Bioavailability and toxicity of nanoscale/bulk rare earth oxides in soil: Physiological and ultrastructural alterations in *Eisenia fetida*

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Supplementary information

1.1 Cast enzyme activities

Three enzymes were evaluated: acid phosphatase, β-glucosidase, and lkaline phosphatase[1]. The substrates used for the phosphatase and β-glucosidase protocols were p- Nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO) and 4-Nitro- phenyl β-D-glucopyranoside (≥98%, Sigma-Aldrich, St. Louis, MO), respectively. The phosphatase samples were incubated for 45 min and β-glucosidase samples for 1 h. The reactions in the supernatants were then terminated by adding 1 N of NaOH. After adding ultrapure DI water for dilution, a Microplate Reader was used to read the developed colors at 410 nm. The phosphatase and β-glucosidase activity were both calculated as:

Activity (μmol $h^{-1}g^{-1}$) =Final OD=1⁄[(EC=μmol=mL)/(1.5 mL/assay) *(incubation time, h)*(g sample suspension)(0.75 ml sample suspension)

where g DOM was the oven dried mass from 2 g of wet soil sample. The final OD value was calculated by subtracting the OD values of the sample controls and the substrate controls from the sample assays. The EC value for phosphatase and β-glucosidase was 2.2369 mL/μmol.

2.1 NMs characterizations

TEM results revealed the size of of nanoscale La_2O_3, bulk- La_2O_3, nanoscale Yb_2O_3 and bulk-Yb_2O_3 to be 39±9 nm, 756±200 nm, 30±7 nm and 580±100 nm, respectively. TEM observation showing that both nanoparticles are aggregated, but that the bulk particles were not aggregated (Figure S1 A). Zeta potential values of nanoscale La_2O_3, bulk La_2O_3, nanoscale Yb_2O_3 and bulk Yb_2O_3 were 15±2, 19±1.5, 14±1.4 and 17±0.2 mV (Table S1). These results suggest moderate stability of both nanoscale and bulk materials. All bulk and nanoparticles readily dispersed into DI water. The dissolution percentage was significantly greater in acidic conditions (pH 4.5). However, both rare earth based NMs were only slightly dissolved in DI water at pH 7 (Figure S1 B) .

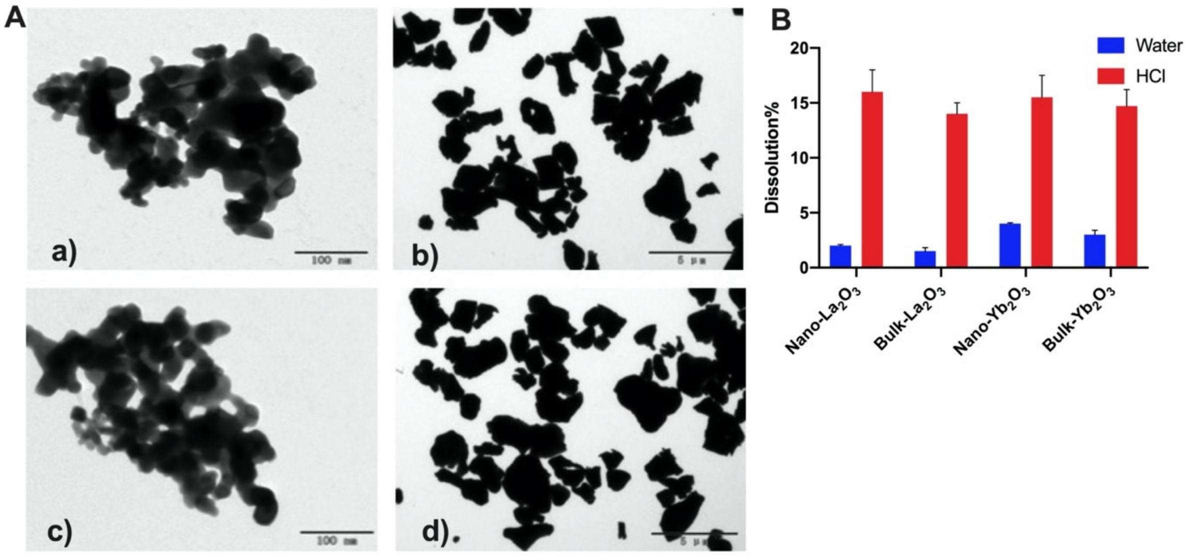

Figure S1 Morphological characterization. **(A)** TEM images of (a)nanoscale La_2O_3 NPs, (b) bulk La_2O_3, (c) nanoscale Yb_2O_3, and (d)bulk Yb_2O_3**.** **(B)** Dissolution of nanoscale and bulk particles in DI water (pH=7) and acidic solution (pH=4.5)

