

BIOAVAILABILITY OF METAL/METAL OXIDE NANOMATERIALS AND
THEIR EFFECTS ON THE PHYSIOLOGY OF PLANTS

NUBIA ZUVERZA-MENA

Doctoral Program in Materials Science & Engineering

APPROVED:

Jorge L. Gardea-Torresdey, Ph.D., Chair

José R. Peralta-Videa, Ph.D.

Devesh Misra, Ph.D.

Stephen W. Stafford, Ph.D.

Charles Ambler, Ph.D.
Dean of the Graduate School

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Dedication

To my role models:

Luis F. Zuverza-Meza

Patricia Mena-Sierra

Jorge L. Zuverza-Mena

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by

NUBIA ZUVERZA MENA, M.S.

DISSERTATION

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Chapter 1. Introduction

In 1959, Richard Feynman introduced to the world the concept of nanotechnology with his breakthrough speech “There’s plenty of room at the bottom.” In 1986, K. Eric Drexler predicted “the coming era of nanotechnology” in his book *Engines of Creation*. However, it was not until the year 2000 when nano-technological applications bloomed and started to grow exponentially (Roco 2003). Nanomaterials are commonly referred to as small objects of less than 100 nm in width, length and/or height and have existed long before we discovered their applications. Materials behave in a different manner when reaching small sizes (below 100 nm) exhibiting unique properties. What makes nanomaterials (NMs) special and different from the “bulk” or above micron-size particles, is mostly attributed to their huge surface area to volume ratio, which makes NMs highly reactive. A piece of material interacts with its surroundings through its outer atoms. If this is broken down in smaller pieces, there will be more of the previously inner atoms now exposed to the environment, capable of reacting. The smaller the pieces, the higher the surface area. Therefore, NMs are not only more reactive, but also exhibit characteristics not observed in bigger “chunks” of the same composition. Figure 1.2 depicts how the surface area to volume ratio (SA:V) increases as size is diminished:

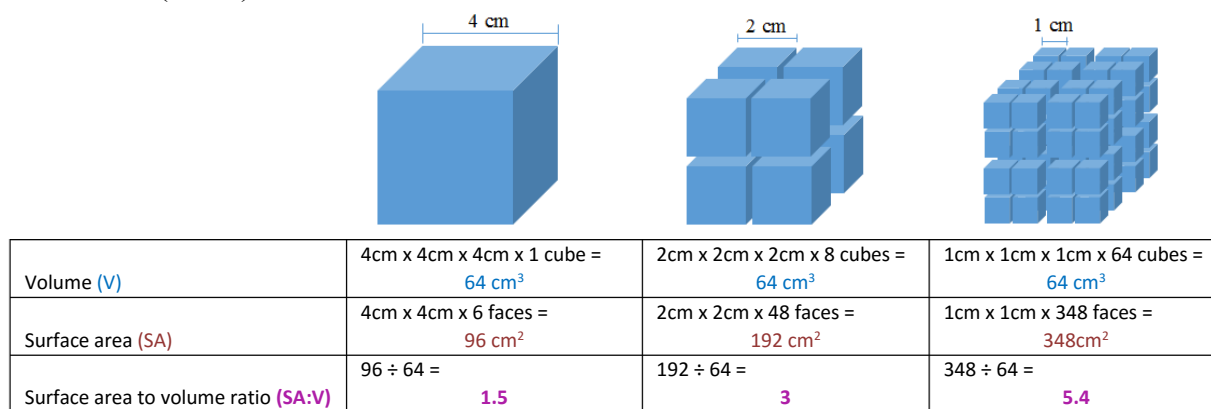


Figure 1.1 A cube broken down in smaller pieces from left to right. The table below the cubes provides the volume, surface area and surface area to volume ratio. Note that the SA and SA:V increases and the pieces become smaller. Modified from: The importance of nanotechnology¹

¹<http://bbc.in/1VSswSH2>

Man-made nanomaterials (engineered nanomaterials, ENMs) are classified as organic (fullerenes and carbon nanotubes, CNTs) and inorganic (metals, metal oxides and quantum dots (Peralta-Videa et al., 2011). Figure 1.2 provides a schematic classification of NMs.

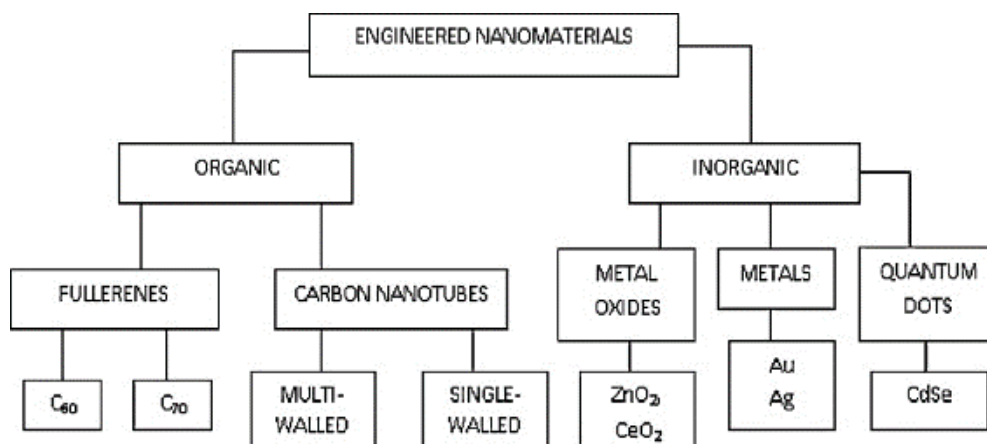


Figure 1.2 Classification of nanomaterials given their physical and chemical properties (Peralta-Videa et al., 2011).

Naturally occurring nanoparticles (NPs, entities with at least two dimensions less than 100 nm in the nanoscale) include debris from dust storms, volcano ashes, and forest fires (Buzea et al., 2007), but it's the deliberate production of non-regulated engineered nanomaterials (ENMs) in which debate arises.

Various fields are being benefited from nanomaterials, and products containing nanoparticles are already commercially available. The Project on Emerging Nanotechnologies (2015) provides an inventory of NM-enabled items marketed in different countries. The list includes goods that claim to include NPs, but are not proven to, as well as articles that do not state they contain NPs, but were confirmed to have NPs. The inclusion of NMs enhances the performance of commercial products. For example, titanium dioxide (TiO₂) NPs are found in Pantene® shampoo and Sensodyne® toothpaste as anticaking agents (additive in granulated materials to enhance dispersion). Carbon nanotubes provide hardness and strength to bicycle frames, while make them light in weight. Silicon dioxide (SiO₂) NPs are additives in anti-graffiti

paints. Silver NPs prevent bacterial infection in wound dressings and are also encountered in cotton sheets, anti-odor socks, towels, air filters, hair brushes, combs, and suspensions marketed to enhance the immune system, among other products². Figure 1.3 shows the constituent NMs in the inventory. Silver is the most widely incorporated NM given its proven antimicrobial properties, comprising 24% of the 1814 products listed (Vance et al., 2015).

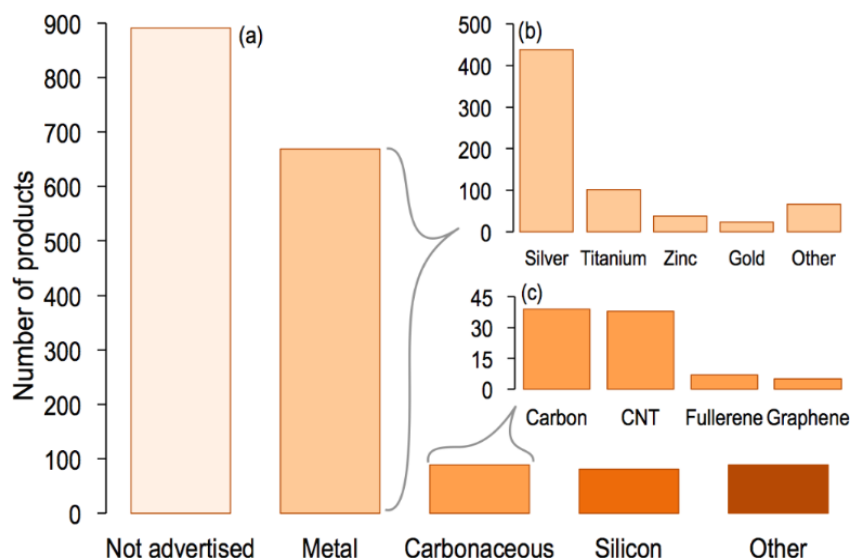


Figure 1.3 NMs present in the inventory listed items. (a) Unknown NMs contained (not advertised), metal (metals and metal oxides), carbon based, silicon-based (silicon and silica), and other NMs (organics, polymers, ceramics, etc.). (b) Metal NMs' element composition. (c) Carbon based NMs (Vance et al., 2015).

Despite the marvelous NM applications in many industries, a gap still exists in understanding the effects of NMs in the environment. This is of concern because NPs will ultimately end up in the air, water or soil once the functionality of the devices/systems containing them is over or the NPs themselves are used (Keller et al., 2013).

²<http://www.nanotechproject.org/cpi/>

Few researchers have assessed the amount of NPs that enter into the soil, air and water (Keller et al., 2013; Keller and Lazareva, 2014; Gottschalk et al., 2013). Keller and Lazareva (2014) estimated a flow of approximately 52,000 metric tons yr^{-1} of ENMs into the soil and about 69,000 metric tons yr^{-1} into water streams, globally, obviating the urge to study the effects of NPs on the environment.

While NPs bring positive applications, they come with unknown consequences from their use. One of the characteristics of NPs is that their miniature dimensions allow them to enter passively through the cell membranes of living organisms. This is true for “very small” NPs, while larger NPs are engulfed (by endocytosis) or internalized by other mechanisms (Chen et al 2013). By using electron microscopy, Bandyopadhyay et al. (2015) observed clusters of ZnO NPs agglomerated along the cell wall of alfalfa stem tissue (Figure 1.4). Figure 1.4 also provides a sense of scale that illustrates the comparison in size between NPs within plant tissue.

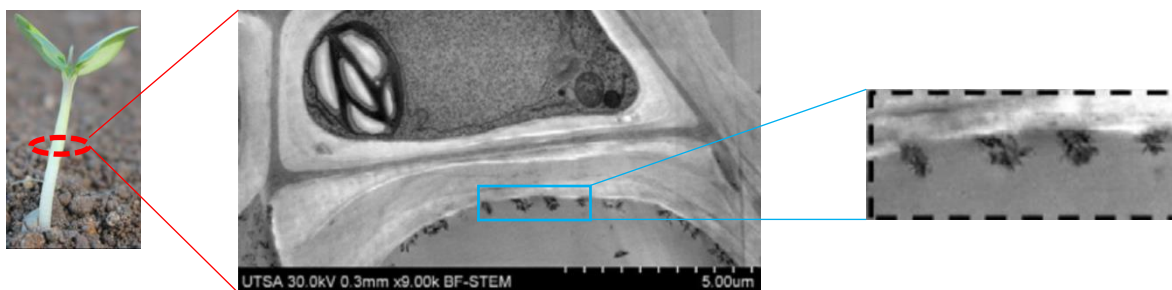


Figure 1.4 Size comparisons of nanoparticles inside a plant. The scanning/transmission electron micrograph (bright field) shows ZnO NPs agglomerates along an alfalfa cell wall from stem tissue (modified from Bandyopadhyay et al., 2015).

STATEMENT OF THE PROBLEM

The applications of nanomaterials grew faster than their regulations and faster than our understanding on the consequences of their use. Even with the benefits that products provide by including nanomaterials in their composition, like transparent sunscreen with ZnO NPs, protests arose. People claim that having unlabeled items (products not stating they contain nano-sized materials) is unsafe and not wanted. In 2007, nanotechnology opponents sprayed a “no-nano” sign at the Bastille fortress in Grenoble, France, while others gathered and exhibited in public their concerns (Figure 1.5).



Figure 1.5 Expressions of reject against nanotechnology in different regions of the world. Left: France, 2007³ and right: Australia, 2010⁴

As previously mentioned, it is assumed that all of the employed NMs will eventually end up in the environment (Keller et al., 2013). Even though the impact of nanomaterials in the environment has been explored, there is still lack of information on their effects in terrestrial plants (Anjum et al., 2015). Among other reasons, edible plants are being studied as carriers of NPs into the food chain (Gardea-Torresdey et al., 2014). However, exposure concentrations for plants within the design of experiments is a topic of discussion, given that it has been difficult to establish relevant/realistic NPs amounts already found in the environment (Holden et al., 2014).

³<http://bit.ly/1T1vmkY>

⁴<http://bit.ly/24vLSlO>

Plants are a main character in the ecosystem that withstand life, and are conducts through which nanoparticles (NPs) could enter other living organisms (Gardea et al., 2014). The plants involved in this research were cilantro (exposed to Cu compounds), radish (treated with Ag NPs), cucumber and corn (grown in soil with ZnO or CeO₂ NPs). Cilantro (*Coriandrum sativum*) is a popular cuisine ingredient. The herb is eaten fresh and its dried fruit, containing two seeds, is consumed as a spice commonly known as coriander. In addition, cilantro has been known for its medicinal and antioxidant properties (Laribi et al., 2015). In 2008, the highest exporter country was Mexico with 42 million kg (Morales-Payan, J.P., 2011). In the US, California was the largest producer state in 2009 with 34 million kg of cilantro (<http://bit.ly/23jIPY1>). Radish (*Raphanus sativus*) is another vegetable eaten raw. Radish sprouts are used as research models because they are grown easily in a short period of time (Létondor et al., 2015). Radish sprouts are consumed worldwide as a good source of carbohydrates and also due to their antioxidant properties (Xiao et al., 2014; Baenas et al., 2015). Corn (*Zea mays*) was selected because of its importance as the third most popular cereal crop produced in the world (Smith et al., 2011). We also studied cucumber plants (*Cucumis sativus*) because, in comparison to corn, cucumber has shown higher sensitivity to pollutants as exhibited by the plant physiological responses (Zhao et al., 2013; Zhao et al., 2015). Studying the nanoparticles' effects in plants is important given that the fate and behavior of nanomaterials in the environment are still lacking understanding (Peijnenburg et al., 2015).

OBJECTIVES

The purpose of this work is to provide deep insights on the interaction of nanometals (Cu and Ag) and nanometal oxides (CuO, ZnO, CeO₂) on the physiology and biochemistry of plants.

Specific objectives

1. Compare the physiological and biochemical effects of Cu nanomaterials and conventional Cu compounds in cilantro plants (Chapter 2).
2. Determine the changes in the nutritional quality of radish sprouts caused by a colloidal silver suspension (Chapter 3).
3. Evaluate the alterations in macromolecules and nutritional elements' composition of cucumber and corn plants exposed to ZnO and CeO₂ NPs. (Chapter 4).

HYPOTHESES

This investigation was performed under the working hypotheses that:

1. Copper-based nanomaterials affect cilantro more than conventional copper compounds.
2. Silver nanoparticles alter the nutritional quality of radish sprouts and inhibit seedlings' development.
3. Nanoparticles of ZnO and CeO₂ differentially affect cucumber and corn plants.

REFERENCES

- Anjum, N.A.; Adam, V.; Kizek, R.; Duarte, A.C.; Pereira, E.; Iqbal, M.; Lukatkin, A.S.; Ahmad, I.; Nanoscale copper in the soil–plant system – toxicity and underlying potential mechanisms. *Environ Res.* **2015**, *138*, 306–325. <http://dx.doi.org/10.1016/j.envres.2015.02.019>
- Baenas, N.; Ferreres, F.; García-Viguera, C.; Moreno, D. A. Radish sprouts—characterization and elicitation of novel varieties rich in anthocyanins. *Food Res. Int.* **2015**, *69*, 305–312. doi: 10.1016/j.foodres.2015.01.009
- Bandyopadhyay, S.; Plascencia-Villa, G.; Mukherjee, A.; Rico, C.M.; José-Yacamán, M.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. *Sci. Total Environ.* **2015**, *515*, 60–69. doi:10.1016/j.scitotenv.2015.02.014
- Buzea, C.; Pacheco, I.I.; Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity *Biointerphases* **2007**, *4*, MR17–MR172.
- Chen, X.; Tian, F.; Zhang, X.; Wang, W. Internalization pathways of nanoparticles and their interaction with a vesicle. *Soft Matter* **2013**, *9*, 7592–7600. doi: 10.1039/c3sm50931a
- Gardea-Torresdey, J. L.; Rico, C. M.; White, J. C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* **2014**, *48*, 2526–2540. DOI 10.1021/es4050665
- Gottschalk, F.; Sun, T.Y.; Nowack, B. Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environ. Pollut.* **2013**, *181*, 287–300.
- Holden, P.A.; Klaessig, F.; Turco, R.F.; Priester, J.H.; Rico, C.M.; Avila-Arias, H.; Mortimer, M.; Pacpaco, K.; Gardea-Torresdey, J.L. Evaluation of exposure concentrations used in assessing manufactured nanomaterial environmental hazards: Are they relevant? *Environ. Sci. Technol.* **2014**, *48*, 10541–10551. dx.doi.org/10.1021/es502440s
- Keller, A.A.; Lazareva, A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* **2014**, *1*:65–70. dx.doi.org/10.1021/ez400106t
- Keller, A.A.; McFerran, S.; Lazareva, A.; Suh, S. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* **2013**, *15*, 1692. doi 10.1007/s11051-013-1692-4
- Laribi, B.; Kouki, K.; M'Hamdi, M.; Bettaieb, T. Coriander (*Coriandrum sativum* L.) and its bioactive constituents. *Fitoterapia.* **2015**, *103*, 9-26.
- Létondor, C.; Pascal-Lorber, S.; Laurent, F. Uptake and distribution of chlordecone in radish: different contamination routes in edible roots. *Chemosphere.* **2015** *118*, 20–28.
- Morales-Payan, J.P., 2011. Herbs and leaf crops: Cilantro, broadleaf cilantro and vegetable amaranth. *Soils, Plant Growth and Crop Production*, *3*, pp.1-28.
Retrieved from <http://bit.ly/23jEbcw> on April, 2016

- Peijnenburg, W.J., Baalousha, M., Chen, J., Chaudry, Q., Von Der Kammer, F., Kuhlbusch, T.A., Lead, J., Nickel, C., Quik, J.T., Renker, M. and Wang, Z.,. A review of the properties and processes determining the fate of engineered nanomaterials in the aquatic environment. *Crit. Rev. Environ. Sci. Technol.* **2015**, 45(19), 2084-2134.
- Peralta-Videa, J.R.; Zhao, L.; Lopez-Moreno, M.L.; de la Rosa, G.; Hong, J.; Gardea-Torresdey, J.L. Nanomaterials and the environment: A review for the biennium 2008–2010. *J. Hazard. Mater.* **2011**, 186, 1–15. doi:10.1016/j.jhazmat.2010.11.020
- Project on Emerging Nanotechnologies (2015). Consumer Products Inventory. Retrieved on 11/13/2015, from <http://www.nanotechproject.org/cpi>
- Roco, M.C. Nanotechnology: convergence with modern biology and medicine. *Curr. Opin. Biotechnol.* **2003**, 14, 337–346. doi:10.1016/S0958-1669(03)00068-5
- Smith, R.; Bi, J.; Cahn, M.; Cantwell, M.; Daugovish, O.; Koike, S.; Natwick, E.; Takele, E. “Cilantro production in California.” University of California Agriculture and Natural Resources Communication Services. 2011. Publication 7236. Retrieved from: <http://bit.ly/23jIPY1> on April, 2016.
- Vance, M.E.; Kuiken, T.; Vejerano, E.P.; McGinnis, S.P.; Hochella, M.F.; Rejeski, D.; Hull, M.S.; Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J. Nanotechnol.* **2015**, 6, 1769–1780. doi:10.3762/bjnano.6.181
- Xiao, Z.; Luo, Y.; Lester, G. E.; Kou, L.; Yang, T.; Wang, Q. Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. *LWT-Food Sci. Technol.* **2014**, 55, 551–558. doi: 10.1016/j.lwt.2013.09.009.
- Zhao, L. J.; Sun, Y. P.; Hernandez-Viezcas, J. A.; Servin, A. D.; Hong, J.; Niu, G. H.; Peralta-Videa, J. R.; Duarte-Gardea, M.; GardeaTorresdey, J. L. Influence of CeO₂ and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. *J. Agric. Food Chem.* **2013**, 61 (49), 11945–11951.
- Zhao, L. J.; Sun, Y. P.; Hernandez-Viezcas, J. A.; Hong, J.; Majumdar, S.; Niu, G. H.; Duarte-Gardea, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Monitoring the environmental effects of CeO₂ and ZnO nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ μ -XRF mapping of nutrients in kernels. *Environ. Sci. Technol.* **2015**, 49 (5), 2921–2928.

Chapter 2. Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*)¹

ABSTRACT

The environmental impacts of Cu-based nanoparticles (NPs) are not well understood. In this study, cilantro (*Coriandrum sativum*) was germinated and grown in commercial potting mix soil amended with Cu(OH)₂ (Kocide and CuPRO), nano-copper (nCu), micro-copper (μCu), nano-copper oxide (nCuO), micro copper oxide (μCuO) and ionic Cu (CuCl₂) at either 20 or 80 mg Cu per kg. In addition to seed germination and plant elongation, relative chlorophyll content and micro and macroelement concentrations were determined. At both concentrations, only nCuO, μCuO, and ionic Cu, showed statistically significant reductions in germination. Although compared with control, the relative germination was reduced by ~50% with nCuO at both concentrations, and by ~40% with μCuO, also at both concentrations, the difference among compounds was not statistically significant. Exposure to μCuO at both concentrations and nCu at 80 mg kg⁻¹ significantly reduced ($p \leq 0.05$) shoot elongation by 11% and 12.4%, respectively, compared with control. Only μCuO at 20 mg kg⁻¹ significantly reduced (26%) the relative chlorophyll content, compared with control. None of the treatments increased root Cu. These results showed that Cu-based NPs/compounds depress nutrient element accumulation in cilantro, which could impact human nutrition.

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INTRODUCTION

“There is plenty of room at the bottom,” a speech given by Richard Feynman in 1959 led to the development of what we know today as nanotechnology. The formation of the National Nanotechnology Initiative (NNI) in January 2000 began the “Nano-era” (Roco 2003) and the steadily increasing production of engineered nanomaterials (ENMs). ENMs exhibit different behaviors in comparison with equivalent bulk materials because they have higher surface area to volume ratio and are highly reactive. Unique properties of ENMs, not found in microsize materials, allowed the development of nanotechnologies. However, ENMs have shown to impact the environment in a different way than their corresponding microsize materials. The primary difference is likely due to ion release. For example, copper (Cu) is considered a nonhazardous stable solid, insoluble in water (Copper MSDS). However, nanoparticulate Cu (nCu) is designated as flammable and very toxic to aquatic life². nCu is gaining industrial popularity in the medical field as an antimicrobial agent (Mary et al., 2009), antibacterial paint (Cioffi et al., 2005) or bactericidal coating in biocompatible devices (Cometa et al., 2013), while nCuO is used in printable conductive inks³. Cu(OH)₂, which forms nanodrops when suspended in water, is marketed as CuPRO[®] 2005 and Kocide[®] 3000 (Adeleye et al., 2014) and is used as fungicide/bactericide in agriculture. Estimates indicate that in 2010, about 200 metric tons of nanosize Cu and Cu oxides were produced by different industries, 36 of which ended in soil and 11 in water (Keller et al., 2013). Therefore, increasing amounts of nano-sized Cu are entering the environment after end user application or by intentional release in agricultural production.

²<http://bit.ly/1WcH6W2>

³<http://bit.ly/1WcH9Rw>

Moreover, it is still unknown if nanoparticulate Cu will enter into the food chain through agricultural uses (Holden et al., 2013; Gardea-Torresdey et al., 2014). The interaction of Cu-based nanoparticles (NPs) with living organisms is not well understood (Anjum et al., 2015). It is well known that interactions of chemical products with plants are modulated by growth media components. Thus, it is expected that the effects of specific NPs will be environment-and-species-dependent.

Musante and White (2012) reported that humic acid was able to inhibit the phytotoxic effects of microsize Cu, but not those of nanosize Cu. Dimkpa et al. (2012) found that nCuO had greater phytotoxic effects on wheat (*Triticum aestivum*) than nZnO. Nair and Chung (2015) reported that nCuO reduced chlorophyll, carotenoids, and shoot elongation in Indian mustard (*Brassica juncea* L.). Hong et al. (2015) found that Cu-based ENMs altered nutrient content in lettuce and alfalfa. In addition, a few studies have shown that nCuO reduce seed germination in plants such as rice (*Oryza sativa*) (Shaw et al., 2013) and cucumber (*Cucumis sativus*) (Moon et al., 2014). Cilantro (*Coriandrum sativum*) is a culinary herb consumed fresh worldwide (Morales et al., 2013). Statistics for world production are scarce, but in 2008, Mexico was the highest cilantro exporter⁴ with 42 million kg and in 2009, more than 34 million kg were produced by California⁵. Besides its popularity as a culinary herb, cilantro is also known as a medicinal plant (Laribi et al., 2015). Morales et al. (2013) exposed cerium oxide NPs (nCeO₂) at 0–500 mg kg⁻¹ to cilantro in organic soil. The authors did not report effects on seed germination but they observed a 10% increase in shoot elongation at 125 mg kg⁻¹ and a 15% reduction in shoot biomass at 250 mg kg⁻¹.

