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Toxic effects of nanomaterial-adsorbed cadmium on *Daphnia magna*

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ABSTRACT

Chemical immobilization technologies involving the use of chemical absorbents such as nanomaterials have been recommended for the remediation of Cd-contaminated water and soil. The impact of nanomaterials or nanomaterials coexisting with other contaminants on aquatic organisms has been reported, but information on the toxic effects of nanomaterial-adsorbed cadmium (Nano-Cd) on aquatic organisms is lacking. This study aimed to investigate the acute and sub-acute toxicity of Nano-Cd on *Daphnia magna* by using a method developed based on the standard Organisation for Economic Co-operation and Development (OECD) 202 guidelines. The toxicity of cadmium chloride (Cd^{2+}), nano-manganese dioxide-cadmium ($nMnO_2$-Cd), 20 nm nano-hydroxyapatite-cadmium ($nHAP_{20}$-Cd), and 40 nm nano-hydroxyapatite-cadmium ($nHAP_{40}$-Cd) to *D. magna* was in the following order: Cd^{2+} > $nMnO_2$-Cd > $nHAP_{20}$-Cd > $nHAP_{40}$-Cd. Further, $nMnO_2$-Cd, $nHAP_{20}$-Cd, and $nHAP_{40}$-Cd showed acute toxicity to *D. magna* of level II grade according to the Commission of the European Communities and OECD standards. Exposure to low and medium, but not high, Nano-Cd concentrations increased the activities of peroxidase, superoxide dismutase, catalase, and anti-superoxide anion. Thus, Nano-Cd, particularly at high concentrations, could exert oxidative damage in *D. magna*. An increase in Cd^{2+} and Nano-Cd concentrations gradually increased the malondialdehyde content, indicating cell membrane damage caused by the production of excessive O_2^-. Thus, the use of nanomaterials after adsorption of Cd is associated with a potential risk to aquatic organisms.

1. Introduction

Heavy metals have become major environmental pollutants that can cause permanent and potential hazard (Feng, 2013). Cadmium (Cd) is a typical heavy metal element that is mainly released from industrial processes, phosphate fertilizers, and zinc extraction. It has been found in the soil, water, and atmosphere. Feng et al. (2010) reported that the concentration of Cd in polluted waters was as high as 4500 μg L^{-1} in China; this level far exceeded the national Cd safety standard of 30 μg L^{-1}. Xie et al. (2012) found that the accumulation index of Cd in the Pearl River sediments was 7.41, and the pollution degree was very strong. The European Union indicated that Cd, the main pollutant in the environment, showed high toxic and carcinogenic effects and could accumulate in organisms via the food chain after long-term exposure at low levels (Zhao et al., 2003), stimulate the respiratory tract of organisms, and damage the liver (Chakraborty et al., 2010) or kidney of animals (Prozialeck and Edwards, 2012).

At present, many technologies and methods are available for removing heavy metals from water and soil environments, such as ion-exchange, chemical precipitation, reverse osmosis, adsorption, and electrodialysis. In particular, chemical immobilization technologies have been recommended because they are effective and economic (Wang et al., 2010). Nanomaterials as adsorbent have a great potential to remediate heavy metal contamination owing to their adsorbent properties (Jin et al., 2016; Wang et al., 2016; Tamez et al., 2015). For example, Huang et al. (2016) reported that $nMnO_2$ could remove Cu^{2+} and Cd^{2+} from aqueous solution, and the sorption maxima was 104.5 and 89.1 mg g^{-1}, respectively. Fernando et al. (2015) found rod nano hydroxyapatite (nHAP) had an adsorption capacity in the range of 138–83 mg g^{-1} for Pb in aqueous solutions.

