

Magnetite (Fe₃O₄) Nanoparticles Alleviate Growth Inhibition and Oxidative Stress Caused by Heavy Metals in Young Seedlings of Cucumber (*Cucumis Sativus* L)

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Abstract: Accumulation of heavy metals in the ecosystem and their toxic effects through food chain can cause serious ecological and health problems. In the present study, experiments were performed to understand how the addition of magnetite (Fe₃O₄) nanoparticles reduces the toxicity caused by Cd, Pb, Cu, and Zn in cucumber plants. Plant growth parameters, lipid peroxidation, and antioxidant enzymes were measured in seedling samples treated with either metals or metals supplemented with Fe₃O₄ to demonstrate the reduction in metal-induced oxidative stress conferred by Fe₃O₄. Results showed that the toxic effect of metals on seedling growth parameters can be arranged in the rank order of inhibition as follows: Cu > Cd > Zn > Pb. Exposure to metals significantly decreased the seedlings growth, the activities of superoxide dismutase (SOD) and peroxidases (POD), while the malondialdehyde (MDA) content significantly increased in cucumber seedlings. The reducing activity of nano-Fe₃O₄ against heavy metals stresses was confirmed in this study by the decrease in MDA content. The correlation between the decrease of MDA concentration and the increase in SOD and POD activities in the presence of nano-Fe₃O₄ suggest that the MDA reduction in the tested seedlings can result from the increased enzyme activity.

1 Introduction

Accumulation of heavy metals (e.g Cd, Cu, Zn and Pb) in the ecosystem and its toxic effects through food chain can cause serious ecological and health problems. At higher concentrations, all heavy metals are toxic to living organisms including plants. The excess accumulation of these metals in plant tissues may disturb various physiological processes such as inhibition of root growth and damage to plasma membrane permeability [8]. Previous studies [23] have demonstrated that heavy metals can enhance lipid peroxidation (MDA) and cause oxidative stress in plants by generating reactive oxygen species (ROS), superoxide anion radicals (O⁻²), hydroxyl radicals (•OH) and hydrogen peroxide (H₂O₂). To remove ROS and reduce the toxic effect of heavy metals, plants have evolved a series of antioxidant enzymatic mechanisms including superoxide dismutase (SOD) and peroxidase (POD). Due to their potential application in the remediation of polluted soils and water as well as biomedicine, magnetic (Fe₃O₄) nanoparticles are gaining more interest in research [17]. Many studies have shown that iron compounds have a high ability for heavy metal adsorption. According to Hu et al.[7], magnetite nanoparticles could be potential adsorbents to reduce heavy metals concentration in the natural environment (contaminated water or soil). The key drivers in the adsorption of heavy metals by iron oxide nanoparticles are their surface properties. Innovative approaches are desirable in order to prevent or reduce the toxic effects of heavy metals on plants. The knowledge about the use of nanoparticles such as nano-Fe₃O₄ to reduce the root growth inhibition and oxidative stress induced by heavy metals in terrestrial plants is

very limited [29, 12]. Limited beneficial effects of nano-Fe₃O₄ on the Cd-induced growth have been observed in wheat and cucumber plants [29]. However, several biological roles of nano-Fe₃O₄ on heavy metals stress in plants are needed to be studied. According to Shen et al.[21], nano-Fe₃O₄ can be used to adsorb metal ions. Investigation into how nano-Fe₃O₄ can be used as an intervention strategy to respond to oxidative stress caused by heavy metals could be essential to protect the environment and public health. We hypothesized that the inhibitory effects of the selected metals may be reduced by nano-Fe₃O₄ and the reducing effect would vary depending on the type of the metal. Root elongation, accumulation of metals, SOD and POD activities, and MDA content were investigated in this study to test these hypotheses. The aim of the present study was to determine the effects of selected heavy metals (Pb, Zn, Cd and Cu) on cucumber seedlings and subsequently to determine the effects of reducing metal toxicity in plants by magnetite (Fe₃O₄) nanoparticles.

2 Materials and Methods

2.1 Nano-Fe₃O₄, Heavy Metals, and Plant

We synthesized magnetite (Fe₃O₄) nanoparticles with a size of about 6nm following the procedures from Xiao et al.[27]. The four heavy metals (PbCl₂, ZnSO₄·H₂O, CdCl₂ and CuCl₂·2H₂O) were purchased from Xilong Chemical Co., Ltd., China. The analytical grade chemicals were purchased from Beijing Chemical Works. Seeds of Cucumber (*Cucumis sativus* L. var.zhongnong No.16) were obtained from Chinese Academy of Agricultural Sciences. A sample of the

seeds was tested in a preliminary experiment to determine the germination rate (greater than 95%). The seeds were refrigerated at 4 °C before use. Dynamic Light Scattering (DLS) equipment (ZETA SIZER 90, Nano series, Malvern, UK) and Tecnai G2 20 S-Twin transmission electron microscope 119 (FEI Co., Japan) operating at 200 KV were used to evaluate the agglomeration of nano-Fe₃O₄ in water

2.2 Preparation of Nano-Fe₃O₄ Suspensions and Metal Solutions

The growth of seedlings was investigated at 1mM, 10 mM (Pb, Zn, Cd, and Cu), and 2000mg/L (nano-Fe₃O₄) using water as solvent and control. We chose 2000 mg/L concentration as the upper limit in this study because, according to the US EPA [26] guidelines, this level could be regarded as the phytotoxicity level on tested plant species if there is no negative effect on root growth under such a high concentration. The accumulation of metals and the oxidative stress were investigated only at 1 mM of the selected metals with or without nano-Fe₃O₄ (2000mg/L). Thus, nano-Fe₃O₄ particles were suspended directly in deionized water or the metal (1 mM) solution, and then an ultrasonic vibration was applied for 40 min to obtain properly dispersed and stable nano-Fe₃O₄ suspensions of each selected metal concentration.

