Toxicity and trophic transfer of P25 TiO2 NPs from Dunaliella salina to Artemia salina: Effect of dietary and waterborne exposure

M. Bhuvaneshwari, Vignesh Thiagarajan, Prateek Nemade, N. Chandrasekaran, Amitava Mukherjee
Centre for Nanobiotechnology, VIT University, Vellore 632014, India

ABSTRACT

The recent increase in nanoparticle (P25 TiO2 NPs) usage has led to concerns regarding their potential implications on environment and human health. The food chain is the central pathway for nanoparticle transfer from lower to higher trophic level organisms. The current study relies on the investigation of toxicity and trophic transfer potential of TiO2 NPs from marine algae Dunaliella salina to marine crustacean Artemia salina. Toxicity was measured in two different modes of exposure such as waterborne (exposure of TiO2 NPs to Artemia) and dietary exposure. The toxicity and accumulation of TiO2 NPs were observed to be more in waterborne exposure as compared to dietary exposure. Waterborne exposure seemed to cause higher ROS production and antioxidant enzyme (SOD and CAT) activity as compared to dietary exposure of TiO2 NPs in Artemia. There were no observed biomagnification (BMF) and trophic transfer from algae to Artemia through dietary exposure. Histopathological studies confirmed the morphological and internal damages in Artemia. This study reiterates the possible effects of the different modes of exposure on trophic transfer potential of TiO2 NPs and eventually the consequences on aquatic environment.

1. Introduction

P25 TiO2 nanoparticles (TiO2 NPs) are photocatalysts, which find applications in cosmetics, sunscreens, paints, water purifying system, and self-cleaning agents (Browning et al., 2014; Robertson et al., 2010). Occurrence of both anatase and rutile forms increases the photocatalytic activity of TiO2 NPs (Hurum et al., 2003). Their release into the marine environment is inevitable and can occur due to urban activities, surface runoff, sewage and waste discharge, and coatings on marine structures (Zhu et al., 2016). Nanoparticles can significantly contribute to the marine ecotoxicity irrespective of their aggregation and agglomeration in seawater (Keller et al., 2010). The predicted levels of TiO2 were about 1.6 μg L−1 in surface water, 1.2 mg L−1 in water treatment plant effluent, and 6 mg kg−1 in sediments (Gottschalk et al., 2013). The risk evaluations of nanomaterials suggested that organisms at both lower and higher trophic levels are greatly affected (Wang et al., 2016).

As model test species, marine algae D. salina and crustacean Artemia have been used in the current investigation to study the effect of TiO2 NPs in artificial sea water medium (ASW). Marine algae, being highly susceptible to engineered nanomaterials, can be utilized as a pollution indicator in marine systems owing to their high bioaccumulation ability (Barhoumi and Dewez, 2013). Only a few prior studies have reported the toxicity of P25-type NPs towards marine algae. Miller et al. (2012) have analysed the growth inhibition in four algal species (Thalassiosira pseudonana, Skeletonema costatum, Dunaliella tertiolecta, and Isochrysis galbana) by TiO2 NPs. Sendra et al. (2017b) reported the toxicity of sunscreens and TiO2 NPs towards Chaetoceros gracilis, Amphidinium carterae, Pleurochrysis rosoffensis, and Nannochloropsis gaditana. Under direct sunlight, sunscreens without NPs were found to be less toxic compared to those with TiO2 NPs. A comparative study of TiO2 particles in both NP and bulk forms towards marine algae Phaeodactylum tricornutum and freshwater algae Chlamydomonas reinhardtii was carried out by Sendra et al. (2017a).

