Environmental Pollution 197 (2015) 99–107

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Translocation and biotransformation of CuO nanoparticles in rice (*Oryza sativa* L.) plants

Cheng Peng [a], Dechao Duan [a], Chen Xu [a], Yongsheng Chen [b], Lijuan Sun [a], Hai Zhang [a], Xiaofeng Yuan [c], Lirong Zheng [d], Yuanqiang Yang [e], Jianjun Yang [a], Xiangjun Zhen [f], Yingxu Chen [a], Jiyan Shi [a,*]

[a] Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
[b] School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States
[c] College of Life Science, Zhejiang Chinese Medical University, Hangzhou 310053, China
[d] Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[e] Department of Technology, Beijing Construction Engineering Environmental Remediation Co., Ltd., Beijing 100015, China
[f] Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201204, China

ARTICLE INFO

Article history:
Received 16 September 2014
Received in revised form
1 December 2014
Accepted 2 December 2014
Available online 15 December 2014

Keywords:
CuO NPs
Rice
XAFS
XRF
STXM

ABSTRACT

Metal-based nanoparticles (MNPs) may be translocated and biochemically modified *in vivo*, which may influence the fate of MNPs in the environment. Here, synchrotron-based techniques were used to investigate the behavior of CuO NPs in rice plants exposed to 100 mg/L CuO NPs for 14 days. Micro X-ray fluorescence (μ-XRF) and micro X-ray absorption near edge structure (μ-XANES) analysis revealed that CuO NPs moved into the root epidermis, exodermis, and cortex, and they ultimately reached the endodermis but could not easily pass the Casparian strip; however, the formation of lateral roots provided a potential pathway for MNPs to enter the stele. Moreover, bulk-XANES data showed that CuO NPs were transported from the roots to the leaves, and that Cu (II) combined with cysteine, citrate, and phosphate ligands and was even reduced to Cu (I). CuO NPs and Cu-citrate were observed in the root cells using soft X-ray scanning transmission microscopy (STXM).

1. Introduction

Due to unique electrical, magnetic, and catalytic properties (Kennedy et al., 2010; Meulenkamp, 1998), metal-based nanoparticles (MNPs) have extensive applications in sensors, remediation, fertilizers, herbicides, and pesticides which has a large impact on the agricultural industry (Joseph and Morrison, 2006); however, the concern about the safety of MNPs has been raised because of the inevitable release of MNPs into the environment during the process of production, use, and disposal of MNP-related products (Meng et al., 2009). Several studies have confirmed the toxicity of MNPs on microorganisms, animals, plants and human beings (Blinova et al., 2010; Dietz and Herth, 2011; Fahmy et al., 2008; Shoults-Wilson et al., 2011; Wang et al., 2011a; Zhang et al., 2012b). Plants are an essential component in natural ecosystems and play a critical role in providing food sources for animals and human beings. MNPs absorbed by plants may contaminate food and also have potential risks for human health. Therefore, it is important to understand the interactions between plants and MNPs which may alter the behavior and the fate of MNPs in environmental systems (Holbrook et al., 2008; Judy et al., 2011).

Intimate contact between MNPs and roots can lead to the adsorption and absorption of MNPs by roots and the translocation of MNPs in plants (Gardea-Torresdey et al., 2014; Limbach et al., 2010; Wang et al., 2012). Nonetheless, several studies have reported that some MNPs absorbed by roots were not translocated to the shoots (López-Moreno et al., 2010; Miralles et al., 2012). The conflicting results indicate that the interactions between MNPs and plants greatly depend on the type of MNP, the plants species, and the experimental conditions. The transportation of MNPs via an apoplastic or symplastic pathway once inside plants has been

