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Effects Of Citric Acid Coated And Uncoated Cerium Oxide Nanoparticles In Tomato (Solanum Lycopersicum) Plants

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EFFECTS OF CITRIC ACID COATED AND UNCOATED CERIUM OXIDE NANOPARTICLES IN TOMATO (*SOLANUM LYCOPERSICUM)* PLANTS

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Dedication

This thesis is dedicated to my parents, for their unconditional love and support.
For their guidance, encouragement and for teaching me that any accomplishment, no matter how small, is another step towards success.
I admire and love you.

I also dedicate this to my siblings, for making me a better teacher and guide.
Because even with all the hustle and hard times, they always bring light.

EFFECTS OF CITRIC ACID COATED AND UNCOATED CERIUM OXIDE NANOPARTICLES, BULK CERIUM OXIDE, CERIUM ACETATE AND CITRIC ACID IN TOMATO (*SOLANUM LYCOPERSICUM L.)*

by

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THESIS

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Chapter 1: Introduction

Nanomaterials (NMs) have a width, length and/or height ranging between 1 and 100 nm. When a particle has at least two dimensions between this range, it is considered a nanoparticle (NP) (ASTM, 2012). The main feature of these novel materials is their "nano-size" that brings their unique physicochemical characteristics. Remarkable properties of NPs include higher reactivity due to the large surface area to volume ratio, enhanced electrical conductivity, and strength, among others, in comparison to homologous materials of conventional size (Klaine et al., 2008; Peralta et al., 2011; Hong et al., 2013).

Cerium (Ce) belongs to a family of elements commonly referred to as the rare earth elements (REEs) or lanthanides. Usually REEs form oxide or phosphate complexes (Kabata-Pendias and Pendias, 1992) and are readily available in the Earth's crust. In nature, Ce can be found in a trivalent (Ce^{3+}) or tetravalent (Ce^{4+}) state and it has diverse applications. The interactions of REEs with plants is still not well understood. The application of Ce in soils as a fertilizing agent is of concern (Pang et al., 2001). In China, Yuan et al. (2001) reported that "Changle", a Ce-containing fertilizer, enhanced root growth in rice (*Oryza sativa*). Conversely, Diatloff et al. (2008) stated that Ce, at concentrations higher than 5µM, reduced corn (*Zea mays*) and mungbean (*Vigna radiata*) root elongation.

At nanoparticle level, cerium oxide (CeO_2) has been found to reach crop plants via intentional exposure (Servin and White, 2016). Cerium oxide nanoparticles (NPs) or nanoceria ($nCeO_2$) are one of the most produced metal-oxide NPs, with an estimated annual production of 10,000 metric tons (Lazareva and Keller, 2014). Some applications include catalysts (Reed et al., 2014), polishing agents, UV-coatings, and others (Piccino et al., 2012). These uses suggest that $nCeO_2$ can be widely dispersed in the environment mainly through the air, water, or deposited in

soils. Coatings in NPs are an emerging application to modify the surface and procure more stability. However, the effects that coated NPs have in crops are still unknown. The widespread use of these materials and their release to the environment will inevitably have an impact on organisms, especially plants.

A previous study by Lopez-Moreno et al. (2010) shows that $nCeO_2$ at 2000 mg/L, promoted root elongation in cucumber (*Cucumis sativus*) and corn, but reduced germination rate in tomato (*Solanum lycopersicum* L.), corn, and cucumber. In cilantro (*Coriandrum sativum* L.), Morales et al. (2013) reported conformational changes in macromolecular composition when plants were exposed to 0-500 mg/kg $nCeO_2$. Some of these changes include alterations in lipids, amide, lignin, and carbohydrates. These alterations may cause modifications in the food quality. Wang et al. (2013) reported that second generation seedlings treated with $nCeO_2$ at 10 mg/L, reduced biomass, water transpiration and increased the reactive oxygen species (ROS) content. Similarly, Rico et al. (2015) described that the grain formation was affected in barley (*Hordeum vulgare L.*) when exposed to $nCeO_2$ at 500 mg/kg. The effects observed in the interactions between the $nCeO_2$ and the plants are variable. The outcome depends on the crop species, the NPs concentration, and growth stage of the plants (Gardea-Torresdey et al., 2014). Nevertheless, Hernandez-Viezcas et al. (2013) reported that the majority of the $nCeO_2$ taken up by soybean (*Glycine max*) was stored without biotransformation in the seeds. This finding suggests that nanoceria can translocate into the fruit/grain of plants and, therefore, enter the food chain. There is a limited number of studies on fully mature fruit/grain producing plants and the effects that NPs have in the physiology, biochemistry, yield, and nutritional properties of edible harvests (Gardea-Torresdey et al., 2014).

Tomatoes are the second most produced crop in the United States (U.S) and the eleventh worldwide (FAOSTAT, 2012). As seen in Figure 1.1, China is the main tomato producer

(50,000,000 MT) followed by India (17,500,000 MT) and the U.S. (13,206,950 MT). In the U.S., Florida and California usually account for at least two-thirds of all the commercially available tomatoes produced each year (ERS USDA, 2016). This vegetable is rich in calcium, iron, magnesium phosphorous, potassium, sodium, and zinc. Also, it has a high water content and is a source of carbohydrates like sugars, starch, and fiber, as well as many vitamins (USDA, 2013). The red color is a characteristic trait given to tomatoes by lycopene. When consumed, this phytochemical usually acts as an antioxidant and diminishes the free radicals in the body (USDA, 2010).

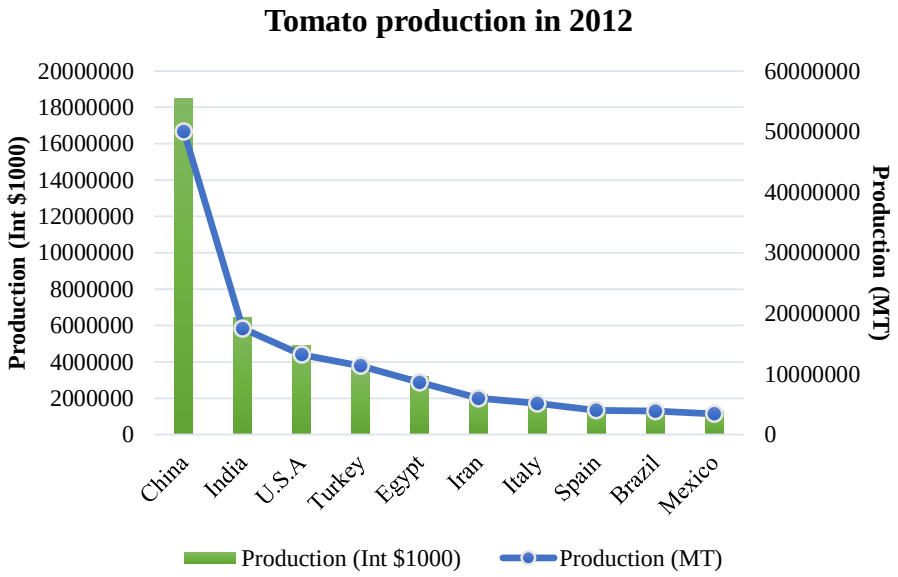

Figure 1.1 World's tomato production in thousand International dollars (Int $1000) and metric tons (MT) in 2012. Data retrieved from FAOSTAT, 2012

Due to the importance of tomato in the dietary needs of many organisms, it is critical to examine its interactions with ENMs. A review by Gardea-Torresdey et al. (2014) reported that by 2014, only 30 studies included the effects of ENMs on fully grown plants throughout their life cycle. Interestingly, only five were about $nCeO_2$. In this study, the research was conducted in two parts. Part I encompassed the development, chlorophyll content, antioxidant analysis, and Ce, aluminum, and nutrient accumulation in the different plant tissues (root, stem, leaf). The plants were grown in a greenhouse (14-h photoperiod, 25/20°C day/night temperature, 70% relative humidity) during a 210-day period. Part II involved the harvesting of the fruits throughout the 210 days of study, and later, agronomical parameters, carbohydrate composition, nutrient accumulation, and lycopene content were reviewed.

Hypothesis

This research project was done under the hypothesis that citric acid coated cerium oxide NPs ($nCeO_2$ + CA) affect in a different way than bare $nCeO_2$ the physiological functions and biochemical composition of tomato plants. The hypothesis was tested throughout the life cycle of the plants.

Research Objectives

The general objective was to study the effects of $nCeO_2$ + CA and $nCeO_2$ in the physiology and biochemistry of fully matured tomato plants.

Specific Objectives

The specific objectives were to:

- Determine the Ce and nutritional elements uptake within the plant tissues and fruits
- Analyze the effects of $nCeO_2$ + CA and $nCeO_2$ in the antioxidant capacity and chlorophyll content in tomato leaves
- Evaluate the fruit's carbohydrate and lycopene content

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Chapter 2: Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants[1]

ABSTRACT

Little is known about the physiological and biochemical responses of plants exposed to surface modified nanomaterials. In this study, tomato (*Solanum lycopersicum* L.) plants were cultivated for 210 days in potting soil amended with uncoated and citric acid coated cerium oxide nanoparticles ($nCeO_2$, CA+ $nCeO_2$) bulk cerium oxide ($bCeO_2$), and cerium acetate (CeAc). Millipore water (MPW), and citric acid (CA) were used as controls. Physiological and biochemical parameters were measured. At 500 mg/kg, both the uncoated and CA+ $nCeO_2$ increased shoot length by ~9 and ~13%, respectively, while $bCeO_2$ and CeAc decreased shoot length by ~48 and ~26%, respectively, compared with MPW ($p \leq 0.05$). Total chlorophyll, chlo-*a*, and chlo-*b* were significantly increased by CA+ $nCeO_2$ at 250 mg/kg, but reduced by $bCeO_2$ at 62.5 mg/kg, compared with MPW. At 250 and 500 mg/kg, $nCeO_2$ increased Ce in roots by 10 and 7 times, compared to CA+ $nCeO_2$, but none of the treatments affected the Ce concentration in above ground tissues. Neither $nCeO_2$ nor CA + $nCeO_2$ affected the homeostasis of nutrient elements in roots, stems, and leaves or catalase and ascorbate peroxidase in leaves. CeAc at 62.5 and 125 mg/kg increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg/kg, increased Ca in stems (84% and 86%, respectively). On the other hand, $bCeO_2$ at 62.5 increased Zn (152%) but reduced P (80%) in stems. Only $nCeO_2$ at 62.5 mg/kg produced higher total number of tomatoes, compared with control and the rest of the treatments.

[1]Reprinted with permission from Barrios, A.C., Rico, C.M., Trujillo-Reyes, J., Medina-Velo, I.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci. Total Environ. (2016), http://dx.doi.org/10.1016/j.scitotenv.2015.11.143

Besides the effect on chlorophyll production, there were no clear differences in the physiological and biochemical effects of uncoated and CA + $nCeO_2$ on tomato plants. Moreover, surface coating reduced the Ce uptake by roots but did not have an effect on its translocation to the aboveground plant parts. In addition, there was no clear effect of surface coating on the fruit production of tomato plants. To our knowledge, this is the first study comparing the effects of coated and uncoated $nCeO_2$ on tomato plants.

Keywords: Cerium oxide nanoparticles; surface coating; tomato plant life cycle; fruit production

2.1 INTRODUCTION

Cerium oxide nanoparticles (NPs) or nanoceria ($nCeO_2$) are amongst the top 10 nanomaterials produced worldwide (Keller and Lazareva, 2014). Similar to the bulk cerium, these nanoparticles (NPs) are mainly used in the automotive industry as catalysts or in electronics and optics. Keller and Lazareva (2014) estimated that in 2010, the global production of $nCeO_2$ reached 10,000 tons of which 100 ended in air, 300 in water and 1,400 in soil. Engineered nanomaterials (ENMs) including $nCeO_2$, have several applications; however, the uncoated forms tend to aggregate and overgrowth, which limit their performance. To improve their stability, ENMs are surface capped with several materials (Niu and Li, 2014). Citric acid (CA) is a common coating agent due to its stability and availability (Masui et al., 2002; Chanteau et al., 2009; Liu et al., 2012). However, coating molecules change the surface chemistry and interaction of ENMs with the environment (Chanteau et al., 2009).

Previous studies have shown that $nCeO_2$ have the potential to alter the physiology and biochemistry of plants. However, there is a lack of uniformity in the reported results and none of the parameters seem to be affected in the same manner when there are variations in species, growth media, and treatment concentration. Lopez-Moreno et al. (2010a) exposed $nCeO_2$ to several seeds

in liquid medium and found that at 2000 mg/L, $nCeO_2$ reduced the germination of tomato (*Solanum lycopersicum*), corn (*Zea mays*), and cucumber (*Cucumis sativus*). Lopez-Moreno et al. (2010a) also reported an increase in cucumber and corn root seedling elongation but a reduction in alfalfa and tomato root length. On the other hand, Ma et al. (2010) reported that at 2000 mg/L, $nCeO_2$ reduced the root elongation in lettuce but not in tomato, radish (*Raphanus sativus*), wheat (*Triticum aestivum*), cabbage (*Brassica oleracea*), cucumber, and rape (*Brassica napus* L.).

A complete assessment of the effects of $nCeO_2$ on plants is difficult due to the lack of studies covering the entire life cycle. A review of current literature reported that by 2014, only 30 studies covered the effects of ENMs over the full life cycle of plants (Gardea-Torresdey et al. 2014). Of those, only five were about $nCeO_2$. Wang et al. (2012) exposed tomato in potting soil to consecutive applications of $nCeO_2$ suspension at 10 mg/L. These researchers reported no effects on plant growth and production; however, high Ce content was found in the fruit. Morales et al. (2013) reported that at 250 mg/kg, $nCeO_2$ decreased biomass and caused conformational changes in the macromolecular composition of cilantro. Rico et al. (2013a, 2014) reported changes in essential elements and other nutritional components in rice (*Oryza sativa*) and wheat (*Triticum aestivum*) grains. Zhao et al. (2014) reported 31.3% reduction in cucumber fruit production under exposure to 800 mg $nCeO_2$/kg; Corral-Diaz et al. (2014) also exposed $nCeO_2$ (500 mg/kg) to radish and reported no effects in production but changes on the antioxidant power of radish tubers. Rico et al. (2015) reported that $nCeO_2$ increased plant biomass in *Hordeum vulgare*, but inhibition of grain formation in plants exposed to 500 mg/kg.

Several reports have also shown that $nCeO_2$ affect the activity of stress enzymes. Zhao et al. (2012b) reported that catalase (CAT) and ascorbate peroxidase (APOX) activities increased up to day 15 in shoots of corn seedlings exposed to $nCeO_2$ at 800 mg/kg soil. Rico et al. (2013b)

found a decrease in CAT activity, yet an increase in APOX activity in rice roots exposed to 500 mg $nCeO_2$/kg soil. Majumdar et al. (2014) reported a decrease in APOX in kidney bean leaves of plants exposed for 15 days to 250 and 500 mg $nCeO_2$/kg .

A few studies have shown the effects of surface coating on the interaction of ENMs with plants. Zhao et al. (2012a) reported that the uptake of Ce by corn plants exposed to alginate coated $nCeO_2$ was driven by the soil organic matter. In a more recent study, Trujillo-Reyes et al. (2013) found that the Ce uptake by radish was significantly lower in plants exposed to citric acid coated $nCeO_2$, compared to uncoated NPs. Continuous increments in the applications of coated CeO_2 NPs increase the chances for their build up in the environment, which could result in unpredicted effects on crop plants. In addition, Hernandez-Viezcas et al. (2013) have shown that $nCeO_2$ taken up by crop plants are stored without changes in plant organs. Tomatoes are berry-type fruits widely consumed in raw form. Thus, they could become a carrier of $nCeO_2$ into the food chain.

In this research, effects of Ce compounds/NPs on the growth, fruit production, uptake of Ce and essential elements, as well as chlorophyll content and the activity of CAT and APOX enzymes were measured in fully developed tomato plants.

2.2 Materials and Methods

2.2.1 Preparation of nanoparticle suspensions and other treatments

Uncoated CeO_2 NPs ($nCeO_2$) (10 nm, Meliorum Technologies, Rochester, NY) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to a previous characterization (Keller et al., 2010), these $nCeO_2$ have primary size of 8 ± 1 nm, particle size of 231± 16 nm in DI water, surface area of 93.8 m^2/g, and 95.14% purity. Citric acid coated CeO_2 NPs (CA+$nCeO_2$, 1:2 ratio) were prepared and characterized according to Trujillo-Reyes et al. (2013). Enough particles were suspended in

an 8:2 v/v water: ethanol solution to reach a 0.001 M concentration. Nanoparticles were sonicated (Crest Ultrasonics, Trenton, NJ) in a water bath for 60 minutes at 20°C with a sonication intensity of 180 watts. Another 8:2 v/v water: ethanol solution was prepared with enough citric acid to reach a concentration of 0.002 M. The reaction was adjusted to pH 7-8 with a 3M NaOH solution. Both solutions were mixed and maintained in reflux for 3 hours. At last, ethanol evaporated, and the coated NPs were oven dried at 65°C for 24 hours. Suspensions/solutions of NPs or compounds including $nCeO_2$, CA+$nCeO_2$, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA) were prepared with MPW in order to add to each pot 0, 62.5, 125, 250 and 500 mg/kg of the respective compound. Each pot was irrigated with 450 ml of the corresponding suspension/solution. These concentrations were selected after Rico et al. (2013b). The calculations were done according to the amount of potting soil used per pot (~450 g). Suspensions were stirred and sonicated for 30 min to avoid aggregation before homogeneous mixing with the soil.

2.2.2 Seed Germination and plant growth

Seeds of tomato (*Solanum lycopersicum*), Roma variety, were purchased from Del Norte Seed & Feed (Vinton, TX). Seeds were placed in a beaker with MPW and stirred for 3 hours until hydrating. One thousand, six hundred and eighty grams of Miracle-Gro® organic potting mix were separated, put in a glass container, and mixed with the Ce treatments. A brief description of the Miracle-Gro® is shown in Table S9 of the supplementary data. Four hundred and twenty grams of the Ce amended soil and control soil were placed in each pot, creating four replicates per treatment, except the MPW control that had 16 replicates, four for each Ce compound/NP. The soil was left for 24 hours for conditioning before planting.

For germination, seeds of approximately the same size and aspect were selected. Five seeds per replicate/treatment were used. The seeds were placed about 2.5 cm deep in the soil and watered

with 100 mL of MPW every day. Pots were placed in a greenhouse with 14-h photoperiod, 25/20°C day/night temperature, 70% relative humidity under light intensity of 340 µmol m^{-2} s^{-1}. The seed germination began on the third day and the stem length was recorded at 15, 30, 60, 120, and 210 days after germination (DAG). At 60 days, four seedlings were removed and only the biggest plant per pot was cultivated to full maturity. The number of fruits per plant and the percent of mature fruits from day 139 to 210 after germination were also recorded.

2.2.3 Quantification of Ce, nutrients, and Al in dry plant tissues

At harvest (210 days), roots, stems and leaves were washed with a 5% $CaCl_2$ solution and rinsed three times with MPW. Samples were dried for 72 hours in an oven at 60° C, and grinded with mortar and pestle until powdered. Samples of 0.2 g of tissues were microwave acid-digested by adding 1 mL of plasma pure HNO_3 and 4 mL of 30% hydrogen peroxide in a microwave oven (MarsX, CEM Corporation Mathews, NC). The digests were diluted to 50 mL with MPW. Micro and macro nutrients, aluminum, and cerium quantification in the acidic solutions was performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 4300 DV, Shelton, CT). Blanks, spikes and standard reference materials NIST 1547 and peach leaves, (Gaithersburg, MD) were used to validate the digestion and analytical methods for Ce, Al, B, Ca, Cu, Fe, K, Mg, Mn, P and Zn. For QA/QC, ICP readings of a blank and a standard were done every 15 samples.

