

Toxicity Assessment of Cerium Oxide Nanoparticles in Cilantro (Coriandrum sativum L.) Plants Grown in Organic Soil

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Supporting Information

ABSTRACT: Studies have shown that CeO₂ nanoparticles (NPs) can be accumulated in plants without modification, which could pose a threat for human health. In this research, cilantro (Coriandrum sativum L.) plants were germinated and grown for 30 days in soil amended with 0 to 500 mg kg⁻¹ CeO₂ NPs and analyzed by spectroscopic techniques and biochemical assays. At 125 mg kg⁻¹, plants produced longer roots ($p \le 0.05$), and at 500 mg kg⁻¹, there was higher Ce accumulation in tissues ($p \le 0.05$). At 125 mg, catalase activity significantly increased in shoots and ascorbate peroxidase in roots ($p \le 0.05$). The FTIR analyses revealed that at 125 mg kg⁻¹ the CeO₂ NPs changed the chemical environment of carbohydrates in cilantro shoots, for which changes in the area of the stretching frequencies were observed. This suggests that the CeO2 NPs could change the nutritional properties of cilantro.

KEYWORDS: coriander, cerium oxide nanoparticles, antioxidant enzymes' activities

INTRODUCTION

Nanotechnology encompasses the fabrication and utilization of materials having at least one dimension less than 100 nm. Nanomaterials (NMs) with at least two dimensions between 1 and 100 nm are known as nanoparticles (NPs). These materials possess distinctive characteristics provided by their high surface area to volume ratio, surface charge, and size. These special characteristics allow their utilization in a variety of consumer products such as medical, food and food packaging, and agricultural products. Some of the applications of NPs in food, and related areas, include biosensors,² plant growth regulators,³ food additives,⁴ genetic improvement of plants and animals,^{5,6} delivery systems for fertilizers,^{7,8} and nanopesticides.^{8,9} In the manufacturing industry, the CeO₂ NPs are among the 13 most used NMs. 10 These NMs are used in the fabrication of products that can easily be in contact with humans, such as polished glass mirrors and ophthalmic lenses, 11,12 fuel additives, solid oxide fuel cells, and catalysts. 13 However, after the end-user applications, these products and residues will be in the landfills and sewage sludge interacting with soil, therefore becoming a risk for plants, humans, and other organisms. 14-16

Previous reports have shown that plants can accumulate CeO₂ NPs. Through the use of X-ray absorption spectroscopy

and microscopy studies, our research group was able to determine the presence of untransformed CeO2 NPs in soybean seedlings,¹⁷ and confocal microscopy was used to image CeO₂ aggregates in corn (Zea mays L.) root seedlings. Wang et al. 19 reported that the CeO₂ NPs can be taken up by the roots and translocated to the shoots in tomato (Solanum lycopersicum L.). Recently, Zhang et al.²⁰ reported that most of the CeO₂ NPs absorbed by cucumber (Cucumis sativus L.) plants grown in hydroponics remained as NPs, and a small percentage was biotransformed to CePO₄, in roots, and to cerium carboxylates in shoots. More recently, Hernandez-Viezcas et al.21 reported, by using synchrotron X-ray techniques, that most of the CeO2 NPs absorbed by soybean from CeO2 NP-treated soil were stored as NPs in the reproductive/edible parts.

The above narrative suggests that CeO2 NPs could be introduced in the food chain through plants eaten fresh, such as tomato and cucumber, ^{19–22} or through dry grains. ²¹ Culinary plants, such as cilantro (Coriandrum sativum L.), could also

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represent a way to introduce CeO₂ NPs in the food chain. Cilantro is a very important culinary and medicinal plant consumed worldwide.²³ It is eaten either as a fresh herb or as a spice.^{24,25} Cilantro has been used for a long time around the world because of its curative abilities.²⁶ It is believed that these qualities in herbs such as cilantro, result from their antioxidant properties.²⁷

While growing and developing, plants have to deal with biotic and abiotic factors that can alter their physiology or life cycle. Plants have developed special mechanisms to deal with negative factors.²⁸ Enzymes such as catalase (CAT) and ascorbate peroxidase (APX) are among the most important antioxidant enzymes that help plants deal with oxidative stress or reactive oxygen species (ROS) production.²⁹ These enzymes scavenge ROS molecules that affect important functions required for healthy growth. In a previous report, Zhao et al.³⁰ have shown that CeO₂ NPs increase CAT and APX activity in corn plants. Another report indicates that ZnO NPs increase CAT and APX activity in some organs of velvet mesquite (*Prosopis juliflora* (Sw.) DC.).³¹ However, there are no studies concerning the stress response to CeO₂ NPs in garden vegetables.

