Effect of Cobalt Ferrite ($CoFe_2O_4$) Nanoparticles on the Growth and Development of *Lycopersicon Lycopersicum* (Tomato Plants)

Martha L. López-Moreno[1*], Leany Lugo Avilés[1], Nitza Guzmán Pérez[2], Bianca Álamo Irizarry[1], Oscar Perales[3], Yarilyn Cedeno-Mattei[4,5], Félix Román[1]

[1]Department of Chemistry, [2]Chemical Engineering, [3]Department of Engineering, University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico 00680, USA. [4]Department of Biology, Chemistry, and Environmental Sciences, Interamerican University of Puerto Rico at San Germán, San Germán, PR 00683. [5]Department of Chemistry and Physics, University of Puerto Rico at Ponce, Ponce, PR 00716

*Corresponding autor: martha.lopez@upr.edu Department of Chemistry, University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico 00680, USA. Phone (787) 832-4040 ext 2171

Abstract

Nanoparticles (NPs) have been synthetized and studied to be incorporated in many industrial and medical applications in recent decades. Due to their different physical and chemical properties compared with bulk materials, researchers are focused to understand their interactions with the surroundings. Living organisms such as plants are exposed to these materials and they are able to tolerate different concentrations and types of NPs. Cobalt ferrite ($CoFe_2O_4$) NPs are being studied for their application in medical sciences because of their high coercivity, anisotropy, and large magnetostriction. These properties are desirable in magnetic resonance imaging, drug delivery, and cell labelling. This study is aimed to explore the tolerance of *Solanum lycopersicum* L. (tomato) plants to $CoFe_2O_4$ NPs. Tomato plants were grown in hydroponic media amended with $CoFe_2O_4$ nanoparticles in a range from 0 to 1000 mg L^{-1}. Exposure to $CoFe_2O_4$ NPs did not affect germination and growth of plants. Uptake of Fe and Co inside plant tissues increased as $CoFe_2O_4$ nanoparticle concentration was increased in the media. Mg uptake in plant leaves reached its maximum level of 4.9 mg g^{-1} DW (dry weight) at 125 mg L^{-1} of $CoFe_2O_4$ NPs exposure and decreased at high $CoFe_2O_4$ NPs concentrations. Similar pattern was observed for Ca uptake in leaves where the maximum concentration found was 10 mg g^{-1} DW at 125 mg L^{-1} of

$CoFe_2O_4$ NPs exposure. Mn uptake in plant leaves was higher at 62.5 mg L^{-1} of $CoFe_2O_4$ NPs compared with 125 and 250 mg L^{-1} treatments. Catalase activity in tomato roots and leaves decreased in plants exposed to $CoFe_2O_4$ NPs. Tomato plants were able to tolerate $CoFe_2O_4$ NPs concentrations up to 1000 mg L^{-1} without visible toxicity symptoms. Macronutrient uptake in plants was affected when plants were exposed to 250, 500 and 1000 mg L^{-1} of $CoFe_2O_4$ NPs.

Keywords: nanoparticles; cobalt ferrite; *Solanum lycopersicum* L; toxicity; oxidative stress

1. Introduction

Nanoparticles (NPs) are ultrafine particles whose dimensions are in the range between 1 and 100 nm. Due to their physical and chemical properties, nanomaterials are being studied for their use in several different fields of science and engineering (Ghormade et al., 2011; Chen and Wang, 2011). NPs are also being integrated to industrial and manufacturing processes in textile coating, as additives in paints, varnishes, as catalysts in diesel fuels or semiconductors in electronics (Garcia et al., 2011). In medicine, NPs are investigated for their application in cancer therapy, drug delivery, or diagnostic and imaging (Baldi 2007, Amiri et al., 2013, Romih et al., 2015). In the environmental field, Fe NPs have been studied for the removal of contaminants from waters and soils; however there is still concern related to the fate and transport of these NPs in soils (Rajan, 2011). Additionally, NPs are gaining industry attention for their potential applications in agricultural and food related sectors, or as prospective materials in food preservation and packaging (Duncan, 2011, Begum et al., 2011). Magnetite NPs are being investigated for the treatment of polluted waters with heavy metals or metalloids (Tang and Lo, 2013). Regarding other magnetite oxides, cobalt ferrite ($CoFe_2O_4$) NPs exhibit magnetic properties suitable for their use as nanomagnets, microwave and high-density information storage devices. Moreover,

these NPs can be incorporated in human biological fluids in combined cancer therapies due to its chemical stability (Di Guglielmo et al., 2010). $CoFe_2O_4$ NPs have been recently studied as heat source in cancer treatment for drug delivery (magnetic hyperthermia). In addition, these NPs offer an excellent contrast in MRI cancer diagnostics (Amiri et al., 2013). Kooti et al. (2015) reported the antibacterial activity of $CoFe_2O_4/SiO_2$/Ag NPs composite (in combination with streptomycin) on gram-positive and gram-negative bacteria. Due to the envisioned extensive use of $CoFe_2O_4$ NPs in medicine and electronics, uncontrolled release of these NPs into the environment would become inevitable (Chattopadhyay et al., 2015).

NPs do not behave same as bulk when they enter to the environment. External factors such as light, temperature, pH, etc. may change their fate and transport (Arruda et al., 2015, Nowack and Bucheli, 2007). Knowledge about interaction of nanomaterials with the impacted environment is critical to establish precedents and insights about the possible toxic effects of NPs to the different ecosystems and living organisms. At present, there is a lack of regulations for the synthesis, manipulation, and disposal of engineered nanomaterials (ENMs) and as a consequence, nanoparticles present in the environment are being accumulated (Ma et al., 2010). Some types of NPs are able to stimulate plant root growth while others tend to decrease root length (Faisal et al., 2013, Boonyanitipong et al, 2011). Several researchers have found evidence that some plants can uptake nanoparticles and accumulate them in their tissues (Rico et al., 2011; Prasad et al., 2012; Schwab et al., 2011; Lee et al., 2010). NP accumulation in plant tissues will depend on the NP size and composition, interacting plant species, and NP availability in the media (Ghormade et al., 2011; C. Rico et al., 2011). Lee et al. (2010) reported the effect of Al_2O_3, SiO_2, Fe_3O_4, and ZnO nanoparticles on the germination, growth, and development of *Arabidopsis thaliana* in hydroponic systems. The authors found that Al_2O_3 nanoparticles produced an in-

crease in root length while Fe_3O_4 and ZnO nanoparticles decreased plant root growth. Besides, plant species may respond different to the same types of NPs (Haghighi et al., 2014); edible plants may be able to accumulate NPs in their tissues and transport them to the aerial parts or fruits (Rico et al., 2011).

Reactive Oxygen Species (ROS) are free radicals containing oxygen atoms. These species are normally produced as result of several metabolic pathways inside plant structures and membranes (Rao and Shekhawat 2014, Sharma et al., 2012). Increase of ROS inside cellular membranes is due to environmental factors that induce stress to the plant. Heavy metals increase ROS inside cellular membranes provoking several damages and disrupting the normal cellular activity in plants. Variation in catalase activity can be attributed to the metal concentration and plant tolerance to the metal (Nair and Chung 2015, Pandey et al., 2009). Based on the above considerations, oxidative stress has been studied in roots and leaves of tomato plants in presence of $CoFe_2O_4$ NPs; particularly, catalase (CAT) enzyme was evaluated. Heavy metals also induced inhibition of the photosynthesis process lowering the chlorophyll and carotenoid content (Pandey et al, 2009; John et al, 2009).

Tomato is one of the major crops consumed by humans around the world; for this reason, this plant was selected to evaluate the effect of $CoFe_2O_4$ NPs and their possible toxicity. To the author's knowledge, there is no report on the literature about the effect of $CoFe_2O_4$ NPs on tomato plants. Concentration range was chosen based on previous literature for other type of NPs such as CeO_2, TiO_2, and ZnO among others. Growth and development, metal and mineral concentrations, CAT activity, and chlorophyll content in tomato leaves were evaluated in this study. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) was used to quantify Co, Fe, macro, and micronutrient concentrations inside different plant tissues.