2.2.4 Catalase (CAT) and ascorbate peroxidase (APOX) assays

A previous study showed differential effects of the $nCeO_2$ concentrations on different stress enzymes in rice plants (Rico et al. 2013b). In this study we determined the activity of catalase (EC 1.11.1.6) and ascorbate peroxidase (EC 1.11.1.11) in leaves of 210-day old tomato plants grown

in potting soil amended with the different Ce-based compounds/NPs. Fresh leaves were washed with a 5% $CaCl_2$ solution and MPW three times to remove external contaminants. For each sample, 0.2 g of fresh leaves were grinded in a mortar and pestle and extracted with 1800 µL of a phosphate buffer solution (25 mM KH_2PO_4 at pH 7.4). Extracts were centrifuged for 10 min at -4°C and 9,600 rpm (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). The supernatants were then transferred to 2 mL Eppendorf tubes to continue with the assay. Catalase (CAT) activity was done according to Gallego et al. **(**1996). A 950 µL aliquot of 10 mM H_2O_2 was placed in a quartz cuvette, and an aliquot of 50 µL of the sample was added to obtain a final volume of 1 mL. The mixture was shaken three times by hand, and the absorbance at 240 nm was recorded for three min in a Perkin Elmer Lambda 14 UV/Vis Spectrometer (single-beam mode, Perkin-Elmer, Uberlinger, Germany). The amount of protein for CAT/APOX was determined by the fresh weight of the tissue employed.

The APOX activity was evaluated according to Murguia et al. (2004). Extract of fresh tomato leaves were prepared as described previously Rico et al. (2013b). The supernatant was separated by centrifugation. An aliquot of 4 µL of 25 mM ascorbate, 10 µL of 17 mM H_2O_2, 886 µL of 0.1 M KH_2PO_4 buffered at pH 7.4 and 100 µL of fresh leaf extract were placed in a quartz cuvette and mixed three times. The absorbance was recorded at 265 nm for 2 min in a Perkin Elmer Lamda 14 UV/Vis Spectrometer. The absorbance was recorded as described above.

2.2.5 Chlorophyll content

Total chlorophyll, chlorophyll *a* and *b* (chlo-*a* and chlo-*b*) contents were determined as per Porra et al. (2002). Fresh tomato leaf tissue was cryogenized with liquid nitrogen, and later employed for extractions. A sample of 0.5 g of leaf tissue was grinded with 80% acetone for chlorophyll extraction. The extracts were kept in a freezer at -80°C until the assay was performed.

The absorbance at 663 and 646 nm was measured using a Perkin Elmer Lamda 14 UV/Vis Spectrometer.

2.2.6 Statistical Analysis

Four replicates of each treatment concentration were allocated in a completely random design in the greenhouse facility. Data was analyzed using one-way ANOVA (PASW Statistics 18 software) and the Tukey's HSD test at $p \leq 0.05$ was used to determine statistical differences between treatment means. Data presented are mean ± standard errors (SE) of four replicates.

2.3 Results and Discussion

2.3.1 Cerium concentration in tissues

Figure 2.1 shows the cerium concentration in roots, stems and leaves of 210-day old tomato plants grown in soil amended with uncoated and coated $nCeO_2$, $bCeO_2$, and cerium acetate at 0 to 500 mg/kg. In this study, no Ce was detected in plants exposed to citric acid. As seen in Figure 2.1, the Ce accumulation in vegetative organs was affected in roots, stems and leaves in all or some of the treatments with respect to their MPW controls. In roots, there was a concentration-dependent increase of Ce that was statistically higher in plants exposed to $nCeO_2$ at 125, 250 and 500 mg/kg (~41 ± 8.1, 130 ± 18.0 and 197 ± 20 mg/kg d wt, respectively), compared to control. Moreover, the 250 and 500 mg/kg concentrations from the same compound are statistically higher, compared to 62.5 and 125 mg/kg. At all concentrations CA + $nCeO_2$, $bCeO_2$ and CeAc showed statistically higher Ce concentrations in root tissues. (Figure 2.1A). The data suggests that the concentration of Ce in roots was not associated with the solubility of the compounds. The solubility of $nCeO_2$ is 1.28 g/L (Dahle and Arai, 2015) and the solubility of CeAc is 3.5 g/L (http://www.gelest.com/goods/pdf/metalOrganicCatalog/58.pdf). The difference could be due to a

high absorption of uncoated NPs plus particles adsorbed to the root surface that were not removed by the washing process. The surface coating significantly reduced the Ce uptake by roots (Fig 1A). The Ce concentration in roots of plants exposed to coated NPs was seven times lower than in plants exposed to uncoated NPs (Table S5). This could be a result of the different interactions of coated and uncoated NPs with the root surface, due to the ζ potential of the particles. Uncoated $nCeO_2$ had a ζ potential of 20.1 ± 1.2 mV and the ζ potential of coated $nCeO_2$ was −57 ± 0.6 mV (Trujillo-Reyes et al. 2014). Thus, the negative surface charge of the root plasma membrane (Wang et al. 2014) repelled the negatively charged coated NPs. Trujillo-Reyes et al. (2014) found similar results in radish exposed to citric acid coated $nCeO_2$. Similar results were also reported by Zhao et al. (2012a) in corn roots exposed to alginate coated $nCeO_2$.

Previous studies have shown that $nCeO_2$ tend to remain in roots (Wang et al. 2012; Zhao et al. 2012a; Schawbe et al. 2013; Chichiricco and Pomma, 2015). Zhao et al. (2012a) reported that the translocation of Ce in corn plants exposed to alginate coated $nCeO_2$ was driven by the soil organic matter. These authors found that shoots of plants grown in low organic matter soil amended with 200 and 400 mg/kg of alginate coated $nCeO_2$ "had 104 and 106%, respectively, more Ce compared with plants grown in organic soil." Trujillo-Reyes et al. (2014) exposed uncoated and citric acid coated $nCeO_2$ to radish seedlings in hydroponics. Authors did not report translocation as they measured the whole seedling; however, they found 94% less Ce in plants exposed to coated NPs. In our study, none of the treatments showed high Ce translocation to the above ground tissues. In stems, Ce concentrations were, in general, < 0.8 mg/kg d wt, while in leaves were ≤ 2 mg/kg d wt (Table S5). In stems, only the $nCeO_2$ at 125 and 500 mg/kg (~ 0.67 ± 0.03 and 0.61 ± 0.07 mg/kg d wt, respectively) showed a statistical difference with respect to the MPW control (~ 0.36 ± 0.07 mg/kg d wt). Uncoated $nCeO_2$ also increased at 62.5 mg/kg in tomato leaves when

compared to the control (2.1± 0.7 and 0.9 ± 0.2 mg/kg, respectively) (Figures 2.1B-C). In addition, none of the treatments showed Ce accumulation in fruit. This result differs from the result reported by Wang et al. (2012) who reported "substantially higher Ce concentrations" in fruit of plants that were fed with 130 mg of CeO_2/kg of dry potting mix during the entire life cycle. Perhaps the difference was due to the exposure methodology. In our study, the whole amount of NPs was applied to the soil 24 hours before seeding, while Wang et al. fed the plants twice a week until harvesting with a NP suspension at 10 mg/L. In addition, the substrate we used has a high content of organic matter (50-60 percent forest products as shown in Table S9) that has been shown to bind NPs (Grillo et al. 2015).

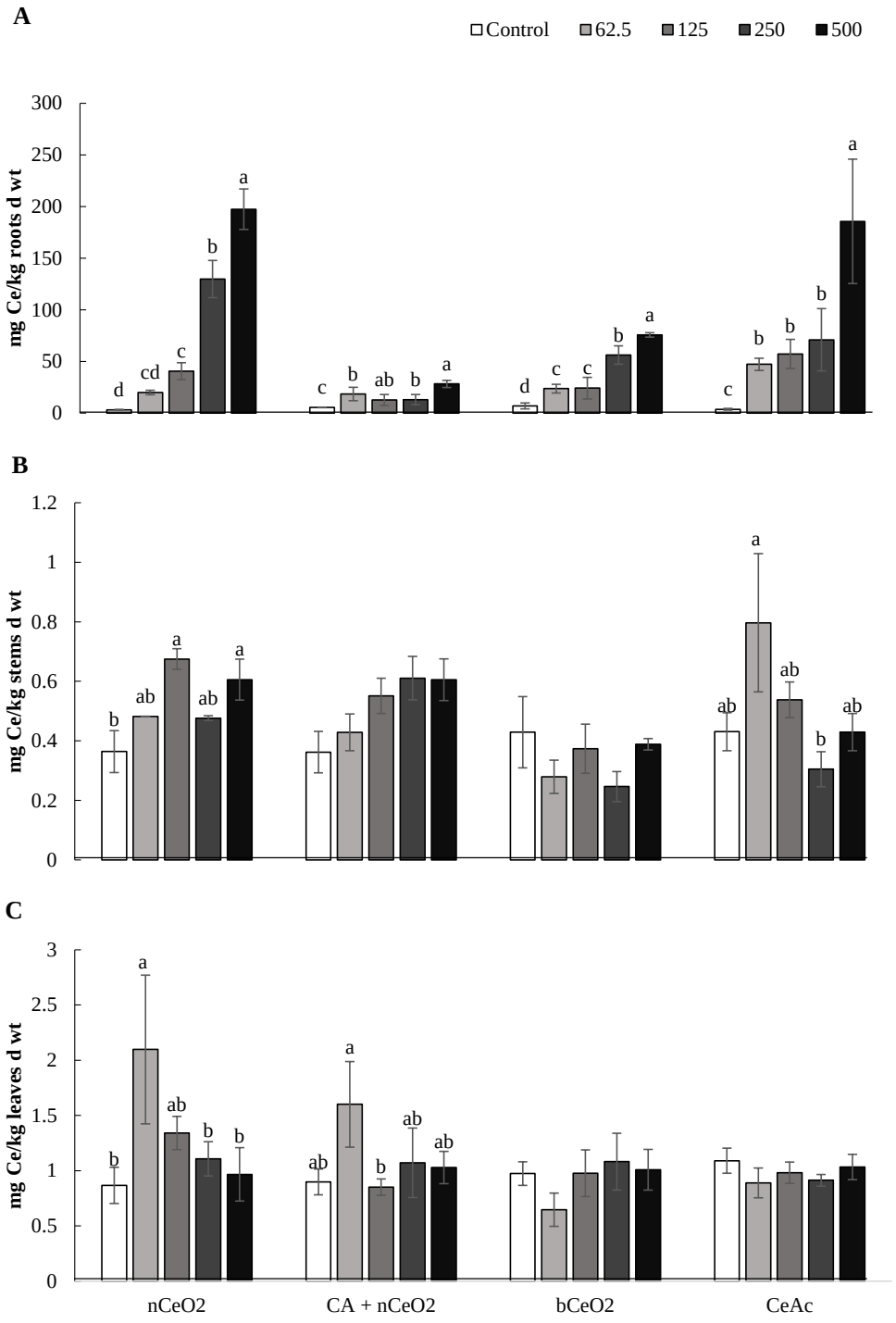

Figure 2.1 Ce concentration in roots (A), stems (B), and leaves (C) of tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between concentrations of the same treatment at ($p \leq 0.05$); n = 4. Citric acid was not included in the figure as it does not contain cerium.

2.3.2 Chlorophyll content in leaves

Figure 2.2 shows total chlorophyll, chlo-*a*, and chlo-*b* in leaves of tomato plants cultivated in soil amended with $nCeO_2$, CA + $nCeO_2$, $bCeO_2$, CeAc and citric acid (Table S6). As seen in Figure 2.2 (A-C) only the $bCeO_2$ at 62.5, 250 and 500 mg/kg affected the chlorophyll production. At 62.5 mg/kg the $bCeO_2$ treatment significantly reduced total chlorophyll and chlo-*a*, compared with control, 250 and 500 mg/kg. This suggests less production of ATP that can affect the general performance of the plants (Rabinowitch and Govindjee, 1965). However, at 250 and 500 mg/kg from bCeO2 increased total chlorophyll and chlo-*a* with respect to the MPW control. Our results concur with previous reported results with $nCeO_2$. Zhao et al. (2014, 2015) reported that $nCeO_2$ did not alter leaf net photosynthetic rate, gas exchange, stomata conductance, transpiration rate and total chlorophyll content in cucumber and corn. These results contrast with previous studies which note that nanoparticles procure a negative effect on the chlorophyll content (Perreault et al., 2010; Rico et al., 2013b; Mohammed et al., 2011; Mazumdar et al., 2014). This corroborate that the response to NPs varies with several factors, including the growth medium, the environment, and plant species. In the present study, the surface modification of $nCeO_2$ did not change the impact on chlorophyll production.

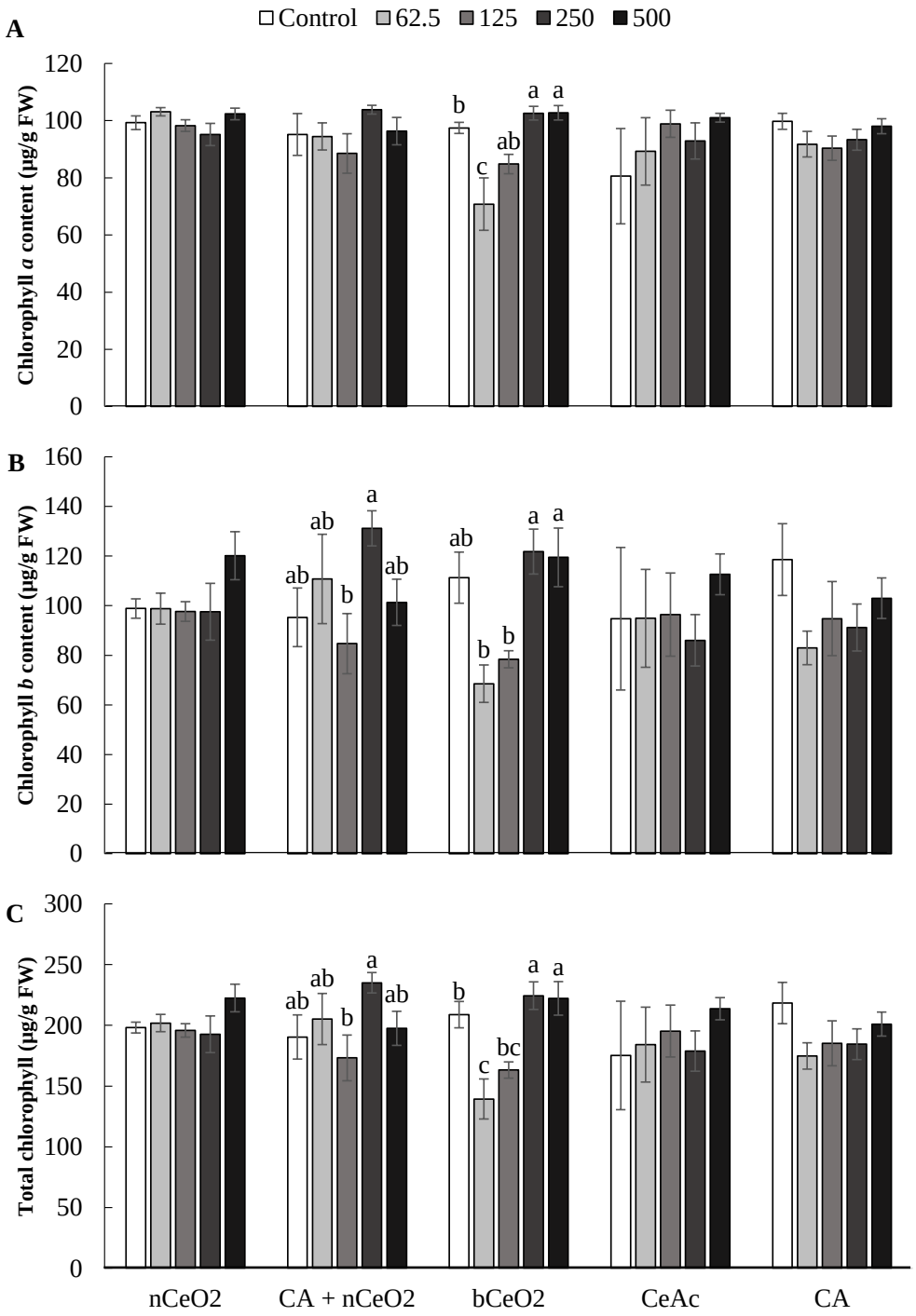

Figure 2.2 (A) Chlorophyll *a*, (B) chlorophyll *b*, and (C) total chlorophyll contents in leaves of 210 day-old tomato plants grown in soil amended with uncoated ($nCeO_2$), citric acid coated ($CA + nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of three replicates ± SE. Different letters indicate statistically significant differences between concentrations from the same treatment at ($p \leq 0.05$); $n = 3$

2.3.3 Catalase and Ascorbate peroxidase activities.

The defense mechanism of plants is sometimes activated by environmental, biological, or chemical stress. Catalase and ascorbate peroxidase are enzymes that deal with stress by fighting the reactive oxygen species (ROS) generated by the plants in the form of H_2O_2 (Panda, 2005). Figure 2.3 (A-B) shows the activity of CAT and APOX in leaves of 210-day old tomato plants. As seen in this figure, none of the concentrations of $nCeO_2$ significantly affected CAT activity, compared with control; while coated NPs at 500 mg/kg, significantly increased CAT, compared with control. It is possible that at concentrations < 500 mg/kg the plant could cope with the stress. On the other hand, the activity of APOX showed the same reduction pattern under exposure to both uncoated and coated NPs, except at 62.5 mg/kg, where coated NPs did not affect APOX activity. Differences in CAT activity on plants exposed to uncoated and coated NPs suggest that the coating reduced the CAT mimetic activity $nCeO_2$ (Pirmohamed et al., 2010). However, it seems that the coating does not reduce the peroxidase-like activity of $nCeO_2$, as APOX activity in tomato showed, in general, the same pattern.

Catalase activity was significantly reduced by $bCeO_2$ at 125 mg/kg, but surprisingly, increased at 250 and 500 mg/kg, although the difference at 500 g/kg did not reach statistical significance, compared with control. Similarly, at 125 mg/kg, CeAc significantly increased CAT activity, compared with control (Figure 2.3A). The effects of bCe and CeAc were different on APOX activity. While practically all $bCeO_2$ concentrations significantly reduced the activity, only the highest concentrations of CeAc (250 and 500 mg/kg) significantly reduced APOX activity, compared with control. In CeAc, cerium is in the trivalent state (Ce(III)), which easily binds to phosphates and hydroxides and has lower catalase mimetic activity (Pirmohamed et al., 2010), while in $bCeO_2$ it exist as both Ce(III)/Ce(IV) that has shown to have superoxide dismutase and

peroxidase scavenging activity (Rico et al., 2015). This can explain the effects of both compounds on CAT and peroxidase activities.