2.3 Seedling Exposure

Cucumber seeds were immersed in 10% sodium hypochlorite solution for 10 min and then rinsed four times with DI-water to ensure surface sterility (USEPA.[26]). One piece of wet filter paper was put into each 100 mm × 15 mm Petri dish. The seeds were transferred onto the filter paper in groups of 50 in the Petri dishes. Finally, distilled water was added into the Petri dishes. The seeds were left on the wet filter paper in the covered Petri dishes to produce radicles within 24h in a dark growth chamber at 25 °C with 75% relative humidity. Once approximately 95% of the cucumber seeds had produced radicles, the seedlings were exposed to the test solutions as described below.

2.3.1 Effects of nano-Fe₃O₄ and heavy metals on seedling growth.

Seeds with radicles (at 1 d) were transferred onto another filter paper (10 seedlings) in a Petri dish at about 1 cm apart from each other [13]. 5 mL of nanoparticles suspension or metal solution was subsequently added. Only distilled water was added to the Petri dishes of the control group. All the Petri dishes were covered, sealed with parafilm, and placed in the dark in a growth chamber at 25 °C with 75% relative humidity. After 5 days, the seedlings were removed from the growth chamber and the seedling root and shoot lengths were measured by a millimeter ruler. Tolerance index, seedling viability, seedling growth and the inhibitory effects of nanoparticles and metals were

also determined. A completely randomized design with four replicates per treatment was used for all experiments.

2.3.2 Effects of nano-Fe₃O₄ particles on metal toxicity and their accumulation in seedling

In order to investigate the effects of nano-Fe₃O₄ on heavy metal-induced root growth inhibition and their accumulation in the seedlings, the first experiment was repeated. 5 mL of a test medium (metal solution or metal solution+ nano-Fe₃O₄) was added. In each case, metal solution (1mM) was used as a solvent and control. The seedling root length was measured by a millimeter ruler after 5 d. The roots and shoots were then separated and dried at 60 °C. The dry samples were used to determine the total Fe, Pb, Zn, Cd and Cu contents in the tissues of cucumber seedlings grown in the metal solutions with or without nano-Fe₃O₄ by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo X7, USA) following the methods previously described by Le et al.,[11]. All the experiments used a completely randomized design with four replicates per treatment.

2.3.3 Effects of nano-Fe₃O₄ on the oxidative stress induced by heavy metals in the seedlings

To examine the oxidative stress induced by heavy metals and investigate the effects of nano-Fe₃O₄ on the reduction of metal stress in the seedlings, we repeated the second experiment with the same concentrations: metal (1mM) and nano-Fe₃O₄ (2000 mg/L). Firstly, the effects of each metal and nanoparticles were compared to those of water. Secondly, the alleviation effects of nano-Fe₃O₄ on the oxidative stress induced by metals to the cucumber seedlings were examined. Thus, seedlings of cucumber treated with metals solution or metal+ nano-Fe₃O₄ for 5 d were used to determine the antioxidant activities (SOD and POD) and MDA concentration as described by Le et al.,[11]. All experiments used a completely randomized design with four replicates per treatment.

2.4 Statistical Analysis

Each treatment was conducted with four replicates and the results were expressed as mean ± SD (standard deviation). One-way ANOVA followed by Tukey's HSD (equal group size) or Bonferroni (unequal group size) test was employed to determine the statistical differences. P < 0.05 was considered to be a significant difference. All statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS) Version 20.0 and OriginPro 8. Other relevant calculations on the exposure of the selected metals were made based on Ahmad et al.[1] and Shaikh et al.[19]. The length of shoot and root were determined using a centimeter scale.

$$T.I. = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in distilled water}} \times 100 \quad (1)$$

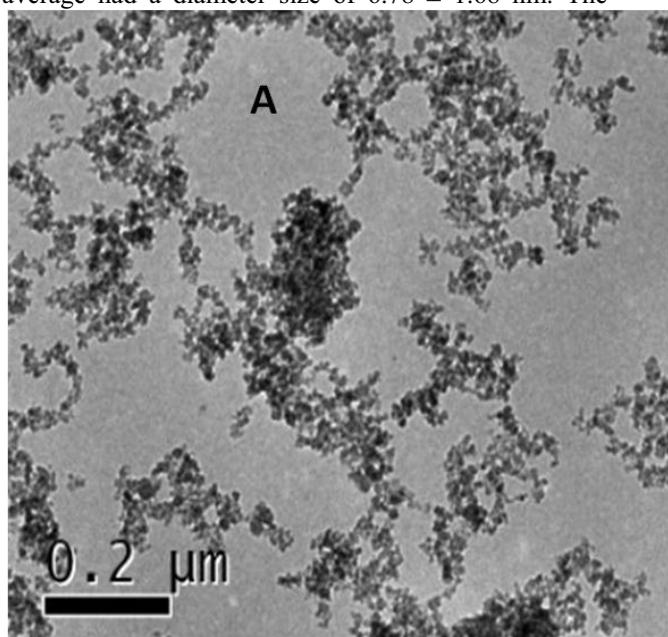
$$\text{Inhibitory rate (\%)\ of Shoot} = \frac{\text{Shoot length of control} - \text{shoot length of treatment}}{\text{Shoot length of control}} \times 100 \quad (2)$$

$$\text{Inhibitory rate (\%)\ of Root} = \frac{\text{Root length of control} - \text{Root length of treatment}}{\text{Root length of control}} \times 100 \quad (3)$$

3 Results and Discussion

3.1. Characterization of Nano-Fe₃O₄

The Fig. 1 shows the TEM image and the particle size distribution histograms for nano-Fe₃O₄ (Fig. 1A-a). The TEM image showed that the nano-Fe₃O₄ on average had a diameter size of 6.78 ± 1.68 nm. The



hydrodynamic size and zeta potential of 50 mg/L nano-Fe₃O₄ in deionized water were measured by dynamic light scattering (DLS) equipment. The hydrodynamic size was 251 ± 7.87 nm and the NPs were well dispersed with a negative zeta-potential (-13.9 ± 0.26). The hydrodynamic diameters and their corresponding TEM image sizes were different due to the agglomeration in water and dry state of the sample particles, respectively.

