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Uptake and Accumulation of Bulk and Nano-sized Cerium Oxide Particles and Ionic Cerium by Radish (*Raphanus sativus L.*)

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Abstract

The potential toxicity and accumulation of engineered nanomaterials (ENMs) in agricultural crops has become an area of great concern and intense investigation. Interestingly, while below ground vegetables are most likely to accumulate the highest concentrations of ENMs, little work has been done investigating the potential uptake and accumulation of ENMs for this plant group. The overall objective of this study was to evaluate how different forms of cerium (bulk cerium oxide, cerium oxide nanoparticles, and the cerium ion) affected the growth of radish (*Raphanus sativus L.*) and accumulation of cerium in the radish tissues. Ionic cerium (Ce^{3+}) had a negative effect on radish growth at 10 mg $CeCl_3$ /L while bulk cerium oxide (CeO_2) enhanced plant biomass at the same concentration. Treatment with 10 mg/L cerium oxide nanoparticles (CeO_2 NPs) had no significant effect on radish growth. Exposure to all forms of cerium resulted in the accumulation of this element in radish tissues, including the edible storage root. However, the accumulation patterns and their effect on plant growth and physiological processes varied with the characteristics of cerium. This study provides a critical frame of reference on the effects of CeO_2 NPs vs. their bulk and ionic counterparts on radish growth.

Keywords

Cerium oxide, Nanomaterials, Nanoparticles, Radish, Phytotoxicity, Plant uptake

Introduction

Nanotechnology is a rapidly expanding global industry. Engineered nanomaterials (ENMs) with their size smaller than 100 nm in at least two dimensions are increasingly found in commercial products. Due to their small size and large specific surface area, ENMs exhibit novel and different physical, chemical and biological properties from their bulk or ionic counterparts. These unique properties provide new opportunities to fight diseases, enhance energy efficiency and improve the environment (*1,2,3*).

While the synthesis of ENMs adds desirable physical and/or chemical properties over the bulk or ionic forms, the potential environmental health and safety implications of ENM uses have become a serious concern. Previous research has shown that some ENMs used in consumer products are released into the environment and many of these materials are detected in wastewater streams (*4,5,6*). As one of the most commonly employed nanomaterials, cerium oxide nanoparticles (CeO_2 NPs) have attracted great attention. The potential toxicity of CeO_2 NPs (6-40 nm, unmodified) to bacteria, fish, and mammalian cells has been reported (*7,8*). Plants play a critical role in maintaining ecosystem health and function and as a food source for humans. Plant uptake of ENMs represents an important pathway for human exposure to these nanoparticles through food consumption (*9*). Consequently, investigation of the uptake and accumulation of ENMs by agricultural crops is not only warranted but also critical to food safety and human health. However, there are only a small number of studies in the literature that have addressed the interactions of CeO_2 NPs with terrestrial plants (*10-16*). In one study, Wang et al. (*10*) found that uncoated CeO_2 NPs (< 25nm) at 0.1-10 mg/L had a slightly positive effect on tomato (*Solanum lycopersicum* L.) growth and yield (*e.g.* increased production of tomato

fruit at 10 mg/L). However, cerium was reportedly transported from roots to shoots and accumulated in edible tissues, although the chemical form of Ce was not determined. These authors further investigated the trans-generational effect of CeO_2 NPs and found that second generation seedlings grown with seeds from treated parental plants with 10 mg/L CeO_2 NPs were significantly smaller and accumulated more cerium as compared to seedlings generated from control plants (*11*). Ma et al. (*12*) studied how rare earth oxide NPs affected root elongation and found that bare CeO_2 NPs with an average diameter of 7.2 $\pm$ 0.7 nm had no effects on rape (*Brassica napus* L.), radish (*Raphanus sativus* L.), wheat (*Triticum aestivum* L.), cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* L.), and cucumber (*Cucumis sativus* L.) at 2,000 mg/L. Rico et al. (*13*) demonstrated that exposing rice seedlings to bare CeO_2 NPs with an average size of 231 $\pm$ 16 nm up to 500 mg/L for ten days caused no visible signs of toxicity. However, CeO_2 NPs induced a concentration-dependent modification of the oxidative stress and antioxidant defense system in the rice seedlings. Several studies have reported the uptake and accumulation of CeO_2 NPs by agricultural crops. For instance, Zhang and colleagues (*14*) showed that 7 nm and 25 nm bare CeO_2 NPs were detected in cucumber tissues but the transport of cerium from roots to shoots was limited. Zhao et al. (*15*) investigated the effects of bare and alginate coated CeO_2 NPs on corn plants and reported that surface coating and soil organic matter could promote the translocation of cerium in higher plants. A recent study demonstrated that intact CeO_2 NPs (7 nm) were taken up by soybean roots (*16*). In summary, CeO_2 NPs can be taken up by plants and accumulated in plant tissues, but the majority of the NPs appeared to remain in the root tissues, raising concerns on the heightened accumulation of ENMs by root vegetables.

Interestingly, even though the edible tissues of belowground vegetables often have direct contact with soil-borne ENMs and present the highest potential for ENMs accumulation in food crops, little attention has been paid to this important group of food plants. In this study, radish (*Raphanus sativus* L.) was adopted as a model plant in that it is a popular vegetable with high global consumption. In addition, radishes mature rapidly in full sun and light, and can be harvested in 3-4 weeks, making it an ideal plant to study the fate and impact of environmental chemicals on the development of belowground vegetables. The objectives of this study were two-fold: 1) how does cerium in different chemical forms (e.g. ionic cerium vs. cerium particles) and physical sizes affect the growth of radish? (2) How extensively and differently will the radish tissues take up and accumulate cerium in different forms and sizes? With these two objectives, we aimed to fill some of the current knowledge gap on the possible differential accumulation of cerium with different forms and particles sizes by plants. Even though detailed studies on the cerium effect of essential physiological and biochemical processes are not the concentration of this study, their interactions are important for mechanistic understanding of the interactions of plants and nanoparticles and warrant further investigations.

