



Quantifying the adsorption and uptake of CuO nanoparticles by wheat root based on chemical extractions

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Abstract

Extensive application of metal nanoparticles is attracting more attention because of their potential environmental risks. Many studies have focused on the uptake of metal nanoparticles (NPs) by plant, but the adsorption of nanoparticles on root surface is often mistakenly regarded as their uptake. This study optimized the methods to distinguish the adsorption and uptake of CuO-NPs on the wheat root by applying different metal competing ions (Na^+ , Mg^{2+} , and La^{3+}), surfactant (i.e., sodium dodecyl benzene sulfonate, SDBS), or complexing agents like NaOAc and Na_4EDTA , as well as ultrasonic technique. The results indicated that some CuO-NPs is strongly adsorbed on the plant root surface, and part of them by mechanical adhesion. Competing ions could not desorb the CuO-NPs from the root surface, while NaOAc and Na_4EDTA well dissolved the adsorbed CuO-NPs. In addition, the uptake and adsorption of CuO-NPs increased with increasing exposure concentrations of CuO-NPs in the range of 5–200 mg/L. The amount of CuO-NPs adsorption is always lower than that of their uptake.

Key words: CuO-NPs; root surface; adsorption; uptake

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Introduction

Along with the rapid development of nanotechnology, metal nanoparticles are widely used in electronic, pharmaceutical, cosmetic, chemical catalyst and new materials development fields (Nowack and Bucheli, 2007). Thus, they will inevitably enter into the air, water and soil, which cause environmental risks. Their security to organisms has caused increasing attention in recent years. Metal nanoparticles with high specific surface and surface reactivity cannot only be easily adsorbed on the general physical interface (Wang et al., 2005), but also react with biological proteins, and are even absorbed to cells. Nanometer cerium oxide can react with bovine serum albumin, and be absorbed by lung adenocarcinoma cells (Patil et al., 2007). In addition, Lin and Xing (2008) found that root surface adsorbed ZnO-NPs, at the same time the uptake of ZnO-NPs occurred. However, the effective quantitative analysis of their adsorption and uptake is deficient. They adopted tap and deionized water for washing the root of *Lolium perenne* seedlings after exposed to ZnO-NPs, and determined the Zn content in the seedlings as the content of Zn uptake. The adsorbed ZnO-NPs could not be totally desorbed from the root surface by such method, so that the

ZnO content in the seedlings was overestimated. Therefore, it is necessary to develop a method to distinguish the uptake and adsorption of metal nanoparticles on the plant root surface.

The interaction of nanomaterials with organisms in general can be roughly divided into mechanical adhesion, electrostatic and biological interactions. Nanoparticles have been proven to be interacted with the ligands on the surface of animal cells (Gubin and Kataeva, 2006), and the combined effect has been used for specific targeting medicine (Chavanpatil et al., 2006). But, it is still worth investigating if the coordination effect of nanomaterials exists on plant root surface, or machinery adhesion or electrostatic adsorption of nanomaterials occurs with the plant root.

To distinguish the adsorption and uptake of CuO-NPs on plant root surface, this study chose different valent cations (Na^+ , Mg^{2+} , and La^{3+}), surfactant (sodium dodecyl benzene sulfonate, SDBS), metal complexing agents like NaOAc and Na_4EDTA for desorbing CuO-NPs. Different cationic and anionic surfactants were used based on their effects on the surface potential of CuO-NPs, which could be expected for identifying the electrostatic interaction of metal nanoparticles with plant roots. Due to strong complexation with heavy metal ions, EDTA has been

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widely used in eluting the adsorbed metal ions from solid and biological surface (Hassler et al., 2004), but it is still unclear whether metal nanoparticles can be eluted by EDTA from plant root surface. The aim of this study is to screen out eluants for efficiently desorbing metal nanoparticles from plant root surface, and distinguish the proportions of CuO-NPs uptake and adsorption.

1 Materials and methods

1.1 Nanomaterials and chemical reagents

Nano copper oxide (CuO-NPs) was purchased from Nanjing Emperor Nano Materials Co. Ltd., China with purity > 99.8%. Its original particle sizes ranged from 25 to 80 nm, with average particle size of 55 nm, and specific surface area of 23.9 m²/g. The specific morphological feature of the CuO-NPs is examined by transmission electron microscopy (TEM) (H-7650, Hitachi, Japan), as shown in Fig. 1. No preprocessing was applied before use.

