INTERACTION OF NANOPARTICLES (ZNO & CEO_2) AND COEXISTENCE OF HEAVY METALS/ METALLOIDS (CD, PB & AS) WITH DIETARY PLANTS, A FOOD SAFETY PERSPECTIVE

A Dissertation

by

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ABSTRACT

Crops and leafy vegetables are a rich source of protein, essential minerals, and fibers for the human diet, which may turn to toxic plants if exposed to heavy metals or metalloid contamination. Cadmium (Cd), Lead (Pb) and Arsenic (III/V) are extremely toxic elements to humans even at trace concentrations. Elevated levels of such compounds in agricultural soil due to various industrial activities lead to their hyper accumulation in a variety of dietary plant tissues and food products, which raise food safety concerns for consumers. Due to advances in nanotechnology-facilitated agrichemicals, engineered nanoparticles (ENPs) are substantially entering into agricultural systems. Exposure of edible plants with the coexistence of ENPs and heavy metals can alter their uptake and localization of essential minerals such as Fe through different mechanisms. However, the change of this uptake pattern is very critical for the leafy vegetable compared to crops, because of their direct consumption by humans. The goal of this research is to understand the mutual effects of metallic oxide nanoparticles (CeO_2 & ZnO) and coexisting divalent heavy metals Pb^{2+} and Cd^{2+} and metalloids (As(III/V) on their uptake and accumulation as well localization of essential minerals in biological systems of a grain plants, Soybean (*Glycine max (L.) Merr*.), and four leafy vegetables including Spinach (*Spinaciae oleracea*), Parsley (*Petroselinum sativum*),Romaine lettuce (*Lactuca sativa L. var. Longifolia*), cilantro (*Coriandrum sativum*)by Romaine lettuce (*Lactuca sativa L. var. Longifolia*) in soil and hydroponic systems in both pre-harvesting and post-harvesting stages of plant life. At termination, shoots were gently separated from the roots, and the concentrations of Pb, Cd, Fe, Cu, Ce and Zn in all plant tissues were quantified by

inductively coupled plasma-mass spectrometry (ICP-MS). In addition, microbial density analysis in the growth media was performed for the treatments in some studies. The last chapter focused on the role of ZnO nanoparticles on the shelf life of tomato (*Solanum Lycopersicum*). The results will shed light on the role of ENPs on governing the essential elements translocation as well as their key role in alleviating the bioavailability of heavy metals to leafy vegetables and crops which are produced in hydroponics and soil.

DEDICATION

I dedicate my dissertation work to God, my source of inspiration, wisdom, knowledge and understanding, to children whose parents have divorced, and they are affected in many ways through their entire life. To my Mom who sacrificed her life, health and young age to raise me alone. To my wife Melina that without her support, I may have given up, who loves me unconditionally and supports me in all situations. To my grandmother and grandfather for all their support. To my American parents, Jalaldin and Fay Martin Mansourzadeh, for all their love and support. Also, to my many friends including Fahad Asiri and Dr. Ali Ardalan and church family, especially Doug and Dian Chandler who have supported me throughout this journey.

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Contributors

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All other work conducted for the dissertation was completed by the student independently.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1. Application of nanotechnology in food safety

Due to the rapid advancement of nanotechnology in industrial areas, engineering nanoparticles have established their role as an engineered indicator for many fundamental implications including medicine, nutrition, agriculture, personal care, electronics, energy, transport, and communications. The global investment in nanotechnologies market was estimated up to $10 billion by 2005 [1], the growth of this market led to an annual rate of $1 trillion from 2011 to 2015 [2].

The massive growth of this sector is due to the novel physicochemical properties of nanomaterials, which characterize their applicability in different fields [3]. Chemically, nanomaterials are provided from organic sources (i.e., quantum dots, nanotubes, and fullerene) or inorganic (i.e., zero-valent metals and metal oxides/salts). Physical properties include size range (<100 nm), shape, surface area, aggregation state, zeta potential, density and color [4, 5]. The combination of both physical and chemical properties put nanomaterials in three fundamental classes: i) nanoparticulates; ii) nanoplatelets; and iii) nanofibers [6].

Engineered nanoparticles (ENPs) represents a class of manufactured based materials with especial physicochemical characteristics and coatings; unique mechanical, electrical, thermal, and imaging

characteristics, which make them highly suitable for a variety of applications within the agriculture, medical and environmental engineering [7].

Presence of nanoparticles in foods can naturally occur through the environmental contamination or intentionally through different engineering applications. The properties of the engineering nanoparticles (ENPs) offer many new opportunities for food industries, such as more efficient coloring of the foods, adding a variety of flavors and nutritional elements[8]. Nanoparticles can also apply as antimicrobial ingredients for food packaging, anti-fungal for post harvested agro-commodities and controlled release of pesticides and fertilizers[9, 10].

There are many nanoparticles designed and implemented for diverse food applications at industrial scale. For example, titanium dioxide and zinc oxide nanoparticles have been widely used as food additive and antimicrobial agents for food packaging and in storage vessels[8, 11]. Some studies, reported the applications of silver (Ag) nanoparticles as antimicrobial agents for the food storage packaging, and in coating of the chopping boards and refrigerators[8, 12].

Providing the direct use of safe irrigation and healthy crops during the flooding events is one of the innovative applications of the nanoparticles in food safety prospective. It can economically and environmentally benefit the Texas State Water Plan and further reduce the load of wastewater treatment for urban planning. ENPs possess special properties, such as high surface area and adsorption capacity, easy operation, and cost-effective production [9, 13, 14]. To increase the food safety during the flood events as well as many other environmental incidents, there is high interest in the use of ENPs for the removal of heavy metals in aqueous systems such as flooded condition and immobilizing them into soil composite or retain their uptake by dietary plants[14, 15]. ENPs can change the hazardous state of the toxic metal ions into a stable or safe state and reduce the need for the treatment of flooding water for agriculture and immobilize the heavy metals in the

soil [16, 17]. Further, ENPs have demonstrated to increase crops tolerance to the water stress [18, 19]. In addition, large-scale applications of ENPs in crop lands to reduce the uptake of heavy metals is economically feasible.

Several studies indicated the high adsorption capacity of heavy metals and organic compounds onto ENPs surfaces, and strong attachment of ENPs on the sediment properties [10, 20]. For example, CeO_2-ENPs display high adsorption capacity for hazardous heavy metals such as arsenic (As), lead (Pb) and cadmium (Cd) [13, 16]. The CeO_2-ENPs surface by immobilizing the Cd^{2+} can reduce its uptake into plant tissues or retain Cd^{2+} in plant rhizosphere when and hold them in soil composite [14, 21]. In such a biological matrix, sediment morphology and composition of soil play a critical role in adsorption efficiency of heavy metals on ENPs.

In food safety prospective, metal-ENPs (Au, Ag, Ni, Zn, Cu, and Fe) and metal oxides-ENPs (TiO_2, SiO_2, CeO_2, Fe_3O_4, and Al_2O) are the most frequently used in agriculture as nano-pesticide, nano-fertilizers, and nano-sensors[3, 22, 23].

Many research studies and critical reviews have been focused on behavior, effects and environmental fate of ENPs in different media including water and soil. Studies on the application of ENPs in agricultural fields is missing. This chapter aims to discuss the application of the ENPs in the most recent revolutionary use of nanotechnology in increasing the agricultural food safety and security.

1.2. Nano-fertilizers

Agriculture plays a critical role in the economy of developing countries where the increasing global population growth calls for efficient agriculture products. Conversely, climate and environmental

changes are affecting agriculture production. As a result, the agriculture system of any country is a point of concern to deal with sustainable development. In this regard, nanotechnology is a promising tool for sustainable agriculture by providing of slow and controlled release fertilizers [24].

The natural composition of soil consists of organic and inorganic nanomaterials including aluminosilicate minerals; oxides and hydroxides of iron (Fe), aluminum (Al), and manganese (Mn); humic substances and mobile colloids [25]. The hydrodynamic diameters of most ENPs fall inside the range of these natural nanoparticles. Therefore, ENPS exhibit high natural compatibility within the soil structure [26]. Several researchers have suggested that the presence of ENPs can be beneficial to soil matrix by adding the meta-pedogenetic factors [27]. In fact, metallic ENPs in soil increase wettability of agricultural formulations, and positively impact the soil microbial structure and function via influencing nutrient turnover in soil over long time exposure [28]. Also, metallic ENPs may directly or indirectly alter the formation of symbiotic associations with root fungi and bacteria; influencing nutrient availability, uptake and plant growth [28]. For example, Zhang et al. 2017 reported the ENPs-CeO_2 increased the growth of radish storage roots [29].

Several studies indicated the beneficial application of ENPs in the germination phase of an agricultural process. In this process, the main critical factor in the stimulation of seed germination to grow faster is the diffusion of ENPS into the seed [30]. Zheng et al. 2005, reported a 73% more dry weight of spinach seeds when treated by ENPs-TiO_2 (at an optimum dose of 2.5‰). In addition, the photosynthetic rate increased three times higher than control, and an increase of 45% in chlorophyll *a*, and 28% chlorophyll *b* were observed in comparison to the control seeds (with no TiO_2) during 30 days of germination [31].

A different study demonstrated the carbon nanotubes/materials (CNMS) improve the water uptake in seeds of the tomato (*Solanum lycopersicum)*. As a result, the multi-walled carbon nanotubes (MWCNTs) at a concentration range of 10-40 ug/ml increased the moisture uptake (which was three times greater than control) leading to an increase up to 90% of the germination rate within 20 days [32]. In the same way, applying fullerol [C_{60} (OH) $_{20}$] at a productive dose of (47 nM) through a CNMS- seed treatment increased the biomass yield of bitter melon (Momordica charantia) more than 50% over 85 days of harvesting. [33]. The productivity of this nanoparticle resulted in significant increases in fruit number (60%) and weight (70%), which raised the total fruit yield of bitter melon up to 128%[33]. Due to this beneficial role, the fullerol has been suggested as a potential candidate for drought resistance in sugar beets (*Beta vulgaris* L.) via foliar application (20 full pump amplitudes) during three months of drought condition for the four months aged plants. This approach potentially increases the intercellular water supply, which enables ENPs to bind with available water in cell compartments and easily absorb onto the plant leaf and root tissues [34]. Moreover, the foliar spray application assists in avoiding the toxicity effects of soil for certain ENPS. For example, foliar spray of the of zinc (Zn) and boron (B) nano-fertilizers on pomegranate (*Punica granatum* L. cv. Ardestani) resulted in promoting the quality of the fruit yield by increasing the number of fruits per tree while conserving its physical and nutritional characteristics [35].

In addition to the utilization of ENPS as fertilizer, porous nanostructure media such as prepared nanocomposite (*i.e.,* zeolite and silica-based) were proposed as an efficient low-release of nutrients for soil enrichment, plant growth and controlled delivery of pesticides, guaranteeing long-term bioavailability of macronutrients and micronutrients to plants [24, 36]. Development of such nanostructure composition can bloom agroeconomic by saving more water and land and dealing

with drought condition. However, the research on a hybrid complex between nanostructured porous media and the implication of known ENPS as fertilizer elements can open a new research horizon in AgroSciences. Research in this area can overcome the deficiency of this nano-matrices (i.e., Loading cations). Due to the low mobility of ENPs and their stimulating properties onto the cells of the root, an empirical ratio of organic components can enhance the efficiency of such nanostructure, which has not been researched yet.

1.3. Nano-Pesticides

One of the main concerns in agricultural food production is the applications of efficient pesticides and antibacterial agents with no contamination of water or soil environment. Conventional pesticides sometimes leave their byproducts, which stay in the environment as persistent organic pollutants (POPs) and cause serious risks. For instance, by-products of a organochlorines pesticides (including DDT, methoxychlor, lindane, chlordane, dieldrin, and benzene hexachloride), which are frequently used in agriculture to control insects are associated with endocrine disruption impacts on human health, the high annual rate of 1 million cases has been reported [37, 38]. ENPs (*i.e.*, TiO_2, zero-valent Fe and carbon nanotubes) were introduced in agricultural practice as an effective tool in the removal of such POPs through photocatalytic degradation [37, 39]. In addition, metallic ENPs are demonstrated as a promising solution for sustainable agriculture due to their effective pesticide characteristics [28].

Also, metallic ENPs can alleviate the toxicity of biotoxins by changing the bioavailability of nutrient or toxic substances or acts as an antioxidant through the elimination of free radical before

oxidative damage to the living organisms[40, 41]. This underlying mechanism is a key concept behind their application as antimicrobial, antifungal or pesticides. In addition, in the context of sustainable agriculture metallic ENPs are capable to effectively enhance the nutrient diffusion and wettability of agricultural soils [28]. As an example, silver nanoparticle (ENPs-Ag) was proposed as antifungal due to the release of Ag^{+} ions to the medium [5, 41].

In addition, several types of research on gold nanoparticle (ENPs-Au) indicated the antibacterial characteristics of this nanoparticle, which significantly reduce the growth rate of gram-positive and negative bacteria (*i.e., Escherichia coli* and *Staphylococcus aureus*) [42]. Arokiyaraj et al., 2013, showed about %50 enhancement of antibacterial activity on *Argemone mexicana* (planted for medical purposes) when it was treated with iron oxide ENPs (50 µg/disc) against a widely distributed bacteria (*Proteus mirabilis*) in the environmental matrices, including soil and contaminated water [43]. In a similar study, a high dose of iron oxide ENPs (3 mg/mL) considerably inhibited, the growth rate of *Staphylococcus aureus* compared to the control samples [44].

Recently, nanosilica application was successfully developed for controlling a wide range of agricultural pest insects for a variety of horticultural and crop plants [45]. This nonmetallic ENPs are capable of being absorbed onto the cuticular cell lipids, and effectively through a biological mechanism that kills the pest insects. The advantage of this nano-bio technique is the conservation of physical and biological properties of photosynthesis or respiration from the leaf and stem surface of the plants [45].

Stadler et al. 2010, for the first time, proved the applicability of nanostructured alumina (250 mg kg^{-1}) on two worldwide predominant pest insects' species (*Sitophilus oryzae* L. and *Rhyzopertha*

dominica) in food supplies including wheat. Their results illustrated a substantial mortality rate as high as %80 between the adult population of both species within nine days of exposure to treated wheat; the mortality rate could reach up to 95% within three days at a higher dose at 1000 mg kg^{-1} concentration level [46].

However, despite many research aimed to understand the positive implication of certain ENPs as antibacterial or pesticide, but due to the thermodynamic behavior of such ENPs will influence by the composition of soil matrices (i.e., NOM and other ionic and cationic elements). For example, Grillo et al. (2015) explained in detail the possible confounding behavior of ENPs in the presence of NOM. As the soil profile is a mixture of different trace and macro elements (i.e., cu^{+2}, mg^{+2}, etc.) and comprise a variety of naturally deposited ENPs as well as those that have been introduced in the soil as nano-fertilizers, there is a question mark, whether a chemical interaction between ENPs fertilizers (i.e., CeO_2) and antibacterial nanoparticles (i.e., ZnO) exist due to changing the electrophoretic mobility in soil. For example, the repulsion force between two nano-metal particles as well as CNPs may incorporate in their bioavailability and mobility into a receiving porous media. This is a new field of research that has not been seriously addressed. In order to illustrate the potential affecting the energy of the nanoparticles, figure 1 compares the levels of repulsion energy between a metallic ENPs and a carbon-ENPs (CNPs) based on reported data from Tian et al. (2010). This comparison is an identical comparison between different ENPs, in this case, the Ionic Strength and Zeta Potential were considered constant for both ENPs. As it can be interpreted the interaction energy is considerable in affecting the collision of two ENPs and their affinity with NOM. This remarkable difference can be explained by different effective diameter, variable surface potential, and other corresponding parameters.

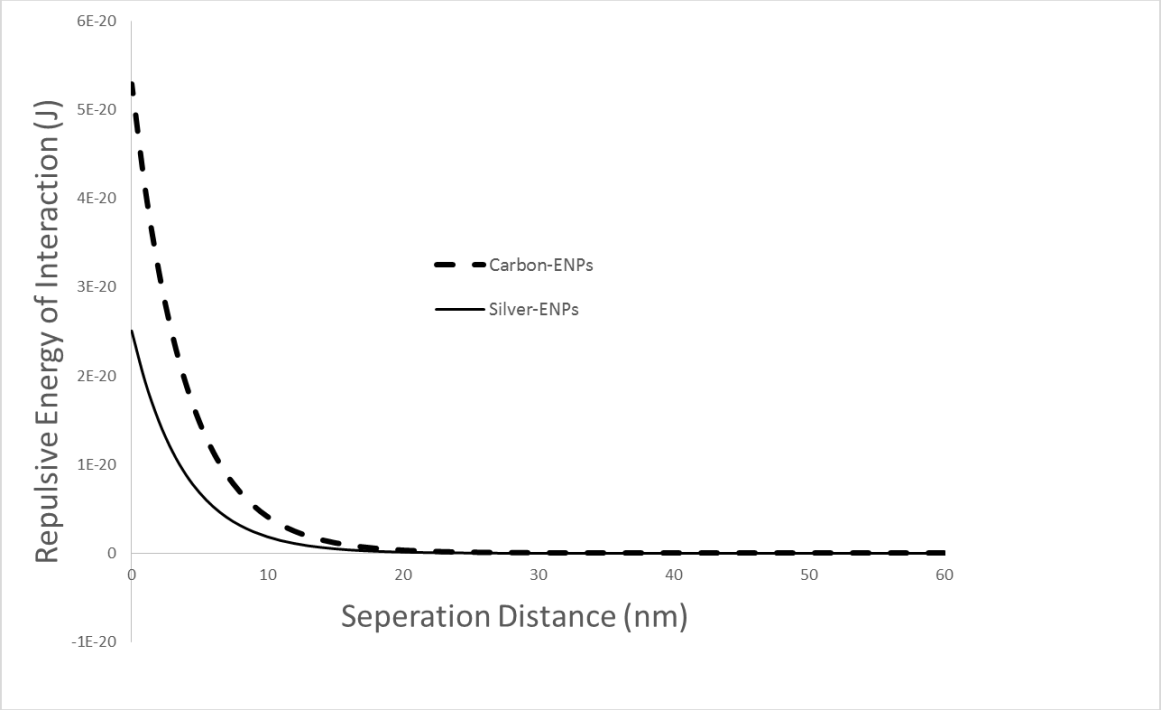

Figure 1.1. A comparison of the levels of repulsion energy between a metallic ENPs and a carbon-ENPs (CNPs) based on reported data from Tian et al. (2010).

The ENPs have a high susceptibility to environmental factors (i.e., chemical stability, dispersion, and surface functionalization) through their implication as pesticide or fertilizer as discussed earlier. It may most likely occur due to unique surface-to-volume ratio, which made them prone to be attacked by oxidative or corrosive environmental matrices including soil, leading to deformation of their nanostructure properties[47].

In order to improve the quality of ENPs implication in soil composition, several techniques have been developed to create protective coating materials around nanoparticles[47]. Wang et al. (2016) deliberately summarized a list of most frequently used coating materials including fluorescein and citrate[48].

However, this coating is beneficial for the functionality of ENPs, but it may considerably change the thermodynamic behavior of the particles as the size may increase as high as 15 to 45 folds (sphere particle) according to reported data from Wang et al. (2015). The matter of coating seems to be very critical instability of attraction force of the ENPs as well as their EPM characteristics. Figure 1-2 represents an example of an ENPs with potential impacts of coating with the assumption of increasing the size of the ENPs. This graph was calculated on the basis of classic DLVO theory specified for the behavior of ENPs in soil matrices. The detail about this model is out of the scope of this literature review (detail presented by [49, 50]). However, regardless of Ionic strength and chemical composition of the soil matrices, this figure is a clear picture of coating effect (i.e., Fe^{+2}) on increasing the energy barrier of an ENPs to target the porous media. This energy barrier will decrease upon to type of coating and redox condition in the soil.

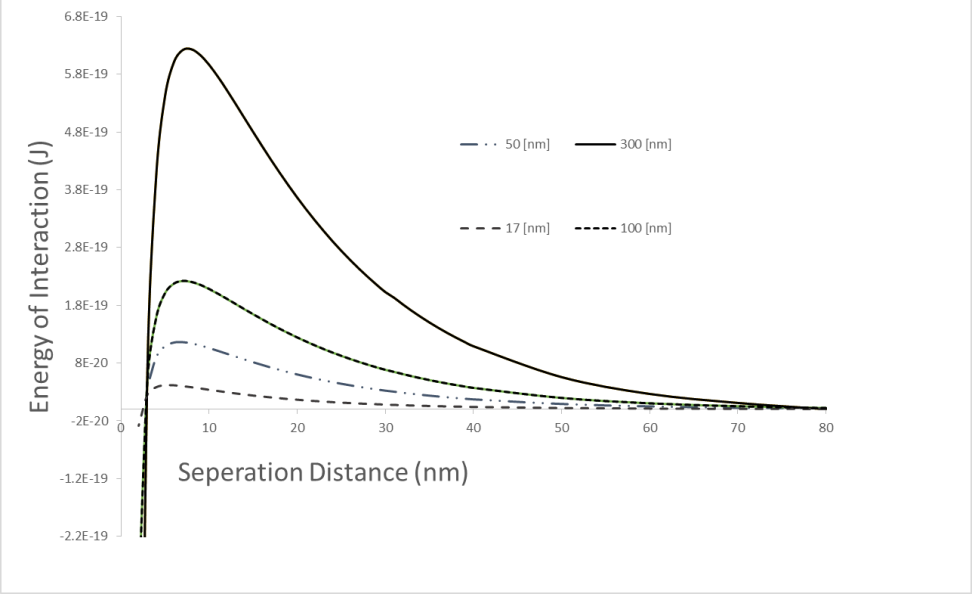

Figure 1.2. Potential impacts of coating with the assumption of increasing the size of the nanoparticles on their interaction energies. This graphs were calculated on the basis of classic DLVO theory specified for the behavior of engineered nanoparticles in soil matrices.

1.4. Nano-Sensors

In the context of agriculture and its direct relation to human health, it is essential to protect the quality and safety of water resources and food supplies from their production points. In this regard, there are increasing demands for reliable field-deployable devices for on-site monitoring of various contaminants in the environment particularly for agriculture and food supplies[30, 51]. For example, the residue of organophosphorus chemicals as derivatives of used pesticides or chemical weapons can contaminate the water and soil and put human and animal life at risk [52]. Sensing such a chemical by conventional microbiological techniques, analytical methods, and in some cases live animal tests are costly, time-consuming, require more complicated instrumentation and substantial scientific expertise [53, 54].

Development of nanotechnology into sensing science can effectively overcome these shortcomings in precise nanoscale levels. The selected ENPs including nanowire (proficiency of very high detection sensitivity), carbon nanotubes (large surface area) or magnetic nanoparticle-based are promising novel approaches to detect such contaminants as a nano-biosensor element [55]. The applications of ENPS as nanosensors can be defined by their roles in three fundamental principles; i) as receptors that explicitly bind to the target analyte, ii) to provide a physical interface for biological activities, and iii) amplifying the received signal from a nano-transducer element [56]. Due to high sensitivity, simplicity, capture efficiency, low price, and nanosize, ENPs have been widely used in nano-biosensors [52].

For example, a combination of nanowires and metallic nanoparticles (i.e., Au, Ag) represent identical biosensor elements in electrochemical labels to sense the DNA [56, 57], which ultimately can incorporate in genetically modified agricultural plant species.

The nanosensors based on ENPs-TiO_2 has been successfully applied for detecting the redox state on a variety of herbals including chamomile (*Matricaria chamomilla*), green/black tea, mint (*Lamiaceae*), sage (*Salvia*) and fennel (*F. Vulgare*) [58].

For the first time, Wang et al. 2015 developed a nanosensor using a conjugate form of graphene quantum dots (GQDs) and ENPS-Au for sensing the highly toxic element of Cyanide (CN^-) in the plants such as cassava roots [59]. In another study, Chaiyo et al. 2015, successfully applied a nanosensor designed on ENPS-Au application for highly sensitive and selective detection of Cu^{2+} over 15 of most available macro and micro-nutritional elements in a variety of agricultural and food products including rice, tomato, groundwater and mineral water [60].

The pyrophosphate is an additive to agricultural products, with potential health threat at high doses, ENPs-Au and Ag have been applied for monitoring of this compounds in a wide variety of products including white flour, sugar, vegetable fat, soya flour and dry wheat gluten [61]. Table 1.1 represents some of the frequently implemented ENPs in nanosensors structures for the monitor, detect and qualify the certain compounds in agricultural products.

Table1.1. Frequently implemented Engineering nanoparticles in nanosensors structures for the monitor, detect and qualify the certain compounds in agricultural products.

ENPs	**Detection Technique**	**Sensing element**	**Agricultural benefits**	**Ref**
Carbon dots coated with vitamin B_{12}	fluorescence resonance	N-methyl carbamates	Detecting a highly toxic residue of pesticide *	[62]
Cellulose nanofibers (CNFs) coated with silver ENPs-Ag	surface-enhanced Raman spectroscopy (SERS)	thiabendazole	thiabendazole (TBZ) pesticides in various food products (i.e., apples)	[63]
Graphene quantum dots (QDs)	fluorescence	fenoxycarb carbamate i	Detecting toxic residue of pesticide	[64]
ENPs-Au/Ag	graphene-enhanced Raman spectroscopy (GERS)	Direct GERS response	Assessment of the freshness of fruits and vegetables (Wax apple, Lemon, Tomato, Red Pepper, and Carrot)	[65]

*Carbofuran is considered as a toxic pesticide by FAO (Food Agriculture Organization)

1.5. More insight into the interaction of nanoparticles with plants

Proteins consist of a chain of amino acids, in which through a different sequence of the amino acids the physical shape, structure, and function of proteins form. The primary compartments of protein secondary structure are known as α-helices and β-sheets, which their three-dimensional arrangement forms the tertiary structure[66].

The functional conformation of a protein is strictly governed by the shape of the hydrophobic compartments. The intrusion of the nanoparticles into a biosystem is associated with the interruption of the protein established conformation and, therefore, the protein is prone to dysfunction[67]. This event corresponds to one of the primary biological impacts of nanoparticles.

The effect of the surface chemistry of nanoparticle on its interaction with the protein is a very complex process which incorporated many factors including the electrostatic and hydrophobic interactions, as well as specific chemical interactions between the protein and the nanoparticle surface charges [67, 68].

It is highly possible that the surface charge of nanoparticles will be partially or entirely affected by the presence of root exudate and associated amino acids in rhizosphere zones of the plants before plant uptake [13]. Because the pathways of the nanoparticle to the root cells are entrapped by high concentration of amino acids and low molecular chains. As results, amino acids in the rhizosphere are able to indirectly change the enthalpy of certain protein interaction with nanoparticles [66]. In addition, the proteins possess a very wide range of affinities for different nanoparticle surface, leading to a varied range of residence times for proteins at a nanoparticle surface.

1.6. Interaction of root exudate Amino Acids with Nanoparticles

The agglomeration of the nanoparticle is a critical phenomenon that occurs upon entrance of nanoparticle in a biological system which may be affected by the particle shape, size, surface area, and charge, as well as the adsorption properties of the nanoparticles[14, 69]. Furthermore, key factors including pH, ionic strength, water quality and the presence of organic/inorganic matter potentially modify nanoparticle aggregation, which govern the toxicity and reactivity of the nanoparticles [70].

In the plant root zone (the rhizosphere), amino acids are the second most significant component of the root exudates. The release of such molecules has been reported in numbers of plants by 50% (w v^{-1}) through the root exudates [71].

Representative amino acid solutions hold distinctive charges at a pH range (5.5–8.5) characterizing the natural environment. For example, four frequently reported amino acids in rhizosphere include positively charged (Histidine), neutral (Glycine), and negative charges (Aspartic acid and Glutamate) [71].

The nanoparticle colloidal stability in exposure to amino acids solution and their tendency to agglomerate corresponds to the surface charge of the nanoparticles as well as functional groups of the amino acids which overall interaction can be measured in the framework of multiple forces of electrostatic, steric, and van der Waals, which can be articulated through application of the Derjaguin–Landau–Verwey– Overbeek (DLVO) theory[71].

In copresene of heavy metals and nanoparticles, several processes can lead to the lower adsorption of Cd on CeO_2NPs such as competitive adsorption of root exudates that occupy part of the adsorption sites for Cd; the formation of root exudate and Cd complexes which display lower

adsorption on CeO_2NPs than free Cd^{2+} or less available adsorption sites caused by CeO_2NPs aggregation.

In the presence of root exudates, if the adsorption density of Cd^{2+} is greater than that of other organic ions and charged organic ligands, the imbalance of adsorbed ions will contribute a net positive charge to the surface. Therefore, the charge imbalance might be described either in terms of unequal adsorption of the Cd^{2+} or nonstoichiometric dissolution of the CeO_2. The higher feasibility of Cd^{2+} adsorption in a substitution mechanism with Ce^{3+} with respect to higher dissolution of CeO_2 in co-presence of root exudates was further supported by reducing the trend of zeta potential and a higher concentration of cerium after equilibrium [13].

In the presence of the root exudate amino acids, the surface complexes form with Cd ions, in which the process is one of adsorption. Examples include binding of organic molecules on the surface of CeO_2 and formation of surface complexes between cadmium ions from solution and organic acid functional groups on the surface of NPs[50, 72].

However, these complexes in natural matrices seem to be more complicated. For example, Krishnamurti et al., 1997 reported that the complex of oxalic acid as a root exudate compound with cadmium as Cd-(oxalate)n complexes could easily be decomposed by microbial activities and the resulting the readsorption of free Cd by the ENPs [73]. In contrary, D-serine is a rare amino acid which cannot be quickly metabolized by bacterial activities of the rhizosphere in soil [74]. Other ionizable functional groups in root exudates also impact the surface charge of the CeO_2NPs. For example, organic acids molecules (i.e., succinic acid and D-Serine) with both carboxyl ($-COOH$) and amino ($-NH_2$) groups [50, 72]. In a liquid medium, the carboxyl group is a stronger acid than the amino group. As a result, at low pH, both types of groups tend to be protonated ($-COOH$ and $-NH_3^+$), whereas, at high pH, both tend to be deprotonated ($-COO^-$ and $-NH_2$); in

the intermediate pH range, most of the carboxyl groups are likely to be deprotonated, while most amino groups are protonated.

At high pH range, (pH>5) the surface concentration of the organic acids far exceeds that of the amino groups. Hence, CeO_2NPs are generally negatively charged in this range of pH. Therefore, at lower pH values when CeO2NPs that exposed to both types of functional groups, the surface carries more positive charges compared to negative charges at higher pH [50].

However, as it is illustrated in Figure 1, I proposed that adsorption of succinic acid and D-glucose occur through its two carboxyl groups binding to two adjacent cadmium surface sites, and not only one. Also, l-malic acid adsorption binds via one carboxyl group and one hydroxyl, which forms a "claw" on CeO_2NPs surface. This mechanism was in a good agreement with the proposed mechanism of the other scientists[75]. However, in such medium, the strength of the surface complex of -malic acid is thought to be far stronger than a weak surface complex of succinic acid. To support this mechanism, it is worth to note that l-malic acid adsorption can reach the equilibrium state four times faster than succinic acid (12/48 h)[75].

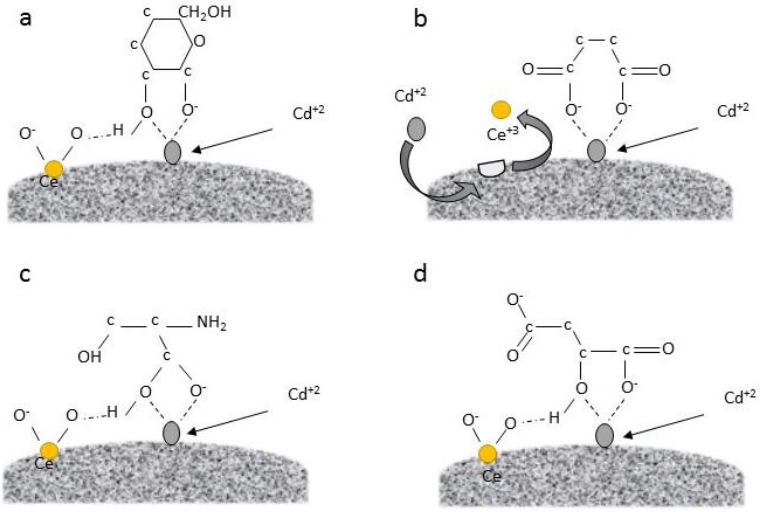

Figure1.1. The schematic diagram for the CeO_2NPs and possible binding between synthetic root exudate molecules and Cd^{2+} on the surface of CeO_2NPs during adsorption. a) Illustrate the binding of the oxygen molecule in D-glucose with Cd and Ce. B) Succinic acid. c) D-serine amino acid and d) L-malic acid

In addition to the behavior of CeO_2NPs in exposure to root exudate, a similar correlation was found between the ZnO nanoparticles behavior and four commonly found amino acids in rhizosphere area (Glycine, L-Glutamic, L-Aspartic, and L-Histidine) at different concentrations. It was shown that the nanoparticle size distribution was reduced as the pH changed into more acidic and nanoparticles became less agglomerated [71].

Further, in exposure to the ZnO, the charge of the amino acid functional groups affected the colloidal stability of the ZnO nanoparticle and its size distribution (agglomeration effects). The critical finding suggested that the amino acids holding more positively charged seems to be more effective in lowering the agglomeration rate compared to the neutral or negative charged amino

acids. Overall, the more significant concentration of amino acids is associated with the reduction of the zeta potential at a broad range of environmental pH [71].

In soil complex, the most critical example of adsorption that affects the surface charge of particles is that of large organic molecules, such as those identified as natural organic matter (NOM). Such molecules are often strongly adsorbed onto both inorganic minerals and manufactured nanoparticles. The functional groups that are part of these molecules are often charged in the dominant pH of the environment so that when the molecules adsorb, they contribute charge to the particle surface. It is now widely accepted that the adsorption of NOM onto particles in natural waters is almost always the principal determinant of their surface charge. The fact is accepted that the charge on nanoparticles in natural waters is dictated by NOM.

