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Effects of rare earth oxide nanoparticles on root elongation of plants

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ABSTRACT

The phytotoxicity of four rare earth oxide nanoparticles, nano-CeO_2, nano-La_2O_3, nano-Gd_2O_3 and nano-Yb_2O_3 on seven higher plant species (radish, rape, tomato, lettuce, wheat, cabbage, and cucumber) were investigated in the present study by means of root elongation experiments. Their effects on root growth varied greatly between different nanoparticles and plant species. A suspension of 2000 mg L^{-1} nano-CeO_2 had no effect on the root elongation of six plants, except lettuce. On the contrary, 2000 mg L^{-1} suspensions of nano-La_2O_3, nano-Gd_2O_3 and nano-Yb_2O_3 severely inhibited the root elongation of all the seven species. Inhibitory effects of nano-La_2O_3, nano-Gd_2O_3, and nano-Yb_2O_3 also differed in the different growth process of plants. For wheat, the inhibition mainly took place during the seed incubation process, while lettuce and rape were inhibited on both seed soaking and incubation process. The fifty percent inhibitory concentrations (IC_{50}) for rape were about 40 mg L^{-1} of nano-La_2O_3, 20 mg L^{-1} of nano-Gd_2O_3, and 70 mg L^{-1} of nano-Yb_2O_3, respectively. In the concentration ranges used in this study, the RE^{3+} ion released from the nanoparticles had negligible effects on the root elongation. These results are helpful in understanding phytotoxicity of rare earth oxide nanoparticles.

1. Introduction

Due to their rapidly increasing applications in various areas of industry and commerce, engineered nanoparticles (NPs) have received much attention recently. The unique properties of these materials, such as a large specific surface area and greater reactivity, have raised questions concerning potential adverse effects on human and environmental health (Oberdörster et al., 2005; Andre Nel et al., 2006; Maynard et al., 2006) To support sustainable development of nanotechnology, possible risk assessment must be evaluated based on sound research to elucidate all relevant aspects of the concern.

NPs may be intentionally put into the soil to facilitate soil and groundwater remediation (Zhang, 2003). Information on the fate of NPs in water and soil is limited, and they might be bioaccumulated through the food chain and finally accumulated in higher-level organisms. Plants are an important component in the ecological system and may serve as a potential pathway for NP transport and a route for bioaccumulation into the food chain (Zhu et al., 2008). Both negative and positive effects of NPs on higher plants have been reported. The first report of negative effects of NPs on several plants (corn, cucumber, soybean, cabbage, and carrot) at relatively low dosage was performed by Yang and Watts (2005). However, the study is problematic as pointed out by Murashov (2006), because the authors did not take into account a fact that soluble Al^{3+} is a potent root toxicant and known to inhibit root growth. The authors warned that care must be taken in toxicity testing when the effects may be related to simple solubility. Lin and Xing (2007) investigated the phytotoxicity of five types of NPs (multi-walled carbon nanotube, aluminium, alumina, zinc and zinc oxide) on seed germination and root growth of six higher plant species. In the root elongation test, all plants were affected when suspended in 2000 mg L^{-1} nano-Zn or nano-ZnO. Lee et al. (2008) adopted a plant agar for homogeneous exposure of NPs. Copper NPs dispersed in plant agar media were toxic to both tested plants, namely wheat and mung bean, and also were bioavailable. The amount of cupric ions released from Cu NPs had negligible effects. Cañas et al. (2008) investigated the effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of six crop species (cabbage, carrot, cucumber, lettuce, onion, and tomato). Both CNTs and fCNTs affected root elongation of four crop species, but phytotoxicity varied between CNTs and fCNTs, with CNTs affecting more species.

On the other hand, there have been reported the positive effects of NPs on plants. For example, several articles (Zheng et al., 2004; Hong et al., 2005; Yang et al., 2007; Gao et al., 2008) have shown that nano-sized TiO_2 can have a positive effect on growth of spinach when administered to the seeds or sprayed onto the leaves. Nano-TiO_2 was shown to increase the activity of several enzymes and to promote the adsorption of nitrate, accelerating the transformation of inorganic into organic nitrogen. Normal-sized TiO_2 does

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not have these effects. In an evaluation on the effect of magnetic NPs coated with tetramethylammonium hydroxide (TMA-OH) on the growth of young popcorn plants, it was found that low concentrations of aqueous ferrofluid stimulated plant growth while high concentrations induced inhibitory or toxic effects (Racuciu and Creanga, 2007).

These researchers have contributed to our understanding of effects on plants for several types of NPs. However, the effects vary depending on the type of NPs, as well as plant species, and are not always consistent between different studies. There are still many unresolved issues and challenges concerning the biological effects of NPs. The types of NPs were chosen according to the extent of their application, rather than systematic studies.

Rare earth elements (REEs) are a series of elements with very similar chemical and physical properties. Most of the REEs, such as La, Gd, and Yb, exist in +3 state. But cerium also occurs in a +4 state and may interexchange between the two states in a redox reaction. Rare earth oxide (REO) NPs generally have magnetic, catalytic, and optic properties and have been widely used in paint coating, polishing powder, automobile exhaust catalysts, and so on (Kosynkin et al., 2000; Fu et al., 2003; Gordon et al., 2004; Karakoti et al., 2008). REO NPs could be released into the environment from various application routes, but their effects on the ecosystem are still unknown.

