

RESEARCH ARTICLE

# Fate and Phytotoxicity of CeO<sub>2</sub> Nanoparticles on Lettuce Cultured in the Potting Soil Environment

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## Abstract

Cerium oxide nanoparticles (CeO<sub>2</sub> NPs) have been shown to have significant interactions in plants. Previous study reported the specific-species phytotoxicity of CeO<sub>2</sub> NPs by lettuce (*Lactuca sativa*), but their physiological impacts and vivo biotransformation are not yet well understood, especially in relative realistic environment. Butterhead lettuce were germinated and grown in potting soil for 30 days cultivation with treatments of 0, 50, 100, 1000 mg CeO<sub>2</sub> NPs per kg soil. Results showed that lettuce in 100 mg·kg<sup>-1</sup> treated groups grew significantly faster than others, but significantly increased nitrate content. The lower concentrations treatment had no impact on plant growth, compared with the control. However, the higher concentration treatment significantly deterred plant growth and biomass production. The stress response of lettuce plants, such as Superoxide dismutase (SOD), Peroxidase (POD), Malondialdehyde(MDA) activity was disrupted by 1000 mg·kg<sup>-1</sup> CeO<sub>2</sub> NPs treatment. In addition, the presence of Ce (III) in the roots of butterhead lettuce explained the reason of CeO<sub>2</sub> NPs phytotoxicity. These findings demonstrate CeO<sub>2</sub> NPs modification of nutritional quality, antioxidant defense system, the possible transfer into the food chain and biotransformation in vivo.

## Introduction

Cerium oxide nanoparticles (CeO<sub>2</sub> NPs), as a typical rare earth metal oxide NPs, are commonly used as a polishing agent for ophthalmic lenses, television tubes and glass mirrors and as fuel additives in order to decrease the emissions of particulates.[1–3] As they can enter soil through atmosphere deposition or from biosolids treated in waste water treatment,[4,5] concerns over

potential health and environmental risks of CeO<sub>2</sub> NPs exposure have been raised.[6] CeO<sub>2</sub> NPs are one of the selected nanomaterial for priority testing by the Working Party of Manufactured Nanomaterials of the Organization for Economic Cooperation and Development (OECD). CeO<sub>2</sub> NPs are generally recognized as stable and insoluble in biological or environment systems, therefore they can exist for a long time.[7]

Previous reports show that CeO<sub>2</sub> NPs exist in nanoparticle form and affect physiological processes and molecular response to plants. Zhang et al. systematically studied the uptake, distribution and biotransformation of CeO<sub>2</sub> NPs in cucumber plants, and verified that Ce presented in the roots as CeO<sub>2</sub> and CePO<sub>4</sub> while in the shoots as CeO<sub>2</sub> and cerium carboxylates.[8,9] Rico et al. showed that CeO<sub>2</sub> NPs modify the antioxidative stress enzyme activities and macromolecule composition in rice seedlings.[10] Ma et al. demonstrated CeO<sub>2</sub> NPs could alter expression of glutathione and sulfated metabolic pathways in *Arabidopsis thaliana* (L.) under Petri dish culture condition.[11] The genotoxicity of CeO<sub>2</sub> NPs on soybean plants was reported by López-Moreno *et al.*[12] Majority of the existed researches were carried out in aqueous[13], sand[14] or agar medium[15], they are generally used as an idealistic environment, without complex components. Soil is a complex system of minerals, organic material, water, gasses, and living organisms. Lee et al. demonstrated that exposure media have significant effects on phytotoxicity of NPs, and suggested that application of NPs in soil is important to understand the terrestrial toxicity of nanoparticles.[16] Zhao et al. found that lipid peroxidation and ion leakage in corn plants were not affected by CeO<sub>2</sub> NP exposure in soil.[17] The results were different from hydroponic medium condition. Priester et al. found that CeO<sub>2</sub> NPs could stunt soil-cultivated soybean growth and pod biomass while shut the nitrogen fixation down.[18] However, Wang et al. reported that CeO<sub>2</sub> NPs had either an inconsequential or a slightly positive effect on plant growth and tomato production cultivated in potting soil at concentrations of 0.1–10 mg·L<sup>-1</sup>. [19] Morales et al. exhibited that CeO<sub>2</sub> NPs could change the nutritional properties of cilantro.[20] These inconsistencies may be caused by the differences in exposure methods, plant species, and especially culture media.

Lettuce is an edible plant and among the 10 species recommended by US EPA (1996) for phytotoxicity assessment. We previously reported that CeO<sub>2</sub> NPs significantly inhibited root elongation of lettuce in both aqueous suspension and plant agar medium. Compared to deionized water, the bioavailability of CeO<sub>2</sub> NPs in the agar medium was reduced, but the sensitivity of asparagus lettuce to CeO<sub>2</sub> NPs was increased. [15] [21,22] The aim of this work was to evaluate the influence of CeO<sub>2</sub> NPs on the growth of lettuce plants in soil medium. Representative parameters such as biomasses, chlorophyll, protein, nitrate and soluble sugar contents, antioxidant enzyme activities, and lipid peroxidation were investigated to understand the plant's defense and response to abiotic stress caused by CeO<sub>2</sub> NPs. In addition, CeO<sub>2</sub> NPS uptake and transformation in lettuce plants were studied using ICP-MS and X-ray absorption near edge structure.

