Role of Titanium Dioxide Nanoparticles in the Elevated Uptake and Retention of Cadmium and Zinc in *Daphnia magna*

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Ⓢ Supporting Information

ABSTRACT: Titanium dioxide nanoparticles (nano-TiO$_2$) are now widely applied in consumer products, and the dispersion of nano-TiO$_2$ may adsorb metals and modify their behavior and bioavailability in the aquatic environment. In the present study, the aqueous uptake, dietary assimilation efficiency (AE), and efflux rate constant (k$_e$) of two toxic metals (cadmium-Cd, and zinc-Zn) adsorbed on nano-TiO$_2$ in a freshwater zooplankton *Daphnia magna* were quantified. The biokinetics was then compared to daphnids that were exposed only to dissolved metals as controls. The aqueous uptake of Cd and Zn involved an initial rapid uptake and then an apparent saturation, and the uptake of metals was accompanied by an ingestion of nano-TiO$_2$. The AEs of Cd and Zn adsorbed on nano-TiO$_2$ were 24.6 ± 2.4−44.5 ± 3.7% and 30.4 ± 3.4−51.8 ± 5.0%, respectively, and decreased with increasing concentrations of nano-TiO$_2$. Furthermore, the difference between the AEs of Cd and Zn indicated that the desorption of Cd and Zn from nano-TiO$_2$ may have occurred within the gut of daphnids. With the use of algae as carrier, the AEs of Cd and Zn adsorbed on nano-TiO$_2$ were significantly higher than those of Cd and Zn directly from nano-TiO$_2$. The efflux rate constants of Cd and Zn adsorbed on nano-TiO$_2$ in the zooplankton were significantly lower than those of Cd and Zn not adsorbed on nano-TiO$_2$. Our study shows that the uptake and retention of toxic metals is enhanced when they are adsorbed on nano-TiO$_2$, and suggests more attention be paid to the potential influences of nano-TiO$_2$ on the bioavailability and toxicity of other contaminants.
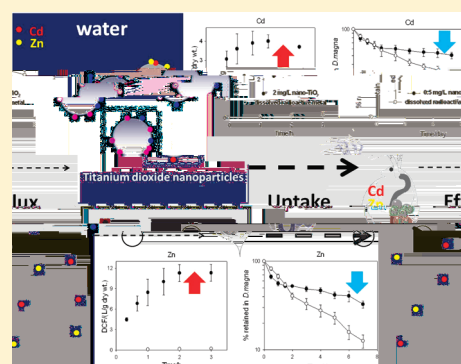
INTRODUCTION

Titanium dioxide nanoparticles (nano-TiO$_2$) have attracted considerable attention because of their unique properties and widespread uses in sunscreens, toothpastes, surface coatings, and water treatments.[1] The estimated worldwide production of nano-TiO$_2$ was 5000 t/year for 2006−2010, 10 000 t/year for 2011−2014, and 2.5 million metric tons/year by 2025.[2,3] Nano-TiO$_2$ has a greater surface area to volume ratio than traditional TiO$_2$ particles. This means that nano-TiO$_2$ have a higher level of catalytic activity and greater UV absorption at certain wavelengths.[4] Inevitably, these nanoparticles are entering into the environmental systems with increasing frequency. Direct evidence of the release of synthetic nanoparticles from urban applications into the aquatic environment has been documented.[5] In Switzerland, the predicted environmental concentration of nano-TiO$_2$ in water was 0.7−16 μg/L, which was close to or higher than the predicted no effect concentration, with a maximum safety limit of 1 μg/L.[6]

Most previous studies on the health risks and environmental impacts of nano-TiO$_2$ have focused on their biological effects and toxicities. Generation of reactive oxygen species (ROS) and their inflammatory effects are considered as the main mechanisms for nano-TiO$_2$ toxicity.[7,8] For aquatic organisms, several studies have considered the effects of nano-TiO$_2$ on water fleas (*Daphnids magna*, *Daphnia pulex*, *Ceriodaphnia dubia*), and the 48-h

mortality and immobility (EC50) was generally greater than 100 mg/L.[9−11] However, nanomaterials can also adsorb metals and organic contaminants because of their large surface areas,[12,13] and the sorption behaviors of nano-TiO$_2$ include physical sorption through electrostatic force and chemical sorption through chemical bonding. Stone et al.[14] found that the neutral and unoccupied surface sites of TiO$_2$ were Ti−(OH)(OH$_2$) in water. In contrast, when the pH of nano-TiO$_2$ is below the pH at the point of zero charge (pH$_{PZC}$), the nano-TiO$_2$ is in the form of the positively charged Ti−OH$_2^{1/2+}$, otherwise the nano-TiO$_2$ is in the form of the negatively charged Ti−OH$^{1/2-}$.[15] Much less is known about the potential influences of nano-TiO$_2$ on the bioaccumulation and bioavailability of other pollutants. Nanoparticles may facilitate the entry of pollutants adsorbed on the nanoparticles themselves into aquatic organisms and promote potential toxic effects. Previously, Baun et al.[16] showed that the toxicity of phenanthrene in *Daphnia magna* increased by 60% in the presence of C$_{60}$ aggregates and that phenanthrene adsorbed on C$_{60}$ was available to the organisms. Kim et al.[17] found that the bioavailability of Cu was enhanced by the presence of

multiwalled carbon nanotubes (MWCNTs) interacting with natural organic matter (NOM). In a recent study,[18] we found that nano-TiO$_2$ enhanced the toxicity of copper in natural water to *Daphnids magna* and inhibited detoxification by metallothioneins, indicating that Cu complexed with nano-TiO$_2$ was toxic as well. Therefore, free ions and ions complexed with nanomaterials may have apparently different effects on aquatic organisms. However, there is essentially no information on the influences of nanomaterials on the biokinetics of contaminants in aquatic organisms.