2. Materials and Methods

2.1. Nanoparticle synthesis and characterization

The precursor salts employed in the synthesis of cobalt ferrite nanocrystals were $CoCl_2 \cdot 6H_2O$ (ACS, 98–102%, Alfa Aesar) and $FeCl_3 \cdot 6H_2O$ (ACS, 97–102%, Alfa Aesar). A NaOH (pellets, 98%, Alfa Aesar) solution acted as precipitant agent. All reagents were used without further purification.

Synthesis of cobalt ferrite nanocrystals was carried out using a modified co-precipitation method (Cedeño-Mattei et al., 2008). Stoichiometric amounts of Co (II) and Fe (III) chlorides were contacted with an excess of NaOH solution which is mechanically stirred at 500 rpm, under intensive heating conditions. The excess of NaOH provides a net negative surface charge with the aim of minimize agglomeration. The conventional co-precipitation approach was modified by controlling the flow-rate at which the solution containing the cations is added to the precipitant agent (Cedeño-Mattei et al., 2009; Cedeño-Mattei et al., 2008). The purpose of this control was to evaluate the possibility of promoting heterogeneous nucleation and hence, crystal growth. The hydrolysis reaction produced a mixed paramagnetic Fe-Co hydroxide, which undergoes de-hydration and atomic re-arrangement conducive to stoichiometric ferrite.

2.2. Nanocrystals characterization

The structural and magnetic characterization of cobalt ferrite nanocrystals was carried out using X-Ray Diffraction (XRD) and Vibration Sample Magnetometry (VSM), respectively. A Siemens D500 diffractometer, using Cu K_α radiation was used to confirm the ferrite formation and the average crystallite size was calculated using the Scherrer's equation. A Lakeshore 400

series vibrating sample magnetometer was used to measure the magnetic properties of cobalt ferrite nanocrystals at room temperature.

2.3. Preparation of $CoFe_2O_4$ NP suspensions

Suspensions of $CoFe_2O_4$ NP were prepared at 0, 62.5, 125, 250, 500, and 1000 mg L^{-1} in deionized water and sonicated for 30 min in a bath sonicator with power of 125 Watts (Smith-Kline Co, Parrot Drive Shelton, CONN USA) to avoid NP aggregation. Suspensions were transferred to 250 mL beakers for the hydroponic experiments.

2.4. Plant germination

Tomato seeds (*Solanum lycopersicum* L.*)* were purchased from Eden Brothers (Asheville, NC, USA). Seeds were disinfected with 4% sodium perchlorate ($NaClO_4$) solution for 30 min and rinsed three times with sterilized deionized water. Thirty seeds were placed in each Petri Dish (three replicates per treatment) and covered by germination paper. 5 mL of each $CoFe_2O_4$ NP suspension were added to every Petri dish and a second germination paper covered the seeds following the Lin and Xing procedure (Lin and Xing, 2008). Petri dishes were covered with aluminum paper to protect seeds from light and were placed at room temperature (25ºC) to allow germination. Seeds were considered germinated in Petri dishes when 65% of the root controls were at least 5 mm long (USEPA, 1996).

2.5. Hydroponic experiments

Tomato seeds were placed in germination paper for 5 days in a dark place at room temperature (25ºC). Seedlings were transferred to 250 mL beakers containing 200 mL of modified

Hoagland nutrient solution according to Peralta et al (2002). After one week, plants were transferred to 250 mL beakers containing suspensions at 0, 62.5, 125, 250, 500, and 1000 mg L^{-1} of $CoFe_2O_4$ NPs. Deionized water was used in control plants. Plants were exposed to the treatments for 15 days. Plants were harvested, separated in roots, stems and leaves and saved for further experiments. Roots and stems measurements (in cm) were taken from 30 plants of each treatment.

2.6. Quantification of Co, Fe, macro and micronutrients in dry plant tissues

Plant tissues were dried in an oven at 70ºC for three days and digested on a microwave oven (CEM Corporation Mathews, NC; USA) following the USEPA 3051 method. Dry samples were weighed and placed into microwave vessels with 3 mL of plasma pure HNO_3. After digestion, samples were diluted to 50 mL with deionized water and Co, Fe, macro and micronutrient concentrations were determined by ICP-MS (Agilent 7500 ICP-MS, Yokogawa Analytical Systems Inc. Tokyo Japan). For quality control/quality assurance (QC/QA), ICP-MS readings of a blank and a standard were taken every 10 samples.

2.7. Determination of CAT activity

CAT essay was performed in leaves of tomato plants exposed $CoFe_2O_4$ nanoparticles according to the method described by Gallego et al. (1996) with minor variations. A ratio of 10% w/v of leaf samples was homogenized with phosphate buffer. (0.100 mg of leaves mixed with 900 µL of 25 mM KH_2PO_4 at pH 7.4). The mixture was centrifuged for 10 minutes at 10,000 rpm. The supernatant was transferred to a micro tube for the assay. A sample of 1800 µL of hy-

drogen peroxide (H_2O_2, 10 mM) was placed in a quartz cuvette and 200 µL of the sample was added to obtain a final volume of 2 mL. This was mixed by hand shaking three times and the absorbance was recorded at 240 nm in an UV/Vis Spectrometer (Perkin Elmer Lambda single-beam mode, Perkin-Elmer, Uberlinger, Germany). A serum bovine albumin (BSA) was used as standard to determine the protein content in the plant leaves.

2.8. Determination of chlorophyll content

Chlorophyll content was measured in 30 tomato leaves from each treatment by a SPAD 502 chlorophyll meter (Minolta Ltd., Osaka, Japan).

2.9. Statistical analysis

A completely randomized design with triplicate treatments was set in all experiments. Statistical analysis of data was performed using the Minitab software (Minitab Inc, Pine Hall Road, State College PA, USA). Means were compared using Tukey's HSD test assuming a two-tailed distribution and equal variance. Data were reported as mean (n=3) ± standard error (SE). Different letters mean significant ($p < 0.05$) differences between treatments.

3. Results and Discussion

3.1. XRD analyses

Figure 1A) shows the XRD pattern of cobalt ferrite nanocrystals synthesized after 2 hours of reaction and using a flow-rate of 0.85 mL min^{-1} of Fe solution to contact a 0.315 M NaOH. The diffraction peaks corresponding to the ferrite structure were clearly identified. The average crystallite size, determined using the Scherrer's equation, was estimated at 17 nm ± 1 nm and the

lattice parameter, a, 8.3836 Å. The lattice parameter is in good concordance with the crystallographic data reported in the literature for the cobalt ferrite structure (8.3919 Å) (Goldman, 1990).

3.2. Room temperature Magnetic Hysteresis (M-H) measurements

Figure 1B) shows the M-H loop of 17 nm cobalt ferrite nanocrystals synthesized by the modified coprecipitation method. The powdered sample exhibited high coercivity and moderate maximum magnetization when an external magnetic field of 1.5 x 10^6 A m^{-1} was applied. The control in flow-rate during the addition of reactants to boiling NaOH solution should have promoted heterogeneous nucleation conditions leading to an increase in average crystallite and, hence, in the corresponding coercivity values within the single domain region (Cedeño-Mattei et al., 2008). A coercivity value as high as 3.2 x 10^5 A m^{-1} and a maximum magnetization of 57 A m^2kg^{-1} were attained using the above described synthesis conditions.