Materials and Methods

Chemicals

Dispersions of CeO_2 NPs (10 wt. % in H_2O) and cerium chloride powder were purchased from Sigma-Aldrich (St. Louis, MO). The bulk powder of CeO_2 was obtained from Strem Chemicals, Inc. (Newburyport, MA). Hoagland solution (one-quarter strength) was prepared by dissolving an appropriate amount of the modified Hoagland basal salt mixture (Phytotechnology Laboratories, Lenexa, KS) with deionized water. The

size and morphology of CeO_2 NPs and the bulk suspensions were characterized by transmission electron microscopy (TEM). The hydrodynamic size and zeta potential of CeO_2 NPs in quarter strength Hoagland solution were measured with a dynamic light scattering instrument (Malvern Zetasizer Nano-ZS90, NY).

Seed germination and growth conditions

The radish seeds [Cherriette (F1)] were obtained from Johnny's Selected Seeds (Winslow, ME). Seeds were surface sterilized with 1.25% sodium hypochlorite solution for 10 minutes and then rinsed with deionized water three times. The sterilized seeds were germinated on moist filter paper in a Petri dish for 7 days. Healthy young seedlings with similar size (7.5 -8 cm in height from the root tip to the tip of cotyledons) and stage of development were transferred to 50 mL polypropylene centrifuge tubes containing quarter strength Hoagland solution and were incubated in a growth cart with a 16 hrs-light/8 hrs-dark cycle (28 °C) to allow the seedlings to further develop. The growth cart was equipped with four T5 fluorescent tubes, providing a light intensity of approximately 104 umol $m^{-2}.s^{-1}$ of visible light (400 ~ 700 nm) at the height of plant leaves. After 7 days, the seedlings were transferred from the centrifuge tubes to 100 mL glass jars containing 10 mg/L of bulk or nano-sized cerium oxide (CeO_2) or cerium chloride ($CeCl_3$). Each jar had a floating lid made by Hareline 2 mm thin fly foam (Fishwest, Sandy, UT) so that the plant roots were constantly submerged in the treatment solutions. Due to the scarcity of information on the potential adsorption of cerium on foam surface, it was assumed that the potential impact of foam on cerium bioavailability was insignificant in this study. Four treatments were prepared, all in quarter strength Hoagland solution: (1) control (no cerium treatment), (2) 10 mg/L CeO_2 NPs, (3) 10

mg/L CeO_2 bulk, and (4) 10 mg/L $CeCl_3$. The concentration of 10 mg/L was chosen because this value is considered environmentally relevant (*18*) and our previous studies also showed that CeO_2 NPs at this concentration slightly enhanced plant growth (*10*,*11*). Each treatment had 12 replicates. The solutions in the jars were replenished every other day with the same treatment solution to compensate for evapotranspiration, with the assumption that Ce would be taken up concurrently with water by plants. However, if plants preferably take up water, it is possible that cerium would accumulate in the growing solution. Plants were harvested 35 days after germination (i.e., 21 days after treatment). Additionally, a separate set of radish plants were grown and treated exactly as above. The harvested tissues from these plants were used to determine the distribution of cerium across the fine root tips and storage roots microscopically (see below for details).

Plant physiological responses

For the first batch of plants, daily transpiration rate was recorded for each seedling after they were transferred to 100 mL glass jars by measuring the water surface drop before the solution replenishment. The cumulative transpiration of each treatment was then calculated by summing the daily transpiration over the 21 d treatment period. Relative chlorophyll content was measured with a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc. Aurora, IL) one day prior to harvest and was expressed as a percent of control. An OS1p chlorophyll fluorometer (Opti-sciences, Inc. Hudson, NH) was used to measure the yield of quantum efficiency of PSII (light-adapted Y(II)) and the photochemical efficiency of PSII (dark-adapted F_V/F_M) on the same day the relative chlorophyll was measured. Five of the 12 replicates from each treatment were used in a leakage test to assess root membrane integrity. The leakage test followed the published

procedures with some modifications (*19*). Briefly, the entire fine root system was submerged in 50 mL of deionized water and the initial conductivity C_w was measured immediately (Orion ROSS Ultra pH/ATC Triode Orion Star A325 Thermo Fisher Scientific, Waltham, MA). The conductivity of the solution was measured again as C_0 after 3 hours of incubation at room temperature. The entire fine roots were then autoclaved at 121°C for 20 min with a Tuttnauer Brinkman 3850M autoclave to release all electrolytes. The final conductivity C_t was measured after the samples cooled to room temperature. The percentage of electrolyte leakage was calculated as: $EL=(C_0 - C_w)/(C_t - C_w)\times 100$.

Uptake, accumulation and distribution of cerium

At harvest, radish plants were separated into fine roots, storage roots (the edible radish bulb), and shoots. The tissues were then dried in an oven at 75 °C for 7 days and their dry weights determined. For each treatment, the 12 storage roots and 12 shoot tissues were divided into to four groups respectively. The shoot and storage root tissues in each group were then ground together into fine powders, from which 0.25 g of the ground tissues were weighed and digested in 4 mL of 70% nitric acid and swirled to mix. For the fine roots, the remaining seven replicates (five replicates used for the electrolyte leakage test were excluded) were divided into three groups and each group contained either two or three of the fine root systems. Due to the smaller biomass of the fine roots, all ground tissues from each group were used for the digestion. The nitric acid digest was heated at 95 °C for 20 minutes and then 45 °C for 4 minutes, and the cycle was repeated 5 times until all the dry tissues were dissolved. Afterwards, 2 mL of hydrogen peroxide was added to the mixture and heated using the same temperature cycle until the solution

was clear. The digest solution was then analyzed by inductively coupled plasma – mass spectrometry (ICP-MS) to obtain the concentration of cerium in each sample.