Utilizing Artemia as an experimental model in ecotoxicological testing is noteworthy, considering the advantages it possesses namely, their short life span, compliance to various salinity and temperature conditions, and as it is a non-selective filter feeder (Nunes et al., 2006).
Owing to their filter feeding behaviour, ability to accumulate metals, and sensitivity to environmental pollutants, Artemia is often used as a bio-indicator (Viarengo et al., 2007). Previous studies about the effects of TiO2 NPs on Artemia showed significant accumulation of NPs in both Artemia nauplii and adults. Higher concentrations (LC50 > 100 mg L\(^{-1}\)) and longer exposure duration caused mortality in both nauplii and adults (Ates et al., 2013). Clemente et al. (2014) reported 48 h EC50 of 285 mg L\(^{-1}\) for anastase and rutile mixture of nTiO2 NPs in the presence of visible light. Under UV illumination, EC50 decreased to 4 mg L\(^{-1}\), highlighting the significance of UV in exacerbating the toxic effects. Absence of light decreased the toxicity effects of nTiO2 NPs on Artemia franciscana larvae, whereas in the case of starvation condition, toxicity increase for both nTiO2 and TiCl4 was reported (Minetto et al., 2017).

Toxicity of nanoparticles in the lower trophic level may greatly affect the organisms at higher levels via food web. Thus, it is important to study the transfer of nanomaterials across the trophic levels. Uptake–deposition studies of TiO2 NPs in Chlamys farrelli were performed for waterborne and dietary exposure. Waterborne exposure of the particles reportedly resulted in an increased content of NPs in the gills, digestive gland, and mantle of scallops compared to the dietary exposure (Wang et al., 2017). A study of trophic transfer of Ag NPs from Artemia nauplii to marine Oryzias melastigma showed a trophic transfer efficiency of less than 6% for a 24 h treatment period (Wang and Wang, 2014). The lack of information on the trophic transfer of TiO2 NPs from algae to Artemia necessitated further investigation.

Herein, we report the toxicity of TiO2 NPs on algae (D. salina) and subsequent trophic transfer to marine crustacean Artemia. The influence of two different modes of exposure such as waterborne (nanoparticles exposure to Artemia via aqueous media) and dietary exposure (NP-accumulated algal cells are used to feed the Artemia) on the toxicity, uptake, and trophic transfer of TiO2 NPs was evaluated.

2. Materials and methods

2.1. Materials

Titanium dioxide nanoparticles (Aeroxide P25, particle size: 21 nm (TEM), ≥ 99.5% trace metals basis) and 2', 7'-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich. Hydrogen peroxide solution 30% w/v (H2O2) and nitroblue tetrazolium chloride (NBT) were purchased from SDFCL (Mumbai, India). MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) and hydroxylamine hydrochloride were obtained from Hi-Media Pvt. Ltd (Mumbai, India).

2.2. Test organism

Marine algae Dunalella salina was procured from CMFRI, Mandapam, Tamil Nadu, India, and the culture was grown along with supplements (Supplemental Table 1) at 23 ± 2 °C (day/night rhythm of 16 h/8 h, under white fluorescent lighting with a photon concentration of 40.5 μmol m\(^{-2}\) s\(^{-1}\)). Artemia brine shrimp cysts were obtained from Ocean Star International Inc., USA, and stored at 4 °C. Prior to hatching, the brine shrimp cysts were hydrated in deionized water for 12 h at 4 °C. Sinking cysts were rinsed with distilled water. Approximately, 1 g of pre-cleared cysts was incubated in 2 L of sterilized natural seawater in a round-bottom glass tank at 30 ± 1 °C. Aeration and light illumination were provided using an aquarium air pump and a fluorescence lamp (10 W, 0.44 mW/cm²), respectively. Hatching of Artemia brine shrimp cysts commenced within 24 h of incubation. The hatched nauplii were transferred into fresh seawater medium, and the 48-h-old nauplii were used for toxicity studies. Toxicity assessment of nanoparticles was performed according to OECD (2004) guidelines. All the experiments were carried out using ASW.

