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RESEARCH PAPER

Influence of titanium dioxide nanoparticles on cadmium and lead bioaccumulations and toxicities to *Daphnia magna*

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Abstract Titanium dioxide nanoparticles (TiO_2 NPs) have attracted considerable concerns due to the increasing production and widespread applications, while their influences on other co-existing pollutants in real environment are not well studied. In this paper, the colloidal stability of TiO_2 NPs in the exposure medium was first evaluated, and then, the medium was modified so that TiO_2 NP suspension remained stable over the exposure period. Finally, using the optimized exposure medium, the effects of cadmium (Cd) and lead (Pb) on *Daphnia magna* both in the absence and presence of TiO_2 NPs were investigated. Results showed that 2 mg L^{-1} of TiO_2 NPs was well dispersed in 1:20 diluted Elendt M7 medium without EDTA, and no immobility was observed. The presence of the nanoparticles increased the bioaccumulation and toxicity of Cd to the daphnias. On the contrary, while Pb bioaccumulation was enhanced by three to four times, toxicity of Pb was reduced in the presence of TiO_2 NPs. The decreased toxicity of Pb was more likely attributed to the decreased bioavailability of free Pb ion due to adsorption and speciation change of Pb in the presence of TiO_2 NPs. Additionally, surface-attached TiO_2 NPs combined with adsorbed heavy metals caused adverse effects on daphnia swimming and molting behavior, which is supposed to lead to chronic toxicity.

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Introduction

Titanium dioxide nanoparticle (TiO_2 NPs) is globally one of the most popular nanoparticles due to its high commercial relevance. It has been widely used for example as a UV blocker in sunscreen creams, as a catalyst in water treatment, and as a photocatalyst in degradation of air pollutants (Ju-Nam and Lead 2008). As a consequence of the large production and widespread use of TiO_2 NPs, the release of synthetic TiO_2 NPs from various applications into the aquatic environment is inevitable (Klaine et al. 2008). Gottschalk et al. (2013) reported that the TiO_2 NP concentrations in rivers ranged from 3 ng L^{-1} to 1.6 μg L^{-1}, but the proportion of engineered TiO_2 NPs could not be quantified.

To date, the health risks and environmental impacts of TiO_2 NPs have been studied to some extent in different media and to different model organisms (Li et al. 2013; Mansfield et al. 2015; Xiong et al. 2011). Most studies have focused on biological effects and toxicity of TiO_2 NPs alone. Although the exact mechanisms remain unclear, generation of reactive oxygen species (ROS) and consequently oxidative stress are considered as the main mechanisms for TiO_2 NP toxicity (Okupnik and Pflugmacher 2016; Xiong et al. 2011). Regardless of the direct environmental effects of engineered nanomaterials (ENMs) themselves, they often co-exist with different types of inorganic

and organic pollutants in the natural waters. There may be synergistic toxic effects when ENMs are present in mixtures of other chemicals (Kim et al. 2016; Qin et al. 2014; Wang et al. 2011; Zhang et al. 2007), which will lead to changes in toxicity and bioavailability of other pollutants.

TiO_2 NPs have a net negative surface charge in most water conditions and will therefore bind cationic pollutants such as metals, indicating that the bioavailability and toxicity of other pollutants may be influenced (Handy et al. 2008). Hu et al. (2012) found that the bioavailability and toxicity of lead to *Ceriodaphnia dubia* was significantly enhanced by the presence of nano-CeO_2 and TiO_2 NPs. In another study, the presence of TiO_2 NPs significantly increased the accumulation of Cd by 146% in carp after 25 days of exposure (Zhang et al. 2007).

The toxicity of bare ENMs and the effects on toxicity of other pollutants are associated with their physicochemical properties, such as particle size and crystal structure. Both in a laboratory test and in the environment, the actual concentration and chemistry of ENMs likely change over exposure time due to aggregation and/or sedimentation (Li et al. 2013). Therefore, monitoring of nanoparticle stability during the entire test period is critical for the interpretation of the results of ecotoxicity studies. In order to properly assess the environmental risk of TiO_2 NPs and its combination with different pollutants, the aim of this study was first to seek an exposure medium that was able to maintain TiO_2 NPs stable, and then to investigate the possible effects of TiO_2 NPs on toxicity of cadmium (Cd) and lead (Pd) to *Daphnia magna*. The stability of TiO_2 NPs was evaluated by means of a dynamic light-scattering device. The toxicity of TiO_2 NPs, Cd, and Pb separately on *D. magna* in optimized medium was also studied to provide as background information for toxicity comparisons. To our best knowledge, this is the first study that examines the influence of TiO_2 NPs on toxicity and bioaccumulation of heavy metals to *D. magna* under maintained nanoparticle colloidal stability.

Materials and methods

Chemicals

Titanium oxide (TiO_2-P25) with a nominal primary diameter of 21 nm, and a specific surface area of 50 ± 15 m^2 g^{-1}, was obtained from Evonik Industries AG (Essen, Germany). A stock solution of 1 g L^{-1} was prepared by dispersing TiO_2 powder in deionized water (Millipore, Finland, resistance 18.2 MΩ cm). The stock solution was stored at 4 °C and sonicated at ultrasonic bath (Ultrasonic Cleaners, Bronson, USA) two times for 30 min prior to further dilution and exposure experiments. Cadmium chloride ($CdCl_2$; 99.99%) was purchased from Acros Organics (Geel, Belgium). Lead (II) nitrate ($Pb(NO_3)_2$; 99.999%) was purchased from Merck KGaA (Darmstadt, Germany). Cd stock solution (100 mg L^{-1}) and Pb stock solution (100 mg L^{-1}) were prepared by dissolving $CdCl_2$ and $Pb(NO_3)_2$ in deionized water. Further working solutions were freshly prepared from the stock solutions for each experiment.