The radish roots used to determine the localization and distribution of cerium in their fine roots and storage roots were harvested at day 21 after treatment. To obtain a reference for the anatomy of the radish storage root, a cross section of the radish storage root taken at the equator was cut with a razor blade and observed under a Kruss MBL3000 light microscope (A.KRÜSS Optronic, Hamburg, Germany) (Supplementary Figure 1). The radish fine root tips and sections of the storage roots from each treatment were also examined using a Zeiss LSM 510 META confocal microscope. A laser excitation wavelength of 543 nm was used and an emission filter band pass was set between 530-590 nm to collect both the laser reflection and autofluorescence in this region. To image the storage root, the root was first cut in half horizontally from the thickest point and then a thin slice (~ 1mm) of the storage root was cut from the bottom half of the storage root. That slice was then divided into four quarters and one of the quarters was randomly selected to determine the radial distribution of cerium toward the midpoint of the storage root. A schematic illustration of the slices preparation is shown in Supplementary Figure 1. For each sample, a serial scanning along the z-axis of the sample was conducted and the numbers of scanning planes varied from 8 to 11. The distance between two optical planes was approximately 10.2 µm.

Data Analysis

A one-way ANOVA was performed in this study for data analysis. The Duncan test was conducted for post hoc comparisons.

Results

Characterization of CeO_2 NPs and the bulk

Supplementary Figure 2 shows the TEM images of CeO_2 NPs and bulk CeO_2 in quarter strength Hoagland solution. The nanoparticles displayed variable shapes and sizes. Individual nanoparticles possessed triangular, rectangular and other irregular shapes. The images indicate that the average diameter of individual CeO_2 NPs ranged from 10 – 30 nm. The nanoparticles aggregated considerably in the Hoagland solution, due to the high ionic strength. The hydrodynamic diameter of the nanoparticle aggregates was ~600 nm as measured by the DLS. The zeta potential of CeO_2 NPs in the Hoagland solution was approximately -11.9 mV, suggesting that the nanoparticles were not stable. Bulk CeO_2 were mostly at the micron scale but the sizes were not uniform. Particles at the nanoscale were also detected in the bulk solutions. The sizes of bulk CeO_2 ranged approximately from 100 nm to 4,000 nm.

Plant physiological status

The radish exposed to CeO_2 bulk had the highest total dry biomass and was significantly greater than all other treatments (Figure 1a). The biomass of the nanoparticle treated plants was not significantly different from the control plants. The plant biomass exposed to cerium ions was significantly lower than all other treatments. When the plant tissues were examined separately, the bulk cerium treated radishes, which had similar shoot biomasses as CeO_2 NPs-treated radishes, had significantly higher dry shoot biomass than control and cerium ions treatment (Figure 1b). The dry weight of storage roots across the treatments exhibited similar patterns as the total dry biomass (Figure 1c), but the dry biomass of fine roots did not differ significantly as a function of

188 treatment (Figure 1d). In addition to the total biomass, the distribution of the biomass
189 between the root (fine + storage root) and shoot compartments was significantly different
190 in response to treatment. The shoot/root ratio of dry biomass of cerium ion treated radish
191 (1.34 $\pm$ 0.11) was significantly higher ($p<0.05$) than all other treatments, which had
192 similar ratios (control: 1.00 $\pm$ 0.10; bulk CeO_2: 0.95 $\pm$ 0.06; CeO_2 NPs: 1.07 $\pm$ 0.07).
193 Visually, there was no apparent adverse effect of any of the cerium treatments on growth
194 and development of the radish plants except for the size differences (Figure 1e).
195 In addition to the root biomass, the fine root membrane integrity was significantly
196 affected by different forms of cerium. Figure 2 indicates that 10 mg/L of CeO_2 NPs and
197 ionic cerium resulted in significantly greater electrolyte leakage when compared to the
198 control roots. Leakage from bulk cerium treated roots was not significantly different from
199 control roots. The accumulative transpiration of radish for all treatments was comparable
200 until day 21, since then the accumulative transpiration of cerium ion treated radish
201 became significantly lower than other treatment groups (Supplementary Figure 3). The
202 relative chlorophyll content expressed in percentage is shown in Table 1. Although all
203 treated radishes had lower chlorophyll content, only the bulk CeO_2 and CeO_2 NPs treated
204 leaves had significantly lower chlorophyll content compared to the controls. The average
205 quantum yield of photosystem II (Y(II)) and the F_V/F_M ratio for plants from different
206 treatments are also shown in Table 1. The results indicated that the Y(II) was unaffected
207 by the treatments. In contrast, only bulk CeO_2 treated radishes displayed an F_V/F_M ratio
208 significantly lower value than the control. No significant differences were observed
209 between the other cerium treatments.
210 **Cerium uptake and accumulation**

Not surprisingly, exposure to cerium resulted in significantly greater concentrations of this element in plant tissues. For the treated plants, the cerium concentration and content were significantly higher in the fine roots than in other tissues (Figure 3a-d). Among different treatments, the concentration of cerium in the storage root was not significantly different between cerium treatments (Figure 3a). In the shoot tissues, cerium ion treated radish had highest cerium concentration, followed by bulk cerium and then CeO_2 NPs treated radish (Figure 3a). The fine roots of CeO_2 NPs treated radish had significantly higher concentration of cerium than the bulk and ion treated radish (Figure 3b). When the cerium content rather than the concentration in different tissues was compared, cerium content in the storage roots of different treatments was still similar. The shoot cerium content of bulk and ion treated radishes was not significantly different but was markedly higher than nanoparticle treated radishes (Figure 3c). In the fine roots, the cerium content demonstrated similar patterns as the cerium concentration for different treatments (Figure 3d).

