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Effects of TiO_2, SiO_2, Ag and CdTe/CdS quantum dots nanoparticles on toxicity of cadmium towards *Chlamydomonas reinhardtii*

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ABSTRACT

Nanoparticles (NPs) are inevitably released into the aquatic environment for being widely used and may affect the toxicity of other contaminants already present in the environment, such as trace metals. However, the effects of NPs on the ecotoxicity of cadmium (Cd), a common environmental trace metal pollutant, are not well explored. In this study, effects of four widely used NPs TiO_2 (n-TiO_2), SiO_2 (n-SiO_2), Ag (n-Ag) and CdTe/CdS core/shell quantum dots (QD) on the toxicity of Cd to the freshwater algae *Chlamydomonas reinhardtii* were assessed respectively. Cd reduced the algae biomass, impaired the photosynthetic activities, and led to intracellular oxidative stress of algae. At non-toxic concentrations, both n-TiO_2 (100 mg L^{-1}) and n-SiO_2 (400 mg L^{-1}) attenuated the toxicity of Cd towards the algae for reducing the intracellular Cd contents, and the former was more pronounced. QD (0.5 mg L^{-1}) increased the toxicity of Cd to algae, but n-Ag (0.2 mg L^{-1}) had no significant influence on the Cd toxicity to algae. The microscopic observations on the ultrastructure of algae cells presented the same phenomena and n-TiO_2, n-SiO_2 aggregations were clearly observed outside the cell wall. Furthermore, the regulation of NPs to the Cd toxicity towards algae was related to the intracellular nitric oxide (NO), an important signaling molecule, rather than the phototaxis of algae. Above all, this study provided a basic understanding about the difference in joint toxicity of different kinds of NPs and Cd to aquatic organisms.

1. Introduction

Microalgae are the first trophic level of the food chain, providing biological energy and oxygen for other trophic levels and playing very important roles in keeping the material balance and energy cycle of aquatic ecosystems. Then, the microalgae are commonly used to assess the toxicity of a hazardous substance in aquatic environment (Jagadeesh et al., 2015). *C. reinhardtii*, a unicellular green microalgae regarded as one of the most used model system, is very sensitive to contaminants and rapidly responses to environmental stresses, such as heavy metal cadmium (Cd) (Da Costa et al., 2016).

Cadmium (Cd), a nonessential and toxic trace element, enters the environment through various anthropogenic activities such as lead-zinc mining and disposal of rechargeable nickel-cadmium batteries (Jamers et al., 2013). In China, Cd can reach concentrations as high as 5.6 mg L^{-1} in freshwaters associated with e-waste recycling activity (Wu et al., 2015). In aquatic environment, Cd is easily absorbed by organisms in lower trophic levels, such as microalgae, and transferred to higher trophic levels in food chain (Jamers et al., 2013). For similarity in molecular size and charge, Cd absorbed into cells can substitute for essential metals as calcium in several biochemical pathways, resulting toxicity (Faller et al., 2005).

Besides Cd, there are many other kinds of pollutants existing in the aquatic environment, such as nanoparticles (NPs), the ultrafine particles with many unique properties different from bulk materials, such as small size, large specific surface area, large proportion of the surface atoms, etc. (Suh et al., 2009). NPs are released into the environment unavoidably and cause potential eco-toxicity for being widely used in cancer therapy, targeted drug delivery, electronics, cosmetic industry and biosensors (Nel et al., 2006) Mechanistically, the toxicity of NPs to organisms can be divided into direct and indirect effects (von Moos and Slaveykova, 2014). Direct effects are mediated by the increased production of ROS and can lead to the oxidative damage of cellular compounds. Indirect effects are mainly mediated by the release of toxic ions (Ivask et al., 2014; Cheloni et al., 2016). Additionally, the effects on adsorption of organisms to ambient pollutants are also involved in the

Abbreviations: NPs, nanoparticles; Cd, cadmium; n-TiO_2, NPs TiO_2; n-SiO_2, NPs SiO_2; n-Ag, NPs Ag; QD, quantum dots; NO, nitric oxide; TEM, transmission electron microscope; PSII, photosystem II; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; GSH/GSSG, reduced glutathione/glutathione disulphide; EC, electrical conductivity

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indirect toxicity effects of NPs when co-exist (Miao et al., 2015). Previous works on NPs toxicity mainly focused on their direct effects (von Moos et al., 2016), while their potentially indirect effects especially the effects on the eco-toxicity of conventional pollutants such as heavy metals have been underestimated.

