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Exploring The Effects Of Zinc Nanoparticle Concentration, Antioxidant, And Media On Cilantro (coriandrum Sativum), And Radish (raphanus Sativus) Plants Growth

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Dedication

To almighty God, although there are many smarter, capable people out there.

You gave me this wonderful life as well as opportunity. You have answered my every prayer.

Thank you for all blessings.

To my parents and siblings, every moment of my life is filled with your support and love.

Indebted to you all forever.

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by

VENKATA LAXMA REDDY PULLAGURALA

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Abstract

Engineered nanomaterials (ENMs) have proven to be one of the emerging chemicals of concern in the contemporary times. Soil acts as one of the major sinks of these ENMs. Reports have shown that ENMs have varied effects on soil biota. Particularly, their effects on plants are inconsistent. Amongst these ENMs, ZnO nanoparticles (nZnO) are the fourth largest raw materials in the nanotechnology industry. Globally, it is estimated that around 34,000 tons of n ZnO are utilized per year. The nZnO exposure on terrestrial plants yielded both beneficial as well as detrimental effects. Recently, there is an emerging evidence about the scope of nZnO as a nanofertilizer. The beneficial effect is attributed to sustained Zn^{2+} release property of the nZnO compounds. On the other hand, at elevated concentrations it has proven to exert oxidative stress upon plants. Thus affecting their ambient growth and development. The factors that contribute to these conflictory findings are yet to be known. It is also necessary to determine if application of antioxidants such as L-ascorbic acid would potentially alleviate oxidative stress exerted by nZnO exposure. Most studies reported in literature are been carried out in soil-grown plants. Considering the changes in recent agricultural practices, we lack knowledge about the ZnO exposure towards plants grown in soil-less media such as hydroponics and nutrient media studies. Thus, there are more questions than answers concerning the role of concentrations, media, and plant response in overall assessment of nZnO exposure.

Cilantro (*Coriander sativum*) and radish (*Raphanus Sativus*) are herb plants widely used since immemorial times in various cuisines across the globe. These plants are edible both cooked as well as in raw form (roots, leaves, and seeds). Besides nutritional components, the herbs possess anti-oxidant and metal chelating properties that help maintain a good health. In order to enhance

our knowledge about the impact of nZnO exposure on these plants, the research project was carried out in three parts.

In the first part, cilantro plants were cultivated for 35 days in soil amended with ZnO nanoparticles (nZnO), bulk ZnO (bZnO) and ionic $ZnCl_2$ (Zn^{2+}) at 0-400 mg/kg. This study was aimed to assess the metallomics, ^{1}NMR metabolic profiling and biochemical alterations upon the aforementioned exposure.

Part two was aimed to study the impact of soil amended nZnO and Zn $(NO_3)_2$ (Zn^{2+}) and foliar L-Ascorbic acid (Asc) exposure on cilantro at concentrations 500 mg/kg and 200 mg/L respectively. At the seed development stage (35 days), the plants were harvested. The biomass, pigment contents, stress enzymes, and metallomics were evaluated. In the third part, the radish seeds were exposed to nZnO and $ZnCl_2$ (Zn^{2+}) suspensions/solutions at concentrations 0-400 mg/kg and the sprouts allowed to growth for 8 days. At harvest, germination, biomass, metallomics and FTIR-based biomolecule conformational changes in plant tissues were evaluated.

Results from the first part, indicates n400 and b400 treatments increased chlorophyll content in cilantro at least by 50%, compared with control ($p \geq 0.05$). Additionally, nZnO at 400 mg/kg decreased the lipid peroxidation by 70%, compared with control. The highest Zn uptake in roots was observed with b400, while and shoots and Zn^{2+} 100 treatments, respectively. Finally, the ^{1}NMR data showed alterations in carbinolic regions (pertaining to lipids) of the plant metabolites. In part two, all the treatments nZnO, Asc + nZnO, Zn^{2+} and Asc+ Zn^{2+} did not affect the chlorophyll or lipid peroxidation compared with control. Asc+nZnO decreased carotenoid content by 47% in comparison to control ($p \leq 0.1$). Furthermore, the same treatment increased the dry biomass and catalase content by 300% compared with control. The highest Zn uptake

was observed for the Zn^{2+}treatment, while the lowest Zn uptake was obtained with Asc+ nZnO treatment. In part three, all treatments biomass accumulation of radish seedlings by 70 and 58% were observed for Zn^{2+} 200 and Zn^{2+} 400 treatment ($p \geq 0.05$). The nZnO and Zn^{2+} at 400 mg/L reduced seed germination by 50%, compared with control. Highest Zn uptake in radish seedlings was observed at Zn^{2+}100 and Zn^{2+} 400 treatments. Finally, FTIR spectra of all plant tissues have revealed functional group based conformational changes pertaining to lipids, carbohydrates and proteins. The Zn^{2+} at concentrations of 200 and 400 mg/kg had clearly caused band shifts in the spectra.

Above results suggest that ENMs such as nZnO at concentrations ($\leq$ 400 mg/kg) may be beneficial to soil grown cilantro as it can improve pigment content as well induce enhanced stress response.. Furthermore, the application of ascorbic acid in combination with nZnO can enhance the reduce the metal uptake and enhance the plant biomass to a good extent. However, aforementioned beneficial results may be plant and media specific. The beneficial results may not be replicated in soil-less medium such as hydroponics and media cultures. In the case of radish seedlings study, the nano and ionic treatments at 400 mg/L have led to deleterious effects on seed germination, biomass and caused band shifts in the regions pertaining to lipids, proteins and carbohydrates of FTIR spectra.

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Chapter 1: Introduction

Engineered nanomaterials (ENMs) are integral parts of a multitude of nanotechnology industries. These industries are projected to reach $75.8 billion by 2020 (Dyachenko 2017). Commercial production of ENMs can be achieved through the engineering and manipulation of structures with dimensions ranging from one to 100 nanometers (Kim et al., 2014). These ultrafine particles have special properties such as high surface area, quantum effects, and functionalization among others. These unique properties allow their applications in various fields such as agriculture, energy, medicine, and personal care products. The large-scale utilization of ENMs leads to higher disposal in environmental compartments including water and soil. However, our knowledge about the impact of these ENMs in living organisms, such as plants, is still far from being complete.

ZnO nanoparticles (nZnO) are the fourth largest employed engineered nanomaterial in the industry. It has unique properties such as low bandgap and piezoelectric effect. have wide applications in textile, energy, and personal care products (Pullagurala et al., 2018). According to Keller et al. (2013), by 2010, the average global utilization of nZnO was around 21,000 metric tons/year, 8-28% of which end in soil. Considering the high amount of nZnO disposal in the soil environment, there is a need of impact assessment through the entire life cycle of plants. The impact assessment comprises studies regarding fate, transport, as well as effects in living organisms. Various physiochemical properties of soil such as organic matter, clay, and salinity tend to affect the retention, dissolution, and transport behavior of nZnO in soil (Cruz et al., 2017). Recent findings suggest that the most bioavailable form of Zn in soil is Zn^{2+}. Upon the uptake, plants can have unique responses to Zn exposure (Table 1) (Pullagurala et al., 2018).

Table 1.1: Impact of nZnO exposure and its effect on terrestrial plants

S.NO	Plant	Media	Concentration	Size(nm)	Effect	Reference
1	Lettuce	Soil	10 mg/kg	90 ± 10	Increased biomass and photosynthesis	Xu et al., 2017
2	Cucumber	Soil	1000 mg kg-1		root tip deformation and decreased biomass	Moghadassi et al., 2017
3	Fenugreek	Soil	500 µg g-1		decrease root nodule formation and biomass	Siani et al., 2017
4	Soybean	Soil	0.5 g kg- 1	10	Leaf damage and genotoxicity	Priester et al., 2017
5	Tomato/Egg plant	Foliar	1.0 mg ml^{-1}	10–30	Reduced disease of fusarium	Elmer and white 2016
6	Spinach	Soil	1000 mg/L	<100	Reduction in root length and shoot length and transgenerational impact	Singh and Kumar 2016
7	Maize	Soil	800 mg/kg		Inhibited growth and AMF colonization	Wang et al., 2016
8	Bean	Soil	1000 mg/kg	<100	Root growth inhibition	Dimpka et al., 2015
9	Maize	Soil	800 mg/kg	90 ± 10	Oxidative stress	Liu et al., 2015
10	Alfalfa	Soil	750 mg/kg	10	reduced root and shoot biomass	Bandyopadhyay et al., 2015
11	Corn	Soil	800 mg/kg	10	Reduced photosynthesis, stomatal conductance and chlorophyll content	Zhao et al., 2014
12	Green peas	Soil	500 mg/kg	10	decreased chlorophyll and H_2O_2	Mukherjee et al., 2014a
13	Soybean	Soil	500 mg/kg	10	Alteration in nutritional value	Peralta-videa et al., 2014
14	Cucumber	Soil	800 mg/kg	10	Increased starch content	Zhao et al., 2014
15	Soybean	soil	500 mg/kg	<50	Lack of formation of seeds	Yoon et al., 2014
16	Buckwheat	Soil	2000 mg/kg	<50	Genotoxicity	Lee et al., 2013

Table note: Plant species common and biological name in the order of appearance in the table. Lettuce: *Lactuca sativa*, cucumber: *Cucumis sativus,* fenugreek:*Trigonella foenum-graecum,* kidney beans: *Phaseolus vulgaris,* tomato: *Solanum lycopersicum/ Lycopersicon esculentum,* Spinach*: Spinacia oleracea*, Maize: *Zea mays*, Alfalfa: *Medicago sativa,* Green pea*: Pisum sativum,* Buck wheat*: Polygonum convolvulus*

Data in Table 1 indicates that nZnO exposure has yielded both beneficial as well as detrimental effects on plants under different cultivation conditions. For instance, Xu et al. (2017) reported that 10 mg/kg of nZnO led to an increase in biomass of soil-grown lettuce (*Lactuca sativa*) plants. On the other hand, nZnO at concentration of 800 mg/kg induced oxidative stress in the corn (*Zea mays*) plants (Liu et al., 2017). It must be noted that Zn is an essential plant nutrient and it plays a key role in around 300 metalloenzymes of plants. On the other hand, the Zn uptake at higher concentrations can trigger abiotic stress through the reactive oxygen species generation (ROS) inside plant cells. The examples shown in Table 1 suggest that nZnO exposure effects in soil-grown plants could be concentration-dependent. For instance, nZnO at lower concentrations (0-400 mg/kg) are beneficial, while at higher concentrations (>500 mg/kg) they are detrimental. It is yet to be tested whether similar results will be achieved on exposure towards all plants, or whether they may be plant-specific. In the worst-case scenario, if nZnO (> 500 mg/kg) is detrimental towards plants, the tools to alleviate these detrimental effects need to be explored. It needs to be tested whether external application of antioxidants can neutralize ROS and alleviate the detrimental effects.

Most of the nZnO exposure studies towards plants have been carried out in soil-supported plants. There are few studies about plants grown in soil-less media such as hydroponics. Per the reports, the nZnO exposure at 1000 mg/L was able to decrease biomass of hydroponically grown brown mustard (*Brassica juncea)* (Zafar et al., 2016). Other researchers have reported about genotoxicity and germination inhibition by nZnO at higher concentrations in hydroponics grown plants (Lin and Xing 2008; Lopez-Moreno et al., 2010). However, Awasthi et al. (2017) has reported that nZnO at 50 mg/L improved seed germination and plant biomass of wheat (*Triticum aestivum*). Agriculture practices are changing a lot in modern times. There has been a strong

emergence of soil-less agricultural practices. Some of the plant seedlings, which are eaten as sprouts, are commercially grown in soil-less media. Thus, we have many knowledge gaps about nZnO exposure assessment in such cultivation conditions.

Cilantro (*Coriander sativum*) and radish (*Raphanus sativus*) are included in all well-known cuisines across the globe. Every part of these plants including leaves and seeds are consumed. Radish is one the few species consumed as sprouts. In addition, both plant species have unique antioxidant and metal chelating properties. The nZnO at concentrations below 400 mg/kg is predominantly beneficial towards various plants. To date, there are no studies about the impact of the nZnO exposure to cilantro plants. Furthermore, the application of antioxidants such as L-ascorbic acid is expected to neutralize the ROS and alleviate detrimental effects of nZnO at higher concentrations (500 mg/kg). To the best of the author's knowledge, there are no studies reported in the literature, which have tested the foliar application of anti-oxidants to alleviate nZnO exposure effects.

This study was performed in three parts. The aims of part 1 and part 2 were to test the hypothesis mentioned below. The effects of treatments were determined by evaluating the pigments, metabolic profiling, metallomics, and stress response. Part 3 was aimed to answer the question if nZnO at 0-400 mg/kg would be beneficial to hydroponically grown radish seedlings. To the best of the author's knowledge, nZnO exposure effects on radish seedlings has never been reported in the literature.

Specific objectives

The objectives of this research are:

[1] To determine the pigments, lipid peroxidation content and metabolite profiling in soil-grown cilantro plant exposed to nZnO, bZnO, and Zn^{2+} at concentrations ranging from 0 to 400 mg/kg soil.

[2] To investigate the role of L-ascorbic acid in minimizing the ROS stress induced by nZnO exposure (500 mg/kg) on soil-grown cilantro plants.

[3] To examine whether nZnO at lower concentrations (0-400 mg/L) would be beneficial towards radish sprouts in soil-less medium.

Working hypotheses

The working hypotheses of this research were:

[1] nZnO at concentrations less than < 400 mg/kg will induce beneficial effect on cilantro plants.

[2] The application of the L-ascorbic acid will alleviate the nZnO-exerted toxicity on cilantro plants.

[3] The nZnO exposure at < 400 mg/L in soil-less media will yield detrimental effects in radish seedlings

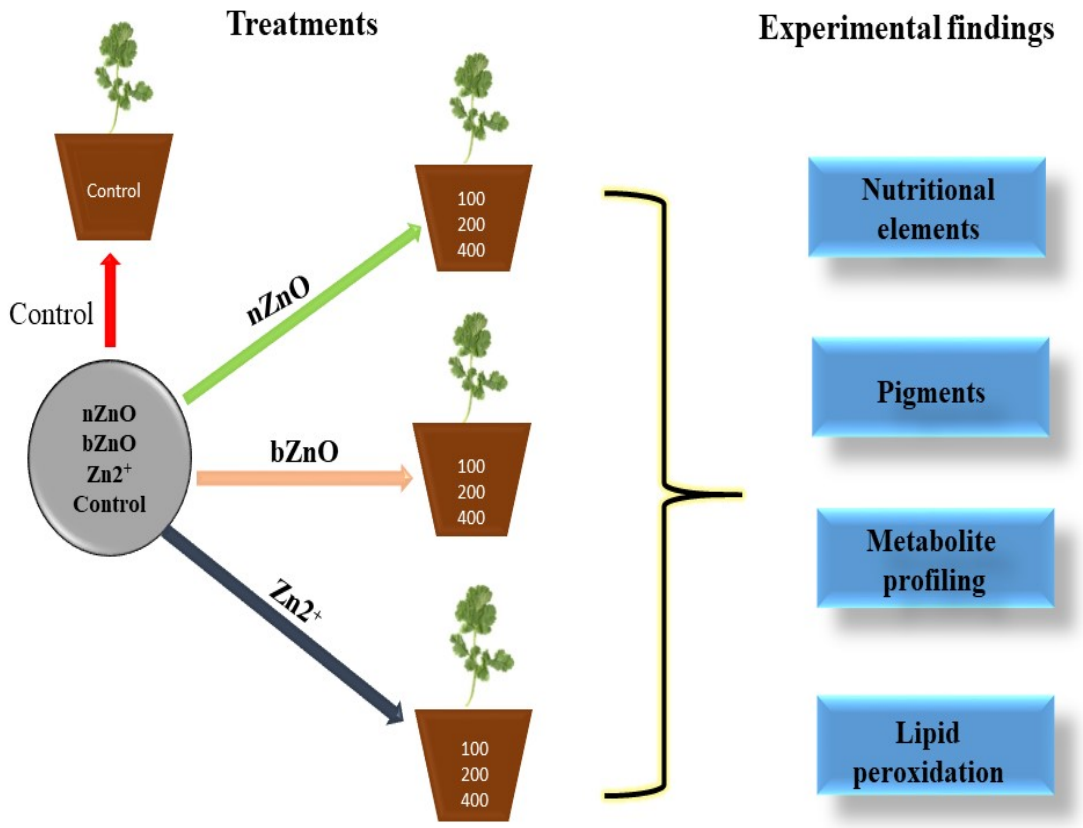

Figure 1.1 Schematic diagram for the nZnO, bZnO, Zn^{2+} exposure studies carried on cilantro at concentrations 0,100, 200 and 400 mg/kg

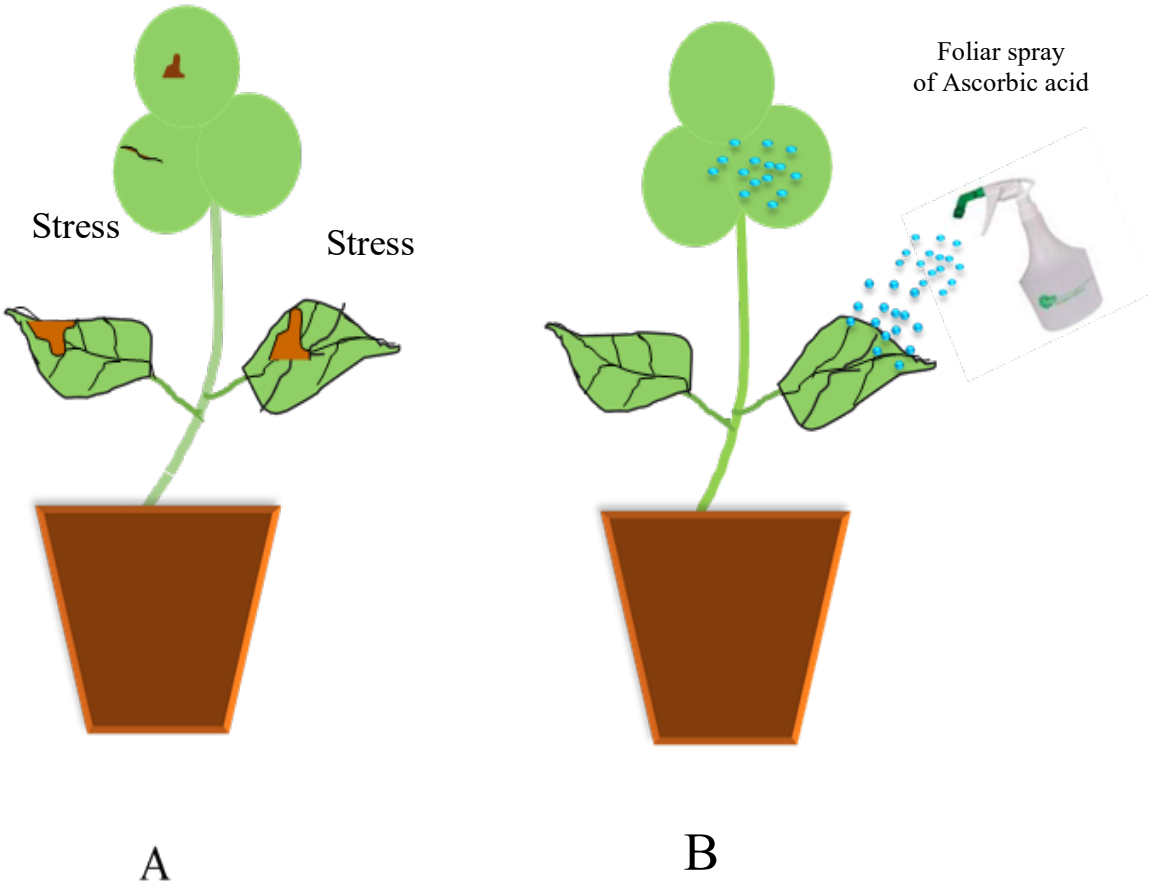

Figure 1.2 Schematic diagram for effects of foliar application of L-ascorbic acid on cilantro grown in nZnO amended soil

Foot Note: A] nZnO amended soil-grown plant with stress response; B] Asc+ nZnO amended soil-grown plant showing enhanced plant growth due to foliar spray of L Ascorbic acid.

Chapter 2: ZnO nanoparticles increase photosynthetic pigments and decrease lipid peroxidation in soil grown cilantro (*Coriandrum sativum*)

Abstract

The growth of the nanotechnology industry has raised concerns about their environmental impact. In particular, the effect on terrestrial plants which are the primary producers of the global food chain is widely debated. In this study, cilantro plants (*Coriandrum sativum)* were cultivated for 35 days in soil amended with ZnO nanoparticles (nZnO), bulk ZnO (bZnO) and ionic $ZnCl_2$ (Zn^{2+}) at 0-400 mg/kg. Photosynthetic pigments, lipid peroxidation, ^{1}NMR-based metabolic, and ICP-based metallomic profiles were evaluated. All Zn compounds increased the chlorophyll content by at least 50%, compared to control. Only nZnO at 400 mg/kg decreased lipid peroxidation by 70%.^{1}NMR data showed that all compounds significantly changed the carbinolic-based compounds, compared with control. Highest root and shoot uptake of Zn was observed at bZnO 400 and Zn^{2+} at 100 mg/kg respectively. Results of this study corroborates that nZnO at a concentration < 400 mg/kg improve photosynthetic pigments and the defense response in cilantro plants cultivated in organic soil.

Keywords: Nano ZnO, nanofertilizer, lipid peroxidation, nanoparticles, NMR metabolomics, plant uptake, omics

2.1. Introduction

Nanotechnology has taken root in all aspects of modern life. Materials synthesized at nanoscale wherein at least one of its dimensions are less than 100 nm are referred to engineered nanomaterials (ENMs). These engineered nanomaterials (ENMs) are the basic building blocks of nanotechnology. Upon utilization, ENMs are proven to be disposed into various environmental compartments such as soil, water, and air (Pullagurala *et al*., 2018). The impact of ENMs exposure on living organsims surviving in the aforementioned environmental compartments, specially terrestrial plants, has attracted attention in recent times (Reddy *et al*., 2016; Ruotolo *et al.,* 2018; Verma *et al.,* 2018; Pullagurala *et al.,* 2018). Studies dealing with the effects of TiO_2, ZnO, CeO_2, CNTs, and CuO-based ENMs on edible plants have shown different, sometimes contrasting, results (Montes et al., 2017; Rawat et al., 2018; Cota-Ruiz et al., 2018; Adisa *et al.,* 2018). As of 2010, the nZnO global production reached 30,000 metric tons/year (Keller and Lazareva 2013), which are used in coatings, cosmetics, energy and environmental applications (Ong *et al.,* 2018). Upon utilization, nZnO have been shown to find their way into soil, wastewater plants, and landfills (Smeraldi *et al.,* 2017).

Although the majority of findings have reported detrimental effects of nZnO in plants, there are few studies which have shown beneficial effects (Liu and lal 2015; Reddy *et al.,* 2018). For instance, Lopez-Moreno *et al.* (2017) showed that nZnO at 400 mg/kg affected seed germination and root length by 40 and 47% in maize (*Zea mays*), compared with control. Conversely, Awasthi *et al.* (2017) reported that nZnO at 50 mg/L was able to improve seed germination and plant biomass in wheat (*Triticum aestivum*). Thus, it can be inferred that the impact of the nZnO exposure on terrestrial plants has led to inconsistent findings. Therefore, it is imperative to find out the impact of nZnO on various aspects of plant growth and development. The chlorophyll pigment has an essential role in photosynthesis as it acts as the light capturing center.

Any alterations in this could affect the plant growth in a detrimental way. Furthermore, carotenoids are also an important plant pigments and they have a vital role in the defense of the plants against biotic as well as abiotic induced stress. They are considered to be the plant's first line of defense against harmful singlet oxygen ($^1O_2$) species which is an integral part of the reactive oxygen species (ROS) exerted by plant upon stress (Ramel *et al.,* 2012). According to Wang *et al.,* (2016), nZnO at 200 and 300 mg/L concentration reduced chlorophyll content by 50%; whereas, the carotenoid content was unaffected in *Arabidopsis* plants.