Cerium localization and distribution in radish root and storage root

For the fine root tips, the confocal microscopic images were captured both on the surface and at different depths from the surface. The control had some weak signals from either the cerium content in control tissues or from background excitation (Figure 4a). In contrast, plant roots from treated plants all generated stronger signals (Figure 4b-f). However, the signal patterns were noticeably different. On the bulk CeO_2 treated root, the signals were only detected from the mucilage surrounding the root tip in both surface and deeper scanning images (Figure 4b,c). CeO_2 NPs were detected on larger areas of the root surface as well as the mucilage on the root tip of the nanoparticle treated plants (Figure

4d). The signal was even more prominent in the deeper scanning planes (Figure 4e). For cerium ion treated radish root, the signals were predominantly detected in the surrounding areas. Neither the surface scan nor the deep scan detected significantly stronger signals than the controls within the root itself (Figure 4f).

Figure 5 shows the confocal images of cut slices of radish storage roots. The control storage root showed little signal (Figure 5a). In comparison, storage roots from treated plants had strong signals. For bulk CeO_2 treated radish, all signals came from the pigmented periderm with a random pattern (Figure 5b). For CeO_2 NPs treated radish, stronger signals were observed in the pigmented periderm. In addition, the nanoparticles appeared to penetrate further into the storage root (Figure 5c). Cerium was not only detected in the periderm but also in the secondary vascular tissues in the storage root of cerium ion treated radish (Figure 5d,e).

Discussion

Accompanied with the ever expanding applications of engineered nanomaterials are the increasing concerns about their toxicity to humans and the environment. A major question scientists are trying to ascertain is whether the reduction of micro-sized particles to nano-sized particles will significantly increase their toxicity. Several previous studies have shown that nanoparticles typically exhibit stronger effect on plants than their bulk counterparts (*20*, *21*). For example, following a 15-day hydroponic exposure, the biomass of zucchini plant exposed to silver nanoparticles was 75% less than plants treated with same concentrations of bulk silver powder (*21*). For CeO_2 particles, it is well accepted that the presence of highly mobile lattice oxygen on the surface will cause oxygen

257 vacancy on the surface (22). With the decrease of nanoparticle size, the specific surface
258 area and consequently the density of the oxygen vacancy increase. The separation of
259 oxygen from the lattice structure generates electrons which can be used to reduce Ce^{4+} to
260 Ce^{3+}. With increasing oxygen vacancy, the ratio of Ce^{3+}/Ce^{4+} will increase on the surface
261 of nanoparticles (23). Because Ce^{3+} is about 14% larger than Ce^{4+} (22), the conversion of
262 Ce^{4+} to Ce^{3+} will strain the lattice structure and increase the reactivity and the superoxide
263 dismutase (SOD) mimetic activity of the CeO_2 particles (24). Therefore, particle size is
264 an important consideration in the assessment of the environmental toxicity of CeO_2.
265 Unfortunately, information on the size effect of CeO_2 particles on plant development in
266 the literature is very limited.
267 Due to the potential dissolution of some metallic nanoparticles, another major
268 question actively investigated in the scientific community of nanotoxicology is the
269 comparative toxicity of nanoparticles and the ionic form of the particles. Because of the
270 general acceptance that CeO_2 NPs are stable in liquid solutions, ionic cerium was
271 generally not included in the treatment paradigms (*13-16*). However, the broad
272 applications of different forms and sizes of cerium require a comprehensive
273 understanding and comparison of their fate and phytotoxicity. Our investigation provides
274 an assessment of the differential fate and phytotoxicity of cerium in its ionic, nanoscale
275 and bulk particle forms. Several physiological parameters including the root membrane
276 integrity photosynthesis-related measurements, and biomass parameters were affected by
277 certain forms of cerium at the tested concentration.
278 While the specific mechanisms by which cerium compounds may compromise
279 membrane integrity are not known and may differ, all forms and sizes of cerium resulted

280 in some damage to root membrane integrity as indicated by an increase in electrolyte
281 leakage. The effect was significant however only for the nanoparticle and ionic forms.
282 The changes in the integrity of root membrane could also alter the membrane potential
283 and potentially the function of the membrane (*24*). It has been reported that altered
284 plasma membrane integrity and potential is associated with changes in the ion fluxes into
285 plant roots (*25*). Whether this alteration of membrane integrity influenced the
286 concentration of any essential macronutrients or micronutrients in radish was not
287 examined but would be a reasonable question for future studies. For the bulk CeO_2 and
288 CeO_2 NPs, in addition to their impact on the membrane, physical adsorption on root
289 surface and blockage of nutrient uptake by plant roots may also occur. It is possible that
290 such impacts on the roots may have affected the uptake of elements such as magnesium
291 or iron, two nutrients associated with the synthesis of chlorophyll. A decrease in the
292 concentration of either of these essential nutrients might have contributed to the decrease
293 in relative chlorophyll content observed in some treatments. Other aspects of chlorophyll
294 synthesis or degradation could have been affected as well and a more detailed study will
295 be required to understand the extent or severity of effects of cerium on chlorophyll
296 metabolism. The significantly lower F_V/F_M values observed for the bulk cerium treatment
297 as compared to the control plants suggested that photosynthetic electron transport
298 associated with photosystem II was stressed in those plants, but not for the other cerium
299 treatments. These results differ from a study with plantlets of *Medicago arborea* in
300 which nanoceria was found to have a more negative effect on the F_V/F_M ratio than the
301 same concentrations of bulk cerium (25). Other studies have shown that the influence of
302 cerium compounds on plant photochemistry differs depending on factors such as plant

