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Exposure of cerium oxide nanoparticles to kidney bean shows disturbance in the plant defense mechanisms

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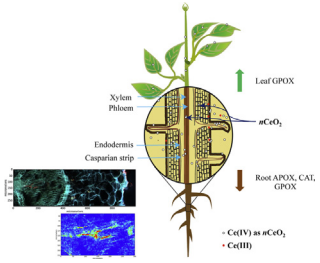
HIGHLIGHTS

- Kidney bean roots uptake nCeO$_2$ primarily without biotransformation.
- Cerium reached the root vascular tissues through gaps in the Casparian strip.
- On longer exposure to high concentration, roots demonstrate stress response.
- In leaves, guaiacol peroxidase plays a major role in ROS scavenging.

GRAPHICAL ABSTRACT



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ABSTRACT

Overwhelming use of engineered nanoparticles demands rapid assessment of their environmental impacts. The transport of cerium oxide nanoparticles (nCeO$_2$) in plants and their impact on cellular homeostasis as a function of exposure duration is not well understood. In this study, kidney bean plants were exposed to suspensions of ~8 ± 1 nm nCeO$_2$ (62.5 to 500 mg/L) for 15 days in hydroponic conditions. Plant parts were analyzed for cerium accumulation after one, seven, and 15 days of nCeO$_2$ exposure. The primary indicators of stress like lipid peroxidation, antioxidant enzyme activities, total soluble protein and chlorophyll contents were studied. Cerium in tissues was localized using scanning electron microscopy and synchrotron μ-XRF mapping, and the chemical forms were identified using μ-XANES. In the root epidermis, cerium was primarily shown to exist as nCeO$_2$, although a small fraction (12%) was biotransformed to Ce(III) compound. Cerium was found to reach the root vascular tissues and translocate to aerial parts with time. Upon prolonged exposure to 500 mg nCeO$_2$/L, the root antioxidant enzyme activities were significantly reduced, simultaneously increasing the root soluble protein by 204%. In addition, leaf's guaiacol peroxidase activity was enhanced with nCeO$_2$ exposure in order to maintain cellular homeostasis.

1. Introduction

Over the last decade, globalization of nanomaterial research initiatives have progressed nanotechnology towards a general-purpose technology [1]. The unregulated usage and disposal of

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engineered nanoparticles (ENPs) has resulted in a dilemma among environmentalists, industrialists, and regulatory agencies on the possible sinks and associated environmental risks. Plants are at maximum risk due to the concentration build-up of ENPs in natural sediments, agricultural soils, and aquatic environments [2–4]. Cerium oxide nanoparticles (nCeO$_2$) are one of the most produced metal oxide nanoparticles worldwide, accounting for around 10,000 metric tons/year [5]. They are used as catalysts for augmenting fossil fuel oxidation, scratch-resistant glass polishing, petrochemical processing, and UV radiation protectants [6,7]. Studies have confirmed that the reduction of Ce(IV) in nCeO$_2$ and nCe$_{1-x}$Zr$_x$O$_2$ is easier than in bulk ceria [8]. Thus, nanoscale cerium compounds are expected to behave differently in environmental matrices and biological systems. Dissolution of ENPs and redox conditions at the nano–bio interface are critical parameters to determine their toxicity [9–11].

Previous reports suggest that nCeO$_2$ have positive effects in terms of growth and yield at low concentrations (10-125 ppm) [12,13]; whereas, at higher concentrations, adversely affect the plant physiology [14], metabolism [15], and yield [15,16]. The available literature on the toxicity of nCeO$_2$ in edible plants is contradictory. For instance, in tomato seeds (*Solanum lycopersicum*) exposed to nCeO$_2$ at 2000 mg/L, Ma et al. [17] reported no toxicity symptoms, whereas in another study, López-Moreno et al. [14] reported decrease in germination rate and root length. In soybean (*Glycine max*), at similar exposure concentration, the plants were shown to experience genotoxicity [18], and at 1000 mg/kg soil the nitrogen assimilation, growth, and yield was negatively affected [15]. Thus, this area is still in its infancy and needs more standardized studies to conclude their effects depending on the exposure medium. Hydroponic studies are preferred when precise information on the interaction of the ENPs with the plant is being explored. This is to avoid multitude of variables that come into play when the ENPs interact with a complex exposure medium like soil [3]. The impact of nCeO$_2$ on plant physiology and their defense mechanisms, as a function of time, has not been well explored. Also, there are very few studies on the protein rich edible crops which deserve special attention due to their contribution to our daily nutrition.