None of the citric acid concentrations modified CAT activity, and only at 250 mg/kg, there was a significant reduction in APOX activity, compared with control (Figure 2.3B). However, the difference was not clear as the average obtained at 250 mg/kg, overlap with the averages observed at other concentrations, and these overlapped with control. Previous reports have shown different effects of $nCeO_2$ on plants. Further comparisons between the treatments can be observed in the Table S7.Morales et al. (2013) did not report changes on CAT activity in cilantro exposed to 0 to 500 mg/kg in similar soil to the one used in this study. Zhao et al. (2012b) cultivated corn in soil amended with 400 and 800 mg $nCeO_2$/kg. These researchers reported an increase in H_2O_2, concomitant with increases in CAT and APOX, but only in 10-day old plants, suggesting that corn rapidly generated an adaptive response to the stress imposed by the NPs.

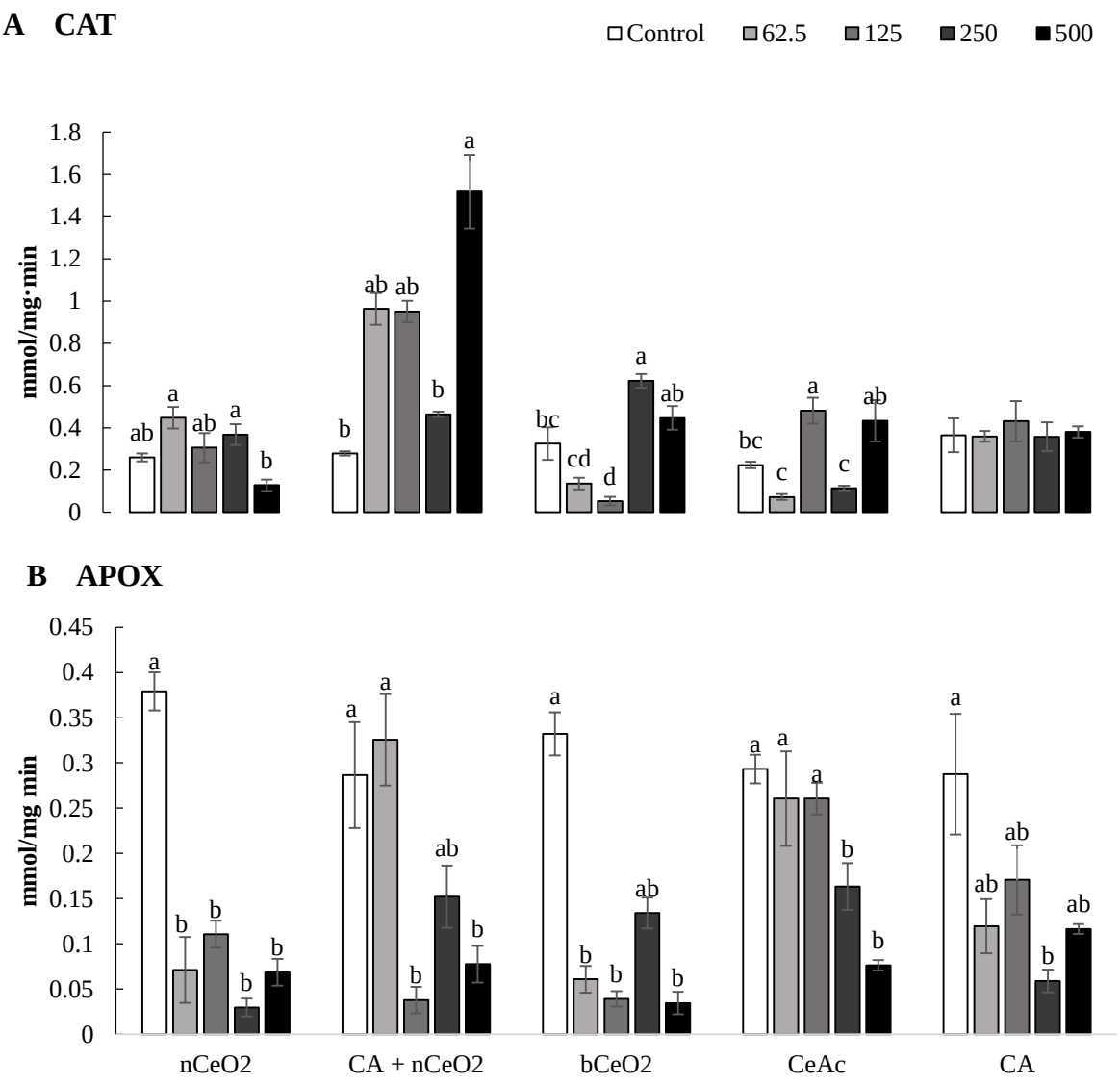

Figure 2.3 Antioxidant activity of (A) catalase and (B) ascorbate peroxidase in fresh leaves of 210 day-old tomato plants grown in soil amended with uncoated ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between concentrations from the same treatment at ($p \leq .05$); $n = 4$.

2.3.4 Nutrient element accumulation

Previous studies have shown that ENMs alter the uptake and translocation of nutrient elements. In the present study, all macro and microelements were analyzed in root, stem, leaf, and fruit (Table 2-1 and Supplementary data Tables S1-S3). However, only Al (a non-essential element), B, Ca, Fe, P and Zn showed statistically significantly differences, compared with control (Table 2-1). As seen in this table, CeAc at 62.5 and 125 mg/kg increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg/kg, increased Ca in stems (84% and 86%, respectively). On the other hand, bCeO_2 at 62.5 increased Zn (152%) but reduced P (80%) in stems. Coated nCeO_2 increased Al in roots (175%) and leaves (180%). CeAc has a Ksp of 0.35 g/100 g H_2O; this means that the acetate ion may function as a chelating agent for cations, increasing their absorption. It is not clear how the coated NPs increased Al absorption; however, in a previous study Trujillo-Reyes et al. (2013) found that citric acid coated nCeO_2 increased Al uptake by 93% in radish. Perhaps the negative surface charge of the coated NPs bound Al, facilitating its uptake. More studies are needed in order to elucidate these results.

Table 2-1 Micro- and macro- elements altered in 210 day-old tomato plants exposed to uncoated ($nCeO_2$) and citric acid coated (CA + $nCeO_2$) nanoparticles, cerium acetate (CeAc), and bulk CeO_2 ($bCeO_2$). Data are average ± SE of four replicates, except control (Millipore water) that had 16 replicates. Comparisons were made with respect to the controls and symbols + and – stand for percent of increase and decrease in nutrient concentration.

Organ	Element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%
Roots	**Al**	Control	1732.3 ± 171.6	100
		Coated NP 62.5	4760.0 ± 1122.2	174.8 +
		CeAc 62.5	5130.4 ± 820.0	196.2 +
	Fe	Control	1268.1 ± 104.2	100
		CeAc 62.5	3470.1 ± 488.9	173.6 +
	B	Control	30.5 ± 2.7	100
		CeAc 125	55.0 ± 3.2	80.5 +
Stems	**Ca**	Control	10473.5 ± 540.3	100
		CeAc 250	19295.1 ± 2587.2	84.2 +
		CeAc 500	19501.0 ± 255.82	86.2 +
	P	Control	39993.1 ± 255.8	100
		$bCeO_2$ 125	7853.1 ± 594.8	80.4 -
	Zn	Control	71.9 ± 7.7	100
		$bCeO_2$ 62.5	181.6 ± 17.5	152.5 +
Leaves	**Al**	Control	189.2 ± 13.4	100
		Coated NP 250	530.1 ± 131.8	180.2 +

2.3.5 Stem growth

Table 2-2 shows the stem length of tomato plants at 15, 60, and 210 days after germination. As one can observe in this table, both the uncoated and coated NPs affected the growth at 60 days after germination. At this stage, uncoated NPs at 62.5 and 125 and coated at 62.5 mg/kg reduced stem growth, compared with control. Mixed results were observed at 210 days; however, at 500 mg/kg uncoated and coated NPs increased stem length by 9 and 13%, respectively, compared with MPW control. The current data is not enough to explain the effects on stem elongation as, practically, the NPs did not affect chlorophyll contents and nutrient uptake. A previous study showed that $nCeO_2$ reduced radish root biomass and stem length, while citric acid coated $nCeO_2$ increased root biomass (Trujillo-Reyes et al. 2013). The results observed with coated NPs in tomato do not seem to be driven by the external citric acid that could be released by the coated NPs. Mudunkotuwa and Grassian (2010) have shown that at the pH used in this study, citric acid is fully deprotonated and tightly bound to NPs. Then, it could be due to surface modifications that interfered with other functions of the plants not analyzed in this study.

Citric acid showed no significant effect at 15 days but mixed results at 60 days, while concentrations of 250 and 500 mg/kg produced significant reduction in stem elongation at 120 days, but the plants recovered at 210 days (Table S8). Citric acid is normally synthesized by tomato plants and can protect the plants by chelating excess of elements (Table S4), or it can help the plant to uptake some elements found at low concentrations in the soil solution (Senden et al., 1995). In this study, citric acid did not increase elements in tissues, neither affect chlorophyll nor CAT and APOX. Thus, this should be the reason why at the end of the cycle, plants were not affected. Bulk cerium also showed mixed results at 30 days. However, at 120 and 210 days, all concentrations showed a consistent and significant stem reduction, compared with control and the other

treatments. At 210 days, $bCeO_2$ and CeAc decreased shoot length by ~48 and ~26%, respectively, compared with MPW ($p \leq 0.05$). Our data concurs with the data reported by Majumdar et al. (2014) who found that in red kidney bean there was a decrease in stem biomass in plants exposed to $bCeO_2$, compared to $nCeO_2$. Majumdar et al. (2014) also reported a correlation among the stress and the reduction in biomass. In tomato, $bCeO_2$ at 250 and 500 mg/kg significantly increased CAT but reduced APOX, which could be the reason for the stem growth reduction. Cerium acetate also reduced stem length in adult plants exposed to 250 and 500 mg/kg. Cerium acetate (Ce^{3+}), has shown to have superoxide scavenging activity but not catalase activity (Pirmohamed et al., 2010). Due to that, in general, CeAc did not affect CAT activity, but reduced APOX activity at 250 and 500 mg/kg. This reduction on APOX activity prevents the reduction of H_2O_2 generated by SOD into H_2O (Rico et al., 2015). Excess of H_2O_2, one of the reactive oxygen species, is translated in toxicity, which in turn reduced the growth of stems.

Table 2-2 Shoot length of 15, 60, and 210 day-old tomato plants grown in soil amended with uncoated ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between concentrations at ($p \leq 0.05$); $n = 4$.

		Control	62.5	125	250	500
$nCeO_2$	15	17.33 ± 0.30	15.94 ± 0.07	14.73 ± 1.00	16.33 ± 0.19	16.96 ± 0.74
	60	49.65 ± 0.21a	40.49 ± 0.93 b	39.55 ± 0.84 b	44.11 ± 1.94 ab	46.10 ± 0.90 ab
	210	146.31 ± 0.27 b	135.99 ± 0.03b	112.21 ± 0.15c	142.21 ± 1.30 b	162.20 ± 1.80 a
CA + $nCeO_2$	15	14.38 ± 0.22	14.57 ± 0.16	14.66 ± 0.46	16.13 ± 0.48	14.76 ± 0.04
	60	47.22 ± 1.02 a	41.66 ± 1.51 b	43.25 ± 1.05 ab	44.53 ± 0.67 ab	43.26 ± 0.43 ab
	210	145.64 ± 1.12 c	136.06 ± 2.41 d	130.09 ± 1.07 d	158.61 ± 1.85 b	168.49 ± 1.73 a
$bCeO_2$	15	13.26 ± 0.20	13.54 ± 0.87	14.48 ± 1.15	15.91 ± 0.26	16.46 ± 0.79
	60	49.76 ± 0.21 a	44.54 ± 0.75 b	39.68 ± 0.72 b	31.62 ± 0.48 c	30.57 ± 0.36 c
	210	149.19 ± 1.47 a	89.48 ± 0.55 b	83.11 ± 0.02 bc	81.55 ± 0.20 bc	78.13 ± 0.05 c
CeAc	15	15.03 ± 0.44	15.19 ± 0.18	15.11 ± 0.04	16.37 ± 0.22	15.04 ± 0.85
	60	48.68 ± 0.47	46.91 ± 0.71	45.05 ± 1.49	46.33 ± 1.12	47.75 ± 0.68
	210	144.38 ± 0.25 a	140.31 ± 0.02 ab	135.10 ± 1.16 bc	127.00 ± 0.49 c	106.31 ± 1.53 d
CA	15	15.27 ± 0.29	15.43 ± 0.09	16.28 ± 0.43	16.39 ± 0.81	16.03 ± 0.37
	60	49.33 ± 1.05 bc	40.22 ± 0.82 c	45.28 ± 0.73 ab	54.55 ± 0.30 a	52.88 ± 0.32 a
	210	144.38 ± 0.21	148.65 ± 1.47	144.74 ± 1.65	156.04 ± 0.77	156.81 ± 1.23

2.3.6 Fruit production

Table 2-3 shows the absolute number of ripe fruits collected from the tomato plants exposed to the different treatments. Although the greenhouse had good conditions for the plant to grow, the light intensity (340 µmol/m^2s) was not high enough to support good fruit production as tomatoes grow better under full light exposure. Thus, this table shows the total number of fruits, but the data was not representative for a statistical analysis. The data gathered showed that most of control plants got mature fruits in normal period (http://tchester.org/analysis/tomatoes/). The percent of ripe tomatoes in MPW control treatments at 151 days varied from 57% (controls for coated NPs) to 100% (controls for CeAc treatments). Interestingly, all uncoated and coated NP treatments, except 500 mg/kg, had ripe tomatoes at 151 days. However, plants exposed bCeO_2 at 62.5 did not produced ripe tomatoes at 151 days, plants exposed to 125 mg/kg did not produce any tomatoes, while plants exposed to 250 and 500 mg/kg had 50% and 40% ripe tomatoes, respectively, at 151 days. It is worth noting that plants exposed to coated NPs, except at 125 mg/kg, had blossom end rot. This is a serious tomato disorder associated with Ca deficiency that can affect more than 50% of production (http://ohioline.osu.edu/hyg-fact/3000/pdf/3117.pdf). However, none of the NPs interfered with Ca accumulation in fruit (data not shown); in addition, there was a regular water supply, and the pH was around 6.5 (Table S9). This suggests that other factors were involved in the induction of the blossom end rot, which deserves additional investigation.

Table 2-3 Total production, percentage of mature fruits at 151 and 210 days, and fruits with blossom end rot at 210 days in tomato plants grown in soil amended with 0 (control) to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). The control treatment was watered with Millipore water. DAG stands for days after germination.*Total number of tomatoes for 4 replicates.

			Mature (139-151 DAG)	**Never ripened (210 DAG)**	**Blossom end rot**
Treatment	**mg/kg**	**No. tomatoes***	**%**	**%**	**%**
$nCeO_2$	0	6	83.3	16.7	0
	62.5	18	38.9	27.8	0
	125	6	50	16.7	0
	250	9	66.7	11.1	0
	500	5	0	40	0
CA + $nCeO_2$	0	7	57.14	14.3	14.3
	62.5	8	37.5	37.5	12.5
	125	5	40	0	0
	250	8	25	0	25
	500	6	0	0	16.7
$bCeO_2$	0	4	75	25	0
	62.5	3	0	100	0
	125	0	0	0	0
	250	4	50	0	0
	500	5	40	20	0
CeAc	0	5	100	0	0
	62.5	4	25	0	0
	125	5	0	0	0
	250	3	0	33.3	0
	500	6	33.3	16.7	16.7
CA	0	8	62.5	12.5	0
	62.5	6	16.7	0	0
	125	1	0	100	100
	250	12	50	25	0
	500	1	0	0	0

2.4. CONCLUSIONS

The data of this study suggests that tomato stem elongation, in fully developed plants, was enhanced at the highest concentration of both coated and uncoated nanoparticle treatments, but was reduced by bulk cerium and acetate compounds after 210 days of germination. The citric acid coating did not have effect on chlorophyll a, b and total chlorophyll contents of tomato. However, the surface coating had effect on the biochemical response of the plant as coated NPs increased CAT activity at 500 mg/kg. On the other hand, at 125 mg/kg, bCeO2 decreased CAT activity by 83.90%. Both coated and uncoated NPs showed similar reducing effects on APOX, except at 62.5 mg/kg, where coated NPs did not affect APOX. In addition, all bCeO2 concentrations and CeAC at 250 and 500 mg/kg reduced APOX activity. ICP-OES results demonstrated that the coating reduced Ce uptake by roots but did not have effect on its translocation to the aboveground plant parts. Neither uncoated nor coated $nCeO_2$ affected the homeostasis of nutrient elements in roots, stems, and leaves, and there was no clear effect of surface coating on the fruit production of tomato plants. To our knowledge, this is the first life cycle study comparing the effects of coated and uncoated $nCeO_2$ on tomato plants.

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Chapter 3: Nutritional quality assessment of tomato fruits after exposure to uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate and citric acid[1]

ABSTRACT

Little is known about the effects of surface modification on the interaction of nanoparticles (NPs) with plants. Tomato (*Solanum lycopersicum* L.*)* plants were cultivated in potting soil amended with bare and citric acid coated nanoceria ($nCeO_2$, $nCeO_2$+CA), cerium acetate (CeAc), bulk cerium oxide ($bCeO_2$) and citric acid (CA) at 0-500 mg/kg. Fruits were collected year-round until the harvesting time (210 days). Results showed that $nCeO_2$+CA at 62.5, 250 and 500 mg/kg reduced dry weight by 54, 57, and 64% and total sugar by 84, 78, and 81%. At 62.5, 125, and 500 mg/kg, $nCeO_2$+CA decreased reducing sugar by 63, 75, and 52%, respectively and at 125 mg/kg reduced starch by 78%, compared to control. The $bCeO_2$ at 250 and 500 mg/kg increased reducing sugar by 67 and 58%. In addition, when compared to controls, $nCeO_2$ at 500 mg/kg reduced B (28%), Fe (78%), Mn (33%), and Ca (59%) and at 125 mg/kg decreased Al by 24%; while $nCeO_2$+CA at 125 and 500 mg/kg increased B by 33%. On the other hand, bCeO2 at 62.5 mg/kg increased Ca (267%), but at 250 mg/kg reduced Cu (52%), Mn (33%), and Mg (58%). Fruit macromolecules were mainly affected by $nCeO_2$+CA, while nutritional elements by $nCeO_2$; however, all Ce treatments altered, in some way, the nutritional quality of tomato fruit. To our knowledge, this is the first study comparing effects of uncoated and coated nanoceria on tomato fruit quality.

Keywords: Nanoceria, Surface coating, Tomato fruits, Nutritional quality, Essential elements

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3.1 INTRODUCTION

Lanthanides, also known as rare earth elements (REEs), are abundant in the Earth's crust; however, they tend to coexist, making single element acquisition quite challenging. In nature, they are present as oxide or phosphate complexes (Kabata-Pendias and Pendias, 1992). REEs are widely used in several applications, and their demand is estimated to increase around the world (EPA, 2012; Gonzalez et al., 2014). Cerium (Ce) and other REEs have found application in agriculture (Pang et al., 2001); however, the effects of these elements on plants are still not well understood. Even though Ce is nonessential to plants, previous studies have shown that it stimulates root growth and impacts other plant functions. According to Yuan et al. (2001), "Changle," a fertilizer composed mainly of Ce (50.2%), improved root growth in rice (*Oryza sativa*) seedlings. Similarly, Shyam and Aery (2012) reported that Ce, at low concentrations (0.713-17.841 µM), promoted chlorophyll content, dry matter production, and nitrate reductase activity in cowpea (*Vigna unguiculata*) plants. Liang et al. (2011) reported that Ce (20 mg/L) could alleviate ultraviolet-B-induced inhibition of photochemical reaction activity and photosynthetic pigments in soybean (*Glycine max*) seedlings. On the other hand, Diatloff et al. (2008) reported that Ce, at concentrations > 5 µM, inhibited corn (*Zea mays*) or mungbean (*Vigna radiata*) root elongation. Hu et al. (2002) also reported that Ce (0.5-25 mg/L) reduced root elongation, shoot, and root dry weight and mineral content in wheat (*Triticum aestivum*). Another study by Thomas et al. (2014) showed that Ce (978 mg/kg soil) at low pH decreased germination in four crops, including tomato.