## Materials and Methods

### Plant culture and nanoparticle application

The potting soil for this research was obtained from Scotts Miracle-Gro Company and air-dried at room temperature prior to use. Plastic pots with 12 cm in diameter and 10.8 cm in height were filled out with 150 g of potting soil. CeO<sub>2</sub> NPs (Sigma Aldrich, USA) suspensions were sonicated for 30 min then applied to the soil to obtain final concentrations of 0, 50, 100, 1000 mg NPs per kg soil with 8 replications. Ten seeds of butterhead lettuce (*Lactuca sativa*) were sown at a depth of approximately 1 cm. At 5 d after planting, the seedlings were thinned to 3 plants per pot. The minimum and maximum greenhouse temperature during the test was

26 and 18 C. Each pot was supplied 100 mL deionized water every other day. To avoid interferences with the NPs, no additional fertilizer was added to the pots. After 30 d of growth, chlorophyll contents in the leaves were analyzed using a chlorophyll meter (Konic Minolta SPAD-502 Plus, Japan). Then the plants were harvested.

### Determination of Cerium by ICP-MS

Plant roots and shoots were separated thoroughly washed with deionized water, and lyophilized with a freeze dryer (Alpha 1–2 LD plus, Christ, Germany). The dried tissues were digested with a mixture of concentrated plasma-pure HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> (v/v, 4:1) on a heating plate. The obtained residual solutions were then diluted with deionized water and analyzed by ICP-MS (Thermo X7, USA). A standard reference (bush branches and leaves, GBW07602) was also digested and analyzed by ICP-MS to examine the recovery. Indium of 20 ng·mL<sup>-1</sup> was used as an internal standard to compensate for the matrix suppression and signal drifting. Analytical runs include calibration verification samples, spike recovery samples and duplicate dilutions. The linearity was from 0.1 to 50 ng·mL<sup>-1</sup>, Recovery from GBW07602 was 99%. Spike recovery was 102%. Detection limit was 0.01 ng·mL<sup>-1</sup>.

### Speciation Analysis by X-ray Absorption Near Edge Fine Structure (XANES)

Lyophilized roots of butterhead lettuces were grinded to powder and pressed into slices with a diameter of 10mm and a thickness of 2 mm for XANES analysis. The Ce L<sub>III</sub> XANES spectra were recorded in fluorescence mode on beamline 1W1B at Beijing Synchrotron Radiation Facility (BSRF). The ring storage energy of the synchrotron radiation accelerator during data collection was 2.5 GeV with current intensity of 50 mA. Cerium phosphate CePO<sub>4</sub>, cerium oxalate Ce<sub>2</sub>(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub> and cerium acetate Ce(CH<sub>3</sub>COO)<sub>3</sub> as well as the three types of CeO<sub>2</sub> NPs were used as the standard compounds. Data processing and the linear combination fitting (LCF) of the collected spectra were performed in the software program ATHENA. Samples of lettuce treated with the three types of CeO<sub>2</sub> NPs (1000 mg L<sup>-1</sup>) were also prepared for the XANES analysis.

### Determination of Protein, Nitrate and Soluble Sugar

Fresh leaf and root samples (0.1g) were homogenizing with 1 mL phosphate buffer (50 mM KH<sub>2</sub>PO<sub>4</sub> at pH 7.4) by the Mixer Mills (MM400, RETSCH, German), and then centrifuged at 4000×g for 15 min at 4°C. The supernatant were quantified for protein according to the method by Olson et al. [23]

Fresh leaf tissues (0.2 g) were cut in tiny pieces and aced in screw capped tubes to have a water bath at 100°C for 30 min. After cooling, they were centrifuged at 1500\* g for 5 min at 4°C. The method of determination nitrate and soluble sugar was conducted following by Cataldo et al and Irigoyen et al. [24,25] Lastly, the absorbance at 625 nm and 410 nm was determined for soluble sugar and nitrate in a UV-visible spectrophotometer (TU-1901, Beijing)

### Stress Response of Butterhead Lettuce to CeO<sub>2</sub> NPs

Fresh roots and shoots of lettuce separately homogenized with phosphate buffer solution (PBS, 50 mM, pH 7.8) under ice bath, and then centrifuged at speed of 10 000g and 4°C for 10 min. The supernatants were kept for analyses of SOD, POD activities and MDA contents using the assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

## Statistical Analysis

The results are expressed as mean ± SE (n = 8). When data are homogeneity of variance, a one-way ANOVA using General Linear Model, followed by Turkey's Honestly Significant Difference (HSD) test; when data are heterogeneity of variance, Kruskal-Wallis one-way ANOVA be used, These were performed using SPSS statistical package, version 16.0 (SPSS, Chicago, IL). Statistical significance was based on a probability of  $p \leq 0.05$ .