Daphnids are exposed to nanomaterials through external contact or ingestion by filter-feeding.[19−22] They can even feed on particles in the nanosize range directly by drinking the water surrounding them. One question of interest is whether metals bound with nano-TiO$_2$ were more available for biological uptake by the animals. Meanwhile, nano-TiO$_2$ can be adsorbed on biogenic particles [23] which crustaceans consume as food, resulting again in the uptake of metals. Previous study showed that nano-TiO$_2$ had a strong sorption capacity for cadmium-Cd and arsenate-As, and the presence of nano-TiO$_2$ (10 mg/L) significantly enhanced the bioaccumulation of Cd and As in carp.[24,25] Therefore, the effects of ambient nano-TiO$_2$ levels on the influx and efflux of Cd and Zn need to be quantified in aquatic animals.

The aims of this study were therefore to quantify the uptake, dietary assimilation, and efflux of Cd and Zn adsorbed on nano-TiO$_2$ using the radiotracer technique. We focused on two metals (Cd and Zn) mainly because of their environmental importance as metal contaminants, and because the biokinetics of these two metals in *D. magna* has been extensively studied previously.[26−28] *Daphnia magna*, an ecologically important freshwater zooplankton, was used as the model organism. These biokinetic measurements can provide a better understanding of the influx and efflux processes of Cd and Zn adsorbed on nano-TiO$_2$, and reveal the potential hazards of nano-TiO$_2$ as carrier of pollutants.

■ MATERIALS AND METHODS

Organisms, Water, And Radioisotopes. *Daphnia magna* have been cultured in our laboratory for over ten years and were used in this study. The animals were cultured in glass-fiber filtered unpolluted pond water (GF/C Whatman, Maidstone, U.K.) at a temperature of 23.5 °C with a light to dark cycle of 14:10 h. Green algae *Chlamydomonas reinhardtii* were provided as food daily at a density of 10^5 cells/mL, and the water was changed every two days. The algae were grown in an artificial WC medium (containing CaCl$_2$: 0.25 mM, MgSO$_4$: 0.15 mM, NaHCO$_3$: 0.15 mM, K$_2$HPO$_4$: 0.05 mM, NaNO$_3$: 1 mM, H$_3$BO$_3$: 0.1 mM, and trace metals and vitamins).[29] At the exponential growth stage, algae were collected by centrifugation to remove the growth medium, and stored in filtered pond water at 4 °C.

In all experiments, the synthetic water Elendt M7 [30] and the simplified Elendt M7 medium (SM7, containing only CaCl$_2$, MgSO$_4$, K$_2$HPO$_4$, KH$_2$PO$_4$, NaNO$_3$, NaHCO$_3$, Na$_2$SiO$_3$, H$_3$BO$_3$, and KCl and without disodium ethylenediaminetetraacetic acid, trace metals, or vitamins) were used instead of filtered pond water in order to control the medium conditions. M7 was used in the depuration experiment, mainly because it was better than SM7 for growing *D. magna* over a relatively long period of time.

The radioactive isotopes ^{109}Cd and ^{65}Zn were used in this study. The former was obtained from New England Nuclear (Boston, MA, USA) and the latter was obtained from Riso National Laboratory (Roskilde, Denmark). Both isotopes were diluted in 0.1 N HCl. The radioactivity was determined by a Wallac 1480 NaI (T1) γ detector (Turku, Finland), at 88 keV for ^{109}Cd and at 1115 keV for ^{65}Zn. The counts were related to appropriate standards and calibrated for spillover. The counting time was 3 min and was sufficient to yield propagated counting errors of less than 5%.

Characterization of Nano-TiO$_2$. Nano-TiO$_2$ was purchased from Nanjing High Technology Material Co., Ltd. (P.R. China). According to the manufacturer, the nanoparticles were synthesized using a chemical vapor deposition (CVD) method. Powder X-ray diffraction (XRD) was used to identify the crystal phase, and was performed using RigaKu D/max-3C. The instrument was operated at 50 kV and 300 mA, with a dwell time of 1 s per each angle and a step of 0.02°. The particle morphology was analyzed using transmission electron microscopy (TEM, JEOL 2010F) at an acceleration voltage of 100 kV. Nano-TiO$_2$ were dispersed in SM7 medium followed by sonication for 20 min (50 w/l at 40 kHz), dripped onto cleaned 200 mesh Cu carbon grid, and dried at room temperature for one day before the TEM analysis. The average diameter and the ζ potential of nano-TiO$_2$ (0.5, 1, and 2 mg/L) in SM7 were determined by dynamic light scattering with a ZetaPLAS (DLS, Brookhaven Instruments). The changes of average diameter of nano-TiO$_2$ in SM7 with 0.1 μM EDTA (before and after aqueous uptake experiment) and the ζ potentials of nano-TiO$_2$ at different pH conditions were also determined. Because an important property of nano-TiO$_2$ was the high surface area to volume ratio, the surface area was measured with a Nova 2200e BET surface area analyzer (Quantachrome, Boynton Beach, FL). Nitrogen was used as the adsorbate at 77.4 K under isothermal conditions, and nano-TiO$_2$ was degassed overnight before analysis.