Although the concentration of pollutants (e.g., heavy metals or organic pollutants) significantly declined by immobilization with nanomaterials, their toxicity could be enhanced in the biomass (Zhu et al., 2008). The presence of nanomaterials significantly increases the absorption of Cu^{2+} by zebrafish larvae and enhances Cu^{2+} toxicity in zebrafish larvae and embryos (Zhang et al., 2013). Nano-TiO_2 exacerbates Cu^{2+} toxicity in *Daphnia magna* (Fan et al., 2011). These studies indicate that interaction with nanomaterials could aggravate the

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adverse effects of heavy metals in organisms.

Previous studies have mainly focused on the effects on aquatic organisms of nanomaterials alone or in combination with other contaminants. However, few studies have investigated the toxic effects of nanomaterials-adsorbed Cd (Nano-Cd) on aquatic organisms. Do such nanomaterials change the morphological characteristics of organisms? Is Nano-Cd safe to aquatic organisms? To answer these questions, the present study was designed to (1) investigate the acute toxicity of nano-manganese dioxide -adsorbed Cd ($nMnO_2$-Cd) and nHAP-adsorbed Cd (nHAP-Cd) on *Daphnia magna*; (2) determine the antioxidant defense responses of *D. magna* to $nMnO_2$-Cd and nHAP-Cd complexes; and (3) elucidate the mechanism of toxicity of these complexes in *D. magna*.

2. Materials and methods

2.1. Chemicals

2.1.1. Nanomaterials

Nano-hydroxyapatite of 20 nm and 40 nm ($nHAP_{20}$ and $nHAP_{40}$; purity, > 99%) was purchased from Nanjing Xian Feng Nanomaterials Technology Ltd. (Nanjing, China). The 20 nm $nMnO_2$ (purity, > 99%) was provided by the Agro-environmental Protection Institute, Ministry of Agriculture, Tianjin, China. Enzyme kits of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), and anti-superoxide anion (ASA) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents were analytical grade.

2.1.2. Reconstituted water

Reconstituted water, used for all exposure experiments, was prepared according to Organisation for Economic Co-operation and Development (OECD) 202 guidelines (OECD, 1992). The pH was adjusted to 7.6 by using sodium hydroxide solution or hydrochloric acid solution before use.

2.2. Cultivation of D. magna

Daphnia magna were provided by the School of Environmental Science and Safety, Tianjin University of Technology, Tianjin, China. They were cultivated at a temperature of 23 ± 1.0 °C under natural light and grown on *Scenedesmus obliquus* as specified in OECD 202 (OECD, 1992). Variation among clones was eliminated by collecting the third-generation neonates (< 24 h) from the same female for testing. Individual neonates were of similar size.

2.3. Experimental design

2.3.1. Acute toxicity of Cd^{2+} and Nano-Cd to D. magna

The acute toxicity experiments were performed according to OECD 202 (OECD, 1992). According to the preliminary experiment results, concentrations of Cd^{2+} ($CdCl_2$) were 0, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mg L^{-1}. The Nano-Cd suspensions were prepared as follows: 2.0 mg of $nHAP_{20}$, $nHAP_{40}$, and $nMnO_2$ was suspended in 1 L reconstituted water and dispersed with ultrasonic vibration for 30 min. Subsequently, different concentrations $CdCl_2$ solutions (0.98, 1.47, 1.96, 2.45, 2.94 and 3.43 mg L^{-1}) were successively added to the nanomaterial suspensions and stirred for 30 min by using a magnetic stirrer to prepare Nano-Cd. Finally, those of Cd^{2+} in every Nano-Cd composite all were 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 mg L^{-1} (Cd^{2+} concentration), respectively. A variety of exposure concentrations of Cd^{2+} in single Cd^{2+} and Nano-Cd suspensions were determined using an iCAP Q series ICP-MS (Thermo Scientific, Germany). Nano-Cd solutions were centrifuged at 3000 r min^{-1}, and then Cd^{2+} in the supernatant was filtered by 0.22 μm and detected with a triple quadrupole spectrometer. Auxiliary gas flow (Ar) and nebulizer gas flow (Ar) is 0.79 and 1.05 L min^{-1}, respectively. RF power is 1600 W. Detector model is single. The wash time is 20 s. The analysis results showed the concentrations of in Nano-Cd solutions all were 0 mg L^{-1}, indicating Cd^{2+} has been loaded on nanoparticles.