1.2 Tested plant

Wheat (*Triticum aestivum* L.), as adopted in the OECD (2003) toxicity testing standards, was used to study the adsorption and uptake of CuO-NPs, which was purchased from Shenzhou Seeds Company, Nanjing Agricultural University, China. Its variety was Nannong 9918.

1.3 Wheat seedling cultivation and exposure to CuO-NPs

Full and uniform wheat seeds were disinfected with 0.3% NaClO for 10 min, and then were washed with deionized water for 30 min. After that, the sterilized seeds were planted in the perlite saturated with nutrient medium, with the embedding depth about 1 cm from surface to ensure the normal growth of wheat (Li et al., 2011). Then the wheat seedlings were incubated in an incubator (MLR-351H, Sanyo Corporation, Japan) at constant temperature.

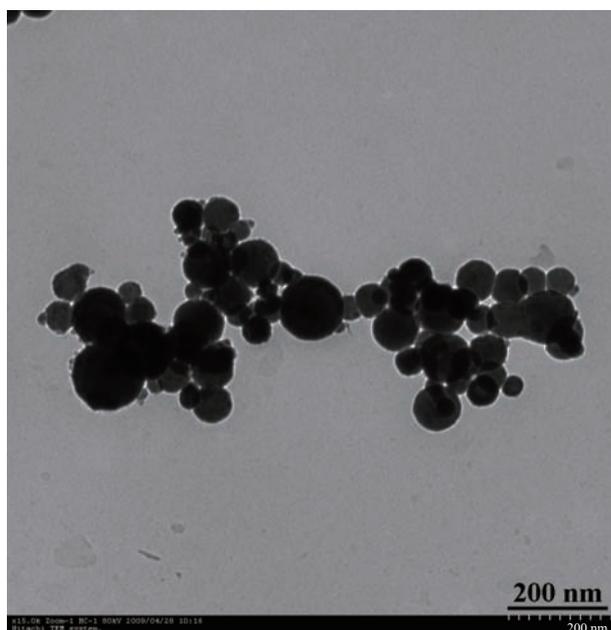


Fig. 1 TEM image of CuO nanoparticles.

The incubator temperature was maintained at (25±1)°C, and incubation time was 48 hr. During the tests nutrient solution was supplied due to water lose.

Dispersions of CuO-NPs were prepared by adding 2.0% of normal melting agarose (Sigma-Aldrich, USA) and a suitable amount of NPs to deionized (DI) water, heated up to 80°C. The dispersions were sufficiently shaken after sonication for 1 hr to break up agglomerates. The agarose medium was then poured into plastic containers. After cooling down and solidifying, the agar gel was cut into small cubes (2 mm). Each NP treatment concentration was prepared separately, without dilution, by weighing particles and dispersing them in agar. CuO-NPs (5, 10, 50, 100 and 200 mg/kg) were prepared in three replicate test units per treatment. Agar media without NPs was used as the negative control. Each test unit contained 1000 mL agar with a specific CuO NP concentration.

After the wheat seedlings grew for 48 hr, they were planted in the above agar gels containing 20 or 200 mg/L CuO-NPs suspension. The setting temperature was (25±1)°C. During the experiment, the suspension was mixed with glass rod for every 8 hr, according to the method introduced by Lin and Xing (2008). The dissolution of CuO-NPs is limited, and only 0.76 mg/L Cu ions were observed with CuO NP concentration of 200 mg/kg in the agar media (Jin et al., 2010a, 2010b). Thereby, it means that the adsorption and uptake of Cu by wheat seedlings were mainly ascribed to the CuO-NPs (Li et al., 2011).

