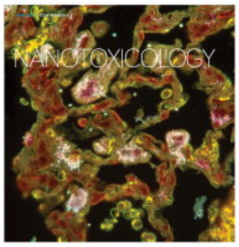

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RESEARCH ARTICLE

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Toxicity of lanthanum and yttrium oxide nanoparticles and bulk forms on *Folsomia candida*: a study of single *versus* mixture exposures

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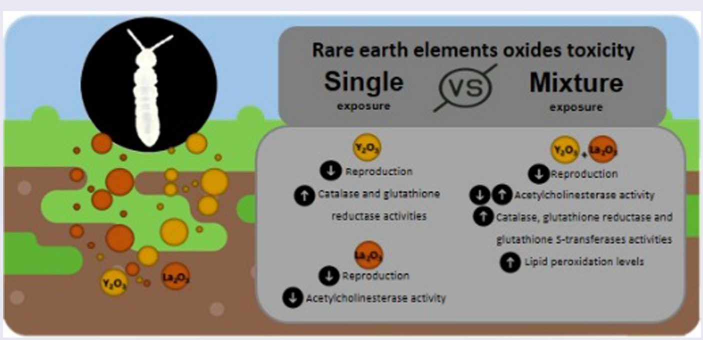
ABSTRACT

The intensive use of rare earth elements (REEs) raises concerns about their effects on soil organisms, particularly under mixture exposure scenarios. This study evaluated the toxicity of lanthanum oxide (La_2O_3) and yttrium oxide (Y_2O_3) nanoparticles (NPs) and bulk forms on *Folsomia candida*. Single (0–2500 mg/kg) and dual mixture exposures were tested for effects on survival, reproduction, avoidance behavior, and biochemical markers. No effects on survival and avoidance behavior were observed. NPs were more toxic than bulk forms. La_2O_3 NPs reduced reproduction (≥ 1250 mg/kg) and acetylcholinesterase (AChE) activity (2500 mg/kg), whereas Y_2O_3 NPs exhibited greatest toxicity, reducing reproduction (≥ 313 mg/kg) and increasing catalase (CAT) (156 and 625 mg/kg) and glutathione reductase (GR) (625 and 2500 mg/kg) activities. Mixture exposures revealed complex interactions (synergism, antagonism, or no interaction), with toxicity depending on concentration, endpoint, and material form. Besides, higher number of biochemical endpoints were affected by mixture exposures, but dissimilar responses were observed with different concentrations: 2500 mg/kg Y_2O_3 NPs + 2500 mg/kg La_2O_3 NPs decreased reproduction and increased GR, glutathione S-transferases (GST) and AChE activities; 2500 mg/kg Y_2O_3 NPs + 625 mg/kg La_2O_3 NPs increased CAT, GR, GST and AChE activities; 625 mg/kg Y_2O_3 NPs + 625 mg/kg La_2O_3 NPs increased GR activity; 156 mg/kg Y_2O_3 NPs + 2500 mg/kg La_2O_3 NPs decreased AChE activity, increased GR activity and lipid peroxidation levels. This study highlights that REE exposures, particularly mixtures, can pose risks to soil organisms and emphasizes the need to include mixture interactions in risk assessments.



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Nanotoxicology; rare earth elements; mixture effects; collembolan; nano versus bulk

1. Introduction

The presence of rare earth elements (REEs) in terrestrial environments has been increasing due to anthropogenic activities, such as mining and processing of REEs-containing ores, the use of REEs-containing fertilizers, oil refining, and fossil fuel combustion (Kotelnikova, Rogova, and Volkov 2022). REEs-based materials are widely applied across high-technology industries, as catalysts in oil cracking, and in the production of glass, ceramics, and in metallurgical products (Egler et al. 2023; Sysolyatina and Olkova 2023). While REEs

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concentrations in soils are typically in the range of hundreds of milligrams per kilogram, higher levels are observed in agricultural soils (928 mg/kg in China farmland soils), and mining areas, where concentrations can reach several thousands of milligrams per kilogram (~4670 mg/kg in Chinese mining areas) (Gong et al. 2021; Kotelnikova, Rogova, and Volkov 2022). Pollution from REEs mixtures has been reported in sediments, water bodies, and soils impacted by mining activities (He et al. 2020). Given the prevalence of REEs mixtures in soils, it is crucial to investigate their combined toxic effects on terrestrial organisms to better predict the risks posed by intensified REEs use in natural ecosystems (Kotelnikova, Rogova, and Volkov 2022; Sysolyatina and Olkova 2023).

Although research in this area remains scarce, it is expected that when in a mixture, REEs exhibit interactions that either enhance toxicity (synergism) or reduce it (antagonism), thus altering the overall impact (Kotelnikova, Rogova, and Volkov 2022). In aquatic organisms, synergistic effects have been observed, such as increased genotoxicity in *Oncorhynchus mykiss* juveniles exposed to a mixture of five REEs (including lanthanum chloride in bulk form) (Hanana, Kleinert, and Gagné 2021). Conversely, no interaction was reported in the algae *Skeletonema costatum,* where a mixture of thirteen REEs (including lanthanum chloride and yttrium nitrate in bulk form) showed the same inhibitory effect on growth as single REEs exposures (Tai et al. 2010). In plants, antagonistic effects were reported for root elongation of *Triticum aestivum* exposed to yttrium chloride-cerium chloride mixtures (bulk form), while additive effects were observed for yttrium chloride-lanthanum chloride and lanthanum chloride-cerium chloride mixtures (Gong et al. 2019). For soil organisms, only one study has investigated the combined toxicity of REEs mixtures. Gong et al. (2021) reported that a mixture of lanthanum chloride and cerium chloride (both bulk form) caused antagonistic to non-interactive effects on the survival of *Enchytraeus crypticus,* depending on the exposure duration.

Folsomia candida, a widely distributed soil species, is a terrestrial model organism commonly used in ecotoxicological studies due to its significant role in decomposition and mineralization processes (Jośko et al. 2022; Santos et al. 2023). Given the high levels of REEs reported in soil, including agricultural soils, is of the most importance to evaluate the effects of REEs mixtures in ecological relevant organisms such as *F. candida*. However, studies evaluating the effects of REEs, in either nano or bulk form, on *F. candida* under mixture exposure conditions are still lacking. We predict that mixture exposures results in higher toxicity levels. Thus, this study aims to unravel the impact of REEs-based materials, specifically yttrium oxide (Y_2O_3) and lanthanum oxide (La_2O_3), on *F. candida* by comparing the effects of single and dual mixture exposures. Previous research has utilized *F. candida* to investigate the effects of various nanoparticles (NPs), including gold, zinc, copper and molybdenum disulfide NPs (Guimarães et al. 2022; Jośko et al. 2022; Santos et al. 2023). Toxicity will be assessed with endpoints such as survival, reproduction, avoidance behavior, and biochemical markers related to oxidative stress and neurotransmission. This work seeks to determine the relative toxicity of Y_2O_3 and La_2O_3 in their nano and bulk forms, given the known influence of particle size on toxicity outcomes.