3.3. Germination and growth of tomato exposed to $CoFe_2O_4$ NPs

Different types of NPs have a different impact on seed germination in plant species. From the author's knowledge, there is no information reported in the literature about the effect of $CoFe_2O_4$ NPs on tomato seed germination. Begum et al. (2011) reported that some graphene NPs concentration delayed germination of *Solanum Lycopersicon* seeds though this effect was not observed in *Lactuca sativa* seeds. López-Moreno et al. (2010) reported that germination was decreased by about 30% when tomato seeds were exposed to 2000 mg L^{-1} CeO_2 NPs. On the other hand, Siddiqui and Al-Whaibi (2014) revealed that nano-SiO_2 increased tomato germination at concentrations up to 8 g L^{-1}. Song et al. (2013) did not find significant differences in germination with exposure to TiO_2 and Ag NPs in a concentration range from 50 – 5000 mg kg^{-1}. In the

present study, there was not a significant difference in germination in tomato seedlings exposed to all $CoFe_2O_4$ NP concentrations. After germination (seedlings with cotyledon and roots developed), number of seedlings were counted and the germination percentage compared to controls was about 99 % in all treatments.

3.4. $CoFe_2O_4$ NPs influence in tomato root and stem growth

Root and stem length (cm) from tomato plants exposed to different $CoFe_2O_4$ NPs concentrations (mg L^{-1}) are shown in figure 2A. When the concentration of NPs available in the media increases, there is a slight increase in root growth. Treatment of 1000 mg L^{-1} of $CoFe_2O_4$ NPs resulted in a significant increase ($p < 0.05$) of tomato roots (6.9 ± 0.3 cm) compared to roots of control plants (5.3 ± 0.2 cm). There is no information reported in the literature about the effects of $CoFe_2O_4$ NPs on tomato plants growth or mineral uptake. However, Lee et al. (2010) found that nFe_3O_4 NPs did not have any effect on germination of *Arabidopsis thaliana* seeds in a concentration range of 400 - 4000 mg L^{-1}; nevertheless, they affect the root growth in all concentrations. The effect of Fe nanoparticles on plant root growth can be attributed to the partial release of Fe from the NPs, which is a micronutrient needed for plant development (Wang et al., 2011). Internalization of NPs on roots depends upon the apoplastic transport through the exodermis and layers from the inner cell walls which is related to the NP size and chemical properties (Dietz and Herth, 2011). There was no significant difference ($p < 0.05$) between stem lengths of tomato plants in all treatments (except for the 1000 mg L^{-1} $CoFe_2O_4$ NPs) with respect to stem from control plants (4.6 ± 0.1 cm).

3.5. Iron and Cobalt uptake by plant tissues

Fe and Co contents in tomato roots are shown in figure 2B. Highest concentration of Fe and Co was found in roots of plants exposed to 1000 mg L^{-1} of $CoFe_2O_4$ NPs (18.14 ± 0.4 mg g^{-1} and 9.82 ± 0.1 respectively). Fe and Co concentrations in stems were found in an average concentration of 0.30 mg g^{-1} (for both elements) and 0.20 mg g^{-1} in tomato leaves. Wang et al. (2011) reported the uptake of Fe_3O_4 NPs by *Lolium perenne L* (ryegrass) and *Cucurbita mixta* (pumpkin) plants in liquid media. The authors stated that due to the size of Fe_3O_4 NPs and aggregates present in suspensions, ryegrass and pumpkin plants uptake and transport these NPs through K^+ and Ca^{+2} channels across cell membranes rather than pores from water channels. Canivet et al. (2015) studied the uptake of elemental Fe NPs by *Physcomitrella patens* (moss) via foliar application. They observed that Fe NPs did not have a significant effect in lipid peroxidation of leaf membranes which is commonly associated with the production of reactive oxygen species (ROS) after exposure to abiotic stress. Due to NP agglomeration and low concentrations used by Canvey et al. (0.005 – 50 µg/plant), cytotoxic effects were not found after 7 days. We suggest that Co found in plant tissues would come from stable nanoparticles instead of Co^{+2} ions because there is no dissolution of $CoFe_2O_4$ NPs in deionized water after 15 days. $CoFe_2O_4$ NPs suspensions were analyzed by voltammetry (BASi EPSILON Analyzer, Bioanalytical Systems Inc, USA) on time 0, 1, and 2 weeks after their preparation. Fe and Co ions were not found in solution at all times. $CoFe_2O_4$ dissolution release to Co^{+2} ions is expected to take place under acidic conditions (Romih et al., 2015), which was not the case in our experiments (pH = 6.5 – 7.9 ± 0.1).

3.6. Mg, K, and Ca content in plant tissues

Figure 3 shows the uptake of A) Mg, B) K, and C) Ca by tomato plants exposed to $CoFe_2O_4$ NPs. Mg was translocated to the leaves in higher amounts when plants were exposed to 62.5 and 125 mg L^{-1} of $CoFe_2O_4$ NPs (4.78 ± 0.03 mg g^{-1} and 4.92 ± 0.05 mg g^{-1} respectively) as shown in figure 3A. The same behavior was found in Ca uptake where translocation was higher in plants exposed to same treatments (9.50 ± 0.7 mg g^{-1} and 10.05 ± 0.7 mg g^{-1} (figure 3C). However at higher $CoFe_2O_4$ NPs concentrations (250, 500 and 1000 mg L^{-1}), translocation of Mg and Ca ions to the leaves and roots was lower compared to control plants. Translocation factor (concentration of nutrient in shoots/concentration of nutrient in roots) was higher that controls in all treatments except for 1000 mg L^{-1} of $CoFe_2O_4$ NPs. Peralta-Videa et al. (2014) reported than CeO_2 and ZnO NPs affect K and Mg accumulation in soybean plant tissues while CeO_2 NPs reduced Ca uptake in plants exposed to 1000 mg L^{-1} of CeO_2 NPs. Figure 3B displays K accumulation in tomato tissues. There was a significant reduction in K uptake in tomato roots in all treatments compared to control roots. However, K translocation in stems and leaves was not affected by $CoFe_2O_4$ NPs. Some NPs may damage the endodermis cell layer in plant roots. Lee et al. (2008) reported that Cu NPs caused the movement of K and other solutes to cell outside due to the damage caused to epidermal cells by Cu NPs. Transport of K^+ ions through root cells is also mediated by concentration gradient and H^+-ATPase activity. Accumulation of $CoFe_2O_4$ NPs on roots cell membrane may block free K^+ ion transport.

3.7. Zn and Mn content in plant tissues

Figure 4A shows Mn uptake by tomato plants exposed to different NPs concentration. Mn was translocated to leaves in higher concentrations in all treatments compared to Mn concen-

tration in control plants. Peralta-Videa et al. (2014) and Corral-Diaz and co-workers (2014), have reported Mn uptake in soybean and radish plants under the influence of CeO_2 NPs. They found that 500 mg L^{-1} of CeO_2 NPs increased Mn translocation to the leaves in both plant species. In this study, translocation of Mn at 1000 mg L^{-1} of $CoFe_2O_4$ NPs in tomato was twice the translocation in control plants.

Zn uptake by roots, stems, and leaves of tomato plants exposed to $CoFe_2O_4$ NPs is represented in figure 4B. Roots exposed to NPs accumulated less Zn compared to control plants. Addition of 62.5 mg L^{-1} $CoFe_2O_4$ NPs significantly ($p < 0.05$) decreased Zn uptake by roots compared with roots from controls and other treatments. Zn uptake in stems was higher when 1000 mg L^{-1} of $CoFe_2O_4$ NPs was added to the media. Zn movement from roots to leaves was higher only in plants exposed to 62.5 and 1000 mg L^{-1} $CoFe_2O_4$ NPs compared with control plants. Trujillo-Reyes et al. (2014) reported that the addition of 10 or 20 mg L^{-1} of Fe_3O_4 NPs decreased Zn uptake in *Lactuca sativa* roots. Previous studies have reported less translocation of Zn from roots to shoots in *Prosopis juliflora* plants not only in natural growing plants but also in plants exposed to other type of NPs such as ZnO (Hernandez-Viezcas et al., 2011). Pinto and Ferreira (2015) stated that Zn in Arabidopsis, barley, and rice is mobilized from roots by several transporters such as P-typeATPase, AtPCR2, AtFRD3, HvYSL2, etc. which move Zn to vascular tissues, xylem, or pericycle cells. YS and YSL are also considered transporters related to Zn movement to aerial parts (shoots and leaves). Presence of NPs may alter transporters role inside plant tissues decreasing Zn translocation.

