Environmental Pollution 238 (2018) 631–637

Contents lists available at ScienceDirect

Environmental Pollution

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

# Titanium dioxide nanoparticles enhance inorganic arsenic bioavailability and methylation in two freshwater algae species☆

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ARTICLE INFO

*Article history:*
Received 27 December 2017
Received in revised form
17 March 2018
Accepted 20 March 2018

*Keywords:*
Bioaccumulation
Engineered nanomaterials
Dissociation
Algae
Trojan horse

ABSTRACT

The effect of titanium dioxide nanoparticles (nano-$TiO_2$) on the bioaccumulation and biotransformation of arsenic (*As*) remains largely unknown. In this study, we exposed two freshwater algae (*Microcystis aeruginosa* and *Scenedesmus obliquus*) to inorganic *As* (arsenite and arsenate) with the aim of increasing our understanding on *As* bioaccumulation and methylation in the presence of nano-$TiO_2$. Direct evidence from transmission electron microscope (TEM) images show that nano-$TiO_2$ (anatase) entered exposed algae. Thus, nano-$TiO_2$ as carriers boosted *As* accumulation and methylation in these two algae species, which varied between inorganic *As* speciation and algae species. Specifically, nano-$TiO_2$ could markedly enhance arsenate (*As*(V)) accumulation in *M. aeruginosa* and arsenite (*As*(III)) accumulation in *S. obliquus*. Similarly, we found evidence of higher *As* methylation activity in the *M. aeruginosa* of *As*(III) 2 mg $L^{-1}$ nano-$TiO_2$ treatment. Although this was also true for the *S. obliquus* (*As*(V)) treatment, this species exhibited higher *As* methylation compared to *M. aeruginosa*, being more sensitive to *As* associated with nano-$TiO_2$ compared to *M. aeruginosa*. Due to changes in pH levels inside these exposed algae, *As* dissociation from nano-$TiO_2$ inside algal cells enhanced *As* methylation. Accordingly, the potential influence of nanoparticles on the bioaccumulation and biotransformation of their co-contaminants deserves more attention.



## 1. Introduction

Nanotechnology is developing rapidly under various applications. Subsequently, it is inevitable that the release of engineered nanoparticles (ENPs) into the environment has been considerable throughout the world (Gao et al., 2013). As a result, the environmental and human health implications of ENPs is of significant concern (Quik et al., 2011). Titanium dioxide nanoparticles (nano-$TiO_2$) have attracted substantial attention because of their unique properties and extensive uses in surface coatings, toothpastes, sunscreens, food products, and water treatments (Luo et al., 2014; Tian et al., 2014).

Nano-$TiO_2$ has been demonstrated to facilitate the uptake of contaminants into aquatic organisms, such as fish and crustaceans, adsorbed on the nanoparticles themselves (Deng et al., 2017; Li et al., 2016). In carp, Sun et al. (2009, 2007) demonstrated that when exposed to arsenate (As(V)) and arsenite (As(III)) contaminated water in the presence of $TiO_2$, arsenic (*As*) concentrations significantly increased, which subsequently increased *As* bioavailability. Zhang et al. (2007) also showed that cadmium (Cd) concentrations in carp increased by 146% in the presence of $TiO_2$ nanoparticles, and a positive correlation was found between Cd and $TiO_2$ concentrations. Similarly, Yang et al. (2014) found that $TiO_2$ nanoparticles act as a carrier to enhance Cd accumulation in the ciliate *Tetrahymena thermophila*. Furthermore, Tan et al. (2012) demonstrated that the uptake and retention of Cd and zinc (Zn) in *Daphnia magna* were enhanced when adsorbed on nano-$TiO_2$. Alga, composed of a group of autotrophic organisms, are widely distributed at the very bottom of the aquatic food chain throughout

☆ This paper has been recommended for acceptance by B. Nowack.

