



Effect of titanium dioxide nanoparticles on the accumulation and distribution of arsenate in *Daphnia magna* in the presence of an algal food

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Abstract

The impact of titanium dioxide nanoparticles (nano-TiO₂) on the bioavailability of metals in aquatic filter-feeding organisms has rarely been investigated, especially in the presence of algae as a food source. In this study, we quantified the accumulation and subcellular distribution of arsenate (As^V) in *Daphnia magna* in the presence of nano-TiO₂ and a green alga (*Scenedesmus obliquus*) food source. Results showed that *S. obliquus* significantly increased the accumulation of total arsenic (As) and titanium (Ti) in *D. magna*. The presence of this food source increased As in metal-sensitive fractions (MSF) and as biologically detoxified metals (BDM), while it decreased Ti levels in MSF but increased levels as BDM. The difference in the subcellular distribution of As and Ti demonstrates the dissociation of As from nano-TiO₂ during digestion at subcellular partitioning irrespective of food availability. In turn, the presence of algae was shown to increase metal-based toxicity in *D. magna* due to the transfer of As from BMD to MSF. Furthermore, *S. obliquus* significantly increased the concentration of As and Ti in soluble fractions, indicating that As and nano-TiO₂ ingested by *D. magna* could be transferred more readily to their predators in the presence of *S. obliquus*. Our study shows the potential of algae to increase the toxicity and biomagnification of As^V. Furthermore, it highlights food as an important factor in the toxicity assessment of nanomaterials and co-existing pollutants.

Keywords Nanoparticles · Arsenic · Bioavailability · Subcellular distribution · Algae

Introduction

Along with the development of nanotechnology, titanium dioxide nanoparticles (nano-TiO₂) have been progressively used in increasing numbers of disciplines, such as medicine and healthcare, but also wastewater treatment. The

mass production of nano-TiO₂ may lead to their release into the environment. Therefore, its potential contamination and its subsequent safety have attracted considerable attention. At the same time, arsenic (As) is known to be one of the most hazardous heavy metal pollutants in the environment (Smedley and Kinniburgh 2002; Wang et al. 2017). Released into the environment, earlier studies have shown that nano-TiO₂ can potentially interact with As and has adverse effects on aquatic organisms (Guan et al. 2012; Rosenfeldt et al. 2014). In this light, our previous study showed that a 20 mg/L nano-TiO₂ concentration increased As accumulation and toxicity in *Daphnia magna* (Li et al. 2016).

Daphnia are widely used in standardized toxicity tests. In the environment, algae are a food source for daphnids and, as a result, they inevitably co-occur in nature. Different studies have shown the occurrence of interactions between algae and nanoparticles, which may affect the physical parameters of nanomaterials, such as aggregate size, surface charge, and surface functional groups (Bundschuh et al. 2016; Ji et al.

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2011; Lin et al. 2012). Furthermore, Gong et al. (2011) reported that the microalgae *Chlorella vulgaris* can effectively enhance the aggregation of nickel oxide nanoparticles. At the same time, Metzler et al. (2011) found that nano-TiO₂ can adsorb onto algal cell surfaces in multiple layers. Similarly, Ma et al. (2015) studied algal cell aggregation in the presence of oxide nanoparticles as affected by ionic strength and pH. Algae may thus act either as a source of/or a sink for contaminants to aquatic filter feeders (Deng et al. 2017; Zhang et al. 2017). In this study, we hypothesized that the presence of algae as a food source could be a key factor for the variability in results among toxicity tests (Balmès and Corasaniti 1990). In terms of bioavailability and biomagnification, even though it is of considerable interest to understand the influence of algae on the accumulation and distribution of contaminants associated with nanoparticles in daphnids, it is also of considerable interest to understand the influence of algae for the potential predators of these crustaceans (Barry et al. 1995; Deng et al. 2017; Mangas-Ramírez et al. 2001). In our previous study, we showed how nano-TiO₂ can change the subcellular and spatial distribution of As in *D. magna*, further inducing a change in As toxicity (Li et al. 2016). Therefore, we proposed that algae could affect the distribution of As and nano-TiO₂ in *D. magna*, subsequently resulting in a change in their bioavailability (Deng et al. 2017; Luo et al. 2018). Only limited information, however, is available on the impact of nano-TiO₂ on the bioavailability of As in *D. magna* with the concurrence of algae as a food source.

