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Impact Of Cerium Oxide Nanoparticles On Cilantro (coriandrum Sativum)

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(*CORIANDRUM SATIVUM*)

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IMPACT OF CERIUM OXIDE NANOPARTICLES ON CILANTRO (CORIANDRUM SATIVUM)

By

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Abstract

Studies have shown that plants exposed to ENPs suffer different types of stress. Other studies have revealed that plants can take up and accumulate CeO_2 NPs without modification. Thus, these NPs could enter the food chain through edible plants, posing a threat for human health. Cilantro (*Coriandrum sativum*) is a worldwide culinary and medicinal plant consumed either as a fresh herb or a spice. In this research, cilantro plants were germinated and cultivated for 30 days in organic soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Subsequently, plant organs were analyzed by using spectroscopic techniques and biochemical assays. Results indicate that at 125 mg kg^{-1}, the CeO_2 NPs significantly increased the root size compared with the other treatments. The ICP-OES results showed that plants exposed to 500 mg kg^{-1} had significantly ($p \leq 0.05$) more Ce in shoots and roots compared to the other treatments. Results from the biochemical assays showed that at 125 mg kg^{-1}, catalese activity significantly increased in shoots and ascorbate peroxidase in roots ($p \leq 0.05$). In addition, the FTIR analyses revealed that at 125 mg kg^{-1}, the CeO_2 NPs changed the chemical environment of the carbohydrates within the cilantro shoots, for which changes in the area of the stretching frequencies were observed. Moreover, analyses of antioxidant compounds showed a significant ($p \leq 0.05$) reduction on total phenolic content in shoots of cilantro plants treated with 500 mg CeO_2 NPs kg^{-1}. This suggests that the CeO_2 NPs have the potential to diminish the ability of cilantro plants to scavenge reactive oxygen species. The multi-elemental analysis showed that plants treated with CeO_2 at the 500 mg kg^{-1} treatment had a significant ($p \leq 0.05$) reduction in shoots' sulfur, silicon, and zinc accumulation. The results of this research indicate that the CeO_2 NPs at 500 mg CeO_2 kg^{-1} concentration cause a reduction in the antioxidant ability and nutritional properties of cilantro plants.

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Chapter 1: Introduction

NANOPARTICLES

Nanotechnology encompasses the fabrication and utilization of materials having at least one dimension less than 100 nm.[1] Nanomaterials (NMs) with at least two dimensions between 1 and 100 nm are known as nanoparticles (NPs).[1] These materials posse 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 like 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] Carbonaceous nanoparticles, nanopolymers, quantum dots, zero-valent metals and, metal oxides are the five distinctive manufactured NPs better known to date.[10] CeO_2, ZnO, and TiO_2 are among the most used metal oxide NPs in the area of food, material, chemical, and biological sciences.[11] The fact that several products containing these and other NPs are widely used raises a very important point: our knowledge about the fate, transport, and ecological impact of NPs is still incomplete.

Cerium Oxide Nanoparticles

Cerium is one of the most abundant elements among the Rare Earth elements. It can be found in the nature as a cerium(III) or cerium(IV). Cerium oxide NPs (CeO_2 NPs) are manufactured with different sizes, but the most used are those with size of 10 nm or less, because of their size-dependent properties.[12] In the manufacturing industry, the CeO_2 NPs are among the thirteen most used NMs. [13] 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,[14,15] fuel additive, solid oxide fuel cells, and catalysis. [16] However, after the end-user applications, these products

and residues will be in the ecosystems; therefore becoming a risk for plants, humans, and other organisms. [17- 19] Reports indicate that CeO_2 NPs induced apoptosis and mitochondrial damage in human monocytes.[20] Other reports have shown that CeO_2 NPs induce cytotoxicity by promoting oxidative stress and chronic inflammatory response in rats.[21] The CeO_2 can also affect microorganisms. Studies have demonstrated that CeO_2 NPs can penetrate the outer membrane of *Escherichia coli* and *Sinorhizobium meliloti* internalizing in the bacterial periplasm. [22,23]

Concerning the interaction of CeO_2 NPs with plants, there are no consistent reports. While some studies have shown that these NPs are beneficial for plant growth as they increase seed germination and plant elongation,[24] other reports have shown that the CeO_2 NPs are toxic to plants. For instance, toxic effects of CeO_2 NPs on germinating seeds of *Lactuca sativa*, *Solanum lycopersicum*, *Cucumis sativus* and *Spinacia oleracea* are reported.[25] In addition, Lopez-Moreno et al.[26] reported that the CeO_2 NPs at 2000 and 4000 mg/L produced the appearance of three and four new bands, respectively, in the genomic DNA of soybean seedling. Though, there are not reports about the effect of CeO_2 NPs on culinary herbs.

CILANTRO (*CORIANDRUM SATIVUM*)

Cilantro (*Coriandrum sativum*) is a very important herb used in several traditional dishes of many cultures around the world. Cilantro plant classification is shown on Table 1. It is consumed either as a fresh herb or as a spice because of its flavor and aroma. Cilantro's origin is unclear but some literature suggested that its origin is the Near East.[27] It is worldwide produced but the principal exporters of cilantro are India, Mexico, Central America, South America, and the Caribbean.

Cilantro is an annual herb with indented leaves that can grow fast and it is a relative small plant. The word cilantro refers to stems and leaves freshly consumed. When cilantro is used as a spice, it is termed coriander.[28] Cilantro seeds are reach in essential oil, and its extract is used by industry as a flavor for food. For thousands of years, cilantro has been consumed as a medicinal plant, since it is reach in mineral and antioxidant compounds (phenolic compounds).[27] Some compounds provided by cilantro are vitamins C, B_1, B_2, protein, fibers, carbohydrates, and water among others.[28]

Figure 1. Cilantro (*Coriandrum sativum*) plants

Table 1. Botanical classification of cilantro plant

Kindom	Plantae (plants)
Subkingdom	Tracheobionta (vascular plants)
Superdivision	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledons)
Subclass	Rosidae
Order	Apiales
Family	Apiaceae (the carrot family)
Genus	Coriandrum L.
Species	Coriandrum sativum

BIOCHEMICAL METHODS

Biochemical assays are commonly used in the analyses of biological samples. These assays are used to determine the activity of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX).[29] The radical scavenging capacity is another important biochemical assay used to determine the presence of antioxidant compounds such as phenolic compounds.

Plants are multisystem organisms. They possess specialized systems that protect them in stressing conditions produced by abiotic or biotic factors. Under abiotic stress conditions, plants produce or are exposed to reactive oxygen species (ROS). ROS can be hydroxyl radical (OH^-), superoxide radical ($O_2\cdot$) and hydrogen peroxide (H_2O_2), among others. ROS molecules are used as markers when a plant is under stress. The activity of enzymes like APX and CAT is an indicative of stress induced by ROS.[29] APX has many biological functions including the removal of H_2O_2 in chloroplast and cytosol. CAT enzymes are located in the peroxisomes. CAT catalyzes H_2O_2 decomposition into water and oxygen in the cells.

