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Effect of titanium dioxide nanoparticles on copper toxicity to *Daphnia magna* in water: Role of organic matter

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ABSTRACT

Inevitably released into natural water, titanium dioxide nanoparticles (nano-TiO_2) may affect the toxicity of other contaminants. Ubiquitous organic matter (OM) may influence their combined toxicity, which has been rarely reported. This study investigated the effect of nano-TiO_2 on Cu toxicity to *Daphnia magna* and the role of OM (dissolved or particle surface bound) in inducing combined effects. The effect of nano-TiO_2 on heavy metal accumulation depended on the adsorption capacity for heavy metals of nano-TiO_2 and the uptake of nano-TiO_2–metal complexes by organisms. Nano-TiO_2 significantly decreased Cu accumulation in *D. magna*, but the reducing effect of nano-TiO_2 was eliminated in the presence of humic acid (HA, a model OM). In the Cu and HA solution, nano-TiO_2 slightly affected the bioavailability of Cu^{2+} and Cu–HA complexes and thus slightly influenced Cu toxicity. The nanoparticle surface-bound HA reduced the effect of nano-TiO_2 on the speciation of the accumulated Cu; therefore, the combined effects of nano-TiO_2 and Cu on biomarkers similarly weakened. HA-altered Cu speciation may be the main factor responsible for the influence of HA on the combined effects of nano-TiO_2 and Cu. This study provides insights into the combined effects of nano-TiO_2 and heavy metals in natural water.

1. Introduction

Titanium dioxide nanoparticles (nano-TiO_2) have been widely used in various industries and materials, such as sunscreens, paints, papermaking, wastewater treatment, solar cells, and food additives, because of their unique physical and chemical properties (Robichaud et al., 2009). With the increasing production, nano-TiO_2 is inevitably released into natural water. The predicted nano-TiO_2 concentration in rivers ranges from 3 ng/L to 1.6 μg/L (Gottschalk et al., 2013). Once in an aquatic environment, nano-TiO_2 with unique properties inevitably interacts with other contaminants; thus, the behavior, bioavailability, and toxicity of contaminants to aquatic organisms are modified (Rosenfeldt et al., 2014; Torre et al., 2015; Yang et al., 2014). The behavior and bioavailability of nano-TiO_2 can also be altered by background components in natural water (Pavagadhi et al., 2014; Romanello and de Cortalezzi, 2013; Tong et al., 2013).

Heavy metals are common contaminants in natural water. Heavy metal toxicity is influenced by nano-TiO_2 to varying degrees. Nano-TiO_2 decreases the concentration of free heavy metal ions through adsorption, as a result, the bioaccumulation and toxicity of heavy metals are reduced (Liu et al., 2015; Rosenfeldt et al., 2014, 2015b; Yang et al., 2012). Nano-TiO_2 can also act as a heavy metal carrier and enhance heavy metal bioavailability when nano-TiO_2–metal complexes penetrate organisms (Fan et al., 2011, 2012b; Hartmann et al., 2012; Miao et al., 2015; Tan et al., 2012; Yang et al., 2014). Nano-TiO_2 may cause differences in heavy metal subcellular distribution (Yang et al., 2014) and synergistically affect heavy metal toxicity (Hartmann et al., 2010). These various findings may be attributed to different exposure conditions and types of nano-TiO_2, heavy metals, and organisms. However, limited information on the comparative analysis of these various results has been published.

Moreover, the influence of background components in natural water on behavior of nanoparticles and heavy metals has been disregarded in most studies. Organic matter (OM), which is ubiquitous in natural water, contains numerous functional groups and has high reactivity. OM plays an important role in metal toxicity in an aquatic environment (Kramer et al., 2004; Lamelas and Slaveykova, 2007; Richards et al., 2001). OM can also alter the surface properties and sorption behavior of nano-TiO_2 (Liu et al., 2008; Yang et al., 2009). OM can significantly influence the

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interaction of heavy metals and nano-TiO_2 (Chen et al., 2012; Tang et al., 2014). For example, Chen et al. (2012) found that Cd adsorption on humic acid (HA)–TiO_2 was higher than that on nano-TiO_2. OM can also change the combined bioaccumulation and toxicity of nano-TiO_2 and heavy metals. Hu et al. (2011) investigated the combined effects of nano-TiO_2 and HA on Cd accumulation in zebrafish; they demonstrated that Cd bioaccumulation in zebrafish in the mixed system of HA and TiO_2 nanoparticles was significantly lower than that in the corresponding TiO_2 solution but comparable to that in the corresponding HA solution. Rosenfeldt et al. (2015a) reported that Cu toxicity was weakened by nano-TiO_2 and further reduced in the presence of OM because of the adsorption and complexation by nano-TiO_2 and OM, respectively. After entering natural waters, nano-TiO_2 is coated by OM. Nanoparticle surface-bound OM may affect differently from dissolved OM on combined effect of nano-TiO_2 and heavy metals (Lin et al., 2012). However, studies have yet to investigate the effect of nanoparticle surface-bound OM on the combined toxicity of nano-TiO_2 and heavy metals, and the mechanisms by which OM affect the combined toxicity have yet to be elucidated.

This study further investigated nano-TiO_2-related factors affecting the heavy metal toxicity and the role of HA (a model of OM) in this process. The nanoparticle surface-bound HA and dissolved HA were applied on the basis of different forms of OM in natural water. As a commonly detected metal in water, Cu was used as a heavy metal model. *Daphnia magna* was selected as test organism. The interaction of HA, Cu, and nano-TiO_2 was analyzed to elucidate the mechanism of their combined effects on Cu toxicity. This study provided additional information on the combined effects of nano-TiO_2 and heavy metals in natural water.