1.7. Cerium oxide nanoparticles (CeO_2)

Cerium oxide nanoparticles (CeO_2 NPs) is one of those ENPs that have been extensively applied in various industries over the past several years particularly as a fuel additive [76, 77]. The main introduction pathway of the CeO2 into soil and plants is reported through deposition of vehicle exhausts and diffusion of municipal runoff into the top soil [76]. It is very important to understand the uptake mechanism of such ENPs into crop plants due to their bioaccumulation in the human food, their chemical interaction with essential trace elements, delivering toxic ions to the plants, biotransformation, and potential biological inherence to the next plant generations [76, 78].

Cerium oxide possesses the lowest solubility against acid among the rare earth metal oxides and does not elute during the removal of harmful ions in water[79]. Also, natural organic matter

(NOM) including root exudates in a growth medium can enhance in the stability of CeO_2 [5]. Elemental Ce belongs to a lanthanide family and is the most abundant and reactive rare element of the earth (20-60 mg/kg)[80]. It is a soft, ductile metal with silver color, which acts as a strong oxidizer in exposure to air — cerium with a molecular weight of the 140.116 g mol-1 represents density of 6.770 g cm $^{-3}$. Its melting point has been reported as of 798 °C and respectively a boiling point of 3443 °C. It also solves in dilute, mineral acids.

Elemental Ce tarnishes gradually in exposure to air and actively react at 150 °C to form CeO_2[81]. CeO_2 is the most common form for Ce in the earth's crust. CeO_2 nanoparticles are massively applied in different industries due to their catalytic ability, which is basically subjected to redox behavior depending on the Ce's redox state[82]. The CeO_2 nanoparticles are mainly contained of Ce(III) (up to 60%) and Ce(IV), which the higher ratio of Ce(IV) cause the stronger the catalytic activity. In the lattice structural form of CeO2, the cationic form of Ce will be coordinated via eight oxygen anions at the corner of the crystal form while the anionic oxygen tetrahedrally corresponds to four Ce cations. Once the oxygen anions (O^{2-}) gets off from the lattice structure, the oxygen vacancies will increase the reactivity of the nanoparticle, concerning the temperature and environmental parameters [83]. Thus it is believed to be a promising alternative nano-adsorbents to eliminate toxic elements. In environmental remediation, cerium oxide had demonstrated a high adsorption capacity for hazardous anions, such as fluoride, bichromate, [79] and cations including As(V), Cr(VI) and Pb (II) [84].

The loss of O^{2-} creates an electrostatic balance which is associated with the reduction of two Ce^{4+} to Ce^{3+} [85]. On the surface of CeO_2 nanoparticle, Ce^{3+} can potentially oxidize to Ce^{4+} in exposure to environmental oxidants. At smaller CeO_2 nanoparticle sizes, the density of oxygen vacancy, as well as Ce^{3+}/Ce^{4+}, will increase[83, 85].

1.8. Zinc oxide (ZnO)

Zinc oxide with the formula of ZnO is an inorganic compound, which typically appears in the form of insoluble white powder[86]. ZnO has been massively applied as an additive into many products including glass, plastics, sunscreen, ceramics, rubber, cement, lubricants, paints, and batteries[14, 87]. ZnO is currently being used as food additives and has been recognized as safe material by the Food and Drug Administration[88]. ZnO can be found on the Earth crust as a natural mineral zincite, but mostly available as a commercial product through chemical synthetases. ZnO is also known as II-VI semiconductor due to representing the groups of II and VI in the periodic table, respectively for zinc and oxygen[89]. It exhibits specific properties which make it favorable for many implications; this properties includes decent transparency, high electron mobility as well as strong room temperature luminescence[90]. Also, ZnO is a multipurpose functional material that is available within diverse morphologic groups such as nanorings, nanocombs, nanosprings, nanobelts, and nanowires[91]. Such ZnO nanostructures can be economically synthesized at very low cost. Therefore it has a high promising potential in the development of nanotechnology [92].

As and an amphoteric oxide, ZnO is almost insoluble in water and alcohol, but it can be solved in many acidic solutions, such as hydrochloric acid:

$ZnO + 2\ HCl \rightarrow ZnCl_2 + H_2O.$

Also, basic solutions can be dissolute the ZnO and lead to the production of soluble zincates:

$ZnO + 2NaOH + H_2O \rightarrow Na_2(Zn(OH)_4)_2.$

ZnO nanostructures are also attractive for biological and agricultural application because of their safe properties and large surface area[92, 93]. For example, in biological systems, ZnO enters into a slow reaction with fatty acids and produce carboxylates compounds, such as oleate or

stearate[94]. Also, ZnO nanoparticle represents strong antibacterial characteristics which may stem from the generation of reactive oxygen species (ROS)[95, 96]. Such ROS could be induced on the nanosurface of ZnO, which have been investigated through a conductometric technique. In this regard, ZnO being exhibit a wide band-gap semiconductor (3.36 eV), which make it an ideal nanoparticle for agricultural applications[69]. Its application as an antimicrobial agent against various pathogens including *Escherichia coli*, *Campylobacter jejuni*, *Pseudomonas aeruginosa* has been frequently reported[70, 97].

The benefits of using ZnO as antimicrobial agents is that Zn is an essential mineral element for humans and crops, which exhibit strong activity in the form of oxide at very small amounts. For example, Zn is the sole metal formulated in all six enzyme classes (transferases, viz. oxidoreductases, hydrolases and ligases, lyases, isomerases)[70]. Zn plays a vital role in different integral metabolic processes, promotes the synthesis of carotenoids and chlorophylls leading to higher photosynthetic apparatus of the crops [94]. However, it has been reported that particle size and surface charge of coating materials can affect the activity of the ZnO in plants[98, 99].

Different studies reported the impact of ZnO ENPs on crop plants. For instance, it has demonstrated to enhance germination rate, pigments synthesis, the contents of carbs and protein as well as activities of antioxidant enzymes[100, 101].

1.9. Inhibitors of biological process in plants

1.9.1. Lanthanum (La^{3+}) and Gadolinium (Gd^{3+})

Calcium-permeable channels represent an extremely high affinity for divalent cations like Ca^{2+}, Sr^{2+}, and Ba^{2+} [102]. The trivalent lanthanide gadolinium (Gd^{3+}) and lanthanum (La^{3+}) are chemically very close to the divalent calcium ion (Ca^{2+}) in terms of size (radius of Gd^{3+} 1.05–1.11

Å and Ca^{2+} 1.00–1.06 Å), donor atom preference, bonding, and coordination [103, 104]. These trivalent cations can indicate high potency and completely block the current through an open channel at concentration levels less than 10 μM [102]. Several studies indicated VIC channels that exist in all root cell-types, as well as voltage dependent channels, could be inhibited by Gd^{3+} and La^{3+} [105]

The inhibitory behavior of $Gd^{3+}$and La^{3+}, primarily stems from inhibiting the influx of extracellular calcium into cells through calcium channels in the plasma membrane[106] because they are competing with Ca^{2+} for binding at the same site [102, 107]. The regulation of calcium flux through this class of lanthanide is a highly concentration dependent. For example, Liu et al. (2012) indicated that at lower concentration levels, La^{3+} could inhibit Ca^{2+} flux, while the higher concentration levels induce Ca^{2+} in the root cells[108]. It has been reported that in certain cell types, $La^{3+}$and Gd^{3+} can passively diffuse into the cytosol where their intracellular inhibitory effects take place stronger in comparison to extracellular effects [107]. In this case, lanthanides may appear in two forms of free ions or complexes through binding with negatively charged compounds [107].

1.9.2. 2,4-Dinitrophenol (DNP)

Calcium-permeable channels require energy (in the form of ATP) for their maintenance and activities including gate action and transport of certain divalent cations. These channels can be activated directly through i) depolarization ii) cyclic-AMP-mediated phosphorylation and iii) fluoride-mediated inhibition of phosphatase-induced dephosphorylation [106].

The formation of Adenosine triphosphate (ATP) is the ultimate product of the tricarboxylic acid (Krebs) cycle in mitochondria with simultaneous formation of CO_2 and H_2O. Through glycolysis,

there is a net production of two ATP molecules, but the majority of energy-rich phosphate bonds are produced during the final oxidative phosphorylation process[109].

Within the final phase, ATP synthetase converts adenosine diphosphate to ATP with the addition of an inorganic phosphate molecule [110]. 2,4-Dinitrophenol (DNP), reduces the production of high-energy phosphate bonds in mitochondria and simultaneously encourages systemic oxygen intake. This dissociative progression is recognized as uncoupling of oxidative phosphorylation [109, 110].

In oxidative phosphorylation, the current of electrons from NADH and $FADH_2$ to oxygen occur through exporting of H^+ from the matrix to the inner mitochondrial membrane. This gradient of H^+ can yield to the synthesis of ATP by flowing through ATP synthetase in the mitochondrial inner membrane. DNP interfere with the H^+ gradient and decrease the synthesis of required cellular ATP [110].

Also, DNP interferes with the synthesis of ATP at the final energy production phase by preventing the uptake of inorganic phosphate molecules into the mitochondria. Consequently, all energy-requiring processes block and the extra-mitochondrial accumulation of inorganic phosphate occur[110].

The effects of DNP vary with the age of the plants as well as applied concentration [111, 112]. For example in a 6 day barley plant, DNP inhibits the absorption of phosphate by approximately %40 while in 18 days plant it increases as high as 98%. This observation implies a possible correlation between glycolytic phosphorylation, oxidative phosphorylation and root development [111]. This can be strongly liked to species of leguminous plants that possess phosphomannoseisomerase activities which may display higher sensitivity to DNP on phosphate absorption.

DNP also inhibit the transport of inorganic phosphate to the shoots, indicating that the inhibition of metabolic mechanisms is critically effective not only within the entry process but also through the exit process into the shoot[111].

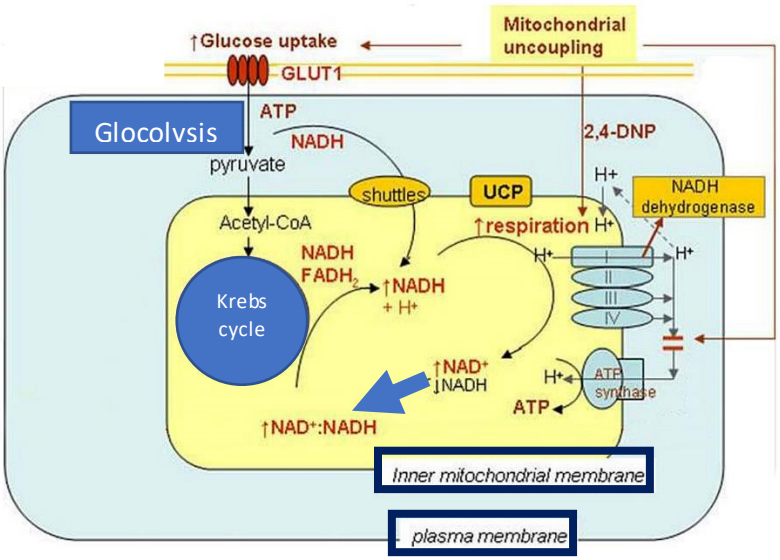

Figure 1.3. Proposed model by Liu et al. 2008[113] for the uncoupling effects of DNP which induces metabolic stress through interruption of the hydrogen ion shuttling across the inner membrane.

1.9.3. Cycloheximide (CHX)

Cycloheximide (CHX) represents the most common laboratory reagent used to block protein synthesis and is widely used in plant physiology. Cycloheximide was at first isolated from Streptomyces griseus and later was recognized as an inhibitor of eukaryotic translation blocking the translational elongation phase. Elongation is the step by step addition of amino acids to the growing protein chain. The order of amino acids is quantified by the sequence of codons in the mRNA, CHX is capable of binding the ribosomal E-site and inhibits eukaryotic elongation factors (eEF2)-mediated translocation[114].

Therefore, CHX blocks the synthesis of proteins in cell signaling. The use of CHX may represent other side effects. For example, inhibition of protein synthesis by CHX restrict the hydraulic conductivity of root through reducing cellular water transport from one sell to other[115]

Generally, effects of CHX on plants can be envisaged in three interdependence categories, i) inhibition of DNA synthesis ii), disruption of ion absorption, and iii) interfere the uptake of required amino acids. These effects are directly or indirectly governing by the ability of CHX concentration on blocking the protein synthesis[114].

1.10. Research Hypothesis and Goal

The applications of metallic oxide engineered nanoparticles (MONPs) in agriculture and food processing sectors including biosensors, nano-pesticides, plant growth stimulator, food additives, nano-fertilizers, delivery agents for micronutrient has massively increased [51, 116, 117]. Such nanoparticle can benefit the cultivation of dietary plants including crops and leafy vegetables by reducing the chance of heavy metals uptake or improve their post harvesting qualities such as chlorophyll content and anti-microbial properties [80, 118]. In addition, nanoparticles may contribute to increase the shelf life of vegetables by reducing the heavy metal content or act as a strong anti- microbial/fungal [118, 119]. The rationale to imply the MONPs in agrichemicals can be articulated into three specific goals: (i) efficient harvest of agricultural products with higher yield; (ii) to reduce the consumption of irrigation water and associated energy and (iii) minimizing the agri-waste production.

As a result of advances in nanotechnology-facilitated agrichemicals, MONPs are directly applied to agricultural systems [120, 121]. In addition, the progressive development of nano-biotechnology has resulted in noteworthy unintentional releases of MONPs into the agricultural soil as a primary

sink due to water irrigation and the collide deposition. Therefore, interactions of crops and leafy vegetables with MONPs with is fully expected.

Due to the different size-specific properties, surface charge, and phytotoxicity potential, the risk of bioaccumulation of MONPs in dietary plants and edible crop tissues, there is a need to understand the impacts of MONPs on plants, their uptake pathways and underlying mechanisms in plant tissues. In this regard, most ongoing research is centered on the potentially detrimental effects of MONPs [122, 123]. While, the effects of MONPs on plant uptake in exposure to a group of highly toxic contaminants, heavy metals, and metalloids, have not been adequately addressed.

In exposure to organic or inorganic contaminants, plant roots undertake different uptake mechanisms. For organic chemicals, hydrophobicity is a key parameter which mediates the entrance of compounds into the plant and dictates the primary mechanism for MONPs when they co-exist with the organic compounds[124]. This pathway is known as the "Trojan horse effect" in which adsorption of the organic compounds onto the MONPs surface lead to concomitantly uptake by plants[125, 126].

For inorganic elements including heavy metals and metalloids, sophisticated plant root systems can regulate their transport into root cytoplasm. Two pathways are well known, first, actively regulating the shuttle of heavy metals through various ion and protein channel which embedded in the cell membrane, second, passively transporting via passive diffusion which occurs through the cell membranes [94, 127].

Therefore, the interactions between MONPs and co-existing heavy metals and metalloids can be different from their interactions with organic compounds. In addition, MONPs have been observed to alter the gene expressions, interrupt the bilayer membrane of the cell, adsorb on cell wall and

interact with root exudate[128]. As a result, exposure to MONPs expected to alter plant uptake and accumulation of co-existing heavy metal and metalloid ions.

MONPs can be taken up by crops roots and translocated into edible parts of plants including leaf and fruits[129]. However, uptake and accumulation of MONPs in co-existence with heavy metals as well as the physiological effects of this co-exposure on plants are affected by the interference of various abiotic stresses [80, 130].

For example, the chemistry in the growth media has a strong impact on ENPs fate and transport and their interactions with coexisting pollutants. In particular, the presence of phosphate has been shown to drastically affect the speciation and uptake of MONPs by plants[131]. Addition of phosphate was shown to inhibit the redox cycle between Ce(III) and Ce(IV) on the CeO_2NPs surface due to the formation of $CePO_4$[132]. A similar phenomenon was also observed in plant roots that the transformation of Ce(IV) to Ce(III) was substantially reduced in the presence of 1 mM PO_4^{3+} in the growth media[133]. The presence of PO_4^{3+} also led to the formation of needle-like $CePO_4$ nanoparticles and different subcellular localizations of Ce in plant root cells[133]. A recent study found that the presence of PO_4^{3+} significantly increased the adsorption of Cd on graphene oxide (GO) at pH 5.3-10.0 due to the formation of a ternary surface complex [134]. Given the abundance of phosphate in biological systems and plant growth media and the strong impact of surface chemistry on MONPs fate and impact, it is imperative to understand the role of MONPs surface properties and the biogeochemical conditions in plant growth media on the mutual interactions of ENPs and co-occurring environmental chemicals.

There are several critical questions which raised from the previous studies: What biological mechanisms are involved in uptake of MONPs from media into root cells? Does the physiochemical properties of either MONPs or heavy metals/metalloid change in the presence of

root exudate? Does the nanoparticle surface charge impact the uptake and translocation of themselves and coexisting heavy metals? How the chemistry of growth medium can impact the uptake of both MONPs and heavy metals?

Mechanistic understanding of these research questions is imperative to elucidate of the altered plant heavy metal/metalloid uptake, determine the underling physiological mechanism in the accumulation of co-existing heavy metals and MONPs.

This dissertation aimed to investigate the interaction, involved mechanism in uptake and change of physiochemical properties of two important metallic oxide nanoparticles, CeO_2 and ZnO nanoparticles in biological systems of two crops; rice (Oryza sativa L.), Soybean (*Glycine max* (L.) Merr.) and four leafy vegetables including Spinach (*Spinaciae oleracea),* Parsley (*Petroselinum sativum),*Romaine lettuce (*Lactuca sativa L. var. Longifolia),* cilantro (*Coriandrum sativum)*in coexistence to heavy metals (Cadmium (Cd) and Lead (Pb)) and metalloids Arsenic III, V (As) under different growth conditions (soil and hydroponics).

The rationale to select these plants as the study model is due to their importance in human dietary system. Soybean was reported by the Food and Agricultural Organization (FAO) as the fifth most produced crop worldwide that provides about 30% vegetable oil and 77% nitrogen fixation[129]. Rice grains is one of the most staple food in the world[135]. The selected four leafy vegetables for this research are rich in bioactive compounds, essential minerals, and dietary fibers. The dry or fresh consumption of these vegetables through a variety of foods have been extensively increased due to rich flavor, anti-cancer properties, and high vitamin content. Therefore, these crops and vegetables are formulated in dietary supplements and are a proposed diet for infants, older adults, and patients[136, 137].

The rationale to select ZnO and CeO_2 nanoparticle due to their advancement in agriculture and food processing. CeO_2 has been introduced as a priority contaminant by the Organization for Economic Cooperation and Development (OECD). It has high potential impacts on dietary plants, strong stimulating impacts on the plant growth (i.e. chlorophyll content), and represented a high adsorption capacity of heavy metal contamination[129, 138].

ZnO ENPs are closely related to agricultural food processing because Zn is an essential nutrient for crops and many vegetables and progressing advancement aims to formulate ZnO into the composition of nano-fertilizers. ZnO ENPs represent high efficiency, low toxicity, and act as antimicrobial/ fungicides which have been extensively applied to protect vegetables, fruit, and grain crops among many promising organic antimicrobial agents [116, 139].

To achieve the overall goal of this research, six research hypotheses were developed:

Hypothesis 1: heavy metals of Cd and Pb with ZnO nanoparticles can represent competitive behavior on ZnO surface adsorption and may alter the heavy metals uptake pattern and microbial community in root zone and physiology of plants.

Hypothesis 2:. The response of the leafy greens to the co-contamination of two divalent heavy metals Cd and Pb may differ from plants to plants, leading to alteration of the nutritional values of minerals (Fe, Cu).

Hypothesis 3: Co-existence of CeO_2 ENPs and Cd, ENPs surface charge and chemistry of growth media can alter their uptake by soybean plants and change the defense mechanism of plants such as the release of root exudates.

Hypothesis 4: The release of root exudate can lead to the transformation of CeO_2 ENPs and co-existing heavy metals and metalloids in terms of nanoparticle size and shape and impact the adsorption potential of the nanoparticle for reducing the heavy metals

Hypothesis 5: Coexistence of CeO_2 ENPs and Cd can impact active or passive transport of the chemicals. Suspecting the active transport will play a role in the transport of heavy metals, CeO_2

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CHAPTER II

INVESTIGATION ON THE PLANT ROOT EXUDATE EFFECTS ON THE MODIFICATION OF PHYSICOCHEMICAL PROPERTIES OF CERIUM OXIDE NANOPARTICLES THROUGH ADSORPTION OF CD AND AS(III)/AS(V)[1]

2.1. Summary

Contamination of agricultural soils by cadmium (Cd) and arsenic (As) has caused many concerns on the production of safe food crops. Rapid development and application of nanotechnology in the recent decade raise new concerns on the accumulation of emerging nanoparticles in crop tissues. Evidence has also been reported that in the co-presence of heavy metals and engineered nanoparticles (ENPs), plant uptake of these chemicals are mutually affected by each other. Adsorption of heavy metal ions on the surface of ENPs has been identified as an important mechanism for the altered plant metal uptake by ENPs. However, quantitative information on the extent of adsorption in the environmentally relevant conditions such as in the presence of root exudates has not been reported. Information is also lacking on how the interaction of ENPs and various heavy metals through adsorption may alter the physicochemical properties of ENPs, and consequently change the plant uptake of these ENPs. This study investigated the adsorption of Cd and As(III)/As(V) onto 100 mg L^{-1} cerium oxide nanoparticles (CeO_2NPs) both in the presence and absence of synthetic root exudates (SRE). The results indicated that both As and Cd displayed strong adsorption on CeO_2NPs, and the presence of SRE reduced the adsorption for both metals,

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but to different extents. Importantly, the interactions of heavy metal ions with CeO_2NPs significantly changed the physicochemical properties of CeO_2NPs including their zeta potential and size distribution. Different effects on CeO_2NPs properties were noticed for Cd and As at different oxidation states. The results of shed new lights on the reciprocal effect of CeO_2NPs and these two metals on their plant uptake and accumulation.

2.2. Introduction

Heavy metals are ubiquitous in the environment and their concentrations in agricultural soils are steadily increasing because of their excessive uses in industries. Arsenic (As) and cadmium (Cd) are of high concern due to their carcinogenic and mutagenic effects [2]. As and Cd are listed as the first and fourth most problematic substances on the 2013 Priority List of Hazardous Substances developed by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), (http://www.atsdr.cdc.gov/spl). The background Cd in U.S soil is in the range of 0.1 to 0.5 mg/kg in most cases, increased by 43% in the past century due to various industrial activities (*e.g.*, plastic manufacturing, mining, paint pigments, production of household appliances, automobiles and fungicides). The background concentration of As in soil ranges from 0.4-40 mg/kg, with an average concentration of >10 mg/kg in many agricultural soils [3]. Both chemicals can be easily accumulated in edible tissues of plants, exposing humans to them through diet [2]. Prolonged exposure to these chemicals has been shown to be damaging to human health.

Nanotechnology is a rapidly growing industry and its contribution to the global economy was estimated to be $3 trillion in 2015 [4]. Nanotechnology is used in a wide variety of industries including water and wastewater treatment, agriculture, medicine, and manufacturing. In the agricultural sector, ENPs have been incorporated into fertilizers, nanosensors and pathogen combating formulas, and directly applied to crop plants throughout the world [5, 6]. In addition, the

rapid development of nanotechnology has resulted in significant unintentional releases of ENPs into the environment, with agricultural soil as a primary sink due to water irrigation and biosolid land application [7, 8]. Cerium oxide nanoparticles (CeO_2NPs) is a widely used metallic ENPs in a wide range of industries and holds great potential as an agrichemical stimulator to enhance plant's capacity to fight against various abiotic stresses such as salt stress [9]. Consequently, the accumulation of CeO_2NPs in agricultural soils is highly expected and numerous studies have been conducted to evaluate the physiological and biochemical impact of CeO_2NPs on plants from bench scale hydroponic studies to large-scale field conditions [10-12]. These studies have demonstrated strong impact of CeO_2NPs on plant physiology such as plant net photosynthesis rate and plant root anatomy. The previous studies also demonstrated the uptake and accumulation of elemental Ce in plant tissues through both direct uptake of CeO_2NPs and their dissolved ions [7, 13].

In addition to the direct impact of CeO_2NPs on plants, an emerging topic concerning the CeO_2NPs plant interactions is to understand the impact of CeO_2NPs on the fate and transport of co-existing environmental pollutants [14, 15]. Some studies have shown that CeO_2NPs can alter the plant uptake and accumulation of several freshly added or historic pesticides in soil [16-18]. The extent of impact depends on both the physiochemical properties of the pesticides and the plant species. Recently, we showed that CeO_2NPs can modify the plant uptake and accumulation of co-existing Cd in both hydroponic and soil systems [10, 19]. Several mechanisms have been identified for the altered Cd uptake and accumulation by CeO_2NPs including the adsorption of Cd on CeO_2NPs to form Cd-CeO_2NPs complexes, the altered excretion of root exudates and plant root anatomical structure changes in the co-presence of Cd and CeO_2NPs[10]. Even though the adsorption of Cd on CeO_2NPs has been identified as an important mechanism, quantitative information on the extent of adsorption of Cd on CeO_2NPs is lacking. Similarly, As is another concerning heavy metal in soil

and water and evidence has shown that As has high adsorption capacity on ENPs at both oxidation states, including CeO_2NPs, but quantitative information on the adsorption of As on CeO_2NPs in typical plant growing conditions is not available [20]. It is well recognized that the environment in the root region is very different from the most test systems (e.g. in DI water) used to quantify the adsorption of As and other heavy metals on CeO_2NPs. For instance, there are a high amount of organic acids in the root region excreted from plant roots and these compounds could have a significant impact on the adsorption process. Studies on the adsorption of Cd and As on CeO_2NPs in an environment relevant for plant growth is critical to obtain accurate information on the impact of ENPs on the fate and plant uptake of co-occurring heavy metal ions.

One interesting phenomenon we have noticed in our previous studies with the co-existing CeO_2NPs and Cd was that the alteration of plant uptake and accumulation of these chemicals was mutual. We have investigated the plant root physiology in the co-presence of Cd and CeO_2NPs and partially explained the altered plant uptake of CeO_2NPs from that perspective [10, 20], however, possibilities exist that the co-present Cd or As may modify the physiochemical properties of CeO_2NPs, leading to a different plant uptake and accumulation of Ce. Such possibility has not been explored in the literature. The objectives of this study therefore were: (1) to determine the adsorption of Cd and As on CeO_2NPs, both in the presence and absence of a synthetic root exudate (SRE) and (2) to evaluate the potential impact of Cd and As on the physiological and chemical properties of CeO_2NPs. Both As(III) and As(V) were investigated in this study due to the broad presence of these two oxidation states for As and their different fate and toxicity to plants.

2.2. Materials and Methods

2.2.1. Reagents

CeO_2NPs coated with polyvinylpyrrolidone (PVP) was purchased from the US Research Nanomaterials, Inc. (Houston, TX) and was extensively characterized through our previous studies [10, 21]. The average size of the primary nanoparticles fell in the range of 30-50 nm, with a dominant hydrodynamic size of 210.1 ±4.6 nm in DI water at 100 mg L^{-1}. The nanoparticles are primarily spherical and have a zeta potential of -51.8 mV at pH 7. Cd sulfate ($CdSO_4$) was obtained from Fisher Scientific Inc. (Pittsburgh, PA). High purity As (V) ($Na_2HAsO_4 \cdot 7H_2O$ >98%) and As (III) ($NaAsO_2$ >99%), was purchased from Sigma Aldrich (St. Louis, MO) and Lab Chem, (Zelienople, PA) respectively. D-Glucose was purchased from Fisher Scientific, the succinic acid and L-malic acid (>99%) were bought from Acros Organics (Fair Lawn, NJ) and D-serine (>99%) from Alfa Aesar (Lancashire, UK).

2.2.2. Synthetic root exudates (SRE) preparation

SRE, consisting of L-malic acid (25 mM), D-glucose (50 mM), succinic acid (25 mM), and D-serine (12.5 mM), was prepared according to a published recipe [13, 22]. Different amounts of those four chemicals were added to a liter of sterilized DI water and stirred vigorously at room temperature with a plastic-coated magnetic bar for 2 hours until they were fully dissolved. To minimize the oxidation of As (III), the SRE was prepared using boiled water for As (III) treatments.

2.2.3. Adsorption of Cd and As on CeO_2NPs without synthetic root exudates

CeO_2NPs solution was freshly prepared at a concentration of 100 mg L^{-1} in sterilized ultra-pure water (18.3 MΩ). $CdSO_4$ and both forms of arsenic were dissolved in a solution containing 100 mg L^{-1} of CeO_2 to obtain eight final elemental concentrations of Cd or As (III/V) (0, 0.2, 0.5, 1,

1.5, 3, 4, 5 mg L^{-1}). To minimize the oxidation of As (III), the deionized water (DI) was boiled in a beaker for two hours to remove the dissolved oxygen. Immediately after boiling, the beaker was covered with a glass lid and was allowed to cool down to room temperature before As (III) was added at the above-mentioned concentrations. The prepared solutions (30 mL) containing different concentrations of Cd or As and 100 mg L^{-1} of CeO_2NPs were transferred to 50 mL centrifuge tubes and each treatment had three replicates. The tubes were shaken at 180 rpm on a shaking table for 48 h at 25 °C (New Brunswick Scientific, Edison, NJ), adequate for the system to reach equilibrium [23, 24]. Afterward, the pH of the solution was measured with a pH probe. To separate the remaining dissolved metal ions from the ions adsorbed onto CeO_2NPs, 10 mL of the solution was centrifuged (7000 x g) for 45 minutes with a syringe centrifuge (NMWL = 3 kDa, Merck Millipore) and the concentrations of heavy metals in the filtrate was considered as the un-adsorbed heavy metal ions and were determined by an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Briefly, 2 mL of each filtrate was diluted with 10 mL of 1% nitric acid in a 15 mL centrifuge tube first. The diluted solutions were directly introduced to ICP-MS (Perkin Elmer mod. DRCII, Waltham, MA) for the quantification of Cd and As as well as the dissolved Ce ion. The adsorbed heavy metals on CeO_2NPs (q_e) were calculated based on the mass balance as follows:

$$q_e = \frac{(C_0 - C_e)V}{m}$$

Where C_e is the concentration of Cd or As(III)/As(V) in solution (mg L^{-1}) at equilibrium, C_0 is the initial concentration of the heavy metal ions; V is the volume of the solution (30 mL), and m is the mass of CeO_2NPs, (100 mg L^{-1} x 30 mL). The adsorption data were fitted with the Freundlich model as shown below:

$$q_e = K_f C_e^{1/n}$$

Where, K_f (mg g^{-1})(L g^{-1})^n indicates the adsorption affinity and capacity and, 1/n refers to the Freundlich exponential coefficient.

2.2.4. Adsorption of Cd and As on CeO_2NPs with synthetic root exudates

The experimental setup was similar to what was described above without SRE. The major difference was that the SRE compounds were dissolved into the sterilized water before adding the heavy metal ions and CeO_2NPs. In order to further investigate mechanisms for the impact of root exudates on the adsorption of heavy metals on CeO_2NPs, two additional experiments were carried out with Cd. In the first treatment, SRE was first mixed with 4 mg L^{-1} of Cd in a 50 mL centrifuge tube for 48 hours, and then 100 mg L^{-1} CeO_2NPs was added to the mixture and the total mixture was shaken for additional 48 hours. In the second experiment, 100 mg L^{-1} of CeO_2NPs was first mixed with SRE for 48 hours. Then 4 mg L^{-1} of Cd was added to the mixture and shaken for another 48 hours.

2.2.5. Analysis of the particle size distribution and zeta potential changes after adsorption

At the end of the adsorption, 2 mL of solution from each sample was transferred to a 4.5 mL polystyrene cuvette with 10 mm optical path length (Fisher Scientific, USA) and the hydrodynamic size and zeta potential of CeO_2NPs were analyzed using a DelsaNano C, (Beckman Coulter Inc., Miami, FL). The hydrodynamic particle size distribution for each sample was determined through two 5-min sequences and the reported values were the average of five continuous measurements. Part of the solutions was also examined under a transmission electron microscope (TEM) (JEOL JEM-1200EX, JEOL Ltd., Tokyo, Japan).

Zeta potential of CeO_2NPs was measured with the same instrument. Solutions containing 100 mg L^{-1} of CeO_2NPs and different concentrations of As or Cd were analyzed using a glass cell with the

optical surface of 1 mm at the scattering angle of θ = 90° (633 nm) for 15 min to determine the zeta potential of CeO_2NPs.Three replicates were measured for each treatment.

2.2.6. Statistical analysis

All results were subjected to analysis of variance, means and standard deviation. One-way ANOVA was performed and means separation between treatments was obtained by the Tukey's post-hoc test. Data were analyzed using the Minitab 18 Statistical Software (Minitab Inc., State College, PA).

2.3. Results

2.3.1. Adsorption of cadmium on CeO_2NPs

Cd adsorption isotherms in the presence and absence of SRE are shown in **Figure 2.1**. Cd^{2+} displayed strong adsorption onto CeO_2NPs in the absence of synthetic root exudates. The presence of SRE significantly reduced the adsorption of Cd^{2+}. For instance, the K_f values from the Freundlich model fitting dropped from 0.082 to 0.015 after SRE was included in the system.

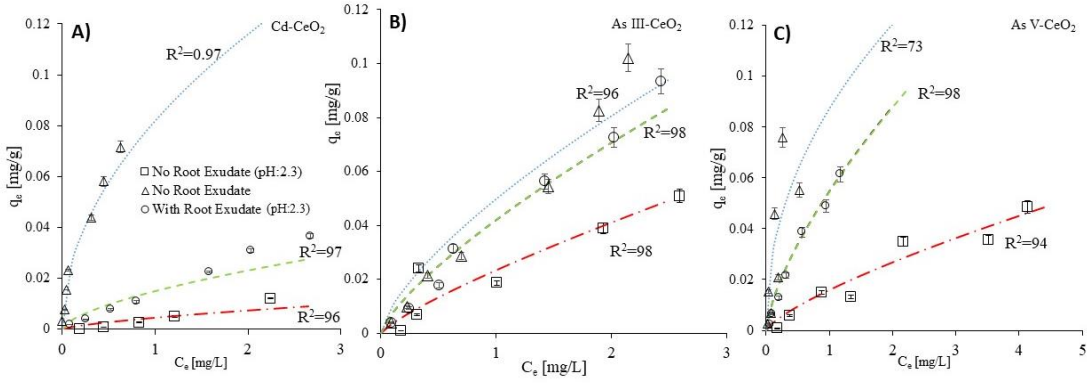

Fig. 2.1. The adsorption isotherms of A) Cd, B) As (III) and C) As (V) onto CeO_2NPs. q_e (mg g^{-1}) is the amount of Cd or As adsorbed per unit weight of CeO_2NPs at equilibrium, and Ce (mg L^{-1})

is the adsorbate concentration in solution at equilibrium. Error bars indicate standard deviation (n = 3). Data points with no error bars designate very small error bars. Dotted lines indicate Freundlich isotherm fittings.