* Corresponding author.
E-mail addresses: zjupcsnow@gmail.com (C. Peng), duandechao111@163.com (D. Duan), smilechenbanban@126.com (C. Xu), yongsheng.chen@ce.gatech.edu (Y. Chen), sunliuliu2012@126.com (L. Sun), zhangh@zhdec.com (H. Zhang), sjyxf.ok@163.com (X. Yuan), zhenglr@ihep.ac.cn (L. Zheng), yangyuanqiang@bceer.com (Y. Yang), youngjianjun.cn@gmail.com (J. Yang), zhenxiangjun@sinap.ac.cn (X. Zhen), yingxuc@gmail.com (Y. Chen), shijiyan@zju.edu.cn (J. Shi).

http://dx.doi.org/10.1016/j.envpol.2014.12.008
0269-7491/

proposed (Rico et al., 2013a). Zhao et al. (2012a) indicated that ZnO NPs penetrated the root epidermis and the cortex, and finally passed the endodermis by fluorescein isothiocyanate (FITC)-labeled ZnO NPs; however, the modification of NPs can affect the physic-chemical properties of NPs, including the solubility and surface charge, which may induce a difference between the stained NPs and the bare NPs in the translocation and transformation of NPs in the plant (López-Moreno et al., 2010; Wang et al., 2013). The confocal detection was only used for the observation of labeled MNPs rather than the bare MNPs, and what is more, it would not provide any information about the biotransformation of the MNPs in the plants. In fact, MNPs may be transformed after being absorbed by plants. The biotransformation process may involve redox, dissolution, sulfidation, aggregation, and the adsorption of macromolecules and molecules/ions; however, the occurrence of the reactions depends on the metallic features, the nanometer–scale interactions of MNPs and plants, and even the mode of exposure (Lowry et al., 2012). For example, CeO_2 NPs kept the original speciation in the soybeans and corn (*Zea mays*) roots (Hernandez-Viezcas et al., 2013; Zhao et al., 2012b) but were transformed into $CePO_4$ in the cucumber (*Cucumis sativus*) roots (Zhang et al., 2012a). In addition, in soybeans and cowpeas (*Vigna unguiculata*) roots, only Zn-nitrate or Zn-acetate forms of Zn were observed after the plants treated with ZnO NPs (Hernandez-Viezcas et al., 2013; López-Moreno et al., 2010; Wang et al., 2013). $Ni(OH)_2$ NPs were transformed into Ni^{2+} in plant shoots but not in roots (Parsons et al., 2010). Thus, the fate of various MNPs in different plants merits further investigation.

As one of the most important metal oxide NPs, CuO NPs are widely used in sensors, catalysts, surfactants, and antimicrobials (Cioffi et al., 2005; Saison et al., 2010). Rice is the most consumed basic food for much of the world's population, particularly in Asia and the West Indies. It cannot be ignored that the translocation and biotransformation of CuO NPs in rice plants could be a means of metal contamination in the food chain (Rico et al., 2013a). Dimkpa et al. (2013) studied the speciation of Cu in the wheat shoot treated with CuO NPs, but knowledge concerning the possible translocation pathway of CuO NPs within the roots of plants and the biotransformation of CuO NPs at organ, tissue, and even cellular levels remains limited. Moreover, the structures and compositions of the plants can induce differences in the transformation of Cu or even affect the translocation of Cu. Compared with wheat, which is planted in dry farmland, rice is an aquatic plant, so it is more vulnerable to contamination from MNPs because a waterlogged condition can promote the mobility, solubility, bioavailability, and toxicity of MNPs. In addition, the specific aerenchyma in rice allows for the exchange of gases, such as oxygen, between the shoot and root so that the rice root can secrete oxygen and form a specific micro-interface in the rhizosphere. All of this information implies that the aquatic environment may strengthen the influence of MNPs on the rice. Thus, in this research, we investigated the translocation pathway of CuO NPs and Cu species in rice plants grown with CuO NPs under hydroponic conditions at the organ, tissue and cellular levels using several synchrotron-based techniques, including bulk-X-ray absorption near edge structure (XANES) spectroscopy, micro X-ray fluorescence (μ-XRF), micro XANES (μ-XANES) spectroscopy, and soft X-ray scanning transmission microscopy (STXM).