1.4 Desorption of CuO-NPs with different chemical eluants

Elution test of CuO-NPs from root surface: NaCl, MgCl₂, LaCl₃, NaOAc, SDBS, or Na₄EDTA at 0.1, 1.0, or 10.0 mmol/L was respectively diluted with deionized water, and used as desorbing solutions. The solution pH was adjusted to 6.0. The wheat seedlings after exposed to CuO-NPs were washed with deionized water for three times at first, and then ultrasonically treated with the above eluants. After that, the wheat seedlings were taken out, washed with water, and the water solution was also collected. These desorbed solutions were put altogether, and then digested with concentrated HNO₃. The solution Cu concentration was measured with Z-2000, Hitachi Corporation, Japan.

1.5 Effect of different chemical eluants on the surface zeta potential of CuO-NPs

CaCl₂, MgSO₄, NaCl, and KCl were used to prepare the solution containing 0.20 mmol/L Ca²⁺, 0.05 mmol/L Mg²⁺, 2.5 mmol/L Na⁺ and 0.08 mmol/L K⁺. The solution pH was adjusted to 6.0 with 2.0 mmol/L 2-(N-morpholino) ethanesulfonic acid (MES) buffer and diluted NaOH. The amount of CuO-NPs was added to 10 mmol/L NaCl, MgCl₂, LaCl₃, NaOAc, SDBS, or Na₄EDTA. The concentration of CuO-NPs was 200 mg/L, and deionized water was used as control. After ultrasonic dispersing of the CuO-NPs suspension at 45 Hz and 150 W for 30 min, the suspension was adjusted to pH 6.0 with NaOH and HCl, and stood for 3–5 days at room temperature. The surface zeta potentials of CuO-NPs were measured with micro

electrophoresis apparatus (JS94G+, Zhongchen Corporation, China).

1.6 Effect of shaking and ultrasonic on the desorption of CuO-NPs from root surface

After the wheat seedlings were exposed to 20 or 200 mg/L CuO-NPs for 24 hr, they were washed with DI water and treated by shaking (120 r/min, 30 min) or ultrasonic (45 Hz, 300 W, 30 min) for comparison. In addition, the seedlings exposed to 200 mg/L CuO-NPs were ultrasonically washed at different frequencies or powers (45 Hz, 150 W; 45 Hz, 300 W; and 100 Hz, 150 W) for 30 min in the presence of EDTA. Finally, two pieces of wheat roots were randomly selected without and with dilution, and were vacuum freeze-dried. Root meristems with 0.5 mm length were cut for the observation of CuO-NPs on root surface with Scanning Electron Microscopy (Quanta 200, the Netherlands) and X-ray Spectrum (INCA Energy 250 X, Oxford, England).

1.7 Uptake of CuO-NPs by wheat root

After thorough desorption of the CuO-NPs from root surface, the wheat root was collected, dried, and digested with $\text{HNO}_3 + \text{H}_2\text{O}_2$. Standard wheat samples were used for guaranteeing the analytical accuracy. Copper concentration in the root was measured with Z-2000.

1.8 Statistical analysis

All experiments were performed in triplicates. The data included in this study are presented as the mean \pm SD, and the differences between groups were tested for significance using one-way analysis of variance (ANOVA) at $p < 0.05$.

2 Results

2.1 Desorption of CuO-NPs from wheat root surface with different elution agents

Figure 2 shows the desorbed amounts of CuO-NPs from the root surface with the different elution agents. It is clear that large amounts of CuO-NPs was adsorbed on the wheat

root surface, and increased with the increasing exposure concentrations of CuO-NPs. At the CuO-NPs exposure concentration of 20 mg/L, the desorption of CuO-NPs from root surface did not show any difference as desorbed with cations Na^+ , Mg^{2+} , La^{3+} , and SDBS. Instead, the two kinds of organic elution agents, NaOAc and Na_4EDTA , significantly desorbed the CuO-NPs from the wheat root. A more effective desorption was observed for Na_4EDTA than NaOAc, and the desorption amount of CuO-NPs also increased with increasing Na_4EDTA concentrations. The desorption percentage of CuO-NPs from root surface increased 22.4%, 64.3% and 87.2% in the presence of 0.1, 1.0 and 10 mmol/L Na_4EDTA in comparison with the control, respectively.