Additionally, investigating elemental distributions in organisms helps in the identification of accumulation and detoxification mechanisms of heavy metals (Klerks and Bartholomew 1991; Mason and Jenkins 1995; Wallace et al. 2000a). Wallace et al. (2003) proposed that the accumulation and loss of cadmium (Cd) and zinc (Zn) in *Macoma balthica* and *Potamocorbula amurensis* could be explained not only by subcellular partitioning into individual fractions, but also by subcellular compartmentalization as metal-sensitive fractions (MSF) and biologically detoxified metals (BDM). We used the latter in this study to interpret the ecotoxicological effects of As and nano-TiO₂ on *D. magna* with the occurrence of algal food availability, focusing on the effects of nano-TiO₂ on the accumulation and subcellular distribution of arsenate (As^V) in *D. magna* using *Scenedesmus obliquus* as a food source. Accordingly, the objectives of this study were to examine to what extent the availability of algal food alters the accumulation and distribution of As^V in *D. magna* in the presence of nano-TiO₂ and to explore the potential consequential ecological risks in the food chain. This information could provide valuable insight into the bioavailability of As in the presence of nano-TiO₂ in conjunction with the availability of algae as a food source.

Material and methods

Source and cultivation of *Daphnia magna*

D. magna were continuously cultured in our laboratory, which obtained from the Sun Yat-sen University (Guangzhou, China). We used *S. obliquus* as a food source, provided on a daily basis at a density of 0.5×10^6 cells/mL, while we changed the culture medium (simplified Elenndt M7 medium; SM7) every 2 days. The culture was maintained at a constant temperature (22 ± 2 °C) within a natural light–dark (16 h:8 h) cycle.

Preparation of stock solutions

We obtained uncoated and powdered nanoscale Crenox A-100 TiO₂ (nano-TiO₂, CAS No. 1317-70-0) from the Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA), whence we obtained an average surface area of 50 m²/g and an average primary particle size of 25 nm. A nano-TiO₂ stock solution of 1.0 g Ti/L was prepared by dispersing the nanoparticles in ultrapure water (Millipore, Billerica, MA, USA), applying sonication for 10 min (50 W/L at 40 kHz), prior to its application; an additional 10 min sonication was conducted immediately afterward. In our previous study, dynamic light scattering (DLS) measurements showed that the average nano-TiO₂ diameter increased from approximately 250 to 600 nm with increasing concentrations from 2.0 to 20.0 mg Ti/L in the SM7 medium (Li et al. 2016). At the same time, we found that nano-TiO₂ tended to aggregate for sizes from a few hundred nanometers (nm) to several microns (μm) in diameter in the SM7 medium using a scanning electron microscope (SEM, S-4800, Hitachi, Japan). Furthermore, Na₃AsO₄·12H₂O (Sigma-Aldrich Chemicals Co.) was used for the preparation of the As^V stock solutions at a concentration of 100 μM. The stock solutions were stored at 4 °C in the dark until use.

Sorption of arsenate onto algae and nano-TiO₂

We prepared the algae suspension using BG-11 (algal culture medium). The BG-11 and algae were separated by centrifugation at 9000 r/min for 10 min. Following this, the BG-11 was carefully removed using a pipette. For actual toxicity testing, the algae were resuspended in SM7 using a centrifuge tube (BD Falcon). After thoroughly mixing, the nano-TiO₂ solution was added immediately and was followed by the addition of an As^V solution. The chosen density of algae was similar to the density used for culturing, which ensured good growth of *D. magna*. At the same time, we set up treatments with nano-TiO₂ and As^V without algae. The two treatments (with and without algae as a food source) were combined with a corresponding As^V concentration of 1 μM and nano-TiO₂ concentrations of 0, 2, and 20 mg/L, respectively. For comparison,

we used the same As^V and nano-TiO₂ concentrations as our previous study (i.e., nano-TiO₂ concentrations of 0, 2, and 20 mg/L and an As^V concentration of 1 μM) (Li et al. 2016). In total, three replicates of each treatment were prepared. At intervals of 0.5, 1, 2, and 3 h, 1.5 mL of the nano-TiO₂ suspension was centrifuged twice for 10 min at 12000g using a high-speed centrifuge (attaining 91.8–95.0% removal of algae and nano-TiO₂). Following this, 1 mL of the collected supernatant and 1 mL of the original nano-TiO₂ suspension were used to measure As concentrations. Finally, the percentage of As adsorbed onto algae and nano-TiO₂ was calculated.