In addition, it has been reported that ENMs, such as nZnO, tend to induce oxidative stress in plants (Mukherjee *et al.,* 2016). MDA (Malondialdehyde) is a byproduct of the oxidation of the polyunsaturated lipids caused by ROS species (Gaschler and stock well 2017). Thus, the levels of the MDA concentrations are a direct parameter of lipid peroxidation, which is an eventual outcome of the ROS generation inside the cell. Foliar application of nZnO at 1.5 mg/L reduced MDA in chickpea (*Cicer arientinum*) (Burnman *et al.,* 2013). ^{1}H NMR Nuclear magnetic resonance spectroscopy is a versatile method used to analyse any molecule containing atoms with a non-zero magnetic moment. The spectra obtained is used to determine the presence of molecules in a sample. This nondestructive technique has been extensively used to identify and quantify metabolites in plant tissues. According to Zhao *et al.* (2017), the nCuO exposure at concentrations 400 and 800 mg/kg has led to variations in metabolite profile pertaining to sugars, amino acids and fatty acids. To the author's knowledge, there are no experimental studies reported in the literature, which have tested the impact of nZnO on plant metabolite profiles. Furthermore, the method employed in this experimental study is easier to operate and very reliable.

In this work, the nZnO exposure, at concentrations 0, 100, 200 and 400 mg/kg, was tested to determine the impact on the chlorophyll, carotenoids, and MDA content. Furthermore, 1NMR metabolomics were performed to check the variations in metabolic profiles. In the process the ^{1}NMR obtained peaks have been assigned and compared with the published data (Guadagno *et al.,* 2013). Any visible changes in the NMR spectra can be attributed to the possible metabolomics changes of the ZnO based exposure. The downfield region along with the signal obtained at 7.26 ppm (corresponding to solvent used in extraction ($CDCl_3$)) were excluded from the analysis. The major focus of the analysis was concentrated on the chemical shifts ranging from 0.5 ppm to 7.0 ppm.

2.2 Materials and methods

2 2.1 Zinc based materials and cilantro seeds

The nanoZnO (nZnO), bulk ZnO (bZnO) and ionic $ZnCl_2$ (Zn^{2+}) were obtained from University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). The nZnO were 24 $\pm$ 3 nm in size (meliorum Technologies, Rochester). TEM images of the aggregated n ZnO indicated average size of 322 ± 187 nm included in supplementary material (Keller et al., 2010). All the charecterisations of the nZnO was included in supplementary material. The bZnO (ACS reagent ≥99.0% purity) and I $ZnCl_2$ (ACS reagent 97 % purity) were purchased from Sigma-Aldrich and Acro organics respectively. The cilantro plant seeds were obtained from Del Norte Seed and Feed (Vinton, TX, USA).

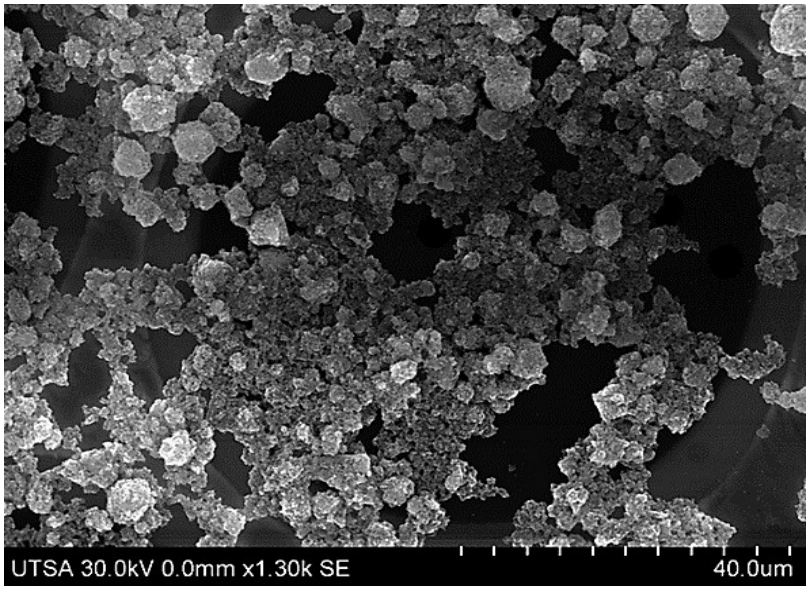

Figure 2.1 The TEM image characterization of aggregated nZnO (Bandhyopadhyay et al., 2015).

Table 2.1. Physicochemical Characteristics of the nZnO

S NO	Properties	Technique	Units	Characterization results
1	Primary size	TEM	nm	24 ± 3
2	Phase and structure	DLS	nm	100% zincite hexagonal
3	Shape/ morphology	TEM		Spheroid
4	Surface area	BET	$m^2\ g^{-1}$	42.1
5	Purity	TGA	wt %	97.27%

Abbreviations: Transmission and scanning electron microscopy (TEM), dynamic light scattering (DLS), X-ray powder diffraction (XRD), Brunauer-Emmett-Teller analysis (BET) and thermogravimetric analysis (TGA) (Adapted from keller et al., 2010)

2.2.2 Plant growth conditions and harvest

Suspensions from the three Zn-based compounds were prepared at 100, 200 and 400 mg/kg. All of the aforementioned suspensions/solution were sonicated at 25ºC for 30 min at 120 volts/3 amps, 50 to 60 Ghz to ensure uniform dispersion.

The resultant suspensions/solutions were added and manually mixed with soil to obtain final concentrations of 100, 200 and 400 mg/kg of the Zn/kg soil. The soil used in this study was commercially available organic potting mix. A brief description as well as characterization of the potting soil is shown in supplementary data which was adapted from previous published literature (Barrios *et al.,* 2016)

General-purpose plastic pots (4 × 2.5 inch) containg 350 grams of soil were amended with the Zn-based compounds and placed in dark conditions for 24 h. The seeds were hydrated and washed using 2% sodium hypochlorite and deionized water 18 MOhms (DI). The experiment was carried out in triplicates for every treatment along with the control (bare soil with no Zn-based compounds). Thirty seeds of cilantro were placed in each pot and incubated in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) at 25/20º C day/night, 14/10 h photoperiod and 60 % relative humidity. Plants were watered with 40 mL DI water for 34 days until the seed development stage. On the 34^{th} day (harvest), roots and shoots were separated, rinsed with tap water to remove excess soil, soaked in 0.01 M HNO_3 for about 15 seconds, rinsed twice with DI water, and prepared for enzymatic studies.

2.2.3 Chlorophyll in leaves of cilantro

At harvest, the cilantro leaves were tested for relative chlorophyll content. The measurements were performed in the center of the three plant leaves of the three replicates/treatment by using a single-photon avalanche diode (SPAD) chlorophyll meter (Minolta, Japan SPAD, Spectrum Technologies).

2.2.4 Carotenoid content

Carotenoid content was determined as per Shaw and Hossain (2013). According to this method, 50 mg of leaf tissue was homogenized in 5 mL chilled methanol (100%). The homogenate was centrifuged using eppendorf centrifuge 5417R at 4000 rpm/min for 15 min; the absorbance supernatants was recorded at 470 nm. The attained value was expressed as mg carotenoids g^{-1} freshweight.

2.2.5 Malonidialdehyde (MDA) content

The MDA content in shoots was determined according to Wang *et al.* (2017). The plant tissues were homogenized in 5 mL of 10 % trichloroacetic acid (TCA) and subjected to centrifugation using Eppendorf 5417R Hamburg, Germany at 12,000 rpm for 10 min. Two mL of clear supernatant was collected, added to 4 mL of 0.6 % thiobarbituric acid (TBA, in 10 % TCA), and incubated at 100 °C in a water bath for 15 min. Upon cooling at room temperature, the absorbance of the supernatant (containing MDA) was measured at 450, 532 and 600 nm using a UV–vis spectrophotometer (Cary 50 Agilent technologies, California, USA). The overall MDA content was calculated with the following formula: 6.45(OD532–OD600) − 0.56(OD450). The MDA content was expressed as μmol g^{-1} FW.

2.2.6 ^{1}NMR sample preparation and measurements

The ^{1}NMR sample extraction was carried out after Guadagno *et al.* (2013). The leaves were, frozen in liquid nitrogen and powdered in a ceramic mortar with a pestle. Ground samples (0.4 ± 0.15 g of dry weight) were homogenized with 4 ml of acetone (100%) in order to obtain a lipidic fraction extract. All extraction procedures were rapidly executed at a low temperature (0 to 4°C) in dim light using only glassware. No attempt at purifying single components was made throughout the experiments. Immediately before every measurement, the extract was suspended into the NMR tubes with 0.7 ml of $CDCl_3$ (0.1 % TMS). NMR spectra of the vegetal extracts

were recorded at 297 K on a Bruker spectrometer (Massachusetts, USA) operating at the ^{1}H frequency of 400.1 MHz. 1 H spectra of the extracts were obtained using the following parameters: 64 K data points, recycle delay of 2.0 s. The bruker 3.2 top spin software was utilised for NMR spectra data analysis. Lastly, the molecules of interest were identified by comparison with previously published data. Previous plant metabolic studies have shown that the regions can be attributed to specific metabolites. For instance, the regions between 7.2.6 ppm is assigned to the solvent $CDCl_3$ whereas the region from 7.0 to 5.4 olefenic due to the presence of signals from a long chain conjugation of double bonds of pigments such as carotenoids. The signals at region 5.26 is assigned for the double bond protons originated from fatty acids. The region from 5.0 to 3.0 is called carbinolic region. In this region, all proton resonances for the glycerolipids sterols are pheophytins are usually present. Lastly, the upfield region is attributed to the presence of aliphatic proton signals.

2.2.7 Elemental quantification via inductively coupled plasma optical emission spectrometry (ICP-OES)

Upon harvest, the plant tissues were dried at 65^{o}C in an oven for at least 72 h before digestion and elemental analysis. Samples of oven dried roots and shoots were grinded until powdered. Samples of approximately 0.2 g of tissue were digested by adding 4 mL of trace pure HNO_3 in a mid-temperature graphite digestion block (Digi PREP MS, SCP Science, NY) for 45 min at 115 °C. The obtained digested samples were diluted to 50 mL with DI water. Samples were then analyzed for Zn content along with metal based micro and macro nutrients (Mg, Ca, Mn, Cu, Fe) by using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). The ICP-OES parameters were as follows: nebulizer flow, 0.50 L/min; power, 1150 W; peristaltic pump rate, 45 rpm; and flush time, 45 s. For QC of the ICP-OES readings, a multielemental standard solution of 1 mg/L was employed. In addition, the National Institute of

Standards and Technology (NIST) standard reference material 1570a (spinach leaves) was used to validate the digestion and analytical method; a Zn analyte recovery of 94% was achieved.

2.2.8 Statistical analysis

Three replicates of each treatment were allocated in a completely random design in an environmental growth chamber facility. Experimental data was analysed using SPSS22 software. One-way ANOVA was utilized to evaluate the experimental variance; whereas, differences between treatments were scrutinized with the multi comparison Tukey's HSD test at a $p < 0.05$. Data represented here has a mean $\pm$ std errors of all the three replicates.

2.3. Results and discussion

2.3.1 Chlorophyll and carotenoid determination

The chlorophyll data is shown in Figure 2.2. As seen in this figure, all compounds, at all concentrations significantly increased the chlorophyll content, however only nZnO and bZnO at 400 mg/kg had statistical significance compared with control ($p \leq 0.05$). The nZnO at 100, 200 and 400 mg/kg increased the relative chlorophyll by 41%, 37% and 58%. This is in accordance with the findings reported in the literature. According to the experimental studies carried out by Gurmani *et al.* (2012), the soil application of Zn has consistently improved the chlorophyll content in the tomato plants. Similar results were found in hydroponic cultures. According to Samreen *et al.* (2013), the exposure of the Zn has improved the chlorophyll, protein, and mineral contents of the hydroponic grown mung bean plants. Studies have shown that even the priming of the seeds with nZnO has proven to increase the chlorophyll and other photosynthetic pigments levels in plants (Latef *et al.,* 2017). Zn plays an essential part of plant metabolism by influencing the activities of key important enzymes such as carbonic anhydrase. The enzymes carbonic anhydrases are a zinc metalloenzymes and plays key role in the photosynthesis through the

process of the facilitating the carbondioxide utilization in all plants. However, there are some alternative findings in the literature, which have reported that the nZnO, in fact, decreased the chlorophyll content in higher plants such as soybean and kidney bean plants (Preiester *et al.,* 2017; Medina-Velo *et al.,* 2017). This ambiguity may be due to the fact that the higher plants may have different responses to the Zn exposure than smaller plants. More studies are needed in this area to understand why different plants behave in different ways in regard to the exposure of the Zn based compounds.

Fig 2.3 shows the carotenoids content in the leaves. As shown in this figure, the carotenoid content was decreased for most treatments compared to control. However only nZnO at 100 mg/kg had an increase, which was also statistical significance in comparison to control ($p \leq 0.05$). Similar behavior upon nZnO exposure has been reported in the literature. In a study carried out by Mohsenzadeh and Moosavin (2017), the exposure of nZnO on *Rosmanrinus officinalis* plant increased carotenoids content at nZnO 100 mg/kg. This enhanced carotenoid content could be possibly attributed to the plants response to inhibit oxidative damage, which could be caused by oxygen ($^1O_2$) species on the photosynthesis aspect of the plant. Likewise, the carotenoid content was also reported to be increased upon exposure of nZnO towards the plants such as green pea, peanut, and cluster bean (Prasad *et al.,* 2012) (Mukherjee *et al.,* 2016). More studies are needed to determine if the impact of the nZnO on the carotenoid levels of higher plants is different. Lastly, the carotenoid content was consistently increased in the case of the Zn^{2+} exposure. However, the increase was not statistically significant.

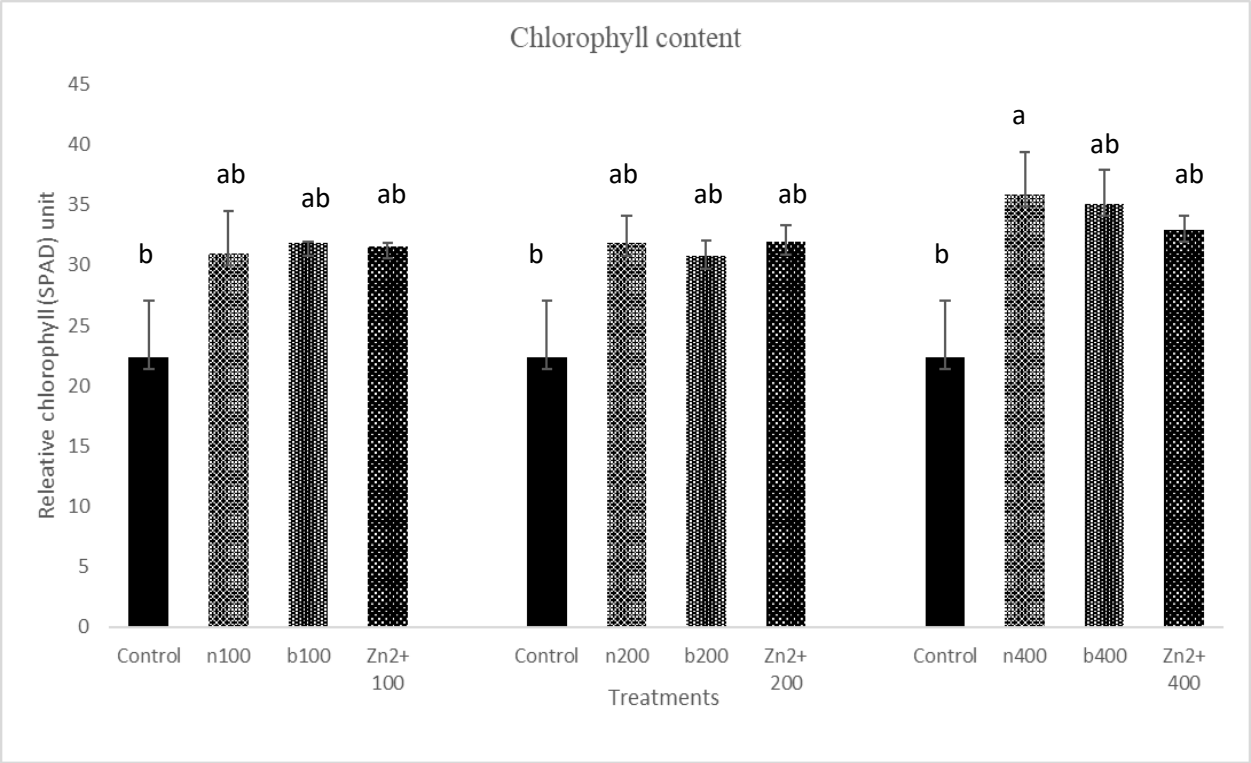

Figure 2.2 Chlorophyll content in cilantro shoots grown for 35 days in soil amended with nZnO, b ZnO, Zn^{2+} at 0, 100, 200, 400 mg·kg-1 soil. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

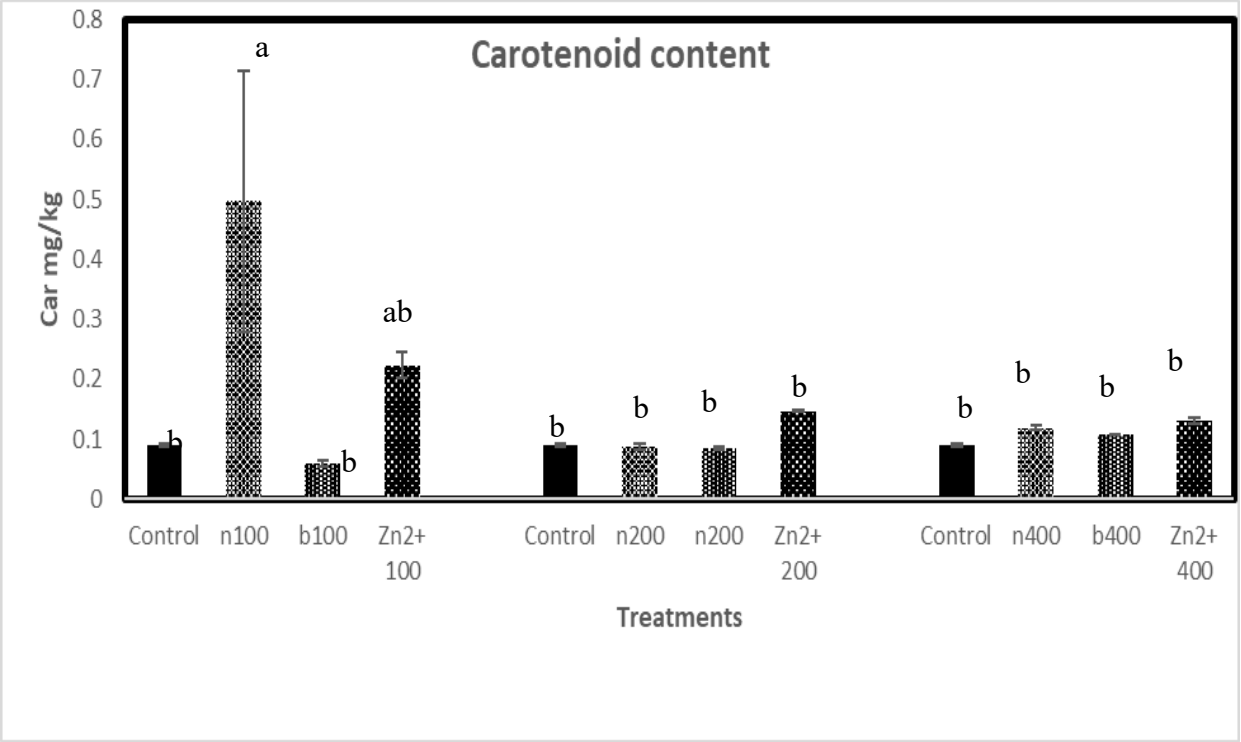

Figure 2.3. Carotenoid content in shoots of cilantro grown for 35 days in soil amended with nZnO, b ZnO, Zn+ at 0, 100, 200, 400 mg·kg-1 soil. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$)

2.3.2 Malondialdehyde (MDA) content

As reported in previous results (according to fig 2.4), the MDA content in the leaves of the cilantro has exhibited significant results in most of the treatments in comparison to the control ($p \leq 0.05$). This indicates that all the treatments have induced stress on the plants and it is believed to trigger the lipid peroxidation in the cilantro shoot. The nZnO exposure at 400 mg/kg witnessed the dramatic decrease in MDA levels by 72%. It is interesting to study in detail if the higher exposure has indeed triggered the anti-stress enzyme production, which could potentially decrease the lipid peroxidation of the cilantro leaves. Previous literature studies have reported similar findings; wherein, the foliar application of the Zn at concentrations as low as 10 mg/kg

has indeed decreased the MDA levels in chickpea seedlings (Burman *et al.,* 2013). In addition, this kind of behavior was also reported in higher plants such as cotton, where the nZnO has induced enhanced anti-stress enzymes such as superoxide dismutase (SOD), peroxidases (POX), which are expected to balance the stress exerted by the plant and consequentially resulting in reduced lipid peroxidation (Venkatachalam *et al.,* 2017). This reduced lipid peroxidation aspect of the plant behavior towards the application of the nZnO supports the proposed use of this compound as a nanofertilizer in the future (Singh *et al.,* 2013). On the other end, the highest levels of the MDA concentrations were witnessed upon the exposure of the bZnO at all concentrations. It is not certain why the bZnO has completely contrasting role in terms of triggering lipid peroxidation in a plant cell. Dissolution of the Zn^{2+} from both bZnO and nZnO might be the deciding factor here. Similar results were observed when bZnO and nZnO were exposed to liver tissues of Zebra fish at a concentration of 50 mg/L. According to the findings, the bZnO exposure increased MDA levels compared to nZnO (Xiong *et al.,* 2011). The increase was very high fold; it was around 142, 200 and 173% in comparison to control ($p \leq 0.05$). More studies are needed to confirm if the nZnO and bZnO have similar tendencies on all circumstances. Lastly, the ionic Zn^{2+} which was expected to be more active due to its Zn^{2+} ion forming capacity had an increase of the 44, 53 and 38% of the increase at three concentrations which are in descending order. Although Zn^{2+} exposed plants had higher MDA levels, in comparison to control and nZnO, it was not as elevated as in the case of the bZnO exposure. It can be concluded that bZnO has the highest lipid peroxidation potential than the other two Zn-based compounds. This enhanced levels of the MDA production in a living cell by the bZnO in comparison to the nZnO was also reported in the animal studies. According to the study carried out by the Xiaong *et al.,* (2011) the exposure of 5mg/L exposure of the bZnO towards zebra fish

triggered higher MDA levels, in comparison to the nZnO. The beneficial aspect of the nZnO could probably be attributed to its size and enhanced capacity to induce the anti-stress enzyme production such as catalase and ascorbate peroxidase.