Oxides of some REEs and other metal elements, at nanoparticle level, have been found to reach crop plants through intentional exposure (Servin and White, 2016) or soil amended with ENP-loaded biosolids (Rico et al., 2011; Miralles et al., 2012). Cerium oxide nanoparticles (NPs) or nanoceria ($nCeO_2$) are amongst the top 10 NPs produced worldwide (Piccino et al., 2012; Keller and Lazareva 2014). One of the most common uses of $nCeO_2$ includes fuel additives and catalysts

(Johnson and Park, 2012). This suggests a high probability of environmental dispersion and interaction of plants with $nCeO_2$.

Previous studies have shown controversial effects of $nCeO_2$ in crop plants (Gardea-Torresdey et al. 2014). However, findings by Lopez-Moreno et al. (2010) and Hernandez-Viezcas et al. (2013) seem to apply to all plants. Lopez-Moreno et al. (2010) reported that most of the $nCeO_2$ taken up by soybean (*Glycine max*) plants was stored without modification in the roots, while Hernandez-Viezcas et al. (2013) reported the translocation of $nCeO_2$ to soybean seeds. Other reports have shown that $nCeO_2$ affects crop production in several ways. Peralta-Videa et al. (2014) studied the alterations that $nCeO_2$ and ZnO NPs have on the nutritional value of soybean plants cultivated in farm soil. Rico et al. (2013a) reported that $nCeO_2$ at 500 mg/kg altered the grain quality in three varieties of rice and inhibited the grain formation in barley (Rico et al., 2015). Zhao et al. (2014) reported that $nCeO_2$ at 400 mg/kg increased starch, globulin, and nonreducing sugar, but at 800 mg/kg reduced phenolic content in cucumber fruits. Micronutrients were also affected in cucumber seeds (Zhao et al., 2014). Rico et al. (2014) also reported that $nCeO_2$ at 500 mg/kg improved wheat grain yield by 36.6% and modified S and Mn storage in grains. In a trans-generational tomato study, Wang et al. (2013) reported that $nCeO_2$ (10 mg/L) treated second generation seedlings showed a reduction in biomass, water transpiration, and higher reactive oxygen species (ROS) content.

Next to potatoes, tomatoes are the most consumed vegetables in the United States. Mostly, tomatoes are eaten either fresh or canned (USDA, 2013) and are a primary source of sugars, proteins, carbohydrates, and many essential nutrients like: calcium, magnesium, iron, phosphorous, potassium, sodium, and zinc[2].

[2]https://ndb.nal.usda.gov/ndb/foods/show/3223?manu=&fgcd=

Tomatoes also have a high lycopene content, a carotenoid with antioxidant properties. Lycopene is present in chromoplasts during ripening (Hornero-Mendez and Britton, 2002). In humans, lycopene scavenges peroxy and singlet oxygen radicals and aids in the deactivation of agents that break DNA-chains (Stahl et al., 1997). The present study is a follow-up of a previous study where the effects of five different compounds: cerium oxide nanoparticles, citric acid coated cerium oxide nanoparticles, cerium oxide bulk, cerium acetate, and citric acid in soil grown tomato plants were reported (Barrios et al., 2016). The hypothesis of this work is that $nCeO_2$ + CA affect in a different way than $nCeO_2$ the physiological and biochemical parameters of tomato fruits. In this manuscript, the changes in macro and micronutrient accumulation, carbohydrate (sugar and starch) content, and the lycopene content in tomato fruits of plants exposed to the Ce compounds mentioned above were studied. To the authors' knowledge, this is the first study comparing the effects of coated and uncoated cerium oxide NPs in the nutritional quality of tomato fruits.

3.2 Materials and Methods

3.2.1 Nanoparticle suspensions and other treatments.

The $nCeO_2$ (Meliorum Technologies, NY, USA) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to Keller et al. (2010), these nanoparticles have a primary size of 11 ± 0.2 nm, particle size of 231 ± 16 nm in deionized water and a surface area of 93.8 m^2/g and a ζ potential of 20.1 ± 1.2 mV (Trujillo-Reyes et al., 2013). Citric acid coated CeO_2 NPs ($nCeO_2$ + CA) on a 1:2 ratio were prepared and characterized by Trujillo-Reyes et al. (2013). Briefly, these NPs have an average primary size of 12.1 nm, particle size of 189 ± 2 nm in deionized water, and a ζ potential of -57 ± 0.6 mV. According to the manufacturer (Sigma-Aldrich), Cerium acetate (CeAc) and bulk cerium oxide ($bCeO_2$) have a size above 5 μm (Figure S1). The pH in soil of all suspensions was 6.12 ±

0.03 and the average temperature was 21.63 ± 0.06 °C. Citric acid (CA), CeAc, $nCeO_2$ and $nCeO_2$ + CA solutions/suspensions were prepared with Millipore water (MPW) accordingly to have final concentrations of 0, 62.5, 125, 250 and 500 mg/kg of each compound. The concentrations were selected from previous studies by Rico et al. (2013b) and Barrios et al. (2016). The dispersed nanoparticle suspensions were sonicated in a water bath for 30 min at 20°C with a sonication intensity of 180 watts and immediately applied to the soil. Each compound had their individual set of MPW controls (no chemical added).

3.2.2 Experimental design and growth conditions

Roma tomato (*S. lycopersicum*) seeds were grown in Miracle-Gro® Organic potting mix and exposed to five different chemicals: $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$, CeAc, and CA at the five concentrations mentioned above. Each treatment had four replicates, and each pot contained five seeds. After 60 days, the biggest plant per pot was selected and cultivated to full maturity. Plants were watered daily and kept in a greenhouse for 210 days. Tomato fruits were collected starting from 139 to 210 days after germination. Further details on the greenhouse conditions, soil composition, and experimental design are described in Barrios et al. (2016).

3.2.3 Nutrient content

After harvesting, tomato fruits were cut into halves. One-half was cryogenized in liquid nitrogen and stored at -20°C for further analysis. The second half was oven dried for 72 h at 60°C. Once dried, samples were ground to a powder with mortar and pestle, and 0.2 g were acid-digested with one mL of plasma pure nitric acid and four mL of 30% hydrogen peroxide in a microwave system (MarsX, CEM Corporation Mathews, NC, USA) as described by Packer et al. (2007). After digestion, tomato samples were diluted to 50 mL with MPW. Quantification of Ce, Al, B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn was conducted using inductively coupled plasma-optical emission

spectroscopy (ICP-OES, PerkinElmer Optima 4300 DV, Shelton, CT). For quality assurance/ quality control (QA/QC) purposes, blank and spikes containing Ce at 1 and 5 mg/L were read every 15 samples. Blanks, spikes, and standard reference materials NIST 1547 peach leaves, (Gaithersburg, MD) were used to validate the quantification.

3. 2.4 Determination of total and reducing sugars

3.2.4.1 Total sugar

Total sugar was quantified following the method of Dubois et al. (1956). For sugar extraction, 100 mg of oven dried tomato samples were homogenized in 10 mL of 80% ethanol, boiled in a water bath (80 °C/ 30 min) and centrifuged at 5,000 rpm for 20 min. The extraction was repeated three times per sample and supernatants were collected together, the volume was reduced to 3 mL through evaporation, and diluted up to 25 mL with MPW. The dry residue was kept for starch analysis. In a test tube, 100 µL of the extract was diluted to 1 mL, and one mL of 5% phenol + 5 mL 96% H_2SO_4 were added, mixed, and let to cool down at room temperature for 30 min. Glucose standards (Sigma-Aldrich, 99.9% pure) and water (blank) were treated with the same protocol to obtain the calibration curve at concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 g mL^{-1}. The absorbance of the samples was recorded using a UV-Vis spectrometer (Perkin-Elmer Lambda 14 UV/Vis Spectrometer, Uberlinger, Germany) at 490 nm, and total sugar was quantified from the standard calibration curve.

3.2.4.2 Reducing sugars

Sample preparation was done following the same procedure as total sugar. Reducing sugar content was done according to Nelson-Somogyi (1952). In a test tube, 100 µL of the extract was diluted to 2 mL with water and 1 mL alkaline copper tartrate was added, then placed in a boiling

water bath for 30 min. After samples had cooled down, 1 mL of arsenomolybdolic acid reagent was added. The mixture was diluted with a final volume of 10 mL with MPW and after 10 minutes absorbance was read at 620 nm in UV-vis (PerkinElmer Lambda 14 UV-Vis spectrometer), using the same calibration curve as total sugar.

3.2.5 Determination of starch in fruit

Starch content was determined according to Verma and Dubey (2001).The dry residue from sugar extraction was diluted with 2 mL of MPW and boiled in a water bath for 15 min, and then cooled to room temperature. Then, 2 mL of 96% H_2SO_4 were added, stirred for 15 min and diluted to 10 mL with MPW. Diluted samples were centrifuged for 20 min at 5,000 rpm, and the supernatant was collected. A second extraction was performed with 50% H_2SO_4, and the supernatants were collected together and diluted to 40 mL with MPW. For estimating the starch content, the same method as total sugars was followed (Dubois et al., 1956), where 100 µL of the extract were reacted and read at 490 nm using a calibration curve of potato starch.

3.2.6 Lycopene content.

The lycopene content was determined after Barrett and Anthon (2001). Firstly, tomatoes were frozen in liquid nitrogen and stored at -20 °C were pureed with a mortar and pestle. Samples were centrifuged at -4 °C and 9600 rpm for 10 min (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). One hundred microliters (100 µL) of the supernatant were transferred to a 15 mL conical centrifuge Falcon® tube. Then, eight mL of hexane: ethanol: acetone (4:2:2) were added using a micropipette. The tubes were capped, vortexed, incubated out of bright light for 1 h, 1 mL of MPW was added, and briefly vortexed. Samples were allowed to stand for 10 min to ensure phase separation and to dissipate any air bubbles. The absorbance of the upper layer (1 mL)

was recorded at 503 nm in a PerkinElmer Lambda 14 UV-Vis spectrometer. Lycopene content was then calculated according to Barrett et al. (2007):

$$\text{mg lycopene/kg fresh wt.} = A_{503} \times 537 \times 8 \times 0.55)/ (0.10 \times 172)$$

$$= A_{503} \times 137.4$$

where 537 g $mole^{-1}$ is the molecular weight of lycopene, 8 mL is the total volume of the solvent mixture, 0.55 is the volume ratio of the upper layer, 0.10 g is the weight of the sample added, and 172 mM^{-1} is the extinction coefficient for lycopene in hexane.

3.2.7 Statistical analysis.

Four replicates of each treatment and concentration were allocated in a completely random design in the greenhouse facility. However, every replicate produced different amounts of tomatoes; therefore *n* had a range from 3 to 12 samples per replicate. The treatments 125 mg/kg $bCeO_2$ and 250 mg/kg CeAc had none or insufficient samples to perform any statistical analysis. The rest of the data was evaluated using one-way ANOVA (PASW Statistics 18 software) and the Tukey's HSD test at $p \leq 0.05$ was used to assay statistical differences between the means of each treatment. The data presented are means ± standard errors (SE).

3.3. RESULTS AND DISCUSSION

3.3.1 Effects on agronomical parameters

Table 3-1 and supplementary data Table S1 show the fruit dimensions including length and width, fresh and dry weights, and water content of tomato fruits exposed to the different treatments. The fruits started ripening at 139 days after seed germination. The fruits had the characteristic ellipsoid-plum shape of Roma variety. As seen in the table, there were no differences in fruit dimensions, fresh and dry weight, and water content in tomatoes from $nCeO_2$ treated plants.

However, the dry weight of tomatoes from $nCeO_2$ + CA decreased by 54, 57, and 64% at 62.5, 250 and 500 mg/kg, respectively, compared with control. Cerium acetate at 125 mg/kg was the only treatment that increased the water content by 58%, compared to 500 mg/kg and by 72%, with respect to its control.

Table 3-1 Size, weight, and water content of fruits harvested from tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated ($nCeO_2$ + CA) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Data are means ± SE, where n has a range from 3 to 12 replicates. Different letters indicate statistically significant differences between concentrations of the same treatment at $p \leq 0.05$.

Parameter	mg/kg	$nCeO_2$	$nCeO_2$ + CA	$bCeO_2$	CeAc	CA
Length (mm)	Control	34.13 ± 3.15	37.04 ± 1.63	34.71 ± 2.61	31.59 ± 1.95 ab	32.51 ± 2.67
	62.5	30.51 ±1.62	36.59 ± 3.33	25.52 ± 4.25	37.51 ± 0.60 a	35.67 ± 3.74
	125	28.94 ± 1.30	39.75 ± 3.61		38.03 ± 0.84 a	39.05 ± 4.08
	250	35.14 ± 1.69	32.88 ± 2.59	33.07 ± 0.06		34.95 ± 2.83
	500	27.01 ± 8.58	30.24 ± 2.24	32.91 ± 2.18	28.81 ± 2.59 b	39.89 ± 4.68
Width (mm)	Control	25.81 ± 2.63	26.80 ± 1.53	28.25 ± 2.83	24.36 ± 1.44 ab	23.20 ± 1.96
	62.5	21.65 ± 1.10	26.04 ± 2.64	18.60 ± 2.57	29.99 ± 0.27 a	24.96 ± 3.00
	125	22.25 ± 1.44	25.87 ± 1.57		28.80 ± 0.96 a	23.59 ± 1.08
	250	25.09 ± 1.34	24.08 ± 1.72	23.88 ± 1.60		25.15 ± 2.02
	500	27.05 ± 3.50	22.42 ± 1.12	22.57 ± 2.02	22.44 ± 1.63 b	24.36 ± 2.15
Fresh wt (g)	Control	6.58 ± 1.19	7.15 ± 0.98	7.67 ± 1.60	5.35 ± 1.13 ab	5.24 ± 0.99
	62.5	4.70 ± 0.71	6.72 ± 1.58	2.19 ± 0.91	6.75 ± 0.39 ab	7.09 ± 2.12
	125	3.90 ± 0.67	7.70 ± 1.02		8.57 ± 0.56 a	7.59 ± 1.87
	250	5.14 ± 0.56	5.23 ± 0.99	5.89 ± 0.89		6.59 ± 1.06
	500	4.78 ± 3.90	3.97 ± 0.64	4.74 ± 1.17	3.71 ± 0.73 b	8.60 ± 3.11
Dry wt (g)	Control	0.19 ± 0.03	0.61 ± 0.13 a	0.27 ± 0.01	0.55 ± 0.26	0.28 ± 0.07
	62.5	0.20 ± 0.02	0.28 ± 0.05 b	0.09 ± 0.04	0.21 ± 0.001	0.35 ± 0.11
	125	0.20 ± 0.03	0.33 ± 0.05 ab		0.31 ± 0.03	0.25 ± 0.15
	250	0.18 ± 0.02	0.26 ± 0.05 b	0.33 ± 0.04		0.21 ± 0.03
	500	0.19 ± 0.07	0.22 ± 0.04 b	0.25 ± 0.07	0.21 ± 0.06	0.32 ± 0.12
Water content (mL)	Control	6.38 ± 1.16	6.63 ± 0.91	7.41 ± 1.59	4.79 ± 0.93 b	4.96 ± 0.93
	62.5	4.50 ± 0.69	6.45 ± 1.54	4.10 ± 0.87	6.54 ± 0.39 ab	6.74 ± 2.03
	125	3.70 ± 0.64	7.37 ± 0.98		8.26 ± 0.53 a	6.15 ± 1.28
	250	4.96 ± 0.55	4.97 ± 0.94	5.56 ± 0.85		6.39 ± 1.04
	500	4.60 ± 3.83	3.75 ± 0.61	4.49 ± 1.10	3.50 ± 0.69 b	8.18 ± 2.04

A study by Takayama et al. (2012) reported that tomato plants under controlled greenhouse conditions emit different volatile organic compounds (VOCs) including n-hexanal, 2-carene, β-caryphyllene and (3E,7E)-4,8,12-trimethyl-1, 3,7,11-tridecatetraene (TMTT). Although VOCs were not determined in this study, it is possible that $nCeO_2$ + CA increased the release of these compounds, reducing the dry weight. Wang et al. (2012) reported no changes in size and average weight of tomato fruits after watering tomato plants twice a week with suspensions of 0.1, 1 and 10 mg/L of $nCeO_2$ (total of 130 mg/L) in contrast to the controls. Raliya et al. (2015) reported that fruit biomass of tomato increased by about 70% in plants exposed to 250 mg/kg of aerosol TiO_2 NPs. Changes found in our study could be attributed to the form of NPs' application and varietal differences.

3.3.2 Effects of the different compounds in fruit carbohydrates

Carbohydrates are the most abundant organic constituents of plants. They are a source of chemical energy (sugars and starch especially) and components of supporting tissues (Solomons and Fryhle, 2011). Sugars, starches, and fibers are the main forms of carbohydrates in plants and play an important role when determining the nutritional quality of fruits (Ruiz and Romero, 1998; Ho, 1996). Figure 3.1 shows the concentration of sugars in fruits of plants exposed to $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$, CeAc, and CA; while Table S2 shows statistical comparisons among concentrations. The $nCeO_2$ did not affect the total sugar content; however, $nCeO_2$ +CA at 62.5, 250 and 500 mg/kg reduced total sugar by 84, 78, and 81% (Figure 3.1A); while at 62.5, 125, and 500 mg/kg decreased reducing sugars by 56, 63, and 75%, respect to control (Figure 3.1B). Reducing sugars were decreased by CeAc at 62.5 mg/kg (58%) and CA at 125 (55%) and 500 mg/kg (77%), but increased by $bCeO_2$ at 250 (67%) and 500 mg/kg (58%). Results suggest that $nCeO_2$ +CA, CeAc, and CA modified the sweetness of the tomato fruit. Paleg et al. (1959) reported

that citric acid inhibits the color formation in the reducing sugar assay described in Somogyi's method. As the concentration of citric acid increases, the absorbance values decreases showing that "citrate has a depressing effect on the absorbance produced by all three reducing sugars" (Paleg et al., 1959). Due to its chelating properties, citrate may replace equal amounts of tartrate, forming citrate-copper complexes, instead of the required tartrate-copper complexes needed for the sugar reduction to occur (Paleg et al., 1959). Sucrose, the most common non-reducing sugar in plants, is a contributor to stress-related responses (Moghaddam and Ende, 2012). Zhao et al. (2014) found that an upregulation of sucrose produced by $nCeO_2$ in cucumber is a possible sign of stress. However, in this study, none of the treatments had an impact on the non-reducing sugars (Fig. 1C), when compared to their controls. Carbohydrates are synthesized in plant leaves by photosynthesis. Prior studies stated that a reduction in the photosynthetic rate leads to a decrease in the sugar content but an increase in starch content (Goodman, et al., 1986). Recently, Barrios et al. (2016) found that none of the NPs' treatments affected the chlorophyll, but $bCeO_2$ at 500 mg/kg increased chlorophyll and sugar content, conversely to what has been reported. Rico et al. (2013b) showed that in rice exposed to the same concentrations of $nCeO_2$, there was no change in sugar content, but starch was impacted. Modifications in sugar and starch content in plants treated with NPs have been reported as toxicity indicators; however, these changes may also be attributed to varietal differences.

