Toxicity of CuO nanoparticles on the root of Arabidopsis thaliana

Lina Xu*[1], Mou Yu[2], Jing Zhao[3]

1.College of Environmental Science and Engineering, Ocean University of China, Qingdao,Shandong, China

2.Sichuan Mianzhu Economic Development Zone,Mianzhu,Sichuan,China

3.Qingdao TianyeGenyuan Real Estate Development Co., Ltd，Qingdao,Shandong，China

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Abstract. CuOnanoparticles (NPs) are widely used in commercial applications. With increasing CuO NPs production, CuO NPs are likely to present in the environment and pose a potential threaten to ecosystem. In this work, 20 and 50 mg/L CuO NPs and 0.15 mg/L Cu^{2+} were used to test the toxicity to *Arabidopsis thaliana* roots. Results showed that CuO NPs can inhibit the root dry weight androot water content at 96 h. ROS and MDA content were increased after exposure to CuO NPs for 96 h. Cu^{2+} released from CuO NPs exhibited no toxicity on the *Arabidopsis thaliana* roots.

1INTRODUCTION

With the rapid development and versatile application of nanotechnology, a large quantity of nanoparticles (NPs) has been released into the environment. Due to the novel physicochemical properties such as tiny size, large specific surface area and abundant reactive sites on the surface, NPs could be interaction with organisms and pose a potential threaten to organisms even to the whole ecosystem(Handy et al., 2008). CuO NPs as one of the most important engineered NPs has a dual characteristic of metal materials and nano materials (Ivasket al., 2010). CuO NPs are widely used in catalysts, gas sensor, heat transfer fluids, semiconductors, photovoltaic cells, and so on (Wang et al., 2012). Many available data showed that metal oxide NPs could cause inhibition on plant (Dimkpa et al., 2012).Ma et al. (2010) observedthat rare earth oxide nanoparticles, suchas CeO2、La2O3、Gd2O3、Yb2O3 inhibited the root elongation of plants. Lee et al.(2010) reported that ZnO NPs had the more toxicity than Al2O3、SiO2、Fe3O4 on the generation of Arabidopsis thaliana, owing to the Zn2+ released from ZnO NPs. However, the toxicity of CuO NPs on the Arabidopsis thaliana rootsis still unknown. The main objective of this study was to investigate the toxicity and the related toxic mechanism of CuO NPs to Arabidopsis thaliana roots.

2 MATERIALS AND METHODS

2.1 Plant growth and growth inhibition test

CuO NPs and CuO bulk particles (BPs) were purchased from Beijing Nachen S&T Ltd. Seeds ofArabidopsisecotypes (Col-0) were provided by Prof. Xing at Agricultural University of Hebei, China. The seeds were surface-sterilized by 75% alcohol and then placed in the agar-containing Murashige and Skoog (MS) medium for germination. After 10-day, the seedlings in the stage of four leaves were moved to dicotyledonous nutrient solution. After additional 3-day culture, the seedlings were moved to distilled water-prepared CuO NPs (20 and 50 mg/L), CuO BPs (50 mg/L) suspensions or Cu2+ ions solution (0.15 mg/L), respectively. “0.15 mg/L” was selected as the test Cu2+ concentration based on the dissolution kinetics of CuO NPs in distilled water. The root dry weight was measured by micro balance (MX/UMX, Mettler Toledo). Root water content (%) = (M1-M2)/M1, where M1 and M2 are the root fresh weight and root dry weight, respectively.

2.2 Detection of root ROS and MDA

The generation of reactive oxygen species (ROS) in roots was measured by the DCF method. In brief, after exposure to CuO NPs for 96 h, the ROS in roots was examined with 2’, 7’-dichlorofluoresceindiacetate (H2DCF-DA, Beyotime, China). The fluorescence intensities were

determined using a fluorescence microscopy (Olympus IX70). The lipid peroxides was quantized in terms of the Methane Dicarboxylic Aldehyde (MDA) content, the MDA were measured by the thiobarbituric acid reactive substance (TBARS) assay (Vavilin et al., 1998).

2.3 Statistical analysis

Statistical analysis was analyzed 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 NPs and CuO BPs

Characterization of CuO NPs and CuO BPs were analyzed by transmission electron microscopy (TEM JEM-2100, Japan). The diameters of CuO NPs and CuO BPs were 20-40 nm and 1500 nm obtained from TEM imaging.

Table 1. Characterization of CuO NPs and CuO BPs

Particle	Diameter (nm)
50 mg/LCuO NPs	20-40
50 mg/LCuO BPs	1500

3.2 Inhibition of CuO NPs on root growth

CuO NPs at both 20 mg/L and 50 mg/L significantly inhibited the root dry weight of Col-0seedlings compared to the unexposed control, Cu2+ ion (0.15 mg/L) and CuO BPs (50 mg/L) treatments after 96 h exposure(Figure 1A).The observed growth inhibition of CuO NPs on seedlings was exposure time-dependent. The seedling roots exposed to 50 mg/LCuO NPs for 96 h, root water content was decreased significantly (Figure 1B).

