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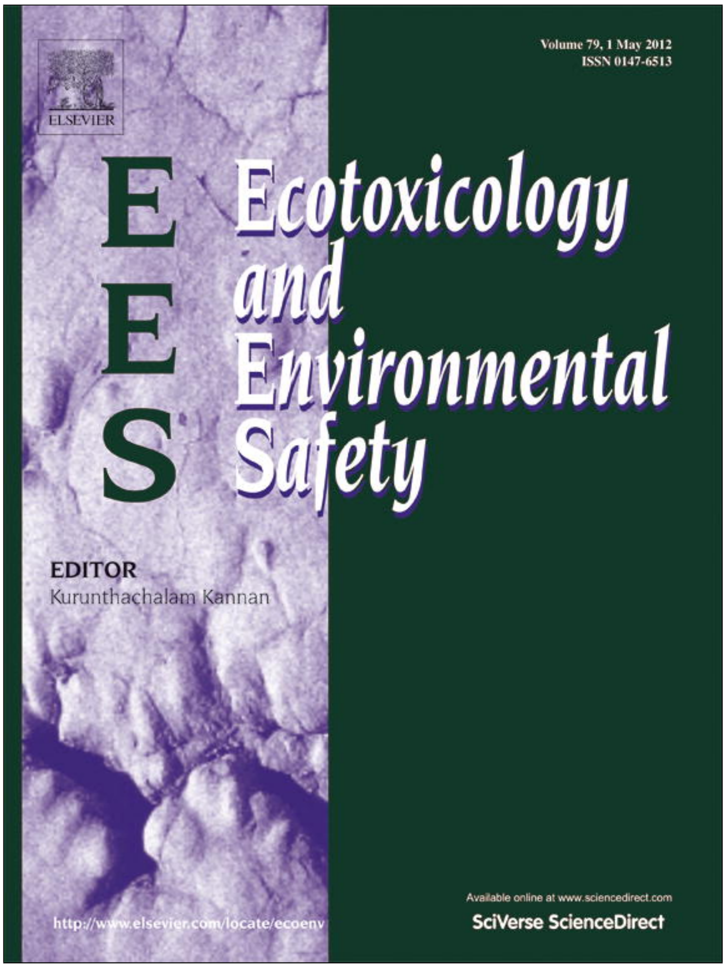

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Alleviation of cadmium-induced root growth inhibition in crop seedlings by nanoparticles

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ABSTRACT

The short-term effects of six types of nanoparticles (NPs) (Kaolin, montmorillonite, hydroxyapatite, Fe_3O_4, α-Fe_2O_3 and γ-Fe_2O_3) on the EC50s (Cd) for root growth of four plant species (i.e. tomato, cucumber, carrot and lettuce) were investigated using standard toxicity testing. NPs and Cd influencing on growth of the plant were as well as tested, respectively. Scanning-electron microscopy (SEM) equipped with the element dispersive spectrometer (EDS) was used to observe the interaction of NPs prepared with EC50s (Cd) as the solvents with the root surface and identify the mechanisms of Cd toxicity reduction to the root growth induced by NPs additives. The results showed that the seedling growth was negatively related to the exposure concentration of Cd, among the tested plants, the sensitive endpoint appeared in the order of tomato $>$ carrot $\approx$ lettuce $>$ cucumber according to the ECx measured. The root growth was not significantly inhibited by the presence of NPs except for HAP on tomato, but was noticeably promoted by particular NPs suspensions prepared with EC50s (Cd) as the solvents at higher test concentrations compared with the controls (Cd, EC50s) with one exception for Kaolin. Microscopy images showed roots of tested plants exposed to Cd exhibited a decrease in root diameter and root wilt, and the disintegration of the root epidermis, the clutter root surface showed the evident stress under Cd solution, after the addition of NPs, many root hairs and no disintegration on the surfaces of the root system can be observed, NPs crystal also occurred on the plants root surface. The element dispersive spectrometer (EDS) analysis showed that the precipitation mainly contributed to phytotoxicity reduction by the NPs.

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1. Introduction

Development of high-efficient and environment-friendly amendments and their potential application in site remediation have received a considerable attention in the last decade (Chen et al., 2009). With the fast development of environmental molecular science and engineering, the application of nanoscale materials (<100 nm) in remediating polluted soils and waters has gained even more attention in recent times. Their small size and large surface area per unit mass make them important binding phases for both organic and inorganic contaminants. Additionally, high surface energy, quantum confinement, and conformational behavior are likely to be important in the application of remediating polluted mediums. However though it has been claimed that nanotechnology has great potential for environmental cleaner technology, the effect is required to be considered as to application of NPs (Reijnders, 2006; Dickinson and Scott, 2010).

According to the application of NPs in environmental remediation, nanoscale materials can be broken down into a number of different compound classes, including metal oxides such as Fe_3O_4, Fe_2O_3, TiO_2; clays, usually including montmorillonite (MMT), hydroxyapatite (HAP), Kaolin; zero-valent metals such as iron, silver and gold; carbonaceous nanomaterials; semiconductor materials, including quantum dots, etc. (Klaine et al., 2008). The first two types of NPs are very cheap, abundant and environmentally friendly because of their main components, which have been proved to own an extensive application prospect in environmental remediation.