One piece of information essential for the biokinetic study of metals is their sorption on and desorption from nano-TiO$_2$. A stock solution of 1 g/L nano-TiO$_2$ was first prepared by dispersing the nanoparticles in Milli-Q ultrapure water (Barnstead, Dubuque, IA, U.S.) followed by sonication for 20 min (50 w/l at 40 kHz). To study the sorption of Cd and Zn onto nano-TiO$_2$, a 2 mg/L nano-TiO$_2$ suspension was prepared using the nano-TiO$_2$ stock in a high-density polyethylene beaker containing 100 mL of SM7. Three replicates were prepared. Radioactive ^{109}Cd and ^{65}Zn were then added into the nano-TiO$_2$ suspension (4 μCi/L Cd and 4 μCi/L Zn), and the pH was adjusted to 8.2 immediately by adding 0.1 N NaOH. At 0.5, 1, 2, 3, 4, and 5 h, 1.5 mL of the nano-TiO$_2$ suspension were centrifuged twice for 10 min at 12 000 ×g using a high-speed centrifuge (91.8−95.0% removal of nano-TiO$_2$ from this centrifugation), then 1 mL of the collected supernate and 1 mL of the original nano-TiO$_2$ suspension were used to measure the radioactivity of ^{109}Cd and ^{65}Zn. The percentage of ^{109}Cd or ^{65}Zn adsorbed on nano-TiO$_2$ was then calculated.

To study the desorption behavior of metals from the nano-TiO$_2$, we first prepared the radiolabeling stock of nano-TiO$_2$ by adding radioactive ^{109}Cd and ^{65}Zn into the 1 g/L nano-TiO$_2$ stock solution. After 12 h, the radiolabeling efficiency was 99.6% for Cd and 98.4% for Zn, suggesting that almost all of the metals had adsorbed onto TiO$_2$. The radiolabeled nanoparticles were then added to 100 mL of SM7 at a concentration of 2 mg/L nano-TiO$_2$ suspension. Again three replicates were prepared. At 0.5, 1, 2, 3, 4, and 5 h, 1.5 mL of the nano-TiO$_2$ suspension were centrifuged twice for 10 min at 12 000 ×g using the same high-speed centrifuge, and then 1 mL of the collected supernate and 1 mL of the original nano-TiO$_2$ suspension were used to measure

the radioactivity of ^{109}Cd and ^{65}Zn. The percentage of ^{109}Cd or ^{65}Zn remaining on nano-TiO$_2$ was then calculated.

Uptake of Cd and Zn Adsorbed on Nano-TiO$_2$. Test solutions of three different nano-TiO$_2$ concentrations, 0.5, 1, and 2 mg/L, were prepared in beakers by diluting the ^{109}Cd- and ^{65}Zn-radiolabeled nano-TiO$_2$ stock containing 0.1 μM EDTA. The control treatments were the supernatant of a 1 mg/L nano-TiO$_2$ suspension and the free ions solution. For each treatment, three replicates were employed, each containing ten 14-day old daphnids (1 individual/10 mL). Daphnids were first removed and allowed to evacuate their guts for 3 h in unspiked SM7 medium without the presence of food particles. Food was not added to the exposure medium during the 3 h uptake period (see Table S1 of the Supporting Information, SI, for experimental purposes, time conditions, and main results). EDTA was added in the uptake medium to ensure that only metals absorbed on nano-TiO$_2$ were taken up by the daphnids (see Results). At the beginning and end of the exposure, a 1-mL water sample was drawn from each replicate for radioactivity measurement, and the average value was used for the calculations. At 20, 40, 60, 90, 120, and 180 min, the daphnids were collected and placed in unspiked SM7 water for approximately 1 min to remove the spiked medium and the loosely adsorbed metals from their carapace, and then their radioactivity was measured. Short-term exposure was used to measure the unidirectional influx assuming that the efflux was negligible during the initial phase of exposure. The daphnids were immediately returned to the previous SM7 medium after measurement. At the end of the 3 h exposure period, the animals were again collected and dried at 80 °C overnight and their dry weights were determined. Meanwhile, at 0, 1, 2, and 3 h, the percentage of ^{109}Cd or ^{65}Zn adsorbed onto nano-TiO$_2$ was also quantified, using methods described above. The dry-weight concentration factor (DCF, L/g dry wt.) was calculated as the radioactivity in daphnids (ccpm/mg dry wt.) divided by the radioactivity in the water (ccpm/mL). In addition, in order to thoroughly understand the Cd and Zn uptake process, the accumulation of nano-TiO$_2$ in daphnids was also quantified after 1 and 3 h of exposure. All of the other experimental conditions were the same as those used in the ^{109}Cd and ^{65}Zn uptake experiments described above. Methods to measure the concentrations of nano-TiO$_2$ in daphnids are described in the SI.