As exposed to 200 mg/L CuO-NPs, the results are similar with those exposed to 20 mg/L CuO-NPs. The desorption percentage of CuO-NPs from root surface increased 23.8%, 68.7% and 118.5% with 0.1, 1.0 and 10 mmol/L Na_4EDTA in comparison with the control, respectively. Therefore, it can be concluded that 10 mmol/L Na_4EDTA could be screened as an ideal elution agent for desorbing CuO-NPs from root surface.

2.2 Zeta potential of CuO-NPs surface as affected by different eluants

Figure 3 shows that 10 mmol/L of different cations increased the surface positive charges of CuO-NPs. It also indicated that this effect was more effective for the cations with higher valence, while the organic eluants did not change the surface electronegativity. For example, 10 mmol/L NaOAc did not influence the zeta potential of CuO-NPs. SDBS increased the electronegativity of CuO-NPs, meaning that the desorption of CuO-NPs from root surface is not related with the change of surface charges of CuO-NPs as affected by the eluants.

2.3 Effects of shaking and ultrasonic on the desorption of CuO-NPs from root surface

Figure 4 shows the effects of shaking and ultrasonic processing on desorbing CuO-NPs from wheat root surface. Both shaking and ultrasonic increased the desorption of

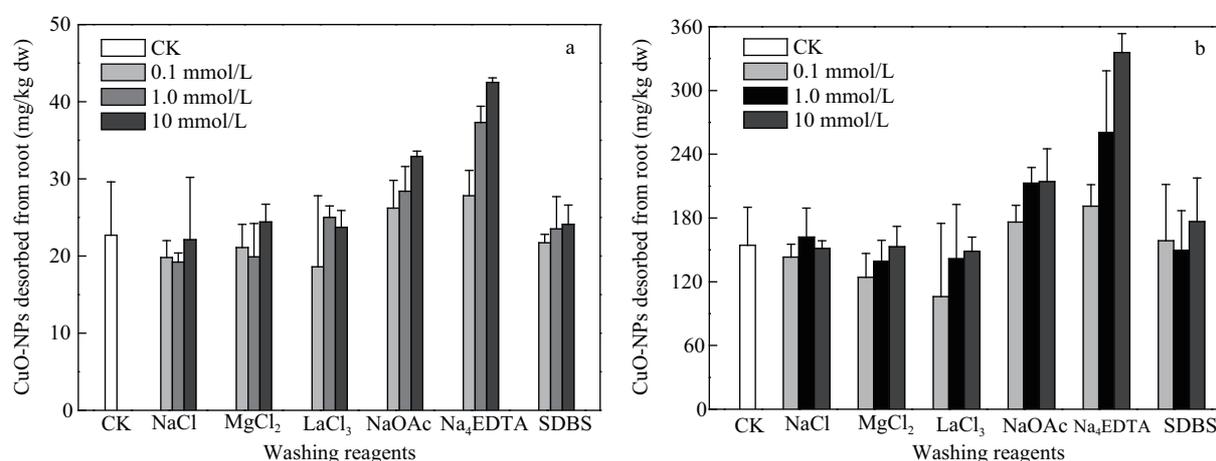


Fig. 2 Effect of washing reagents on desorbing CuO-NPs from wheat root after pre-exposure to 20 mg/L (a) and 200 mg/L (b) of CuO-NPs. dw: dry weight.

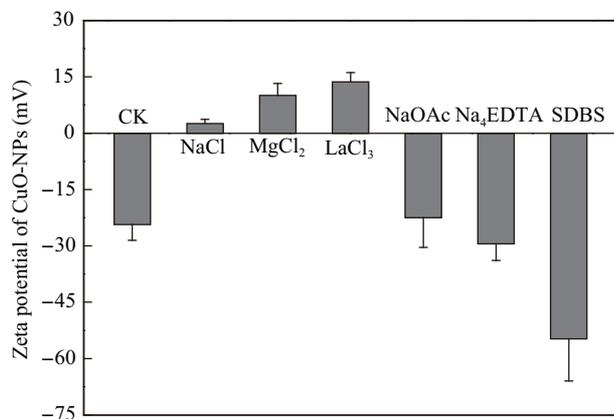


Fig. 3 Effect of washing reagents (10 mmol/L, pH 6.0) on zeta potential of CuO-NPs.