3.8. CAT activity

Changes in enzymatic activity depend on NP concentration and exposure time. Several studies reported an increase in CAT or ascorbate peroxidase activity (APOX) when plants are exposed to different nanoparticles. Figure 5 displays the catalase enzymatic activity in tomato roots, (A), and leaves, (B), exposed to 62.5, 250, and 1000 mg L^{-1} $CoFe_2O_4$ NPs (low, medium and high concentrations). In both cases there was a decrease in CAT activity as the NPs concentration increased in the media. This trend can be attributed to the accumulation of NPs in plant tissues which should have induced an oxidative stress. According to Canivet et al., (2015), there is evidence that elemental Fe-NPs can induce oxidative stress due to the internalization of NPs to the plant cells. Mukherjee et al., (2014) stated that concentrations of 250 and 500 mg L^{-1} of Fe-ZnO NPs caused an increase in CAT activity in green pea plants grown in soil. Variations in CAT activity inside plant tissues have been correlated to the abiotic stress produced by different types and concentrations of NPs. Faisal et al., (2013) demonstrated by flow cytometry an increase of ROS levels inside tomato protoplasts when these plants were exposed to NiO NPs. Fe_3O_4 NPs have been shown to have an effect on the stability of the cell membrane from ryegrass and pumpkin plants due to the increase of ROS species (Wang et al., 2011). Nair and Chung (2015) reported that CuO NPs increases superoxide dismutase activity (SOD) and decreases APOX activity in *Brassica juncea L.*(Indian mustard) roots. SOD indicates the conversion of superoxide to H_2O_2 while APOX catalyzes H_2O_2 to H_2O. There are many other plant defense systems against oxidative stress such as phytochelatins, metallothioneines, pectins, etc which are in charge of cell restoration and ROS scavenging. Evidently, further studies need to be done in order to clarify the internalization of $CoFe_2O_4$ NPs and their effect on cell oxidative stress.

3.9. Chlorophyll content

Chorophyll content in tomato leaves did not show any significant difference ($p \leq 0.05$) in all treatments (data not shown). These results suggest that there was not a physical symptom of phytotoxicity of $CoFe_2O_4$ NPs in a range of 62.5 to 1000 mg L^{-1}. Nair and Chung (2015) reported the effect of CuO NPs on Indian mustard (*Brassica juncea L.*). A significant decrease in chlorophyll content was observed in plants exposed to CuO NPs. These researchers suggest that high concentration of CuO NPs may induce structural changes in chloroplast membranes by the action of ROS species. Rao and Shekhawat (2014) also reported a decrease in chlorophyll content in *Brassica juncea* exposed to ZnO NPs may be due to the decrease of thylakoid and grana in chloroplasts, however, Trujillo et al. (2014) observed that 10 mg L^{-1} of nano-Fe/Fe_3O_4 did not affect chlorophyll content in *Lactuca sativa* plants. This may be attributed to the low concentration of nano-Fe/Fe_3O_4 used in their study.

4. Conclusions

There was no significant difference in germination in tomato seedlings exposed to all $CoFe_2O_4$ NP concentrations. This may suggest that $CoFe_2O_4$ NPs did not pass through seed coat or root system at early germination stage. Treatment of 1000 mg L^{-1} of $CoFe_2O_4$ NPs resulted in a significant increase ($p < 0.05$) of tomato roots compared to roots of control plants (average of 30 %). $CoFe_2O_4$ NPs may stimulate root growth at 1000 mg L^{-1} in hydroponic media. Uptake and translocation of macro nutrients was different for each element and $CoFe_2O_4$ NPs concentration in the media. High $CoFe_2O_4$ NPs concentrations may interfere with the transport of nutrients through protoplasts and cell membranes. Transport channels on the lipid membrane of cells also contribute to the ion movement across plant tissues. In tomato plants, Mg was translocated to the

leaves in higher amounts than Mg in control plants when they were grown in 62.5 and 125 mg L^{-1} of $CoFe_2O_4$ NPs. However, there was a significant reduction in K uptake in tomato roots in all treatments compared to control roots. Micronutrient uptake can also be affected by addition of $CoFe_2O_4$ NPs. NP concentration of 62.5 mg L^{-1} decreased Zn uptake by roots compared with control roots. However translocation of Mn to leaves was higher in plants exposed to $CoFe_2O_4$ NPs. Oxidative stress is related to production of ROS inside plant tissues when plants are exposed to heavy metals. Inhibition of CAT enzymatic activity by $CoFe_2O_4$ NPs could be related to the increase in ROS inside plant tissues. Further experiments need to be done to determine whether $CoFe_2O_4$ NPs can be transported to fruits.

Acknowledgements

The authors acknowledge Center for Education and Training in Agricultural and Related Sciences (CETARS) Program through USDA (award number 2011-38422-30835).

References

Amiri, S., Shokrollahi, H. 2013. The role of cobalt ferrite magnetic nanoparticles in medical science. Mat.Sci.Eng.C-Biomim. c33, 1-8.

Arruda, S.C.C., Silva, A.L.D., Galazzi, R.M., Azevedo, R.A., Arruda, M.A.Z. 2015. Nanoparticles applied to plant science: A review. Talanta 131, 693-705.

Baldi, G., Bonacchi D., Innocenti, C., Lorenzi, G., Sangregorio, C. 2007. Cobalt ferrite nanoparticles: The control of the particle size and surface state and their effects on magnetic properties. J.Magn.Magn.Mater. 311, 10-16.

Begum, P., Ikhtiari, R., Fugetsu, B. 2011. Graphene phytotoxicity in the seedling stage of cabbage, tomato, red spinach, and lettuce. Carbon. 49, 3907-3919.

Boonyanitipong, P., Kositsup, B., Kumar, P., Baruah, S., Dutta, J. 2011. Toxicity of ZnO and TiO2 Nanoparticles on Germinating Rice Seed Oryza sativa L. Int.J.Biosci.Biochem.Bioinform. 1 (4), 282-285.

Canivet, L., Dubot, P., Garcon, G., Denayer, F.O. 2015. Effects of enigneered iron nanoparticles on bryophyte, Physcomitrella patens (Hedw.) Bruch & Schimp, after foliar exposure. Ecotox.Environ.Safe. 113, 499-505.

Cedeño-Mattei, Y., Perales-Pérez, O. 2009. Synthesis of high-coercivity cobalt ferrite nanocrystals. Microelectron. J. 40, 673-676.

Cedeño-Mattei, Y., Perales-Pérez, O., Tomar, M. S., Roman, F., Voyles, P. M., Stratton, W. G. 2008. Tuning of magnetic properties in cobalt ferrite nanocrystals. J. Appl. Phys. 103, 07E512.

Chattopadhyay, S., Dash, S.K., Tripathy, S., Das, B., Mandal, D., Pramanik, P., Roy, S. 2015. Toxicity of cobalt oxide nanoparticles to normal cells; an *in vitro* and *in vivo* study. Chem-Biol. Interact. 226, 58-71.

Chen, Y., Wang J. 2011. Preparation and characterization of magnetic chitosan nanoparticles and its application for Cu(II) removal. Chem. Eng. J. 168, 286-292.

Corral-Diaz, B., Peralta-Videa, J.R., Alvarez-Parrilla, E., Rodrigo-Garcia, J., Morales, M.I., Osuna-Avila, P., Niu, G., Hernandez-Viezcas, J.A., Gardea-Torresdey, J.L. 2014. Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). Plant Physiol. Bioch. 84, 277-285.

Di Guglielmo, C., Ramos López, D., De Lapuente, J., Llobet Mallafre, J.M., Borras Suarez, M. 2010. Embryo toxicity of cobalt ferrite and gold nanoparticles: A first in vitro approach. Reprod.Toxicol. 30, 271–276.

Dietz, K.J., Herth, S. 2011. Plant nanotoxicology. Trends.Plant.Sci. 16 (11), 1360-1385.