In past decades, the widespread accumulation of metals in the environment is increasingly becoming a problem for kinds of organisms (Munzuroglu and Geckil, 2002). Cadmium, an environmental threat, has been recognized to have strong toxic effects and considered as one of the most hazardous pollutant in environment, being included on the US Environmental Protection Agency's (EPA) list of priority pollutants (Cameron, 1992; Nedelkoska and Doran,

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2000). The effects and mechanisms of Cd stress on plant organisms have been widely studied over past decades (Barman et al., 2000; Nedelkoska and Doran, 2000; Oncel et al., 2000). Recently, there is a growing body of literature that report various treatment methods and techniques of Cd-polluted soils, mainly including the electrokinetic remediation (Reddy and Chinthamreddy, 1999), biosorption (Lin and Xing, 2007), bioleaching (Davis et al., 2003), phytoremediation, etc. (Mulligan et al., 2001). However, few researches have reported about reducing the phytotoxicity of Cd to plant using NPs in environmental mediums. More recently, the authors have been interested in the possibility of considering NPs as cost effective amendments for reducing the phytotoxicity of cadmium to plants. To help understand the effects of different NPs amendments on reducing the phytotoxicity of Cd to plants, we conducted a preliminary study regarding effects of six types of NPs on the EC50s (Cd) for root growth of four higher plant species determined by the early seedling growth. Scanning-electron microscopy (SEM) equipped with the element dispersive spectrometer (EDS) was used to observe the interaction of NPs prepared with EC50s (Cd) as the solvents with the root surface, in order to determine if NPs can decrease the toxicity of Cd to the root growth of the plant species after 4 day of exposure.

2. Materials and methods

2.1. Nanoparticles (NPs) and Cd

Six different types of NPs were used in this study. The two nanoscale clays, i.e., Kaolin and Montmorillonite (MMT) were provided by Nanjing Emperor Nano Material Co., Ltd., China. The hydroxyapatite (HAP) and magnetic nanoscale metal oxides (Fe_3O_4, α-Fe_2O_3 and γ-Fe_2O_3) were from Shenzhen Gem Hi-tech Co., Ltd, China. The surface area was determined using the multi-point Brunauere–Emmette–Teller (BET-N_2) method (Chen et al., 2007). $3CdSO_4 \cdot 8H_2O$ (GR, guaranteed reagent) was purchased from Beijing Yili Fine Chemicals Co., Ltd., China. Their characteristics are listed in Tables 1 and 2.

2.2. Seeds

Seeds of four crop species: tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*) and carrot (*Daucus carota*), were provided by Beijing Jiahe Seed Co., Ltd., China. The four species (tomato, cucumber, carrot and lettuce) were chosen as toxicity testing plants because they represented commonly used species recommended in USEPA terrestrial plant toxicity guidelines (USEPA, 1996) and belonged to four different plant families and, thus, can provide great genetic diversity. The average germination rates of the tested seeds were greater than 90% according to our preliminary study. The seeds were refrigerated (4 °C) until use.

Table 1
Characteristics of NPs used for the experiments.

Nanoparticles	Average size (nm)	Purity (%)	Surface area (m^2/g)
Kaolin	103.2 ± 11.7	95.2	92.4
MMT	86.5 ± 9.3	94.6	112.8
HAP	89.3 ± 4.9	96.5	127.4
Fe_3O_4	20.1 ± 2.4	99.2	252.4
α-Fe_2O_3	30.2 ± 4.1	99.4	207.5
γ-Fe_2O_3	20.5 ± 1.7	99.6	262.8

Table 2
Typical chemical composition (105 °C, 3 h) of the selected nanoparticles (in wt%).

Sample	SiO_2	Al_2O_3	CaO	MgO	Fe_2O_3	Fe_3O_4	$CaCO_3$	K_2O	P_2O_5	Na_2O
Kaolin	59.32	14.27	2.01	2.69	2.84	2.60	2.69	0.82	–	–
MMT	73.12	14.87	0.34	1.23	2.71	–	–	1.92	–	0.67
HAP	23.12	5.72	41.31	–	1.38	–	–	–	17.22	–

2.3. Preparation of NPs suspensions and cadmium ion solution

Cadmium ion solution was prepared by dissolving $3CdSO_4 \cdot 8H_2O$ in deionized water (DI-water). The NPs suspensions were prepared as follows: NPs (200 mg) were placed in a 250-mL jar with 100 mL of DI-water or the median effective concentrations (EC50s; i.e., the concentration that reduced root growth of selected crop species by 50% based on Cd concentration in solution). The final concentrations of Cd in the solutions were determined using the atomic absorption spectrophotometry, AAS), and then sonicated for 30 min. No settling of the NPs was observed for all suspensions. The suspensions were agitated again just before application to the Petri dishes to ensure a homogeneous mixture in solution.

2.4. Seedling exposure

The toxicity of Cd to the plant root growth was examined using a solution culture system. Based on this solution culture system, the effect of the NPs on seedling plants and the phytotoxicity of Cd affected by NPs were also investigated as follows. Seeds were soaked in 10% sodium hypochlorite solution for 10 min and then rinsed several times with DI-water to ensure surface sterility (USEPA, 1996). Seeds (in groups of 80 or 100, depending on the species size) were then placed on one piece of wet filter paper (Hangzhou Special Paper Industry CO, LTD., China) in 150 mm × 25 mm Petri dishes. Next, seeds were left on wet filter paper in covered Petri dishes and produced radicals within 1– 2 d (depending on the species) in the dark in a growth chamber at 25 °C with 75% relative humidity. Once approximately 90% of the seeds of the particular species produced a radical, the seedlings were then exposed to the test solutions as described below.

In experiment 1, seedlings of different plant species (tomato, cucumber, lettuce and carrot) were exposed to NPs suspensions or Cd solution. For the different crop species, eight Cd solution concentrations (0, 1, 2, 5, 10, 20, 50 and 100 mg/L) and five NP suspensions concentrations (0, 100, 500, 1000 and 2000 mg/L, using DI-water as the solvent) were used. After the addition of Cd in solution, the actual concentrations of Cd in solution were determined using the AAS (Perkin-Elmer, AAnalyst 300). One piece of filter paper was put into each 90 mm × 15 mm Petri dish, and 5 mL of a test medium was added. Seedlings were then transferred onto the filter paper, with 10 seedlings per dish and 1 cm or larger distance between each seedling (Yang and Watts, 2005). The test solution was applied in concentric circles over the seedlings, completely covering the filter paper. Covered Petri dishes were placed in the dark in a growth chamber at 25 °C with 75% relative humidity (Cañas et al., 2008). Seedling root length was measured after 4 day following the treatment.