Cultivation of *D. magna*

The cultivation was carried out following the OECD TG 202 (OECD 2004) in a Versatile Environmental Test Chamber (MLR-350H, Sanyo, Japan). During cultivation, temperature was maintained at 20 ± 2 °C with a light cycle of 16-h light/8-h darkness. The green alga *Scenedesmus obliquus* was fed to the daphnias three times a week. Elendt M7 medium contains ethylenediaminetetraacetic acid (EDTA) which can strongly bind with various metal ions and result in a reduction on metal ion concentration (Sorvari and Sillanpää 1996; Tan et al. 2012). Therefore, Elendt M7 medium without EDTA was used for cultivation in this study. The OECD TG 202 was followed and appropriate amounts of chemicals except EDTA were dissolved in deionized water as shown in Table 1. The final medium had a pH of 7.8 ± 0.5 and an ionic strength of 8.88 mM.

Characterization and behavior of TiO_2 NPs in test medium

The characterization of size and surface charge of TiO_2 NPs in test medium were performed on a Malvern Instruments (Malvern Zetasizer Nano ZS, UK). Dynamic light scattering (DLS) analyzes the particle hydrodynamic diameter (HDD) and derived count rate (DCR). DCR is obtained by DLS by measuring of the amount of light scattered by the particles in solution per second; thus, it could indicate changes on particle concentration. Zeta potential was determined by measuring electrophoretic mobility of NPs using a laser Doppler velocimetry (LDV). A wavelength of 633-nm He-Ne laser light and a detection angle of 173° were used in the measurements.

Table 1 Composition of Elendt M7 medium without EDTA (OECD TG 202, 2004)

Macronutrients	Concentration (μg L^{-1})	Micronutrients	Concentration (μg L^{-1})
$CaCl_2 \cdot 2H_2O$	293,800	H_3BO_3	357.438
$MgSO_4 \cdot 7H_2O$	123,300	$MnCl_2 \cdot 4H_2O$	90.125
KCl	5,800	LiCl	76.500
$NaHCO_3$	64,800	RbCl	17.750
$Na_2SiO_3 \cdot 9H_2O$	10,000	$ZnCl_2$	13.000
NaNO3	274	KI	3.250
KH_2PO_4	143	NaBr	4.000
K_2HPO_4	184	Na_2SeO_3	2.190
		NH_4VO_3	0.575
		$CoCl_2 \cdot 6H_2O$	10.000
		$CuCl_2 \cdot 2H_2O$	4.188
		$SrCl_2 \cdot 6H_2O$	38.000
		$Na_2MoO_4 \cdot 2H_2O$	15.750

Three replicate measurements were made immediately after dispersing TiO_2 NPs in test medium, and after 1-, 3-, 7-, and 24-h incubation under exposure conditions without organisms. The Elendt M7 medium without EDTA was used at full strength and after dilution by a factor of 10 and 20 (labeled Med0, Med10, and Med20, respectively). All dilutions were made with deionized water and final pH was adjusted to 7.7. The concentration of TiO_2 NPs was 2 mg L^{-1}.

Acute toxicity test

The toxicity tests were carried out according to the OECD TG 202 (OECD 2004) with some modifications. Med20 was used as dilution water. The acute toxicity of TiO_2 NPs to *D. magna* was first evaluated, and was found to be non-toxic at a concentration of 2 mg L^{-1} during 48 h (data not shown).

The toxicity of Cd and Pb was tested both in the absence and presence of 2 mg L^{-1} TiO_2 NPs. The test concentration of Cd and Pb ranged from 66 to 200 and 98–300 μg L^{-1}, respectively. All the dilutions were prepared by diluting the stock solution using Med20. TiO_2 NPs were spiked into the test medium just before the toxicity test was started. Each test involved six test concentrations and one control group with four replicates. For each replicate, five *D. magna* neonates with an age of less than 24 h were transferred into a 25-mL glass test vessel containing 10 mL test solution. The number of immobile organisms was counted after 24- and 48-h exposure.

Chemical analysis

For the analysis of titanium, cadmium, and lead concentrations, medium samples were collected at the end of the test (one sample per concentration and one sample from the control). After removing the daphnias, the test medium was allowed to stand without mixing for about 1 h, and then, 4 mL was pipetted from the middle of the test medium into a test tube containing 1 mL of sulfuric acid (H_2SO_4) diluted 1:1 with deionized water. The metal analyses were done by an inductively coupled plasma-optical emission spectrometer (ICP-OES; Varian Vista-Pro, Australia).

