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Bioavailability of Cerium Oxide Nanoparticles to *Raphanus sativus* L. in Two Soils

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Abstract

Cerium oxide nanoparticles (CeO_2 NP) are a common component of many commercial products. Due to the general concerns over the potential toxicity of engineered nanoparticles (ENPs), the phytotoxicity and *in planta* accumulation of CeO_2 NPs have been broadly investigated. However, most previous studies were conducted in hydroponic systems and with grain crops. For a few studies performed with soil grown plants, the impact of soil properties on the fate and transport of CeO_2 NPs was generally ignored even though numerous previous studies indicate that soil properties play a critical role in the fate and transport of environmental pollutants. The objectives of this study were to evaluate the soil fractionation and bioavailability of CeO_2 NPs to *Raphanus sativus* L (radish) in two soil types. Our results showed that the silty loam contained slightly higher exchangeable fraction (F1) of cerium element than did loamy sand soil, but significantly lower reducible (F2) and oxidizable (F3) fractions as CeO_2 NPs concentration increased. CeO_2 NPs associated with silicate minerals or the residue fraction (F4) dominated in both soils. The cerium concentration in radish storage root showed linear correlation with the sum of the first three fractions ($r^2 = 0.98$ and 0.78 for loamy sand and silty loam respectively). However, the cerium content in radish shoots only exhibited strong correlations with F1 ($r^2 = 0.97$ and 0.89 for loamy sand and silty loam respectively). Overall, the results demonstrated that soil properties are important factors governing the distribution of CeO_2NPs in soil and subsequent bioavailability to plants.

Keywords: cerium oxide nanoparticles, radish, bioavailability, soil fractionation

Introduction

As the world's most abundant rare earth element, cerium is widely used in industries both in free metal and oxide form (Naumov, 2008; Masui et al., 2002). Thanks to the large specific surface area and rich redox chemistry, cerium oxide nanoparticles (CeO_2 NPs) have been used as catalysts, electrolyte materials and fuel additives (Zhang et al., 2002). The increasing popularity of CeO_2 NPs in industry has caused concern over their potential toxicity in the environment. There have been many reports that indicate potential toxicity of CeO_2 NPs to bacteria, fish, and mammalian cells (Pelletier et al., 2010; Rosenkranz et al., 2012). The potential risks of CeO_2 NPs to plants, a critical food source for humans, have also been investigated. However, previous studies were mainly focused on the uptake and accumulation of CeO_2 NPs by grain crops and aboveground vegetables in hydroponic systems. For instance, López-Moreno et al. (López-Moreno et al., 2010) showed that intact CeO_2 NPs were taken up by soybean roots in hydroponic systems without subsequent biotransformation. Zhang et al. (Zhang et al., 2011) also reported that cucumber (*Cucumis sativus* L.) root could take up CeO_2 NPs and transport them to the shoots. However, later investigations suggested that CeO_2 NPs may release Ce^{3+} on root surface and uptake of Ce^{3+} rather than CeO_2 NPs might be the primary pathwyay for plant upktae of CeO_2 NPs (Rui et al., 2015; Ma et al., 2015; Schwabe et al., 2015). Although hydroponic studies provide valuable information on the potential mechanisms of plant uptake and accumulation of CeO_2 NPs, increasing efforts are dedicated to elucidating the fate and impact of CeO_2 NPs in soil to obtain a more realistic understanding of the fate and impact of CeO_2 NPs.

For example, after tomato plants were irrigated with 0.1 to 10 mg/L of CeO_2 NPs solutions, Wang et al. (Wang et al., 2012) reported that Ce was accumulated in tomato (*Solanum lycopersicum* L.) roots and shoots, including the edible tissues, with the root being the primary tissue of accumulation. Zhao et al. (Zhao et al., 2015) also reported low translocation of CeO_2 NPs from root to shoot in corn plants (*Zea mays* L.) and noticed that 800 mg/kg CeO_2 NPs did not affect plant photosynthesis throughout the exposure but significantly reduced the corn yield. Another recent study demonstrated that CeO_2 NPs did not affect the growth of lettuce (*Lactuca sativa* L.) at low concentrations (50 mg/kg and 100 mg/kg) in potting soil, but significantly inhibited biomass production and disrupted plant stress responses at 1000 mg/kg (Gui et al., 2015). While these soil-based studies provide significant new information on the fate and impact of CeO_2 NPs in the ecosystem, none of the previous studies has closely examined the impact of soil properties on the toxicity and bioavailability of CeO_2 NPs to terrestrial plants. Plant uptake of metals in soil depends on both the soluble fraction of total metal and the capability of soil to release the metals and both factors are considerably affected by the soil properties (Backes et al., 1995). Previous research has shown that metal mobility in soil is governed by many factors including the soil characteristics (e.g. soil texture, pH, and organic matter content); the nature of the contaminants (e.g. the chemical forms of pollutants and the binding state); and the environmental conditions (e.g. acidification, redox processes, temperature, and water regime) (Sahuquillo et al., 2003).

In recent decades, several extraction methods have been developed to evaluate the mobility of metals in soil. Sequential selective extraction is defined as the use of a series of selective reagents to solubilize the solid material successively into specific fractions (Gleyzes et al., 2002). A three-step sequential extraction procedure for soil and sediment analysis known as the BCR (Bureau Commune de Reference of the European Commission) method, proposed in 1993 (Ure et al., 1993) and later modified by Rauret et al in 1999 (Rauret et al., 1999) is widely used for the determination of extractable trace metals in soils and sediments. This three-step sequential extraction method separates the metal of interest into four fractions: the exchangeable, water/acid soluble metal (F1); the metal bound to Fe-Mn oxides (F2); the metal bound to organic matter (F3) and the metal bound to silicate minerals in the residual fraction (F4) (Rao et al., 2010; Sahuquillo et al., 2003; Li et al., 2010). According to the research of Li et al. (Li et al., 2010), F1 represents the most active, mobile and bioavailable phase of the metal. These authors used the BCR method to study the bioavailability of Zn, Cu, Pb Cd, Hg, and As in topsoil and found that soil physicochemical properties (e.g. pH, organic matter, and clay content) affected metal fractionation in soil and their bioavailability to plants. Zhong et al. (Zhong et al., 2011) suggested that the first three fractions of the metals in soil were the potentially bioavailable and hazardous fractions to plants. The successful application of the BCR method to estimate the bioavailability of heavy metals in soil to plants provides a potentially useful method to evaluate the availability of engineered metallic nanoparticles under similar exposure scenarios.