Accumulation experiments

Based on the data obtained from our previous acute toxicity tests, this experiment was conducted at a concentration of 1 μM As^V , three concentrations of nano-TiO₂ (0, 2, and 20 mg/L) and two food levels (0 and 0.5×10^6 cells/mL) (Li et al. 2016). The algae suspension was prepared as described above (“Sorption of arsenate onto algae and nano-TiO₂”). Following this, all resuspended algae were transferred to exposure beakers containing daphnids. The nano-TiO₂ suspension was added immediately, and it was followed by the As^V solution. For each treatment, we prepared three replicates, each containing 200 7-day-old daphnids of similar size (1 individual/10 mL). Prior to the test, the daphnids were transferred in clean SM7 medium to empty their guts. Short-term exposure was used to measure the unidirectional influx, assuming that the efflux was negligible during the initial phase of exposure. After 20, 40, 60, 90, 120, and 180 min, 10 daphnids were collected from each of the exposure beaker replicates to analyze the total content of As and Ti (i.e., the body burden of As and Ti in daphnia). Additionally, after 180 min, 100 daphnids were collected from each of the three exposure beaker replicates to analyze the subcellular distribution. First, to remove traces of the exposure medium, daphnids were washed for a few seconds in ultrapure water and then washed in a 0.1 M potassium phosphate buffer (pH 7.0) to remove external nanoparticles, aggregates, and inorganic metal species (Li et al. 2016). Following this, daphnids were carefully washed in deionized water to remove the potassium phosphate buffer before being placed in Eppendorf tubes. This study used the same method as our previous study to analyze total As and Ti concentrations, applying an Agilent 7500a ICP-MS instrument (Li et al. 2016). Briefly, after adding 50 μL of HNO₃ (69%) and (after 12 h) 50 μL of HF (40%), we dissolved daphnids (after an additional 4 h) by means of microwave digestion (4 min 100 W, 3 min 180 W, 2 min 180 W, 2 min 300 W, 2 min 300 W, and 2 min 450 W) under the proviso of a 20 min suspension period between each application of microwave heat. Thereafter, the samples were diluted with 2% HNO₃ to measure total arsenic (TAs) and titanium (Ti).

Subcellular distribution experiments

Given the different accumulations observed upon exposure to As^V and nano-TiO₂ in the presence and absence of algae, we hypothesized whether the presence of an algal food source would change the subcellular partitioning of As and nano-TiO₂ within *D. magna* even during short-term exposure. To test this hypothesis, a 3-h acute exposure experiment was conducted, and the effects of algal food on the subcellular partitioning of As and nano-TiO₂ within *D. magna* were evaluated. In this experiment, the subcellular partitioning of TAs and Ti in *D. magna* body tissue was determined using the methods described by Wang and Guan (2010). In total, five different fractions were obtained, including cellular debris (containing cell membranes), organelles (containing the nucleus, mitochondria, microsome, and lysosome), heat-sensitive (denatured) protein (HDP) (containing enzymes), heat-stable protein (HSP), or metallothionein-like proteins (MT), and metal-rich granules (MRG). All five fractions were separately assayed for their As and Ti concentrations to allow for the estimation of As and nano-TiO₂ subcellular partitioning. Results were expressed as milligrams (dry weight) per gram. The rate of recovery of the subcellular fraction was approximately from 86 to 105%.

Statistical analysis

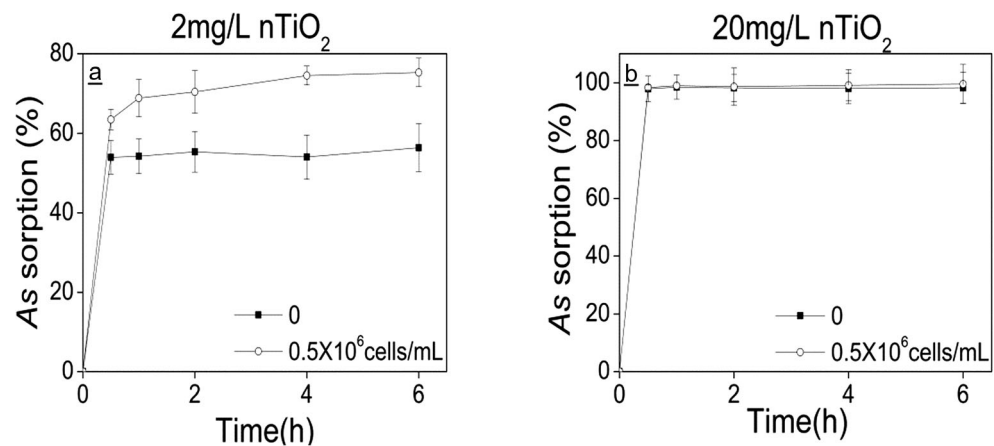
All experiments were independently repeated three times, and data were recorded as means with their corresponding standard deviations (SD). Homogeneity of variance and normal distribution were evaluated, and one-way analysis of variance (ANOVA) with the Tukey’s range test were used to detect significant differences between the control and treated groups. For all data analyzed in SPSS 12.0, a p value < 0.05 was considered statistically significant. Figures were created using Origin 8.5.