In addition to antioxidant enzymes, many plants also have antioxidant compounds that help with the scavenging of ROS molecules. Phenolic compounds are among the most common natural antioxidant compounds. The Folin–Ciocalteau reagent is regularly used to determine total phenolic content in extracts. Through the experiment, oxidation of phenolic compounds produces a blue color which intensity is directly proportional at the phenolic compounds concentration in the samples. The scavenging capacity of antioxidant compounds can be assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation assays. In the DPPH radical assay, antioxidant compounds interact with DPPH• radicals, reducing them by hydrogen donation. Scavenging capacity percent of antioxidant compounds is inversely proportional to the color intensity

produced by DPPH• radicals.[19] In ABTS radical cation assay, the $ABTS^{\bullet+}$ radical is reduced by antioxidant compounds present in the sample. $ABTS^{\bullet+}$ radicals produce a blue color that is reduced by the radicals' neutralization from electron donors. The reduction of color is directly proportional to the antioxidant capacity percent of antioxidant compounds in the investigated sample.[20]

SPECTROSCOPIC ANALYSIS

Inductively Coupled Plasma-Optical Emission Spectroscopy

In studies where elements need to be measured at trace levels, the selection of the right analytic instruments is very important to avoid chemical interferences. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) is a very accurate analytic instrument for elemental analysis. Dozens of elements can be determined by this technique that is an excellent advantage for multi-elemental analysis. This technique allows the determination in solutions (μg/L) and solids (μg/kg) at ppm level.[30] Because of the higher temperature used by ICP-OES, chemical interference are reduced. Its source of emission is argon plasma that conduces a mixture of cations and electrons. In argon plasma the temperatures can reach 10,000 K.[30] The liquid sample is delivered by a pump into a nebulizer and converted into mist. The mist is then sent inside the plasma flame. The sample is converted into ions that lose electrons and produce a determined signal of the element investigated.[30]

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is used for the characterization of molecular species. The characterization is based on the different energies produced by vibrational and rotational molecules states.[31] FTIR is applied to study organic materials and allows to determine changes in macromolecules of organic samples like plants. Molecules have unique vibrational

spectrum. Thus, the FTIR spectrum is unique for each molecule. FTIR spectra are produced by the radiation absorbed by a sample. The energies where peaks appear are related with the vibrational frequency from a molecule portion. Then, a spectrum can be considered as a fingerprint from the molecule analyzed.[31]

OBJECTIVES OF THE RESEARCH

The general objective of this research was to study the effects of CeO_2 NPs at agronomical and physiological levels in cilantro plants.

Specific Objectives

The specific objectives were to:

- Determine uptake by the roots and translocation to the shoots of CeO_2 NPs.
- Analyze the effect of CeO_2 NPs on antioxidant capacity and nutrient content in cilantro shoots.

Hypothesis

Our working hypothesis was that at least one of the treatments would negatively impact the cilantro plants.

Chapter 2: Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum)* plants grown in organic soil.

INTRODUCTION

Previous reports have shown that plants can accumulate CeO_2 NPs. Through the use of X-ray absorption spectroscopy and microscopy studies, our research group was able to determine the presence of untransformed CeO_2 NPs in soybean seedlings[1] and confocal microscope was used to image CeO_2 aggregates in corn (*Zea mays*) root seedlings.[2] Other studies have shown reported that the CeO_2 NPs can be taken up by the roots and translocated to the shoots in tomato (*Solanum lycopersicum*).[3] In addition, Zhang et al.[4] reported that most of the CeO_2 NPs absorbed by cucumber (*Cucumis sativus*) plants grown in hydroponics remained as NPs and a small percentage was biotransformed to $CePO_4$, in roots, and to cerium carboxylates, in shoots. More recently, Hernandez-Viezcas et al.[5] reported, by using synchrotron X- ray techniques, that most of the CeO_2 NPs absorbed by soybean from CeO_2 NPs treated soil were stored in the reproductive/edible parts of this plant.

The above narrative suggests that the CeO_2 NPs could be introduced in the food chain through plants eaten fresh like tomato and cucumber[3-6] or through dry grains.[5] Culinary plants, like cilantro, could also represent a way to introduce CeO_2 NPs in the food chain. Cilantro is a very important culinary and medicinal plant consumed worldwide.[7- 9] Cilantro has been used for a long time around the world because of its curative abilities[10] and it is believed that these qualities in herbs such as cilantro are the result of its antioxidant properties.[11]

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.[12] Enzymes such as catalase (CAT) and ascorbate peroxidase (APX) are among the most important antioxidant enzymes that help plants to deal with oxidative stress or reactive

oxygen species (ROS) production.[13] These enzymes scavenge ROS molecules that affect important functions required for a healthy growth. In a previous report, Zhao et al.[14] have shown that CeO_2 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*).[15] However, there are no studies concerning the stress response to CeO_2 NPs in garden vegetables.

In this study, cilantro plants were germinated and grown to maturity in organic soil treated with CeO_2 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 demonstrates that the CeO_2 NPs produce significant changes in cilantro plants.

MATERIALS AND METHODS

CeO_2 NP suspensions and soil preparation. The CeO_2 NPs (10 nm, Meliorum Technologies, Rochester, NY) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). These NPs were previously characterized in several aqueous environments by Keller et al.[16] The *n*CeO_2 are rod with primary size of 10 ± 1 nm, surface area of 93.8 m^2g^{-1} and 95.14% purity.[16] For the present study, the size in suspension, zeta potential and the concentration of Ce ions in suspensions are shown in Table 2.1. 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 five 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 were mixed

homogeneously with the CeO_2 NPs suspension and placed in pots of 13.21 cm diameter x 10.16 cm high. 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 2.2.

Table 2.1. Characterization of the CeO_2 NP suspensions. Data are means of three replicates ±SE (standard error). Different letters on spectra indicate statistically significant differences between treatments at $p \leq 0.05$.

CeO_2 NPs	pH	zeta potential (mV)	Diameter (nm)	Ce ion (mg L-1)
62.5	5.44+1.36a	29+1.4b	857.3+7.1a	0.32+0.02b
125	5.48+0.21a	35.3+0.7a	527.4+66.5c	1.08+0.37ab
250	5.24+0.84a	30.8+1b	685.6+35.9b	1.69+0.35ab
500	5.09+0.21a	20.4+0.2c	616.2+8.5bc	2.11+0.32a

Table 2.2. Nitrogen content and elemental analysis for organic potting soil.

Elements & Compounds	Concentration ($g\ kg^{-1}$)
P	6.531
Ca	53.407
S	1.571
Mg	8.383
Mo	0
Fe	7.312
NH_4	0.038
NO_3	0.014
Inorganic N_2	0.052

Seed Germination. Cilantro (*Coriandrum sativum*) seeds (botanical known as fruits) were purchased from Del Norte Seed & Feed (Vinton, TX). The seeds were soaked in MPW and stirred for 3 h for hydration. Approximately 40 seeds of the same size were selected and sown in each pot. The seeds were placed about 1 cm depth in the soil and watered with 50 mL MPW every day. Pots were placed in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with 14-h photoperiod, 25/20 °C day/night temperature and 65% relative humidity. 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 5% $CaCl_2$ solution and DI water to remove NPs adhered to 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, separated 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, the samples were microwave assisted acid digested by using 2 mL of plasma pure HNO_3 and 3 mL of H_2O_2 (30%) in a microwave accelerated reaction system (CEM Corporation Mathews, NC)[17] and diluted to 15 mL with MPW. For QC/QA of the digestion method, the reference material 1547 (NIST, USA) and 10 mg 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). 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 ± 0.2 and 1 ± 0.1 mg L^{-1}. The ICP-OES parameters used were as follows: nebulizer flow, 0.80 L min^{-1}; power, 1450W; 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 and APOX assays. Thirty-day old fresh cilantro plants were washed with 5% $CaCl_2$ solution and MPW three times to remove any external contaminant. Samples of 0.1 g of fresh roots and shoots (stems and leaves) were used to determine CAT and APX activity according to Gallego et al.[18] with minor modifications.[19] The extracts were prepared using a ratio of 10% (w/v) of plant tissues to extraction buffer (25 mM KH_2PO_4 at pH 7.4). Extracts were centrifuged for 8 min at -4 °C and 9,600 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.[20]

For CAT activity assay, an aliquot of 950 µL 10 mM H_2O_2 was placed in a quartz cuvette and added with 50 µL 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.[21] with minor modifications. A volume of 886 µL of 0.1 M KH_2PO_4 buffered at pH 7.4, 4 µL of a 25 mM ascorbate solution, 10 µL of 17 mM H_2O_2, and 100 µL 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.[22]

FTIR studies. Dry tissues from both roots and shoots of all treatments were powdered and analyzed using FT-IR 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 replicate was analyzed per sample.

Statistical Analysis. Three replicates from each treatment were set in a completely random design for statistical analysis. Data (means ± SE) was analyzed by one-way ANOVA, and Duncan 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 other value is stated.