⁴ <http://www.eolss.net/sample-chapters/c10/e1-05a-47.pdf>

⁵ <http://anrcatalog.ucdavis.edu/pdf/7236.pdf>

To the best of the authors' knowledge, there are no other studies describing the interactions of ENMs with cilantro. Thus, nothing is known about physiological and biochemical changes induced by Cu-based nanoproducts/compounds in this popular edible plant. The aims of this study were to elucidate the effects of seven Cu-based NPs/compounds on the growth and nutrient accumulation in cilantro. Plants were cultivated in soil amended with each one of the seven Cu-based chemicals, either at 20 or 80 mg Cu per kg. Growth and biomass production were measured 30 days after germination. In addition, chlorophyll production, Cu enrichment, and concentration of micro and macronutrients were quantified.

MATERIALS AND METHODS

Characterization of Cu NPs/compounds

This study included nanoparticulate CuO (nCuO), micron-size Cu and CuO (mCu and mCuO) (Sigma Aldrich Co., St. Louis, MO), nanoparticulate Cu (nCu, US Research Nanomaterials Inc. Houston, TX), Cu(OH)₂ (Kocide[®] 3000, Dupont, Wilmington, DE), Cu(OH)₂ (CuPRO 2005, SePRO, Carmel, IN), and reagent grade CuCl₂ salt (Sigma Aldrich Co., St. Louis, MO). All these materials were previously characterized in solid and suspension/solution forms (Adeleye et al., 2014; Hong et al., 2015). Primary size, hydrodynamic diameter, zeta potential, and other physicochemical characteristics are shown in Table 2.1 (Hong et al., 2015).

Table 2.1 Physicochemical properties of the particles used in this study (Hong et al., 2015)

Property	nCuO	Bulk CuO	nCu	Bulk Cu	Kocide 3000	CuPRO 2005
Primary particle size (nm)	10 ¹ to 10 ²	10 ² to 10 ⁴	10 ² to 10 ³	<10 ⁴	>10 ⁴	10 ⁴ to 10 ⁶
Hydrodynamic diameter ^a (nm)	280 ± 15	376 ± 26	2590 ± 1138	4546 ± 3940	1532 ± 580	4779 ± 4767
Zeta potential ^a (mV)	-34.4 ± 0.5	-42.7 ± 0.153	-29.4 ± 0.8	-35.4 ± 1.27	-40.9 ± 2.7	-47.8 ± 1.1
Cu content (wt%)	74.3	79.7	83.3	98.7	26.5	34.0
Other elements present	O, C	O	O, C	ND	C, O, Na, Al, Si, S, Cl	C, O, Na, Al, Si, P, Ca
Morphology	Rhombus, irregular	Prism, irregular	Irregular	Dendritic, plate-like, rhombus	Spherical	Spherical
Main copper phase	CuO	CuO	Cu, Cu ₂ O	Cu	Cu(OH) ₂	Cu(OH) ₂
Crystal structure	Monoclinic	Monoclinic	Cubic	Cubic	Orthorhombic	Orthorhombic

^aMeasurement was done at pH 7, ND = non-detect.

Preparation of suspensions/solutions and soil

Cu NPs/compounds' suspensions/solutions were prepared in Millipore water (MPW) and homogenized by sonication in a water bath (Crest Ultrasonics, Trenton, NJ) at 25 °C for 30 min. Sonication intensity was 180 watts. Subsequently, enough volume of each suspension/solution was thoroughly mixed with 240 g of commercial potting mix (MiracleGro[®] with micromax, Marysville, OH, USA) to have 0 (control), 20 (low), and 80 (high) mg Cu per kg. The low concentration was selected because it is very close to the average Cu concentration in agricultural soil (25 mg kg⁻¹) and the high concentration is very close to the maximum Cu concentration (77.4 mg kg⁻¹) in sewage sludge found in a US Environmental Protection Agency report (Georgopoulos et al., 2001). The spiked soil was placed in plastic pots (13.2 cm diameter x 10.2 cm height; Misco Enterprises, Dunellen, NJ) and seeded 24 h after treatment application (Morales et al., 2013). There were four replicates per treatment allocated in a completely random design.

Plant growth and harvest

Cilantro seeds were obtained from Del Norte Seed and Feed (Vinton, TX, USA). Fruits containing the seeds (commonly known as coriander) were hydrated for 24 h in MPW after at least one day of storage at 4 °C. Thirty fruits were placed in each pot and incubated in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) at 25/20 °C day/night, 14/10 h photoperiod, $60 \pm 3\%$ relative humidity, and light intensity of $340 \text{ mol m}^{-2} \text{ s}^{-1}$. Plants were watered with 50 mL MPW for the first three days (the soil was too dry) and with 25 mL per day afterwards.

Plant harvest

Thirty days after germination (Morales et al., 2013), 10 plant/treatment were removed from the soil and measured with a ruler. Plants were then rinsed with tap water to remove excess soil, soaked in 0.01 M HNO_3 for about 10 s, and then rinsed thrice with deionized water (DI). Tissues were stored at 65 °C for at least 72 h before digestion for further elemental analysis.

Agronomic parameters

Percent germination (%G), percent relative germination (%RG) and germination change (%GC) were determined after de la Rosa et al. (2011). The equation for %G was modified, given that cilantro fruits usually contain two seeds:

$$\%G = \frac{\text{no. germinated seeds}}{2(\text{total no. of fruits})} \times 100\%$$

$$\%RG = \frac{\% \text{ germination in treatment}}{\% \text{ germination in control}} \times 100\%$$

$$\%GC = \text{Relative germination in treatments} - \text{Relative germination in controls}$$

where, %G = percent germination, %RG = relative germination percentage, %GC = germination change percentage. The fresh and dry weights of the plants were used to calculate the water content (fresh weight – dry weight) and dry biomass (dry weight).

Relative chlorophyll content and elemental analysis

The relative chlorophyll content was determined in fresh plants, at harvest, with the aid of a portable chlorophyll meter (SPAD- 502 chlorophyll meter; Minolta Camera, Osaka, Japan). Data reported are averages of three measurements per leaf from three leaves per pot. For elemental analysis, samples of 100 mg of oven dried tissues were microwave-assisted acid digested (MarsX, CEM, Mathews, NC) with 4 mL H₂O₂ (BDH®; VWR Chemicals, Radnor, PA) and 1 mL of plasma pure HNO₃ (SPC Science, Champlain, NY). Digested samples were decanted to 15 mL centrifuge tubes and volume adjusted to 15 mL with MPW. For quality control, vessels without plant samples (blanks, n = 3), vessels with spinach leaves as standard reference material (1507a NIST, Gaithersburg, MD), and Cu spikes (0.5 mg L⁻¹) (SPC Science, Champlain, NY) were digested as samples. Digests were analyzed for Cu, macroelements (Ca, Mg, K, P, and S) and microelements (B, Mn, Fe, Mo, Ni, and Zn) by inductively coupled plasma – optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV; Shelton, CT). Acid digested Cu spikes were included every five samples.

Statistical analysis

Agronomic and physiological measurements were analyzed using the Statistical Package for the Social Sciences 22.0 (SPSS, Chicago, IL). Variance was evaluated by one-way analysis of variance (one-way ANOVA) and the difference between treatment means was compared by

Tukey's honest significant difference (Tukey's HSD) test at a p value of 0.05, unless otherwise is stated.

RESULTS AND DISCUSSION

Effect of Cu-based treatments on seed germination

Changes in seed germination (%) are shown in Fig. 2.1. As seen in this figure, all treatments showed reduction in germination, compared with control. However, at both concentrations, only nCuO, μ CuO, and ionic Cu, showed statistically significant reductions. Although compared with control, the relative germination was reduced by $\sim 50\%$ with nCuO at both concentrations, and by $\sim 40\%$ with μ CuO, also at both concentrations, the difference among compounds was not statistically significant. However, this could indicate an effect of particle size. Similar results on %GC were observed with nCu at 20 mg kg^{-1} and CuCl_2 at both concentrations. Notably, less effects were observed with both $\text{Cu}(\text{OH})_2$ compounds, CuPRO at both concentrations and Kocide at low concentration. More biochemical data is needed in order to explain the differences between compounds; nevertheless, it is possible that variations in hydrodynamic particle size ($4779 \pm 4767 \text{ nm}$ for CuPRO and $1532 \pm 580 \text{ nm}$ for Kocide) caused the differences (Hong et al., 2015). However, it is worth noting that the hydrodynamic size of these particles could change over the course of the exposure; thus, the effects could also change. Wu et al. (2012) reported a reduction in the germination of lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) and cucumber seeds exposed to nCuO, being lettuce the most affected of the three species. However, Moon et al (2014). reported no effects of μ CuO in cucumber germination and 23.35% reduction with nCuO. Stampoulis et al. (2009) reported that nCu at 1000 mg L^{-1} did not affect zucchini seed germination. This suggests that the effects of Cu-based NPs or compounds are associated with plant species and cultivation conditions.

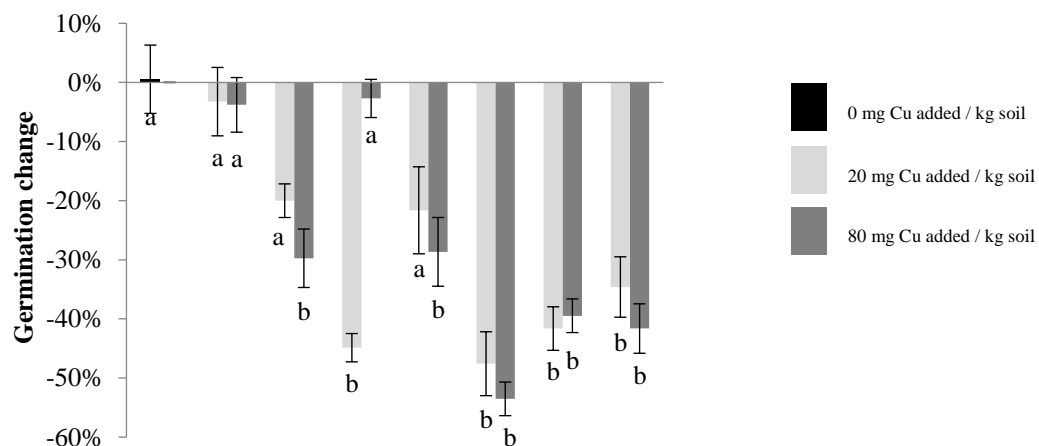


Figure 2.1 Changes in seed germination (% GC) of cilantro exposed to Cu-based nanoparticles or compounds in soil amended with 0 (control), 20, and 80 mg Cu per kg. Different letters denote statistically significant differences between groups. Each data point is me mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$). Negative values refer to a reduction in GC.

Cu uptake and translocation

Concentrations of Cu in roots and shoots of cilantro exposed to Cu-based NPs or compounds are shown in Fig. 2.2. Unexpectedly, none of the Cu treatments increased the Cu concentration in roots, according to the Tukey's HSD test (Fig. 2.2A). This could be due to the native Cu concentration in experimental soil ($27.1 \pm 0.4 \text{ mg kg}^{-1}$, Table 2.2), and the complexation of Cu with the soil organic matter (Karlsson et al., 2006).

Table 2.2 Concentration of elements in soil before treatment application. Values are mean \pm standard error (n = 4)^a

Element	Mean (mg kg ⁻¹)
Cu	27.1 ± 0.4
B	8.9 ± 0.2
Mn	245.6 ± 5.5
Fe	5849.2 ± 462.1
Ni	10.5 ± 0.5
Zn	51.8 ± 1.7
Mo	ND
Na	533.4 ± 80.1
Mg	1886.0 ± 51.4
P	2413.1 ± 82.3
S	3540.4 ± 192.0
Ca	$35\ 153.4 \pm 727.0$

^aND = non detected

Accumulation of Cu in shoots did not show a tendency (Fig. 2.2B). Though, μCuO exposed plants had the higher shoot Cu concentration. In wheat, the shoot Cu concentration from $n\text{CuO}$ was 32% higher compared with μCuO (Dimpka et al., 2012). This could be due to a species-specific response. A comparison within compounds showed no difference in Cu accumulation, except with CuCl_2 that at low concentration resulted in higher accumulation, compared with μCuO and CuPRO ; while at high concentration μCuO was statistically lower. Perhaps the difference with CuPRO was due to its huge hydrodynamic size (4779 ± 4767 nm) that avoided the uptake, although the high error would leave some small particles in suspension (Adeleye et al., 2014). More studies are needed in order to understand the mechanism of Cu uptake from different Cu species.

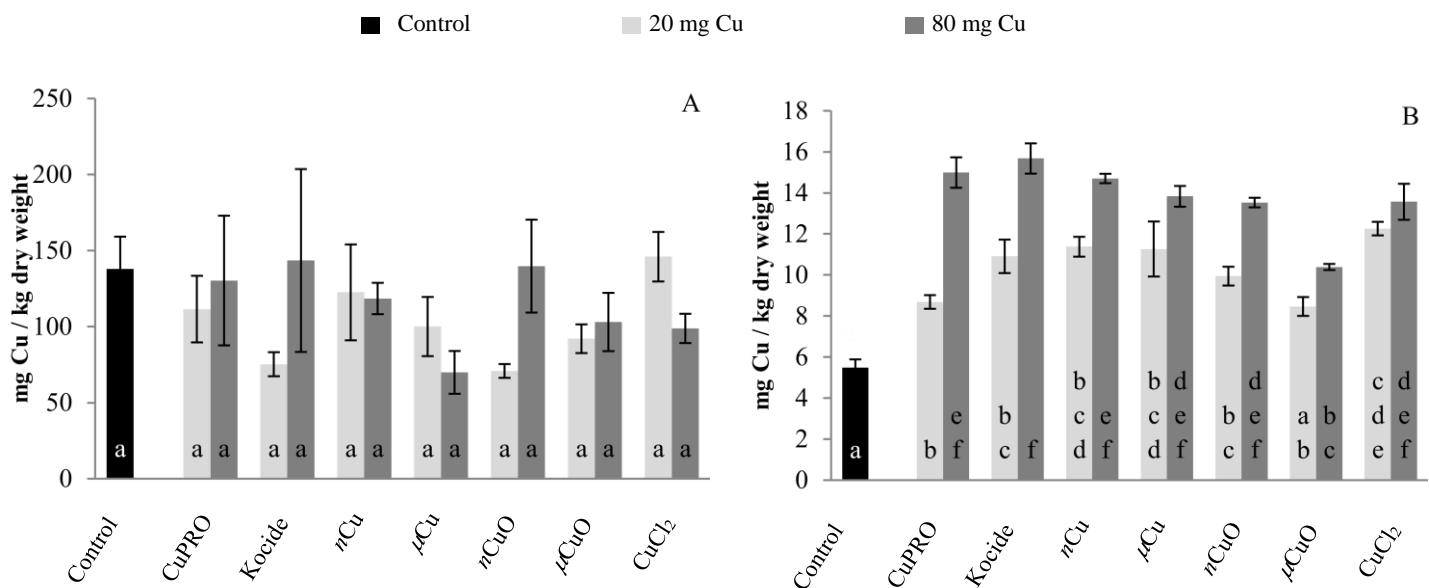


Figure 2.2 Copper concentration in roots (A) and shoots (B) of cilantro plants grown for 30 days in soil amended with Cu-based nanoparticles or compounds at 0 (control), 20, and 80 mg Cu per kg soil. Each data point is mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$)

Root and shoot elongation

Plant elongation is an important agronomic parameter, mainly for fresh produces. Fig. 2.3 shows the length of roots and shoots of plants exposed to nanosize, microsize, and ionic copper. As seen in Fig. 2.3A, none of the treatments significantly affected cilantro root elongation. However, the effects on shoot elongation were varied (Fig. 2.3B). Only nCu at 80 mg kg⁻¹ and μ CuO at both concentrations significantly reduced shoot length in cilantro (12.4%, 11.2 and 11.0%, respectively). Hong et al. (2015) reported that at 20 mg L⁻¹, nCu reduced root length in hydroponically grown alfalfa by 47.65%, compared with control. Nair and Chung (2015) observed that nCuO, at concentrations varying from 20 mg L⁻¹ to 500 mg L⁻¹, diminished root and shoot length in Indian mustard. In addition, Atha et al. (2012) reported that nCuO inhibited plant growth in radish, perennial ryegrass (*Lolium perenne*), and annual ryegrass (*Lolium rigidum*). Song et al. (2015) assessed the toxicity of nCu in *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza*, three aquatic plant species. These researchers reported that the toxicity was species dependent and attributed to both the nCu and Cu ions released by the NPs. *W. arrhiza* was the most affected species, in term of total frond development. There are several potential reasons preventing the alignment of our data with the literature. However, plant species and growth media are considered more important. Previous studies have shown that the toxicity of microsize and nanosize Cu is species dependent (Hong et al., 2015; Song et al., 2015). The growth media is another factor that can lead to different results. Most of the reported results were obtained from plants grown in hydroponics (Musante et al., 2012; Hong et al., 2015), in vitro (Nair and Chung, 2015), or sand (Dimpka et al., 2012).

Our study was performed in potting soil with high content of organic matter. Previous studies have shown that Cu is complexed with soil organic matter. According to Karlsson et al. (2006) less than 0.2% of total Cu can be found as free Cu^{2+} if the pH is in the range of 4.8–6.3.

In the present study, only nCu at high concentration and μCuO at both concentrations significantly reduced shoot elongation ($p \leq 0.05$). It seems that this reduction was not associated with Cu uptake and translocation because plants exposed to other treatments with similar uptake (Fig. 2.2), did not show reduction in shoot elongation. nCu tend to form smaller aggregates compared to nCuO or $\text{Cu}(\text{OH})_2$ (Conway et al., 2015); then, if plants take up and store nCu, this could interfere with enzymatic activities, resulting in growth reduction. Results with μCuO could be due to its higher surface area, compared with the other NPs or compounds.

The surface area⁶ of μCuO is $29 \text{ m}^2\text{g}^{-1}$, while the surface areas of nCuO, nCu, and $\text{Cu}(\text{OH})_2$ are 12.31, 4.86, and $15.71 \text{ m}^2 \text{ g}^{-1}$, respectively (Conway et al., 2015). This suggests that μCuO could physically interfere with the root surface. In addition, its surface area confers higher opportunity to form complexes with other elements, like P, affecting plant growth. A previous study showed that wheat plants grown in hydroponics absorbed more Cu from the nCuO than from μCuO ; however, both compounds had similar reduction in shoot length (Dimpka et al., 2012). More studies are needed in order to determine the mechanism as to why Cu based NPs or compounds interfere with plant growth.

The biomass of experimental cilantro plants is shown in Fig. 2.3C and 2.3D. As seen in Fig. 2.3C, at high concentration μCuO reduced the dry root biomass to 46.9%, compared with control. This difference was statistically insignificant compared with nCu and μCu at high concentration and CuCl_2 at low concentration. In the shoots, nCuO at high concentration yielded

⁶<http://bit.ly/1T45hEU>

more biomass, compared with control ($p \leq 0.05$), while μCuO reduced the biomass, compared with the other treatments, except $n\text{CuO}$ at low concentration (Fig. 2.3D). This could be a direct effect of the reduction of chlorophyll observed with both concentrations of μCuO (Fig. 2.4C). In sand grown wheat, μCuO produced more biomass, compared with $n\text{CuO}$ (Dimpka et al., 2012); perhaps the difference observed in the present work was due to the interactions of the Cu compounds with soil components, which are different in comparison to sand.

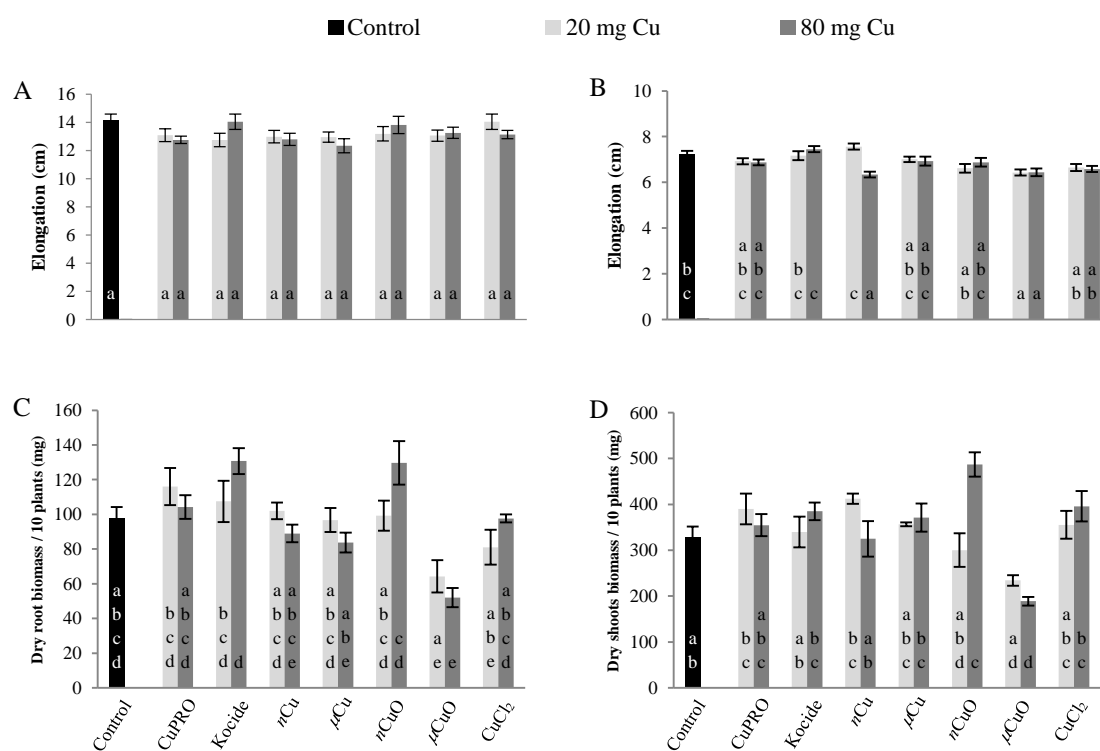


Figure 2.3. Elongation of root (A), shoots (B), dry root biomass (C), and dry shoot biomass (D) of cilantro plants grown for 30 days in soil amended with Cu-based nanoparticles or compounds at 0 (control), 20, and 80 mg Cu per kg soil. Each data point is mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$).

Water content and relative chlorophyll production

Fig. 2.4 shows results for water content and relative chlorophyll production. As seen in Fig. 2.4A, CuPRO and μCuO at high concentration reduced water content in root by 53.9% and 47.4%, respectively, compared with control. However, there were no differences compared with the other treatments. None of the treatments produced statistical differences in shoot water content, although plants exposed to μCuO at low and high concentration had 25.6% and 33.9% less water than control shoots, respectively (Fig. 2.4B). Very likely, the lack of statistical differences was due to a small number of replicates ($n = 4$). However, this could explain the reduction in shoot biomass (Fig. 2.3D). The reduction in water content in plants exposed to Cu treatments has been associated with the toxicity of Cu (Trujillo-Reyes et al., 2014). This suggests that in terms of water absorption, μCuO and CuPRO at the highest concentration imposed more stress on cilantro.

Fig. 2.4C shows the relative chlorophyll content of cilantro exposed to the Cu treatments. As seen in this Figure, μCuO at low concentration reduced the relative chlorophyll content by 26.4%, compared with control and, in general, by more than 25%, compared with the other treatments, except μCuO at high concentration, where the reduction was only 16.5%. A previous study has shown that μCuO can be taken up through roots and translocated to shoots (Dimpka et al., 2012). In addition, Moon et al. (2014) reported that in cucumber, 25% of the differentially expressed proteins under μCuO and nCuO showed “extreme patterns” in μCuO stressed plants. This suggests that in cilantro, μCuO exerts more toxicity than nCuO . As per Moon et al. (2014) the effects on cilantro suggest different mechanisms of toxicity.