Assimilation of Cd and Zn Adsorbed on Nano-TiO$_2$. Daphnids (14 days old) were first removed and allowed to evacuate their guts for 3 h in unspiked SM7 medium without the presence of food particles. ^{109}Cd and ^{65}Zn radiolabeled nano-TiO$_2$ stock (1 g/L) was added to beakers containing 100 mL of the SM7 medium and 0.1 μM EDTA to yield solutions with a range of nano-TiO$_2$ concentrations from 0.5 to 2 mg/L. Thirty individuals were divided equally into three replicates and exposed for 15 min (shorter than the gut passage time), and then the daphnids were rinsed in an M7 medium and analyzed for their radioactivity. We choose 15 min for pulse exposure (or feeding) to ensure that there was negligible egestion of radioactive feces such that the actual intake of radioactive metals could be accurately quantified. Afterward, the daphnids were transferred into an unspiked M7 medium with algae (10^4 cells/mL). The radioactivity remaining in the daphnids was measured over a 38-h depuration period at intervals of 3 to 14 h. The water and food were renewed after each radioactivity measurement. Again, the accumulation and depuration of nano-TiO$_2$ in daphnids were also concurrently quantified in this experiment.

Assimilation of Cd and Zn Adsorbed on Nano-TiO$_2$ with Algae. In this experiment, the algae (*Chlamydomonas reinhardtii*) were first collected onto 1 μm polycarbonate membranes and rinsed with SM7 medium into a centrifuge tube (BD Falcon). Then, the tube was shaken by hand to resuspend the algae in the SM7 medium, to which a ^{109}Cd- and ^{65}Zn-radiolabeled nano-TiO$_2$ stock was then added. The density of algae was 5×10^6 cells/mL in this SM7 medium. After 6 h of radiolabeling, the algae were centrifuged and resuspended in the SM7 medium immediately before the experiment to minimize the amount of weakly adsorbed radioisotopes. In each treatment, three replicates were employed, each containing 10 individuals in 100 mL of the SM7 medium. There were four treatments of food concentrations (5×10^3, 10^4, 2×10^4, and 105 cells/mL), with the corresponding nano-TiO$_2$ concentration of 0.25, 0.5, 1, 5 mg L^{-1}, respectively. However, the actual concentrations of nano-TiO$_2$ in the algal cells were the same among the different algal concentration treatments. After 15 min of feeding and radioactivity measurement, the daphnids were returned to an M7 medium under the same algae density without radioisotopes or nano-TiO$_2$. The radioactivity remaining in the daphnids was measured over a 38-h depuration period at intervals of 3 to 14 h. The water and food were renewed after each radioactivity measurement.

Efflux of Cd and Zn Adsorbed on Nano-TiO$_2$. To determine the efflux of Cd and Zn adsorbed on nano-TiO$_2$, 20 individuals were exposed to the SM7 medium containing ^{109}Cd- and ^{65}Zn-radiolabeled nano-TiO$_2$ (0.5 and 2 mg/L) and 0.1 μM EDTA for 3 days. Each day, the animals were rinsed and placed in a nonradioactive SM7 medium in which they were fed with the unlabeled algae at a cell density of 10^5 cells/mL for 4 h after 20 h of radioactive exposure. There were three replicates in each treatment. After 3 days of radiolabeling to ensure that the metals were distributed in the slowly exchanging compartments (e.g., after absorption across the gut linings), the animals were rinsed, removed, and counted for their radioactivity, and then depurated in 200 mL of the M7 medium with algae at a cell density of 10^5 cells/mL. The radioactivity retained in the animals was measured every 12 h within the first 2 d and once every day for the following 5 days. The water and food were renewed at every time point.

