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Particle-specific toxicity and bioavailability of cerium oxide (CeO_2) nanoparticles to *Arabidopsis thaliana*

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HIGHLIGHTS

- The phytotoxicity and uptake of CeO_2 NPs were examined in *Arabidopsis*.
- CeO_2 NPs stimulated plant growth at low doses but were toxic at high doses.
- The toxicity was due to the NPs per se, rather than from the dissolved Ce ions.
- A similar up-translocation factor was found for CeO_2 NPs, bulk CeO_2 and Ce ions.

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ABSTRACT

The use of manufactured cerium oxide nanoparticles (CeO_2-NPs) in consumer products has increased markedly over the past decade, and their release into natural ecosystems is unavoidable. This study investigated the phytotoxicity and uptake of CeO_2-NPs in *Arabidopsis thaliana* grown in an agar medium. Although low concentrations of CeO_2-NPs had stimulatory effects on plant growth, at higher concentrations, CeO_2-NPs reduced growth and had adverse effects on the antioxidant systems and photosystem. Importantly, the toxicity resulted from the nanoparticles per se, rather than from the dissolved Ce ions. CeO_2-NPs were taken up and subsequently translocated to shoot tissues, and transmission electron microscopy (TEM) showed the presence of a large number of needle-like particle aggregations in the intercellular regions and the cytoplasm of leaf cells. The up-translocation factor to shoots was independent of the concentrations of Ce in the roots and the supplied forms of Ce (i.e. CeO_2-NPs, CeO_2-bulk, and ionic Ce), suggesting that endocytosis is likely to be a general mechanism responsible for the translocation of these Ce compounds. These findings provide important information regarding the toxicity and uptake of CeO_2-NPs in plants, which needs to be considered in environmental risk assessment for the safe use and disposal of CeO_2-NPs.

1. Introduction

Over the past decade, the field of nanotechnology has increased markedly due to rapid innovation and commercialization [1]. As a result, engineered nanoparticles (NPs) are used extensively in a wide range of industrial and commercial applications. Cerium dioxide (CeO_2) NPs are one of the most important engineered NPs. They have special electrical, optical, and thermal properties and thereby are being widely used as a polishing material, additive in glass and ceramic, fuel cell materials, agricultural products, and automotive industry [2]. In the U.S. and Europe, for example, CeO_2-NPs are used to catalyse diesel fuel combustion to reduce NOx emissions [3,4]. Consequently, the release of CeO_2-NPs into the managed and natural ecosystems is unavoidable [5]. It is, therefore, important to assess the ecological risk of CeO_2-NPs.

Plants are an important component of the ecological system, serving both as ecological receptors and as a potential pathway for the transportation of NPs [6,7]. Through the food chain, NPs may potentially accumulate in higher trophic level organisms [8]. Previous studies have showed that CeO_2-NPs can induce compositional modifications in the root xylem of cereals [9] and reduce rice grain quality through decreasing nutrient content (e.g. Fe, S, fatty acids, and starch) [10]. In addition, CeO_2-NPs can be taken up

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by plant roots and subsequently translocated to the above-ground tissues [11–14]. However, few studies have included appropriate controls (for example, a comparison of CeO_2-NPs with bulk CeO_2, or with ionic Ce). Additionally, given that CeO_2-NPs tend to aggregate and precipitate in aqueous solutions due to their size and surface properties, agar-solidified media have been introduced in the phytotoxicity and uptake studies of NPs in order to create homogeneous exposure and prevent aggregation [15].

Cerium has been used as a rare earth fertilizer to improve plant growth and crop production [16] although studies have also shown that it is highly toxic at low concentrations with no beneficial effect [17]. It has been proposed that CeO_2-NPs could be used as a crop fertilizer [18]. Dimkpa [18] reported that CeO_2-NPs stimulated root growth in soybean (*Glycine max*) and cilantro (*Coriandrum sativum*) and induced the activity of antioxidative enzymes, helping to prevent membrane peroxidation and leakage in the plants. However, it remains unclear if CeO_2-NPs exert nano-specific effects on plant growth compared to their counterparts – CeO_2 bulk particles (hereafter termed CeO_2-bulk) or ionic Ce.

In the present study, an agar-solidified medium was used to investigate (i) the phytotoxicity and uptake of CeO_2-NPs in *Arabidopsis thaliana* to discern any nano-specific effects, and (ii) antioxidant systems in response to abiotic stress caused by CeO_2-NPs. To elucidate the potential toxic effects of CeO_2-NPs, we compared their effects to those of CeO_2-bulk, ionic Ce, and a Control (no Ce). Here, we used an ultra-centrifugal unit to separate Ce released from the agar media to disentangle the effects of ionic Ce and CeO_2.

2. Materials and methods

2.1. The characterization of CeO_2 nanoparticles and bulk particles

Both CeO_2-NPs (99.99%, 15–30 nm) and CeO_2-bulk (99.9%, 2–5 μm) used in the present study were purchased from Beijing Dk Nano technology Co., LTD (China). A D/max-rB X-ray diffractometer (XRD) (Rigaku, Japan) was used to determine the crystal forms and confirm the composition of CeO_2-NPs and CeO_2-bulk particles in the range of 3–80° in 2θ with 10 s per step. In addition, a laser particle size analyzer (Beckman, LS13320, USA) was used to determine the hydrodynamic size of particles suspended in deionized water.

