquisition of tolerance to chemical stress. In: Amiard-Triquet, C., Rainbow, P.S., Romeo, M. (Eds.), Tolerance to Environmental Contaminants. CRC Press, Boca Raton, pp. 153–173. https://doi.org/10.1201/b10519-9.

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106–117. https://doi.org/10.1016/j.marenvres.2013.07.006.

Rogowska, J., Olkowska, E., Ratajczyk, W., Wolska, L., 2018. Gadolinium as a new emerging contaminant of aquatic environments. Environ. Toxicol. Chem. 37, 1523–1534. https://doi.org/10.1002/etc.4116.

Romani, R., Antognelli, C., Baldracchini, F., De Santis, A., Isani, G., Giovannini, E., Rosi, G., 2003. Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. Chem. Biol. Interact. 145, 321–329. https://doi.org/10.1016/s0009-2797(03)00058-9.

Ruiz, E., Alonso-Azcárate, J., Rodríguez, L., 2011. *Lumbricus terrestris* L. activity increases the availability of metals and their accumulation in maize and barley. Environ. Pollut. 159, 722–728. https://doi.org/10.1016/j.envpol.2010.11.032.

Saint-Denis, M., Narbonne, J.F., Arnaud, C., Ribera, D., 2001. Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of lead acetate. Soil Biol. Biochem. 33, 395–404. https://doi.org/10.1016/S0038-0717(00)00177-2.

Smolders, E., Buekers, J., Oliver, I., McLaughlin, M.J., 2004. Soil properties affecting toxicity of zinc to soil microbial properties in laboratory-spiked and field-contaminated soils. Environ. Toxicol. Chem. 23, 2633–2640. https://doi.org/10.1897/04-27.

Šrut, M., Drechsel, V., Höckner, M., 2017. Low levels of Cd induce persisting epigenetic modifications and acclimation mechanisms in the earthworm *Lumbricus terrestris*. PLoS One 12, e0176047. https://doi.org/10.1371/journal.pone.0176047.

Suthar, S., Singh, S., Dhawan, S., 2008. Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: is metal bioaccumulation affected by their ecological category? Ecol. Engineer. 32, 99–107. https://doi.org/10.1016/j.ecoleng.2007.10.003.

Tai, P., Zhao, Q., Su, D., Li, P., Stagnitti, F., 2010. Biological toxicity of lanthanide elements on algae. Chemosphere 80, 1031–1035. https://doi.org/10.1016/j.chemosphere.2010.05.030.

Trapasso, G., Coppola, F., Queirós, V., Henriques, B., Soares, A.M.V.M., Pereira, E., Chiesa, S., Freitas, R., 2021. How *ulva Lactuca* can influence the impacts induced by the rare Earth element gadolinium in *Mytilus galloprovincialis*? The role of macroalgae in water safety towards marine wildlife. Ecotoxicol. Environ. Saf. 215, 112101. https://doi.org/10.1016/j.ecoenv.2021.112101.

van Gestel, C.A.M., Dirven-van Breemen, E.M., Baerselman, R., 1992. Influence of environmental conditions on the growth and reproduction of the earthworm *Eisenia andrei* in an artificial soil substrate. Pedobiologia 36, 109–120. https://doi.org/10.1016/s0031-4056(24)00779-0.

Vlachogianni, T.H., Valavanidis, A., 2007. Heavy-metal effects on lipid peroxidation and antioxidant defence enzymes in mussels *Mytilus galloprovincialis*. Chem. Ecol. 23, 361–371. https://doi.org/10.1080/02757540701653285.

Wang, K., Qiao, Y., Zhang, H., Yue, S., Li, H., Ji, X., Liu, L., 2018. Bioaccumulation of heavy metals in earthworms from field contaminated soil in a subtropical area of China. Ecotoxicol. Environ. Saf. 148, 876–883. https://doi.org/10.1016/j.ecoenv.2017.11.058.

Wysocka, I.A., Rogowska, A.M., Kostrz-Sikora, P., 2023. Investigation of anthropogenic gadolinium in tap water of polish cities: gdańsk, Kraków, Warszawa, and wrocław. Environ. Pollut. 323, 121289. https://doi.org/10.1016/j.envpol.2023.121289.

Xia, Q., Feng, X., Huang, H., Du, L., Yang, X., Wang, K., 2011. Gadolinium-induced oxidative stress triggers endoplasmic reticulum stress in rat cortical neurons. J. Neurochem. 117, 38–47. https://doi.org/10.1111/j.1471-4159.2010.07162.x.

Yadav, R., Kumar, R., Gupta, R.K., Kaur, T., Kiran, Kour, A., Kaur, S., Rajput, A., 2023. Heavy metal toxicity in earthworms and its environmental implications: a review. Environ. Adv. 12, 100374. https://doi.org/10.1016/j.envadv.2023.100374.

Yan, X., Wang, J., Zhu, L., Wang, J., Li, S., Kim, Y.M., 2021. Oxidative stress, growth inhibition, and DNA damage in earthworms induced by the combined pollution of typical neonicotinoid insecticides and heavy metals Sci. Tot. Environ. 754, 141873. https://doi.org/10.1016/j.scitotenv.2020.141873.