2. Material and methods

2.1. Test species

While testing all materials on all species is ideal, it is impractical—hence, representative terrestrial species like earthworms, enchytraeids, and collembolans are commonly used (Fountain and Hopkin 2005). Our previous work with single exposures of Y_2O_3 and La_2O_3 NPs and bulk reported negative impact (decrease reproduction, avoidance behavior, DNA damage) on the soil-dwelling *E. crypticus* (Santos et al. 2025). Given species-specific differences in contaminant responses (Santos et al. 2020), we selected *F. candida* for a more accurate REEs risk assessment in the terrestrial ecosystems. This widely distributed collembolan is sensitive to soil pollutants being soil pore water its primary exposure route for metals (Waalewijn-Kool et al. 2014). The collembolan cultures were maintained in the laboratory on a moist substrate composed of plaster of Paris and activated charcoal in an 8:1 ratio. The cultures were kept at 20 ± 1 °C under a photoperiod of 16 hours (h): 8 h (light: dark). The organisms were fed dried baker's yeast (*Saccharomyces cerevisae*) once per week. Age-synchronized juveniles (10–12 days (d) old) were selected for reproduction tests, while adults of similar sizes were used for avoidance behavior and biochemical markers assays. According to Directive 2010/63/EU of the European Parliament and of the Council of 22/9/2010, invertebrates, like *F. candida* are permitted biological models for scientific experimentation and consequently free of Ethical Statement.

2.2. Test medium

The experiments were conducted using natural Standard Landwirtschaftliche Untersuchungs-und Forschungs-Anstalt (LUFA) 2.2 soil (Speyer, Germany). The main soil characteristics, provided by the supplier, can be found in the Supplementary Material. Before use, the soil was dried at 60 °C for 48 h.

2.3. Test materials and concentrations

Commercial La_2O_3 and Y_2O_3, in both NPs and bulk forms, were purchased as powders from Sigma-Aldrich. According to the supplier, the NPs sizes were < 50 nm for Y_2O_3 (CAS: 1314-36-9) and < 100 nm for La_2O_3 (CAS: 1312-81-8). Quantification of the test materials in spiked soil at the start of the assays (0 h) was performed using inductively coupled plasma mass spectrometry (ICP-MS). Particle size and morphology were characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Detailed procedures are provided in the Supplementary Material.

The nominal concentrations for single exposure reproduction tests were 0, 156, 313, 625, 1250 and 2500 mg/kg soil dry weight (DW) of La_2O_3 and Y_2O_3, in nano and bulk forms. Given the scarce research on REEs materials toxicity in *F. candida* organisms, these concentrations were chosen based on our previous work with single exposures of the same materials with the terrestrial species *E. crypticus* (Santos et al. 2025). The selected nominal concentrations are included in the set of values found in the agricultural and mine soils (Sager and Wiche 2024; Tang et al. 2022). For mixture exposure reproduction tests, concentrations of 0, 156, 625 and 2500 mg/kg of La_2O_3 and Y_2O_3 (NPs and bulk) were selected (based on the results from the single exposures), and all dual combinations of these concentrations were tested. Based on reproduction test results, further avoidance behavior and biochemical marker assays were performed using only the NPs forms of the materials at selected concentrations: 625 and 2500 mg/kg for La_2O_3 NPs, and 156, 625 and 2500 mg/kg for Y_2O_3 NPs in single exposures. Dual combination tests included the following mixtures: 2500 mg/kg of Y_2O_3 NPs + 2500 mg/kg of La_2O_3 NPs; 2500 mg/kg of Y_2O_3 NPs + 625 mg/kg of La_2O_3 NPs; 625 mg/kg of Y_2O_3 NPs + 625 mg/kg of La_2O_3 NPs; 156 mg/kg of Y_2O_3 NPs + 2500 mg/kg of La_2O_3 NPs. Details of the spiking procedure are provided in the Supplementary Material.

2.4. Reproduction tests

The reproduction test for *F. candida* was conducted in accordance with OECD guidelines (OECD 2016). Four replicates (n = 4) were used for each experimental condition, with ten individuals per replicate. The test lasted 28 d, during which survival (number of adults) and reproduction (number of juveniles) were assessed. Additional details are provided in the Supplementary Material.

2.5. Avoidance tests

The avoidance tests were performed following ISO guidelines (ISO 17512-2, 2011). Five replicates (n = 5) were included for each experimental condition, with 20 individuals per replicate. The test duration was 2 d, and the percentage of avoidance was assessed. The detailed procedure is described in the Supplementary Material.

2.6. Biochemical markers

Biochemical assays were conducted based on optimized protocols for *F. candida* described by Maria, Ribeiro, and Amorim (2014). Five replicates (n = 5) were used for each experimental condition, with 55 individuals per replicate. The test duration was 14 d, evaluating the following endpoints: 1) Catalase (CAT) activity: quantified using Claiborne's method (Clairborne 1985); 2) Glutathione reductase (GR) activity: measured according to the method by Carlberg and Mannervik (1975); 3) Glutathione S-transferases (GST) activity: determined using the protocol by Habig, Pabst, and Jakoby (1974); 4) Lipid peroxidation (LPO) levels: assessed following the methods of Ohkawa, Ohishi, and Yagi (1979) and Bird and Draper (1984), adapted by Wilhelm Filho et al. (2001); 5) Acetylcholinesterase (AChE) activity: measured using Ellman et al. (1961), adapted by Guilhermino et al. (1996). Protein concentration was determined using the Bradford method (Bradford 1976). Further methodological details are provided in the Supplementary Material. AChE activity was assessed due to its potential link to avoidance behavior, as its inhibition may impair the ability to detect and avoid contaminated soil (Barreto et al. 2023; Capitão et al. 2022). CAT, GR, GST and LPO were selected as oxidative stress related biomarkers, given prior associations between REE-based NPs (lanthanum and ytterbium oxides) and oxidative stress in *Eisenia fetida* (Adeel et al. 2021).

2.7. Data analysis

Statistical analyses and graphical outputs were performed using SigmaPlot Version 12.5 software. Shapiro-Wilk (for normality) and Levene (for homoscedasticity) tests were done. For parametric data, one way analysis of variance (ANOVA) was conducted, followed by Dunnett's post hoc test for control *versus* treatments comparisons and Turkey's post hoc test for mixtures *versus* single comparisons. For non-parametric data, the Kruskal-Wallis test was employed. Statistical significance was set at $p < 0.05$.