Results and discussion

Sorption of arsenate

Figure 1 provides the sorption kinetics of As^V at 2 and 20 mg/L nano-TiO₂. The adsorption processes were rapid, and equilibrium was reached within 30 min. For the 2 mg/L nano-TiO₂ suspension treatments, *S. obliquus* significantly increased the sorption of As^V ($p < 0.05$), namely, from 56.4% (when no *S. obliquus* was added) to 75.3%. It is likely that in addition to adsorbing onto nano-TiO₂, a portion of As^V adsorbed onto *S. obliquus*. For the 20 mg/L nano-TiO₂ suspension treatments, *S. obliquus* had little influence on the sorption of As^V . This is likely the result of almost all As^V (greater than 98%) in solutions being adsorbed onto nano-TiO₂. It is important to note

Fig. 1 Sorption kinetics of As onto nano-TiO₂ (a 2 mg/L; b 20 mg/L) and *Scenedesmus obliquus*. Values are means ± SD (n = 3)



that nano-TiO₂ has a much stronger adsorption capacity to As^V compared to *S. obliquus* (Pena et al. 2005; Wang et al. 2013).

Two distinct phases are known in the uptake of metals by algal cells (Ting et al. 1989; Wang et al. 2013). A rapid phase, which is completed within 10 min, involves physical adsorption through passive ion exchanges with cell walls, which is followed by the slower, long-term active transport of metal ions into the cytoplasm (Wang et al. 2013, 2014). Specifically, the physical adsorption process can exhibit a very rapid and reversible uptake of metal ions (Filippis and Pallaghy 1976; Matzku and Broda 1970; Ting et al. 1989), which can be demonstrated via the easy removal of metal ions from the cell surface by chelating agents, such as ethylenediaminetetraacetic acid (EDTA) (Maeda et al. 1990). In this study, we hypothesize that because metal ions are readily adsorbed onto algal cell surfaces, they may also be desorbed under certain conditions. For example, they could desorb in the digestive tract of consumers where enzymes and acids are secreted to support digestion (Weltens et al. 2000), subsequently affecting the subcellular distribution of As and nano-TiO₂ in *D. magna*.

Accumulation of arsenic and nano-TiO₂ in *Daphnia magna*

The total concentration of As and Ti accumulated in *D. magna* after a 3-h exposure is shown in Fig. 2. For all treatments, *S. obliquus* significantly increased the accumulation of TAs and nano-TiO₂ in *D. magna*. For example, in the 0, 2, and 20 mg/L nano-TiO₂ treatments, TAs increased by a factor of 3.3, 4.9, and 1.9, respectively, compared to the treatments where no food was added (Fig. 2 A1, B1, and C1). Similarly, in the 2 and 20 mg/L nano-TiO₂ treatments, nano-TiO₂ increased by 2.4 and 0.9 times respectively compared to the treatments where no food was added (Fig. 2 B2 and C2). Most importantly, in treatments where both nano-TiO₂ and *S. obliquus* were present, As uptake by *D. magna* was higher compared to treatments containing nano-TiO₂ but no food source, and

this was followed by treatments containing *S. obliquus* but no nanoparticulate material (Fig. 2 A1).

