Phytotoxicity of Metal Oxide Nanoparticles is Related to Both Dissolved Metals Ions and Adsorption of Particles on Seed Surfaces

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Abstract

This study assesses the biological effects of nanoparticles (NPs) based on seed germination and root elongation tests. Lettuce, radish and cucumber seeds were incubated with various metal oxide NPs (CuO, NiO, TiO2, Fe2O3, Co3O4), of which only CuO and NiO showed deleterious impacts on the activities of all three seeds. The measured EC50 for seed germinations were: lettuce seed (NiO: 28 mg/L; CuO: 13 mg/L), radish seed (NiO: 401 mg/L; CuO: 398 mg/L), and cucumber seed (NiO: 175 mg/L; CuO: 228 mg/L). Phytotoxicity of TiO2, Fe2O3 and Co3O4 to the tested seeds was not significant, while Co3O4 NP solution (5 g/L) was shown to inhibit root elongation of radish seedling. Metal oxide NPs tended to adsorb on seed surfaces in the aqueous medium and released metal ions near the seeds. Therefore, metal oxide NPs had higher phytotoxicity than free metal ions of the equivalent concentrations. Further, the surface area-to-volume ratio of seeds may also affect NPs phytotoxicity, whereby small seeds (i.e., lettuce) were the most sensitive to CuO and NiO NPs in our experiments.

Keywords: CuO; EC50; Metal ions; NiO; Root elongation; Seed germination

Introduction

As applications for metal oxide nanoparticles (NPs) are employed by industry, the release of nanomaterials into the environment may pose severe threats for ecological systems and human health [1-4]. Risk assessments of nano-toxicities have already attracted public attention [1]. Toxic effects of NPs on microorganisms and animals have also been reported [4-10], where metal oxide nanoparticles are the most extensively studied. Their toxicities are attributed to three mechanisms: 1. Generation of reactive oxygen species (ROS), which can damage the cell membrane; 2. Penetration of nanoparticles into the cell where they interfere with intracellular metabolism (a nano-Trojan horse type mechanism) [11]; 3. Release of metal ions that hinder enzyme functions. Moreover, the phytotoxicity profile of NPs has also been investigated by researchers via seed germination and root elongation tests which evaluate the acute effects of NPs on plant physiologies [12]. For instance, alumina and zinc oxide NPs have been applied to different plant species [13,14]. Inhibition of seed germination and root elongation has been found to be highly dependent on both plant type and NP properties. This paper explores the impacts of additional metal oxide NPs on seed activities. In particular, we investigate three common vegetable seeds after they were incubated in aqueous NP-containing solutions: lettuce (Lactuca sativa) seed (length/width: 3 mm/1 mm); radish (Raphanus sativus) seed (length/width: 3 mm/3 mm) and cucumber (Cucumis sativus) seed (length/width: 8 mm/6 mm). This work aims to increase understanding of both NPs phytotoxicity on various edible plants and the potential impact of NPs on agricultural processes [15,16].

Materials and Methods

Chemicals

All chemicals used were reagent grade and purchased from Sigma (St. Louis, MO, US) or Fisher (Pittsburg, PA, US). TiO2 NPs (30-50 nm), Fe2O3 NPs (20-40 nm), CuO NPs (30-50 nm), NiO NPs (30 nm) and Co3O4 NPs (10-30 nm) were obtained from Nanostructured & Amorphous Materials, Inc. (Houston, TX, US). The pH of germination solutions (containing deionized water and NP suspensions) was adjusted to 7 for all toxicity studies done in aqueous phases.