Several studies have demonstrated that NPs could affect the toxicity of some trace metals to aquatic organisms. For example, n-TiO_2 increased the toxicity of Pb to zebrafish larvae (Miao et al., 2015). CdTe-QD facilitated the accumulation of Cu in zebrafish, leading to higher mortality and more malformations (Zhang et al., 2013). However, the influence of NPs on Cd toxicity to aquatic organisms remains unclear. Based on the limited results from previous literatures that mainly focused on the effects of n-TiO_2 on Cd toxicity to multicellular animals, n-TiO_2 actied as carriers of Cd (Trojan Horse effect) and increased the toxicity of Cd to *Mytilus galloprovincialis* and *Daphnia magna* respectively (Della Torre et al., 2015; Tan and Wang, 2014), while fewer studies were reported in microalgae (Hartmann et al., 2010). In addition, the interferences on Cd toxicity to microalgae of other NPs, including n-SiO_2, n-Ag and CdTe/CdS-QD, are largely unknown. NPs effects on trace metals eco-toxicity to aquatic organisms are linked to their proprieties (von Moos and Slaveykova, 2014). For example, in the presence of n-TiO_2, the toxicity of Cr (VI) to *Scenedesmus obliquus* decreased considerably, while n-Al_2O_3 did not have any significant effect on the Cr (VI) toxicity (Dalai et al., 2014). Therefore, it is essential to compare the effects of different NPs on the toxicity of Cd to microalgae under the same experimental conditions. Furthermore, comprehension of these points above can provide a better understanding on the physiological response of microalgae when were co-exposed to two contaminants and the different roles played by variety of NPs on Cd toxicity in the aquatic environment.

For these purposes, four widely used NPs, n-TiO_2, n-SiO_2, n-Ag and CdTe/CdS-QD, at non-toxic concentrations, were selected to detect the effects of different kinds of NPs on the toxicity of Cd to the freshwater algae *C. reinhardtii*. Firstly, the NPs dissolution and adsorption to Cd were observed in algae medium. Secondly, the cell biomass, chlorophyll contents, major antioxidant enzymes activities, the photosynthetic activities, intracellular oxidative stress levels and observed the ultrastructure of algae cells in all treatment groups were measured to evaluate the variation of Cd toxicity to algae with or without NPs. And then, a comparison of the effects induced by NPs was made in the present study.

2. Material and methods

2.1. Materials

The *Chlamydomonas reinhardtii* culture (wild type, CC-125) was obtained from the *Chlamydomonas* Genetic Center (Duke University, Durham, NC, USA). Stock cultures were grown in 50 mL liquid Tris-acetate-phosphate (TAP) medium (Harris, 1989) in 100 mL Erlenmeyer flasks at 25 ± 1 °C, under a 14 h/10 h light/dark regime with a light density of 100 μmol of photons m^{-2} s^{-1} and were hand-shaken four times a day. The initial pH value of TAP medium was 7.0. All glassware used were soaked in 10% HNO_4 for at least 48 h and rinsed 7 times with ultrapure water.

n-TiO_2 (anatase, particle size: 20 nm) was purchased from Shanghai Chaowei nanotechnology Co., Ltd., China. n-SiO_2 (particle size: 10–20 nm) was purchased from Sigma-Aldrich, USA. Citrate stabilized n-Ag (particle size: 30 ± 3 nm) was purchased from nanoComposix, USA. CdTe/CdS QD (particle size: 1–10 nm) functionalized with carboxyl was purchased from Janus New-Materials Co., Ltd., China. Cd was added as analytical grade cadmium chloride ($CdCl_2$·2½ H_2O) purchased from Sinopharm, Shanghai, China.