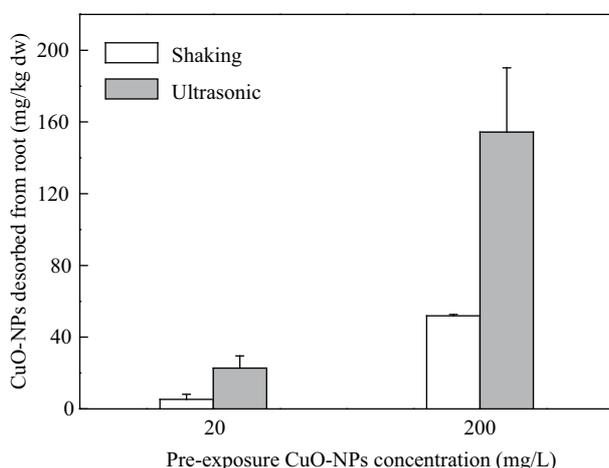


Fig. 4 Effects of shaking and ultrasonic on desorbing CuO-NPs from wheat root surface.

CuO-NPs from root surface, especially for the latter.

2.4 Effect of ultrasonic conditions on the desorption of CuO-NPs from wheat root surface

Besides the screened eluant of 10 mmol/L Na₄EDTA, the effects of ultrasonic power and frequency on the desorption of CuO-NPs (pre-exposure concentration: 200 mg/L) from wheat root surface were studied, as shown in Fig. 5. In comparison with the ultrasonic treatment at 45 Hz and 150 W for 30 min, increasing the ultrasonic power to 300 W facilitated the desorption of CuO-NPs, but increasing the ultrasonic frequency had no effect. It suggests that ultrasonic power is more efficient to desorb CuO-NPs from wheat root surface than ultrasonic frequency.

Figure 6 shows the result of SEM-EDS of wheat root surface after exposed to 200 mg/L CuO-NPs and subsequently washed with 10 mmol/L Na₄EDTA combined with ultrasonic at 45 Hz and 300 W for 30 min. Large amounts of CuO-NPs adsorbed on the wheat root surface, and Cu content was even higher than Mg and Si. After washing with EDTA and application of ultrasonic, CuO-NPs were totally desorbed, and no Cu peak was observed. It means that washing with 10 mmol/L Na₄EDTA and using ultrasonic could efficiently desorb all the adsorbed CuO-NPs from wheat root surface, and distinguish the adsorption and uptake of CuO-NPs by the wheat root.

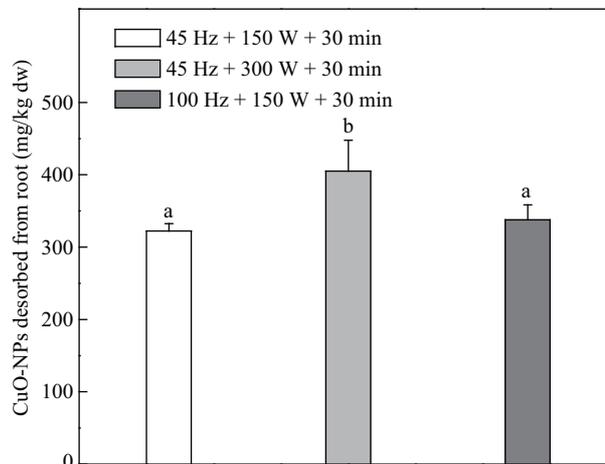


Fig. 5 Effect of different ultrasonic treatments on desorbing CuO-NPs (pre-exposure conc.: 200 mg/L) from wheat root with 10 mmol/L Na₄EDTA. a, b mean that the data have significant difference at $P < 0.5$.

2.5 Adsorption and uptake of CuO-NPs by wheat root exposed to different concentrations of CuO-NPs

Following the above developed method, the adsorption and uptake of CuO-NPs by wheat root as exposed to 5–200 mg/L of CuO-NPs were examined. Table 1 shows that both the adsorption and uptake of CuO-NPs based on dry weight by wheat root increased with increasing CuO-NPs concentrations. The uptake increased 8.2 times, and adsorption increased 11.5 times with the exposure concentration of CuO-NPs increased from 5 to 200 mg/L. In addition, the ratio of uptake to adsorption increased with elevated CuO-NPs concentrations in the range of 5–50 mg/L, which means that the kinetic uptake is higher than the adsorption. But, with further increased CuO-NPs, the ratio decreased, suggesting that the kinetic uptake is slower than the adsorption.