In this study, cilantro plants were germinated and grown to maturity in organic soil treated with CeO₂ NPs at concentrations varying from 0 to 500 mg kg⁻¹. Thirty days post-treatment, plants were sampled and analyzed using spectroscopic and biochemical assays to determine the Ce uptake, CAT and APX activity, and changes in macromolecules. The collected data demonstrate that the CeO₂ NPs produce significant changes in cilantro plants.

MATERIALS AND METHODS

CeO₂ NP Suspensions and Soil Preparation. CeO₂ NPs (8 nm, Meliorum Technologies, Rochester, NY, USA) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). These NPs were previously characterized by Keller et al.³² The $n\text{CeO}_2$ are rods with a primary size of 8 ± 1 nm, surface area of 93.8 m² g⁻¹, and 95.14% purity.³² The size in suspension, zeta potential, and the concentration of Ce ions in suspensions were previously published.³³ Nanoparticle suspensions were prepared at 0, 62.5, 125, 250, and 500 mg kg⁻¹ in Millipore water (MPW). These concentrations were selected based on a screening experiment that shows no visible signs of toxicity at concentrations below 60 mg kg⁻¹. Before the application to the soil, the suspensions were stirred for 5 min and sonicated for 30 min to avoid aggregation. Miracle-Gro organic potting soil was used in this study. Two hundred grams of organic potting soil was mixed homogeneously with the CeO_2 NP suspension and placed in pots of 13.21 cm diameter \times 10.16 cm height. The soil was left for 24 h for conditioning. Three replicates were prepared for each treatment. Some components of the elemental analysis of the soil are shown in Table S1 (Supporting Information).

Seed Germination. Cilantro (*Coriandrum sativum*) seeds were purchased from Del Norte Seed & Feed (Vinton, TX, USA). The seeds were soaked in MPW and stirred for 3 h for hydration. Approximately 40 fruits (each one with 2 to 4 seeds inside) of the same size were selected and sown in each pot. The seeds were placed about 1 cm deep in the soil and watered with 50 mL of MPW every day. Pots were placed in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH, USA) with a 14 h photoperiod, 25/20 °C day/night temperature, 65% relative humidity, and light intensity of 340 µmol s⁻¹ m⁻². The germination began seven days after sowing, and the number of germinated seeds was recorded every five days for 15 days after germination started.

Plant Growth and Biomass Production. To determine plant elongation, three cilantro plants were removed from each replicate/concentration and rinsed three times with 95% CaCl₂ solution and DI

water to remove NPs adhered to the root surface. The shoots were measured from the crown to the top of the tallest leaf. The roots were measured from the crown to the main root apex. These measurements were recorded 30 days after germination. To determine the biomass production, all the plants from the pots of each replicate/treatment were removed from the soil, washed as previously described, severed into shoots and roots, and oven-dried at 70 °C for 72 h (Isotemp Oven, Fisher Scientific). Subsequently, the samples were weighed and the weight/plant was calculated.

