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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 ≈ 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.

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