Radish (*Raphanus sativus* L.) is a popular vegetable with high global consumption and can mature in three to four weeks under favorable growth conditions. Radish is also an underground vegetable, with its edible tissues directly exposed to CeO_2 NPs in soil. Therefore, radish may accumulate high concentrations of ENPs in their edible tissues. A previous study indeed demonstrated that the radish tubes grown in a loamy sand soil with 250 and 500 mg/kg of CeO_2 NPs accumulated high concentrations of Ce, posing potential risks for human exposure (Corral-Diaz et al., 2014). However, detailed distribution of Ce in the tubes and the role of soil properties were not reported in that study. The objectives of this investigation were to (1) use the BCR sequential extraction method to evaluate the fractionation of CeO_2 NPs in two types of soil, (2) assess the bioavailability of CeO_2 NPs to radish roots and (3) determine the impact of soil type on the root to shoot translocation of CeO_2 NPs and their distribution in plant tissues.

Materials and Methods

Chemicals

A dispersion of bare CeO_2 NPs (10 wt. % in H_2O, <25 nm particle size) was purchased from Sigma-Aldrich (St. Louis, MO). The shape, size and size distribution were determined by a Tecnai G2 F20 transmission electron microscope (TEM) (FEI, Hillsboro, Oregon) and are shown in Figure 1. Most of the nanoparticles had quadrilateral or polygonal shapes and fell in the size range of 10-25 nm in diameter with an average nanoparticle size of 19.1 nm. The size distribution was obtained by measuring 112 individual nanoparticles on the TEM image with ImageJ. The hydrodynamic diameter and zeta potential of CeO_2 NPs at 500 mg/L in water were 107.3 nm and 45±0.41 mV respectively, as measured by a dynamic light scattering instrument (Malvern Zetasizer Nano-ZS90, Westborough, MA). The surface speciation of CeO_2 NPs was investigated with an X-ray photoelectron spectroscopy (XPS) (Omicron multiprobe MXPS system, Scienta Omicron, Germany). The XPS spectra of the surface of CeO_2 NPs was shown in Figure 1c. The results indicated that 12.4% of Ce on the surface was in the form of Ce^{3+}, as calculated through the XPS peak fitting software XPSPEAK 4.1.

Quarter and half strength Hoagland solution were prepared by dissolving an appropriate amount of the modified Hoagland’s basal salt mixture purchased from Phytotechnology Laboratories (Lenexa, KS) in deionized (DI) water.

Soil characterization

Two types of soil were used in this study: (1) commercially-purchased topsoil (Timberline Top Soil, Oldcastle Inc., Atlanta, GA); (2) an agricultural soil collected from a farmland associated with Southern Illinois University (Carbondale, IL). Due to the different weight percentages of sand, silt and clay in these two soils, the topsoil was classified as loamy sand and the local soil was classified as silty loam according to the USDA soil texture classification. The weight percentages of sand, silt, and clay were determined through wet sieve analysis and hydrometer test (Bouyoucos, 1962). The results for both soils are shown in Supplementary Table 1.

The Deutsches Institut für Normung (DIN) 19684-1 method was adopted for the measurement of soil pH. One hundred mL deionized water was mixed with 40 g of air-dried soil at the speed of 250 rpm (solid-liquid mass ratio 1:2.5). The mixture was shaken for five minutes and allowed to settle for two hr. The pH was then measured with a pH meter (Thermo Scientific Orion ROSS Ultra pH/ATC Triode, Orion Star A325). The pH of loamy sand was 6.87 and the pH of silty loam was 6.58.

The ASTM D 2974 method (Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Organic Soils) was used to determine the content of organic matter in soil. The soil was first dried in an oven at 105 °C for 24 h. The dry soil was weighed and then combusted at 440 °C for 24 h. The loss in mass was assumed to be due entirely to oxidation of organic matter. Three replicates were prepared for each type of soil. The average organic matter contents were 11.87% ± 0.56% for loamy sand and 2.21% ± 0.04% (average ± standard error, n=3) for silty loam.

Experimental Setup

Soil preparation

The growing pots were established by adding 150 g of dry soil to a plastic container (~266 mL total volume). CeO_2 NPs dispersion and deionized water were added to the container in different proportions so that the soil was saturated to 100% of field capacity and at the same time reached the targeted concentration of CeO_2 NPs homogeneously. Four concentrations of CeO_2 NPs were prepared for each type of soil: control (no treatment), 100, 500 and 1000 mg Ce /kg dry soil. The concentrations were chosen based on the most frequently used concentrations in the literature for the fate and phytotoxicity study of metal oxide nanoparticles to terrestrial plants (Holden et al., 2014). Each treatment had six replicates. Altogether, 24 such containers were prepared for each soil. The soil were incubated for one day before radish seeds were sowed.

Seed germination and growth conditions

Radish seeds [Cherriette (F1)] were purchased from Johnny's Selected Seeds (Winslow, ME). Three seeds were placed approximately 15 mm beneath the soil surface in each container with soils containing different concentrations of CeO_2 NPs. After germination, each container was thinned to one seedling.

Plants were irrigated with quarter strength Hoagland's solution to a constant mass (230 g after irrigation) daily from Day 6 to Day 15 after sowing. The soil was then irrigated to the same constant mass with half strength Hoagland's solution until harvest (Day 31). Plants were incubated on a growth cart with a 16 h photoperiod at 28 °C and ambient humidity. The growth cart was equipped with four T5 fluorescent bulbs, providing a light intensity of approximately 104 umol m^{-2} s^{-1} at the height of plant shoots. Relative chlorophyll content was measured with a SPAD 502 Plus Chlorophyll Meter at Day 26 and was expressed as a percentage of the control plants.