2.2. Preparation of exposure solution and NPs characterization

NPs suspension was prepared by suspending NPs into the TAP medium through a 30 min sonication in a water bath. $CdCl_2$ solution was then added to the dispersed NPs solution to create a NPs-Cd suspension as the exposure solution. The speciation of Cd in the TAP medium was performed using Visual MINTEQ version 3.0 with a fixed pH of 7.0 in the presence of Cd. The state of NPs in TAP medium were observed using a transmission electron microscope (TEM) (Tecnai G2 F30, FEI, USA) after the NPs suspension were dripped onto formvar-coated copper grids, and the hydrodynamic particle size and zeta potential were monitored with Zetasizer APS (Nano ZS, Malvern, England). The dissolutions of NPs in TAP medium were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 5100, Santa Clara, California, USA) (Supporting information (SI) method 1).

2.3. Toxicity tests

C. reinhardtii cells were harvested at the logarithmic growth phase and translated into the exposure solution. The initial concentration of algae cells used in the tests was 5×10^4 cells mL^{-1}. The experimental design consisted of ten treatment groups as follows (i) CK, (ii) Cd, (iii) n-TiO_2, (iv) n-TiO_2 + Cd, (v) n-SiO_2, (vi) n-SiO_2 + Cd, (vii) n-Ag, (viii) n-Ag + Cd, (ix) QD, (x) QD + Cd. Currently, the concentration of Cd was fixed at 49.745 μM L^{-1}, EC_{50} of Cd to *C. reinhardtii* of 96 h duration exposure (SI Fig. S1) and the concentrations of NPs selected were nontoxic doses to *C. reinhardtii* according to Fig. 2. The free Cd ion concentration in TAP medium predicted from Visual MINTEQ was 1.037 μM L^{-1} (SI Table. S1). Negative controls containing the same concentrations of NPs and Cd but no algae cells were applied to eliminate any interference from the NPs. Experiments were carried out for 96 h in a climate chamber at 25 ± 1 °C, under a 14 h/10 h light/dark regime with a light density of 100 μmol of photons m^{-2} s^{-1} and were hand-shaken four times a day. Each experiment was conducted three times with three replicates in each group.

2.4. Cell numbers and chlorophyll contents determination

The whole experiment lasted for 96 h with 4 time points (24, 48, 72, 96 h) and cell numbers and photosynthetic pigments were measured at each time points. Cell numbers were determined in a hemocytometer chamber under a light microscope (Shanghai Opital Instrument Factory, Shanghai, China) using the standard procedure and growth inhibition (EC_{50}) studies were of 96 h duration as detailed in standard test guidelines (OECD, 2002). The photosynthetic pigments were extracted by 80% acetone and left over night at 4 °C. Supernatants were collected by centrifugation at 4226 × g, 4 °C for 5 min and measured the absorbance at 470, 646 and 663 nm with an ultraviolet spectrophotometer (METASH, Shanghai, China). The equations given by Lichtenthaler (1987) were used to calculate the contents of photosynthetic pigments.

2.5. Evaluation of the photosynthetic activity

After 72 h treatment, chlorophyll a fluorescence transients and Q_A^- reoxidation kinetics were measured using a double-modulation fluorometer (FL3500, PSI, Inc., Brno, Czech) to evaluate the photosynthetic activity of photosystem II (PSII). Negative controls containing the same concentrations of NPs and Cd but no algae cells were applied to eliminate the interference from the autofluorescence of NPs. Before measurement were started, the treatment and control algae samples were dark adapted for 15 min to ensure closure of all PSII reaction centers and estimate the maximum fluorescence yield (Fm). The chlorophyll a fluorescence was measured according to Li et al. (2010) under an actinic light of 3000 μmol photons m^{-2} s^{-1} and the fluorescence signals were recorded from 10 μs to 1 s. When plotted on a logarithmic time