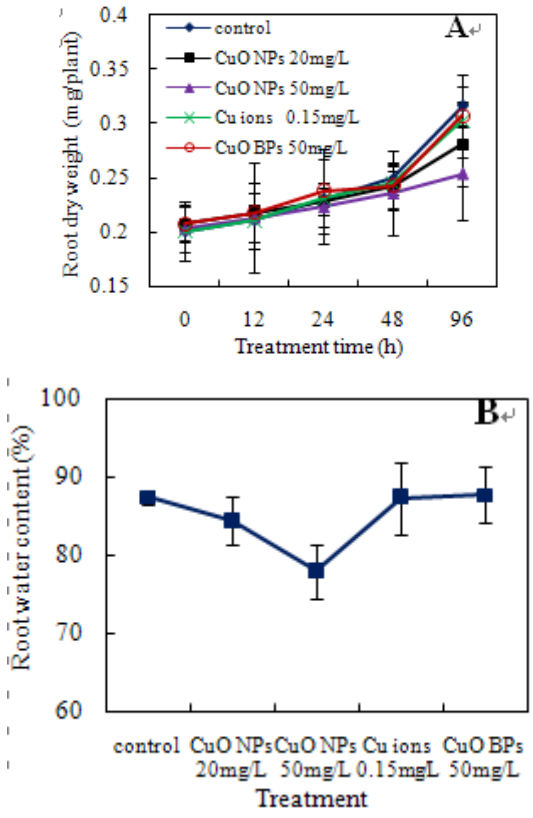

Figure 1. The inhibition of CuO NPs on the root dry weight at different treatment time (A) androot water content at 96 h (B) of Col-0 seedlings. Col-0 seedlings were cultured for 96 h in the distilled water with control, 20 mg/LCuO NPs, 50 mg/LCuO NPs, 0.15 mg/L Cu^{2+}ions or 50 mg/LCuO BPs.

3.3 Oxidative stress

ROS was accumulated after exposed to CuO NPs for 24 h and 96 h, The ROS level in Col-0 roots exposure into CuO NPs showed both time and dose dependent. Exposed to 50 mg/L CuO NPs for 96 h, the ROS level in the roots raised 1.56 times compared with control.

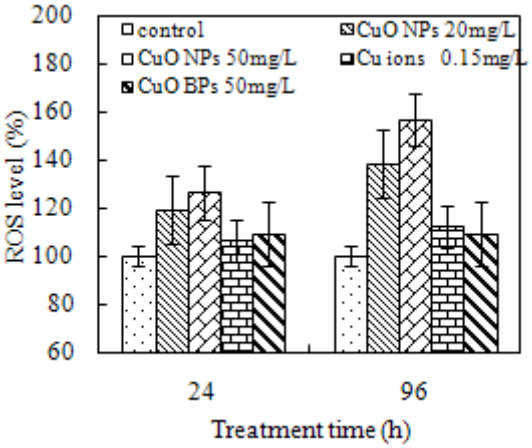

Figure 2. The ROS level in Col-0 roots after exposure to control, 20 mg/L CuO NPs, 50 mg/LCuO NPs, 0.15 mg/L Cu^{2+} ions and 50 mg/LCuO BPs for 24 h and 96 h. For a given treatment time, significant difference among different treatments compared control was marked with "*".

3.4 Lipid peroxidation

Lipid peroxidation in cell membrane caused by oxidative stress could result in the cell damage and even the plant died. As shown in figure 3, there are more MDA contents in roots treated by 20 and 50 mg/LCuO NPs than treatments without CuO NPs within 96 h. However, the content of MDA was not significantly increase treated by Cu2+ and CuO BPs.

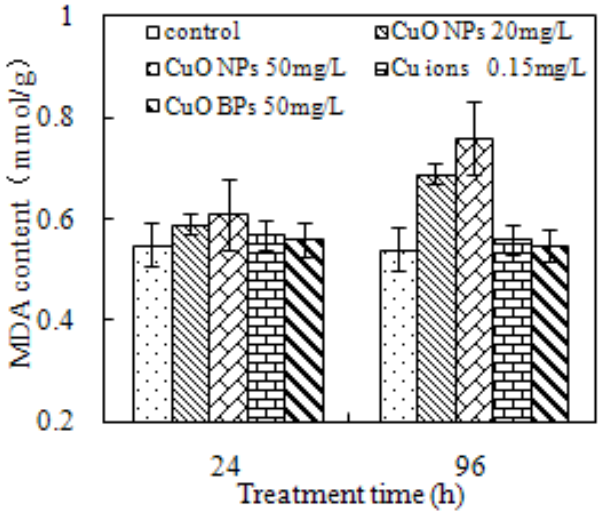

Figure 3. The MDA content in Col-0 roots after exposure to control, 20 mg/L CuO NPs, 50 mg/L CuO NPs, 0.15 mg/L Cu^{2+} ions and 50 mg/LCuO BPs for 24 h and 96 h. For a given treatment time, significant difference among different treatments compared control was marked with "*".

4 CONCLUSIONS

The results of this study demonstrated that toxicity of CuO NPs to Col-0 roots is time-effect relationship and dose-effect relationship. CuO BPsandCu2+ released from CuO NPs exhibited no toxicity on the Arabidopsis thaliana roots. Oxidative stress is responsible for the toxicity of CuO NPs.

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