2.3. Stability and aggregation

The stability and aggregation of nanoparticles (0.1, 1, and 10 mg L\(^{-1}\)) after being sonicated (20 kHz, 750 W, 20 min) in artificial seawater were analysed using dynamic light scattering method (90 Plus Particle Size Analyzer, Brookhaven instruments Corporation, USA) at different time intervals of 0, 24, and 48 h.

2.4. Effect of TiO2 NPs on algae

Exponential phase algal cultures were harvested and centrifuged at 7000 rpm for 10 min at 4 °C. The pellet obtained was washed with sterilized ASW. A series of concentrations of nanoparticles (0.1, 1, and 10 mg L\(^{-1}\)) was allowed to interact with the algal cells of 0.1 optical density at 610 nm (2.5 × 10⁶ cells/mL) under visible light conditions. Interaction was carried out for 72 h according to OECD guidelines (OECD, 1984). MTT assay was performed to determine the cellular viability, wherein yellow colour MTT was reduced to purple colour formazan in the mitochondria of viable cells (Pakrashi et al., 2013). After an interaction period of 72 h, 500 µL of the sample was mixed with 20 µL of MTT solution (5 mg in 1 µL PBS) and incubated in dark for 4 h. After incubation, the samples were centrifuged at 8000 rpm for 8 min. The pellet obtained was washed with 500 µL of ASW, and 200 µL of dimethyl sulfoxide was added. The absorbance was measured at 570 nm using a microplate reader (ELISA plate reader, Biotek, Powerwave XS2).

2.5. Chlorophyll estimation

Chlorophyll, a photosynthetic pigment, is essential in determining the growth of algae and is an important parameter in determining the cellular viability after nanoparticle interaction (Fargasova, 2001). Ten mL of cell suspension was centrifuged at 7000 rpm for 10 min at 4 °C. The pellet was washed using ASW and suspended in 1 mL N, N-di-methyl formamide (DMF) and incubated in dark for 24 h. After the incubation time period, the samples were centrifuged again, and the supernatant containing extracted chlorophyll was measured at 475, 649, and 665 nm using an UV–Vis spectrophotometer (Hitachi, U-2910, Japan).

Chlorophyll content in algal cells was measured at two different stages of interaction: I) After 48 h exposure of algal cells to different concentrations of TiO2 NPs (0.1, 1, and 10 mg L\(^{-1}\)), the chlorophyll a, b, and total content were measured, II) Chlorophyll content was measured during the dietary exposure study. Briefly, algal cells were initially exposed to different concentrations of TiO2 NPs (0.1, 1, and 10 mg L\(^{-1}\)) for 48 h. Then, Artemia salina were fed with algal cells (Dunalella salina) that were pre-treated with TiO2 NPs. After the 48 h of feeding, the remaining algal cells in the medium were measured for chlorophyll content.

2.6. Toxicity, uptake, and depuration of TiO2 NPs on Artemia: waterborne exposure

The 48-h-old hatched Artemia nauplii were interacted with nanoparticles of different concentrations (0.1, 1, and 10 mg L\(^{-1}\)) for 48 h. After the interaction period, the numbers of live nauplii were counted under an optical microscope (Zeiss Axiosstar Optical Microscope, USA). No feed was provided to Artemia during the course of the experiment. For the nanoparticle uptake study, interacted Artemia nauplii were washed with Milli-Q water and transferred to pre-weighted tubes. The samples were dried at 70 °C in a hot-air oven. The uptake of TiO2 NPs was determined by acid digestion (HNO3) of dried nauplii and subsequent analysis for TiO2 concentration using a graphite furnace method (Analyst 400, PerkinElmer). Threshold for the graphite furnace technique for Ti measurement is 10 µg/L. For accumulation study, the interacted nauplii were transferred to fresh ASW medium to evacuate
the nanoparticles (departure) from the gut for 24 h. Later, the nauplii were separated, dried, acid digested, and measured for Ti concentration.