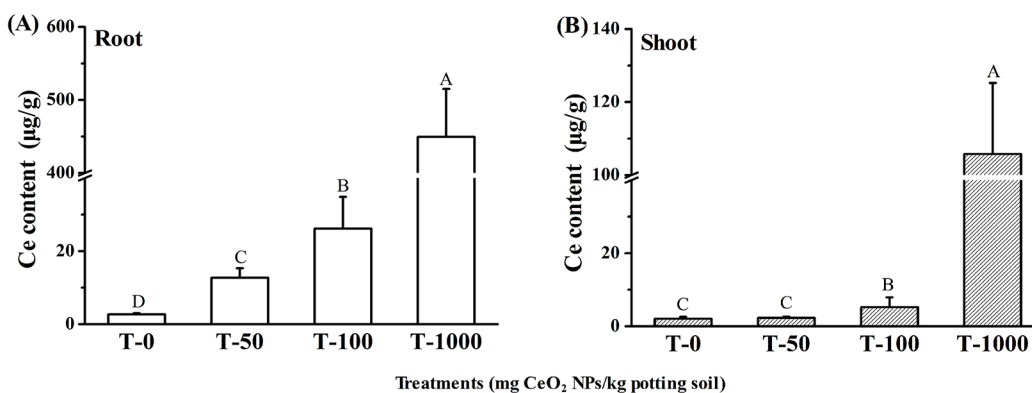
## Results and Discussion

### Ce Contents in Tissues of Lettuce

Ce contents in the roots and shoots of 30-day-old lettuce plants treated with CeO<sub>2</sub> NPs were determined by ICP-MS. As shown in Fig 1A, the Ce contents in roots increased as the external CeO<sub>2</sub> NPs increased. From Fig 1B, plants in shoot exposed to 100 mg·kg<sup>-1</sup> and 1000 mg·kg<sup>-1</sup> CeO<sub>2</sub> NPs had significantly higher Ce content compared to control. Ce content in the root (12.7–449.4 μg·g<sup>-1</sup>) was much higher than that in the shoots (2.3–105.8 μg·g<sup>-1</sup>). Compared to previous results[15], Ce concentrations in butterhead lettuce roots and shoots in this study were lower than in agar medium or hydroponic medium. As a consequence, it is difficult to find the CeO<sub>2</sub> NPs in the roots by TEM.

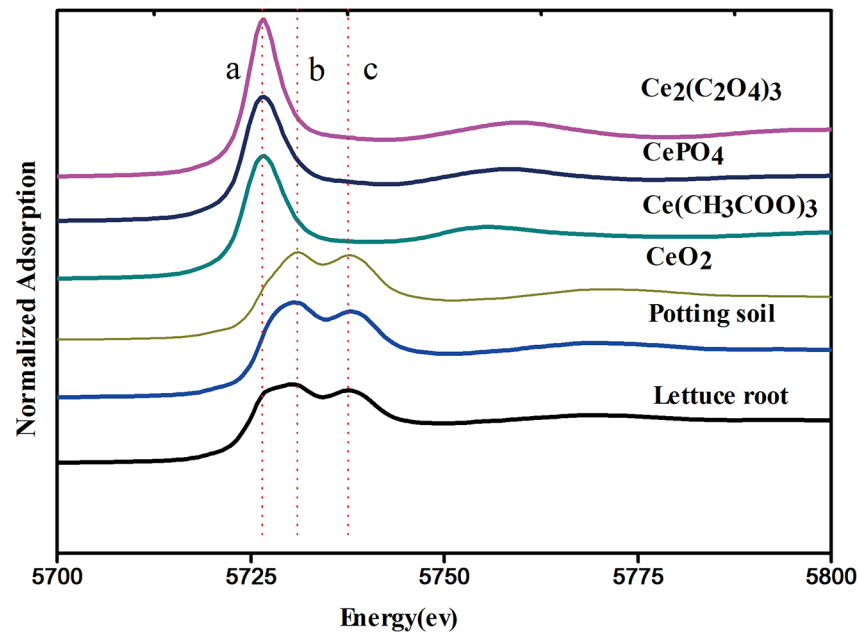
### Speciation Analysis of Cerium in the Lettuce Plants by XANES

The biotransformation of CeO<sub>2</sub> NPs determines the ultimate fate and toxicity of manufactured nanoparticles in living organisms. By XANES, the oxidation state of Ce in the butterhead lettuce root can be identified. The XANES spectra of normalized Ce L<sub>III</sub> edge in the reference Ce compounds and roots of butterhead lettuce are shown in Fig 2. Peak a in the spectra represents the characteristic peak of Ce (III), which come from CePO<sub>4</sub>, Ce (CH<sub>3</sub>COO)<sub>3</sub> and Ce<sub>2</sub> (C<sub>2</sub>O<sub>4</sub>)<sub>3</sub> here. Double peaks B and C indicate the characteristic peak of Ce(IV), which come from CeO<sub>2</sub> in this study.[26] Compared with standard references, the spectra from roots of treated asparagus lettuce showed the mixed features of peaks A, B and C. These indicate that Ce in roots presents a mixed oxidation state of Ce (IV) and Ce (III). To obtain the quantitative information of Ce species in the roots, LCF was performed on the normalized spectra of samples using CeO<sub>2</sub>, CePO<sub>4</sub>, Ce (CH<sub>3</sub>COO)<sub>3</sub> and Ce<sub>2</sub> (C<sub>2</sub>O<sub>4</sub>)<sub>3</sub> as the standard compounds. The fitted lines and fitting parameters indicate that the results are satisfying and convincing.