For analyses of Ti, Cd, and Pb in *D. magna*, the animals were collected at the end of exposure, and prepared for ICP-OES analysis. The daphnias from each exposure concentration were combined and rinsed with Med20 for two times. They were then transferred into small basin and dried at room temperature to remove excess water. After drying, concentrated nitric acid (HNO_3; 0.25 mL) and water (0.75 mL) were added to the reaction vessel containing daphnias, which were subsequently subjected to a microwave digestion at 240 °C, 45 bar for 1 h (Milestone Ultrawave MA149–003, Milestone srl, Italy). After digestion, 50 μL of rhodium (1 mg L^{-1}) was added as an internal standard,

and the resulting solution was diluted to a total volume of 5 mL with deionized water.

Results and discussion

Aggregation of TiO_2 NPs in test conditions

Parameters indicating colloidal stability of TiO_2 NPs are summarized in Table 2. TiO_2 particles were characterized in the absence of *D. magna*, but otherwise under identical conditions as the exposure experiments. The zeta potential values showed that TiO_2 aggregates had negatively charged surface in all test media. Particles had a zeta potential between −12 and −19 mV when in diluted media, which was less negative than in deionized water alone at relevant pH value. The lower zeta potential after spiked into exposure media is likely due to the presence of polyvalent ions, as they can decrease, neutralize, or even reverse the surface charge (Adam et al. 2016). The HDD was much larger (2438 nm) when suspended in Med0 than in dioinized water (276 nm) after 24 h. This tendency to aggregate was consistent with previous results (Hartmann et al. 2012; Tan et al. 2012). In addition, the increase in HDD and polydispersity index (PDI), and decrease in DCR obtained by DLS measurments, signified that the TiO_2 NPs have a clear tendency to aggregate and settle out in Med0. Dilution of the medium reduced the aggregation of TiO_2 NPs. Dilutions 1:10 and 1:20 showed lower PDI over time when compared to the undiluted medium. However, the average HDD increased to >300 nm after 1 h in Med10, whereas the HDD was less than 300 nm in Med20 still after 48 h. In Med20, both HDD and PDI were stable over time, and slightly decreased DCR indicated stable particle concentration. Because different particle size and size distribution contribute to different nanoparticle toxicity, these results clearly demonstrate the importance of monitoring the particle concentrations and size during the whole test period. Based on these measurements, the Med20 was used as test solution for the following exposure experiments. Additional experiment with neonates cultured in this medium showed that no immobility

Table 2 Hydrodynamic diameter (HDD), derived count rate (DCR), and zeta potential of TiO_2 nanoparticles (2 mg L^{-1}) in deionized water, Elendt M7 medium without EDTA (Med0, OECD TG 202, 2004), and diluted Med0 by a factor of 10 (Med10) and 20 (Med20) at different time interval

Dilution factor	Time of measurement (h)	PDI	HDD (nm)	DCR (kcps)	Zeta potnetial (mV)
Deionized water	0	0.30	225	6122	−19.5
	1	0.31	274	5771	−20.6
	3	0.31	276	5165	−22.7
	7	0.30	274	4969	−24.1
	24	0.30	276	4174	−21.8
Med0	0	0.34	333	5977	−15.0
	1	0.47	734	5842	−15.1
	3	0.61	1153	5706	−16.6
	7	0.72	1535	5421	−18.3
	24	1.00	2438	4068	−19.0
Med10	0	0.24	230	6242	−12.4
	1	0.33	373	6296	−13.4
	3	0.39	459	6056	−14.6
	7	0.50	669	5762	−16.2
	24	0.80	1403	4256	−18.8
Med20	0	0.31	245	5985	−15.5
	1	0.29	248	5933	−15.8
	3	0.30	260	5818	−17.3
	7	0.31	257	5585	−17.9
	24	0.31	277	5695	−18.8

was observed up to 72 h, demonstrating that Med20 was well tolerated by *D. magna*.

TiO_2 NP dispersion in the presence of *D. magna*, Cd, or Pb

When daphnias were in the test medium, TiO_2 NPs showed a tendency to adhere onto the exoskeleton of *D. magna*, especially at the antennas and filtering apparatus (thoracic legs and abdominal claw). Large surface area and constant movement of these organs cause high numbers of encounters with NPs. This adhesion phenomenon has been previously described by Baun et al. (2008) and Dabrunz et al. (2011). In addition to the surface adhesion, accumulation of TiO_2 NPs in the gastrointestinal tract was also observed under the microscope (image not shown). In this study, the TiO_2 NPs suspended in Med20 had a particle size range of 202–404 nm (Fig. 1a), which falls into the lower end of the range 100–50,000 nm that could be ingested by daphnia species (Geller and Müller 1981). Seitz et al. (2015) also demonstrated that *D. magna* could ingest TiO_2 NPs that fell into the range of from ~90 to ~500 nm. Therefore, TiO_2 NPs suspended in the test medium could be readily ingested by daphnia. Microscopic observation confirmed that TiO_2 NPs were mainly located in the gut and some attached to the carapaces and abdominal appendages of *D. magna* (image not shown).