Cerium fractionation in soil

At harvest, plants were gently removed from the soil for further analysis (details described below). The soil was homogenized and then three samples were randomly collected from three containers in each treatment and extracted with the modified BCR method to determine the fractionation of CeO_2 NPs in soil. The sample was first extracted with 20 mL of 0.11 M acetic acid solution by shaking at 250 rpm for 16 hours at 22±5 °C and centrifuged at 3,000 g for 20 minutes to obtain the exchangeable fraction (F1). The residue was then resuspended and extracted by 20 mL of 0.5 M hydroxylamine hydrochloride solution at pH 1.5 and shaken at 250 rpm for 16 hours at 22±5 °C. The mixture was centrifuged similarly as described above to obtain the reducible fraction (F2). The residue was then resuspended and mixed with 30% H_2O_2 and shaken at 250 rpm for 1 hour at room temperature, followed by another hour of shaking at 250 rpm at 85±2 °C with a closed cap. The volume of the mixture was reduced to less than 1.5 mL by further heating at the same temperature without cap. Following the volume reduction, an aliquot of 5 mL of 30% w/v H_2O_2 was added and the heating process was repeated until the volume was reduced to about 0.5 mL. Afterwards, 25 mL of 1 M ammonium acetate solution at pH 2 was mixed with the residue for 16 hours at 22±5 °C and the mixture was centrifuged at 3,000 g for 20 minutes to extract the oxidizable fraction (F3). The residue fraction (F4) was extracted by aqua regia following the ISO 11466 protocol;

4.5 mL of HCl (12.0 M) and 1.5 mL of HNO_3 (15.8 M) was added drop-wise to 0.5 g of residue from the third fraction. The mixture was left at room temperature for 16 hours and then was transferred to a 50 mL reaction vessel connected to a reflux condenser. The reaction vessel was heated until reflux conditions were reached and was continuously heated for 2 hours (the condensation zone is lower than 1/3 of the height of the condenser). The condenser was further rinsed with 10 mL HNO_3 (0.5 M) and the rinsing solution and additional HNO_3 (0.5 M) were collected and added to the reaction vessel until they reached the 50 mL scale line. The supernatant solution of each fraction was analyzed for Ce by an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Santa Clara, CA).

Scanning electron microscope characterization of cerium in soil

To determine the physicochemical characteristics of CeO_2 NPs in soil, air dried control and 1000 mg/kg treated loamy sand and silty loam soils were fixed on a double-sided adhesive tape, which was adhered to the specimen holder, and were analyzed using FEI Quanta FEG450 scanning electron microscope (SEM) equipped with an Energy Dispersive X-ray Spectroscopy (EDS). The SEM imaging of soil samples was performed by applying accelerating voltages of 10 kV. The concentration of 1000 mg/kg CeO_2 NPs, the highest concentration used in this study, was selected to ensure the detectability of CeO_2 NPs by SEM.

Plant uptake and accumulation of cerium

After plants were carefully removed from the soil, they were separated into shoots, storage root (the edible radish bulb) and fine roots. The separated tissues were rinsed with DI water to remove all adhering soil particles and dried in an oven at 105 °C for 30 minutes, then at 75 °C for seven days prior to dry weight determination. After drying in the oven, three replicates in each treatment were randomly chosen. The dried shoot, storage root, and fine root tissues were ground into fine powders and digested in 4 mL of 70% (v/v) nitric acid. The nitric acid digest was heated at 95 °C for 20 minutes and then at 45 °C for 4 minutes. The cycle was repeated until all the dry tissues was dissolved. Afterwards, 2 mL of H_2O_2 was added to the mixture. The mixture was heated using the same temperature cycle until the solution was clear. The digest solutions of storage roots and shoots were then analyzed by ICP-MS. The digest solution of fine roots was analyzed by a Thermal Scientific iCAP 6500 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) due to the high cerium concentration in the fine root tissue.

Distribution of cerium in radish shoots and storage roots

Three replicates from the control and 500 mg/kg treatment group grown in both soils were used as representatives to illustrate the cerium localization in the radish storage roots and shoots. The whole storage root was divided into three layers with a precision knife: the periderm (Peri), the intermediate layer (L1), and the inner layer (L2). The thicknesses of the periderm and the intermediate layer were approximately 1 mm and 5 mm respectively (Figure 2). Each shoot was divided into two sections: the edges (S1) and the main leaf area (S2). The width of the edges was about 5- 7 mm (Figure 2). The subsections of the storage roots and shoots were oven dried and digested as described above for the whole tissues. The digest solutions were analyzed by ICP-MS.

Data analysis

The statistical analysis of experimental data was performed by means of one-way and two-way ANOVA using IBM SPSS Statistics 20.0. The Duncan test was conducted for post hoc comparisons. A student t test was conducted to determine the significance of soil impact at the same concentration. Statistical significance was accepted when $p<0.05$.

Results

Plant physiological status

The dry biomass of storage roots and shoots are shown in Supplementary Figure 1. For both soils, treatment with 100 and 500 mg/kg CeO_2 NPs did not cause any significant differences between the treated plants and their controls. Exposure to 1000 mg /kg CeO_2 NPs resulted in significantly greater dry biomass of the storage root than all other treated and control plants in loamy sand. The same treatment, however, led to significantly lower dry biomass of storage roots than that of 500 mg/kg treated radishes in silty loam. When the biomass of radishes grown in two soils at the same concentration was compared, the storage roots of control, 100 mg/kg, and 500 mg/kg CeO_2 NPs treated radishes were significantly greater in silty loam than in loamy sand. At the highest concentration, the difference of the storage root biomass between the two soils was not significant.

In contrast to the storage root biomass, the shoot biomass was not affected by CeO_2 NPs exposure for either soil. However, significant differences were noticed between the soil types at control and 100 mg/kg treatment. Radishes grown in silty loam soil from the two concentration groups had significantly higher shoot biomass than the plants grown in loamy sand. The relative chlorophyll contents, expressed as percentages of controls, are shown in Supplementary Table 2. No significant differences were observed across the treatments.

Cerium fractionation in soil

The percentage of each fraction in the two soils is illustrated in stacked columns in Figure 3. F4 was the dominant fraction of CeO_2 NPs in both soils, and the percentage was invariably higher in silty loam (60.8-78.2%) than in loamy sand (58.6-70.5%) at the same concentration. F1 was the smallest fraction and accounted for less than 0.11% in loamy sand and 0.22% in silty loam. While the relative percentage of F2 was comparable between the two soils, the loamy sand always contained higher oxidizable fraction (F3) than silty loam at the same concentration (15.8-17.8% for loamy sand vs. 9.07-11.8% for silty loam). The distribution of CeO_2 NPs among these four fractions changed with concentration. In general, with the increase of concentration, the percentage of F1 and F2 decreased while the percentage of F4 increased in both soils. The percentage of F3 was relatively stable across the concentration ranges employed in this study.