2.3.2. Impact of Cd adsorption on the physicochemical properties of cerium oxide nanoparticles

Figure 2.2 shows the dissolution of CeO_2NPs in the presence of Cd with and without SRE after 48 hours of mixing. As indicated, Cd alone did not affect the dissolution of CeO_2NPs. However, the mixture with both Cd and SRE significantly increased the dissolution of CeO_2NPs (p <0.05).

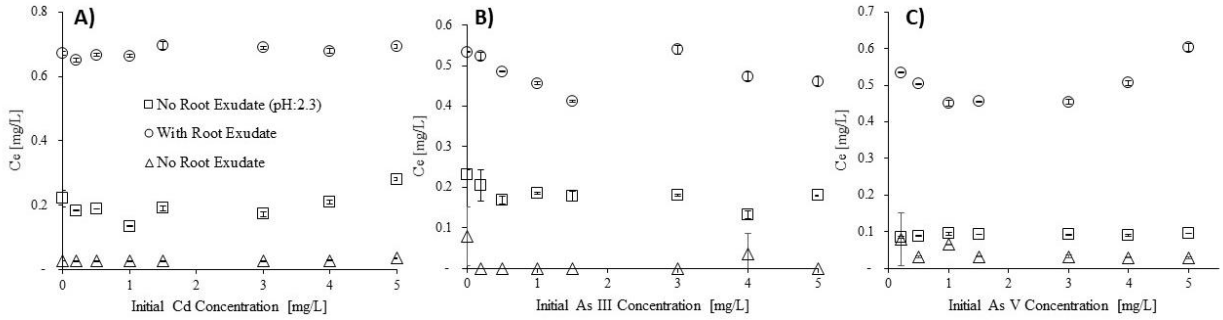

Fig. 2.2. Average concentrations of Ce^{3+} (mg L^{-1}) in solution after CeO_2NPs were mixed with A) Cd B) As (III) and C) As (V), alone or in combination with the synthetic root exudates (SRE). The concentration level in both scenarios (with and without SRE) were statistically meaningful (p <0.05).

Both Cd and SRE significantly modified the zeta potential of CeO_2NPs, Figure 2.3. In the absence of SRE, the zeta potential of CeO_2NPs increased from -45 mv to about -5 mV when the Cd concentration increased from 0 to 5 mg L^{-1}. SRE alone drastically increased the zeta potential of CeO_2NPs from -45 mV to + 20 mV. The zeta potential of CeO_2NPs became less positive with the

increase of Cd concentration in the presence of SRE. The solution pH was not significantly affected by Cd and the addition of SRE significantly lowered the pH from 5-6.0 to 1.9-2.5.

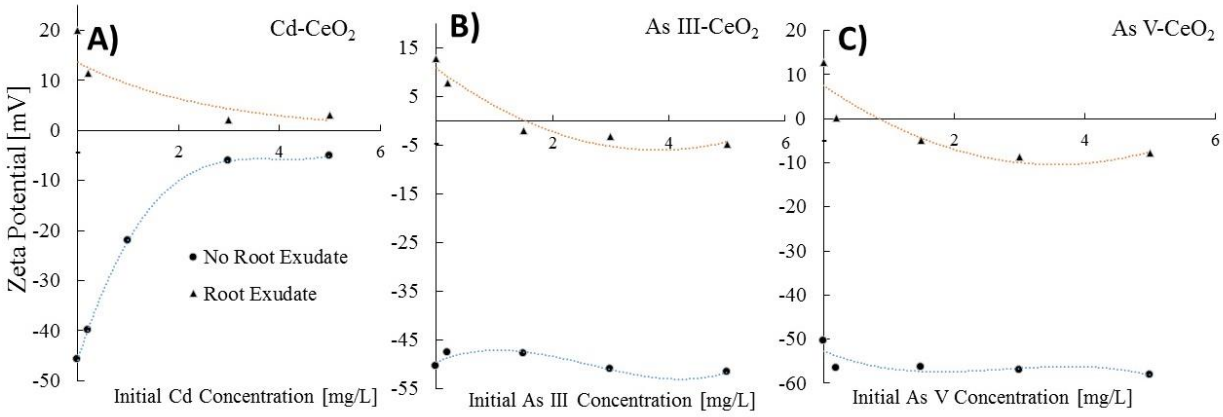

Fig. 2.3. Zeta potential of CeO_2NPs as a function of the adsorbate doses in the presence and absence of SRE. Reported values are the average of three replicates with A) Cd B) As (III) and C) As (V) alone or in combination with the synthetic root exudates (SRE).

Figure 2.4 illustrates the mean hydrodynamic diameter of CeO_2NPs after equilibrium. Without SRE, Cd increased the hydrodynamic size of some CeO_2NPs as indicated by the fractions of larger CeO_2NPs in solutions with the increase of Cd. However, the predominant sizes of CeO_2NPs remained unchanged by Cd. In contrast, SRE drastically increased the hydrodynamic size of CeO_2NPs, Figure 2.5. The results indicated that SRE led to the formation of two dominant size groups of CeO_2NPs (100-2000 nm) and (46-100 µm). The appearance of multiple peaks with varying sizes did not correlate with the concentration of Cd, suggesting that SRE is the dominant factor leading to the aggregation of CeO_2NPs.

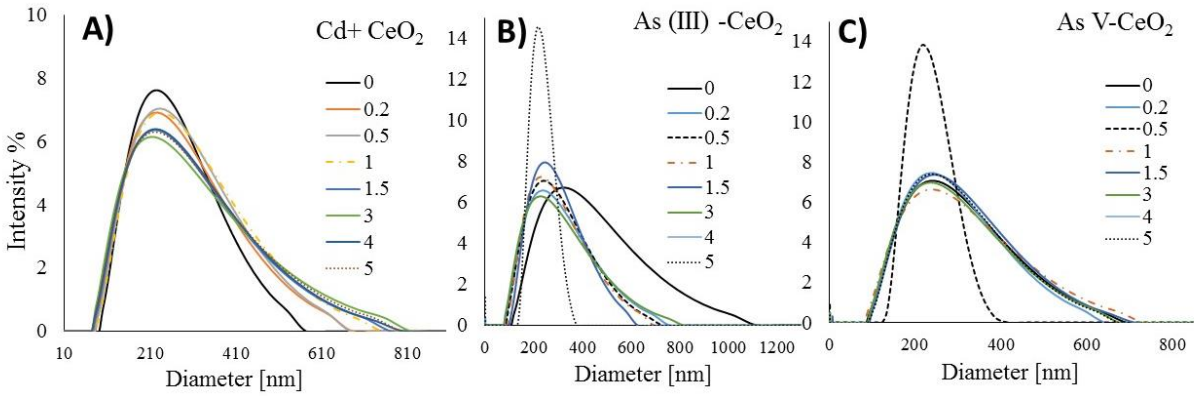

Fig. 2.4. Distribution of the hydrodynamic sizes of CeO_2NPs after exposure to different concentrations of A) Cd; B) As (III) and C) As (V) in DI water. The reported curves are representative of three replicates at each concentration.

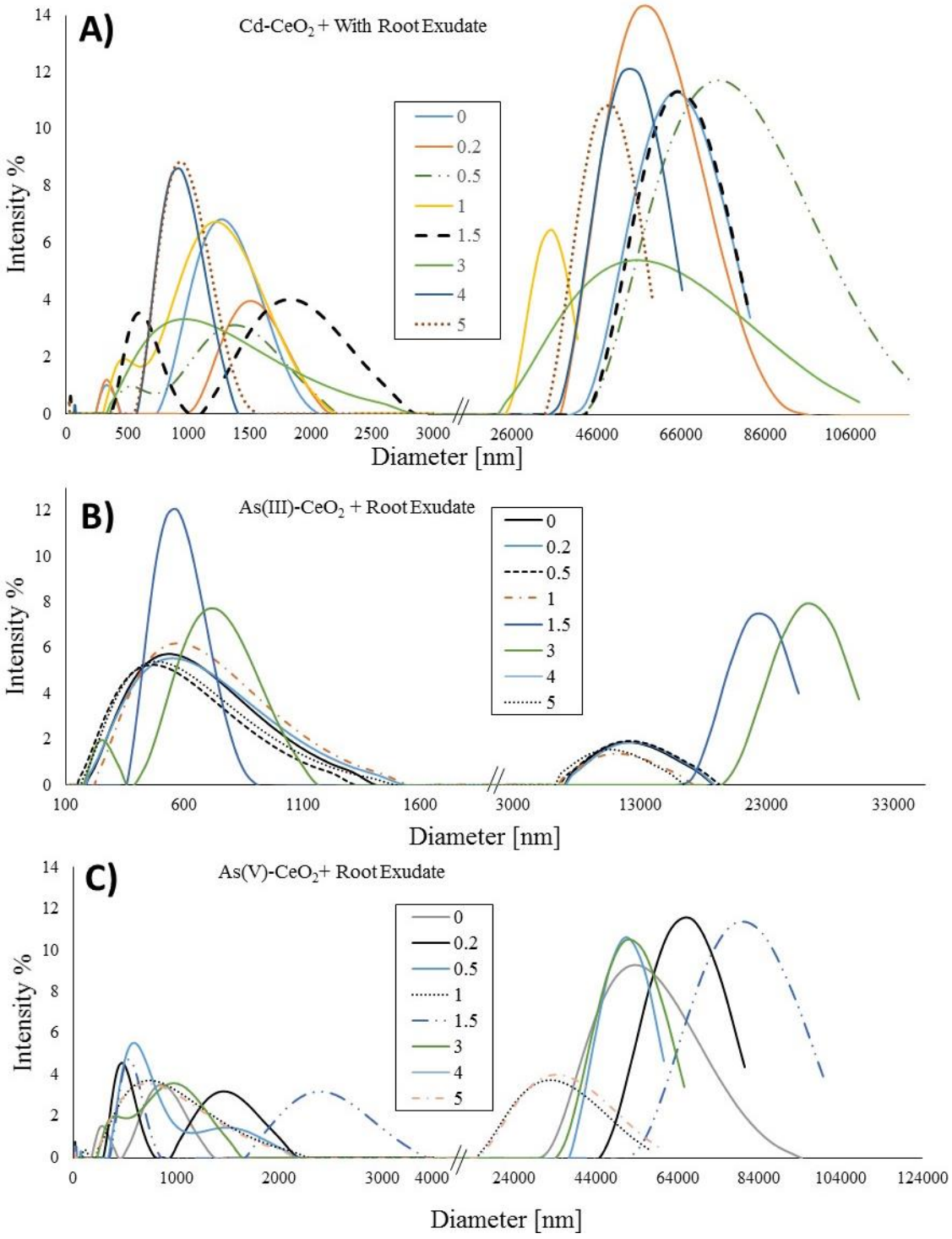

Fig. 2.5. Distribution of the hydrodynamic sizes of CeO_2NPs after exposure to A) Cd; B) As (III) and C) As (V) concurrently with SRE. The reported curves are representative of three replicates at each concentration.

2.3.3. Adsorption of Arsenic on CeO_2NPs

As shown in Figure 2.1, both As (III) and As (V) exhibited strong adsorption on CeO_2NPs without SRE and the adsorption was greater for As (III) than As (V). As observed for Cd, the presence of SRE reduced the adsorption of both As (III) and As (V), but to a less extent compared to its impact on Cd adsorption. In particular, the impact of SRE on the adsorption of As (III) on CeO_2NPs was minimal. For instance, the K_f from Freundlich fitting was 0.049 and 0.042 respectively for As (III) in the absence and presence of SRE. Other fitting parameters for As(III), As(V) and Cd are summarized in the Table 2.1.

Table. 2.1. Freundlich isotherm fitting parameters for the adsorption of Cd and As (III)/As(V) onto cerium oxide nanoparticles (CeO_2NPs) with and without presence of SRE.

Adsorbent	CeO_2NPs								
Adsorbate	Cd			As (III)			As (V)		
	No SRE	With SRE	No SRE	No SRE	With SRE	No SRE	No SRE	With SRE	No SRE
PH	5.9	2.3	2.3	5.9	2.3	2.3	5.9	2.3	2.3
K_f [mg g^{-1} $(L\ mg^{-1})^{1/n}$]	0.082	0.015	0.0045	0.049	0.042	0.0165	0.08	0.05	0.016
1/n	0.5	0.62	0.68	0.69	0.75	0.77	0.33	0.68	0.75
R^2	0.97	0.97	0.96	0.96	0.98	0.98	0.73	0.98	0.94

2.3.4. Impact of Arsenic adsorption on the physiochemical properties of CeO_2NPs

Figure 2.2 illustrates the concentrations of dissolved Ce from CeO_2NPs after 48h of mixing with different concentrations of As (III) and As (V). Without SRE, As (III) appeared to completely stop the dissolution of Ce^{3+} from the nanoparticle surfaces while As (V) displayed minimal effect on Ce dissolution. In the presence of SRE, Ce dissolution was significantly increased to a level similar to what was observed in the mixture with Cd and SRE, suggesting that enhanced dissolution of CeO_2NPs was primarily attributed to the presence of SRE.

As observed in the Cd study, SRE drastically changed the zeta potential of CeO_2NPs from around -50 mV to about +13 mV and the adsorption of As gradually reduced the zeta potential to the negative territory with increasing As concentration, Figure 2.3. However, the As alone at both oxidation states did not markedly change the zeta potential of CeO_2NPs. The zeta potential of CeO_2NPs was around -45 to -50 mV and -47 to -57 mV in the presence of up to 5 mg/L of As(III) and As(V) respectively.

The hydrodynamic size distributions of 100 mg L^{-1} of CeO_2NPs at equilibrium with different concentrations of As (III) and As (V) are presented in Figures 2.4 and 2.5. The average hydrodynamic size of CeO_2NPs was about 233±10 nm after contact with As (V) or As (III) for 48 hours without SRE. Interestingly, unlike the impact of Cd which increased the fractions of larger CeO_2NPs, both As (III) and As (V) appeared to reduce the hydrodynamic sizes of CeO_2NPs even though the predominant sizes were similar at different concentrations of As. The observation was particularly obvious at the highest concentration of As (III) and As (V). No specific correlation was observed between the increasing doses of As and the hydrodynamic size of CeO_2NPs.

In agreement with the study with Cd, the presence of SRE significantly increased the hydrodynamic size of CeO_2NPs. However, the impact of SRE seemed to be far greater in the presence of As (V) than As (III). For example, while SRE led to larger aggregates in both cases, the size of larger aggregates in the presence of As (V) was about five times bigger than that in the presence of As (III). In addition, the large CeO_2NPs aggregates accounted for a smaller fraction of the total CeO_2NPs in the mixture with As (III) than As (V).

2.4. Discussion

As expected, both Cd and As displayed strong adsorption on the surface of CeO_2NPs and this can be a significant mechanism affecting the plant uptake of heavy metals in their co-presence with CeO_2NPs. Plant root exudates is a considerable factor in the interactions of Cd and As with CeO_2NPs and generally reduced their adsorption on CeO_2NPs. The reduction is particularly significant for Cd, and to a less extent to As (V). To gain more insights into the effects of root exudates on the adsorption of Cd, two hypotheses were formulated: one is that in the mixture of SRE and Cd, SRE occupied most of the available spaces for Cd adsorption and the other one is that Cd and SRE form complexes and the complexes had lower adsorption affinity and capacity to CeO_2NPs surfaces. Based on our results shown in Figure 2.6, the Cd and SRE complexes (if they were formed) showed similar adsorption capacity on CeO_2NPs, hence the second hypothesis was not correct. But when we mixed the SRE with CeO_2NPs first, the adsorption of Cd was drastically reduced, suggesting that the first hypothesis is more plausible.

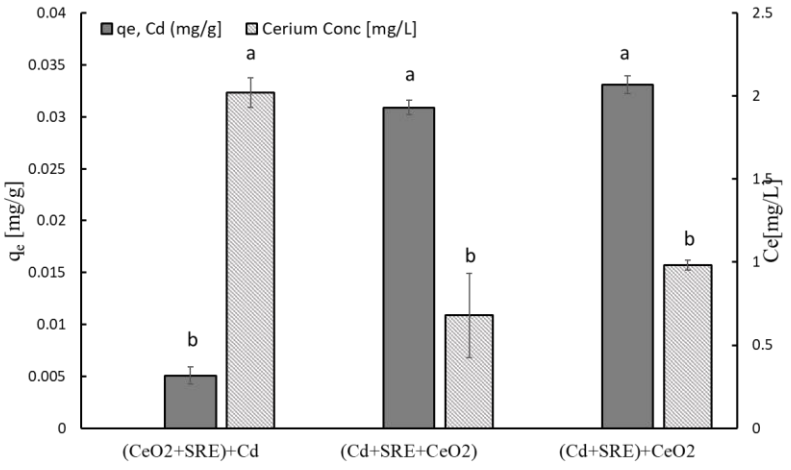

Fig. 2.6. Liquid concentration of Ce^{3+} (mg L^{-1}) and solid concentration of Cd on CeO_2NPs in three different treatments with an initial concentration of 4 mg L^{-1} of Cd; (CeO_2+SRE)+Cd refers to the treatment that CeO_2NPs were first mixed with SRE for 48 hours before the introduction of Cd; (Cd+SRE)+ CeO_2NPs refers to the treatment that Cd and SRE were first mixed for 48 hours before the addition of CeO_2NPs; (Cd+SRE+CeO_2) indicates that the three components were mixed simultaneously. Error bars represent standard deviation (n = 3). Different letters indicate significant differences between treatments ($p < 0.05$).

In addition to these two hypotheses, the reduced accessibility of adsorption sites by Cd due to the aggregation of CeO_2NPs in the presence of SRE may contribute to the lower Cd adsorption on CeO_2NPs. However, the aggregation of CeO_2NPs was greater for both As species and the effect of SRE on their adsorption on CeO_2NPs was weaker, implying that lower accessibility of adsorption sites was not a dominant reason for the reduced Cd adsorption.

For the adsorption of As, two observations stood out as particularly interesting. One is the much weaker effect of SRE on the adsorption of As compared to Cd, in particular the adsorption of As

(III) on CeO_2NPs, indicating that the effects of SRE on As and Cd adsorption might be different. This weaker effect of SRE on As may be ascribed to the chemistry of As. Unlike Cd, As did not exist as free-standing cations, instead, As (III) was predominantly in the form H_3AsO_3 ($pK_a = 9.17$) and As (V) a mixture of H_3AsO_4 ($pK_{a1}=2.20$) and $H_2AsO_4^-$ in this study. It is likely that the adsorption of neutral As(III) and part of the neutral As (V) occupied different adsorption sites from the charged ions of SRE, therefore competition was weak between the neutral molecules and SRE molecules. In the SRE mixture, the NH_2 functional group in D-serine molecule would be protonated to carry a positive charge at pH 1.9-2.5, which might attract the negatively charged $H_2AsO_4^-$ and reduce its adsorption to CeO_2NPs. They would have minimum effect on the adsorption of H_3AsO_3. The second interesting observation was the stronger adsorption of As (III) on CeO_2NPs than As (V). The general consensus about the fate of As species in the environment is that As (III) is more mobile and bioavailable than As (V) based on their adsorption to iron hydroxides minerals[25, 26], however, such conclusion may not hold true in the presence of ENPs, depending on whether ENPs loaded with As are more bioavailable to plants or not. If the ENPs loaded with As are less bioavailable to plant uptake, then As associated with these ENPs would also be retained in the root region, without being taken up by plants. In this scenario, the stronger adsorption of As (III) on ENPs may make it less bioavailable than As (V). Therefore, the availability of these heavy metal ions in the presence of emerging ENPs needs more detailed evaluation.

A key finding of this study was that not only the fate of heavy metal ions was affected by CeO_2NPs, co-existing heavy metal ions could also modify the key properties of CeO_2NPs. In this study, three aspects of the physicochemical properties of CeO_2NPs were closely examined, including their size distribution, zeta potential and the dissolution rate. Dissolution of Ce^{3+} from CeO_2NPs was mostly

unaffected by Cd and As (V) and was reduced by As (III), possibly because the adsorption of neutral H_3AsO_3 blocked some sites for dissolution. SRE alone markedly increased the dissolution rate of Ce^{3+} because once SRE was complexed with Cd, they did not affect CeO_2NPs dissolution, Figure 2.6. The enhanced dissolution was attributed to the redox reactions between low molecular weight acids in SRE mixture with CeO_2NPs in a previous study and our results agree with that conclusion.

Surprisingly, the zeta potential of CeO_2NPs was significantly changed by either SRE or Cd, but not by As species. We attribute the SRE effect to the adsorption of NH_2-containing molecules on the surface of CeO_2NPs, because at acidic condition, these molecules carry a positive charge and their adsorption to the negatively charged CeO_2NPs neutralized the surface charge and bestowed the surface some positive charge, leading to a positive zeta potential. For Cd^{2+}, similar reasoning can be made. For example, Cd^{2+} may bind to the =Ce-O^- group on CeO_2NP surface to form =Ce-O-Cd^+ complexes or form a claw structure with two of the surface groups as =$(Ce\text{-}O)_2$-Cd [27, 28]. Consequently, the adsorption of Cd^{2+} on CeO_2NPs steadily increased the zeta potential of CeO_2NPs, but the modified zeta potentials were both in the unstable range (-30 mV < zeta potential < 30 mV). Neutral H_3AsO_3 or H_3AsO_4 were not expected to significantly change the zeta potential of CeO_2NPs, consistent with what was observed in our study.

The zeta potential of CeO_2NPs play a significant role in nanoparticle aggregation and as shown in Figure 2.5, CeO_2NPs aggregated to a great extent in the presence of SRE and to a less extent, in the presence of Cd^{2+}. The result was supported by TEM images, Figure 2.7. No CeO_2NPs were detected in the solution with SRE by TEM, likely due to the precipitation of micro-sized particles and greater dissolution caused by SRE.

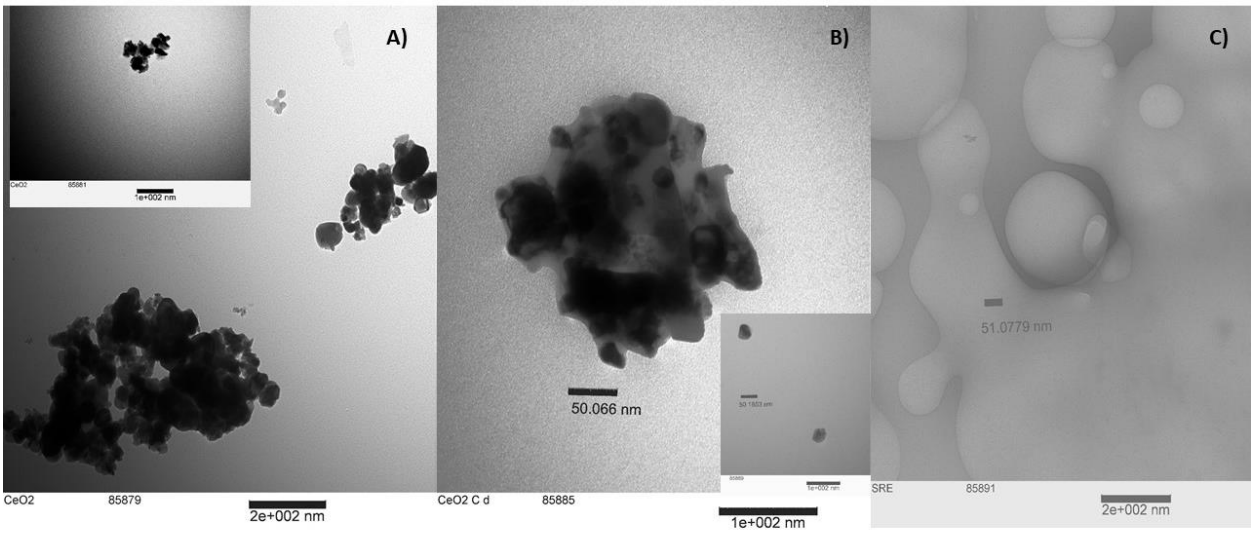

Figure 2.7. High resolution of Transmission electron microscopy TEM (JEOL JEM-1200EX, JEOL Ltd., Tokyo, Japan) studies of 100 mg/L CeO2 ENP (A) the TEM image of intact CeO2 ENPs. (B) It represent the physical transformation of CeO2ENPs after adsorption of Cd. (C) It indicate the macro transformation of ENPs that are not observable at nanoscale when the SRE was introduced in adsorption batch.

2.5. Conclusion

The size distribution of CeO_2NPs narrowed at high As concentrations which might be associated with the even more negative zeta potential of these nanoparticles at high As concentrations, but additional investigation is needed for a mechanistic understanding. CeO_2NPs aggregation, zeta potential and dissolution rate have a significant impact on their fate and accumulation in agricultural systems and this study is probably the first study to demonstrate that co-existing heavy metals can significantly modify the physicochemical properties of CeO_2NPs, providing new insights into the mechanisms of altered plant uptake of CeO_2NPs and co-existing heavy metals.

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CHAPTER III

IMPACT OF NANOPARTICLE SURFACE CHARGE AND PHOSPHATE ON THE UPTAKE OF COEXISTING CERIUM OXIDE NANOPARTICLES AND CADMIUM BY SOYBEAN (*GLYCINE MAX. (L.) MERR.*)[2]

3.1. Summary

Engineered nanoparticles (ENPs) often interact closely with co-existing environmental pollutants; however, the effects of nanoparticle properties, such as surface charge, on the interactions of ENPs with co-present environmental pollutants in a plant system has not been examined. This study investigated the roles of surface charge of nanoparticles and growth media chemistry on the mutual effects of cerium oxide nanoparticles (CeO_2NPs) and cadmium (Cd) on their plant uptake and accumulation in a hydroponic system. Soybean seedlings were exposed to five nanoparticle/Cd treatments including: 100 mg L^{-1} $CeO_2NPs^{(+)}$;100 mg L^{-1} $CeO_2NPs^{(-)}$; 100 mg L^{-1} $CeO_2NPs^{(+)}$ + 1 mg L^{-1} Cd; 100 mg L^{-1} $CeO_2NPs^{(-)}$ + 1 mg L^{-1} Cd; and 1 mg L^{-1} Cd only. The previous treatments were imposed in the presence and absence of 50 mg L^{-1} phosphate. After four days of exposure to the treatments, concentrations of Cd and Ce in plant tissues were quantified by inductively coupled plasma-mass spectrometry (ICP-MS). Roots exposed to $CeO_2NPs^{(+)}$ contained 87% higher Ce than plants exposed to $CeO_2NPs^{(-)}$. The presence of phosphate significantly increased the root

[2] Taylor & Francis is pleased to offer reuses of its content for a thesis or dissertation free of charge contingent on resubmission of permission request if work is published. Impact of nanoparticle surface charge and phosphate on the uptake of coexisting cerium oxide nanoparticles and cadmium by soybean (Glycine max. (L.) merr.),. Hamidreza Sharifan, , Xiaoxuan Wang, X Ma,. International Journal of Phytoremediation,. aylor & Francis 1522-6514 (Print) 1549-7879 (Online) Journal homepage: https://www.tandfonline.com/loi/bijp20

concentration of Ce exposed to both CeO_2NPs. As previously reported, CeO_2NPs and Cd mutually affected their plant uptake and accumulation. The interactions of CeO_2NPs and Cd are heavily affected by nanoparticle surface charge, and phosphate, a common nutrient in the growth medium, plays a significant role in the plant uptake and accumulation of Cd and CeO_2NPs.

Key Words: surface charge, phosphate, cerium oxide, nanoparticle, cadmium, soybean

3.2. Introduction

Nanotechnology is a driving force for innovations in the food-water-energy nexus (Rossi et al. 2019). Accompanying the rapid advancement of nanotechnology is the increasing manufacturing and discharge of engineered nanoparticle (ENPs). Agricultural soil is a primary sink of ENPs in the environment (Meesters et al. 2016). Direct applications of ENP-incorporated agrichemicals further increase the accumulation of ENPs in agricultural soils (Dasgupta, Ranjan, and Ramalingam 2017). As a result, numerous studies have evaluated the effects of ENPs on plants and their accumulation in edible tissues of agricultural crops (Ma and Yan 2018; Ma et al. 2015). In addition to the direct impact and accumulation of ENP elements in plant tissues, however, ENPs may affect plant growth and food safety through their alteration of plant uptake and accumulation of coexisting environmental pollutants (Ma and Yan 2018). Co-occurring ENPs change the plant uptake of both organic (De La Torre-Roche et al. 2012) and inorganic pollutants (Wang et al. 2018) in soil and hydroponic systems. In particular, we have shown that cerium oxide nanoparticles (CeO_2NPs) and cadmium (Cd) mutually affect their plant uptake and accumulation in soybeans through a variety of mechanisms including the adsorption of Cd on CeO_2NPs surface (Sharifan et al. 2018), the modification of rhizosphere environments through greater excretion of root exudates (Rossi et al. 2018) and modified root endodermal barriers (Rossi et al. 2017). Despite an increased understanding of the mutual effects of these two chemicals, the impact of surface properties of CeO_2NPs on their interaction with Cd has not been examined.

Surface charge plays a distinctive role in the fate and transport of CeO_2NPs. For example, after wheat seedlings were treated with CeO_2NPs possessing different surface charges for 34 h, positively charged CeO_2NPs were mostly retained on the root surface while the neutral and negatively charged CeO_2NPs showed much higher accumulation in wheat shoots, despite that the

Ce concentrations in roots exposed to neutral and negatively charged ENPs were much lower (Spielman-Sun et al. 2017). Similarly, positively charged gold nanoparticles (AuNPs) are readily retained by plant roots while negatively charged AuNPs are more efficiently translocated from roots to shoots (Zhu et al. 2012). Different surface charge of ENPs also leads to different subcellular localization in plant roots and different biochemical impacts (Ma and Quah 2016). Using an ultra-high resolution X-ray fluorescence imaging technique, large quantity of negatively charged CeO_2NPs was found in root cell cytoplasm in cortex tissues in addition to their presence in intercellular spaces while positively charged CeO_2NPs were mostly associated with the root epidermal cells, indicating the predominant impact of surface charge on ENP fate and transport (Li et al. 2019). The surface charge also affects the adsorption of Cd on CeO_2NP surfaces due to the significant role of electrostatic forces in the adsorption process (Sharifan et al. 2018).

In addition to the physiochemical properties of ENPs, the chemistry of the growth media has a strong impact on ENPs fate and transport. In particular, the presence of phosphate affects the speciation and uptake of CeO_2NPs by plants. Addition of phosphate inhibits the redox cycle between Ce^{3+} and Ce^{4+} on CeO_2NPs surface due to the formation of $CePO_4$ (Singh et al. 2011). Also, in plant roots, the transformation of Ce^{4+} to Ce^{3+} was markedly reduced in the presence of 1 mM PO_4 in the growth media (Wang et al. 2017). The presence of PO_4 also led to the formation of needle-like $CePO_4$ nanoparticles and different subcellular localizations of Ce in plant root cells. A recent study found that the presence of PO_4 significantly increased the adsorption of Cd on graphene oxide (GO) at pH 5.3-10.0 due to the formation of a ternary surface complex (Ren et al. 2016). Given the abundance of phosphate in biological systems and plant growth media and the strong impact of surface chemistry on ENPs fate and impact, it is important to understand the role of ENP surface properties and the biogeochemical conditions in plant growth media on the mutual

interactions of ENPs and co-occurring environmental chemicals. The primary goal of this study was to understand the effects of surface charge of CeO_2NPs and the presence of phosphate on the uptake and accumulation of coexisting CeO_2NPs and Cd by soybeans (*Glycine max* (L.) Merr. var. 'Tohya'). We chose CeO_2NPs as a model ENP because of its broad applications in industry and our previous experiences with this ENP, and Cd due to its prevalence in agricultural soils. Soybean was used in this study because of its global importance as a food crop.

3.3. Materials and Methods

3.3.1 Materials

An aqueous dispersion of negatively charged CeO_2NPs ($CeO_2NPs^{(-)}$) (20% by weight, 10-30 nm) was purchased from the US Research Nanomaterials, Inc. (Houston, TX). Positively charged CeO_2NPs ($CeO_2NPs^{(+)}$) (10% by weight, <25 nm) was obtained from Sigma Aldrich (St. Louis, MO). Both ENPs were fully characterized through our previous studies (Rossi et al. 2018; Zhang et al. 2018). Both ENPs have irregular polygonal shapes, the zeta potential for $CeO_2NPs^{(-)}$ was approximately -51.8 mV and for $CeO_2NPs^{(+)}$, +47.3 mV in 200 mg/L suspensions (Zhang et al. 2018). Cd sulfate ($CdSO_4$) was purchased from Fisher Scientific Inc. (Pittsburgh, PA). High purity sodium phosphate monobasic (NaH_2PO_4 >98%) was purchased from Sigma Aldrich (St. Louis, MO).

3.3.2. Plant growth

The seeds of *Glycine max* (L.) Merr. var. 'Tohya' (soybean) were purchased from Johnny's Selected Seeds (Fairfield, MN). The seeds were disinfected with 2.7% sodium hypochlorite solution for 5 min, washed with deionized water (DI) three times and then germinated in a moist potting soil mix (Miracle Gro®, the Scotts Company, Marysville, OH) for four days. The seedlings were transplanted to 50 mL

centrifuge tubes containing quarter strength Hoagland solution. The plant seedlings were cultivated in the nutrient solution at room temperature under a 16/8 h fluorescent illumination cycle (250 µmol m^2 s^{-1}) for 14 days before any treatment. The seedlings were randomly rotated every other day during the cultivation stage to reduce possible differences in light reception. The nutrient solution was replenished every morning and evening. Similarly-sized seedlings were selected for different treatments as described below.