■ RESULTS AND DISCUSSION

Characterization of Nano-TiO$_2$ Nanoparticles. The XRD results of nano-TiO$_2$ (Figure S1 of the SI) showed that the peaks observed in the sample indeed matched with those expected for the crystal phase of anatase (number: 21−1272, space group: 2/m/2) and no apparently irrelevant peaks existed, indicating that nano-TiO$_2$ used in this study was only anatase as described by the manufacturer. TEM image (Figure S2 of the SI) showed that nano-TiO$_2$ tended to aggregate, but in general was well-dispersed in SM7 medium. The average diameter of nano-TiO$_2$ measured by DLS increased from 189.0 to 266.1 nm with increasing concentrations of nano-TiO$_2$ from 0.5 to 2 mg/L in SM7 medium (Figure S3A of the SI), indicating that aggregate formation was concentration-dependent. Meanwhile, this tendency to aggregate was explained by the reduction in the zeta potential (Figure S3B of the SI). After adding 0.1 μM EDTA into the SM7 medium, the average diameter of nano-TiO$_2$ increased from 180.5 to 215.6 nm with increasing concentrations of nano-TiO$_2$ from 0.5 to 2 mg/L. Therefore, using EDTA in our experiments did not enhance the additional aggregation of
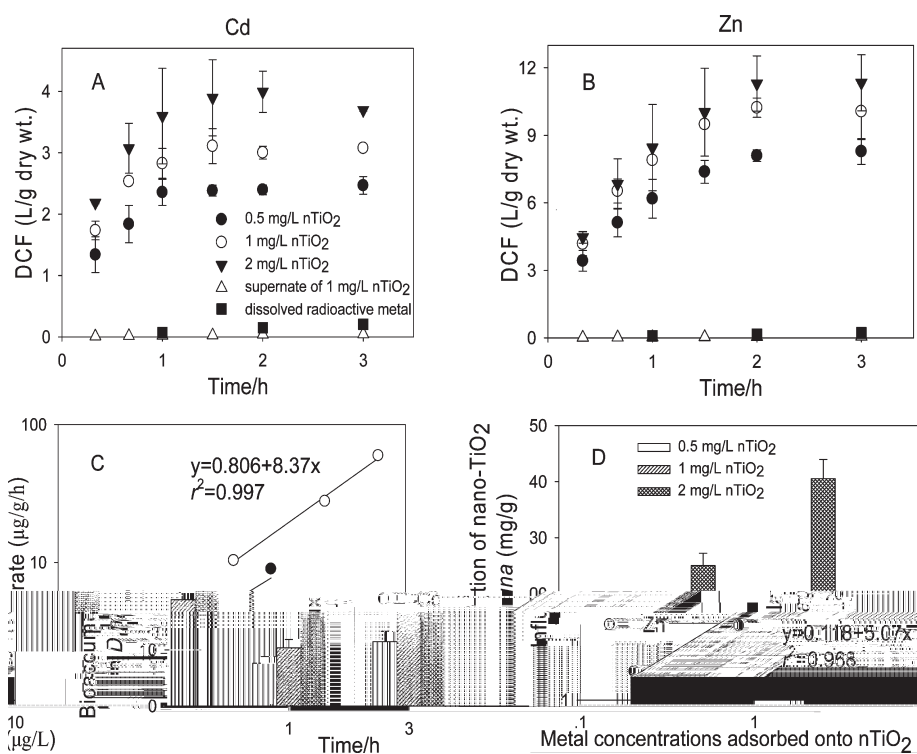
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dx.doi.org/10.1021/es202110d |*Environ. Sci. Technol.* 2012, 46, 469–476

Figure 1. (A) and (B) Uptake of [109]Cd and [65]Zn adsorbed on nano-TiO$_2$ (0.5, 1, and 2 mg/L) and from the supernate of nano-TiO$_2$ suspension by *D. magna* in simplified Elendt M7 medium containing 0.1 μM EDTA during the 3-h exposure. The dissolved uptake of [109]Cd and [65]Zn by *D. magna* without EDTA was the control. (C) Relationship between the metal influx rate (I) and the concentration of metal adsorbed on nano-TiO$_2$ during the first hour of exposure. (D) Accumulation of nano-TiO$_2$ in *D. magna* at different concentrations after 1 and 3 h of exposure. Values are mean $\pm$ SD (n = 3).

nano-TiO$_2$. However, the average diameter of nano-TiO$_2$ decreased significantly after 3 h exposure, which may have been due to the selective ingestion of larger size of nano-TiO$_2$ by the daphnids. The surface area of nano-TiO$_2$ was 114.7 m^2/g, and such relatively large surface area provided a great potential for sorption of Cd and Zn in this study.

The sorption and desorption kinetics of metals at 2 mg/L nano-TiO$_2$ are shown in Figure S4 of the SI. Both the adsorption and desorption processes were rapid, such that the equilibrium was reached within the first 30 min. In subsequent experiments, the radiolabeled nano-TiO$_2$ stock was used to make the required nano-TiO$_2$ suspension. However, since metals were desorbed from the nano-TiO$_2$ during the exposure (32% for Cd and 9% for Zn in the 2 mg/L nano-TiO$_2$ suspension), and were available for uptake by daphnids, 0.1 μM EDTA was used to complex with any released free ions.

Uptake of Cd and Zn Adsorbed on Nano-TiO$_2$. When the free Cd and Zn ions were complexed with EDTA, the uptake of such complexes in daphnids was very slow (Figure 1A,B) due to the unavailability of the Cd-EDTA and Zn-EDTA complex. Previous study also found that the EC$_{50}$ values (24 h) in *D. magna* of EDTA-Cd or EDTA-Zn were 316 times and 165 times higher than those of the free Cd and Zn ions, respectively.[31] Thus, with the use of EDTA, it was possible to quantify the uptake of [109]Cd and [65]Zn adsorbed nano-TiO$_2$ directly. Vohra and Davis[32] demonstrated that the amount of EDTA adsorbed on P-25 TiO$_2$ (2 mg/L, 20/80 mixture of rutile and anatase) decreased with an increase in pH, reaching approximately zero at pH 8. In addition, Yang and Davis[33] also demonstrated that the amount of EDTA-Cd and EDTA-Cu (EDTA binding Cd and Cu) absorbed on P-25 TiO$_2$ reached approximately zero when