To investigate the effect of NPs on the reduction of Cd phytotoxicity to the root growth of crop species tested, another experiment was conducted according to the result of the phytotoxicity of Cd to root growth of plants (EC50s) in experiment 1, The 4-d EC10, EC50s (Cd) for seedling root growth are presented in Table 3. The first experiment was repeated with all species receiving NPs at 0, 100, 500, 1000 and 2000 mg/L, using EC50s (Cd) solution as the solvents. All experiments used a completely randomized design with three replicates per treatment.

2.5. Measurement of Cd in solution

The analysis of Cd in solution prior to and at the end of 4-days' exposure period was carried out using atomic absorption spectrophotometry. At the beginning and the end of exposure experiment, the solution was separated by centrifuging the suspension at 4500 rpm for 20 min and filtered through a quantitative filter paper (< 0.45 µm). The concentrations of Cd in the solutions were determined using the AAS (Perkin-Elmer, AAnalyst 300) and standard Cd solutions in analytical procedure were used for quality control.

Table 3
Effective concentration (EC10, EC50) for seeding root growth of selected crop species exposed to Cd solution in test medium for 4 day and 95% confidence interval (CI).

Test species	EC50 (mg/L)	95% CI		EC10 (mg/L)	95% CI	
		Min.	Max.		Min.	Max.
Lycopersicon esculentum	4.39	2.890	5.879	1.06	1.61	1.98
Cucumis sativus	6.60	6.609	10.278	1.48	0.78	3.45
Lactuca sativa	8.06	5.76	11.34	1.51	0.68	3.35
Daucus carota	6.65	5.38	8.21	1.76	1.04	2.98

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2.6. Scanning-electron microscopy (SEM)

To obtain direct evidence of the phytotoxicity of Cd on the seedlings and probable mechanism of phytotoxicity alleviation induced by NPs additives, selected roots of the species grown in Cd solution with and without 2000 mg/L NPs were examined using a scanning electron microscope (SEM). Root samples were collected at harvest and immediately fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 7 h. The samples were then freeze-dried and coated with a thin layer of carbon. The samples were observed under a thermal field-emission environmental scanning electron microscope (SEM, JSM-6400/TN500, JEOL, USA) equipped with X-ray EDS during SEM observations (Chen et al., 2007).

2.7. Statistical analysis

Each treatment was conducted with three replicates, and the results were presented as mean ± SD (standard deviation). The data were subjected to ANOVA analysis using SPSS Version 12.0 software (SPSS Inc., USA) and differences ($p<0.05$) between means were determined using the Duncan–Waller test. Each of the experimental values was compared to its corresponding control. The effective concentrations (EC10, EC50) for the seedling growth affected by Cd were determined based on the actual determined concentrations using logistic (sigmoidal) by minimizing unweighted squared residuals sum.

3. Results

3.1. Cadmium effect on early seedling growth

The phytotoxicity of Cd solution on the four plant species was assessed using growth inhibition experiments. Dose–response relationships (logistic curves) were established for the root growth of the plants in the range of Cd actually measured in solution, which is used to highlight the range of root length (RL) values under conditions of varied concentrations of Cd added (Table 3). The results showed that the seedling root length of tested species was negatively related to the exposure concentration of Cd in solutions. For all the plants, root growth was suppressed by the treatment of about 5–10 mg/L Cd, when samples were exposed to about 20–50 mg/L Cd, the root length was strongly retarded, while the shoot survived but grew slowly. With the increment of Cd applied, all samples died when treated with 100 mg/L Cd after four days culture.

In the present study, the seedling growth was related to the exposure concentration of Cd as well as plant species. The 4-day EC10s and EC50s values for root growth are listed in Table 3. The EC50s-root in mg/L Cd solution for the tested plant species were in the range 4.39–8.06, the lowest EC50 was observed with tomato and the highest with cucumber. Among the tested plants, the sensitive endpoint appeared in the order of tomato > carrot ≈ lettuce > cucumber according to the ECx measured.

3.2. Effect of NPs suspensions on root growth

Seedling root growth is a rapid and widely used acute phytotoxicity test with several advantages: sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Lin and Xing, 2007). In order to evaluate the effects of different NPs on the root growth of tested plants, the concentrations of 0, 100, 500, 1000, 2000 mg/L of the nanoscale Kaolin, MMT, HAP, Fe_3O_4, α-Fe_2O_3 and γ-Fe_2O_3 suspensions were adopted in this investigation. The highest addition level of 2000 mg/L was selected because those NPs have been reported with minimal phytotoxicity on tested plants if they have no negative effect on root growth at such a high concentration according to the USEPA guidelines (USEPA, 1996). It is worth noting here that few studies have been done using these NPs in phytotoxicity test. Therefore, we hypothesized that the tested species would respond differently to these NPs, and responses among the species did, in fact, vary differently (Fig. 1). The results indicated that Kaolin suspension had no obvious phytotoxicity on all tested plants; MMT suspension had no obvious effect on cucumber and carrot, but promoted the root growth of tomato and lettuce; HAP suspension had also no obvious effect on cucumber and carrot, but, promoted the root growth of lettuce, and retarded root length of tomato at a higher concentrations. In the case of oxide NPs, Fe_3O_4, α-Fe_2O_3 and γ-Fe_2O_3, did not show significant difference ($p<0.05$) from the controls (DI-water) except that the seedling root growth of lettuce was promoted by α-Fe_2O_3 and γ-Fe_2O_3 (Fig. 1).