The actual concentrations of each individual fraction are presented in Supplementary Figure 2. As the most abundant rare earth element on the earth's crust, both soils contained high background concentration of cerium. The total background cerium was 52.5 ± 1.87 mg/kg dry soil in the loamy sand and 77.2 ± 5.25 mg/kg dry soil in the silty loam. Due to the high background concentrations of cerium, the fractionation of dosed CeO_2 was calculated by subtracting the cerium concentration in each individual fraction of the control soil from the concentrations in the corresponding fractions of the treated soil. The results are presented in Figure 4. Both the dosing concentration and soil characteristics were significant factors affecting the fractionation of CeO_2 NPs in soil according to the two-way ANOVA analysis. In general, the silty loam contained higher F1 than the loamy sand and the difference was significant for 500 mg/kg treatment

(Figure 4a). The silty loam contained significantly lower F2 and F3 than the loamy sand in 500 and 1000 mg/kg treatment. The silty loam had significantly higher F4 than the loamy sand in 100 mg/kg but the differences in F4 were not significant in higher concentrations (Figure 4d). It has been reported that CeO_2 NPs cannot be fully dissolved in aqua regia (Antisari et al., 2011). Therefore, it is likely that some cerium residues remained in the soil and was not included in the four fractions reported here.

To further probe the differences of CeO_2 NPs behaviors in the two soils, SEM analysis was conducted. The SEM images shown in Figure 5 were acquired with samples from control and 1000 mg/kg treatment. EDS analysis was conducted in the selected area (red frames in the images) to detect the component elements. The main components of the two soils were silica and oxygen. In control samples from both soil types, no cerium was detected by the EDS even though ICP-MS analysis showed that both soils contained high background cerium. However, in 1000 mg/kg treatment, the cerium weight percentages were 7.23% and 8.05% in loamy sand and silty loam, respectively. The cerium signals in both soil indicate that the CeO_2 NPs were mainly attached to the edge of soil particles. Individual particle aggregates could be seen in the treated loamy sand, but not in the silty loam soil.

Cerium uptake and accumulation

Cerium was detected in all plant tissues even though the total accumulation of cerium in plant biomass was relatively small compared with the total cerium added to the system. The concentrations and the total mass of cerium in different plant tissues are presented in Supplementary Figure 3. Due to the high background cerium concentration in control plants, the accumulation of the dosed cerium in different plant tissues was calculated by subtracting the cerium concentration in different plant tissues of the control plants from the corresponding tissues of treated ones and the results are presented in Figure 6. Even though the accumulation of cerium in all tissues increased with increasing concentration in general, a dose response relationship was not apparent, especially for the shoot tissues.

The comparison of cerium accumulation by plants grown in two soil types indicated that the radish fine roots and storage root from the loamy sand usually possessed higher cerium concentration than the same tissues collected from the silty loam. Interestingly, the cerium concentration in the shoot showed opposite trend between these two soils. However, none of these differences were significant except for the cerium in the fine roots from 100 mg/kg treatment.

Cerium localization in radish storage roots and shoots

The cerium concentrations in different sections of radish storage roots and shoots are shown in Table 1. The average cerium concentration in the periderm (Peri) of radish storage roots from 500 mg/kg was more than ten times higher than that of control in both soils. However, large variations were observed between replicates from the same treatment group. Cerium concentrations in the intermediate layer (L1) and the inner layer were comparable to the control plants in both soils. In radish leaves, the cerium concentrations in the edge section (S1) of treated and control plants were similar for both soils. However, the average cerium concentration in the main leaf area (S2) was significantly higher (almost three times) from 500 mg/kg treated radish than from control plants in the silty loam. No difference was observed for the main leaf area in control plants and 500 mg/kg treated plants in loamy sand.

Discussion

Although plant uptake of CeO_2 NPs from soil has been observed previously (Rico et al., 2013; López-Moreno et al., 2010; Wang et al., 2012; Wang et al., 2013; Zhang et al., 2011), the influence of soil properties on CeO_2 NPs bioavailability has not been examined. However, once cerium enters soil through wastewater irrigation or biosolid amendment, particle bioavailability may depend heavily on the physical and chemical properties of soil, as noted for other elements (Ernst, 1996). The results of this study confirmed that the accumulation and translocation of CeO_2 NPs in plant tissues depend heavily on soil type due to the impact of soil on CeO_2 NPs fractionation.

Even though CeO_2 NPs are generally perceived as stable in the environment, dissolution does occur and Cornelis et al. (Cornelis et al., 2011) reported that about 0.25% of total CeO_2 NPs in soil was released as ions at pH 7 and 9 in soil. The presence of chelating agents in the soil may further enhance the dissolution by forming complexes with Ce^{3+} on the surface of CeO_2 NPs (Schwabe et al., 2014). F1 was considered to include both the dissolved ions and dissolved nanoparticles. Due to the low solubility of CeO_2 NPs and possibly the rapid adsorption of dissolved ions to the solid phase, F1 represented a negligible fraction in both soils in this study even though the concentration of F1 increased with concentrations (<0.16% for the dosed CeO_2 NPs). Water soluble cerium at low concentration is generally not considered as toxic and is sometimes used as fertilizer (Hu et al., 2002). The F1 in silty loam was invariably higher than that in loamy sand at the same concentration. Therefore, the differences of F1 may partially explain the generally higher dry biomass of radish storage roots and shoots in silty loam than in loamy sand (Supplementary Figure 1).