2.7. Trophic transfer of TiO2 NPs from algae to Artemia: dietary exposure

About 10 Artemia were fed with algal cells previously exposed to TiO2 NPs for 48 h. After feeding, the number of live nauplii was determined as a ratio of TiO2 concentration in algal cells to the concentration in Artemia. A factor value greater than 1 indicates a BMF trend, whereas value less than 1 specifies biomagnification trend.

2.8. Estimation of reactive oxygen species in algae and Artemia

DCFH-DA is a cell permeable indicator of reactive oxygen species in cells. ROS measurements in algae were carried out using the protocol described by Wang and Joseph (1999). Five mL of control and treated algal cell suspensions were added to 5 µL of DCFH-DA (100 µM) and incubated in dark at room temperature for 30 min. The fluorescence intensity of DCFH-DA was measured at an excitation and emission wavelength of 485 and 530 nm, respectively, using a fluorescence spectrophotometer (SL174, ELICO, India). A. salina nauplii treated with TiO2 NPs were washed with Milli-Q water. About 10 nauplii were incubated with 10 µM DCFH-DA dye for 30 min at 37 °C in a 24-well plate. The nauplii were washed with PBS (pH 7.4) and homogenized with phosphate-buffered solution using a probe sonicator. The fluorescence intensity of DCFH-DA was measured as described earlier.

2.9. Biochemical changes

Interacted algal cell suspensions were centrifuged at 7000 rpm for 7 min at 4 °C. The cells were homogenized in 0.5 M phosphate buffer (pH 7.5) and centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was collected for further biochemical assays. A. salina nauplii were washed twice with Milli-Q water and homogenized in 0.5 M phosphate buffer (pH 7.5) for 3 min using a probe sonicator. The homogenized samples were centrifuged at 13,000 rpm for 10 min at 4 °C, and the supernatant was collected for biochemical assays.

2.9.1. Superoxide dismutase (SOD) activity

SOD activity was quantified using the protocol described by Kono (1978). About 50 mM pH 10 Na2CO3, 96 mM NBT, 0.6% Triton X-100, and 20 mM hydroxylamine hydrochloride were added to 70 µL of the collected supernatant. The reaction mixture was incubated in the presence of light at 37 °C for 20 min, and the SOD activity was measured by recording the absorbance values at 560 nm using UV–vis spectroscopy (Hitachi, U-2910, Japan).

2.9.2. Catalase (CAT) activity

CAT activity was determined by the method described previously by Yilanciglu et al. (2014). To the recovered supernatant (100 µL), 2 mL of hydrogen peroxide solution (10.8 mM) and 100 µL of 50 mM potassium phosphate buffer (pH 7.0) were added, and the reaction mixture was measured for catalase activity using a UV–vis spectrophotometer at 240 nm by considering the same reaction mixture without H2O2 as the blank.

2.10. Histopathological studies

Transmission electron microscopic image of TiO2 NPs in Milli-Q water shows uniformly dispersed homogeneous spherical-shaped particles in the size of 24.6 ± 4.5 nm (Fig. 1). The stability of TiO2 NPs in artificial seawater medium was studied using dynamic light scattering technique (Supplemental Figure 1). Stability of different concentrations (0.1, 1, and 10 mg L^-1) of TiO2 NPs in ASW at 0, 24, and 48 h was measured. The hydrodynamic diameter was found to increase with increasing concentrations and exposure time. At lower exposure concentration (0.1 mg L^-1), the particles were in the submicron size range. As the interaction time increased (24 and 48 h), the effective diameter of TiO2 nanoparticles (1 and 10 mg L^-1) reached micron size range. Increase in nanoparticle concentration seemed to have increased the hydrodynamic diameter significantly (P < 0.01).

