Aquatic Toxicology 197 (2018) 89–97

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Trophic transfer potential of two different crystalline phases of TiO_2 NPs from *Chlorella* sp. to *Ceriodaphnia dubia*

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ARTICLE INFO

Keywords:
Algae-daphnia system
Biomagnification
Crystallinity
Irradiation
Mixture
Trophic transfer

ABSTRACT

Owing to the increase in the usage of titanium dioxide nanoparticles (TiO_2 NPs), their release into the aquatic environment is inevitable. In the aquatic ecosystem, TiO_2 NPs can bio-magnify at various trophic levels in the food chain through dietary exposure. In the current study, the trophic transfer potential of two crystalline phases of TiO_2, anatase and rutile nanoparticles (individual as well as a binary mixture) has been evaluated in the lake water matrix using algae–daphnia system. *Chlorella* sp. and *Ceriodaphnia dubia* were used as test organisms to represent the algae–daphnia food chain of the freshwater ecosystem. Other than crystallinity, the effect of irradiation (visible and UV-A) was also investigated at the test concentrations, 75, 300, and 1200 μM. TiO_2 NPs treated algal diet produced significant mortality only at the test concentrations, 300 and 1200 μM. The type of irradiation and crystallinity doesn't have any impact on the mortality of daphnids through the dietary exposure of TiO_2 NPs. Comparing the mixture with individual NPs, binary mixture induced less mortality on *C. dubia* which signifies the antagonistic effect of NPs when they coexist. Statistical modeling confirmed the antagonistic effect of the binary mixture on *C. dubia*. As individual NPs, anatase and rutile forms showed a maximum Ti accumulation under UV-A and visible irradiation, respectively. BMF of TiO_2 NPs has been in validation with the bioaccumulation noted in *C. dubia*. Individual NPs (75 μM) showed higher BMF value of ~23 under both UV-A (anatase) and visible (rutile) irradiation. Individual NPs showing higher BMF confirmed their trophic transfer potential in the aquatic food chain, primarily through the diet. In contrast, the binary mixture obtained a higher BMF of 1.9 and 0.79 at 75 and 300 μM under visible and UV-A irradiation, respectively. The plausible reason behind this decrement was the antagonistic effect of the mixture which significantly reduced their Ti bioaccumulation on *C. dubia*.

1. Introduction

The nanotechnology consumer products inventory (CPI) indexes 1814 nanoparticles (NPs) incorporated consumer products as on March 2015. It was also reported that the metal oxide and metal NPs occupies 37% of the NPs incorporated products in the inventory. Among these products, titanium dioxide (TiO_2) nanoparticles employ about 30 products both individually as well as in combination with other NPs like silver (10) and ZnO (10) (Vance et al., 2015). TiO_2 NPs is a metal oxide nanoparticles which gained increased attention as powerful semiconductor and opted as a substance enriched with high photocatalytic activity (Huang et al., 2010). The crystallinity of TiO_2 differentiated them into three various main forms namely, anatase, rutile, and brookite. Based on the applicability, utility and occurrence of TiO_2 NPs, anatase and rutile forms gained specific attention owing to their respective properties such as higher photocatalysis and refractive index. They have been widely employed in consumer products like sunscreens, paints, plastics, paper, foods, and electronics, etc. (Baiqi et al., 2006; Ferguson et al., 2005; Mueller and Nowack, 2008; Winkler, 2003). On other side, the photocatalytic property of TiO_2 NPs enabled its usage in environmental oriented applications like purification of air (Paz, 2010), wastewater treatment (Yuzer et al., 2016), soil remediation (Yang and Xing, 2009), and photodegradation of contaminants in various industries (Mahlambi et al., 2015). Thus, TiO_2 NPs release into the aquatic environment becomes unavoidable nowadays due to its over usage, especially in the consumer products. In the aquatic system, TiO_2 NPs undergoes numerous transformations with their abiotic components like natural organic matter (NOM) and interacts with biotic organisms present in the freshwater. Therefore, it is essential to determine the impact of TiO_2 NPs in aquatic organisms at each trophic level.