RESULTS AND DISCUSSION

Effects of CeO_2 NPs on cilantro plant elongation and biomass production. The root and shoot lengths and biomass production of 30-day old cilantro plants treated with CeO_2 NPs are displayed in Figure 1 and 2, respectively. As shown in Figure 2.1, plants treated with 125 mg kg^{-1} of the CeO_2 NPs had significantly longer roots (5.2 ± 0.1 cm, $p \leq 0.05$) than control plants (~4.9 ± 0.07 cm) and plants treated with 62.5 mg kg^{-1} (~4.9 ± 0.03 cm) or 500 mg kg^{-1} (~4.8 ± 0.06 cm). Similarly, plants treated with 125 mg kg^{-1} had significantly ($p \leq 0.05$) larger shoots (~12.9 ± 0.6 cm) compared with the other treatments (`~7.0 ± 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 2.2). The results suggest that CeO_2 NPs at 125 mg kg^{-1} helps plants grow better. This also suggests that at that concentration, the CeO_2 NPs have fertilizer effects, as the amount of Ce ions released from the CeO_2 NP suspensions were very low (~ 1 mg l^{-1}, Table 2.1). Previous reports indicate that a fertilizer with high cerium concentration (> 50%) was found to increase

rice seedlings growth.[23] Lopez-Moreno et al.[24] also reported enhancement of growth in cucumber plants after exposure with CeO_2 NPs at 2000 mg CeO_2 L^{-1}. In addition, it has been reported that CeO_2 NPs at 10 mg CeO_2 L^{-1} enhanced the growth and increased fruit production by 10% in tomato.[3] 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.[25] This suggests less enzymatic stress in the growing zone, which promotes the growth of the plants.

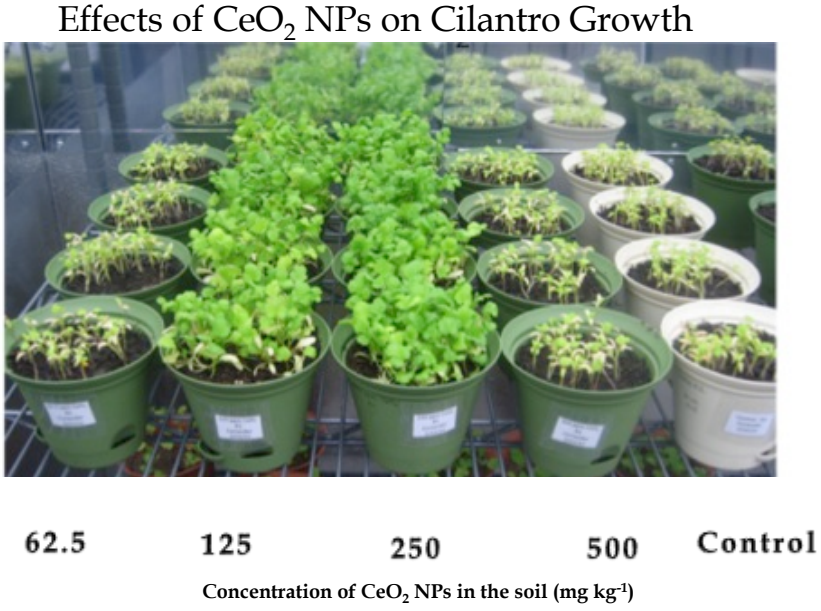

Figure 2.1. Cilantro plant grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}.

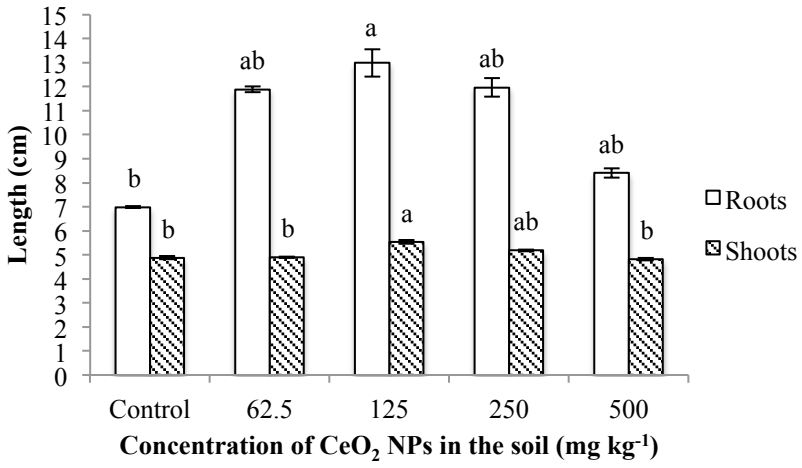

Figure 2.2. Root and Shoot lengths of cilantro plant grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ±SE (standard error). Different letters between columns indicate statistically significant differences at ($p \leq 0.05$).

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 3.3. 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)] ($p \leq 0.05$). 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 biomass).

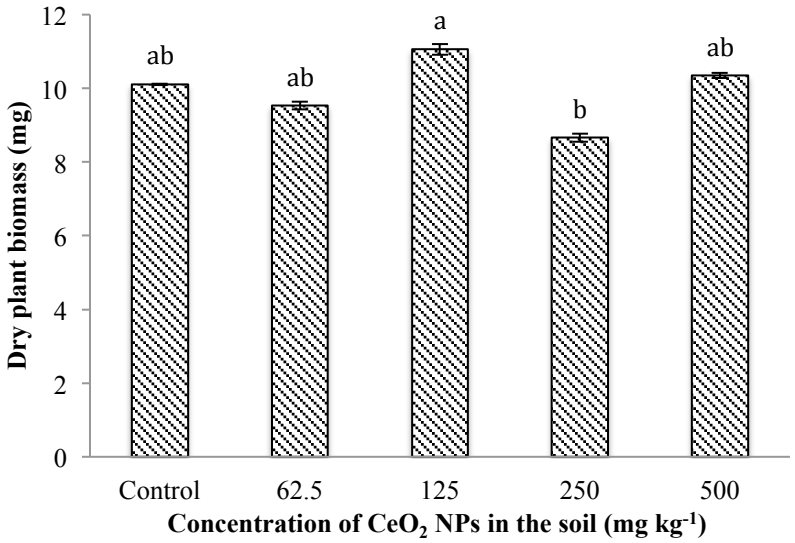

Figure 2.3. Dry biomass of cilantro plants grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ±SE (standard error). Different letters between columns indicate statistically significant differences in at ($p \leq 0.05$).

Our previous work with corn plants has shown that the CeO_2 NP aggregates are taken up by roots via apoplats, and very few of them reached the transport system; [2] 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.[3] Nevertheless, more recent reports have demonstrated that a high portion of the Ce absorbed by plants remains as CeO_2 NPs within tissues, [2, 4, 5, 24] which suggests that, although at low concentration, cilantro plants exposed to CeO_2 NPs may enter these NPs in the food chain.

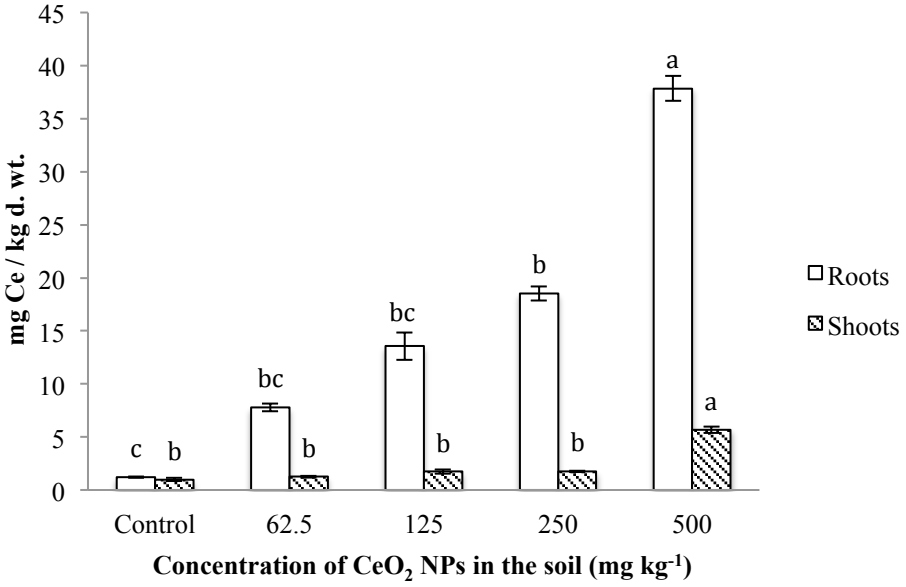

Figure 2.4. Ce concentration in roots and shoots from cilantro plants treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters among columns indicate statistically significant differences in Ce content in each treatment at ($p \leq 0.05$).