the pH was more than 8. Therefore, in this study, EDTA and EDTA-Cd (or EDTA-Zn) could not adsorb on nano-TiO$_2$ in SM7 medium, in which the pH was about 8.2. In addition, EDTA could compete with nano-TiO$_2$ in binding Cd and Zn, resulting in a decreasing percentage of Cd and Zn absorbed on nano-TiO$_2$ (Figure S5 of the SI). After the daphnids were exposed to nano-TiO$_2$ at a series of concentrations for 3 h, the dry-weight concentration factor (DCF) of [109]Cd and [65]Zn increased following a nonlinear pattern (Figure 1A,B) with apparent saturation, and the uptake process was biphasic. A linear uptake was observed within the first hour, and the uptake constant (k$_u$) was calculated as the slope of the linear regression between the metal influx rate (I) and the concentration of metals adsorbed on nano-TiO$_2$ in the suspension (Figure 1C). The y-intercept of the linear regression was considered as the initial nonexchangeable adsorption. The k$_u$ of Cd was 5.07 $\pm$ 0.79 L/g/h and that of Zn was 8.37 $\pm$ 0.97 L/g/h (values in 95% confidence intervals), which were 80.6 and 185 times higher than the k$_u$ of Cd and Zn based on dissolved uptake in a previous study.[26] Such significant increases can be explained by the direct ingestion of nano-TiO$_2$ by the daphnids into the gut (Figure 1D). *D. magna* were able to feed on particles of 0.4—40 μm in size, a big range indeed,[34,35] and nano-TiO$_2$ tended to aggregate and agglomerate in the exposure medium, which could easily be seized by daphnids. It was also reported that *D. magna* fed on bacteria of about 200 nm in size.[36] The k$_u$ of Cd and Zn was also consistent with the recent finding of the high k$_u$ (2.2 L/g/h) of silver nanoparticles (AgNP) in *D. magna*.[37]

Uptake of metals apparently reached saturation after 1 h of exposure. As the concentration of nano-TiO$_2$ increased from 0.5 to 2 mg/L, the DCF of Cd increased by 11.0—16.9 times, and
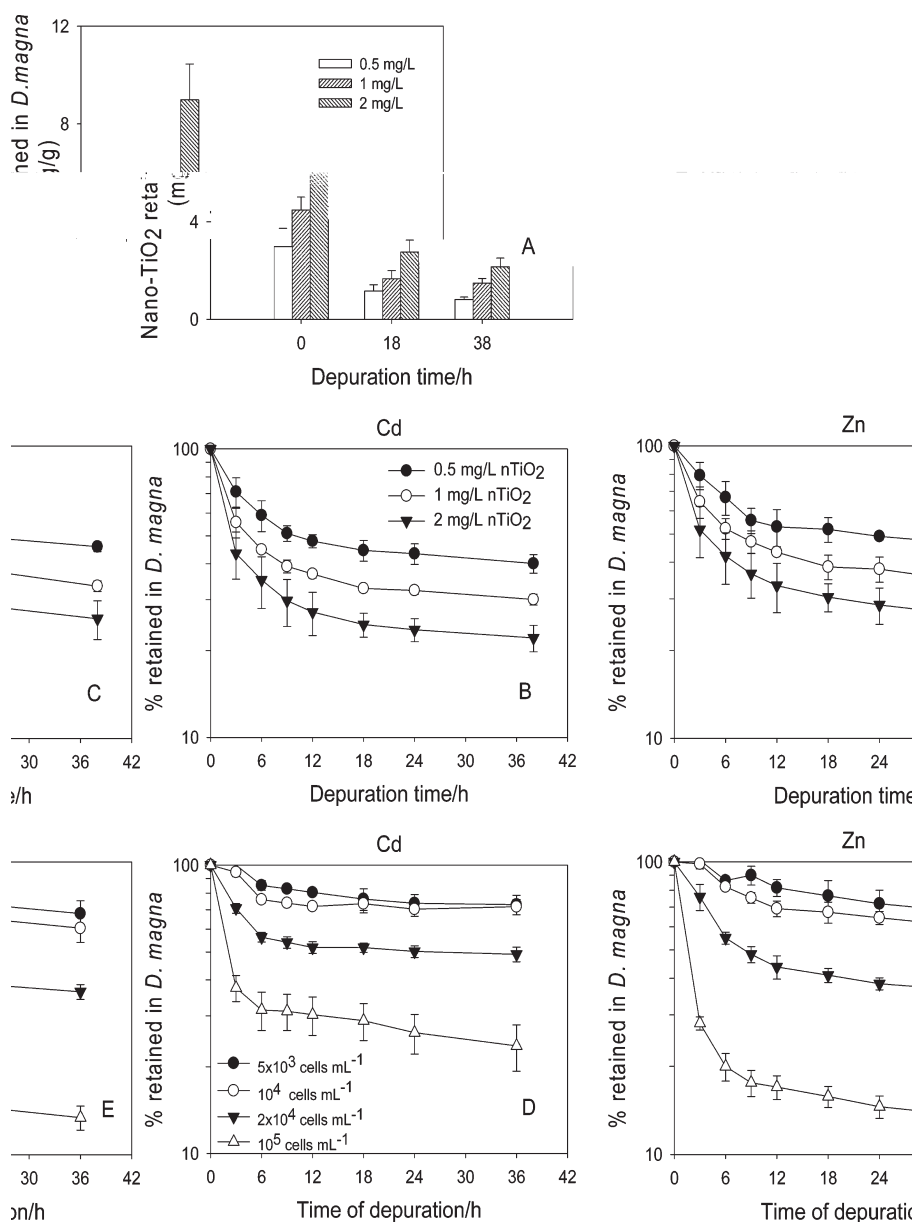
Figure 2. (A) and (B) and (C) Retention g2235O

that of Zn increased by 37.2 to 51.3 times compared with the uptake from the dissolved phase (Figure 1A,B) after 3 h exposure. Such increases in DCF with increasing nano-TiO$_2$ concentration could also be explained by the ingestion of these nanoparticles (Figure 1D). In contrast, DCF of metals from the dissolved phase in D. magna is generally independent of the metal concentrations in the water.[26] Saturation of uptake observed in this study was not due to the limitation of nano-TiO$_2$ available to daphnids. When the exposure medium was renewed at 3 and 6 h of exposure, the DCF of Cd and Zn still increased slightly after 3 h of exposure (Figure S6 of the SI).