2. Materials and methods

2.1. Characterization of CuO particles

CuO NPs (pure, >99.9%, 40 nm) with a spherical shape and a specific surface area of 131 m^2/g were procured from Nachen Sci & Tech Ltd., China. The particle size and morphology of these CuO NPs in deionized water were characterized previously (Shi et al., 2014). In the present study, the hydrodynamic size and zeta potential of these particles in a nutrient solution (see below) at pH 6.0 (Yoshida et al., 1976) were determined using a Zetasizer-Nano ZS instrument (Malvern Instrument Ltd., UK) after a 30 min sonication treatment and a 1 h post-sonication waiting period. After CuO NPs (100 mg/L) were kept in the nutrient solution for 48 h, the actual dissolved Cu concentration of CuO NPs was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP 6300 DUO, Thermo, USA) after being centrifuged at 10,000 rpm for 30 min and being filtered with 0.22 μm glass filters (Wang et al., 2012).

2.2. Plant culture and treatment

Rice (*Oryza sativa* L.) seeds, cv. Xiuyou No. 5, were purchased from the Wuwangnong group in China. Details regarding seed disinfection and germination, transplantation, pretreatment with the CuO NP suspension, and greenhouse conditions were described in a previous study (Shi et al., 2014). In brief, after being sterilized by NaClO, the seeds were placed on moist gauze and germinated for one week in a dark environment, and then were transferred into beakers filled with a half-strength nutrient solution for one week and a full-strength nutrient solution for another week. The nutrient solution with a pH of 6.0 contained the following elements (in ppm): 40 N, 10 P, 40 K, 40 Ca, 40 Mg, 0.5 Mn, 0.05 Mo, 0.2 B, 0.01 Zn, 0.01 Cu, 2 Fe, which were added in the form of NH_4NO_3, $NaH_2PO_4 \cdot 2H_2O$, K_2SO_4, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, H_3BO_3, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $FeCl_3 \cdot 6H_2O$, and citric acid (monohydrate), respectively (Yoshida et al., 1976). Twenty-one-day-old uniform seedlings were treated with 100 mg/L CuO NPs in 500 mL beakers (six seedlings per beaker) containing 400 mL of a full-strength nutrient solution. The control plants were grown in a pure nutrient solution for comparison. The vessels were placed in the growth chamber (60–70% relative humidity, 25 °C for 16 h during the day and at 20 °C for 8 h at night), and the seedlings were allowed to grow further hydroponically for two weeks. All beakers that were covered with tinfoil for light blocking were replenished with a nutrient solution every two days to maintain a constant solution volume.

2.3. Cu content determination

At the end of the exposure period, the seedlings were washed with deionized water to remove any CuO NPs adhered to the roots. The dried plants were ground and digested in a microwave digestion unit (MARS5, CEM Microwave Technology Ltd., USA) using a mixture of HNO_3–H_2O_2 (1:4, V/V), and their Cu content was analyzed using ICP-OES (Rico et al., 2013b).

2.4. Bulk-XANES analysis

For the bulk-XANES analysis, the rice plants were washed, separated, pre-frozen in a −70 °C lab freezer overnight, and lyophilized at −56 °C and 0.280 mbar pressure for 3 days in a freeze-dryer. The dried tissues were then ground to a fine powder, pressed into thin slices, placed in aluminum sample holders, and covered with 3M tape (Scotch 810, 3M, USA). The XANES data at the Cu K-edge of the samples exposed to CuO NPs were recorded on the beamline 14W1 at the Shanghai Synchrotron Radiation Facility (SSRF, Shanghai, China). The storage-ring current during the data acquisition was in the range of 130–210 mA operating at 3.5 GeV. The station was operated with a Si (311) double-crystal monochromator, and the photon energy was calibrated with the first