■ Control ■ 20 mg Cu ■ 80 mg Cu

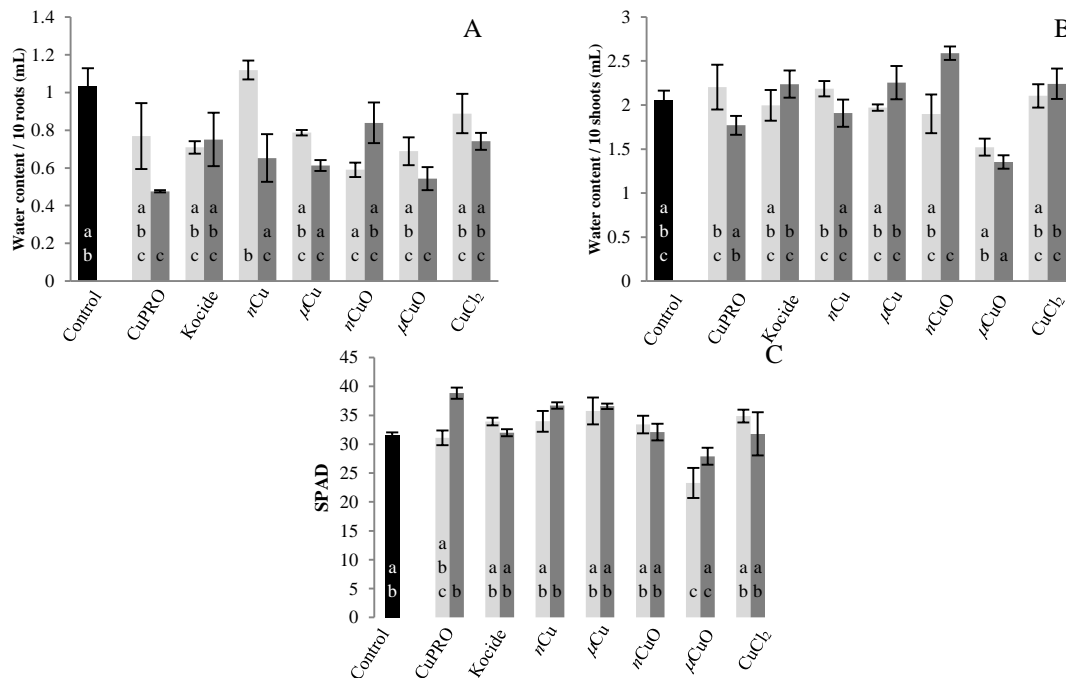


Figure 2.4 Root water content (A), shoot water content (B), and chlorophyll (C) of cilantro plants grown for 30 days in soil amended with Cu-based nanoparticles or compounds at 0 (control), 20, and 80 mg Cu per kg soil. Each data point is mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$).

Element uptake by roots

Fig. 2.5 shows the concentration of microelements (B, Fe, Mn, Ni, and Zn) and macroelements (Ca, Mg, P, and S) in cilantro roots exposed to Cu-based NPs or compounds. Microelement Mo was not detected in soil and digests (Table 2.2). Cu absorption was previously discussed. As seen in Fig. 2.5, none of the treatments affected the accumulation of Zn in roots. Root Fe was reduced by 52.2%, 46.4%, and 39.9% by μ Cu at 80 mg kg⁻¹, and ionic Cu at both concentrations, respectively. Ionic Cu and μ Cu may compete with Fe for the uptake channel (Kochian, 1991), thus, reducing Fe uptake. In addition, Fe can form insoluble hydroxides that are retained in root surface (Trujillo-Reyes et al., 2014). Different results were reported by Hong et al. (2015) who found that the same Cu NPs or compounds used in the present study, except CuCl₂, significantly reduced Fe uptake in alfalfa and lettuce. However, Hong et al. (2015) exposed the

plants to the Cu-based NPs/compounds in hydroponics, where the elements easily form complexes. Root Ni was decreased by 43.3%, 48.3%, and 43.2% with the highest concentration of CuPRO, μCu and CuCl_2 , respectively. A previous study has shown that Cu^{2+} and Zn^{2+} inhibit Ni^{2+} absorption and translocation in soybean because they share the same transporter (Cataldo et al., 1978). Contrary to Fe and Ni, B increased in roots of plants treated with Kocide at high concentration and nCu at low concentration. When the growth medium contains B at similar concentration as the one of this experiment, it is taken up by an active mechanism (Pfeffer et al., 1999) associated with cell permeation by boric acid, which is transported by aquaporins (Dordas et al., 2000). In addition, Robinson et al. (2007) suggested that B is taken up by plants in the form of B(OH)_3 . To this end, it is not possible to determine the effect of Cu NPs/compounds on aquaporins and B uptake in cilantro. CuPRO, the other Cu hydroxide, also increased B uptake; however, the difference with control did not reach statistical significance. Kocide has lower particle size than CuPRO and less Cu content, which could make the difference. Manganese concentration in roots increased by 30.4%, 20.9%, and 46.1%, with the low concentration of nCu, nCuO, and μCuO , respectively, compared with control; however, only μCuO produced a difference statistically significant. A previous report has shown that Mn concentration in wheat tissues increased at lower levels of Cu (Kumar et al., 2009). More experiments are needed in order to determine the mechanisms as to why nCu, nCuO, and μCuO increased Mn in cilantro roots.

With regard to macronutrients, Mg was significantly reduced in roots treated with μCu at 80 mg kg^{-1} , nCuO at 20 mg kg^{-1} , μCuO at both concentrations, and CuCl_2 at 20 mg kg^{-1} . Trujillo-Reyes et al. (2014) reported a reduction on Mg accumulation in lettuce roots exposed to core/shell Cu/CuO NPs at 10 and 20 mg L^{-1} . More experiments are needed in order to determine why only these Cu forms affected Mg uptake by cilantro roots. The effects of the Cu-based NPs or

compounds on P uptake in cilantro were similar to the effects described by Hong et al. (2015) in lettuce. All treatments at all concentrations, except CuPRO at low concentration, reduced P accumulation. Hong et al. (2015) proposed that P could be forming phosphates, which complex with Cu, making a compound that is not available to be taken up by the plants. Another explanation could be that P, in the form of phosphate ion (PO_4^{3-}), is being exchanged by hydroxyl (OH) groups in Fe and Al hydroxides, binding strongly with these cations, limiting its availability for the plant uptake (Taiz and Zeiger, 1998). Sulfur concentration in roots of Cu treated plants was similar to control, except in plants exposed to 80 mg kg^{-1} of μCu and 20 mg kg^{-1} ionic Cu. The presence of sulfur in the form of thiol ($-\text{SH}$) groups in phytochelatins (metal binding polypeptides synthesized from glutathione, GSH) is attributed to stress factors such as trace metals (Taiz and Zeiger, 1998; Cai et al., 2004; Castillo-Michel et al., 2009). Our results suggest that cilantro roots were able to cope with Cu stress. This finding is contrary to the results reported by Hong et al. (2015) who encountered that Cu increased sulfur uptake in alfalfa and lettuce roots; however, these researchers exposed the plants to Cu compounds/NPs in hydroponics and at lower concentrations. Calcium decreased in all treatments except CuPRO at low concentration, Kocide, nCu, and CuCl_2 at high concentration. Österas and Greger (2006) reported that in Norway spruce, Cu^{2+} inhibited Ca^{2+} uptake by roots and vice versa, suggesting that both elements use the same uptake channels. However, according to Lock et al. (2007), the competition for binding sites between Cu^{2+} and Ca^{2+} does not determine the Cu toxicity in barley (*Hordeum vulgare*).

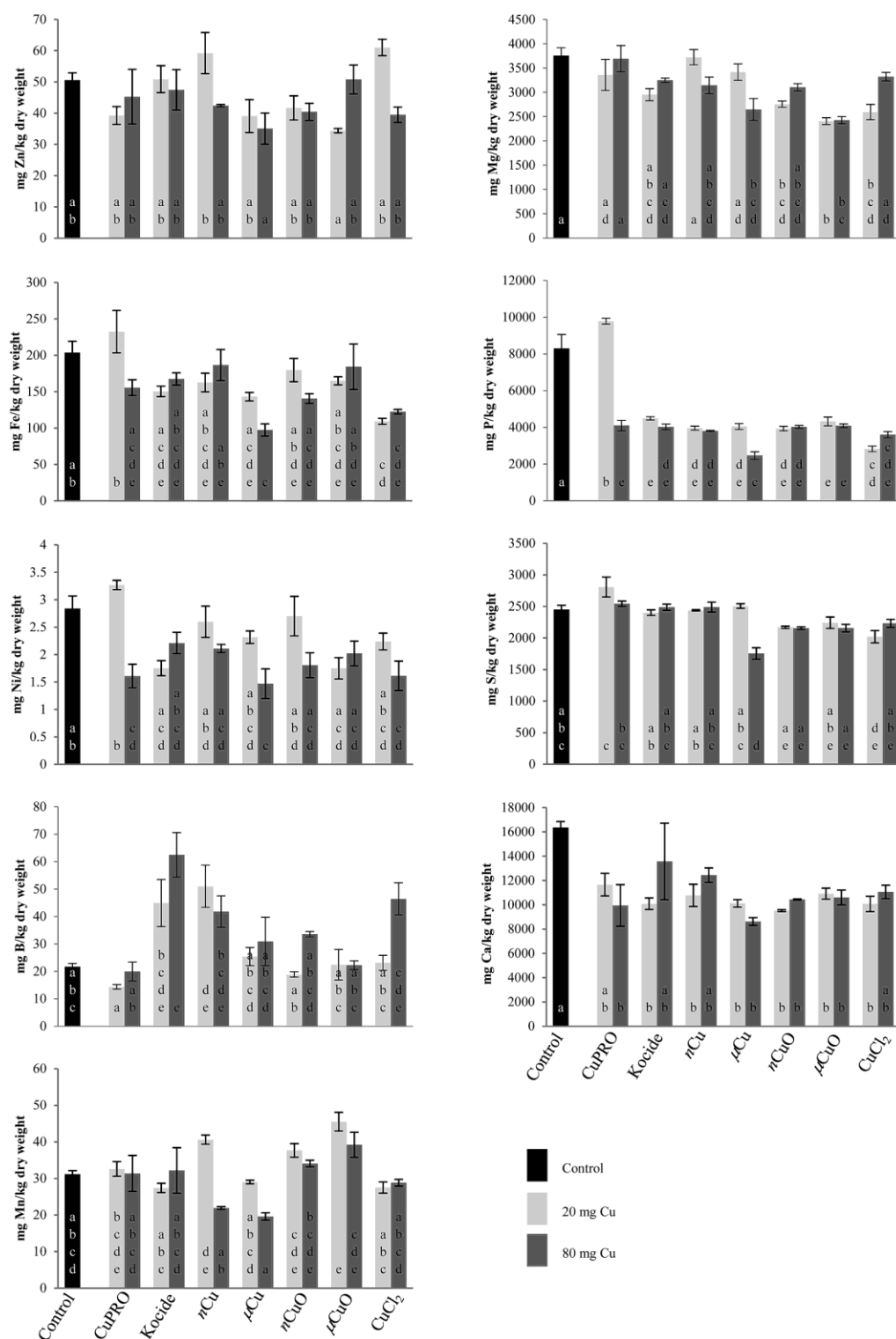


Figure 2.5 Concentration of nutritional elements in roots of cilantro plants grown for 30 days in soil amended with Cu-based nanoparticles or compounds at 0 (control), 20, and 80 mg Cu per kg soil. Each data point is mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$).

Element uptake by shoots

Concentrations of macro and microelements in cilantro shoots are shown in Fig. 2.6. As seen in this figure, none of the treatments impacted Fe and Ni concentration at shoot level. Zinc was reduced by μCu at both experimental concentrations as well as by μCuO at 80 mg Cu per kg, compared with control and CuPRO. However, there were no clear differences between treatments. Previous results with other species showed no interference between Cu NPs/compounds on Zn translocation to lettuce (Trujillo-Reyes et al., 2014), alfalfa (Hong et al., 2015), and field bindweed (*Convolvulus arvensis*) leaves (Gardea-Torresdey et al., 2004). In the case of B, this element was significantly augmented only on plants grown with nCuO at 80 mg Cu per kg. Manganese translocation to shoots was differentially affected by the Cu NPs or compounds. Shoot Mn increased in plants exposed to nCu at 20 mg kg⁻¹, ionic Cu at both concentrations, and CuPRO at 80 mg Cu per kg, but was decreased by Kocide at high concentration. This suggests an effect of the Cu species. The differences between Kocide and CuPRO could be due to the differences in composition and hydrodynamic size of both Cu(OH)₂ compounds (Hong et al., 2015). In addition, Zhao et al. (2012) reported that in the Mn hyperaccumulator *Phytolacca americana*, the interaction of Cu with Mn uptake varied with the Cu concentration. At 25 μM , Cu competed with Mn uptake but not at 100 μM . The translocation of macroelements under the stress of Cu-based NPs or compounds followed similar tendencies as micronutrients. Shoot Mg was reduced by both concentrations of Kocide, nCuO, and μCuO , and by μCu at 20 mg kg⁻¹ and CuCl₂ at 80 mg kg⁻¹, compared with control. The highest reduction was found in plants exposed to μCuO at 20 mg kg⁻¹ (31.9%). This explains the reduction in chlorophyll content shown in plants exposed to such compound (Fig. 4C). All Cu treatments decreased P in shoots. Similar results were reported by Hong et al. (2015) in lettuce, but contrary to their results in alfalfa. This suggest that in lettuce and

cilantro, Cu affects the Pht2; 1 transporter, which is responsible for the translocation and internal distribution of P (Smith et al., 2003). Sulfur accumulation in shoots decreased at both concentrations of CuPRO, nCu, nCuO and Cu^{2+} treatments, and increased only at 20 mg kg⁻¹ μCuO . After root absorption, S is mobilized and stored as sulfate (Buchner et al., 2004). Specific sulfate transporters deliver S into assimilation or storage places. This suggests that the nano-forms of Cu interfered with sulfate transporters. Again, the difference between the two $\text{Cu}(\text{OH})_2$ compounds could be due to differences in hydrodynamic size and other elements present. The response in Ca accumulation at shoot level was not clear. While there was a reduction in plants exposed to Kocide, μCu and μCuO at both concentrations, there was an increase in plants treated with CuPRO at both concentrations, nCu at 20 mg kg⁻¹, and CuCl_2 at both concentrations. It has been proposed that Ca^{2+} is moved with the water flow through the apoplast and by aquaporins through symplasts (Gilliam et al., 2011). Trujillo-Reyes et al. (2014) reported a reduction of Ca in leaves of lettuce treated with CuSO_4 and nano-Cu/CuO in hydroponics. However, Hong et al. (2015) reported no effects in Ca accumulation in lettuce and alfalfa leaves exposed to the same Cu NPs/ compounds. In the present study, there were no differences in water content in shoots that suggest no water stress. Thus, it is proposed that the species of plants and the growth medium affect the expression of aquaporins that regulate movement of Ca^{2+} across membranes (Gilliam et al., 2011).

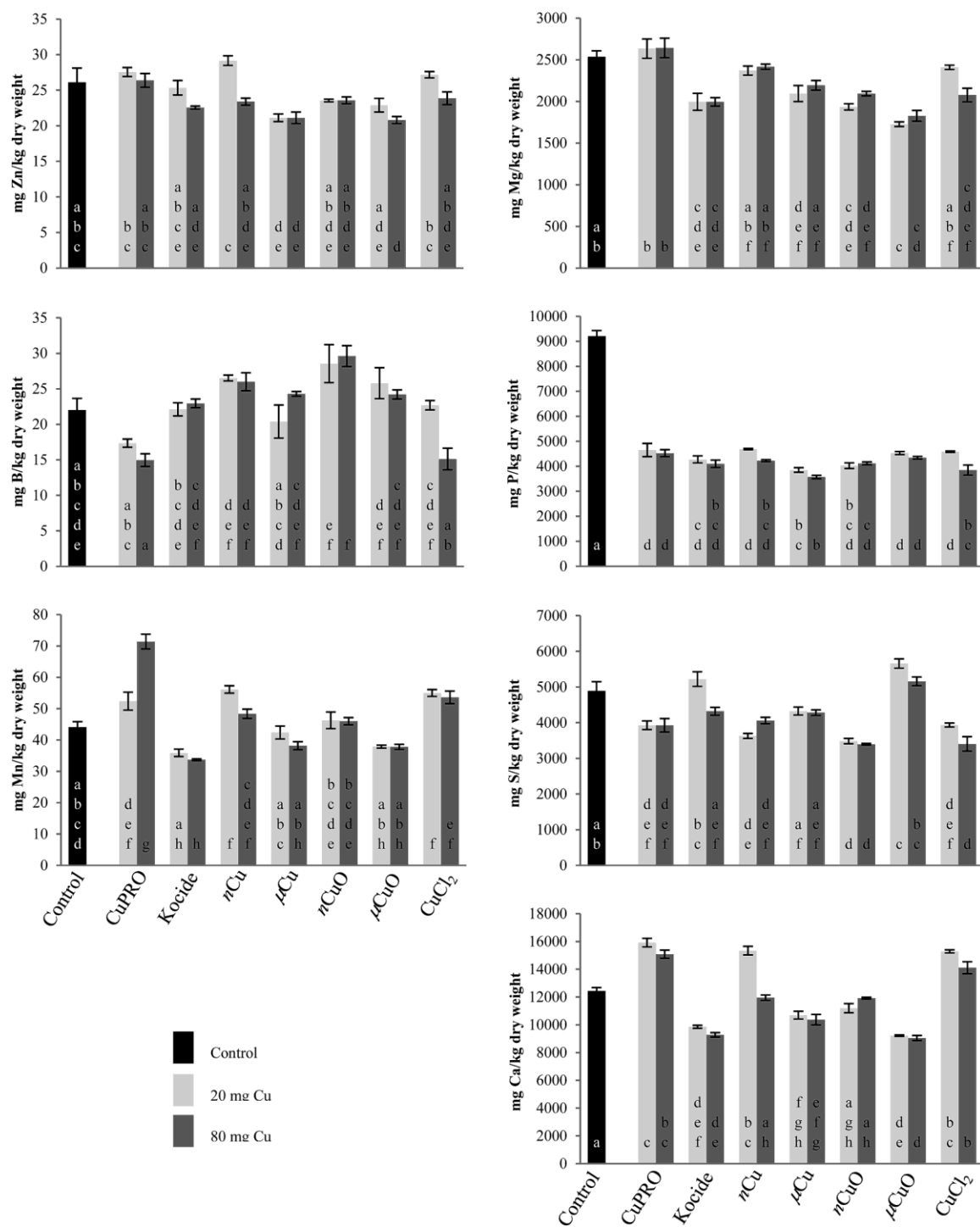


Figure 2.6 Concentration of nutritional elements in shoots of cilantro plants grown for 30 days in soil amended with Cu-based nanoparticles or compounds at 0 (control), 20, and 80 mg Cu per kg soil. Each data point is mean \pm SE ($n = 4$). Means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$).

CONCLUSION

This study has shown that at both concentrations nCuO, μ CuO and ionic Cu significantly reduced cilantro seed germination, compared with control. In addition, μ CuO at 20 mg kg⁻¹ reduced the relative chlorophyll content, while at 80 mg kg⁻¹ significantly reduced plant biomass. Shoot elongation was reduced by nCu at 80 mg kg⁻¹ (12.4%), and μ CuO at both concentrations (11.2 and 11.0%, respectively). Notably, all treatments reduced P accumulation, except CuPRO at 20 mg/kg in roots. Micro and macro elements B, Zn, Mn, Ca, Mg, P, and S were significantly reduced in shoots ($p \leq 0.05$). These results showed that Cu-based NPs/compounds depress nutrient elements accumulation in cilantro, which could impact human nutrition. Results of this study have shown that, even at a low concentration (20 mg kg⁻¹) these compounds impact the nutritional quality of cilantro. However, studies in other types of soils are needed in order to generalize about the effects of Cu-based NPs/compounds in cilantro.

REFERENCES

- Adeleye, A.S.; Conway, J.R.; Perez, T.; Rutten, P.; Keller, A.A. Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. *Environ. Sci. Technol.* **2014**, *48*, 12561–12568.
- Anjum, N.A.; Adam, V.; Kizek, R.; Duarte, A.C.; Pereira, E.; Iqbal, M.; Lukatkin, A.S.; Ahmad, I.; Nanoscale copper in the soil–plant system – toxicity and underlying potential mechanisms. *Environ Res.* **2015**, *138*, 306–325. <http://dx.doi.org/10.1016/j.envres.2015.02.019>
- Atha, D. H.; Wang, H.; Petersen, E. J.; Cleveland, D.; Holbrook, R. D.; Jaruga, P.; Dizdaroglu, M.; Xing, B.; Nelson, B.C. Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environ. Sci. Technol.*, **2012**, *46*, 1819–1827.
- Buchner, P.; Takahashi, H.; Hawkesford, M.J. Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. *J. Exp. Bot.*, **2004**, *55*, 1765–1773.
- Cai, Y.; Su, J.; Ma, L.Q. Low molecular weight thiols in arsenic hyperaccumulator *Pteris vittata* upon exposure to arsenic and other trace elements. *Environ. Pollut.*, **2004**, *129*, 69–78.
- Castillo-Michel, H.A.; Zuverza-Mena, N.; Parsons, J.G.; Dokken, K.M.; Duarte-Gardea, M.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Accumulation, speciation, and coordination of arsenic in an inbred line and a wild type cultivar of the desert plant species *Chilopsis linearis* (Desert willow). *Phytochemistry*, **2009**, *70*, 540–545.
- Cataldo, D.A.; Garland, T.R.; Wildung, R.E.; Drucker, H. Nickel in plants II. Distribution and chemical form in soybean plants. *Plant Physiol.*, **1978**, *62*, 563–565.
- Cioffi, N.; Ditaranto, N.; Torsi, L.; Picca, R. A.; De Giglio, E.; Sabbatini, L.; Novello, L.; Tantillo, G.; Bleve-Zacheo, T.; Zambonin, P.G. Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. *Anal. Bioanal. Chem.* **2005**, *382*, 1912–1918.
- Cometa, S.; Iatta, R.; Ricci, M.A.; Ferretti, C.; De Giglio, E. Analytical characterization and antimicrobial properties of novel copper nanoparticle-loaded electrosynthesized hydrogel coatings. *J. Bioact. Compat. Polym.* **2013**, *28*, 508–522.
- Conway, J.R.; Adeleye, A.S.; Gardea-Torresdey, J.L.; Keller, A.A. Aggregation, dissolution, and transformation of copper nanoparticles in natural waters. *Environ. Sci. Technol.*, **2015**, *49*, 2749–2756.
- de La Rosa, G.; López-Moreno, M.L.; Hernandez-Viezcas, J.A.; Montes, M.O.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Toxicity and biotransformation of ZnO nanoparticles in the desert plants *Prosopis juliflora-velutina*, *Salsola tragus* and *Parkinsonia florida*. *Int. J. Nanotechnol.* **2011**, *8*, 492–506.

- Dimkpa, C.O.; McLean, J.E.; Latta, D.E.; Manangón, E.; Britt, D.W.; Johnson, W.P.; Boyanov, M.I.; Anderson, A.J. CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res.* **2012**, *14*, 1125–1139.
- Dordas, C.; Chrispeels, M.J.; Brown, P.H. Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiol.*, **2000**, *124*, 1349–1362.
- Feynman, R.P. There's plenty of room at the bottom. *Engineering and science*, **1960**, *23*, 22-36.
- Gardea-Torresdey, J.L.; Peralta-Videa, J.R.; Montes, M.; De la Rosa, G.; Corral-Diaz, B. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements. *Bioresour. Technol.*, **2004**, *92*, 229–235.
- Gardea-Torresdey, J.L.; Rico, C.M.; White, J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* **2014**, *48*, 2526–2540
- Georgopoulos, P.G.; Roy, A.; Yonone-Lioy, M. J.; Opiekun R.E.; Lioy, P.J. Environmental copper: its dynamics and human exposure issues. *J. Toxicol. Environ. Health*, **2001**, *4*, 341–394.
- Gilliham, M.; Dayod, M.; Hocking, B.J.; Xu, B.; Conn, S.J.; Kaiser, B.N.; Leigh, R.A.; Tyerman, S.D. Calcium delivery and storage in plant leaves: exploring the link with water flow. *J. Exp. Bot.*, **2011**, *62*, 2233–2250.
- Holden, P.A.; Nisbet, R.M.; Lenihan, H.S.; Miller, R.J.; Cherr, G.N.; Schimel, J.P.; Gardea-Torresdey, J.L. Ecological nanotoxicology: integrating nanomaterial hazard considerations across the subcellular, population, community, and ecosystems levels. *Acc. Chem. Res.* **2013**, *46*, 813–822.
- Hong, J.; Rico, C.M.; Zhao, L.; Adeleye, A.S.; Keller, A.A.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environ. Sci.: Processes Impacts*, **2015**, *17*, 177–185.
- Karlsson, T.; Persson, P.; Skyllberg, U. Complexation of copper (II) in organic soils and in dissolved organic matter-EXAFS evidence for chelate ring structures. *Environ. Sci. Technol.*, **2006**, *40*, 2623–2628.
- Keller, A.A.; McFerran, S.; Lazareva, A.; Suh, S. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* **2013**, *15*, 1692–1708.
- Kochian, L.V. Mechanisms of micronutrient uptake and translocation in plants. In *Micronutrients in Agriculture*, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman and R. M. Welch, Soil Science Society of America, Madison, WI, 2nd edn, 1991, pp. 229–296.