Table 1 Uptake and adsorption of CuO-NPs by wheat root

CuO-NPs concentration (mg/L)	CuO-NPs uptake (mg/kg dw)	CuO-NPs adsorption (mg/kg dw)	Uptake /adsorption
5	27.1±2.0	14.6±1.9	1.9±0.3
10	45.2±1.8	21.3±4.2	2.1±0.3
50	147.0±6.3	59.0±2.4	2.5±0.2
100	212.0±9.4	109.0±17.4	1.9±0.2
200	250.0±23.2	183.0±5.1	1.4±0.1

3 Discussion

In general, the adsorption of granular material on biological surface mainly includes three major mechanisms: electrostatic interactions, machinery adhesion, and biological complexation. Our results show that although the examined different valent cations (Na⁺, Mg²⁺, and La³⁺) and SDBS affected the surface charges of CuO-NPs, the change had little correlation with the desorption of CuO-NPs from root surface. It means that no matter what change of surface charges occur, that would not affect the des-

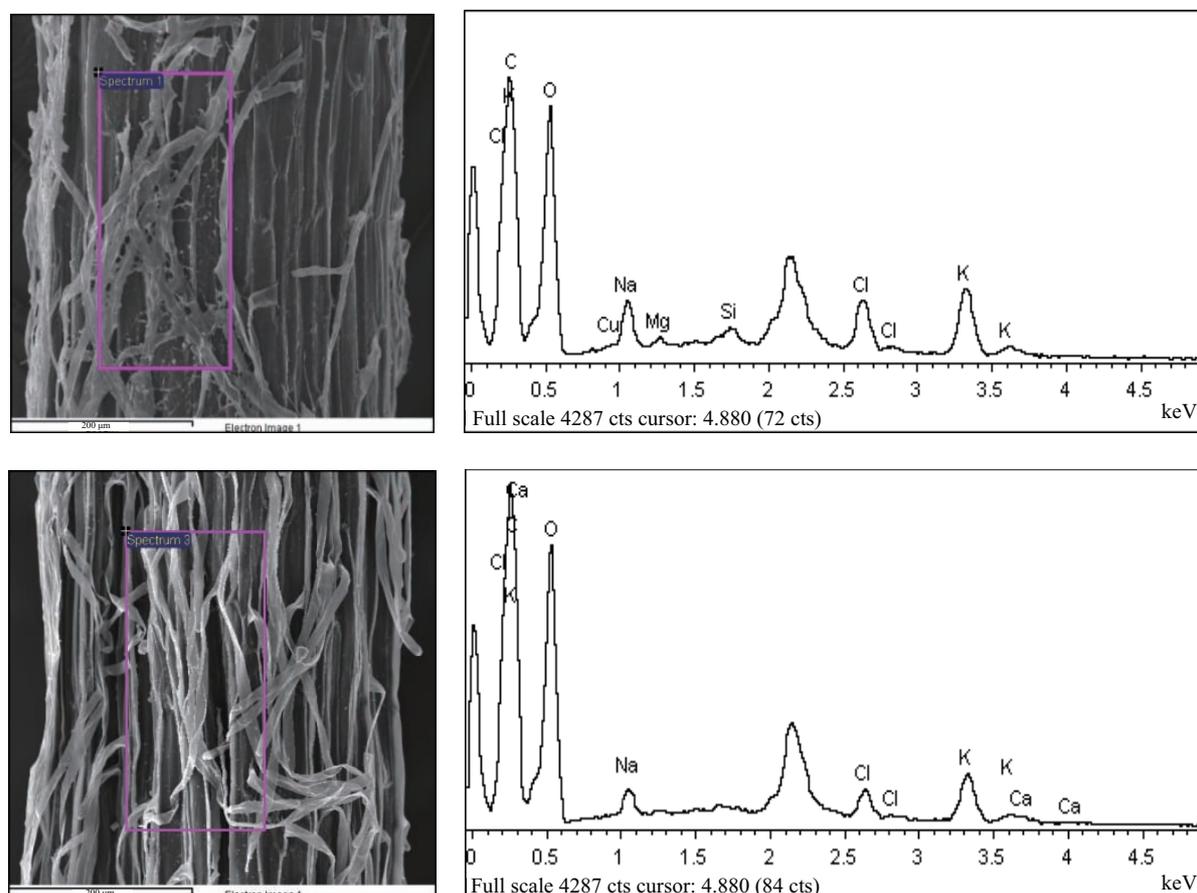


Fig. 6 SEM image and EDS of wheat root surface after exposure to 200 mg/L CuO-NPs (above) and then washed with 10 mmol/L Na₄EDTA and ultrasonic (bottom).

orption of CuO-NPs from wheat root surface. NaOAc and Na₄EDTA had no significant effect on the zeta potential of CuO-NPs, but obviously increased their desorption. Thus, it can be concluded that electrostatic interaction of CuO-NPs on the wheat root surface can be neglected.

Figure 4 shows that whatever the adsorption amount of CuO-NPs on wheat root surface, shaking the root with only DI water results in only part of adsorbed CuO-NPs desorbed from the wheat root surface. It implied that some of CuO-NPs were mechanically adhered with wheat root surface. The desorption of CuO-NPs from root surface can significantly be enhanced through the unique cavitation of ultrasound treatment, which revealed a strong mechanical adhered part of CuO-NPs on the wheat root surface.

In this study, Na₄EDTA elution of CuO-NPs from wheat root surface is the most powerful eluant (Fig. 2). Combined with ultrasonic at 45 Hz and 300 W for 30 min, the adsorbed CuO-NPs can be completely removed from the wheat root surface (Fig. 6). As we know, Na₄EDTA could form stable and water-soluble complex with metal ions, and it has been used for leaching Cu contaminated soil (Maja and Domen, 2009).