3. Results

To simplify the treatments nominations on the graphics and tables of the 'Results' section, La_2O_3 and Y_2O_3 was abbreviated to La and Y, respectively.

3.1. Materials characterization and quantification

Both nanoparticulated materials exhibited significant aggregation/agglomeration (Figure 1). The average particle sizes were determined to be 14.4±3.6 nm for Y_2O_3 NPs and 37.4±11.9 nm for La_2O_3 NPs. These measurements, obtained via electron microscopy, align with the manufacturers' specifications (< 50 nm for Y_2O_3 NPs and < 100 nm for La_2O_3 NPs). Bulk Y_2O_3 exhibited irregular or cuboid structures, while bulk La_2O_3 presented irregular and fractured morphologies (Figure 1). The average lengths measured were 9.63±4.09 µm for bulk Y_2O_3 and 1.54±0.84 µm for bulk La_2O_3.

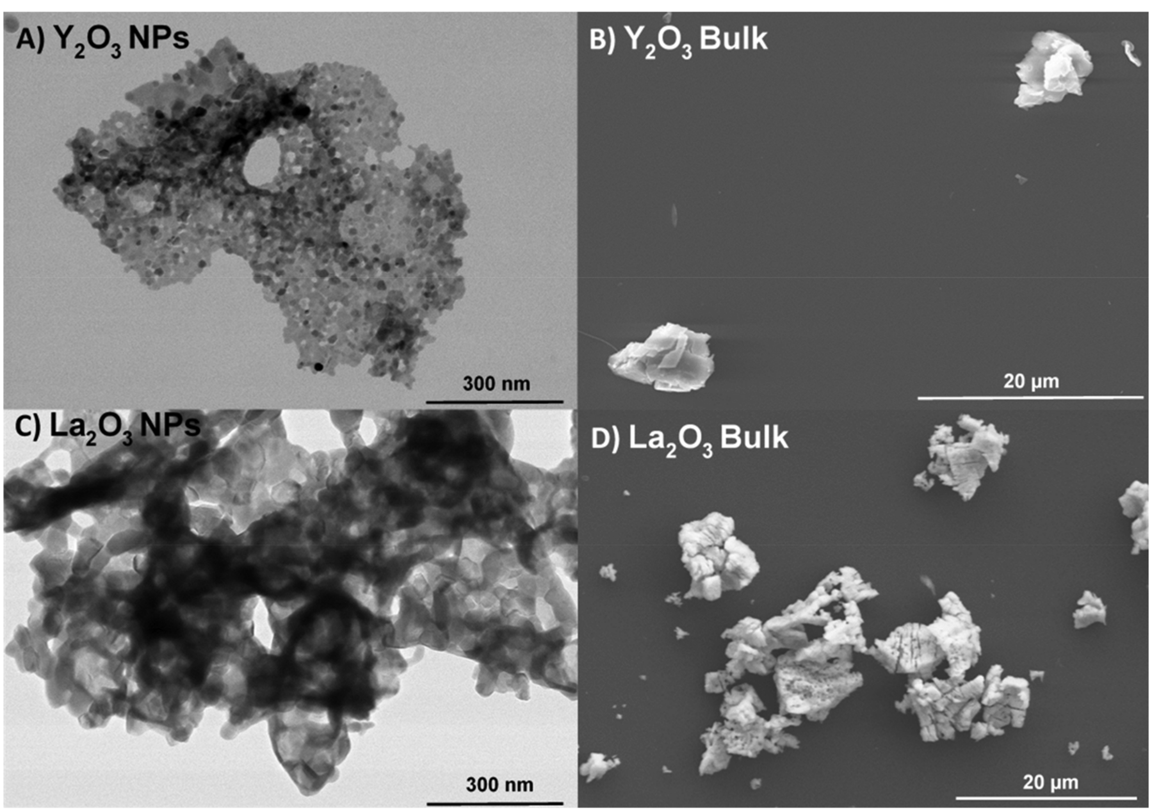

Figure 1. Transmission electron microscopy (TEM) images of yttrium oxide (Y_2O_3) (A), and lanthanum oxide (La_2O_3) (C) nanoparticles (NPs), alongside scanning electron microscopy (SEM) images of bulk yttrium oxide (Y_2O_3) (B) and bulk lanthanum oxide (La_2O_3) (D).

Table 1. Measured concentrations (mg/kg) of lanthanum oxide (La_2O_3) and yttrium oxide (Y_2O_3) in nanoparticles (NPs) and bulk forms in LUFA 2.2 soil at the beginning of the exposure tests (day 0).

Nominal concentrations (mg/kg)	Measured concentrations (mg/kg)			
	La_2O_3 NPs	La_2O_3 Bulk	Y_2O_3 NPs	Y_2O_3 Bulk
Single exposures				
0	5.1 ± 0.09		4.4 ± 0.09	
156	149 ± 2.1	145 ± 2.1	155 ± 2.8	156 ± 0.9
313	306 ± 4.1	311 ± 6.4	315 ± 9.7	313 ± 11
625	599 ± 8.8	615 ± 11	616 ± 6.4	619 ± 6.8
1250	1249 ± 28	1208 ± 24	1206 ± 7.5	1236 ± 5.3
2500	2343 ± 17	2442 ± 29	2469 ± 24	2509 ± 45
Mixture exposures				
0	4.8 ± 0.05		3.8 ± 0.09	
156 Y			153 ± 0.3	158 ± 3.2
625 Y			606 ± 8.8	620 ± 10
2500 Y			2425 ± 24	2464 ± 36
156 La	155 ± 3.8	151 ± 2.6		
625 La	625 ± 13	611 ± 7.1		
2500 La	2472 ± 61	2466 ± 39		
156 Y + 156 La	154 ± 1.4	156 ± 1.8	150 ± 1.3	152 ± 2.0
156 Y + 625 La	612 ± 14	622 ± 11	150 ± 1.5	152 ± 0.5
156 Y + 2500 La	2417 ± 3.3	2532 ± 9.5	155 ± 3.7	155 ± 4.8
625 Y + 156 La	150 ± 1.0	151 ± 1.8	628 ± 8.0	616 ± 9.2
625 Y + 625 La	627 ± 8.4	618 ± 1.4	616 ± 5.3	621 ± 8.5
625 Y + 2500 La	2416 ± 28	2410 ± 42	619 ± 14	613 ± 4.2
2500 Y + 156 La	154 ± 1.2	157 ± 1.8	2449 ± 52	2431 ± 67
2500 Y + 625 La	618 ± 8.9	610 ± 2.0	2418 ± 26	2424 ± 26
2500 Y + 2500 La	2482 ± 19	2424 ± 32	2424 ± 49	2443 ± 27

Results are expressed as mean ± standard deviation (n = 3).