- Kumar, R.; Mehrotra, N.K.; Nautiyal, B.D.; Kumar, P.; Singh, P.K. Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.). *J. Environ. Biol.*, **2009**, 30, 485–488.
- Laribi, B.; Kouki, K.; M'Hamdi, M.; Bettaieb, T. Coriander (*Coriandrum sativum* L.) and its bioactive constituents. *Fitoterapia*, **2015**, 103, 9–26.
- Lock, K.; Criel, P.; De Schampheleere, K.A.C.; Van Eeckhout, H.; Janssen, C.R. Influence of calcium, magnesium, sodium, potassium and pH on copper toxicity to barley (*Hordeum vulgare*). *Ecotoxicol. Environ. Saf.*, **2007**, 68, 299–304.
- Mary, G.; Bajpai, S. K.; Chand, N. Copper (II) ions and copper nanoparticles-loaded chemically modified cotton cellulose fibers with fair antibacterial properties. *J. Appl. Polym. Sci.* **2009**, 113, 757–766.
- Moon, Y.S.; Park, E.S.; Kim, T.O.; Lee, H.S.; Lee, S.E. SELDI-TOF MS-based discovery of a biomarker in *Cucumis sativus* seeds exposed to CuO nanoparticles. *Environ. Toxicol. Pharmacol.* **2014**, 38, 922–931.
- Morales, M.I.; Rico, C.M.; Hernandez-Viezas, J.A.; Nunez, J.E.; Barrios, A.C.; Tafoya, A.; Flores-Marges, J.P.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *J. Agric. Food Chem.* **2013**, 61, 6224–6230.
- Musante, C.; White, J C. Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. *Environ. Toxicol.* **2012**, 27, 510–517.
- Nair, P.M.G.; Chung, I.M. Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). *Ecotoxicol. Environ. Saf.*, **2015**, 113, 302–313.
- Österås, A.H.; Greger, M. Interactions between calcium and copper or cadmium in Norway spruce. *Biol. Plant.*, **2006**, 50, 647–652.
- Pfeffer, H.; Dannel, F.; Römheld, V. Are there two mechanisms for boron uptake in sunflower? *J. Plant Physiol.*, **1999**, 155, 34–40.
- Robinson, B.H.; Green, S.R.; Chancerel, B.; Mills, T.M.; Clothier, B.E. Poplar for the phytomanagement of boron contaminated sites. *Environ. Pollut.*, **2007**, 150, 225–233.
- Roco, M.C. Nanotechnology: convergence with modern biology and medicine. *Curr. Opin. Biotechnol.* **2003**, 14, 337–346.
- Shaw, A.K.; Hossain, Z. Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere*, **2013**, 93, 906–915.
- Smith, F.W.; Mudge, S.R.; Rae, A.L.; Glassop, D. Phosphate transport in plants. *Plant and Soil*, **2003**, 248, 71–83.

- Song, L.; Vijver, M.G.; Peijnenburg, W.J. Comparative toxicity of copper nanoparticles across three Lemnaceae species. *Sci. Total Environ.* **2015**, 518, 217–224.
- Stampoulis, D.; Sinha, S. K.; White, J. C. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.*, **2009**, 43, 9473–9479.
- Taiz, L.; Zeiger, E. Plant Physiology, Sinauer Associates, Sunderland, MA, 2nd edn, **1998**, ch. 5, pp. 104–116.
- Trujillo-Reyes, J.; Majumdar, S.; Botez, C.E.; Peralta-Videa, J.R.; and Gardea-Torresdey, J.L. Exposure studies of core–shell Fe/Fe₃O₄ and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard? *J. Hazard. Mater.*, **2014**, 267, 255–263.
- Wu, S. G.; Huang, L.; Head, J.; Chen, D.; Kong, I. C.; Tang, Y. J. Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. *J. Pet. Environ. Biotechnol.* **2012**, 3, 126.
- Zhao, H.; Wu, L.; Chai, T.; Zhang, Y.; Tan, J.; Ma, S. The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. **2012**, 169, 1243–1252.

Chapter 3. Effects of Silver Nanoparticles on Radish Sprouts: Root Growth Reduction and Modifications in the Nutritional Value¹

ABSTRACT

Reports indicate that silver nanoparticles (nAg) are toxic to vegetation, but little is known about their effects in crop plants. This study examines the impacts of nAg on the physiology and nutritional quality of radish (*Raphanus sativus*) sprouts. Seeds were germinated and grown for 5 days in nAg suspensions at 0, 125, 250, and 500 mg L⁻¹. Seed germination and seedling growth were evaluated with traditional methodologies; the uptake of Ag and nutrients was quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and changes in macromolecules were analyzed by infrared (IR) spectroscopy. None of the nAg concentrations reduced seed germination. However, the water content (% of the total weight) was reduced by 1.62, 1.65, and 2.54 % with exposure to 125, 250, and 500 mg L⁻¹, respectively, compared with the control. At 500 mg L⁻¹, the root and shoot lengths were reduced by 47.7 and 40 %, with respect to the control. The seedlings exposed to 500 mg/L had 901 ± 150 mg Ag kg⁻¹ dry wt and significantly less Ca, Mg, B, Cu, Mn, and Zn, compared with the control. The infrared spectroscopy analysis showed changes in the bands corresponding to lipids (3000–2800 cm⁻¹), proteins (1550–1530 cm⁻¹), and structural components of plant cells such as lignin, pectin, and cellulose. These results suggest that nAg could significantly affect the growth, nutrient content and macromolecule conformation in radish sprouts, with unknown consequences for human health.

Keywords: silver nanoparticles, radish, FTIR, elemental analysis, macromolecules

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INTRODUCTION

The “Nano-Era” started in the late 1990’s propelled by the worldwide increase in government investments into nanomaterials (NMs) and their applications. In the United States, the National Nanotechnology Initiative, created at the beginning of 2000, had coordinated the research and development of nanotechnology (Roco, 2003). Since then, a number of carbon-based and metal-based NMs have been produced and are currently being used in many fields. NMs are commonly referred to as small objects with one or more external dimensions in the size range 1–100 nm. At these dimensions, materials exhibit a distinctive behavior in comparison to larger particles of the same composition. Silver is an important component in the area of NMs, with over 450 metric tons of silver nanoparticles (nAg) produced in 2010 (Keller et al., 2013). In November 2014, fifty percent of the nano-enabled products on the “Project on Emerging Nanotechnologies” inventory contained nAg. Among other goods, listed products included wound dressings with bactericidal effects that enhance healing, antibacterial door knobs and anti-odor socks; all articles containing nAg that provide antimicrobial properties (Kim et al., 2007). At some point, it is assumed that all the nanoparticles (NPs) in different items will end up in the soil, air or water (Keller et al., 2013). Even though some studies have reported the effects of nAg in the environment (Daniel et al., 2010; Beer et al., 2012), their toxicity on crop plants is not yet well understood. Previous investigations indicate that the effects of nAg on seed germination vary with NP characteristics and plant species. Kumari et al. (2009) reported that nAg decrease the mitotic index and cause multiple chromosomal breaks and cell disintegration in onion (*Allium cepa*). Stampoulis et al. (2009) found that nAg at 500 and 100 mg L⁻¹ reduced plant biomass and transpiration in zucchini (*Cucurbita pepo*) by 57 and 41%, respectively. In another experiment, *C. pepo* sp. *ovifera* was exposed in hydroponics to 0, 100, and 500 mg nAg L⁻¹ and corresponding bulk Ag (Ag

powder). Results showed more negative effects in plants exposed to nAg than bulk Ag (Musante and White, 2012). It has also been observed that nAg reduced growth in mung bean (*Phaseolus radiatus*) and sorghum (*Sorghum bicolor*) cultivated in soil or agar-based medium (Lee et al., 2012). In addition, Pokhrel and Dubey (2013) found that coated nAg promoted histological changes in maize (*Zea mays* L.) by inducing elongation of root cells, and reduced root growth in cabbage (*Brassica oleracea* var. *capitata* L.) by 24%. In tomato (*Solanum lycopersicum*), nAg did not affect germination even at 5000 mg L⁻¹ (Song et al., 2013). Thuesombat et al. (2014) reported that the toxicity of nAg in rice (*Oryza sativa* cv. KDML 105) increased directly with the particle size and concentration. A more recent report indicates that nAg at a concentration of 100 mg L⁻¹ reduced germination in *Brassica nigra* (Amooaghaie et al., 2015). However, the effects of nAg on radish have not been reported yet. Radish (*Raphanus sativus* L.) sprouts are widely consumed worldwide due to their nutritional content and antioxidant properties (Xiao et al., 2014; Baenas et al., 2015). More than two decades ago, radish was proposed as a model plant for the study of environmental stresses, mainly atmospheric contaminants (Kostka-Rick and Manning, 1993). More recently, due to its short growing period, this plant has been considered as a model of edible roots for the study of the interaction of plants with soil contaminants (Létondor et al., 2015). A few reports have shown different responses of radish seedlings exposed to NMs. Ma et al. (2010) reported that nLa₂O₃, nGd₂O₃, and nYb₂O₃ at 2000 mg L⁻¹ inhibited root elongation. However, Trujillo-Reyes et al. (2013) found that citric acid coated nCeO₂, at 200 mg L⁻¹, increased root biomass and seedlings' water content. In addition, Corral-Diaz et al. (2014) reported that nCeO₂ at 250 mg kg⁻¹ soil increased radish tubers' antioxidant capacity. There is concern about the trophic transfer of NPs from edible plants into the food chain (Gardea-Torresdey et al., 2014). The present investigation addresses the effects of a nAg suspension, intended for human ingestion, in a

terrestrial plant. In this study, radish seeds were exposed to different nAg concentrations from a commercially available nAg suspension to test its effects on radish sprouts. The marketed nAg product, at 500 mg L⁻¹ per serving, is indicated as a dietary supplement for immune support². Even though the environmental concentrations of nAg are lower than the amounts used for this study (Gottschalk et al., 2013), we chose 500 mg L⁻¹ as the highest concentration for the experiment, assuming the worst case scenario for this product. The effects on seedlings' development, nutrient uptake and changes of macromolecules were studied by using spectrophotometric analytical techniques.

MATERIALS AND METHODS

Silver Nanoparticles and Radish Seeds

Silver nanoparticles (nAg) from Natural Path/Silver Wings (Nashville, TN, USA) came suspended in deionized water at 500 mg L⁻¹. According to the manufacturer, the majority of the nAg are 2 nm in size forming colloids in the range of 1-10 nm. The hydrodynamic size of the suspended particles in water and the zeta potential (ζ , the electrostatic charge between particles) was analyzed by dynamic light scattering (DLS) using a Malvern Zetasizer (Nano-ZS90, Malvern Instruments, UK). Radish (Champion variety) seeds were obtained from Del Norte Seeds and Feed (Vinton, TX, USA).

Seed Germination

Thirty seeds were directly incubated without previous treatment in sterilized standard Petri dishes (10 cm diameter) over germination paper, modified from López-Moreno et al. (2010). Treatments consisted of nAg suspensions at 0 (control), 125, 250, and 500 mg L⁻¹; four

²<http://www.npswsilver.com/>

replicates per treatment. The concentrations for the study were selected considering that the worst case scenario at which plants could be exposed is the commercially available product of nAg at 500 mg L⁻¹. Suspensions were prepared by diluting the stock suspension of 500 mg L⁻¹ (as supplied by the vendor) with Millipore water (18 MΩ cm). We utilized Millipore water for the experiments because it has similar resistivity than deionized water (Yin et al., 2012). Aliquots of five milliliters of nAg suspension were administered to each Petri dish, except for control seeds that received five milliliters of Millipore water. The dishes containing the seeds were covered with aluminum foil for 3 days. Then, they were set into a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH, USA), where seedlings grew for a total of 5 days before analysis. Environmental conditions inside the chamber were 25/20°C day/night temperature, 14/10 h light/dark photoperiod, 60 ± 3% relative humidity, and 340 μmol/m²s light intensity. The percent germination (%G), relative germination (%RG), and germination change (%GC) were calculated as per de la Rosa et al. (2011). The length of the roots and shoots was measured on 15 plants per replicate. Water content, fresh and dry weights (dry wt) were also determined on 15 plants per replicate.

Elemental Analysis

At harvest, seedlings were washed with 0.01 M HNO₃ and rinsed with Millipore water to remove the nAg adhered to tissues. After washing, seedlings were oven dried at 70°C for 72 h (Corral-Diaz et al., 2014). Dried samples were prepared for analysis, according to the EPA method 3051. Briefly, the seedlings were powdered using mortar and pestle and acid digested (0.1 g per sample) with a 1:4 ratio of HNO₃:H₂O₂ in a microwave oven (MarsX, CEM Mathews, NC, USA). Digested samples were placed in 15 mL polypropylene centrifuge tubes and the final volume was adjusted to 15 mL. The digests were analyzed for macronutrients, micronutrients, and Ag content by using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer

Optima 4300 DV, Shelton, CT, USA). For quality assurance/quality control, a standard of 0.5 mg L⁻¹ from the calibration curve that contained Ag, micro and macro elements was analyzed every five samples.

FT-IR Analysis

Changes in lipids, proteins, carbohydrates and other organic polymers (e.g., lignin) were studied by using Fourier transform infrared (FT-IR) spectroscopy. The sprouts were washed with 0.01 M HNO₃ and rinsed with Millipore water, separated into roots, stems and leaves and oven dried at 70°C for at least 72 h. Samples of roots, stems, and leaves were analyzed by using a Perkin-Elmer, Spectrum 100 with a Universal Attenuated Total Reflectance (ATR) sampling accessory. Background correction was performed by acquiring a spectrum without sample. Powdered samples were placed on the spectrometer stand and spectra were recorded. The data was collected in a frequency range from 4000 to 600 cm⁻¹ at a resolution of 1 cm⁻¹ and three scans per reading. Results are averages of triplicate determinations, as similarly reported by Servin et al. (2013). The amide I peak is commonly taken as an internal standard to normalize biological samples' spectra (Yu and Irudayaraj, 2005). Data was normalized at wavenumber 1650 cm⁻¹ for roots, stems, and leaves. This allowed us to compare the spectra within the same plant tissue at different nAg concentrations.

Statistical Analysis

Data was analyzed using the Statistical Package for the Social Sciences 20.0 (SPSS, Chicago, IL, USA). Variance was evaluated by one-way analysis of variance (one-way ANOVA) and the difference between treatment means was compared by Tukey's honest significant difference (Tukey's HSD) test at a *p*-value of 0.05, unless otherwise stated. Regression analysis was performed on growth data.

RESULTS AND DISCUSSION

Characterization and Effects of nAg on Seed Germination

The nAg suspended in Millipore water had (at the highest concentration) a hydrodynamic size of 77 ± 2.44 nm and a zeta potential (ζ) of -24.4 ± 12.6 mV. Results suggest that nAg may be aggregating given that the manufacturer specifications indicate a colloid size of 1–10 nm. Note that our recorded dimensions (77 ± 2.44 nm) include any layer that forms around the nAg due to interactions with the aqueous media, where the inorganic complex is suspended (Supplementary Figure S1).

Supplementary Figure S2 shows an overall view of the experimental setup, while Supplementary Figure S3 shows enlarged views of one Petri dish/treatment. The germination percent and changes in germination of radish seeds exposed to nAg are shown in Table 3.1. As seen in this table, at 125 mg L^{-1} nAg increased the germination by 3%, while at 250 and 500 mg L^{-1} reduced the germination by 3 and 6%, respectively. However, none of the treatments reached a statistical significant difference in comparison to the control.

Table 3.1. Germination percent (%G), relative germination percent (%RG) and germination change (GC%) of radish seeds after 5 days of incubation in nAg suspension at 0, 125, 250, and 500 mg/L.

	(Control) 0 mg/L	125 mg/L	250 mg/L	500 mg/L
%G	93 \pm 1	96 \pm 3	90 \pm 4	88 \pm 1
%RG	100 \pm 1	103 \pm 3	97 \pm 4	94 \pm 1
%GC	0 \pm 1	3 \pm 3	-3 \pm 4	-6 \pm 1

Nanoparticles tend to agglomerate in suspension due to their size, composition of the medium and ionic strength, among other variables (Murdock et al., 2008). nAg showed moderate agglomeration (77 ± 2.44 nm) and negative surface charge when suspended in Millipore water (Murdock et al., 2008). However, these characteristics did not appear to interact with the cellulosic component of the radish seed coat (Esau, 1977). A previous study has shown that radish is a robust plant, in terms of germination under environmental stresses, due to the hard coat of its seeds that may prevent the entrance of contaminants, like heavy metals and nanoparticles (Koul et al., 2000). Previous studies have also shown no effects of heavy metal solutions or NP suspensions on radish germination. Lane and Martin (1977) reported no penetration of Pb within radish seeds exposed to 95 mg L^{-1} of “Analar,” a lead nitrate solution. Lin and Xing (2007) reported that radish germination was not altered by nAl_2O_3 , nAl, nZn, and nZnO, even at the very high concentration of 2000 mg L^{-1} . Wu et al. (2012) found that the EC_{50} for radish germination exposed to nNiO and nCuO was 401 and 398 mg L^{-1} , respectively. Trujillo-Reyes et al. (2013) reported no effects on radish seed germination exposed up to 200 mg L^{-1} of citric acid coated and uncoated nCeO₂. Corral-Diaz et al. (2014) reported that germination of radish seeds sown in soil amended with nCeO₂ at 0–500 mg kg^{-1} was retarded, but not reduced. Thus, it is not surprising that nAg, even at 500 mg/L , did not affect radish seed germination.

Seedling Growth, Silver Uptake, Biomass Production, and Water Content

Figure 3.1 shows the root and shoot elongation, dry biomass production, water content, and Ag concentration in seedlings exposed to nAg at 0 (control), 125, 250, and 500 mg L^{-1} . Individual images of seedlings from the different treatments are shown in Supplementary Figure S4. As seen in Figure 3.1A, accumulation of Ag was concentration dependent, but there was no difference between 125 and 250 mg L^{-1} treatments (114 and $204 \text{ mg Ag kg}^{-1}$ dry tissue, respectively).

However, at 500 mg L⁻¹, Ag accumulation was significantly higher (900 mg Ag kg⁻¹ dry tissue) compared to the other treatments ($p \leq 0.05$).

The elongation of roots and shoots is shown in Figure 3.1B. As shown in this figure, there was a concentration-dependent reduction in root elongation ($r^2 = 0.9626$) that reached statistical significance in seedlings exposed to 250 and 500 mg L⁻¹ with respect to control. The percent reductions in the two treatments were 27.3 and 47.7%, respectively. In addition, the root length of seedlings exposed to 500 mg L⁻¹ (5.2 cm) was statistically lower compared to the length of roots exposed to 250 mg L⁻¹ (7.2 cm). Figure 3.1B also shows the shoot length of radish seedlings. Similar to root length, there was a concentration-dependent significant reduction ($r^2 = 0.9677$) in shoot elongation. However, in the case of shoots, all nAg concentrations significantly reduced shoot elongation, although the reduction at 125 and 250 mg L⁻¹ was statistically similar. At 500 mg L⁻¹ nAg exposure, the reduction in shoot length reached 38%.

The effects of nAg on dry biomass are shown in Figure 3.1C. Plants exposed to 250 mg L⁻¹ had less biomass, compared with control and the other treatments. The reduction was about 10%, compared with control, and about 7% compared with the other treatments.

Water content of the whole seedlings is shown in Figure 3.1D. As shown in this figure, the water content (expressed as % of the total seedlings' weight) was reduced by 1.62, 1.65, and 2.54% with exposure to 125, 250, and 500 mg L⁻¹, respectively, compared with the control. In all cases the differences were statistically significant, compared with the control; however, there were no differences between the 125 and 250 mg L⁻¹ treatments ($p \leq 0.05$).

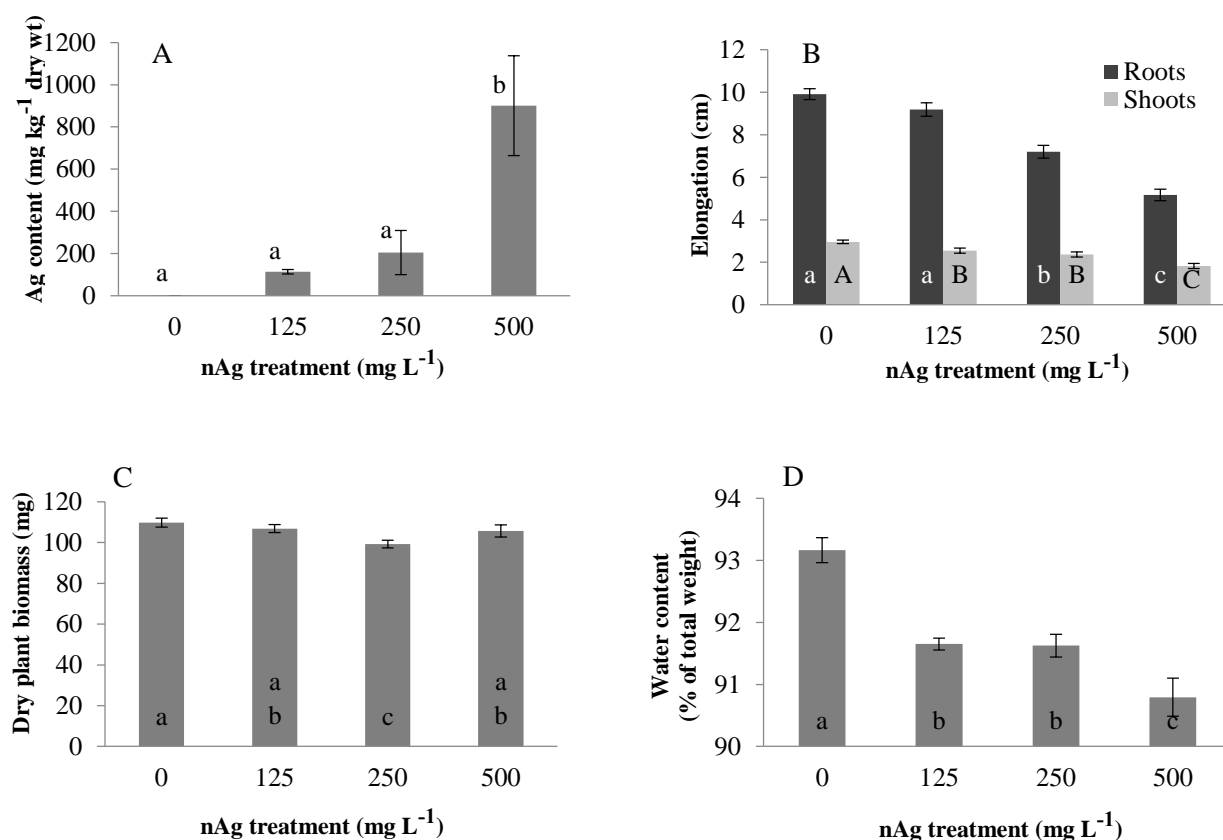


Figure 3.1 Concentration of Ag, mg per kg of dry plant tissue (A), root and shoot length (B), dry biomass (C), and water content (D) in radish seedlings exposed for 5 days to nAg at 0 (control), 125, 250, and 500 mg L⁻¹. Values are means of four replicates per treatment (15 plants each replicate) \pm standard error. Different letters denote statistically significant differences according to the Tukey's HSD test ($p < 0.05$). In (B), small case letters are for roots and upper case letters for shoots.

The measurement of heavy metals or NPs' uptake by plant roots through ICP includes particles/elements adsorbed/ absorbed by the root system (Larue et al., 2012). Hong et al. (2015) showed that washing the tissues with CaCl₂ and HNO₃ removed about 80% of the nCeO₂ sprayed to the leaves of cucumber. Thus, although radish seedlings were washed with HNO₃ to remove the nAg adhered to the seedlings surface, some particles that could have remained were absorbed by

the epidermis. Consequently, the reported data points include both the nAg adsorbed, plus the Ag taken up by the roots. The data reported in the present study, mainly at the highest concentration treatment (500 mg L⁻¹), differs from previously reported data. For instance, Song et al. (2013) reported no differences in silver uptake by tomato seedlings developed under exposure to 100 and 1000 mg L⁻¹ of colloidal silver. These researchers determined that nAg hardly penetrated the hard coat of tomato seeds, and an analogous result could be expected with the hard coat of radish seeds (Koul et al., 2000). In specimens not protected by a hard coat, like rice seeds, penetration of the nAg has been associated with particle size and concentration. Thuesombat et al. (2014) soaked rice seedlings for 24 h in 1000 mg L⁻¹ of either 20 or 150 nm nAg and germinated the seeds in a sand bed. Seven days after germination, concentrations of Ag in roots were 22 and 12 mg kg⁻¹, respectively. In our study, at 500 mg L⁻¹ the uptake was higher than that reported by Thuesombat et al. (2014) at 1000 mg L⁻¹; however, the particles used in the present study were of a smaller size, which could explain the difference. In addition, radish seedlings were in contact with the nAg suspension during the entire experimental period. Results from our study and reports from the literature indicate that the uptake of Ag from nAg depends on a series of factors such as treatment concentration, particle size, plant species, and exposure media.