Fe-Mn oxides, considered as secondary minerals, exist primarily in the clay (Allen and Hajek, 1989; Fieldes and Swindale, 1954; Post, 1999). Therefore, the higher reducible CeO_2 (F2) in silty loam with higher clay content may be expected. Interestingly, the expectation was only consistent with the observations at lower concentrations (<100 mg/kg). At higher concentrations (500 and 1000 mg/kg), the opposite trend was observed. Two processes may have contributed to the seemingly inconsistent observations of CeO_2 NPs fractionation in these two soils. Firstly, the CeO_2 NPs used in this study were positively charged, as indicated by their surface zeta potential. At neutral pH, the surface charges of quartz and feldspars, which are the main components of sand and silt, are negative (Jada et al., 2006; Yin and Drelich, 2008). Previous research showed that electrons can accumulate at the edges of clay particles (Bolland et al., 1976). Therefore, CeO_2 NPs can be electrostatically attracted to the electrons on clay edges and precipitate (Cornelis et al., 2011). The strong affinity between CeO_2 NPs and some soil particles is supported by the SEM images (Figure 5). The electrostatic forces present may therefore restrain the direct contact of CeO_2 NPs with Fe-Mn oxides in the clay. Secondly, the extractant (hydroxylamine hydrochloride) used to recover F2 may lead to higher cerium concentration in loamy sand due to its high reducing capacity. It has been reported that hydroxylamine hydrochloride can reduce Ce^{4+} in CeO_2 to Ce^{3+} ions ($2CeO_2 + NH_2OH + NH_3OH^+ + 2H_2O \rightarrow 2Ce(OH)_3 + NO_2^- + NH_4^+ + H^+$. $E^o = 0.232V$) (Tamilmani et al., 2003). The reaction might be stronger between the extractant and the more mobile CeO_2 NPs in the loamy sand, leading to high measurement of F2 in the loamy sand than in silty loam. This hypothesis needs further evaluation. Different hydrodynamic sizes of CeO_2 NPs at different concentrations might also affect their precipitation and association with different fractions of soil particles. Future studies should aim to characterize ENPs in the actual environment in addition to the characterization of primary particles.

The oxidizable fraction (F3) of CeO_2 is believed to be associated with organic matter in soil. The higher organic matter content in loamy sand soil is consistent with the generally higher F3 in this soil than in the silty loam. Natural organic matter can enhance the mobility of NPs in porous media by increasing charge and steric stabilization (Lin et al., 2010). Zhao et al. (Zhao et al., 2012) studied the uptake of CeO_2 NPs by corn grown in soils and concluded that organic matter improved the mobility and bioavailability of CeO_2 NPs to corn, resulting in higher accumulation of Ce in corn roots. The consistently higher cerium concentration in the fine roots and storage roots of radish grown in loamy sand was consistent with the relative organic matter contents in these two soils. These findings support the theory that natural organic matter plays an important role in regulating the mobility and bioavailability of engineered nanoparticles to plants (Antisari et al., 2011).

One intriguing observation of this study was the disparity of roots and shoots with regard to CeO_2 NPs accumulation from different soils. As described above, the radish storage roots and fine roots generally contained higher cerium concentration in loamy sand. However, the concentrations of cerium in shoot tissues followed the opposite trend between the soils. It is postulated that the low translocation of cerium in the loamy sand is associated with the low F1 in that soil. Previous research suggested that engineered nanoparticles in plant roots are translocated up through the xylem tissues along with water (Allen and Hajek, 1989), which makes the water soluble fraction more readily transferred to the shoot tissues. A recent study also demonstrated that negatively charged humus colloids in soil could chelate with positively charged CeO_2 NPs and reduce their mobility and bioavailability in soil (Majumdar et al., 2015). Consequently, the upward transport of CeO_2 NPs from root to shoot will be limited in soil grown plants and the extent of transport may depend significantly on the amount of water soluble fraction. Our results agreed with the observation of the low root to shoot translocation of CeO_2 NPs in organic matter enriched soil, but contradicted a previous study which indicated that organic matter enriched soil facilitated the uptake and translocation of CeO_2 NPs by corn (Zhao et al., 2012). The discrepancies may derive from the use of different CeO_2 NPs and different plant species and require further investigation.

Following the uptake of cerium, we further evaluated whether the different soil fractionation would affect the distribution of cerium in different plant tissues. Consistent with our previous investigation (Zhang et al., 2015), cerium was predominantly accumulated in the pigmented periderm of radish storage roots for both soils (Table 1). Another recent study on the interactions between CeO_2 NPs and carrot (*Daucus carota* L.) also reported that the accumulation of cerium element principally in the taproot peel and the shoots, with significantly lower cerium concentration in the edible flesh (Ebbs et al., 2015). Notably, even though the average concentration in the periderm was ten times higher in the 500 mg/kg treated radish than the control radish in this study, high variability between the replicates of treated radish was noticed (51.7-217 mg/kg dry tissue for loamy sand and 45.5-236 mg/kg dry tissue for silty loam). It is likely that the high variability was due to the unequal adsorption of CeO_2 NPs on the skin surface of the storage root and the rinsing process during harvest. The similar cerium concentration in the intermediate and inner layers of the treated and control plants suggested that cerium accumulation in the flesh is limited. Altogether, the results indicate that a primary pathway for cerium accumulation in radish storage roots was physical adsorption on the surface and radial diffusion toward the center which is minimal in this study. Interestingly, the cerium concentration in S2 section of the shoot tissue grown in silty loam was three times higher than their corresponding controls, but such difference was not observed in the sandy loam. Our finding is consistent with the higher shoot concentration in CeO_2 NPs treated radish in silty loam and substantiates our earlier contention that F1 was more readily translocated from radish roots to shoots. A previous study indicated that the cerium taken up from roots is transported to leaves through leaf vein vasculature with the transpiration stream (Zhao et al., 2013) and our results appeared to support that conclusion. It is yet to know, however, whether the translocated cerium was in the CeO_2 NPs form or other chemical forms.

In summary, soil characteristics were shown to be an important factor affecting the soil fractionation and subsequent bioavailability of CeO_2 NPs to plants. The accumulation of cerium in radish belowground tissues correlated well with the sum of the first three fractions, suggesting that these fractions were bioavailable to plant roots. However, only the exchangeable fraction correlated well with the element amounts shown to transport from roots to shoots. In addition to their bioavailability, the distribution of cerium in different plant tissues was also affected by the physicochemical properties soils, indicating that the specific soil properties must be an important consideration in the assessment of the fate and transport of engineered nanoparticles in the environment.

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Author’s Contribution

X.M conceived and supervised the experiment. W. Z and Q. W conducted the experiment and W. Z also prepared the first draft of the manuscript. C. M conducted ICP-MS analysis. J.W., P. S., S. E, and X. M contributed to data analysis and interpretation and X. M also contributed to the writing of the manuscript. All authors read and approved the final version of the manuscript.