Daphnids are a planktonic crustacean which serves as a food and energy link between the primary producers (algae) and secondary consumers (fish and fish larvae). Once the daphnids were affected by the nanoparticles, it will affect the other organisms that feed these NP

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https://doi.org/10.1016/j.aquatox.2018.02.003
Received 20 November 2017; Received in revised form 4 February 2018; Accepted 5 February 2018
Available online 07 February 2018

accumulated organisms. Thereby, it moves up through the food chain and possesses a risk for human beings. Hence, it is crucial to understand the flow of nanoparticles from lower trophic level organism to the higher trophic level organisms exist in the food chain system. Cedervall et al. (2012) investigated the potency of polystyrene nanoparticles to transfer across the trophic levels from algae to fish through the zooplankton. The feeding behaviour and fat metabolism of fish have been greatly altered due to the consumption of polystyrene NPs contaminated zooplankton, to which the NPs were chiefly transferred through algae – a diet of zooplankton. Only a few researchers have investigated the efficiency of NPs to pass their effects from algae to daphnia, especially through their diet (Bouldin et al., 2008; Bundschuh et al., 2016). Li et al. (2011) stated that depletion of food upon interaction with TiO_2 NPs induced a negative impact on the energy budget of daphnia. Dalai et al. (2014) investigated the three different modes of TiO_2 NPs transfer from *Scenedesmus obliquus* to *Ceriodaphnia dubia*. Among the three different modes namely water, diet and water + diet, the principal mode of TiO_2 NPs transfer was the dietary exposure, which showed significant bioaccumulation in daphnids, especially at their lower concentrations. Similarly, the marine scallop, *Chlamys farreri* has accumulated TiO_2 NPs in higher amount through the microalgae, *Nitzschia closterium* upon both aqueous and dietary exposures (Wang et al., 2017). They also observed a significant biomagnification of TiO_2 NPs in daphnids which confirmed their trophic transfer potential through the algal diet. Though the reports have mentioned the trophic transfer potential of TiO_2 NPs, the influence of crystallinity on their trophic transfer efficiency has not been addressed so far.

In nature, the freshwater system gets exposed to multiples of contaminants (here referred to nanoparticles) rather than single contaminants. While, the laboratory studies on the risk assessment of nanoparticles have focused only on single NPs, which doesn't mimic the realistic contaminant system that concurrently exposes to different types of nanoparticles. In such an environmental scenario, it is mandatory to assess the effect of nanoparticles both individually as well as a mixture towards various aquatic organisms to be exposed. Few studies have been reported regarding the toxicity studies on nanoparticles as a mixture. In a study by Hua et al. (2016), the influence of TiO_2 NPs on the toxicity of ZnO NPs and Zn ions were investigated on the embryos of zebra fish. They have noticed an increase in the EC_{50} value of both ZnO NPs and Zn ions upon the addition of TiO_2 NPs, which signifies the antagonistic effect of TiO_2 NPs on the toxicity of both zinc forms. Still, no reports were addressing the issue of trophic transfer of nanoparticles when they exist as a mixture. Besides, a solitary report was available for the trophic transfer potential of TiO_2 NPs in the presence of other pollutants. Chen et al. (2015) investigated the effect of sodium dodecyl benzene sulfonate (SDBS) on the transmission of TiO_2 NPs across the aquatic food chain. The presence of SDBS has increased the bioavailability of TiO_2 NPs and thereby inclined the accumulation of TiO_2 NPs on *Daphnia magna* through the NP contaminated algal diet (*S. obliquus*).

As the TiO_2 NPs are photocatalytic, they exhibit variations in their toxicity based on the type of irradiation exposed, either UV or visible. Marcone et al. (2012) noticed a difference in the EC_{50} value of TiO_2 NPs on *Daphnia similis* owing to the variation in the irradiation. The change observed had a considerable effect for P25 NPs in comparison with the other forms of TiO_2 NPs such as anatase and rutile. It was also noted that the phototoxicity of TiO_2 NPs on *D. magna* had been increased by two to fourfold upon exposure to simulated solar radiation than the ambient laboratory light (Ma et al., 2012). Hence it is obligatory to assess the changes in the trophic transfer potential of TiO_2 NPs due to the variance in the irradiation.