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https://doi.org/10.1016/j.envpol.2018.03.070
0269-7491/

the world. It has been reported that nano-$TiO_2$ may influence algal accumulation of co-contaminants (i.e., organochlorine contaminants) by affecting cell surface hydrophobicity and membrane integrity and mobility (Zhang et al., 2017). To date, however, the effect of nano-$TiO_2$ on bioavailability and biotransformation of co-contaminants remains largely unknown given that a number of contaminants could be significantly transformed in the presence of algae (Wang et al., 2014; Zhang et al., 2013).

Being a major hazardous metalloid, *As* is of great concern due to its serious global contamination (Wang et al., 2013b). Although *As* has been identified in a number of oxidation states in the environment, the primary species is inorganic *As*, including *As*(V) and *As*(III). It is important to note that the toxicity, uptake, and accumulation of *As* in algae depend on the chemical speciation of *As* in the environment (Wang et al., 2015). In laboratory studies, algae exhibit high *As* bioaccumulation in ambient solutions, demonstrating a critical role that algae plays in *As* cycling in aquatic environments (Wang et al., 2013a, 2015; Yan et al., 2014; Zhang et al., 2013). Additionally, nano-$TiO_2$ acts as an effective adsorbent of *As* because of its high affinity to surface hydroxyl groups and comparatively large surface area (Pena et al., 2006). Within a neutral pH range, the amount of *As*(V) absorbed on nano-$TiO_2$ is higher than *As*(III) (Pena et al., 2006). Therefore, it is assumed that nano-$TiO_2$ possesses different capacities as a potential carrier for *As*(V) and *As*(III) transport to algae. Similarly, the mobility and toxicity of *As*(V) and *As*(III) associated with nano-$TiO_2$ would largely vary, depending in part on the exposed algae species in question (Wang et al., 2015). This variation could result in different stress on algae growth, and to some extent cause different levels of *As* methylation in algae (Nunes et al., 2017; Qin et al., 2006). Furthermore, nano-$TiO_2$ could transform *As*(III) into *As*(V) through photocatalytic oxidation, resulting in an important distinction in adsorption, mobility, and toxicity between *As*(III) and *As*(V) in the environment (Yao et al., 2012). Taken together, the association between *As* and nano-$TiO_2$ could substantially alter *As* bioavailability and methylation, which is likely influenced by *As* speciation, the specific algal species involved, and nano-$TiO_2$ level. However, it remains unclear as to what extent and how *As* bioavailability and methylation are influenced by this association.

The main objective of this study was therefore to identify the changes in accumulated and methylated *As* resulting from nano-$TiO_2$ exposure to algae. We selected two common freshwater algae, *Microcystis aeruginosa* and *Scenedesmus obliquus*, which are widely used as model alga species, each exhibiting different tolerance to *As*, in *As* cycling studies of aquatic environments (Vocke et al., 1980; Wang et al., 2013b). As the two primary inorganic *As* found in freshwater, we used *As*(V) and *As*(III) to test the effects of *As* speciation on *As* bioavailability and methylation influenced by nano-$TiO_2$. Furthermore, we explored why *As* bioavailability is facilitated by nano-$TiO_2$, and why *As* methylation is stimulated by *As* associated with nano-$TiO_2$ inside algae using direct TEM examination and simulated experiments of arsenic dissociation from nano-$TiO_2$. Our results are intended to provide new insight into the effects of engineered nano-$TiO_2$ on *As* cycling and the environmental risk of ENPs associated with co-contaminants.

## 2. Materials and method

### 2.1. Nano-$TiO_2$ and arsenic preparation

We used the anatase form of nano-$TiO_2$ purchased from the Sigma-Aldrich Corporation with a particle size of less than 25 nm and a purity of >99.7%. A nano-$TiO_2$ stock suspension (1 g/L) was prepared by first suspending nanoparticles in ultrapure water. We then sonicated the solution at 33 W for 30 min. The average hydrodynamic size of nano-$TiO_2$ was 193 ± 10 nm, as measured by the dynamic light scattering technique (DLS, Malvern Instruments, UK) at automatic attenuator mode. Experimental nano-$TiO_2$ concentrations of 100 μg $L^{-1}$ and 2 mg $L^{-1}$ were diluted from the stock suspension. The aggregate morphology of nano-$TiO_2$ was observed by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). We used $Na_3AsO_4 \cdot 12H_2O$ and $NaAsO_2$ to prepare *As* stock solutions at 1 mM, which were stored at 4 °C in the dark until further use. Additionally, we measured the average hydrodynamic diameter ($d_H$) and zeta potential (ζ) using DLS, and pH levels of nano-$TiO_2$ in BG-11 culture media with *As*(III) and *As*(V) of 10 μM at 0.1 mg $L^{-1}$ and 2 mg $L^{-1}$ $TiO_2$ concentrations.