Effect of CeO_2 NPs on CAT and APX activity. It was expected that CeO_2 NPs would affect the production of ROS molecules in cilantro plants, triggering stress response. CAT and APX are important enzymes used by the plants to cope with excess of H_2O_2.[26] Thus, the activity of both enzymes was analyzed in order to know if the CeO_2 NPs caused stress in cilantro (Figure 2.4). As seen in Figure 2.4A, the activity of CAT in the roots at 62.5 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 size of the plants at this NP concentration. An increase in size implies higher cellular activity and H_2O_2 generation; consequently, 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}.[14]

APX is other important enzyme that helps to control ROS molecules in the cell's cytosol or mitochondria. [27] It has a high affinity for H_2O_2 and helps the plants to deal better with excess of ROS molecules generated under stress conditions. Figure 2.4B shows the results for APX activity in cilantro plants treated with CeO_2 NPs at different concentrations. As shown in this figure, the APX activity significantly increased ($p \leq 0.05$) in roots of plants treated with 125 mg CeO_2 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 CeO_2 NPs kg^{-1} treatments. This suggests that the CeO_2 NPs down regulated the production of this defensive enzyme, which could compromise the defense mechanism of cilantro. Zhao et al.[14] reported that in corn seedlings (root plus shoot) of 10 day-old corn plants, there was an increase in APX activity at 800 mg CeO_2 NPs kg^{-1} treatment, compared to control. However, the difference disappeared at 20 days. Although it has been reported that the CeO_2 NPs have antioxidant activity,[28, 29] these results show that the CeO_2 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 (FTIR) spectroscopy is a well-established tool for the identification of specific functional groups in plant tissues. In this research, the FTIR was used to identify any changes in specific functional groups in cilantro plants exposed to CeO_2 NPs. Comparisons of FTIR spectra from cilantro roots and shoots treated at 0-500 mg CeO_2 NPs kg^{-1} are displayed in Figure 2.5.

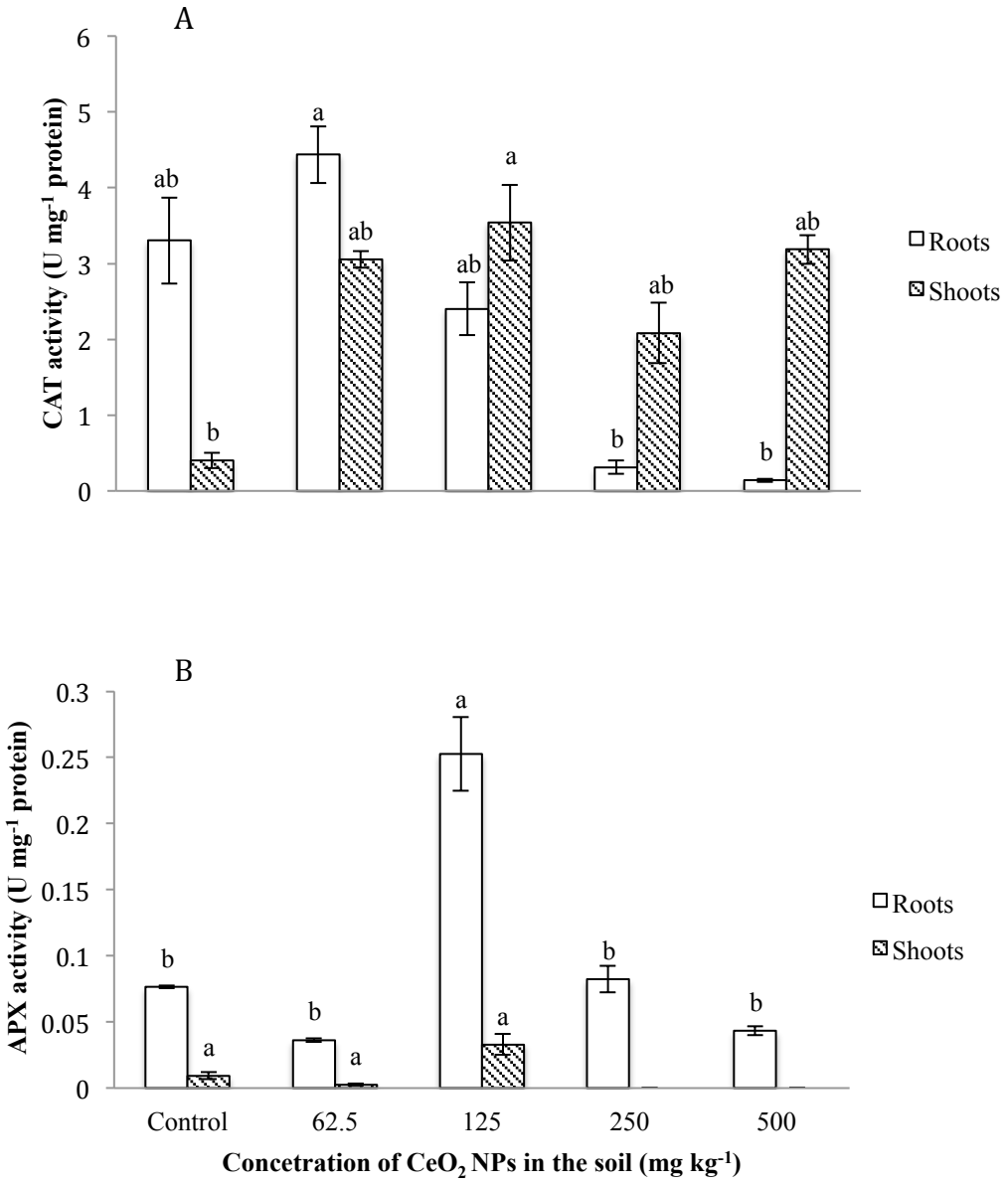

Figure 2.5. Catalase (A) and ascorbate peroxidase (B) activity in roots and shoots from cilantro plants treated with 0-500 mg CeO_2 NPs kg^{-1}. Data are means of three replicates ±SE (standard error). Different letters among columns indicate statistically significant differences at ($p \leq 0.05$).

