Environmental Pollution 231 (2017) 311–318

Contents lists available at ScienceDirect

Environmental Pollution

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

Influences of TiO_2 nanoparticles on dietary metal uptake in *Daphnia magna*☆

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ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form
30 July 2017
Accepted 6 August 2017
Available online 12 August 2017

Keywords:
Nano-TiO_2
Metal assimilation
Food quantity
Food quality
Daphnia

ABSTRACT

Increasing applications of titanium dioxide nanoparticles (nano-TiO_2) have intensified the risk of environmental contamination. Since nano-TiO_2 can absorb metals and be consumed as 'food' by zooplankton but also can interact with phytoplankton, they could significantly disturb the existing metal assimilation patterns. In the present study, we quantified the dietary assimilation of Cd and Zn from nano-TiO_2 and algae (*Chlamydomonas reinhardtii*) at comparable particle concentrations as well as in complex food environment (variable food quality and quantity) in a freshwater zooplankton *Daphnia magna* using the radiotracer technique. For both nano-TiO_2 and algae as food, the feeding food quality and depuration food quantity significantly affected the assimilation efficiencies (AEs) of Cd and Zn. At feeding food quantity of 1 mg/L to 10 mg/L without food in depuration, the AEs of Cd and Zn from nano-TiO_2 were lower than those from algae. When food was added during depuration, the influences of nano-TiO_2 on metal AEs were variable due to the differential effects of food quantity on the gut passage of nano-TiO_2 and algae. Furthermore, mixed nano-TiO_2 and algae had the lowest metal AEs compared to sole nano-TiO_2 or algae as a result of interaction between nano-TiO_2 and algae during feeding. Overall, this study showed the distinguishing metal AEs between nano-TiO_2 and algae, and that nano-TiO_2 could significantly reduce the existing metal AEs from algae. More attention should be paid to the potential roles of nano-TiO_2 in disturbing metal assimilation in the environmental risk assessments of nanoparticles.

1. Introduction

With increasing global production of nanoparticles/nanomaterials due to their special properties, it is necessary to understand the behavior of nanoparticles and their impacts on the environment. Titanium dioxide nanoparticle is one of the most commonly used nanoscale materials. It is estimated that by 2025 the worldwide production of nano-TiO_2 will reach nearly 2.5 million metric tons (Robichaud et al., 2009). Applications of nano-TiO_2 include food industry, personal care products (Weir et al., 2012), catalysis, purification agents (Li et al., 2009), antimicrobial agents (Hossain et al., 2014) and numerous coatings of building materials (Shandilya et al., 2015). Weir et al. (2012) found TiO_2 in food both in its bulk form and nano form (at least 36% of total TiO_2), and estimated a human consumption of 1 mg/kg body weight per day. As the consequence of large production and wide use of nano-TiO_2 particles, from the early stages of production to later use, recycling, and disposal, their release into aquatic environment is unavoidable. Previously, with a probabilistic material flow model Gottschalk et al. (2009) predicted that the environmental concentrations of nano-TiO_2 were 21 ng/L in surface waters and 4 μg/L in sewage treatment effluents. Gondikas et al. (2014) used several approaches to detect nano-TiO_2 released from sunscreen products into the Old Danube Lake, and showed that nano-TiO_2 from sunscreen was possibly released into the lake. Therefore, the mission to fully understand the risk posed by nano-TiO_2 in aquatic environment is urgent and critical for safe use and management of nano-TiO_2.

Many studies have focused on the adverse effects of nano-TiO_2 and indicated that generation of reactive oxygen species (ROS) may be the main mechanism of nano-TiO_2 toxicity in aquatic organisms such as water flea and fish (Ma et al., 2013; Federici et al., 2007; Marcone et al., 2012). Meanwhile, interests also stemmed from the roles of nano-TiO_2 as carriers of other contaminants, which may

☆ This paper has been recommended for acceptance by Baoshan Xing.