**Fig 1. (A) Ce contents in roots (A) and leaves (B) of lettuce plants.** Error bars stand for standard errors. Bar with the same letters show no statistically significant differences at  $p \leq 0.05$ . n = 8.

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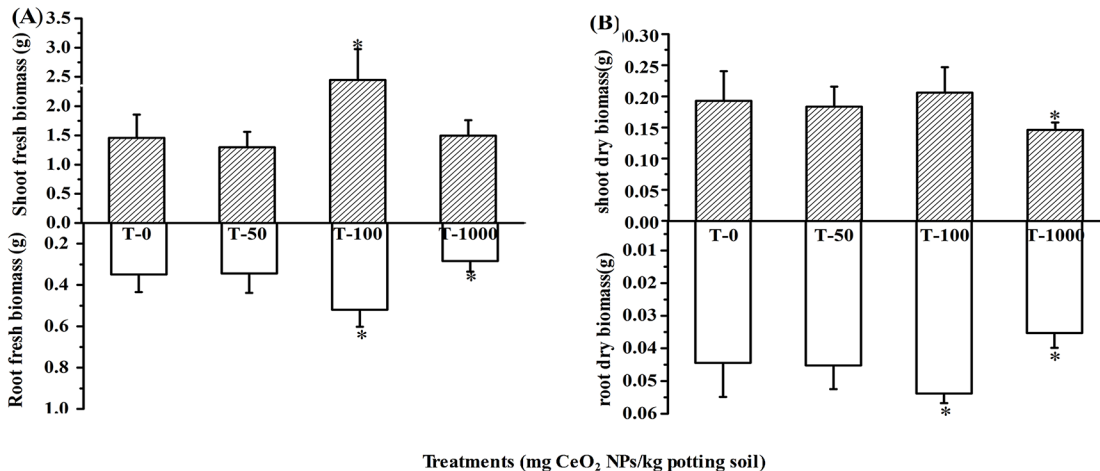
**Fig 2. XANES Ce LIII-edge spectra (5723 eV) in roots of butterhead lettuce treated with CeO<sub>2</sub> NPs.** The dotted line indicates the feature a, b and c.

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The Ce species in the potting soil emerged as 95.1% CeO<sub>2</sub> and 4.2% Ce<sub>2</sub>(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>, without CePO<sub>4</sub> and Ce(CH<sub>3</sub>COO)<sub>3</sub>. In the root samples, the Ce species presented as 77.3% CeO<sub>2</sub> and 22.7% Ce carboxylates (included 0.5% CePO<sub>4</sub>, 7.5% Ce(CH<sub>3</sub>COO)<sub>3</sub> and 12.3% Ce<sub>2</sub>(C<sub>2</sub>O<sub>4</sub>)<sub>3</sub>). According to our previous report[22], Sigma CeO<sub>2</sub> NPs with non-uniform size contained a large number of small particles less than 25 nm (S1 Fig), so they have higher reactivity and released more Ce<sup>3+</sup> than the dimension of 25 nm CeO<sub>2</sub>. Particles with smaller size have higher specific surface area and can be expected to show higher reactivity. The release of Ce<sup>3+</sup> may play a key role in phytotoxicity of metal-based NPs.[27]

### Growth of Lettuce Plants

In this study, butterhead lettuce plants were treated with CeO<sub>2</sub> NPs in potting soil at different concentrations (0, 50, 100, 1000 mg NPs per kg soil). After 30 days, wet and dry mass of the root and shoot of lettuce plants were weighed and shown in Fig 3). It is observed that plants treated with 100 mg·kg<sup>-1</sup> grew significantly faster than others. The lower concentration (50 mg·kg<sup>-1</sup>) treatment had no impact on plant growth, compared with the control. However, the higher concentration (1000 mg·kg<sup>-1</sup>) treatment significantly decreased the dry masses of root and shoot. The results indicated that CeO<sub>2</sub> NPs are phytotoxic to plants at a high concentration, and the effect to dry biomass of lettuce plants is more obvious than the fresh ones (more than 94% water in the fresh plant). Rare earth elements (REEs) including cerium can exert positive or negative physiological effects on plants depending on the dosage and other conditions. Much evidence has accumulated in support of the view low concentrations of REEs exert positive effects on growth and yield of crops, whereas high concentrations of REEs seem to be harmful for plants, although the exact mechanism is still unknown.[28] According to our previous results, the effects of CeO<sub>2</sub> NPs on lettuce plants were probably attributed to the released Ce<sup>3+</sup> in the roots.