From our study, it was found that the desorption solution became blue after 10 mmol/L Na₄EDTA eluting solution desorbed the adsorbed CuO-NPs from wheat root surface, which indicate that Na₄EDTA accelerated the dissolution of CuO-NPs surface by forming complex with the released Cu²⁺, and finally make CuO-NPs to

be completely dissolved. It is also said that Na₄EDTA had strong complexation with Cu²⁺, so, the equilibrium of CuO-NPs and Cu²⁺ was destroyed, and the dissolution of CuO-NPs to Cu²⁺ enhanced. NaOAc also can desorb part of the adsorbed CuO-NPs from wheat root surface because of its weak complexation compared with Na₄EDTA (Fig. 2). Thus, it was speculated that CuO-NPs were mainly coordinated with the wheat root surface.

Nanoparticles at first was adsorbed to plant root surface, and then transferred into the cell or cell membranes through apoplast, and were finally absorbed. When the nanoparticles exists in a particular area of the biological cells (e.g., mitochondria, vesicles), they can produce certain of the toxic effects (Nel et al., 2006).

This study found that the uptake and absorption of CuO-NPs by wheat root increased with elevated CuO-NPs concentrations of 5–200 mg/L, and the amount of uptake was higher than that of adsorption. However, the uptake/adsorption ratio increased with increased exposure concentration of CuO-NPs at first, and then decreased. After exposure to different concentrations of CuO-NPs, the change of uptake/adsorption of CuO-NPs confirmed and reflected their surface interaction to certain extent. With low CuO-NPs concentration, the CuO-NPs adsorbed at the wheat root surface are easily transported to cells. However, when the exposed CuO-NPs concentration was high, they were easily absorbed on the root surface, and only part of them was transferred into cell.

4 Conclusions

It can be concluded that most of the CuO-NPs that adsorbed on wheat root surface was strongly interacted with the root surface, and part of them was adhered mechanically. Na₄EDTA and NaOAc can desorb the adsorbed CuO-NPs by facilitating their dissolution. With increasing exposure concentration of CuO-NPs, the uptake of CuO-NPs increased, while the absorption/adsorption ratio increased at first, and then decreased, confirming the kinetic surface coordination and transport of CuO-NPs on the wheat root surface.

Acknowledgments

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