At the start of the exposure tests (0 h), the measured concentrations of Y_2O_3 and La_2O_3 in the soil were generally consistent with the nominal concentrations for both NPs and bulk forms (Table 1).

3.2. Reproduction tests

After 28 d, no significant effects on *F. candida* survival were observed following single exposures to any of the tested materials or forms compared to the control group ($p > 0.05$; Figure 2A). Similarly, dual combinations of Y_2O_3 and La_2O_3 (in both NPs and bulk forms) did not impact survival in this terrestrial species ($p > 0.05$; Figure 2C). In contrast, reproduction output was more sensitive, with a significant decrease in the number of juveniles observed at Y_2O_3 NPs concentrations ≥ 313 mg/kg ($p < 0.05$; Figure 2B). Exposure to La_2O_3 NPs also led to reduced reproduction at concentrations ≥ 1250 mg/kg ($p < 0.05$; Figure 2B). However, bulk forms of both La_2O_3 and Y_2O_3 did not affect reproduction ($p > 0.05$; Figure 2B). During dual exposures, reproduction significantly decreased at the highest tested concentration (2500 mg/kg) of both Y_2O_3 and La_2O_3 NPs ($p < 0.05$; Figure 2D). Other dual combinations did not affect reproductive output ($p > 0.05$; Figure 2D). Regarding interaction effects, the dual exposure of 2500 mg/Kg Y_2O_3 NPs + 2500 mg/Kg La_2O_3 NPs showed no interaction, as the reproduction decrease was comparable to their respective single exposures (Figure 2D; Table 2). An antagonistic effect was observed in other NPs combinations involving 2500 mg/kg La_2O_3 NPs and 625 mg/kg or 2500 mg/kg of Y_2O_3 (Figure 2D; Table 2).

3.3. Avoidance test

After 2 d, no significant avoidance responses by *F. candida* were observed in any treatment, whether for single or mixture exposures to Y_2O_3 and La_2O_3 NPs, compared to the control group ($p > 0.05$; Figure 3).

3.4. Biochemical markers

After 14 d, single exposure to La_2O_3 NPs affected *F. candida* AChE activity, leading to a significant decrease at 2500 mg/kg ($p < 0.05$; Figure 4E). Exposure to Y_2O_3 NPs caused significant increases in CAT activity at lower concentrations (156 and 625 mg/kg) and GR activity at higher concentrations (625 and 2500 mg/kg) ($p < 0.05$; Figure 4A, B). Dual NPs exposures elicited significant increases in CAT (2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3), GR (all tested conditions), AChE (2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3, 2500 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3) and GST activities (2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3, 2500 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3) and LPO levels (156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3) ($p < 0.05$; Figure 4A–E). A significant decrease in AChE activity was also observed for the combination 156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3 ($p < 0.05$; Figure 4E).

Interaction effects varied, showing patterns of synergism, antagonism or no interaction (Table 2). For CAT activity, antagonism was observed in treatments 156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3 and 625 mg/kg Y_2O_3 + 625 mg/kg La_2O_3, as no significant effect was detected despite the single concentrations of Y_2O_3 NPs significantly increasing CAT activity (Figure 4A; Table 2). Synergism was detected for the treatment 2500 mg/kg Y_2O_3 + 625 La_2O_3, with a significant increase in CAT activity where single exposures showed only a tendency (Figure 4A; Table 2). For GR activity, synergism occurred in the combinations of 156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3, 2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3 and 2500 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3, as activity levels were significantly elevated (Figure 4B; Table 2). No interaction was detected for 625 mg/kg Y_2O_3 + 625 mg/kg La_2O_3, as the GR activity increase matched that of Y_2O_3 NPs single exposure (Figure 4B, Table 2). Significant synergism was observed for GST activity in mixtures 2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3 and