303 Mn status (26,) and the presence of salt stress (27). Definitive conclusions about the
304 comparative phytotoxicity of the cerium ion and nanoceria cannot be made without
305 further investigation. Even so, the overall effect of all treatments on the two
306 photosynthetic parameters measured were modest and perhaps not indicative of a
307 significant stress imposed on the plants, particularly given that there were no overt visible
308 effects observed for any treatments, including the ionic cerium and the CeO_2 NPs
309 treatments. The only other indication of a negative effect of treatment with cerium was
310 the decrease in biomass observed for the cerium ion treatment.
311 The shoot/root ratio of radish was also affected by cerium, primarily through the
312 change of the biomass of the storage roots. Because the root thickening is a result of the
313 combined cell division and enlargement of secondary xylem and phloem cells which
314 depends on the activity of the vascular cambium (*28*), it is possible that the cerium of
315 different forms have different impacts on the activity of the vascular cambium. One could
316 speculate that the bulk CeO_2 might have enhanced the activity of the vascular cambium
317 while ionic Ce inhibited it. Metabolically, according to the "sink capacity" theory, the
318 storage root represents a major reservoir for radish and sucrose transported within radish
319 is the main carbohydrate for the growth of sinks. As such, photosynthate distribution into
320 different tissues is heavily affected by the activity of sucrose synthase (SuSy) (*29-32*). If
321 future studies examined the expression of SuSy genes and/or measured the activity of this
322 enzyme in the radish hypocotyl in response to different forms of cerium, it might be
323 possible to ascertain whether the changes in the mass of the storage root in response to
324 bulk or ionic cerium treated radish plants were due to changes in sink strength. The
325 specific mechanisms by which these cerium compounds influence the biomass of radish

storage roots has implications for the agricultural production of radish and related vegetable species and are therefore worth further attention.

It should be pointed out that concentration of $CeCl_3$ used in this study was very low and the impact of chloride ion is not expected to be substantial. Parida and Das (*33*) investigated plant salt tolerance and salinity effects on plant growth and the authors reported that under 100 mM NaCl (3.55 g/L Cl^-), chloride demonstrated limited influence on the osmotic adjustment of cell membrane. Scialabba and Melati (*34*) also reported that sodium chloride up to a concentration of 0.1% positively affected radish growth. The Cl^- in the ionic cerium solution used in this study was significantly lower than those reported values and was not expected to significantly contribute to the negative effect observed in the ionic treatment group. Consequently, the negative effect observed in the ionic treatment should be attributed to the ionic cerium. Another caveat about the results is that 10 mg/L was the concentration of the compounds of CeO_2 and $CeCl_3$, not the concentration of cerium as an element. Due to the different molecular weight percentage of cerium in CeO_2 and $CeCl_3$, the actual concentration of cerium as an element was 8.14 mg Ce/L in CeO_2 NPs and the bulk and was only 5.68 mg Ce/L in the ionic form. Cerium in CeO_2 was 43.5% higher than in the ionic form. If the equivalent concentration of cerium as an element was used, the ionic cerium may display an even stronger effect on plant physiology.

In addition to the yield of edible storage root, the potential accumulation of cerium was examined. Exposed plants had detectable cerium in all plant tissues, including the edible storage roots and leaves even though the forms of cerium in these tissues are unknown. However, the forms of cerium in plant tissues may affect both their

toxicity and potential availability to humans and they deserve detailed investigation in future studies. In current study, the significantly higher cerium detected in the shoot tissues of exposed plants indicated that cerium translocation from roots to shoot had occurred. The upward transport of bulk cerium to radish shoots was unexpected given the size of the particles and the low dissolution rate of bulk CeO_2. It is most likely that the cerium content detected from the bulk treated shoot tissues was from the nanoscale particles present in the bulk mixture (Supplementary Figure 2). The upward transport of CeO_2 NPs and ionic cerium from roots to shoots was expected and has been reported in the literature (*10, 14, 35*). Interestingly, however, when the cerium localization in the storage root was investigated with the confocal microscope, signals of cerium in the vascular tissues were only observed in cerium ion treatment, suggesting that active transport may function as an important pathway of cerium accumulation only for ion treated radishes. In contrast, signals from the CeO_2 NPs and bulk treated radish roots were mainly located on the periderms. The results suggest that adsorption and diffusion of particulate cerium along the radial direction might be a more important pathway for CeO_2 NPs and bulk accumulation in radish storage roots. The diffusion may possibly occur from the lenticels on the periderm, but more precise techniques are needed to confirm this assumption. From the food safety point of view, the cerium accumulation in the edible storage root is more concerning and our results showed that while cerium concentration and content were similar across the cerium treatment, the distribution of cerium in the storage roots varied and consequently their availability to humans would vary. For example, the majority of particulate cerium accumulated in the edible tissue could be removed in the food preparation process while ionic cerium in the storage roots

is more likely to be consumed by humans with the storage root.

In closing, our results suggested that 10 mg/L cerium as cerium oxide or cerium chloride could affect the growth of radish and could accumulate in the edible storage root and shoot tissues. However, the impact and accumulation patterns varied significantly by the size and chemical form of cerium. Ionic cerium displayed the strongest impact on radish root membrane integrity and growth, followed by CeO_2 NPs and then the bulk. While cerium of different forms all accumulate in radish tissues, their accumulation potential and distribution patterns varied greatly. As a result, potential exposure and risk to human health through diet exposure to different sizes and forms of cerium may vary and these differences should be considered when evaluating the food safety of cerium in the environment.

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Supporting Information Available

The transmission electron microscopic images of bulk CeO_2 and CeO_2 NPs, the accumulative transpiration of radish plants and the anatomy of radish storage root were provided as supporting information. This information is available free of charge via the Internet at http: //pubs.acs.org.