3.3.3. Treatments with CeO_2NPs, Cd and phosphate

Five treatments without the presence of phosphate salt were prepared in deionized (DI) water as follow: 100 mg L^{-1} $CeO_2NPs^{(+)}$;100 mg L^{-1} $CeO_2NPs^{(-)}$; 100 mg L^{-1} $CeO_2NPs^{(+)}$ + 1 mg L^{-1} Cd; 100 mg L^{-1} $CeO_2NPs^{(-)}$ + 1 mg L^{-1} Cd; and 1 mg L^{-1} Cd without nanoparticles. A parallel series of treatments containing 50 mg L^{-1} phosphate salt (NaH_2PO_4) in addition to the above-mentioned CeO_2NPs and/or Cd were also prepared. 100 mg L^{-1} of CeO_2NPs were freshly prepared in sterilized ultra-pure water before use (18.3 MΩ). A known amount of $CdSO_4$ or NaH_2PO_4 were then dissolved in a solution containing 100 mg L^{-1} of CeO_2NPs to obtain concentrations of 1 mg L^{-1} Cd or 50 mg L^{-1} phosphate in the solution. The phosphate concentration was selected based on the recommended concentrations in literature (Lv et al. 2012). All solutions were mixed on a shaker table for ten minutes before use. A negative control with seedlings growing in DI water was also prepared.

After solution preparation, 14-day old seedlings were removed from Hoagland solutions and rinsed five times with DI water to remove the residue of the nutrient solution from seedling roots. These seedlings were then transferred to 50 mL polypropylene centrifuge tubes containing 50 mL of different treatment solutions as described above. Three replicates were prepared for each treatment. Altogether, 33 seedlings were grown in 11 different treatment solutions for four days.

During the period of treatment, the centrifuge tubes were replenished with DI water twice per day (morning-evening) to compensate for the transpired water. The water transpiration in all treatments was recorded.

3.3.4. Plant uptake of cerium and cadmium

At termination, the seedlings were removed from the tubes and the roots were rinsed three times with DI water. The seedlings were then dried with a paper towel. Afterward, the seedlings were separated into roots and shoots, dried in an oven at 75 °C for 48 hours and acid digested following published protocols (Wang et al. 2018). Briefly, 5 mL of 70% (w/w) nitric acid (Certified ACS Plus) was added to a 50 mL digestion tube containing 0.5 g (shoots) or 0.25 g (roots) of dry biomass and heated at 95 °C for 5 h on a DigiPREP MS hot block digester (SCP Science, Clark Graham, Canada). After being cooled down to room temperature, 2 mL of hydrogen peroxide (30%, Certified ACS) was added to each tube and re-heated on the hot block to 95 °C until the biomass was fully digested (Zhang et al. 2015; Zhang et al. 2017). The amount of Ce and Cd in each sample was then quantified by an inductively coupled plasma−mass spectrometry (ICP-MS) (Agilent 7500i, Agilent Technologies Co. Ltd, USA). Detailed information on the operational parameters of ICP-MS and sample quality control can be found from our previous publications (Sharifan et al. 2018).

3.3.5. Statistical Analysis

Statistical analysis of Ce and Cd concentrations in plant tissues was performed using the Minitab 18 Statistical Software (Minitab Inc., State College, PA, USA). The comparison between the mean values of different treatments was carried out using One-Way ANOVA, followed by Tukey's test at the significance level of 5% ($p < 0.05$).

3.4. Results and Discussion

The dry biomass of soybean roots and shoots exposed to different treatments of Cd and CeO_2NPs in the presence and absence of the 50 mM phosphate is shown in **Figure 1.** No significant differences were detected between different treatments. For the plant transpiration (**Figure S1**), the only significant difference was the $CeO_2^{(-)}$-Cd transpiration was higher than that for $CeO_2^{(+)}$-Cd treated plants.

In agreement with a previous study (Spielman-Sun et al. 2017), soybean roots exposed to $CeO_2NPs^{(+)}$ had 87% higher Ce than plants exposed to the same concentration of $CeO_2NPs^{(-)}$ in the absence of phosphate, **Figure 2**. However, Ce concentrations in soybean shoots were statistically equivalent in most treatments, but indicating greater transport of $CeO_2NPs^{(-)}$ compared to $CeO_2NPs^{(+)}$. The presence of PO_4 increased the root concentration of Ce exposed to CeO_2NPs, regardless of the surface charge, and some of the increases were significant. The Ce concentration in soybean roots increased by 61% and 66% for plants exposed to $CeO_2NPs^{(+)}$ and $CeO_2NPs^{(-)}$, respectively. The presence of PO_4 displayed different impacts on Ce concentration in soybean shoots exposed to CeO_2NPs with different surface charges. While 50 mg/L of PO_4 significantly increased Ce concentration by 58% in soybean shoots exposed to $CeO_2NPs^{(-)}$, this increase was insignificant in soybean shoots exposed to $CeO_2NPs^{(+)}$. The impact of PO_4 on the transformation of CeO_2NPs is well recognized due to the formation of $CePO_4$ by Ce^{3+} and PO_4, which can be either deposited back on nanoparticle surface (Schwabe et al. 2014), or immobilized on root epidermis and intercellular spaces (Zhang et al. 2012). It is likely that the formation of $CePO_4$ reduced the potential migration of dissolved Ce^{3+} away from the root surface and hence increased the root Ce concentration in the presence of PO_4. While similar processes might have occurred in the soybean rhizosphere exposed to $CeO_2NPs^{(+)}$, the electrostatic interactions between $CeO_2NPs^{(+)}$

and negatively charged root surfaces may be stronger than the potential effects of PO_4, leading to an insignificant impact of PO_4 on the root concentration of Ce in plants exposed to $CeO_2NPs^{(+)}$.

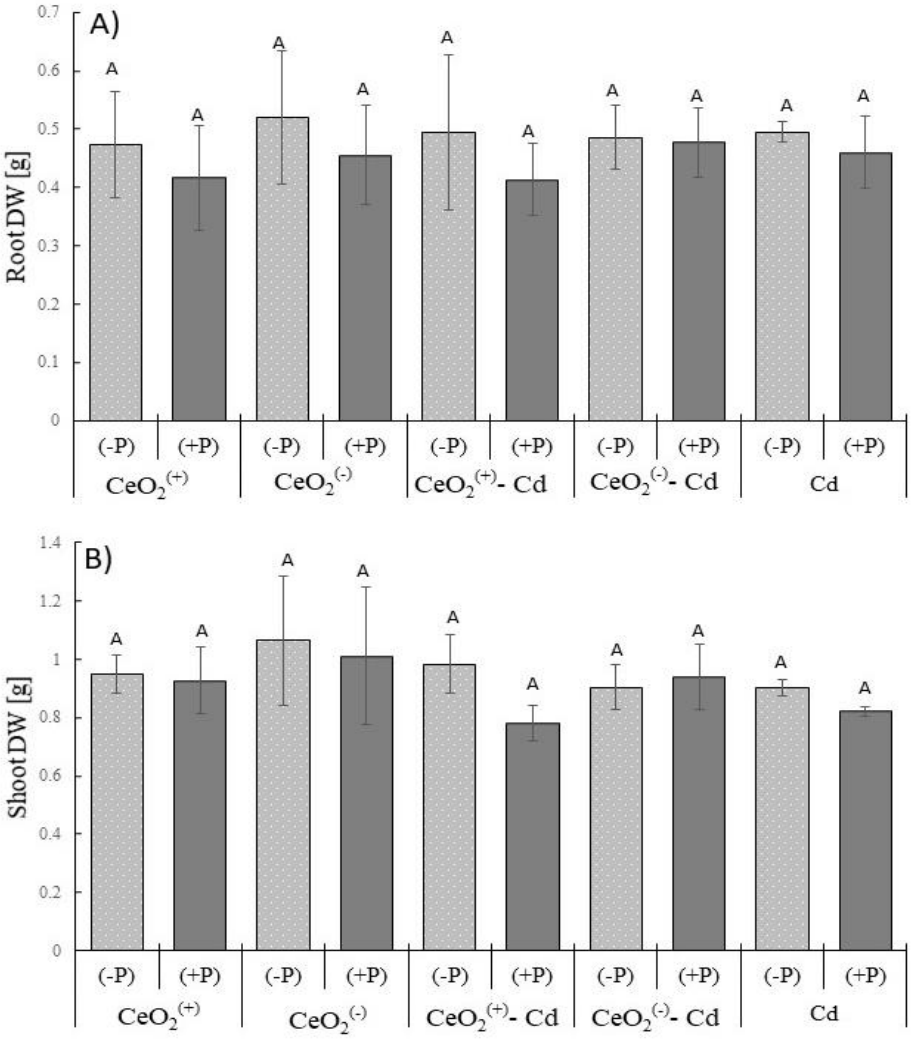

Figure 3.1: Dry weight (g) of soybean roots (A) and shoots (B) grown in 1.0 mg L^{-1} Cd and 100 mg L^{-1} $CeO_2NPs^{(+)}$ or $CeO_2NPs^{(-)}$ separately or in combination. (-P) and (+P) indicates the absence and presence of 50 mg L^{-1} of phosphate in the growth media. Different letters above each column indicate significant differences by Tukey's post hoc test ($p < 0.05$) and the error bars represent standard deviation ($n = 3$).

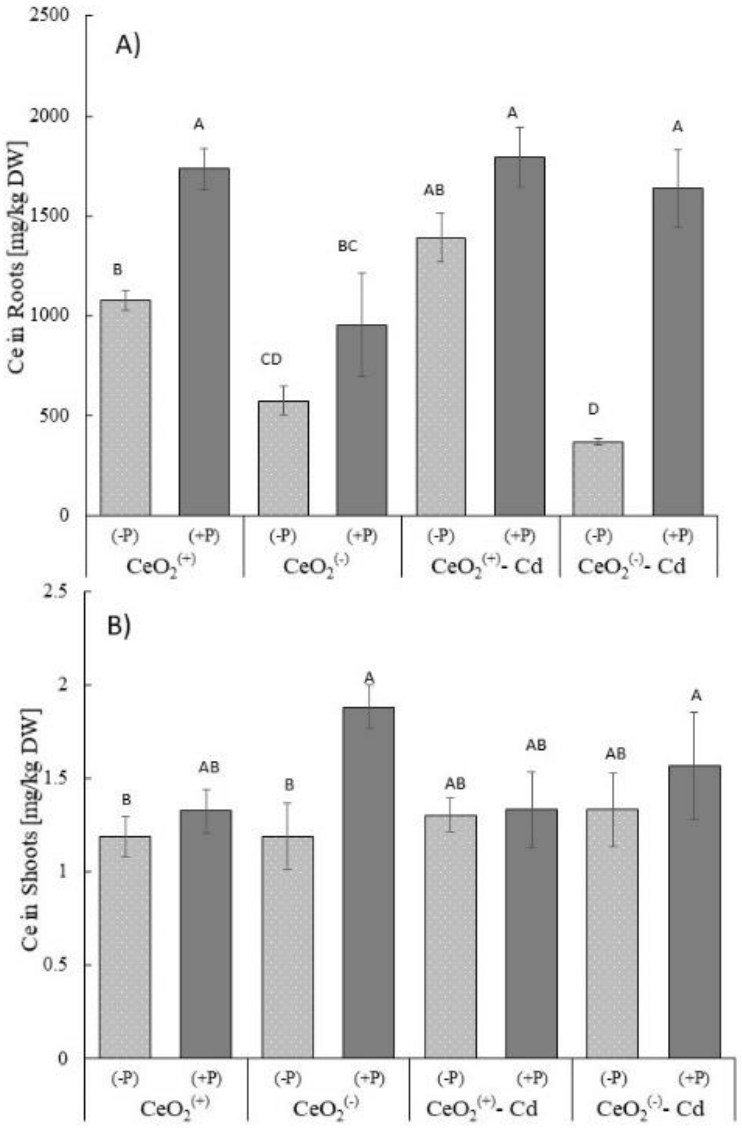

Figure 3.2: Cerium concentrations in soybean roots (A) and shoots (B) grown in 1.0 mg L^{-1} Cd and 100 mg L^{-1} $CeO_2NPs^{(+)}$ or $CeO_2NPs^{(-)}$ separately or in combination. (-P) and (+P) indicates the absence and presence of 50 mg L^{-1} of phosphate in the growth media. Different letters above each column indicate significant differences by Tukey's post hoc test ($p < 0.05$) and the error bars represent standard deviation ($n = 3$).

Cadmium affected plant uptake of Ce in the absence of PO_4. Herein, we further demonstrate that the impact was dependent on the surface charge of CeO_2NPs. The co-presence of 1.0 mg/L of Cd and $CeO_2NPs^{(+)}$ increased the Ce concentration in roots by 29% compared to the absence of Cd but decreased Ce concentration in roots by 35% in $CeO_2NPs^{(-)}$-exposed soybean seedlings. The presence of PO_4 resulted in a slightly but not significantly higher Ce concentration in soybean roots which concurrently exposed to Cd and $CeO_2NPs^{(+)}$. However, the presence of PO_4 increased Ce concentration in soybean roots exposed to both Cd and $CeO_2NPs^{(-)}$ by almost 4 times compared with soybean plants exposed to the same concentrations of Cd and Ce but without PO_4. Cadmium could affect plant Ce accumulation from coexisting $CeO_2NPs^{(-)}$ through the modification of nanoparticle physicochemical properties such as their size, surface charge and dissolution (Sharifan et al. 2018), the anatomical structures of plant roots (Rossi et al. 2017), and the chemistry around root rhizosphere (Rossi et al. 2018). However, the overall effect of Cd on Ce concentration in soybean roots hydroponically grown in the medium with both Cd and $CeO_2NPs^{(-)}$ was not significant due to the competing effects of affected processed on Ce accumulation. The underlying mechanism on PO_4 behavior in exposure to Ce in soybean roots rhizosphere in the co-presence of Cd and $CeO_2NPs^{(-)}$ is unclear, but may be ascribed to the formation of $CeO_2NPs^{(-)}$-Cd-PO_4^{3-} ternary complex (Ren et al. 2016) which had larger hydrodynamic size than $CeO_2NPs^{(-)}$ and therefore, greatly enhanced their deposition on soybean roots. Similar $CeO_2NPs^{(+)}$-PO_4^{3}-Cd complex might have occurred too, but to a lesser extent. If the postulation of the ternary complex is correct, lower transport of Ce from roots to shoots should be expected. Indeed, we observed insignificant impact of Ce concentration in soybean shoots by co-present Cd in the presence of PO_4, regardless of the surface charge of the nanoparticles. In comparison, the Ce concentration in soybean shoots in a previous study was

increased by 60% by co-occurring Cd when the plants were jointly exposed to $CeO_2NPs^{(-)}$ and Cd hydroponically without PO_4, while the Ce concentration in the shoots is reduced by 15% in the presence of PO_4 in the $CeO_2NPs^{(-)}$-Cd treatment.

Soybean seedlings showed high uptake of Cd in plant roots and shoots. The presence of PO_4 significantly increased Cd concentration in soybean roots but only slightly increased its concentration in soybean shoots (**Figure 3)**. The formation of a Cd-phosphate solid phase might be the primary reason for the elevated Cd concentration in soybean roots because the particulate Cd-phosphate is more likely to be retained by soybean roots, and thus higher Cd concentration in soybean roots did not lead to higher Cd concentration in the shoots. Consistent with a previous study, $CeO_2NPs^{(-)}$ had little impact on Cd root concentration in soybean seedlings hydroponically grown in the absence of PO_4 (Rossi et al. 2018). However, $CeO_2NPs^{(+)}$ significantly increased Cd concentration in soybean roots by approximately 48%. The greater attachment of $CeO_2NPs^{(+)}$ on root surfaces could have lowered the pore sizes of root epidermal cells and increased their adsorption on the root surface, however, this is unlikely a dominant reason because of the relative insignificance of apoplastic transport for Cd (Song, Jin, and Wang 2017) and comparable water transpiration by plants from different treatment groups in this study, **Figure 41**. It is more likely that the symplastic pathways of Cd uptake, regulated by various active transporters, are more heavily affected by $CeO_2NPs^{(+)}$ than $CeO_2NPs^{(-)}$. Detailed studies concerning the impact of ENPs on heavy metal transporters in plant root cells are rare, and future studies are needed to elucidate the interactions of heavy metals with co-existing ENPs with different physicochemical properties. Neither nanoparticles significantly affected Cd concentration in soybean shoots in this study, in contrast to a previous study that $CeO_2NPs^{(-)}$ reduced Cd concentration in soybean shoots by almost 70% (Rossi et al. 2018).

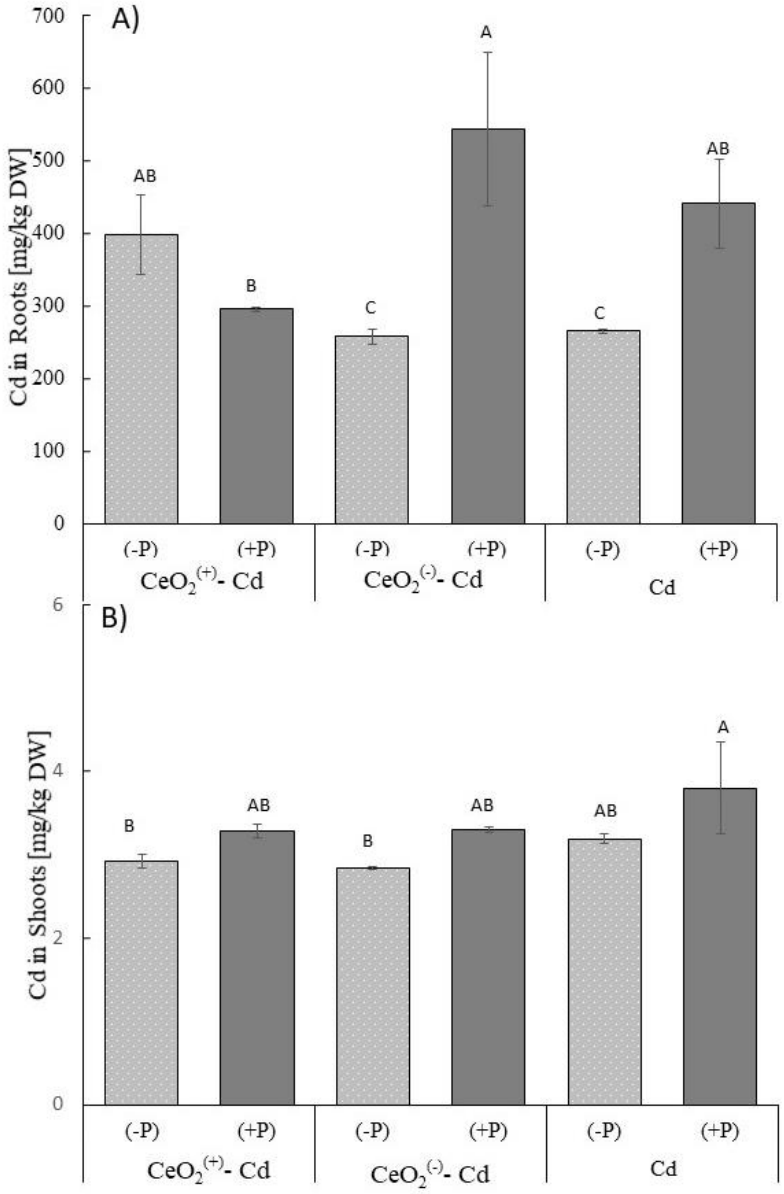

Figure 3.3: Cadmium concentrations in soybean roots (A) and shoots (B) grown in 1.0 mg L^{-1} Cd and 100 mg L^{-1} $CeO_2NPs^{(+)}$ or $CeO_2NPs^{(-)}$ separately or in combination. (-P) and (+P) indicates the absence and presence of 50 mg L^{-1} of phosphate in the growth media. Different letters above each column indicate significant differences by Tukey's post hoc test ($p < 0.05$) and

the error bars represent standard deviation (n = 3).

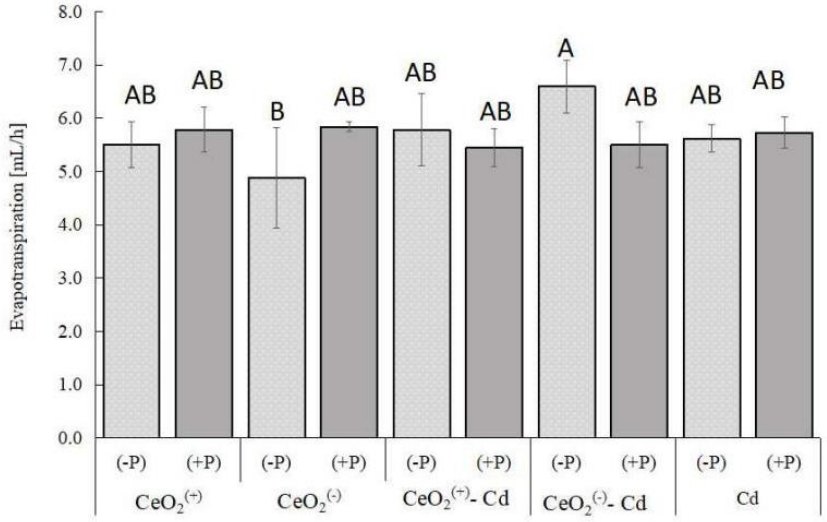

Figure 3.4: Accumulative transpiration of soybean seedlings exposed to 1.0 mg L^{-1} Cd and 100 mg L^{-1} $CeO_2NPs^{(+)}$ or $CeO_2NPs^{(-)}$ separately or in combination. (-P) and (+P) indicates the absence and presence of 50 mg L^{-1} of phosphate in the growth media. Different letters above each column indicate significant differences by Tukey's post hoc test ($p < 0.05$) and the error bars represent standard deviation (n = 3).

Interestingly, the presence of PO_4 almost doubled the Cd concentration in soybean roots grown in the media containing $CeO_2NPs^{(-)}$ and Cd, compared with soybean seedlings co-exposed to these two chemicals but without PO_4. On the contrary, the presence of PO_4 lowered the root Cd concentration by about 30% in the system containing $CeO_2NPs^{(+)}$ and Cd, suggesting that PO_4 interfered the interactions between CeO_2NPs and Cd and the interference differed with the surface charge of CeO_2NPs.

3.5. Conclusion

Understanding the mutual interactions of ENPs with co-occurring environmental chemicals and how these interactions affect their fate and transport in different environmental systems is an emerging topic in sustainable nanotechnology. Several previous studies have demonstrated that the close interactions of coexisting CeO_2NPs and Cd in a plant system reciprocally affect their plant uptake and accumulation. Our present study confirmed those interactions and demonstrated for the first time that the mutual effect of CeO_2NPs and Cd is heavily affected by the nanoparticles surface charge. In addition, our study revealed that PO_4, a common nutrient in the growth medium, plays a significant role in the plant uptake and accumulation of Cd and CeO_2NPs.

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CHAPTER IV

MUTUAL EFFECTS AND *IN-PLANTA* UPTAKE OF CERIUM OXIDE NANOPARTICLES AND CADMIUM IN HYDROPONICALLY GROWN SOYBEAN (*GLYCINE MAX* (L.) MERR.)[3]

4.1. Summary

Cadmium (Cd) is an extremely toxic metal to humans even at very low concentrations. Elevated Cd in soil due to various anthropogenic activities has led to higher Cd concentration in various crop tissues, making it a serious food safety concern. With the progressive production and continued accumulation of engineered nanoparticles (ENPs) in agricultural soils, it is imperative to understand how ENPs may affect the plant metal uptake and accumulation. The goal of this study was to understand the mutual effects of cerium oxide nanoparticles (CeO_2NPs) and Cd^{2+} on their uptake and accumulation by soybean seedlings (*Glycine max* (L.) Merr.) in a hydroponic system. Soybean seedlings were exposed to four treatments (control, 1.0 mg L^{-1} Cd^{2+}, 1.0 mg L^{-1} Cd^{2+} + 100 mg L^{-1} CeO_2NPs, 100 mg L^{-1} CeO_2NPs) for 10 days. At termination, plant roots and shoots were separated and the concentrations of Cd and Ce in these tissues determined. In addition, the amount of ionic Ce and particulate Ce within soybean roots were analyzed. Significant interactions between co-existing CeO_2NPs and Cd were found concerning their accumulation in plant tissues. While CeO_2NPs did not affect the total Cd associated with soybean roots, they significantly reduced the translocation of Cd from roots to shoots ($p<0.05$). In contrast, the co-

[3] 1. Rossi, L.; Sharifan, H.; Zhang, W.; Schwab, A. P.; Ma, X., Mutual effects and in planta accumulation of co-existing cerium oxide nanoparticles and cadmium in hydroponically grown soybean (Glycine max (L.) Merr.). *Environmental Science: Nano* **2018,** *5*, (1), 150-157.; Reproduced by permission of The Royal Society of Chemistry (RSC) on behalf of the Centre National de la Recherche Scientifique (CNRS) and the RSC

presence of Cd lowered the concentration of Ce in soybean roots but significantly increased the concentration of Ce in soybean shoots more than 90%. The altered plant accumulation of co-existing Cd and Ce was attributed to various physical, chemical and biological processes occurred in plant rhizosphere. Specifically, the co-presence of Cd and CeO_2NPs led to higher excretion of plant root exudates, which lowered the pH in plant rhizosphere, enhanced CeO_2NPs dissolution and likely facilitated Cd complexation with large biomolecules, leading to altered uptake accumulation of both chemicals.

Keywords: heavy metals, cerium oxide nanoparticles, cadmium, soybean

4.2. Introduction

Cadmium (Cd) is a ubiquitous environmental pollutant extremely toxic to humans. Chronic exposure to Cd has been shown to be associated with a wide variety of health problems such as renal dysfunction, cancer, osteoporosis and cardio vascular disease [2, 3]. Consumption of Cd-tainted food is a predominant pathway for human exposure to Cd due to its highly efficient soil-to-plant transfer [4, 5]. A recent study indicated that food consumption accounts for almost 90% of Cd exposure in the general non-smoking population, rendering it an urgent task to understand processes which may potentially aggravate the plant accumulation of Cd [3].

With the current embrace of nanotechnology in many industries including the agricultural industry, engineered nanoparticles (ENPs) are increasingly detected in agricultural soils. They have been shown to affect plant physiological and biochemical processes at various levels and altered plant uptake of co-existing environmental pollutants [6, 7]. Earlier efforts to investigate the ENP and co-occurring environmental pollutants, mostly focused on organic contaminants [7, 8]. Few studies have evaluated the effects of ENPs on plant uptake of co-existing heavy metals. Plant uptake of heavy metals involves complex transport processes and some are highly regulated. For example, Cd^{2+} is

taken up by plant roots both apoplastically and symplastically [4, 9]. In the symplastic pathway, Cd^{2+} may diffuse into plant root cells passively or be actively transported via different protein transporters embedded in root plasma membrane [4, 9, 10]. Two types of Cd^{2+} transporters have been identified including the more specific Cd^{2+} ZIP transporters (Zn/Fe regulated transporter protein) and the less selective calcium permeable channels [9, 11, 12]. If Cd^{2+} forms complexes with surrounding chelates such as chemicals in plant root exudates, the complexes are transported into plant roots through YSL (Yellow-Stripe 1-Like) proteins [9, 13]. Once Cd species are in plant root cells, they can be translocated to the leaves and grain tissues. The translocation from roots to shoots is greatly affected by the vacuolar compartmentation of Cd-phytochelatin complexes as well as the transporters involved in xylem loading of Cd [4, 10, 14, 15].

Scientific evidences dictate that the ENPs are capable to may potentially interrupt many of the physiological processes involved in heavy metal uptake and transport. Consequently, it is of great interest to investigate the impact of ENPs on plant uptake of co-existing heavy metals. Cerium oxide nanoparticles (CeO_2NPs) are one of the ENPs that have been broadly used over the past years, particularly as a fuel additive [16, 17]. The main pathways for CeO_2NPs to enter into agricultural soils include deposition of vehicle exhausts, municipal runoff and land application of bio-solids [16]. Simultaneous exposure to both CeO_2NPs and heavy metals such as Cd^{2+} is increasingly possible for crops. Due to the disruptive impacts of CeO_2NPs on the cellular membrane as well as the plant physiology and root anatomy [18, 19], CeO_2NPs may substantially change the plant uptake and accumulation of heavy metals.

In addition, CeO_2NPs display high adsorption capacity for hazardous heavy metals such as arsenic (As), lead (Pb) and Cd [20, 21] that implies the carrier role ofCeO_2NPs in surface immobilizing the Cd^{2+} to penetrate into plant tissues or retain Cd^{2+} in plant rhizosphere when they are not taken up

by plants [16, 22]. The complex interactions of CeO_2NPs and other metals suggest that CeO_2NPs are likely to alter the plant uptake and accumulation of co-present heavy metals. A few available studies in the literature with different ENPs corroborate this assumption. For example, TiO_2NPs (100 mg L^{-1}) were shown to significantly reduce the phytotoxicity of Cd to rice (*Oryzasativa L*), likely due to the adsorption of Cd on TiO_2 [23]. However, comprehensive studies and mechanistic insight into ENP-heavy metal interactions in plant rhizosphere are lacking.

Meanwhile, evidence has been reported that plant uptake of ENPs may be affected by various abiotic stresses. In general, higher abiotic stresses such as salt stress, nutrient shortage stress appeared to increase plant uptake of ENPs elements[24]. ENPs accumulation in plant tissues has been a great food safety concern due to multiple dietary exposure routes.. As a result, the plant uptake of ENPs in the presence of heavy metals (as a source of abiotic stress) is also pivotal to investigate. The objectives of this research were to investigate the reciprocal effects of CeO_2NPs and Cd on their uptake by soybeans and to explore the underlying physical and chemical mechanisms of the their uptake.

Soybean (*Glycine max*. (L.) Merr.) is a strong study model because it is a main protein source of human diet that can accumulate metals (including Cd) in the grains [25]. Soybean was reported by the Food and Agricultural Organization (FAO) as the fifth most produced crop worldwide that provides about 30% vegetable oil and 77% nitrogen fixation. The global demand for soybean products increases by 2.2% annually [26]. The concentration of Cd in soybean seeds is positively correlated with its concentration in soil and has surpassed the maximum permissible concentration (0.2 mg/kg) administrated by the Codex Alimentarius Commission in some studies (FAO) [27], making it a great choice for this study as a model plant.

4.3. Material and Methods

4.3.1. *CeO_2NPs and Cd*

CeO_2NPs coated with Poly Vinyl Pyrrolidone (PVP) were purchased from the US Research Nanomaterials, Inc. (Houston, TX). The average size of the nanoparticles was 41.7 $\pm$ 5.2 nm, calculated by averaging the diameter of over 100 individual nanoparticles measured with ImageJ on transmission electron microscopic images of the NPs. The pH of the CeO_2NPs dispersed in water at 100 mg L^{-1} was 7.1 and the zeta potential was −48.6 mV, determined by a dynamic light scattering Zetasizer (Malvern, UK). X-ray photoelectron spectroscopy (XPS) analysis indicated that about 9.7 % of Ce on the nanoparticle surface exists as Ce^{3+} (Fig. 6).

The concentration of CeO_2NPs (100 mg L^{-1}) was chosen based on many previous nanotoxicity studies with terrestrial plants, which used concentrations mostly in the range of 1−1000 mg L^{-1} [28, 29]. In addition, the literature suggested that CeO_2NPs at this concentration display significant impact on plant physiology and biochemistry but not lethal effects[30]. Cd sulfate ($CdSO_4$) was purchased from the Fisher Scientific Inc. (Pittsburgh, PA) and was dissolved in water to obtain the final concentration of 1.0 mg L^{-1} Cd. This concentration falls in the typical range of Cd in agricultural soils [25].

4.3.2. *Plant species and growth conditions*

Glycine max (L.) Merr. (soybean) cv. 'Tohya' seeds were purchased from Johnny's Selected Seeds (Winslow, ME). Soybean seeds were germinated in moist sand for 5 days. After germination, young seedlings were individually transplanted into 50 mL plastic centrifuge tubes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) filled with 25% Hoagland solution [31] (Phyto Technology Lab, Shawnee Mission, KS). The transpiration rate was recorded by daily water loss

(mL h^{-1}) shown in Suppl. Fig. 2. When the water transpiration reached 50 mL day^{-1} (after 10 days), plants were transferred from the Hoagland solution to new tubes filled with solutions containing 1.0 mg L^{-1} Cd^{2+} and/or 100 mg L^{-1} CeO_2NPs. Soybean seedlings were cultivated in these solutions for additional 10 days at room temperature under fluorescent bulbs providing 250 µmol m^2 s^{-1} photosynthetic photon flux density (16 h light – 8 h dark photoperiod). Seedlings grown in just tap water were used as controls. Six seedlings were grown under each treatment.

After the exposure, pH of the growth medium and root surface were measured respectively with a pH-meter (Thermo Orion Star A121, Fisher Scientific Int., Pittsburgh, PA) and pH-strips (Fisher Scientific Int., Pittsburgh, PA).

4.3.3. *Growth analyses and biomass partitioning*

Immediately after the exposure, plant roots were gently removed from the tubes and washed with 50 mL $CaCl_2$ solution (5 mM) five times to remove the Ce and Cd elements deposited on root surface. The washing solutions and the growth media were collected for further analysis. Fresh leaves and root samples of three replicates from each treatment were collected for cerium speciation analysis. Detailed descriptions of the analytical parameters and procedures are provided below. The rest three replicates from each treatment were rinsed with DI three times, divided into roots and shoots and dried in an oven at 70 ºC for 7 days to determine the total concentrations of Cd and Ce associated with soybean roots and shoots.

4.3.4. *Total Organic Carbon determination*

Total organic carbon (TOC) in the growth media was measured as an indicator of the excretion of plant root exudates. The growth medium at termination was first diluted by tap water to 50 mL. The diluted solution was then shaken for 12 hours on a shaker table. Subsequently, TOC in those

samples was measured by a TOC analyzer (Shimadzu TOC-VWP Analyzer, Japan) following reported procedures [32, 33]. TOC in the growth medium was then obtained by correcting the measured TOC with the dilution factor for each sample. Three replicates from each treatment were measured.