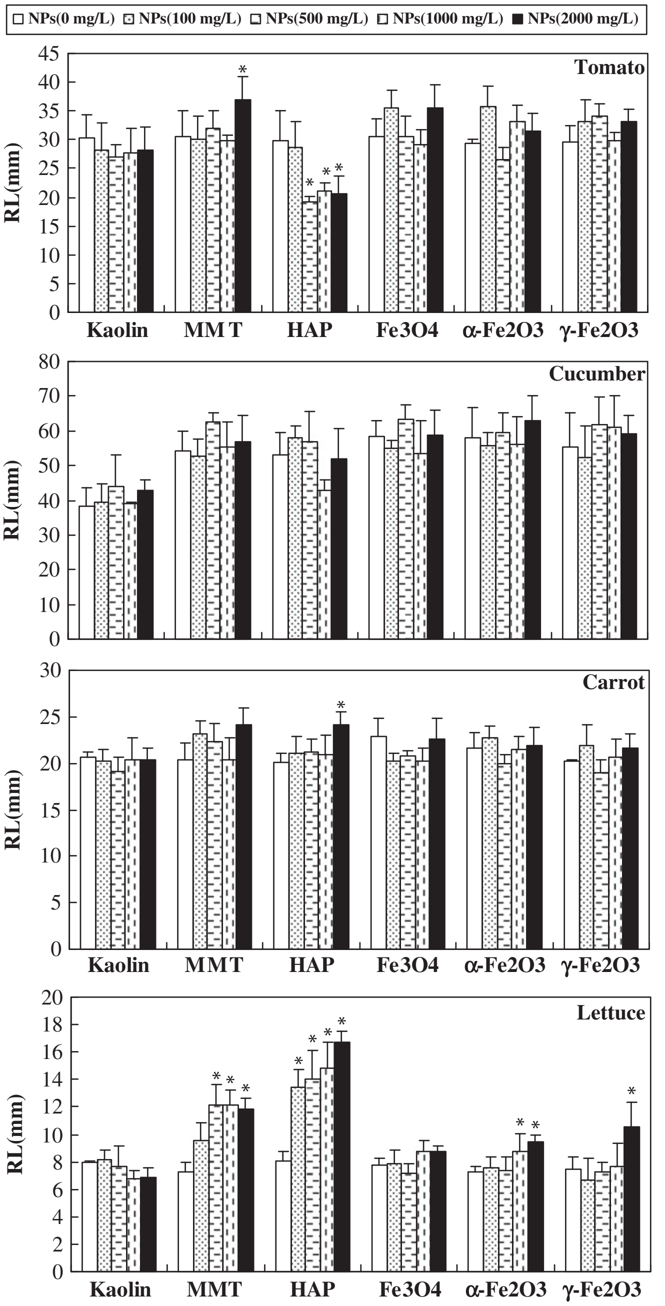

Fig. 1. The root length (RL) of seedlings of four plant species after their exposure for 4 day to 0, 100, 500, 1000, 2000 mg/L NPs. The values were expressed as mean ± SD (standard deviation) of triplicate samples with 10 seedlings each. Significant differences from controls were marked with "asterisk" ($p<0.05$).

3.3. Effect of NPs on the toxicity of cadmium to root growth

In order to examine the effect of NPs on the toxicity reduction of Cd to root growth of tomato, cucumber, carrot, and lettuce,

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a further experiment was conducted based on the result of the toxicity of Cd to root growth of plants (EC50s), in which all plant species received NPs at 0, 100, 500, 1000 and 2000 mg/L, using EC50s (Cd) of each plant as the solvents. The main objective of the present experiment was to test the hypothesis concerning if the NPs can be used as high-efficient and environment-friendly amendments to alleviate the toxicity of Cd to the root growth of the plant species. The result showed that the root growth was all promoted in the presence of the NPs suspensions compared with the controls with one exception for Kaolin treatment, moreover, the seedling length of tested species was positively related to the exposure concentration of NPs (Fig. 2). In detail, MMT suspension had no statistically obvious effect on tomato and cucumber, but promoted the root growth of lettuce and carrot at higher test concentrations (1000 and 2000 mg/L). Similar response was found in HAP suspension test, but HAP had better efficiency than that of MMT. Oxide NPs (Fe_3O_4, α-Fe_2O_3 and γ-Fe_2O_3) had also showed positive significant difference ($p < 0.05$) at test concentrations 500, 1000, 2000 mg/L from the controls (Cd, EC50s) except for the seedlings of cucumber, so obviously oxide NPs displayed the ability to amend the phytotoxicity of Cd to plants. In general, there is an increase in the root growth for NPs mentioned above compared with that of the controls (Cd, EC50s), which indicates that these NPs do alleviate the toxicity of Cd to the growth of the plants.