There is no consistency in the reports of the effects of nAg on seedlings growth. For instance, Lee et al. (2012) reported a reduction of about 60% in mung bean and 75% in sorghum seedlings exposed in agar for 2 days to nAg at 10 mg L⁻¹. These reduction rates are considerable larger than those observed in radish, although their support (agar) was different than the suspension used. However, the reduction in radish growth was similar to the reduction in sorghum, but different from that found with mung bean exposed in soil to 800 mg kg⁻¹ of nAg (Lee et al., 2012), which does not support a comparison between liquid medium or agar. On the other hand, Nair and

Chung (2014) exposed rice seedlings for 1 week to nAg in hydroponics (similar conditions of the present study), but at lower concentrations (0.2–1.0 mg L⁻¹) and did not report changes in root elongation. This corroborates that the effects of nAg on root growth depend on a series of factors. In addition, differences in treatment concentrations and exposure media made it difficult to compare the results.

Our results on the effects of nAg in biomass production concurs with the reports found in 4-week old rice seedlings exposed to nAg at 0.1–1000 mg L⁻¹ (Thuesombat et al., 2014). However, they differ from the results with tomato exposed for 15 days to a similar nAg concentration, where the reduction was about 75% (Song et al., 2013). The mechanisms for reduction in biomass production by nAg are not known; however, Nair and Chung (2014) found differential transcription of genes associated with stress tolerance, which could explain the reduction in biomass production.

The reduction in seedlings' water content under NP exposure has been previously reported. Trujillo-Reyes et al. (2014) found that nCu at 10 and 20 mg L⁻¹ significantly reduced water content in lettuce. According to the literature, both Cu and Ag block water permeability in roots cells (Niemietz and Tyerman, 2002; Chang et al., 2012), which in turn reduce water absorption. In addition, Qian et al. (2013) proposed that nAg have the potential to change the transcription of antioxidant and aquaporin genes, affecting the balance of water in Arabidopsis. It is possible that nAg block aquaporins, reducing the water uptake in radish seedlings. This raises a question which requires more studies for a complete understanding of the aquaporins blockage by nAg.

Effects of nAg on Macro and Micronutrient Accumulation

Figure 3.2 shows the concentration of elements that were affected by nAg in the whole seedling. Amongst macroelements, only Ca and Mg were significantly reduced by the highest concentrations of nAg (Figure 3.2A). Calcium was reduced by 20 and 33% and Mg by 10 and 19% at 250 and 500 mg L⁻¹, respectively, compared with control.

With regard to micronutrient absorption, none of the nAg concentrations impaired the absorption of Fe, Ni, and Mo. However, all treatments significantly reduced ($p \leq 0.05$) absorption of B, Mn, and Cu, while Zn was only reduced at 500 mg L⁻¹, compared with control (Figure 3.2B). Percent reductions at 125, 250, and 500 mg L⁻¹ were 32, 36.8, and 44.6% for B; 11.6, 14.8, and 26.9% for Mn, and 47.5, 52.5, and 52.5% for Cu, respectively. Zinc was reduced by 12.6% at the highest concentration treatment.

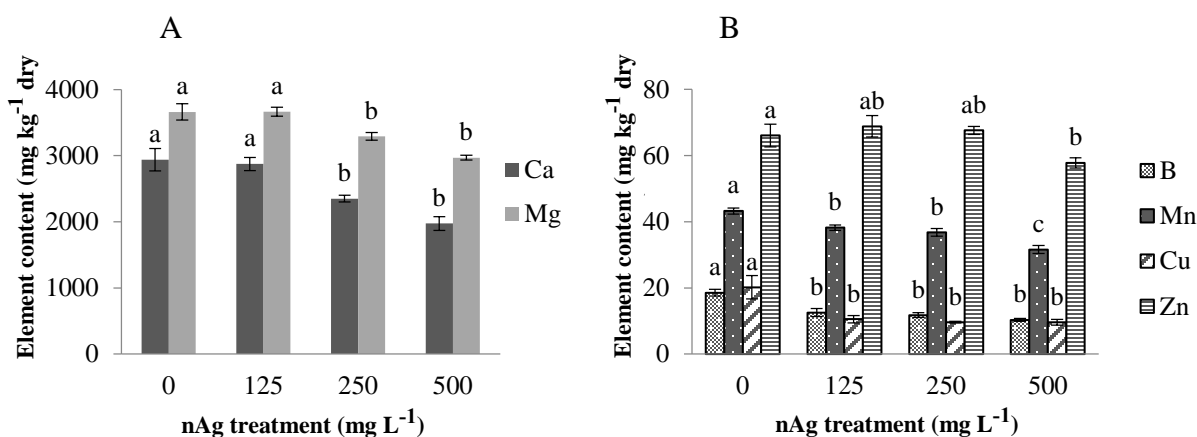


Figure 3.2 Concentration of macroelements (A) and microelements (B) in radish seedlings germinated and grown for 5 days in nAg suspensions at 0 (control), 125, 250, and 500 mg L⁻¹. Values are means of four replicates per treatment (15 plants each) \pm standard error. Different letters denote statistically significant differences according to the Tukey's HSD test ($p < 0.05$).

Previous studies have shown that the uptake of nutrient elements is affected by both the NM and the species of plant. For instance, Servin et al. (2013) reported increases in Ca, K, and Mg in cucumber exposed to nTiO₂. Trujillo-Reyes et al. (2014) reported changes on the accumulation of Mn and Zn in lettuce leaves exposed to core-shell Fe/Fe₃O₄ and Cu/CuO NPs. Hong et al. (2015) exposed alfalfa and lettuce to several Cu-based NPs/compounds in hydroponics and found changes in the absorption of some macroelements such as K, Mg, and Cu. In addition, Trujillo-Reyes et al. (2013) reported that CeO₂ NPs modified the content of Mn and Ni in hydroponically grown radish. In the present study, it was found that nAg reduced the uptake of macroelements Ca and Mg and microelements like B, Mn, and Zn. There is the possibility that nAg decrease the expression of the Ca channel protein, reducing Ca uptake. Magnesium is absorbed in a similar way as bacterial transporters CorA Mg²⁺ (Maathuis, 2009). It is very likely that at high concentrations, nAg are physically blocking the channels, reducing the absorption of Mg.

Boron uptake mechanisms include passive transport through the uncharged boric acid molecule, active transport through boron transporter 1 (BOR1) that uploads B into the xylem, and facilitated diffusion through channels belonging to intrinsic proteins (Dordas et al., 2000; Miwa and Fujiwara, 2010; Wimmer and Eichert, 2013). Trujillo-Reyes et al. (2013) reported that citric acid coated nCeO₂ reduced the uptake of B in radish; however, to the best of the authors' knowledge, there is no explanation about the interference of NPs on B uptake. The uptake of Mn and Zn by roots is mediated by putative transporters, Nramp and ZIP family (Guerinot, 2000). The current information is not sufficient enough to get a clear idea of how nAg could affect the uptake of these microelements. As explained above, it is possible that nAg physically block the diffusion pathway or the channels for active absorption. In addition, Magesky and Pelletier (2015)

mentioned that silver is a membrane disruptor that breaks down cellular homeostasis. Very likely, this disruption affected the uptake of essential elements. It is also possible that nAg down regulate the genes encoding for metal transporters. Further investigation is needed in order to unravel the mechanism of nAg interference with the uptake of micronutrients.

FT-IR Analysis of Roots, Stems, and Leaves

Fourier transform infrared spectroscopy has been used to identify conformational changes in the macromolecules of plants exposed to contaminants, including NPs. Table 3.2 provides a summary of previously compiled FT-IR data from plant samples that relates the functional groups identified and the macromolecules involved (Dokken and Davis, 2007; Lammers et al., 2009; Rico et al., 2015). For comparison purposes, we chose specific spectral regions where lipids (3000–2800 cm^{-1}), proteins and lignin (1700–1500 cm^{-1}), lipids and pectin (1790–1720 cm^{-1}) cellulose, and hemicellulose (1300–1180 cm^{-1}) and other carbohydrates (fingerprint region 1200–900 cm^{-1}) are presumably found. Changes in the FT-IR spectra of radish seedlings (Figures 3.3–3.5) were compared with the data shown in Table 3.2. There are no apparent band shifts in the FT-IR spectra of the roots, stems or leaves treated with nAg at different concentrations as seen in Figures 3.3–3.5. However, changes in band intensities were found in all studied regions.

Table 3.2 Summary of FT-IR band frequencies found in plants exposed to NPs and other contaminants (Dokken and Davis, 2007; Lammers et al., 2009 and Rico et al., 2015).

Frequency (cm ⁻¹)	Literature freq. (cm ⁻¹)	Functional Group	Molecule/tissue component	From
3350-3250	3300	OH	Polysaccharides, alcohols	Lammers et al., 2009
3100-2800	3100-3000	C–H aromatic	-	Lammers et al., 2009
	3000-2800	C–H aliphatic	-	Lammers et al., 2009
	2960-2940	CH ₃ asymmetric	lipids	Dokken and Davis, 2007
	2930-2910	CH ₂ asymmetric	lipids	Dokken and Davis, 2007
	2885-2860	CH ₃ symmetric	lipids	Dokken and Davis, 2007
	2860-2840	CH ₂ symmetric	lipids	Dokken and Davis, 2007
1790-1720	1790-1744	C=O	carboxyl ester, lipids, esterified pectins	Rico et al., 2015
	1749	COOH	carboxylate COOH, pectin	Lammers et al., 2009
	1742, 1732	C=O	ester carbonyls, polysaccharides	Lammers et al., 2009
	1740	C=O (alkyl)	lipids, esterified pectins	Dokken and Davis, 2007
1700-1500	1664-1648	C=O, C–N	protein	Rico et al., 2015
	1650	C=O, C–N	protein	Dokken and Davis, 2007
	1635	Aromatic C=C	lignin	Dokken and Davis, 2007
	1632	Aromatic C=C	lignin	Rico et al., 2015
	1630-1605	COOH	carboxylate COOH, pectin	Lammers et al., 2009
	1568-1536	N–H, C–N	protein	Rico et al., 2015
	1550	N–H, C–N	protein	Dokken and Davis, 2007
1300-1180	1250-1240	Asymmetric C–O–H	cellulose, hemicellulose	Dokken and Davis, 2007
	1248-1216	Asymmetric C–O–H	cellulose, hemicellulose	Rico et al., 2015
1200-900	1200-900	-	carbohydrate	Dokken and Davis, 2007
	1150-1060	C–O–C (ether)	lignin	Lammers et al., 2009
	1150-980	C–O	starch	Lammers et al., 2009
	1130-1050, 1370	-	cellulose	Lammers et al., 2009
	1072-1040	C–O	cellulose, hemicellulose	Rico et al., 2015
	1024-992	-	carbohydrate	Rico et al., 2015
	928-912	-	carbohydrate	Rico et al., 2015

Changes in the regions of bands from radish sprouts were compared with data shown in this table

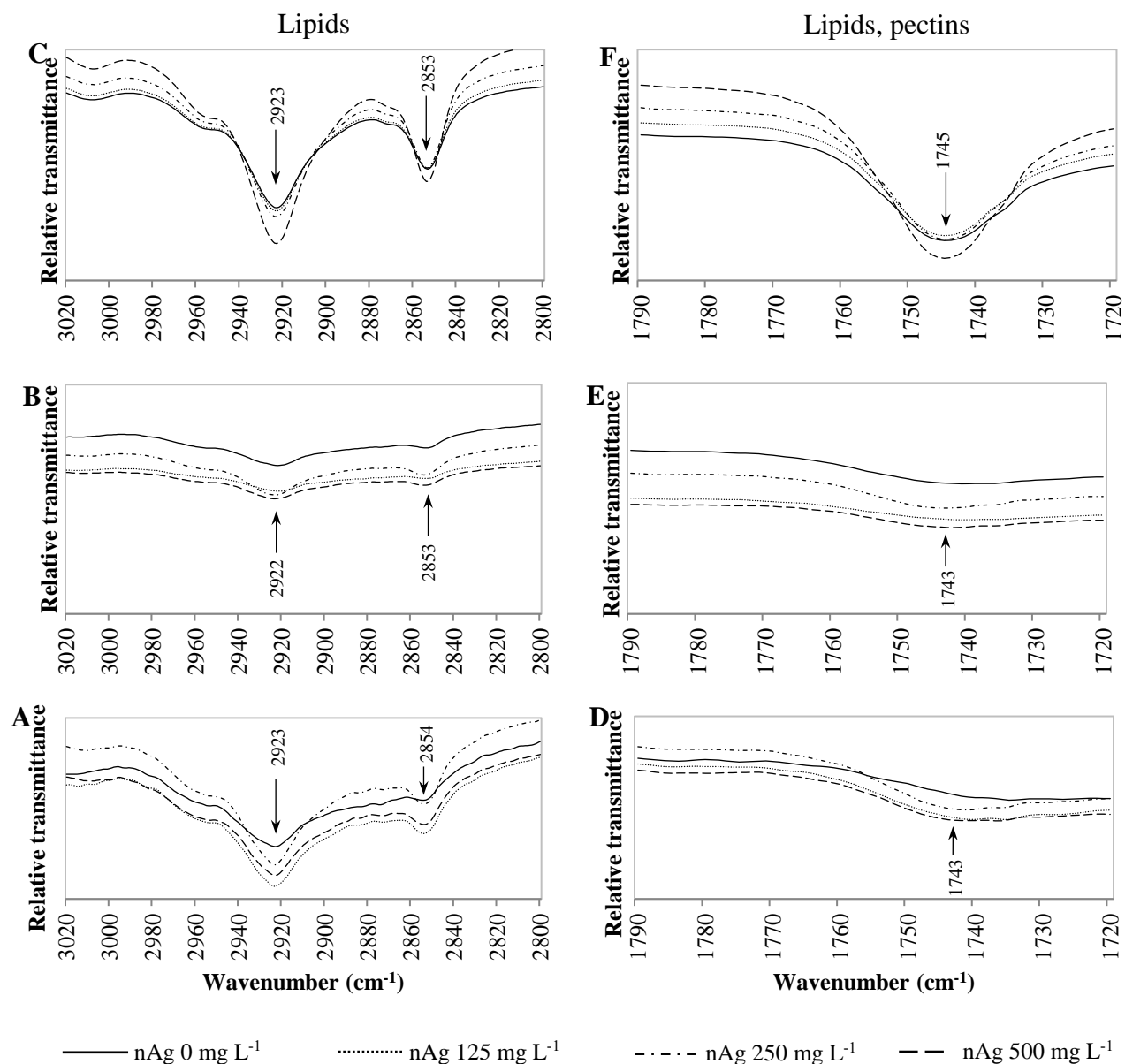


Figure 3.3. Overlap ATR-FTIR spectra of radish sprouts exposed to nAg at 0, 125, 250, and 500 mg L⁻¹. Spectral region associated with lipids in seedlings' roots (A), stems (B) and leaves (C); and regions related to lipids and pectins in sprouts' roots (D), stems (E), and leaves (F).

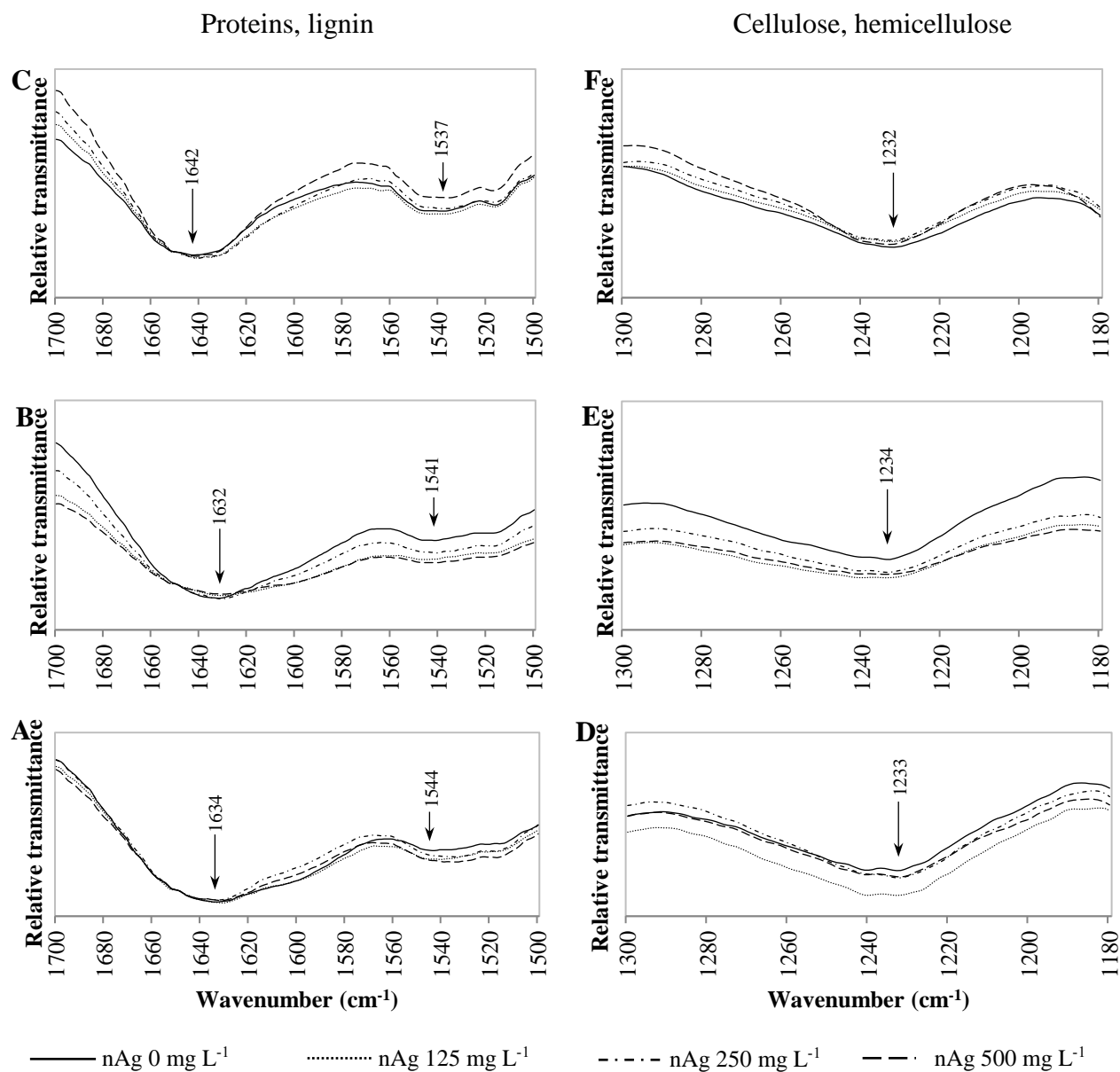


Figure 3.4 Overlap ATR-FTIR spectra of radish sprouts exposed to nAg at 0, 125, 250, and 500 mg L⁻¹. Spectral region associated with proteins and lignin in seedlings' roots (A), stems (B), and leaves (C); and regions related to cellulose and hemicellulose in sprouts' roots (D), stems (E) and leaves (F).

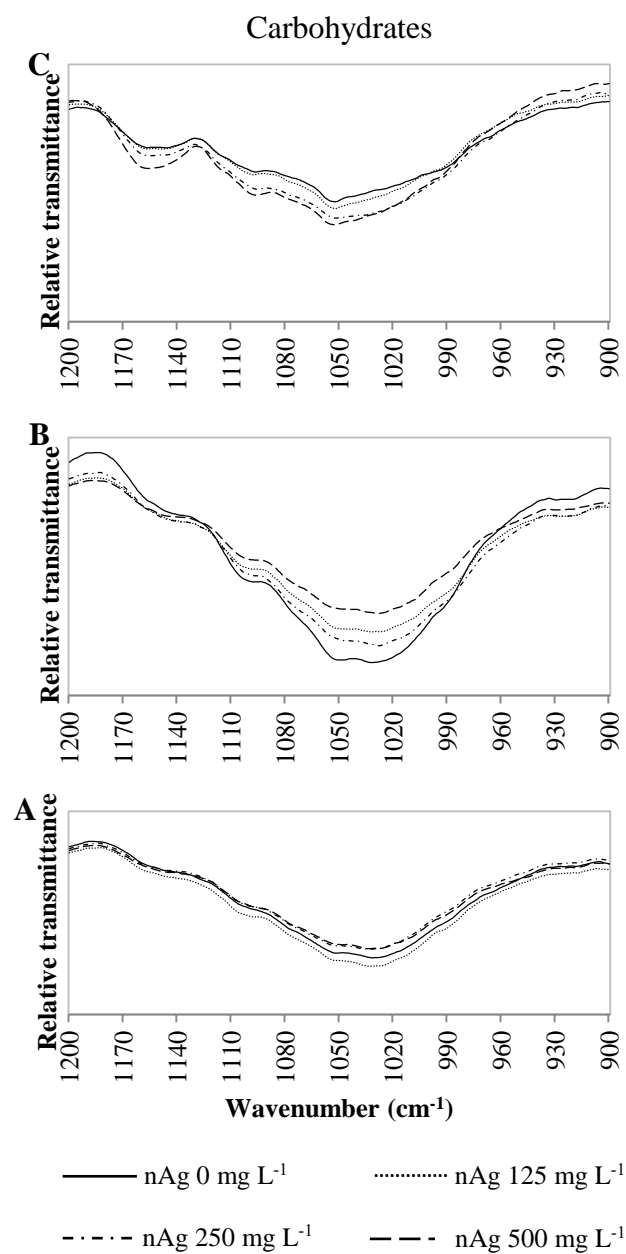


Figure 3.5 Overlap ATR-FTIR spectra of radish sprouts exposed to nAg at 0, 125, 250, and 500 mg L^{-1} . Spectral region associated with carbohydrates in seedlings' roots (A), stems (B), and leaves (C).

According to Table 3.2, the C–H bond in CH₂–CH₃ groups associated with lipids is found in the region of 3000–2800 cm⁻¹. These macromolecules are constituents of the lipid bilayer found in cell membranes. Figures 3.3A–C shows the relative transmittance of bands in the spectra obtained for roots, stems and leaves assigned to lipids in radish sprouts. Bands in the region between 1790 and 1720 cm⁻¹ (Figures 3.3D–F) are associated to C=O and COOH groups assigned also to lipids and pectins, these last are made of polysaccharides and are accountable for the structure of the primary cell wall.

Figures 3.4A–C shows the peaks found at 1650–1630 cm⁻¹ for lignin and bonds identified for proteins at 1650 cm⁻¹, 1549–1530 cm⁻¹ corresponding to C=O, N–H and C–N (Dokken and Davis, 2007; Rico et al., 2015). Changes in cellulose and hemicellulose are observed at 1242–1230 and 1054–1051 cm⁻¹ in Figures 3.4D–F and 3.5 respectively. Also, carbohydrates not attributed to a specific biopolymer are shown from 1200–900 cm⁻¹ in Figure 3.5.

Alterations in lipids and carbohydrates were similar to those reported for cilantro exposed to nCeO₂ (Morales et al., 2013). While hemicellulose, cellulose and pectin are structural components of primary cell walls, lignin provides rigidity to terrestrial plants (Cabane et al., 2012). Disruption in these macromolecules may lead to changes in morphology that could impair the normal development of the plants. The fact that no band shifts were observed suggests that there are no chemical changes in the macromolecules studied, only shape alterations in the plant tissue components (Morales et al., 2013).

In summary, concentrations of nAg used in this study did not affect radish seed germination. However, there was a concentration-dependent reduction in seedling elongation and water content. In addition, at 250 mg L⁻¹ the biomass was reduced by 10%, compared with control ($p \leq 0.05$). Silver NPs also impaired the absorption of nutritional elements in radish seedlings.

Important macroelements such as Ca and Mg and microelements B, Cu, Mn, and Zn were reduced by the highest concentration of nAg. Moreover, nAg induced conformational changes in carbohydrates, lignin, and lipids. The impacts of such changes in the nutritional value of radish sprouts are not known yet. In addition, as per Holden et al. (2014), “it may be premature for manufactured NMs risk research to sanction information on the basis of concentration ‘environmental relevance’.”