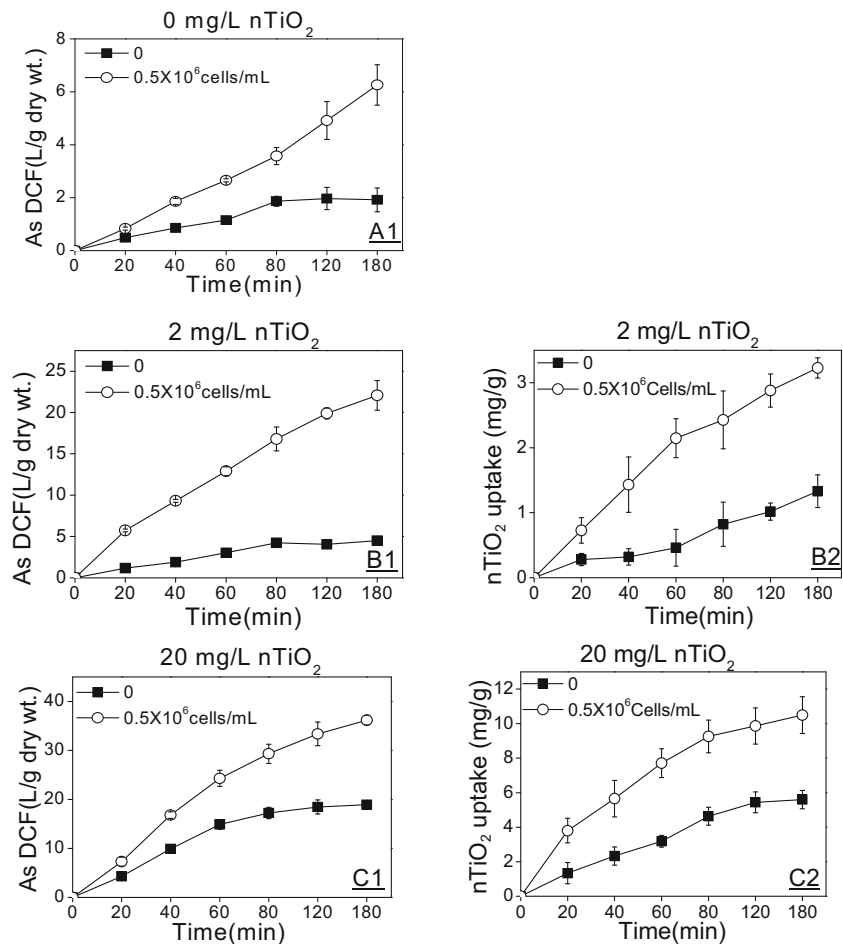
Such increases in As^V accumulation with increasing nano-TiO₂ concentrations and the addition of *S. obliquus* as a food source could be explained by the co-ingestion of nanoparticles (Fig. 2 B2 and C2) and *S. obliquus* by the test organism (*D. magna*). Moreover, *D. magna* is able to feed on a sizable particulate range (from 0.4 to 40 μm) (Geller and Müller 1981; Gophen and Geller 1984). Similarly, *D. magna* feeds on bacteria of an approximate size of 200 nm (Hartmann and Kunkel 1991; Rosenkranz et al. 2009). In our previous study, when 1 μM As^V was added to 20.0 mg Ti/L of nano-TiO₂, the average diameter of nano-TiO₂ was 580 nm (Li et al. 2016). Most likely, nano-TiO₂ and *S. obliquus* tended to form heteroagglomerates with a size range from 0.2 to 40 μm, which could be easily ingested by *D. magna* (Seitz et al. 2013).

Subcellular distribution in *Daphnia magna*

For treatments that excluded the food source, As in daphnids was largely distributed into organelles, cellular debris, and HDP. In contrast, As was largely distributed into five subcellular fractions for treatments that included the food source. At the same time, Ti in daphnids was largely distributed into HSP for treatments that excluded the food source, but largely distributed into metal-rich granules, cellular debris, and HSP for treatments that included the food source (Fig. 3). The differences in subcellular distribution between As and Ti indicated that As dissociated from nano-TiO₂ irrespective of food source availability. This dissociation could further cause toxic effects to *D. magna* due to an increase in free As ions (Li et al. 2016).

Previous studies have proposed that the subcellular partitioning of metals within aquatic invertebrates reflect internal processes that occur during metal accumulation, and this could provide valuable insight into metal toxicity and tolerance (Jenkins and Mason 1988; Klerks and Bartholomew 1991; Roesijadi 1981). Metals associated with organelles and enzymes could be viewed together as a

Fig. 2 Uptake of As adsorbed onto nano-TiO₂ (0, 2, and 20 mg/L) by *Daphnia magna* in simplified Elenct M7 medium containing 0 or 0.5 × 10⁶ cells/mL *Scenedesmus obliquus* at a 3-h exposure (A1, B1, and C1). The corresponding accumulation of nano-TiO₂ in *Daphnia magna* at different concentrations (B2 and C2). Values are means ± SD (n = 3)



subcellular compartment containing metal-sensitive fractions (MSF) (Brown and Parsons 1978; Jenkins and Mason 1988; Roesijadi 1981; Wallace et al. 2000b). At the same time, if metals sequestered in MT and MRG are considered collectively as a subcellular compartment that provides metal tolerance and perhaps resistance, metals within this compartment could be defined as BDM. Results demonstrated that the presence of algae significantly increased the As concentration partitioned to MSF, but it decreased the Ti concentration of this fraction (Fig. 4). For example, for the 0, 2, and 20 mg/L nano-TiO₂ treatments, As concentrations in MSF increased by 9, 29, and 23 times, respectively, compared to treatments that excluded the food source. Also, the presence of algae significantly increased As and Ti concentrations partitioned to BDM (Fig. 4). For example, As concentrations in BDM increased by 35, 24, and 7 times, respectively, compared to treatments that excluded the food source for the 0, 2, and 20 mg/L nano-TiO₂ treatments, while Ti concentrations in BDM increased by 51 and 103, respectively, compared to treatments that excluded the food source for the 2 and 20 mg/L nano-TiO₂ treatments. This indicated that Ti was mainly distributed in BDM (Fig. 4). Our previous study demonstrated that the transfer of metals to MSF following BDM saturation could cause a direct

biological toxicological response in *D. magna* (Li et al. 2016). Additionally, As was found to dissociate from nano-TiO₂ at a subcellular level irrespective of food source availability in this study, potentially inducing toxic effects in *D. magna* due to the presence of free As (Li et al. 2016). As a result, the presence of algae (food) should increase *D. magna* toxicity.