Fig. 1. Transmission electron micrograph of TiO2 NP dispersion in Milli-Q water.
3.2. Toxicity and uptake of TiO$_2$ NPs on marine algae Dunaliella salina

The toxicity of TiO$_2$ NPs on marine algae Dunaliella salina in ASW medium was measured after 72 h of interaction. TiO$_2$ NPs showed a significant concentration-dependent loss in cell viability of Dunaliella salina with respect to the control ($P < 0.001$). The lethal concentration (LC$_{50}$) of TiO$_2$ NPs on algal cells was found to be 11.35 mg L$^{-1}$ (with 95% CI of 7.61–20.07 mg L$^{-1}$), (Supplemental Figure 2A). Depending on the lethal concentrations (LC$_{50}$), the range of sublethal test concentrations (0.1, 1, and 10 mg L$^{-1}$) was selected for further toxicity study. A concentration-dependent increase in the loss of cell viability of Dunaliella salina was observed upon exposure to 0.1, 1, and 10 mg L$^{-1}$ of TiO$_2$ NPs (Fig. 2A). The effect of TiO$_2$ nanoparticles on chlorophyll a (CA), chlorophyll b (CB), and total chlorophyll content of Dunaliella salina was also studied (Supplemental Figure 3A). The decrease in chlorophyll content was found to be dependent on the exposure concentrations of TiO$_2$ NPs ($P < 0.01$), which correlated well with the cell viability loss results. Chlorophyll a (CA) content decreased significantly as compared to chlorophyll b (CB) with respect to the control ($P < 0.01$). The uptake of Ti content into Dunaliella salina was found to be dependent on the exposure concentration (Fig. 2B).

3.3. Waterborne exposure of Artemia salina to TiO$_2$ NPs

3.3.1. Toxicity of TiO$_2$ NPs on Artemia salina

The toxicity of TiO$_2$ NPs on the marine crustacean Artemia (48-h-old nauplii) in ASW medium was determined after 48 h of interaction. TiO$_2$ NPs showed a concentration-dependent increase in mortality of Artemia with respect to the control ($P < 0.001$). The LC$_{50}$ of TiO$_2$ NPs on Artemia was found to be 4.21 mg L$^{-1}$ (with 95% CI of 3.75–5.32 mg L$^{-1}$), (Supplemental Figure 2B). The toxicity of TiO$_2$ NPs on Artemia was found to be concentration dependent, and almost 60% of mortality was observed at 10 mg L$^{-1}$ treatment (Fig. 3A).

3.3.2. Uptake and depuration of TiO$_2$ NPs on Artemia salina

The uptake and accumulation concentration of Ti were based on the dry weight of Artemia. The accumulated concentration reflects the total body burden (tissue residual). After exposure to 0.1, 1, and 10 mg L$^{-1}$, the Ti content in Artemia was observed to be dependent on TiO$_2$ concentration (Fig. 3 B). Uptake of Ti was found to be 15.15 ± 3.47 mg/g dry weight upon exposure to 10 mg L$^{-1}$ of TiO$_2$ NPs, whereas after the depuration of 24 h, the accumulated Ti was only about 0.476 ± 0.001 mg/g dry weight. Uptake was measured to be higher as compared to that of accumulation, which signifies the higher elimination of the ingested particles by Artemia.

3.4. Dietary exposure of Artemia salina to TiO$_2$ NPs

3.4.1. Toxicity of TiO$_2$ NPs on Artemia salina through dietary exposure

For dietary exposure, Artemia were fed with algal cells (D. salina) that were pre-treated with TiO$_2$ NPs of 0.1, 1, and 10 mg L$^{-1}$ concentrations. The mortality of Artemia upon exposure to Ti-accumulated algal cells was found to be dependent on the exposure concentration (Fig. 4A). After the dietary exposure, the feeding rate of Artemia exposed to Ti-accumulated D. salina cells was studied in terms of decrease in chlorophyll a (CA), chlorophyll b (CB), and total chlorophyll content (Supplemental Figure 3B). The decrease in chlorophyll content was found to be dependent on the exposure concentrations of TiO$_2$ nanoparticles ($P < 0.01$). Chlorophyll a (CA) content decreased significantly as compared to chlorophyll b (CB) with respect to the control ($P < 0.01$).
3.4.2. Uptake and depuration of TiO₂ NPs by Artemia salina through dietary exposure