REFERENCES

- Amooaghaie, R., Tabatabaei, F., and Ahadi, A.M. (2015). Role of hematin and sodium nitroprusside in regulating *Brassica nigra* seed germination under nanosilver and silver nitrate stresses. *Ecotoxicol. Environ. Saf.* 113, 259-270. doi: 10.1016/j.ecoenv.2014.12.017.
- Baenas, N., Ferreres, F., García-Viguera, C., and Moreno, D.A. (2015). Radish sprouts—Characterization and elicitation of novel varieties rich in anthocyanins. *Food Res. Int.* 69, 305-312. doi: <http://dx.doi.org/10.1016/j.foodres.2015.01.009>.
- Beer, C., Foldbjerg, R., Hayashi, Y., Sutherland, D.S., and Autrup, H. (2012). Toxicity of silver nanoparticles—Nanoparticle or silver ion? *Toxicol. Lett.* 208, 286-292. doi: <http://dx.doi.org/10.1016/j.toxlet.2011.11.002>.
- Bindhu, M.R., and Umadevi, M. (2015). Antibacterial and catalytic activities of green synthesized silver nanoparticles. *Spectrochim. Acta Part A: Mol. Biomol. Spectros.* 135, 373-378. doi: <http://dx.doi.org/10.1016/j.saa.2014.07.045>.
- Cabane, M., Afif, D., and Hawkins, S. (2012). "Chapter 7 - Lignins and abiotic stresses," in *Advances in Botanical Research*, eds. J. Lise & L. Catherine. Academic Press), 219-262.
- Chang, Y.-N., Zhang, M., Xia, L., Zhang, J., and Xing, G. (2012). The toxic effects and mechanisms of CuO and ZnO nanoparticles. *Materials* 5, 2850.
- Corral-Diaz, B., Peralta-Videa, J.R., Alvarez-Parrilla, E., Rodrigo-García, J., Morales, M.I., Osuna-Avila, P., Niu, G., Hernandez-Viezcas, J.A., and Gardea-Torresdey, J.L. (2014). Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). *Plant Physiol. Biochem.* 84, 277-285. doi: <http://dx.doi.org/10.1016/j.plaphy.2014.09.018>.
- Daniel, S.C.G.K., Tharmaraj, V., Sironmani, T.A., and Pitchumani, K. (2010). Toxicity and immunological activity of silver nanoparticles. *Appl. Clay Sci.* 48, 547-551. doi: <http://dx.doi.org/10.1016/j.clay.2010.03.001>.
- de La Rosa, G., López-Moreno, M.L., Hernandez-Viezcas, J.A., Montes, M.O., Peralta-Videa, J., and Gardea-Torresdey, J. (2011). Toxicity and biotransformation of ZnO nanoparticles in the desert plants *Prosopis juliflora-velutina*, *Salsola tragus* and *Parkinsonia florida*. *Int. J. Nanotechnol.* 8, 492-506.
- Dokken, K.M., and Davis, L.C. (2007). Infrared imaging of sunflower and maize root anatomy. *J. Agric. Food Chem.* 55, 10517-10530. doi: 10.1021/jf072052e.
- Dordas, C., Chrispeels, M.J., and Brown, P.H. (2000). Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiol.* 124, 1349-1362.

- Esau, K. (1997). *Anatomy of Seed Plants*, 2nd ed. (Santa Barbara: John Wiley) 455-473.
- Gardea-Torresdey, J. L., Rico, C. M., White, J.C. (2014). Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 48, 2526-2540.
- Gottschalk, F., Sun T.Y., Nowack, B. (2013). Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environ. Pollut.* 181, 287-300.
- Guerinot, M.L. (2000). The ZIP family of metal transporters. *Biochim, Biophys. Acta (BBA) - Biomembranes* 1465, 190-198. doi: [http://dx.doi.org/10.1016/S0005-2736\(00\)00138-3](http://dx.doi.org/10.1016/S0005-2736(00)00138-3).
- Holden, P., Klaessig, F., Turco, R., Priester, J.H., Rico, C., Arias, H., Mortimer, M., Pacpaco, K., Gardea-Torresdey, J. (2014). Evaluation of exposure concentrations used in assessing manufactured nanomaterial environmental hazards: Are they relevant? *Environ. Sci. Technol.* 48, 10541–10551.
- Hong, J., Rico, C.M., Zhao, L., Adeleye, A.S., Keller, A.A., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2015). Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environ. Sci.: Process Impacts* 17, 177-185. doi: 10.1039/c4em00551a.
- Keller, A.A., McFerran, S., Lazareva, A., and Suh, S. (2013). Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1-17.
- Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y.H., Hwang, C.Y., Kim, Y.K., Lee, Y.S., Jeong, D.H., and Cho, M.H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine* 3, 95-101. doi: 10.1016/j.nano.2006.12.001.
- Kostka-Rick, R., and Manning, W.J. (1993). Radish (*Raphanus sativus* L.): a model for studying plant responses to air pollutants and other environmental stresses. *Environ. Pollut.* 82, 107-138.
- Koul, K., Nagpal, R., and Raina, S. (2000). Seed coat microsculpturing in *Brassica* and allied genera (subtribes *Brassicinae*, *Raphaninae*, *Moricandiinae*). *Ann. Bot.* 86, 385-397.
- Kumari, M., Mukherjee, A., and Chandrasekaran, N. (2009). Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci. Total Environ.* 407, 5243-5246. doi: <http://dx.doi.org/10.1016/j.scitotenv.2009.06.024>.
- Lammers, K., Arbuckle-Keil, G., and Dighton, J. (2009). FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning. *Soil Biol. Biochem.* 41, 340-347. doi: <http://dx.doi.org/10.1016/j.soilbio.2008.11.005>.
- Lane, S., and Martin, E. (1977). A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytol.* 79, 281-286.

- Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., and Carriere, M. (2012). Accumulation, translocation and impact of TiO₂ nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. *Sci. Total. Environ.* 431, 197-208. doi: 10.1016/j.scitotenv.2012.04.073.
- Lee, W.M., Kwak, J.I., and An, Y.J. (2012). Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: media effect on phytotoxicity. *Chemosphere* 86, 491-499. doi: 10.1016/j.chemosphere.2011.10.013.
- Létondor, C., Pascal-Lorber, S., and Laurent, F. (2015). Uptake and distribution of chlordecone in radish: Different contamination routes in edible roots. *Chemosphere* 118, 20-28. doi: <http://dx.doi.org/10.1016/j.chemosphere.2014.03.102>.
- Lin, D., and Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ. Pollut.* 150, 243-250. doi: 10.1016/j.envpol.2007.01.016.
- López-Moreno, M.L., De La Rosa, G., Hernández-Viezcas, J.A., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2010). X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO₂ nanoparticles and assessment of their differential toxicity in four edible plant species. *J. Agric. Food Chem.* 58, 3689-3693.
- Ma, Y., Kuang, L., He, X., Bai, W., Ding, Y., Zhang, Z., Zhao, Y., Chai, Z. (2010). Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere* 78, 273-279.
- Maathuis, F.J.M. (2009). Physiological functions of mineral macronutrients. *Curr. Opin. Plant Biol.* 12, 250-258. doi: <http://dx.doi.org/10.1016/j.pbi.2009.04.003>.
- Magesky, A., and Pelletier, E. (2015). Toxicity mechanisms of ionic silver and polymer-coated silver nanoparticles with interactions of functionalized carbon nanotubes on early development stages of sea urchin. *Aquat. Toxicol.* 167, 106-123.
- Miwa, K., and Fujiwara, T. (2010). Boron transport in plants: co-ordinated regulation of transporters. *Ann. Bot.* 105, 1103-1108. doi: 10.1093/aob/mcq044.
- Morales, M.I., Rico, C.M., Hernandez-Viezcas, J.A., Nunez, J.E., Barrios, A.C., Tafoya, A., Flores-Marges, J.P., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2013). Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *J. Agric. Food Chem.* 61, 6224-6230. doi: 10.1021/jf401628v.
- Murdock, R.C., Bradich-Stolle, L., Schrand, A.M., Schlanger, J.J., and Hussain, S.M. (2008). Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique. *Toxicol. Sci.* 101, 239-253. doi:10.1093/toxsci/kfm240
- Musante, C., and White, J.C. (2012). Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. *Environ. Toxicol.* 27, 510-517. doi: 10.1002/tox.20667.

- Nair, P.M.G., and Chung, I.M. (2014). Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. *Chemosphere* 112, 105-113. doi: <http://dx.doi.org/10.1016/j.chemosphere.2014.03.056>.
- Niemietz, C.M., and Tyerman, S.D. (2002). New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. *FEBS Lett.* 531, 443-447.
- Pokhrel, L.R., and Dubey, B. (2013). Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles. *Sci. Total Environ.* 452-453, 321-332. doi: <http://dx.doi.org/10.1016/j.scitotenv.2013.02.059>.
- Qian, H., Peng, X., Han, X., Ren, J., Sun, L., and Fu, Z. (2013). Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. *J. Environ. Sci.* 25, 1947-1956. doi: [http://dx.doi.org/10.1016/S1001-0742\(12\)60301-5](http://dx.doi.org/10.1016/S1001-0742(12)60301-5).
- Rico, C.M., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2015). Differential effects of cerium oxide nanoparticles on rice, wheat, and barley roots: a fourier transform infrared (FT-IR) microspectroscopy study. *Appl. Spectrosc.* 69, 287-295. doi: 10.1366/14-07495.
- Roco, M.C. (2003). Nanotechnology: convergence with modern biology and medicine. *Curr. Opin. Biotechnol.* 14, 337-346. doi: [http://dx.doi.org/10.1016/S0958-1669\(03\)00068-5](http://dx.doi.org/10.1016/S0958-1669(03)00068-5).
- Servin, A.D., Morales, M.I., Castillo-Michel, H., Hernandez-Viezcas, J.A., Munoz, B., Zhao, L., Nunez, J.E., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2013). Synchrotron verification of TiO₂ accumulation in cucumber fruit: a possible pathway of TiO₂ nanoparticle transfer from soil into the food chain. *Environ. Sci. Technol.* 47, 11592-11598. doi: 10.1021/es403368j.
- Song, U., Jun, H., Waldman, B., Roh, J., Kim, Y., Yi, J., and Lee, E.J. (2013). Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*). *Ecotoxicol. Environ. Saf.* 93, 60-67. doi: 10.1016/j.ecoenv.2013.03.033.
- Stampoulis, D., Sinha, S.K., and White, J.C. (2009). Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* 43, 9473-9479. doi: 10.1021/es901695c.
- Thuesombat, P., Hannongbua, S., Akasit, S., and Chadchawan, S. (2014). Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol. Environ. Saf.* 104, 302-309. doi: 10.1016/j.ecoenv.2014.03.022.
- Trujillo-Reyes, J., Majumdar, S., Botez, C.E., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. (2014). Exposure studies of core-shell Fe/Fe₃O₄ and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard? *J. Hazard. Mater.* 267, 255-263. doi: <http://dx.doi.org/10.1016/j.jhazmat.2013.11.067>.

- Trujillo-Reyes, J., Vilchis-Nestor, A., Majumdar, S., Peralta-Videa, J., and Gardea-Torresdey, J. (2013). Citric acid modifies surface properties of commercial CeO₂ nanoparticles reducing their toxicity and cerium uptake in radish (*Raphanus sativus*) seedlings. *J. Hazard. Mater.* 263, 677-684.
- Wimmer, M.A., and Eichert, T. (2013). Review: mechanisms for boron deficiency-mediated changes in plant water relations. *Plant Sci.* 203-204, 25-32. doi: 10.1016/j.plantsci.2012.12.012.
- Wu, S.G., Huang, L., Head, J., Chen, D.R., Kong, I.C., and Tang, Y. J. (2012) Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. *J. Pet. Environ. Biotechnol.* 3,126. doi:10.4172/2157-7463.1000126
- Xiao, Z., Luo, Y., Lester, G.E., Kou, L., Yang, T., and Wang, Q. (2014). Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. *LWT-Food Sci. Technol.* 55, 551-558.
- Ying, L., Colman, B.P., McGill, B.M., Wright, J.P., Bernhardt, E.S. (2012). Exposure of silver nanoparticloe exposure on germination and early growth of eleven wetland plants. *PloS One*, 7, e47674.
- Yu, C., and Irudayaraj J. (2005) Spectroscopic characterization of microorganisms by Fourier transform infrared microspectroscopy *Biopolymers.* 77, 368-377.

SUPPLEMENTAL INFORMATION FOR CH. 3

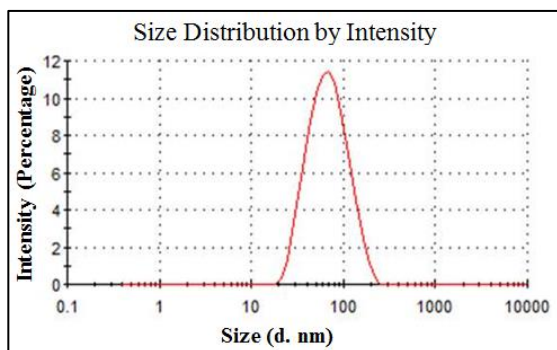


Figure S1. Dynamic light scattering (DLS) determination of the silver nanoparticles' size distribution in colloidal suspension.

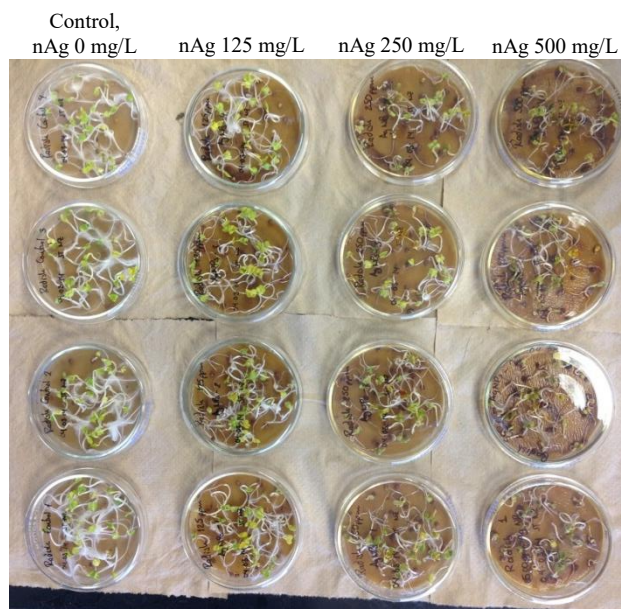


Figure S2. Experimental setup: radish seedlings exposed nAg at 0, 125, 250 and 500 mg/L. Each treatment had four replicates with 30 seeds per Petri dish. Petri dishes are 10 cm in diameter.

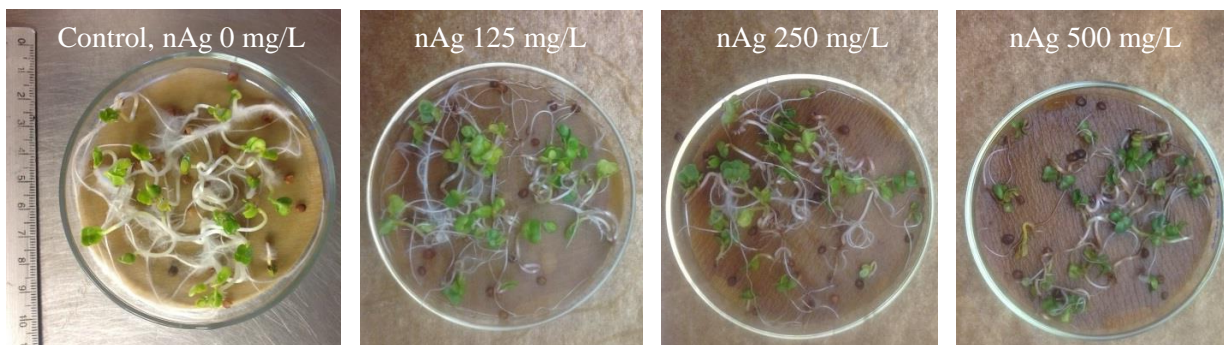


Figure S3. Five day-old radish seedlings exposed to nAg at 0, 125, 250 and 500 mg/L.

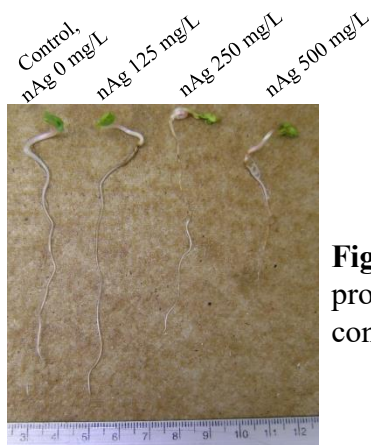


Figure S4. Radish sprouts unraveled from petri dish. Image provides a visual perception of the effects that different nAg concentrations have on radish seedlings growth.

Chapter 4. Effects of ZnO and CeO₂ nanoparticles on sugar, starch, and essential elements in cucumber (*Cucumis sativus*) and corn (*Zea mays*) plants¹

ABSTRACT

Although the effects of nanoparticles (NPs) in plant growth and stress response have been previously reported, information concerning their impacts on carbohydrates and essential elements' uptake is still limited. In this study, cucumber and corn plants were grown until maturity in soil amended with either ZnO NPs or CeO₂ NPs at 0 (control), 400, and 800 mg kg⁻¹. At harvest, sugar and starch contents were analyzed in the leaves, while essential elements were determined in roots, stems and leaves by inductively coupled plasma – optical emission spectroscopy (ICP-OES). The greatest alterations on essential elements were observed in cucumber plants exposed to ZnO NPs, where Mn was lower in all tissues at 400 and 800 mg kg⁻¹, compared to control, except for roots of plants exposed to ZnO NPs at 400 mg kg⁻¹. In cucumber, CeO₂ NPs at 800 mg kg⁻¹ reduced Cu in stems and Ca and Mg in leaves. Nanoparticles of ZnO (at both concentrations) increased Cu and Mn (at 400 mg kg⁻¹) in corn roots. On the other hand, CeO₂ NPs at 400 mg kg⁻¹ increased B in corn stems, while at 800 mg kg⁻¹, Mn and S were increased. None of the treatments affected sugar contents, while only CeO₂ at 800 mg kg⁻¹ increased starch in corn tissues. Overall, CeO₂ NPs presented less toxicity to both crop plants than ZnO NPs.

Keywords: CeO₂, ZnO, nanoparticles, cucumber, corn, mineral elements

¹Zuverza-Mena, N.; Trujillo-Reyes, J.; Hong, J.; Zhao, L.J.; Barrios, A.C.; Sun, Y.; Niu, G.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. To be submitted to the Journal of Plant Physiology and Biochemistry.

INTRODUCTION

The widespread applications of nanoparticles (NPs) raise concerns because significant portions of them end up in the environment after end user applications (Keller 2013). For example, cerium-based nanomaterials (nCe-based) are utilized as additives in diesel to catalyze and improve the fuel combustion process (Erdakos et al., 2014). Exhaust emission tests have detected the release of particulate cerium oxide into the environment (HEI 2001), known to be toxic (Cassee et al., 2011; Snow et al., 2014). According to Erdakos et al (2014), nCe-based compounds in additives could contribute to the increase of fine-particulate Ce from 69 ton yr⁻¹ to 1750 ton yr⁻¹ in the US. Regarding zinc oxide nanoparticles (ZnO NPs), Keller et al (2013) estimated a production of over 30,000 metric ton yr⁻¹. The Project on Emerging Nanotechnologies (2013) reported that ZnO NPs constitute ~2% of the products in the market containing nanoparticles (38 out of 1825 products) which are mainly found in sunscreens. ZnO NPs are released from sun block lotions and find their way through wastewater treatment facilities into wastewater sludge or are directly applied to soil as a fertilizer (Brar et al., 2010; Ma et al., 2013). The impact of CeO₂ and ZnO NPs on plant growth has been previously reported (Rico et al., 2013; Ma et al., 2013, Du et al., 2015). Rico et al. (2014, 2015) encountered that CeO₂ NPs reduced the amounts of S and Mn in wheat grains, but promoted the accumulation of Ca, K, Zn, Mg, Cu, Al, Fe, P and S in barley grains. More recently, Barrios et al. (2015) reported that tomato plants had higher concentrations of B and Fe in roots and more P and Zn in the stems of tomato plants exposed to 500 mg kg⁻¹ of CeO₂ NPs. The availability of nutrient elements can be a major restraint to plant growth because the adequate supply of mineral nutrients is important for internal signaling transport that controls plant development processes (Dakora and Phillips, 2002). Plants take up most mineral nutrients from the rhizosphere. Nanoparticles may have effects in the rhizosphere pH, affecting the uptake of nutrients. In addition

to nutrients, the sustainment of plant development depends on energy sources. Carbohydrates are formed by reducing CO₂ in chloroplasts during photosynthesis. Sugar molecules are translocated through the phloem, and if not used, they are stored as starch for later use (Taiz and Zeiger, 1998). In previous reports we have shown effects of CeO₂ and ZnO NPs on physiological markers, Ce and Zn accumulation in cucumber fruit and corn kernels (Zhao et al., 2013; Zhao et al., 2015), but the impacts of these NPs on the uptake of nutrients by these plants have yet to be reported (Zhao et al., 2014). The objective of this study was to determine the effects of the ZnO NPs and CeO₂ NPs on the elemental composition, sugar and starch concentrations in vegetative tissues of full developed plants. Inductively coupled plasma – optical emission spectrometry and ultrafast UV/Vis were used as analytical techniques.

MATERIALS AND METHODS

ZnO and CeO₂ nanoparticles

The CeO₂ and ZnO NPs (Meliorum Technologies, NY, U.S.) used in this study were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to the supplier, the primary size for those two NPs is 10 nm. The characterization of both NPs are shown in Table 4.1, as previously reported by Keller et al. (2010). Stock solutions were prepared by adding 1000 mg of nanoparticles to 1.0 L of deionized water. The dispersed nanoparticles were sonicated for 30 min in a water bath (Crest Ultrasonics, Trenton, NJ) at 25 °C, 9 watt, for 30 min. Suspensions were immediately applied to the soil substrate at final concentrations of 400 or 800 mg NPs kg⁻¹ soil.

Table 4.1 Physicochemical characterization of the metal oxide NPs

Properties	Technique	Unit	CeO ₂	ZnO
Primary size	TEM ^a	nm	rods: $(67 \pm 8) \times (8 \pm 1)$ ($\leq 10\%$ polyhedra: 8 ± 1 nm)	24 ± 3
Particle size in DI water	DLS ^a	nm	231 ± 16	205 ± 14
Phase and structure	XRD ^a		100 % cubic ceria	100 % zincite hexagonal
Shape/morphology	TEM ^a		rods ($\leq 10\%$ Polyhedra)	Spheroid
Surface area	BET ²	m ² g ⁻¹	93.8	42.1
IEP	zetaPALS ^a		7.5	9.2
EPM in 1mM KCl	zetaPALS ^a	10 ⁻⁸ m ² V ⁻¹ s ⁻¹	2.19 ± 0.04	1.83 ± 0.11
Purity	TGA ^a	wt. %	95.14	97.27
Moisture content	TGA ^a	wt. %	4.01	1.61

^aTransmission and scanning electron microscopy (TEM), dynamic light scattering (DLS), X-ray powder diffraction (XRD), isoelectric point (IEP), electrophoretic mobility (EPM), and thermogravimetric analysis (TGA) were done by the UC-CEIN at UCLA. ²Brunauer-Emmett-Teller analysis (BET) was conducted by Dr. Ponisseril Somasundaran's lab at Columbia University.

Plant growth

Two plants per pot (22.5×19.5 cm²) were grown in a soil substrate (with a density of 0.76 g·cm⁻³) consisting of a 1:1:3 mixture ratio by volume of local loam sand soil (3.7% clay, 12.2% silt, 84.1% sand, and 0.04% organic matter content, pH 7.9, collected from Texas A&M experimental station, Texas, U.S.A and air-dried and sieved through a 2 mm sieve), sand (Quikrete Premium Play Sand, Atlanta, GA), and potting mix (Sunshine Mix No. 4, SunGro Hort., Bellevue, WA). Four replicates were prepared per treatment and plants were allowed to grow in a greenhouse under controlled conditions: photosynthetically active radiation of 17.3 ± 3.6 mol·m⁻²·d⁻¹, daylight temperature of 30.5 ± 4.7 °C (mean \pm standard deviation) and 25.8 ± 2.8 °C at night temperature. Cucumber and corn plants were harvested at 53 and 84 days, respectively. At harvest, plants were

removed from the pots, washed with tap water and rinsed with distilled water three times, separated into roots, stems and leaves, and oven dried at 70 °C for 14 days.

Micro and macronutrient concentrations in tissues of cucumber and corn plants

Dry tissues were digested in 1:4 (v/v) HNO₃ and H₂O₂ using a microwave oven (CEM Corp, Mathews, NC, U.S.). Analyses were compared against standard reference samples of 1547 and 1570a from the National Institute of Standards and Technology (USA). The macro and micro nutrient elements (Al, B, Cu, Fe, Mn, Zn, Ca, Mg, P, S, Na, K) were analyzed using inductively coupled plasma optical emission spectrometry (Perkin-Elmer Optima 4300 DV).

Sugar and starch analysis in corn and cucumber leaves.