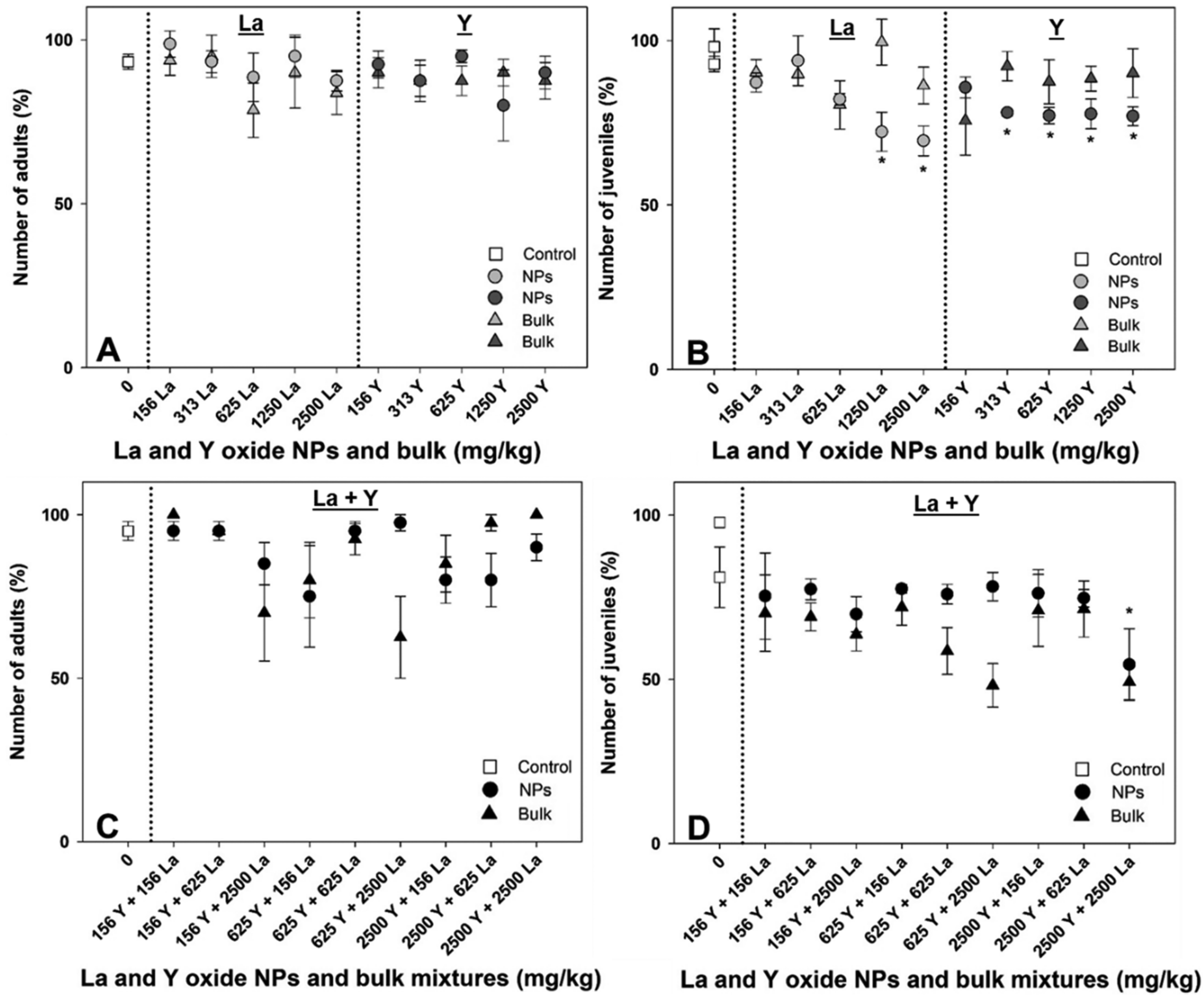

Figure 2. Effects on survival (number of adults) and reproduction (number of juveniles) of *Folsomia candida* after 28 days of exposure to lanthanum (La) oxide and yttrium (Y) oxide nanoparticles (NPs) and bulk forms, under single (A, B) or dual exposures (C, D) in LUFA 2.2 soil. Data are expressed as mean ± standard error (n = 4). *Significant differences with control group – 0 mg/kg ($p < 0.05$).

Table 2. Different interaction effects detected for the mixture exposures of lanthanum (La) oxide and yttrium (Y) oxide nanoparticles (NPs).

Endpoints	Mixtures interaction effects		
	No interaction	Antagonism	Synergism
Survival	–	–	–
Reproduction	2500 Y + 2500 La	156 Y + 2500 La 625 Y + 156 La 625 Y + 625 La 625 Y + 2500 La 2500 Y + 156 La 2500 Y + 625 La	–
Avoidance	–	–	–
Catalase	–	156 Y + 2500 La 625 Y + 625 La	2500 Y + 625 La
Glutathione reductase	625 Y + 625 La	–	156 Y + 2500 La 2500 Y + 625 La 2500 Y + 2500 La
Glutathione S-transferases	–	–	2500 Y + 625 La 2500 Y + 2500 La
Lipid peroxidation	–	–	156 Y + 2500 La
Acetylcholinesterase	156 Y + 2500 La	–	2500 Y + 625 La 2500 Y + 2500 La

2500 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3, with no effects detected in single exposures (Figure 4C; Table 2). The mixture 156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3 synergistically increased LPO levels (Figure 4D; Table 2). Synergism in AChE activity was also detected for 2500 mg/kg Y_2O_3 + 625 mg/kg La_2O_3 and 2500 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3 (Figure 4E; Table 2). No interaction was detected for 156 mg/kg Y_2O_3 + 2500 mg/kg La_2O_3, as the decrease in AChE activity mirrored that of Y_2O_3 NPs single exposure (Figure 4E; Table 2).

4. Discussion

4.1. Single exposure

At the tested concentrations, exposure to La_2O_3 was not lethal to *F. candida*, but the nano form

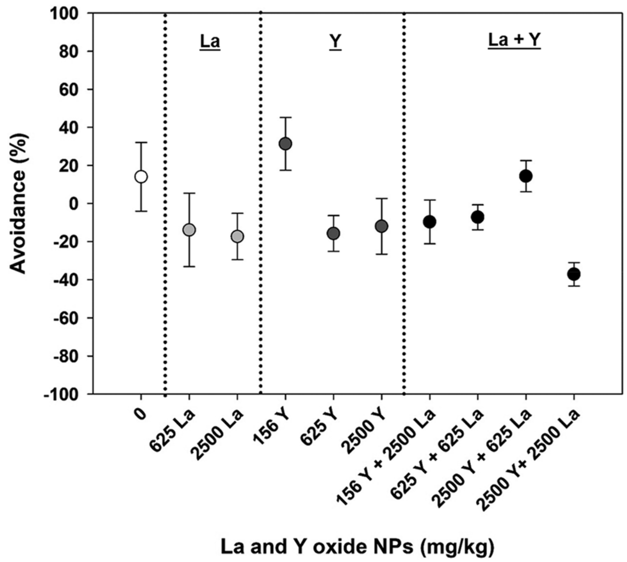

Figure 3. Avoidance responses (%) of *Folsomia candida* after 2 days of exposure to lanthanum (La) oxide and yttrium (Y) oxide nanoparticles (NPs), under dual or corresponding single exposures, in LUFA 2.2 soil. Data are expressed as mean ± standard error (n = 5).

significantly reduced the organisms' reproductive output (≥ 1250 mg/kg). A study on *F. candida* exposed to 12.4 – 10000 mg/kg of La (Lanthanum nitrate hexahydrate - $La(NO_3)_3.6H_2O$) reported an LC_{50} (50% lethal concentration) of 1690 mg/kg and an EC_{50} (50% effect concentration) for reproduction of 1220 mg/kg after 28 d of exposure (Li, Verweij, and van Gestel 2018). Compared to our work, more pronounced toxic effects were detected in the previous study. This can be attributed to differences in experimental design (equilibration of spiked soil for 2 weeks *versus* 1 d; and spiking with solutions *versus* powder) and tested chemical ($La(NO_3)_3.6H_2O$ *versus* La_2O_3). To the best of our knowledge, no other study has assessed La toxicity to *F. candida*. However, our prior work with the same material (La_2O_3 NPs and bulk) on the earthworm *E. crypticus* showed no significant effects on survival and reproduction (Santos et al. 2025) at the same concentration range used in the present study. These results suggest that collembolans were more susceptible to La_2O_3 exposure than enchytraeids, with reproductive effects observed at concentrations ≥ 1250 mg/kg. AChE activity results showed that 2500 mg/kg La_2O_3 NPs inhibited this enzyme's activity in *F. candida*. Neurotoxic effects, indicated by AChE inhibition in invertebrates, have previously been linked to REEs exposures (Sager and Wiche 2024). In *E. fetida,* exposure to ≥ 100 mg/kg of La_2O_3 in nano and bulk forms inhibited AChE activity in a concentration-dependent manner (Adeel et al. 2021). For this endpoint, *E. fetida* appeared to be more sensitive than *F. candida,* as effects in the present study were only observed at the highest tested concentration and exclusively for NPs form. The authors also reported that La_2O_3 NPs effects on AChE activity were more pronounced than the bulk form, suggesting that NPs adsorption or physical interaction with the enzyme's structure might explain this result (Adeel et al. 2021). AChE inhibition can impair organisms' ability to avoid contaminated soil (Barreto et al. 2023; Capitão et al. 2022). Thus, the lack of avoidance behavior in *F. candida* at 2500 mg/kg La_2O_3 NPs may have been due to AChE inhibition observed at the same concentration. However, at concentrations lower than 2500 mg/kg, no avoidance was observed, and no AChE effects were detected, possibly indicating other neurotoxic mechanism. Santos et al. (2025) also reported no avoidance response in *E. crypticus* exposed to 625 and 2500 mg/kg of La_2O_3 in both NPs and bulk forms. No other biochemical marker assessed in this study was affected by single exposure to La_2O_3 NPs. In *E. fetida,* 14-d exposure to lanthanum(III) chloride ($LaCl_3$) (up to 1200 mg/kg) stimulated antioxidant defense systems (initial increase in superoxide dismutase activity, later decrease at higher concentrations with prolonged exposure) and induced LPO (Tang et al. 2022). However, in this study, La_2O_3 NPs induced no significant effects on oxidative stress related enzymes (CAT, GR and GST) or LPO in *F. candida*. This discrepancy might relate to differences in the tested material ($LaCl_3$ *versus* La_2O_3 NPs) or the intrinsic characteristics/sensitivities of the test species (*E. fetida versus F. candida*).