**Fig 3. (A) Root and shoot fresh biomass of lettuce plants grown for 30 days in potting soil, treated with 0 (control)-1000 mg·kg<sup>-1</sup> CeO<sub>2</sub> NPs. (B) Root and shoot dry biomass of lettuce plants grown for 30 days in potting soil treated with 0 (control)-1000 mg·kg<sup>-1</sup> CeO<sub>2</sub> NPs.** Error bars stand for standard errors. Bar with this asterisk (\*) symbol shows statistically significant differences at  $p \leq 0.05$ ,  $n = 8$ .

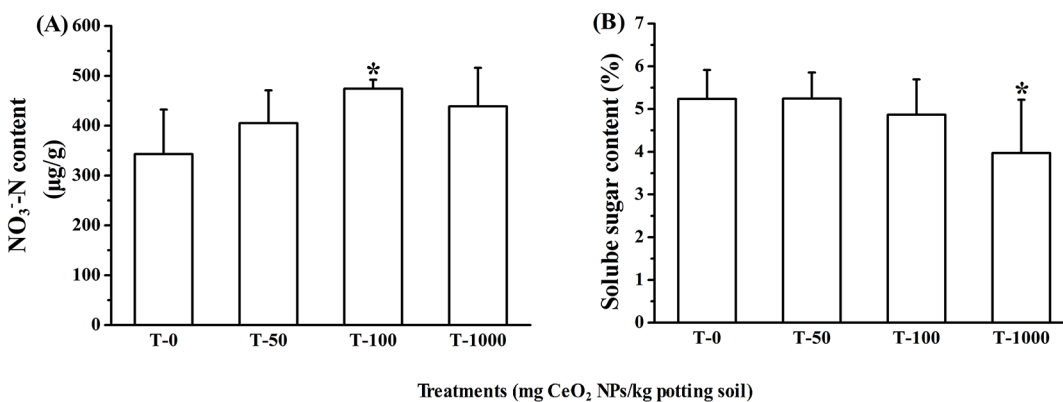
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### Chlorophyll and Protein Content

The contents of chlorophyll and protein are important physiological parameters in the development of plant growth. In this study, no significant differences among of treatments were observed, even at a high concentration (1000 mg·kg<sup>-1</sup>) exposure (S2 and S3 Figs) Several studies have demonstrated that ENPs induce modifications on the protein levels in plants at early seedling stage.[16],[29,30] Zhao *et al.* have shown that neither CeO<sub>2</sub> nor ZnO NPs impacted cucumber chlorophyll content at concentrations of 0, 400, and 800 mg kg<sup>-1</sup>, however, at 800 mg kg<sup>-1</sup> treatment, CeO<sub>2</sub> NPs reduced the yield by 31.6%.[31] Different plant species may act differently to the exposure of nanoparticles.

### Nitrate and Soluble Sugar Contents

Physiological effects of CeO<sub>2</sub> NPs on nitrate and soluble sugar contents in lettuce plants were shown in Fig 4. In 100 mg·kg<sup>-1</sup> group, CeO<sub>2</sub> NPs significantly increased the nitrate content by 38.2%, compared with the control. Nitrate is one of the major N sources for high plants; it promotes vegetative development and yield. Recently, there are numerous studies evaluating the



**Fig 4. NO<sub>3</sub><sup>-</sup>-N (A) and soluble sugar (B) contents content in the leaves** Error bars stand for standard errors. Bar with this asterisk (\*) symbol shows statistically significant differences at  $p \leq 0.05$ ,  $n = 8$ .