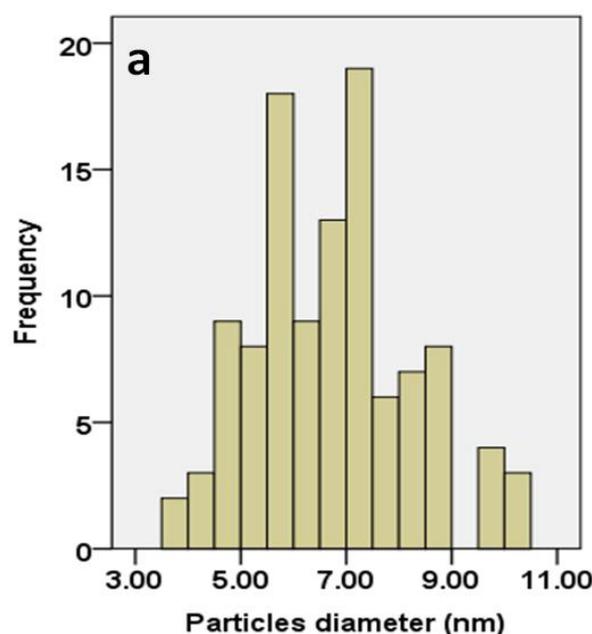


Figure 1. TEM image and particle size distribution histogram of nano-Fe₃O₄.

3.2 Effects of Nano-Fe₃O₄ and Heavy Metals on Seedling Growth

Table 1, showed the effects of nano-Fe₃O₄ and metals (Pb, Zn, Cd and Cu) on the growth of cucumber

seedlings. The results showed that the synthesized nanoparticles did not have an effect on the growth of cucumber seedlings when compared to control (Table 1)

Table 1. Effect of nano-Fe₃O₄ or metals on cucumber seedling growth

NPs or Heavy metals	Concentration	Root length(Cm)	Inhibitory rate (%)	Shoot length(cm)	Inhibitory rate (%)
H ₂ O	0	5.57±1.86	0	6.71± 1.49	0
NPs	2000mg/L	5.71±1.74	-2.47	6.97±1.50	-3.99
Pb		1.86±0.66*	66.55	4.69±1.11*	30.09
Zn		1.64±0.89*	70.48	3.55±1.27*	46.96
Cd	1mM	0.45±0.06*	91.80	2.52±0.92*	62.34
Cu		0.31±0.22*	94.48	1.68±0.46*	74.97

The values were expressed as mean ± SD (standard deviation) of four replicated samples with 10 seeds for

each replication. * indicated significant difference from the control (P< 0.05).

The effect of nanoparticles on plant growth is thus dependent on various factors such as the particle size and the tested concentration of nano-Fe₃O₄ or the plant species under study [17]. In the present study, the most important response of plants to heavy metals toxicity is the inhibition of seedling growth and the induction of oxidative stress in cucumber seedlings. There were great morphological differences between the metal-treated and control seedlings. An important difference was also found between the treatments of the four tested metals (Cd, Cu, Pb and Zn). Growth inhibition is one of the most common agriculture indices of heavy metal tolerance but also a response to heavy metal stress [14]. Growth inhibition by the heavy metals (1mM concentration) was in the order: Cu > Cd > Zn > Pb (Table 1). These results were in agreement with those of Fargašová[3], who reported that trace elements had toxic inhibitory effects of on the mustard seedling in a descending order of Cu > Se > Cd > Zn > Pb. Unlike our experiment the later authors, included Se in their study. It has been demonstrated that Cd can interfere with the uptake, transport and the use of some nutrients

elements (e.g., ca, P, K, etc.) and water by plants [2]. Cadmium can also reduce plant cellular activities due to the generation of oxidative stress and the inhibition of the enzyme activity [29]. In plants, heavy metals can be transported across membranes nutrients transporters. During the transportation phase of heavy metals within the plant from roots to the shoots, the most important site of toxic action might be the root [25]. The inhibition rank order of root and shoot elongation was similar. However, inhibition of growth by the four metals was greater in roots than shoot (Table 1). This could be attributed to the high accumulation of the metals in roots than in the shoots. Our results showed that the shoot and root length decreased significantly under the treatments of Cd and Cu as compared with Zn and Pb. Zinc and Pb are not generally considered as an essential element for plant growth. Their effects on seedlings can change according plants species, organs and metabolic process [20].