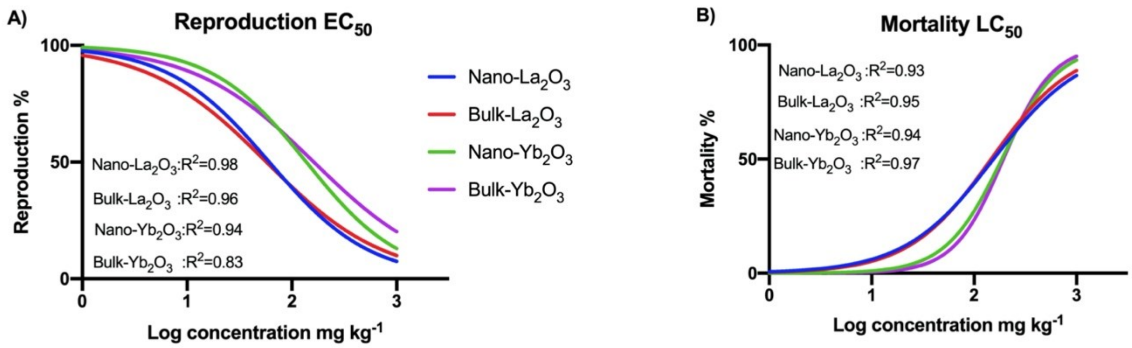

Figure S2 Dose–response curves for *Eisena fetida* exposed to nanoscale and bulk REO for 28 days. (a) Reproduction (28-day median effective concentration (EC_{50}) of nano La_2O_3, bulk La_2O_3, nano Yb_2O_3 and bulk Yb_2O_3 was. 60, 57, 138 and 162 mg kg^{-1}, respectively (b) Mortality (28-day median lethal concentration (LC_{50}) of nano La_2O_3, bulk La_2O_3, nano Yb_2O_3

and bulk Yb_2O_3 was 155, 146, 186 and 193 mg kg^{-1}. Results are presenting as average values and curves represent the models fit to data. $p < 0.05$