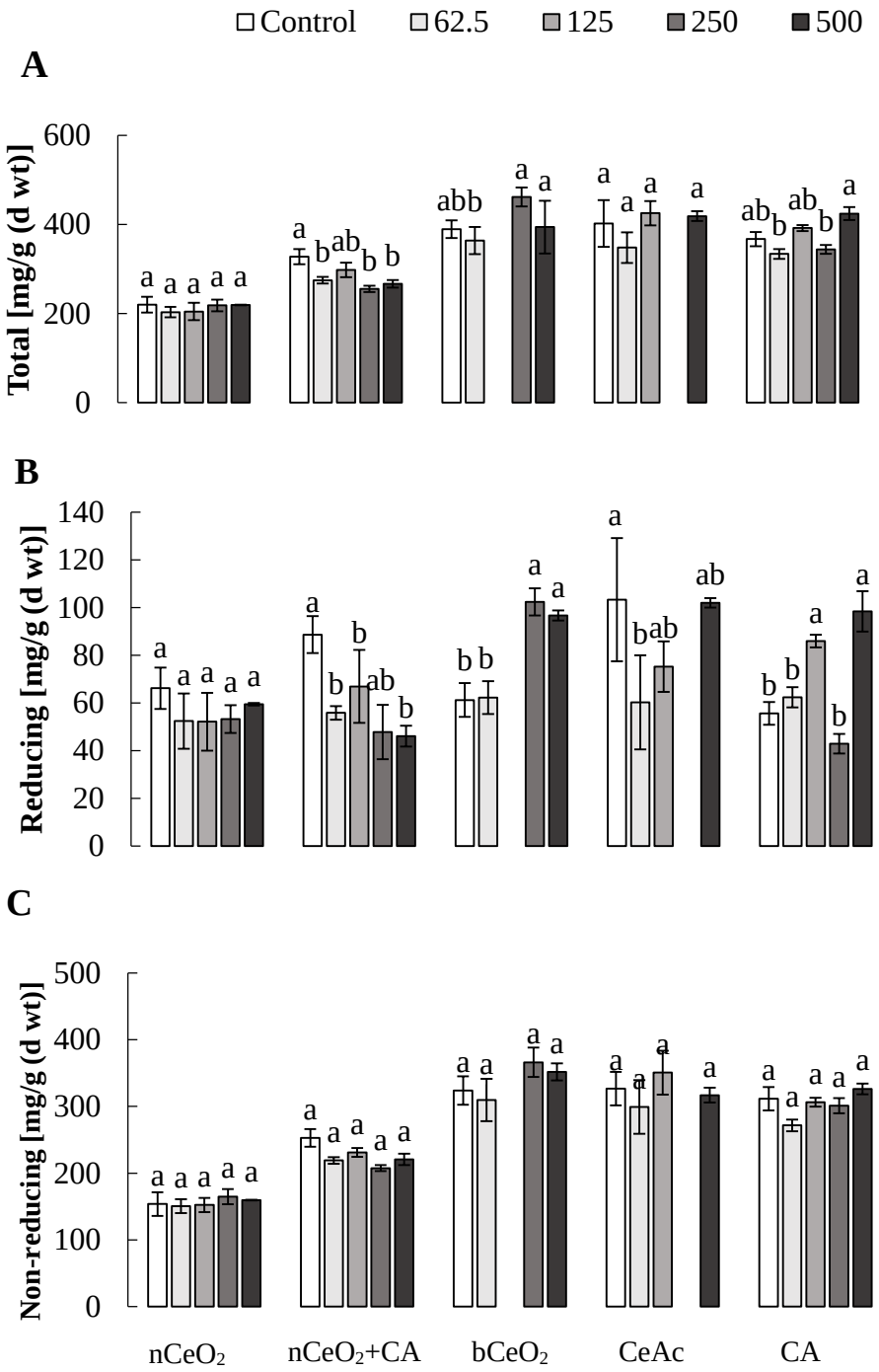

Figure 3.1 Total sugar (A), reducing sugar (B), and non-reducing sugar (C) contents of fruits harvested from tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated ($nCeO_2$ + CA) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). At 125 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Data are means ± SE, where n has a range from 3 to 12 replicates. Different letters indicate statistically significant differences between concentrations of the same treatment at $p \leq 0.05$

Figure 3.2 shows the starch concentration in fruits of plants exposed to $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$, CeAc, and CA; while Table S3 shows the statistical comparisons between concentrations. As seen in Figure 3.2, $nCeO_2$ +CA at 125 mg/kg and CA at 500 mg/kg reduced the starch content, when compared to their controls (78 and 68%, respectively). Previous studies have shown that stress caused by copper produces an accumulation of carbohydrates in cucumber plants (Alaoui-Sosse et al., 2004). Wang et al. (2013) reported that sugar and starch contents increased in *Thellungiella halophile* leaves due to salinity stress. Zhao et al. (2014) found an increase in starch content in cucumber when exposed to $nCeO_2$. These authors stated that an increment in starch could indicate stress produced by $nCeO_2$. Rico et al. (2013b) showed that high and low amylose rice varieties exposed to 500 mg/kg of $nCeO_2$ had a decrease in starch content of 9.2 and 7.9%, respectively. In this study, none of the nanoparticle treatments produced an over accumulation of starch in tomato fruit. This might be attributed to the species-specific responses. Further studies with other tomato varieties in similar conditions are required to fully understand the response of this plant to Ce compounds.

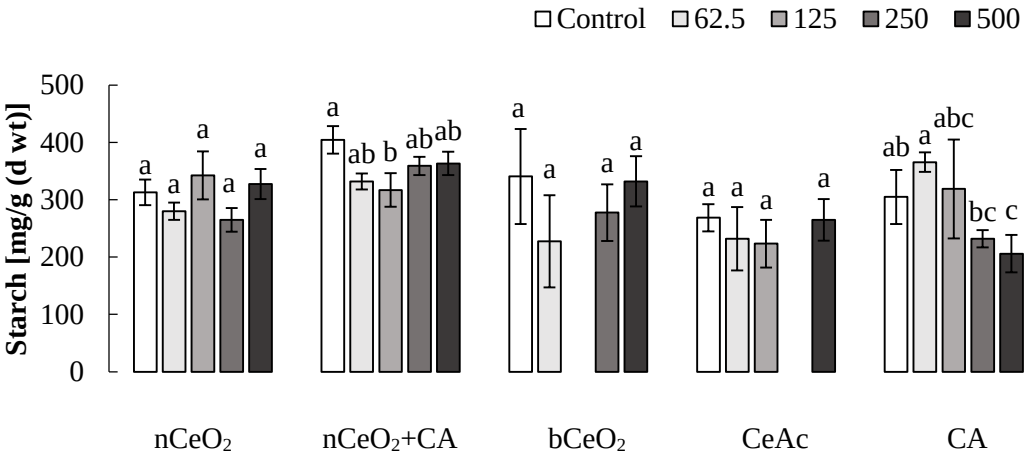

Figure 3.2 Starch content of fruits harvested from tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated ($nCeO_2$ + CA) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). At 125 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Data are means ± SE, where n has a range from 3 to 12 replicates. Different letters indicate statistically significant differences between concentrations of the same treatment at $p \leq 0.05$.

3.3.3 Effects of the treatments in fruits micro and macro elements accumulation.

Micro and macro elements are essential components of functional and structural molecules of living organisms. Plants acquire these nutrients, mainly through the roots, from the soil or growth medium. Previous reports have shown that NPs alter the root uptake and translocation of essential elements. Macro and micronutrients were previously determined in roots, stems, and leaves of tomato plants (Barrios et al., 2016). In the previous study, there was a concentration-dependent increase of Ce in tomato roots exposed to $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$ and CeAc. However, the translocation from roots to shoots and shoots to leaves was minimal. Additionally, the Ce uptake in $nCeO_2$ + CA treated plants was lower than in $nCeO_2$. The mechanisms that deal with the transport of NPs from soil to root and aboveground tissues are still elusive. Nonetheless, Zhao et al. (2012) reported $nCeO_2$ embedded within the root tissues in the epidermis, endodermis, cortex and xylem. These authors also suggested that the $nCeO_2$ aggregates are moved via the apoplastic pathway, in which particles translocate between cell to cell from the outer layer (epidermis) to the inner layer (endodermis) all the way to the vascular tissue (phloem and xylem) (Zhao et al., 2012). In this report, we analyzed the element composition of fruits (Table 3-2 and Table S4). As seen in Table 3-2, the cerium treatments altered the fruit ionome. Elemental Ce was analyzed in fruit, but the concentrations were below the detection limits of the ICP-MS. On the other hand, concentrations of Al (a non-essential element), B, Cu, Fe, Mn, Ca, and Mg showed statistically significant differences, compared to controls. The $nCeO_2$ at 125 mg/kg decreased Al by 24% and at 500 mg/kg decreased B (28%), Fe (78%), Mn (33%), and Ca (59%), with respect to control.

Table 3-2 Micro- and macro- nutrients altered in tomato fruits harvested from tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated ($nCeO_2$ + CA) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means ± SE, where n has a range from 3 to 12 replicates. Comparisons were made with respect to the controls and symbols + and – stand for percent of increase and decrease in nutrient concentration.

	Element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%
	Al	Control	42.07 ± 3.56	100
		$nCeO_2$ 125	31.95 ± 2.84	24.05 -
Micro	B	Control	13.89 ± 0.65	100
		$nCeO_2$ 500	9.91 ± 3.27	28.65 -
		Control	12.34 ± 1.13	100
		$nCeO_2$ + CA 125	16.48 ± 0.62	33.55 +
		$nCeO_2$ + CA 500	16.44 ± 0.94	33.23 +
	Cu	Control	13.87 ± 1.63	100
		$bCeO_2$ 250	6.65 ± 0.86	52.01 -
	Fe	Control	46.69 ± 4.11	100
		$nCeO_2$ 500	10.16 ± 5.24	78.24 -
		Control	17.34 ± 5.16	100
		CeAc 125	32.50 ± 3.74	87.43 +
	Mn	Control	17076.96 ± 602.23	100
		$nCeO_2$ 500	11506.42 ± 1896.28	32.62 -
		Control	18731.29 ± 1918.23	100
		$bCeO_2$ 250	12800.00 ± 966.31	31.67 -
		$bCeO_2$ 500	12388.29 ± 860.14	33.86 -
Macro	Ca	Control	5699.71 ± 799.20	100
		$nCeO_2$ 500	2317.22 ± 810.81	59.34 -
		Control	1748.72 ± 237.77	100
		$bCeO_2$ 62.5	6413.14 ± 822.53	266.73 +
		Control	2351.61 ± 544.83	100
		CeAc 62.5	6053.16 ± 1007.64	157.41 +
		CeAc 500	6053.16 ± 1007.64	157.41 +
	Mg	Control	2175.08 ± 275.19	100
		$bCeO_2$ 250	917.03 ± 237.66	57.84 -

The zeta potential of $nCeO_2$ was 20.1 ± 1.2 mV; this suggests that the positive surface of $nCeO_2$ repelled other cations, reducing their uptake. Conversely, $nCeO_2$ + CA at 125 and 500 mg/kg increased B by 33%. The negative surface (-57 ± 0.6 mV) of $nCeO_2$ + CA (Trujillo-Reyes et al. 2013) attracts B, facilitating its uptake. The $bCeO_2$ at 62.5 mg/kg increased Ca by 267%; whereas at 250 mg/kg decreased Cu (52%), Mn (33%), and Mg (58%), and at 500 mg/kg, also decreased Mn by 34%, compared to control. At 62.5 and 500 mg/kg, CeAc increased Ca by 157% and 125 mg/kg increased Fe by 87%. In the earlier study, none of the treatments altered the concentration of essential elements in stems and leaves, except Ca that was increased by CeAc and P and Zn that were reduced and increased by $bCeO_2$, respectively. There is not enough information to explain these results; however, it is possible that the concentration of these ions in the stems and leaves drove the translocation to the fruits.

3.3.4 Effects of the treatments on lycopene content

Figure 3.3 shows the concentration of lycopene in fruits of plants exposed to $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$, CeAc, and CA; while Table S5 shows the statistical comparisons between concentrations. As seen in the Figure, at 62.5, 250 and 500 mg/kg, $bCeO_2$ decreased lycopene by 92, 61, and 72%, respectively, compared to control. Interestingly, at 62.5 mg/kg all the fruits obtained were green. Therefore, lycopene, a red pigment, was almost absent in this concentration. Similarly, CeAc at 62.5, 125, and 500 mg/kg decreased lycopene by 69, 79 and 81%, with respect to control. However, the analysis of the data (Table S5) did not show statistical significance between compounds. Very likely this was due to the great variability of the data.

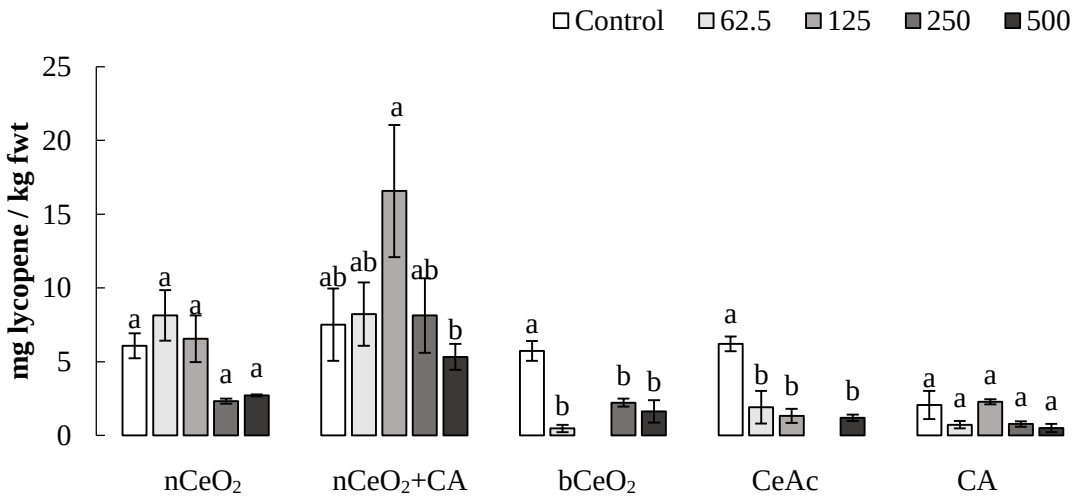

Figure 3.3 Lycopene content of fruits harvested from tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated ($nCeO_2$), citric acid coated ($nCeO_2$ + CA) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). At 125 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Data are means ± SE, where n has a range from 3 to 12 replicates. Different letters indicate statistically significant differences between concentrations of the same treatment at $p \leq 0.05$.

Previous studies have shown that lycopene concentration is affected by other NPs. Kole et al. (2013) reported that lycopene increased by 82% in melon plants exposed to carbon-based fullerol NPs. Raliya et al. (2015) exposed through soil and foliar application, TiO_2 and ZnO NPs at 100 mg/L to tomato plants. They found an increase in lycopene of foliar treated plants, but not in soil exposed plants. A decrease in lycopene might be a sign of toxicity produced by the bulk and cerium acetate treatments. In plants, lycopene synthesis usually derives from the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways. These pathways synthesize isopentenyl diphosphate (IPP) and diethylallyl diphosphate (DMAPP), which function as precursors for carotenoid synthesis (Botella-Pavia et al., 2004; Collins and Perkins-Veazie, 2006). There are also a number of enzymes involved in this process, and even though the synthesis of carotenoids is well understood, the behavior of these regulatory enzymes still require further research (Fraser et al.,

2001). More research is still needed to understand fully the effects of Ce compounds in the lycopene synthesis.

3.4. CONCLUSIONS

The results of this study have shown that cerium compounds affect the chemical constitution of tomato fruits. While $nCeO_2$ + CA decreased fruit dry weight, total sugar, reducing sugars, and increased B concentration, $nCeO_2$ reduced the essential elements B, Fe, Mn, and Ca. This suggests that the citric acid coating in the NPs mainly affected the macromolecules, while pristine NPs altered the fruit ionome. On the other hand, $bCeO_2$ decreased Cu, Mn, and Mg, but increased Ca, suggesting that the size of the particle had differential effects in the content of essential elements in the fruit. Overall, the three Ce compounds tested demonstrated to affect the physiology and biochemistry of tomato fruits. To our knowledge, this is the first study reporting the effects of coated and uncoated $nCeO_2$ on the quality of tomato fruits.

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Chapter 4: Summary and Conclusions

The impacts of $nCeO_2$ in terrestrial plants are still not well understood. The aim of this study was to reveal some physiological and biochemical responses of tomato plants exposed to environmentally relevant nanoceria concentrations. The effects of $nCeO_2$ + CA, $bCeO_2$, CeAc, and CA were also assessed to compare the influences that surface modifications and particle size have on the tomato plant grown to maturity. The full life cycle study showed varied responses in the development, Ce and nutrient uptake, biochemistry, productivity, and macromolecular changes of tomato plants to the different compounds. Table 4-1 provides the summarized findings in the plant and fruit tissues. Interestingly, the amount of Ce that was translocated to the aerial part of the plant was minimum, and most of the Ce from the different compounds remained in the roots. ICP-MS data also showed that Ce was below the limit of detection in the fruit tissues, suggesting that it was not translocated to the edible part of the plant. In the plant tissues, results revealed that both NPs, coated and uncoated, increased stem length by 9 and 13%, respectively, whereas $bCeO_2$ and CeAc reduced it when compared to control.

Essential elements in the plant were not impacted by either of the NPs or CA. However, in the fruit, $nCeO_2$ at 500 mg/kg significantly reduced B, Fe, Mn, and Ca and $nCeO_2$ + CA increased B. Interestingly, the $bCeO_2$ and CeAc treatments had a repercussion in the essential elements in both plant tissues and fruits. Previous studies have reported that $nCeO_2$ exhibits mimetic activity to both superoxide dismutase (SOD) (Heckert et al., 2008) and catalase (CAT) (Pirmohamed et al., 2010). However, this study reveals that, at high concentrations, $nCeO_2$ reduces the antioxidant activity of ascorbate peroxidase (APOX). In the leaves, all treatments at 500 mg/kg halted the APOX activity. These findings suggest that the Ce compounds/NPs alter the biochemical composition of tomato leaves by diminishing the ROS scavenging ability of the antioxidant enzyme. In the fruits, the

carbohydrates investigated: sugar and starch, were mainly affected by $nCeO_2$ + CA. Lycopene, one of the most important antioxidants in tomato, was not impacted by any of the NPs but was significantly reduced by $bCeO_2$ and CeAc at all concentrations. This suggests that particles at microscale size or their ions, interfere in lycopene synthesis. Overall, $nCeO_2$ mainly affected the fruit's ionome; whereas, $nCeO_2$ + CA disturbed the macromolecular composition. However, all Ce compounds altered the nutritional quality of tomato fruits in one way or another. Further studies are required to assess a safer use of $nCeO_2$ in crops. The possible environmental/health implications are still in their infancy. The nutritive value of tomato fruits was impacted by $nCeO_2$. Therefore, these modifications may affect human health and nutrition. Additionally, due to their nanoscale size, $nCeO_2$ may enter the food chain through trophic transfer. In this study, Ce was not detected in the tomato fruit. However, Wang et al. (2012) did a similar study where, after a chronological exposure of tomato plants to $nCeO_2$ (10 mg/L twice a week for 70 days), Ce was found in the fruits. The route (aerial, soil, solution) and time (continuous, periodically) of exposure are critical to identify the movement of $nCeO_2$ within the plants. This suggests the need for more studies in order to fully understand the interactions of $nCeO_2$ and plants. Special attention has to be dedicated to the mechanisms implicated in the entry and sequestration of $nCeO_2$ into the plant tissues. The study of macromolecular changes (carbohydrates, proteins, lipids, nucleic acids) through omics techniques is another critical area where knowledge is missing in order to understand how NPs affect fruit quality.