Y_2O_3 was notably more toxic to *F. candida* than La_2O_3. Fertility and enzyme activity alterations were evident with Y_2O_3 NPs: decreased reproduction (≥ 313 mg/kg) and increased CAT (156 and 625 mg/kg) and GR (625 and 2500 mg/kg) activities. This suggests that Y_2O_3 exposure stimulated *F. candida's* antioxidant defense system. No LPO alterations were detected, indicating the increased CAT and GR activity was sufficient to prevent oxidative damage. To our knowledge, only one previous study (Santos et al. 2025) addressed Y_2O_3 effects on a terrestrial species (*E. crypticus*) and this study is the first to assess its effects on *F. candida*. Y_2O_3 reduced *E. crypticus* reproduction at 1250 mg/kg (bulk and NPs) and 2500 mg/kg (NPs) (Santos et al. 2025). Compared to bulk forms, *F. candida* was more sensitive to Y_2O_3 NPs, with reproduction effects at lower concentrations (≥ 313 mg/kg). Conversely, bulk Y_2O_3 affected *E. crypticus* reproduction at 1250 mg/kg (Santos et al.

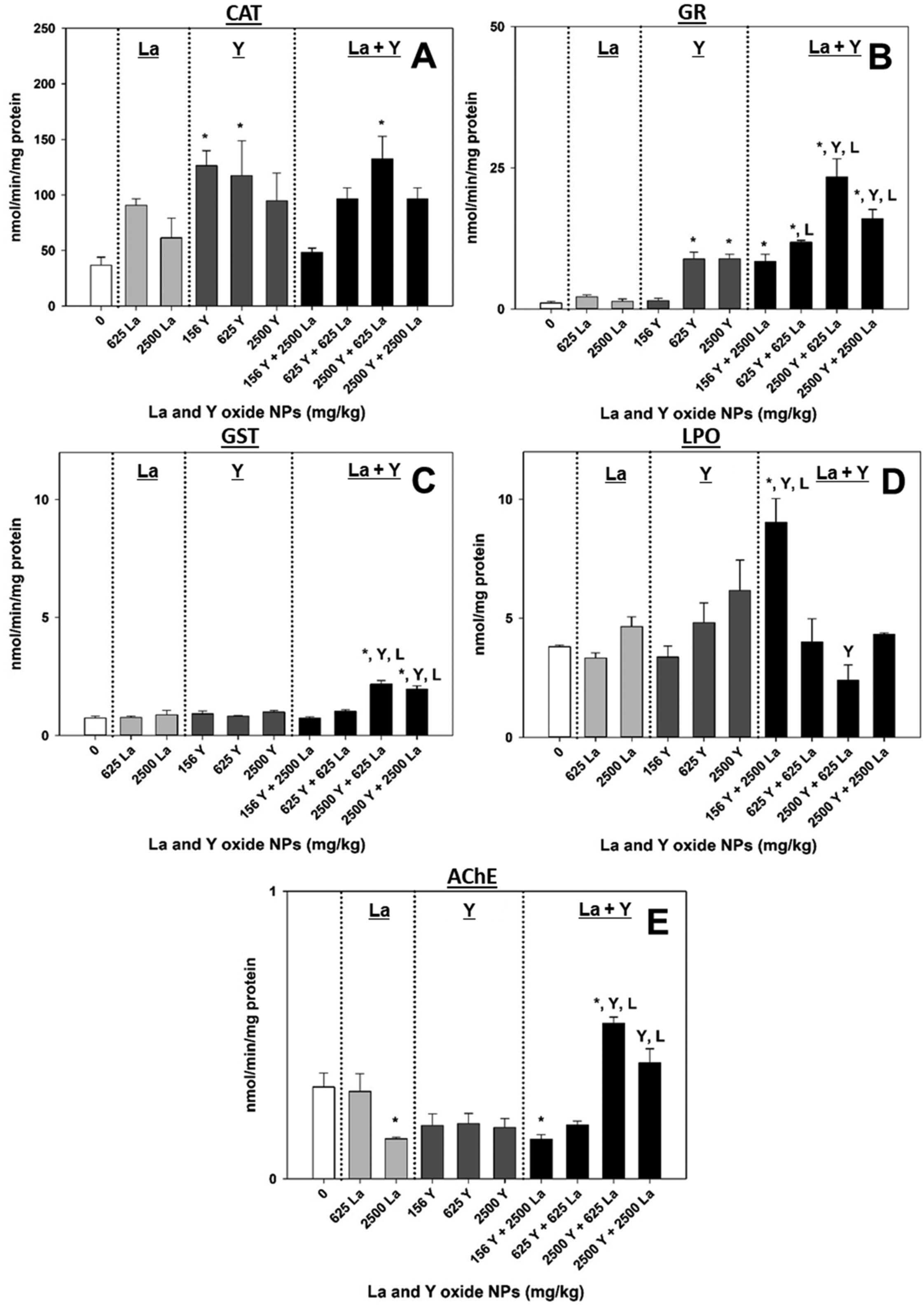

Figure 4. Effects on catalase (CAT, A), glutathione reductase (GR, B), glutathione S-transferases (GST, C) activities, lipid peroxidation (LPO, D) levels and acetylcholinesterase (AChE, E) activity in *folsomia candida* after 14 days exposure to lanthanum (La) oxide and yttrium (Y) oxide nanoparticles (NPs), under dual or corresponding single exposures, in LUFA 2.2 soil. Data are expressed as mean ± standard error (n = 5). *Significant differences with the control group – 0 mg/kg ($p < 0.05$); Y significant differences with the correspondent yttrium oxide single concentration ($p < 0.05$); L significant differences with the correspondent lanthanum oxide single concentration ($p < 0.05$).