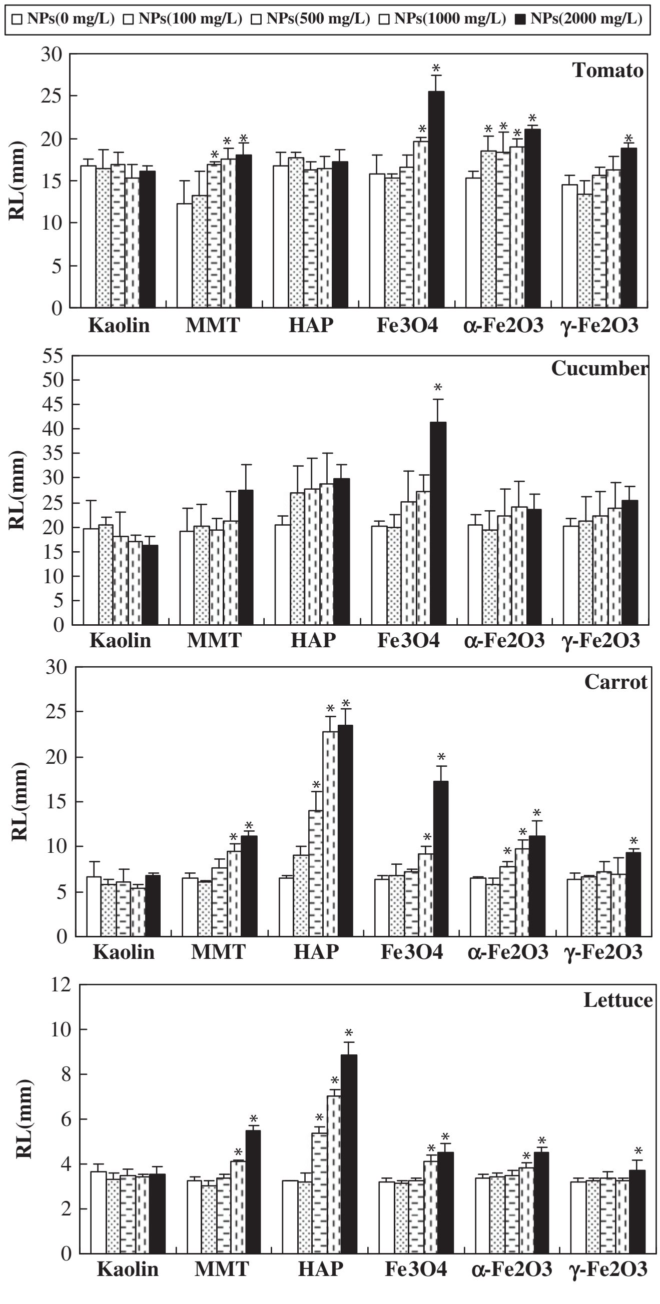

Fig. 2. The root length (RL) of seedlings of four plant species after their exposure for 4 day to 0, 100, 500, 1000, 2000 mg/L NPs prepared with EC50s (Cd) of each plant as the solvents. The values were expressed as mean ± SD (standard deviation) of triplicate samples with 10 seedlings each. Significant differences from controls were marked with "asterisk" ($p < 0.05$).

3.4. Scanning-electron microscopy (SEM)

To study the possible mechanism of Cd-phytotoxicity decrease induced by NPs additives, scanning-electron microscopy micrographs were conducted using root samples taken from HAP, Fe_3O_4 and γ-Fe_2O_3 treatments after their exposure for 4 day to 2000 mg/L NPs prepared with EC50s (Cd) as the solvents. Roots of carrot (Fig. 3) and tomato (Fig. 4) after treated with HAP, Fe_3O_4 and γ-Fe_2O_3, exhibited considerable physiological differences when compared to the controls. Carrot roots exposed to Cd exhibited a decrease in root diameter and root wilt, the clutter root surface showed the evident stress under Cd in solution (Fig. 3A), however, many root hairs on the surfaces of the root system can be observed after the addition of HAP, Fe_3O_4 NPs (Fig. 3B and C). In terms of tomato, the disintegration of the root epidermis was observed on the control plants (Fig. 4A). In roots of treatments with HAP, Fe_3O_4 and γ-Fe_2O_3 NPs (Fig. 4B–D), no disintegration was the most important alteration observed when compared with the controls. Besides of physiological differences, the SEM maps also documented the occurrence of nanoparticle crystal on the carrot and tomato root surface after the addition of NPs. In general, the phytotoxicity will occur if the NPs penetrate cell walls and plasma membranes of epidermal layers in roots to enter vascular tissues (xylem) (Li et al., 2005). However, the pore sizes of plant walls are typically in the range of 3–8 nm, which is much smaller than the tested NPs for this study. The element dispersive spectrometer (EDS) analysis showed that the particles adhered to the root contained a significant amount of FeO, P_2O_5, CaO and CdO (data not shown), indicating the occurrence of precipitation associated with Cd on the root surface, the authors concluded that the precipitation mainly contributed to phytotoxicity reduction by the NPs.

4. Discussion

In this study, the effects of Cd on root growth of different plant species and its relation with NPs were investigated. The dose–response curves showed that the seedling root length of tested species was negatively related to the exposure concentration of Cd in solutions, the response of the plant species to Cd stress differs significantly. Among the tested plants, tomato (*Lycopersicon esculentum*) was the most sensitive endpoint according to the ECx measured. In general, 20–50 mg/L Cd began to cause serious damage to the root growth of the plants.

In higher plants, cadmium ion or its complex compounds are likely to be transported across membranes via nutrient transporters or channels that are not completely selective, which is assumed to enter root cells via either the high affinity uptake system for iron or low affinity system for Ca or Zn uptake (McLaughlin and Singh, 1999; Suzuki, 2005). During the transportation of Cd within the plant from the roots to the shoots, the site of toxic action might be the root (Suzuki, 2005). Cd has been shown to interfere with the uptake, transport and use of several