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http://dx.doi.org/10.1016/j.envpol.2017.08.024
0269-7491/

further enhance the undesired effects (Fan et al., 2011; Hartmann et al., 2012; Rosenfeldt et al., 2014; Fang et al., 2015, 2016). Previously, we investigated the biokinetics of metals (Cd and Zn) binding with nano-TiO_2 (Tan et al., 2011), and demonstrated that nano-TiO_2 ingested into the gut of *Daphnia magna* (*D. magna*) after exposure could provide potential binding sizes and increase the uptake of dissolved metals (Tan and Wang, 2014). However, the apparent increased bioaccumulation of these metals did not necessarily account for their bioavailability or toxic effects. Rosenfeldt et al. (2014) found that the presence of nano-TiO_2 increased the Ag toxicity for juveniles (*D. magna*) resulting in a 40% lower 72-h EC50, nevertheless the toxicities of As and Cu were decreased by up to 80%. The toxicity data could not be fully explained by the bioaccumulation of the metals, which was elevated by 2-fold for Ag and 6-fold for As, and decreased by 14-fold for Cu in the presence of nano-TiO_2. For *D. magna*, direct ingestion of nano-TiO_2 as food may account for the uptake of metals absorbed on nano-TiO_2 (Hartmann et al., 2012; Tan et al., 2011).

Previous study showed that metal bioavailability from food particles can be quantified by the dietary assimilation efficiency (AE), which represents the percentage of ingested metal that is transported across gut membrane to retain in soft tissue of an organism (Wang and Fisher, 1999). Quantifying metal AEs from nano-TiO_2 is essential to understand their bioavailability and effects. Furthermore, when nano-TiO_2 are released into water, they can coexist and interact with traditional diets such as phytoplankton, resulting in the possible change of dietary assimilation. It is thus important to quantify metal assimilation in a complex food environment (e.g., variable food quality and quantity), and further understand the influences of the interaction between nano-TiO_2 and algae on metal assimilation. However, essentially no systematic information is available on metal assimilation from single nano-TiO_2 compared with algae and from nano-TiO_2 and algae together.

Therefore, the objectives of this study were to quantify the dietary assimilation of Cd and Zn from nano-TiO_2 and algae at comparable particle concentration and under complicated food environment using the radiotracer technique. Four factors (feeding food quality/quantity, and depuration food quality/quantity) were investigated for their influences on the AEs of Cd and Zn. Within their feeding range, *D. magna* can randomly collect and ingest nano-TiO_2 in the same manner as algae into guts, thus the word 'food' was used to broadly refer to nano-TiO_2 and/or algae in this study. Cd and Zn were chosen in this study mainly due to their importance in the environment. Cd as a priority contaminant has no known biological function whereas Zn is essential to *Daphnia*. The dietary assimilation of these two metals in *D. magna*, an ecologically important freshwater zooplankton as the model organism, has been quantified in earlier studies (Yu and Wang, 2002; Guan and Wang, 2004). Measurements of dietary assimilation can provide a better understanding of bioavailability of Cd and Zn absorbed on nano-TiO2 and the influences of nano-TiO2 on dietary assimilation of Cd and Zn in *Daphnia*.