Trophic transfer of arsenic

The trophic transfer of metals to predators can be influenced by the subcellular distribution of metals within an organism (Wallace and Lopez 1997; Wallace et al. 1998). Specifically, the bioavailability of metals in soluble fractions of prey (i.e., cytosols and proteins) is higher for predators than metals bound to non-soluble fractions (i.e., cell walls, exoskeletons, and metal concretions) (Reinfelder and Fisher 1994; Wallace and Lopez 1997; Wallace et al. 1998; Wallace and Luoma 2003). In this study, algae significantly increased As and nano-TiO₂ concentrations in soluble fractions (Fig. 5). For example, As concentrations increased by a factor of 15, 16, and 10, respectively, compared to treatments that excluded the food source for the 0, 2, and 20 mg/L nano-TiO₂ treatments.

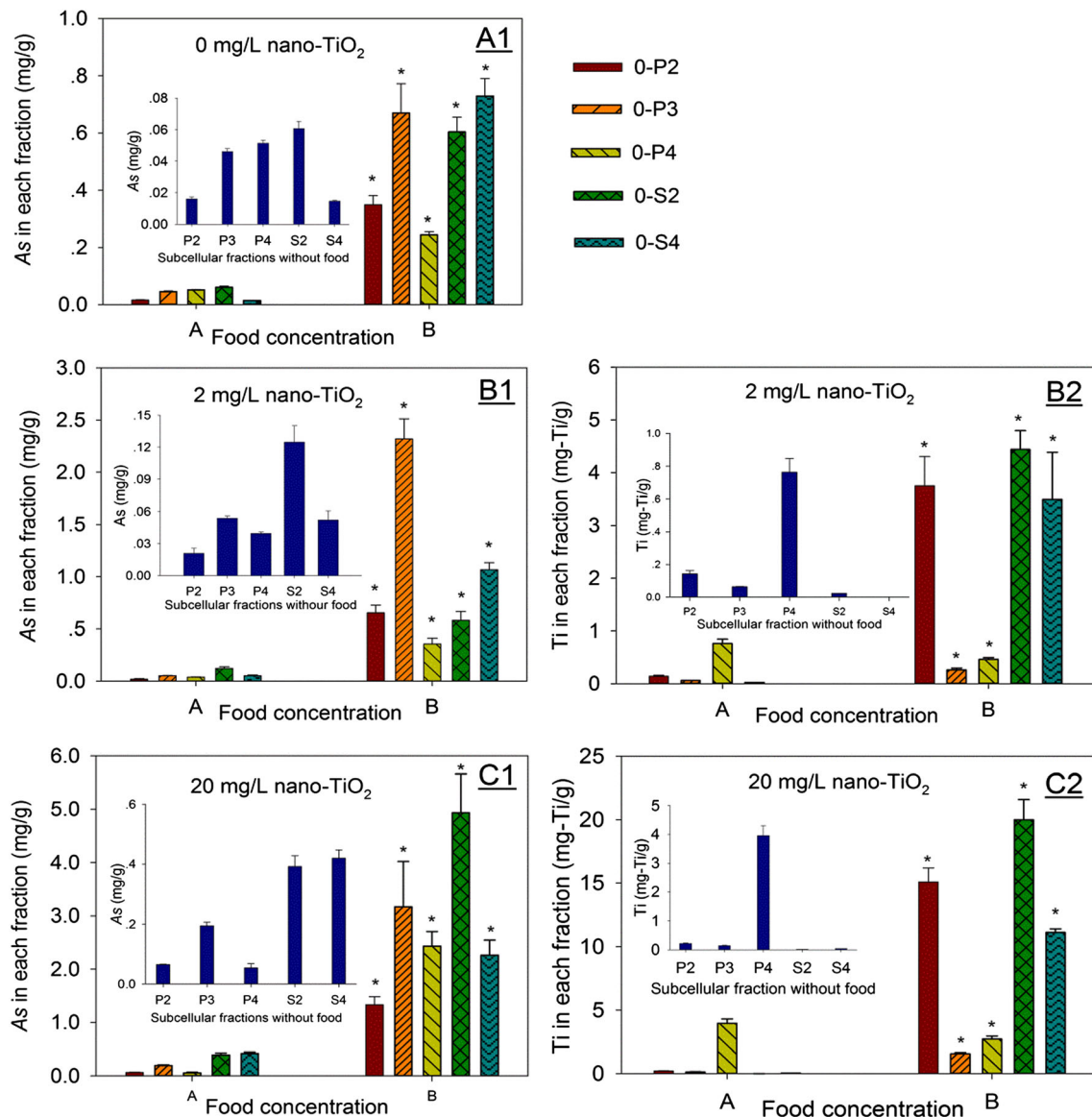


Fig. 3 Subcellular distribution of As and nano-TiO₂ in *Daphnia magna* after exposure to a sublethal As concentration (1 μ M) and different nano-TiO₂ concentrations (0, 2, and 20 mg/L) containing 0 (A) or 0.5×10^6