As shown in Fig. 1a, the HDD of TiO_2 NPs increased from 228 to 404 nm after 48 h when *D. magna* was present. A 55% reduction on the suspended TiO_2 NP concentration compared to its nominal concentration was found. The loss of TiO_2 NPs could be explained by ingestion by *D. magna*, and attachment on *D. magna* body surface and exuviae. Moreover, in a study of mussel exposed to TiO_2 NPs, a reduction on the suspended TiO_2 NP concentration was also observed due to the agglomeration/adsorption processes in the presence of organic particles produced by the mussels (Della Torre et al. 2015). When Cd or Pb was present in the medium, no overt changes in the HDD of the TiO_2 NPs were observed, whereas the zeta potential values were found to be less negative (Fig. 1). This observation could be explained by the adsorption of Cd or Pb cations onto the negatively charged surface of TiO_2 NPs. Many studies have shown the strong sorption ability of TiO_2 NPs for Pb and Cd (Engates and Shipley 2011; Hartmann et al. 2012; Hu et al. 2012; Hua et al. 2012; Liu et al. 2013; Recillas et al. 2011; Xie and Gao 2009; Zhang et al. 2007). In the study of Xie and Gao (2009), zeta potential of TiO_2 NPs also showed slight decreases due to the adsorption of Cd and Pb on the NPs surface. The surface charges of TiO_2 NPs decreased markedly when both Cd and *D. magna* were present (Fig. 1b), especially when both Pb and *D. magna* were present, which suggested an unstable NP colloidal suspension. After 48 h of exposure to Pb and *D. magna*, large aggregates (915 nm) were formed (Fig. 1a), and the suspended TiO_2 NP concentrations significantly decreased (Fig. 2).

Effects of TiO_2 NPs on *D. magna*

In our experiment, the EC_{50} of TiO_2 NPs at 24 h was 8.0 ± 0.8 mg L^{-1}, well above the concentration of

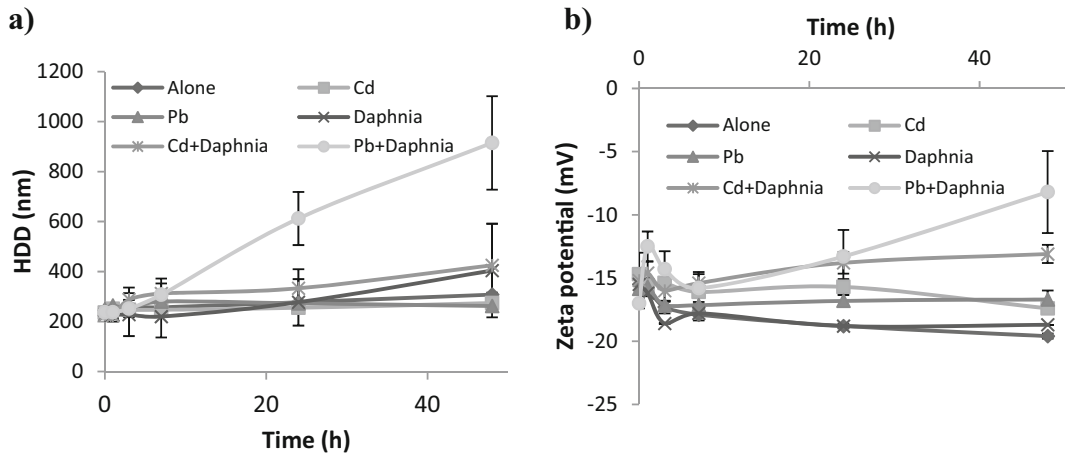

Fig. 1 Effect of *D. magna*, Cd, and Pb on the hydrodynamic diameter (HDD) (**a**) and zeta potential (**b**) of TiO_2 NPs. TiO_2 = 2 mg L^{-1}, Cd = 200 μg L^{-1}, Pb = 300 μg L^{-1}

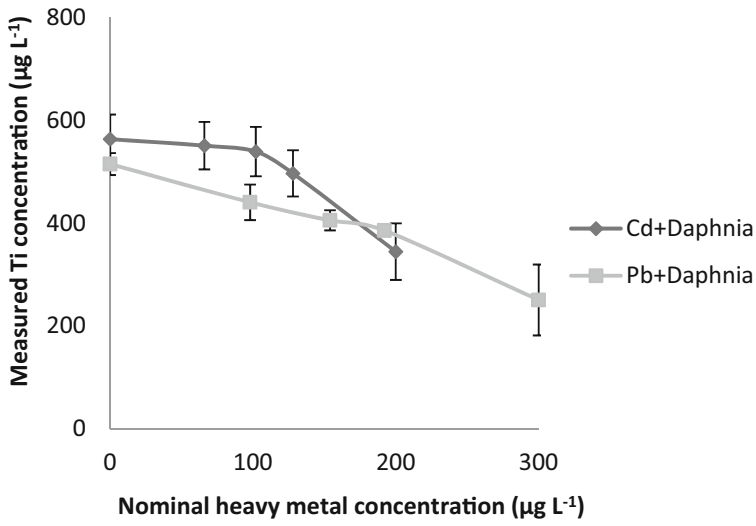

Fig. 2 Measured Ti concentration in exposure medium (μg L^{-1}) when exposed to different concentrations of Cd and Pb in 1:20 diluted Elendt M7 medium without EDTA for 48 h ($n = 3$). *D. magna* was present in all cases