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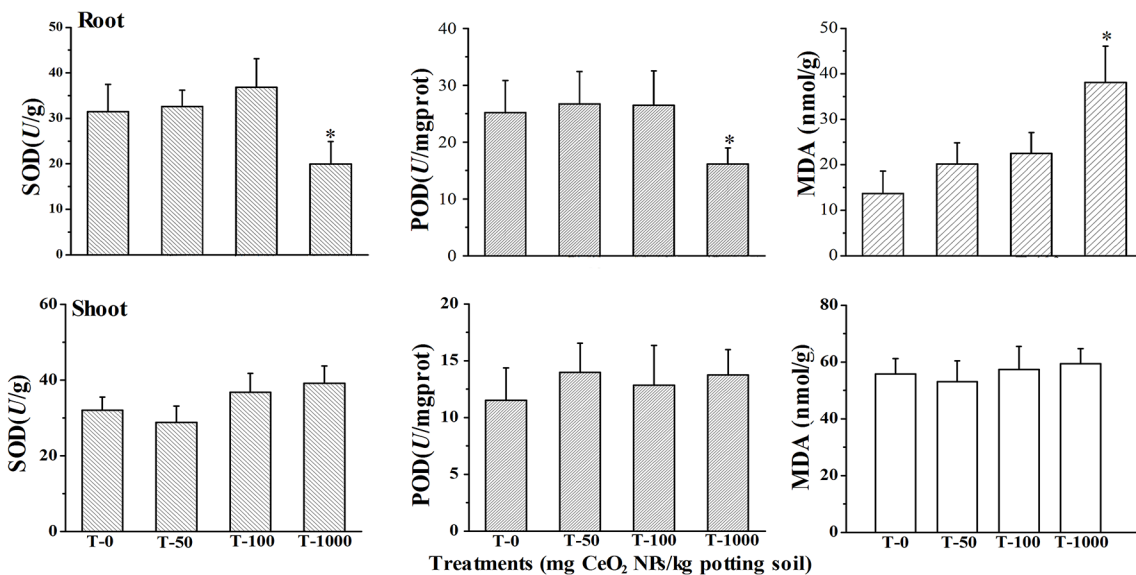
impact of CeO<sub>2</sub> NPs on quality of plants, but few reports with nitrate. The finding explained why CeO<sub>2</sub> NPs promoting lettuce growth at certain concentrations. However, excessive nitrogen could influence the quality of vegetable.[32] Nitrate itself is relatively non-toxic but its metabolites may produce a number of negative health effects.

Sugar is one of the three major nutrients of plant. CeO<sub>2</sub> NPs had a significantly inhibitory effect on the soluble sugar content of at the concentration of 1000 mg·kg<sup>-1</sup>. CeO<sub>2</sub> NPs at a concentration of 50 mg·kg<sup>-1</sup> had no impacts on biomass, chlorophyll content, protein, enzyme activity; probably due to the fact that CeO<sub>2</sub> NPs were absorbed by potting soil, leading to reduction of Ce bioavailability. It is not surprising to see that at 100 mg·kg<sup>-1</sup>, CeO<sub>2</sub> NPs stimulated the lettuce growth, which is in consistent with an earlier study that Yuan et al. reported a fertilizer containing Ce increased root elongation of rice seedlings, attributed to the Ce<sup>3+</sup>.[33]

### Antioxidant defense system in roots and shoots

The plant cells have antioxidant defense system composed of enzymes, such as SOD and POD that are known to be involved in the detoxification of H<sub>2</sub>O<sub>2</sub> by converting the H<sub>2</sub>O<sub>2</sub> to water and oxygen. These root enzymes were differently affected by the concentrations of CeO<sub>2</sub> NPs used in this study. As seen in Fig 5A, SOD and POD activities of lettuce roots were slightly up-regulated by 50 and 100 mg kg<sup>-1</sup> CeO<sub>2</sub> NPs exposure, but significantly down-regulated at 1000 mg kg<sup>-1</sup>. Additionally, the high concentrations of CeO<sub>2</sub> NPs led to high levels of MDA. The antioxidant defense system of plant cells is composed of SOD, POD and other enzymes, the MDA is a product of degradation of cell membrane components damage of free radicals.<sup>15</sup> And it can be accepted that the toxicity of CeO<sub>2</sub> NPs may be caused by excessive production of reactive oxygen species (ROS). These excessive ROS would be cleared by antioxidant enzymes, such as SOD and POD. But if the ROS had not been cleared timely, they would have some damages to the plant cells membrane, also damage the antioxidant defense system, means the increase of MDA levels and the decrease of SOD and POD contents.

Similar to the roots, SOD and POD activities in the shoots were also only slightly up-regulated at all test concentrations of CeO<sub>2</sub> NPs (Fig 5B). But MDA levels remained constant. These suggested that the CeO<sub>2</sub> NPs had little impacts on the physiology of the shoots.



**Fig 5. SOD and POD activities and MDA levels in the roots and shoots of lettuce plants** Error bars stand for standard errors. Bar with this asterisk (\*) symbol shows statistically significant differences at  $p \leq 0.05$ .

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## Conclusion

In this research, we investigated the fate and phytotoxicity of CeO<sub>2</sub> NPs amended in soil. Results show that CeO<sub>2</sub> NPs were uptake by lettuce plant, and had a positive effect on plant growth at 100 mg kg<sup>-1</sup>, whereas inhibited plant growth at 1000 mg kg<sup>-1</sup>. Meantime, SOD and POD activities and MDA levels was also disrupted by 1000 mg kg<sup>-1</sup> treatment. The toxicity was probably attributed to the biotransformation of CeO<sub>2</sub> NPs and the high sensitivity of *Lactuca* plants to the released Ce<sup>3+</sup> ions.