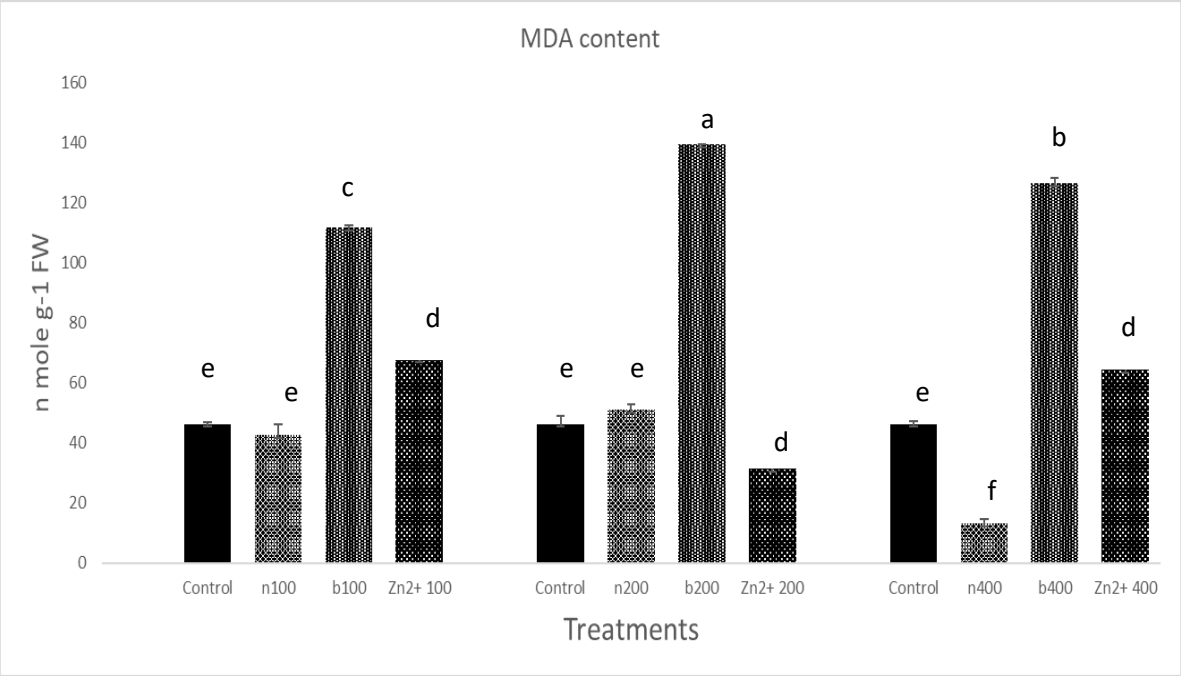

Figure 2.4 The MDA content in the shoots of cilantro grown for 35 days in soil amended with nZnO, b ZnO, Zn^{2+} at 0, 100, 200, 400 mg·kg-1 soil. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$)

2.3.3 Analysis of the ^{1}H NMR spectra and impact of the ZnO-based exposure on the metabolic fingerprinting of cilantro

The ^{1}H NMR peaks regarding the metabolites of interest are identified based on the previously published literature (Fig 2.5). Per our findings, changes have been observed in all nano, bulk, and ionic treatments in the regions of the carbinolic region (Fig 2.6, 2.7, 2.8). Thus, we can clearly

identify metabolic changes in the biomolecules corresponding to the glycerolipids, sterols, and pheophytins. Tan *et al*. (2018) has reported similar results in the fatty acid and lipid content upon the GC-MS based analysis of foliar-based exposure of copper-based compounds on *Ocimum basilicum*.

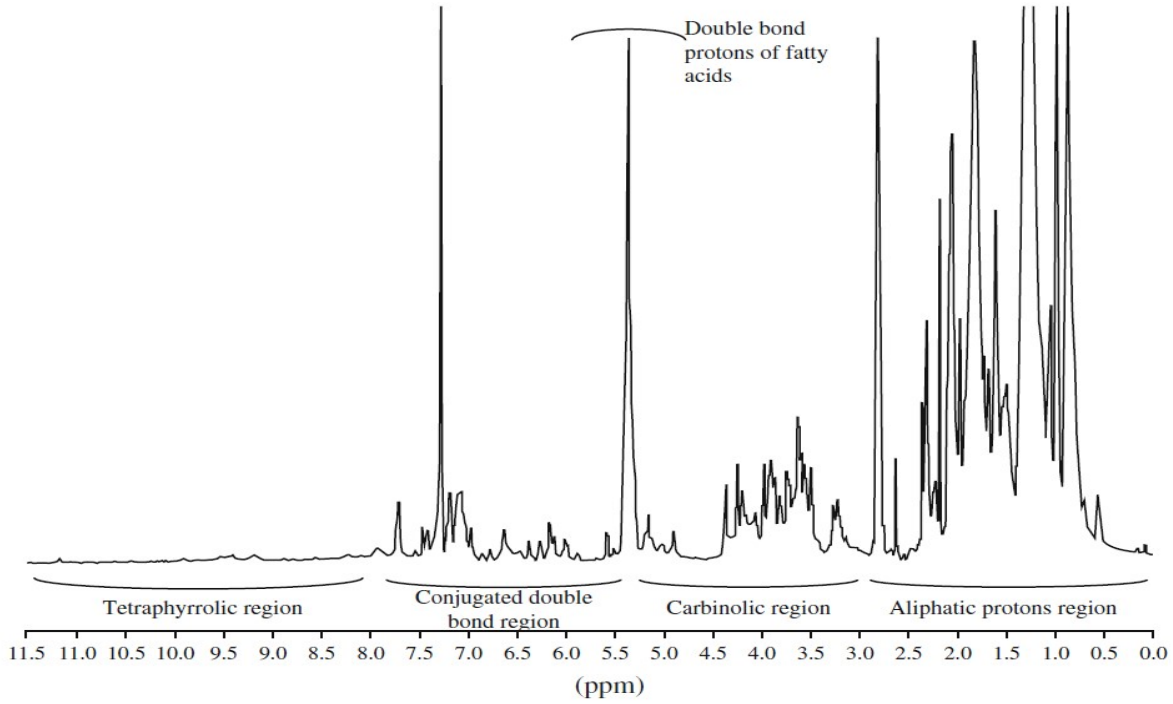

Figure 2.5 The chemical shifts of the 1NMR spectrum and corresponding regions of plant metabolites (Adapted from Guadagno et al., 2013)

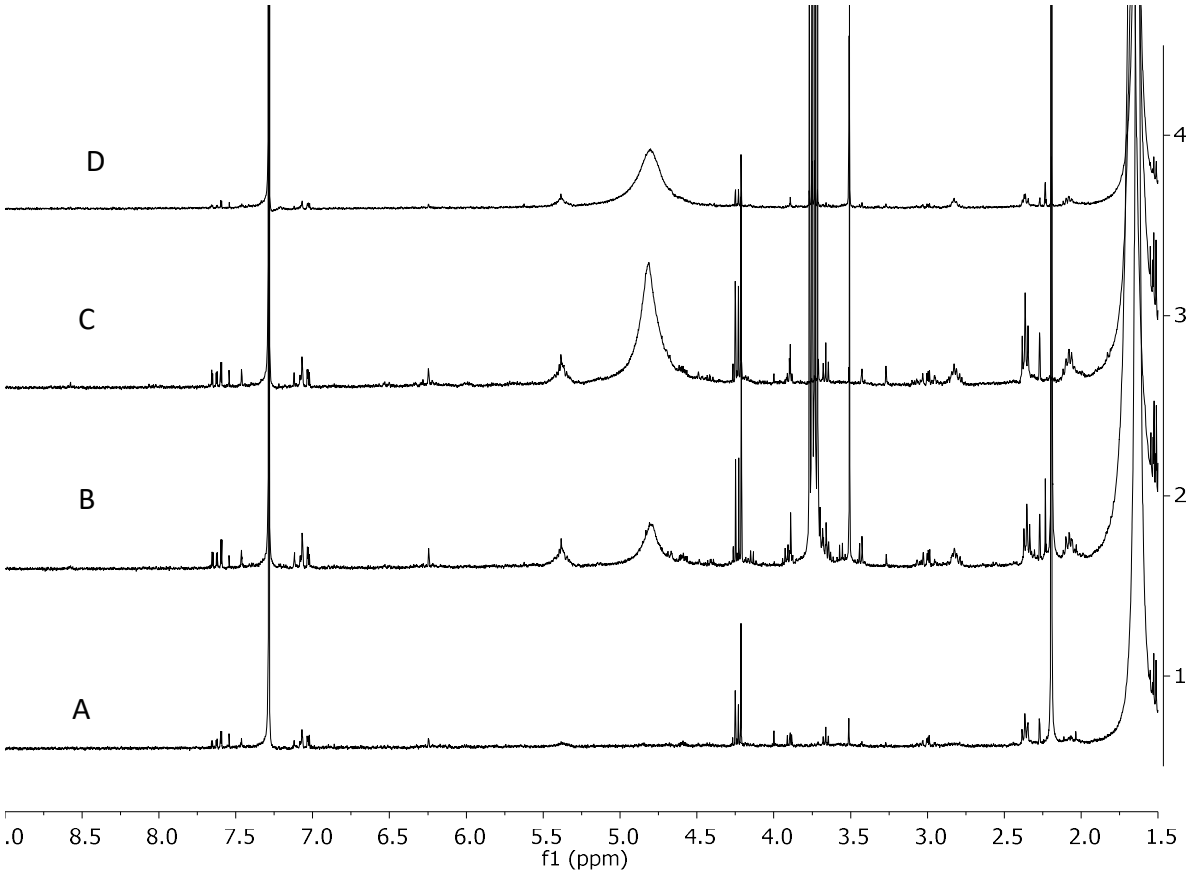

Figure 2.6. 1 NMR spectra of the comparision of nZnO based treatments at concentrations 100,200, and 400 ppm in comparison to control. A- Control, B-n100, C-n200, D-n400 respectively.

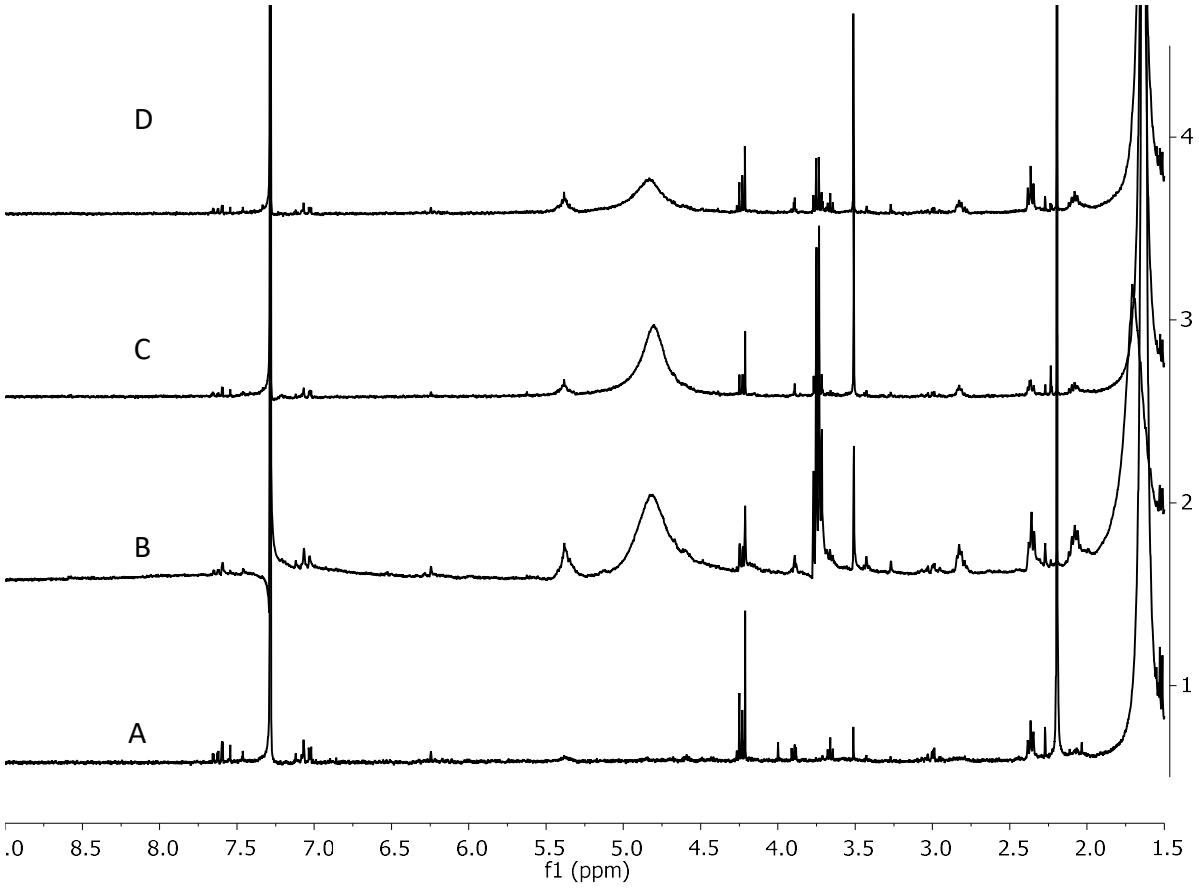

Figure 2.7. 1 NMR spectra of the comparision of bZnO based treatments at concentrations 100,200, and 400 ppm in comparision to control. A- Control, B-b100, C-b200, D-b400 repectively.

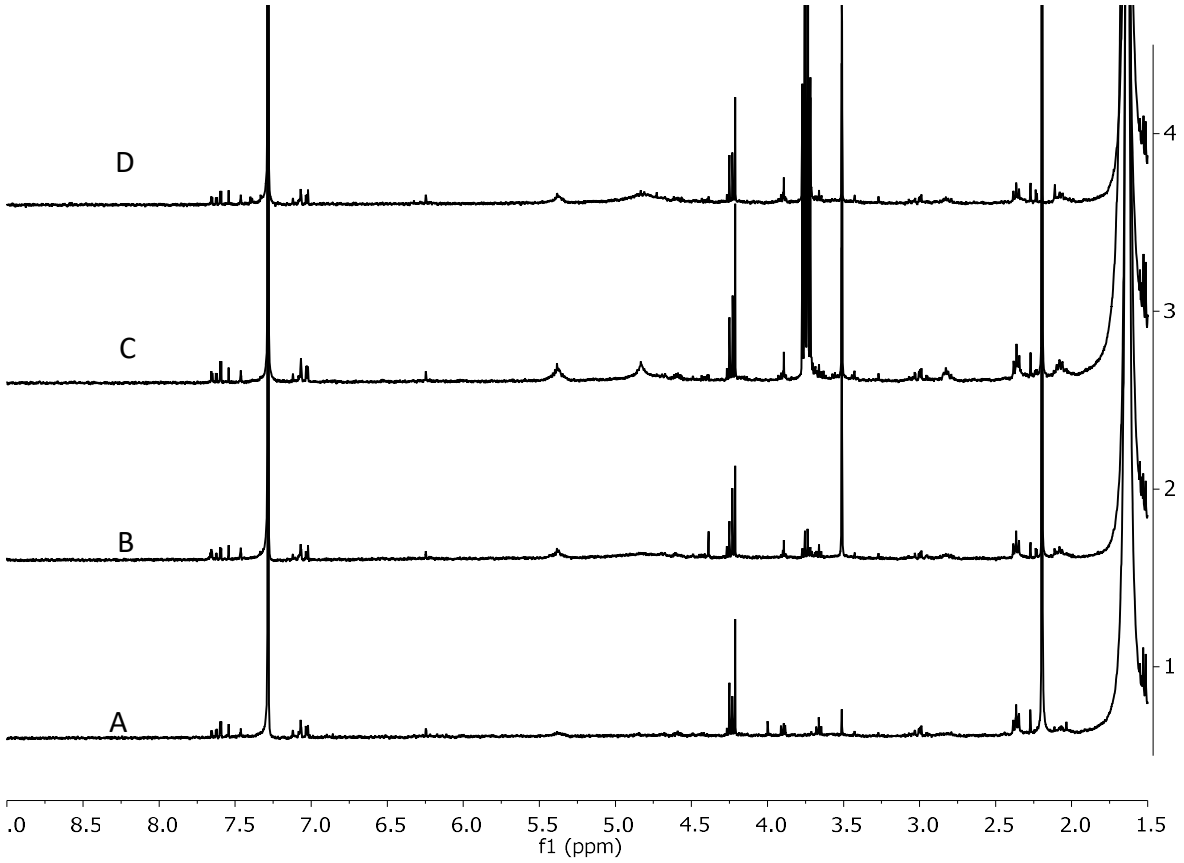

Figure 2.8. [1] NMR spectra of the comparision of Zn^{2+} based treatments at concentrations 100,200, and 400 ppm in comparision to control. A- Control, B-Zn+100, C-Zn+200, D- Zn+ 400 repectively

2.3.4 Zinc uptake in both root and shoot

Figure 2.9 and 2.10 shows the concentration of Zn in booth roots and shoots of the exposed cilantro plant. None of the treatments at 100 mg/kg concentration had statistical significance compared with control ($p \leq 0.05$). The highest concentration of the Zn accumulation was observed in the root samples of bZnO at 400 mg/kg concentration. All the treatments have a concentration-dependent increase of the Zn in the roots of the cilantro except the nZnO. In the case of the nZnO the Zn accumulation is higher at the 200 mg/kg concentration than in

comparison to the 400 mg/kg. In the case of the Zn accumulation in the stem tissues of the cilantro plant, the results of the root to shoot ratio has some interesting findings. The overall releative percentage of the Zn root and shoot levels at highest concentration such as 400 mg/kg were calculated. As per the findings, nZnO (30%), bZnO (16%) and Zn^{2+} (42%) were observed. Based on this calculation, it is clearly evident that the Zn^{2+} treatment has resulted in the higher uptake of the Zn bioavailable form. This could be probably because Zn^{2+} is most bioavailable form of Zn in nature. It is experimentally proven that major Zn based available form in shoot is zinc phosphate. Furthermore, it is also worth mentioning that at higher concentrations such as 400 mg/kg^{-1}, the nZnO has higher Zn bioavailability (30%) in the stem of the cilantro in comparison to the bZnO (16%). It could be due to the easier translocation of the Zn from the roots to shoots due to the smaller size of the nZnO. Thus, it can be summarized that the nZnO may form chelates in the soil and have less bioavailability for the plants root uptake, however once it is uptaken through the roots, the translocation rate from the root to shoot is higher than the bZnO.

On the other end, the bZnO and Zn^{2+} based treatments followed a linear concentration increase in the roots of the cilantro tissues. Similar trend of the results are already reported in the literature wherein the bZnO tend to have more accumulation than the nZnO (Medina-velo *et al.,* 2017). These observations could question the rationale over whether the translocation of the Zn in the soil is either size dependent or the size independent. It has to be noted that bZnO properties are size independent whereas the nZnO are more size independent. Furthermore, as the nZnO is more reactive due to its higher surface area, it could also have resulted in the chelates formation upon the interaction with organic matter of the soil (Watson *et al.,* 2015). This evidence is based on the fact that natural soil indeed facilitates the higher uptake of the nZnO than bZnO on three

plant species wheat, radish, wetch (Garcia-Gomez *et al.,* 2015). However, there are reports that mentioning that nZnO can be accumulated in a great extent than the bulk under acidic soil. So the basis of the higher Zn accumulation of nZnO than bZnO could be attributed to the soil properties such as pH (Garcia-Gomez *et al.,* 2017).

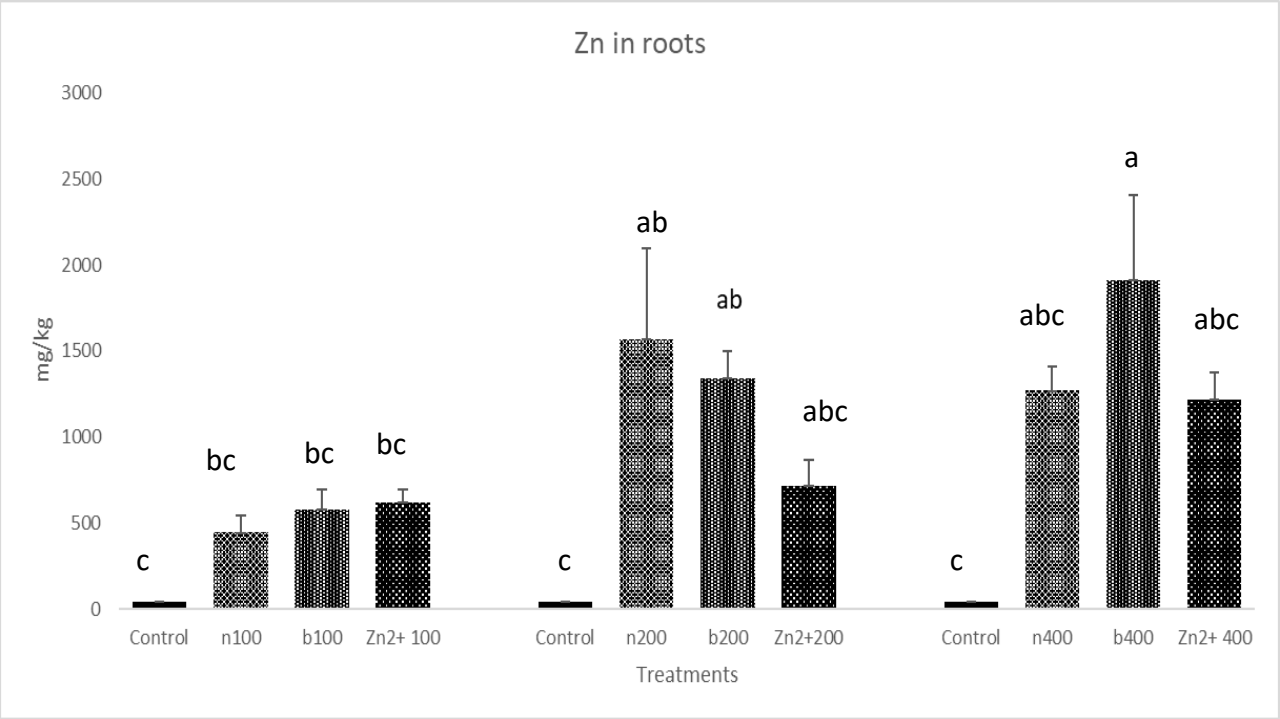

Figure 2.9. The Zn uptake in roots of cilantro plant grown for 35 days in soil amended with nZnO, b ZnO, Zn^{2+} at 0, 100, 200, 400 mg·kg-1 soil.

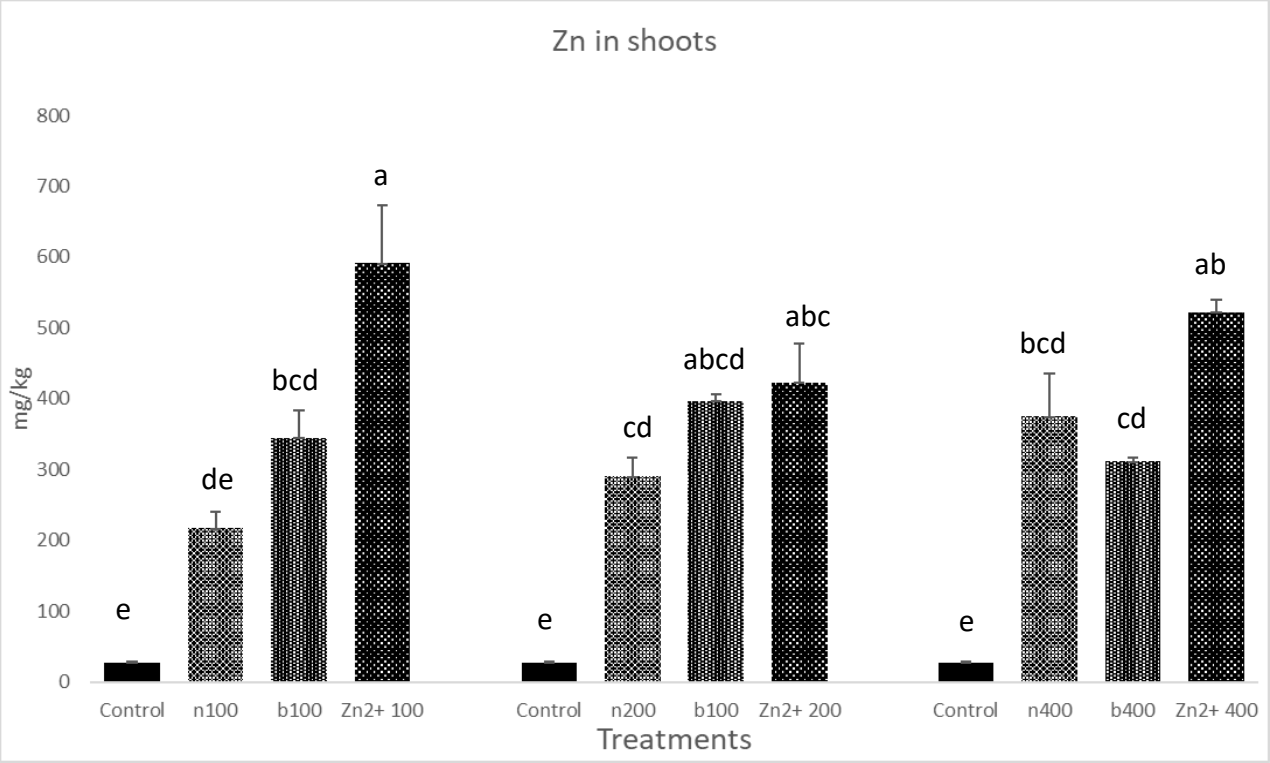

Figure 2.10 The Zn uptake in shoot of cilantro plant grown for 35 days in soil amended with nZnO, b ZnO, Zn^{2+} at 0, 100, 200, 400 mg·kg-1 soil.