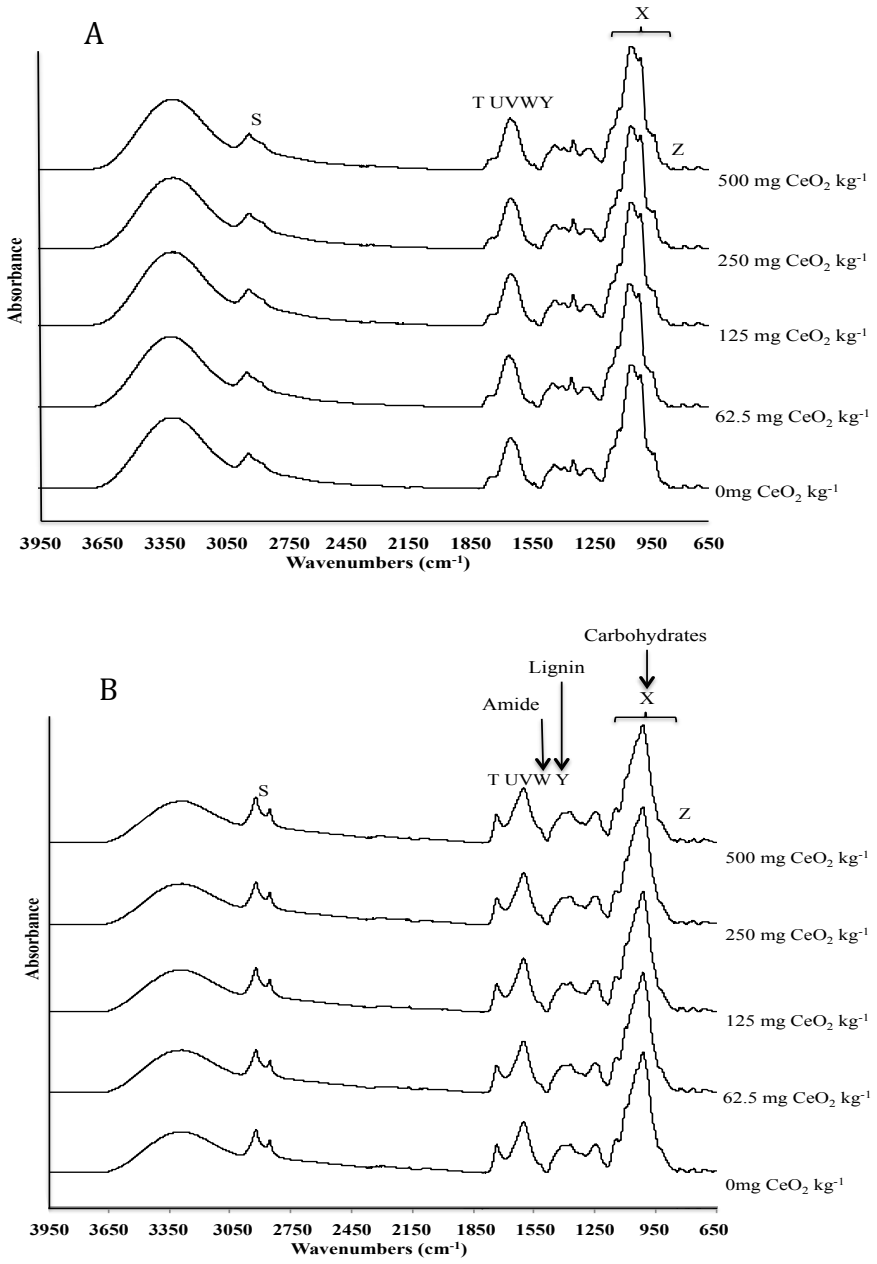

Figure 2.6. FTIR spectra of cilantro roots (A) and shoots (B) tissues treated with 0-500 mg CeO_2 NPs kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters on spectra indicate the band area from each frequency range; S and T lipids, U and W amide, V, Y, and Z lignin.

The FTIR bands in spectra of plants are shown in Table 2.3 and spectra and area for root and shoot from cilantro plants are shown in Tables 2.4 and 2.5. 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 CeO_2 NPs kg^{-1} treatments in the lipid area located between 2840 and 2960 cm^{-1} (Figure 2.5A). 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 2.4). Cilantro roots from 125 CeO_2 NPs kg^{-1} treatment were different from control in amide and lignin areas from 1630 to 1650 cm^{-1} (Tables 2.4). In addition, the roots from 125 and 500 mg CeO_2 NPs kg^{-1} treatments were significantly different from control in the amide area 1550 cm^{-1}. Moreover, at 125 and 500 mg CeO_2 NPs kg^{-1}, the cilantro plants presented differences from control in the carbohydrate area between 900 and 1200 cm^{-1}. However, roots from 62.5 and 250 mg CeO_2 NPs kg^{-1} were different among themselves but not different from control in the lignin area of 1515 cm^{-1}. In the lignin area (845 cm^{-1}), only the 250 mg CeO_2 NPs kg^{-1} treatment produced differences compared to control roots.

The FTIR band area data from cilantro shoots are shown in Table 2.5. 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 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 more changes in their infrared spectrum as compared to shoots, most likely due to their direct exposure to the CeO_2 NPs.

Table 2.3. FTIR bands in spectra of plants.[30]

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

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 2.6 and 2.7) and minimal shifting (within 10 cm^{-1}) within the lipid (1720 cm^{-1}-1740 cm^{-1}) and carbohydrate (900 cm^{-1}-1200 cm^{-1}) areas of the roots. Therefore, CeO_2 seems to induce conformational changes within the plant as opposed to chemical changes, for which vibrational shifting would be observed. This indicates that the influence of the CeO_2 affects a type of aggregation, or conformational change, within the components of the roots, but does 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 also support the analysis above of higher cellular activity within the cell by CAT and APX, 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 CeO_2 (250 mg kg^{-1} and 500 mg kg^{-1}).

Table 2.4. FTIR band area from roots of cilantro plants germinated and grown in organic soil treated with 0-500 mg CeO_2 NPs kg^{-1}. Data are means of three replicates ±SE (standard error). Different lower case letters on spectra indicate statistically significant difference between treatments at $p \leq 0.05$.

CeO_2 NPs (mg kg^{-1})	Band area (area units)							
	S	T	U	V	W	X	Y	Z
0	29.689±0.019c	1.968±0.006a	0.787±0b	0.859±0b	0.148±0b	155.104±0.188c	0.091±0ab	0.011±0b
62.5	29.880±0.078c	1.991±0.013a	0.806±0.001ab	0.880±0.001ab	0.152±0b	156.027±0.084bc	0.096±0a	0.013±0b
125	29.880±0.083a	1.985±0.005a	0.816±0a	0.888±0.001a	0.160±0a	157.483±0.159a	0.092±0ab	0.017±0.001ab
250	30.442±0.089cb	1.860±0.016b	0.79±0.003b	0.862±0.003b	0.150±0.001b	155.856±0.016bc	0.089±0b	0.020±0.001a
500	30.974±0.047ab	1.975±0.005a	0.804±0.001ab	0.877±0.001ab	0.163±0a	156.261±0.043b	0.095±0ab	0.015±0ab

Table 2.5. FTIR band area from cilantro shoot tissues treated with 0-500 mg CeO_2 NPs kg^{-1}. Data are means of three replicates ±SE (standard error). It was not found significant differences among treatments on data from S, T, U, V and Z band areas. Different letters on spectra indicate statistical significant difference between treatments at $p \leq 0.05$.

CeO_2 NPs (mg kg^{-1})	Band area (area units)		
	W	X	Y
0	0.298±0.008b	150.471±0.427b	0.144±0.004b
62.5	0.290±0.002b	150.317±0.013b	0.158±0.002b
125	0.292±0.004b	153.741±0.256a	0.155±0.003b
250	0.333±0.006ab	149.848±0.192b	0.179±0.005b
500	0.413±0.014a	148.950±0.366b	0.265±0.015a

Table 2.6. FTIR vibrational shifts on bands from cilantro root tissues treated with 0-500 mg CeO_2 NPs kg^{-1}.

CeO_2 NPs concentration (mg kg^{-1})	Vibrational Shifts (cm^{-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 2.7. FTIR vibrational shifts on bands from cilantro shoot tissues treated with 0-500 mg CeO_2 NPs kg^{-1}.

CeO_2 NPs concentration (mg kg^{-1})	Vibrational Shifts (cm^{-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

Chapter 3: Changes in the antioxidant properties and nutrient value of cilantro plants grown in cerium oxide nanoparticles treated soil

INTRODUCTION

Recent reports have shown different degrees of interaction of CeO_2 NPs with plants such as corn (*Zea mays*),[1] tomato (*Solanum lycopersicum*),[2] cucumber (*Cucumis sativus*),[3] and soybean (*Glycine max*),[4] among others. Although the responses are varied, all these reports have shown that CeO_2 NPs can be taken up by the roots, affecting plant growth in different ways.[1-6] These investigations greatly contributed to better understanding the effects of CeO_2 NPs on edible plants. However, the effects of CeO_2 NPs in vegetable gardens and herbs are not well known. Wang et al.[2] reported that these NPs at 1 -10 mg L^{-1} concentrations affect fruit production in tomato plants. These researchers also reported the presence of CeO_2 NPs in the fruits of tomato exposed to the CeO_2 NPs spiked to growth media. However, no reports were found about the effects of CeO_2 NPs in herb or their effects in the antioxidant capacity of plants.

