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TiO_2 nanoparticles in the marine environment: Impact on the toxicity of phenanthrene and Cd^{2+} to marine zooplankton *Artemia salina*☆

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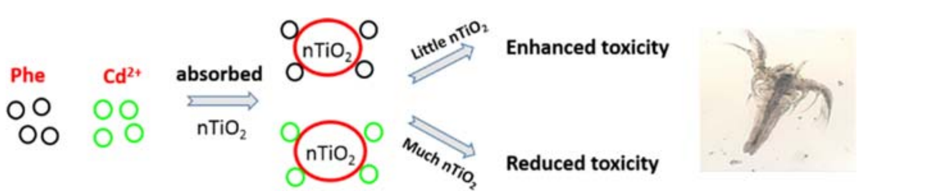
HIGHLIGHTS

- How $nTiO_2$ impacts Phe and Cd^{2+} toxicity to marine zooplankton are proposed.
- The roles of $nTiO_2$ on toxicities of pollutants appear concentration-dependent.
- The formation and bioaccumulation of Phe/Cd^{2+}-$nTiO_2$ complexes determine the toxicity.
- $nTiO_2$ poses more significant influence on the toxicity of Cd^{2+} than Phe.

GRAPHICAL ABSTRACT



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ABSTRACT

The impact of manufactured nanoparticles on the toxicity of co-existing pollutants in aquatic environments has raised increasing concerns. However, the toxicity of polycyclic aromatic hydrocarbons or metal ions in the presence of titanium dioxide nanoparticles ($nTiO_2$) to marine zooplankton has been rarely reported. In the present study, the impacts of $nTiO_2$ on the toxicity of phenanthrene (Phe) and cadium (Cd^{2+}) to *Artemia salina*, a model marine zooplankton, were investigated. Although $nTiO_2$ alone exerted a limited toxicity to *A. salina* within 48 h of exposure, $nTiO_2$ strongly altered the toxicity of Phe and Cd^{2+} to *A. salina*. Compared with the individual toxicities of pollutants to *A. salina*, the toxicities of Phe and Cd^{2+} increased by 2.0% and 12.2%, respectively, in the presence of 5 mg/L $nTiO_2$, but decreased by 24.5% and 57.1%, respectively, in the presence of 400 mg/L $nTiO_2$. These concentration-dependent impacts of $nTiO_2$ on the toxicity of Phe or Cd^{2+} might be attributed to the concurrent functions of several interrelated factors including the adsorption of pollutants on $nTiO_2$, the $nTiO_2$-faciliated bioaccumulation of pollutants, the limited gut volume in organisms, and the aggregation and sedimentation behaviors of $nTiO_2$. These results presented in the study could help understand the effects of manufactured nanomaterials in marine environments.

1. Introduction

Since titanium dioxide nanoparticle ($nTiO_2$) is widely used as ingredients for commercial products (pigment, sunscreens, paints, ointments and toothpaste), it has attracted a lot of attention (Chen and Mao, 2007). It is estimated that the worldwide production of $nTiO_2$ will reach 2.5 million tons by 2025 (Robichaud et al., 2009). $nTiO_2$ may enter marine systems either directly through aerial deposition, effluents, dumping and run-off or indirectly e.g. via river systems (Baker et al., 2014). The

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discharge of products with $nTiO_2$ into coastal waters can make an impact on the marine food chain, especially for algae and zooplankton (Farré et al., 2009; Moore, 2006). Risk assessment for $nTiO_2$ releases to marine environments is essential to assure environmental safety and human well-being (Zhu et al., 2016). However, until now, most studies focused on ecological toxicity of $nTiO_2$ in fresh water, including fish (Qi et al., 2015), water fleas (Heinlaan et al., 2008) and green algae (Hall et al., 2009). Quite a few researches probed the ecological toxicity of $nTiO_2$ in sea water, in which high ionic strength may cause different toxic effects (Ates et al., 2013; Ozkan et al., 2016).

Marine environments contain a variety of contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and metal ions (Cd^{2+}, As^{3+} and Pb^{2+}). Due to their high surface area, $nTiO_2$ can absorb these contaminants and form nanoparticle-toxin complexes (Klaine et al., 2008). The $nTiO_2$ firstly acts as an accumulator and then as a carrier of the contaminants in marine environment. Tian et al. indicated that $nTiO_2$ can act as carrier to facilitate bioaccumulation of phenanthrene (Phe) in marine ark shell (Tian et al., 2014). Zhu et al., confirmed that the presence of 2 mg/L $nTiO_2$ increased the toxicity of tributyltin (TBT) up to 20-fold compared with TBT alone to abalone embryos (Zhu et al., 2011). In a recent study, Fang et al., reported that the enhanced bioconcentration of bisphenol A in the presence of TiO_2 nanomaterials led to adverse reproductive outcomes in zebrafish (*Danio rerio*) (Fang et al., 2015). However, most of the previous studies explored the impact of $nTiO_2$ on the toxicity of pollutants to organisms residing in higher trophic levels of marine environments. To date, there are limited data that have been reported about the influence of $nTiO_2$ on the toxicity of pollutants to marine zooplankton.

Marine zooplanktons are the most abundant organisms in the marine environments and play an important role in the marine food chain. Zooplanktons usually use mechanical sieving to obtain food particles (diameter < 50 μm) from water system (Ates et al., 2013; Hund-Rinke and Simon, 2006). Therefore, zooplankton may act as a mediator to transfer the particulate pollutant, including engineered naomaterials (ENMs) to higher trophic levels (Zhu et al., 2010b). *Artemia salina* (brine shrimp) is a representative type of zooplankton that is used to feed larval fish in aquacultures like copepods and daphnids (Sorgeloos, 2000). It has also been recognized as a suitable biological model in ecotoxicology (Nunes et al., 2006) and nanoecotoxicology (Libralato, 2014) due to its known biological and ecological knowledge (Kos et al., 2016).