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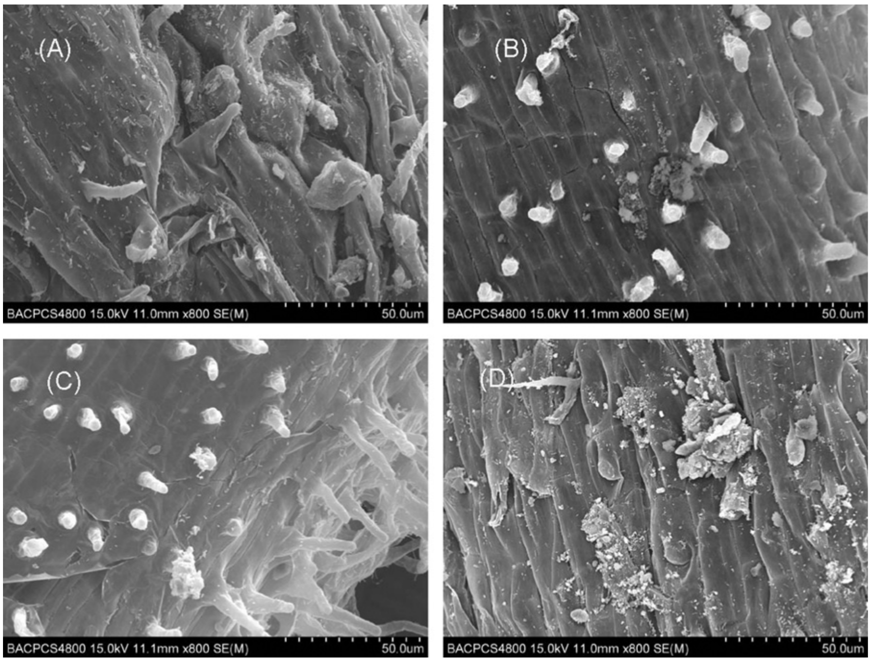

Fig. 3. Root epidermis of carrot (*Daucus carota*) grown in Cd solution with and without 2000 mg/L NPs, observed by scanning electron microscopy. (A) Control (EC50-Cd); (B) treated with HAP; (C) with Fe_3O_4 and (D) with γ-Fe_2O_3, respectively. Bars = 50 μm.

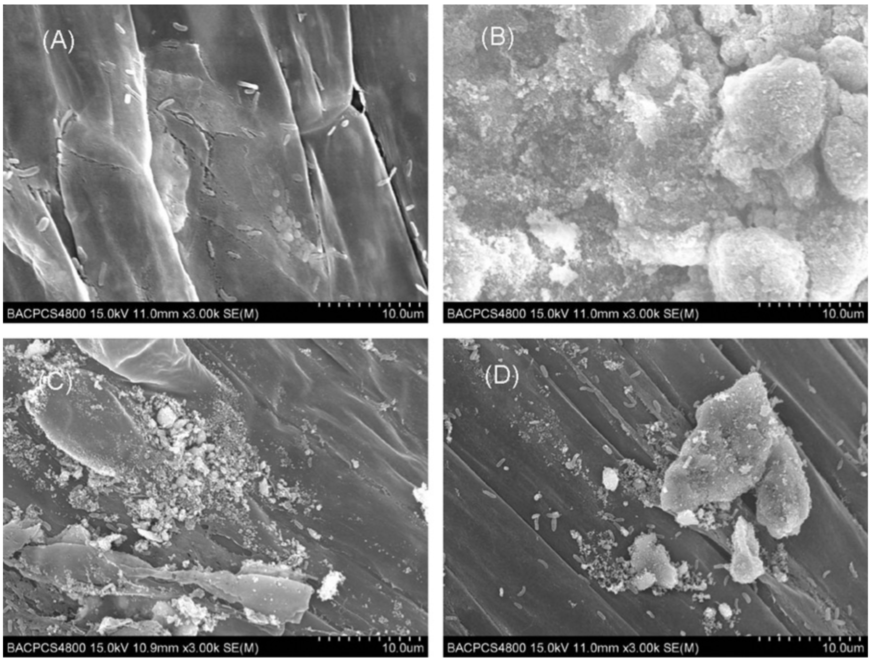

Fig. 4. Root epidermis of tomato (*Lycopersicon esculentum*) grown in Cd solution with and without 2000 mg/L NPs, observed by scanning electron microscopy. (A) Control (EC50-Cd); (B) treated with HAP; (C) with Fe_3O_4 and (D) with γ-Fe_2O_3, respectively. Bars = 10 μm.

nutrition elements (Ca, Mg, P, K, etc.) and water by plants (Das et al., 1997) while the reaction of plants to Cd stress is also firstly expressed in root system (Kollmeier et al., 2000). The rooting test is normally used to assess the effect of chemicals such as cadmium on plants; root growth of plants is particularly sensitive to metals. In the present study, we observed that under the Cd stress in solution, the sensitive endpoint appeared in the order of tomato $>$ carrot $\approx$ lettuce $>$ cucumber according to the ECx measured. A similar phenomenon was observed in a previous study about phytotoxicity of Cd on wheat (*Triticum aestivum L.*) and cucumber (*Cucumis sativus L.*) (Munzuroglu and Geckil, 2002). It is readily apparent that Cd can reduce plant cellular activities; this might be attributed to the generation of oxidative stresses, the inhibition of enzyme reactions, etc.; however, multiple molecular mechanisms may exist since the responsiblity for Cd uptake is very complex among different plant species.