## Supporting Information

**S1 Fig. TEM image of sigma CeO<sub>2</sub> NPs.**  
(JPG)

**S2 Fig. Chlorophyll contents in the leaves of lettuce plants grown for 30 days in potting soil, treated with 0 (control)-1000 mg kg<sup>-1</sup> CeO<sub>2</sub> NPs.**  
(TIF)

**S3 Fig. Root and shoot protein of lettuce plants grown for 30 days in potting soil treated with 0 (control)-1000 mg kg<sup>-1</sup> CeO<sub>2</sub> NPs.** Error bars stand for standard errors. Bar with this asterisk (\*) symbol shows statistically significant differences at  $p \leq 0.05$ .  
(TIF)

**S1 Data. The data for all of the experiments.**  
(XLSX)

## Acknowledgments

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## Author Contributions

Conceived and designed the experiments: ZZ YR XG HL. Performed the experiments: XG SL. Analyzed the data: XG YM PZ YL. Contributed reagents/materials/analysis tools: XH JZ ZZ LL WC. Wrote the paper: XG YR.

## References

1. Cassee FR, van Balen EC, Singh C, Green D, Muijser H, et al. (2011) Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Critical reviews in toxicology* 41: 213–229. doi: [10.3109/10408444.2010.529105](https://doi.org/10.3109/10408444.2010.529105) PMID: [21244219](https://pubmed.ncbi.nlm.nih.gov/21244219/)
2. Saadat-Monfared A, Mohseni M (2014) Polyurethane nanocomposite films containing nano-cerium oxide as UV absorber; Part 2: Structural and mechanical studies upon UV exposure. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 441: 752–757.
3. Lawrence NJ, Brewer JR, Wang L, Wu T-S, Wells-Kingsbury J, et al. (2011) Defect engineering in cubic cerium oxide nanostructures for catalytic oxidation. *Nano letters* 11: 2666–2671. doi: [10.1021/nl200722z](https://doi.org/10.1021/nl200722z) PMID: [21627100](https://pubmed.ncbi.nlm.nih.gov/21627100/)
4. Kunhikrishnan A, Shon HK, Bolan NS, El Saliby I, Vigneswaran S (2015) Sources, Distribution, Environmental Fate, and Ecological Effects of Nanomaterials in Wastewater Streams. *Critical Reviews in Environmental Science and Technology* 45: 277–318.
5. Gantt B, Hoque S, Willis RD, Fahey KM, Delgado-Saborit JM, et al. (2014) Near-Road Modeling and Measurement of Cerium-Containing Particles Generated by Nanoparticle Diesel Fuel Additive Use. *Environmental science & technology* 48: 10607–10613.