2025) but did not impact *F. candida* reproduction. Organisms' response to contaminants vary with their ecology, physiology and exposure routes (Santos et al. 2020). Collembolans primarily interact with soil pore water, while earthworms encounter both pore water and soil particles via dermal and oral contact, potentially altering chemical bioavailability (Santos et al. 2020). Such differences can partially explain varying species responses to REEs contamination. The different sensitivities of the organisms to REEs exposure highlight the need to test a set of soil organism's species for a more accurate REEs risk assessment in the terrestrial ecosystems, since the species used greatly influence the resulting toxicity.

The comparison between bulk and NPs forms revealed that NPs of both La_2O_3 and Y_2O_3 were more toxic to *F. candida* than their bulk counterparts. Previous studies have shown that metal oxide NPs (CeO_2, Al_2O_3, CuO, NiO, and TiO_2) are generally more soluble than their bulk counterparts due to their higher surface area (Avramescu et al. 2022, 2020). Enhanced dissolution and subsequent leaching of metal ions may partly explain the higher toxicity observed for NPs in the present study when comparing with bulk forms. Dahle and Arai (2015) also noted that NPs such as silver, silicon dioxide, and titanium dioxide are generally more toxic due to heightened reactivity from their high surface area to volume ratio. Similarly, Adeel et al. (2021) found that REEs-based NPs (e.g. La and Ytterbium oxides) induced more toxic effects than their bulk forms, causing histopathological changes and reactive oxygen species (ROS) production, which disrupt antioxidant systems. Due to their smaller size, NPs can penetrate organisms more efficiently, entering cells and tissues (Ekner-Grzyb et al. 2023). Noordhoek et al. (2018) demonstrated that *F. candida* can ingest NPs, as evidenced by the red coloration of the midgut, following exposure to organic pigmented NPs (Noordhoek et al., 2018), suggesting direct uptake via ingestion. Furthermore, in vitro studies also suggested that cellular uptake of NPs involve endocytosis-based uptake pathways (Toscano and Torres-Arias, 2023). Nonetheless, further investigations are required to confirm the uptake of Y_2O_3 and La_2O_3 NPs by *F. candida* and the subsequent entrance of NPs inside the cells. The previous research discussed above explores possible reasons for the higher toxicity of NPs reported in the present study, including greater metal ion dissolution, increased reactivity, and easier cellular uptake. However, further in-depth studies are needed to confirm these hypotheses and clarify the underlying mechanisms for La_2O_3 and Y_2O_3. Studies with cerium oxide (CeO_2) demonstrated greater NPs accumulation in zucchini than bulk forms (Hawthorne et al. 2014). However, research on La_2O_3 demonstrated that La element undergoes trophic transfer (*Lactuca sativa* → *Acheta domesticus* → *Tenodera aridifolia sinensis* and *Sphodromantis centralis*), but NPs levels were equivalent to or lower than bulk forms (De la Torre Roche et al. 2015). Santos et al. (2025) reported that Y_2O_3 NPs were more toxic to *E. crypticus* than bulk forms, but for La_2O_3, the bulk form had greater effects. No consistent pattern emerges for REEs toxicity based on form (nano *versus* bulk). However, material form significantly influences toxicity, depending on the chemical type, species, and endpoint evaluated.

The electron microscopy characterization of the NPs presented in this study revealed significant aggregation/agglomeration. However, this analysis was based on NPs suspensions, and the observed aggregation may partly result from the drying process during sample preparation on the microscopy support. As such, the aggregation observed under these conditions may not accurately reflect the behavior of the particles in soil environments. Consequently, the characterization results cannot be directly linked with the toxicity outcomes, as the behavior of the particles in soil media may differ substantially. Unfortunately, the characterization of NPs in complex environmental matrices, such as soils, remains a major challenge as most available techniques are limited to aqueous systems (Tourinho et al. 2012). However, if aggregation or agglomeration of Y_2O_3 and La_2O_3 NPs also occurred in soil, this could influence their toxicological effects. Generally, aggregation/agglomeration is thought to reduce the toxicity by decreasing the specific surface area, which can limit the nano-cell interactions and metal ion leaching (Liu et al. 2022). However, some studies have reported increased toxicity associated with larger aggregates/agglomerates, potentially due to more extensive damage to the cell structures (Liu et al. 2022). Therefore, further studies are needed to better assess the behavior of Y_2O_3 and La_2O_3 NPs in soil media and the consequent impact on their toxicity.

4.2. Mixture exposure

Under realistic environment conditions, single-element contamination is rare, whereas contamination with mixtures of REEs is more widespread. However, the toxic effects of these mixtures remain understudied (Gong et al. 2019; Li et al. 2020). In this study, REEs mixtures exhibited a different toxicity pattern toward *F. candida* compared to single exposures. For some evaluated endpoints the effects were similar regardless of whether the REEs were in a mixture or individually, indicating no interaction and resulting in a simple additive effect from each component (Gong et al. 2019). Additive effects were observed for GR activity at 625 mg/kg of Y_2O_3 NPs + 625 mg/kg of La_2O_3 NPs (a similar increase in activity compared to the single exposure of Y_2O_3 NPs), as well as for AChE activity at 156 mg/kg of Y_2O_3 NPs + 2500 mg/kg of La_2O_3 NPs (a similar decrease in activity to the single exposure of La_2O_3 NPs). Previous studies have also reported additive effects in REEs mixtures: He et al. (2020) found no interaction in binary mixtures of Y, La and Cerium, showing an additive effect on wheat root elongation (*Triticum aestivum*); similarly, Tai et al. (2010) observed additive effects on *Skeletonema costatum* algae, with 50% reduction in growth in both single and mixed exposures of various REEs (La, Cerium, Neodymium, Samarium, Europium, Gadolinium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium, Lutetium, Scandium, and Y).