2. Materials and methods

2.1. Organisms, water and radioisotopes

D. magna previously cultured in our laboratory were used in this study. Daphnids were cultured in glass-fiber with filtered and unpolluted pond water collected from a hill covered by forest on the campus of the Hong Kong University of Science and Technology (GF/CWhatman, Maidstone, UK). The culture condition was at a temperature of 23.5 °C with a light to dark cycle of 14:10 h. Green algae *Chlamydomonas reinhardtii* as foods were fed to daphnids at a density of 10^5 cells/mL, and the water was changed every two days. An artificial WC medium (containing $CaCl_2$: 0.25 mM, $MgSO_4$: 0.15 mM, $NaHCO_3$:0.15 mM, K_2HPO_4: 0.05 mM, $NaNO_3$: 1 mM, H_3BO_3:0.1 mM, and trace metals and vitamins) was used as growth medium of algae (Guillard and Lorenzen, 1972). Algae were centrifuged to remove the growth medium at the exponential growth stage, and stored in filtered pond water at 4 °C. The simplified Elendt M7 medium (SM7, containing only $CaCl_2$, $MgSO_4$, K_2HPO_4, KH_2PO_4, $NaNO_3$, $NaHCO_3$, Na_2SiO_3, H_3BO_3, and KCl and without disodium ethylenediaminetetraacetic acid, trace metals, or vitamins) was used in this study (Samel et al., 1999), and the pH of the SM7 medium was adjusted to 8.2 by adding 0.1 N NaOH before all experiments.

The assimilation efficiencies (AEs) of the two metals were measured using the gamma radiotracer ^{109}Cd (in 0.1 N HCl, from New England Nuclear, Boston) and ^{65}Zn (in 0.1 N HCl, from Riso National Laboratory, Denmark). The radioactivity was determined with a Wallac 1480 NaI (T1) gamma detector (Turku, Finland) at 88 keV for ^{109}Cd and at 1115 keV for ^{65}Zn. All analyses were related to appropriate standards and calibrated for spillover and radioisotope decay. The counting time was 3 min which yielded less than 5% propagated counting errors.

2.2. Characterization of nano-TiO_2

Nano-TiO_2 (product number: 637254, <25 nm in particle size, 99.7% trace metals basis) was purchased from Sigma-Aldrich Corporation, USA. Based on the manufacturer's specification, the crystal phase of nano-TiO_2 was anatase, and the specific surface area was 45–55 m^2/g with a density of 3.9 g/mL. The particle morphology and elemental composition were analyzed using transmission electron microscopy (TEM, JEOL, 2010F) with energy-dispersive X-ray spectrometry (EDX) capability. The TEM equipment was operated at an acceleration voltage of 100 kV. The nano-TiO_2 were dispersed in Milli-Q ultrapure water (Barnstead, Dubuque, IA, USA), followed by sonication for 20 min (50 w/l at 40 kHz), and were dripped onto a cleaned 200 mesh Cu carbon grid at room temperature for one day before the TEM and EDX analysis. Dynamic light scattering (DLS) with a zeta potential analyzer (ZetaPALS, Brookhaven Instruments) were used to measure the average diameter and the zeta potential of nano-TiO_2 (1 and 10 mg/L) in SM7.

2.3. Radiolabeling of nano-TiO_2 and algae

The algae and nano-TiO_2 used in the pulse-feeding were respectively radiolabeled by radioactive ^{109}Cd and ^{65}Zn. For nano-TiO_2, 1 g/L nanoparticles stock was prepared by adding nano-TiO_2 in Milli-Q ultrapure water (Barnstead, Dubuque, IA, U.S.) which was then subjected to 20 min sonication (50 w/l at 40 kHz). Radioactive ^{109}Cd and ^{65}Zn were added into the 1 g/L stock solution. After 24 h radiolabeling, the labeling efficiency of nano-TiO_2, i.e., the percentage of metals adsorbed on nano-TiO_2, was >99% for Cd and Zn, and this radiolabeled nano-TiO_2 stock solution was used in the pulse-feeding of AE experiments. For algae, the cells at the exponential phase were collected by centrifugation at 3000*g* and resuspended in the modified WC medium (without the addition of Cu, Zn, and EDTA), which was spiked with ^{109}Cd (20 μCi/L) and ^{65}Zn (30 μCi/L) at an initial cell density of 5×10^4 cells/mL. After 5 days of growth, the cells were again centrifuged at 3000 *g* and resuspended in the SM7 medium. This process was repeated twice to remove the weakly bound ^{109}Cd and ^{65}Zn. After the cell density measurement using a hemocytometer, the algae were immediately used in the pulse-feeding of AE experiments.