2 mg L^{-1} used in the exposure experiments with Cd and Pb. TiO_2 NP toxicity to daphnia has been investigated before (Hall et al. 2009; Hartmann et al. 2012). Reported 24-h EC_{50} values range from 7.6 to 143.4 mg L^{-1} depending on the different properties of TiO_2 NPs (such as size, crystal composition, and surface modification), and also variability in TiO_2 NP dispersion methods and exposure protocols. The study by Cupi et al. (2016) used an unmodified OECD Elendt M7 medium, soft EPA medium, and a very soft EPA medium to investigate the stability and toxicity of TiO_2 NPs on *D. magna*. Similar to the observation in our study, TiO_2 NPs were found to be unstable in M7 medium (pH 6–9) and formed large agglomerates in the micrometer range. No toxicity was found in this medium, 48-h $EC_{50} > 100$ mg L^{-1}. A TiO_2 NP suspension kept stable with small HDD (~200 nm) in very soft EPA medium (pH 7.0) caused a higher toxicity than that found in M7 medium, 48-h EC_{50} value of 13.7 mg L^{-1}. Study results indicate the imporant influence of media composition and ionic strength levels on the stability of TiO_2 NP suspensions and the immobilization of *D. magna*.

Although 2 mg L^{-1} of TiO_2 NPs showed no overt toxic effect on mobility, *D. magna* was found to accumulate TiO_2 NPs at this concentration over the test period, through surface attachment and ingestion as discussed earlier. In addition, *D. magna* showed abnormal behavior such as slow and sporadic swimming during the exposure test. Such physical defects and a loss of mobility were also observed as result of the surface adhesion in earlier reports (Baun et al. 2008; Charde Manoj et al. 2014). Accumulation of NPs on the body surface could cause an increase in specific weight and physical resistance during swimming movements, and thus lead to an increase in energy consumption.

Toxicity and bioaccumulation of Cd in the presence of TiO_2 NPs

The calculated Cd 24- and 48-h EC_{50} based on nominal Cd concentration were 160 ± 34.5 and 104 ± 6.1 μg L^{-1}, respectively. The toxicity of Cd determined here is lower than the values of 331 μg L^{-1} (Guilhermino et al. 1997), and 299 to 348 μg L^{-1} (Hartmann et al. 2012). However, the media composition was different, which influences cadmium speciation and is likely to cause differences in toxicity. Higher 48-h EC_{50} values were found in tests performed using Elendt M7 medium than in tests using more simple media without chelators (Guilhermino et al. 1997). Protective effects of Ca and Mg on Cd toxicity were also observed by Clifford and McGeer (2010). Hence, the absence of EDTA and dilution of Elendt M7 medium mitigated the protective effects and resulted in a higher Cd toxicity in this study. When the 24- and 48-h EC_{50} were expressed on the basis of measured Cd concentration (analyzed by ICP-OES) in the medium, the values decreased to 147 ± 35.3 and 97 ± 2.7 μg L^{-1}, respectively. The measured concentration of Cd dissolved in medium was close to the nominal concentration (Fig. 3a); no obvious precipitation was observed though phosphate and sulfate were present in the test medium.

Figure 3b shows the Cd bioaccumulation in *D. magna* body at different test concentrations. After

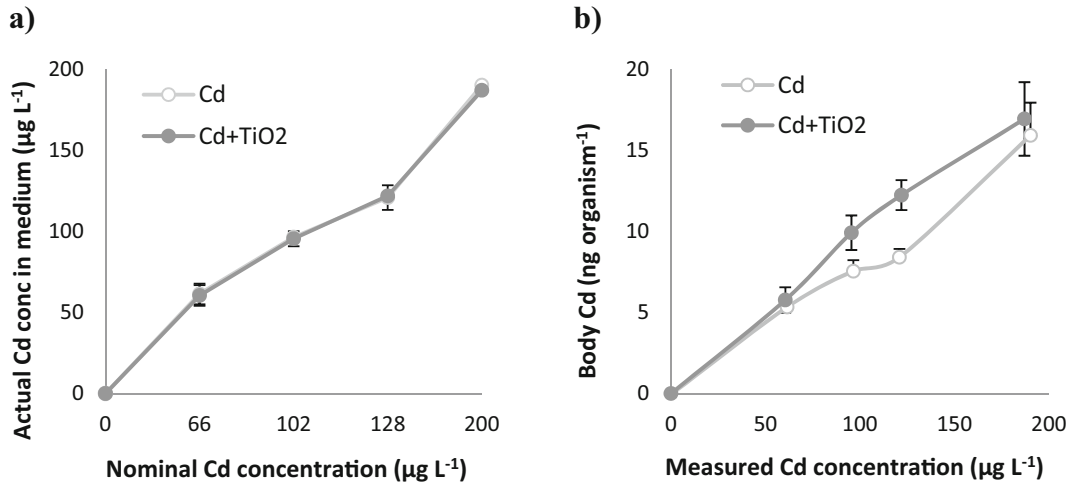

Fig. 3 Measured Cd concentration in exposure medium (**a**) and bioaccumulation of Cd in *D. magna* (**b**) in the absence and presence of 2 mg L^{-1} TiO_2 NPs ($n = 3$)

48-h exposure, the Cd concentration accumulated in *D. magna* increased following a linear pattern with the increase of exposure Cd concentration. No saturation in Cd accumulation was observed. The uptakes of Cd and Zn by *D. magna* were reported to be proportional to their concentrations up to 20 μg L^{-1} in ambient water (Tan and Wang 2014). Guan and Wang (2004) also demonstrated that the accumulated Cd concentrations in *D. magna* increased with ambient Cd concentration.