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Table 1: The relative chlorophyll content expressed as percentage of control of each treatment, the average Y(II), F_V/F_M ratio, n=12. Different letters in the table represent significant differences between the treatments ($p<0.05$).

Treatment	Relative Chlorophyll (%)	Standard error	Y(II)	Standard error	F_V/F_M	Standard error
Control	100.00 [a]	2.67	0.774	0.007	0.830 [a]	0.003
Bulk	87.22 [b]	3.84	0.728	0.022	0.757 [b]	0.026
Nanoparticle	83.69 [b]	4.24	0.731	0.020	0.780 [ab]	0.016
Cerium ion	91.51 [ab]	4.68	0.697	0.060	0.797 [ab]	0.020

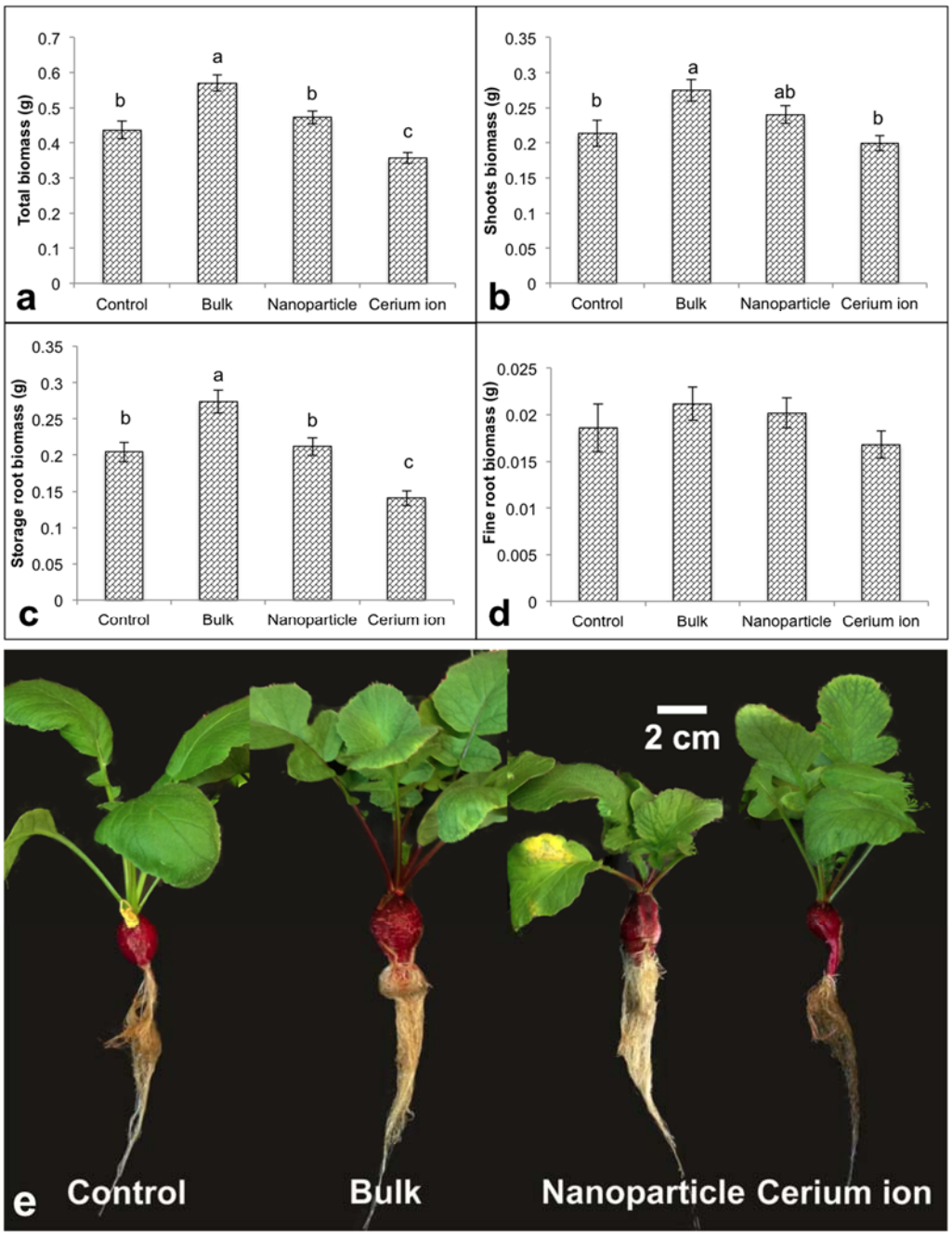


Figure 1: Dry biomass of total radish and different radish tissues treated with 10 mg/L of different forms of cerium (a-d). The reported values are the mean of 12 replicates and the error bars represent standard error. Different letters represent significant differences between the treatments ($p<0.05$). (e) Images of typical radish plants from the different treatments.

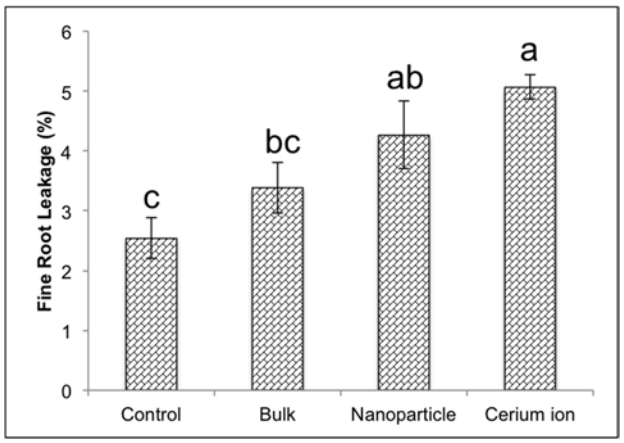


Figure 2: Electrolyte leakage from radish fine roots grown hydroponically in different solutions. The reported values are the average of 5 replicates in each treatment and the error bars represent standard error. Different letters represent significant differences between the treatments ($p<0.05$).