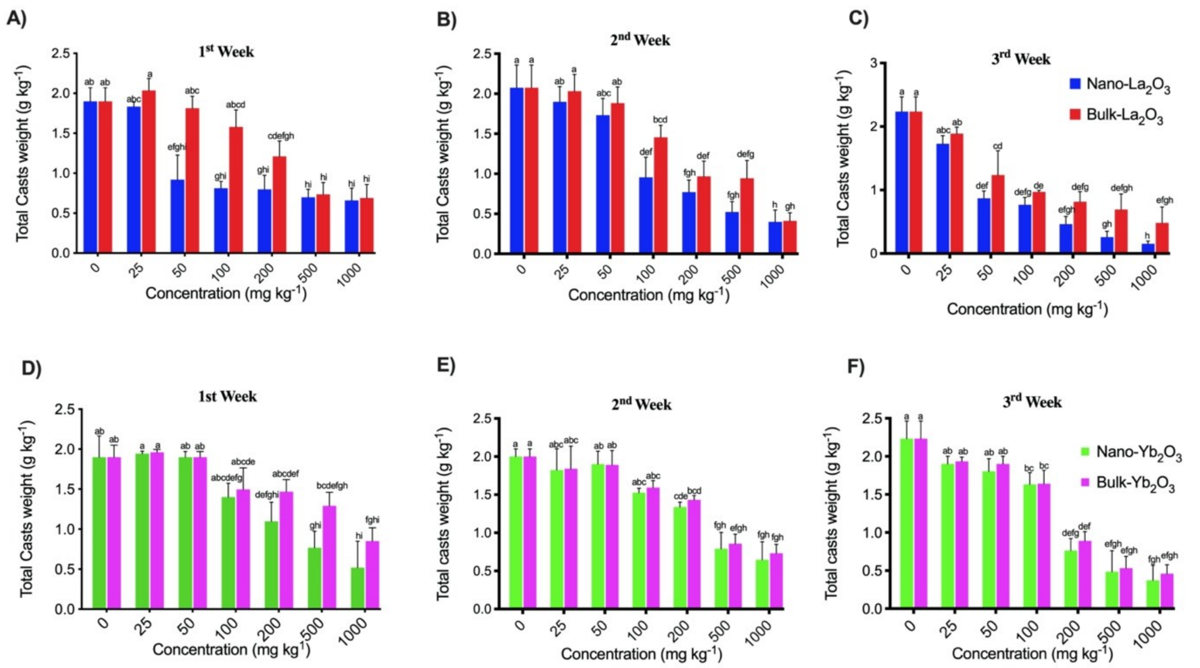

Figure S3. Weekly earthworm cast production under exposure to nanoscale and bulk REO based material (n=4, $p < 0.05$)

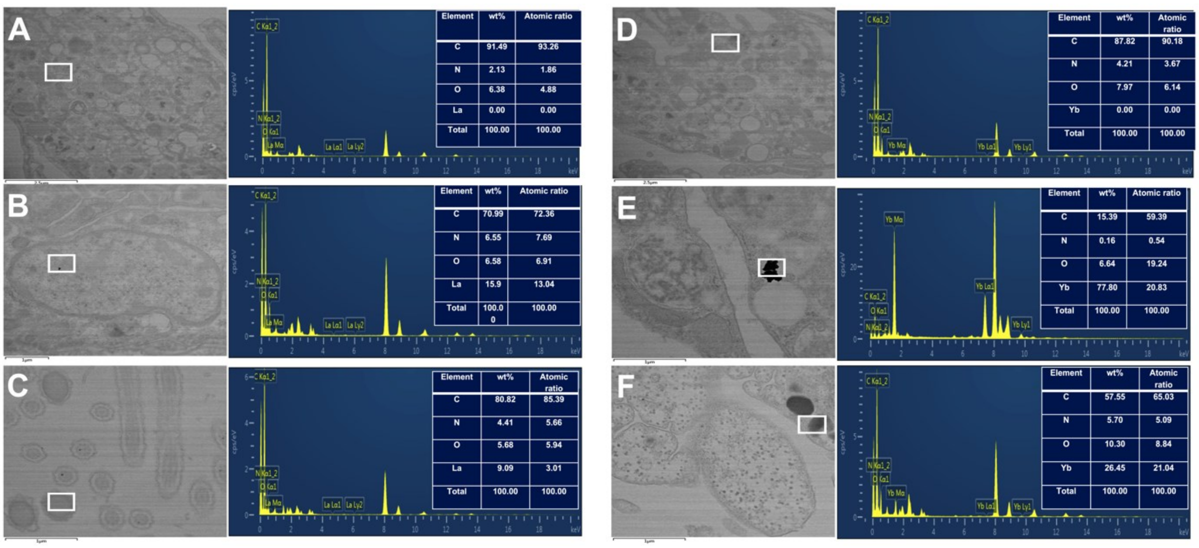

Figure S4. EDS analysis of earthworm tissue (A) Control (B) Nanoscale La_2O_3 (C) Bulk La_2O_3 (D) Control (E) Nanoscale Yb_2O_3 (F) Bulk Yb_2O_3