When 2 mg L^{-1} TiO_2 NP was added to the test medium, Cd concentration in the aqueous phase at the end of test was negligible no matter the absence or presence of TiO_2 NPs (Fig. 3a), whereas changes in heavy metal acute toxicity and bioaccumulation were observed. When TiO_2 NP was present, the organism Cd concentration was increased by up to 46% (Fig. 3b), and 48-h EC_{50} (calculated based on measured Cd concentration) of Cd to *D. magna* decreased by 48% to 50 ± 9.3 μg L^{-1}. Similarly, TiO_2 NPs were found to increase the toxicity of Cd and Zn to *D. magna* due to enhanced uptake (Tan and Wang 2014). Due to their small particle size, large specific surface area, and the presence of high-affinity hydroxyl groups on their surface, TiO_2 NPs have a strong adsorption ability for heavy metals, and may act as carriers for them (Miao et al. 2015; Sun et al. 2009; Tan and Wang 2014). The mechanism of adsorption Cd was suggested to be the chemical sorption through chemical bonding and physical sorption through electrostatic force, of which the reversible physical sorption was dominant (Lin et al. 2016; Tan et al. 2012; Xie and Gao 2009). The ingested Cd adsorbed onto TiO_2 NPs may have finally been released within the gastrointestinal tract of *D. magna*, as hypothesized by Tan and Wang (2014). Similar mechanisms have been proposed for Ag in *D. magna*, and As in *Cerodaphnia dubia* at the presence of TiO_2 NPs (Rosenfeldt et al. 2014; Wang et al. 2011). Given that TiO_2 NP was not toxic to *D. magna* at 2 mg L^{-1}, the accumulated Cd bound on NPs was still bioavailable, and could be assumed to be released from the NP surface into free ions and eventually contributed to an increased toxicity of Cd.

Interestingly, a delay and difficulty in molting was found when both Cd and TiO_2 NPs were present (Fig. 4). Regularly, a juvenile *D. magna* kept at 20 ± 1 °C molts once within 48 h when starved (Smith 1963). As shown in Fig. 4, daphnias molted normally when exposed to 2 mg L^{-1} of TiO_2 NPs alone, whereas in the presence of TiO_2 NPs and Cd, even the first molting could not be completed thorougly; daphnias struggled with exuvia which were still connected with the body tightly. Molting is highly relevant to growth and reproduction, and a delay or disturbance of molting ultimately leads to reduced reproduction rates and long-term toxicity (Dabrunz et al. 2011).

Toxicity and bioaccumulation of Pb in the presence of TiO_2 NPs

The calculated 24- and 48-h EC_{50} based on nominal Pb concentration were 167 ± 18 and 143 ± 47.9 μg L^{-1}, respectively. The 48-h EC_{50} value is markedly lower than that reported earlier by Hu et al. (2012) (EC_{50} value

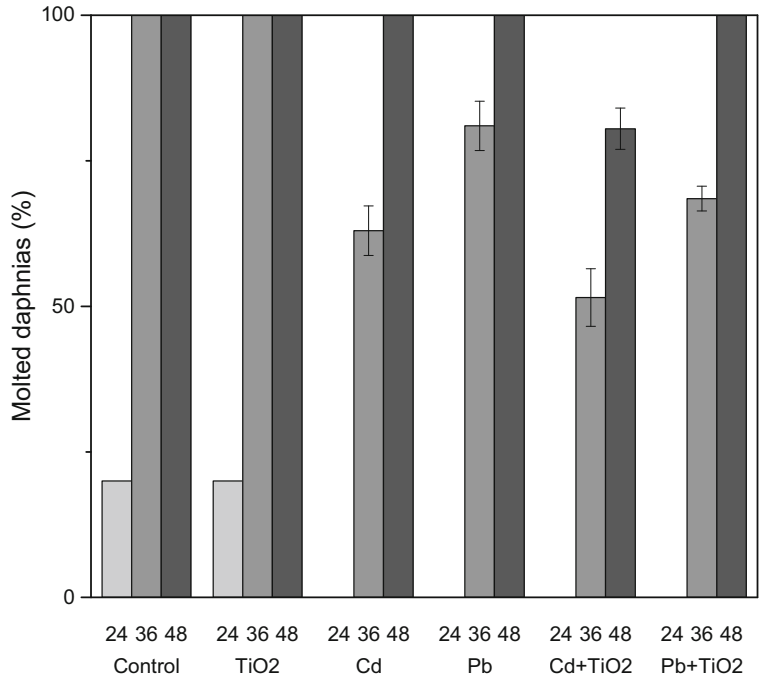

Fig. 4 Molting success of *D. magna* at hour of 24, 36, and 48 ($n = 3$)

606 μg L^{-1}), but it is close to the reported value from Cooper et al. (2009) (EC_{50} value 208.8 μg L^{-1}). Depending on various water chemistry parameters (such as pH, ionic strength, calcium, and dissolved organic carbon), the toxicity may vary significantly due to the speciation of Pb (Mager et al. 2011; Qin et al. 2014). Exposure guidelines and medium composition used in these studies were different compared with the present one, and attributed to the higher toxicity of Pb (OECD 2004; USEPA 2002). Interestingly, a decrease of about 20–42% of the nominal Pb concentration was observed at the end of toxicity test (Fig. 5a). This could be explained by the precipitation of Pb in the presence of phosphate and sulfate in the test medium (Hughes and Poole 1991; Marani et al. 1995). Thus, using nominal concentration to calculate EC_{50} would lead to underestimate of the toxicity of Pb. On the basis of measured Pb concentration in medium, the 24- and 48-h EC_{50} values