2.4 Conclusions

This experimental study about the impact of the Zn-based compounds on the cilantro plant has yielded some interesting findings. According to the study, the nZnO is infact potentially less toxic in comparison to its bulk and ionic counterparts. It can be witnessed that the treatment has indeed produced higher photosynthetic pigments concentrations. Furthermore, the ability of the nZnO to induce the generation of the anti-stress enzymes could be very interesting area as it is improving the defense aspects of the plant to encounter unfavorable conditions. Despite all its beneficial effects, it must be noted that the higher concentrations of the nZnO and other Zn-based compounds have caused impediments in the overall nutrition of the plants. This could cause more damage to the plant as these metal-based nutrients have pivotal role in various enzymatic

and other biochemical aspects of the plant growth. Thus, we conclude that the nZnO amended organic soil at concentrations between 100 and 200 mg/kg is proven to improve the photosynthesis of the plant. Furthermore, nZnO at 400 mg/kg improve the defense mechanism of the cilantro plant. Our experimental results have proven that nZnO can act as beneficial nutrient rather than a toxin. However, these results may be plant-specific. The results might also vary based on soil composition, ENMs physiochemical properties and other experimental conditions. Thus, we call upon research to determine whether nZnO exposure at lower concentrations (< 500 mg/kg) could lead to the beneficial effects on plants.

Chapter 3: Foliar application of L-Ascorbic acid improves stress tolerance and increase biomass in cilantro (Coriandrum sativum) grown in ZnO nanoparticle amended soil

Abstract

Reports indicate that most engineered nanomaterials (ENMs) may be toxic to plants at concentrations > 500 mg/kg. Foliar application of antioxidants, such as L ascorbic acid (Asc), may have the potential to alleviate detrimental effects. Cilantro was cultivated in soil amended with ZnO nanoparticles (nZnO) and Zn $(NO_3)_2$ (Zn^{2+}) at concentrations of 500 mg/kg and foliar applied with ascorbic acid (Asc) at 200 mg/L. At the seed development stage, 45 days after treatment application, none of the treatments affected chlorophyll, while carotenoid content was decreased on an average by 40% across all treatments. The highest decrease of 47% was witnessed at Asc + nZnO treatment in comparison to control ($P \leq 0.1$). Conversely, Asc + nZnO treatment increased the dry biomass content by over 300%. The overall highest uptake of Zn in both roots and shoots was observed in the case of Zn^{2+} treatment; while the lowest was observed in Asc + nZnO treatment. Results of this study suggests that foliar application of antioxidants improve the biomass and defense response of cilantro grown in organic soil.

Keywords: Antioxidant, ZnO nanoparticles, Biomass, Foliar application, Zn exposure, nanofertilizer

3.1. Introduction

Engineered nanomaterials (ENMs) have a wide range of applications covering health, energy, agriculture, cosmetics. After the end user application, ENMs tend to concentrate in landfills, air, soil and water. (Keller *et al*., 2017; Reddy et al., 2017; Pullagurala et al., 2018). The environmental impact assessment of the ENMs in all these aforementioned media is imperative, especially the impact on soil biota, such as plants, the primary producers in terrestrial food chain. Previous reports indicate that ENMs, such as nZnO, nCuO, $nCeO_2$ at concentrations >500 mg/kg have proven to be detrimental to the growth and development of the terrestrial plants (Pullagurala *et al*., 2018). For instance, according to Mukherjee *et al*. (2014), the nZnO (ZnO nanoparticles) exposure at 500 mg/kg was able to induce stress response and decreased chlorophyll in pea (*Pisum Sativum* L.) plants. In addition, nZnO exposure at 500 and 750 mg/kg was able to decrease the root and shoot biomass of alfalfa (*Medigo Sativa* L) by 80% (Bandhopadhyay *et al*., 2015). Much higher exposure concentrations such as at 2000 mg/kg, nZnO was able to decrease chlorophyll and carotenoids and increase lipid peroxidation in rosemary plant (*Rosmarinus officinalis*) (Mohsenzadeh & Moosavian 2018). Thus, it can be inferred that nZnO at higher concentrations are majorly detrimental to plant growth. The aforementioned detrimental effects of nZnO are primarily attributed to the Zn^{2+} dissolution and reactive oxygen species generation (ROS) (Reddy *et al*., 2016).

Although ROS is an essential component of plant growth and defense, it can act counterproductive towards plant at elevated levels. Elevated levels of ROS leads to stress and eventually leads to programmed cell death. Thus, it is imperative to maintain ideal levels of ROS in plant cells. Antioxidant-based applications might be able to reduce the excessive ROS generated inside the plant cells. According to recent literature, antioxidants such as L-ascorbic acid and some amino acids have potential to neutralize ROS generated through their anti-

oxidative action. For instance, according to Athar and Ashraf (2006), foliar application of ascorbic acid was able to alleviate salt stress in hydroponically grown wheat (*Triticum aestivum* L). Furthermore, other modes of application such as seed priming with L-Ascorbic acid has also proven to induce better metal-based stress tolerance in okra plants (*Abelmoschus esculentus*) (Hussain et al., 2017). Ascorbic acid has a multi-facted role in plants. It is an antioxidant, co-factor in various enzymes, and acts as first line of defense in the cell wall of plants (Foyer, 2017). In this study, we have tested the impact of the foliar application of ascorbic acid on the Zn amended soil grown cilantro plant at concentrations of 500 mg/kg. We hypothesize that ascorbic acid application will quench the harmful ROS generated by the Zn exposure, as it will improve enzyme functioning in the plants. The results were compared to control and soil amended nZnO and Zn^{2+} treatments (500 mg/kg) wherein no foliar application of Asc was performed. The impact of all aforementioned exposures on plant growth are determined by analyzing the photosynthetic pigment (chlorophyll, carotenoids), stress markers (malondialdehyde and catalase), metallomics, and plant biomass. We believe these studies will enhance our knowledge about the possible role of anti-oxidants in nanoparticle induced abiotic stress management in plants. To the author's knowledge, there are no studies reported yet wherein antioxidants were tested to alleviate detrimental effects of ENMs exposure.

3.2. Materials and methods

3.2.1 Zn-based materials and radish seeds

The two Zn-based compounds used in this experiment were ZnO NPs (nZnO) and Zn $(NO_3)_2$ $6H_2O$ (Zn^{2+}). The nZnO was obtained from University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). The size of nZnO used in this study was 24 $\pm$ 3 nm (Meliorium technologies, Rochester). TEM images indicated the aggregated average size of

nZnO as 322 ± 187 nm. All other characterization of nZnO was included in supplementary material (Keller et al., 2010; Bandhyopadhyay et al., 2014). Zn $(NO_3)_2$. $6H_2O$ was obtained from (MCB manufacturing chemists, USA); L- Ascorbic acid was obtained from (Mallinckrodt chemicals, MO, USA). The seeds of cilantro were obtained from Del Norte seed and Feed (Vinton, TX, USA).

3.2.2 Plant growth conditions and harvest

The focus of this study was to test the role of antioxidants in alleviating high concentrations of ENMs induced-stress in plants. Thus, concentrations of Zn at 500 mg/kg were used for all treatments except control. Since Zn was used at this concentration, it is believed that ENMs exert abiotic stress on plants at this level. On the other hand, highest concentration of ascorbic acid a human body can intake is 200 mg/day. Here in this study, suspensions of the two Zn-based compounds (e.g. ZnO and Zn $(NO_3)_2$) were prepared at concentrations of 500 mg/kg. Similarly, the anti-oxidant L-Ascorbic acid solutions were prepared at concentration 200 mg/L. All the aforementioned suspensions/solution were subjected to sonication at 25^o C for 30 min at 120 volts/3 amps, 50 to 60 Ghz to ensure uniform dispersion. Zn-based suspensions/solutions were added and manually mixed with potting soil to obtain final concentrations of 500 mg/kg of the Zn content to soil. Commercially available organic potting mix was used in this experimental study. Description and characterization of the soil is shown in supplementary data, which has been adapted from previous published research studies (Barrios et al., 2016).

Three fifty grams of soil amended with Zn-based compounds were placed in general purpose plastic pots (4 × 2.5 inch) and kept in dark conditions for 24 h. Seeds were disinfected and washed using 2% sodium hypochlorite and Deionized water (DI) 18MOhms, respectively. Triplicates were used for every treatment along with the control. Thirty seeds were placed per

pot and incubated in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) at 25/20^{o} C day/night, 14 h. Photoperiod, 60 % relative humidity. All the plants were watered with 35 mL DI water for 35 days until the day of harvest. Foliar spraying of L-ascorbic acid was carried out on 7, 14, 21, 28 and 34th day of plant growth on treatments wherever it is applicable. The multiple applications are intended to ensure Zn exposure-based ROS is kept in control through all the stages of plant growth. At the seed development stage, (35th day) roots and shoots were separated and rinsed with tap water to remove excess soil adhered to plant tissues. Furthermore, the obtained both roots and shoot tissues were soaked in 0.01 M HNO_3 for about 15 s, and then rinsed twice with DI water. The fresh harvested tissues were prepared for enzymatic studies.

3.2.3 Chlorophyll in leaves of cilantro

The leaves of cilantro were tested for relative chlorophyll content. The measurements were performed in the center of the three cilantro leafs per each replicate by using a single-photon avalanche diode (SPAD) chlorophyll meter (Minolta, Japan SPAD, Spectrum Technologies) (Medina-Velo et al., 2017).

3.2.4 Carotenoid content

The carotenoids content was calculated by the method of Shaw and Hossain (2013). Fifty mg of leaf tissues were homogenized in 5 mL chilled methanol (100%). The obtained homogenate was centrifuged using Eppendorf centrifuge 5417R at 4000 rpm/min for 15 minutes. The absorbance of supernatant was recorded at 470 nm. The value attained was expressed as mg/g fresh weight.

3.2.5 Malondialdehyde (MDA) content

MDA content of the plants shoots were determined according to the methodology described by Wang et al. (2017). The shoots were homogenated in 5 ml of 10 % trichloroacetic acid (TCA). The obtained homogenate was centrifuged by using Eppendorf 5417R Hamburg, Germany at 12,000 rpm for 10 min at 12,000 rpm for 10 min. Two mL of supernatant obtained was added to 4 mL of 0.6 % thiobarbituric acid (TBA, in 10 % TCA). Upon the addition, it was incubated at 100 °C in a water bath for 15 min. The reaction tubes were cooled down to room temperature; the absorbance reading of the supernatant (containing MDA) was measured at 450, 532, and 600 nm using a microplate format33 using a Spectra Max 190 Microplate Reader (Molecular Devices, San Jose, CA). The overall MDA contents was calculated with the following formula: 6.45(OD532–OD600) − 0.56(OD450). This MDA content was expressed as μmol g^{-1} FW.

3.2.6 Catalase activity.

Catalase activity was determined according to the methodology described by (Gallego et al., 2012). In this procedure, reaction mixture containing 950 μL of 10 mM H_2O_2 and 50 μL of the enzyme extract were shaken three times in a quartz cuvette. The absorbance of the mixture was read and recorded for 3 min at 240 nm using a PerkinElmer Lambda 14 UV/vis spectrophotometer (single-beam mode, PerkinElmer, Uberlingen, Germany). Catalase activity was expressed as the overall amount of the enzyme required to degrade 1 μmol of H_2O_2 per minute.

3.2.7 Biomass yield

The biomass yield was calculated based on the modified method employed by Alam et al (2015). The 35-day-old harvested shoots of cilantro plants were measured using electrical balance and fresh weight (FW) was calculated. Since the fresh biomass included water content (WC), the fresh weight weighed shoots were kept in oven at 72° C for 72 hours to get the dry weight (DW). Thus, the mean dry weights of oven-dried samples were again calculated using electric balance. The results of the FW, WC, and DW of the cilantro plant was included in supplementary material

3.2.8 Elemental quantification via ICP-OES

Fresh harvested plant roots and shoots were oven dried and grinded until powdered. The grinded samples of approximately 0.2 g of both roots and shoot tissue were digested by adding 5 mL of trace pure HNO_3 in a mid-temperature graphite digestion block (Digi PREP MS, SCP Science, NY) for duration of 45 min at 115 °C. The obtained digested samples were diluted to 50 mL with DI water. Upon the digestion, the samples were then analyzed for Zn content along with metal-based micro and macro nutrients (Mg, Ca, Mn, Cu, Fe) by using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). The instrumental parameters of ICP-OES were as follows: nebulizer flow, 0.50 L/min; power, 1150 W; peristaltic pump rate, 45 rpm; and flush time, 45 s. For the quality control of the ICP-OES readings, a multi-elemental containing standard solution of 1 mg/L was used. Furthermore, the National Institute of Standards and Technology (NIST) standard reference material 1570a (spinach leaves) was employed to validate the overall digestion and analytical method; a total Zn analyte recovery of about 94% was achieved.

3.2.9 Statistical analysis

Triplicates of each treatment were allocated in a completely random design in the environmental growth chamber facility. The obtained experimental data was analyzed using SPSS22 software. To calculated experimental variance, one-way ANOVA was utilized; whereas, differences between treatments were scrutinized with the multi comparison Tukey's HSD test. There was no statistical differences against control at probability levels 0.01 or 0.05, but there was significance at 0.1 (10%). Thus the statistical significance shown are p = 0.1. All the data values represented here has a mean $\pm$ standard errors of all the three replicates.

3.3. Results and discussion

3.3.1 Chlorophyll and carotenoid content

The chlorophyll data has not yielded any significant differences for all treatments when compared with control ($p \leq 0.1$). On the other hand, the carotenoid content yielded some interesting findings (Figure 3.1). All the exposure treatments have led to decreased carotenoid content; however only Asc+nZnO treatment had statistically significant difference in comparison to control ($p \leq 0.1$). Although statistically not significant, all other treatments had approximately 40% decrease as well. The possible significant decrease in carotenoid content could be due to synergetic effect of both nZnO and Asc application. Ascorbic acid has potential to deteriorate carotenoid content through the acid-elicted isomerization reactions on foliar parts of plants (Martinez et al., 2009). There are also instances reported in the literature, wherein the rice plants exposed to the 200 mg/L of ZnO nanoparticles had decreased carotenoid content in comparison to control (Samart et al., 2017). Similar results were reported even in the case of other plants exposure studies such as tomato and wheat at similar concentrations (Amooaghaie et al., 2016). In addition to the effects on plants, similar results were also observed 200 mg/L nZnO was exposed to micro algae grown in spirulina medium. 76% of decreased carotenoid content in

microalgae was reported in this study. Decrease in carotenoid contents is a biological indicator of zinc-based heavy metal stress in plants. It is a category of anthocyanins, which has a pivotal role in protection of plant organs against stress (Karanjealker et al., 2017). Furthermore, carotenoids also has an antioxidant role in alleviation of stress and protection of chlorophyll in plants (Khoroshyy et al., 2018).

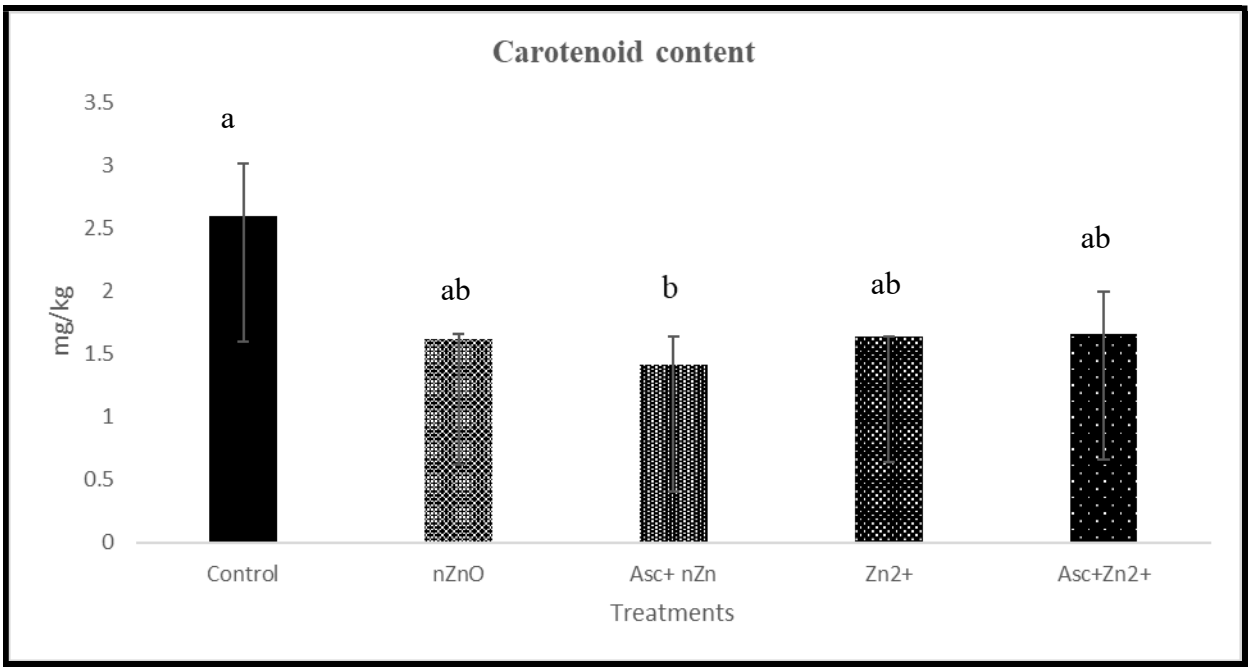

Figure 3.1 Carotenoid content in cilantro shoots grown for 35 days in various treatments viz. control, nZnO, Asc + nZnO, Zn2+, and Asc+ Zn^{2+} at concentrations of Asc (200 mg/L) and Zn (500 mg/kg) respectively. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.1$).

3.3.2 Malondialdehyde (MDA) content

None of the treatments altered MDA content in comparison to control ($p \leq 0.1$) (Fig 3.2). Although MDA is a bio-indicator of stress in plants, we believe the concentrations of Zn 500 mg/kg in all aforementioned treatments was not able to induce lipid peroxidation in cilantro plants. Furthermore, unlike Cu or Ni, the heavy metals such as Zn has comparatively less potential to induce lipid peroxidation. This was experimentally proven when 1 mM of heavy metals such as Zn, Cu, and Ni were exposed to hydroponically- grown winter wheat. According to the results, the MDA content in leaves of wheat increased for other two metals exposure except for Zn (Sazanova et al., 2012). Similarly, the green synthesized N Au (Gold nanoparticles) even at very high exposures such as 2000 mg/L was not able to induce the MDA-based changes in the rice plants (Ndeh et al., 2017).

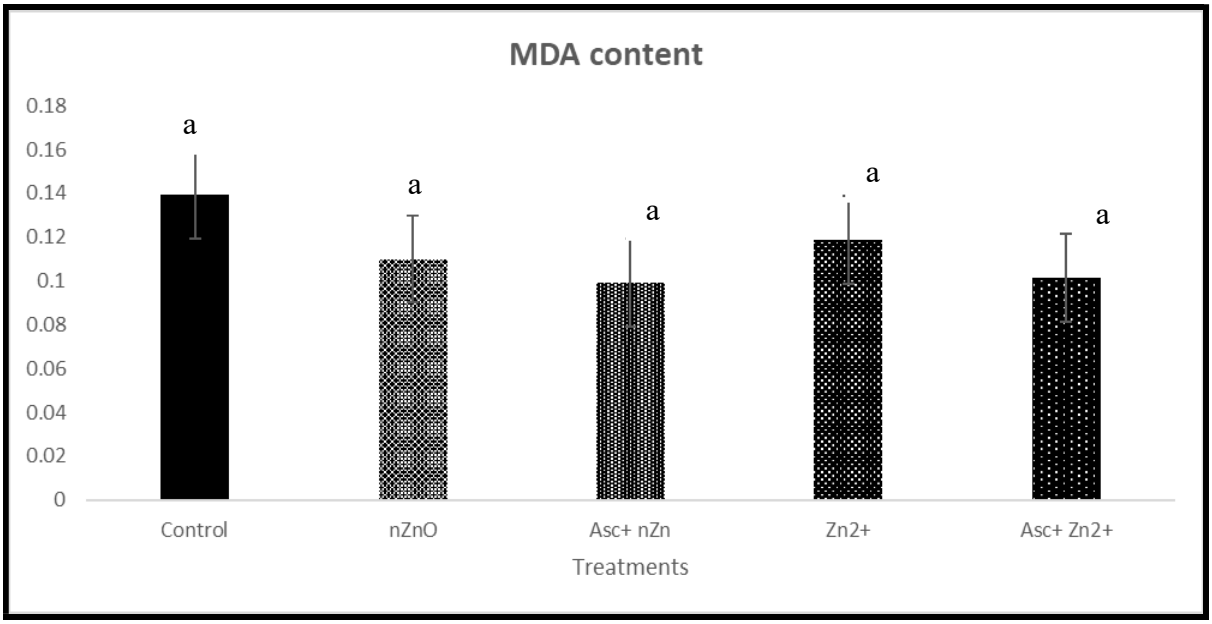

Figure 3.2 MDA content in cilantro shoots grown for 35 days in various treatments viz. control, nZnO, Asc + nZnO, $Zn^{2+,}$ and Asc+ Zn^{2+} at concentrations of Asc (200 mg/L) and Zn (500 mg/kg) respectively. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.1$).

3.3.3 Catalase activity

The catalase activity across the treatments is shown in the Fig 3.3. As per the findings, almost all the exposed treatments have shown elevated levels of catalase content. The results obtained are statistically significant in comparison to control ($p \leq 0.1$). None of the treatments had statistical significance in comparison with control. However, Asc + nZnO exposed cilantro plants has highest catalase content increase (18%), in comparison to control, whereas the nZnO treatment decreased catalase by (17%). There was statistical significance between nZnO and Asc+ nZnO treatment. Other two treatments had no significant effect in comparison to control. Increased levels of catalase content in the case of Asc+nZnO might be beneficial to plant. Increased catalase content upon nZnO exposure is often related to increased biomass and growth in plants. This is in accordance to the studies reported in literature. However, these results were obtained in a seedling experiment wherein 25 mg/L of nZnO was exposed to *Leucaena leucocephala* (Venkatachalam et al., 2017). These results indicate the role of ascorbic acid in alleviating the high concentration effects of nZnO exposure. On other hand, decrease in catalase content in N ZnO exposed plants has always led to detrimental effects on plants. According to Du et al. (2011), application of higher nZnO concentrations 5g/kg was able to induce decrease catalase content and inhibited ambient wheat plant growth. In the case of Asc+ nZnO treatment, the application of antioxidants L- Ascorbic acid probably helped in increased catalase, which contributes, to improved defense of cilantro plants. Catalase is an important anti-stress enzyme in alleviation of ROS stress. Based on our results, we can infer that L-ascorbic acid might have developed plants defense against H_2O_2 which is a very powerful oxygenated radical. Similar results were reported in the case of insects, wherein the supplementation of ascorbic acid improved catalase content and life span (Garg & Mahajan 1993).