Uptake of Ti content in Artemia was measured after feeding with TiO₂ NP (0.1, 1, and 10 mg L⁻¹)-pre-treated algal cells (D. salina). Ti uptake was gradually increased with increasing concentrations (P < 0.01) with respect to the control (Fig. 4B). Uptake of Ti was measured to be 0.119 ± 0.02 mg/g dry weight upon exposure to 10 mg L⁻¹, while after the depuration of 24 h, the accumulated Ti was rapidly decreased to 0.045 ± 0.005 mg/g dry weight. The accumulation of Ti content in Artemia was found to be below the detection limit (BDL) after feeding with algal cells exposed to 0.1 mg L⁻¹ of TiO₂ NPs.

3.5. Biomagnification of TiO₂ NPs from algae to Daphnia

The biomagnification factor (BMF) of TiO₂ NPs from algae to Artemia was measured. The calculated BMF was found to be 0.029, 0.048, and 0.016 upon exposure to 0.1, 1, and 10 mg L⁻¹ of TiO₂ NPs, respectively. BMF was calculated to be less than 1 signifying that no biomagnification of nanoparticles occurred across the trophic level.

3.6. Oxidative stress (ROS) and antioxidant enzyme activity (CAT and SOD) in algal cells and Artemia (waterborne and dietary exposure)

Reactive oxygen species generation (ROS) and antioxidant enzyme activity (CAT and SOD) in algal cells and Artemia (waterborne and dietary exposure) on interaction with TiO₂ NPs were measured (Fig. 5 A, B and C). As compared to the control group, a significant increase in the CAT activity was observed, which was dependent on the concentration (TiO₂ NPs) and species (P < 0.05). CAT activity was observed to be higher for Artemia as compared to algal cells at all exposure concentrations (P < 0.05). At lower exposure concentration (0.1 mg L⁻¹), there was no significant difference (P > 0.05) observed between species and exposure conditions (waterborne and dietary exposure). A significant difference was observed at 1 and 10 mg L⁻¹ of TiO₂ NP exposure to algae and Artemia.

The inhibition of the antioxidant enzyme, SOD, was observed to decrease with respect to exposure concentrations (Fig. 5 C). As the nanoparticle concentration increased, SOD inhibition decreased with respect to the control group of algae for waterborne exposure of Artemia (P < 0.05), whereas, dietary exposure showed an insignificant decrease in SOD inhibition (P > 0.05). Artemia showed higher SOD inhibition as compared to algae at all exposure concentrations (P < 0.05).

3.7. Histopathology studies

Exposure of Artemia to TiO₂ NPs caused significant changes in the morphology. The longitudinal and transverse section of mid and hind gut regions of the control Artemia nauplii are shown in Fig. 6A (a and c). The circularly arranged cuboidal, columnar cells, and longitudinal muscle layers of mid and hind gut region could be observed. Exposure to 10 mg L⁻¹ of TiO₂ NPs caused deformation of cells in the epithelial lining of hind gut region (d and e). Agglomerated nanoparticles in the mid gut region were also evident (e and f). Under dietary exposure, the ingested Ti-contaminated algal cells were observed in the mid gut of
Fig. 6. A: Longitudinal and transverse section of mid and hind gut region of waterborne exposure of control (a and b) and TiO2 NP (10 mg L$^{-1}$)-treated Artemia nauplii (c, d, e and f) after 48-h treatment. The following numbering shows the different sections of the gut region. 1, foregut content; 2, foregut midgut transition; 3, foregut cells; 4, mid gut content; 5, midgut lumen; 6, mid gut epithelial cells. B: Histopathological sectioning of dietary exposed Artemia a. Accumulation of algal cells contaminated with 10 mg L$^{-1}$ TiO2 NPs in mid gut region (b and c).
4. Discussion