### 2.2. Exposed algae species

The two freshwater alga species (*S. Obliquus* and *M. Aeruginosa*) used in our experiments were inoculated under sterile conditions in BG-11 media in Erlenmeyer flasks at 25 °C. The light-dark cycle used was 16:8 with a light intensity of 115 μmol photons $m^{-2}$ $s^{-1}$. For the following experiments, exposed algae were shaken at 100 rpm using a shaker to avoid settling. In the tables and figures, M and S denote *M. Aeruginosa* and *S. Obliquus*, respectively.

### 2.3. Algae toxicity and stress

Toxicity of *As*(III) and *As*(V) was determined using 96 h growth rate bioassays. Algal cell at the exponential growth phase were added separately into a final concentration of *As*(III) (10 μM) and *As*(V) (10 μM) under increasing nano-$TiO_2$ levels (from 0 to 200 mg $L^{-1}$). The initial cell density of exposed algae was $10^6$ cells $mL^{-1}$. We used a hemocytometer and a microscope to measure algal cell density every 24 h until the end of the exposure experiment. The specific growth rate (μ) of cells was thus calculated according to the method reported by Zeng et al. (2009). Afterwards, the 96 h EC50 was calculated based on μ values of tested algal cells using a probability unit graphical method (Li et al., 2016).

We examined alga stress from nano-$TiO_2$ associated with 10 μM of *As*(III) or *As*(V). Final concentrations of nano-$TiO_2$ were 0 mg $L^{-1}$ (control), 0.1 mg $L^{-1}$, and 2 mg $L^{-1}$. Cells at the exponential growth phase were added individually to *As*(V) and *As*(III) under the three separate aforementioned nano-$TiO_2$ concentrations. The initial cell density was $1 \times 10^6$ cells $mL^{-1}$. At the same time, we conducted parallel experiments with final nano-$TiO_2$ concentrations of 0 mg $L^{-1}$, 0.1 mg $L^{-1}$, and 2 mg $L^{-1}$ (without the addition of *As*). We conducted chlorophyll *a* (Chl-a) quantification after 96 h exposure. Additionally, we measured methane dicarboxylic aldehyde (MDA) to indicate the degree of lipid peroxidation (LPO) in this experiment, representing alga stress from *As* associated with nano-$TiO_2$. We detected MDA content using the thiobarbituric acid reactive substances (TBARS) method by applying a reagent kit (the Nanjing Jiancheng Biotechnology Institute, China) according to the manufacturer's instructions (Rocchetta et al., 2006).

Furthermore, we characterized nano-$TiO_2$ in algae and the culture media after both 0.1 mg $L^{-1}$ and 2 mg $L^{-1}$ of nano-$TiO_2$ exposure associated with 10 μM of *As*(III) and *As*(V) using TEM as well as energy-dispersive X-ray spectroscopy (TEM, H-7650, Hitachi, Japan; EDX, Genesis XM2) (Lin et al., 2012). In brief, we fixed exposed algal cells using 2.5% glutaraldehyde and then refrigerated them for 12 h. Afterwards, the treated algal cells were washed thrice in a 0.1 M phosphate buffer (pH 7.0), then postfixed in 1% osmium tetroxide for 1 h, dehydrated through a graded series of ethanol (30%, 50%, 70%, 90%, 95%, and 100%) and embedded for 12 h. The resultant algal cells were incised using a diamond blade in an ultramicrotome (Leica UC7, Germany) to obtain cell ultrathin sections (approximately 70 nm in thickness) for TEM and EDX