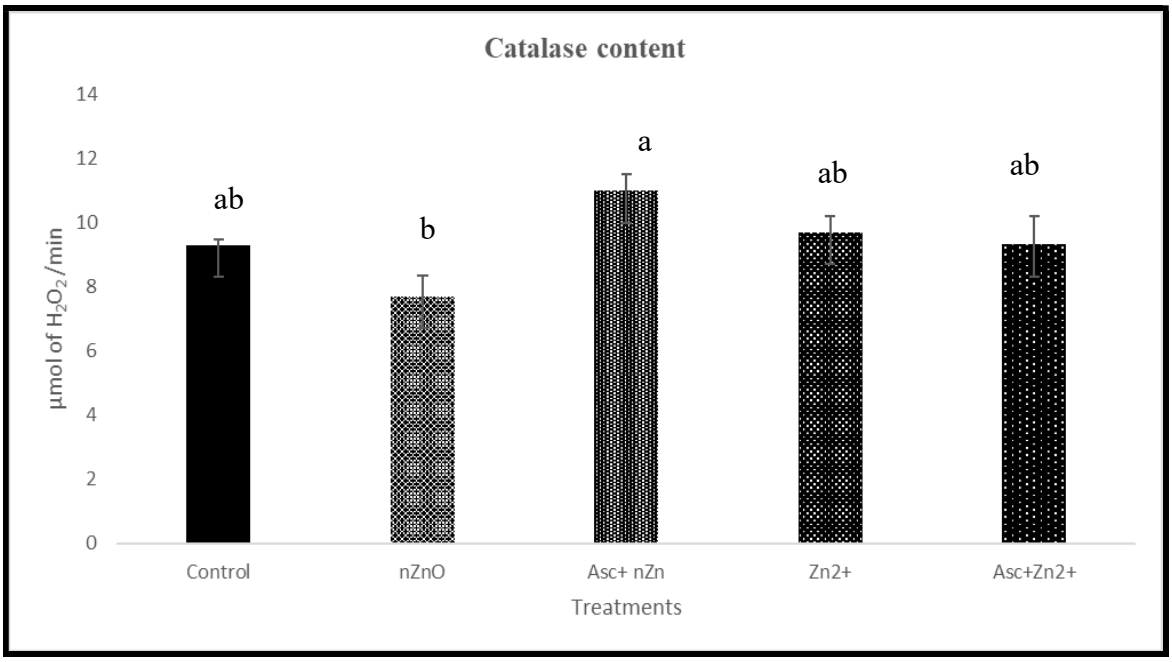

Figure 3.3 The catalase content in the shoots of cilantro grown for 35 days in various treatments viz. control, nZnO, Asc + nZnO, $Zn^{2+,}$ and Asc+ Zn^{2+} at concentrations of Asc (200 mg/L) and Zn (500 mg/kg) respectively. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.1$).

3.3.4 Biomass yield

The biomass yields across the treatments are shown in the Fig 3.4 and 3.5. According to the results, the fresh biomass weight of the treated plants did not show any statistical significance in comparison to control ($p \leq 0.1$). However, the dry biomass weight was proven to increase upon all aforementioned exposures. The Asc+ nZnO treatment has shown statistically significance in comparison to control ($p \leq 0.05$). The dry biomass is the absolute yield without water content. As per results, Asc + nZnO treatment had the highest increase of the dry biomass wt. An almost 350% of the increased dry biomass weight was observed in comparison to control. Oxidative stress is usually regarded as a growth suppressant. It stunts plant growth and development in various ways (Kasote et al., 2015). In this situation, the mitigation of the oxidative stress by

ascorbic acid could be possible reason for better plant biomass yield. Furthermore, the antioxidants also act as secondary metabolites in plants. There are some literature findings wherein nZnO application was capable of improving biomass in plants. This beneficial effect is due to the fortifying properties of the nZnO. For instance, the foliar application of nZnO at 15 ppm was able to improve the biomass of the chickpea plants. In another study, the nZnO at 10 mg/kg concentration was able to improve the biomass content in lettuce. However, all these studies have shown the beneficial results on plants at smaller doses. Thus, it can be inferred that increased biomass could also be due to other factors such as foliar application of ascorbic acid. Ascorbic acid might have played a stress suppressant role in this scenario. There are experimental studies available in the literature that support this finding. According to Noman et al (2015), the foliar application of ascorbate was able to improve the biomass yield of drought stressed maize plant. This enhanced biomass yield could be due to the alleviation of the N ZnO-based abiotic stress and metabolite role of ascorbic acid in plants. There are strong evidences about the role of ascorbic acid in the alleviation of different kinds of abiotic stress in plants. According to Agami (2014), the 1mM application of ascorbate was able to salt stress in barley seedlings.

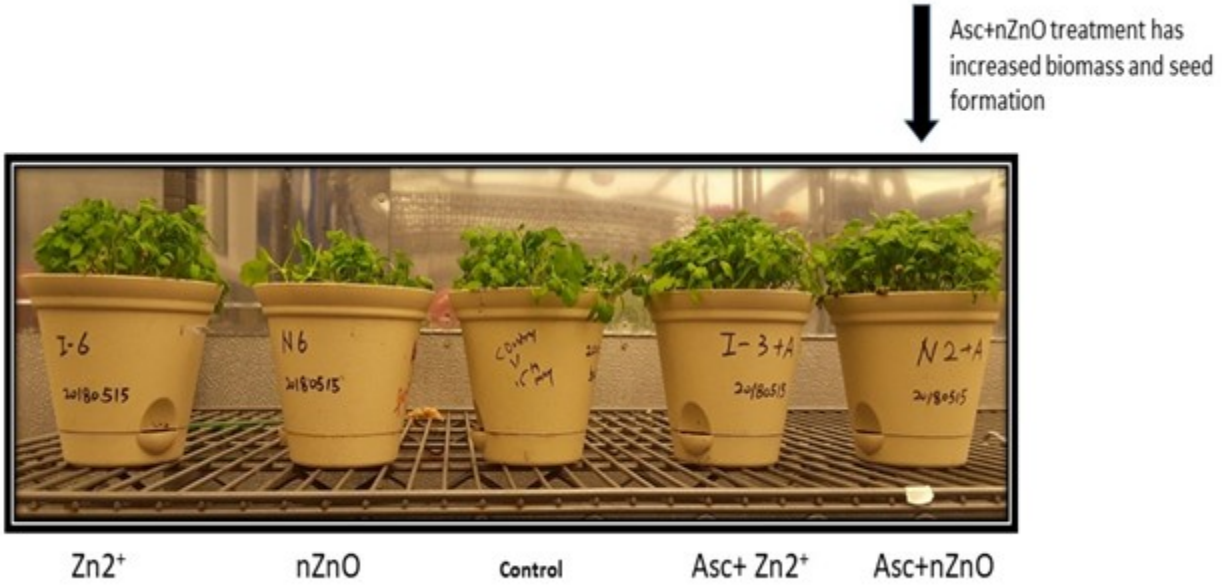

Figure 3.4 The figure showing enhanced biomass and seed formation with Asc+ nZnO treatment

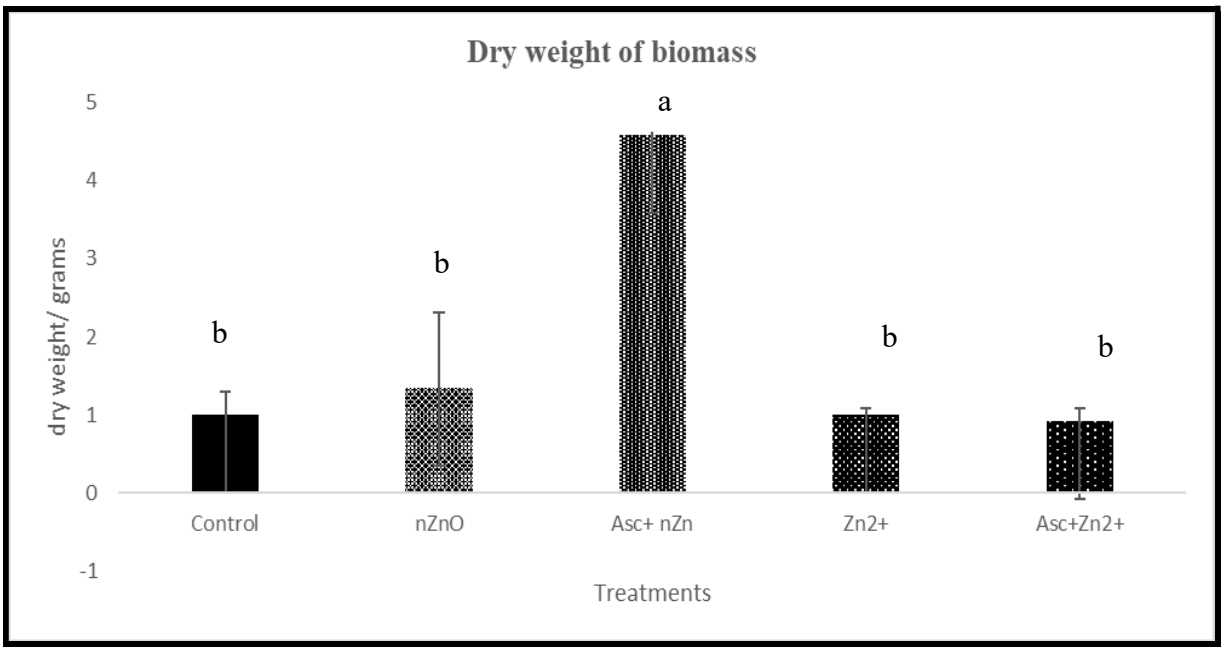

Figure 3.5 The biomass content in the shoots of cilantro grown for 35 content in the shoots of cilantro grown for 35 days in various treatments viz. control nZnO, Asc + nZnO, $Zn^{2+,}$ and Asc+ Zn^{2+} at concentrations of Asc (200 mg/L) and Zn (500 mg/kg) respectively . Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.1$).

3.3.5 Zinc and other metal uptake in both root and shoot

Fig 3.6A and 3.6 B shows the concentrations of the Zinc uptake in roots as well as shoots of cilantro plant. The highest concentration of the Zn uptake in both roots and shoots was observed in the case of Zn^{2+} treatment at 400 mg/kg. These findings are statistically significant in comparison to control ($p \leq 0.05$) Zn^{2+} ions are the only bioavailable form of zinc in soil. Thus, it can be understood that the highest uptake of Zn in the case of Zn^{2+} treatment is probably due to its easy bioavailability in plants. This is in agreement with previous literature studies wherein it was reported that the all zinc-based compounds undergo dissolution prior plant uptake (Lv et al., 2015). Lin and Xing (2008) have reported similar findings about the uptake and translocation of

Zn exposed at higher concentrations (1000 mg/L). However, it must be noted these results were obtained in hydroponically grown *Lolium perenne* (rye grass). According to the findings, the Zn^{2+} treatment had higher uptake compared to nZnO treatment. In our study, the lowest Zn uptake among the treatments was observed in the case of the Asc + nZnO treatment. It can be inferred that ascorbic acid might probably have a role in decreased uptake of Zn in the plants. Probably, the ascorbic acid application led to the formation of the Zn metal chelates formation leading to decrease uptake. Overall, very high Zn uptake was observed in all treatments. Cilantro is a very well-known biological metal chelator, thus very high uptake of the Zn was observed in the plants (Sears, 2013).

In the case of Zn shoot uptake, all the treatments had statistical significance in comparison to control ($p \leq 0.05$). The root to shoot ratio of the Zn in the exposed cilantro are as follows. The nZnO (51%), Asc + nZnO (65%), Zn^{2+} (38%), Asc + Zn^{2+} (48%). Based on these results we can infer that foliar application has indeed improved the root to shoot ratio of the Zn in both the nano and ionic forms. Since ascorbic acid has potential to reduce transition metals, such as Fe and Cu, it probably had a role in the reduction of Zn as well. All the other metals such as Ca, Fe, Mn, Mg and Cu both in root and shoot have shown no statistical significance, in comparison to control.

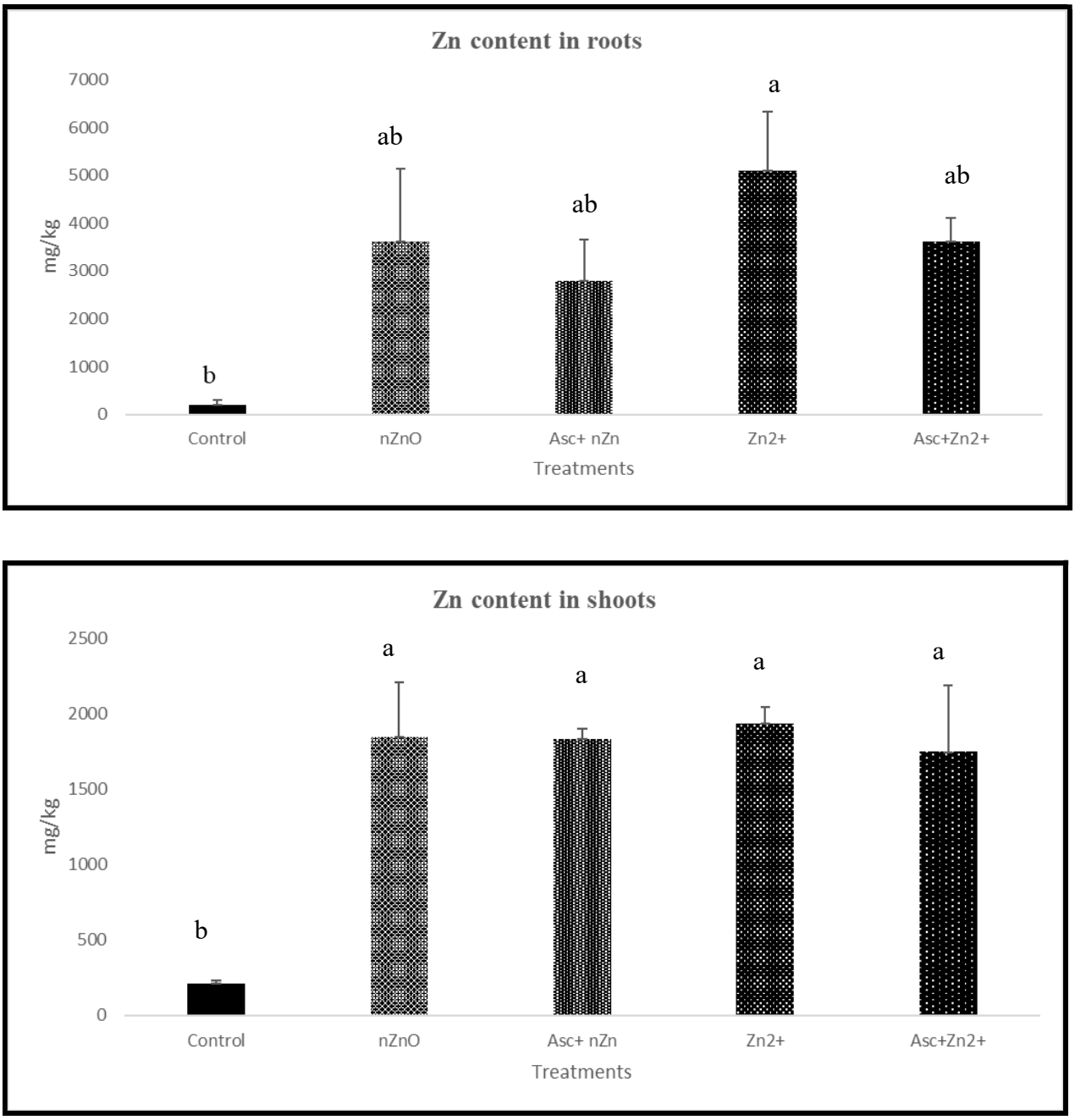

Figure 3.6 The Zn uptake A) Root B) shoot of cilantro plant grown for 35 days in various treatments viz. control, nZnO, Asc + nZnO, $Zn^{2+,}$ and Asc+ Zn^{2+} at concentrations of Asc (200 mg/L) and Zn (500 mg/kg) respectively .Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.1$).

3.4. Conclusions

This experimental study about the impact of foliar application of ascorbic acid in nZnO soil amended cilantro has yielded some interesting findings. At concentration of 500 mg/kg, the Zn in both nano and ionic forms is neutral or mildly detrimental. However, the application of antioxidants such as L ascorbic acid has yielded some beneficial effects such as increased biomass and enhanced abiotic stress tolerance. However better results might be achieved when the nZnO is amended at lower concentrations (< 200 mg/kg). However, these findings might be plant specific. Thus, more research is needed to be carried out through the application of antioxidants to enhance the plant growth and defense mechanisms. Furthermore, the other parameters such as soil, ENM properties and other experimental conditions might have to be considered. Thus, we conclude that 500 mg/kg of nZnO is neutral or mildly toxic. Application of antioxidants might enhance the beneficial effects while simultaneously ameliorating the detrimental effects of nZnO based abiotic stress.

Chapter 4: Comparative effects of ZnO nanoparticles and ionic zinc compounds on germination, biomass production and conformational changes in biomolecules of radish (*Raphanus raphanistrum subsp. Sativus*) seedlings

Abstract

The impact of the ZnO nanoparticles (nZnO) on food crops is still debatable. In this study, radish seeds were germinated and the seedlings cultivated for 8 days in aqueous suspensions/solutions of nZnO and $ZnCl_2$ (Zn^{2+}) at 100, 200, and 400 mg/L. Germination, biomass production, Zn uptake, and conformational changes in biomolecules were analyzed. Results showed that all treatments reduced seed germination in the range of 20-50%, compared with control. Similarly, all treatments reduced the biomass production (58 – 70%), being Zn^{2+} at 200 mg/L the treatment that affected the most. The highest Zn uptake was observed with Zn^{+} at 100 mg/L. FTIR data revealed that both the nZnO and Zn^{2+} produced conformation changes in the functional groups carbohydrates, lipids, and proteins. The highest conformational changes were observed in the shoots from Zn^{2+} treatments. Results from this study suggest that, in soil-less medium, at the concertation tested, ionic forms of Zn are more detrimental than nanoparticulate forms.

Key words: FTIR-based studies, seedlings, ZnO nanoparticles, radish, biomass

4.1. Introduction

The expansion of nanotechnologies has resulted in rapid increase of ENMs' utilization in various industries. Upon utilization, the environmental fate and transport of ENMs have raised concerns. According to the literature, ENMs can have an impact on all major environmental compartments, such as air, water, and soil (Garner *et al.,* 2017). Although ENMs exposure can have an impact on living organisms, the degree of the effects is still not well understood. Several papers have depicted the effects of ENMs on plants, which are primary producers in terrestrial food chain (Zuverza-Mena et al., 2017; Du et al., 2017; Pullagurala et al., 2018)

Zinc oxide nanoparticles (nZnO) are one of the most extensively used ENMs. According to Keller et al. (2013), by 2010 the global production of nZnO was 21,100 metric tons, which makes it the fourth largest ENM utilized in the industry. Recent studies have reported conflictive findings regarding the nZnO exposure towards plants. For instance, at 10 mg/kg nZnO improved biomass and net photosynthetic rate of soil-grown lettuce (*Lactuca sativa* L.) (Xu et al., 2018), while at 100 mg/kg it increased the yield of soil-grown carrot (*Daucus carota* L,) (Elizabeth et al., 2017). On the other hand, at 1000 mg/kg nZnO reduced the biomass yield of soil-grown sweet potato (*Ipomoea batatas)* (Bradfield et al., 2017). This suggest that nZnO can produce beneficial effect to plants in soil amended with <400 mg/kg. However, data from plants exposed to nZnO in soil-less media is still incomplete. Considering the emergence of soil-less agriculture practices, and the possible fertilizer effect of nZnO, it is essential to understand the response of plants exposed such nanomaterial. Up to now, most of the hydroponic studies have reported detrimental effects of nZnO towards plants. For instance, according to Lopez-Moreno *et al.* (2010), the nZnO at 2000 and 4000 mg/L induced genotoxicity in soybean *Glycine max*. Similarly, Lee et al. (2013) reported stunted root growth and genotoxic effects on buckwheat (*Fagopyrum esculentum*) seedlings exposed to nZnO at 2000 and 4000 mg/L. It is important to

highpoint that the negative effects have been obtained from exposure to extremely high concentrations. Thus, data from lower exposure concentrations are needed.

Plants such as radish are eaten at their sprout stage. Radish seedlings are consumed raw as they are packed with well-nourished nutrients such as vitamins and minerals. Commercially, radish sprouts are grown by soaking seeds in water media. It is hypothesized that the nutritional value of the sprouts can be enhanced if they are exposed to suspensions of microelement nanomaterials, like nZnO. Thus, in this study, radish seeds were exposed to water-based suspension/solutions of nZnO and Zn^{2+} at 100- 400 mg/L. The impact of the treatments were evaluated through growth parameters, as well as FTIR-based conformational changes in tissues.

4.2. Materials and methods

4.2.1 Zn based materials and radish seeds

The nZnO (Meliorum Technologies, Rochester, NY) were obtained from University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). They were 24 + 3 nm in size (Keller et al., 2010). The characterizations of the nZnO is available in supplementary material (SI). Transmission electronic microscope (TEM) images indicated an average size of 322 + 187 (Bandhyopadyay et al., 2015, SI). On the other hand, $ZnCl_2$ (ACS reagent 97+% purity) was procured from Acro organics. The radish seeds (champion variety) were obtained from Del Norte Seed and Feed (Vinton, TX, USA).

4.2.2 Radish seedlings growth conditions and harvest

The seeds were hydrated and washed with 2% sodium hypochlorite and deionized water 18 MOhms (DI). Later on, thirty seeds were placed in sterilized Petri dishes (60 x 15 mm) over a standard filter paper. Since previous studies have shown that nZnO at 400 mg/kg have shown beneficial effects on soil-grown plants, this concentration was selected as the maximum for the

soil-less study. In addition, 100 and 200 mg/L were used in order to see the effects of lower concentrations. All of these the aforementioned suspensions/solutions were subjected to sonication at 25 ºC for 30 min at 120 volts/3 amps, 50 to 60 Ghz to ensure uniform dispersion.

A control treatment whit only millipore water (MW) was also set. All the treatments were carried out in triplicates. Petri dishes containing control and Zn exposed seeds were covered with aluminum foil and kept in darkness for 3 days. Then, all the dishes were moved into a growth chamber (environmental growth chamber, Chagrin falls, OH, USA) at 25/20^{O}C day/night, 14/10 h photoperiod and 60 % relative humidity and 340 mmole $m^{-2}s^{-1}$. According to the reports, the average germination times for radish at room temperature are 4-5 days (Ciska et al., 2008). Thus, the seedlings were allowed to grow for 5 days prior harvest.

4.2.3 Biomass yield

The fresh weight of the 8-day old sprouts were calculated after Alam et al. (2015). Since the fresh weight includes the biomass as well as water content (WC), the dry weight (DW) was measured after drying the fresh seedlings in an oven at 70 ºC for 72 h.

4.2.4 Seed germination

The number of germinated seed was counted three days after treatment application (Breen and Richards 2008). The seeds of radish are considered as germinated when a radical is protruded beyond the seed coat (Schoper et al., 2001).

4.2.5 Elemental quantification via ICP-OES

Before drying, the seedlings were washed with 0.1 M HNO_3 and three times with DI water, in order to remove debris and metals adhered on the surface. The oven-dried samples were grinded until powdered using mortar and pestle. Samples of approximately 0.1 g of tissues were acid digested by adding 4 mL of trace pure HNO_3 assisted with a mid-temperature graphite digestion

block (Digi PREP MS, SCP Science, NY) for 45 min at 115 °C. Upon digestion, the samples were volume adjusted to 50 mL with DI water. The obtained samples were analyzed for Zn content by using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). The ICP-OES instrumental parameters were as follows: nebulizer flow, 0.50 L/min; power, 1150 W; peristaltic pump rate, 45 rpm; and flush time, 45 s. For the quality control of the ICP-OES metal analysis, a multielemental standard solution of 1 mg/L was employed. Furthermore, the National Institute of Standards and Technology (NIST) standard reference material (SRF) 1570a (spinach leaves) were also used to validate the ICP digestion and analytical method; overall the Zn analyte recovery of 99% was achieved.

4.2.6 ATR-FTIR

The oven dried radish roots and shoots were grounded, and the powder were analyzed using FTIR spectroscopy (Perkin-Elmer, Spectrum 100, Universal ATR Sampling Accessory) in a range of 450−3950 $cm^{-1.}$ The derived spectrum was collected using EFTIR software. The experiment was carried out in triplicates and the obtained spectra was compared with previously reported data and other libraries available in the literature.

4.2.7 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.