Quantification of Ce in Dry Plant Tissues. For Ce determination in tissues, 30-day-old plant samples were microwaveassisted acid digested by using 2 mL of plasma pure HNO3 and 3 mL of H₂O₂ (30%) in a microwave-accelerated reaction system (CEM Corporation Mathews, NC, USA),³⁴ and the volume was adjusted to 15 mL with MPW. For quality control/quality assurance of the digestion method, the reference material 1547 (NIST, USA) and 10 ${\rm mg}~{\rm L}^{-1}$ Ce spikes were treated as samples, obtaining recoveries of 89% and 97%, respectively. Cerium quantification in the acidic solutions was performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer Optima 4300 DV, Shelton, CT, USA) equipped with a Burgener PEEK MiraMist nebulizer with argon flow. The wavelength used for the Ce ICP-OES analysis was 413.764 nm. Every 10 samples a blank and spiked samples containing Ce at 5 and 1 mg L^{-1} were analyzed. The average readings of the spiked samples were 5 \pm 0.2 and 1 \pm 0.1 mg L⁻¹. The ICP-OES parameters used were as follows: nebulizer flow, 0.80 L min ⁻¹; power, 1450 W; peristaltic pump rate, 1.5 mL min⁻¹; flush time, 15 s; delay time, 20 s; read time, 15 s; wash time, 50 s; and every sample was read in triplicate.

CAT/APX Assays. Thirty-day-old fresh cilantro plants were washed with 5% CaCl₂ solution and three times with MPW to remove any external contaminant. Samples of 0.1 g of fresh roots and shoots (stems and leaves) were used to determine CAT (EC 1.11.1.6) and APX (EC1.11.1.11) activities according to Gallego et al.,³⁵ with minor modifications.³⁶ The extracts were prepared using a ratio of 10% (w/v) of plant tissues to extraction buffer (25 mM KH₂PO₄ at pH 7.4). Extracts were centrifuged for 8 min at -4 °C and 9600 rpm in a refrigerated centrifuge (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). The supernatants were then transferred to microcentrifuge tubes and stored at -20 °C until analysis.³⁷

For CAT activity assay, an aliquot of 950 μ L of 10 mM H₂O₂ was placed in a quartz cuvette and added with 50 μ L of the enzyme extract to obtain a final volume of 1 mL. The absorbance at 240 nm was recorded for 3 min. The APX activity was evaluated according to Murguia et al. ³⁸ with minor modifications. A volume of 886 μ L of 0.1 M KH₂PO₄ buffered at pH 7.4, 4 μ L of a 25 mM ascorbate solution, 10 μ L of 17 mM H₂O₂, and 100 μ L of the sample were placed in a quartz cuvette and mixed three times. The absorbance was recorded at 265 nm for 2 min. The absorbance reading was performed using a Perkin-Elmer Lambda 14 UV/vis spectrometer (single-beam mode, Perkin-Elmer, Uberlinger, Germany). The protein content was determined by the Bradford method using serum albumin as a standard. ³⁹

FTIR Studies. Thirty-day-old dry tissues from both roots and shoots of all treatments were powdered and analyzed using Fourier transform infrared (FTIR) spectroscopy (Perkin-Elmer, Spectrum 100, Universal ATR sampling accessory) with a range of 650–3950 cm⁻¹. Each powdered sample was placed on the sample plate, and one spectrum from each sample (3 replicates per treatment) was obtained; three replicates per sample were considered to perform the area integration. The samples were normalized from 4000 cm⁻¹ to 100 cm⁻¹ absorption.

Statistical Analysis. Three replicates from each treatment were set in a completely random design for statistical analysis. Data (means \pm SE) were analyzed by one-way ANOVA, and Duncan's test was used to determine statistical significance for enzyme assays and FTIR analysis, while Tukey's HSD test was used for the rest of the analysis. The significance was determined with $p \leq 0.05$, unless another value is stated.

■ RESULTS AND DISCUSSION

Effects of CeO₂ NPs on Cilantro Plant Elongation and Biomass Production. The root and shoot lengths and

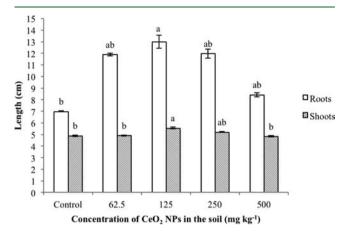


Figure 1. Root and shoot length of cilantro plants grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg⁻¹. Data are means of three replicates \pm SE (standard error). Different letters between columns indicate statistically significant differences at $p \le 0.05$.