Total sugar and starch contents were analyzed according to the methodology described by Verma and Dubey (2001) and quantified as described by Dubois et al. (1956). In short, 80% v/v ethanol was added to 0.1g of oven dried samples in 15 mL centrifuge tubes and placed in a water bath at ~80 °C for 30 min and then centrifuged. The procedure was repeated three times and the supernatants were collected in 50 mL centrifuge tubes. Supernatants of the same treatment were collected together for sugar quantification. For starch analysis, the pellets from the sugar extraction were left to oven dry for 24 h at 70 °C. Then, 2 mL of Millipore water (18 MΩ cm) were added and boiled for 15 min in a water bath, cooled to room temperature and added 2 mL of concentrated H₂SO₄. Samples were stirred for 15 min, followed by adjusting the volume with Millipore water to 10 mL and then centrifuged (model 5804R, Eppendorf™) at 5,000 rpm for 20 min. Extractions were repeated three times and supernatants of the same treatment were collected in 50 mL centrifuge tubes. Samples were placed in 96-well plates and absorbance was read (SPECTROstar Nano, BMG Labtech) at a wavelength of 490 nm, 100 rpm for 30 s.

Data Analysis

The treatments (four replicates each) with NPs at the two concentrations and the control were allocated in a completely random design. Mineral nutrients were analyzed by using PROC GLM in SAS software (Version 9.1.3, SAS Institute, Cary, NC). Total sugar and starch were evaluated with the Statistical Package for the Social Sciences 22.0 (SPSS, Chicago, IL). One-way analysis of variance (one-way ANOVA) and Tukey's honest significant difference (Tukey's HSD) tests were performed to compare the differences between treatment means at a p value ≤ 0.05 .

RESULTS AND DISCUSSION

Elemental analysis

The present investigation assesses the composition of essential elements in cucumber and corn plants. Although Al is not an essential element, at low concentration has shown to have a stimulatory effect in plant growth (Taiz and Zeiger, 1998). In addition, it was found in a previous report that accumulation of Al is affected by NPs (Zhao et al. 2015; Trujillo-Reyes et al. 2013). On the contrary, Mo was not included in the report because it was below the detection limits of the instrument.

Effects of ZnO and CeO₂ NPs on cucumber plants nutrient composition

Table 4.2 shows the mineral nutrient concentration in roots, stems and leaves of cucumber plants treated at different levels of ZnO NPs exposure. Table 4.2 displays that Al, B, Fe, Ca and S were unaffected in all tissues of cucumber plants.

With regard to micronutrients, manganese was significantly increased in every tissue at both concentrations, except in roots of plants treated with ZnO NPs at 400 mg kg⁻¹. The normal Mn concentration in cucumber leaves is between 100 to 300 mg kg⁻¹ (Adams, 2002). Our findings

of 108 and 176 mg kg⁻¹ in the leaves of plants exposed to ZnO NPs at 400 and 800 mg kg⁻¹, respectively, suggest that Mn in leaves was within the normal range. Therefore, ZnO NPs at the concentrations tested in this study did not lead to toxicity due higher levels of Mn in the leaves.

Copper, considered an immobile element, was diminished in stems with increasing concentration of ZnO NPs, while Cu in leaves decreased only in plants exposed to 800 mg kg⁻¹. Cu is an essential nutrient involved in redox reactions. Copper deficiency could negatively affect the photosynthesis process and therefore, plant growth. However, the development of cucumber plants was not affected (Zhao et al. 2013).

Sodium was decreased in stems of plants treated with ZnO NPs at 800 mg kg⁻¹. As expected, Zn significantly increased in plant tissues as external ZnO NPs increased. According to Tzerakis et al. (2012), the first symptoms of Zn toxicity appeared when the concentrations of Zn in cucumber leaves reached 450 mg kg⁻¹ dry weight. In this study, cucumber leaves had 470 and 649 mg Zn kg⁻¹ dry weight in plants exposed to 400 and 800 mg kg⁻¹ ZnO NPs, respectively. This suggest that ZnO NPs did not induce toxicity due to Zn accumulation in tissues.

Accumulation of macronutrients Mg, P, and K was also affected by ZnO NPs in cucumber plants. At 800 mg kg⁻¹ ZnO NPs reduced P in roots, stems, and leaves, and K in stems. Phosphorous is commonly found in plants as phosphate ion (PO₄³⁻) and it is involved in photosynthesis, energy production (Taiz and Zeiger, 1998), and it is also an essential component of the phospholipid bilayer that makes up plant membranes. Therefore, a reduction in P can lead to a lack of structural strength of cells. Phosphorus, Na, and K, among others, are classified as “mobile minerals” meaning that plants relocate them to places where they are required (Taiz and Zeiger, 1998). Therefore, plants react to deficiencies by translocating such elements. With regards to Na and K,

more studies will be needed to understand why they were deficient only in stems and not in other tissues. Magnesium is the central atom in the chlorophyll, and it is also involved in the activation of enzymes. Our results show that Mg increased only in cucumber stems by 11% at both ZnO NPs concentrations. This suggests that, under the growth conditions of this experiment, a higher portion of Mg was retained in xylem. The xylem loading of Mg is through active and passive transport systems that operate with the external Mg concentration. It is possible that ZnO NPs interferes with the active transport system (Tanoi et al. 2011); thus, Mg moved slowly from the xylem to the leaves tissues; then, at the time of analysis, there was more Mg in stems than leaves. Tzerakis et al. (2012) reported that in cucumber, an excess of external Zn reduced the uptake of Mg, Ca, Fe, and Cu, while that of K and P remained unaffected. But the real mechanism of interference of ZnO NPs with the Mg transport system is still missing.

Nanoparticles of CeO₂ appeared to be less toxic to cucumber than ZnO NPs. It can be seen in Table 4.3 that Al, B, Fe, Mn, Zn, P, S, Na and K remained unaffected by CeO₂ NPs, even at the highest exposure level. However, at 800 mg kg⁻¹, CeO₂ NPs affected the accumulation of Cu, Ca, and Mg. While Cu increased in the stems, Ca and Mg were reduced in leaves. Calcium has an essential role in plant growth and development, mostly related to cell wall structure and permeability (Hepler, 2005). Magnesium has a number of key functions in plants, e.g., chlorophyll formation and photosynthetic carbon dioxide fixation (Maguire and Cowan, 2002). Both Ca and Mg, as divalent cations, utilize transport proteins to move across membranes, but they are also passively transported (Taiz and Zeiger, 1998). It is possible that at so high concentration (800 mg kg⁻¹), CeO₂ NPs might have physically blocked the cations' gates. It is worth noting that the decrease observed with Ca and Mg in cucumber leaves did not result in a reduced growth rate or

chlorophyll deficiency (Zhao et al., 2013). More research should be conducted to confirm the interaction of nano-CeO₂ with transport system of Ca and Mg.

Table 4.2 Elemental analysis of cucumber plants exposed to ZnO NPs

	Treatment / Element	Al	B	Cu	Fe	Mn	Zn
Roots	Control	1574.6 ± 313.5 a	37.4 ± 0.7 ab	34.3 ± 6.3 a	1057.3 ± 223.7 a	127.2 ± 16.7 a	175.8 ± 15.9 a
	ZnO NPs 400	1773.8 ± 392.2 a	42.5 ± 1.1 b	29.1 ± 2.7 a	1170.6 ± 244.6 a	169.7 ± 9.9 a	1722.1 ± 149.5 b
	ZnO NPs 800	864.1 ± 201.0 a	36.6 ± 2.1 a	33.3 ± 3.2 a	582.5 ± 106.0 a	252.0 ± 15.3 b	2560.2 ± 115.3 c
Stems	Control	71.5 ± 7.9 ab	34.7 ± 0.9 a	6.2 ± 0.2 b	66.0 ± 7.4 ab	37.3 ± 1.6 a	39.0 ± 2.5 a
	ZnO NPs 400	94.6 ± 10.4 b	35.9 ± 0.8 a	2.5 ± 0.3 a	78.1 ± 5.8 b	61.2 ± 1.0 c	233.7 ± 8.6 b
	ZnO NPs 800	57.3 ± 6.1 a	33.5 ± 1.3 a	1.9 ± 0.2 a	47.7 ± 4.4 a	53.3 ± 2.2 b	301.7 ± 20.0 c
Leaves	Control	111.8 ± 9.8 a	83.7 ± 3.1 a	11.0 ± 0.4 b	144.7 ± 8.7 a	105.0 ± 4.7 a	48.9 ± 2.4 a
	ZnO NPs 400	114.5 ± 13.6 a	83.7 ± 1.3 a	8.9 ± 2.0 ab	144.1 ± 11.9 a	180.8 ± 10.7 b	470.4 ± 22.8 b
	ZnO NPs 800	115.8 ± 13.9 a	90.3 ± 4.7 a	5.3 ± 0.3 a	134.4 ± 12.1 a	176.8 ± 5.4 b	649.1 ± 32.2 c
		Ca	Mg	P	S	Na	K
Root	Control	14579.2 ± 1410 a	3576.7 ± 274.8 a	8029.0 ± 823.2 b	3762.6 ± 65.5 a	3468.8 ± 383.9 a	33854.4 ± 1690.5 a
	ZnO NPs 400	13146.2 ± 815.5 a	4076.6 ± 246.2 a	6497.9 ± 424.9 ab	3560.5 ± 213.3 a	3519.2 ± 157.0 a	33663.0 ± 3841.9 a
	ZnO NPs 800	11767.2 ± 352.2 a	3915.3 ± 229.4 a	5456.7 ± 190.8 a	3338.5 ± 164.1 a	3288.0 ± 229.5 a	31687.0 ± 1693.0 a
Stem	Control	22025.0 ± 723.6 a	6084.2 ± 80.0 a	7891.1 ± 526.6 b	3512.5 ± 73.4 a	1618.8 ± 29.1 b	74192.4 ± 2429.4 b
	ZnO NPs 400	22793.6 ± 435.0 a	6766.0 ± 139.6 b	6867.1 ± 289.1 ab	3281.3 ± 48.5 a	1390.2 ± 87.2 ab	65920.2 ± 2120.1 ab
	ZnO NPs 800	20699.9 ± 684.0 a	6753.0 ± 128.3 b	6013.7 ± 205.4 a	3268.6 ± 219.7 a	1275.9 ± 70.4 a	64326.5 ± 2005.8 a
Leaf	Control	53757.3 ± 747.2 a	11924.1 ± 203.0 a	5386.3 ± 357.8 b	7101.5 ± 218.2 a	500.0 ± 41.3 a	26820.4 ± 1512.4 a
	ZnO NPs 400	48793.6 ± 1471.6 a	11286.4 ± 372.8 a	4782.5 ± 90.8 ab	6324.4 ± 498.2 a	463.1 ± 38.5 a	25654.6 ± 1299.5 a
	ZnO NPs 800	49029.4 ± 1916.2 a	11610.4 ± 349.5 a	4388.3 ± 75.4 a	6649.1 ± 296.5 a	417.7 ± 28.1 a	24600.4 ± 714.9 a

Table 4.3 Elemental analysis of cucumber plants exposed to CeO₂ NPs

	Treatment / Element	Al	B	Cu	Fe	Mn	Zn
Roots	Control	1574.6 ± 313.5 a	37.4 ± 0.7 a	34.3 ± 6.3 a	1057.3 ± 223.7 a	127.2 ± 16.7 a	175.8 ± 15.9 a
	CeO₂ NPs 400	1563.2 ± 337.9 a	35.1 ± 0.8 a	39.9 ± 4.9 a	1082.2 ± 307.3 a	149.5 ± 18.5 a	191.7 ± 20.4 a
	CeO₂ NPs 800	1215.0 ± 358.4 a	34.5 ± 0.9 a	32.3 ± 3.9 a	877.7 ± 259.7 a	112.1 ± 14.9 a	156.6 ± 23.5 a
Stems	Control	71.5 ± 7.9 a	34.7 ± 0.9 a	6.2 ± 0.2 a	66.0 ± 7.4 a	37.3 ± 1.6 a	39.0 ± 2.5 a
	CeO₂ NPs 400	71.6 ± 3.1 a	35.4 ± 1.4 a	6.5 ± 0.4 ab	68.3 ± 3.9 a	42.5 ± 3.9 a	34.2 ± 1.7 a
	CeO₂ NPs 800	70.0 ± 8.9 a	34.4 ± 2.0 a	7.7 ± 0.3 b	66.1 ± 8.2 a	35.4 ± 5.6 a	36.3 ± 1.2 a
Leaves	Control	111.8 ± 9.8 a	83.7 ± 3.1 a	11.0 ± 0.4 a	144.7 ± 8.7 a	105.0 ± 4.7 a	48.9 ± 2.4 a
	CeO₂ NPs 400	93.7 ± 7.5 a	79.3 ± 2.1 a	11.5 ± 0.3 a	135.0 ± 7.1 a	104.0 ± 2.2 a	45.3 ± 1.3 a
	CeO₂ NPs 800	87.6 ± 3.4 a	75.5 ± 2.8 a	12.2 ± 0.2 a	131.7 ± 4.9 a	88.3 ± 5.7 a	45.6 ± 1.9 a
		Ca	Mg	P	S	Na	K
Root	Control	14579.2 ± 1410 a	3576.7 ± 274.8 a	8029.0 ± 823.2 a	3762.6 ± 65.5 a	3468.8 ± 383.9 a	33854.4 ± 1690.5 a
	CeO₂ NPs 400	13255.5 ± 643.1 a	3586.8 ± 254.7 a	6580.6 ± 264.8 a	3406.4 ± 63.2 a	3033.2 ± 347.5 a	31962.8 ± 1153.2 a
	CeO₂ NPs 800	12979.0 ± 844.9 a	3259.9 ± 136.3 a	6702.7 ± 384.5 a	3335.9 ± 198.0 a	3126.1 ± 327.1 a	31714.2 ± 1966.0 a
Stem	Control	22025.0 ± 723.6 a	6084.2 ± 80.0 a	7891.1 ± 526.6 a	3512.5 ± 73.4 a	1618.8 ± 29.1 a	74192.4 ± 2429.4 a
	CeO₂ NPs 400	24673.1 ± 2028.3 a	6429.0 ± 169.1 a	7410.8 ± 489.8 a	3268.1 ± 176.6 a	1430.3 ± 65.3 a	75754.7 ± 6144.3 a
	CeO₂ NPs 800	21771.7 ± 1715.2 a	6207.1 ± 363.1 a	7754.3 ± 127.0 a	3264.4 ± 298.8 a	1471.9 ± 111.7 a	66826.4 ± 1889.0 a
Leaf	Control	53757.3 ± 747.2 b	11924.1 ± 203.0 b	5386.3 ± 357.8 a	7101.5 ± 218.2 a	500.0 ± 41.3 a	26820.4 ± 1512.4 a
	CeO₂ NPs 400	50850.8 ± 1064.3 ab	11113.1 ± 87.5 ab	5119.4 ± 463.9 a	6517.7 ± 237.1 a	477.1 ± 26.9 a	26587.5 ± 1456.2 a
	CeO₂ NPs 800	46725.4 ± 1715.2 a	10598.6 ± 394.0 a	5327.7 ± 321.2 a	6672.1 ± 294.8 a	562.2 ± 112.2 a	29403.0 ± 1485.8 a

Effects of ZnO and CeO₂ NPs on corn nutrient composition

Different from cucumber, ZnO and CeO₂ NPs increased the concentrations of all elements altered by these NPs in corn plants (Tables 4.4 and 4.5). Table 4.4 shows that Cu and Mn increased in roots of plants exposed to ZnO NPs at 400 and 800 mg kg⁻¹, but not in stems or leaves. It has been previously reported that Cu is strongly adsorbed to cell walls (Sentenac and Grignon, 1981; Allan and Jarrell, 1989). Jarvis and Robson (1982) also found that “at low solution Cu concentrations, wheat, ryegrass, and red clover can retain Cu bound to root cell walls even when the shoots become Cu-deficient.” Manganese is absorbed by epidermal root cells through an active transport system. Takahashi and Sugiura (2001) suggested that the tomato root protein binds Mn helping it to be taken up by root cells. Within the plant, Mn is transported by Nramp proteins that also transport other divalent cations (Pittman, 2005). This suggest that ZnO NPs did not interfere with the uptake, but they did with transport within plants. The interference could be by sharing the transport Nramp protein with Zn²⁺. In agreement with the expectations, exposure to ZnO NPs at 400 or 800 mg kg⁻¹ increased Zn content in corn plant tissues, comparison with the control. All other elements (Al, B, Fe, Ca, Mg, P, S, Na and K remained statistically equivalent to the control and within the suggested concentrations for healthy field-grown corn (Reuter and Robinson, 1986).

Table 4.5 shows the essential elements of corn plants exposed to 400 or 800 mg kg⁻¹ CeO₂ NPs. As seen in Table 4.5, CeO₂ NPs did not reduce the mineral content of corn roots, stems and leaves. Moreover, at 400 mg kg⁻¹ increased B in stems, while at 800 mg kg⁻¹ increased Mn and S, also in stems. A previous report has shown that in barley (*Hordeum vulgare*) CeO₂ NPs at 500 mg kg⁻¹ reduced sulfur accumulation in leaves, but at 250 mg kg⁻¹ increased Mn and S in grains (Rico et al., 2015). Unfortunately, there are no mechanistic studies explaining the effects of CeO₂ NPs

on the uptake of nutrient elements. However, it is possible that CeO₂ NPs interfere with the transport systems of the affected elements.

Table 4.4 Elemental analysis of corn plants exposed to ZnO NPs

	Treatment / Element	Al	B	Cu	Fe	Mn	Zn
Roots	Control	792.5 ± 119.5 a	5.1 ± 0.7 a	8.6 ± 0.3 a	480.9 ± 68.6 a	41.4 ± 5.5 a	34.7 ± 4.5 a
	ZnO NPs 400	898.6 ± 99.5 a	4.2 ± 0.5 a	14.5 ± 1.4 b	566.0 ± 66.6 a	144.3 ± 27.1 b	1383.0 ± 159.4 b
	ZnO NPs 800	887.7 ± 68.6 a	4.0 ± 0.5 a	14.9 ± 1.4 b	583.2 ± 53.0 a	100.4 ± 17.3 ab	1280.5 ± 68.6 b
Stems	Control	16.4 ± 2.2 a	9.6 ± 0.7 a	0.7 ± 0.2 a	38.6 ± 12.6 a	23.3 ± 1.7 a	52.4 ± 5.6 a
	ZnO NPs 400	13.1 ± 2.4 a	10.9 ± 2.1 a	0.3 ± 0.1 a	22.3 ± 5.6 a	29.9 ± 4.8 a	300.5 ± 36.0 b
	ZnO NPs 800	11.5 ± 1.5 a	10.5 ± 0.9 a	0.3 ± 0.2 a	16.3 ± 1.7 a	24.8 ± 3.3 a	343.2 ± 57.2 b
Leaves	Control	59.5 ± 4.3 a	179.2 ± 3.9 a	3.6 ± 0.1 a	68.9 ± 0.9 a	215.6 ± 16.3 a	58.7 ± 33.1 a
	ZnO NPs 400	71.2 ± 7.8 a	163.1 ± 17.3 a	2.4 ± 0.6 a	87.0 ± 14.4 a	205.4 ± 22.4 a	113.0 ± 36.5 ab
	ZnO NPs 800	67.2 ± 6.4 a	184.6 ± 12.3 a	2.2 ± 0.9 a	71.0 ± 7.1 a	223.3 ± 31.4 a	191.1 ± 36.9 b
		Ca	Mg	P	S	Na	K
Roots	Control	5769.6 ± 619.2 a	2244.8 ± 212.3 a	2130.0 ± 129.6 a	3587.1 ± 586.7 a	2159.1 ± 429.6 a	21520.8 ± 813.3 a
	ZnO NPs 400	4237.0 ± 113.9 a	2555.4 ± 134.8 a	2664.6 ± 156.8 a	3132.2 ± 256.8 a	1598.3 ± 82.4 a	21506.9 ± 940.7 a
	ZnO NPs 800	4318.7 ± 423.9 a	2676.8 ± 180.8 a	2641.4 ± 180.1 a	3474.3 ± 246.7 a	1656.7 ± 215.2 a	21795.7 ± 228.1 a
Stems	Control	1663.9 ± 121.0 a	1687.1 ± 61.1 a	3198.0 ± 169.6 a	1012.1 ± 13.1 a	41.1 ± 8.3 a	24377.7 ± 583.8 a
	ZnO NPs 400	1578.1 ± 183.2 a	1905.9 ± 157.4 a	3472.6 ± 182.0 a	1092.9 ± 55.9 a	32.0 ± 6.4 a	24056.3 ± 913.8 a
	ZnO NPs 800	1403.2 ± 179.2 a	1765.4 ± 55.0 a	3517.6 ± 261.1 a	1142.9 ± 29.9 a	27.9 ± 3.9 a	25550.4 ± 1023.4 a
Leaves	Control	8034.5 ± 582.8 a	4977.3 ± 194.7 a	3207.8 ± 144.8 a	2227.4 ± 107.9 a	131.3 ± 33.7 a	27860.0 ± 1186.1 a
	ZnO NPs 400	7247.5 ± 421.7 a	4457.0 ± 238.9 a	3063.4 ± 452.1 a	2165.6 ± 122.9 a	86.5 ± 17.7 a	26324.6 ± 1554.7 a
	ZnO NPs 800	7240.3 ± 549.6 a	4570.6 ± 280.5 a	3572.9 ± 116.7 a	2277.2 ± 52.7 a	55.4 ± 12.7 a	26007.4 ± 800.6 a

Table 4.5 Elemental analysis of corn plants exposed to CeO₂ NPs.

	Treatment / Element	Al	B	Cu	Fe	Mn	Zn
Roots	Control	792.5 ± 119.5 a	5.1 ± 0.7 a	8.6 ± 0.3 a	480.9 ± 68.6 a	41.4 ± 5.5 a	34.7 ± 4.5 a
	CeO ₂ NPs 400	762.2 ± 83.0 a	4.7 ± 0.6 a	14.5 ± 5.1 a	443.8 ± 52.0 a	45.1 ± 6.6 a	50.0 ± 7.2 a
	CeO ₂ NPs 800	1223.2 ± 360.4 a	2.9 ± 0.8 a	10.5 ± 1.7 a	836.2 ± 294.4 a	52.9 ± 15.2 a	40.4 ± 5.7 a
Stems	Control	16.4 ± 2.2 a	9.6 ± 0.7 a	0.7 ± 0.2 a	38.6 ± 12.6 a	23.3 ± 1.7 a	52.4 ± 5.6 a
	CeO ₂ NPs 400	19.9 ± 3.4 a	13.0 ± 0.2 b	0.3 ± 0.2 a	21.7 ± 2.6 a	29.4 ± 1.2 ab	54.2 ± 3.8 a
	CeO ₂ NPs 800	14.9 ± 3.2 a	11.2 ± 1.2 ab	0.4 ± 0.1 a	21.8 ± 3.3 a	30.6 ± 2.1 b	71.8 ± 15.1 a
Leaves	Control	59.5 ± 4.3 a	179.2 ± 3.9 a	3.6 ± 0.1 a	68.9 ± 0.9 a	215.6 ± 16.3 a	58.7 ± 33.1 a
	CeO ₂ NPs 400	65.6 ± 2.0 a	161.6 ± 12.8 a	2.6 ± 0.7 a	73.7 ± 2.2 a	178.2 ± 8.8 a	17.4 ± 1.4 a
	CeO ₂ NPs 800	68.5 ± 9.1 a	166.3 ± 17.8 a	3.8 ± 1.1 a	95.2 ± 13.7 a	205.8 ± 13.3 a	26.0 ± 3.3 a
		Ca	Mg	P	S	Na	K
Roots	Control	5769.6 ± 619.2 a	2244.8 ± 212.3 a	2130.0 ± 129.6 a	3587.1 ± 586.7 a	2159.1 ± 429.6 a	21520.8 ± 813.3 a
	CeO ₂ NPs 400	4995.2 ± 566.5 a	2396.1 ± 158.3 a	2135.7 ± 78.7 a	3589.5 ± 354.2 a	1965.7 ± 253.2 a	18458.1 ± 494.4 a
	CeO ₂ NPs 800	5904.0 ± 1144.2 a	2477.1 ± 332.9 a	2140.4 ± 113.4 a	4067.6 ± 486.4 a	2352.0 ± 341.3 a	21001.2 ± 1388.4 a
Stems	Control	1663.9 ± 121.0 a	1687.1 ± 61.1 a	3198.0 ± 169.6 a	1012.1 ± 13.1 a	41.1 ± 8.3 a	24377.7 ± 583.8 a
	CeO ₂ NPs 400	1907.8 ± 219.7 a	1969.1 ± 90.7 a	3296.5 ± 267.2 a	1149.5 ± 13.0 ab	46.3 ± 19.5 a	26178.5 ± 2944.2 a
	CeO ₂ NPs 800	1632.6 ± 41.6 a	1968.1 ± 137.0 a	3364.2 ± 226.7 a	1255.0 ± 95.3 b	23.6 ± 4.7 a	25429.7 ± 2330.5 a
Leaves	Control	8034.5 ± 582.8 a	4977.3 ± 194.7 a	3207.8 ± 144.8 a	2227.4 ± 107.9 a	131.3 ± 33.7 a	27860.0 ± 1186.1 a
	CeO ₂ NPs 400	7751.9 ± 463.5 a	5050.6 ± 130.8 a	3499.9 ± 405.6 a	2285.9 ± 43.1 a	106.3 ± 27.9 a	25606.7 ± 1999.2 a
	CeO ₂ NPs 800	7229.7 ± 400.7 a	5087.8 ± 254.3 a	3897.2 ± 176.4 a	2505.1 ± 121.9 a	76.7 ± 17.5 a	25154.8 ± 651.2 a

Effects of ZnO and CeO₂ NPs on sugar and starch contents

Figure 4.1 shows the sugar content in corn and cucumber plants exposed to ZnO or CeO₂ NPs. The amount of sugar was not altered by any of the concentrations of the two NPs in comparison to the control.