4.3. Results and discussion

4.3.1 Biomass of the radish sprouts

As shown in Figure 4.1, The Zn^{2+} at 200 and 400 mg/L resulted in a significant decreased of biomass (70 and 58%, respectively, compared with control; ($p \leq 0.05$). Similar results were observed in soil grown carrot plant where Zn^{2+} was more detrimental in comparison to the nanoparticulate form (Ebbs et al., 2016). The biomass variation is one of the consequence of the metabolic changes in the plants (Hermans et al., 2016). According to Poorter and Nagel (2000), the biomass allocation in plants are influenced by the availability of carbon dioxide, light, and nutrients. It must be noted that nZnO did not affect the biomass as negatively as the Zn^{+}. In fact, there are some instances wherein the nZnO can be beneficial. According to Mahajan et al. (2011), the nZnO at concentrations of 20 mg/L increased the biomass of agar-cultured mung bean (*Vigna radiata*) by 76%. The beneficial aspect of Zn exposure is probably attributed to its role as co-factor of around 300 enzymes, which play a crucial role in plant growth and development.

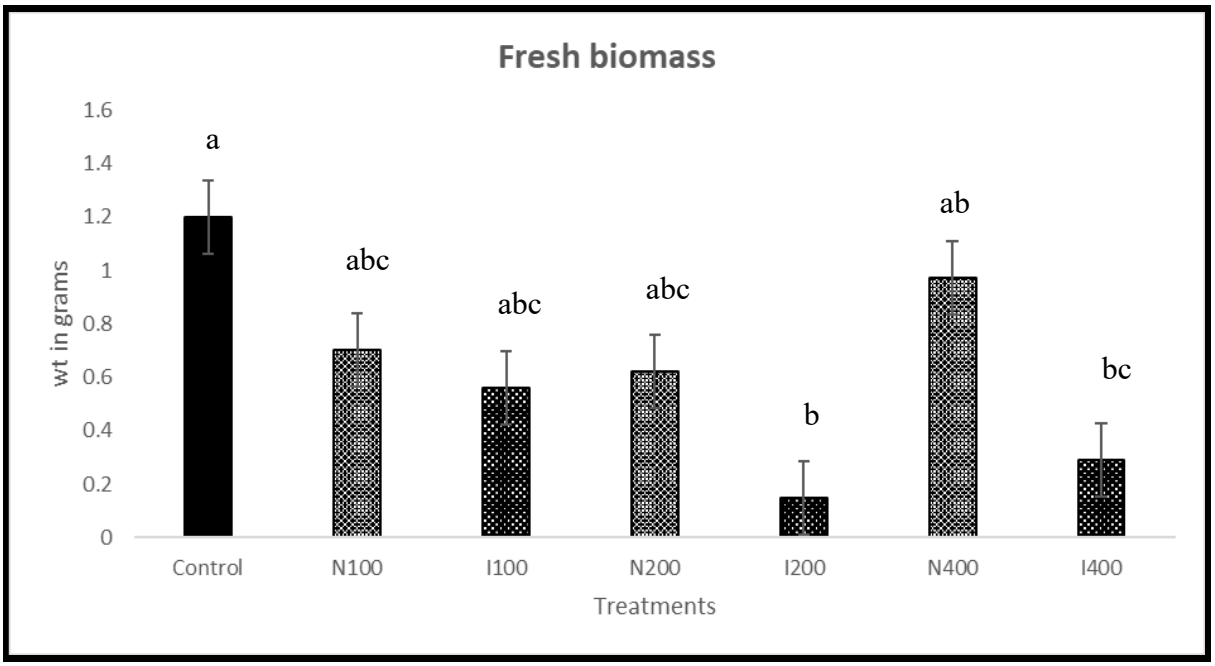

Figure 4.1 Biomass content in radish seedlings grown for 8 days in petri dishes amended with nZnO and Zn+ at 0, 100, 200, 400 mg/L. Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

4.3.2 Seed germination

Figure 4.2 shows the germination rate of the radish seeds. As seen in this figure, all the treatments reduced the germination, but the difference was statically significant (p <0.05) only at 400 mg/L of nZnO (43%) and Zn^{2+} (39%). The seed dormancy observed at these aforementioned concentrations is probably due to alterations in plant hormones and genes involved in seed germination (Bentsink and Koomneel 2008).

Similar findings about seed germination at higher nZnO concentrations reported for nZnO at 750 mg/L on seed germination of pigeon pea (*Cajanus cajan*) (Korishettar et al., 2016). Although the suppression of seed germination can be attributed to the heavy metal stress. It must be noted that, compared to other metals such as Cu and Pb, the impact of Zn induced stress might

be lower. Furthermore, in some instances, the Zn might even promote germination of plant seeds. However, this effect might also be plant specific. For instance, nZnO at concentrations of 1000 mg/L was able to increase the germination of peanut (*Arachis hypogea*) (Prasad et al., 2012). This statement is further supported by other experimental studies reported in literature. According to de la Rosa et al. (2013), the nZnO at 1600 mg/L improved the germination of cucumber (*Cucumis sativus*), whereas at the same concentration decreased germination for alfalfa (*Medigo sativa*) and tomato (*Solanum lycopersicum*) plants. The seed germination in higher plants seems to be positively influenced by the Zn nutrition. Zn is a key component in an enzymatic system, which triggers the biosynthesis of the plant growth hormone auxin (Aglar et al., 2016). Thus, it can be concluded that the germination of the plants might be affected by both the concentration as well as plant species.

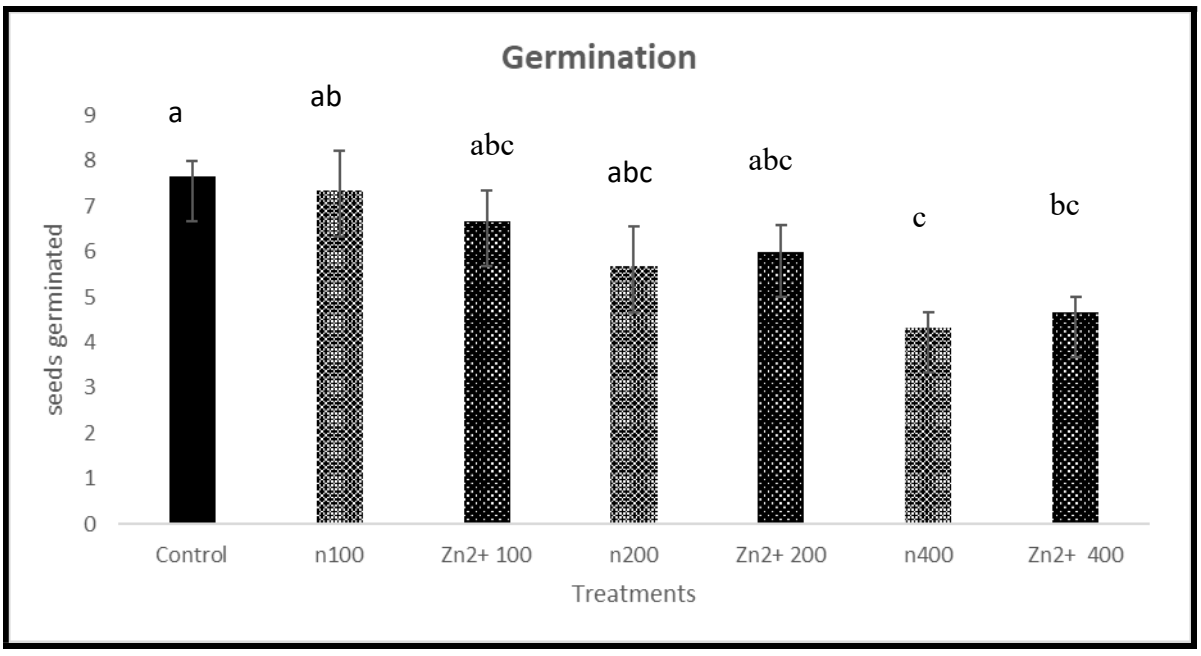

Figure 4.2 Germination of radish seedlings study radish seedlings grown for 8 days in petri dishes amended with n ZnO and Zn^{2+} at 0, 100, 200, 400 mg/L Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$)

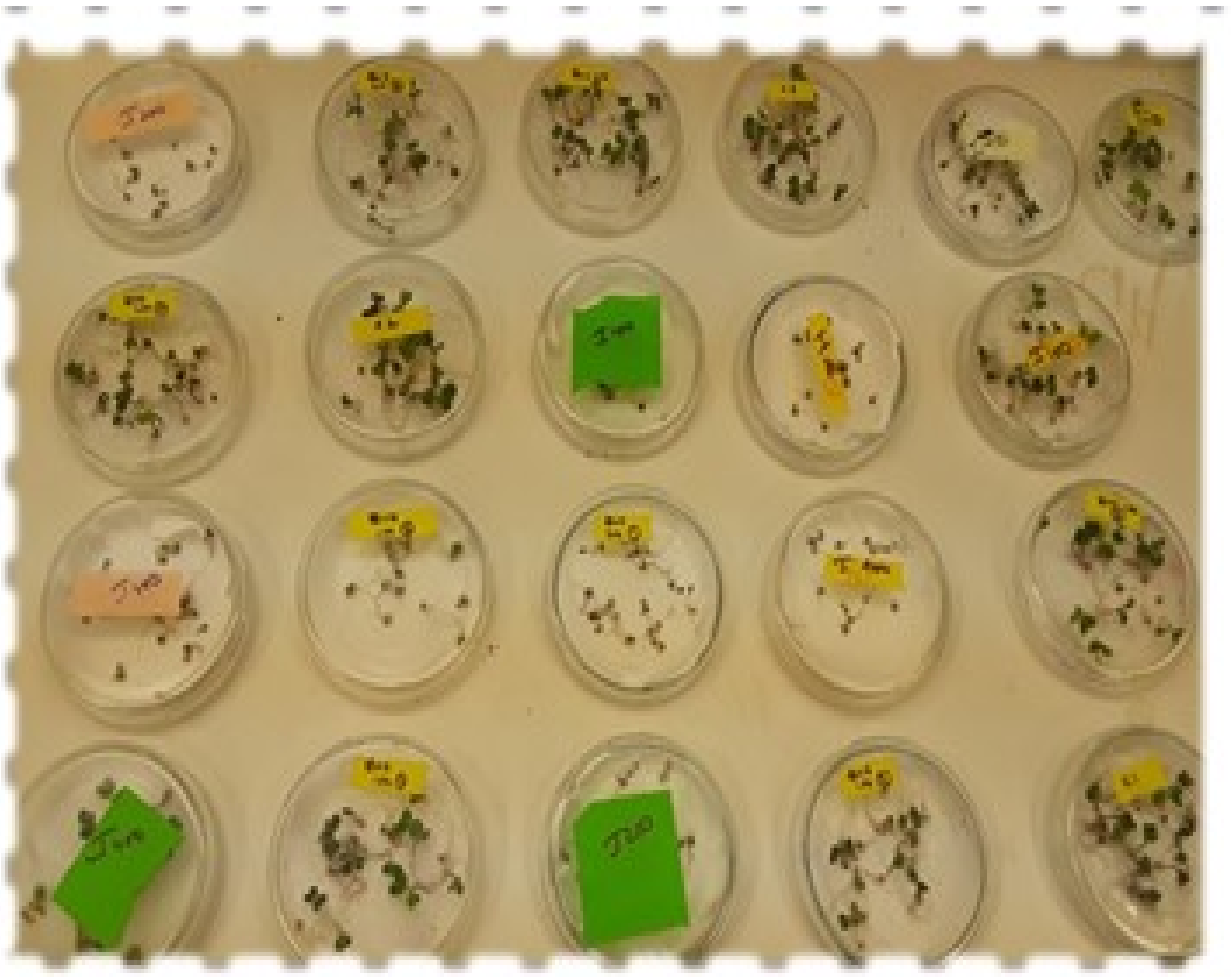

Figure 4.3 Germination of radish seedlings study.

4.3.3 Zn uptake in radish seedlings

Figure 4.4 shows the Zn uptake of radish seedlings exposed to nZnO and Zn^{2+} (0, 100, 200, and 400 mg/L). As seen in this figure, seedlings exposed to nZnO at 100 and 400 mg/L had similar Zn concentrations than control. While all Zn^{+} exposed seedlings had significantly more Zn than control (p<0.05). The highest concentration (2300 mg/kg) was found with Zn^{2+} at 100 mg/L. Interestingly, there were no differences in Zn uptake between nZnO and Zn^{+} treatments. Several

reports have shown higher uptake from ionic Zn, in comparison to nanoparticulate ZnO. The enhanced Zn^{2+} uptake is probably attributed to Zn homeostasis network in plants. This is maintained by regulatory network of membrane and Zn-binding proteins inside the plant cell (Moreira et al., 2018). Furthermore, the interactions of Zn-plant tissues can also play a crucial role. According to Lin and Xing (2008), the nZnO uptake in plants usually is more confined to adhering to the outer root surface, and their translocation is much less compared to Zn^{+} treatments. These above mentioned findings about the adherence of nZnO on root surface were observed in hydroponic grown rye grass (Lin and Xing 2008). Since this study is soilless media, the impact of the soil and its properties will not have any effect. Furthermore, the results may not be in agreement with ones we observe in soil-grown plant studies. The aggregation and dissolution of the nZnO and Zn^{+} play a crucial role. There are also evidences about the role of Zn dissolution properties of root exudates (Chen et al., 2017). With less interfering factors such as humic acid and soil particles, the root exudates might have played a crucial role in the dissolution in this petri dish study. Similar behavior of higher Zn^{2+} compared to nanopartcilaute form was previously reported in literature. For instance, according to Amooaghaie et al. (2016), the uptake of Zn^{2+} was higher in tomato (*Solanum lycopersicum*) and wheat (*Triticum Aestivum*) compared to the nanoparticulate form at 23 mg/L concentrations. It must be also noted that Zn uptake in plants is a complex process and it involves the role of Zn transporters and natural metal chelator compounds inside plant tissues (Gupta and Kumar 2016). Furthermore, the plants maintain the Zn homeostasis through their vascular sequestration and detoxification mechanisms inside plants.

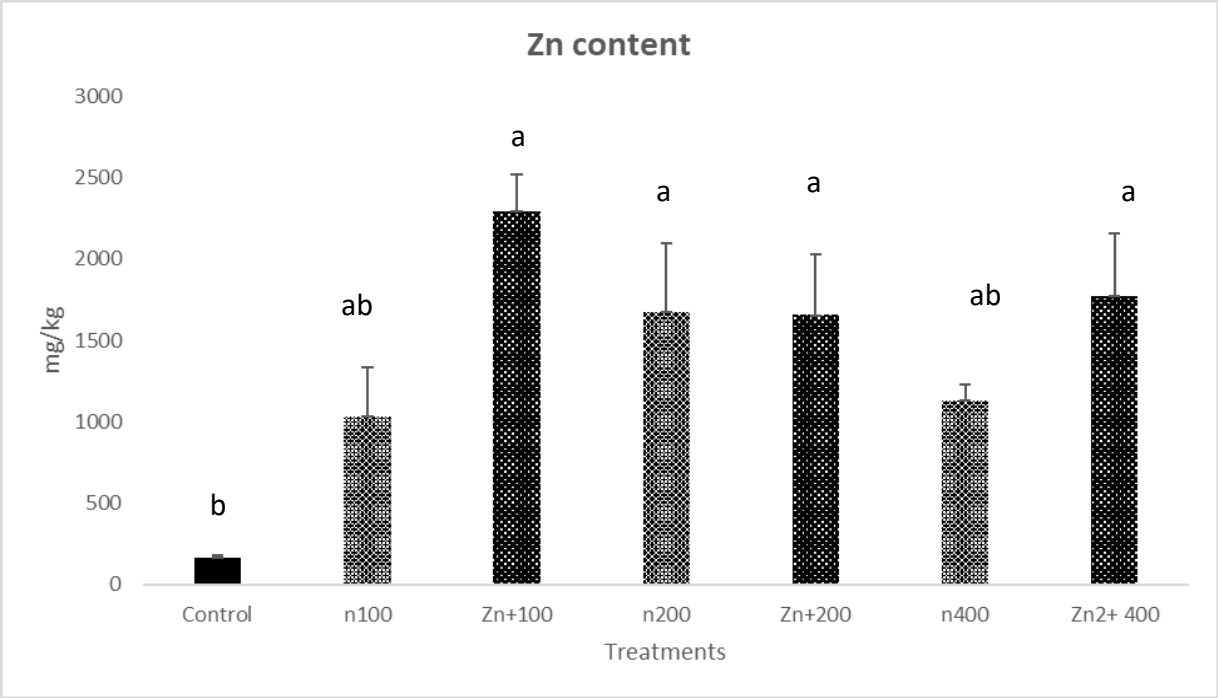

Figure 4.4 Zn uptake of radish seedlings grown for 8 days in petri dishes amended with n ZnO and Zn^{2+} at 0, 100, 200, 400 mg/L Each value is mean ± SE of three replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$)

4.3.4 FT-IR analysis of roots and shoots

Figures 4.5, 4.6, 4.7, and 4.8 show the FTIR spectra of nZnO and Zn^{2+} exposed radish seedlings at aforementioned concentrations. Table S4 shows a summary of all previously compiled FT-IR data from various plant samples that corresponds to the functional groups identified in plants (Rico et al., 2015). For the comparison of the results, three major regions are been focused. The spectral regions corresponding to lipids (300-2800 cm^{-1}) proteins (1700-1500 cm^{-1}), and carbohydrate region (1200-900 cm^{-1}). The results obtained in this study were compared to the existing literature. In the present study, the changes were observed in the band frequencies

ranging from 1200-900 cm^{-1} pertaining to the carbohydrates such as lignin, starch, and cellulose. Changes were also observed in the band frequencies 1790-1500 cm^{-1}, pertaining to protein and lipids. The possible changes in the radish plants were probably due to the presence of additional Zn inside plant cells, which could have affected various metallozyme enzymatic functions. (McCall et al., 2000). Similar results were obtained in the study carried out Zuverza-Mena et al. (2017), wherein the seedlings were exposed in the Petri dish cultures to colloidal Ag. According to the report, the carbohydrates and lipids were the most affected by colloidal Ag (Zuverza-Mena et al., 2017).

Results showed slight alterations in the transmittance spectra for all the treatments; however, the spectra for the Zn^{2+} 200 and 400 mg/L had clear band shifts for the shoot samples (Fig 6). Similar results about other nano exposures have been reported in the literature. According to Suresh et al. (2016), the variations in the regions corresponding to the lipids, proteins and carbohydrates were observed in leaves of CuO nanoparticles exposed peanut plant. Similar results were observed for the FTIR analysis carried out in soil grown higher plants. Zhao et al. (2014) reported that nZnO at 400 and 800 mg/kg induced changes in the carbohydrate regions of the cucumber plants. Thus, it can be inferred that irrespective of media or size of plant, the carbohydrates region are more prone to be affected by the nZnO exposure. It must also be noted that the effect on carbohydrates region is not just applicable to nZnO exposure alone. According to the studies carried out by Morales et al. (2013), the FTIR spectra showed that $nCeO_2$ was able to affect the carbohydrates region of the cilantro shoots. In conclusion, most ENMs, irrespective of the media or the type, have a potential to affect the carbohydrates region of the plants

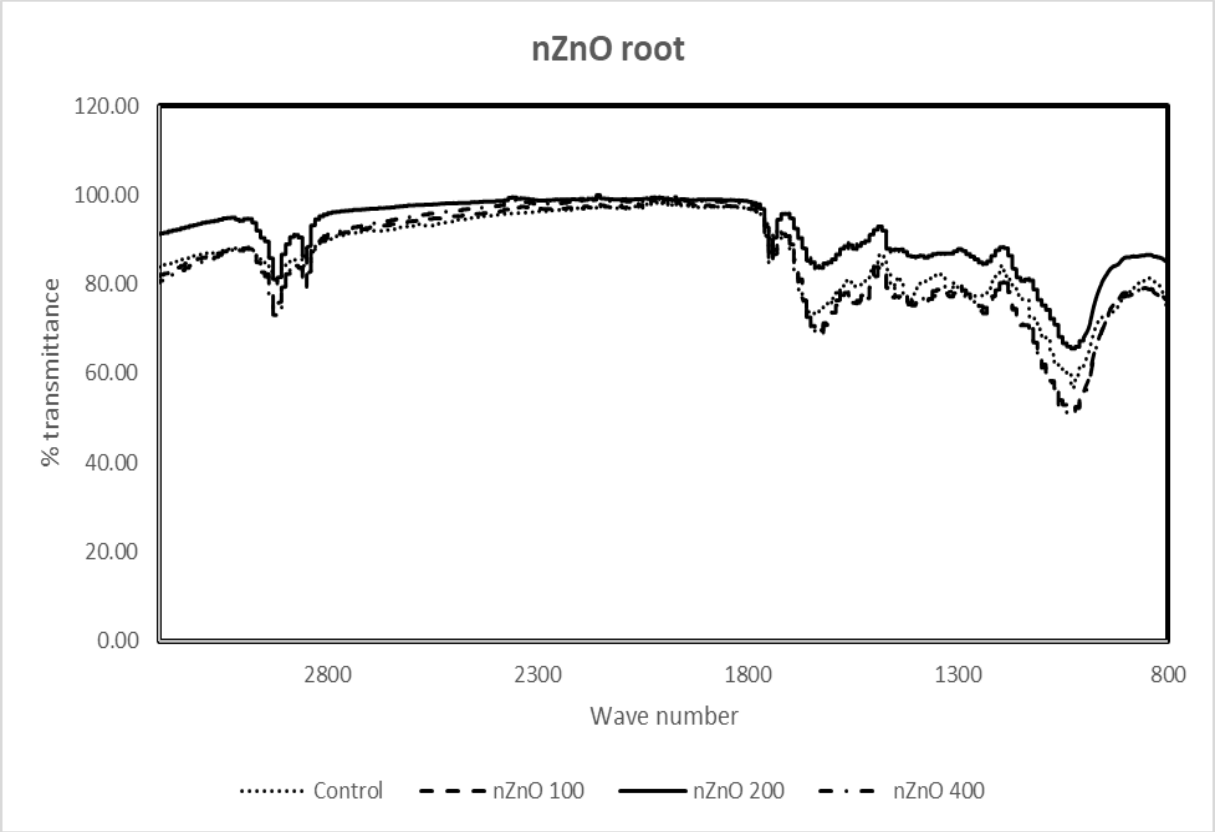

Figure 4.5 FTIR spectra of the comparision radish seedlings shoots A] nZnO based treatments at concentrations 100,200, and 400 mg/L in comparision to control. B] Zn^{2+} treatments at concentrations 100, 200, and 400 mg/L

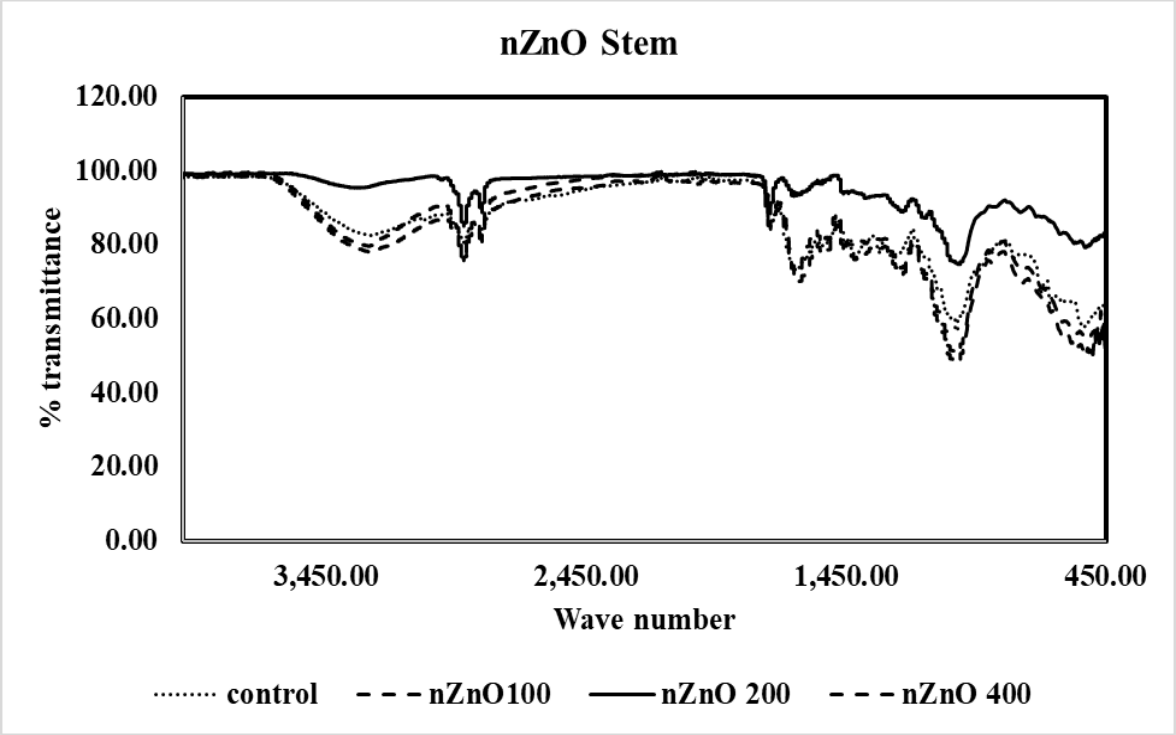

Figure 4.6 FTIR spectra of the comparision radish seedlings shoots A] nZnO based treatments at concentrations 100,200, and 400 mg/L in comparision to control. B] Zn^{2+} treatments at concentrations 100, 200, and 400 mg/L