4.3.5. *Investigation on the Association of Cd with CeO_2NPs and Plant Root Exudates*

A parallel experiment was performed to evaluate the potential chemical interactions Cd with CeO_2NPs and biomolecules in plant exudates. To begin with, forty-two soybean seedlings were grown in 20% strength Hoagland solution in 50 mL centrifuge tubes for 21 days. Transpired water was replenished with the same strength Hoagland solution during cultivation. At termination, plant seedlings were removed, and the growth medium containing root exudates was centrifuged at 10,000 rpm for 10 min. Afterwards, the supernatant was decanted and the pellet at the bottom of each centrifuge tube was gently collected and transferred to a new tube in which the pellet was combined with pellets from six other tubes to further concentrate them. The pellets were assumed to contain large molecular weight molecules from exudates such as proteins, polysaccrides and border cells. Low molecular weight molecules in exudates such as short-chain aliphatic acids were expected to remain in the supernatant and were decanted. The combined pellets were re-suspended in 40 mL DI water and altogether six such tubes were prepared. They were divided into three groups. The first group was injected with 30 mg L^{-1} of Cd^{2+}, the second group 100 mg L^{-1} of CeO_2NPs and the third group the combination of these two materials at the same concentrations. The tubes were shaken for 24 h on a shaker table and then centrifuged at 10,000 rpm for 15 min. The supernatant was then decanted, and the residual was desiccated at 90 °C of heat blocker for 4 hours to form a dried pellet for X-ray photoelectron spectroscopy (XPS) analysis. XPS was carried out using an Omicron ESCA (Scientia Omicron GmbH, Germany) connected to a probe

spectrometer with polychromatic Mg Ka X-rays (hν = 1253.6 eV). The grounded sample was taken on a stainless-steel sample holder using double sided adhesive conductive carbon tape. The X-ray run was performed at the power of 225 W where the pass energy was set to 100 eV for survey scans and 50 eV for specific regions to ensure sufficient sensitivity under a base pressure of 10^{-6} Pa. The calibration of the binding energies of the core levels was fixed at 284.8 eV for the main C 1s B.E peak. The peaks of XPS spectrum were fitted by Gaussian function using Origin software 2017 (OriginLab, Northampton, MA).

4.3.6. *Statistical analyses.*

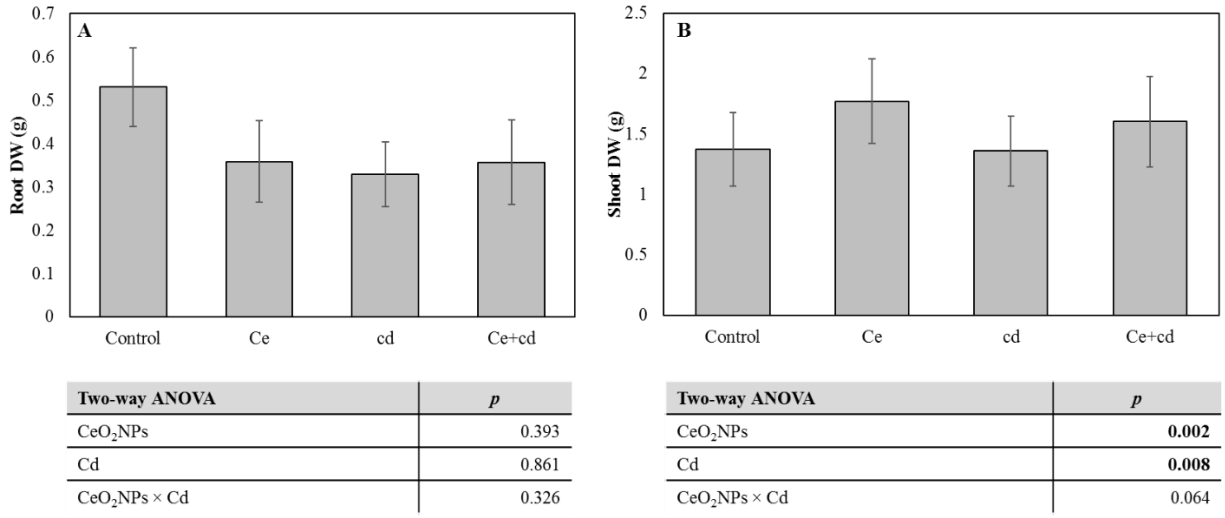
Data were subjected to analysis of variance using a completely randomized experimental design. Since the experiment was set up as a 2 × 2 factorial experiment, CeO_2NPs vs. Cd, a two-way ANOVA analysis was carried out to differentiate the significance of independent variables: CeO_2NPs and Cd. In addition, one-way ANOVA was performed and means separation between treatments was obtained by the Tukey's post-hoc test. Data were analyzed using the Minitab 18 Statistical Software (Minitab Inc., State College, PA).

4.4. Results

4.4.1. Growth analysis and plant uptake of CeO_2NPs and Cd

Neither CeO_2NPs nor Cd at the applied concentrations, separately or in combination, caused any significant changes in the root and shoot dry biomass of soybean seedlings (Fig. 1). As expected, Ce concentrations increased in roots and shoots in CeO_2NPs treated plants compared to the controls (Fig. 2). Interestingly, the co-existence of Cd markedly decreased the concentration of Ce in soybean roots but not in shoots (Fig. 2). The Ce associated with soybean roots decreased by

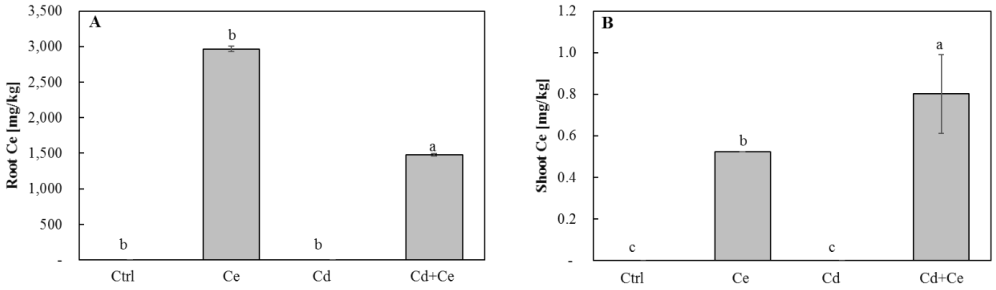
51% in CeO_2NPs + Cd treated plants but increased by 44% in the shoots compared to plants treated with CeO_2NPs alone. Two-way ANOVA showed a high level of interactions between CeO_2NPs and Cd for Ce accumulation in soybean tissues. Total Cd associated with soybean roots was unaffected by the presence of CeO_2NPs (Fig. 3). By contrast, the presence of CeO_2NPs led to a significant decrease of Cd in plant shoots by 78%. Significant interactions between CeO_2NPs and Cd were only found in shoots for Cd accumulation.



Two-way ANOVA	*p*
CeO_2NPs	0.393
Cd	0.861
CeO_2NPs × Cd	0.326

Two-way ANOVA	*p*
CeO_2NPs	**0.002**
Cd	**0.008**
CeO_2NPs × Cd	0.064

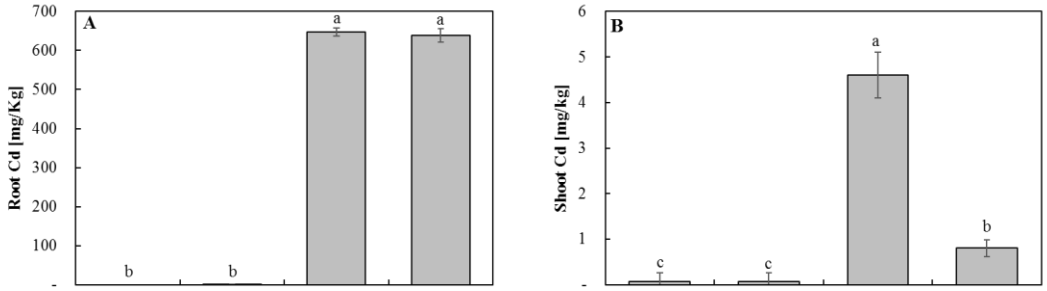
Fig. 4.1. Dry weight of *Glycine max* grown in the presence of 100 mg L^{-1} CeO_2NPs and 1 mg L^{-1} Cd. A: Root dry weigh, B: Shoot dry weight. Means labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n=3). Two-way ANOVA analysis are reported in tables.



Two-way ANOVA	*p*
CeO_2NPs	**<0.001**
Cd	**<0.001**
CeO_2NPs × Cd	**<0.001**

Two-way ANOVA	*p*
CeO_2NPs	**<0.001**
Cd	0.078
CeO_2NPs × Cd	0.079

Fig. 4.2. Total cerium concentration in roots (A) and shoots (B) of *Glycine max* grown in the presence of 100 mg L^{-1} CeO_2NPs and 1 mg L^{-1} Cd. Means labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n=3). Two-way ANOVA analysis are reported in tables.



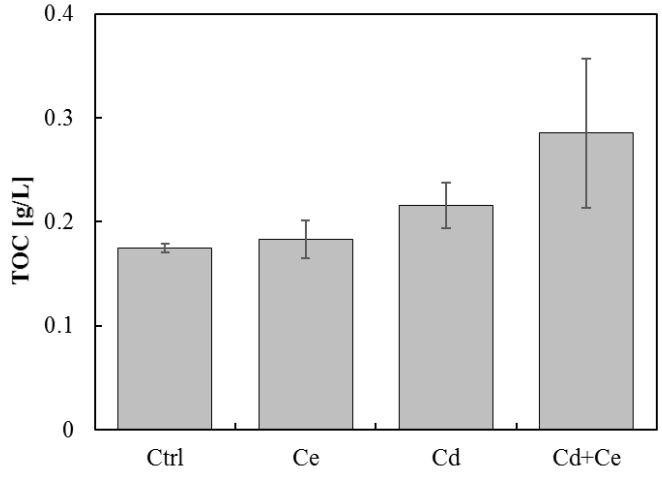
Two-way ANOVA	*p*
CeO_2NPs	0.742
Cd	**<0.001**
CeO_2NPs × Cd	0.716

Two-way ANOVA	*p*
CeO_2NPs	**<0.001**
Cd	**<0.001**
CeO_2NPs × Cd	**<0.001**

Fig. 4.3. Cadmium concentration (mg/kg) in root (A) and leaves (B) of *Glycine max* grew in the presence of 100 mg L^{-1} CeO_2NPs and 1 mg L^{-1} Cd. Means labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n=3). Two-way ANOVA analysis are reported in tables.

4.4.2. Total organic carbon and pH

TOC in the growth media exposed to CeO_2NPs and/or Cd was higher than control plants. TOC in the growth medium from the joint treatment of CeO_2NPs and Cd was noticeably higher than that from the treatments with CeO_2NPs or Cd alone, even though the differences were not statistically significant (Fig. 4).



Two-way ANOVA	*p*
CeO_2NPs	0.114
Cd	**0.017**
CeO_2NPs × Cd	0.147

Fig. 4.4. Total organic carbon (TOC) in *Glycine max* grown in the presence of 100 mg L^{-1} CeO_2NPs and 1 mg L^{-1} Cd. A and D: Total biomass, B and E: Leaf biomass, C and F: Root biomass. Means labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n=3). Two-way ANOVA analysis are reported in tables.

The pH values in the growth media and on root surface were significantly reduced by the joint exposure of CeO_2NPs and Cd (Table 1), and interactions between CeO_2NPs and Cd were found for pH on root surface. However, no significant interactions were found between CeO_2NPs and Cd in the growth media (Table 1).

Table 4.1. Root surface and growth medium pH values of *Glycine max* grown in the presence of 100 mg L^{-1} CeO_2NPs and 1 mg L^{-1} Cd. Means ± SD (n = 3) labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$).

Treatments	***Root pH***	***Medium pH***
Control	7.40 ± 0.10 *b*	7.69 ± 0.15 *ab*
CeO_2NPs	7.53 ± 0.06 *ab*	7.80 ± 0.06 *a*
Cd	7.40 ± 0.10 *ab*	7.66 ± 0.13 *ab*
Ce NPs + Cd	7.20 ± 0.10 *a*	7.50 ± 0.07 *b*
Two-way ANOVA	*p*	*p*
CeO_2NPs	0.527	0.633
Cd	0.014	0.025
CeO_2NPs × Cd	0.015	0.057

4.4.3. X-ray photoelectron spectroscopy

Cadmium spectra were shown in Fig. 5. Two fitting peaks (u and u') at the binding energy of 404.82 eV, and 411.82 eV were identified and they were assigned to Cd3d5/2 and Cd3d3/2 respectively [34, 35]. No Cd specific peaks were found in CeO_2 samples, but Cd peaks were detected in samples containing both root exudates and 30 mg L^{-1} of Cd alone and samples containing part of the root exudates as well as 30 mg L^{-1} of Cd and 100 mg L^{-1} of CeO_2NPs. . The results suggest that some Cd might have complexed with large molecules such as proteins and polysaccharides in plant root exudates or adsorbed on the surfaces of border cells, and some Cd might have adsorbed on the surface of CeO_2NPs. The evidence provided here is only qualitative, and quantitative characterization on the interactions between Cd and exudates and CeO_2NPs requires additional study, yet the results provided here do shed lights on the possible mechanisms for the interactions of CeO_2NPs and Cd in plant root rhizosphere.

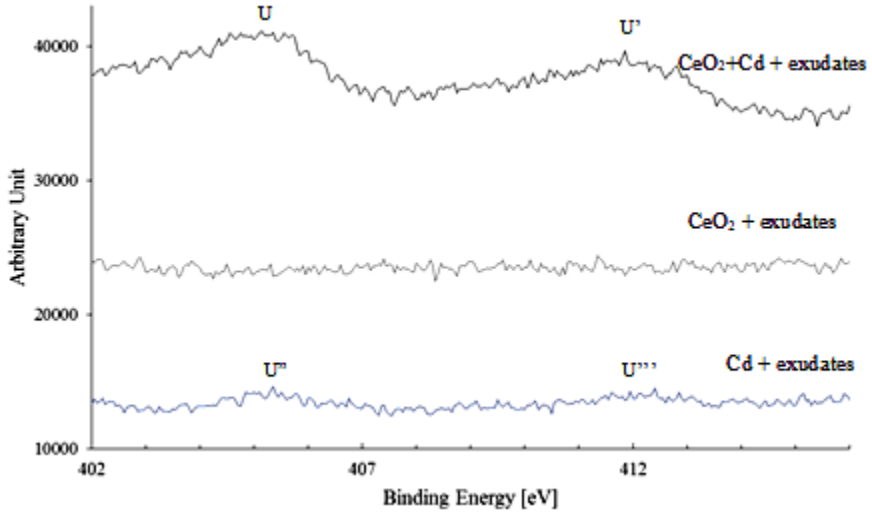


Fig. 4.5. The XPS spectrum of the presence of cadmium (30 mg/L) on the surface of CeO_2NPs (75 mg/L). Two peaks at B.E 404.82 eV, and 411.82 eV correspond to Cd3d5/2 and Cd3d3/2, respectively.

4.5. **Discussion**

Interests in the interactions between ENPs and co-present environmental pollutants in different environmental systems are growing [28, 36, 37]. Understanding their interactions in plant rhizosphere and the implications of these interactions for their accumulation in plant tissues have significant implications for food safety. In fact, consumption of food crops contaminated by various environmental pollutants represent a significant pathway for human exposure to these materials [38-40].

In this study we examined the reciprocal effects of CeO_2NPs and Cd in an aqueous system, avoiding many of the complications caused by soil particles, and allowing a mechanistic investigation on the interactions of CeO_2NPs and Cd in soybean rhizosphere. As shown in the results, CeO_2NPs, Cd and their interactions did not affect the overall dry weight and water transpiration, confirming that CeO_2NPs and Cd at the tested concentrations do not have any detrimental effects on plant growth and biomass, separately or together. Similar results have been reported in literature. In fact, Gui, Zhang, Liu, Ma, Zhang, He, Li, Zhang, Li, Rui, Liu and Cao [39] reported that lettuce (*Lactuca sativa* L.) treated with 100 mg kg^{-1} CeO_2NPs grew much faster than the controls. Similarly, Cao, et al. [41] demonstrated that 100 mg kg^{-1} of CeO_2NPs improved the net photosynthesis rate and the Rubisco activity of soybean seedlings and enhanced the soybean water use efficiency (WUE).

A general observation in our study is that plant uptake and accumulation of Cd and Ce were affected by the co-presence of the other chemical, but the extent and pattern of alteration by co-existing chemicals differed. Cd led to a significant decrease of Ce in roots but a significant increase in leaves. For Cd, however, the co-occurrence of CeO_2NPs significantly reduced its concentration

in shoots but did not affect Cd associated with roots. This interesting observation was attributed to the complicated physical, chemical and biological processes co-occurring in soybean rhizosphere. First of all, as a result of the enhanced excretion of plant exudates as indicated by the higher TOC in the joint presence of CeO_2NPs and Cd, the pH in the rhizosphere and on plant root surface dropped in the co-presence of these two materials. The lower pH generally favors the dissolution of metals. But more importantly, in the cocktail of plant root exudates are many lower molecular weight acids, which can behave as reducing agents and enhance the reduction of Ce^{4+} to Ce^{3+}, facilitating Ce uptake and transport to plant leaves [42]. Previous research has demonstrated that ionic Ce is much more efficiently transported from roots to shoots than CeO_2NPs [17, 43]. Consequently, higher Ce was detected in soybean shoots exposed to both CeO_2NPs and Cd than those exposed to CeO_2NPs alone, even though the total Ce within root tissues was lower when plants were exposed to both materials simultaneously.

As described above, uptake of Cd is complicated , both apoplastic and symplastic pathways contribute to the entrance of Cd in plant root cells and then in the vascular system. The presence of CeO_2NPs can affect both pathways. In a recent study, we have found that the co-occurrence of CeO_2NPs and Cd altered the formation of "root apoplastic barriers" by depositing suberin on the cell wall of endodermal cells, compared to plants exposed to them separately [19]. The altered apoplastic barriers could affect Cd entrance through cellular hydraulic conductivity. It is also possible that CeO_2NPs may affect plant symplastic pathway by altering the functionality of ion transporters or the integrity of root cell membranes, leading to different Cd accumulation in plants. Such mechanisms have not been extensively explored, but some previous studies have shown that CeO_2NPs disrupt root membrane integrity [30, 44]. Additionally, previous research with ENPs and co-existing organic contaminants has shown that the adsorption of organic compounds on ENPs

surfaces play a crucial role for the altered plant accumulation of organic compounds [8, 45, 46]. Similar process could occur between ENPs and heavy metal ions and indeed there have been many reports which showed the high adsorption capacity of heavy metals on ENP surfaces [20, 47]. Particularly, Contreras, Garcia, Gonzalez, Casals, Puntes, Sanchez, Font and Recillas [21] showed that the adsorption capacity of Cd on CeO_2NPs was four times higher than that on TiO_2NPs. In our study, while detailed adsorption study was not performed, we have conducted a qualitative study in which CeO_2NPs and Cd were mixed in growth media containing some actual root exudates. Our results (Fig. 6) indicated that Cd was detected in dry pellets containing CeO_2NPs, suggesting a likely adsorption of Cd on CeO_2NPs. Because of the relatively less efficient root-to-shoot transport of CeO_2NPs, the adsorbed Cd would penetrate into roots with CeO_2NPs, but was less effectively transported to shoots. This possible interaction between Cd and CeO_2NPs is consistent with the lower Cd in plant shoots in the co-presence of CeO_2NPs and Cd, while Cd in the roots was unaffected. In addition, the co-existence of CeO_2NPs and Cd resulted in greater release of exudates as shown above, Cd may form complexes with organic exudates outside plant roots. Previous studies have shown that the Cd chelates are transported into plant root cells through different protein transporters such as YSL protein [48, 49]. Earlier research also demonstrated that chelated Cd is more effectively sequestered in the vacuole of plant root cells, reducing the loading efficiency to xylem tissues and therefore less efficient translocation to shoots [4, 10, 14, 15]. The formation of Cd-exudate complexes is supported by the XPS analysis (Fig. 6). The adsorption of Cd into biomolecules was shown to be strongest at pH>5 [34]. In this study, pH in the growth media was above 6, suggesting that affiliation between Cd and large size biomolecules in root exudates was possible.

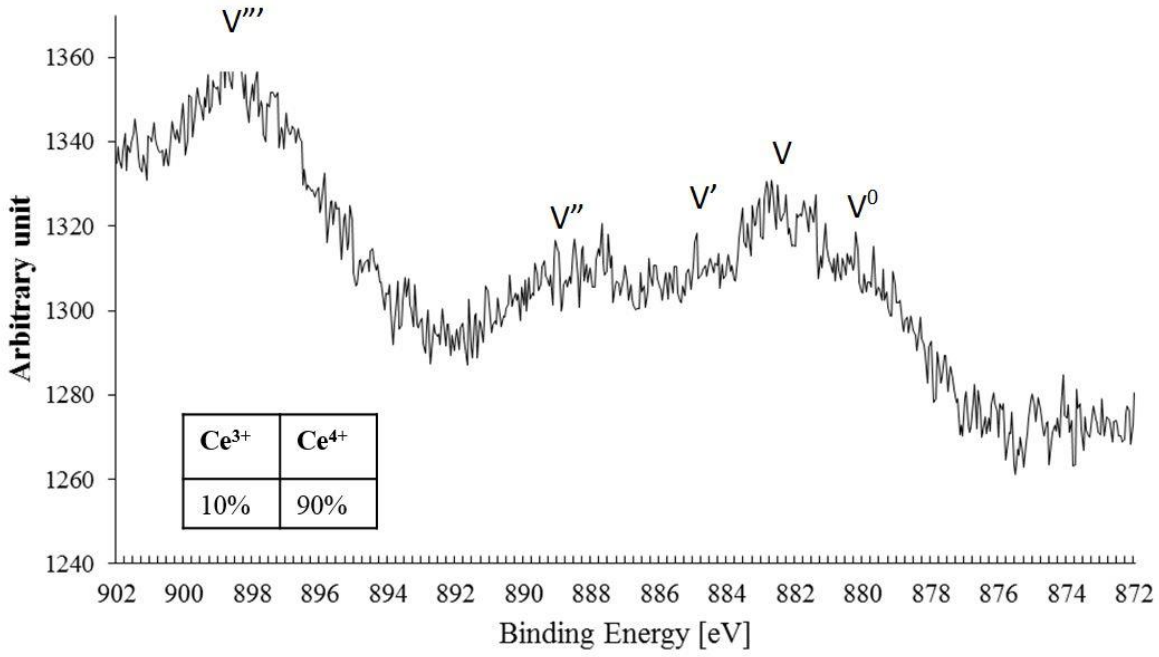


Fig. 4.6. illustrates the Ce $3d_{3/2, 5/2}$ spectra obtained for the CeO_2 compound and dissociated ions. The CeO_2 spectrum is composed of two multiplets in five peaks Ce (IV) = v,v'',v''' and Ce(III) = v^o, v' with a ratio of Ce^{4+}: C^{3+}, 90%:10%. These multiplets attributed to the spin-orbit split $3d_{5/2}$ and $3d_{3/2}$ core holes [4]. The five identical peaks corresponding to the pairs of spin-orbit doublets were consistent with results from other authors[4]. The highest binding energy peaks, v'" located at B.E 898.3 ± 0.1 eV is the result of a Ce $3d^94f^0$ O $2p^6$ final state. The low binding energy states v and v" respectively located at 882.7and 888.5 ± 0.1 eV are the result of Ce $3d^94f^2$ O $2p^4$ and Ce $3d^94f^1$ O $2p^5$ final states.

4.6. Conclusions

Overall, we have observed significant interactions between co-existing CeO_2NPs and Cd on plant uptake of these metal elements. It appears that the co-presence of Cd with CeO_2NPs led to higher excretion of plant root exudates, lowered the pH values on root surface and enhanced CeO_2NPs dissolution, therefore resulted in lower accumulation of Ce in plant roots but higher accumulation

of Ce in plant shoots. This statement is supported by the higher Ce content in the root washing solution from the CeO_2NPs + Cd treated plants which indicated that higher amount of Ce was attached to plant root surface but did not penetrate into the tissues. Alternatively, the presence of CeO_2NPs may provide an adsorption site for Cd and reduces it is uptake into plants. The elevated release of root exudates may be another possible reason for the reduced Cd in plant shoots. The study provides important new insights in the chemical mechanisms for Cd and CeO_2NPs interactions in plant root region. Due to the complex processes involved in both materials, future studies should aim to elucidate more molecular mechanisms on the mutual effects of these chemicals on their uptake and accumulation by plants.

4.7. References

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CHAPTER V

ZINC OXIDE NANOPARTICLES ALLEVIATED THE BIOAVAILABILITY OF CADMIUM AND LEAD AND CHANGED THE UPTAKE OF IRON IN HYDROPONICALLY GROWN LETTUCE (*LACTUCA SATIVA L. VAR. LONGIFOLIA*[4])

5.1. Summary

Leafy vegetables are a rich source of iron and fibers for the human diet, which may become hazardous if exposed to heavy metals contamination. Cadmium (Cd) and lead (Pb) are two highly toxic metals even at trace concentrations. Engineered nanoparticles (ENPs) can alter the uptake of heavy metals and localization of essential minerals such as iron (Fe) through different mechanisms. The goal of this study was to understand the mutual effects of zinc oxide nanoparticles (ZnONPs) and coexisting heavy metals Pb^{2+} and Cd^{2+} on their uptake and accumulation as well as their affects on Fe concentrations in romaine lettuce (*Lactuca sativa L. var. Longifolia)* in a hydroponic system. At termination, shoots were gently separated from the roots, and the concentrations of Pb, Cd, Fe, and Zn in all plant tissues were quantified by inductively coupled plasma-mass spectrometry (ICP-MS). In addition, microbial density analysis in the growth media was performed for each treatment. The results indicated active interactions between ZnONPs and coexisting divalent heavy metals. ZnONPs significantly reduced the accumulation of Cd and Pb in roots by 49% and 81%. In shoots, Cd was reduced by 30%, and Pb elevated by 44%. Fe concentration in shoots was strongly affected by the presence of ZnONPs, and the total Zn in shoots was negatively correlated with the microbial population in the growth media. Exposure to ZnONPs alone increased the total Fe in shoots by 80% compared to controls, and the copresence of ZnONPs and heavy metals increased Fe concentration by about 77%. The results revealed the

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role of ENPs in governing the uptake and translocation of some essential elements and toxic heavy metals in plants.

Keywords: Zinc oxide nanoparticles; Cadmium; Lead; Iron, Lettuce

5.2. Introduction

The applications of engineered metallic oxide nanoparticles in agriculture, and food processing sectors have massively increased [1-3]. These engineered nanoparticles (ENPs) can promote the cultivation of dietary plants, including crops and leafy vegetables by reducing the uptake of heavy metals or improving their post harvesting qualities [4, 5]. ENPs may also increase the shelf life of vegetables by reducing the heavy metal content or act as a strong anti- microbial/fungal agent [4, 6].

However, the mechanistic interactions of ENPs with biological systems needs further investigation. For example, several studies have been conducted to elucidate the interactions of ENPs with leguminous plants, but there is a serious lack of studies on ENPs interactions with leafy vegetables that are directly consumed by humans [1, 7]. Leafy vegetables allegedly accumulate higher concentrations of heavy metals in their edible tissues compared to legumes [8]. Consumption of leafy vegetables is recommended to pregnant women because of its high iron (Fe) content [9]. Recent studies showed a significant role of leafy vegetables in the birth weight of newborns by comparing mothers who consumed leafy vegetable and those who did not [9, 10]. Therefore, controlling the level of microelements in leafy vegetables is of high interest for food scientists and engineers. Production of leafy vegetables has advanced to a fast production track through the application of large urban hydroponic systems [11-13]. Such production systems greatly increase the exposure of leafy vegetables to heavy metals and emerging chemicals such as ENPs through irrigation, atmospheric fallouts or direct applications of nanoagrichemicals [14, 15]. Co-exposure of

leafy vegetables to ENPs and heavy metals affects plant growth and food safety through their mutual effects on plant uptake and accumulation of these chemicals [16].

Cadmium (Cd) and lead (Pb) are two ubiquitous carcinogenic contaminants [17, 18]. Major sources of these metals include mining, emission of fuels, municipal wastes, fertilizers, and pesticide applications [19]. Consumption of contaminated vegetables by Cd and Pb is an important pathway for human exposure [15]. For instance, food consumption corresponds to approximately 90% of Cd and Pb ingestion in non-smoking people around the world [20, 21]. Pb intake by pregnant women may lead to reduced birth weight, length, and head circumference of new born babies [22, 23]. Therefore, efforts to alleviate the uptake of heavy metals by dietary plants are of high interest. Potential effects of ENPs interactions with leafy vegetables that are exposed to heavy metals may also alter the bioavailability of essential minerals such as Fe, and the microbial community, which has not been explored as a critical factor in plant ENPs interactions. Microbial communities in hydroponics can be highly beneficial for plant nitrogen fixation, phosphate mobilization, and mediating nutrient uptake [24].

ZnONPs is a frequently used metallic ENPs in a variety of industrial and agricultural products [4, 6]. For example, ZnONPs have been introduced as an effective nanofertilizer due to its capability to enrich agricultural soils with Zn deficit [25]. ZnO is also currently used as a food additive [26]. In addition, it acts as an antimicrobial agent, which exhibits strong activity at low concentrations [6]. Also, it plays a vital role in plant metabolic processes, promoting the synthesis of carotenoids and chlorophylls, resulting in higher photosynthetic performance [25]. Some studies reported the toxicity of ZnONPs at high concentrations, and the effects of ZnONPs on the upregulation of Fe in plants is suspected because both ions share the same transporting membrane proteins (Zinc / Iron Regulatory Transporter Proteins family) [27]. Therefore, the primary goal of this study was to

investigate the interactions of romaine lettuce (*Lactuca sativa L. var. Longifolia*) with the coexisting ZnONPs, Cd, and Pb and to monitor the translocation of Fe in plant tissues.

Romaine lettuce (*Lactuca sativa L. var. Longifolia)* is a frequently consumed leafy vegetable which is rich in bioactive compounds, essential minerals, and dietary fibers [28, 29]. The dry or fresh consumption of lettuce through a variety of foods has been extensively increased due to its rich flavor, anti-cancer properties, and high vitamin content [28, 30]. Therefore, it is formulated in dietary supplements and is a proposed diet for infants [31]. A hydroponic system was used because mass vegetable productions are obtained through this system. Furthermore, it avoids the interfering effects of soil and the microorganisms in the soil.

5.3. Materials and Methods

5.3.1. Materials

Negatively charged ZnONPs (20% by weight, 10-30 nm) was purchased from the US Research Nanomaterials, Inc. (Houston, TX). High purity Pb ($Pb(NO_3)_2$ >99%) was obtained from Alfa Aestar (Ward Hill, MA), and Cd sulfate ($CdSO_4$) was purchased from Fisher Scientific Inc. (Pittsburgh, PA). Dextrose agar was purchased from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany. All analytical standards of Pb, Cd, Zn, Fe (10000 ppm, %3 nitric acid) were obtained from RICCA Chemical Company, Arlington, TX.

5.3.2. ZnONPs Characterization

The size and shape of the ZnO were fully characterized in our previous study by a Tecnai G2 F20 transmission electron microscope (TEM) [7]. Briefly, the ZnONPs is primarily spherical, but a small fraction of the particles has triangular or other irregular shapes. The mean diameter of ZnONPs

was around 68.1 nm, within a range of 15 to 137 nm. The hydrodynamic size in 100 mg L^{-1} water solution was 621.1±7.6 nm determined via dynamic light scattering (DLS). The zeta potential was about -28.8±2.0 mV.

5.3.3. Plant Culture and Exposure Experiments

The lettuce seeds were purchased from Johnny's Selected Seeds (Winslow, ME). The seeds were germinated in moist sand in a greenhouse at a controlled temperature (25°C) under fluorescent light providing 250 µmol m^2 s^{-1} photosynthetic photon flux density (16/ 8 h light/dark cycle). Germinated lettuce seedlings were transferred to clay soil in 12 cm x 10 cm square pots for 14 days. Afterward, the plant seedlings were gently removed from the soil, and roots were thoroughly washed with DI water to remove soil residual, and each plant was immediately transplanted to a 50 mL volume hydroponic system (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) containing one fourth strength Hoagland solution (Phyto Technology Lab, Shawnee Mission, KS). To keep the root area dark, the tubes were covered by aluminum foil.

When the transpiration rate reached 10 mL /day (after 10 days), the plants were transferred from the Hoagland solution to new tubes, which were filled with solutions containing 100 mg L^{-1} Pb^{2+} + 1.0 mg L^{-1} Cd^{2+} and/or 100 mg L^{-1} ZnONPs in DI water. During the exposure period, the Hoagland solution was avoided due to the high ionic strength of the nutrient solution that induces aggregation of the ZnONPs. The hydroponic system was used to prevent sorption of the dosed ZnONPs to soil particles and to ensure that the added ENPs and heavy metals were fully bioavailable [13]. Four replicates for each treatment as well as three replicates of blank controls were grown. To determine water evaporation, two tubes with no plants were placed among the plants. Lettuce seedlings were cultivated in the hydroponic system containing the target solution for an additional 10 days. During the

exposure time, growth media were replenished with DI water two times per day (morning and evening). The plants were randomly rotated and relocated to ensure equal light exposure. After the heavy metal/ENPs exposure, the pH of the growth medium was measured with a pH meter (VWR symphony – SB90M5) at termination.

5.3.4. Growth analyses and biomass partitioning

Immediately after the exposure, plant roots were removed from the hydroponic system and washed with 50 mL $CaCl_2$ solution (5 mM) five times to remove the Zn, Pb and Cd elements deposited on the root surface [20]. The growth media were collected for further analysis. Harvested plants were separated into leafy tissues and roots. The fresh weight was measured for three or four replicates of leaves and roots from each treatment. Stem and root lengths were measured with a ruler. To prepare the samples for metal content analysis, fresh samples were dried in an oven at 75°C for 5 days to ensure that the tissues are fully dehydrated. The dry biomass was then determined [7, 20].

5.3.5. Heavy metals and trace elements of content analyses

An aliquot of 0.15 g of dry leaf and 0.05 g of dry root tissues were homogenized via a household blender (Braun Multiquick MX 2050). Next, the samples were placed into a DigiPREP MS hot block digester (SCP Science, Clark Graham, Canada) and pre-digested with 3 mL of 70% (v/v) nitric acid (Certified ACS Plus) overnight [32]. Then the samples were heated at 95 °C for 4 hours following the EPA method 3050b [32,33]. After being cooled down to room temperature, 2 mL of 30% (w/v) H_2O_2 were added to the samples and re-heated in the hot block at 95 °C for an additional 2 hours until fully digested [7]. To quantify Zn, Pb, Cd and Fe, the digest solution was diluted 15 times in ultra-pure water (18.2 MΩ cm, P.LAB Option-Q) containing 1% nitric acid and the element concentrations were quantified by an

inductively coupled plasma-mass spectroscopy (ICP-MS) (Agilent 7500i, Agilent Technologies Co. Ltd, USA) [32,33].