Table 4-1 Responses of tomato plant/fruit after a long-term exposure to $nCeO_2$, $nCeO_2$ + CA, $bCeO_2$, CeAc and CA.

Parameters	$nCeO_2$	$nCeO_2$ + CA	$bCeO_2$	CeAc	CA
Development	Increased stem length	Increased stem length	Reduced stem length at harvesting time	Reduced stem length at harvesting time	No apparent changes
Ce accumulation	Heavy accumulation of Ce in roots, no uptake in fruits	No accumulation in fruits	No accumulation in fruits	Significant accumulation of Ce in roots, no uptake in fruits	No Ce
Plant nutrients	No apparent changes in plant	No apparent changes	Reduction of P in stems, increase of Zn	Accumulation of Al, Fe, and B in roots and Ca in stems	No apparent changes
Fruit nutrients	Reduced B, Fe, Mn, Ca	Increased B	Reduced Cu, Mn, Mg and accumulated Ca	Increased Fe and Ca	No apparent changes
Enzymes in leaves	Reduced APOX activity	Increased CAT activity but reduced APOX	Reduced APX activity	Reduced APX activity	Only a few changes in APOX
Chlorophyll in leaves	No apparent changes	No apparent changes	Increase in total chlorophyll, chloro-a, and chloro-b at 250 and 500 mg/kg	No apparent changes	No apparent changes
Fruit production	No apparent changes	Blossom end rot	No tomato production at 125 mg/kg	Blossom end rot	Blossom end rot
Fruit carbohydrates	No apparent changes	Reduced total and reducing sugar	Increased reducing sugar	Few changes in reducing sugar	Increased reducing sugar but reduced starch
Fruit lycopene	No apparent changes	No apparent changes	Reduced at all concentrations	Reduced at all concentrations	No apparent changes

4.1 REFERENCES

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Appendix

1 SUPPORTING INFORMATION FOR CHAPTER 2: EFFECTS OF UNCOATED AND CITRIC ACID COATED CERIUM OXIDE NANOPARTICLES, BULK CERIUM OXIDE, CERIUM ACETATE, AND CITRIC ACID ON TOMATO PLANTS

Table S1 Nutrient composistion of dry tomato roots after 210 days of germination. Different letters indicate statistically significant differences between treatments. Data are means ±SE (standard error) of four replicates ($p \leq 0.05$). Only the elements that indicate statistical differences are shown.

		0	62.5	125	250	500
Al	$nCeO_2$	1401.15 ± 207.90 b	1609.42 ± 138.81 c	1857.51 ± 372.00	2042.44 ± 332.88	1414.39 ± 148.63 ab
	CA + $nCeO_2$	1186.48 ± 222.51 b	4760.04 ±1122.17 ab	3146.49 ± 1017.96	1846.00 ± 352.87	1866.05 ± 355.82 ab
	$bCeO_2$	2048.75 ± 472.02 ab	1965.02 ± 326.44 bc	1323.32 ± 415.14	1648.01 ± 400.32	905.86 ± 29.45 b
	CA	1283.31 ± 153.04 b	1784.66 ± 314.74 c	1375.66 ± 284.68	1100.33 ± 222.85	1319.32 ± 361.25 b
	CeAc	2741.87 ± 131.09 a	5130.44 ± 820.02 a	3839.57 ± 1186.55	2802.32 ± 1096.95	3453.77 ± 907.34 a
B	$nCeO_2$	23.07 ± 1.36 b	25.34 ± 1.05 b	25.08 ± 2.29 b	16.35 ± 3.08 b	21.44 ± 0.56 c
	CA + $nCeO_2$	18.93 ± 0.89 b	12.71 ± 2.59 c	26.33 ± 3.53 b	25.28 ± 1.61 ab	22.06 ± 1.96 c
	$bCeO_2$	23.61 ± 2.64 b	26.50 ± 0.42 b	25.12 ± 0.81 b	33.78 ± 4.21 ab	47.36 ± 0.55 a
	CA	41.19 ± 4.65 a	31.27 ± 1.67 b	26.65 ± 1.96 b	29.21 ± 1.81 ab	23.03 ± 1.72 c
	CeAc	45.57 ± 2.16 a	49.06 ± 2.61 a	55.01 ± 3.20 a	40.84 ± 8.21 a	33.98 ± 2.44 b
Ca	$nCeO_2$	20894.12 ± 2563.87 ab	23003.80 ± 1154.02 ab	23020.50 ± 1598.67 a	24763.09 ± 3173.54 a	20351.76 ± 907.12
	CA + $nCeO_2$	12026.75 ± 493.85 b	23893.96 ± 2502.59 ab	21168.33 ± 1500.43 a	19890.41 ± 1102.75 ab	18932.14 ± 1710.09
	$bCeO_2$	20475.76 ± 3265.74 ab	17375.47 ± 553.02 b	12456.57 ± 1142.87 b	14307.31 ± 721.59 b	15337.98 ± 831.18
	CA	18674.81 ± 3151.43 ab	22446.07 ± 2640.90 ab	24738.00 ± 3254.75 a	16667.37 ± 2120.23 ab	21375.04 ± 3153.92
	CeAc	25841.09 ± 798.50 a	26308.72 ± 1415.43 a	26371.36 ± 1155.35 a	17209.60 ± 3044.60 ab	18836.46 ± 1643.71
Cu	$nCeO_2$	91.01 ± 11.83 ab	83.47 ± 7.38	62.42 ± 5.72	136.74 ± 14.48	180.75 ± 7.90 a
	CA + $nCeO_2$	163.44 ± 30.87 a	78.36 ± 33.19	56.77 ± 10.43	81.82 ± 18.87	114.39 ± 27.11 b
	$bCeO_2$	56.87 ± 10.25 b	72.43 ± 1.93	84.18 ± 11.46	84.58 ± 13.78	62.44 ± 9.35 b
	CA	60.59 ± 15.64 b	66.92 ± 9.82	59.29 ± 11.45	135.73 ± 48.67	67.24 ± 8.45 b
	CeAc	83.45 ± 5.81 b	85.97 ± 8.53	87.98 ± 6.49	58.96 ± 11.97	53.82 ± 5.65 b
Fe	$nCeO_2$	998.61 ± 157.84 b	1129.99 ± 98.57 c	1139.46 ± 189.75	2036.56 ± 529.28	995.00 ± 91.09 ab
	CA + $nCeO_2$	1018.64 ± 125.45 b	2987.90 ± 711.24 ab	1979.52 ± 665.35	1185.87 ± 259.68	1427.32 ± 438.14 ab
	$bCeO_2$	1410.69 ± 195.62 ab	1519.08 ± 215.58 bc	986.58 ± 320.82	1250.35 ± 229.59	676.65 ± 21.07 b
	CA	1007.74 ± 188.17 b	1175.01 ± 172.04 c	903.91 ± 164.30	803.09 ± 169.54	1031.90 ± 285.97 ab
	CeAc	1904.65 ± 141.08 a	3470.10 ± 488.89 a	2414.92 ± 727.56	1852.88 ± 687.75	2435.48 ± 641.01 a
Mg	$nCeO_2$	1328.46 ± 142.88	1700.55 ± 128.05 b	1718.74 ± 251.40	1196.55 ± 61.55 bc	1215.84 ± 128.79 b
	CA + $nCeO_2$	1709.92 ± 140.75	1431.09 ± 268.10 b	1983.64 ± 340.08	1539.84 ± 104.76 abc	1874.43 ± 311.23 ab
	$bCeO_2$	1812.43 ± 358.55	2512.21 ± 139.32 a	1991.88 ± 290.51	2253.24 ± 397.37 a	2203.86 ± 172.56 a
	CA	1531.27 ± 305.50	1388.81 ± 159.26 b	1198.46 ± 240.28	2117.29 ± 196.26 ab	1249.41 ± 140.90 b
	CeAc	1267.44 ± 113.58	1755.84 ± 144.30 ab	1564.29 ± 235.25	1007.80 ± 219.67 c	1196.66 ± 180.38 b

Mn	$nCeO_2$	72.42 ± 11.39 b	78.16 ± 9.32 b	78.18 ± 13.46	161.96 ± 41.28	71.70 ± 10.36 ab
	CA + $nCeO_2$	77.87 ± 20.84 b	191.40 ± 21.56 a	97.03 ± 21.05	78.82 ± 27.40	90.65 ± 13.52 ab
	$bCeO_2$	95.61 ± 15.99 ab	113.75 ± 10.21 ab	75.91 ± 47.21	76.89 ± 6.92	53.20 ± 4.00 b
	CA	66.62 ± 9.23 b	156.37 ± 50.88 ab	71.75 ± 19.67	54.59 ± 14.64	63.18 ± 22.39 ab
	CeAc	148.68 ± 15.83 a	180.91 ± 9.81 ab	182.77 ± 49.79	111.42 ± 37.73	124.23 ± 19.04 a
Zn	$nCeO_2$	45.61 ± 5.76	61.89 ± 2.69	51.87 ± 7.87	45.15 ± 2.82 ab	64.27 ± 8.15
	CA + $nCeO_2$	58.49 ± 9.02	44.30 ± 3.90	47.42 ± 4.61	40.95 ±3.26 ab	44.49 ± 2.51
	$bCeO_2$	40.11 ± 2.81	54.26 ± 11.03	34.33 ± 7.21	36.51 ± 5.22 b	57.27 ± 4.45
	CA	35.20 ± 4.98	51.37 ± 11.16	47.23 ± 5.76	69.29 ± 14.29 a	49.15 ± 15.69
	CeAc	49.18 ± 2.64	46.39 ± 4.88	48.77 ± 4.02	31.01 ± 3.57 b	36.05 ± 7.20

Table S2. Nutrient composistion of dry tomato stems after 210 days of germination. Different letters indicate statistically significant differences between treatments. Data are means ±SE (standard error) of four replicates ($p \leq 0.05$). Only the elements that indicate statistical differences are shown.

		0	62.5	125	250	500
Ca	$nCeO_2$	9897.35 ± 1022.62	14522.06 ± 1437.71	14531.23 ± 2891.58 a	12007.70 ± 465.7	14637.73 ± 1341.33
	CA + $nCeO_2$	9234.43 ± 1640.94	15518.3 ± 1457.22	15833.14 ± 1664.2 b	14004.17 ± 1980.43	12349.32 ± 1225.09
	$bCeO_2$	10029.28 ± 701.8	11620.85 ± 348.75	9937.53 ±203.07 b	8982.86 ± 1442.07	9650.32 ± 872.30
	CA	10757.48 ± 1341.71	16254.66 ± 2931.89	14472.55 ± 2228.15 b	8621.44 ± 280.73	13412.45 ± 1516.61
	CeAc	12449.07 ± 1093.99	14065.02 ± 698.19	17131.67 ± 440.91 ab	19295.06 ± 2587.24	19501.02 ± 1653.22
Mg	$nCeO_2$	2446.12 ± 236.2	1892.06 ± 260.35 b	2372.25 ± 406.7 ab	1497.49 ± 204.0	1806.28 ± 291.96 b
	CA + $nCeO_2$	2965.16 ± 422.15	1992.75 ± 125.13 b	1789.1 ± 221.71 b	1997.61 ± 315.3	2484.78 ± 354.21 ab
	$bCeO_2$	2498.24 ± 471.08	3039.15 ± 157.79 a	3742.33 ± 574.09 a	2937.96 ± 640.83	3518.74 ± 346.4 a
	CA	2813.46 ± 440.73	2192.68 ± 370.49 ab	2550.14 ± 179.67 ab	2534.00 ± 324.52	2211.64 ± 200.23 ab
	CeAc	2298.79 ± 393.78	1766.43 ± 132.67 b	1854.5 ± 260.01 b	2051.75 ± 86.94	2212.74 ± 360.91 ab
P	$nCeO_2$	3788.87 ± 392.94	3347.61 ±480.75 b	3550.15 ± 263.23 b	3031.09 ± 429.59 b	2991.59 ± 312.66 b
	CA + $nCeO_2$	4894.61 ± 843.57	3956.35 ± 505.88 b	3073.31 ± 600.82 b	2978.54 ± 415.20 b	3891.28 ± 389.9 b
	$bCeO_2$	4135.30 ± 463.21	6937.05 ± 301.23 a	7853.13 ± 594.81 a	6249.96 ± 747.80 a	6201.65 ± 479.79 a
	CA	4091.13 ± 534.54	3762.59 ± 518.68 b	3582.60 ± 340.83 b	4695.42 ± 290.22 ab	4156.9 ± 310.28 b
	CeAc	3055.7 ± 344.11	3003.28 ± 328.32 b	3009.98 ± 616.28 b	5039.41 ± 472.22 ab	3090.27 ± 241.37 b
Zn	$nCeO_2$	58.06 ± 13.38	81.31 ± 8.78 b	85.79 ± 21.32	71.83 ± 1.67	80.04 ± 4.49
	CA + $nCeO_2$	83.13 ± 24.21	96.82 ± 21.12 b	111.74 ±15.52	75.36 ± 16.75	75.26 ± 12.49
	$bCeO_2$	81.24 ± 24.29	181.58 ± 17.54 a	98.96 ± 22.73	116.5 ± 26.21	64.48 ± 9.68
	CA	62.22 ± 9.91	116.9 ± 17.93 ab	80.09 ± 11.21	132.44 ± 0.86	88.5 ± 9.79
	CeAc	74.96 ± 15.82	103.59 ± 22.61 ab	101.74 ± 7.77	113.03 ± 11.88	102.53 ± 16.15

Table S3. Nutrient composition of dry tomato leaves after 210 days of germination. Different letters indicate statistically significant differences between treatments. Data are means ±SE (standard error) of four replicates ($p \leq 0.05$). Only the elements that indicate statistical differences are shown.

		0	62.5	125	250	500
Al	$nCeO_2$	214.98 ± 38.12	308.49 ± 91.81	237.59 ±	171.18 ± 44.81 b	141.39 ± 25.66
	CA + $nCeO_2$	192.06 ± 48.49	228.71 ± 42.13	165.80 ± 29.04	530.10 ± 131.83 a	184.00 ± 36.07
	$bCeO_2$	185.39 ± 19.83	165.32 ± 16.32	230.18 ± 43.68	196.42 ± 42.10 b	212.17 ± 31.58
	CeAc	177.69 ± 28.61	146.02 ± 25.41	214.96 ± 45.68	137.73 ± 13.67 b	165.60 ± 36.23
	CA	175.97 ± 17.85	205.41 ± 43.62	200.75 ± 38.61	169.05 ± 23.27 b	172.29 ± 31.81
B	$nCeO_2$	49.19 ± 1.17 b	52.76 ± 3.55	65.04 ± 4.06 a	47.46 ± 2.07 b	54.49 ± 1.74
	CA + $nCeO_2$	48.28 ± 4.05 b	40.00 ± 2.81	39.59 ± 3.20 b	36.19 ± 5.16 b	49.96 ± 3.70
	$bCeO_2$	51.73 ± 5.41 b	53.78 ± 5.88	64.05 ± 0.89 a	69.55 ± 4.77 a	65.84 ± 4.22
	CeAc	44.04 ± 5.34 b	50.26 ± 4.92	47.97 ± 1.39 b	41.43 ± 2.86 b	50.90 ± 2.64
	CA	50.38 ± 11.50 a	59.00 ± 6.043	65.17 ± 1.00 a	49.81 ± 3.02 b	59.44 ± 6.60
Cu	$nCeO_2$	12.20 ± 0.45	17.63 ± 2.13	16.03 ± 1.49 ab	13.61 ± 1.54	14.16 ± 2.76
	CA + $nCeO_2$	12.47 ± 2.80	12.18 ± 0.89	11.19 ± 1.48 b	14.61 ± 2.04	12.43 ± 0.86
	$bCeO_2$	11.06 ± 0.91	13.24 ± 0.65	12.97 ± 1.13 ab	13.33 ± 1.09	13.18 ±1.25
	CeAc	11.26 ± 1.02	12.81 ± 0.83	13.42 ± 0.75 ab	9.84 ± 1.28	12.27 ± 1.34
	CA	13.02 ± 0.99	14.77 ± 1.82	19.04 ± 1.33 a	12.69 ± 0.92	12.46 ± 0.94
Fe	$nCeO_2$	196.52 ± 27.34	340.71 ±103.88	322.23 ± 43.10 a	185.03 ± 33.19	159.71 ± 23.68
	CA + $nCeO_2$	222.15 ± 41.57	274.71 ± 46.42	198.77 ± 25.69 b	419.69 ± 142.67	192.82 ± 26.90
	$bCeO_2$	185.74 ± 29.39	205.77 ± 9.82	178.45 ± 20.66 b	233.93 ± 35.76	203.46 ± 25.56
	CeAc	201.10 ± 38.12	187.57 ± 15.90	185.68 ± 13.37 b	167.97 ± 15.92	184.14 ± 27.82
	CA	198.25 ± 21.81	240.71 ± 29.61	222.36 ± 18.25 ab	238.51 ± 68.43	200.15 ± 31.40
P	$nCeO_2$	5061.03 ± 332.41	6361.86 ± 337.52 a	7236.67 ± 913.70 a	6012.14 ± 367.61	5659.15 ± 312.80 ab
	CA + $nCeO_2$	6373.97 ±530.93	4906.02 ± 466.44 b	4888.44 ± 362.73 ab	3944.37 ±771.89	5309.20 ± 304.12 ab
	$bCeO_2$	5259.18 ± 437.29	5586.04 ± 17.94 ab	4860.46 ± 215.86 ab	5198.52 ± 460.38	4634.12 ± 479.79 b
	CeAc	4761.31 ± 529.12	5454.13 ± 372.81 ab	4315.21 ± 441.73 b	5672.56 ± 344.66	6344.46 ± 261.80 a
	CA	5011.54 ± 323.79	4830.18 ± 255.99 b	5846.02 ± 605.61 ab	5044.74 ± 306.03	4403.51 ± 86.38 b
S	$nCeO_2$	13806.60 ± 424.71	17273.80 ± 1552.6	17173.40 ± 453.65 ab	14333.72 ± 785.58	15621.24 ± 851.17
	CA + $nCeO_2$	13399.42 ± 567.93	13697.21 ± 935.81	14386.23 ± 1396.80 bc	11583.64 ± 1738.00	14594.61 ± 1217.12
	$bCeO_2$	15431.21 ± 1111.25	12495.87 ± 243.77	11079.34 ± 700.67 c	10920.74 ± 1055.20	10688.90 ± 594.04
	CeAc	11945.17 ± 917.44	14647.25 ± 2005.60	14358.73 ± 879.32 bc	13700.58 ± 998.93	15085.12 ± 507.39
	CA	14018.21 ± 1674.85	16021.43 ± 2209.67	19318.93 ± 919.73 a	12486.76 ± 1289.81	16391.44 ± 3271.32

Table S4. Stability constants of citric acid with different metals

Metal (to right) Ligand (below)	Al(III)	Ca	Cu	Fe(II)	Fe(III)	Mg	Mn	Zn
Citric acid	11.7 b	3.5 a	6.1 a	3.2 a	11.9 a	2.8 a	3.2 a	4.5 a

a. Furia, T.E. 1972. CRC Handbook of food additives. Chapter 6: Sequestrants in foods. 2, 275-278

b. Martin, R.B. 1994. *Accounts of Chemical Research*, 27(7), 204-210

Table S5. Ce concentration in roots, stems, and leaves of tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of bare ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between treatments at ($p \leq 0.05$); $n = 4$. Citric acid was not included in the figure as it does not contain cerium.