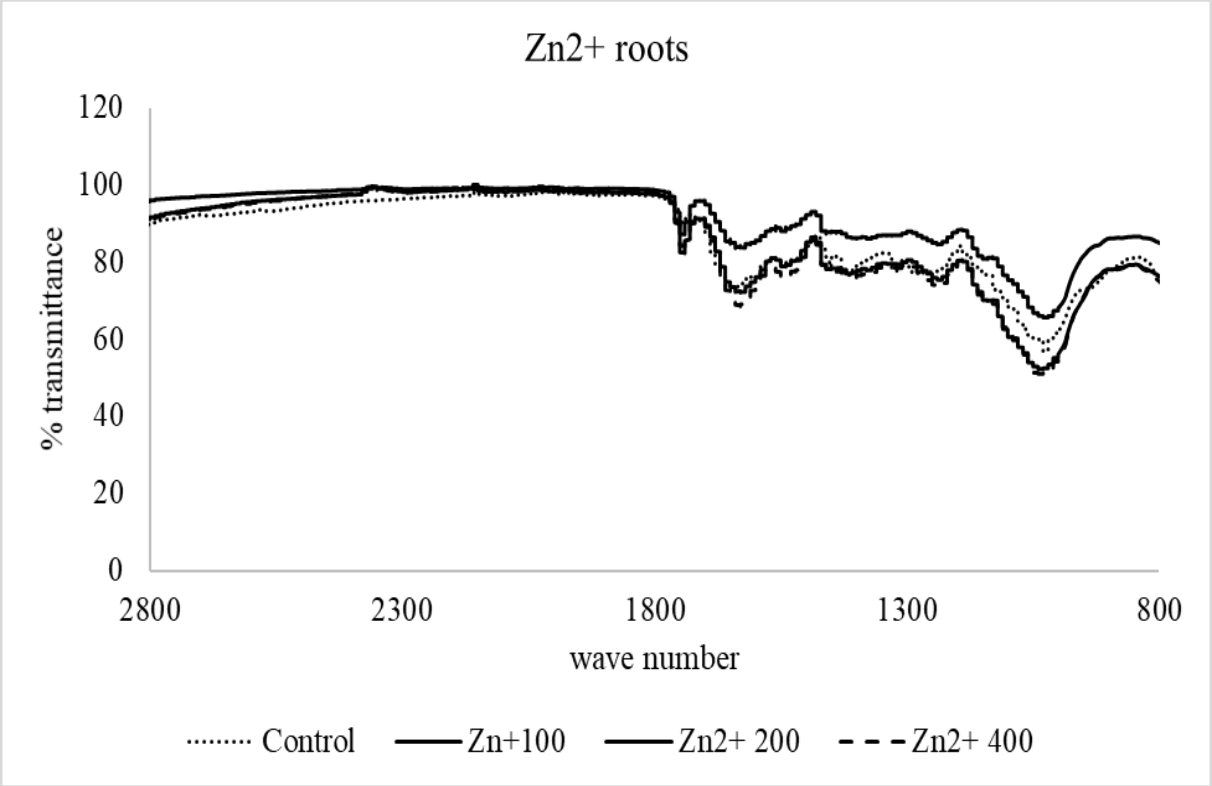

Figure 4.7 FTIR spectra of the comparision radish seedlings shoots A] nZnO based treatments at concentrations 100,200, and 400 mg/L in comparision to control. B] Zn^{2+} treatments at concentrations 100, 200, and 400 mg/L

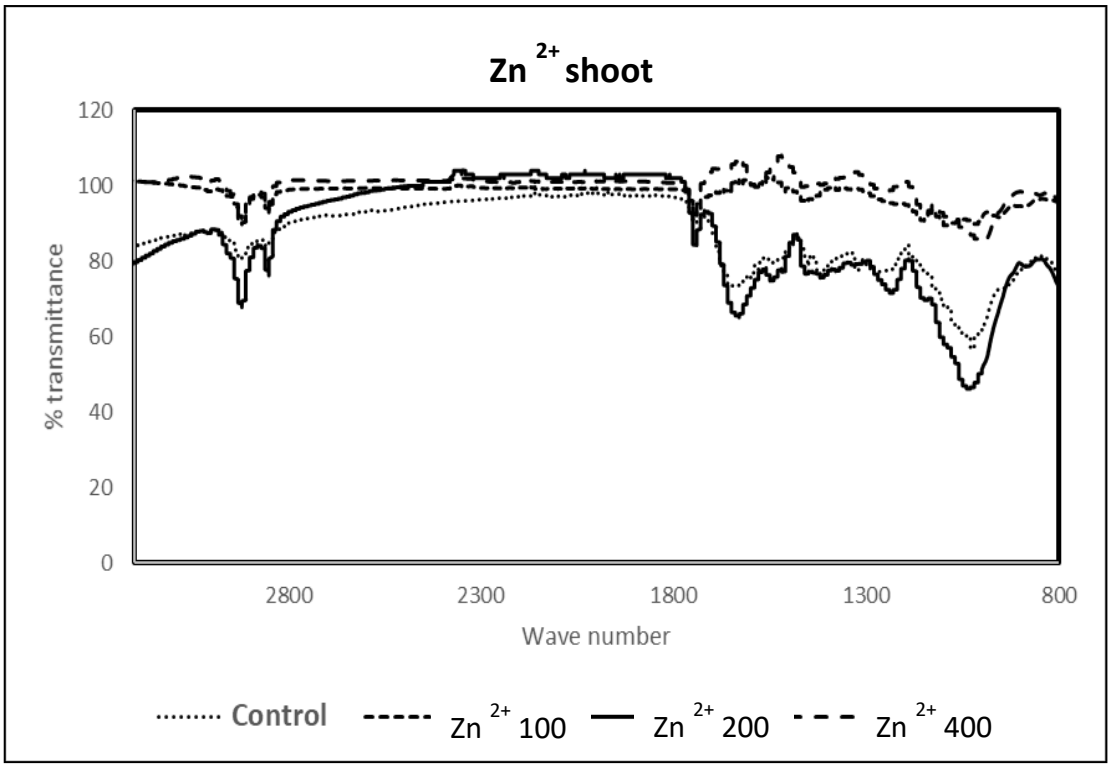

Figure 4.8 FTIR spectra of the comparision radish seedlings shoots A] nZnO based treatments at concentrations 100,200, and 400 mg/L in comparision to control. B] Zn^{2+} treatments at concentrations 100, 200, and 400 mg/L

4.4. Conclusions

Results of this study showed that nZnO and Zn^{2+}, both at 400 mg/L, had detrimental effects on the radish germination. Furthermore, biomass decrease was observed for both compounds at all concentrations; however, the Zn^{2+} treatment at 200 and 400 mg/L was proven to exhibit statistical significance compared with control. The FTIR-based analysis showed that proteins, carbohydrates, and lipids of the radish seedlings were altered. The Zn^{2+} at 400 mg/L had very strong effect on the conformational changes of shoots. The Zn^{2+} at 100 mg/L had higher uptake than 400 mg/L indicated the role of aggregation kinetics of suspension/solution at higher concentrations. The aforementioned findings are in disagreement with the reports obtained from soil-grown plants exposed to similar Zn concentrations. In the present study, nZnO even

concentrations as low as 100 mg/L, did not show beneficial effects on radish seedlings. More research is needed in order to find the beneficial effects of the nZnO exposure studies in soil-less media.

5. Summary and conclusions

This research was carried out to understand the impact of different nZnO plants grown in different media.

First objective of our study was to evaluate the effects of nZnO, bZnO and Zn^{+} (0,100, 200, 400 mg/kg) towards soil grown cilantro plant. Results from these studies have shown that nZnO at 400 mg/kg increased photosynthetic pigment content and decreased lipid peroxidation. The ^{1}NMR based metabolic profiling indicated significant changes in carbinolic regions of plant metabolites. Highest Zn uptake in the roots was observed in plants exposed to bZnO at 400 mg/kg; whereas in the shoots it was at obtained with Zn^{2+} at 100 mg/kg. nZnO at 400 mg/kg treatment increase chlorophyll and improve stress response through less lipid peroxidation. Data suggest that nZnO is beneficial in comparison to its bulk and ionic counterparts. However, these results might be only confined to soil medium. Furthermore, they may change in other type soil, or might be plant specific.

The second objective aimed to evaluate the impact of foliar application of ascorbic acid on cilantro cultivated in nZnO amended soil. The results of these studies have proven that even at 500 mg/kg the n ZnO is neutral or mildly detrimental. The foliar application of ascorbic acid + nZnO treatment decreased carotenoid and improved plant biomass by 300%. Additionally, ascorbic acid decreased Zn uptake in plants exposed to the same concentration of nZnO. Data suggest that the antioxidant application on plants exposed to less than 200 mg/kg of nZnO might enhance the beneficial effects of these nanoparticles.

The third objective of this research was to determine whether the nZnO at 0-400 mg/L would be beneficial to radish seedlings grown in soil-less medium. Results showed that nZnO and Zn^{2+} exposure had detrimental effects on germination of radish seedlings. Zn^{2+} exposure at 200 and 400 mg/L decreased the biomass accumulation of the seedlings. The highest Zn uptake was observed with Zn^{2+} treatments as well. Lastly, the FTIR analysis revealed band shifts in spectra pertaining to proteins, lipids and carbohydrates. At the concentration tested, nZnO did not show beneficial effects on hydroponically grown radish seedlings. However, these results might be plant specific; more research must be carried out at lower concentrations in order to clarify the results.

Overall, the data suggest that nZnO has potential prospects to be used as nanofertilizer on soil-grown plants. Furthermore, application of antioxidants, such ascorbic acid, might enhance the yield of the plants.

A summary of the results are shown in Table 5.1, Table 5.2, and Table 5.3

Table 5.1: Effects of nZnO, bZnO and Zn+ (0,100, 200, 400 mg/kg) exposure towards soil grown cilantro plant.

Parameters	nZnO	bZnO	Zn^{2+}
Chlorophyll	400 (+)	400 (+)	-
Carotenoid	100	-	-
MDA	400 (-)	100 (+), 200 (+), 400(+)	100 (+), 200 (-), 400(+)
Zn content (roots)	200 (+)	200 (+), 400 (+)	-
Zn content (Shoots)	400 (+)	-	100 (+)
Mg	-	-	200 (+)
Mn	400 (+)	-	100 (+)
Cu	-	400 (+)	100(+), 200 (+)
Fe	-	100 (+)	200 (+)

Table note: Each number in the column represent the concentration (100,200,400 mg/kg) of treatment type; the (+) and (-) represent increase and decrease compared to control ($p \leq 0.05$)

Table 5.2: Foliar application of L-ascorbic acid on nZnO amended (500 mg/kg) soil-grown cilantro plants.

Parameters	n ZnO	Asc+ n ZnO	Zn^{2+}	Asc+ Zn^{2+}
Chlorophyll	-	-	-	-
Carotenoid		(-)		
MDA	-	-	-	-
Catalase	-	-	-	-
Biomass	-	(+)	-	-
Zn content (roots)			(+)	
Zn content (shoots)	(+)	(+)	(+)	(+)

Table 5.3: Comparative effects of ZnO nanoparticles and ionic zinc compounds on germination, biomass production of radish seedlings at concentration 0,100,200, and 400 mg/L

Parameter	nZnO	Zn^{2+}
Biomass	-	200 (-)
Germination	400 (-)	400 (-)
Zn content	200 (+)	100 (+) 200 (+) 400 (+)

References

Chapter 1

Awasthi, A., Bansal, S., Jangir, L. K., Awasthi, G., Awasthi, K. K., Awasthi, K. (2017, December). Effect of ZnO Nanoparticles on Germination of *Triticum aestivum* Seeds. In Macromolecular Symposia (Vol. 376, No. 1, p. 1700043).

Bandyopadhyay, S., Plascencia-Villa, G., Mukherjee, A., Rico, C. M., José-Yacamán, M., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2015). Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. Science of the Total Environment, 515, 60-69.

Dimkpa, C. O., Hansen, T., Stewart, J., McLean, J. E., Britt, D. W., Anderson, A. J. (2015). ZnO nanoparticles and root colonization by a beneficial pseudomonad influence essential metal responses in bean (*Phaseolus vulgaris*). Nanotoxicology, 9(3), 271-278.

Dyachenko, V. "Nanotechnologies of Future." (2017).

Elmer, W. H., White, J. C. (2016). The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. Environmental Science: Nano, 3(5), 1072-1079.

Keller, A. A., McFerran, S., Lazareva, A., Suh, S. (2013). Global life cycle releases of engineered nanomaterials. Journal of Nanoparticle Research, 15(6), 1692.

Kim, Y. H., Boykin, E., Stevens, T., Lavrich, K., Gilmour, M. I. (2014). Comparative lung toxicity of engineered nanomaterials utilizing in vitro, ex vivo and in vivo approaches. Journal of nanobiotechnology, 12(1), 47.

Lee, S., Chung, H., Kim, S., Lee, I. (2013). The genotoxic effect of ZnO and CuO nanoparticles on early growth of buckwheat, *Fagopyrum esculentum*. Water, Air, & Soil Pollution, 224(9), 1668.

Lin, D., & Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution, 150(2), 243-250.

Liu, X., Wang, F., Shi, Z., Tong, R., Shi, X. (2015). Bioavailability of Zn in ZnO nanoparticle-spiked soil and the implications to maize plants. Journal of Nanoparticle Research, 17(4), 175.

López-Moreno, M. L., de la Rosa, G., Hernández-Viezcas, J. Á., Castillo-Michel, H., Botez, C. E., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. Environmental science & technology, 44(19), 7315-7320.

Moghaddasi, S., Fotovat, A., Khoshgoftarmanesh, A. H., Karimzadeh, F., Khazaei, H. R., Khorassani, R. (2017). Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicology and environmental safety, 144, 543-551.

Mukherjee, A., Peralta-Videa, J. R., Bandyopadhyay, S., Rico, C. M., Zhao, L., Gardea-Torresdey, J. L. (2014). Physiological effects of nanoparticulate ZnO in green peas (*Pisum sativum* L.) cultivated in soil. Metallomics, 6(1), 132-138.

Peralta-Videa, J. R., Hernandez-Viezcas, J. A., Zhao, L., Diaz, B. C., Ge, Y., Priester, J. H., Holden, P.A., 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, 128-135.

Priester, J. H., Moritz, S. C., Espinosa, K., Ge, Y., Wang, Y., Nisbet, R. M., Holden, P. A., (2017). Damage assessment for soybean cultivated in soil with either CeO_2 or ZnO manufactured nanomaterials. Science of The Total Environment, 579, 1756-1768.

Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kim, B., Barrios, A. C., Medina-Velo, I. A., Gardea-Torresdey, J. L. (2018). Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environmental Pollution.

Siani, N. G., Fallah, S., Pokhrel, L. R., Rostamnejadi, A. (2017). Natural amelioration of zinc oxide nanoparticle toxicity in fenugreek (*Trigonella foenum-gracum*) by arbuscular mycorrhizal (*Glomus intraradices*) secretion of glomalin. Plant Physiology and Biochemistry, 112, 227-238.

Singh, D., Kumar, A. (2016). Impact of irrigation using water containing CuO and ZnO nanoparticles on Spinach oleracea grown in soil media. Bulletin of environmental contamination and toxicology, 97(4), 548-553.

Wang, F., Liu, X., Shi, Z., Tong, R., Adams, C. A., Shi, X., (2016). Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants–a soil microcosm experiment. Chemosphere, 147, 88-97.

Xu, J., Luo, X., Wang, Y., Feng, Y., (2018). Evaluation of zinc oxide nanoparticles on lettuce (*Lactuca sativa* L.) growth and soil bacterial community. Environmental Science and Pollution Research, 25(6), 6026-6035.

Yoon, S. J., Kwak, J. I., Lee, W. M., Holden, P. A., An, Y. J. (2014). Zinc oxide nanoparticles delay soybean development: a standard soil microcosm study. Ecotoxicology and environmental safety, 100, 131-137.

Zafar, H., Ali, A., Ali, J. S., Haq, I. U., Zia, M. (2016). Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and antioxidative response. Frontiers in plant science, 7, 535.

Zhao, L., Peralta-Videa, J. R., Rico, C. M., Hernandez-Viezcas, J. A., Sun, Y., Niu, G., Servin, A., Nunez, J.E., Gardea, M.D., 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), 2752-2759.

Chapter 2

Awasthi, A., Bansal, S., Jangir, L. K., Awasthi, G., Awasthi, K. K., Awasthi, K., 2017. Effect of ZnO Nanoparticles on Germination of Triticum aestivum Seeds. In Macromolecular Symposia 376, 1700043.

Adisa, I. O., Pullagurala, V. L., Rawat, S., Hernandez-Viezcas, J. A., Dimkpa, C., Elmer, W. H., White, J.C., Peralta-Videa, J.R., Gardea-Torresdey, J. L. (2018). Role of cerium compounds in Fusarium wilt suppression and growth enhancement in tomato (*Solanum lycopersicum*). Journal of agricultural and food chemistry.

Barrios, A. C., Rico, C. M., Trujillo-Reyes, J., Medina-Velo, I. A., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2016. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci. Total. Environ. 563, 956-964.

Burman, U., Saini, M., Kumar, P., 2013. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Environ. Toxicol. Chem.95, 605-12.

Cota-Ruiz, K., Delgado-Rios, M., Martínez-Martínez, A., Núñez-Gastelum, J. A., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2018. Current Findings on Terrestrial Plants-Engineered Nanomaterial Interactions: Are Plants Capable of Phytoremediating Nanomaterials from Soil? Current Opinion in Environmental Science & Health. https://doi.org/10.1016/j.coesh.2018.06.005

Fontanili, L., Lancilli, C., Suzui, N., Dendena, B., Yin, Y. G., Ferri, A., Ishii, S., Kawachi, N., Lucchini, G., Fujimaki, S., Sacchi, G. A., Nocito, F.F., 2016. Kinetic analysis of zinc/cadmium reciprocal competitions suggests a possible Zn-insensitive pathway for root-to-shoot cadmium translocation in rice. Rice, 9, 16.

Gaschler, M.M., Stockwell, B.R., 2017. Lipid peroxidation in cell death. Biochem. Biophys. Res. Commun. 482, 419-25.

García-Gómez, C., Obrador, A., González, D., Babín, M., Fernández, M. D. (2017). Comparative effect of ZnO NPs, ZnO bulk and ZnSO4 in the antioxidant defences of two plant species growing in two agricultural soils under greenhouse conditions. Science of the Total Environment, 589, 11-24.

García-Gómez, C., Babin, M., Obrador, A., Álvarez, J. M., & Fernández, M. D. (2015). Integrating ecotoxicity and chemical approaches to compare the effects of ZnO nanoparticles, ZnO bulk, and $ZnCl_2$ on plants and microorganisms in a natural soil. Environmental Science and Pollution Research, 22(21), 16803-16813.

Guadagno, C.R., Della Greca, M., De Santo, A.V., D'Ambrosio, N., 2013. NMR (1H) analysis of crude extracts detects light stress in beta vulgaris and *spinacia oleracea* leaves. Photosynthesis. Res. 115,115-22.

Gurmani, A.R., Khan, S.U., Andaleep, R., Waseem, K., Khan, A., 2012. Soil application of zinc improves growth and yield of tomato. Int. J. Agric. Biol. 14.

Keller AA and Lazareva A., 2013. Predicted releases of engineered nanomaterials: From global to regional to local. Environ. Sci. Technol. Lett. 1, 65-70.

Keller A.A., McFerran, S., Lazareva, A., Suh, S., 2013. Global life cycle releases of engineered nanomaterials. J. Nanoparticle Res 15, 1692.

Keller, A. A., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., Miller, R., Ji, Z. 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ. Sci. Technol. 44, 1962-1967.

Kobayashi, N. I., Tanoi, K., 2015. Critical issues in the study of magnesium transport systems and magnesium deficiency symptoms in plants. Int. J. Mol. Sci. 16 23076-23093.

Latef, Arafat Abdel Hamed Abdel, Alhmad MFA, Abdelfattah KE., 2017. The possible roles of priming with ZnO nanoparticles in mitigation of salinity stress in lupine (*lupinus termis*) plants. J Plant Growth Regul 36(1):60-70.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'neill, B., Skjemstad, J.O., Thies, J., Luizao, F.J., Petersen, J., Neves, E. G., 2006. Black carbon increases cation exchange capacity in soils. Soil. Sci. Soc. Am. J, 70, 1719-1730.

Liu, R., Lal, R., 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Sci Total Environ. 514,131-9.

López-Moreno, M. L., de la Rosa, G., Cruz-Jiménez, G., Castellano, L., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2017. Effect of ZnO nanoparticles on corn seedlings at different temperatures; X-ray absorption spectroscopy and ICP/OES studies. Microchem. J. 134, 54-61.

Medina-Velo, I.A., Barrios, A.C., Zuverza-Mena, N., Hernandez-Viezcas, J.A., Chang, C.H., Ji, Z., Zink, J.I., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2017. Comparison of the effects of commercial coated and uncoated ZnO nanomaterials and Zn compounds in kidney bean (*phaseolus vulgaris*) plants. J Hazard Mater 332, 214-22.

Montes, A., Bisson, M. A., Gardella, Jr J. A., Aga, D. S., 2017. Uptake and transformations of engineered nanomaterials: Critical responses observed in terrestrial plants and the model plant Arabidopsis thaliana. Sci. Total. Environ. 607, 1497-1516.

Mukherjee, A., Sun, Y., Morelius, E., Tamez, C., Bandyopadhyay, S., Niu, G., White, J.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016. Differential toxicity of bare and hybrid ZnO nanoparticles in green pea (*pisum sativum* L.): A life cycle study. Front. Plant. Sci. 6, 1242.

Ong, C. B., Ng, L. Y., Mohammad, A. W., 2018. A review of ZnO nanoparticles as solar photocatalysts: synthesis, mechanisms and applications. Renew. Sust. Energ. Rev. 81, 536-551.

Prasad, T., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R., Sreeprasad, T., Sajanlal, P., Pradeep, T., 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutr 35, 905-27.

Priester, J.H., Moritz, S.C., Espinosa, K., Ge, Y., Wang, Y., Nisbet, R.M., Schimel, J.P., Goggi, A.S., Gardea-Torresdey, J.L., Holden, P.A., 2017. Damage assessment for soybean cultivated in soil with either CeO_2 or ZnO manufactured nanomaterials. Sci Total Environ 579,1756-68.

Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kim, B., Barrios, A. C., Medina-Velo, I. A., Hernandez-Viezcas, J.A., Peralta-Videa, J.A., Gardea-Torresdey, J. L., 2018. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environmental Pollution. https://doi.org/10.1016/j.envpol.2018.06.036

Pullagurala, V. L. R., Rawat, S., Adisa, I. O., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2018. Plant uptake and translocation of contaminants of emerging concern in soil. Sci Total Environ 636, 1585-1596.

Ramel, F., Birtic, S., Ginies, C., Soubigou-Taconnat, L., Triantaphylidès, C., Havaux, M., 2012. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc. Natl. Acad. Sci. 109, 5535-5540.