5.3.6. Rhizosphere microbial population assay

Immediately after removing the seedlings from the hydroponic system and pH measurement, 100 µL of the growth medium of each treatment were collected for the microbial analysis. In total, 12 subsamples containing three replicates for each treatment were prepared. Subsequently, the aliquots were ten times diluted (1:10) in ultra-pure water (18.2 MΩ), and strains in 100 µL of the subsamples with no turbidity were loop-inoculated over 100 mm × 15 mm sterile Petri-dishes containing potato dextrose agar. Next, agar plates were incubated for two days at 37 °C [34]. Finally, the colony-forming unit (CFU) was counted [35].

5.3.7. Statistical Analysis

Statistical analysis of Zn, Pb, Cd, and Fe in both shoot and root tissues were performed using the Minitab 18 Statistical Software (Minitab Inc., State College, PA, USA). The One-Way ANOVA followed by Tukey's test at a significance level 5% ($p < 0.05$) was applied to compare the mean values between different treatments of dry weight, pH, CFU and metal concentrations.

5.4. Results

5.4.1. Biomass and Plant Growth

Figure 5.1A, B illustrates the measured dry weight of both roots and shoots under four different treatments. Error bars in each column correspond to the standard deviation. Both the shoot and root dry weight were strongly impacted by ZnONPs. This impact is more profound in shoot dry

weight than root dry weight. ZnONPs alone significantly (0.05<P) reduced the dry shoot biomass by 57%. The co-exposure of Pb and Cd with ZnONPs lowered the dry shoot biomass by 46%. There were no statistically significant impacts of ZnONPs on the dry biomass of roots, but the average root dry biomass was reduced by 50% in the sole presence of ZnONPs compared to the control. The dry biomass data showed strong correlations with the stem and root lengths.

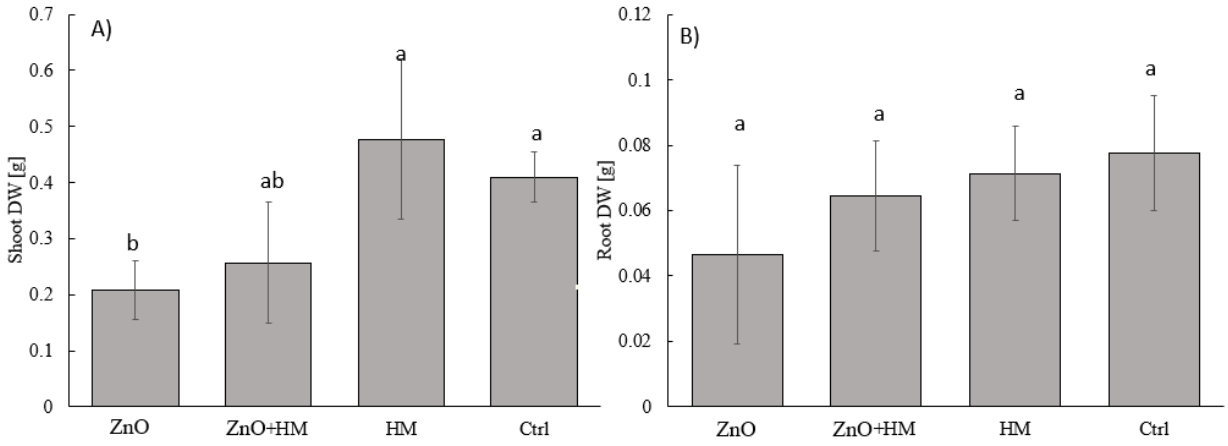

Fig.5.1. Dry biomass of lettuce tissues under different treatments including combinations of 1 mg/L of Cd,100 mg/L Pb and/or 100 mg/L ZnONPs. A) Shoot dry biomass of lettuce treated with different treatments and B) Root dry biomass of lettuce exposed to different combinations of heavy metals and ZnONPs. The column represents the mean value. The error bars indicate standard deviation (n = 3 or 4). Different letters designate significant differences ($p \leq 0.05$) analyzed via one-way ANOVA followed by Tukey's test. HM: heavy metals.

Figure 5 A shows images of lettuce from different treatments. Figure 5 B reports the root and shoot length of lettuce from four different treatments. Similar to what was observed for the dry biomass, shoot length was strongly impacted by the presence of the ZnONPs. Heavy metals alone reduced

the shoot length insignificantly by 7%. When (co)-exposed to ZnONPs, the shoot length was significantly reduced by approximately 30% compared to control, with and without the heavy metals ($p<0.05$)

A similar pattern of root elongation was observed. Introduction of ZnONPs significantly ($0.05<p$) reduced the length of the roots by 28% in the co-presence of heavy metals and 36% in its sole exposure. The impact of heavy metals on root elongation was insignificant.

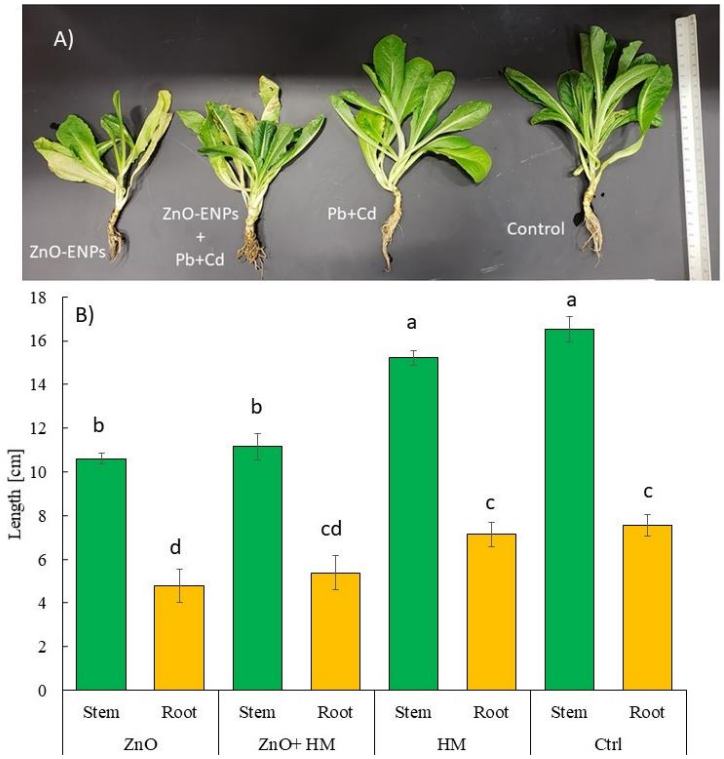

Fig. 5.2. (A) Phenotype of *Lactuca sativa L. var. Longifolia* plants grown in hydroponic systems after exposure to different combinations of 1 mg/L of Cd+100 mg/L Pb and 100 mg/L ZnONPs, ; (B) Illustrates the impacts of the four different treatments on plant root and shoot length. Error bars represent standard deviation. Letters above each column differentiate the significance level ($p \leq 0.05$).

5.4.2. **Total Heavy Metals and Nanoparticle accumulation**

Concentrations of total heavy metals of Cd and Pb in root and shoot tissues of lettuce from different treatments are shown in Figure 5.3. Co-presence of ZnONPs with both Cd and Pb significantly changed the shoot uptake of Cd and Pb. The Cd level in lettuce shoot was significantly reduced by 30% and in contrast, the Pb concentration was increased by 44%. The impact of ZnONPs was significant in the bioaccumulation of heavy metals in lettuce roots, in which both Cd and Pb concentration was significantly reduced by 49% and 81%, respectively.

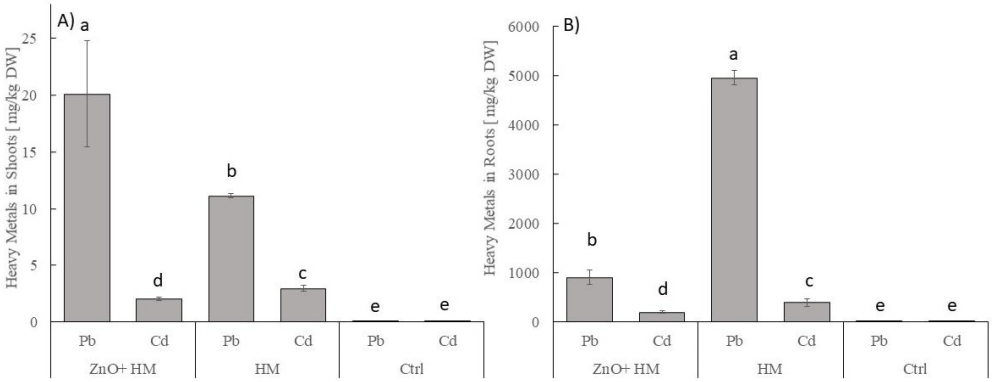

Fig. 5.3. Concentrations of heavy metals (Cd and Pb) in A) lettuce shoot and B) lettuce root tissues from different treatments. Error bars indicate standard deviation, and the alphabetic letters designate the statistical significance of each treatment.

Figure 5.4 shows the total Zn contents in plant tissues. In the co-presence of nanoparticles and both Cd and Pb, the total Zn concentration in shoots was significantly decreased by 73%. The same impact was observed in roots but to a lower extent, in which the total Zn was insignificantly dropped by 7%.

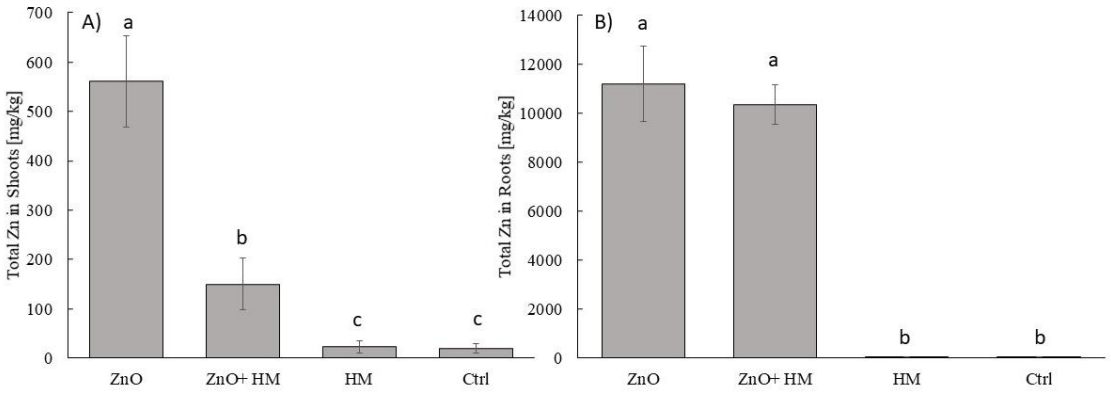

Fig.5.4. The mean concentrations of total Zn in (A) shoots (B) roots of lettuce exposed to four different treatments of heavy metals (Pb and Cd) and nanoparticles (ZnO). Error bars indicate standard deviation (n = 3).

ZnONPs has significantly altered the amount of Fe in shoot tissues. The total Fe concentration in lettuce shoot was elevated as high as 80% when plants were exposed only to ZnONPs compared to the control. In the co-presence of heavy metals and ZnONPs, Fe level in lettuce shoot was about 77% of that in control plants. The Fe concentration in roots responded differently to ZnONPs than that in shoots. All treatments led to significantly higher Fe than in control roots. Combination of heavy metals and ZnONPs reduced the amounts of total Fe by approximately 23% compared to treatments exposed only to ZnO in roots.

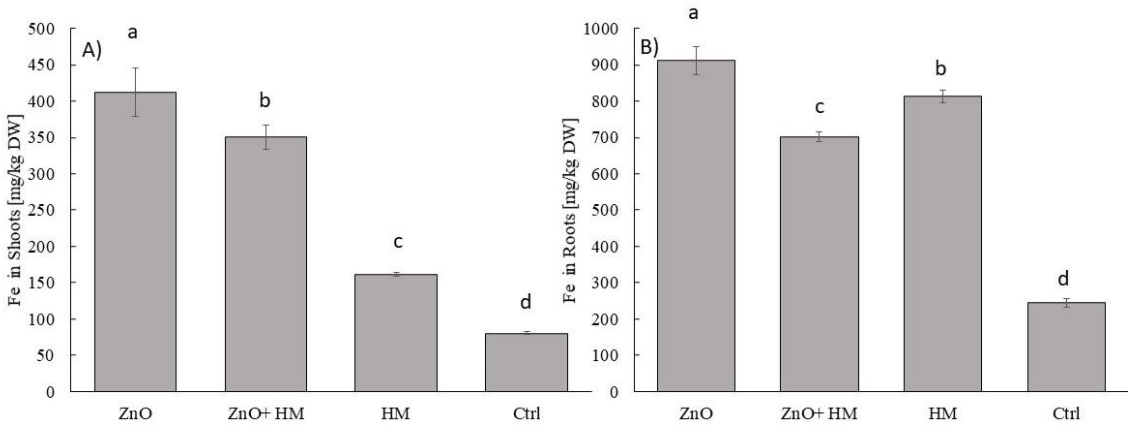

Fig.5.5. Concentrations of Fe in A)lettuce shoots and B) roots exposed to 100 mg/L of ZnONPs alone or in combination with 1 mg/L Cd+ 100 mg/L Pb (. Reported values indicate mean ± SD (n = 3), and different letters display significant differences ($p \leq 0.05$) conferring to one-way ANOVA post analyzed by Tukey's test.

5.5. Discussion

ZnONPs negatively affected the growth of romaine lettuce at the used concentration as indicated by the lower biomass and short elongation of both roots and shoots, likely due to the phytotoxicity ZnONPs or/and its dissociated Zn ions. The phytotoxicity of ZnONPs to various crops has been reported in a number of previous studies [36, 37], but not to leafy vegetables. The phytotoxicity of ZnONPs to romaine lettuce in this study can likely be attributed to the reactive oxidative species (ROS) induced by ZnONPs and their dissociated ions [36, 37].

ROS can affect plant physiology via the interruption of chlorophyll synthesis, protein denaturation and over-expression of antioxidant enzymes (i.e., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)) [38, 39]. Together they could lead to slower growth; as shown in Figure 5.2. For example, treatment of *C. sativus* with 100 mg/L ZnONPs reduced the plant biomass by about 43%. Some studies hypothesized that the phytotoxicity of ZnONPs is associated with the competition of

the dissociated Zn with Fe ions, leading to the interference of Fe-related physiological processes [39-41]. The results shown in Figure 5.5 indicate that excessive Zn could mediate the Fe concentration in shoots (shown in Fig 5.6), which can be beneficial to enhance the nutritional values of lettuce. This enhancement may stem from the reduction of the Fe efflux through the root exudate. The results of the translocation pattern of the Fe in relation to Zn concentration provide some evidence that ZnONPs has mitigated the efflux of the Fe. However, the mechanistic interpretation of these results needs further investigations. Figure 5.6 derived from ICP- analysis of four treatments further illustrates the correlation between Fe detected in shoots and available Zn. The results suggest that the physiological impact of ZnONPs can be, at least partially, attributed to its interference on plant Fe uptake. However, the underlying mechanisms for this connection are not well understood.

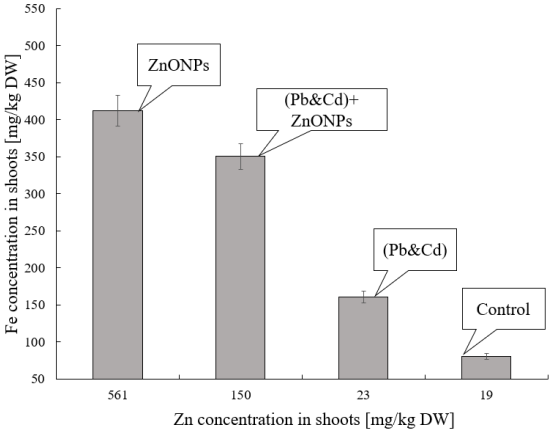

Fig.5.6. Correlation between concentrations of Fe and Zn in *L. sativa* upon exposure to 100 mg/L of ZnONPs alone or in combination with 1 mg/L Cd+ 100 mg/L Pb. Each bar represents the mean (n = 3).

The co-presence of Cd and Pb mitigated the impact of ZnONPs. A possible reason for this observation could be the aggregation of ZnONPs in the presence of Cd and Pb. Co-present heavy

metals such as Cd, and metallic ENPs were reported to induce greater excretion of root exudates, which prompted the aggregation of ENPs [42]. Therefore, the bioavailability of ZnONPs might have been significantly reduced when plants were co-contaminated by ZnONPs and heavy metals. This interpretation is consistent with the lower observed Zn concentration of lettuce shoots in the copresence of ZnONPs with heavy metals, compared to plants exposed only to ZnONPs (Fig 5.4). The impact on the microbial community is another very critical consideration to understand the interactions of ZnONPs and heavy metal ions in plant system. Because ZnONPs can act as an anti-microbial agent, and the microbial community affects the Zn uptake, the correlation between Zn accumulation in shoots and numbers of bacterial colonies was investigated, Figure 5.7. A higher concentration of Zn in shoots is negatively correlated to the colony formation units of the microbial community in the rhizosphere area. The results indicate that the microbial community may also play an important role in plant uptake of Zn and other co-contaminants. More comprehensive microbial studies with soil-grown plants are needed to identify and characterize the precise role of the microbial community.

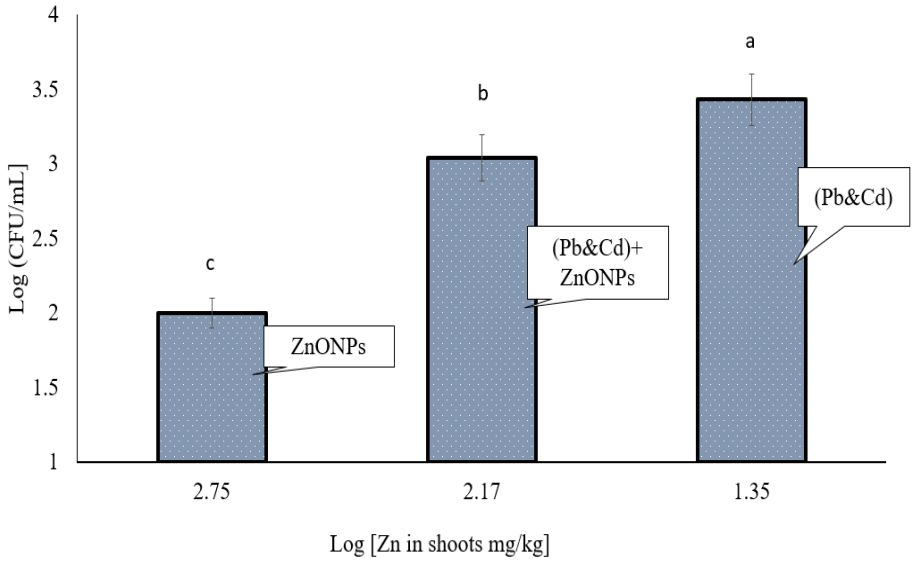

Fig.5.7. A relationship between the log of the colony forming units (CFU) in the growth media and the log of total Zn in shoots. Letters indicate significant differences. Error bars represent the standard deviation between treatments.

One of the interesting findings of this study was the key role of ZnONPs in changing the uptake of Pb and Cd. In an overall biomass analysis, both metals were significantly reduced in the presence of ZnONPs. However, this reduction was more prominent in root tissues. In fact, the concentration of Pb in lettuce shoots was higher in the system with both ZnONPs and heavy metals, compared with plants exposed to heavy metals only. The different accumulation patterns between Cd and Pb suggest that the properties of metals such as their complex chemistry are important factors affecting the interaction of these metals with co-occurring ZnONPs. Different heavy metals also have different adsorption capacity on ZnONPs, and our previous studies suggest that the adsorption of metal ions on metallic ENPs is a significant phenomenon affecting the plant uptake of these metals in a system with both heavy metals and ENPs[20].

The overall significant reduction of total Cd and Pb by ZnONPs was attributed to two primary processes. First, higher excretion of root exudates could lead to greater adsorption of heavy metals on ZnONPs and stronger aggregation of ZnONPs. Precipitation of larger particles may reduce the bioavailability of heavy metals. This interpretation was supported by the observation of the lower pH in the solution compared to the control due to greater organic acid secretion from plant roots (Shown in Fig 5.8). In a previous study, higher root exudation was observed in the co-presence of cerium oxide nanoparticle and Cd [20], which was consistent with the lower pH observed in the medium containing coexistence of ZnONPs and heavy metals in this study.

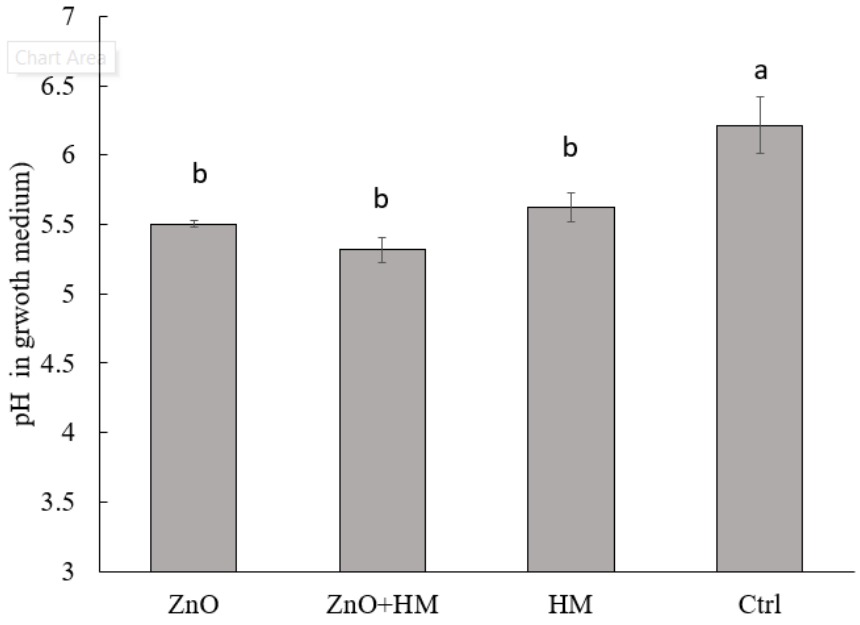

Fig.5.8. pH in the growth media of lettuce grown in the presence of 100 mg/L ZnONPs and/or 1 mg/L Cd +100 mg/L Pb. The reported values are mean ± SD (n = 3). Different letters reflect significant differences as analyzed by ANOVA followed by Tukey's test ($p < 0.05$).

5.6. Conclusion

In summary, the strong effect of coexisting ZnONPs on the bioavailability of Pb and Cd accumulation and concentration of Fe in lettuce tissues were observed. The explicit effects of ZnONPs on heavy metals uptake depend on the properties of heavy metals. Various biological and chemical processes might be responsible for the altered Fe, Pb and Cd uptake by lettuce in the presence of ZnONPs. Further investigations are necessary to elucidate whether similar uptake pattern in a soil-plant system will occur. Interestingly, Zn is a key biological inorganic compound in the nutritional properties of leafy vegetables, and its concentration was associated with altered Fe accumulation in lettuce. These critical findings suggest that proper concentration of ZnONPs may be applied as an effective agrichemical to simultaneously enhance plant growth and limit Cd and Pb accumulation in the leafy vegetable. Due to the strong dissolution of ZnONPs, a dose-response relationship relating ZnONPs and Zn ions as well as their impacts on plant accumulation of different micronutrients and co-existing environmental pollutants need to be investigated. . Even though underlying mechanisms with regard to the interactions of ZnONPs and coexisting heavy metals, this study highlights the critical role ZnONPs may play in plant accumulation of toxic heavy metals as well as essential micronutrients. Because ZnONPs are receiving increasing acceptance as a novel nanofertilizer, this study shed light on the potential food safety and health impact of nanotechnology applications in agriculture. .

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CHAPTER VI

IMPACTS OF ZNO NANOPARTICLES ON THE MOBILIZATION OF ESSENTIAL MICRONUTRIENT (IRON AND COPPER) AND BIOAVAILABILITY OF HEAVY METALS (LEAD AND CADMIUM) IN VEGETABLES

6.1. Summary

The advances in large hydroponic production systems, easy adoption to urban agriculture and higher hydrophobic surface area of leafy greens, highly expose them to atmospheric fallout of heavy metals and nanoparticles. Cadmium (Cd) and lead (Pb) are extremely toxic metallic contaminants, which at trace level threaten humans and livestock health through consumption of edible plant tissues. The uptake of both Cd and Pb may occur through the same protein transporters of essential minerals such as iron (Fe^{2+}) and copper (Cu^{2+}). This study aims to understand the role of zinc oxide nanoparticles (ZnONPs) in uptake pattern of both Cd and Pb and their impacts on re-localization of the iron and copper as the key micronutrient in edible tissues of three species; spinach (*Spinaciae oleracea),* parsley (*Petroselinum sativum)* and cilantro (*Coriandrum sativum).* Pre-grown plant seedlings were exposed to four hydroponic treatments (1.0 mg L^{-1} Cd^{2+}+100.0 mg L^{-1} Pb^{2+}, 1.0 mg L^{-1} Cd^{2+}+100.0 mg L^{-1} Pb^{2+} + 100 mg L^{-1} ZnONPs, 100 mg L^{-1} ZnO-ENPs and a control with no chemical exposure) for two weeks. At termination, shoots were gently separated from the roots, and the concentrations of Pb, Cd, and Fe in all plant tissues were quantified by ICP-MS. The results revealed the mitigation of heavy metal root uptake in coexposure to ZnONPs. The response of each plant was different in relocalization of the Cu and Pb in edible tissues. The translocation trend of heavy metals was identical in edible tissues of three species. Fe concentration in edible tissues exposed to ZnONPs compared to control was evaluated as follow spinach(+10%)> cilantro(+9%)>

parsley(-8%). The trend of re-localization of the Cu was different from the Fe contents, and the Cu level in edible tissues of three species were affected in copresence of both heavy metals as follow cilantro (+8%)> spinach (+4%)> parsley (+1.5%).

6.2. Introduction

With the rapid advancement of nanotechnology and release of engineering nanoparticles, the production of healthy food products became a critical concern for many scientists and engineers[1, 2]. Leafy greens with a wide variety of vegetables are the main ingredients of healthy diets for humans, which are at high risk of contamination by heavy metals and nanoparticles. The large hydroponic production systems[3, 4], easy adoption to urban agriculture and higher leaf surface area of leafy greens, highly expose them to atmospheric fallout of heavy metals and nanoparticles[5]. In addition, their growth in hydroponic systems mediates the bioavailability of organic and inorganic elements for the plants[6, 7].

Copresence of the heavy metals and nanoparticles is an inevitable event that impacts the physiological and biological systems of the plants[8]. Through these interactions, the localization of essential minerals in dietary tissues may change, leading to varying the nutritional values of the plants. A number of studies have investigated the influence of the nanoparticles, heavy metals or their coexistence on the physiological characteristics of the plants[9, 10]. For example, the root elongation of corn roots was observed a 17% reduction at a high level of ZnO nanoparticle (ZnONPs)1000 mg L^{-1} [11]. However, at lower concentration (100-500 mgL^{-1}), positive impacts of the ZnONPs on seed germination, growth, and height of roots and shoots have reported [12, 13]. The change of plant physiological properties in exposure to ENPs or heavy metals may stem from delocalization and alteration of the essential minerals dynamics inside the plant tissues. Rossi et al. 2018 found that the coexistence of CeO_2NPs was associated with mitigating the bioavailability

of Cd and positive impacts of the chlorophylls contents[6, 9]. On the contrary, De la Rosa et al. 2013 observed signs of the ZnONPs toxicity in alfalfa and tomato at a concentration greater than 800 mg L^{-1} in hydroponic system[10].

Cadmium (Cd) and lead (Pb) are the second and third major metallic contaminants of high risk to the environment, which at generally not phytotoxic level threaten humans and livestock health through consumption of edible plant tissue. The uptake of both Cd and Pb ions may occur via the same protein transporters, which are involved in the transport of the essential minerals including Fe^{2+}, $Cu^{2+,}$ and Zn^{2+}. Therefore, the copresence of heavy metals and nanoparticles is of high importance to elucidate their mutual impacts on the mobilization of essential minerals in edible tissues of the plants.

Zinc Oxide nanoparticle (ZnONPs) has a wide application range, including sunscreen products, paint additive, and nano-fertilizers. Due to their specific physicochemical properties, low toxicity, and fungicides properties [1, 14], their application have progressively advanced into the agriculture and food processing sectors. ZnONPs represent substantial stimulating impacts on the dietary plant's growth, such as chlorophyll content. It also contains 80% of the essential nutrient of Zn for leafy greens, and possess a high adsorption capacity for heavy metals intrusion[2, 6]. Bringing all the potential agricultural benefits, the US Food and Drug Administration (FDA) has been recognized as a safe food additive[15].

Therefore, it is very imperative to understand the underlying mechanism of ZnONPs interaction with leafy greens and their behavior in the coexistence of the heavy metals. Type of plant species in such nano-effects studies is a key factor that influences the interaction of heavy metals or nanoparticles with the plants as well as their mutual impacts on the mobilization of the essential minerals[16]. Plants have developed various strategies against the intrusion of heavy metals or

nanoparticles to sustain their homeostasis system. Rossi et al. 2018 found the different response of soybean plant through the release of root exudate in exposure to Cd, ENPs, or their coexistence[6]. Such dynamic response will change the uptake and translocation patterns of heavy metals and micronutrients for the plants. This study aims to understand the mutual interaction of leafy greens with the coexistence of ZnONPs and two heavy metals (Cd and Pb) and their impacts on the mobilization of the three essential minerals of Zn, Fe, and Cu. Three frequently consumed vegetables in the human diet, including spinach (*Spinaciae oleracea),* parsley (*Petroselinum sativum)* and cilantro (*Coriandrum sativum)* were selected as study models.

6.3. Materials and Methods

6.3.1. Materials

Zinc oxide nanoparticle with an average size range of 10-30 nm and negative surface charge (ZnO NPs, 20% by weight) was obtained from the US Research Nanomaterials, Inc. (Houston, TX). Lead nitrate ($Pb(NO_3)_2$ >99%) and cadmium sulfate ($CdSO_4$) in high purity were purchased respectively from Alfa Aestar (Ward Hill, MA) and Fisher Scientific Inc. (Pittsburgh, PA). ICP-MS standard solutions (Pb, Cd, Zn, Fe, and Cu) at a concentration of 10000 ppm, in 3% nitric acid were supplied by RICCA Chemical Company (Arlington, TX).

6.3.2. ZnO Characterization

ZnO physiochemical properties (size, zeta potential, and surface structure) were fully characterized through our previous study[7]. Briefly, the image analysis of the Tecnai G2 F20 transmission electron microscope (TEM) showed; a recorded mean diameter of 68.1 nm (15- 137 nm), dominant

spherical shape at high density, as well existence of the triangular or irregular shapes at low density. The average hydrodynamic size of dispersed ZnO NPs in ultra-pure water (18.3 MΩ) was recorded by 621.08±7.63 nm through the dynamic light scattering (Delsa Nano C, Beckman Coulter Inc. Miami, FL) at a concentration of 100 mg L^{-1}. Respectively, the surface charge (zeta potential) was measured -28.80±2.04 mV.

6.3.3. Plant Culture and Exposure Experiments.

The seeds of parsley, cilantro, and spinach were obtained from Johnny's Selected Seeds (Winslow, ME). Before germination, seeds surface were sterilized for about 20 min applying 30% H_2O_2 then carefully rinsed with ultra-pure water[17]. Germination took place in moist sand. All cultivation and transplantation steps were performed in a controlled temperature greenhouse (25°C), supplied by a fluorescent light (250 µmol m^2 s^{-1} photosynthetic photon flux density) cycle of 16/8.

Germinated seedlings were transplanted to a clay-soil square pot (12 cm x 10 cm) for two weeks to naturally regulate the essential mineral uptake from the soil. Next, the plant species were carefully detached from the soil using a smooth flow of DI water, roots were fully cleared off from the soil residual, and each plant was immediately transferred to a hydroponic system: a series of 50 mL tubes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), covered by aluminum foil. Each tube contained 1/4th strength of the Hoagland solution (Phyto Technology Lab, Shawnee Mission, KS).

Through the hydroponic system's potential interaction of soil compartments with the applied doses of nanoparticle and heavy metals were avoided, and heavy metals and nanoparticles became fully

bioavailable to plant roots. Also, this system has been extensively applied for the production of leafy greens in greenhouses [18].

Once the rate of transpiration was about 10 mL/day (after 10-15 days), plant species were separated into four groups. Each group was assigned to treatments with four replicates. Treatment solutions were prepared in DI water containing i) only heavy metals (100 mg L^{-1} Pb^{2+} + 1.0 mg L^{-1} Cd^{2+}) ii) heavy metals and 100 mg L^{-1} ZnO-ENPs, iii) solution only with 100 mg L^{-1} ZnO-ENPs and iv) control with no external elements. At the exposure step, the Hoagland solution was not applied to avoid aggregation of the nanoparticles as results of its high ionic strength.

To differentiate water evaporation from evapotranspiration, three tubes were filled only with water and monitored among the other plants. During the uptake experiment time, water loss was supplied with the DI water twice a day (morning and evening). To homogenize the effects of light intensity, plants were randomly relocated and rotated. The pH of the growth medium was recorded before and after the uptake experiment using a pH meter (VWR symphony – SB90M5).

6.3.4. Growth analyses and biomass partitioning

Immediately following the uptake experiment, plants were gently isolated from the hydroponic system and submerged in DI water for 30 min, then washed with 50 mL $CaCl_2$ solution (5 mM) five times to possibly eliminate outer root surface deposition of the Zn, Pb and Cd elements [6]. Each plant species was cut into 1) roots and 2) leafy tissues (shoots). The fresh biomass was measured for both roots and shoots of all species. For the element analysis by ICP-MS, fresh biomasses were dried at 80°C for four days to ensure the biomasses were fully dehydrated through the analytical procedure, and respectively, the dry biomass weight was recorded[6,7].