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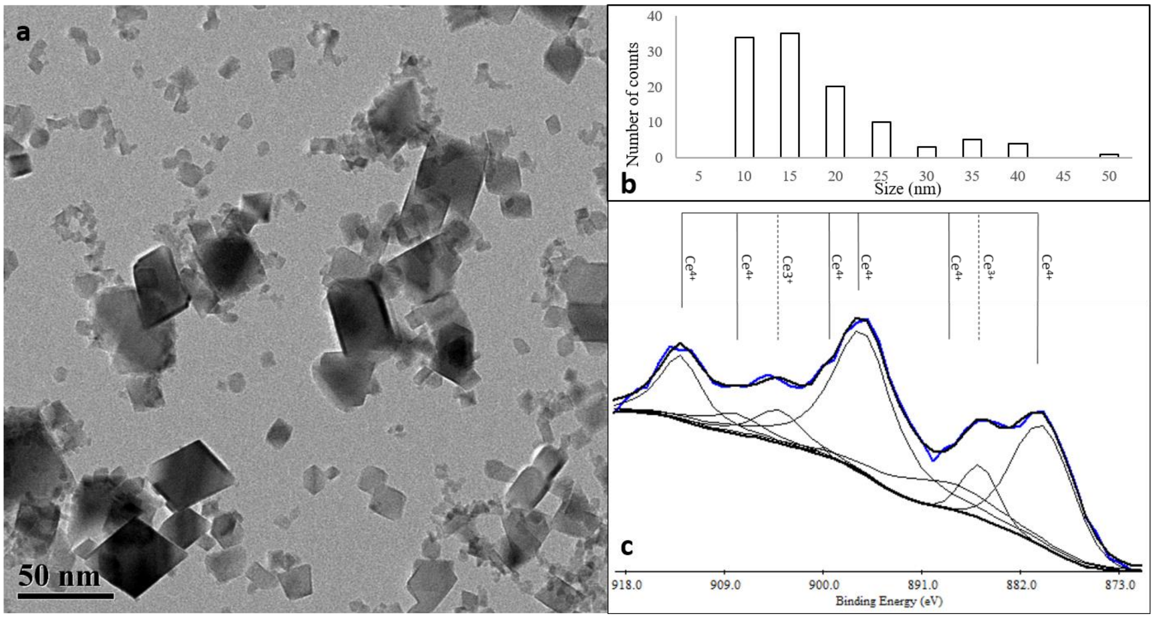


Figure 1: Characterization of CeO2 NPs. (a) TEM image of CeO_2 NPs; (b) The size distribution of the NPs; and (c) The XPS spectra of cerium on the surface of CeO_2 NPs.

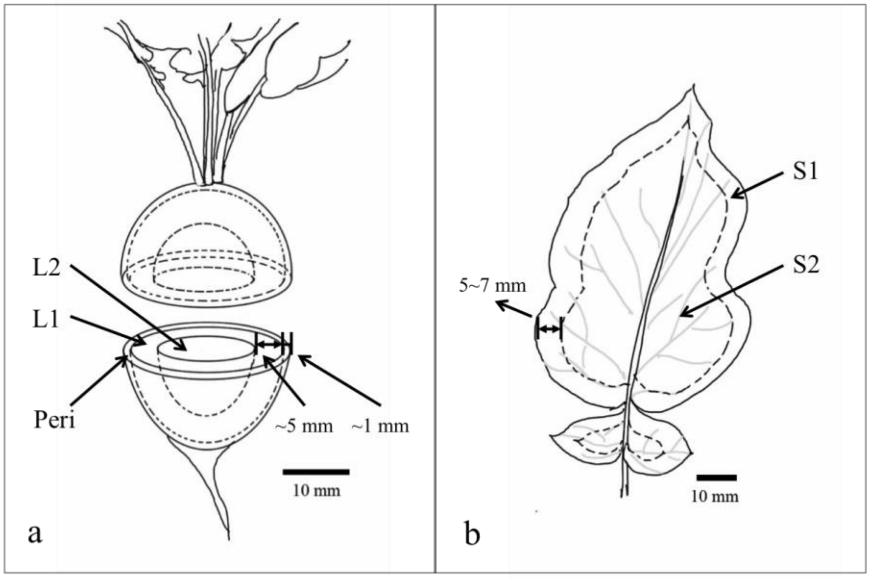
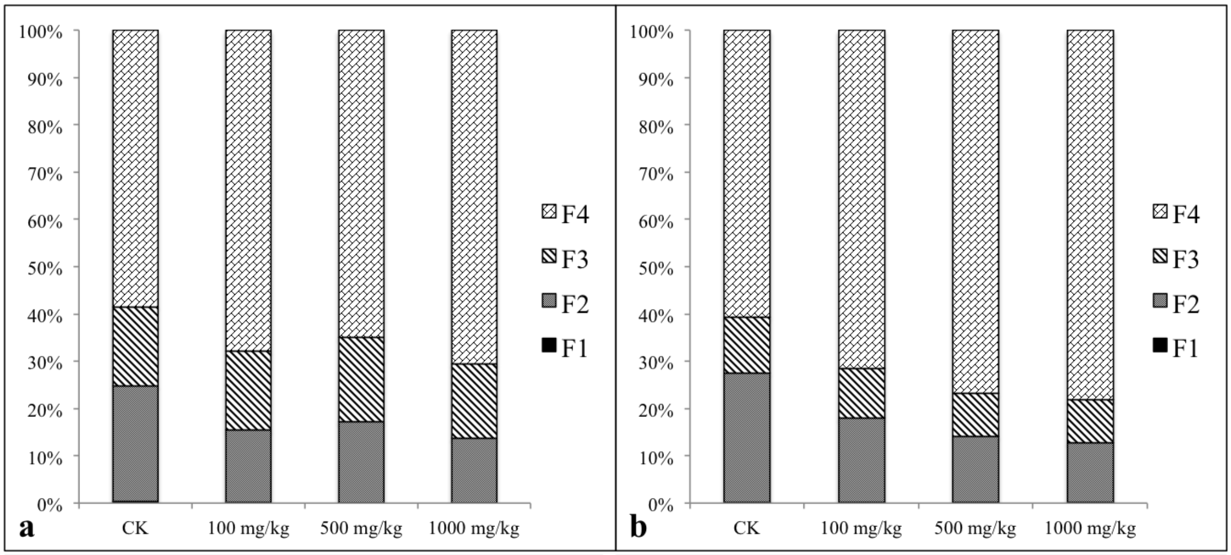


Figure 2: Schematic illustration of the cutting method of the radish storage root and shoot used for cerium uptake distribution.