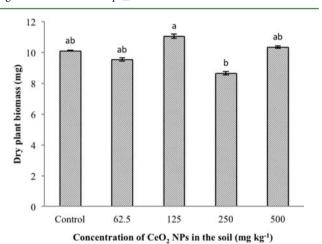


Figure 2. Dry biomass of cilantro plants grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg⁻¹. Data are means of three replicates \pm SE (standard error). Different letters between columns indicate statistically significant differences at $p \leq 0.05$.

biomass production of 30-day-old cilantro plants treated with CeO₂ NPs are displayed in Figures 1 and 2, respectively. As shown in Figure 1, plants treated with 125 mg kg⁻¹ of the CeO₂ NPs had significantly longer shoots (5.2 \pm 0.1 cm, $p \leq$ 0.05) than control plants (~4.9 \pm 0.07 cm) and plants treated with 62.5 mg kg⁻¹ (~4.9 \pm 0.03 cm) or 500 mg kg⁻¹ (~4.8 \pm 0.06 cm). Similarly, plants treated with 125 mg kg⁻¹ had significantly ($p \leq$ 0.05) larger roots (~12.9 \pm 0.6 cm) compared with the other treatments (~7.0 \pm 0.03 cm) (Figure 1). However, the biomass production at 125 mg kg⁻¹ was statistically higher only compared to the 250 mg kg⁻¹ treatment (Figure 2). The results suggest that CeO₂ NPs at 125 mg kg⁻¹ help plants grow better. This also suggests that at that concentration the CeO₂ NPs have fertilizing effects, as the amount of Ce ions released from the CeO₂ NP suspensions was very low (~1 mg L⁻¹). Previous reports indicate that a fertilizer with high cerium

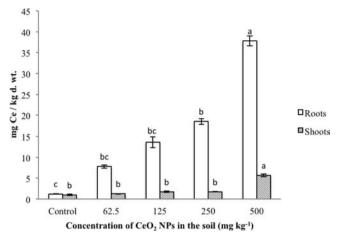


Figure 3. Ce concentration in roots and shoots from cilantro plants treated with CeO₂ NPs at concentrations varying from 0 to 500 mg kg⁻¹. Data are means of three replicates \pm SE (standard error). Different letters among columns indicate statistically significant differences in Ce content at $p \le 0.05$.

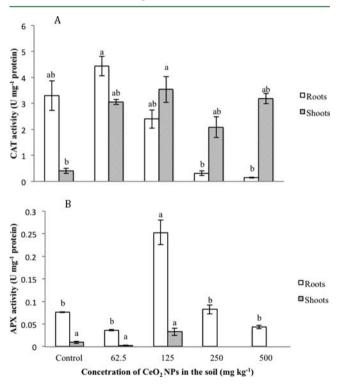
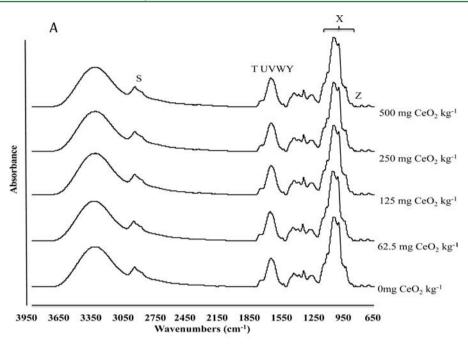


Figure 4. Catalase (A) and ascorbate peroxidase (B) activity in roots and shoots from cilantro plants treated with 0 to 500 mg CeO₂ NPs kg⁻¹. Data are means of three replicates \pm SE (standard error). Different letters among columns indicate statistically significant differences at $p \leq 0.05$.

concentration (>50%) was found to increase rice seedling growth. ⁴⁰ Lopez-Moreno et al. ⁴¹ also reported enhancement of growth in cucumber plants after exposure to CeO₂ NPs at 2000 mg CeO₂ L⁻¹. In addition, it has been reported that CeO₂ NPs at 10 mg CeO₂ L⁻¹ enhanced the growth and increased fruit production by 10% in tomato. ¹⁹ Another report indicates that Ce accumulates in the form of cerium perhydroxide in cell walls and intercellular spaces of epidermal and cortical cells, but not in meristematic cells. ⁴² This suggests less enzymatic stress in the growing zone, which promotes the growth of the plants.