Duncan, T. 2011. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. J.Colloid.Interf .Sci. 363, 1-24.

Faisal, M., Saquib, Q., Alatar, A.A., Al-Khedhairy, A.A., Hegazy, A.K., Musarrat, J. 2013. Phytotoxic hazards of NiO-nanoparticles in tomato: A study on mechanism of cell death. J.Hazard.Mater. 250-251, 318-332.

Gallego, S.M., Benavides, M.P., Tomaro, M.L. 1996. Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. Plant Sci. 121, 151-159.

García, A., Espinosa R., Delgado L., Casals E., González E., Puntes, V., Barata, C., Font, X., Sánchez, A. 2011. Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. Desalination 269, 136-141.

Ghormade, V., Deshpande, M.V., Paknikar, K.M. 2011. Perspectives for nano-biotechnology enable protection and nutrition of plants. Biotechnol.Adv. 29, 792-803.

Goldman, A. *Modern Ferrite Technology*; Van Nostrand Reinhold: New York, 1990.

Haghighi, M., Abolghasemi, R., Teixeira da Silva, J.A. 2014. Low and high temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment. Sci. Hortic. 178, 231-240.

Hernandez-Viezcas, J.A., Castillo-Michel, H. Servin, A.D., Peralta-Videa, J.R., Gardea-Torrey, J.L. 2011. Spectroscopic verification of zinc absorption and distribution in the desert plant Pro-

sopis juliflora-velutina (velvet mesquite) treated with ZnO nanoparticles. Chem.Eng.J. 170, 346-352.

John, R., Ahmad, P., Gadgil, K., Sharma, S. 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by Brassica juncea L. Int.J.Plant.Prod. 3(3), 1735-8043.

Kooti, M., Gharieneh, S., Mehrkhah, M., Shaker, A., Motamedi, H. 2015. Preparation and anti-bacterial activity of CoFe2O4/SiO2/Ag composite impregnated with streptomycin. Chem.Eng.J. 259, 34-42.

Lee, C.W., Mahendra, S., Zodrow, K., Li, D., Tsai, Y.C. 2010. Developmental Phytotoxicity of metal Oxide nanoparticles to Arabidopsis Thaliana. Environ.Toxicol.Chem. 29 (3), 669-675.

Lee, W.M., An, Y.J., Yoon, H., Kweon, H.S. 2008. Toxicity and bioavailability of copper nano-particles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestitvum): plant agar test for water-insoluble nanoparticles. Environ.toxicol.Chem. 27(9), 1915-1921.

Lin, D., Xing, B. 2008. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ.Pollut. 150(2), 243-250.

López-Moreno, M.L., De la Rosa, G., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2010. X-ray Absorption Spectroscopy (XAS) Corroboration of the Uptake and Storage of CeO_2 Nanoparticles and Assessment of Their Differential Toxicity in Four Edible Plant Species. J. Agr. Food. Chem. 58, 3689-3693.

Ma, Y., Kunag, L., He, X., Bai, W., Ding, Y., Zhang, Z., Zhao, Y., Chai, Z. 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere. 78, 273-279.

Mukherjee, A., Pokhrel, S., Bandyopadhyay, S., Madler, L., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2014. A soil mediated phyto-toxicological study of iron doped zinc oxide nano-particles (Fe@ZnO) in green peas (Pisum sativum L.). Chem.Eng.J. 258, 394-401.

Nair, P.M.G., Chung, M. 2015. Study on the correlation between copper oxide nanoparticles in-duced growth suppression and enhanced lignification in Indian mustard (Brassica juncea L.). Ecotox.Environ.Safe. 113, 302-313.

Nowack, B., Bucheli, T.D. 2007. Occurrence, behavior and effects of nanoparticles in the envi-ronment. Environ.Pollut. 150, 5-22.

Pandey, N., Pathak, G.C., Pandey, D.K., pandey, R. 2009. Heavy metals, Co, Ni, Cu, Zn, and Cd produce oxidative damage and provoke differential antioxidant responses in spinach. Braz.J.Plant.Physiol. 21(2), 103-111.

Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiemann, K.J., Parsons, J.G., Carrillo, G. 2002. Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environ.Pollut. 119, 291-301.

Peralta-Videa, J.R., Hernandez-Viezcas, J.A., Zhao, L., Corral-Diaz, B., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L. 2014. Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. Plant.Physiol.Bioch. 80, 128-135.

Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Raja Reddy, K., Sreeprasad, T.S., Sajanlal, P.R., Pradeep, T. 2012. Effect of Nanoscale Zinc Oxide Particles oon the Germination, Growth and Yield of Peanut. J. Plant Nutr. 35, 905-927.

Pinto, E., Ferreira, I.M.P.L.V.O. 2015. Cation transporters/channels in plants: Tools for nutrient biofortification. J.Plant.Physiol. 179, 64-82.

Rajan, C.S. 2011. Nanotechnology in Groundwater Remediation. Int.J.Environ.Sci. Dev. 2 (3), 16-21.

Rao, S., Shekhawat,. 2014. Toxicity of ZnO engineered nanoparticles and evaluation of their effect in growth, metabolism and tissue specific accumulation in *Brassica juncea*. J. Environm. Chem. Engineer. 2, 105-114.

Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2011. Interaction of Nanoparticles with Edible Plants and Their Possible Implications in the Food Chain. J.Agric.Food.Chem. 59, 3485-3498.

Romih, T., Drasler, B., Jemec, A., Drobne, D., Novak, S., Golobic, M., Makovec, D., Susic, R., Kogej, K. 2015. Bioavailability of cobalt and iron from citric-acid-adsorbed CoFe2O4 nanoparticles in the terrestrial isopod Porcellio scaber. Sci.Total.Environ. 508, 76-84.

Schwab, F., Bucheli, T.D., Lukhele, L.P., Magrez, A., Nowack, B., Sigg, L., Knauer, K. 2011. Are Carbon Nanotube Effects on Green Algae Caused by Shading and Agglomeration? Environ. Sci. Technol. 45, 6136-6144.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M. 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative DefenseMechanism in Plants under Stressful Conditions. J.Bot. 2012, 1-26.

Siddiqui, M.H., Al-Whaibi, M.H. 2014. Role of nano-SiO2 in germination of tomato (Lycopersicum esculentum seeds Mill.). 21, 13-17.

Song, U., Heeju J., Waldman, B., Roh, J., Kim, Y., Yi, J., Ju Lee, E., 2013. Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO2 and Ag on tomatoes (Lycopersicon esculentum). 93, 60-67.

Tang, S.C.N., Lo, I.M.C. 2013. Magnetic nanoparticles: Essential factors for sustainable environmental applications. Water.Res. 24, 2613-2632.

Trujillo-Reyes, J., Majumdar, S., Botez, C.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2014. Exposure studies of core-shell Fe/Fe3O4 and Cu/CuO NPs to lettuce (Lactuca sativa) plants: Are they a potential physiological and nutritional hazard? J.Hazard.Mater. 267, 255-263.

US Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, OPPTS 850.4200, Seed germination/root elongation toxicity test, EPA 712-712-C96-154, p.6.

Wang H., Kou, X., Pei, Z., Xiao, J.Q., Shan, X., Xing, B. 2011. Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. 2011. Nanotoxicol. 5(1), 30-42.

Figure Legends

Figure 1. A) X-Ray Diffraction pattern of 17-nm cobalt ferrite nanocrystals produced by the modified coprecipitation method, B) Room temperature M−H loop of 17-nm cobalt ferrite nanocrystals produced by the modified coprecipitation method.

