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Phytotoxicity, uptake and transformation of nano-CeO_2 in sand cultured romaine lettuce☆

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ABSTRACT

Toxicity and uptake of nano-CeO_2 ($nCeO_2$) in edible vegetables are not yet fully understood. In the present study, we grew romaine lettuce in sand amended with $nCeO_2$. At high concentrations (1000 and 2000 mg/kg), $nCeO_2$ diminished the chlorophyll content by 16.5% and 25.8%, respectively, and significantly inhibited the biomass production. $nCeO_2$ (≥100 mg/kg) altered antioxidant enzymatic activities and malondialdehyde levels in the plants. $nCeO_2$ (≥500 mg/kg) triggered a remarkable increase of nitrate-N level in the shoots, which can be converted to toxic nitrite in humans thereby posed risk to human health. Concentration dependent accumulation of Ce in the plant tissues was observed. X ray absorption near edge spectroscopy (XANES) results indicate that Ce presented as $nCeO_2$ and $CePO_4$ in the roots while as $nCeO_2$ and Ce carboxylates in the shoots. Chelation of Ce^{3+} by citric acid or precipitation of Ce^{3+} by PO_4^{3-} reduced the translocation and toxicity of $nCeO_2$, indicating that release of Ce^{3+} played a critical role in the toxicity $nCeO_2$.

1. Introduction

Wide production and application of engineered nanomaterials (ENMs) inevitably lead to their entering and accumulation in the environment (Nel et al., 2006; Oberdörster et al., 2005). Intentional use of ENMs in agriculture (e.g., application of ENMs-bearing biosolids, plant growth enhancer, nanopesticide, and nanosensor for detection of pesticide, herbicide and plant pathogen) especially impose the potential threat to environmental organisms and human health *via* the food chains (Khot et al., 2012). Therefore, extensive studies on the interaction of ENMs and higher plants is essential for fully understanding the environmental impact of ENMs. $nCeO_2$ is among the most promising ENMs with a wide application as fuel additives (Cassee et al., 2011), polishing agents (Stanek et al., 2008), UV protection additives in cosmetics (Kang et al., 2011), and potential antioxidant agent *etc* (Li et al., 2015). A number of studies concerning the environmental impact of $nCeO_2$ have been reported (Cassee et al., 2011; Ma et al., 2015b; Peng et al., 2014; Zhang et al., 2011a, 2012), given its increasing production with an estimated global production of 100–1000 tons per year (Piccinno et al., 2012). In recent few years, interaction of $nCeO_2$ with plants has also aroused increasing concerns (Aslani et al., 2014; Deng et al., 2014; Gardea-Torresdey et al., 2014; Zhang et al., 2015a).

There have been many reports regarding to the impact of $nCeO_2$ on higher plants. $nCeO_2$ may exhibit low toxicity or non-significant effects to higher plants (López-Moreno et al., 2010a, 2010b; Ma et al., 2015b). On the other hand, reports also indicate that $nCeO_2$ may impair the plant growth, e.g., inhibiting seed germination and root development (López-Moreno et al., 2010b; Zhang et al., 2015b), reducing the chlorophyll content (Du et al., 2015), modify the antioxidant system (Ma et al., 2013; Rico et al., 2013a, 2013b), and altering the nutritional quality (Peralta-Videa and Gardea-Torresdey, 2015; Zhao et al., 2014). A recent study shows that $nCeO_2$ at high concentrations can shut down the nitrogen fixation system in soybean, which poses risks to the agriculture of leguminous crops (Priester et al., 2012). In addition to the direct impact on plant growth, accumulation of $nCeO_2$ in plant tissues including

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plant root, leaf, flower and fruit has been evidenced by a number of researches (Wang et al., 2012; Zhang et al., 2011b), which impose risks to environmental organisms and human health *via* food chains (Bradfield et al., 2016; Ebbs et al., 2016b; Hawthorne et al., 2014; Majumdar et al., 2015). Hawthorne et al. (2014) treated zucchini in soil with 1228 μg/g $nCeO_2$ and used zucchini leaves to feed crickets. They found that $nCeO_2$ was transferred to crickets with a content of 33.6 ng/g and can be transferred to wild spiders in a higher trophic level which fed with crickets. Recently, Bradfield et al. evaluated the projected dietary intake of Ce from consumption of carrot and sweet potato by performing a dietary intake modeling (Bradfield et al., 2016; Ebbs et al., 2016a). Results indicated that the dietary intake of Ce for young children from sweet potato which was treated with 1000 mg/kg $nCeO_2$ was higher than the median oral references dose (5 μg kg^{-1} d^{-1}) of rare earth elements established by US EPA (2012). The projected dietary intake in carrot was higher than sweet potato, where the value from carrot treated with 500 mg/kg $nCeO_2$ can reach up to 200 μg kg^{-1} d^{-1}. The author also suggested that peeling significantly reduced the intake of Ce. Although the evaluation was based on a high exposure concentration of $nCeO_2$, the possible accumulation in human body *via* food chain and the potential risk to human health shouldn't be ignored.