3.3 Effects of Nano-Fe₃O₄ on Metal-induced Root Growth Inhibition

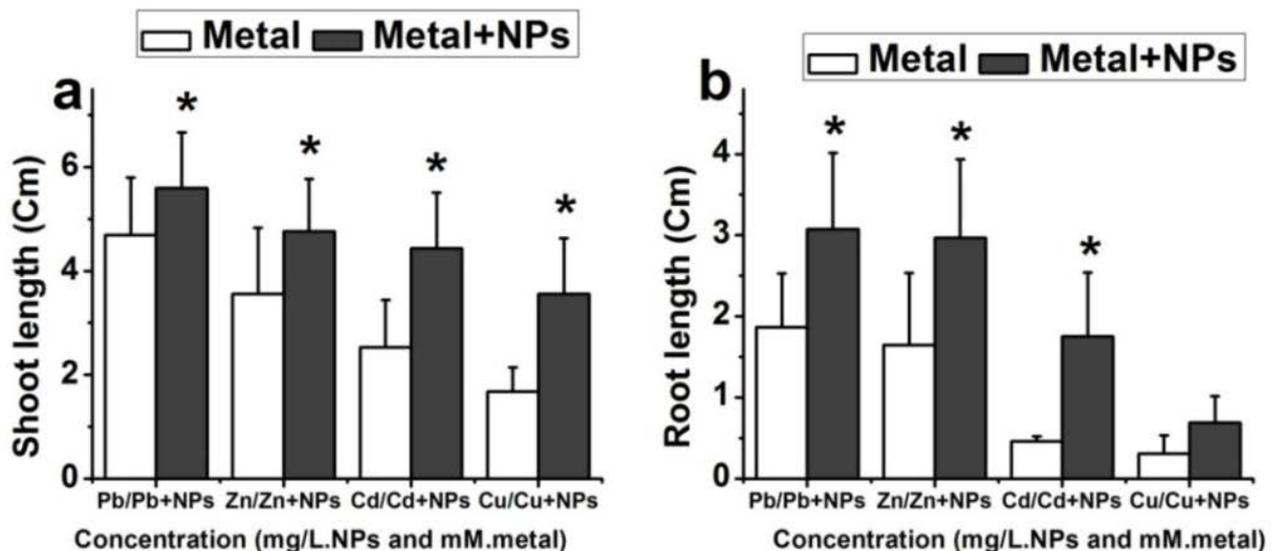


Figure 2. Shoot length (a) and root length (b) of cucumber seedlings after their exposure for 5 days to 1mM of four different metals (Pb, Zn, Cd, or Cu) solutions or 2000mg/L of nano-Fe₃O₄ (6 nm) prepared with solution (1mM) of these four different metals (Pb, Zn, Cd, or Cu) as solvents. The values were expressed as mean \pm SD (standard deviation) of four replicated samples with 10 seedlings for each replication. Significant differences from control (1mM) were marked with "asterisk" (P<0.05)

The utilization of metal oxides nanoparticles in decontaminating the polluted media have been reported in different studies [21]. The types of nonmaterial have been proposed as sorbents due to their large surface area but also because they are easy to synthesize. The electronic properties and the unique structure of magnetic (Fe₃O₄) nanoparticles make them the powerful adsorbents in decontaminating process of polluted soils and water. In order to understand more on the effect of nanoparticles, each heavy metal (1mM) with or without nano-Fe₃O₄ (2000mg/L) was compared (Fig 2). Root and shoot growth were positively affected by the presence of nano-Fe₃O₄ when compared with metal solutions (1mM) without nano-Fe₃O₄, except for roots of seedlings exposed to Cu solution where no significant effect of nano-Fe₃O₄ was noted (Fig. 2b).

The presence of nano-Fe₃O₄ significantly reduced inhibition on root growth induced by the selected heavy metals (Pb, Zn, Cd and Cu) by 39.32%, 44.50%, 73.95%, 37% in the root, and 16.17%, 25.30% 43% and 52.79% in the shoot, respectively (Fig. 2a-b). In agreement to our results, Wang et al.[28] observed that, among the oxide nanoparticles used in their study, Fe₃O₄ was not different from the control (DI-water). However, the addition of Cd solution showed positive effects. The seedling root growths of the tested species were promoted at the concentration (2000mg/L Fe₃O₄) as seen in the present study. In general, the increase in seedling growth in our study, clearly demonstrates that presence of nano-Fe₃O₄ affected the uptake of heavy metals and reduced their toxicity in cucumber seedlings.

3.4 Tolerance Index and Seedling Viability

Fig. 3 shows the effects of nano-Fe₃O₄ and the tested metals (Pb, Zn, Cd and Cu) on seedling viability (Fig.

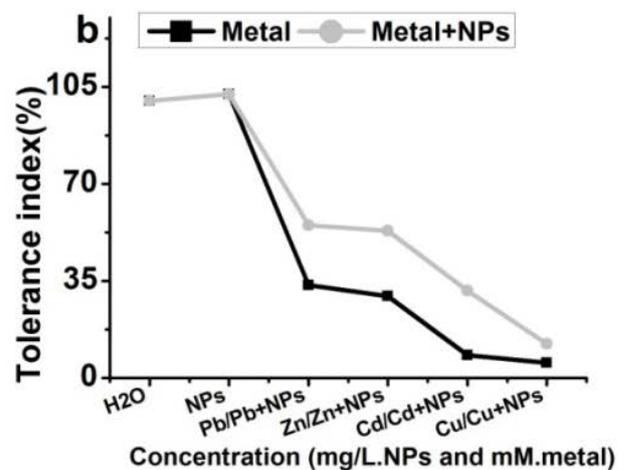
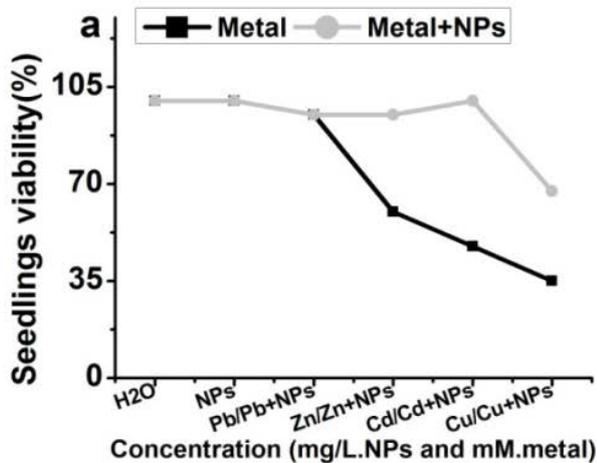