	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Control	0.11±0.057	24.62±1.12	16.72±0.78	58.55±1.60
100 mg/kg	0.093±0.0048	15.32±0.21	16.68±0.61	67.92±1.21
500 mg/kg	0.048±0.0034	17.15±0.75	17.87±0.53	64.93±1.33
1000 mg/kg	0.027±0.0034	13.64±0.44	15.80±0.74	70.54±1.27

	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Control	0.22±0.0053	27.27±0.47	11.82±0.48	60.69±5.85
100 mg/kg	0.16±0.024	17.74±0.54	10.58±0.69	71.51±0.49
500 mg/kg	0.11±0.0031	14.04±0.79	9.07±1.09	76.78±12.18
1000 mg/kg	0.08±0.014	12.68±0.1.90	9.08±1.31	78.16±17.26

Figure 3: Percentage of cerium fractionation in (a). loamy sand and (b). silty loam determined by the modified BCR sequential extraction procedure. The results shown on the table beneath the figures represent the average and standard error of three replicates.

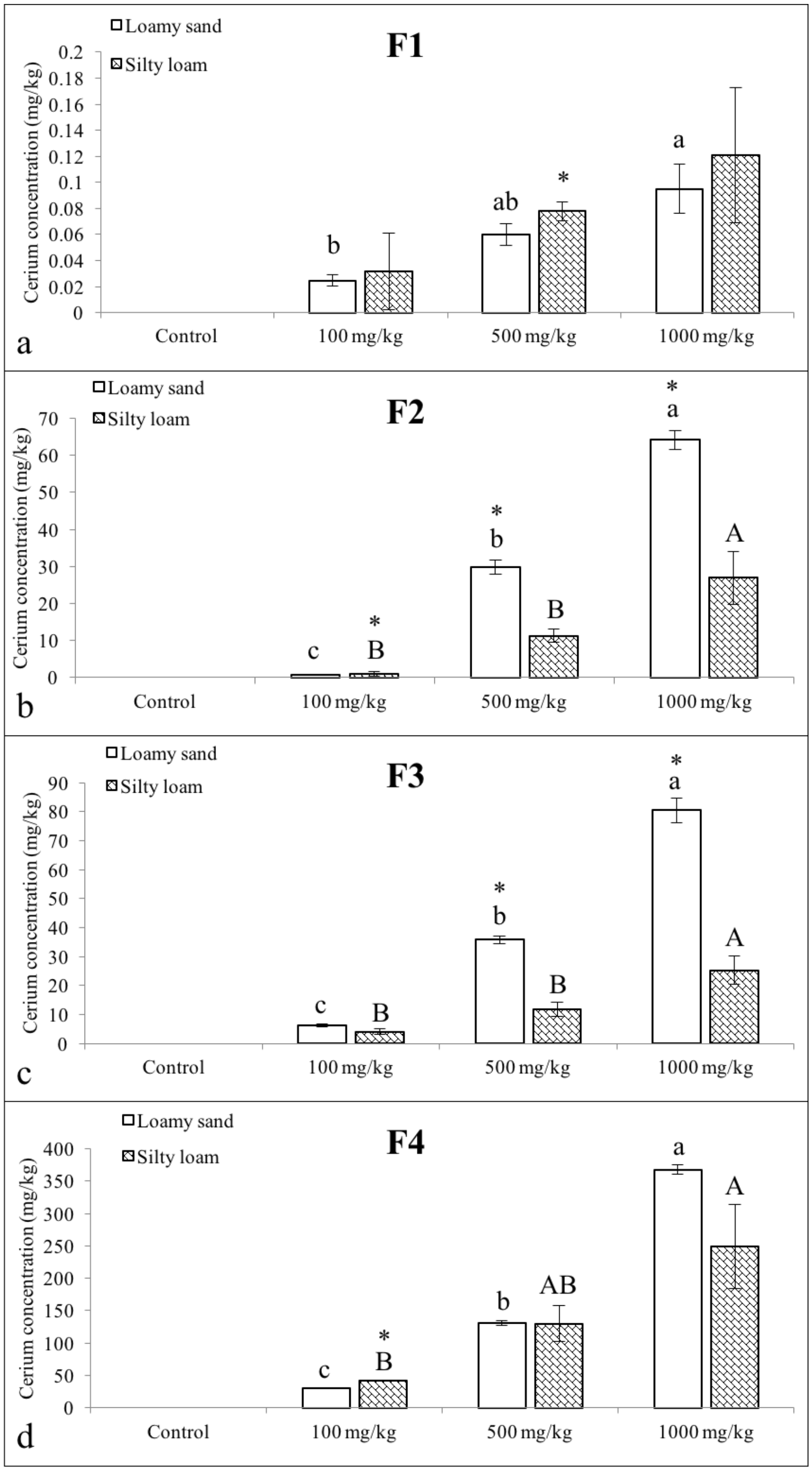
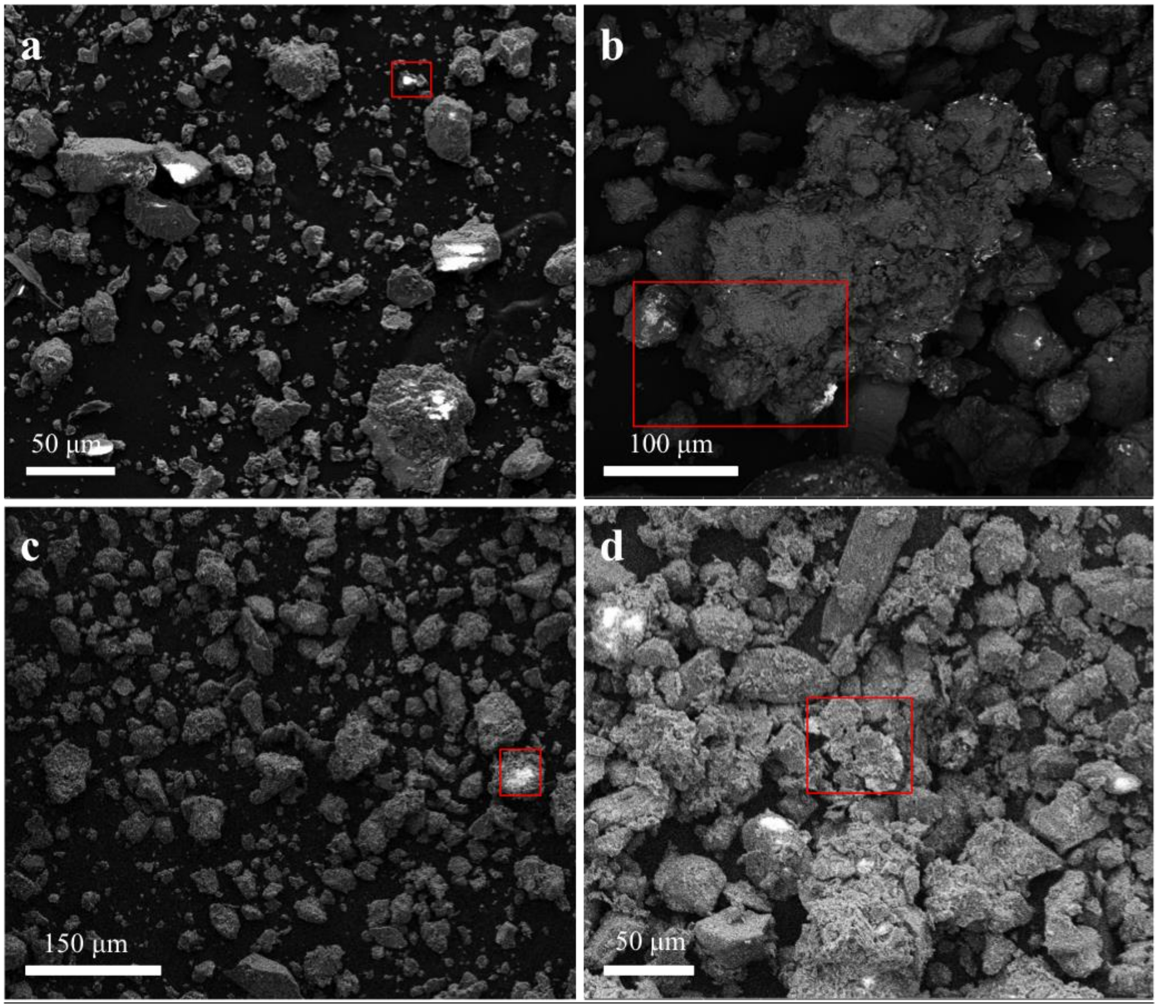