Rawat, S., Pullagurala, V. L., Hernandez-Molina, M., Sun, Y., Niu, G., Hernandez-Viezcas, J. A., Peralta-Videa, J.R., Gardea-Torresdey, J. L. 2018. Impacts of copper oxide nanoparticles on bell pepper (*Capsicum annum* L.) plants: a full life cycle study. Environ. Sci: Nano 5, 83-95.

Reddy, P.V.L., Hernandez-Viezcas, J., Peralta-Videa, J., Gardea-Torresdey, J., 2016. Lessons learned: Are engineered nanomaterials toxic to terrestrial plants? Sci Total Environ 568, 470-9.

Ruotolo, R., Maestri, E., Pagano, L., Marmiroli, M., White, J. C., Marmiroli, N. (2018). Plant response to metal-containing engineered nanomaterials: an omics-based perspective. Environ. Sci. Technol. 52(5), 2451-2467.

Samreen, T., Shah, H.U., Ullah, S., Javid, M., 2017. Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*vigna radiata*). Arab. J. Chem. 10, S1802-7.

Shaw, A.K., Hossain, Z., 2013. Impact of nano-CuO stress on rice (*oryza sativa* L.) seedlings. Chemosphere 93, 906-15.

Singh, N., Amist, N., Yadav, K., Singh, D., Pandey, J., Singh, S., 2013. Zinc oxide nanoparticles as fertilizer for the germination, growth and metabolism of vegetable crops. Journal of Nanoengineering and Nanomanufacturing 3, 353-64.

Smeraldi,, J., Ganesh, R., Hosseini, T., Khatib, L., Olson, B. H., Rosso, D., 2017. Fate and Toxicity of Zinc Oxide Nanomaterial in Municipal Wastewaters. Water Environ Res. 89, 880-889.

Spencer, H., Norris, C., Williams, D., 1994. Inhibitory effects of zinc on magnesium balance and magnesium absorption in man. J Am Coll Nutr 13, 479-84.

Tan, W., Gao, Q., Deng, C., Wang, Y., Lee, W., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey JL. 2018. Foliar exposure of cu $(OH)_2$ nanopesticide to basil (*ocimum basilicum*): Variety-dependent copper translocation and biochemical responses. J Agric Food Chem.

Venkatachalam, P., Priyanka, N., Manikandan, K., Ganeshbabu, I., Indiraarulselvi, P., Geetha, N., Muralikrishna, K., Bhattacharya, R., Tiwari, M., Sharma, N., 2017. Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (*gossypium hirsutum* L.). Plant Physiol Biochem. 110,118-27.

Verma, S. K., Das, A. K., Patel, M. K., Shah, A., Kumar, V., Gantait, S., 2018. Engineered nanomaterials for plant growth and development: A perspective analysis. Sci Total Environ 630, 1413-1435.

Wang, C., Yang, Z., Yuan, X., Browne, P., Chen, L., Ji, J., 2013. The influences of soil properties on cu and zn availability in soil and their transfer to wheat (*triticum aestivum* L.) in the yangtze river delta region, china. Geoderma 193,131-9.

Wang, X., Yang, X., Chen, S., Li, Q., Wang, W., Hou, C., Gao, X., Wang, L., Wang, S., 2016. Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in arabidopsis. Front. Plant. Sci. 6, 1243.

Wang, J., Liu, S., Li, C., Wang, T., Zhang, P., Chen, K., 2017. PnLRR-RLK27, a novel leucine-rich repeats receptor-like protein kinase from the Antarctic moss *Pohlia nutans*, positively regulates salinity and oxidation-stress tolerance. PloS one, 12, e0172869.

Watson, J., Fang, T., Dimkpa, C.O., Britt, D.W., McLean, J.E., Jacobson, A., Anderson, A.J., 2015. The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. Biometals. 28,101-12.

Xiong, D., Fang, T., Yu, L., Sima, X., Zhu, W., 2011. Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409,1444-52.

Zhao, L., Hu, J., Huang, Y., Wang, H., Adeleye, A., Ortiz, C., Keller, A. A., 2017. 1 H NMR and GC–MS based metabolomics reveal nano-Cu altered cucumber (*Cucumis sativus*) fruit nutritional supply. Plant Physiol Biochem. 110, 138-146.

Chapter 3

Agami, R. A. (2014). Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. Biologia plantarum, 58(2), 341-347.

Alam, M., Juraimi, A. S., Rafii, M. Y., Abdul Hamid, A. (2015). Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of Purslane (*Portulaca oleracea* L.) accessions. BioMed research international, 2015.

Amooaghaie, R., Norouzi, M., & Saeri, M. (2016). Impact of zinc and zinc oxide nanoparticles on the physiological and biochemical processes in tomato and wheat. Botany, 95(5), 441-455.

Athar, R., Ashraf, M. (2006). Interactive effect of foliarly applied ascorbic acid and salt stress on wheat (*Triticum aestivum* L.) at the seedling stage. Pak. J. Bot, 38(5), 1407-1414.

Bandyopadhyay, S., Plascencia-Villa, G., Mukherjee, A., Rico, C. M., José-Yacamán, M., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2015). Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. Science of the Total Environment, 515, 60-69.

Barrios, A. C., Rico, C. M., Trujillo-Reyes, J., Medina-Velo, I. A., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2016. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci. Total. Environ. 563, 956-964.

Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J., Guo, H. (2011). TiO 2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. Journal of Environmental Monitoring, 13(4), 822-828.

Foyer, C. H. (2017). Ascorbic acid. In Antioxidants in higher plants (pp. 31-58). CRC press.

Garg, S. K., Mahajan, S. (1993). Effect of ascorbic acid on longevity, catalase and lipid peroxidation in *Callosobruchus maculatus* F. Age, 16(3), 87-92.

Hussain, I., Siddique, A., Ashraf, M. A., Rasheed, R., Ibrahim, M., Iqbal, M., Akbar, S., Imran, M. (2017). Does exogenous application of ascorbic acid modulate growth, photosynthetic pigments and oxidative defense in okra (*Abelmoschus esculentus* (L.) Moench) under lead stress?. Acta Physiologiae Plantarum, 39(6), 144.

Karanjalker, G. R., Ravishankar, K. V., Shivashankara, K. S., Dinesh, M. R., Roy, T. K., Rao, D. S. (2018). A study on the expression of genes involved in carotenoids and anthocyanins during ripening in fruit peel of green, yellow, and red colored mango cultivars. Applied biochemistry and biotechnology, 184(1), 140-154.

Kasote, D. M., Katyare, S. S., Hegde, M. V., Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. International journal of biological sciences, 11(8), 982.

Keller, A. A., Adeleye, A. S., Conway, J. R., Garner, K. L., Zhao, L., Cherr, G. N., Hong, J., Gardea –Torresdey, J. L., Godwin, H, A., Hanna, S., Ji, Z., Kaweeterawat, C., Lin, S., Lrniham, H.S., Miller, R.J., Nel, A, E., Perlata, J.R., Walker, S.L., Taylor, A.A., Torres-Duarte, Ac., Zink, J.I., Torres-Duarte, C., Zink, J.I., Zuverza-Mena, N., (2017). Comparative environmental fate and toxicity of copper nanomaterials. NanoImpact, 7, 28-40.

Keller, A. A., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., ... & Ji, Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environmental science & technology, 44(6), 1962-1967.

Khoroshyy, P., Bína, D., Gardian, Z., Litvín, R., Alster, J., Pšenčík, J. (2018). Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. Photosynthesis research, 135(1-3), 213-225.

Lin, D., & Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. Environmental science & technology, 42(15), 5580-5585.

Lv, J., Zhang, S., Luo, L., Zhang, J., Yang, K., & Christie, P. (2015). Accumulation, speciation and uptake pathway of ZnO nanoparticles in maize. Environmental Science: Nano, 2(1), 68-77.

Medina-Velo, I.A., Barrios, A.C., Zuverza-Mena, N., Hernandez-Viezcas, J.A., Chang, C.H., Ji, Z., Zink, J.I., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2017. Comparison of the effects of commercial coated and uncoated ZnO nanomaterials and Zn compounds in kidney bean (*phaseolus vulgaris*) plants. J Hazard Mater 332, 214-22.

Mohsenzadeh, S., & Moosavian, S. S. (2017). Zinc Sulphate and Nano-Zinc Oxide Effects on Some Physiological Parameters of *Rosmarinus officinalis*. American Journal of Plant Sciences, 8(11), 2635.

Mukherjee, A., Peralta-Videa, J. R., Bandyopadhyay, S., Rico, C. M., Zhao, L., & Gardea-Torresdey, J. L. (2014). Physiological effects of nanoparticulate ZnO in green peas (Pisum sativum L.) cultivated in soil. Metallomics, 6(1), 132-138.

Ndeh, N. T., Maensiri, S., & Maensiri, D. (2017). The effect of green synthesized gold nanoparticles on rice germination and roots. Advances in Natural Sciences: Nanoscience and Nanotechnology, 8(3), 035008.

Noman, A., Ali, S., Naheed, F., Ali, Q., Farid, M., Rizwan, M., Irshad, M. K. (2015). Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. Archives of Agronomy and Soil Science, 61(12), 1659-1672.

Noman, A., Ali, S., Naheed, F., Ali, Q., Farid, M., Rizwan, M., Irshad, M. K. (2015). Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. Archives of Agronomy and Soil Science, 61(12), 1659-1672.

Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kim, B., Barrios, A. C., Medina-Velo, I. A., Hernandez-Viezcas, J.A., Peralta-Videa, J.A., Gardea-Torresdey, J. L., 2018. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environmental Pollution

Pullagurala, V. L. R., Rawat, S., Adisa, I. O., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). Plant uptake and translocation of contaminants of emerging concern in soil. Science of The Total Environment, 636, 1585-1596.

Reddy, P. A. K., Manvitha, C., Reddy, P. V. L., Kim, K. H., & Kumari, V. D. (2017). Enhanced hydrogen production activity over BiOXTiO2 under solar irradiation: Improved charge transfer through bismuth oxide clusters. Journal of energy chemistry, 26(3), 390-397.

Reddy, P. V. L., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2016). Lessons learned: are engineered nanomaterials toxic to terrestrial plants?. Science of the Total Environment, 568, 470-479.

Samart, S., Chutipaijit, S., & Phakamas, N. (2017). Evaluating the effect of zinc oxide nanoparticles on the physiological responses of nine non-photoperiod sensitive rice cultivars. Materials Today: Proceedings, 4(5), 6430-6435.

Sazanova, K. A., Bashmakov, D. I., BRAZAITYTĖ, A., BOBINAS, Č., Duchovskis, P., & Lukatkin, A. S. (2012). The effect of heavy metals and thidiazuron on winter wheat (*Triticum aestivum* L.) seedlings. Žemdirbystė= Agriculture, 99(3), 273-278.

Sears, M. E. (2013). Chelation: harnessing and enhancing heavy metal detoxification—a review. The Scientific World Journal, 2013.

Shaw, A.K., & Hossain, Z., 2013. Impact of nano-CuO stress on rice (*oryza sativa* L.) seedlings. Chemosphere 93, 906-15.

Venkatachalam, P., Priyanka, N., Manikandan, K., Ganeshbabu, I., Indiraarulselvi, P., Geetha, N., Muralikrishna, K., Bhattacharya, R., Tiwari, M., Sharma, N., 2017. Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (*gossypium hirsutum* L.). Plant Physiol Biochem. 110,118-27.

Wang, J., Liu, S., Li, C., Wang, T., Zhang, P., Chen, K., 2017. PnLRR-RLK27, a novel leucine-rich repeats receptor-like protein kinase from the Antarctic moss *Pohlia nutans*, positively regulates salinity and oxidation-stress tolerance. PloS one, 12, e0172869.

Xu, J., Luo, X., Wang, Y., & Feng, Y. (2018). Evaluation of zinc oxide nanoparticles on lettuce (*Lactuca sativa* L.) growth and soil bacterial community. Environmental Science and Pollution Research, 25(6), 6026-6035.

Chapter 4

Alam, M., Juraimi, A. S., Rafii, M. Y., & Abdul Hamid, A. (2015). Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of Purslane (*Portulaca oleracea* L.) accessions. BioMed research international, 2015.

Amooaghaie, R., Norouzi, M., Saeri, M. (2016). Impact of zinc and zinc oxide nanoparticles on the physiological and biochemical processes in tomato and wheat. Botany, 95(5), 441-455.

Awasthi, A., Bansal, S., Jangir, L. K., Awasthi, G., Awasthi, K. K., Awasthi, K. (2017, December). Effect of ZnO Nanoparticles on Germination of *Triticum aestivum* Seeds. In Macromolecular Symposia (Vol. 376, No. 1, p. 1700043).

Bandyopadhyay, S., Plascencia-Villa, G., Mukherjee, A., Rico, C. M., José-Yacamán, M., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2015). Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. Science of the Total Environment, 515, 60-69.

Bentsink, L., Koornneef, M. (2008). Seed dormancy and germination. The Arabidopsis Book/American Society of Plant Biologists, 6.

Bradfield, S. J., Kumar, P., White, J. C., Ebbs, S. D. (2017). Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: yield effects and projected dietary intake from consumption. Plant Physiology and Biochemistry, 110, 128-137.

Breen, A. N., Richards, J. H. (2008). Irrigation and fertilization effects on seed number, size, germination and seedling growth: implications for desert shrub establishment. Oecologia, 157(1), 13-19.

Chen, Y. T., Wang, Y., Yeh, K. C. (2017). Role of root exudates in metal acquisition and tolerance. Current opinion in plant biology, 39, 66-72.

de la Rosa, G., López-Moreno, M. L., de Haro, D., Botez, C. E., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2013). Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: root development and X-ray absorption spectroscopy studies. Pure and Applied Chemistry, 85(12), 2161-2174.

Du, W., Tan, W., Peralta-Videa, J. R., Gardea-Torresdey, J. L., Ji, R., Yin, Y., Guo, H. (2017). Interaction of metal oxide nanoparticles with higher terrestrial plants: physiological and biochemical aspects. Plant Physiology and Biochemistry, 110, 210-225.

Ebbs, S. D., Bradfield, S. J., Kumar, P., White, J. C., Musante, C., Ma, X. (2016). Accumulation of zinc, copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and metal ions. Environmental Science: Nano, 3(1), 114-126.

Elizabath, A., Bahadur, V., Mistra, P., Prasad, V. M., Thomas, T. (2017). Effect of different concentrations of iron oxide and zinc oxide nanoparticles on growth and yield of carrot (Daucus carota L.). J Pharmacogn Phytochem, 6, 1266-1269.

Garner, K. L., Suh, S., Keller, A. A. (2017). Assessing the risk of engineered nanomaterials in the environment: development and application of the nanoFate model. Environmental science & technology, 51(10), 5541-5551.

Hart, J. J., Norvell, W. A., Welch, R. M., Sullivan, L. A., Kochian, L. V. (1998). Characterization of zinc uptake, binding, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiology, 118(1), 219-226.

Hermans, C., Hammond, J. P., White, P. J., Verbruggen, N. (2006). How do plants respond to nutrient shortage by biomass allocation?. Trends in plant science, 11(12), 610-617.

Keller, A. A., McFerran, S., Lazareva, A., Suh, S. (2013). Global life cycle releases of engineered nanomaterials. Journal of Nanoparticle Research, 15(6), 1692.

Keller, A. A., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., Miller, R., Ji, Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environmental science & technology, 44(6), 1962-1967.

Korishettar, P., Vasudevan, S. N., Shakuntala, N. M., Doddagoudar, S. R., Hiregoudar, S., Kisan, B. (2016). Seed polymer coating with Zn and Fe nanoparticles: An innovative seed quality enhancement technique in pigeonpea. Journal of Applied and Natural Science, 8(1), 445-450.

Lee, S., Chung, H., Kim, S., Lee, I. (2013). The genotoxic effect of ZnO and CuO nanoparticles on early growth of buckwheat, *Fagopyrum esculentum*. Water, Air, & Soil Pollution, 224(9), 1668.

Li, W., Khan, M. A., Yamaguchi, S., Kamiya, Y. (2005). Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. Plant growth regulation, 46(1), 45-50.

Lin, D., Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. Environmental science & technology, 42(15), 5580-5585.

López-Moreno, M. L., de la Rosa, G., Hernández-Viezcas, J. Á., Castillo-Michel, H., Botez, C. E., Peralta-Videa, J. R., Gardea-Torresdey, J. L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (Glycine max) plants. Environmental science & technology, 44(19), 7315-7320.

Mahajan, P., Dhoke, S. K., Khanna, A. S. (2011). Effect of nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. Journal of Nanotechnology, 2011.

McCall, K. A., Huang, C. C., Fierke, C. A. (2000). Function and mechanism of zinc metalloenzymes. The Journal of nutrition, 130(5), 1437S-1446S.

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., 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), 6224-6230.

Moreira, A., Moraes, L. A., dos Reis, A. R. (2018). The Molecular Genetics of Zinc Uptake and Utilization Efficiency in Crop Plants. In Plant Micronutrient Use Efficiency (pp. 87-108).

Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Functional Plant Biology, 27(12), 1191-1191.

Prasad, T. N. V. K. V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K. R.. Sreeprasad, T.S., Sajanlal, P.R., Pradeep, T. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of plant nutrition, 35(6), 905-927.

Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kim, B., Barrios, A. C., Medina-Velo, I. A., ... & Gardea-Torresdey, J. L. (2018). Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environmental Pollution.

Pullagurala, V. L. R., Rawat, S., Adisa, I. O., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). Plant uptake and translocation of contaminants of emerging concern in soil. Science of The Total Environment, 636, 1585-1596.

Rico, C. M., Peralta-Videa, J. R., 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. Applied spectroscopy, 69(2), 287-295.

Suresh, S., Karthikeyan, S., Jayamoorthy, K. (2016). FTIR and multivariate analysis to study the effect of bulk and nano copper oxide on peanut plant leaves. Journal of Science: Advanced Materials and Devices, 1(3), 343-350.

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., 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), 2752-2759.

Zuverza-Mena, N., Armendariz, R., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2016). Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. Frontiers in plant science, 7, 90.

Zuverza-Mena, N., Martínez-Fernández, D., Du, W., Hernandez-Viezcas, J. A., Bonilla-Bird, N., López-Moreno, M. L., ... & Gardea-Torresdey, J. L. (2017). Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. Plant Physiology and Biochemistry, 110, 236-264.

Appendix

Table S1: The metal content in shoots of cilantro exposed to all three treatments ((nZnO, Zn^{2+} and bZnO) at three different concentrations 100,200,400 ppm. The results are compared to control and different letters indicate statistically significant differences between treatments. Data calculated are means ± SE, whole analysis was carried out using three replicate and compared to control ($p \leq 0.05$).

Concentrations	Mn	Cu	Mg	Fe
Control	53.3333±2.66c	3.6667±0.33c	3042.6667±128.73ab	45.6667+6.3c
n100	408±45.61abc	6.2333±0.712c	2500.6667±249.71b	84.6667+8.83bc
b100	207.3333±91.82bc	7.0667±1.88c	2631.3333±677.419b	410.6667+130.2a
Zn^{2+} 100	796.6667±205.90a	16.2±2.620b	4726± 601.87b	178.6667+20.66abc
n200	452±27.46abc	5.7±0.305c	2942.6667±28.9ab	103.6667+6.69bc
b200	314±11.71abc	6.4333±0.145c	3768.6667±77.76ab	95.6667+5.23bc
Zn^{2+} 200	542.3333±64.31abc	16.8±1.307a	4726± 601.8a	312+56.41ab
n400	567± 194.76ab	8.5167±0.58bc	4060.6667± 528.11ab	148.6667+24.05bc
b400	410.6667± 47.06abc	14.2667±0.033ab	3142±18.50ab	158.3333+4.33bc
Zn^{2+} 400	298± 24.51bc	7.7333±0.233c	4449.3333± 86.9ab	102.3333+11.1bc

Table S2: The metal content in roots of cilantro exposed to all three treatments ((nZnO, Zn^{2+} and bZnO)) at three different concentrations 100,200,400 ppm. The results are compared to control and different letters indicate statistically significant differences between treatments. Data calculated are means ± SE, whole analysis was carried out using three replicate and compared to control ($p \leq 0.05$).

Concentrations	Cu	Fe	Mn	Mg
Control	12.6667+7.68	611.3333+334.12	56.3333+19.15c	2738+271
n100	17.6667+1.45	1171+305.47	163.3333+47.86abc	1374.3333+114.44
b100	17+3.0	2218+109	185.3333+90.87bc	1320.6667+517.66
Zn^{2+} 100	40+1.15	1542.6667+1105.79	148+0	2216.6667+492.41
n200	43.6667+19.66	1186.3333+192.33	371.3333+94.333	2653.6667+562.6
b200	26.6667+12.66	440+112	142.3333+73.33	1573.6667+324.6
Zn^{2+} 200	31.3333+7.33	775.3333+264.66	102+32.0	1321+557
n400	28+9.53	973.3333+322.16	125.6667+37.97	1250.3333+403.25
b400	27+3.0	323.6667+65.33	52.6667+5.66	1898.3333+188.33
Zn^{2+} 400	27+3.0	320.6667+63.88	52.6667+5.66	1898.3333+188.33

Table S3. The soil characterization (adapted from Barrios et al. (2016))

Miracle-Gro® Potting Mix	**%**	**Concentration (mg/kg)** **Average ± SE**	
Forest products, compost, sphagnum peat moss, perlite, wetting agent and fertilizer	50-60	7551.28 ± 447.58	Al
Total nitrogen (N)*	0.21	29570.39 ± 3406.41	Ca
ammoniacal nitrogen	0.12	30.52 ± 4.97	Cu
nitrate nitrogen	0.09	4653.38 ± 404.12	Fe
Available phosphate (P_2O_5)	0.07	1868.65 ± 92.83	K
Soluble potash (K_2O)*	0.14	3110.12 ±789.19	Mg
Iron (Fe)	0.1	197.67 ± 12.08	Mn
water soluble iron (Fe)	0.1	1818.36 ± 261.48	P
		44.22 ± 5.22	Zn
Derived from: polymer coated: ammonium nitrate, ammonium phosphate, calcium phosphate, and potassium phosphate; and ammonium nitrate, ammonium phosphate, calcium phosphate, potassium sulfate, and ferrous sulfate.			
* A portion of the nitrogen, phosphate and potash has been coated to provide 0.15% coated slow release nitrogen (N), 0.03% coated slow release available phosphate (P_2O_5) and 0.08% coated slow release soluble potash (K_2O)			

Table S4: Summary of FTIR band frequencies in plants exposed to ENMs and other contaminants (Zuverza-Mena et al., 2016)

Frequency	Functional group	Molecular tissue
3100-2800	CH2 asymmetric	Lipids
	CH3 symmetric	Lipids
	CH2 symmetric	Lipids
1700-1500	C=O, C-N	Protein
1200-900	C-O-C	Lignin
	C-O	Starch

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Vitae

Venkata Laxma Reddy Pullagurala, (Reddy) was born in Hyderabad, Telangana, India. He received his bachelors of Science degree and majored in biotechnology and chemistry from Osmania University, Hyderabad, India in the year 2008. His master's degree was obtained in environmental science from Osmania University, Hyderabad in the year 2010. The master dissertation research work was carried out under the supervision of Dr. Valluri Durga Kumari (Scientist) at a prestigious institute "Indian Institute of Chemical Technology" (IICT) at Hyderabad, India. The title of dissertation was "Photocatalytic degradation of isoproturon pesticide by using C, N, and S doped titania. His master's dissertation and collaborative work has resulted in four peer-reviewed publications. In the year fall 2014, he joined doctoral research program in environmental science and engineering (ESE) at the University of Texas at El Paso (UTEP). The doctoral research work was carried out under the supervision of Dr. Jorge Gardea-Torresdey (Dudley professor of chemistry and ESE).

During these 4 years, his collaborative work with other scholars resulted in six peer-review journal papers as a first author and co-author. Additionally he has another five manuscripts and a book chapter, which are either submitted or ready to be submitted.

Reddy is a member of the University of California's center for environmental implications of nanotechnology (UC CEIN) and was invited to attend site visits and workshops. While pursuing his doctoral research in ESE program, Reddy worked as teaching assistant in the chemistry department. He also served as judge for undergraduate research at COURI symposium, UTEP.

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