Figure 3. Viability and tolerance index (%) of cucumber seedlings after their exposure to nano-Fe₃O₄, metals (Pb, Zn, Cd and Cu) and metal+nano-Fe₃O₄ for 5 days

The seedling viabilities were: 47.5%, 35%, 67% and 60% at 1mM for Cd, Cu, Pb and Zn, respectively. Interestingly, the viability of cucumber seedlings increased with the addition of nano-Fe₃O₄ in the metal solutions by 95%, 95%, 100 and 67.5% for Pb, Zn, Cd and Cu respectively. The comparison with metal solutions alone showed that the addition of nano-Fe₃O₄ significantly increased seedling viability which was inhibited by the selected metals (Pb, Zn, Cd and Cu), and the inhibitory rates of seedling viability from the metal+nano-Fe₃O₄ treatments were in the order, Cu >

3a) and the tolerance index (Fig. 3b). The results showed a 100% viability in both control and nano-Fe₃O₄ treated seedlings. The inhibitory rates of seedling viability by the metal treatments decreased in the order, Cu > Cd > Zn > Pb.

Zn = Pb > Cd. The tolerance index of seedlings increased in the presence of nano-Fe₃O₄ compared with metals solutions (1mM) without nano-Fe₃O₄, except in the case of Cu+nano-Fe₃O₄ (Fig. 3b). Nano-Fe₃O₄ suspension had no effect on the tolerance index of seedlings grown in Cu solution. Plant tolerance systems to heavy metals need the coordination of different physiological and biological processes. Different plant species have different mechanisms to tolerate heavy metals.

Table. 2 Effects of nano-Fe₃O₄ on heavy metals uptake by cucumber seedlings

Heavy metals and NPs	Heavy metal concentrations (mg.kg ⁻¹) in seedlings					
	Shoot			Root		
	Metals-NPs	Metals+NPs	Reduction in metal uptake (%)	Metals-NPs	Metals+NPs	Reduction in metal uptake (%)
Pb	32.59±11.58	4.37±1.22	86.59	1141.30±72.75	196.83±50.30	82.75
Fe	85±19.46	119.18±26.11		162.87±20.49	3020.89±280.52	
Zn	64.54±10.07	37.37±12.03	42.09	424.62±18.21	174.96±27.83	58.79
Fe	71.29±22.46	121.97±27.63		184.61±18.39	2987.69±287.79	
Cd	59.20±14.87	6.06±1.09	89.76	563.36±75.52	88.08±18.79	84.36
Fe	60.08±17.45	127.11±12.16		174.92±15.88	3140.59±265.56	
Cu	8.58±2.06	4.45±0.37	48.13	186.78±28.84	54.13±10.75	71.01
Fe	67.06±13.62	116.39±31.26		198.56±23.40	3315.07±308.87	

The values were considered as mean ± SD (standard deviation) of four replicated samples with 10 seedlings for each replication. Significant differences from control (1mM of metal) were marked with "asterisk" (P<0.05)

Table 2 shows the effects of nano-Fe₃O₄ on heavy metal accumulation and their effect on cucumber seedlings. Seedlings received the tested metal solutions (1mM) with or without nano-Fe₃O₄ (2000 mg/L). All the heavy metals (Pb, Zn, Cd and Cu) were taken up by shoot and root of the cucumber seedlings (Table 2).

Most of the tested metals had higher accumulation in the roots compared with the shoots. Previous studies found similar results in *Allium sativum* [34], wheat seedlings [28, 29], native plants [32] and water hyacinths [14]. When cucumber seedlings were exposed to 1mM concentration of tested metal solutions without nano-Fe₃O₄, accumulation of metals in plants were in the order of Pb > Cd > Zn > Cu and Zn > Cd > Pb > Cu for root and shoot samples, respectively. The highest concentration in the root and shoot were of Pb and Zn treatments, respectively. The accumulation of Pb in plants could be depending on the tested concentration in the growth medium [14]. Some plants considered as stable to heavy metals can transport higher concentration of heavy metals from root to leaf [18]. The results were supported by the report of Yilmaz et al. [31] for Pb accumulation in roots and those of Jadia and Fulekar [10] and Wang et al. [28] for Zn accumulation in shoots. In this study, accumulation heavy metals were significantly reduced in cucumber seedlings with the addition of nano-Fe₃O₄ (Table 2). The addition of nano-Fe₃O₄ reduced metal concentrations and changed the accumulation order in the cucumber seedlings as follows: Pb > Zn > Cd > Cu and Zn > Cd > Cu > Pb for root and shoot samples, respectively. The reduction rate of metal concentrations in seedling varied according to the type of heavy metals as follows, Cd > Pb > Cu > Zn (Table 2). The order of metals decreasing in the root was similar with that found in shoot. In detail, the addition of nano-Fe₃O₄ (2000mg/L) significantly ($P < 0.05$) decreased the metal (Pb, Zn, Cd and Cu) uptake by cucumber seedlings by 82.75%, 58.79%, 84.36% and 71.01%, in the roots and 86.59%, 42.09% 89.76% and 48.13% in the shoot, respectively at 1mM concentration (Table 2). Our results show the role of nano-Fe₃O₄ in reducing the uptake of heavy metals by the seedlings of cucumber. In the present study, nanoparticles were visually observed on root surface after 5d, suggesting that their adsorption capacity could inhibit the uptake of heavy metals and thus protect root seedlings. The zeta potential results show that the synthesized nano-Fe₃O₄ (6nm) used in this study was negatively charged, which could increase their adsorption capacity. It has been reported that the adsorption capacity of Fe₃O₄ nanoparticles depends on of various parameters including the different