6.3.5. Quantification of the heavy metals and trace elements in plant tissues

Dry biomass of each species (shoot and root) were carefully crushed and homogenized using a household blender (Braun Multiquick MX 2050), then a subsample aliquot of the dry shoot tissues (0.15 g) and dry root tissues (0.05 g) were transferred into a digestion tube placed on DigiPREP MS hot block digester (SCP Science, Clark Graham, Canada). Pre-digestion was performed within 8 hours of preparation by applying 3 mL of 70% (v/v) nitric acid (Certified ACS Plus) [19]. Next, predigested samples were heated up to fully digestion stage and reheated using additional 2 mL of 30% (w/v) H_2O_2 (95 °C / 6 hours) following the EPA method 3050b. [8, 19 7]. Next, 15 mL volume of digested samples were prepared at the level of 1% nitric acid to detect the Zn, Pb, Cd, Cu by an inductively coupled plasma−mass spectrometry ICP-MS (Agilent 7500i, Agilent Technologies Co. Ltd, USA). [8, 19].

6.3.6. Relocation rate of the Cu and Fe

The relocation rate of essential minerals (Cu and Fe rate) in response to the presence of the ZnONPs was developed by Xiong et al. 2017 as a function of roots exposure time[20]. This rate is an indicator of efflux of Cu and Fe from root pores that was primarily articulated as a transferred dose per day. The higher relocation rate in this experimental design indicates a faster interaction in biosystem of the plant and slower efflux of the essential minerals through root exudates. The Cu and Fe relocation rates (milligrams per day) values during the uptake time were calculated with eq 1:

Eq3.1

$$[EM]_{Relocation\ rate} = \frac{([EM_{Shoot}] \times DW_{Shoot}) + ([EM_{Root}] \times DW_{Root})}{Exposure\ Time\ (days)}$$

Where the [EM] represents the essential mineral (Cu or Fe) concentrations (milligrams per kilogram), quantified by ICP-MS analysis, and DW indicates the dry weights of the tissues (mg).

6.3.7. Statistical Analysis

Statistical analysis of the five elements concentration data (Zn, Pb, Cd, Cu and Fe) in both shoot and root tissues of three species (3 or 4 n), as well as data pertaining to their dry and fresh biomass were performed via the Minitab 18 Statistical Software (Minitab Inc., State College, PA, USA). All data were processed through the One-Way ANOVA, to compare the mean of each data set, followed by Tukey's data processing with a significance level of 5% ($p < 0.05$).

6.4. Results

6.4.1. Growth analysis and plant uptake of Pb and Cd

As shown in Fig 6.1, A and B, the biomass of edible tissues of cilantro and parsley in treatments subjected to ENPs were insignificantly reduced by 1.5% compared to control. The sole exposure of the ENPs to the spinach species has shown a significant reduction ($P<0.05$) in dry shoot biomass by 20% (Fig 6.1 C), while the copresence of ENPs and heavy metals showed positive impacts on the growth (15% increase in shoot yield) compared to control. Regarding the non-edible tissues, as shown in Fig 6.2, the impacts of sole ENPs on dry biomass of the roots were insignificant in all three species. However, in the copresence of heavy metals, a slight increase in dry biomass of roots in all species has been observed.

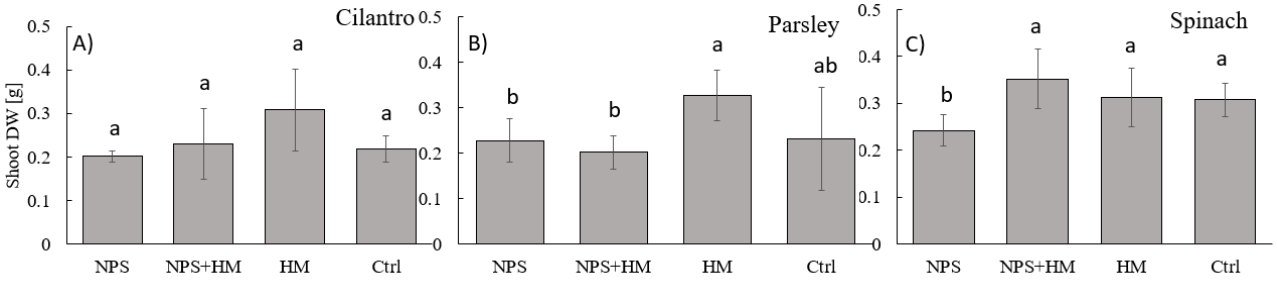

Fig.6.1. Impact of dry biomass of shoot tissues in three species in response to four treatments (ZnONPs. Cd&Pb+ZnONPs, Cd&Pb, Control A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum),* and C) spinach (*Spinaciae oleracea).* Each column indicates the mean of the dataset and the error bars represent standard deviation (n = 3 or 4). Different letters differentiate the significance level ($p \leq 0.05$). HM: heavy metals.

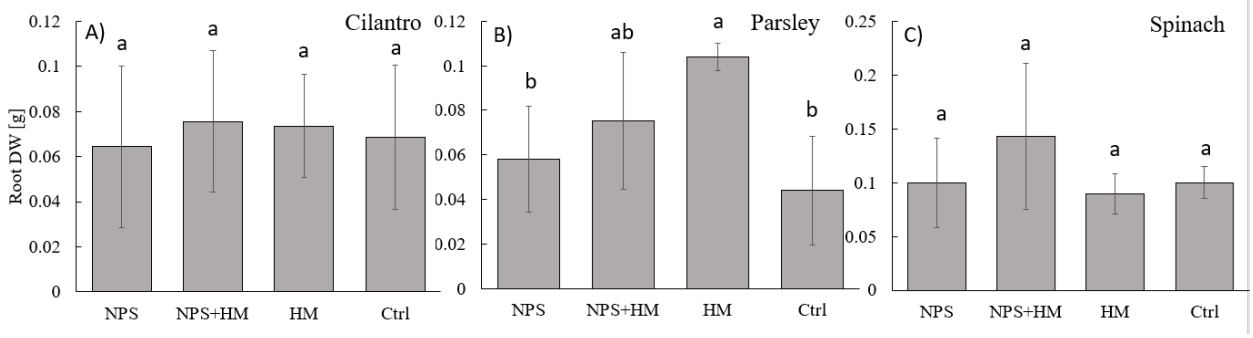

Fig.6.2. Dry biomass of root tissues in three leafy greens under different treatments on the mean basis (ZnONPs. Cd&Pb+ZnONPs, Cd&Pb, Control A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum),* and C) spinach (*Spinaciae oleracea)*). The error bars above each column bar indicate standard deviation (n = 3 or 4) and each letter defines the statistical significance ($p \leq 0.05$). HM: heavy metals.

Fig. 6 depicts the uptake pattern of both Cd and Pb in shoot tissues of all three species under three different treatments. A similar pattern was observed in shoot response to uptake of Pb in all three species. However, the extent of uptake in parsley shoots was at least 10 folds greater than cilantro

and spinach. Copresence of the heavy metals and ENPs have mediated the uptake of Pb in shoot tissues of all three species by approximately 54%, 44%, and 58% respectively for cilantro, parsley and spinach compared to the treatments exposed to only heavy metals. In copresence of ENPs, the Cd level was reduced in edible tissues of all three species compared to treatments subjected to the sole presence of heavy metals. However, this reduction was significant only in cilantro by 27%, and statistically insignificant in both parsley and spinach by approximately 5% and 2%, respectively.

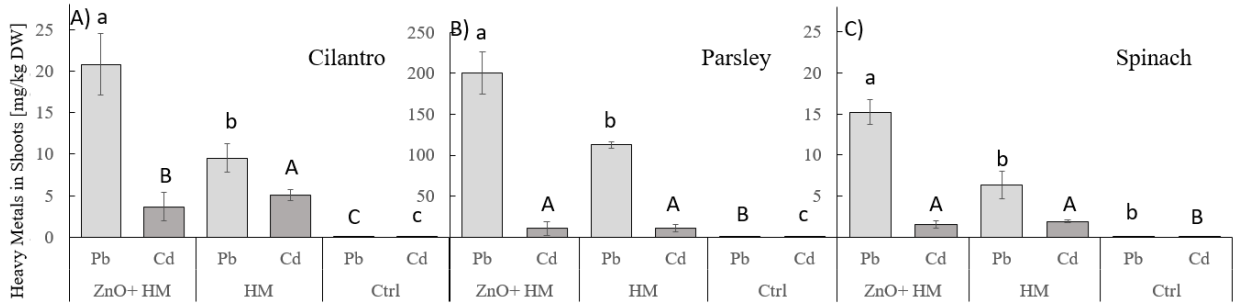

Fig. 6.3. Detected level of heavy metals (Cd and Pb) in shoot tissues A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum)*, and C) spinach (*Spinaciae oleracea)* exposed to different treatments. Error bars designate standard deviation (n = 3), and the letters indicate the significant difference processed by ANOVA-Tukey.

Figure 6.4 illustrates the uptake of both Cd and Pb in non-edible tissues (roots) of the three species under the three treatments. A similar trend of uptake to the shoots was observed for both heavy metals in all plant's roots. Interestingly, the presence of ENPs was associated with a reduction of both Cd and Pb in all species roots, in which, the concentration of Pb in roots was significantly dropped by 39%, 83% and 85% for cilantro, parsley, and spinach, respectively. Cadmium level was mitigated insignificantly in root cells of cilantro coexposed to ENPs by 11%

(Fig 6.4 A), and significantly reduced in parsley and spinach respectively by 30% and 52% (Fig 6.4 B&C).

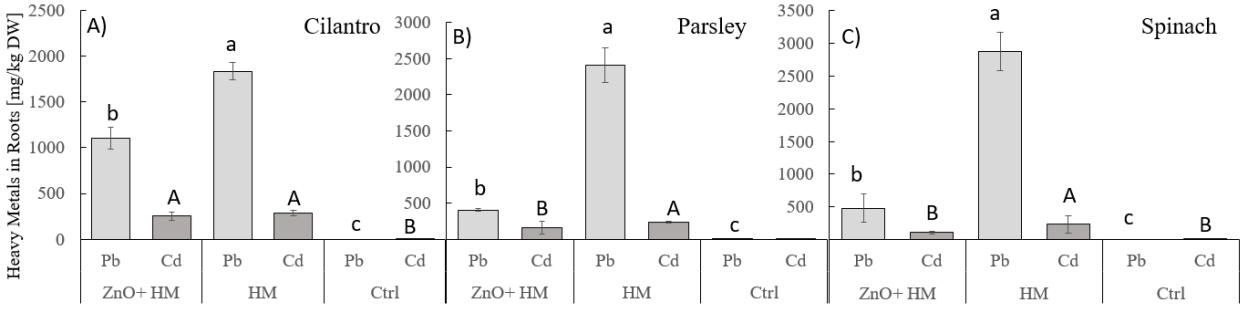

Fig. 6.4. Concentration of cadmium and lead in roots of three species A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum)*, and C) spinach (*Spinaciae oleracea)* exposed to different treatments (combinations of 1 mg/L of Cd+100 mg/L Pb and 100 mg/L ZnONPs). Error bars designate standard deviations (n = 3), and the letters indicate the significant difference processed by ANOVA-Tukey.

6.4.2. Dynamics of plant localization of Fe and Cu and Zn

Described in Fig 6.5 A, the presence of ZnONPs was associated with elevation of Fe in edible tissues of cilantro compared to control. However, the increase of Fe level was not significant in cilantro shoots, in which the ENPs approximately 10% elevated the Fe levels in both treatments. In comparison to cilantro, a slightly different pattern of Fe levels in shoots of parsley was observed (Fig 6.5B). Though the change of Fe in parsley shoots with copresence of heavy metals and ENPs were insignificant, the presence of ENPs lowered the levels of Fe by 8%, and in the sole presence of ENPs, Fe was reduced significantly by 36% compared to control.

The dynamics of Fe in shoot cells of spinach was very identical to cilantro behavior. Presence of ENPs in either treatment has shown an increase in the shoots Fe concentration of spinach at

insignificant level (Fig 6.5 C), in which, Fe was elevated by 13% and 10%, respectively in the sole presence of ENPs and their copresence with heavy metals.

The Cu content in shoots of cilantro was elevated significantly in both treatments exposed to ENPs by approximately 71% on an average basis (shown in Fig 6.5A). However, in the copresence of ENPs and heavy metals, this elevation was at a lower extent by 31% compared to sole ENPs presence. There was no significant change of Cu in parsley shoots compared to control treatment (Fig 6.5 B). Similar to the uptake pattern of Fe in both cilantro and spinach, the levels of Cu in the shoot tissues were also identical. As shown in Fig 6.5 C, exposure to ENPs was associated with significant elevation of Cu content in edible tissues of spinach (37% on an average basis) but at far lower extent compared to what was observed in cilantro.

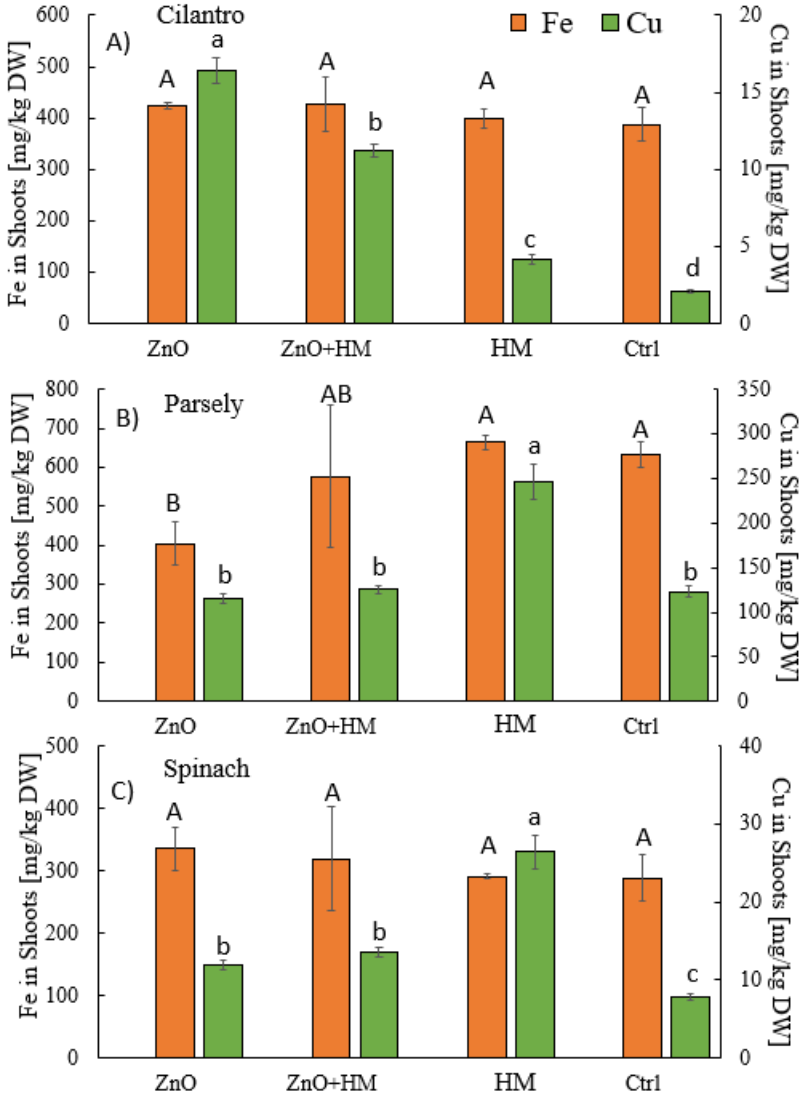

Fig. 6.5. Concentrations of essential minerals (Fe and Cu) in edible biomass (shoots) of A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum),* and C) spinach (*Spinaciae oleracea)* subjected to different treatments (100 mg/L of ZnONPs alone or in combination with 1 mg/L Cd+ 100 mg/L Pb). Detected values represent mean ± SD (n = 3), and different letters differentiate statistical significance ($p \leq 0.05$) conferring to one-way ANOVA post analyzed by Tukey's test.

Figure 6.6 represents the interaction of ZnONPs and heavy metals on the total Zn uptake in both roots and shoots of the plants. The concentration of total Zn in both roots and shoot tissues of all

species which exposed to ENPs were higher than control. However, the detected levels of Zn in spinach shoots (Fig 6.6 C) was relatively higher than two other species.

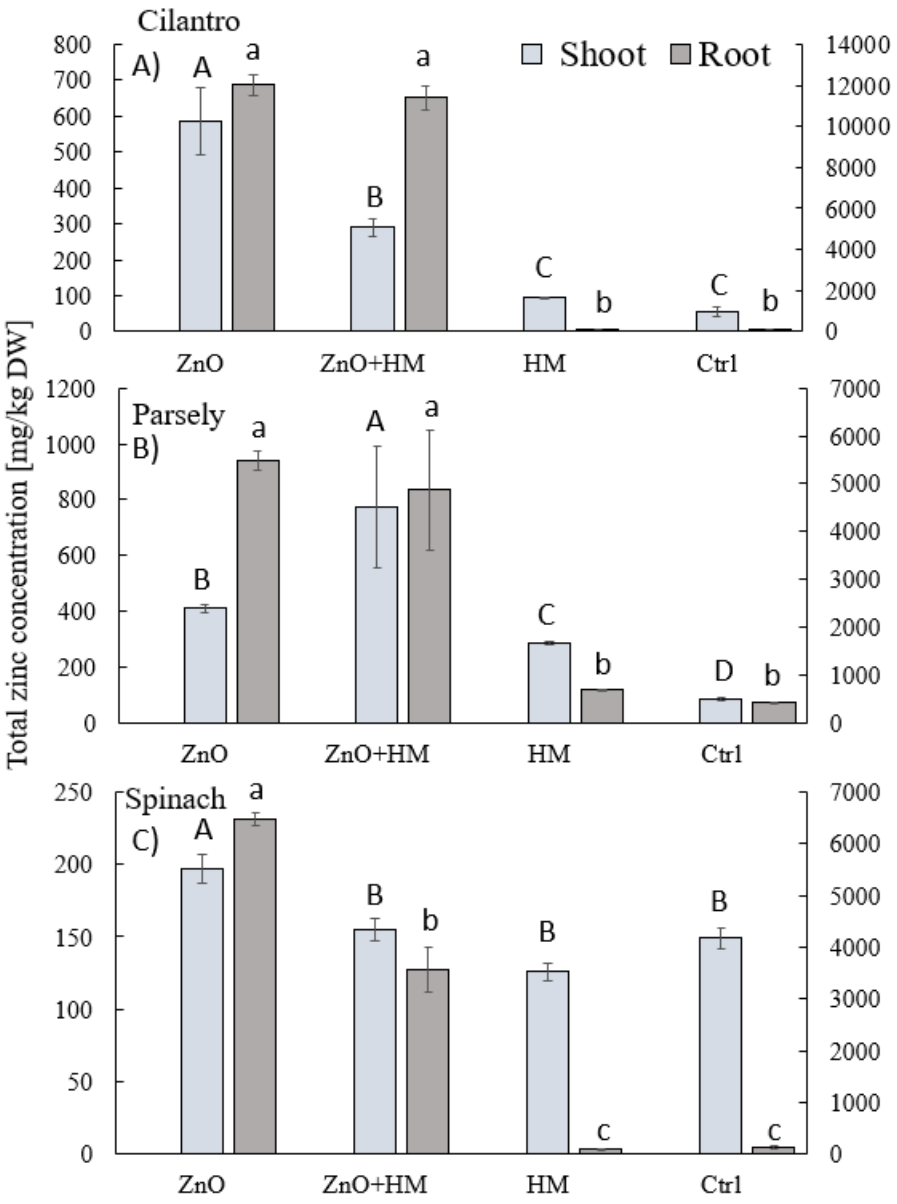

Fig. 6.6. Total Zn concentration on a mean basis in roots and shoots A) cilantro (*Coriandrum sativum)* B) parsley (*Petroselinum sativum),* and C) spinach (*Spinaciae oleracea)* exposed to four different treatments of heavy metals (Pb and Cd) and nanoparticles (ZnO). Error bars specify standard deviation (n = 3).

6.5. Discussion

Considerably increased the biomass of edible tissues in cilantro and spinach at copresence of the ENPs and heavy metals may have resulted from their mutual impacts on the elevation of the Fe levels. Iron plays a fundamental role in photosynthesis, and enzymatic process of the plants, the dynamics of Fe levels can substantially impact the plant's health, and their physiological performance[21]. Slightly reduction of the biomass of edible tissues of all species when exposed to only ZnONPs (Fig 6.1), likely stem from the minor ZnONPs phytotoxicity through their dissociated Zn ions. This interpretation was consistent with total detected levels of Zn in edible tissues, shown in Fig 6.6.

The phytotoxicity impacts of ZnONPs on the biomass production to a variety of the grains and fruits at concentration >>100mg L^{-1} have been reported via soil production system[36, 37], but few studies concentrated in hydroponics production of leafy vegetables. For example, 1,000 mg L^{-1} ZnO NPs reduced root elongation of cucumber (*Cucumis sativus L.*) and corn (*Zea mays L.*) by 51 % and 17 %[22] and at 500 mg L^{-} reduced the roots and shoots elongation in wheat (*Triticum aestivum*)[23]. Some studies hypothesized that the phytotoxicity of ZnONPs is associated with the competition of the dissociated Zn with Fe ions, leading to the interference of Fe-related physiological processes [39-41].

The sole existence of ENPs on the non-edible biomass in all three species was negligible, while by the heavy metal stress, the root biomass was noticeably increased. This difference may be explained by the increasing level of Zn. Our previous study indicated in the presence of heavy metals the release of root exudates may intensify, leading to dissociation of the ZnONPs. Therefore, it mediates the bio-accessibility of the Zn^{2+} supplement that could improve plant growth. This interpretation was consistent with the measurement of the pH values from the

hydroponic system after the uptake experiment. Table 1 represents the change of the pH after adding the heavy metals and in the coexistence of the heavy metals and ZnONPs.

Table 6.1. Measured pH values from the hydroponic system after the uptake experiment

Treatments	pH values		
	Cilantro	Parsley	Spinach
Ctrl	6.19	6.21	6.35
Cd + Pb	6.14	6.1	6.07
Cd+ Pb + ZnONPs	6.06	5.59	6.01

Adequate amounts of the essential minerals, particularly Cu, Fe and Zn is a crucial factor in food quality evaluation of the leafy greens. Unlike many metallic oxide nanoparticles that found to reduce the essential mineral levels[24, 25], the overall impacts of 100 mg L^{-1} in this study on two species of spinach, cilantro were positively evaluated, while its impact slightly lowered the Fe levels in parsley. The order of the positive impacts of ZnONPs in coexistence with Cd and Pb on affecting the Fe concentration in edible tissues was evaluated as follow spinach(+10%)> cilantro(+9%)> parsley(-8%). The trend of re-localization of the Cu was different from the Fe contents, and the Cu level in edible tissues of three species were affected in copresence of both heavy metals as follow cilantro (+8%)> spinach (+4%)> parsley (+1.5%).

Dynamic translocation, uptake, and efflux of essential minerals is very imperative issues that affected by ZnONPs. The efflux of essential minerals through the root pores can be altered by the

presence of either heavy metals or with the coexistence of the ENPs[6, 9]. The obtained results from the detected levels of Cu and Fe suggested the impacts of the ZnONPs on the Cu mobilization is lower than the Fe localizations. Therefore, it was crucial to elucidate the rate of impacts and interaction of ENPs with plant relocalization of the essential minerals of Cu and Fe.

Our calculated results of the mobilization rate (shown in Fig 6.7) represent an indication of the efflux between the roots and hydroponic media. Higher transfer rates suggest the less efflux of the elements. The higher transfer rate in parsley was consistent with the observed concentration of the Cu and Fe in their edible tissues. In a general trend, the mobilization of Cu in copresence of heavy metals is at a far lower rate compared to Fe. For example, in the coexistence of Cd and Pb with ZnONPs exposed to the parsley species, the mobilization of the Cu compared to its control and Fe to the control is 3% and 11%, respectively. However, further investigations of the elemental release into the hydroponic system are needed to elucidate the rate of micronutrient loss in exposure to heavy metals and nanoparticles.

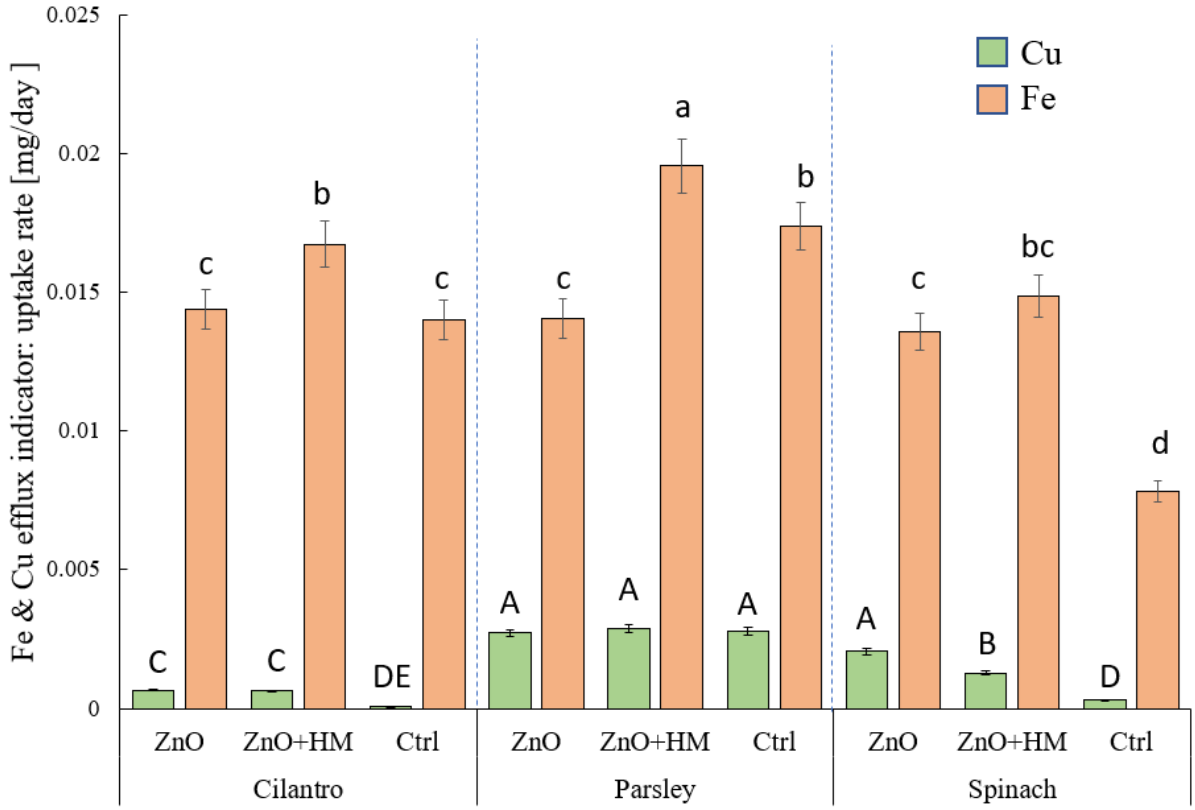

Fig. 6.7. The relocation rate of the essential minerals Fe and Cu on a mean basis in three species of cilantro (*Coriandrum sativum)*, parsley (*Petroselinum sativum)*, and spinach (*Spinaciae oleracea)* during two weeks of exposure time. The results were calculated by Eq.2. Error bars indicate the standard deviation (n = 3).

The co-presence of Cd and Pb limited the overall uptake of ZnONPs in all three species. This phenomenon will impact the dynamics of another essential mineral of Zn as the main component of the ZnONPs. The underlying mechanism for this limitation may be caused by aggregation of ZnONPs in the coexistence with both Cd and Pb. As discussed earlier (Table.1) the stress of heavy metals co-present induce the higher root exudate leading to the aggregation of ZnONPs[26]. Consequently, the bioactive surface of the ZnONPs might be shielded with organic materials, while the simultaneous adsorption of the heavy metals on its surface will occur. Our previous study

revealed the root exudate will limit the bioavailability of metallic oxide nanoparticles and will reduce the extent of heavy metal adsorption[26].

Heavy metals interactions

The most prominent results were the response of ZnONPs that significantly reduced the Cd at low concentration in edible tissues. The higher concentration of Pb in edible tissues suggests that at high contamination levels ZnONPs will not be effective in the reduction of heavy metals. The different uptake patterns between Cd and Pb in shoots of the three species suggest that the properties of each metal and their concentration are the governing factors in their complex chemical interaction with co-occurring ZnONPs.

Heavy metals represent different adsorption capacity on ZnONPs, and our previous studies revealed that the adsorption of metal ions on metallic oxide ENPs is a significant phenomenon altering the plant metals uptake in a system with both heavy metals and ENPs[6, 27]. In the copresence of ZnONPs, lower level of Cd and elevated level of Pb strengthen a scenario in which the Cd represents higher adsorption tendency compared to Pb towards ZnONPs. Another plausible interpretation on higher Pb level in copresence of ZnONPs is attributed to the reactive oxidative species (ROS) generated by ZnONPs and their dissociated ions. ROS can affect plant homeostasis system via the interruption of photosynthesis, protein denaturation and up-regulation of antioxidant enzymes (i.e., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)).

One of the interesting findings of this study was the key role of ZnONPs in changing the root uptake of Pb and Cd. In an overall biomass analysis, both metals were significantly reduced in the presence of ZnONPs. However, this reduction was more prominent in root tissues.

The overall significant impacts of ZnONPs in mitigation of total Cd and Pb was attributed to a primary mechanism; it is highly possible that the surface charge of nanoparticles will be partially or entirely affected by the presence of root exudate and associated amino acids in rhizosphere zones of the plants before their uptake [26]. Because the pathways of the nanoparticle to the root cells are entrapped by high concentration of amino acids and low molecular chains. As results, amino acids in the rhizosphere are able to indirectly change the enthalpy of certain protein interaction with nanoparticles [28].

The agglomeration of the nanoparticle is a critical phenomenon that occurs upon entrance of nanoparticle in a biological system which may be affected by the particle shape, size, surface area, and charge, as well as their adsorption properties [29]. Furthermore, key factors including pH, ionic strength, water quality, and the presence of organic/inorganic matter potentially modify nanoparticle aggregation, which govern the toxicity and reactivity of the nanoparticles [30].

In the plant root zone (the rhizosphere), amino acids are the second most significant component of the root exudates. The release of such molecules has been reported in numbers of plants by 50% (w v^{-1}) through the root exudates [31]. Representative amino acids hold distinctive charges at a pH range (5.5–8.5) characterizing the natural environment. For example, four frequently reported amino acids in rhizosphere include positively charged (Histidine), neutral (Glycine), and negative charges (Aspartic acid and Glutamate) [31].

The ZnONPs colloidal stability in exposure to amino acids solution and their tendency to agglomerate corresponds to the surface charge of the nanoparticles as well as functional groups of the amino acids which their overall interaction can be measured in the framework of multiple forces of electrostatic, steric, and van der Waals [31].

In copresence of Cd and b and ZnONPs, several processes can lessen the potential adsorption of Pb and Cd onto ZnONPs surface such as competitive adsorption of root exudates that occupy part of the adsorption sites for Pb or Cd; the formation of root exudate and heavy metals complexes which display lower adsorption on ZnONPs than free Pb^{2+} or Cd^{2+} or less available adsorption sites caused by ZnONPs aggregation.

In the presence of root exudates, if the adsorption density of Cd^{2+} is greater than Pb^{2+} or other available organic ions induced by amino acids and charged organic ligands, the imbalance of adsorbed ions will contribute a net positive charge to the surface. Therefore, the charge imbalance might be described either in terms of unequal adsorption of the Pb^{2+} or nonstoichiometric dissolution of the ZnONPs. The higher feasibility of Cd^{2+} adsorption in a substitution mechanism with ZnONPs with respect to higher dissolution of ZnONPs in co-presence of root exudates will be further investigated by measuring the trend of zeta potential at different levels of root exudates [26]. Above all, the precipitation of larger particles can reduce the bioavailability of Cd and Pb. This interpretation was consistent with the change of the pH in the solution compared to the control (Shown in Table.1).

6.6. Conclusion

The overall impacts of ZnONPs at 100 mg L^{-1} level on the growth and yield production of all three species were positively evaluated towards increasing the nutritional values and reduction of the heavy metals. This evaluation was achieved over data interpretation of the Fe, Zn and Cu as essential minerals, total mitigation of the Cd and Pb as well as their impacts on the total biomass of each species.

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CHAPTER VII

INVESTIGATION OF EXTERNAL APPLICATION OF ZNO-NANOPARTICLES FOR ENHANCING SHELF-LIFE OF TOMATO (SOLANUM LYCOPERSICUM)

7.1. Summary

Tomato (*Solanum lycopersicum*) is rich source of vitamins, essential minerals and amino acids, that ranked it as the second most consumed vegetable in the world. Also, it is rich of anti-cancer compound of lycopene that is a main carotenoid in the entire human body. It is very imperative to apply cutting edge technologies to extend the shelf life of tomato. Zinc oxide nanoparticles (ZnO ENPs) are used in wide range of agricultural practice. Though general consensus has been reached that ZnO ENPs possess high anti-microbial properties and UV absorption, but their external application on shelf life of tomato has not been investigated. To authors's knowledge, for the first time, this study extensively investigated the external application of ZnO nanoparticles at 100 mg L-1 on the shelf life of tomato. Main shelf life factors of tomato including the microbial spoilage, dehydration and color change were investigated. Additional analytical experiment was conducted to elucidate the diffusion of Zn into the tissues and water removal efficiency of the Zn after washing the tomato by an inductively coupled plasma−mass spectrometry ICP-MS. Application of ZnO ENPs was associated with 47% inhibition of the microbial growth and significant increase of the lycopene antioxidant ($P<0.05$). In summary, results highlight the beneficial application of the ZnO ENPs could enhance the tomato shelf life at room temperature.

7.2. Introduction

Nanotechnology advancement into agricultural practice has developed as a revolutionary breakthrough in food science and engineering. Engineered Nanoparticles (ENPs) have found their ways in post-harvesting technologies through different applications, including food packaging, nanosensors and many more. ENPs possess unique properties, such as high surface area and adsorption capacity, easy operation, and cost-effective production. There is high interest in the use of ENPs towards increasing food safety and agricultural resilience. ENPs can change the precarious state of the toxic metal ions into a stable or safe state and reduce the need for the treatment of contaminated water for agriculture. Also, ENPs can prevent the uptake of heavy metals and enhance the delivery of micronutrient into the edible tissues of the crops.