6. Lee J, Mahendra S, Alvarez PJ (2010) Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. *Acs Nano* 4: 3580–3590. doi: [10.1021/nn100866w](https://doi.org/10.1021/nn100866w) PMID: [20695513](https://pubmed.ncbi.nlm.nih.gov/20695513/)
7. Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, et al. (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *Acs Nano* 2: 2121–2134. doi: [10.1021/nn800511k](https://doi.org/10.1021/nn800511k) PMID: [19206459](https://pubmed.ncbi.nlm.nih.gov/19206459/)
8. Zhang Z, He X, Zhang H, Ma Y, Zhang P, et al. (2011) Uptake and distribution of ceria nanoparticles in cucumber plants. *Metallomics* 3: 816–822. doi: [10.1039/c1mt00049g](https://doi.org/10.1039/c1mt00049g) PMID: [21731965](https://pubmed.ncbi.nlm.nih.gov/21731965/)
9. Zhang P, Ma Y, Zhang Z, He X, Zhang J, et al. (2012) Biotransformation of ceria nanoparticles in cucumber plants. *Acs Nano* 6: 9943–9950. doi: [10.1021/nn303543n](https://doi.org/10.1021/nn303543n) PMID: [23098040](https://pubmed.ncbi.nlm.nih.gov/23098040/)
10. Rico CM, Morales MI, McCreary R, Castillo-Michel H, Barrios AC, et al. (2013) Cerium oxide nanoparticles modify the antioxidative stress enzyme activities and macromolecule composition in rice seedlings. *Environmental science & technology* 47: 14110–14118.
11. Ma C, Chhikara S, Xing B, Musante C, White JC, et al. (2013) Physiological and molecular response of *Arabidopsis thaliana* (L.) to nanoparticle cerium and indium oxide exposure. *ACS Sustainable Chemistry & Engineering* 1: 768–778.
12. López-Moreno ML, de la Rosa G, Hernández-Viezcas JÁ, Castillo-Michel H, Botez CE, et al. (2010) Evidence of the differential biotransformation and genotoxicity of ZnO and CeO<sub>2</sub> nanoparticles on soybean (*Glycine max*) plants. *Environmental science & technology* 44: 7315–7320.
13. Schwabe F, Schulin R, Limbach LK, Stark W, Bürge D, et al. (2013) Influence of two types of organic matter on interaction of CeO<sub>2</sub> nanoparticles with plants in hydroponic culture. *Chemosphere* 91: 512–520. doi: [10.1016/j.chemosphere.2012.12.025](https://doi.org/10.1016/j.chemosphere.2012.12.025) PMID: [23352517](https://pubmed.ncbi.nlm.nih.gov/23352517/)
14. Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, et al. (2012) CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *Journal of Nanoparticle Research* 14: 1–15.
15. Cui D, Zhang P, Ma Y, He X, Li Y, et al. (2014) Effect of cerium oxide nanoparticles on asparagus lettuce cultured in an agar medium. *Environmental Science: Nano* 1: 459–465.
16. Lee W-M, Kwak JI, An Y-J (2012) Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: Media effect on phytotoxicity. *Chemosphere* 86: 491–499. doi: [10.1016/j.chemosphere.2011.10.013](https://doi.org/10.1016/j.chemosphere.2011.10.013) PMID: [22075051](https://pubmed.ncbi.nlm.nih.gov/22075051/)
17. Zhao L, Peng B, Hernandez-Viezcas JA, Rico C, Sun Y, et al. (2012) Stress response and tolerance of *Zea mays* to CeO<sub>2</sub> nanoparticles: Cross talk among H<sub>2</sub>O<sub>2</sub>, heat shock protein, and lipid peroxidation. *ACS nano* 6: 9615–9622. doi: [10.1021/nn302975u](https://doi.org/10.1021/nn302975u) PMID: [23050848](https://pubmed.ncbi.nlm.nih.gov/23050848/)
18. Priester JH, Ge Y, Mielke RE, Horst AM, Moritz SC, et al. (2012) Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. *Proceedings of the National Academy of Sciences* 109: E2451–E2456.
19. Wang Q, Ma X, Zhang W, Pei H, Chen Y (2012) The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) and its implications for food safety. *Metallomics* 4: 1105–1112. PMID: [22986766](https://pubmed.ncbi.nlm.nih.gov/22986766/)
20. Morales MI, Rico CM, Hernandez-Viezcas JA, Nunez JE, Barrios AC, et al. (2013) Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *Journal of agricultural and food chemistry* 61: 6224–6230. doi: [10.1021/jf401628v](https://doi.org/10.1021/jf401628v) PMID: [23799644](https://pubmed.ncbi.nlm.nih.gov/23799644/)
21. Ma Y, Kuang L, He X, Bai W, Ding Y, et al. (2010) Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere* 78: 273–279. doi: [10.1016/j.chemosphere.2009.10.050](https://doi.org/10.1016/j.chemosphere.2009.10.050) PMID: [19897228](https://pubmed.ncbi.nlm.nih.gov/19897228/)
22. Zhang P, Ma Y, Zhang Z, He X, Li Y, et al. (2013) Species-specific toxicity of ceria nanoparticles to *Lactuca* plants. *Nanotoxicology*: 1–8.
23. Olson BJ, Markwell J (2007) Assays for determination of protein concentration. *Current protocols in protein science*: 3.4.1–3.4.29.
24. Cataldo D, Maroon M, Schrader L, Youngs V (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid 1. *Communications in Soil Science & Plant Analysis* 6: 71–80.
25. Irigoyen J, Einerich D, Sánchez-Díaz M (1992) Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum* 84: 55–60.
26. Bianconi A, Marcelli A, Dexpert H, Karnatak R, Kotani A, et al. (1987) Specific intermediate-valence state of insulating 4f compounds detected by L 3 X-ray absorption. *Physical Review B* 35: 806.
27. Miralles P, Church TL, Harris AT (2012) Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants. *Environmental science & technology* 46: 9224–9239.

28. Tyler G (2004) Rare earth elements in soil and plant systems-A review. *Plant and soil* 267: 191–206.
29. Chai M, Shi F, Li R, Liu L, Liu Y, et al. (2013) Interactive effects of cadmium and carbon nanotubes on the growth and metal accumulation in a halophyte *Spartina alterniflora* (*Poaceae*). *Plant Growth Regulation* 71: 171–179.
30. Krystofova O, Sochor J, Zitka O, Babula P, Kudrle V, et al. (2012) Effect of magnetic nanoparticles on tobacco BY-2 cell suspension culture. *International journal of environmental research and public health* 10: 47–71. doi: [10.3390/ijerph10010047](https://doi.org/10.3390/ijerph10010047) PMID: [23343980](https://pubmed.ncbi.nlm.nih.gov/23343980/)
31. Zhao L, Sun Y, Hernandez-Viezcas JA, Servin AD, Hong J, et al. (2013) Influence of CeO<sub>2</sub> and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. *Journal of agricultural and food chemistry* 61: 11945–11951. doi: [10.1021/jf404328e](https://doi.org/10.1021/jf404328e) PMID: [24245665](https://pubmed.ncbi.nlm.nih.gov/24245665/)
32. Ouzounidou G, Paschalidis C, Petropoulos D, Koriki A, Zamanidis P, et al. (2013) Interaction of soil moisture and excess of boron and nitrogen on lettuce growth and quality. *Horticultural Science, Slezská, Czech Republic* 40: 119–125.
33. Yuan D, Shan X-q, Huai Q, Wen B, Zhu X (2001) Uptake and distribution of rare earth elements in rice seeds cultured in fertilizer solution of rare earth elements. *Chemosphere* 43: 327–337. PMID: [11302578](https://pubmed.ncbi.nlm.nih.gov/11302578/)