electrostatic attraction between heavy metal cations and negatively charged adsorption sites [4]. The use of magnetite nanoparticles as adsorbents provides a convenient approach to reduce the uptake of heavy metals and mitigate their toxicity in plants. The particle sizes play an important role in nano-Fe₃O₄ application because their properties such as surface area greatly depend upon the dimension of the particles. Magnetite nanoparticles with small size (less than 30 nm) have a large surface area. These properties of nano-Fe₃O₄ can be strongly useful in the adsorption process. The above-mentioned observations from our study showed that the reduction in the uptake of the tested heavy metals, and the decrease in their toxicity in the cucumber seedlings could be associated with the sizes and surface area of nano-Fe₃O₄, but also their adsorption capacity.

3.5 Antioxidant Enzyme Activities and Lipid Peroxidation Assays

3.5.1 Effect of nano-Fe₃O₄ and heavy metals on antioxidative enzyme activity and MDA content

As seen in the Fig. 4, SOD and POD activities as well as MDA content were not significantly different ($P < 0.05$) between seedlings treated with nano-Fe₃O₄ at 2000mg/L only and control (DI-water). However, the SOD activity decreased significantly in the roots and shoots of cucumber seedlings under the stress of Cd and Cu (Fig. 4a-b). When seedlings were exposed to Pb and Zn solutions, SOD activity increased significantly in roots but did not change in shoot as compared to control (DI-water). Among the tested metals, only Cd treatment reduced the POD activity in root and shoot of cucumber seedlings followed by Cu which only decreased the POD activity in roots (Fig.4c-d). Heavy metals are a risk factor in plants and can induce disturbance in their life cycle and consequently, decrease its growth [18]. The decline in SOD activity under Cd and Cu stress indicated that oxygen scavenging function of SOD was impaired [22]. These observations are similar with the results from *Allium sativum* [34] and *Jatropha curcas* L [22]. In the roots, an increase in the activity of SOD was noted under Pb and Zn stress (Fig. 4b), which could be due to the metal tolerance ability in the cucumber plants.