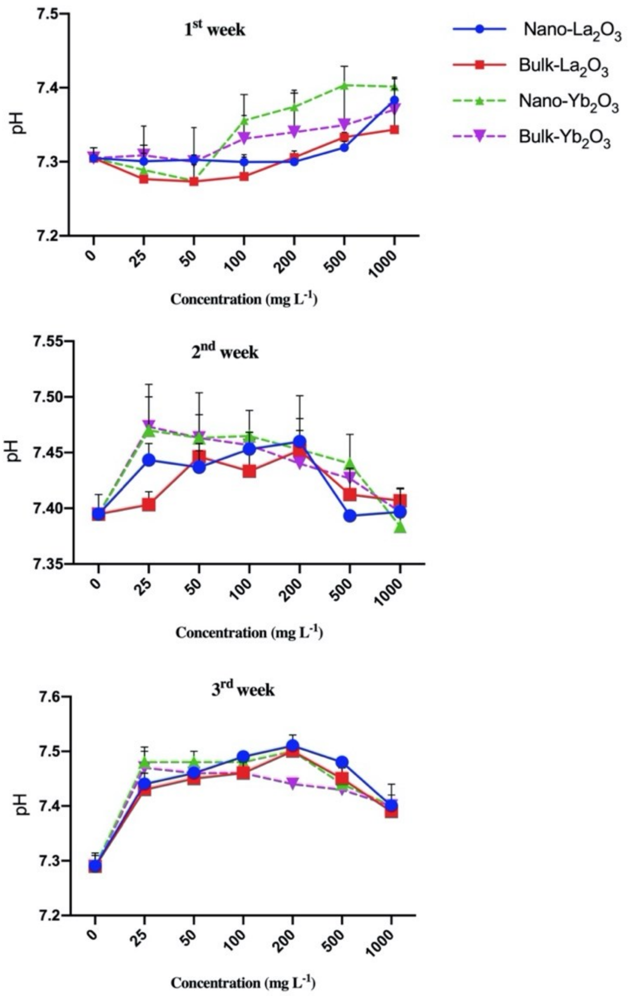

Figure S5. Weekly pH measurements in exposed soil

Table S1. Soil characteristics

Index	Mean Value
Soil property	Silty loam
Dry unit weight (g/cm^3)	1.29
pH	6.9
Electrical conductivity (dS/m)	0.16
Rapidly available N (mg/kg)	20.37
Rapidly available P (mg/kg)	11.21
Rapidly available K (mg/kg)	73.64
Organic matter (mg/kg)	11.31
CEC (cmol/kg)	14.27
$CaCO_3$ (g/kg)	43.19
Rapidly available Fe (mg/kg)	22.91
Rapidly available Mn (mg/kg)	12.77
Rapidly available Cu (mg/kg)	3.17
Rapidly available Ni (mg/kg)	20.22

Table S2. Measurements of REO-NMs

REO	Purity(%)	TEM (nm)	ζ mv	DLS (nm)
Nano-La_2O_3	98.8	39±9	15±2	216±20
Bulk-La_2O_3	98.9	756±200	19±1.5	624±14
Nano-Yb_2O_3	99.9	30±7	14±1.4	207±13
Bulk-Yb_2O_3	99.9	580±100	17±0.2	557±15

Table S3 : Bioaccumulation factor (BAF) of nanoscale and bulk REO in earthworm over 28 days Mean ± SD (n = 3) with different letters in the column indicate significant ($p < 0.005$) difference between the treatments.

mg kg^{-1}	Nao-La_2O_3	Bulk-La_2O_3	Nano-Yb_2O_3	Bulk- Yb_2O_3
25	$0.08±0.00096^{fg}$	$0.07±0.00020^{fg}$	$0.13±0.0033^{a}$	$0.11±0.00035^{b}$
50	$0.11±0.0057^{cd}$	$0.10±0.00030^{de}$	$0.13±0.0070_{a}$	$0.12±0.00035^{a}$
100	$0.10±0.00020^{bc}$	$0.06±0.00061^{h}$	$0.09±0.0002^{e}$	$0.0096±0.47^{e}$
200	$0.06±0.00027^{h}$	$0.04±0.00042^{i}$	$0.08±0.00035^{f}$	$0.07±0.00071^{g}$
500	$0.03±0.00022^{i}$	$0.03±0.00082^{i}$	$0.07±0.0002^{fg}$	$0.06±0.0012^{h}$
1000	$0.03±00045^{i}$	$0.03±0.00021^{i}$	$0.06±0.0004^{h}$	$0.03±0.00287^{i}$

References

1. Li, B.; Chen, Y.; Liang, W.-z.; Mu, L.; Bridges, W. C.; Jacobson, A. R.; Darnault, C. J. G., Influence of cerium oxide nanoparticles on the soil enzyme activities in a soil-grass microcosm system. *Geoderma* **2017,** *299*, 54-62.