Figure 4: Adjusted cerium concentrations in different soil fractions. The error bars represent standard error (n=3). Different letters in lower case and upper case represent significant differences between the treatments in loamy sand and silty loam respectively ($p<0.05$). Asterisks indicate significant differences between two soils at the same CeO_2 dosing concentration ($p<0.05$).



Element	Weight% in a	Weight% in b	Weight% in c	Weight% in d
O	61.56	56.49	62	55.32
Al	2.48	4.04	4.85	4.43
Si	34.85	28.65	30	30.03
K	N/A	0.73	3.15	0.72
Ca	N/A	0.76	N/A	N/A
Fe	1.11	2.1	N/A	1.45
Ce	N/A	7.23	N/A	8.05
Total	100	100	100	100

Figure 5: SEM images of soil samples of. (a): loamy sand control; (b): loamy sand 1000 mg/kg; (c): silty loam control; (d): silty loam 1000 mg/kg. Table below images shows the weight percentage of detected elements in selected area (red frames in images)

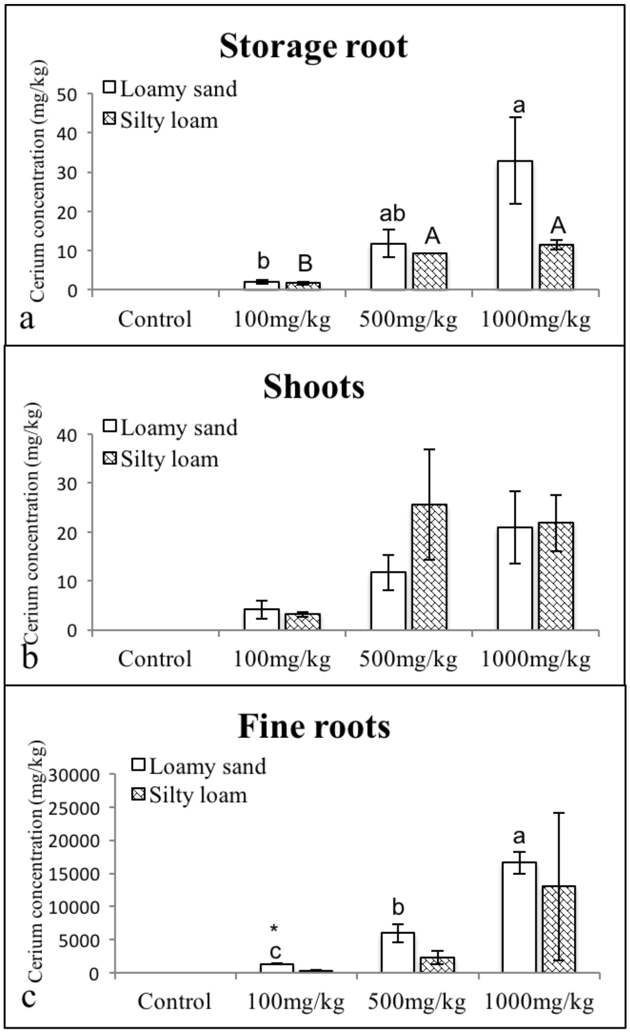


Figure 6: Modified cerium concentrations in different radish tissues after the background cerium concentrations in the control plants were subtracted from the corresponding tissues of treated plants. The error bars represent standard error (n=3). Samples without error bars indicate that the error bars are too small to see on the figures. Different letters in lower case and upper case represent significant differences between the treatments in loamy sand and silty loam respectively ($p<0.05$). Asterisks indicate significant differences between two kinds of soil at same CeO_2 NPs dosing concentration ($p<0.05$).

Table 1: The cerium concentration in different parts of radish, data represented the mean and standard error (n=3). Different letters represent significant differences between the treatments

Soil Type	Treatment	Peri (mg/kg)	L1 (mg/kg)	L2 (mg/kg)	S1 (mg/kg)	S2 (mg/kg)
Loamy sand	Control	11.4±3.06	7.45±1.38	11.09±1.83	18.83±1.67	8.85±0.42^{ab}
	500 mg/kg	112.9±52.35	10.88±1.61	9.4±1.67	23.12±0.49	9.81±1.57^{ab}
Silty loam	Control	8.91±0.76	10.43±2.09	8.07±2.97	22.9±4.23	7.00±0.52^{b}
	500 mg/ kg	127.06±56.25	11.49±1.18	8.61±0.32	18.26±3.14	20.58±7.29^{a}