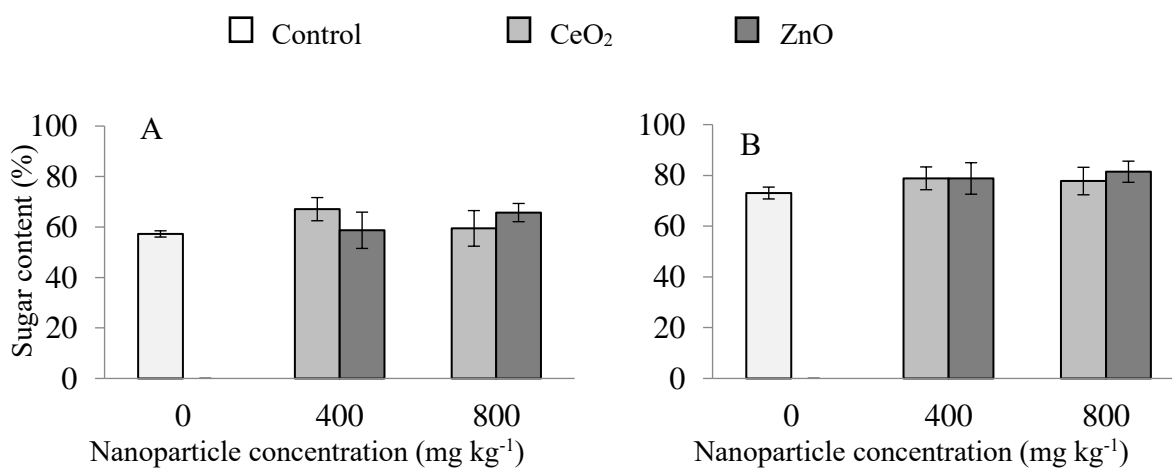


Figure 4.1 Sugar content percent in cucumber (A) and corn (B) plants exposed to different concentrations of ZnO and CeO₂.

On the other hand, starch content (Figure 4.2) increased in corn plants exposed to CeO₂ at 800 mg kg⁻¹. This result supports previous observations where CeO₂ NPs act as a fertilizer, promoting plant growth. Lopez-Moreno et al (2010) reported an increase in plant growth on cucumber and corn treated with CeO₂ NPs at 2000 mg kg⁻¹. Moreover, Wang et al. (2012) found that CeO₂ NPs at 10 mg L⁻¹ enhanced fruit production in tomato plants.

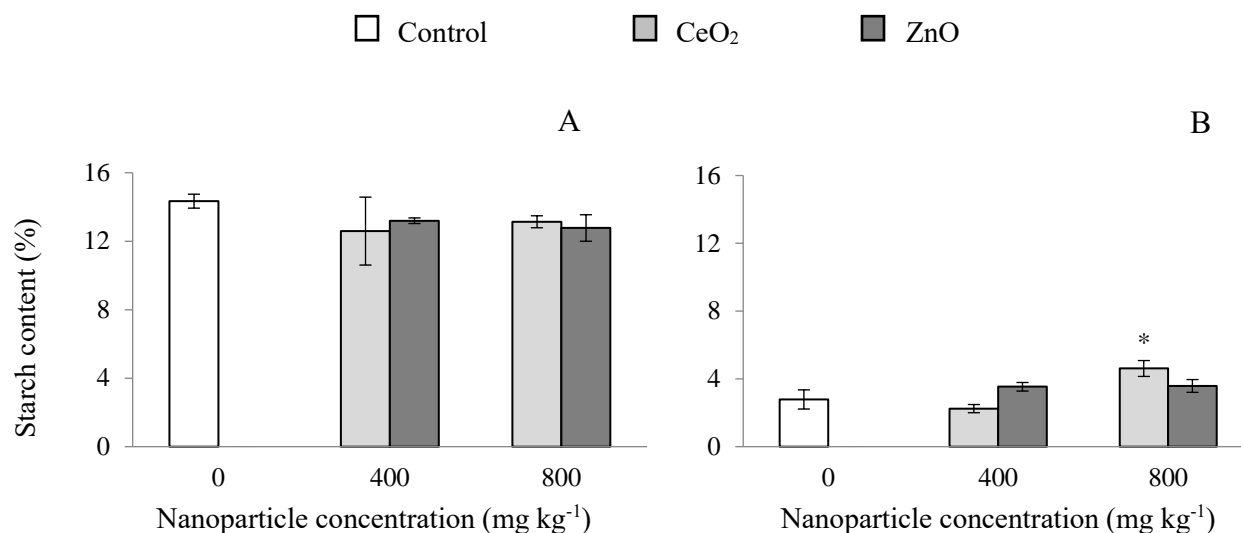


Figure 4.2 Starch content in corn (A) and cucumber (B) leaves exposed to different concentrations of CeO₂ and ZnO.

In summary, ZnO and CeO₂ NPs differentially affected nutritional components of cucumber and corn plants. ZnO NPs imbalanced Cu, Mn, Mg, P, Na and K at different concentrations in various tissues of cucumber. In corn, both concentrations of ZnO NPs increased the amounts of Cu and Mg only in the roots.

CeO₂ NPs increased minerals only in corn stems, and imbalanced Cu, Ca and Mg in cucumber only at the highest NP exposure. The amount of total sugars and starch remained equivalent to that of the controls of both species. The exception was an increase in the starch amounts in leaves of corn plants treated with CeO₂ NPs at 800 mg kg⁻¹. Overall, cucumber plants were less tolerant to ZnO NPs than corn.

REFERENCES

- Adams, P. 2002, Nutritional control in hydroponics, in Savvas, D., Passam, H.C. (eds): Hydroponic production of vegetables and ornamentals. Embryo Publ., Athens, Greece. Pp. 211-261.
- Allan, D.L.; Jarrell W.M. Proton and copper adsorption to maize and soybean root cell walls. *Plant Physiol.* 1989, 89, 823-832.
- 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. *Science of The Total Environment* (2015). <http://dx.doi.org/10.1016/j.scitotenv.2015.11.143>
- Brar SK, Verma M, Tyagi RD, Surampalli RY (2010) Engineered nanoparticles in wastewater and wastewater sludge—evidence and impacts. *Waste Manag* 30:504–520.
- Cassee FR, et al. (2011) Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Crit Rev Toxicol* 41:213–229.
- Dakora F.D.; Phillips D.A. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil.* 245, 35-47.
- Diatloff E, Smith FW, Asher CJ. 1995a. Rare-earth elements and plant growth. 1. Effects of lanthanum and cerium on root elongation of corn and mungbean. *Journal of Plant Nutrition* 18: 1963–1976.
- Du, W.C., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Zhu, J.G., Peralta-Videa, J.R., Guo, H.Y., 2015. Physiological and biochemical changes imposed by CeO₂ nanoparticles on wheat: a life cycle field study. *Environ. Sci. Technol.* 49, 11884–11893.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, 26, 350-356.
- Erdakos, G.B., Bhawe, P.V., Pouliot, G.A., Simon, H. and Mathur, R., 2014. Predicting the Effects of Nanoscale Cerium Additives in Diesel Fuel on Regional-Scale Air Quality. *Environmental science & technology*, 48(21), pp.12775-12782.
- HEI - Health Effects Institute, Research Report, 2001. Evaluation of Human Health Risk from Cerium added to Diesel Fuel. HEI Research Communication 9. North Andover Ma: Flagship Press. <http://pubs.healtheffects.org/view.php?id=172>
- Hepler, P.K., 2005. Calcium: a central regulator of plant growth and development. *The Plant Cell*, 17(8), pp.2142-2155.
- Jarvis SC, Robson AD (1982) Absorption and distribution of copper in plants with sufficient or deficient supplies. *Ann Bot* 50: 151-160
- Keller, A.A., McFerran, S., Lazareva, A., and Suh, S. (2013). Global life cycle releases of engineered nanomaterials. *Journal of Nanoparticle Research* 15, 1-17

- Keller, A.A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R. and Ji, Z., 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science & Technology*, 44(6), pp.1962-1967.
- López-Moreno, M.L., de la Rosa, G., Hernández-Viezcas, J.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2010. X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO₂ nanoparticles and assessment of their differential toxicity in four edible plant species. *Journal of agricultural and food chemistry*, 58(6), pp.3689-3693.
- Ma C.; Chhikara S.; Xing B.; Musante C.; White J.C.; Dhankher O.P. Physiological and Molecular Response of *Arabidopsis thaliana* (L.) to Nanoparticle Cerium and Indium Oxide Exposure. *ACS Sustainable Chem. Eng.* 2013,1, 768-778.
- Maguire ME, Cowan JA (2002) Magnesium chemistry and biochemistry. *Biometals* 15:203–210.
- Morales, M.I., Rico, C.M., Hernandez-Viezcas, J.A., Nunez, J.E., Barrios, A.C., Tafoya, A., Flores-Marges, J.P., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2013. Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *Journal of agricultural and food chemistry*, 61(26), pp.6224-6230.
- Peralta-Videa, J.R., Hernandez-Viezcas, J.A., Zhao, L., Diaz, B.C., Ge, Y., Priester, J.H., Holden, P.A. and Gardea-Torresdey, J.L., 2014. Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. *Plant Physiology and Biochemistry*, 80, pp.128-135.
- Project on Emerging Nanotechnologies (2013). Consumer Products Inventory. Retrieved on 03/28/2016, from <http://www.nanotechproject.org/cpi>
- Rico, C. M., Hong, J., Morales, M.I., Zhao, L., Barrios, A.C., Zhang, J., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2013. Effect of cerium oxide nanoparticles on rice: A study involving the antioxidant defense system and in vivo fluorescence imaging. *Environmental Science and Technology* 47 (11),5635-5642.
- Rico, C. M.; Lee, S. C.; Rubenecia, R.; Mukherjee, A.; Hong, J.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (*Triticum aestivum* L.) *J. Agric. Food Chem.* 2014, 62 (40) 9669– 9675, DOI: 10.1021/jf503526r
- Rico, C.M., Barrios, A.C., Tan, W., Rubenecia, R., Lee, S.C., Varela-Ramirez, A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2015. Physiological and biochemical response of soil-grown barley (*Hordeum vulgare* L.) to cerium oxide nanoparticles. *Environmental Science and Pollution Research*, 22(14), pp.10551-10558.
- Reuter DJ, Robinson JB. 1986. Plant analysis: an interpretation manual. Sydney: Inkata Press.
- Sentenac H, Grignon C (1981) A model for predicting ionic equilibrium concentrations in cell walls. *Plant Physiol* 68: 415-419
- Snow SJ, McGee J, Miller DB, Bass V, Schladweiler MC, Thomas RF, Krantz T, King C, Ledbetter AD, Richards J, Weinstein JP, Conner T, Willis R, Linak WP, Nash D, Wood CE, Elmore SA, Morrison JP, Johnson CL, Gilmour MI, Kodavanti UP (2014) Inhaled diesel emissions generated with cerium oxide nanoparticle fuel additive induce adverse pulmonary and systemic effects. *Toxicol Sci.* 142(2):403-17. doi: 10.1093/toxsci/kfu187

- Taiz, L., Zeiger, E., 1998. Plant Physiology, 2nd. Ed. Sinauer, Sunderland, Massachussets, pp.195.
- Tzerakis C.; Savvas D.; Sigrimis N. Responses of cucumber growth in recirculating nutrient solution to gradual Mn and Zn accumulation in the root zone owing to excessive supply via the irrigation water. J. Plant Nutr. Soil Sci. 2012, 175, 125-134.
- Verma, S.; Dubey, R. S. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biol. Plant. 2001, 44, 117-123.
- Wang, Q.; Ma, X.; Zhang, W.; Pei, H.; Chen, Y. The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) and its implications on food safety. Metallomics 2012, 4, 1105–1112.
- Zhao, L., Sun, Y., Hernandez-Viezcas, J.A., Hong, J., Majumdar, S., Niu, G., Duarte-Gardea, M., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2015. Monitoring the environmental effects of CeO₂ and ZnO nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ μ -XRF mapping of nutrients in kernels. Environmental Science & Technology, 49(5), pp.2921-2928.
- Zhao, L., Peralta-Videa, J.R., Rico, C.M., Hernandez-Viezcas, J.A., Sun, Y., Niu, G., Servin, A., Nunez, J.E., Duarte-Gardea, M. and Gardea-Torresdey, J.L., 2014. CeO₂ and ZnO nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). Journal of agricultural and food chemistry, 62(13), pp.2752-2759.
- Zhao, L., Sun, Y., Hernandez-Viezcas, J.A., Servin, A.D., Hong, J., Niu, G., Peralta-Videa, J.R., Duarte-Gardea, M. and Gardea-Torresdey, J.L., 2013. Influence of CeO₂ and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: a life cycle study. Journal of agricultural and food chemistry, 61(49), pp.11945-11951. DOI: 10.1021/jf404328e

General conclusions

The use of metal and metal oxide nanoparticles continually increase before having clear regulations based on environmentally relevant concentrations. This research evaluated the physiological and biochemical effects of metals and metal oxide nanomaterials in edible plants.


Contrary to the expectations, Cu-based nanomaterials did not affect cilantro plants more than conventional Cu compounds. The agronomic parameters in cilantro were mostly affected by Cu NPs (nCu) at 80 mg kg⁻¹ and both 20 and 80 mg kg⁻¹ bulk CuO (μCuO). Chlorophyll content was only affected by 20 mg kg⁻¹ of μCuO and the nutritional value was affected by all copper compounds. Table 5.1 summarized the effects caused by Cu compounds in cilantro shoots, the most popular consumed part of the plant. Overall all copper compounds altered in some way the physiology of cilantro plants especially in the accumulation of essential elements. Of particular importance is the reduction in P accumulation, which could represent a threat for human health.

Table 5.1. Effects of Cu compounds in soil grown cilantro plants

		CuPRO		Kocide		nCu		μCu		nCuO		μCuO		CuCl ₂	
		20	80	20	80	20	80	20	80	20	80	20	80	20	80
Shoot length							↓					↓	↓		
Chlorophyll			↑				↑					↓	↓		
Nutrients	Mg	↑		↓	↓			↓		↓		↓	↓		↓
	P	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	S			↑						↓		↑			↓
	Ca	↑	↑	↓	↓	↑		↓				↓	↓	↑	
	B						↑	↑		↑		↑			
	Mn		↑			↑								↑	↑
	Zn							↓				↓	↓		

In agreement to our predictions, Ag NPs affected the nutritional quality of radish sprouts and inhibit seedlings' development. Table 5.2 shows the main effects of Ag NPs on radish sprouts.

Table 5.2. Effects of Ag NPs in radish sprouts.



↓ Water content	↓ Water content	↓ Water content
No changes	↓ Root length	↓ Root length
↓ Shoot length	↓ Shoot length	↓ Shoot length
↓ B, Mn, Cu,	↓ Ca, Mg, B Mn, Cu	↓ Ca, Mg, B, Cu, Mn, Zn

The nanoparticles of ZnO and CeO₂ differentially affected corn and cucumber. Table 5.3 exhibits the major changes encountered in leaves of both species. While ZnO NPs reduced minerals at both concentrations, CeO₂ did not alter any elements, but increased starch in leaves of corn plants exposed to 800 mg kg⁻¹.

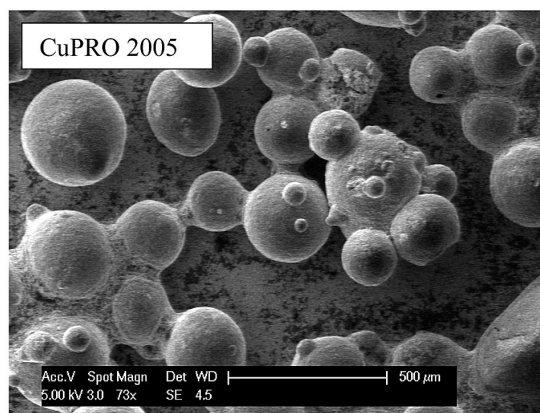
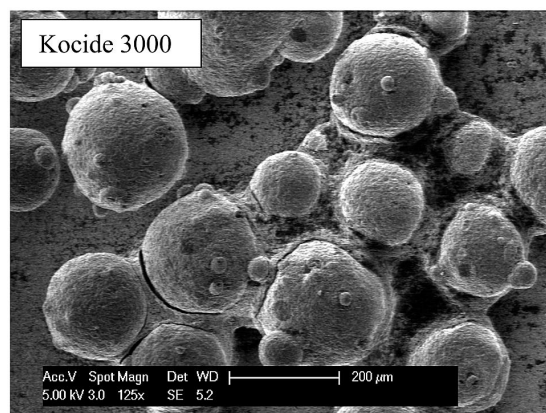
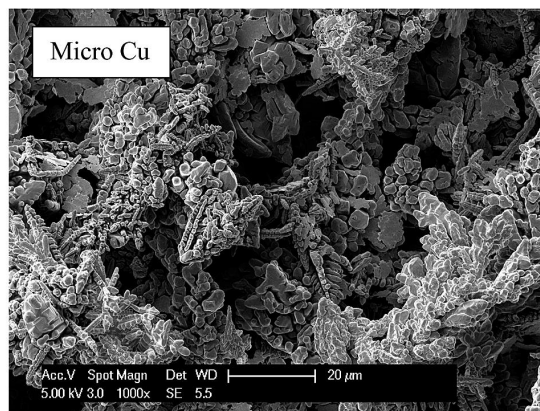
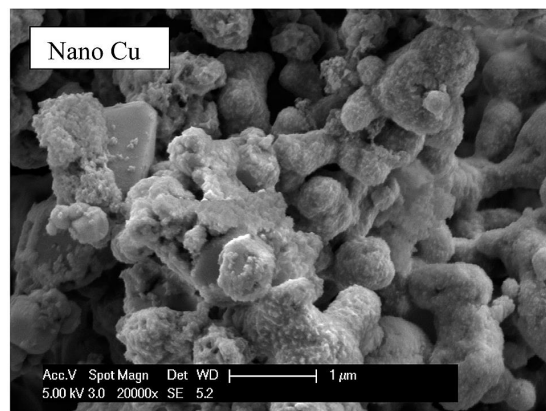
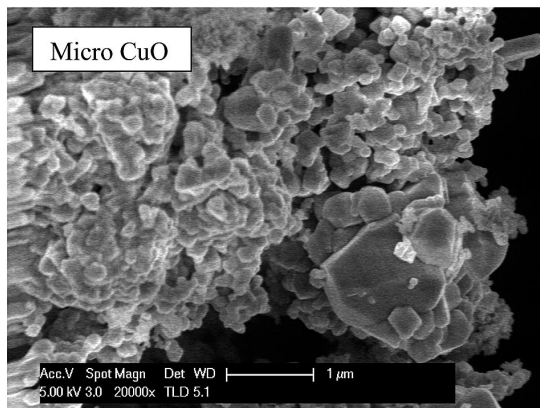
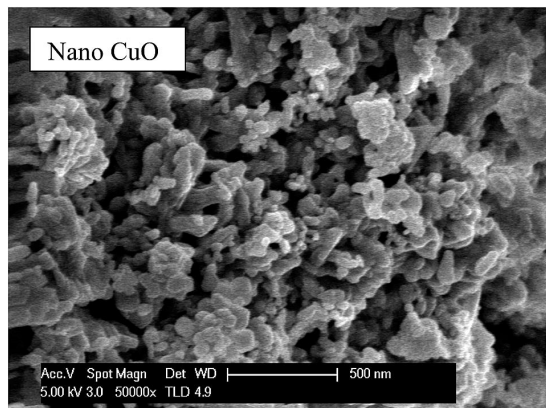
Table 5.3. Effects of ZnO and CeO₂ NPs in leaves of mature cucumber and corn plants.

		ZnO NPs		CeO ₂ NPs	
		400	800	400	800
Cucumber plant leaves	Mn	↓	↓		
	Ca		↓		
	Mg		↓		
Corn plant leaves	Starch				↑

Overall, the effects that NPs cause in plants is variable. In addition to the nanomaterial characteristics, the impacts are also dependent on the plant species and the environmental conditions. More studies are needed to determine the effects of aging and surface modifications on the interaction with plants.

Appendix

Electron micrographs (Hong et al., 2014) of copper compounds utilized in Chapter 2.



Vita

Nubia is originally from Ciudad Juarez, Chihuahua, Mexico. She earned her Bachelor's degree in Chemistry with a minor in Environmental Science in 2006 at The University of Texas at El Paso (UTEP). She conducted undergraduate research on the effects of arsenic in a desert willow plant under the supervision of Dr. Gardea-Torresdey. As a daily international commuter, she volunteered as a paramedic in the Red Cross in Ciudad Juarez between the years of 2002-2003. Moreover, in 2006 she completed an internship as a laboratory analyst in what was then Phelps Dodge. In 2007, Nubia became part of Dr. Ryan Wicker's Tissue Engineering team at the W.M. Keck Center for 3D innovation. In 2009, she pursued her Metallurgical and Materials Engineering Master's degree at UTEP with her thesis entitled: "Exploring poly(ethylene glycol) as a suitable material for peripheral nerve regeneration scaffolds manufactured by stereolithography". Nubia joined the medical industry as a Manufacturing Engineer at Cordis de Mexico (a Johnson and Johnson Co.) and went back to UTEP to pursue a doctoral degree in Materials Science and Engineering. In 2014 she began her research activities in Dr. Gardea's group.

Nubia is part of the University of California's Center for Environmental Implications of Nanotechnology (UC CEIN) and she has presented her broad work at local, national and international conferences in organizations such as the Society for Advancement of Chicanos and Native Americans in Science (SACNAS), the Society of Environmental Toxicology and Chemistry (SETAC), Solid Freeform Fabrication (SFF) and the Sustainable Nanotechnology Organization (SNO). She was recipient of the National Council on Science and Technology (CONACYT) scholarship and 2nd place winner of the "nano-pitch" at the 2015 SNO international conference in Italy.

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Dr. Gardea-Torresdey supervised Nubia's thesis: "Bioavailability of metal/metal oxide nanomaterials and their effects on the physiology of plants". Currently, Dr. Zuverza accepted a postdoctoral position at the University of California, Santa Barbara.

Publications:

- **Zuverza-Mena, N.;** Trujillo-Reyes, J.; Hong, J.; Zhao, L.; Barrios, A.C.; Sun, Y.; Niu G.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L.; Effects of ZnO and CeO₂ nanoparticles on sugar, starch, and absorption of essential elements in cucumber (*Cucumis sativus*) and corn (*Zea mays*) plants. To be submitted to *Plant Physiol. Biochem.*
- **Zuverza-Mena, N.;** Martínez-Fernández, D.; Du, W.C.; Hernandez-Viezcas, J.A.; Bonilla-Bird, N.; López-Moreno, M.L.; Komárek, M.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A Review. Submitted to *Plant Physiol. Biochem.* 2016.
- **Zuverza-Mena, N.;** Armendariz, R.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Effects of silver nanoparticles on radish sprouts: Root growth reduction and modifications in the nutritional value. *Front. Plant Sci.* 2016, 7.
- **Zuverza-Mena, N.;** Medina-Velo, I.A.; Barrios, A.C.; Tan, W.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). *Env. Sci. Process Impact.* 2015. 17, 1783-1793.

- Barrios, A. C.; Medina-Velo, I. A.; **Zuverza-Mena, N.**; Dominguez, O. E.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. 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. *Plant Physiol. Biochem.* 2016, in press.
- Castillo-Michel, H.A.; **Zuverza-Mena, N.**; Parsons, J.G.; Dokken, K.M.; Duarte-Gardea, M.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Accumulation, speciation, and coordination of arsenic in an inbred line and a wild type cultivar of the desert plant species *Chilopsis linearis* (Desert willow). *Phytochemistry*, 2009, 70, 540-545.
- Medina-Velo, I.A.; **Zuverza-Mena, N.**; Tan, W.; Hernandez-Viezcas, J.A.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Chapter 3. Biophysical Methods of Detection and Quantification of Uptake, Translocation and Accumulation of Nanoparticles. In: Plant Nanotechnology-Principles and Practices. Chittaranjan Kole, D. Sakthi Kumar and Mariya V. Khodakovskaya (EDS). Springer, Germany. 2016.

Email: nzuverza@yahoo.com

Permanent address: Privada del Charro 1306-12
Ciudad Juarez, Chihuahua, MX, 32457

This dissertation was typed by Nubia Zuverza Mena.