Recently, ENPs has shown a high potential to increase the shelf life of fresh foods through their anti-fungal/microbial properties. For example, Sing et al 2017 found impregnation of cellulosic food package with silver (Ag) -ENPs at minimum level of 15.3 ug/ml can significantly increase the shelf life of tomato and cabbage [1]. Several metallic nanoparticles have shown high potential in retarding the fungal and microbial activities in fresh foods such as CuO and ZnO ENPs [2, 3].

Losses due to spoilage of the postharvest fresh vegetables are imperative issues in food process engineering. Microbial contamination of post-harvested foods are inevitable. From the field collection and packaging until the vegetable are placed at consumer's cold storage, food quality and shelf life are subjected to reduce [1].

However, the growing demand for less processed foods, extended shelf-life and health concerns for the potential toxicity of food preservatives surge food engineers to find alternatives for a better preservation. Nanotechnology as a promising alternative hailed ENPs application throughout the

food chain supply, which tenders a novel food processing; nano-materials in production efficiency (i.e. packaging), agrochemicals minimization (i.e. preservatives); better flavor and textures; sanitary processing and enhancing the nutritional values. During the past decade, a variety of organic and inorganic ENPs have been introduced in food science.[4, 5] Nanomaterials are generally engineered to increase profitability and sustainability from agricultural field production to the post harvesting processes.

Zinc oxideENPs with the formula of ZnO is an inorganic compound, is currently being used as food additives and has been recognized as safe material by the Food and Drug Administration [2]. It exhibits specific properties which make it favorable for food industries such as decent transparency, high electron mobility as well as strong room temperature luminescence [6]. ZnO nanostructures are also attractive for biological and agricultural application because of their safe properties and large surface area [7, 8]. For example, in biological systems, ZnO enters into a slow reaction with fatty acids and produce carboxylates compounds, such as oleate or stearate[9]. Also, ZnO nanoparticle represents strong antimicrobial characteristics which may stem from the generation of reactive oxygen species (ROS)[10, 11]. Such ROS could be induced on the nanosurface of ZnO [12]. Its application as an antimicrobial agent against various pathogens including Escherichia coli, Campylobacter jejuni, Pseudomonas aeruginosa has been frequently reported [13, 14].

The benefits of using ZnO as antimicrobial agents is that Zn is an essential mineral element for humans and crops, which exhibit strong activity in the form of oxide at very small amounts [11, 15]. For example, Zn is the sole metal formulated in all six enzyme classes (transferases, viz. oxidoreductases, hydrolases and ligases, lyases, isomerases) [13]. Zn plays a vital role in different integral metabolic processes, promotes the synthesis of carotenoids and chlorophylls leading to

higher photosynthetic apparatus of the crops [9]. It has demonstrated to enhance germination rate, pigments synthesis, the contents of carbs and protein as well as activities of antioxidant enzymes [16, 17].

To the authors' knowledge, for the first time this study investigated the external application of ZnO ENPs in shelf life properties of the tomato. Tomato is rich source of vitamins, essential minerals and amino acids, that ranked it as the second most consumed vegetable in the world[18]. Also, it is rich of anti-cancer compound of lycopene that is a main carotenoid in the entire human body [19]. Lycopene possess the highest antioxidant potential among the six major carotenoids of human plasma [19]. Studies have shown the strong correlation between the lycopene level and tomato color [20]. ZnO ENPs with high UV adsorption capacity, may affect the concentration of the concentration of the lycopene in tomato. Through this research, extensive microbiological and analytical analysis was used to study the potential effects of the ZnO ENPs on the shelf life of tomato.

7.3. Materials and Methods

7.3.1. Materials and characterization

Negatively surface charged ZnO ENPs (20% by weight, 10-30 nm) was purchased from the US Research Nanomaterials, Inc. (Houston, TX). Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$>99%) was provided from Acros Organics (Geel, Belgium). Potato dextrose agar was purchased from Sigma Aldrich Inc. (Merck KGaA, Darmstadt, Germany). Peptone water was purchased from Sigma Aldrich (St. Louis, MO). ZnO ENPs was fully characterized for the size and shape in our previous study by a Tecnai G2 F20 transmission electron microscope (TEM); spherical shape at high density was primarily dominant, with less density of triangular or other irregular shapes at the images [21]. The diameter varied between 15 to 137 nm with a recorded mean size of 68.1 nm. Dynamic light

scattering applied to quantify the hydrodynamic ENPs size at dispersed level of 100 mg L-1 in DI water (621.08±7.63 nm) and zeta potential (-28.80±2.04 mV) [21]. A suspension of ZnO ENPs at concentration of 100 mg L-1 was prepared in ultrapure water and sonicated for one minute at Branson B2510 ultrasonic bath (Branson, Danbury, CT) [15].

7.3.2. External applications and water loss

The fresh ripped tomatoes Solanum lycopersicum were purchased from local farmers in College Station, Texas. Before any treatments, tomatoes were disinfected with 3% sodium hypochlorite solution for 15 min, washed with deionized water (DI) three times [1]. Tomatoes were grouped in three treatments, 100 mg L-1 ZnO ENPs, 100 mg L-1 ZnSO4 and control with no chemical exposure. Each treatment series were exposed at three different temperatures, room temperature 25 °C, average summer temperature of Texas 33 °C and refrigerator temperature of 4 °C. tomatoes were hold in climate chambers under a 16/8 h fluorescent illumination cycle (250 μmol m2 s−1) and were randomly rotated every other day during the storage stage to reduce possible differences in light reception [22]. All samples were subjected to weight on a daily basis to determine the water loss factor.

7.3.3. Color measurement

To evaluate the color of tomato under different treatments, HunterLab LabScan XE spectrocolorimeter (Hunter Associates, USA) was used with a port size of 10.1 mm and a view area of 6.35 mm. The CIE illuminant C applied for the color characterization [20, 23]. The colorimeter was calibrated before each measurement, using a black, red and white standard tiles. Three color dimensions of the L, a and b values were read from the processed colors [23, 24]. In each treatment, the final color values were quantified by mean of three replicates. Each measurement was recorded within at 8 am and the treatments were kept under constant climatic

condition at room temperature (16/8 h fluorescent illumination cycle (250 µmol m2 s−1)). The quantity of lycopene was simulated based on the linear model developed by Barret and Anton 2008[20].

7.3.4. Natural microflora analyses of fresh-cut tomato

Before any treatments, intact tomatoes in three replicates were homogenized in 250 mL 3% sterile peptone water (Sigma–Aldrich, Louis, MO) using a sterile Blender bag (VWR, Randor, PA, USA)[25]. Next, triplicates of tomatoes were classified in three treatments groups, 100 mg L ZnOENPs, 100 mg L ZnSO4 and control with no chemical exposure. After the external treatments, tomatoes were subjected to air dry for 24 h at room temperature. At day 4, tomatoes were prepared for microbial analysis on the outer skin. Approximately 1 Cm2 of the outer skin of each tomato (<1 mm depth) were cut and plated on Potato Dextrose Agar (PDA, Difco) petri dish (15 x 5 mm) [1]. The plates in triplicates were incubated at 30 °C for 72 h.

7.3.5. Microbial growth of existing tomato spoilage agents

A spoiled tomato was collected from the shelf of the control treatments at 25 °C. The tomato was submerged and shaken into a sterile Blender bag for 15 min to retrieve homogenate aliquot of the microbial communities for further analysis [26]. On the side, agar plates in three replicate of three treatments were prepared; two were inoculated with 20 µL of either (100 mgL) ZnO ENPs or 100 (mgL) ZnSO4, and a control with no Zn exposure. A 100 µL of the tomato filtrate were spread on agar plates for the enumeration of microbial communities. Plates were kept in incubation chamber at 35 °C for 48 h and colony forming units (CFU) were counted respectively with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK).

7.3.6. Zn Diffusion Analysis

At day four of the exposure, three tomatoes from each treatment were randomly selected and fully washed with DI water three times. Each tomato was chopped and dried in an oven at 75 ºC for 5 days and acid digested following published protocols [4, 27, 28]. Briefly, 5 mL of 70% (w/w) nitric acid (Certified ACS Plus) was added to a 50 mL digestion tube containing 0.5 g of homogenized dry biomass of tomato and heated at 95 °C for 5 h on a DigiPREP MS hot block digester (SCP Science, Clark Graham, Canada). After being cooled down to room temperature, 2 mL of hydrogen peroxide (30%, Certified ACS) was added to each tube and re-heated on the hot block to 95 °C until the biomass was fully digested [28, 29]. The amount of Zn in each sample was then quantified by an inductively coupled plasma−mass spectrometry (ICP-MS) (Agilent 7500i, Agilent Technologies Co. Ltd, USA). Detailed information on the operational parameters of ICP-MS and sample quality control can be found from our previous publications [30].

7.3.7. Statistical Analysis

Statistical analysis of cerium and cadmium concentration in dry plant tissues was performed using the Minitab 18 Statistical Software (Minitab Inc., State College, PA, USA). The comparison between mean values of different treatments was carried out using One-Way ANOVA, followed by Tukey's test at significance level 5% ($p < 0.05$).

6.4. Results and Discussion

6.4.1. Biological analysis

Figure 1 illustrate the impacts of the 100 mg L-1 ZnO ENPs on the microbial growth of tomato. The ZNO ENPs significantly reduced the microbial growth compared to control by approximately

47%, which indicate its high potential for hindering the spoilage of the tomato. However, the impacts of the Zn2+ ions were also statistically significant ($P<0.05$) in reduction of the microbial community in comparison to control (14%) but to less extent in comparison to ENPs. These results provide strong evidence upon anti-microbial/fungal properties of the ZnO NPs, leading to further experimental investigation. It is noteworthy to mention that the anti-microbial properties of the ZnO ENPs may stem from the release of Zn2+ on the outer skin of the tomato because of the significant reduction of of microbial activities over applying the Zn ions instead of ENPs in this experiment. Several studies have shown strong antimicrobial properties of ZnO ENPs at a very low level on retarding the growth of both gram negative and gram-positive bacteria, in which in some cases reach up to 99.9% efficiency [31]. Figure 2 displays the qualitative analysis of the microbial growth of the outer skin of the tomato within 24 and 72 incubation hours. The qualitative results in Figure 2 provides a very critical information upon the growth rate of the microbial comunity that has been substantially limited with the presence of the ZnO ENPs compared to control and Zn^{2+} ions. One of the responsive mechanisms can be the inducing of the ROS by ENPs that strongly interrupt the function of the microbial cell wall [5]and prohibits their further development of the bio-surface of tomato. Studies have proposed that Zn^{2+} at low level might behave as a nutrient supplement for microbial communities leading to enhance the metabolic process of microbial community [32], developing their footprint.

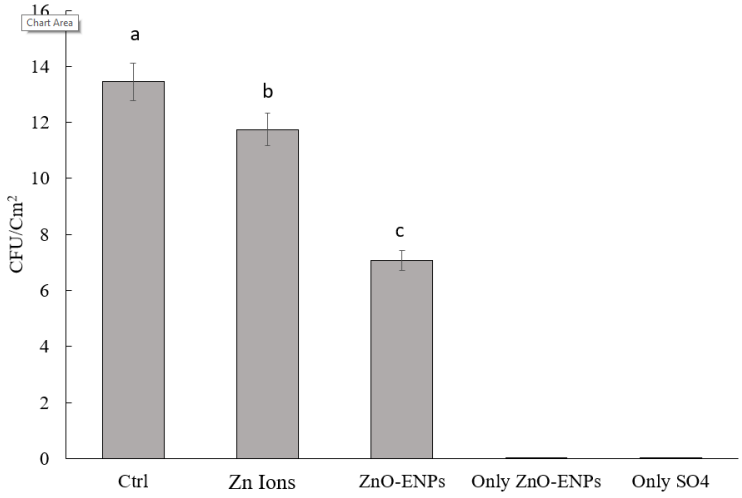

Figure 7.1. The Colony Forming Units (CFU) formed after 100 mg L-1 external application of ZnO ENPs in potato dextrose agar medium to monitor the susceptibility of existing microbial community on tomato skins to presence of ENPs.

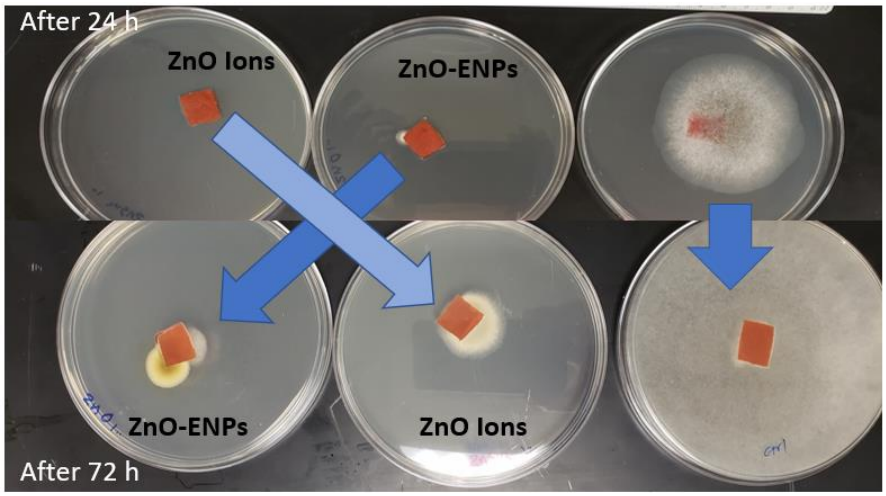

Figure 7.2. Inhibition of microbial growth in presence of the 100 mg L-1 external application of ZnO ENPs or Zn^{2+} on outer surface of tomato. Plates on the top indicate the response of microbial community and 24 hours of incubation at 35 C and the lower plates display their response after 72 hours.

7.4.2. Water loss and color change in shelf life experiment

Figure 3 shows the key parameter in shelf life of tomato as a customer perception factor and indicator of the microbial activities within the bio-surface. Water loss is directly related to the storage temperature of the tomato; however, it can be impacted by applying the ZnO ENPs. The applied ZnO ENPs have shown strong UV absorber that increased its content in formulation of the several sunscreen products [33]. Also, it has shown that increase the photosynthesis of the several of crops [2, 15]. In case of tomato, the higher UV absorption may change the color and reduce the water contents of tomato. The observation of tomato treated with 100 mg L-1 ZNO ENPs under the higher temperature of 33 °C compared to the room temperature in Figure 3 shows stronger relation between the tomato water loss and presence of the ZnO ENPs at higher temperature. ENPs elevated the water loss significantly by 60 % compared to control at 33 °C. This reduction was 50% less at room temperature (25 °C) compared to treatments at higher temperature. However, in case of the room temperature this relation was not significant compared to control. The key point of this observation was the factor of temperature than the presence of the ENPs. Future investigation me be conducted to optimize the temperature and ENPs application regarding the water loss factor. The reaction response of treatments by the Zn2+ ion and increasing the water loss is not clear yet. One reason can be the energy absorption of the metallic ions and promoting the surface evaporation from the tomato skin.

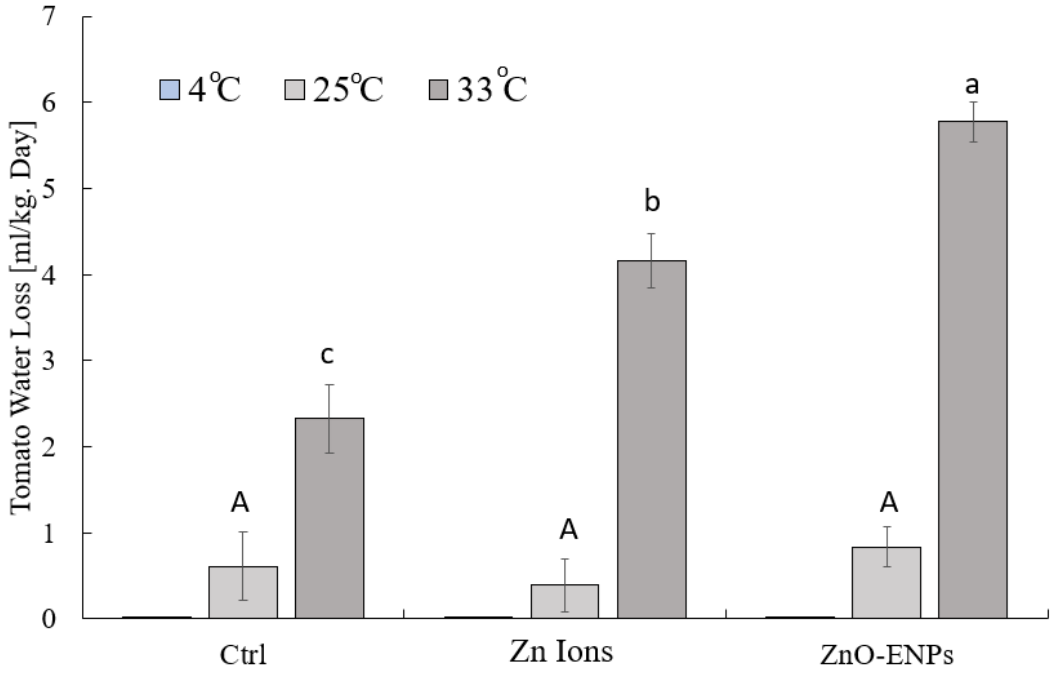

Figure 7.3. Water loss profile of tomato: the mean data set of control, ZnO ENPs and Zn2+ treatments under three storage temperatures were examined by measuring the biomass change over five days of exposure. All treatments were kept under a 16/8 h fluorescent illumination cycle (250 μmol m2 s−1). The letters indicate the statistical significance ($p<0.05$, n=3).

Shelf life results based on water loss measurements have been further coupled with colorimetric analysis of the pigments. Using LabScan XE spectrocolorimeter technique reduces the complexity of the shelf life data-set under ZnO ENPs treatments. The characteristic structure of the data-set is a strong indicator as ZNO ENPs UV absorption and promotional factor in ripening the tomato. As shown in Figure 4, exposure to ENPs significantly increased the redness of the tomato as time proceeds. The scores obtained from the instrument, further indicates nearly neutral impacts of the Zn2+ on the color properties of the tomato. Combination of all treatments dictates the beneficial application of ZnO ENPs in enhancing the red color of tomato while the water loss may off set

this factor. A very important protein of tomato known as lycopene is indicator of nutritional value of tomato shelf life was respectively simulated. As it can be seen from the Figure 4 the contents of lycopene may closely relate to the presence of ENPs. For example, within four days of the storage time, the lycopene content may increase by at least 5 mg/kg compared to control. However, these results call for further investigations of the change of antioxidant level in tomato exposed to external application of ZnO ENPs.

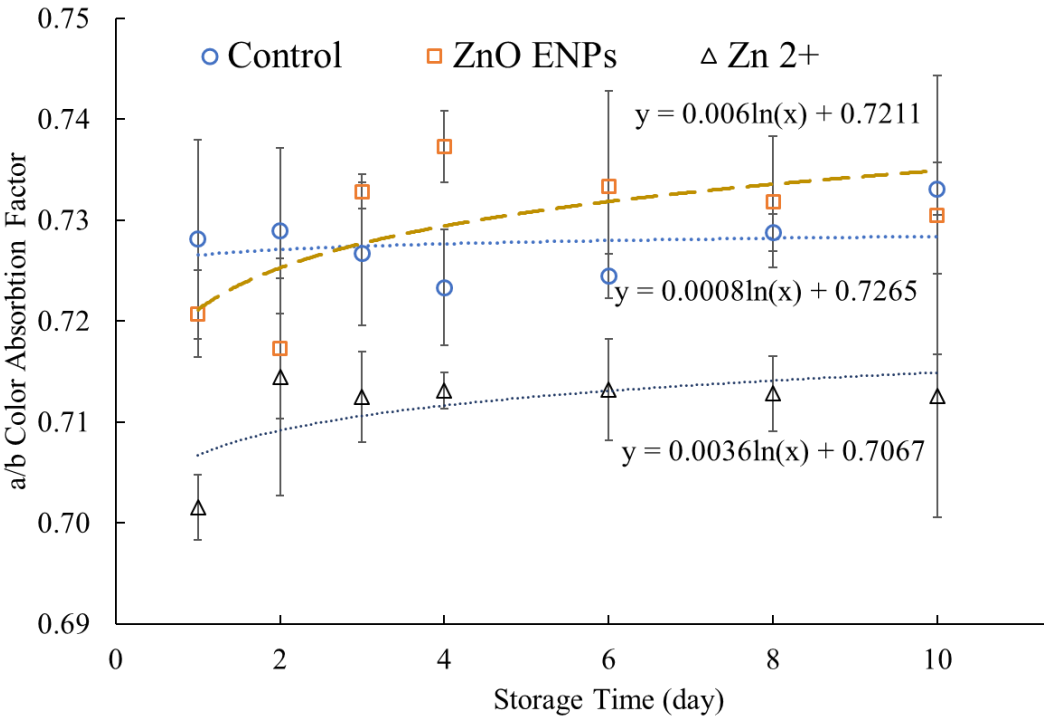

Figure 7.4. The black curves represent the relationship between the a/b color factor of tomatoes under each treatment (control, 100 mg L-1 external application of ZnO ENPs or Zn2+) and storage time. Each curve is representative of a simulated value of the final experiment.

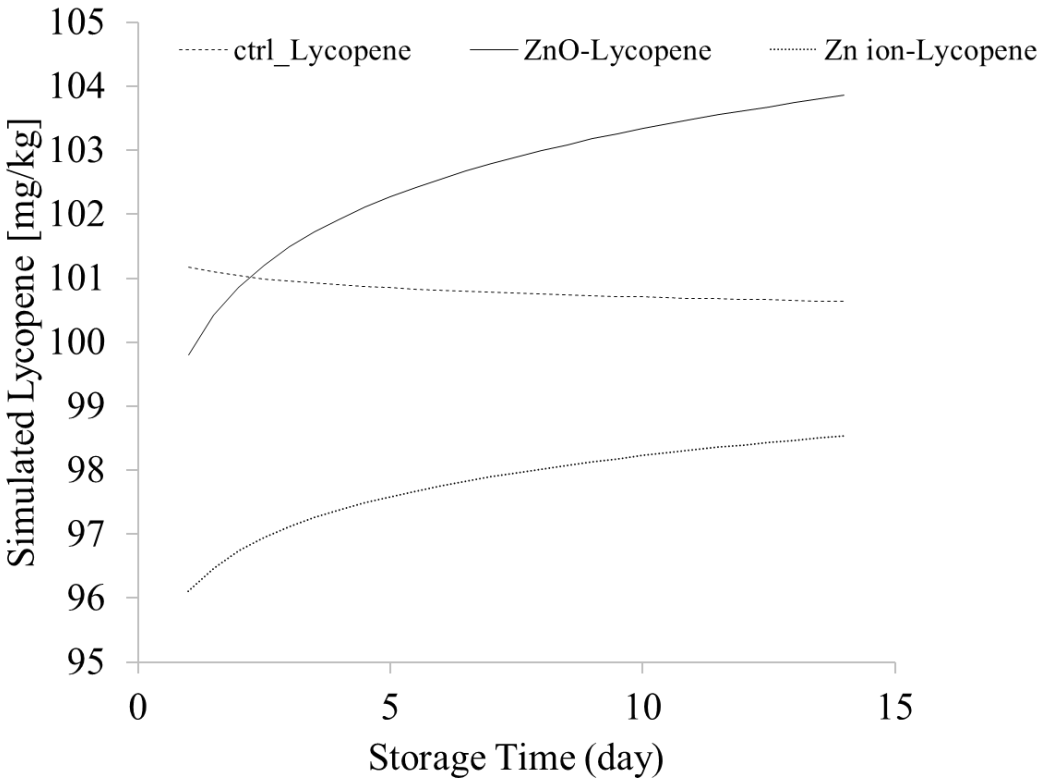

Figure 7.5. Projected concentration of lycopene antioxidant under three different treatments. The colorimetric experimental data were applied through the suggested linear developed model of Barret and Anthon, 2008.

7.4.3. Zn enrichment

Figure 6 illustrate imperative results regarding the essential element enrichment of tomato after the exposure time to ENPs. The amounts of the ZnO ENPs has been remarkably washed off from the tomato surface. The high efficiency of the water removal of this nanoparticle cleared the concerns regarding over dose concentration of Zn after uptake experiment. In addition, the Zn level was enhanced towards the enrichment of the nutritional values, in which treatments exposed to ENPs the total Zn content was increased by 16% and those exposed to only Zn ions, it was increased by 13%. Surface absorption and diffusion of ZnO from the hydrophobic surface of tomato into the edible tissues may strongly relate to the coating material of the ENPs. For example,

there are several ZnO ENPs in the market coated materials with a variety of organic substances such as amino acids which can be further investigate towards enhancing the shelf life of tomato.

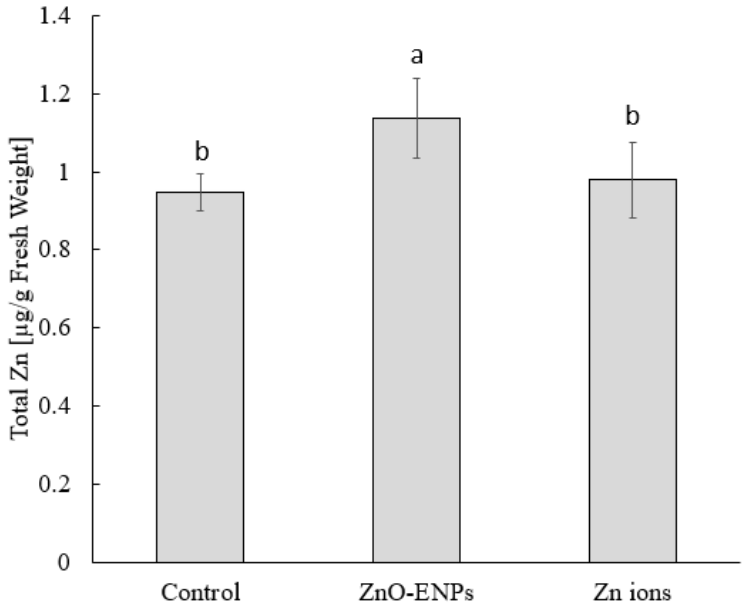

Figure 7.6. illustration of the total Zn contents after washing the tomato at termination stage. The letters indicate the significance level using one-way ANNOVA ($p<0.05$, $n=3$).

7.5. Conclusion

Despite the importance of external application of ZnO ENPs for improving crop nutrition and extending the shelf life, the underlying mechanisms by which external-applied ZnO ENPs move across the outer surface or stays on the surface remain unclear. Using XE spectrocolorimeter, revelaed the role of ZnO ENPs in ripening and color improvement can be further investigated. The microbial analysis indicated the anti-microbial properties of the ZnO ENPs can be main governing factor in extending the tomato shelf life. Finding from the impact of the ZnO on the dehydration may be further investigated under different climatic set up. Enhancing the nutritional value of the tomato by increasing 16% of total Zn content was one of the key finding in this study. This study established a baseline for more investigation on external applications of a variety of metallic oxide

nanoparticles with anti-microbial/fungal properties such as CuO on a variety of susceptible vegetables to spoilage. Overall, this suggests the potential application of ZnO to extend of shelf life. However, further studies are needed to extend and safely apply this ENPs in extending the shelf life.

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CHAPTER VIII

CONCLUSION AND RESEARCH PERSPECTIVE

This dissertation work aimed *to elucidate the underlying mechanisms of interaction between engineering metallic oxide nanoparticles and biosystem of different plant species and possibly finding their potential phytoremediation application.* With embracement of nanotechnology into agrichemicals, the presence of engineered nanoparticles (ENPs) can strongly affect the modulating factors and changing the bioavailability of heavy metals and essential minerals to plants. Exposure of edible plants with the coexistence of ENPs and heavy metals can alter their uptake and localization of essential minerals such as iron (Fe) and copper (Cu) through different mechanisms. Agricultural commodities such as legumes, grains, leafy vegetables, and herbs are a rich source of proteins, essential minerals, and fibers for the human diet and livestock. Heavy metals and metalloids including cadmium (Cd), lead (Pb), and arsenic (III/V) are extremely toxic elements to humans even at trace concentrations that their accumulation in edible plants turns them to phytotoxic foods. Several factors can modulate the availability of such metals and other essential minerals to plants, such as pH, the rhizosphere microbiome, and the presence of organic acids. Characterizing these factors and revealing this role can substantially add to the advancement of nanotechnology. My research area is very interesting for students; it is an interdisciplinary research and can be supported by different source of funding.

In addition, as has been shown in my publication list the *environmental fate of organic and inorganic contaminants in various environmental compartments* has been among my top research interests. I have three peer-reviewed publication regarding the environmental fate of organic Ultraviolet (UV) filters and five printed publications as the outcome of my Ph.D. progress on underlying mechanisms of the interaction of coexisting metallic oxide nanoparticles (CuO, ZnO, and CeO_2) with different crops including rice and soybean.

8.1. The Challenge and Research Needs

Due to different size-specific properties, surface charge, and phytotoxicity potential, the risk of bioaccumulation of ENPs in dietary plants and edible crop tissues is increasing. In addition, different plant species have developed different defense mechanisms towards the intrusion of organic and inorganic contaminants. Coexistence of ENPs and organic and inorganic contaminants change the physiochemistry of ENPs and response of the plants.

For organic chemicals, hydrophobicity is a key parameter which mediates the entrance of compounds into the plant and dictates the primary mechanism for ENPs when they co-exist with the organic compounds[1]. This pathway is known as the "Trojan horse effect" in which adsorption of the organic compounds onto the ENPs surface leads to concomitantly uptake by plants [2]. For inorganic elements including heavy metals and metalloids, sophisticated plant root systems can regulate their transport into root cytoplasm. Two pathways are well known. First, actively regulating the shuttle of heavy metals through various ion and protein channel embedded in the cell membrane. Second, passively transporting via passive diffusion which occurs through the cell membranes [3]. Passive transport of ENPs into plants could occur through cells via Van der Waals forces, steric interactions and electrostatic charges without stimulus on vesicles formation [4]. Taking advantage of advanced analytical techniques, a previous study using confocal microscopic images of fluorescein isothiocyanate-stained (FITC)-labeled CeO_2NPs showed the presence of CeO_2NPs in the intercellular spaces, suggesting a passive apoplastic pathway of CeO_2NPs uptake [5]. Active transport, including signaling from calcium channels and the regulation of plasma membranes are also likely important mechanisms for ENPs uptake [6]. The possibility of active

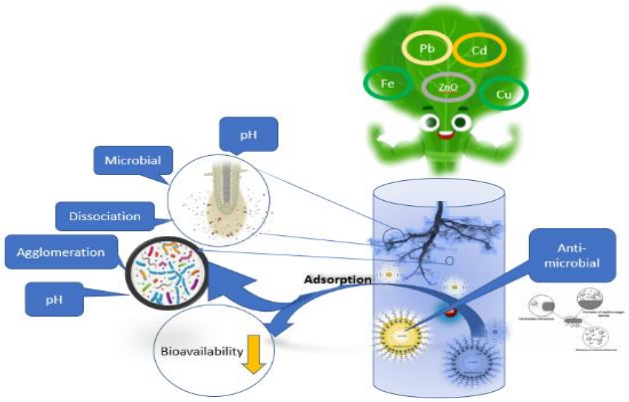

Fig. 8.1. potential interaction of between plant and nanoparticles and heavy metals in a hydroponic system.

transport of ENPs through different ion and protein channels into plant roots was also proposed by other researchers [7]. However, no conclusive evidence has been reported up to now to substantiate the assumption that ENPs are actively transported into plant roots through proteinous channels.

ENPs surface charge also plays a distinctive role in their fate and transport. For example, after wheat seedlings were treated with CeO_2NPs possessing different surface charges for 34 h, positively charged CeO_2NPs were mostly retained on the root surface while the neutral and negatively charged CeO_2NPs showed much higher accumulation in wheat shoots[8]. Similarly, positively charged gold nanoparticles (AuNPs) were readily retained by plant roots while negatively charged AuNPs were more efficiently translocated from roots to shoots [9]. Different surface charge of ENPs also leads to different subcellular localization in plant roots [10]. Overall, the underlying mechanisms for plant uptake of ENPs are still poorly understood.

Therefore, the interactions between ENPs and co-existing heavy metals and metalloids can be different from plants interactions with organic compounds. In addition, ENPs have been observed to alter the gene expressions, interrupt the bilayer membrane of the cell, adsorb on the cell wall,

and interact with root exudate[11]. As a result, exposure to ENPs expected to alter plant uptake and accumulation of co-existing heavy metal and metalloid ions. ENPs can be taken up by crops roots and translocated into edible parts of plants including leaf and fruits[12]. However, uptake and accumulation of ENPs in co-existence with heavy metals as well as the physiological effects of this co-exposure on plants are affected by the interference of various abiotic stresses [13]

Potential effects of ENPs interactions with leafy vegetables that are exposed to heavy metals may also alter the bioavailability of essential minerals such as Fe, and the microbial community, which have not been explored as a critical factor in plant ENPs interactions. Microbial communities in hydroponics can be highly beneficial for plant nitrogen fixation, phosphate mobilization, and mediating nutrient uptake [14]. Fig.1 illustrates some of the possible interaction of heavy metals and ENPs in a hydroponic system.

Therefore, there is a need to elucidate the impacts of ENPs on plants, their uptake pathways, and underlying mechanisms in plant tissues. In this regard, most ongoing research is centered on the potentially detrimental effects of ENPs [15]; while, the effects of ENPs on dietary plant uptake in exposure to a group of highly toxic contaminants, heavy metals, and metalloids, have not been adequately addressed.

8.2. Research Perspective

Understanding the interfacial chemistry of nanostructures in a biosystem which extends their application in environmental remediation efforts and other preventive protocols defines my research perspective. Elucidating the underlying mechanism of the altered uptake pattern by ENPs is a novel approach and a very critical research for the leafy greens (vegetables) compared to legumes and grains, because of their direct consumption by humans and, hydrophobic cuticle surface, higher leaf surface area and large hydroponic production systems, which highly expose them to risk of contamination by atmospheric

industrial fallout. My research plan is to start my initial research on this area that is very cost-effective research, and I have strong expertise, knowledge, and collaborative contacts to develop it through the funding and collaboration opportunities.

Due to the multi-disciplinary nature of my research, research funding through different financial sources is highly expected. The governmental organizations; USDA and EPA funding opportunities are directly related to my research benefit. In addition, NSF (BIO, ENG), NIH (NIBIB, NIHS), and DOE are major source of research funding in the area of my research. Private chemical companies such as BASF that provide research support are a great supplemental source to apply for a grant.

8.3. References

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