4.1. Effect of TiO2 NPs on algae (Dunaliella salina)

Algae, which is a primary producer in the aquatic food chain, plays a major role in maintaining a balanced aquatic ecosystem. Loss in the algal growth and chlorophyll content can directly reflect the effects of pollutants in the aquatic system (Ji et al., 2011). Several earlier studies dealt with the toxicity of TiO2 nanoparticles towards marine algae (Sendra et al., 2017b). The concentration-dependent decrease in the chlorophyll content cA, cM, and total chlorophyll (Supplemental Figure 3A) was in agreement with the toxicity results. The decrease in cell viability could be due to entrapment and adsorption of NP aggregates (Supplemental Figure 4), which reduced the light availability (shading effect) and limited the nutrient uptake (Wang et al., 2016) by the algal cells. TiO2 NPs can also cause cell membrane damage and release exopolymeric substances (EPS) as a plausible defence mechanism against stress (Wang et al., 2012). Release of EPS can also exacerbate the cell–NP interaction through aggregation of the algal cells (Zhou et al., 2016).

The concentration-dependent increase in uptake of TiO2 particles into the algal cells was noted (Fig. 2B). The internalized nanoparticles can promote oxidative damage to algal cells through lipid peroxidation. One of the principal toxicity mechanisms of TiO2 NPs is the generation of ROS radicals under light condition (Xia et al., 2015) through a Fenton-type reaction (Arbab et al., 2003). In this study, the photo-reactive TiO2 NPs resulted in a concentration-dependent generation of ROS radicals in Dunaliella salina (Fig. 5A). The enhanced ROS generation by TiO2 NPs in marine algae Nitzschioa closterium was reported to be the leading cause of toxicity (Xia et al., 2015). These radicals can directly damage the cell membrane as indicated by the oxidative stress–related increase in membrane permeability of algae (Miao et al., 2009). Cells release antioxidant enzymes (SOD and CAT) as a defense system to protect against the oxidative stress (ROS). In this work also, we noted a concentration-dependent changes in the SOD and CAT activities. Similar to our observation, Li et al. (2015) mentioned that an increase in CAT activity and decrease in SOD activity was observed in (Karenia brevis) marine algae exposed to TiO2 NPs.

4.2. The effect of TiO2 NPs on Artemia salina through waterborne exposure

Being the primary consumer, the filter-feeding crustacean Artemia is among the most widely used model organisms to study the impact of environmental toxicants. Ingestion is considered to be the primary route of nanoparticles uptake for most of the micro crustaceans (Bhuvaneshwari et al., 2017). Ingestion of aggregated TiO2 NPs can increase the uptake into Artemia. Also, the uptake of nanoparticles can be facilitated through endocytosis process (Pan and Wang, 2004). The uptake studies (Fig. 3B) in the current investigation revealed that ingestion of nanoparticles by Artemia was a function of concentration. The maximum Ti uptake was measured to be 15 mg/g dry weight upon ingestion of TiO2 for 96 h of exposure. Another study by Ozkan et al. (2016) reported uptake of Ti (250 mg/g) upon exposure to 100 mg L−1 of TiO2 for 24 h. Uptake of Ti might be influenced by the TiO2 NP concentration, duration of exposure, and feeding pattern of Artemia.

Artemia is incapable of deputing all the ingested NPs, which causes high Ti accumulation (Fig. 3B). Higher uptake and accumulation of the particles in Artemia could have significantly contributed to toxic effects. Histopathological results confirmed the accumulation of NPs in the mid gut content and consequent deleterious morphological changes in Artemia (Fig. 6A e and f). Similar to our observation, translocation of accumulated NPs into epithelial cells and across the intestinal barriers was also reported by Arnold et al. (2013). Ingested nanoparticles can bring disruptive changes to the physiological process of Artemia like ROS generation (Fig. 5 A).