Ten *D. magna* were placed in a 50 mL glass beaker containing 30 mL of Cd^{2+} or Cd-loaded nanomaterials for 48 h. Four replicate groups were used for each treatment. Pollution and evaporation were prevented by sealing the beakers with a plastic film having small ventilation holes. Next, the beakers were transferred to a shaker under controlled conditions (23 ± 1.0 °C; 16 h light: 8 h dark). In order to reduce deposition of Nano-Cd composites, a shaker was kept at 140 ± 5 r min^{-1} (Zhu et al., 2008). During the exposure, *D. magna* were not fed. The surviving number of *D. magna* in each beaker were counted at 24 and 48 h. Static *D. magna* without discernible activity and heartbeat after repeated rotation of the beaker for 15 s were considered to have died (Wang et al., 2012). The *D. magna* were also observed after 48 h by using a stereomicroscope (Olympus SZ51-SET; Japan).

2.3.2. Enzyme activity assay

The acute toxicity test results were used to conduct sub-acute toxicity experiments as specified in OECD 202 (OECD, 1992). The concentrations of $Cd^{2+}$and Nano-Cd (Cd^{2+} concentration) were 1/2 EC_{50}, 1/4 EC_{50}, 1/8 EC_{50}, 1/16 EC_{50}, and 1/32 EC_{50}, that is, 0.04, 0.08, 0.16, 0.32, and 0.64 mg L^{-1}, respectively. Fifty *D. magna* were placed in a 150 mL glass beaker containing 100 mL of Cd^{2+} and nanomaterial-adsorbed Cd for 48 h; the other conditions were the same as those for the experiment of acute toxicity. The experiment was performed in triplicate. The *D. magna* were collected and washed with Milli-Q water for 5 min and completely homogenized by using a glass homogenizer containing 300 μL ice-cold Tris-HCl buffer solutions (0.01 M Tris-HCl, 0.1 mM EDTA-2Na, 0.01 M sucrose, 0.8% sodium chloride solution, pH 7.4). Homogenates were centrifuged at 3500*g* for 15 min at 4 °C, and the supernatants were immediately collected for enzyme activity determination. The activities of SOD, CAT, POD, ASA and MDA were assessed to determine the possible influences on oxidative stress and antioxidant defense responses of *D. magna*.

2.3.3. SOD assay

SOD activity was measured using the xanthine oxidase determination method described by Devrima et al. (2008). The enzyme activity was expressed as U mg^{-1} protein. One unit of SOD activity was defined as the amount required to induce 50% inhibition of the decrease rate of tetrazolium. The nitroblue tetrazolium concentration was analyzed using a VIS-7220 spectrophotometer at 560 nm (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China).

2.3.4. CAT assay

The colorimetric determination of CAT activity was performed at 405 nm and 25 °C by using ammonium molybdate (Lv et al., 2009). One unit of CAT activity was defined as the amount of enzyme that catalyzed the dismutation of 1 μM of H_2O_2 per minute.

2.3.5. POD assay

POD activity was determined in accordance with the manufacturer's instructions. The reaction mixture contained 100 μL supernatant and 4 mL reaction system (2.0 mL 0.3% H_2O_2, 1 mL 0.2% guaiacol, and 1 mL phosphate buffer saline, pH 7). The reaction mixture was centrifuged for 10 min at 3500*g*. The POD activity was determined at 470 nm. One unit of POD activity was defined as a change of 0.01 in the absorbance value per minute.

2.3.6. ASA assay

Superoxide anions ($O_2^{\cdot-}$) were produced by the reaction of xanthine and xanthine oxidase. The changes in the absorbance corresponding to the reaction of cytochrome c and Gress's reagent were measured at 550 nm. One unit of ASA was defined as 1 mg of tissue protein inhibiting $O_2^{\cdot-}$, which corresponded to the change of 1 mg vitamin C