In the present study, nano-CeO_2, nano-La_2O_3, nano-Gd_2O_3, and nano-Yb_2O_3 were selected as the test materials. In CeO_2, the apparent chemical valence of Ce is +4. In the other three rare earth oxides, the chemical valence of RE is +3. Moreover, La, Gd, and Yb represent light, medium, and heavy REEs, respectively. From La to Yb, the increase of atomic mass is accompanied by a decrease of ionic radii. The phytotoxicity of the four rare earth oxide nanoparticles was investigated by means of root elongation experiments. The different effects between REO NPs and plant species were compared.

2. Materials and methods

2.1. Chemicals

Nano-La_2O_3 and nano-Gd_2O_3 were purchased from Sigma Chemical Co. and used as received. Nano-CeO_2 and nano-Yb_2O_3 were synthesized in our laboratory. Other chemicals are analytical grade and purchased from Beijing Chemical Plant (China).

2.2. Synthesis of NPs

Cerium oxide NPs used in the experiments were synthesized as the method described by Schubert (2006). Ytterbium oxide NPs were synthesized using a precipitation method. Briefly, a solution of 0.064 M $Yb(NO_3)_3 \cdot 6H_2O$ was dropped into 0.256 M ammonia with stirring. Hydrogen peroxide (35%) was added to disperse the product, the molar ratio of hydrogen peroxide and ammonia was 4:1. The mixture was stirred for 1 h and then the reaction was halted. The precipitates were collected by centrifugation and washed with deionized water and ethanol for three times, separately. The cleaned precipitates were dried in an oven at 110 °C and sintered at 650 °C for 3 h.

2.3. Characterization of NPs

The NPs were dispersed in deionized water and sonicated for more than 15 min before dropped on a Cu grid for TEM observation. The TEM images were obtained with Tecnai G^2 20 S-Twin transmission electron microscope (FEI Company, Japan) operated at 200 keV. Certain concentrations of the four types of NPs were dispersed in deionized water and sonicated for 30 min, and then their size distributions of them were analyzed with dynamic light scattering (DLS), using a Coulter NicompTM 380 ZLS Particle Size Analyzer (Santa Barbara, CA, USA).

2.4. Seeds

Seeds of seven plant species: *Brassica napus* (rape), *Raphanus sativus* (radish), *Triticum aestivum* (wheat), *Lactuca sativa* (lettuce), *Brassica oleracea* (cabbage), *Lycopersicon esculentum* (tomato), and *Cucumis sativus* (cucumber), were purchased from the Chinese Academy of Agricultural Sciences. Four of the plant species (lettuce, cabbage, tomato, and cucumber) are among the 10 species recommended by US EPA (1996) for phytotoxicity, and the other three (radish, rape, and wheat) are commonly used in phytotoxicity studies. The average germination rates of all plant seeds were greater than 90% as shown by a preliminary study. Seeds were kept in a refrigerator (4 °C) until use.

2.5. Preparation of particle suspensions and rare earth ion solution

The NPs were suspended directly in deionized water and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. Rare earth ion (RE^{3+}) solution was prepared by dissolving $LaCl_3 \cdot 6H_2O$, $Gd(NO_3)_3 \cdot 6H_2O$ or $Yb(NO_3)_3 \cdot 6H_2O$ in deionized water. RE^{3+} concentrations in the supernatants of the NP suspensions after centrifugation (11 000 rpm for 30 min) and filtration (0.45 μm filter) were determined by a colorimetric method (Funan, 2000).

2.6. Seed germination and exposure

Seeds were immersed in a 10% sodium hypochlorite solution for 10 min and then rinsed three times with deionized water to ensure surface sterility. Then the seeds were soaked in deionized water or NP suspensions solution for about 2 h. One piece of filter paper was put into each 100 mm × 15 mm Petri dish, and 5 mL of a test medium was added. Seeds were then transferred onto the filter paper, with 10 seeds per dish and 1 cm or larger distance between each seed (Yang and Watts, 2005). Petri dishes were covered and sealed with tape, and placed in the dark in a growth chamber at 25 °C. After 5 d, more than 90% of the control seeds had germinated and the roots were at least 20 mm or longer. Then, the germination was halted, and seedling root length was measured by a millimeter ruler.

To examine the different effects between seed soaking and incubation process on the root elongation, we chose three treatments as described in a previous study (Lin and Xing, 2007). Treatment I, seeds were soaked in 2000 mg L^{-1} nanoparticle suspensions for 2 h, and were then rinsed with deionized water for three times and incubated in deionized water; Treatment II, seeds were incubated in 2000 mg L^{-1} nanoparticle suspensions after being soaked in deionized water for 2 h; and Treatment III, both seed soaking and incubation were performed in 2000 mg L^{-1} nanoparticle suspensions.

2.7. Data analysis

Each treatment was performed in triplicate, and the results were expressed as mean ± SD (standard deviation). Statistical differences of experimental data were examined by the Student's *t*-test. Each of experimental values was compared with its corresponding control. All the statistical analysis was implemented using SPSS 10.0 (SPSS Inc., Chicago, USA). Significant difference