Organ	Treatment	Control	62.5	125	250	500
Stem	$nCeO_2$	0.36 ± 0.07	0.48 ± 0.0	0.67 ± 0.03 a	0.48 ± 0.01 ab	0.61 ± 0.07
	CA + $nCeO_2$	0.36 ± 0.07	0.43 ± 0.06	0.55 ± 0.06 ab	0.61 ± 0.07 a	0.61 ± 0.07
	$bCeO_2$	0.43 ± 0.12	0.28 ± 0.06	0.37 ± 0.08 b	0.25 ± 0.05 c	0.39 ±0.02
	CeAc	0.43 ± 0.06	0.80 ± 0.23	0.54 ±0.06 ab	0.30 ± 0.05 bc	0.43 ± 0.06
Leaf	$nCeO_2$	0.87 ± 0.16	2.10 ± 0.67	1.34 ± 0.15	1.11 ± 0.16	0.97 ± 0.24
	CA + $nCeO_2$	0.90 ± 0.12	1.60 ± 0.40	0.85 ± 0.07	1.07 ± 0.31	1.03 ± 0.15
	$bCeO_2$	0.97 ± 0.11	0.65 ± 0.15	0.98 ± 0.21	1.08 ± 0.26	1.01 ± 0.18
	CeAc	1.09 ± 0.11	0.89 ± 0.13	0.98 ± 0.10	0.91 ± 0.05	1.03 ± 0.11
Root	$nCeO_2$	3.27 ± 0.39	20.03 ± 2.14 b	40.73 ± 8.10 ab	129.84 ± 18.00 a	197.43 ± 19.55 a
	CA + $nCeO_2$	5.60 ± 0.17	18.56 ± 6.55 b	12.75 ± 5.27 b	13.08 ± 4.90 b	28.32 ± 3.42 b
	$bCeO_2$	6.97 ± 2.85	23.81 ± 4.18 b	24.24 ± 10.46 ab	56.31 ± 8.92 ab	75.78 ± 2.10 ab
	CeAc	3.77 ± 0.93	47.38 ± 5.88 a	57.35 ± 14.08 a	71.01 ± 30.19 ab	185.71 ± 60.23 a

Table S6. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in leaves of 210 day-old tomato plants grown in soil amended with bare ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between treatments at ($p \leq 0.05$); $n = 4$.

		Control	62.5	125	250	500
Chlorophyll a	$nCeO_2$	99.26 ± 2.36	103.10 ± 1.43 a	98.23 ± 2.03	95.15 ± 3.86	102.32 ± 1.99
	CA + $nCeO_2$	95.12 ± 7.30	94.45 ± 4.73 ab	88.50 ± 6.91	103.78 ± 1.52	96.28 ± 4.80
	$bCeO_2$	97.41 ± 1.94	70.77 ± 9.17 b	84.81 ± 3.37	102.55 ± 2.39	102.72 ± 2.53
	CeAc	80.57 ± 16.67	89.24 ± 11.78 ab	98.86 ± 4.73	92.86 ± 6.31	101.02 ± 1.53
	CA	99.71 ± 2.80	91.73 ± 4.46 ab	90.37 ± 4.23	93.30 ± 3.65	98.02 ± 2.62
Chlorophyll b	$nCeO_2$	98.89 ± 3.90	98.78 ± 6.27	97.66 ± 3.95	97.55 ± 11.48 ab	120.15 ± 9.68
	CA + $nCeO_2$	95.29 ± 11.83	110.75 ± 18.00	84.74 ± 12.13	131.21 ± 7.08 a	101.34 ± 9.30
	$bCeO_2$	111.32 ± 10.31	68.56 ± 7.52	78.40 ± 3.39	121.84 ± 9.04 ab	119.49 ± 11.82
	CeAc	94.73 ± 28.69	94.92 ± 19.70	96.40 ± 16.80	86.03 ± 10.38 b	112.65 ± 8.19
	CA	118.58 ± 14.43	82.97 ± 6.79	94.78 ± 14.90	91.17 ± 9.48 ab	103.01 ± 8.18
Total chlorophyll	$nCeO_2$	198.14 ± 4.46	201.88 ± 7.12	195.89 ± 5.51	192.70 ± 15.11	222.47 ± 11.37
	CA + $nCeO_2$	190.41 ± 18.25	205.20 ± 21.09	173.24 ± 18.84	234.99 ± 8.39	197.63 ± 14.05
	$bCeO_2$	208.74 ± 10.83	139.32 ± 16.48	163.21 ± 6.72	224.39 ± 11.42	222.21 ± 13.80
	CeAc	175.3 ± 44.64	184.16 ± 30.82	195.26 ± 21.42	178.88 ± 16.54	213.67 ± 9.15
	CA	218.30 ± 17.01	174.7 ± 10.86	185.15 ± 18.46	184.47 ± 12.73	201.04 ± 9.97

Table S7. Antioxidant activity of catalase and ascorbate peroxidase in fresh leaves of 210 day-old tomato plants grown in soil amended with bare ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of three replicates ± SE. Different letters indicate statistically significant differences between treatments at ($p \leq .05$); $n = 4$.

		Control	62.5	125	250	500
CAT	$nCeO_2$	0.26 ± 0.02	0.45 ± 0.05 b	0.31 ± 0.07 ab	0.37 ± 0.05 b	0.13 ± 0.03 b
	CA + $nCeO_2$	0.28 ± 0.01	0.96 ± 0.08 a	0.95 ± 0.05 a	0.46 ± 0.01 ab	1.52 ± 0.17 a
	$bCeO_2$	0.33 ± 0.08	0.14 ± 0.03 c	0.05 ± 0.02 b	0.62 ± 0.03 a	0.45 ± 0.06 b
	CeAc	0.22 ± 0.02	0.07 ± 0.01 c	0.48 ± 0.06 ab	0.11 ± 0.01 c	0.43 ± 0.10 b
	CA	0.36 ± 0.08	0.36 ± 0.03 b	0.43 ± 0.10 ab	0.36 ± 0.07 c	0.38 ± 0.03 b
APOX	$nCeO_2$	0.38 ± 0.02	0.07 ± 0.04	0.11 ± 0.01 bc	0.03 ± 0.01 c	0.07 ± 0.01 ab
	CA + $nCeO_2$	0.29 ± 0.06	0.33 ± 0.05	0.04 ± 0.01 c	0.15 ± 0.03 ab	0.08 ± 0.02 ab
	$bCeO_2$	0.33 ± 0.02	0.06 ± 0.01	0.04 ± 0.01 c	0.13 ± 0.02 ab	0.03 ± 0.01 a
	CeAc	0.29 ± 0.02	0.26 ± 0.05	0.26 ± 0.02 a	0.16 ± 0.03 a	0.08 ± 0.01 ab
	CA	0.29 ± 0.07	0.12 ± 0.03	0.17 ± 0.04 ab	0.06 ± 0.01 bc	0.12 ± 0.01 a

Table S8. Shoot length of 15, 60, and 210 day-old tomato plants grown in soil amended with bare ($nCeO_2$), citric acid coated (CA + $nCeO_2$) NPs, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates ± SE. Different letters indicate statistically significant differences between compounds at ($p \leq 0.05$); $n = 4$.

Treatment	DAG	Control	62.5	125	250	500
$nCeO_2$	15	17.33 ± 0.30 a	15.94 ± 0.07	14.73 ± 1.00	16.33 ± 0.19	16.96 ± 0.74
CA + $nCeO_2$		14.38 ± 0.22 ab	14.57 ± 0.16	14.66 ± 0.46	16.13 ± 0.48	14.76 ± 0.04
$bCeO_2$		13.26 ± 0.20 b	13.54 ± 0.87	14.48 ± 1.15	15.91 ± 0.26	16.46 ± 0.79
CeAc		15.03 ± 0.44 ab	15.19 ± 0.18	15.11 ± 0.04	16.37 ± 0.22	15.04 ± 0.85
CA		15.27 ± 0.29 ab	15.43 ± 0.09	16.28 ± 0.43	16.39 ± 0.81	16.03 ± 0.37
$nCeO_2$	60	49.65 ± 0.21	40.49 ± 0.93	39.55 ± 0.84 b	44.11 ± 1.94 a	46.10 ± 0.90 a
CA + $nCeO_2$		47.22 ± 1.02	41.66 ± 1.51	43.25 ± 1.05 ab	44.53 ± 0.67 a	43.26 ± 0.43 a
$bCeO_2$		49.76 ± 0.21	44.54 ± 0.75	39.68 ± 0.72 b	31.62 ± 0.48 b	30.57 ± 0.36 b
CeAc		48.68 ± 0.47	46.91 ± 0.71	45.05 ± 1.49 a	46.33 ± 1.12 a	47.75 ± 0.68 a
CA		49.33 ± 1.05	40.22 ± 0.82	45.28 ± 0.73 a	54.55 ± 0.30 a	52.88 ± 0.32 a
$nCeO_2$	210	146.31 ± 0.27	135.99 ± 0.03 a	112.21 ± 0.15 b	142.21 ± 1.30 b	162.20 ± 1.80 a
CA + $nCeO_2$		145.64 ± 1.12	136.06 ± 2.41 a	130.09 ± 1.07 a	158.61 ± 1.85 a	168.49 ± 1.73 a
$bCeO_2$		149.19 ± 1.47	89.48 ± 0.55 b	83.11 ± 0.02 c	81.55 ± 0.20 d	78.13 ± 0.05 c
CeAc		144.38 ± 0.25	140.31 ± 0.02 a	135.10 ± 1.16 a	127.00 ± 0.49 c	106.31 ± 1.53 b
CA		144.38 ± 0.21	148.65 ± 1.47 a	144.74 ± 1.65 a	156.04 ± 0.77 c	156.81 ± 1.23 b

Table S9. Soil composition

		Concentration (mg/kg)	
Miracle-Gro® Potting Mix	**%**	**Average ± SE**	
Forest products, compost, sphagnum peat moss, perlite, wetting agent and fertilizer	50-60	7551.28 ± 447.58	Al
Total nitrogen (N)*	0.21	29570.39 ± 3406.41	Ca
ammoniacal nitrogen	0.12	30.52 ± 4.97	Cu
nitrate nitrogen	0.09	4653.38 ± 404.12	Fe
Available phosphate (P_2O_5)	0.07	1868.65 ± 92.83	K
Soluble potash (K_2O)*	0.14	3110.12 ±789.19	Mg
Iron (Fe)	0.1	197.67 ± 12.08	Mn
water soluble iron (Fe)	0.1	1818.36 ± 261.48	P
		44.22 ± 5.22	Zn

Derived from: polymer coated: ammonium nitrate, ammonium phosphate, calcium phosphate, and potassium phosphate; and ammonium nitrate, ammonium phosphate, calcium phosphate, potassium sulfate, and ferrous sulfate.
* A portion of the nitrogen, phosphate and potash has been coated to provide 0.15% coated slow release nitrogen (N), 0.03% coated slow release available phosphate (P_2O_5) and 0.08% coated slow release soluble potash (K_2O)
Soil pH= 6.8-7.2

Figure S1. Different developmental stages of tomato plants. (A) Experimental setup; (B) Young tomato plants after 10 days of germination; (C) Mature tomato plants after 120 days of germination; (D) Close-up view of the tomato fruit and (E) Mature tomato plant after 160 days of germination

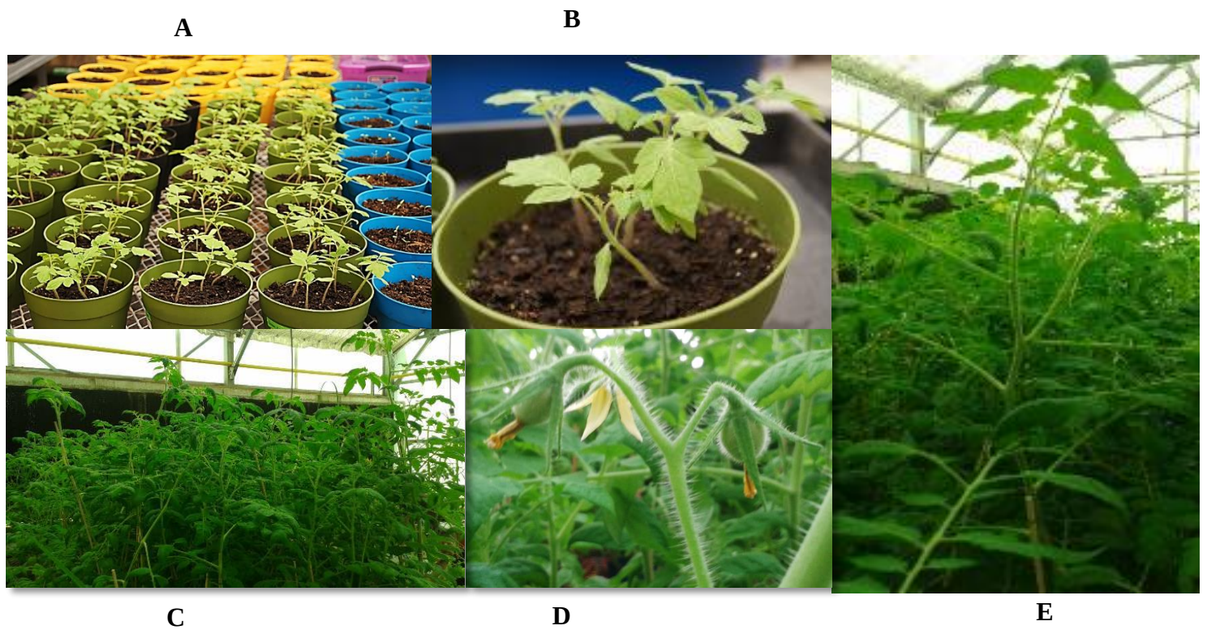

Figure S1.

2 SUPPORTING INFORMATION FOR CHAPTER 3: NUTRITIONAL QUALITY ASSESSMENT OF TOMATO FRUITS AFTER EXPOSURE TO UNCOATED AND CITRIC ACID COATED CERIUM OXIDE NANOPARTICLES, BULK CERIUM OXIDE, CERIUM ACETATE AND CITRIC ACID

Figure S1. Representative transmission electron microscopy (TEM) (left images) and X-ray diffraction (XRD) (Right images) images of: A) $bCeO_2$, B) $nCeO_2$, C) $nCeO_2$ + CA.

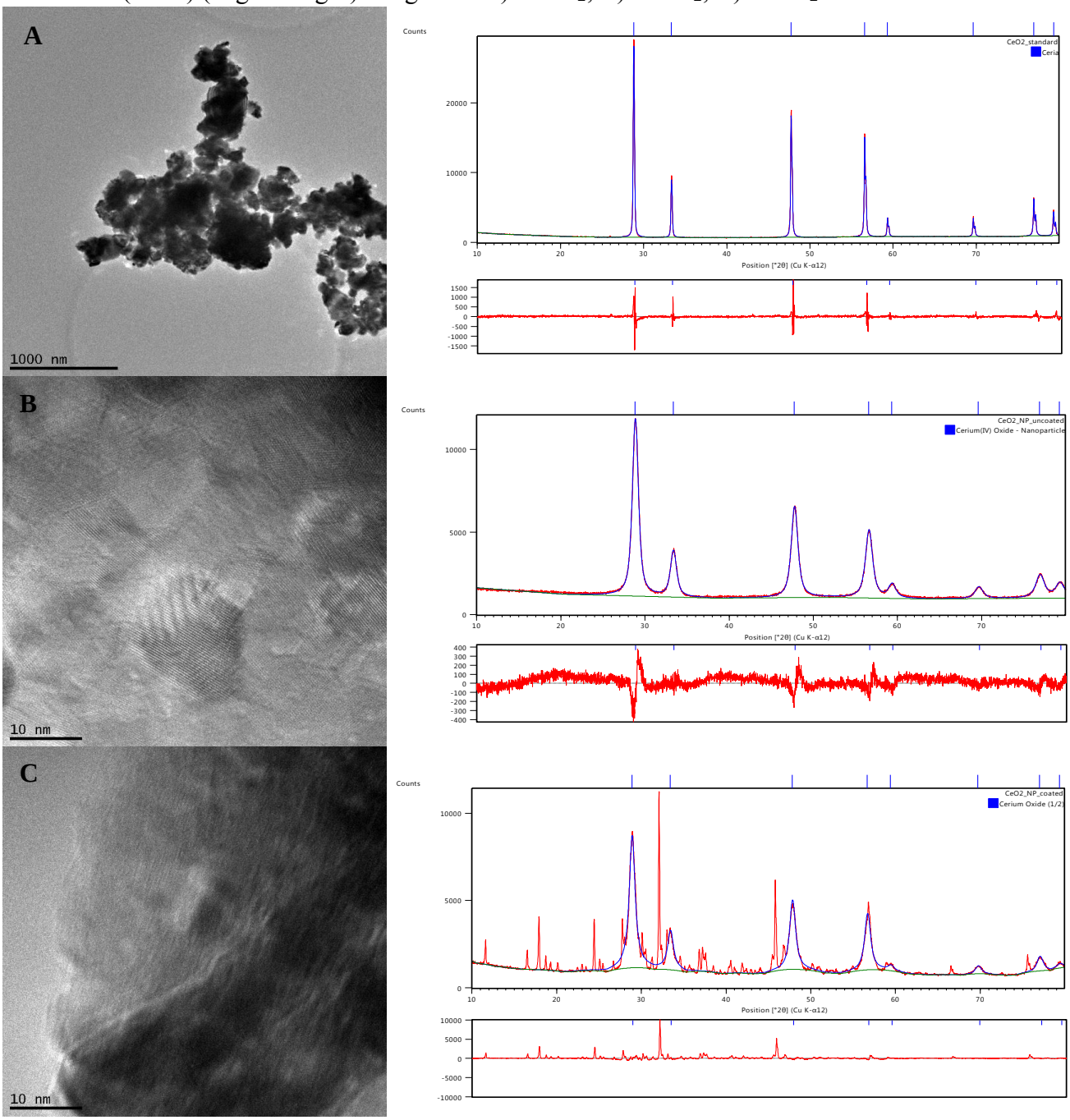

Figure S1.

Table S1. Physiological parameters of fruits harvested from tomato plants grown to full maturity (210 days). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Different letters indicate statistically significant differences between treatments. Data are means ± SE, where n has a range from 3 to 12 replicates ($p \leq 0.05$).