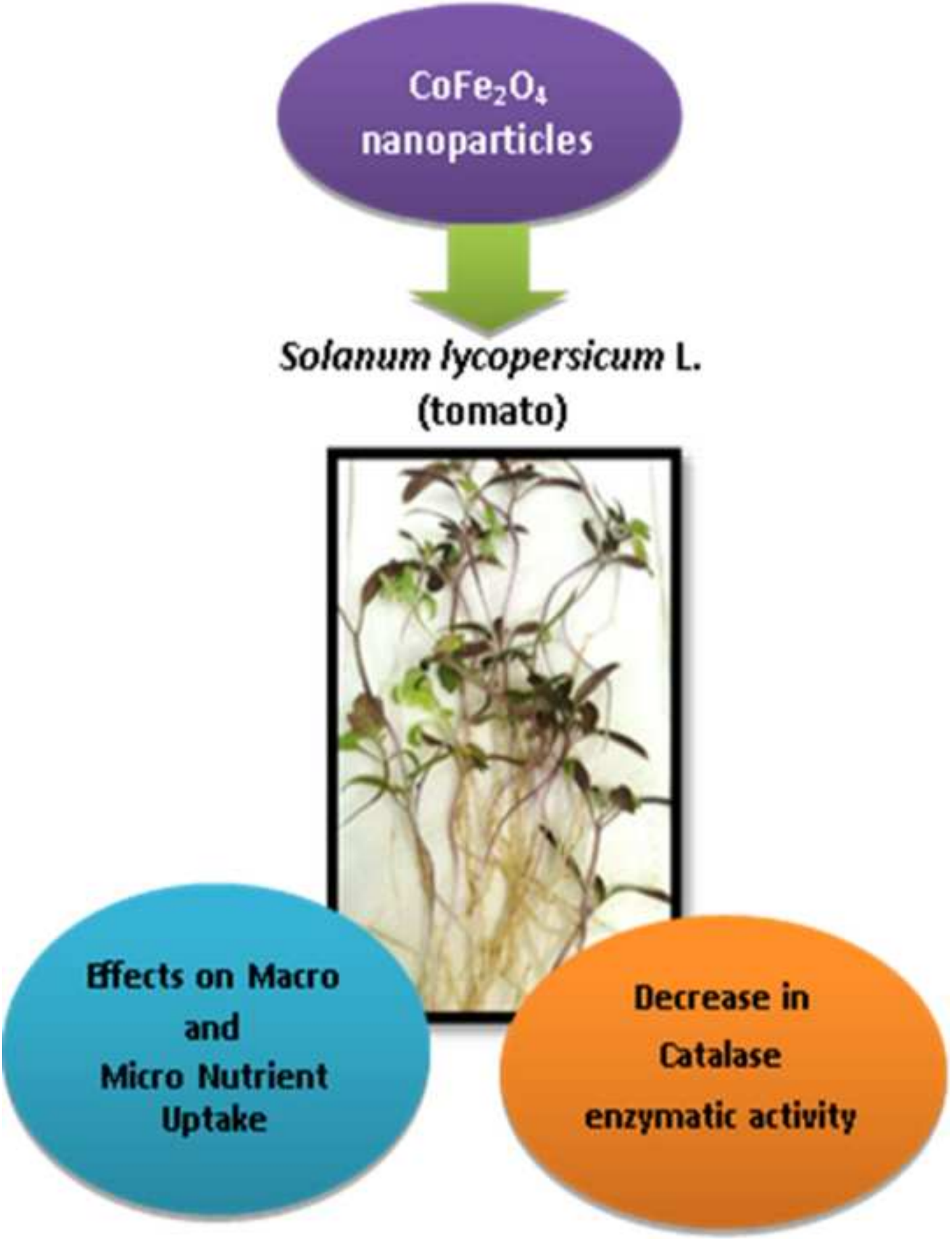
Figure 2. A) Average length of roots and stems (cm) of tomato plants grown for 15 days in hydroponics' media and different $CoFe_2O_4$ NPs concentrations. B) Total Fe and Co concentrations in roots of tomato plants exposed to different $CoFe_2O_4$ NPs concentrations. Error bars represent ± SE of 30 replicates for each treatment.

Figure 3. Uptake of A) Magnesium (Mg), B) Potassium (K), and C) Calcium(Ca) in roots, stems and leaves of tomato plants cultivated in hydroponics' media and exposed to $CoFe_2O_4$ NPs. Error bars represent ± SE of three replicates.

Figure 4. A) Uptake of Manganese (Mn) and B) Zinc (Zn) in roots, stems, and leaves of tomato plants cultivated in hydroponics' media and exposed to $CoFe_2O_4$ NPs. B) Error bars represent ± SE of three replicates.

Figure 5. Catalase activity in A) roots and B) leaves of tomato plants cultivated in hydroponics' media and exposed to $CoFe_2O_4$ NPs. Error bars represent ± SE of three replicates.

*Graphical Abstract



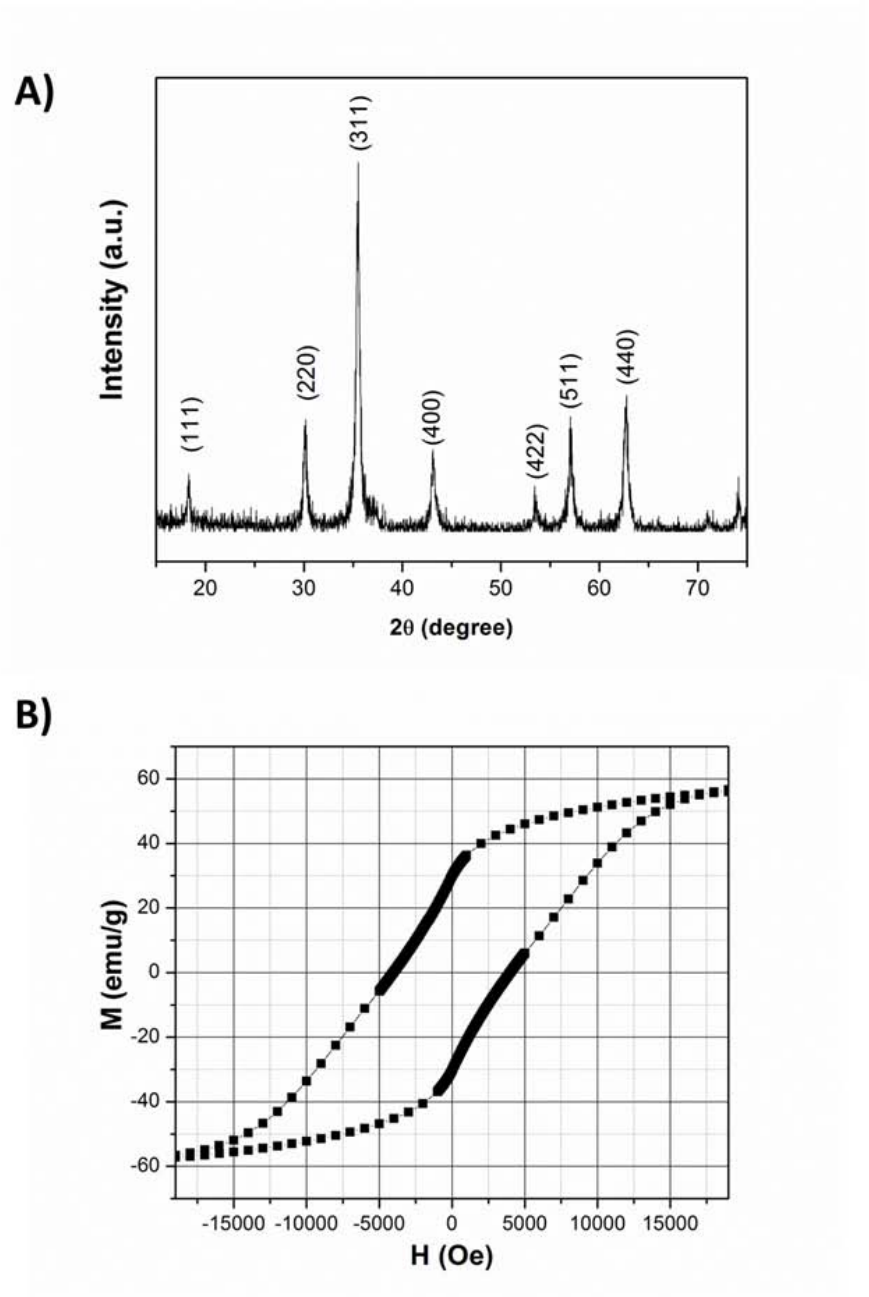

A)
Intensity (a.u.)
(111)
(220)
(311)
(400)
(422)
(511)
(440)
20
30
40
50
60
70
2θ (degree)
B)
M (emu/g)
60
40
20
0
-20
-40
-60
-15000
-10000
-5000
0
5000
10000
15000
H (Oe)