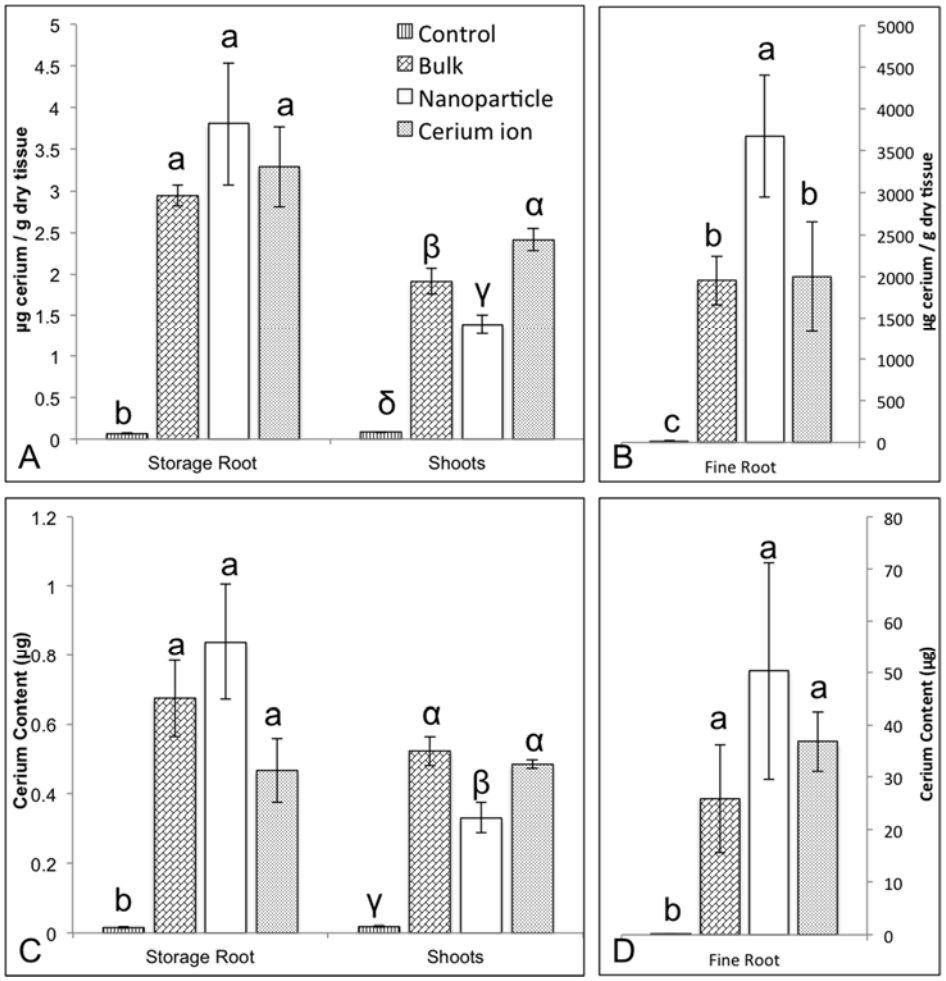


Figure 3: Cerium concentration (A and B) and mass (C and D) in different radish tissues. The reported values in A and C are the average of 4 measurements. The reported values in B and D are the average of 2 or 3 measurements. Errors bars represent standard error. Letters above bars reflect their statistical grouping. Different letters and Greek symbols represent significant differences between the treatments ($p<0.05$).