Lettuce is among the most popular vegetables that is consumed almost over the world. We previously found that $nCeO_2$ aqueous suspension at 2000 mg/L showed species-specific toxicity to the root growth of three different types of lettuce plants in germination stages (Zhang et al., 2015b). Lettuce plants were found highly sensitive to the released Ce^{3+} from $nCeO_2$, which resulted in the toxicity. In our another study, lettuce was found more sensitive to $nCeO_2$ and Ce^{3+} ions in agar medium than in water medium; 500 mg/L $nCeO_2$ and 0.005 mg/L Ce^{3+} ions can remarkably inhibit the root growth (Cui et al., 2014). A recent study showed that 250 mg/L $nCeO_2$ (~25 nm) aqueous suspension inhibited the root growth of lettuce (Andersen et al., 2016). In a potting mix media which is close to the realistic soil, Gui et al. (2015) found that $nCeO_2$ at moderate concentration (100 mg/kg) can promote the growth of head lettuce while showed inhibitory effect at high concentration (1000 mg/kg) after 30 days treatment. A recent report shows that $nCeO_2$ exhibited no significant impact on biomass production in residential and agricultural soil and did not differ as a function of biochar supplement (Servin et al., 2016). However, the uptake of Ce in lettuce was affected by the supplement of biochar. These studies indicate that culture medium is an important factor affecting the phytotoxicity and uptake of $nCeO_2$.

Since lettuce is almost indispensable in many countries, different culture media has been applied in lettuce cultivation including sand and hydroponic solution (Saunby, 1953). This study for the first time investigated the interaction of $nCeO_2$ with lettuce in sand media. Specifically, effect of $nCeO_2$ on the biomass production, chlorophyll content, sugar and nitrate content, and antioxidant enzymatic activities were studied. Transformation of $nCeO_2$ in lettuce was investigated and its correlation with the plant effect of $nCeO_2$ was studied to explore the mechanism involved in the toxicity of $nCeO_2$. Accumulation of $nCeO_2$ in lettuce was also determined and the potential risk to human health was discussed.

2. Materials and methods

2.1. Chemicals and seeds

Seeds of romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam) were purchased from Chinese Academy of Agricultural Sciences, and kept at 4 °C for further use. Average germination rates were examined to be greater than 85%. $nCeO_2$ were purchased from Sigma Aldrich (USA). All the other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd (China).

2.2. Characterization of $nCeO_2$

Transmission electron microscopy (TEM, JEM-2010, Japan) was used to characterize the morphology and particle size of $nCeO_2$. X-ray diffraction (XRD, X'pert PRO MPD, Holland) was used to determine the crystal phase. Hydrodynamic size and Zeta potential in deionized water and nutrient solution were measured on a Tecan Infinite® 200 PRO dynamic light scattering system (Switzerland). Synchrotron radiation XANES technique was applied to determine the chemical speciation of $nCeO_2$.

2.3. Plant culture and nanoparticles application

Silica sands of 40 mesh (Sinopharm Chemical Reagent, China) were used for plant culture. $nCeO_2$ powders were mixed with 70 g sands and homogenized to obtain a final concentration of 10, 100, 500, 1000, and 2000 mg/kg, and poured into a glass beaker. 2000 mg/kg was set as the highest concentration according to US EPA guideline that ENMs could be considered as minimal toxicity on test plants if no toxicity can be observed under such a high concentration (US EPA, 1996). Five replicates were set for each concentration. Each beaker was supplemented with 25 mL deionized water. Seeds of romaine lettuce were sterilized with 10% NaClO solution and seeded in the sand with 5 seeds for each beaker. All the seeds were allowed for vernalization at 4 °C for 24 h and germinated in darkness at 20 °C for 2 days. Seedlings were then allowed to grow in the climate chamber with 16-h photoperiod, 27 °C/18 °C day/light temperature and 50%/70% day/night humidity for three weeks. Germination rates were monitored at the first 5 days, and three seedlings with uniform size were retained for further culture. Hoagland nutrient solution (1/4 strength, 2 mL) was added into each beaker every other day, and deionized water was supplemented every day to maintain the sand humidity. Relative chlorophyll contents of lettuce leaves were monitored using a hand-held SPAD chlorophyll meter (Spectrum Technologies, Inc. USA). Two healthy leaves were chosen for each plant for the measurement.

2.4. Biomass and Ce concentration determination

After cultivation for three weeks, plants were harvested and rinsed with flowing tap water and deionized water thoroughly. Two of the three plants from each plot were used for determination of the biomasses. Shoots and roots were separated, blotted with filtrate paper thoroughly, and the fresh weight were determined. Samples were then lyophilized and the dry weights were measured. To determine the Ce contents, the dry samples were ground to fine powders and digested with a HNO_3/H_2O_2 mixture on a heating plate (80 °C for 1 h, 120 °C for 3 h, and 160 °C for 0.5 h). The residues were diluted with deionized water, and the Ce, Fe, Cu, Zn, Mn concentrations were determined by an inductively coupled plasma mass spectroscopy (ICP-MS, Thermo X7, USA). Bush branches and leaves were used as standard references (GBW07602). Indium solution (20 mg/mL) was applied as an internal standard to compensate for the matrix suppression and signal drifting. Spike recovery, standard deviation and detection limit were calculated.

2.5. Stress response of romaine lettuce to $nCeO_2$

One of the three plants in each pot was collected and the fresh samples were stored in −80 °C for analyses. Samples were excised and homogenized with cold PBS buffer (50 mM, pH 7.8), and