Parameter	Treatment	Control	62.5	125	250	500
Length (mm)	$nCeO_2$	34.13 ± 3.15	30.51 ±1.62	28.94 ± 1.30 b	35.14 ± 1.69	27.01 ± 8.58
	$nCeO_2$+ CA	37.04 ± 1.63	36.59 ± 3.33	39.75 ± 3.61 a	32.88 ± 2.59	30.24 ± 2.24
	$bCeO_2$	34.71 ± 2.61	25.52 ± 4.25		33.07 ± 0.06	32.91 ± 2.18
	CeAc	31.59 ± 1.95	37.51 ± 0.60	38.03 ± 0.84 a		28.81 ± 2.59
	CA	32.51 ± 2.67	35.67 ± 3.74	39.05 ± 4.08 a	34.95 ± 2.83	39.89 ± 4.68
Width (mm)	$nCeO_2$	25.81 ± 2.63	21.65 ± 1.10 ab	22.25 ± 1.44 b	25.09 ± 1.34	27.05 ± 3.50
	$nCeO_2$+ CA	26.80 ± 1.53	26.04 ± 2.64 ab	25.87 ± 1.57 ab	24.08 ± 1.72	22.42 ± 1.12
	$bCeO_2$	28.25 ± 2.83	18.60 ± 2.57 b		23.88 ± 1.60	22.57 ± 2.02
	CeAc	24.36 ± 1.44	29.99 ± 0.27 a	28.80 ± 0.96 a		22.44 ± 1.63
	CA	23.20 ± 1.96	24.96 ± 3.00 ab	23.59 ± 1.08 ab	25.15 ± 2.02	24.36 ± 2.15
Fresh Wt. (g)	$nCeO_2$	6.58 ± 1.19	4.70 ± 0.71	3.90 ± 0.67 b	5.14 ± 0.56	4.78 ± 3.90 ab
	$nCeO_2$+ CA	7.15 ± 0.98	6.72 ± 1.58	7.70 ± 1.02 a	5.23 ± 0.99	3.97 ± 0.64 b
	$bCeO_2$	7.67 ± 1.60	2.19 ± 0.91		5.89 ± 0.89	4.74 ± 1.17 ab
	CeAc	5.35 ± 1.13	6.75 ± 0.39	8.57 ± 0.56 a		3.71 ± 0.73 b
	CA	5.24 ± 0.99	7.09 ± 2.12	7.59 ± 1.87 a	6.59 ± 1.06	8.60 ± 3.11 a
Dry Wt. (g)	$nCeO_2$	0.19 ± 0.03	0.20 ± 0.02 ab	0.20 ± 0.03	0.18 ± 0.02 b	0.19 ± 0.07
	$nCeO_2$+ CA	0.61 ± 0.13	0.28 ± 0.05 ab	0.33 ± 0.05	0.26 ± 0.05 ab	0.22 ± 0.04
	$bCeO_2$	0.27 ± 0.01	0.09 ± 0.04 b		0.33 ± 0.04 a	0.25 ± 0.07
	CeAc	0.55 ± 0.26	0.21 ± 0.001 ab	0.31 ± 0.03		0.21 ± 0.06
	CA	0.28 ± 0.07	0.35 ± 0.11 a	0.25 ± 0.15	0.21 ± 0.03 ab	0.32 ± 0.12
Water Ct. (g)	$nCeO_2$	6.38 ± 1.16	4.50 ± 0.69	3.70 ± 0.64 b	4.96 ± 0.55	4.60 ± 3.83
	$nCeO_2$+ CA	6.63 ± 0.91	6.45 ± 1.54	7.37 ± 0.98 a	4.97 ± 0.94	3.75 ± 0.61
	$bCeO_2$	7.41 ± 1.59	2.10 ± 0.87		5.56 ± 0.85	4.49 ± 1.10
	CeAc	4.79 ± 0.93	6.54 ± 0.39	8.26 ± 0.53 a		3.50 ± 0.69
	CA	4.96 ± 0.93	6.74 ± 2.03	6.74 ± 2.03 a	6.39 ± 1.04	8.18 ± 2.04

Table S2. Total, reducing and non-reducing sugar content (in mg/g (d wt.)) of fruits harvested from tomato plants grown to full maturity (210 days). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Different letters indicate statistically significant differences between treatments. Data are means ± SE, where n has a range from 3 to 12 replicates ($p \leq 0.05$).

	Treatment	Control	62.5	125	250	500
TOTAL	$nCeO_2$	219.95 ± 17.53 b	203.12 ± 11.66 c	204.56 ± 19.55 b	218.18 ± 13.14 c	219.22 ± 0.74 b
	$nCeO_2$+CA	327.71 ± 17.17 a	274.80 ± 7.34 bc	297.98 ± 16.33 b	255.19 ± 7.22 c	266.51 ± 8.37 b
	$bCeO_2$	389.39 ± 19.76 a	363.90 ± 30.78 a		461.96 ± 21.54 a	394.43 ± 59.26 a
	CeAc	402.48 ± 52.43 a	347.97 ± 34.60 ab	425.35 ± 27.40 a		418.82 ± 11.18 a
	CA	367.15 ± 16.15 a	333.83 ± 10.82 ab	392.20 ± 6.76 a	344.09 ± 9.69 b	424.54 ± 14.28 a
REDUCING	$nCeO_2$	66.17 ± 8.67 b	52.36 ± 11.54 a	52.12 ± 12.10 a	53.21 ± 5.82 b	59.45 ± 0.58 b
	$nCeO_2$+CA	88.65 ± 7.72 ab	55.80 ± 2.84 a	66.91 ± 15.32 a	47.78 ± 11.39 b	46.05 ± 4.41 b
	$bCeO_2$	61.23 ± 7.14 b	62.20 ± 6.87 a		102.38 ± 5.70 a	96.70 ± 2.08 a
	CeAc	103.34 ± 25.85 a	60.22 ± 19.72 a	75.24 ± 10.63 a		101.98 ± 1.95 a
	CA	55.58 ± 4.77 b	62.32 ± 4.24 a	85.94 ± 2.65 a	42.89 ± 4.10 b	98.40 ± 8.44 a
NON- RED	$nCeO_2$	153.78 ± 17.85 b	150.76 ± 10.43 c	152.43 ± 10.67 b	164.97 ± 11.30 b	159.76 ± 0.47 b
	$nCeO_2$+CA	252.73 ± 13.36 ab	219.00 ± 5.18 b	231.07 ± 6.77 ab	207.41 ± 4.49 b	220.46 ± 8.51 a
	$bCeO_2$	323.76 ± 21.29 a	309.70 ± 31.80 a		366.11 ± 21.95 a	351.76 ± 12.90 a
	CeAc	326.77 ± 25.10 ab	299.21 ± 40.43 a	350.66 ± 32.88 a		316.76 ± 11.12 a
	CA	311.58 ± 17.49 a	271.51 ± 8.67 ab	306.26 ± 6.67 ab	301.20 ± 11.34 a	326.14 ± 8.19 a

Table S3. Starch content (in mg/g (d wt.)) of fruits harvested from tomato plants grown to full maturity (210 days). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Different letters indicate statistically significant differences between treatments. Data are means ± SE, where n has a range from 3 to 12 replicates ($p \leq 0.05$).

STARCH

Treatment	Control	62.5	125	250	500
$nCeO_2$	312.69 ± 22.20 ab	279.94 ± 15.15 ab	342.55 ± 41.75 a	264.86 ± 20.64 b	327.20 ± 26.10 a
$nCeO_2$ + CA	404.28 ± 24.05 a	331.93 ± 13.80 ab	316.92 ± 29.17 a	359.04 ± 15.84 a	363.19 ± 20.37 a
$bCeO_2$	340.50 ± 82.70 ab	227.47 ± 80.45 b		277.63 ± 49.38 ab	332.03 ± 43.72 a
CeAc	268.55 ± 23.85 b	231.79 ± 55.30 b	223.28 ± 41.40 a		264.82 ± 36.07 a
CA	305.00 ± 47.18 ab	365.53 ± 16.94 a	318.78 ± 86.17 a	231.84 ± 15.15 b	205.93 ± 32.70 a

Table S4. Micro- and macro- elemental composition (in mg/kg d wt.) of fruits harvested from tomato plants grown to full maturity (210 days). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Different letters indicate statistically significant differences between treatments. Data are means ± SE, where n has a range from 3 to 12 replicates ($p \leq 0.05$).

	Treatment	Control	62.5	125	250	500
Al	$nCeO_2$	42.07 ± 3.56 ab	42.07 ± 2.86 a	31.95 ± 2.84 a	40.93 ± 4.62 a	34.71 ± 6.41 a
	$nCeO_2$ + CA	33.36 ± 2.63 b	37.36 ± 3.57 a	34.25 ± 3.10 a	57.21 ± 10.28 a	45.89 ± 6.92 a
	$bCeO_2$	30.30 ± 2.30 b	38.76 ± 1.18 a		36.32 ± 2.99 a	32.44 ± 4.79 a
	CeAc	35.90 ± 5.27 b	47.43 ± 3.26 a	35.90 ± 1.88 a		34.82 ± 3.49 a
	CA	61.84 ± 5.64 a	48.53 ± 8.30 a	35.76 ± 2.91 a	62.08 ± 9.80 a	39.45 ± 9.72 a
B	$nCeO_2$	13.89 ± 0.65 a	15.26 ± 0.56 a	15.88 ± 1.36 a	14.36 ± 0.54 a	9.91 ± 3.27 b
	$nCeO_2$ + CA	12.34 ± 1.13 a	14.18 ± 0.63 a	16.48 ± 0.62 a	16.04 ± 0.81 a	16.44 ± 0.94 a
	$bCeO_2$	14.60 ± 1.03 a	17.38 ± 2.42 a		13.31 ± 2.01 a	16.14 ± 0.73 a
	CeAc	15.09 ± 1.82 a	17.98 ± 4.84 a	13.59 ± 0.18 a		12.52 ± 0.76 ab
	CA	16.88 ± 1.04 a	17.09 ± 1.36 a	13.67 ± 0.57 a	15.35 ± 0.67 a	13.92 ± 1.74 ab
Ca	$nCeO_2$	5699.71 ± 799.20 a	4846.87 ± 635.41 a	3737.05 ± 540.36 ab	5069.54 ± 619.86 ab	2317.22 ± 810.81 a
	$nCeO_2$ + CA	1826.85 ± 360.77 b	3434.73 ± 751.79 a	4043.79 ± 632.81 a	4343.40 ± 666.55 ab	3023.39 ± 425.75 a
	$bCeO_2$	1748.72 ± 237.77 b	6413.14 ± 822.53 a		2886.39 ± 530.33 b	3756.93 ± 1030.3 a
	CeAc	2351.61 ± 544.83 b	6053.16 ± 1007.64 a	3360.71 ± 22.89 ab		2104.82 ± 279.38 a
	CA	5432.76 ± 579.96 a	6028.87 ± 1211.78 a	2020.00 ± 64.11 b	6138.91 ± 666.58 a	2642.09 ± 448.35 a
Cu	$nCeO_2$	12.43 ± 0.42 a	10.88 ± 0.57 a	10.19 ± 0.60 ab	12.77 ± 0.61 ab	11.53 ± 4.14 a
	$nCeO_2$ + CA	13.48 ± 3.19 a	10.41 ± 2.09 a	13.51 ± 1.39 a	9.87 ± 1.13 bc	7.95 ± 0.46 a
	$bCeO_2$	13.87 ± 1.63 a	13.29 ± 2.14 a		6.65 ± 0.86 c	10.06 ± 0.43 a
	CeAc	8.41 ± 1.49 a	9.97 ± 2.11 a	13.28 ± 1.22 a		9.24 ± 0.46 a
	CA	13.06 ± 1.33 a	10.84 ± 0.88 ab	8.48 ± 0.31 b	13.50 ± 0.73 a	10.34 ± 1.12 a
Fe	$nCeO_2$	46.69 ± 4.11 a	39.55 ± 6.12 b	22.43 ± 4.80 ab	42.96 ± 6.46 a	10.16 ± 5.24 b
	$nCeO_2$ + CA	22.74 ± 5.45 a	21.13 ± 9.29 b	20.22 ± 3.05 ab	32.11 ± 4.69 a	24.94 ± 5.69 ab
	$bCeO_2$	45.41 ± 15.38 ab	104.98 ± 65.72 a		20.89 ± 4.58 a	21.40 ± 4.73 b
	CeAc	17.34 ± 5.16 b	21.15 ± 2.97 b	32.50 ± 3.74 a		14.07 ± 2.19 b
	CA	28.97 ± 6.82 ab	19.35 ± 3.21 b	8.86 ± 1.32 b	42.77 ± 21.09 a	44.94 ± 7.81 a
Mg	$nCeO_2$	1605.78 ± 103.36 ab	1655.20 ± 90.93 a	1483.29 ± 175.79 a	1683.58 ± 252.36 a	1515.23 ± 209.47 a
	$nCeO_2$ + CA	1208.21 ± 158.77 b	1335.15 ± 163.14 a	1402.39 ± 212.81 a	1404.65 ± 98.85 ab	1109.21 ± 136.87 a
	$bCeO_2$	2175.08 ± 275.19 a	1410.47 ± 106.65 a		917.03 ± 237.66 b	1513.83 ± 137.27 a
	CeAc	1442.43 ± 288.93 b	1406.11 ± 211.79 a	1383.23 ± 119.17 a		1290.12 ± 127.89 a
	CA	1511.80 ± 90.18 ab	1242.45 ± 72.89 a	1348.86 ± 51.31 a	1727.97 ± 91.60 a	1459.81 ± 267.85 a
Mn	$nCeO_2$	17076.96 ± 602.23 b	15718.78 ± 645.99 a	13176.23 ± 583.42 a	17743.67 ± 1219.06 a	11506.42 ± 1896.28 a
	$nCeO_2$ + CA	15120.34 ± 683.16 ab	13269.56 ± 1418.07 a	12772.55 ± 1210.6 a	15417.28 ± 1068.89 ab	14979.66 ± 1040.2 a
	$bCeO_2$	18731.29 ± 1918.23 b	15565.66 ± 1011.52 a		12800.00 ± 966.31 bc	12388.29 ± 860.14 a
	CeAc	11803.00 ± 1693.75 b	17669.23 ± 6193.72 a	13920.26 ± 815.23 a		11998.95 ± 1450.66 a
	CA	16181.86 ± 1010.84 ab	15309.44 ± 2811.52 a	9354.43 ± 414.9795 b	9416.08 ± 1201.45 c	14883.97 ± 1825.26 a
P	$nCeO_2$	4372.44 ± 226.28 ab	4437.09 ± 301.95 a	3739. 26 ± 283.46 a	4009.37 ± 482.73 a	4843.89 ± 137.74 a
	$nCeO_2$ + CA	4071.49 ± 143.74 ab	3441.43 ± 224.91 a	3831.44 ± 422.67 a	3702.39 ± 182.38 a	3352.01 ± 425.88 a
	$bCeO_2$	4887.30 ± 297.23 a	4746.3 ± 625.50 a		2695.61 ± 571.74 a	4608.03 ± 391.34 a
	CeAc	4667.54 ± 327.72 ab	3604.71 ± 418.53 a	4025.07 ± 274.07 a		3994.62 ± 396.58 a
	CA	3799.88 ± 158.33 b	3398.01 ± 200.05 a	3579.11 ± 111.35 a	3803.36 ± 182.02 a	3641.14 ± 520.53 a
S	$nCeO_2$	2044.58 ± 71.77 a	1965.82 ± 80.66 a	1629.38 ± 109.86 a	1835.67 ± 134.78 ab	2078.16 ± 75.92 a
	$nCeO_2$ + CA	1851.24 ± 109.14 a	1688.96 ± 79.72 a	1701.62 ± 118.13 a	2020.86 ± 111.52 a	1718.53 ± 175.32 a
	$bCeO_2$	2058.60 ± 176.93 a	2132.56 ± 4.38 a		1284.71 ± 244.52 b	1928.46 ± 144.39 a
	CeAc	2105.45 ± 152.73 a	2123.02 ± 454.84 a	1912.75 ± 93.42 a		1682.88 ± 124.06 a

	CA	1832.47 ± 133.51 a	1659.27 ± 125.71 a	1634.05 ± 39.93 a	1841.15 ± 111.87 ab	1904.54 ± 271.92 a
	$nCeO_2$	17.61 ± 0.89 a	18.94 ± 1.17 ab	15.71 ± 1.61 a	19.70 ± 1.51 a	17.56 ± 2.09 a
	$nCeO_2$ + CA	13.84 ± 1.90 a	13.93 ± 1.60 bc	14.02 ± 1.33 ab	16.12 ± 0.80 ab	16.94 ± 1.49 a
Zn	$bCeO_2$	21.81 ± 4.07 a	23.75 ± 3.95 a		15.74 ± 5.66 ab	15.35 ± 2.02 a
	CeAc	16.98 ± 2.86 a	10.71 ± 0.22 c	18.42 2.18 a		10.71 ± 0.87 a
	CA	15.32 ± 2.11 a	14.10 ± 0.75 bc	9.11 ± 0.46 b	11.40 ± 0.72 b	14.13 ± 2.33 a

Table S5. Lycopene content (in mg/kg f wt.) of fruits harvested from tomato plants grown to full maturity (210 days). At 62.5 mg/kg $bCeO_2$ did not produce any tomatoes and CeAc at 250 mg/kg did not produce enough samples for statistical analysis. Different letters indicate statistically significant differences between treatments. Data are means ± SE, where n has a range from 3 to 12 replicates ($p \leq 0.05$).

LYCOPENE

Treatment	Control	62.5	125	250	500
$nCeO_2$	6.08 ± 0.85 a	8.14 ± 1.72 a	6.55 ± 1.59 ab	2.33 ± 0.17 ab	2.72 ± 0.06 ab
$nCeO_2$ + CA	7.50 ± 2.45 a	8.22 ± 2.15 a	16.57 ± 4.48 a	8.13 ± 2.52 a	5.32 ± 0.88 a
$bCeO_2$	5.74 ± 0.67 a	0.48 ± 0.25 a		2.23 ± 0.27 ab	1.62 ± 0.76 b
CeAc	6.20 ± 0.50 a	1.91 ± 1.11 a	1.33 ± 0.47 b		1.20 ± 0.22 b
CA	2.06 ± 0.96 a	0.72 ± 0.25 a	2.28 ± 0.17 b	0.77 ± 0.18 b	0.50 ± 0.28 b

Vita

Ana Cecilia Barrios was born in El Paso, TX, but raised in Ciudad Juarez, Mexico. She graduated High School from Colegio San Patricio as the top senior in her class with an average of 9.8 (on a 1 to 10 scale). Afterwards, she was awarded with the Presidential Excellence Scholarship from the University of Texas at El Paso, where she majored in Biochemistry obtained her Bachelor of Science degree in 2013 with a GPA of 3.69, graduating with *cum laude* honors. She also obtained the Research Excellence for the Undergraduate Student in Biochemistry. That same year, she started her Master's degree in Chemistry, working under the mentorship of Dr. Jorge L. Gardea-Torresdey. In the spring 2013, Ana obtained the Louis Stokes Alliance for Minority Participation (LSAMP), Bridge to the Doctorate Fellowship, which funded her research during two years. Her research focuses on the impacts of citric acid coated and uncoated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate and citric acid on tomato (*Solanum lycopersicum* L.) plants and fruits. Her study comprehends the importance of a full life cycle study to understand the effects on biochemical and physiological parameters in the tomato plant.

Ana has authored and co-authored eight publications during her Master's degree in high impact factor journals. There are three other manuscripts in review process. In August 2016, Ana will be joining Arizona State University (ASU) to pursue a Ph.D. degree in Civil, Environmental and Sustainable Engineering. She was also awarded a Dean's Fellowship by ASU to fund her doctoral studies.

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