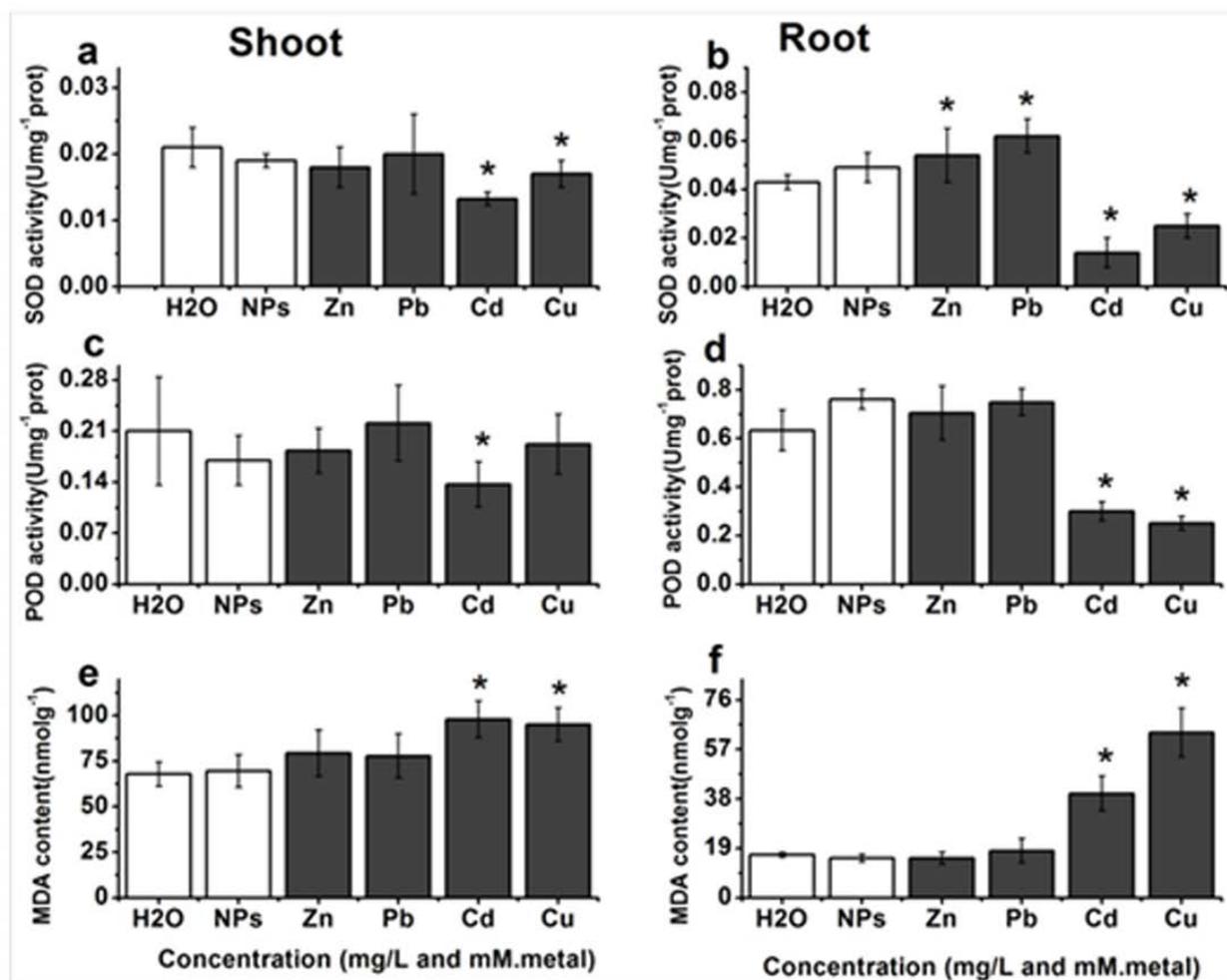


Figure 4. SOD and POD activities and MDA content in the root and shoot of cucumber seedlings treated with H₂O, nano-Fe₃O₄ (2000mg/L) and four heavy metals (Pb, Zn, Cd and Cu) at 1mM concentration. SOD in shoot (a) and root (b); POD in shoot (c) and root (d), MDA content in shoots (e) and roots (f). The values were expressed as mean \pm SD (standard deviation) of four replications. The asterisk "*" indicated the significant differences versus controls at ($P < 0.05$).

MDA concentration is usually used as a biomarker of lipid peroxidation resulting from oxidative stress [24]. In the present study, MDA concentration increased significantly in roots and shoots of cucumber seedlings when exposed to Cd and Cu treatments (Fig. 4e-f). On the other hand, Pb and Zn treatments had no effect on MDA concentration in roots and shoots of cucumber seedlings when compared to control (DI-water). The increase in MDA content suggested that Cd and Cu indirectly lead to production of superoxide radicals, which resulted in increased lipid peroxidation products and caused oxidative stress in the tested seedlings [12]. Our results were supported by the reports on cucumber plants [24] and strawberry [16]. Certain plants can tolerate, uptake and translocate high concentrations of some heavy metals even if they are toxic to other organisms [15]. The treatments of Zn and Pb did not affect the MDA content suggesting that cucumber seedlings have efficient mechanism to tolerate the stress induced by Zn and Pb under the experimental condition noted in the present study[22]

3.5.2 Alleviation of oxidative stress induced by heavy metals in cucumber seedlings by nano-Fe₃O₄

In this experiment, results of metal+nano-Fe₃O₄ were compared to their corresponding metal solutions (Fig. 5). As seen in the Fig. 5a-b and 5c-d, the addition of nano-Fe₃O₄ and Pb or Zn had no obvious effect on SOD and POD activities in cucumber seedlings. The SOD activity significantly increased by 43.02% and 33% in the shoots, and by 76.06% and 53.55% in roots, with the addition of nano-Fe₃O₄ to the solution (1mM) of Cd and Cu, respectively (Fig. 5a-b). The POD activity also, significantly increased by 49.21% and 30.53% in the shoots, and by 69.23% and 65.04% in roots, with the addition of nano-Fe₃O₄ to the solution (1mM) of Cd and Cu, respectively (Fig.5c-d).