cells/mL (B) algae for 3 h. Means \pm SD ($n = 3$). P2, metal-rich granules; P3, organelles; P4, heat-sensitive proteins; S2, cellular debris; S4, heat-stable proteins. * $p < 0.05$

Similarly, nano-TiO₂ concentrations increased by a factor of 5 and 4, respectively, compared to treatments that excluded the food source. This indicated that As in *D. magna* can be more easily transferred to its predators in the presence of *S. obliquus*. This would consequently cause a higher potential for the ecological risk of As released into the food chain.

Our study found that the bioavailability of metal ions bound into or onto algal cells was high for aquatic filter feeders given that algae are fully digested in the gut of daphnids. This may represent an important route of the transfer and uptake of contaminants under both laboratory and natural conditions (Jenkins and Mason 1988; Klerks and Bartholomew 1991). However, for daphnids, it is difficult to transfer As bound to nanoparticles from their guts to soluble

fractions if As does not first dissociate from nanoparticles. This study demonstrated that the presence of *S. obliquus* increased the adsorption of As onto nano-TiO₂ and algae mixture because As is also readily adsorbed onto the surface of algal cells (Wang et al. 2013). Furthermore, in this study, As was observed to dissociate from nano-TiO₂ at a subcellular level irrespective of the presence or absence of the food source. Collectively, increased fractions of As associated with algae could be desorbed in the digestive tract of *D. magna*, enhancing the potential As bioavailability of these organisms. As a result, the presence of an algal food source increased the soluble fraction of As, which both promoted the trophic transfer of As along with its dissociation from nanoparticles in daphnids.