Seed germination and root elongation assay

All seeds in this study were purchased from Ferry-Morse Seed Co. (Fulton, KY, US): Lettuce (Black Seeded Simpson, 3236); Radish (Icicle Short Top, 3236); Cucumber (Marketmore 76, 2646). All three species are commonly used for phytotoxicity tests [17-19]. Seeds were first sterilized by soaking them in 3% H2O2 solution for 1 min and then rinsing twice with deionized water (dH2O). Then seeds were placed in dH2O (control) or certain NP solutions and shaken gently for two-hours [13]. All seeds were subsequently transferred into 15 mm × 100 mm Petri dishes containing one piece of filter paper (90 mm in diameter, Whatman No.1). 10 seeds of radish or cucumber and 15 seeds of lettuce were evenly spaced on top of the filter paper in each Petri dish. The dishes were filled with 5 ml of dH2O or NP solutions and sealed by parafilm tape before being incubated at 25°C in dark conditions [20,21]. After 3 days of incubation, the root length of each seedling was measured. Experimental procedures are summarized in Figure 1. Root length greater than 1 cm for lettuce seeding and 2 cm for
Data analysis

Three parameters were adopted in this analysis to evaluate the conditions of seed germination: Relative germination rate, Germination Index and EC_{so}. Value. They were calculated based on the following equations according to previous reports [22,23]:

Relative germination rate = \frac{Seeds germinated in test sample}{Seeds germinated in control} \times 100

Relative root elongation = \frac{Mean root length in test sample}{Mean root length in control} \times 100

Germination Index = \frac{Relative germination rate \times Relative root elongation}{100}

EC_{so} is the effective concentration of a drug/chemical that reaches half of its maximal effects. We employed the USEPA software to analyze the phytotoxicity data (http://www.epa.gov/eerd/stat2.htm#tsk) [19], which calculates EC_{so} values using the Trimmed Spearman-Karber Method [24]. Student’s t-test was performed to determine the variations in root length and germination rate between different treatment and control group. Statistics Toolbox of Matlab (MathWorks, MA, US) was employed to conduct all statistical analyses, and statistically significant was defined at the level of P < 0.05.

Determination of metal ions released from NP suspensions

To measure the concentration of metal ions released from NP solutions, aliquots of all five NP suspensions were drawn after the suspensions were incubated at room temperature for 2 hours. The extracts were centrifuged at 19,000 g for 20 min, and supernatants were collected and filtered with 0.22 μm nylon filters (GE Water & Process Technologies, CT, US). Inductively coupled plasma mass spectroscopy (ICP-MS, Agilent, CA, US) was used to conduct concentration assays of metal ions, and duplicated samples were measured for each condition.

Protocols for scanning electron microscope (SEM) and dynamic lighting scattering (DLS)

Seeds sprayed with NPs or incubated with NP suspensions were dried overnight in a fume hood. They were then coated with gold by a low vacuum sputter coater (SPI supplies, PA, US) prior to image taking. Images of seed surfaces were taken with a scanning electron microscope (SEM) (Nova 2300 FEI, OR, US). Zeta potential of NP suspensions (the electric potential difference between the culture medium and the interfacial layer of fluid attached to the NPs) was determined by dynamic lighting scattering (Malvern Instruments, Worcestershire, UK) after 30 minutes of incubation in room temperature.

Results and Discussion

The toxicities of different metal oxide NPs at various concentrations on lettuce, radish, and cucumber seeds were tested. Seeds incubated in DI_{2}O (pH=7) were considered as the control upon which all statistical analysis was performed. From results shown in Table 1 and Figure 2, CuO and NiO NPs were far more toxic than the other tested NPs on
all three species of seeds, while lettuce seeds were the most sensitive to NPs in terms of germination. Our results showed that the toxicities of the NPs were also dependent upon the plant species, which was in accordance with a previous report [13]. The relative toxicities based on the germination index (combined seed germination and root elongation) for the tested NPs were listed below:

Lettuce: CuO > NiO >> Fe₂O₃, TiO₂, Co₃O₄
Radish: NiO = CuO >> Fe₂O₃, TiO₂, Co₃O₄
Cucumber: NiO > CuO >> Fe₂O₃, TiO₂, Co₃O₄

The measured EC₅₀ in this study were: lettuce seed (NiO: 28 mg/L; CuO: 13 mg/L), radish seed (NiO: 401 mg/L; CuO: 398 mg/L), and cucumber seed (NiO: 175 mg/L; CuO: 228 mg/L). Interestingly, Co₃O₄ NP solution did not inhibit the germination of cucumber seeds and even improved root elongation of radish seedling at high concentrations (5 g/L). Previous studies have provided similar reports of the positive effects of NPs on germination and growth of plants. For example, TiO₂ and SiO₂ NPs were found to enhance both the germination and growth of Glycine max seeds [25], carbon nanotubes (CNT) were discovered to improve germination and root elongation of tomato seeds [26], and Nano-Al were shown to augment root elongation of radish and rape seedling [13]. Such observations are likely due to an increased water uptake by seeds in the presence of high concentrations of NPs [27].