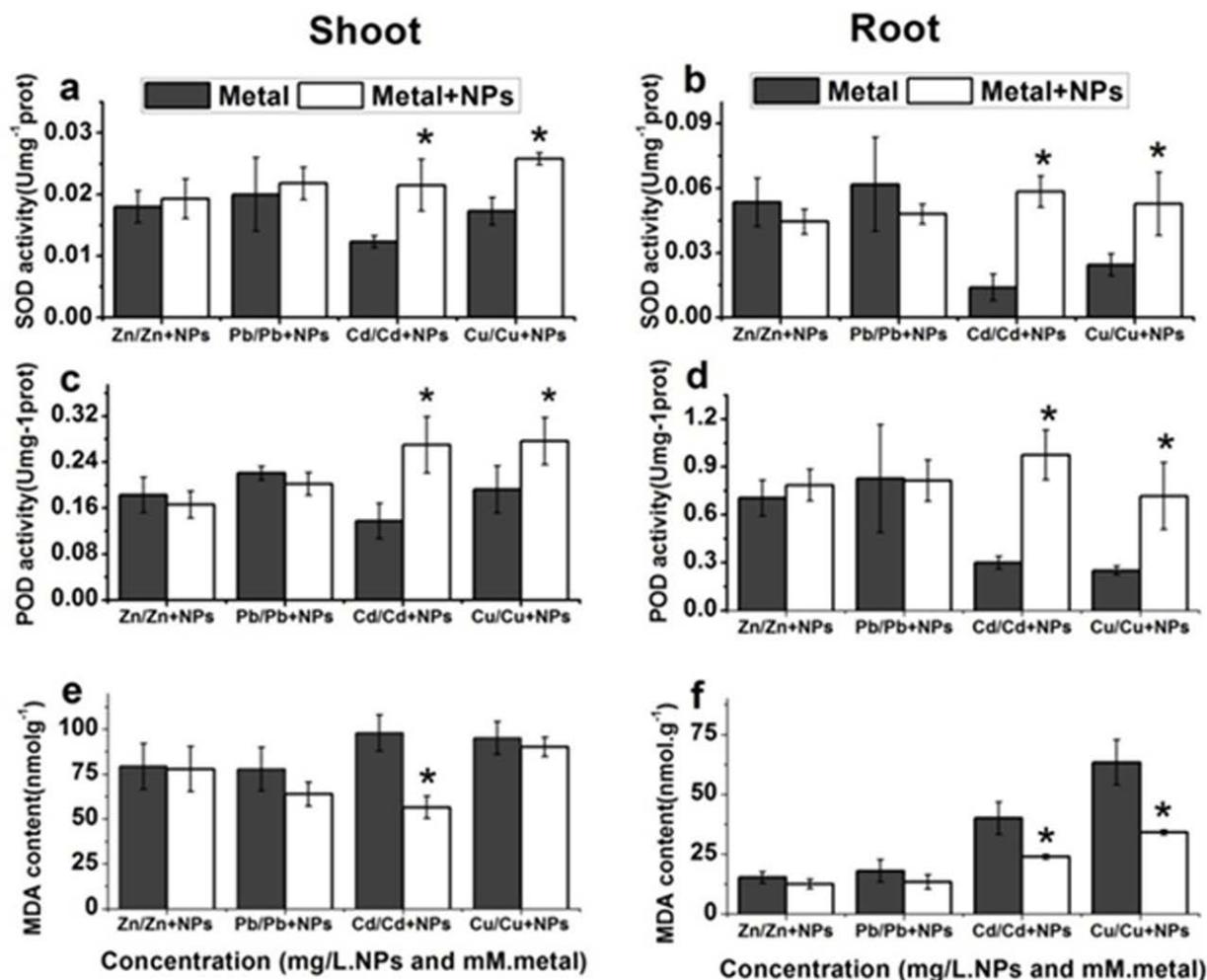


Figure 5. SOD and POD activities and MDA content in the root and shoot of cucumber seedlings treated with 1mM of four heavy metals (Pb, Zn, Cd and Cu) with or without nano-Fe₃O₄ (2000mg/L). SOD in shoot (a) and root (b); POD in shoot (c) and root (d), MDA content in shoots (e) and roots (f). The values were expressed as mean \pm SD (standard deviation) of four replications. The asterisk "*" indicated the significant differences versus controls (Corresponding metal solution without nano-Fe₃O₄) at ($P < 0.05$).

The Cd and Cu treatments without nano-Fe₃O₄ caused decrease in the SOD and POD activities in shoot and root of cucumber seedlings (Fig. 4a-b and 4c-d). The addition of nano-Fe₃O₄ significantly up-regulated the decrease in the activities of SOD and POD under Cu and Cd stresses, suggesting that nano-Fe₃O₄ particles played a protective role by increasing the seedlings ability to scavenge the O₂⁻ radicals and H₂O₂, and prevent the oxidative stress to cells [33, 14]. Heavy metals (e.g., Pb, Cd and Cu) initially induce a severe oxidative stress in living organisms, which in turn causes lipid peroxidation and H₂O₂ accumulation [6]. Here, we performed an experiment to test whether external nano-Fe₃O₄ could be used as a regulator or be capable of initiating antioxidant intervention in response to oxidative stress brought about by heavy metals. The MDA concentration was used as one of the main indicator to evaluate the effects of nano-Fe₃O₄ on the oxidative stress induced by metals. The MDA concentration significantly decreased ($P < 0.05$) in the roots by 40.39% and 46.21%, on addition of nano-Fe₃O₄ to the Cd and Cu solutions, respectively, (Fig. 5f). In the shoot, only the addition of nano-Fe₃O₄ and Cd showed a positive effect with decrease in MDA content

by 42.31% (Fig. 5e). The MDA concentration in the cucumber seedlings did not change significantly with the presence of nano-Fe₃O₄ in the solutions of Pb and Zn. The reduction role of nano-Fe₃O₄ against heavy metals stress was confirmed in this study by the decrease in MDA content on the addition of nano-Fe₃O₄. We observed in our study a correlation between the decrease in MDA concentration and the increase in SOD and POD activities in the presence of nano-Fe₃O₄, suggesting that the decline in MDA content under Cd and Cu could be due to the increase in antioxidative enzyme activities in the presence of nano-Fe₃O₄, which reduced H₂O₂ levels, increased the ability of cucumber to scavenge the radicals and reduced membrane damage. Previous studies found similar results in the seedlings of *Kandelia candel* [33]. It has been reported that the oxidative damage induced by Cd toxicity could be alleviated by enzymatic and non-enzymatic antioxidants in plants [30]. Guo et al.[5] found that the presence of salicylic acid (SA) significantly increased the activities of SOD and POD which had been negatively affected by Cd stress, but also decreased significantly the MDA concentration in roots of rice (*Oryza sativa*) when compared with the Cd treatment alone. The reducing

effects of SA on Cd toxicity were attributed to the increased level in the activities of antioxidant enzymes.

6 Conclusions

In this study, exposure of heavy metals (Pb, Zn, Cd and Cu) inhibited cucumber seedling growth and seedling viability. Copper (Cu) and cadmium (Cd) had the strongest unfavorable effect on the growth of young cucumber seedlings, respectively. Although most of the heavy metals accumulated in roots, lower levels of the same were translocated into and accumulated in the shoots. The total activity of SOD and POD decreased significantly in the roots and shoots of cucumber seedlings under Cd and Cu stress. When exposed to Pb and Zn solutions, SOD activity increased significantly in roots but did not change in shoot as compared to control (DI-water). On the other hand, MDA concentrations increased significantly in roots and shoots of cucumber seedlings when exposed to Cd and Cu treatments. The addition of nano-Fe₃O₄ significantly decreased root growth inhibition, reduced and alleviated oxidative stress induced by the heavy metals tested in the cucumber seedlings. Our study showed that nano-Fe₃O₄ (6nm) could mitigate metal-induced oxidative stress, growth inhibition and enhancing metal tolerance in the seedlings of cucumber.

Acknowledgement

The work was supported by National Natural Science Foundation of China (No. 41371471) and NSFC-Guangdong Joint Fund (U1401234). The Ministry of Science and Technology of China (no. 2011CB933400, 2012CB932504 and 2013CB932703), and National Natural Science Foundation of China (no. 11275215, 11275218, and 11375009).

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