Unexpectedly, the metal DCF in D. magna reached the apparent saturation within 3-h of exposure at the concentrations of 9.71 to 38.8 nM for Cd and 38.5 to 153.8 nM for Zn. In contrast, previous studies found no evidence of saturation or

steady-state condition in daphnids during the 12-h exposure to Cd (<180 nM) and Zn (<769 nM).[26] Regarding the accumulation of nano-TiO$_2$ in daphnids (Figure 1D), there was also evidence of saturation during the 3 h exposure period (7.6 to 25.1 mg/g in daphnids after 1 h exposure, and 11.5 to 40.5 mg/g in daphnids after 3 h exposure). Therefore, nano-TiO$_2$ as carriers facilitated the uptake of Cd and Zn, with a possibility of desorption of Cd and Zn in the gut of daphnids. During the 3 h exposure, the partitioning of Cd and Zn was also quantified at 1, 2, and 3 h (Figure S7 of the SI). Initially, 23—50% of Cd and 46—75% of Zn were adsorbed on nano-TiO$_2$, but these percentages gradually decreased to 10—23% for Cd and 32—56% for Zn during the 3-h of exposure. Besides desorption, such decreases in metal partitioning were also caused by the decrease in the concentration of nano-TiO$_2$ as a result of direct uptake.

Table 1. Assimilation efficiency (AE) of Cd and Zn in *Daphnia magna* Fed on Different Concentrations of Nano-TiO$_2$ without and with Algae Present in the Environment[a]

nano-TiO$_2$ (mg L^{-1})	algal concentration (cells mL^{-1})	Cd AE (%)	Zn AE (%)
0.5	0	44.5 ± 3.7	51.8 ± 5.0
1	0	32.9 ± 0.5	38.6 ± 3.7
2	0	24.6 ± 2.4	30.4 ± 3.4
0.25	5 × 10^3	76.2 ± 6.5	76.5 ± 9.7
0.5	10^4	73.5 ± 5.2	67.3 ± 5.8
1	2 × 10^4	51.8 ± 1.9	41.0 ± 2.3
5	10^5	28.9 ± 4.2	15.8 ± 1.3

[a] Mean±SD (n = 3).

Assimilation of Cd and Zn Adsorbed on Nano-TiO$_2$. In the metal uptake experiment, *Daphnia magna* could ingest nano-TiO$_2$, thus the assimilation efficiency (AE) of Cd and Zn adsorbed on nano-TiO$_2$ was further investigated. In addition, it was observed that nano-TiO$_2$ could be absorbed on algae, and so the AE of Cd and Zn bound to nano-TiO$_2$ but with algae as the carrier was also determined. The depuration of Cd and Zn following the 15-min pulse ingestion of radiolabeled nano-TiO$_2$ without and with algae is shown in Figure 2 (Figure 2B, C for "without" and Figure 2D,E for "with"). In general, ingested Cd and Zn were rapidly released within the first 12 h, after which there was a gradual loss. The AE was calculated as the amount of radioactivity in the daphnids at 18 h divided by the amounts ingested (Table 1). Without the presence of algae, the AE decreased significantly from 44% to 25% for Cd and from 52% to 30% for Zn, as the concentration of nano-TiO$_2$ increased from 0.5 to 2 mg/L (p < 0.01, one-way ANOVA). This trend was similar to that reported for the influence of algal concentration on metal AE—the Cd AE decreased from 65% to 56% and the Zn AE decreased from 26% to 16%, as the concentration of algae (*Chlamydomonas reinhardtii*) increased from 0.55 to 2.73 mg C/L.[27] Again, such relationship also indicated that the bioaccumulation of Cd and Zn adsorbed on nano-TiO$_2$ was due to the direct ingestion of these nanoparticles. In addition, the uptake of nano-TiO$_2$ in daphnids increased from 3.0 to 9.0 mg/g with increasing concentration of nano-TiO$_2$ from 0.5 to 2 mg/L, following 15 min of pulse exposure. After 18 h of depuration, 30.1–38.9% of ingested nano-TiO$_2$ still remained in the daphnids, and such percentage decreased with increasing concentrations of nano-TiO$_2$.