4.3. Dietary exposure of Artemia salina to TiO2 NPs

Trophic transfer is the mobility of nanoparticles from prey (D. salina) to predator (Artemia) through diet in the aquatic food chain (Cardoso et al., 2014). Feeding of NP-contaminated algal cells to Artemia caused significant toxicity (Fig. 4A), uptake, and accumulation of particles (Fig. 4B). Feeding pattern of Artemia on TiO2 NP-contaminated algal cells was measured during dietary exposure through chlorophyll estimation (Fig. S3 B) and estimation of ingested algal cells (Fig. S5). Artemia was found to be more selective towards less contaminated algal cells. The ingestion of algal cells was in the order: Control > 0.1 > 1 > 10 mg L−1, which confirmed the selective feeding pattern of Artemia. Ingested algal cells in the gut of Artemia were confirmed using microscopic studies (Fig. 6c and d). Similarly, Dalai et al. (2014) reported the selective feeding of uncontaminated algal cells by Daphnia.

As compared to the dietary exposure, Artemia was more sensitive towards waterborne exposure. Ti uptake in Artemia was found to be 32.2-fold higher than accumulation through waterborne exposure, while it was only about 2.6-fold increment in the case of dietary exposure. Also, during the starving conditions (waterborne exposure), Artemia ingests more sediment and aggregated TiO2. While in the case of dietary exposure, particle availability was only through the contaminated feed, and accumulated TiO2 NPs in Dunaliella salina required digestion in Artemia. On the other hand, free nanoparticles in Artemia could be directly excreted from the digestive tract, and the eliminated nanoparticles are readily available for re-uptake during starving condition of waterborne exposure. Additionally, the longer gut retention time of NPs can also facilitate the sub-cellular localization/uptake and transportation to different organs (Ward and Koch, 2009). Feswick et al. (2013) reported the translocation of QD NPs into the lipid-storage vesicles of D. magna through the penetrating gut epithelial cells. Faster depuration in dietary exposure (during feeding exposure) could also be another reason for the lesser accumulation as compared to that of waterborne exposure. Similar to our study, Wang et al. (2016), reported enhanced uptake of TiO2 NPs in waterborne exposure as compared to that of dietary exposure.

The calculated BMF factor was found to be < 1 at all exposure concentrations, thereby indicating that no biomagnification occurred from algae to Artemia. Even though the evidence of algal cell accumulation in the gut of Artemia was observed through histopathological study (Fig. 6B), biomagnification from algae to Artemia was absent. Similarly, dietary exposure of Cd QD-contaminated green algae to Daphnia showed presence of nanoparticles in the gut, but still, no biomagnification was observed (Bouldin et al., 2008). Zhu et al. (2010) also reported no indication of biomagnification through dietary exposure of TiO2 NPs from Daphnia to zebraﬁsh. In contrast, Werlin et al. (2011) showed a BMF > 8 upon dietary exposure of TiO2 from algae to Daphnia. It is considered that the BMF of any pollutant in the food web is dependent on the gut passage time, assimilation, elimination ratio, and environmental conditions (Tsuji and Wang, 2004). Together, the observed results indicated that the mode of exposure (waterborne and dietary exposure) of NPs through their interactions with marine aquatic organisms in the ecosystem should not be neglected. Further studies at the molecular level will provide a detailed mechanism of action of TiO2 NPs in the trophic level.

5. Conclusion

Artemia was found to be more sensitive to TiO2 NPs as compared to algal cells. Waterborne exposure was the major route of NP uptake and accumulation than that of dietary exposure. Higher availability of nanoparticles in waterborne exposure enhanced the uptake and
accumulation. During the starving condition, *Artemia* would ingest sediments and aggregated TiO₂. The increase in ROS generation and subsequent antioxidant enzyme (SOD and CAT) activities influenced the phototoxicity of the NPs in both algal cells and *Artemia*. The bioaccumulation (BHF) from algae to *Artemia* was not observed in this study.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.09.022.

References