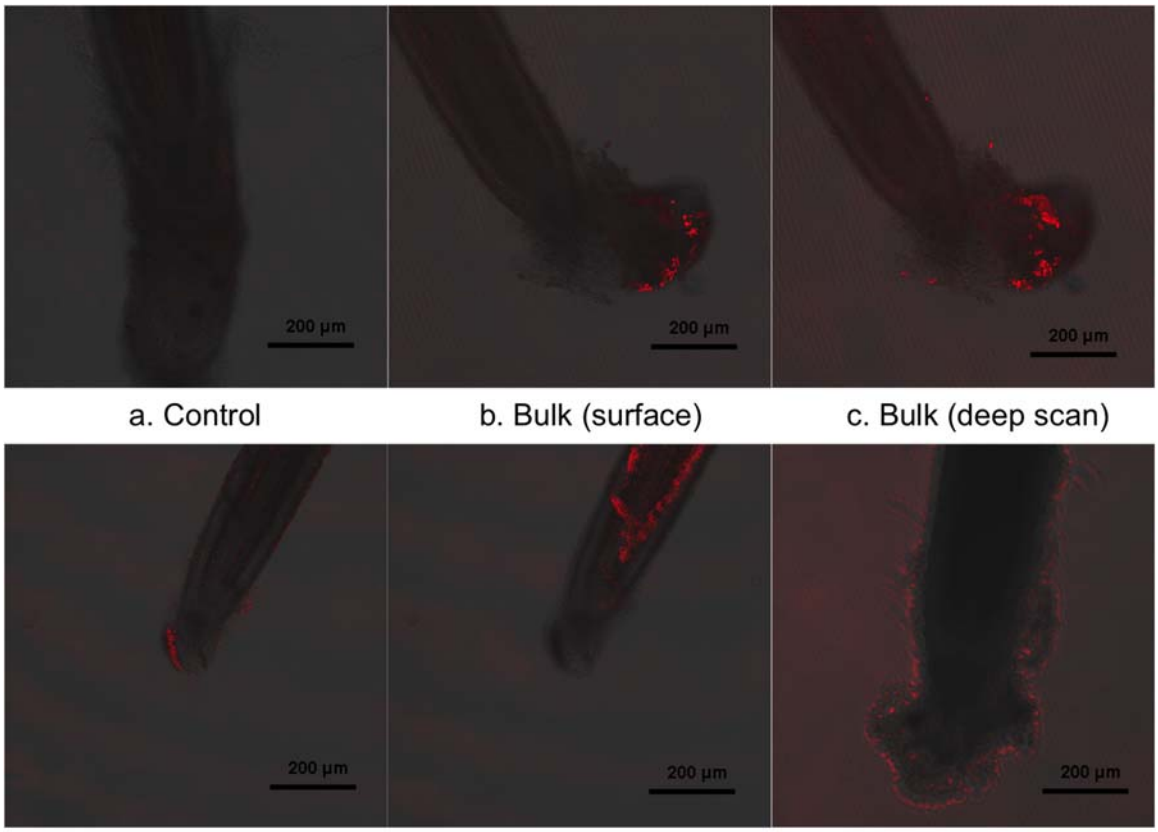


Figure 4: Confocal microscopic images depicting the accumulation of cerium in the fine roots of radish. (a) control root showing weak signals, (b, c) surface and representative deeper scan of fine roots treated by bulk CeO_2, (d, e) surface and representative deeper scan of fine roots exposed to CeO_2 NPs and (f) a deeper scan image of fine roots exposed to cerium ion. The deeper scan images shown were selected from a stack of deep scan images for different roots.

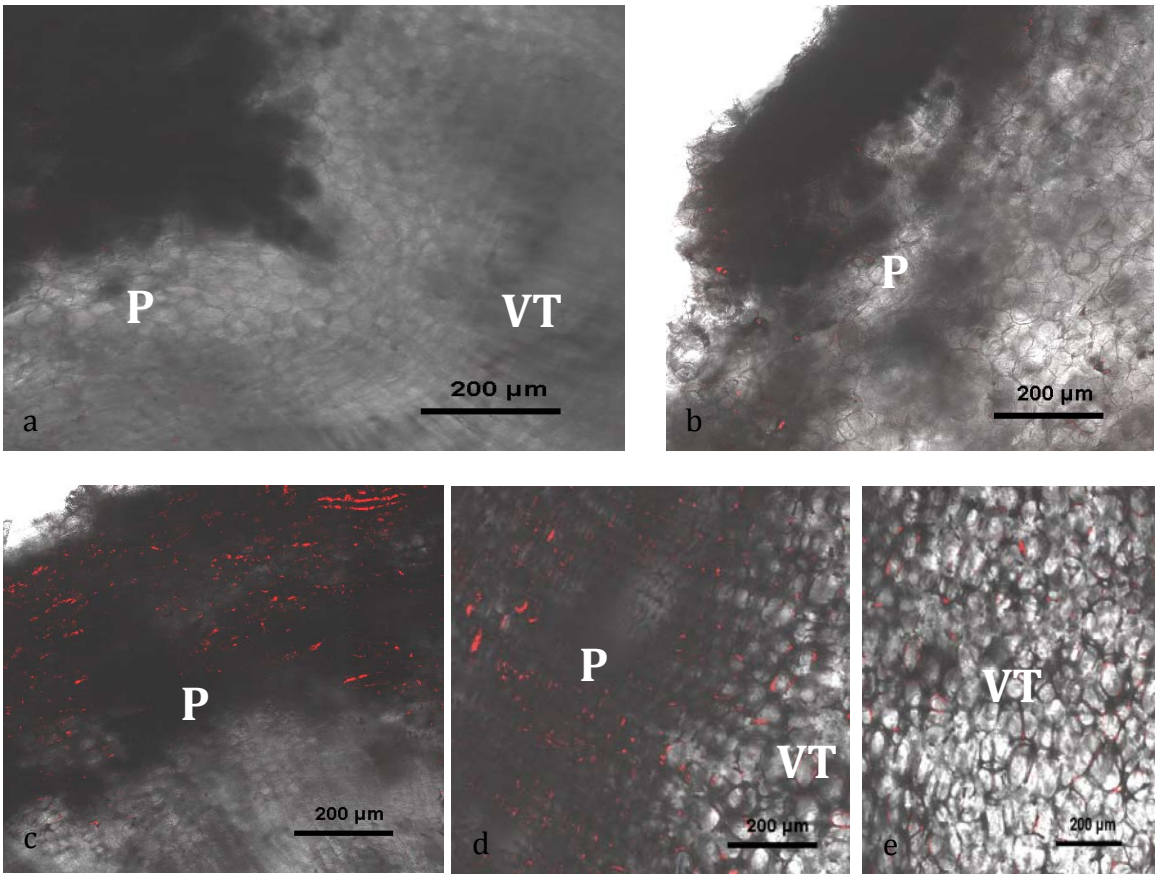
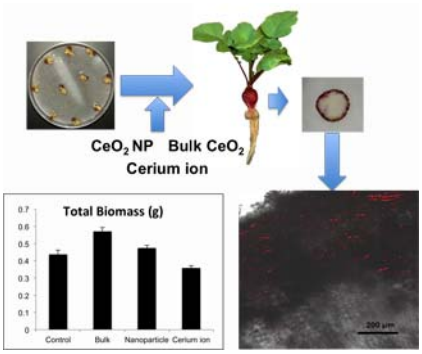


Figure 5: (a) Confocal images of the horizontal slices of radish storage root treated with different types of cerium. (a): control; (b): bulk CeO_2 treated radish; (c): CeO_2 NPs treated radish and (d, e): ionic cerium treated radish. P: Periderm; VT: Vascular tissues.

TOC Graphic


CeO_2 NP
Bulk CeO_2
Cerium ion
Total Biomass (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
Bulk
Nanoparticle
Cerium ion
200 µm