Toxicity and transfer of CuO Nanoparticles on *Arabidopsis thaliana*

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Abstract. CuO engineered nanoparticles (ENPs) are widely used in commercial applications. With increasing CuO ENPs production, CuO ENPs are likely to present in the environment and cause a potential threat to ecosystem. In this work, *Arabidopsis thaliana* (Bay-0) was chosen to take the toxic experiment after exposed to CuO ENPs (0, 20, and 50 mg/L) and Cu²⁺ (0.15 mg/L). And the copper content of shoots at 50 mg/L CuO ENPs was about 20 times of control, indicating that CuO ENPs could be absorbed into *Arabidopsis thaliana* seedlings and transferred from root to shoot in a certain way.

1. Introduction

Recently, the implications of Engineered Nanoparticles (ENPs) have been incorporated into diverse commercial products [1]. With their growing production and usage, ENPs will be increasingly released into the environment including into water and soils [2]. CuO engineered nanoparticles (NPs) as one of the most important ENPs have a mixing characteristic of metal materials and nano materials [3]. As producers, plants play a very important role in the cycle of environmental ecosystems [4]. Therefore, it is of great scientific significance to study the toxic effects of ENPs on plants. And *Arabidopsis thaliana* is a model plant for its completion of genome sequencing projects. In many studies, *Arabidopsis thaliana* was used to study the toxic effects of ENPs on plants, such as Au NPs [5]. However, the effects of CuO ENPs on plant growth and absorption are not clear. So the aim in this work was to study the toxic effect of CuO ENPs on growth and absorption of *Arabidopsis thaliana*.

2. Materials and methods

2.1 Materials

CuO ENPs were purchased from Beijing Nachen S&T Ltd. And seeds of *Arabidopsis thaliana* (Bay-0) were provided by Prof. Xing at Agricultural University of Hebei, China.

2.2 Seedling culture system

The seeds were surface-sterilized by 8% sodium hypochlorite alcohol solution for 6~8 minutes, then rewashed by alcohol for 5 times. The seeds were sowed on a sterile 1/2MS medium, sealed with a sealed membrane, and then put into the light incubator. Incubator is set to 14h for light and 10h for dark, light intensity is about 6000lx, temperature 24/22 ℃. After 10 days growing in incubator, choose the same
size of growing seedlings into hydroponics culture for 4 days, then move seedlings in CuO ENPs solutions (0, 20 and 50mg/L CuO ENPs, 0.15 mg/L Cu$^{2+}$) for 14d.

2.3 Determination of Copper Concentration in Seedlings

Plants were harvested in triplicate and roots and shoots were separated for determination of fresh mass. Roots were rinsed with deionized water at first, then soak roots for 15min with Na$_2$-EDTA solution at a concentration of 20 mmol/L and then rinse thoroughly with deionized water to remove ions and CuO ENPs adsorbed on the root surface[6]. These samples were then dried in a 70 °C for 24h to remove the water content. The dry tissues were milled into powder, then facilitate acid digestion as reported[7]. Finally, the copper content in the samples were analyzed with an Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (Thermo, M6).

2.4 Data analysis

Statistical analysis was analyzed by Microsoft Excel 2016 and SPSS18.0, using a one-way analysis of variance (ANOVA) and compared with LSD test. All treatments included three replicates, and standard error was reported (p<0.05).

3. Results and discussion

3.1 Characterization of CuO ENPs

The basic properties of CuO ENPs and their suspensions were characterized in this experiment.

Characterization of CuO ENPs was analyzed by transmission electron microscopy (TEM JEM-2100, Japan). The diameters of CuO ENPs were 20-40 nm obtained from TEM imaging (Fig. 3-1).

![Fig. 3-1 TEM image of CuO ENPs in this study. Scale bar: 50 nm.](image)

The hydraulic radius of CuO ENPs is 378.1 nm (Fig. 3-2) in the solution culture system in this experiment.
3.2 Effects of CuO ENPs on Biomass of Arabidopsis thaliana Seedlings

The change curve of the fresh weight was shown in Fig. 3-3. Under the treatment of CuO ENPs, the fresh weight of *Arabidopsis thaliana* seedlings increased slowly. The fresh weight of shoots and roots in seedlings were declined with the increasing of concentration of CuO ENPs increasing, leading to the inhibition of the growth. And it was obvious that the inhibitory effect of 50 mg/L CuO ENPs on root was significantly higher than that of 20 mg/L CuO ENPs treatment, while 0.15 mg/L Cu$^{2+}$ had little effect on fresh weight of seedling roots. After 4-day exposure, the root fresh weight of *Arabidopsis thaliana* treated with 50 mg/L CuO ENPs was significantly different and decreased by 36% compared with the control on the second day after treatment.

3.3 The transfer of CuO ENPs in Arabidopsis Seedlings

The content of copper in the roots of *Arabidopsis thaliana* was determined as 50 mg/L CuO ENPs$>$ 20 mg/L CuO ENPs$>$ 0.15 mg/L Cu$^{2+}$ $>$ Deionized water control (Figure 3-4), with copper content of 2.22,
1.08, 0.53 and 0.10mg/g, respectively. The copper contents of *Arabidopsis thaliana* seedlings roots at 20 mg/L CuO ENPs and 0.15 mg/L Cu²⁺ were higher than those of deionized water, but the total copper content at 50 mg/L CuO ENPs was the highest. Indicating that CuO ENPs can enter the roots of *Arabidopsis thaliana* seedlings in some way.

![Fig.3-4 Cu content in Root/Shoot of *Arabidopsis thaliana* after exposed to CuO ENPs.](image)

**4. Conclusions**

The effects of CuO ENPs on the *Arabidopsis thaliana* growth were studied by biomass, absorption and transfer. The results showed that CuO ENPs treatment can lead to a decrease in plant fresh weight and inhibit plant growth. Besides, plants under the treatment of CuO ENPs had a significant increase in total copper content in the shoots and roots of *Arabidopsis thaliana* seedlings, and the copper content of shoots at 50 mg/L CuO ENPs was about 20 times of control, indicating that CuO ENPs could be absorbed into the *Arabidopsis thaliana* seedlings and transfer from root to shoot in a certain way.

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