To better assess the ecological impact of $nTiO_2$ on marine environments, we made an effort to investigate the effects of $nTiO_2$ on the toxicity of marine pollutants, such as PAHs and metal ions, to *A. salina*. Phe and Cd^{2+} were chosen as the model contaminants because both of them are typical pollutants in coastal waters of China. We conducted the acute toxicity tests of TiO_2 nanoparticles, Phe, and Cd^{2+} alone firstly. Then the co-toxicity tests of Phe-$nTiO_2$ and Cd^{2+}-$nTiO_2$ to *A. salina* were performed to evaluate the effect of $nTiO_2$ on the toxicity of Phe and Cd^{2+}. These results may help promote or clarify the potential risks of $nTiO_2$ in marine environments.

2. Materials and methods

2.1. Chemicals and working solutions

The uncoated TiO_2 nanoparticles (particle size ≤10 nm, specific surface area ≥ 150 m^2/g, purity ≥99.5%) used in the current study were purchased from Nanjing High Technology Nano Material Co., Ltd. (Nanjing, China). A stock solution of 1.0 g/L $nTiO_2$ was prepared by dispersing the nanoparticles in ultrapure water (Millipore, Billerica, MA, USA) followed by bath sonication for 10 min (50 W/L, 40 kHz). Testing solutions of $nTiO_2$ were prepared immediately prior to use by diluting the stock solution with artificial seawater (prepared by dissolving a commercial salt purchased from Tianjin Cnsic Marine Biotechnology Co., Ltd., Tianjin, China; salinity 30 ± 2‰, pH 8.0 ± 0.2, dissolved oxygen ≥7.0 mg/L). Further information on the particle size distribution and the morphology characteristics of $nTiO_2$ in seawater is provided in a previous report (Zhu et al., 2011). Concisely, the particle size detected by dynamic light scattering (DLS) ranged in diameter from 562 nm to 22.7 μm. The morphology observed by transmission electron microscopy (TEM) illustrated the highly irregular shape of the aggregates and revealed the presence of particles <500 nm.

A 500 mg/L stock solutions of Phe (99.8% purity; AccuStandard, Inc., New Haven, CT, USA) were prepared in analytical grade dimethyl formamide (DMF). A series of testing solutions (0.05, 0.125, 0.3 and 0.8 mg/L) were obtained by dilution of the stock solution with artificial seawater (salinity 30 ± 2‰, pH 8.0 ± 0.2, dissolved oxygen ≥7.0 mg/L). $CdCl_2$ was purchased from Aladdin ® (Aladdin Industrial Corporation). The stock solutions (2.0 g/L) of Cd^{2+} were prepared in ultrapure water (Millipore, Billerica, MA, USA). A group of testing solutions (20, 40, 80 and 160 mg/L) were obtained by diluting the Cd^{2+} stock solutions with artificial seawater. All other chemicals used in this study were of analytical grade.

2.2. Tested organisms

Certified cysts of the Ebinur Lake Artemia were purchased from Tianjin Hai Ding aquatic product Co., Ltd. (Tianjin, China). Hatching of the cysts was performed in the artificial seawater. In brief, 0.5 g dehydrated Artemia cysts were added to a glass beaker with 0.5 L artificial seawater which was aerated for 1 h before the start of hatching procedure. The beaker was then continuously illuminated by a lamp box. Under these conditions, nauplii of *A. salina* hatched after 24 h.

2.3. Acute toxicity of $nTiO_2$ alone

To investigate the toxicity of $nTiO_2$ alone under different test conditions on *A. salina*, acute toxicity tests were conducted in static and dynamic systems respectively. The static toxicity test adopted by most toxicity studies was intended to provide comparable results, while the dynamic toxicity test was intended to simulate the flows in marine environment. The toxicity tests were performed at a range of $nTiO_2$ concentrations (5, 50, 100, 200 and 400 mg/L) plus a blank control (0 mg/L). For the static system, 10 I instar nauplii (6–24 h old) were placed in a 50 mL glass beaker that contains 20 mL test solution. The beakers were put in an artificial climate incubator (PQX—350H, Shanghai Jiwei test instrument equipment Co. Ltd., China) with the temperature at 25 °C. For the dynamic system, the acute toxicity test was conducted in a conical flask that contains 10 nauplii and 20 mL test solution placed in the thermostat water bath oscillator (SHA—B, Guangzhou Shenhua Biotechnology Co. Ltd., China) at 25 °C. The rotation speed was set at 100 r/min, which simulated the dynamic conditions of the marine environment. Potassium dichromate, $K_2Cr_2O_7$, is usually selected as a reference chemical in various aquatic toxicity tests (Kos et al., 2016; Manfra et al., 2015). So, additional toxicity tests of $K_2Cr_2O_7$ (15, 30, 40 and 60 mg/L) to *A. salina* were performed to verify the suitability of this assay protocol.

The nauplii were not fed during the experiments. A light regime of 16: 8 h light: dark was used. After exposure for 24 and 48 h, the immobilization and the mortality of the individuals in each container were assessed using a Stereo Microscope. *A. salina* that was unable to swim within 15 s of gentle agitation of the test container was considered immobile. The *A. salina* whose heartbeats have stopped were considered dead. All experiments were conducted with five repeats.

2.4. Acute toxicity of Phe or Cd^{2+} without $nTiO_2$

Before assessing the impact of $nTiO_2$ on the toxicity of Phe and Cd^{2+} to *A. salina*, the acute toxicity tests of single Phe with the concentrations (0, 0.05, 0.125, 0.3 and 0.8 mg/L) and Cd^{2+} with the concentrations