Both CeO_2-NPs and CeO_2-bulk were suspended directly in Millipore deionized (DI) water and twice dispersed by an ultrasonic cleaner (KQ-700DE, Kunshan ultrasonic instruments Co., Ltd., China) at 40 kHz (100 W) for 30 min to produce the suspensions of CeO_2-NPs and CeO_2-bulk, both at 3000 mg/L. A few drops of the suspensions were deposited on the copper-coated grids and allowed to dry overnight before examination using a transmission electron microscopy (TEM) (Hitachi H7650, Tokyo, Japan).

2.2. Release of Ce ions from CeO_2-NPs and CeO_2-bulk

The extent to which Ce ions were released from the CeO_2 particles during their preparation (for example, during sonication) was measured. A range of CeO_2-NP or CeO_2-bulk suspensions (100, 200, 500, 1000, 2000, and 3000 mg/L) were sonicated for 30 min (100 W, 40 kHz) twice before being allowed to settle overnight. Dissolved Ce ions were separated from the solid-phase particles by centrifugation for 30 min at 150g, followed by filtration using a Millipore Ultra centrifugal unit (UFC 900396, Millipore, Billerica, USA) with pore diameters of 3–5 nm. The supernatants were subsequently acidified by trace metal grade nitric acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and stored at 4 °C prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer, NexION 300X, USA). Indium (20 μg/L) was added as an internal standard to compensate for matrix suppression and signal drifting. Quality control measures included the use of procedural blanks and repeated analysis of a certified reference.

2.3. Plant culture and treatment

Seeds of *A. thaliana* (ecotype Columbia-0) were surface sterilized by soaking in 8% (v/v) of NaClO for 10 min. Seeds were washed five times with DI water and germinated under sterile conditions in 100 × 10 mm Petri dishes closed with vented lids and filled with 35 ml of semisolid nutrient medium. The semisolid medium is capable of optimizing suspension and preventing aggregation [15] and simulates a soil-like environment.

The medium consisted of autoclaved one-half strength Murashige and Skoog (MS) nutrient solution supplemented with 20 g/L sucrose and 10 g/L agar (pH 5.7). To these media were added appropriate volumes of stock solutions of CeO_2-NPs or CeO_2-bulk to yield one of six nominal concentrations: 0, 100, 200, 500, 1000, and 3000 mg/L. Stock solutions were sonicated at 40 kHz for 30 min before addition. In order to discern the contribution of Ce ions and CeO_2-NPs to the overall toxicity, the dissolved Ce ions were separated from the stock solution of CeO_2-NPs at the volumes which had yielded the nominal concentrations of 1000 mg/L or 3000 mg/L CeO_2-NPs in the one-half MS media (described above). The appropriate amount of supernatants was then added to the MS media to achieve the same concentrations of Ce ions as those in the corresponding CeO_2-NP treatments.

Eighteen sterilized seeds were placed on each Petri dish closed with vented lids and sealed with parafilm, and stratified at 4 °C for 24 h prior to transfer to a controlled environment cabinet (Percival Scientific, Perry, IA) with an illumination flux density of 120 μmol m^{-2} s^{-1}. Temperature was maintained at 22 °C during the day and 18 °C during the night, with 16/8 h light/dark periods. Petri dishes were incubated vertically after germination to facilitate shoot and root growth for a further 25 d. All procedures were conducted under a Steriguard laminar hood to prevent microbial contamination.

2.4. Biomass and Ce content determination

Seedlings were harvested after 25 d and thoroughly rinsed with flowing tap water, DI water, 0.5% HCl, and again with DI water to eliminate any surface contaminants [19]. The seedlings were blotted dry with filter paper before the roots and shoots were separated, and fresh biomass and root length recorded. The tissues were oven-dried, digested with 5 ml of concentrated HNO_3 in a microwave acceleration reaction system (CEM, Mars, USA) prior to analysis for total Ce using ICP-MS.

2.5. Transmission electron microscopy

Samples for TEM were prepared following the procedures outlined by Yin et al. [20]. Briefly, after a two-week treatment, leaves of *A. thaliana* grown in 3000 mg/L CeO_2-NPs or CeO_2-bulk were washed with DI water thoroughly, and the segments were fixed in 2.5% glutaraldehyde. The tissues were rinsed with 0.1 M phosphate buffer (pH 7.2) for three times before being post-fixed with 1% osmium tetroxide, and dehydrated in a graded ethanol series (30, 50, 70, 95, and 100% v/v) with 10 min incubation in each step. LR White Resin was used to infiltrate the tissues with a graded resin: 25, 50, 75, 100, and 100% v/v in ethanol, with 1 h incubation each solution. The tissues were then embedded in 100% LR White Resin in gelatin capsules and polymerized at 60 °C for 40 h. Ultrathin sections (50–90 nm) were cut and post-stained with 2% (v/v)

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