In the present study, the trophic transfer potential of two different crystalline phases of TiO_2 NPs (anatase and rutile) and their binary mixture has been evaluated using a simple algae–daphnia model. In this model, algae refer the primary producer- the base of an aquatic food chain, and daphnia refers the primary consumer – the next to algae in the food chain which consumes algae as food. Besides, the impact of irradiation on the trophic transfer potential of TiO_2 NPs has also been evaluated under two different irradiations such as visible and UV-A. Abbott modeling has been employed to determine the interactions between anatase and rutile NPs when coexists as a mixture. Further, the ultra-structural changes in the *C. dubia* due to the consumption of Ti contaminated algal diet were determined with the help of transmission electron microscopy (TEM). The biomagnification factor (BMF) has been calculated to assess the trophic transfer potential of TiO_2 NPs.

2. Materials and methods

2.1. NPs preparation and characterization

Different crystalline forms of TiO_2 NPs, namely anatase (< 25 nm, 99.7% trace metal basis, CAS no: 1317-70-0) and rutile (< 100 nm, 99.5% trace metal basis, CAS no: 1317-80-2) were obtained in the powder form from the manufacturer Sigma Aldrich, USA. Homogeneous stock dispersion of anatase and rutile NPs (5 mM) were prepared by ultra-sonicating the NPs in Milli-Q water for about 20 min at 20 kHz. After sonication, NPs were utilized for the toxicity studies. Before the toxicity studies, TiO_2 NPs were characterized by various techniques like TEM (Fig. 1) and DLS. The detailed characterization of TiO_2 NPs has been addressed in the supplementary information.

2.2. Test organisms

A freshwater crustacean, *Ceriodaphnia dubia* has been chosen as a test organism of the primary consumer which feeds on algae *Chlorella* sp., a primary producer of the freshwater ecosystem. Information regarding the isolation of the test organisms, *Chlorella* sp. and *C. dubia* has been detailed in our previous studies, Iswarya et al. (2015) and Iswarya et al. (2016). A freshwater alga, *Chlorella* sp. was subcultured and maintained in a BG-11 broth (Himedia Laboratories, Mumbai, India), a specific growth media prepared as described by Dalai et al. (2014). While, *C. dubia* has been maintained in the sterile lake water to which *Chlorella* sp. was given as a diet. A rhythm of about 16 h light and 8 h dark has been followed for both the test species at 24 °C.

2.3. Experimental set up

Freshwater obtained from the freshwater lake situated in the VIT premises were used as a test matrix throughout the study. Then the collected freshwater was processed through a series of filtration with blotting and Whatman no.1 filter paper, followed by sterilization to devoid of microbes. Further, the sterilized lake water has been used for the toxicity studies. Some of the lake water parameters were checked and observed to be in their optimal range of pH: 7.02 ± 0.04, temperature: 29.4 ± 0.3 °C; TDS: 745 ± 95 mg/L; conductivity: 1.05 ± 0.13 mS/cm. Traceable quantities of metal ions were also noticed to exists in the sterile lake water, which has been detailed in our previous study, Iswarya et al. (2016).

Trophic transfer studies have been conducted under two different irradiation conditions such as visible and UV-A irradiation. Visible irradiation was provided by white fluorescent tubes (18 W, Philips) with an intensity of 0.18 mW/cm^2. While, UV-A irradiation was provided by black tubes (18 W, Philips) with an intensity of 0.23 mW/cm^2. The NPs concentrations such as 75, 300, and 1200 μM have been selected to assess the trophic transfer potential of TiO_2 NPs, irrespective of their forms: anatase, rutile, or binary mixture. In case of a binary mixture, the equal concentration of anatase and rutile NPs forms the total concentration of binary mixture. For example, the concentration, 75 μM consists of 37.5 μM of anatase and 37.5 μM of rutile NPs, and thus the total concentration of TiO_2 NPs in the mixture was 75 μM.

A freshwater alga, *Chlorella* sp. cultured in BG-11 broth has been harvested at the exponential phase by centrifugation at 7000 rpm, 10 min at 4 °C. The harvested algal cells were then re-suspended in the