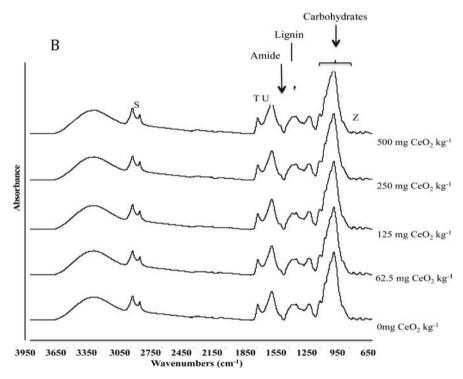


Figure 5. FTIR spectra of cilantro root (A) and shoot (B) tissues treated with 0 to 500 mg CeO_2 NPs kg^{-1} . Spectra are means of three replicates. Different letters on spectra indicate the band area from each frequency range: S and T, lipids; U and W, amide; X, carbohydrates; and V, Y, and Z, lignin.

Cerium Uptake by Cilantro Plants. The cerium concentration in roots and shoots of 30-day-old cilantro plants treated with various CeO_2 NP concentrations is shown in Figure 3. As seen in this figure, the Ce concentration in roots was significantly higher only in plants exposed to 500 mg CeO_2 NPs kg^{-1} [~40 mg kg^{-1} dry weight biomass (d wt b)] ($p \le 0.05$). It is noteworthy that, even at the 500 mg CeO_2 NPs kg^{-1} treatment, the Ce concentration in shoots was very low (about 5 mg kg^{-1} d wt b). Our previous work with corn plants has shown that the CeO_2 NP aggregates are taken up by roots via

apoplasts, and very few of them reached the transport system; 18 thus, the translocation to shoots was expected to be very low. Another report indicates that in soil grown tomato, most of the Ce taken up was stored in roots. 19 Nevertheless, more recent reports have demonstrated that a high portion of the Ce absorbed by plants remains as $\rm CeO_2$ NPs within tissues, 18,20,21,41 which suggests that, although at low concentration, cilantro plants exposed to $\rm CeO_2$ NPs may enter these NPs in the food chain.

Table 1. FTIR Bands in Spectra of Plants⁴⁷

band	frequency range (cm ⁻¹)	assignment	type
S	2840-2960	lipids	C—H symmetric/asymmetric stretch
T	1720-1740	lipids	C=O stretching of carboxylic/ phenolic ester
U	1650	amide	amide C=O and C-N stretch
V	1635	lignin	aromatic C=C stretch
W	1550	amide	N-H deformation and C-N stretch
X	900-1200	carbohydrate	carbohydrate fingerprint region
Y	1515	lignin	C=C phenolic stretch
Z	845	lignin	aromatic C–H wag of aromatic ring associated with lignin

Table 2. FTIR Band Area from Roots of Cilantro (Corinadrum sativum L.) Plants Germinated and Grown in Organic Soil Treated with 0-500 mg CeO₂ NPs kg^{-1a}

	band area (area units)				
CeO ₂ NPs (mg kg ⁻¹)	S	T	X		
0	$29.7 \pm 0 c$	2.0 ± 0 a	$155.1 \pm 0.2 \text{ c}$		
62.5	$29.9 \pm 0.1 c$	2.0 ± 0 a	$156.0 \pm 0.1 c$		
125	$31.6 \pm 0.1 a$	2.0 ± 0 a	$157.5 \pm 0.2 a$		
250	$30.4 \pm 0.1 \text{ cb}$	$1.9 \pm 0 \text{ b}$	$155.9 \pm 0 \text{ bc}$		
500	31.0 ± 0 ab	2.0 ± 0 ab	$156.3 \pm 0 \text{ b}$		

^aData are means of three replicates \pm SE (standard error). Different lowercase letters on spectra indicate statistically significant difference between treatments at $p \le 0.05$.