Figure 1

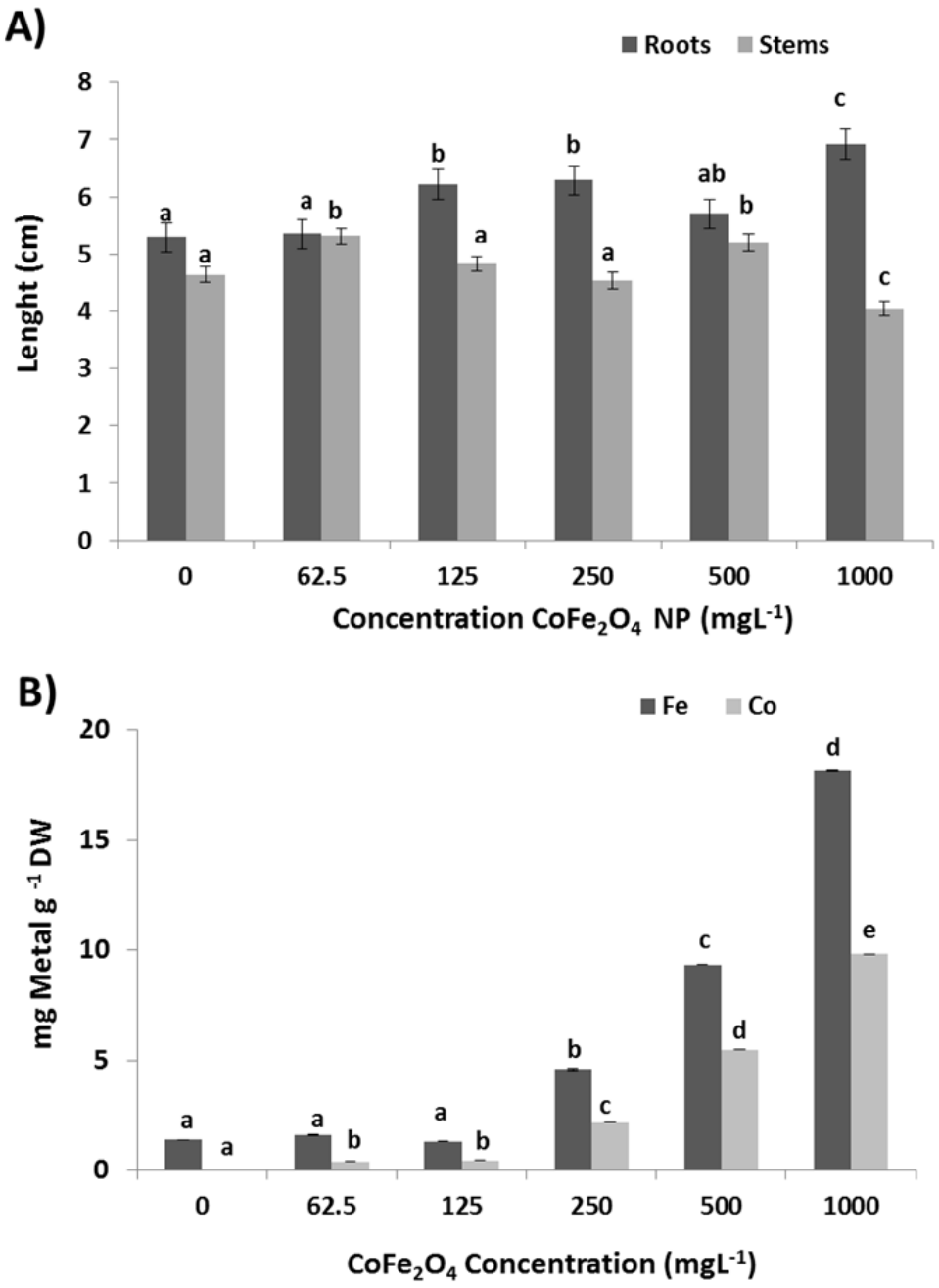

A)
Roots
Stems
Lenght (cm)
0
1
2
3
4
5
6
7
8
a
a
a
b
b
a
b
a
ab
b
c
c
0
62.5
125
250
500
1000
Concentration CoFe2O4 NP (mgL-1)
B)
Fe
Co
mg Metal g -1 DW
0
5
10
15
20
a
a
a
b
a
b
b
c
c
d
d
e
0
62.5
125
250
500
1000
CoFe2O4 Concentration (mgL-1)

Figure 2

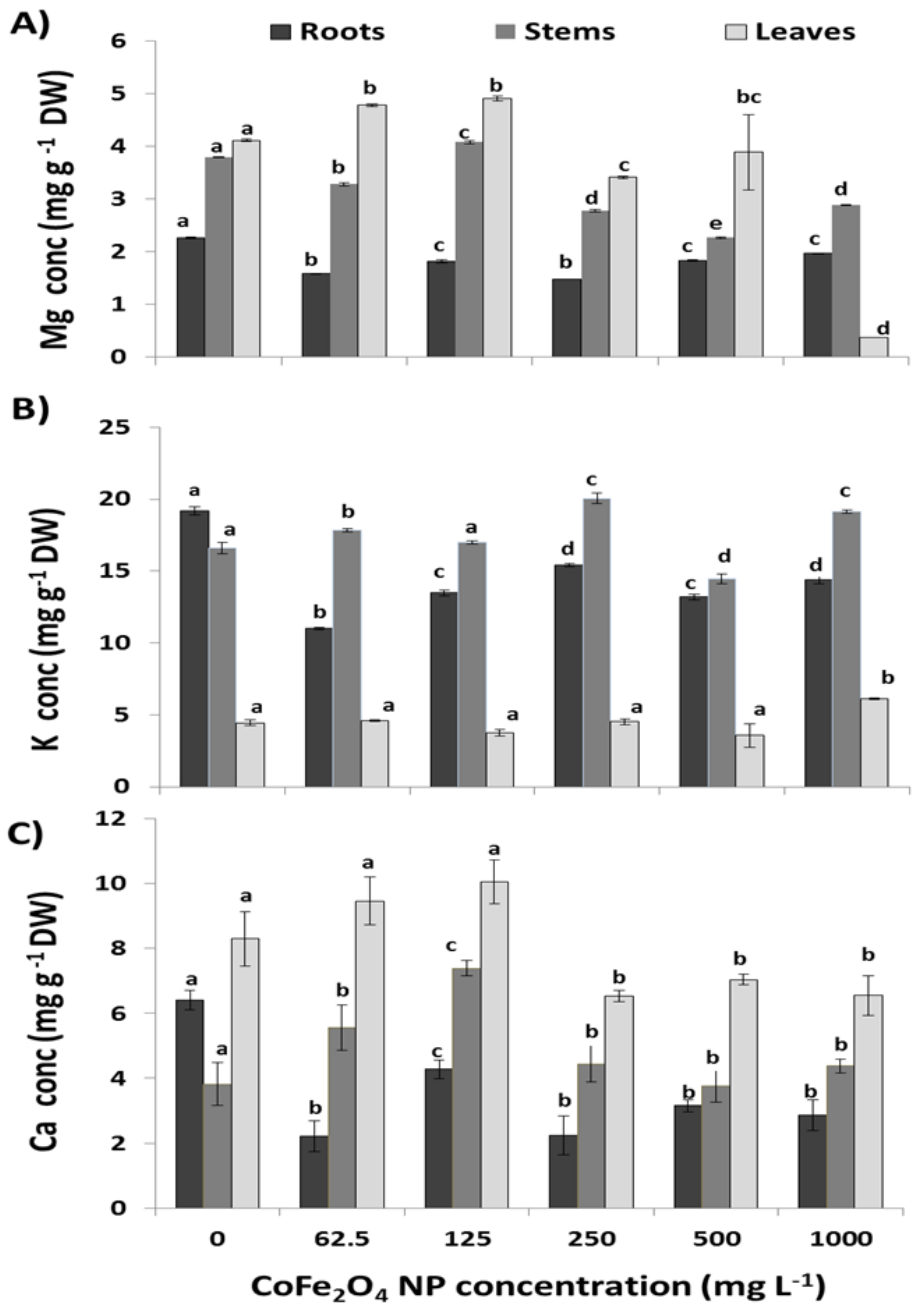

A)
Roots
Stems
Leaves
Mg conc (mg g-1 DW)
B)
K conc (mg g-1 DW)
C)
Ca conc (mg g-1 DW)
0
62.5
125
250
500
1000
CoFe2O4 NP concentration (mg L-1)