Reactive oxygen species (ROS) are molecules containing oxygen; these molecules are important in cell processes like cell signaling.[7] Over production of these molecules due to stress could damage cells. The presence of ROS molecules is associated with the oxidative degradation of lipids.[8] Multiple human diseases like certain types of cancer[9] and Parkinson diseases are associated with excess of ROS molecules.[9] Herbs are rich in essential elements and contain compounds with high capacity to remove ROS molecules. Phenolic compounds are very important metabolites that help to delay the oxidative degradation of lipids by neutralizing ROS molecules.[8] Flavonoids and phenolic acids are some of the major constituents in phenolic compounds.[10] Flavonoids are natural antioxidants in plants and play a similar role like phenolic acid against ROS. They possess multiple hydroxyl groups that have high affinity for peroxyl radicals, reactive intermediates in the oxidation of biological materials.[11] In general, phenolic

compounds are involved in multiple redox reaction acting as electron donors; thus, neutralizing free radicals.[12] Radicals molecules like the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation are used to quantify the scavenging capacity of antioxidant compounds in plants. The ability of antioxidant compounds to neutralize the DPPH• radicals and $ABTS^{\bullet+}$ radical cation is measured in the DPPH and ABTS scavenging assays.

Important nutrients that plants need are designated as micronutrients and macronutrients (also called micro- and macroelements). Micronutrients are those that are needed in trace amounts and macronutrients those that are needed at high levels. Antioxidant activities of plants have also been found related to their nutrient content.[13] For instance, S that is a macronutrient, is present in cysteine, amino acid that helps in the formation of key compounds in cells antioxidant defense.[14] Si is another micronutrient related with the antioxidant defense in plants.[15] Additionally, Zn participate in multiple enzymatic reaction in the cells.[16]

Aromatic herbs such as cilantro (*Coriandrum sativum*) are known for their antioxidant capacity. Cilantro, which is worldwide consumed, is so rich in polyphenolic compounds that is considered a medicinal plant[17-21] Cilantro also contains important micro- and macronutrients that are vital to human health.[22]

A few studies on the uptake, translocation, biotransformation, and plant stress related to CeO_2 NPs have been conducted;[1-4] however, to the author's knowledge, this is the first report on the effects on CeO_2 NPs on the antioxidant properties of culinary herbs. Thus, the objective of this research is to assess the antioxidant capacity of cilantro grown in organic soil treated with CeO_2 NPs at five different concentrations. Antioxidant capacity was examined by DPPH radical and ABTS radical cation assays. The effect of CeO_2 NPs on nutrient uptake by cilantro plants

was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP- OES).

METHODOLOGY

CeO_2 NP suspensions, soil preparation and seed germination. CeO_2 NP suspensions, soil preparation and seed germination followed the methods described in chapter 2.

Plant sample preparation. After 30 days, cilantro plants were harvested and rinsed with 5% $CaCl_2$ solution and MPW to remove any excess nanoparticles remaining on plant surface. Plants were separated into roots and shoots, and lyophilized at -30 °C. After 72 h, the dehydrated plants were weighed and subsequently analyzed.

Preparation of extracts for analysis of antioxidant property. The lyophilized plant tissues (100 mg) were added with 2 mL 80% methanol and placed in a rocker for 12 h. The samples were then centrifuged at 5000 rpm at 4°C for 10 min and the supernatant was recovered. The extraction process was repeated using 1 mL 80% methanol 1 h. The extracts were collected and evaporated to 1 mL before adjusting the volume to 2 mL using MPW.

Total phenolic and flavonoid content determination. Total phenolic content was determined according to the Folin-Ciocalteu's method[23] with some modifications. Briefly, 0.05 mL of each extract was placed into a UV plate, and sequentially added with 0.1 mL Folin-Ciocalteu's regeant and 0.205 mL 5% Na_2CO_3 . After 2 min, the absorbance was recorded at 760 nm using a UV/Vis spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany). Gallic acid was used as a standard, and total phenolic content was expressed as gallic acid equivalents (GAE).

Total flavonoid content was analyzed following the method described by Jia et al.[24] Each sample (500 uL) was mixed with 5% $NaNO_2$ (75 uL), and left to stand at room temperature for 5 min. Then the mixture was sequentially added with 150 uL 10% $AlCl_3$, 500 uL 1 M NaOH, and

275 uL MPW. The absorbance was recorded at 510 nm. Catechin was used as a standard, and total flavonoid was expressed as catechin equivalents (CE).

Scavenging ability by DPPH radical assay. The DPPH scavenging ability of cilantro was analyzed according to Williams et al.[25] Each sample (200 μL) was mixed with 2.8 60 μM DPPH, and kept in the dark for 30 min at room temperature. The absorbance was measured at 515 nm, and BHT was used for comparison. The scavenging ability was calculated using the formula: scavenging ability (%) = $[(Abs_{511nm\ of\ control} - Abs_{511nm\ of\ sample}) / Abs_{511nm\ of\ control}] \times 100$.

Scavenging ability by ABTS radical cation assay. ABTS radical cation scavenging ability of cilantro was determined following the method of Arts et al.[26] The sample (30 μL) was mixed with 3 mL ABTS radical cation solution, and left to stand at the dark for 7 min at room temperature. The absorbance at 734 nm was recorded and BHT was used for comparison. ABTS radical cation solution was prepared according to the method previously described byArts et al.[32] The scavenging ability was calculated using the formula: scavenging ability (%) = $[(Abs_{7341nm\ of\ control} - Abs_{734nm\ of\ sample}) / Abs_{734nm\ of\ control}] \times 100$.

Multielemental Analysis. Elemental analysis was performed according to the method described on chapter 2.

Statistical Analysis. The Data shown are means ± SE and was analyzed by one-way ANOVA, Tukey's HSD test was used for all of the analysis. The significance was determined at $p \leq 0.05$. Pearson correlation was obtained with Statistical Analysis Software (SAS) at $p \leq 0.1$.

RESULTS AND DISCUSSION

Antioxidant Capacity. The scavenging ability of cilantro shoots treated with five different concentrations of CeO_2 NPs was measured by the DPPH radical and ABTS radical cation assays. Data from DPPH analysis of cilantro shoots shows a gradual reduction of the scavenging ability in treatments containing CeO_2 NPs (Figure 3.1). Plants from control exhibited the maximum scavenging ability (37%). Even though the scavenging ability from plants in 65.5, 125, and 250 mg CeO_2 NPs kg^{-1} treatments were not statistically different from those in the control, a decreasing tendency is clearly notable (Figure 3.1). Shoots from the highest CeO_2 NPs concentration (500 mg kg^{-1}) showed the lowest hydrogen donation ability (21%) compared with the control.

Cerium content in cilantro shoots grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1} was displayed in the Figure 2.3. Scavenging data shows that CeO_2 NPs at 500 mg CeO_2 NPs kg^{-1} soil affect hydrogen donation power of cilantro toward radicals. The data suggests that the CeO_2 NPs are reducing the scavenging capacity of antioxidant compounds in the cilantro. Unfortunately, no previous reports about this matter were found to compare. Since CeO_2 NPs are related with the over-production of ROS,[27] the data could indicate that these NPs are stressing the plants, which cause a reduction in hydrogen donation affecting the antioxidant properties of cilantro.

The ABTS radical cation assay is also used to measure the ability of complexes such as flavonoids and phenolic compounds to reduce radicals.[28] Results from ABTS assay are shown in Figure 3.2. Plants from control treatment showed the highest antioxidant capacity with a 95% scavenging ability compared with other treatments containing CeO_2 NPs. The antioxidant property of plants exposed to 62.5 and 125 mg CeO_2 NPs kg^{-1} decreased significantly (10%)

compared with that of control plants. The 250 and 500 mg CeO_2 NPs kg^{-1} treatments also reduced the antioxidant ability of cilantro plants by 8% in relation to the control. The scavenging ability of $ABTS^{\bullet+}$ radical cation of cilantro plants treated with CeO_2 NPs was significantly ($p \leq 0.05$) lower compared to control plants (no CeO_2 NPs). Lower scavenging ability results from the $ABTS^{\bullet+}$ radical cation assay can be associated with the amount of flavonoid and phenolic compounds in cilantro plant samples. Flavonoid and phenolic compounds are considered the major neutralizers of $ABTS^{\bullet+}$ radicals.[29] Results from both scavenging ability assays show a significant decrease in antioxidant capacity associated with the external CeO_2 NPs (Figure 3.2).