Table 3. FTIR Band Area from Shoots of Cilantro (Corinadrum sativum L.) Plants Germinated and Grown in Organic Soil Treated with 0 to 500 mg CeO₂ NPs kg^{-1a}

	band area (area units)				
CeO ₂ NPs (mg kg ⁻¹)	W	X	Y		
0	$0.3 \pm 0 \text{ b}$	$150.5 \pm 0.4 \text{ b}$	0.1 ± 0 b		
62.5	0.3 ± 0 ab	$150.3 \pm 0.0 \text{ b}$	0.2 ± 0 ab		
125	$0.3 \pm 04 \text{ ab}$	$153.7 \pm 0.3 a$	0.2 ± 0 ab		
250	0.3 ± 0 ab	$149.8 \pm 0.2 \text{ b}$	0.2 ± 0 ab		
500	$0.4 \pm 0 \ a$	$149.0 \pm 0.4 \text{ b}$	$0.3 \pm 0 a$		

[&]quot;Data are means of three replicates \pm SE (standard error). Different letters on spectra indicate statistically significant difference between treatments at $p \le 0.05$.

Effect of CeO_2 NPs on CAT and APX Activity. It was expected that the CeO_2 NPs would affect the production of ROS molecules in cilantro plants, triggering a stress response. CAT and APX are important enzymes used by plants to cope with excess H_2O_2 . Thus, the activity of both enzymes was analyzed in order to know if the CeO_2 NPs caused stress in

cilantro (Figure 4). As seen in Figure 4A, the activity of CAT in the roots at $62.5~\rm mg~kg^{-1}$ was statistically higher compared to the 250 and 500 mg CeO $_2$ NPs kg $^{-1}$ treatments and, in shoots, was significantly higher at 125 mg kg $^{-1}$. This coincides with the increase in the size of the plants at this NP concentration. An increase in size implies higher cellular activity and $\rm H_2O_2$ generation; consequently, a higher activity of ROS scavenger enzymes could be expected. An increase in CAT activity was also reported in corn shoots after exposure to 400 and 800 mg CeO $_2$ NPs kg $^{-1}$.

APX is another important enzyme that helps to control ROS molecules in the cell's cytosol or mitochondria.⁴⁴ It has a high affinity for H₂O₂ and helps the plants to deal better with excess ROS molecules generated under stress conditions. Figure 4B shows the results for APX activity in cilantro plants treated with CeO₂ NPs at different concentrations. As shown in this figure, the APX activity significantly increased ($p \le 0.05$) in roots of plants treated with 125 mg CeO₂ NPs kg⁻¹ compared with control and the other treatments. On the other hand, no activity of this enzyme was detected in the shoots of plants exposed to 250 and 500 mg CeO₂ NPs kg⁻¹ treatments. This suggests that the CeO2 NPs down-regulated the production of this defensive enzyme, which could compromise the defense mechanism of cilantro. Zhao et al.³⁰ reported that in corn seedlings (root plus shoot) of 10 -day-old corn plants there was an increase in APX activity at 800 mg CeO₂ NPs kg⁻¹ treatment, compared to the control. However, the difference disappeared at 20 days. Although it has been reported that the CeO₂ NPs have antioxidant activity, ^{45,46} these results show that the CeO₂ NPs produced stress in cilantro plants, as both CAT and APX were increased or decreased at low and high concentrations, respectively.

FTIR Data analysis. Fourier transform infrared spectroscopy is a well-established tool for the identification of specific functional groups in plant tissues. In this research, FTIR was used to identify any changes in specific functional groups in cilantro plants exposed to CeO2 NPs. Comparisons of FTIR spectra from cilantro roots and shoots treated at 0 to 500 mg CeO₂ NPs kg⁻¹ are displayed in Figure 5. The FTIR bands in spectra of plants are shown in Table 1, and spectra and area for root and shoot from cilantro plants are shown in Tables 2 and 3. The band areas were determined using Spectrum software, version 6.0.2.0025 (Perkin-Elmer). The data showed band differences in roots from control compared with roots from 125 and 500 mg CeO₂ NPs kg⁻¹ treatments in the lipid area located between 2840 and 2960 cm $^{-1}$ (Figure 5A). In addition, roots from 250 mg CeO $_2$ NPs kg $^{-1}$ treatment were found to be different from control in the lipid area from 1720 to 1740 cm⁻¹ (band T, Table 2). Moreover, at 125 and 500 mg CeO₂ NPs kg⁻¹, the cilantro plants presented differences from the control in the carbohydrate area between 900 and 1200 cm⁻¹.