To the best of authors' knowledge, there are no studies on the interaction of nCeO$_2$ with kidney beans (*Phaseolus vulgaris*). Kidney bean is a very important leguminous crop and a major global source of proteins and micronutrients, especially zinc and iron [19]. In this study, kidney bean plants were exposed to nCeO$_2$ suspensions for 15 days to follow their uptake and transport to aerial tissues. To determine the probable route of nCeO$_2$ uptake through roots, scanning electron microscopy (SEM) and elemental mapping using synchrotron micro-x-ray fluorescence (μ-XRF) were performed followed by speciation studies using micro-X-ray absorption near-edge structure spectroscopy (μ-XANES). To shed light on the biochemical responses in the plant tissues, activities of major antioxidant enzymes, lipid peroxidation, and contents of chlorophylls and total soluble protein were determined.

2. Materials and methods

2.1. Preparation of nanoparticle suspensions

nCeO$_2$ (≈8 nm, Meliorum Technologies, Rochester, NY) were obtained from The University of California Center for Environmental Implications of Nanotechnology (UC-CEIN) [20]. A detailed characterization of nCeO$_2$ used, has been previously published by Keller et al. [21] and Lopez-Moreno et al. [18]. As previously reported, the nCeO$_2$ are rods, measuring $(67 \pm 8) \times (8 \pm 1)$, (≤10% polyhedra: 8 ± 1 nm) with 95.14% purity and surface area of 93.8 m^2 g^{-1} [21].

Suspensions of nCeO$_2$ at concentrations of 62.5, 125, 250 and 500 mg/L were prepared in modified Hoagland nutrient solution (NS) (pH 5.8) upon 30 min bath sonication (Crest Ultrasonics, Trenton, NJ) at 25 °C. Bulk CeO$_2$ (bCeO$_2$, Sigma–Aldrich) suspensions (125, 500 mg/L) were also prepared similarly. The suspensions were immediately characterized after sonication for pH, size, and zeta-potential (ζ- potential). The size and ζ- potential of the particles in nCeO$_2$ and bCeO$_2$ suspensions were measured by dynamic light scattering (DLS) method using NanoSizer 90 (Malvern Instruments, Worcestershire, UK) at 25 °C. TEM image of the well-dispersed suspension was carried out using a 120 CX Transmission electron microscope (JEOL USA, Inc., MA, USA) at 80 kV.

2.2. Nanoparticle exposure

Phaseolus vulgaris var. red hawk kidney seeds were provided by Dr. James Kelly, Michigan State University. The seedlings grown in sterilized condition were transplanted to NS in plastic containers and placed in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with 14 h photoperiod (340 μmole m^{-2} s^{-1}), 25/20 °C day/night temperature and 65% relative humidity (details in Supplementary data, SD). After seven days, the plant roots were exposed to nCeO$_2$ and bCeO$_2$ suspensions for 15 days. Pure NS served as the control. Quadruplicate sets of four plants per jar per treatment, aerated with aquarium pumps, were installed on magnetic stirrers to reduce the settling of nCeO$_2$ aggregates (Figure S1 SD). Preliminary experiments performed without continuous stirring showed settling of the aggregates within the first few hours. To determine dissolution of nCeO$_2$, Ce ions were quantified on the 1st, 7th and 15th day by centrifuging 50 ml of suspensions for 30 min at 4500 rcf (Eppendorf 5804R, Hamburg). The water loss was replenished everyday using Millipore water (MPW). The experiment was run in three sets that were harvested after one, seven, and 15 days of exposure (DE). Plants exposed to bCeO$_2$ were harvested after 15 days. During sampling, the roots were washed three times with 0.01 M HNO$_3$ and MPW to remove adhered particles prior to all the analyses [14,22].

2.3. Quantification of cerium in tissues

After one, seven, and 15 DE, plants were washed and severed into roots (including hypocotyl), stems, leaves (first true trifoliate with petiole), and other aerial tissues, and dried at 70 °C for 96 h. Ce concentration in the tissues was determined as described by Packer *et al.* [23] using inductively coupled plasma-optical emission spectroscopy (ICP-OES) [14]. Analytical details are provided in SD.

2.4. SEM analysis

After 15 DE to 500 mg nCeO$_2$/L, roots were frozen in liquid nitrogen, freeze dried for 24 h, sectioned, and then mounted on aluminum stubs. The samples were viewed at an accelerating potential of 20 kV under high vacuum mode with backscatter detector using a JSM-5400LV SEM (JEOL, Tokyo, Japan) equipped with IXRF-EDS system with a Moxtek AP3.3 light element entrance window.

2.5. Synchrotron μ-XRF and μ-XANES analysis

After 15 DE to 500 mg nCeO$_2$/L, a portion of the root was excised from the absorption zone, snap-frozen in liquid nitrogen and embedded in Tissue Tek resin (Sakura Finetek USA, Inc., Torrance, CA). The embedded samples were sectioned at 30 μm, mounted on ultralene window film, lyophilized at −53 °C and 0.140 mbar for 1 h (Labcono FreeZone 4.5, Kansas City, MO) and stored in desiccant until analysis at beamline ID21 at the European