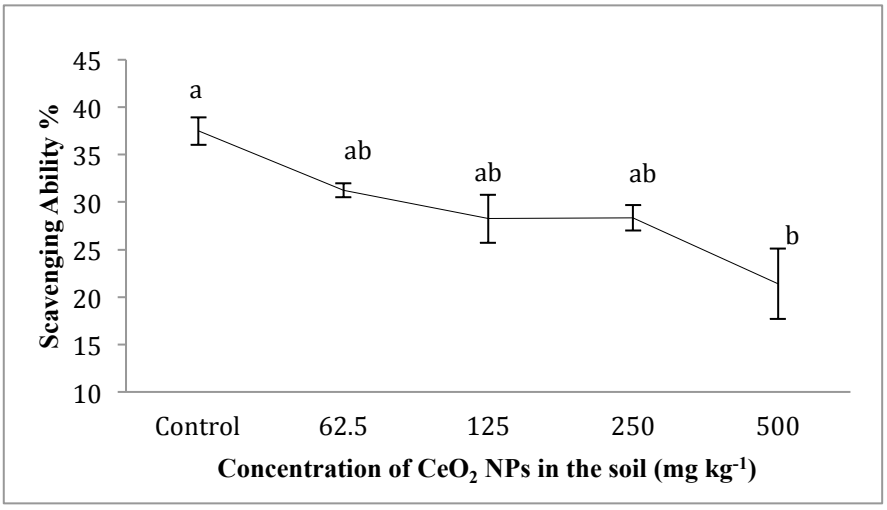

Figure 3.1. DPPH antioxidant scavenging capacity of cilantro grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters indicate statistically significant differences at ($p \leq 0.05$).

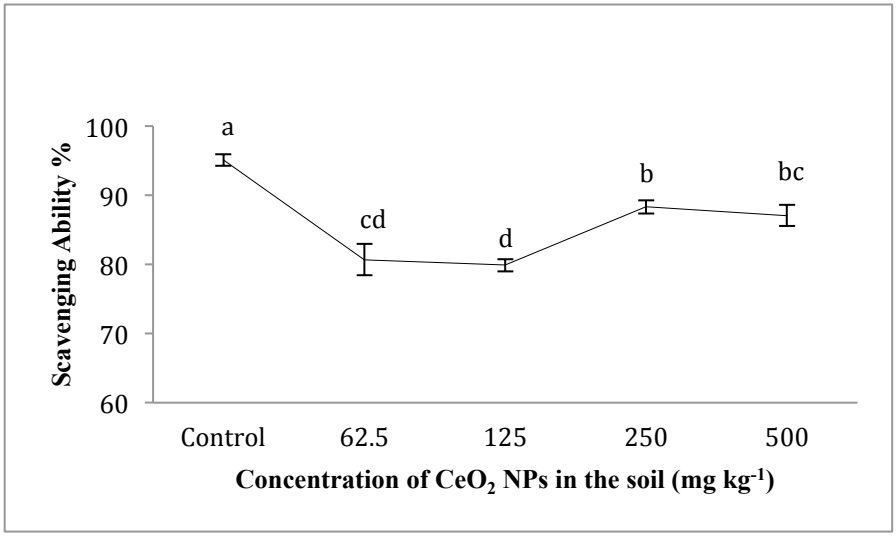

Figure 3.2. ABTS antioxidant scavenging capacity of cilantro grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters indicate statistically significant differences at ($p \leq 0.05$).

Total Phenolic Content. Phenolic compounds represent one of the most important natural antioxidants. They help to reduce free radicals and other ROS that affect healthy development of plants.[29] Phenolic concentrations in cilantro grown with CeO_2 NPs at 0 - 500 mg kg^{-1} are shown in Figure 3.3. The higher concentrations of phenolic compounds 5.959 and 5.890 mg GAE g^{-1} sample were found in control plants and plants treated with 62.5 mg CeO_2 NPs kg^{-1}, respectively (Figure 3.3). The samples from 125, and 250 mg CeO_2 NPs kg^{-1} treatments showed no significant change in phenolic concentration (5.599 and 5.537 mg GAE g^{-1} sample, correspondingly), compared to control plants. However, a reduction in phenolic concentration was observed in samples from the 500 mg CeO_2 NPs kg^{-1} (4.799 mg GAE per g^{-1}) treatment,

relative to the control (5.890 mg GAE). These results suggest that higher concentrations of CeO_2 NPs reduced the phenolic content in cilantro. This is in agreement with the results of scavenging capacity explained in the previous paragraph.

Total Flavonoid Content. Flavonoids are commonly located in the stem and leaves of plants.[31] They are important phenolic compounds with antioxidant activities.[32] Flavonoid content analysis can be used as a marker for the effects of CeO_2 NPs on the antioxidant capacity of cilantro shoots. The flavonoid content in cilantro shoots treated with CeO_2 NPs is displayed in Figure 3.4. Control and plants treated with 62.5 - 250 mg CeO_2 NPs kg^{-1} treatments, had similar (1.381-1.514 mg CE g^{-1} sample). However, a statistically significant difference was found between control and 500 mg CeO_2 NPs kg^{-1} treated samples (1.381 and 1.118 mg CE g^{-1} sample, respectively) ($p \leq 0.05$). These results show that the concentration of cerium reduced flavonoid content in cilantro shoots (Figure 3.4). Thus, the antioxidant capacity of cilantro can be affected by the CeO_2 NPs at 500 mg CeO_2 NPs kg^{-1}.

As previously mentioned, phenolic compounds are main natural antioxidants, and cilantro plants possess a significant content of these metabolites. In other studies, a correlation between total phenolic content and the scavenging ability of plants has been reported.[33] Figure 3.5 shows a statistically significant Pearson's correlation (-0.754) between cerium concentration and scavenging capacity of antioxidant compounds in cilantro. Results indicate that cerium content in cilantro tissues reduce the scavenging capacity of antioxidant compounds in these plants.

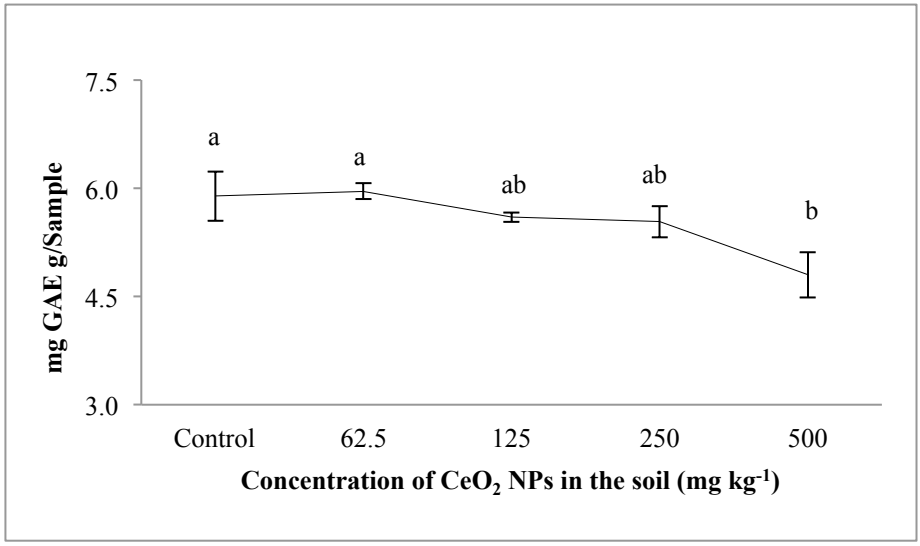

Figure 3.3. Total phenolic content in cilantro grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters indicate statistically significant differences at ($p \leq 0.05$).