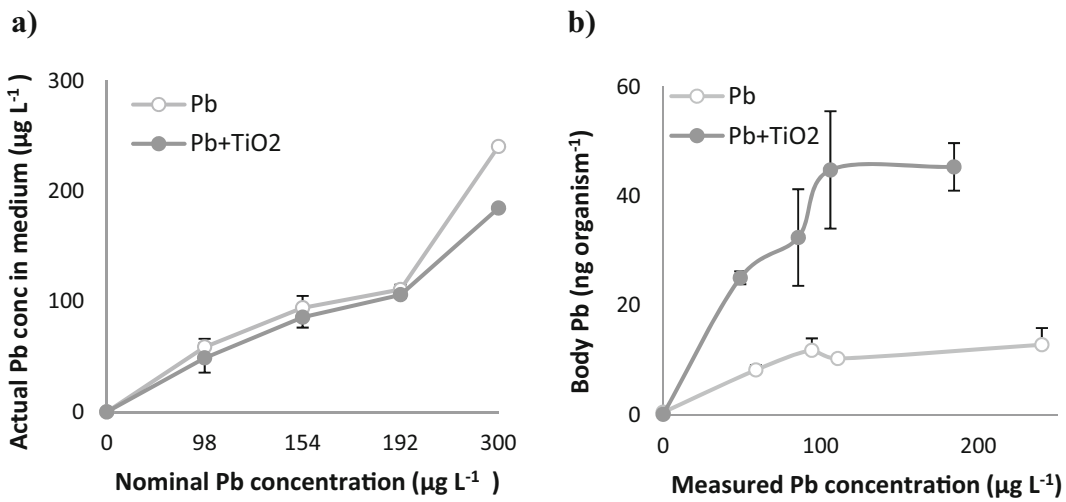

Fig. 5 Measured Pb concentration in exposure medium (**a**) and bioaccumulation of Pb in *D. magna* (**b**) in the absence and presence of 2 mg L^{-1} TiO_2 NPs ($n = 3$)

decreased to 111 ± 11.3 and 62 ± 8.7 μg L^{-1}, respectively. EC_{50} values in the following text are all based on measured metal concentration.

Figure 5b shows the Pb bioaccumulation in *D. magna* body at different test concentrations. After 48-h exposure, bioaccumulation of Pb displayed saturation at concentration of 100 μg L^{-1}. In the study of Miao et al. (2015), no saturation of Pb accumulation in zebrafish was observed, and this was probably due to the lower concentration (30 μg L^{-1}) used in the test. However, a linear increase of Pb body content with increasing ambient Pb concentrations in their study was comparable to the linear increase of accumulated Pb in lower concentration range in this study.

As shown in Fig. 5a, at the end of the toxicity test, the total Pb concentration in the aqueous phase decreased by 23% in the presence of TiO_2 NPs compared to that of Pb alone. This concentration reduction indicated an effect of TiO_2 NPs on the co-transportation of Pb, enhancing either the uptake into the organisms or settle to the bottom of the test vessels. As shown in Fig. 5b, the concentration of Pb accumulated in daphnias after 48 h in the presence of TiO_2 NPs increased three to four times compared to daphnias exposed to Pb alone. Strong sorption capacity of TiO_2 NPs for Pb has been reported in previous reports (Engates and Shipley 2011; Hu et al. 2012; Liu et al. 2013), and the bioaccumulation of Pb in *C. dubia* was significantly enhanced by TiO_2 NPs through nanoparticle uptake. The reduction on the medium concentration of Pb at the presence of TiO_2 NPs was higher than the reduction on Cd, which indicated a higher adsorption capacity of Pd than Cd onto TiO_2 NPs. In addition, the zeta potential of TiO_2 NPs became less negative when Pb was present than when Cd was present, which also indicated a higher adsorption of Pb than Cd. Earlier studies also reported the higher sorption capacity and sorption affinity of TiO_2 NPs for Pb than for Cd (Engates and Shipley 2011; Hua et al. 2012; Liu et al. 2013). The stronger binding of Pd ions onto TiO_2 NPs relative to Cd may result in a much lower portion of Pb ions released from the NP surface and subsequently hamper the bioavailability and toxicity of Pb bound on NPs. Moreover, adsorption onto the TiO_2 NP surface and sedimentation of NPs may reduced the free Pb ion concentration in the medium, which decreases exposure of test organisms. In addition, once free Pb ion was adsorbed onto TiO_2 NPs, potential changes in Pb speciation may have occurred. Sun et al. (2009) previously reported the oxidation of As(III) to As(V) by TiO_2 NP photocatalysis under sunlight. Another study found the photocatalytic reduction of Pb(II) on nanocrystalline TiO_2 coatings under UV irradiation (Yang and Zhang 2010). The toxicity and bioavailability of Pb was dependent on its speciation (Qin et al. 2014). In the present study, when TiO_2 NP was present, the Pb 24- and 48-h EC_{50} values increased by 30% (144 ± 39.2 μg L^{-1}) and 47% (91 ± 2.2 μg L^{-1}) compared to Pb alone, respectively. Considering the lower toxicity of Pb when TiO_2 NP was present, the adsorbed fraction of Pb on TiO_2 NPs could be assumed to be less or no toxic to *D. magna*, even though the bioaccumulation was enhanced through nanoparticle uptake and adhension.