Figure 3

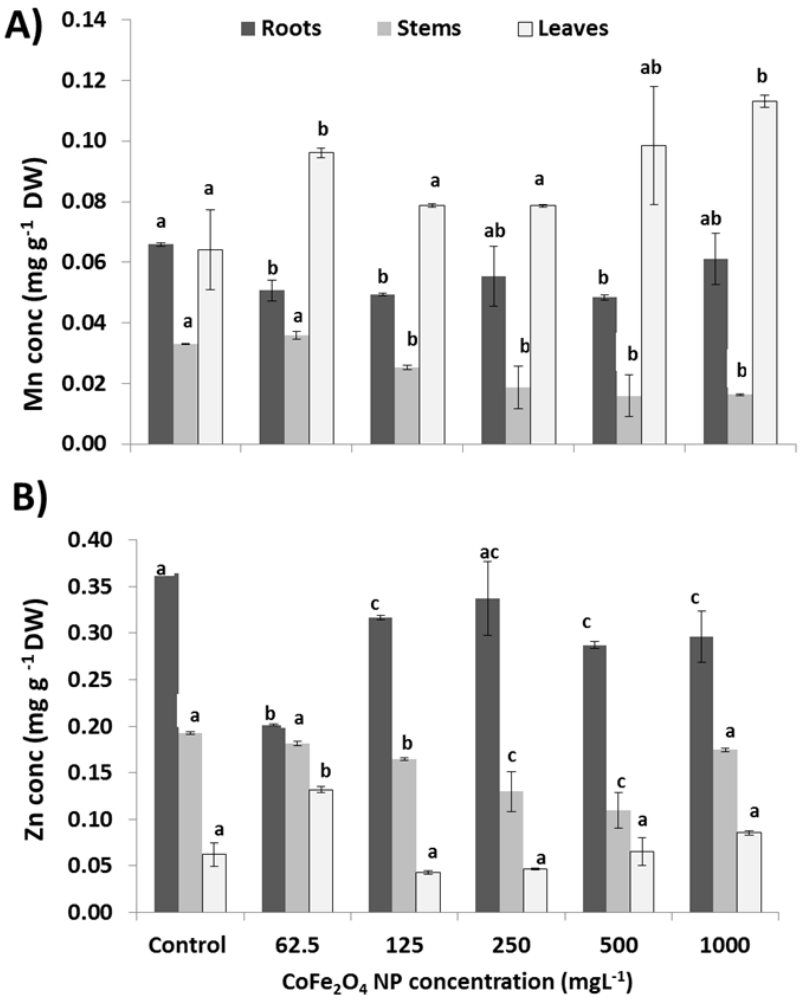

A)
Roots
Stems
Leaves
Mn conc (mg g-1 DW)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
B)
Zn conc (mg g-1 DW)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Control
62.5
125
250
500
1000
CoFe2O4 NP concentration (mgL-1)

Figure 4

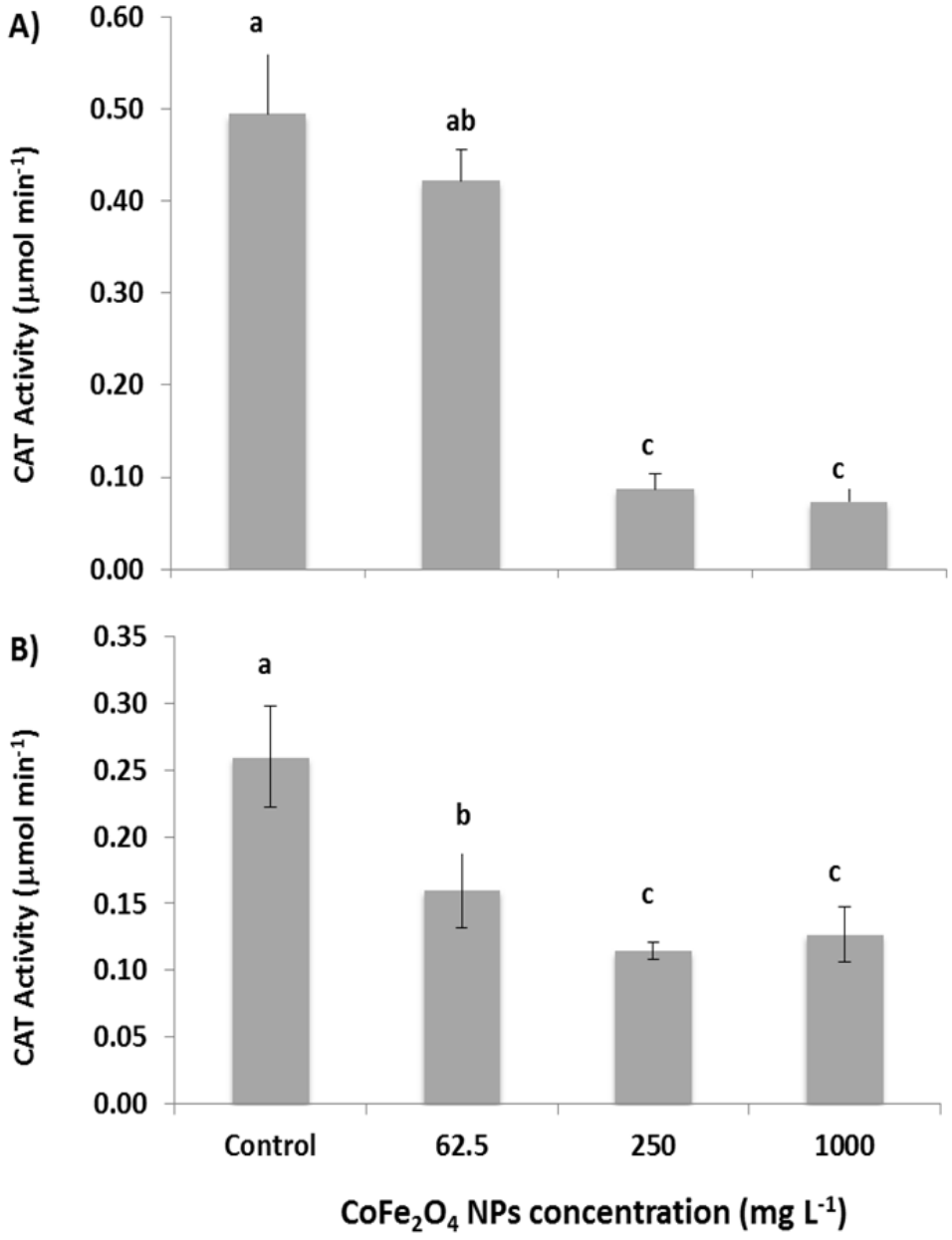

A)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
CAT Activity (µmol min-1)
a
ab
c
c
B)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
CAT Activity (µmol min-1)
a
b
c
c
Control
62.5
250
1000
CoFe2O4 NPs concentration (mg L-1)

Figure 5