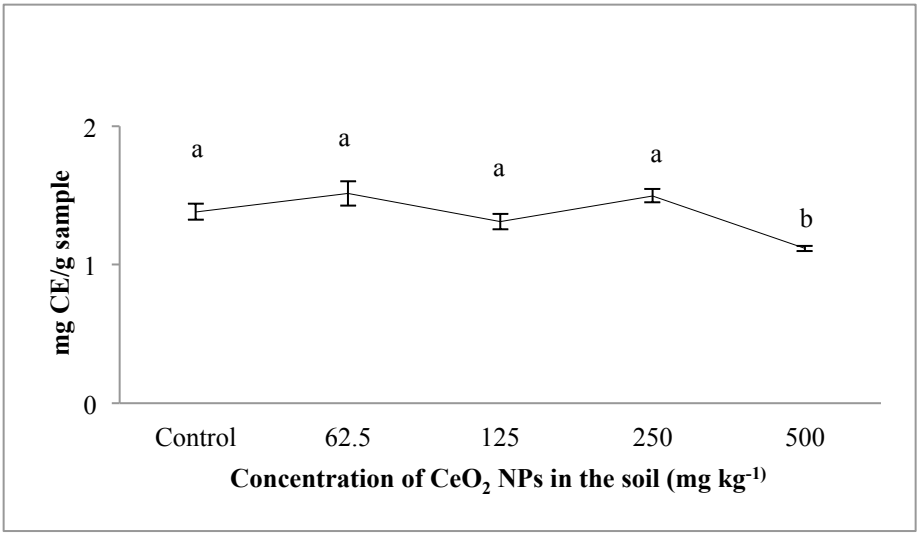

Figure 3.4. Total flavonoid content in cilantro grown for 30 days in potting soil treated with CeO_2 NPs at concentrations varying from 0 to 500 mg kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters indicate statistically significant differences at ($p \leq 0.05$).

Multielemental Analysis. Plants provide important minerals to humans. The uptake of all these elements in cilantro plants exposed to CeO_2 NPs was analyzed by ICP-OES. Table 3.1 presents the amount of micro and macro-elements (S, Si and Zn) in cilantro shoot tissues where a statistically significant difference was found. Other elements were also analyzed but there were no differences among the concentrations in control and NP treated plants.

Sulfur Uptake. Sulfur is considered a macronutrient that is very important in many biochemical processes. The sulfide (S^{2-}) form is the most important speciation of sulfur to overcome metabolic processes in cells.[34] Hence, the right amount of sulfur in an organism is critical. Table 3.1 displays the content of S found in cilantro shoot tissues treated with CeO_2 NPs at different concentration. As seen in this table, all CeO_2 NPs treatments significantly reduced sulfur concentrations lower ($p \leq 0.05$) compared with the control. S content varied from ~1080 in control to ~740 mg kg^{-1} in plants treated with 500 mg CeO_2 NPs kg^{-1}, respectively ($p \leq 0.05$). Metabolites like glutathione, an important antioxidant in plant cells, contain S. This is also found in cysteine and methionine that are very important amino acids. Cysteine is a source of elemental S in the formation of Fe-S clusters that also play an important role in antioxidant defenses.[14] Inorganic S is taken up by plants and integrated into amino acids such as cysteine and methionine. S is found in the human body in many antioxidant molecules such glutathione, mercaptopronylglysine, N-acetylcysteine and others.[35] Thus, this suggest that all concentration of CeO_2 NPs tested significantl reduced y ($p \leq 0.05$) the nutritional value of cilantro plants.

Silicon Uptake. Si is stored in the leaves, stems, and hulls of plants. It is also an important component of plant fibers. It helps plants to deal with biotic and abiotic stresses.[36] Table 3.1 shows the amount of Si in cilantro shoots treated with CeO_2 NPs at five different concentrations (0 - 500 mg kg^{-1}). Si content followed a similar trend than S content at different CeO_2 NP

treatments. Si concentration in control shoots (~917 mg kg^{-1}), was statistically higher ($p \leq 0.05$) compared to the 125, 250 and 500 mg CeO_2 NPs kg^{-1} treatments (~204, 160, and 160 mg kg^{-1}, respectively) (Figure 2.3). Lower content of Si in cilantro tissues from treatments with CeO_2 NPs can be associated with the reduction of antioxidant compounds on cilantro plants, since Si is associated with the production of polyphenolic compound as a stress response mechanism, and in cell wall support as plants response to diseases.[15]

Zinc Uptake. Zn is present as Zn(II) in many enzymatic complexes such us carbonic anhydrase and alcohol dehydrogenase, and participates in multiple enzyme activation in plant cells.[37] Thus, Zn is an essential micronutrient for plants, humans, and animals.[16] In this research, elemental analysis showed a reduction of Zn in cilantro shoot tissues from some treatments containing CeO_2 NPs (Table 3.1). The Zn concentration in cilantro shoots was significantly lower in 250 and 500 mg CeO_2 NPs kg^{-1} treatments, compared with control plants. Concentrations in control and 500 mg CeO_2 NPs kg^{-1} were ~33 and ~24 mg kg^{-1}, respectively (Table 3.1). Reduction of Zn in cilantro tissues is linked with production of ROS, since zinc promotes superoxide dismutase activity that help organism to deal with ROS.[16]

The findings of this research have shown that CeO_2 NPs reduce the concentration of important elements such as S, Si, and Zn involved in the antioxidant capacity of the plants.[14-16] Low S and Zn concentrations increase oxidative stress in plants;[14, 16] while low Si is associated with reduction of polyphenolic compounds.[15] Therefore, these results indicate that the CeO_2 NPs affect the antioxidant capacity of cilantro. To the author's knowledge, there are no other studies about the effect of CeO_2 NPs on the antioxidant capacity and nutrient content in plants, thus a comparison of these results with other studies is not possible.

Table 3.1. Element content in cilantro shoots grown in potting soil treated with 0-500 mg CeO_2 NPs kg^{-1}. Data are means of three replicates ± SE (standard error). Different letters among columns indicate statistically significant differences in element content in each treatment at ($p \leq 0.05$).

Treatments mg CeO_2 NPs kg^{-1}	Elemental Uptake mg kg^{-1} d. wt.		
	S	Si	Zn
0	1079.555±52.957a	916.774±6.010 a	32.584±1.442a
62.5	909.520±6.539b	905.574±10.856a	28.806±1.604ab
125	933.827±8.610b	204.30±34.172b	28.419±0.666ab
250	877.138±5.184b	159.603±10.129b	25.715±1.689b
500	739.995±28.215c	201.149±1.943b	24.274±0.567b

Chapter 4: General Conclusions

The results from this research revealed that cilantro plants grown in organic soil are able to take up and translocate Ce from the roots to the shoots. Although the Ce concentrations found in shoots were relatively low, previous results indicate that the CeO_2 NPs are very stable within plant tissues and most of them will remain as CeO_2 NPs.[4,5] This suggests that cilantro plants could potentially deliver CeO_2 NPs to the food chain. However, no changes in functionality of the components of the macromolecules were observed. The results from antioxidant capacity showed that CeO_2 NPs concentration in cilantro plants is inversely proportional to the content of antioxidant compounds of the plant. These results also showed that CeO_2 NPs reduce the S, Si, and Zn content in cilantro shoots. More studies are required to determine if cilantro plants are able to take up CeO_2 NPs through the foliage; in this case, cilantro plants could be contaminated with CeO_2 NPs released from car exhausts, for example, posing a potential threat to human health. These findings have brought important understanding about the possible effects of CeO_2 NPs on cilantro; however, more studies are needed to elucidate the mechanisms involved in the change of antioxidant capacity and nutrient content in cilantro plants exposed to CeO_2 NPs.

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Curriculum Vita

Maria Isabel Morales was born in Ciudad Juarez, Mexico, and had lived there until 2008. When Maria was in high school she developed a special interest in science classes, especially chemistry and biology. Afterwards, She decided to study chemistry at the Universidad Autonoma de Ciudad Juarez (UACJ), where she maintained an average of 9 (on a 1 to 10 scale), as well as maintained a merit scholarship throughout her enrollment. After that she had the opportunity to attend the University of Texas at El Paso (UTEP), where she completed her bachelor's and master's degree in chemistry. Throughout her master's degree she worked with Dr. Jorge Gardea-Torresdey in the toxicity assessment of cerium oxide nanoparticles on cilantro *(Coriandrum sativum)* in order to understand the fate, transport, and exposure of engineered nanomaterial properties in plants. Maria is going to attend Texas A&M University to pursue a Ph.D. degree in the area of biochemistry.

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