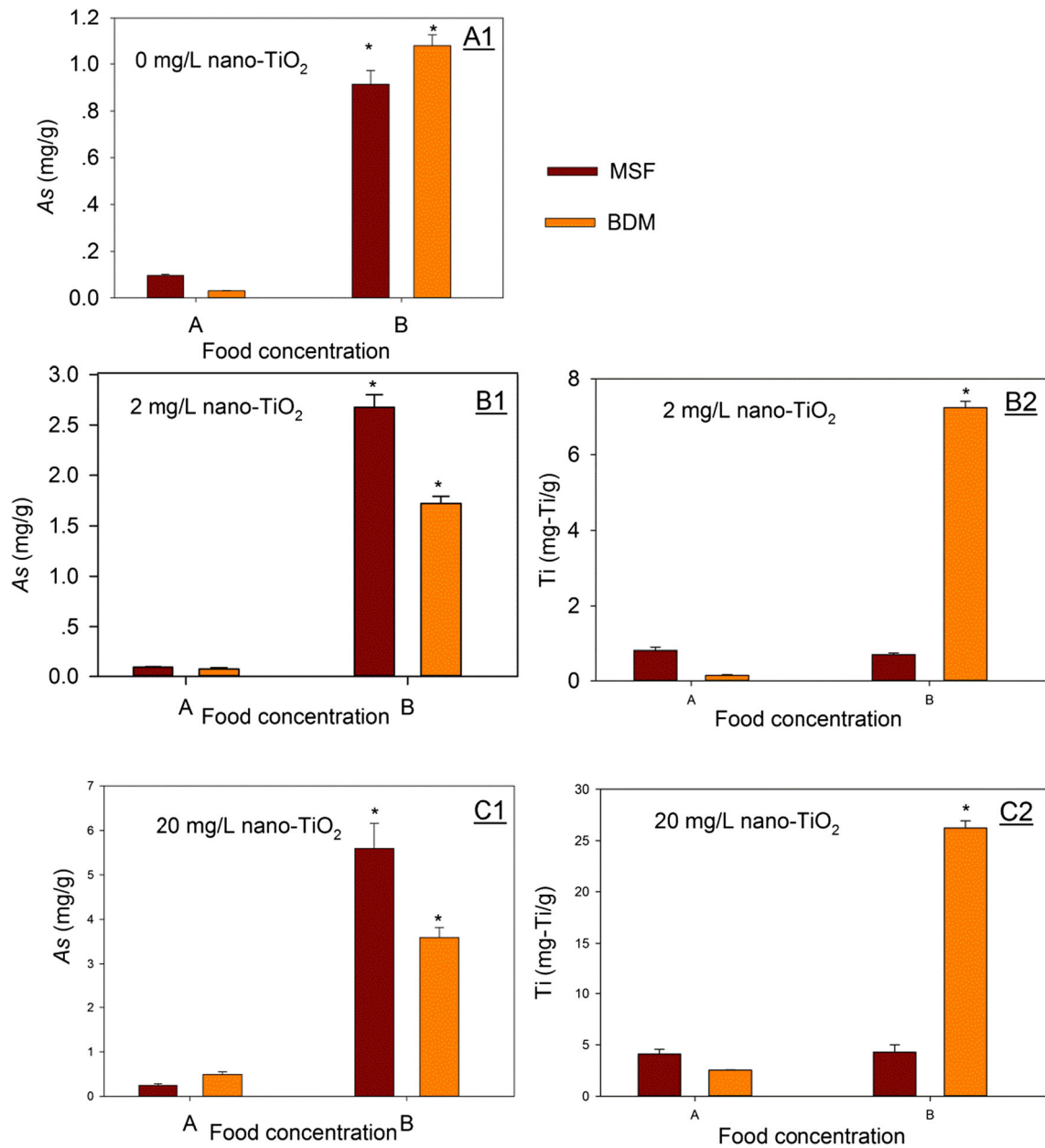
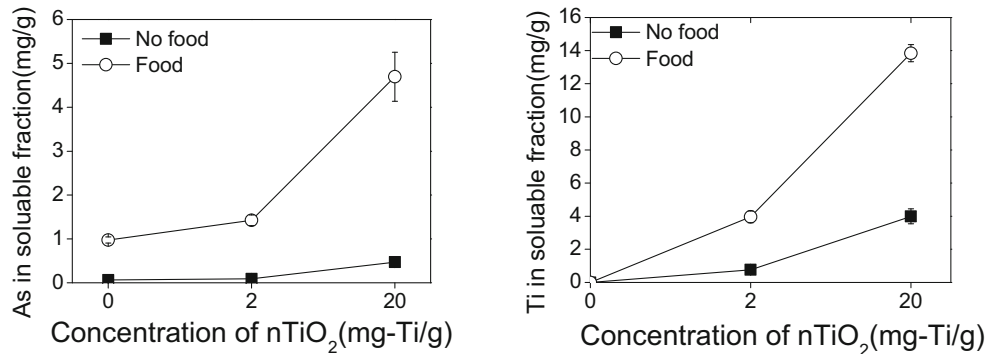


Fig. 4 Subcellular distribution of As and nano-TiO₂ in *Daphnia magna* after exposure to a sublethal As concentration (1 μM) and different nano-TiO₂ concentrations (0, 2, and 20 mg/L) containing 0 (A) or 0.5 × 10⁶ cells/mL (B) algae for 3 h. Means ± SD (n = 3). MSF, metal-sensitive

fraction, including organelles and heat-sensitive proteins; BDM biologically detoxified metals, including metal-rich granules and heat-stable proteins. *p < 0.05

Fig. 5 Changes of As and Ti in soluble fractions after exposure to a sublethal As concentration (1 μM) and different nano-TiO₂ concentrations (0, 2, and 20 mg/L) containing 0 or 0.5 × 10⁶ cells/mL algae for 3 h. Means ± SD (n = 3). Soluble fractions include heat-sensitive proteins and heat-stable proteins. *p < 0.05



Conclusions

In summary, this study found that algae significantly increased the accumulation of *As* and *Ti* in *D. magna*. Specifically, the presence of *S. obliquus* as a food source increased *As* concentrations in MSF and BDM, while it decreased *Ti* concentrations in MSF but increased *Ti* concentrations in BDM of *D. magna*. This study showed that the presence of algae (*S. obliquus*) as a food source increased *D. magna* toxicity, resulting from the dissociation of *As* from nano-TiO₂ and the transfer of *As* from BMD to MSF within *D. magna*. Algae significantly increased both *As* and *Ti* concentrations in soluble fractions, indicating that *As* and nano-TiO₂ in *D. magna* could be more readily transferred to their predators (e.g., fish). These findings indicate the importance of incorporating a food source during laboratory-based toxicity tests as well as the difficulty in predicting environmental outcomes and effects of contaminants using such lab-scale tests. Further research is needed to investigate the bioavailability of *As* in the presence of nano-TiO₂ in conjunction with an algal food source, taking into account the different, complex environmental factors, exposure times, and varying alga species involved.

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