2. Materials and methods

2.1. Preparation and characterization of nanoparticles and HA

Aeroxide P25 TiO_2 with >99.5% purity was provided by Acros Organics (Belgium). HA, from Sigma Aldrich (Germany) was used for the experiments. HA was dissolved in a simplified Elendt M7 medium (SM7) (Samel et al., 1999), containing a mixture of $CaCl_2$ (293.8 mg/L), $MgSO_4$ (123.3 mg/L), K_2HPO_4 (0.184 mg/L), KH_2PO_4 (0.143 mg/L), $NaNO_3$ (0.274 mg/L), $NaHCO_3$ (64.8 mg/L), Na_2SiO_3 (10 mg/L), H_3BO_3 (0.715 mg/L) and KCl (5.8 mg/L). The resulting HA solution was filtered through 0.45 μm cellulose membranes, and then stored in the dark at 4 °C. TiO_2–HA complexes were prepared as described by Yang et al. (2009). In brief, 2 g nano-TiO_2 was added to 800 mL HA solution dissolved in ultrapure water (18 mg TOC/L) and shaken at 120 rpm for 5 days. The suspension was centrifuged at 3500 rpm for 30 min. The precipitate was washed twice with ultrapure water, freeze-dried, ground and stored for subsequent experiments. For nanoparticle stock solutions (1 g/L), the nanoparticles were dissolved in ultrapure water, and stored in the dark. The stock solutions without HA sonicated 20 min before use.

The image of nanoparticles in SM7 was obtained by using a transmission electron microscope (TEM, JEM-2100F, Japan). The C and H contents of the whole nanoparticles were determined with an elemental analyzer (Elementar Analysensysteme Gmbh vario, Germany). Surface areas were calculated via BET method (NOVA4200e, USA). The fourier transform infrared spectra of HA, nano-TiO_2 and TiO_2-HA were obtained from 400 cm^{-1}–4000 cm^{-1} with an infrared microspectrograph (iN10MX, USA). The ultraviolet absorption spectra of the HA solution in the presence or absence of Cu and nanoparticles was obtained by using an ultraviolet spectrophotometer (GBC Cintra 10e, Australia). 3D fluorescence spectra were also obtained with a spectrofluorometer (F-700, Japan). Excitation and emission wavelength respectively ranged from 200 nm to 400 nm and from 220 nm to 550 nm at a scanning speed of 12,000 nm/min.

2.2. Adsorption of Cu^{2+} onto nanoparticles in the presence of HA

The Cu solution (30 μg/L) was prepared by diluting the Cu standard solution (100 μg/mL, National Institute of Metrology) using SM7. The nano-TiO_2 or TiO_2–HA complexes (2 mg/L) were added to 250 mL of Cu only solution or Cu and HA mixture. Three parallels were used. The mixture was shaken at 150 rpm, and 4 mL samples were collected at different times. The samples were then centrifuged at 12,000 × *g* for 10 min to settle the nanoparticles (Tan et al., 2012). The total Cu concentration in the supernatant was determined through inductively coupled plasma mass spectrometry (VGPQ2 Turbo). The amount of Cu adsorbed on the nanoparticles was calculated by mass difference between the initial samples and the supernatant in equilibrium.

2.3. Test organism

D. magna was cultured in a climatic chamber at 22 °C with 16 h light: 8 h dark cycle and fed daily with the green alga *Chlamydomonas reinhardtii*. The culture medium for *D. magna* was collected from the Jingmi River (39°58′5.58″ N, 116°16′53.3″ E) with a pH of 8.50. The concentrations of Ca, Zn, and Cu were 34 mg/L, 6 μg/L and 0.7 μg/L, respectively, and Cd was not detectable. The TOC content was approximately 3 mg/L. The culture medium was uncontaminated, and might slightly influence the sensitivity of organisms. The medium was filtered to remove larger particles before use. The medium was changed every other day.

2.4. Toxicity test

The Cu, nano-TiO_2, and HA concentration for the toxicity test were set at 30 μg/L, 2 mg/L and 1 mg TOC/L, respectively. SM7 (pH = 8.0) was used as test medium. The toxicity test for *D. magna* included the eight following groups: (a) nano-TiO_2 suspension, (b) TiO_2–HA complexes suspension, (c) Cu only solution, (d) mixture of Cu and HA, (e) mixture of Cu and nano-TiO_2, (f) mixture of Cu and TiO_2–HA complexes, (g) mixture of Cu, HA and nano-TiO_2, (h) mixture of Cu, HA and TiO_2–HA complexes. The HA and Cu solution was allowed to reach equilibrium within 12 h before the nanoparticles were added. Three parallels were used for each group. *D. magna* exposure was performed in 500 mL beakers containing 500 mL of test solution for 48 h. Fifty healthy *D. magna* (14 ± 2 days) with similar sizes were added to each beaker. The experimental toxicity conditions were the same as the culture conditions for *D. magna*, as described in Section 2.3, except river water was replaced with SM7. *D. magna* were not fed during exposure.

2.5. Ti and Cu bioaccumulation in D. magna

Ten surviving *D. magna* were removed and washed with ultrapure water after 48 h exposure. *D. magna* were dried at 80 °C to a constant weight and digested in 68% HNO_3 and concentrated sulfuric acid–ammonium sulfate solution (Tan et al., 2012). Cu and Ti concentrations in the digest were determined by the 797 VA computrace system (Metrohm, Switzerland) and an inductively coupled plasma optical emission spectrometer (Optima 5300DV, USA), respectively. The recuperation experiments were performed to evaluate reliability of the digestion method. The average recovery rate of Ti and Cu was approximately 85% and 98%, respectively. Bioaccumulation was expressed on basis of the dry weight of *D. magna*.