Table 4. FTIR Vibrational Shifts on Bands from Roots of Cilantro (Corinadrum sativum L.) Plants Germinated and Grown in Organic Soil Treated with 0 to 500 mg CeO₂ NPs kg⁻¹

	vibrational shifts (cm ⁻¹)							
CeO ₂ NPs (mg kg ⁻¹)	S	T	U	V	W	X	Y	Z
0	2924	1730	1649	1634	1550	1032	1515	845
62.5	2924	1720	1649	1634	1550	1032	1515	845
125	2923	1720	1649	1634	1550	1035	1515	845
250	2924	1720	1649	1634	1550	1043	1515	845
500	2923	1720	1649	1634	1550	1032	1515	845

Table 5. FTIR Vibrational Shifts on Bands from Shoots of Cilantro (Corinadrum sativum L.) Plants Germinated and Grown in Organic Soil Treated with 0 to 500 mg CeO₂ NPs kg⁻¹

	vibrational shifts (cm ⁻¹)							
CeO ₂ NPs (mg kg ⁻¹)	S	T	U	V	W	X	Y	Z
0	2921	1733	1649	1634	1550	1012	1515	845
62.5	2920	1733	1649	1634	1550	1012	1515	844
125	2920	1733	1649	1634	1550	1013	1515	845
250	2921	1732	1649	1634	1550	1012	1515	844
500	2921	1733	1649	1634	1550	1012	1515	844

The FTIR band area data from cilantro shoots are shown in Table 3. As shown in this table, there were differences in amide (1550 cm $^{-1}$) and lignin (1515 cm $^{-1}$) areas between plants treated with 500 mg CeO $_2$ NPs kg $^{-1}$ and control plants. In addition, the shoots of plants from 125 mg CeO $_2$ NPs kg $^{-1}$ treatment were different from the control in the area of carbohydrates (between 900 and 1200 cm $^{-1}$). The FTIR results confirmed that CeO $_2$ NPs at concentrations higher than 62.5 mg kg $^{-1}$ affect different functional groups in roots and shoots of cilantro plants. Concentrations of 125 and 500 mg CeO $_2$ NPs kg $^{-1}$ treatments affected cilantro plants the most. Furthermore, the roots presented similar changes in their infrared spectrum as compared to shoots, most likely due to the uptake of CeO $_2$ NPs by the roots and their translocation to the shoots.

The changes in vibrational shifts, as a function of concentration, were compared for both shoots and roots and demonstrate no shifting for the shoots (Tables 4 and 5) and minimal shifting (within 10 cm⁻¹) within the lipid (1720–1740 cm⁻¹) and carbohydrate (900–1200 cm⁻¹) areas of the roots. Therefore, CeO₂ NPs seem to induce conformational changes within the plant as opposed to chemical changes, for which vibrational shifting would be observed. This indicates that the CeO₂ NPs induced a type of aggregation, or conformational change, within the components of the roots, but do not influence chemical reactions within these components.

This analysis is also supported by the complete lack of vibrational shifting in the infrared spectra of the shoots, for which a lower concentration of Ce is observed, and thus, there is no influence on vibrational shifting. Conformational changes within the plant components are also supported by the higher cellular activity within the cell, as indicated by CAT and APX activities at 125 mg kg⁻¹, as well as the increase in plant size, which would be hindered if the functional groups of the cell's components were greatly affected. These results also correlate with the absence of vibrational shifting in the infrared spectra of the shoots, for which a lack of activity of APX was observed, even at high concentrations of the CeO₂ NPs (250 and 500 mg kg⁻¹).

ASSOCIATED CONTENT

S Supporting Information

Elemental analysis and nitrogen content in organic potting soil. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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