However, REEs mixtures can induce interactive effects, resulting in either antagonistic (less-than-additive) or synergistic (more-than-additive) toxicity (Gong et al. 2019). Antagonism was evident in our study for the reproduction output, where single exposure of Y_2O_3 NPs (≥ 313 mg/kg) and La_2O_3 NPs (≥ 1250 mg/kg) reduced *F. candida* number of juveniles. In mixtures, this reduction was only observed at the dual combination of 2500 mg/kg of Y_2O_3 NPs + 2500 mg/kg of La_2O_3 NPs. Morel et al. (2021) also reported antagonistic effects in a ternary mixture of Cerium, Thulium, and Y in transcriptomic analyses of *Chlamydomonas reinhardtii*. Antagonistic interactions may arise from competition for uptake, translocation, distribution, and detoxification, leading to reduced toxicity (Gong et al. 2019). Since Y_2O_3 and La_2O_3 share similar physicochemical properties, they may compete for common transport sites within the organism, resulting in reduced accumulation of one or both metals and, consequently, lower toxicity (Gong et al. 2021). The biochemical markers assessed in this study revealed alterations in neurotransmission, detoxification and antioxidant responses in *F. candida* that were dependent on the type of exposure (single *versus* mixture). Antagonism was observed in CAT activity under certain experimental conditions. However, synergistic interactions were predominant in the present study, affecting CAT, GR, GST and AChE activities, as well as LPO levels, depending on the concentrations of REEs in the mixtures. Synergistic effects may result from disruptions in physiological processes during the toxicodynamic phase, potentially linked to the combination of stressor factors (Gong et al. 2021; Romero-Freire et al. 2019). Previous studies have also noted synergetic and antagonistic interactions occurred between copper and nickel, depending on their respective concentrations (Gong et al. 2021). Such interactions may influence chemical activity or bioavailability of toxicants, depending on differences in properties and modes of action (He et al. 2020). In the present study, the toxicity of REEs mixture was influenced by the concentration of each REE, the tested endpoint, and the material form (nano *versus* bulk).

The interpretation of toxicity data for REEs mixtures is inherently complex due to potential interactions at the site(s) of toxicity, organismal physiological processes, and other constituents within the organism (Morel et al. 2021). Previous research has shown that the interaction patterns in mixtures may be affected by REEs type/concentration, exposure duration, and test species (Bergsten-Torralba et al. 2020; Gong et al. 2021; Romero-Freire et al. 2019). For example, Gong et al. (2021) reported that the interaction between La and Cerium shifted from antagonistic after 1 – 2 d to nearly additive after 4 – 14 d of exposure, possibly due to equilibrium in metal body concentrations over time. Additionally, species-specific differences influence REEs mixture toxicity. Romero-Freire et al. (2019) observed synergistic interactions in a ternary mixture of Cerium, Gadolinium and Lutetium for *Aliivibrio fischeri* and *Raphidocelis subcapitata*, but antagonistic effects for *Brachionus calyciflorus*. Bergsten-Torralba et al. (2020) observed predominantly synergistic effects in mixtures of REEs (La, Neodymium and Samarium) to algae (*Chlorella vulgaris* and *R. subcapitata)* and antagonistic effects in the primary consumer *Daphnia similis*. These findings highlight the importance of studying REEs mixtures in other terrestrial species to better understand their impact on soil communities.

Our results showed that REEs mixtures decrease reproduction and caused alterations in neurotransmission, detoxification and antioxidant responses in *F. candida*. Negative impacts on *F. candida* populations may have serious consequences for soil ecosystems, including slower decomposition rates, reduced nutrient cycling and availability (which may affect plant productivity), changes in soil microbial diversity and activity, and impact on other species that are higher on the food web (Biryol et al. 2024; Fountain and Hopkin 2005). The well-being of *F. candida* populations is tightly linked to overall soil ecosystems functions, so their toxicity responses after REEs mixture exposure in our study may give some insight about REEs mixtures risk for soil ecosystems. Given the persistence of REE-based materials and the relevance of mixture scenarios, it is crucial to assess their chronic effects under environmentally relevant conditions for long-term exposures. Further research should explore other REEs mixtures and a broader range of terrestrial species, understanding REEs materials behavior in soil, including bioavailability and possible bioaccumulation or biomagnification. In this context, omics methods (transcriptomics, proteomics, metabolomics and genomics) represent promising tools to unravel molecular-level interactions and reveal mechanisms and molecular pathways modulated by mixture exposures, offering a deeper understanding of their effects inside the organisms (Khosrovyan, Vodovnik, and Mortimer 2025). Exploring how the two REEs interact with each other in the mixture and in the complex soil media may also be important to better understand the complex interactions and help to explain the toxicity results.

5. Conclusion

This study investigated the single and binary toxicity of La_2O_3 and Y_2O_3 to *F. candida,* considering different material forms (NPs and bulk). Both La_2O_3 and Y_2O_3, in single exposures, negatively impacted *F. candida* reproduction and biochemical markers, with NPs exhibiting higher toxicity compared to their bulk counterparts. Based on these effects in *F. candida* (some observed at a low concentration; for example, decreased reproduction at 313 mg/kg Y_2O_3 NPs) and considering the REEs concentrations on China agricultural soils (928 mg/kg) and mining areas (~4670 mg/kg), it appears that REEs can pose risks to these terrestrial ecosystems. Between La_2O_3 and Y_2O_3, the latter proved to be more toxic to *F. candida*.

The findings for REEs oxide mixtures (La_2O_3 + Y_2O_3) showed that toxicity was influenced by multiple factors, including the type and concentration of REE oxide and the endpoint evaluated. Mixture exposures resulted in additive, synergistic, or antagonistic effects, with synergistic interactions being more prevalent and generally leading to greater toxicity to *F. candida* than single exposures. These results emphasize the need for further research at biochemical and molecular levels to better understand the mechanisms underlying mixture toxicity and the physiological aspects influencing the toxicity patterns.

To deepen our understanding of REEs mixture toxicity toward soil organisms, future studies should consider other species and endpoints, as prior research has highlighted the role of test species in shaping mixture interactions and their effects. The current toxicity assessment of REEs mixtures offers a foundational perspective on the combined effects of REEs in terrestrial environments. This work aims to support the safer design of NPs, the development of policy-making to minimize REEs future contamination and raise awareness of mixture exposure scenarios in ecological risk assessments.

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Authors' contributions

Joana Santos: Conceptualization, Methodology, Formal analysis, Investigation, Writing—original draft, Writing—review and editing; Ana Capitão: Methodology, Formal analysis, Investigation, Writing—review and editing; Manuel Barbosa: Methodology, Formal analysis, Investigation, Writing—review and editing; Thomás Pires: Methodology, Formal analysis, Writing—review and editing; Edgar Pinto: Methodology, Formal analysis, Resources, Writing—review and editing; Ana L. Daniel-da-Silva: Methodology, Formal analysis, Resources, Supervision, Writing—review and editing; Angela Barreto: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing—review and editing; Vera L. Maria: Conceptualization, Methodology, Investigation, Formal analysis, Resources, Supervision, Writing—review and editing, Project administration, Funding acquisition.

Disclosure statement

The authors report there are no competing interests to declare.

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Data availability statement

Data are contained within the article and supplementary material.

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