With the aim to alleviate toxicity induced by Cd stress, various amendments have been tested for their potential use in *in-situ* remediation of polluted media. Recently, the development of high-efficient and environment-friendly amendments and their application in decontaminating polluted soils and waters have attracted considerable attention. With the fast development of environmental molecular material science and engineering, the application of nanoscale materials (such as oxide NPs and various

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kinds of clay materials) in decontaminating polluted soils and waters has gained even more extensive research in recent times. However, little information is available about NPs' ecotoxicological effects after discharging into environment, the knowledge gaps and associated uncertainties remain unaddressed on the effects of NPs on higher plants, and some previous studies have been just conducted on a limited number of nanoscale materials, which have shown that NPs can be hazardous, for example, a previous study showed that a mixture of nano-SiO_2 and TiO_2 particles could increase nitrate reductase in soybean, enhanced its abilities of absorbing and utilizing water and fertilizer, stimulated its antioxidant system, and apparently hastened its germination and growth (Lu et al., 2002). In another study, Yang and Watts (2005) investigated the phytotoxicity of alumina NPs (13 nm, coated with and without phenanthrene) on plants and concluded that alumina NPs slowed root growth of corn, cucumber, soybean, cabbage and carrot in a soil-free exposure medium, while coating the alumina NPs with an organic compound (phenanthrene), reduced the NPs' effect of root elongation inhibition, but the authors failed to distinguish toxicity caused by application of the Al in NP form and toxicity of solution aluminum derived from the NPs (Murashov, 2006). Recently, Lin and Xing (2007) examined the toxicity of several NPs (multiwalled carbon nanotubes, Al, Al_2O_3, Zn and ZnO) to germination and early root growth of six plant species of radish, rape, ryegrass, lettuce, corn and cucumber, of the five tested NPs, only Zn and ZnO NPs significantly inhibited seed germination and root growth of the tested plants, while Al NPs promoted the root growth of radish and rape. In this report, we found that the effects of different NPs on root growth of plants varied differently depending on plant species and the used NPs. In general, for the six NPs tested in this experiment, no obvious negative effects were observed except the root length of tomato was significant inhibited after the addition of HAP in the concentrations of 2000 mg/L, in addition, some NPs enhanced root growth such as the effects of MMT and HAP on carrot, α-Fe_2O_3 and γ-Fe_2O_3 on lettuce. Similarly, Cañas et al. (2008) studied the effect of functionalized (with poly-3-aminobenzenesulfonic acid) and non-functionalized single-walled carbon nanotubes on root elongation of six crop species (cabbage, carrot, cucumber, lettuce, onion and tomato) routinely used in phytotoxicity testing, they also found that non-functionalized nanotubes inhibited root elongation of tomato and enhanced root elongation of onion and cucumber, while the functionalized nanotubes inhibited root elongation in lettuce. The present results are in agreement with those of previous phytotoxicity testing on NPs, in that the inhibition and promotion of root length were also observed in the present study using the NPs. However, it is rather worthwhile at this stage to highlight that mechanisms of effect of NPs on seedling root growth remain relatively unknown; it would be probably related to the chemical composition, chemical structure, particle size and surface area of the NPs. These effects may be attributed to three different actions: (1) a chemical action based on the chemical composition, e.g., release of ions; (2) stress or stimulation caused by the surface area, size and/or shape of the particles and (3) the factor of seed size (Brunner et al., 2006; Hoecke et al., 2008). Moreover, in most of previous studies, the investigations only reflect potential effects on root growth under aqueous conditions as an initial indicator of the potential toxicity for NPs to affect plant species, however, more realistic exposures including soil as the growth medium and plants at different life stages, such as the early vegetative growth stage normally used in phytotoxicity testing, will be needed.

In recent years, the utilization of nanoscale metal oxides and natural nanosized clay materials in decontaminating the polluted media have been reported (Ngomsik et al., 2005; Chen et al., 2010). These kinds of NPs as sorbents or amendments of heavy metals in polluted soils and waters are being proposed due to their much larger surface area of NPs on a mass basis, easily be synthesized from cheap raw materials, such as natural phosphate rocks, clay materials, red mud, etc. In addition, the unique structure and electronic properties of some NPs can make them especially powerful adsorbents, for example magnetic NPs (Fe_3O_4 and γ-Fe_2O_3) can easily be separated using a magnetic field, this technique allows one to design processes where the particles not only adsorb heavy metals but also can easily be removed again and then be recycled or regenerated (Nowack, 2008; White et al., 2009). Furthermore, our result has showed that these NPs can be used to alleviate Cd-induced root growth inhibition in crop seedlings, the precipitation associated with Cd on the root surface may contribute to phytotoxicity reduction according to the SEM-EDS analysis, the studies on the effect of NPs on bioavailability of metals to plants would be useful for understanding of potential use of NPs as an environmentally friendly, inexpensive way to remove toxic elements or reduce their toxicity of metals in polluted media.

5. Conclusion

Effects of NPs on Cd-induced root growth inhibition in crop seedlings were investigated; acute toxicity data in Cd solution and NPs suspensions for four crop species were obtained. The results indicated that the seedling growth was negatively related to the exposure concentration of Cd, among the tested plants, the sensitive endpoint appeared in the order of tomato $>$ carrot $\approx$ lettuce $>$ cucumber according to the ECx measured. Root growth of all tested plant species was not significantly inhibited by the presence of NPs except for HAP on tomato, but was promoted in the presence of particular NPs suspensions at higher test concentrations compared with the controls (Cd, EC50s) with one exception for Kaolin, which indicated that the NPs can reduce the phytotoxicity of Cd to the plant species effectively. The mechanisms of Cd phytotoxicity reduction induced by added NPs were also identified with scanning electron microscope (SEM) equipped with elemental dispersive spectrometry (EDS). Microscopy images showed roots of tested plants exposed to Cd exhibited a decrease in root diameter and root wilt, and the disintegration of the root epidermis, the clutter root surface showed the evident stress under Cd solution, after the addition of NPs, many root hairs and no disintegration on the surfaces of the root system can be observed, NPs crystal occurred on the plants root surface. The element dispersive spectrometer (EDS) analysis showed that the particles adhered to the root contained a significant amount of FeO, P_2O_5, CaO and CdO (data not shown), indicating the occurrence of precipitation associated with Cd on the root surface, so the authors concluded that the precipitation mainly contributed to phytotoxicity reduction by the NPs. Results obtained in the present study suggest that NPs have the great potential for reducing the phytotoxicity of Cd without obviously negative impact on the plant growth. Clearly, more studies are needed concerning NPs, heavy metals exposures and the plant growth aspects of the tests, the effect of other physiochemical properties of NPs on phytotoxicity such as particle shape and surface charge also needs further investigation.