The biological effects of NPs in aqueous solutions are closely associated to the concentration of released metal ions [6,28]. In this study, we measured the concentrations of metal ions released from all five types of NPs. We did not detect any metal ions released from TiO₂ NP solution, while Fe₂O₃ and Co₃O₄ NPs both released trace metal ions. For example, the aqueous solution with Co₃O₄ NPs contained ~2 mg/L cobalt ion, but its inhibition of seed activity was minimal. Similarly, both Cu and Ni ions were released from the metal oxide NPs during incubation with the seeds (Table 2). To compare phytotoxicity between metal ions and NPs, we assessed seed activity in copper chloride and...
Effects of CuO NPs on seed germination and root elongation

For example, a 13 mg/L CuO NP solution was able to strongly inhibit lettuce seed germination, while the free Cu²⁺ concentration in the culture medium was only ~0.2 mg/L. Therefore, the phytotoxicity of metal oxide NPs is not only due to their dissolved metals ions, but also to their interactions with the seed/root surface.

It has been widely accepted that smaller NPs would have higher surface energy and thus prove more toxic to the cell [29]. However, metal oxide NPs often agglomerate in the aqueous phase to minimize surface energy, and disaggregating is difficult [13,14]. The actual size of our tested NPs in the aqueous solution was therefore up to 1 micrometer due to agglomeration (Table 3 and Figure 3). Previous studies reported that increasing the size of particle aggregates would reduce the toxic effect of the metal oxide particles [13,14]. On the other hand, suspended metal oxide NPs tend to coagulate (Table 3) because of their low zeta potentials in water solution (e.g., 1000 mg/L of CuO NPs: ~ -23.5 mV, determined by DLS) [27]. The phytotoxicity in our tests was not likely caused by mono-dispersed NPs. Instead, we observed that a large amount of NPs (e.g., TiO₂ or CuO) adsorbed on the surface of the seeds/roots in all experiments (Figure 3). The main factors contributing to such adsorption can be physical attachment of particles on a rough seed surface, electrostatic attraction and hydrophobic interactions between seeds and NP agglomerates. For example, variations in the lipid content/the wax of the seed coat would affect the strength of hydrophobic interactions between seeds and NP agglomerates. The adsorption of NPs on the seed surface could generate locally concentrated ions (released from NPs) and enhance NP phytotoxicity. Such adsorption of NPs on the seed’s surface also explains why small-size lettuce seeds are particularly sensitive to NP phytotoxicity: due to the relatively high ratio of surface area to volume, more toxic NPs per unit volume can be adsorbed on the seed surface [29,33]. Figure 4 shows the germination of lettuce seeds was more seriously inhibited by CuO NPs than other larger sized seeds (radish and cucumber seeds).

In conclusion, our experiments determined the impact of five different nanoparticles on common plant seeds. It was discovered that smaller sized seeds, such as lettuce seeds, are more sensitive to toxic NPs. Additionally, this study shows that engineered metal oxide nanoparticles may hold significant potential applications in agriculture and gardening, as they may selectively inhibit unwanted plants (such as weeds), kill harmful fungi and bacteria in plant fields, and release essential metal elements for plant growth.

Acknowledgments

We appreciate valuable suggestions given by Dr. Hui Wei from the University of Illinois at Urbana-Champaign. This research was supported by National Research Foundation of Korea (2011-0026754) through the Ministry of Education, Science and Technology given to I. C. Kong. This research was also supported by Washington University MAGEEP program.

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