Furthermore, abnormal molting pattern was also observed when daphnias exposed to both Pb and TiO_2 NPs (Fig. 4). Compared to the controls, although all survived daphnias completed the first molting, a delay in molting was observed. Similar to Cd, a delay or disturbance of molting behavior will ultimately lead to a long-term toxicity (Dabrunz et al. 2011).

In addition, the bioaccumulation of TiO_2 NPs in *D. magna* in the presence of Cd or Pb after 48 h was also compared (Fig. 6). The presence of Cd or Pb did not affect the suspended TiO_2 NPs in test medium when compared to the control, while it caused a reduction on the bioaccumulation of TiO_2 NPs in *D. magna*. The surface-attached TiO_2 NPs could be removed with the shedding exuviae when daphnia completed the molting, but the suspended TiO_2 NPs continually attached to the new surface of daphnia. When Cd or Pb was present, the molting behavior was disturbed and delayed as discussed earlier, resulting in a less amount of new attached TiO_2 NPs. There was an increase in the body TiO_2 NPs at the Cd concentration of 200 μg L^{-1} (Fig. 6). This increase was likely explained by the non-shedding, surface-attached TiO_2 NPs due to the unsuccessful molting at higher Cd exposure concentration.

Summary and conclusion

This study demonstrated the effect on toxicity and bioaccumulation of Cd and Pb to *D. magna* in the presence of 2 mg L^{-1} TiO_2 NPs following OECD guideline (TG

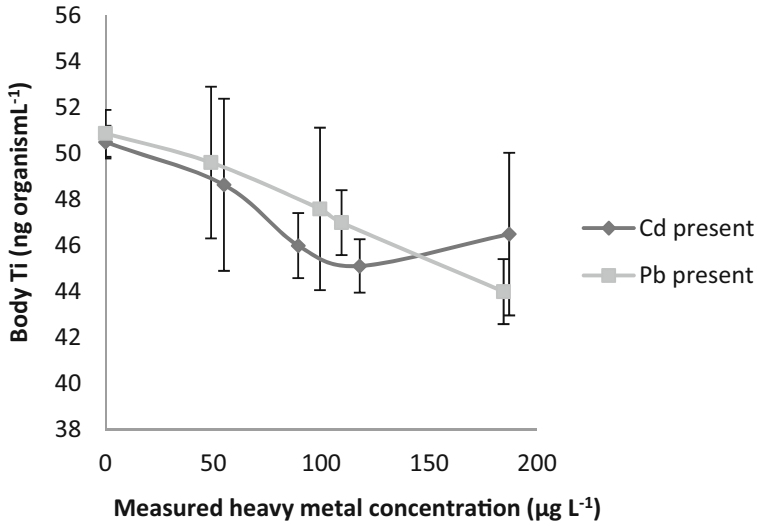

Fig. 6 Bioaccumulation of TiO_2 NPs in *D. magna* (ng organism^{-1}) when exposed to different concentrations of Cd and Pb in 1:20 diluted Elendt M7 medium without EDTA for 48 h ($n = 3$)

202) with some modifications. TiO_2 NP was well suspended in 1:20 diluted Elendt M7 medium without EDTA, providing stable exposure conditions in terms of nanoparticle concentration and size. Of the TiO_2 NPs, 2 mg L^{-1} did not cause death to *D. magna*, but ingestion and attachment to the exoskeleton were observed. When Cd or Pb was co-existing with TiO_2 NPs, TiO_2 NPs could adsorb Cd or Pb from ambient environment and act as carrier, resulting in an enhanced uptake of heavy metals in daphnias. The toxicity of Cd increased by 48% in the presence of TiO_2 NPs, and was supposed to be attributed to the increased body accumulated Cd followed by possible desorption process. Conversely, the Pb toxicity decreased by 30–47% in the presence of TiO_2 NPs. Strong sorption of Pb onto nanoparticles may lead to lower free Pb ion concentration in the exposure medium and lower content of released ions in the organisms. Speciation may also change when Pb adsorb on nanoparticle surface, leading to a low or non-toxic effect to *D. magna*. However, these hypotheses need further investigation before any conclusions on effect mechanisms can be made. Even though no immobility was observed when exposed to 2 mg L^{-1} TiO_2 NPs, daphnias showed abnormal swimming behavior, and this may further affect reproduction, predation, and food intake. When Cd or Pb is present in the medium with TiO_2 NPs, a negative impact on the molting pattern was related to the TiO_2 aggregates attached on daphnia body surface. In real environment, nanoparticles always co-exist with other pollutants and may adsorb and modify their occurance and bioavailability, and ultimatly change their toxicity to water organisms. Therefore, it is necessary not only to evaluate the toxicity of nanopartiles alone but also to investigate the potential interactions with other pollutants in the environmental risk assessments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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