Wang and Guo[38] demonstrated that colloid-bound Zn and Cd were bioavailable to two marine bivalves (*Ruditapes philippinarum* and *Perna viridis*), and suggested that the dissociation of metals from colloidal organic matter was the critical process of metal internalization. Thus, it was possible that Cd and Zn adsorbed on nano-TiO$_2$ dissociated in the gut of daphnids and the free Cd and Zn ions were bound to specific protein transporters. Meanwhile, the AEs of Zn were 16.4−23.5% higher than the AEs of Cd, also indicating that desorption of Cd and Zn from nano-TiO$_2$ occurred in the gut of daphnids. The pH$_{PZC}$ of nano-TiO$_2$ (2 mg/L) in SM7 was between 3.3 and 3.4 (Figure S4 of the SI), which was lower than the pH of the exposure medium SM7 (pH = 8.2). Therefore, the nano-TiO$_2$ were present in the form of the negatively charged Ti−OH$^{1/2−}$ in SM7, leading to Cd (or Zn) complexing with nano-TiO$_2$ to form Ti−OH$^{1/2−}$ Cd(OH)$^+$ by physical sorption and Ti−O−Cd(OH)$^{1/2+}$ by

chemical sorption.[15] However, the pH in the gut of *D. magna* was in the range of 6.8−7.2,[39] which was lower than that of the exposure SM7 medium (pH = 8.2). Thus the change of pH in animal gut may lead to desorption of Cd and Zn, especially the reversible physical sorption through electrostatic force. In addition, other complicated translocations of nanoparticles across the gastrointestinal tract such as endocytosis through "regular" epithelial cells, M-cell-uptake (transcytosis), persorption, and putative paracellular uptake have been observed.[40] It was therefore possible that Cd-nano-TiO$_2$ and Zn-nano-TiO$_2$ as a whole entity may also be assimilated by *Daphnia*.

When nano-TiO$_2$ was adsorbed on algae (5 × 10^3 to 10^5 cell/mL), the AE of Cd decreased from 76.2% to 28.9%, and that of Zn decreased from 76.5% to 15.8%, therefore the metal AEs were negatively correlated with the algal concentration. Among the different algal concentration treatments, the nano-TiO$_2$ concentrations in the algae were comparable, even though its total concentrations in the water increased correspondingly with increasing algal concentration. Such decreases of metal AE with increasing algal concentration can be explained by the changes in filtration activity and the reduced gut passage time for both food and metals.[41] In our experiment, the algae were only radiolabeled with Cd-nano-TiO$_2$ and Zn- nano-TiO$_2$ for 6 h, and it was obvious that the algae enhanced the bioavailability of Cd and Zn absorbed on nano-TiO$_2$.

Efflux of Cd and Zn Absorbed on Nano-TiO$_2$. Efflux experiments were conducted after the daphnids were exposed to Cd and Zn absorbed on nano-TiO$_2$ at two concentrations (0.5 and 2 mg/L) for 3 d. The control treatment involved daphnids radiolabeled with Cd and Zn from the dissolved phase. Within the first 12 h, there was an extremely rapid depuration of both Cd and Zn in the presence of nano-TiO$_2$. The efflux rate constant (k_e) was calculated as the slope of the linear regression between the natural log of the percentage of metal retained in daphnids and the depuration time between 2 and 7d (Figure 3A,B). Therefore, the k_e was 0.038/d for Cd (0.0377 ± 0.008/d for 0.5 mg/L nano-TiO$_2$, 0.0383 ± 0.018/d for 2 mg/L nano-TiO$_2$) and 0.087 ± 0.019/d (0.5 mg/L nano-TiO$_2$)-0.091 ± 0.022/d (2 mg/L) for Zn (values in 95% confidence intervals). Nano-TiO$_2$ concentrations (0.5 and 2 mg/L) had no significant effect on the k_e of Cd and Zn in the SM7 medium (p > 0.05), which was consistent with the results from previous studies which found that k_e did not differ significantly with food density.[27,28] However, the k_e of the control and those of the other treatments were significantly different (p < 0.01, one-way ANOVA), e.g., it was 70% and 62% lower for Cd and Zn, respectively, as compared with the control treatment. Such lower k_e of Cd and Zn adsorbed on nano-TiO$_2$ indicated that it was difficult for the daphnids to depurate the metals and so the metals remained in the animals for a longer period time. Therefore, the efflux behaviors of Cd and Zn were significantly different between different uptake pathways (Cd and Zn from dissolved phase and adsorbed on nano-TiO$_2$). Two possibilities may explain the different efflux behavior of metals. First, nano-TiO$_2$ could generate reactive oxygen species (ROS), which can then damage the cell membrane through lipid peroxidation and the metal transport.[42,43] Second, the whole Cd-nano-TiO$_2$ and Zn- nano-TiO$_2$ entity accumulated by the daphnids may be more difficult to be depurated from the digestive system.

This study has demonstrated the enhanced uptake and retention of Cd and Zn in *D. magna* when the metals are bound to nano-TiO$_2$. The increased bioavailability may also explain the

results of a previous study which found that nano-TiO_2 enhanced the toxicity of copper to *Daphnia magna*.[18] It was clear that metals bound to these nanoparticles may behave fundamentally differently from those in the free ion form, because of the animals' potential ingestion of these nanoparticles directly into their digestive tracts. Cd and Zn adsorbed on nano-TiO_2 may desorb within the gut of daphnids. Clearly, it is necessary to evaluate the potential role of nanoparticles as carrier of other pollutants in the environmental risk assessments of nanoparticles. The much longer and more efficient retention of metals sorbed on nanoparticles could result in chronic toxicity and trophic transfer to the higher levels.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Table S1 shows the experimental purposes, time conditions, and main results. Methods to measure the concentrations of nano-TiO_2 in daphnids and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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