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References

Barman, S.C., Sahu, R.K., Bhargava, S.K., Chaterjee, C., 2000. Distribution of metals in wheat, mustard, and weed grown in field irrigated with industrial effluents. Bull. Environ. Contam. Toxicol. 64, 489–496.

Brunner, T.J., Wick, P., Manser, P., Spohn, P., Grass, R.N., Limbach, L.K., Bruinink, A., Stark, W.J., 2006. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ. Sci. Technol. 40, 4374–4381.

Cameron, R.E., 1992. Guide to site and soil description for hazardous waste site characterization. Metals, vol. 1. Environmental Protection Agency EPA/600/4-91/029.

Caňas, J.E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M.X., Ambikapathi, R., Lee, E.H., Olszyk, D., 2008. Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. Environ. Toxicol. Chem. 27 (9), 1922–1931.

Chen, S.B., Ma, Y.B., Huang, Y.Z., 2009. Can phosphate compounds be used to reduce the plant uptake of Pb and resist the Pb stress in Pb-contaminated soils? J. Environ. Sci. 21, 360–365.

Chen, S.B., Xu, M.G., Ma, Y.B., Yang, J.C., 2007. Evaluation of different phosphate amendments on availability of metals in contaminated soil. Ecotoxicol. Environ. Saf. 67, 278–285.

Chen, S.B., Ma, Y.B., Chen, L., Xian, K., 2010. Adsorption of aqueous Cd^{2+}, Pb^{2+}, Cu^{2+} ions by nano-hydroxyapatite: single- and multi-metal competitive adsorption study. Geochem. J. 44, 233–239.

Das, P., Samantaray, S., Rout, G.R., 1997. Studies on cadmium toxicity in plants: a Review. Environ. Pollut. 98 (1), 29–36.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37, 4311–4330.

Dickinson, M., Scott, T.B., 2010. The application of zero-valent iron nanoparticles for the remediation of a uranium-contaminated waste effluent. J. Hazard Mater. 178, 171–179.

Hoecke, K.V., Schamphelaere, K.A.C.D., Meeren, P.V.D., Lucas, S., Janssen, C.R., 2008. Ecotoxicity of silica nanoparticles to the green alga *Pseuaokirchneriella subcapitata*: importance of surface area. Environ. Toxicol. Chem. 27 (9), 1948–1957.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27, 1825–1851.

Kollmeier, M., Felle, H.H., Horst, W.J., 2000. Genotypic difference in Al resistance of maize are expressed in the distal part of the transition zone. Plant Physiol. 122, 945–956.

Li, W.Q., Khan, M.A., Yamaguchi, S., Kamiya, Y., 2005. Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. J. Plant Growth Regul. 46, 45–50.

Lin, D., Xing, B., 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ. Pollut. 150, 243–250.

Lu, C.M., Zhang, C.Y., Wen, J.Q., Wu, G.R., Tao, M.X., 2002. Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism. Soybean Sci. 21, 168–172 (in Chinese).

McLaughlin, M.J., Singh, B.R.E., 1999. Cadmium in Soils and Plants. Kluwer Academic, Publishers, Dordrecht.

Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng. Geol. 60, 193–207.

Munzuroglu, O., Geckil, H., 2002. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl Growth in *Triticum aestivum* and *Cucumis sativus*. Arch. Environ. Contam. Toxicol. 43, 203–213.

Murashov, V., 2006. Comments on "Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles" by Yang, L., Watts, D.J., 2005. Toxicol. Lett. 158, 122–132 (Toxicol. Lett. 164, 185–187).

Nedelkoska, T.V., Doran, P.M., 2000. Characteristics of metal uptake by plants species with potential for phytoremediation and phytomining. Miner. Eng. 13, 549–561.

Ngomsik, A.F., Bee, A., Draye, M., Cote, G., Cabuil, V., 2005. Magnetic nano- and microparticles for metal removal and environmental applications: a review. C. R. Chim. 8, 963–970.

Nowack, B., 2008. Pollution Prevention and Treatment Using Nanotechnology. Nat. Nanotech. 2, 1–15.

Oncel, I., Keles, Y., Ustun, A.S., 2000. Interactive effects of temperature and metal stress on the growth and some biochemical compounds in wheat seedlings. Environ. Pollut. 107, 315–320.

Reddy, K.R., Chinthamreddy, S., 1999. Electrokinetic remediation of heavy metal-contaminated soils under reducing environments. Waste Manage. 19, 269–282.

Reijnders, L., 2006. Cleaner nanotechnology and hazard reduction of manufactured nanoparticles. J. Cleaner. Prod. 14, 124–133.

Suzuki, N., 2005. Alleviation by calcium of cadmium-induced root growth inhibition in *Arabidopsis* seedlings. Plant Biotechnol. J. 22 (1), 19–25.

U.S. Environmental Protection Agency, 1996. Ecological Effects Test Guidelines (OPPTS 850.4200): Seed Germination/Root Elongation Toxicity Test. Available from: ⟨http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-4200.pdf⟩.

White, B.R., Stackhouse, B.T., Holcombe, J.A, 2009. Magnetic γ-Fe_2O_3 nanoparticles coated with poly-l-cysteine for chelation of As(III), Cu(II), Cd(II), Ni(II), Pb(II) and Zn(II). J. Hazard Mater. 161, 848–853.

Yang, L., Watts, D.J., 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol. Lett. 158, 122–132.