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Combined toxicity of two crystalline phases (anatase and rutile) of Titania nanoparticles towards freshwater microalgae: *Chlorella* sp

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ABSTRACT

In view of the increasing usage of anatase and rutile crystalline phases of titania NPs in the consumer products, their entry into the aquatic environment may pose a serious risk to the ecosystem. In the present study, the possible toxic impact of anatase and rutile nanoparticles (individually and in binary mixture) was investigated using freshwater microalgae, *Chlorella* sp. at low exposure concentrations (0.25, 0.5 and 1 mg/L) in freshwater medium under UV irradiation. Reduction of cell viability as well as a reduction in chlorophyll content were observed due to the presence of NPs. An antagonistic effect was noted at certain concentrations of binary mixture such as (0.25, 0.25), (0.25, 0.5), and (0.5, 0.5) mg/L, and an additive effect for the other combinations, (0.25, 1), (0.5, 0.25), (0.5, 1), (1, 0.25), (1, 0.5), and (1, 1) mg/L. The hydrodynamic size analyses in the test medium revealed that rutile NPs were more stable in lake water than the anatase and binary mixtures [at 6 h, the sizes of anatase (1 mg/L), rutile NPs (1 mg/L), and binary mixture (1, 1 mg/L) were 948.83 ± 35.01 nm, 555.74 ± 19.93 nm, and 1620.24 ± 237.87 nm, respectively]. The generation of oxidative stress was found to be strongly dependent on the crystallinity of the nanoparticles. The transmission electron microscopic images revealed damages in the nucleus and cell membrane of algal cells due to the interaction of anatase NPs, whereas rutile NPs were found to cause chloroplast and internal organelle damages. Mis-shaped chloroplasts, lack of nucleus, and starch-pyrenoid complex were noted in binary-treated cells. The findings from the current study may facilitate the environmental risk assessment of titania NPs in an aquatic ecosystem.

1. Introduction

Titanium dioxide (TiO_2) nanoparticles were extensively used in various industrial applications and consumer products such as water treatment, medicine, cosmetics, and engineering (ICIS Chemical Report, 2010). Excessive usage of TiO_2 nanoparticles have led to their exposure to the aquatic environment and their consequent hazards to the ecosystem (Furman et al., 2013). There are mainly three crystalline phases of TiO_2 *viz.*, anatase (tetragonal), rutile (tetragonal), and brookite (orthorhombic) (Cho et al., 2013). Among these, rutile is the most common and natural form of TiO_2, and it is an integral part of heavy minerals. It is employed in optical elements due to its highest refractive indices and also used as a construct for refractory ceramics, pigments, etc. (Winkler, 2003; Yu et al., 2013). Anatase is extensively used in organic photovoltaics as an electron collecting layer (Small et al., 2012). Anatase is also applied as a catalytic support for the production of nanotubes and nanoribbons (Gregory et al., 2008). Both rutile and anatase phases are being extensively used in sunscreens (due to their high-energy-absorbing property), paints, plastics, paper, foods, electronics, and other applications (Ferguson et al., 2005; Mueller and Nowack 2008; Wang et al., 2006; Winkler, 2003). Since there is scarcity of brookite in nature, this form does not have significant economic importance (Allen et al., 2009).

The released nanomaterials from different industries, consumer products may inevitably end up in the water bodies. They may potentially exert adverse impacts on the aquatic ecosystem due to their unique physical and chemical characteristics such as high reactivity and the photoactivity (Cardinale et al., 2012). Kaegi et al.

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(2008) mentioned about the direct release of TiO_2 NPs of about 16 μg/L into the surface water from aged paints. The predicted environmental concentration (PEC) of TiO_2 NPs in surface water has been stated to be less than 1 μg/mL (Gottschalk et al., 2009). Hence, it becomes necessary to evaluate the toxicity of TiO_2 NPs at its environmentally relevant concentrations. In our previous studies (Dalai et al., 2013; Pakrashi et al., 2013), we have evaluated the toxicity of TiO_2 and Al_2O_3 NPs under environmentally relevant low-exposure concentrations, i.e., 0.05, 0.5, and 1 mg/L, towards freshwater algae. Microalgae are of great importance for the maintenance of aquatic ecosystem. It can be used as a model for the studies of aquatic risk assessment of the nanomaterials (Aruoja et al., 2009). Lubick (2008) and Navarro et al. (2008) reported that the interaction of nanoparticles with algae influenced the aquatic toxicity of nanomaterials. Recently, Ji et al. (2011) noted that ZnO and TiO_2 (anatase) nanoparticles caused severe damage to freshwater green algae. Cardinale et al. (2012) evaluated the toxicity of TiO_2 nanoparticles (Degussa, 82% anatase/18% rutile) on three algal species viz., *Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Chlamydomonas moewusii*. They observed that the gross primary production of these algae were reduced, and the reduction rate varied depending on the species type. Dalai et al. (2013) reported the photoinduced toxicity of TiO_2 anatase NPs on *Scenedesmus obliquus* at low-exposure doses (≤1 μg/mL). They observed reduced cell viability, increased reactive oxygen species (ROS) generation, and membrane damage. Miller et al. (2012) observed the increased toxicity of TiO_2 NPs on marine algae under UV-A irradiation than non-irradiation condition due to their increased photocatalytic activity.

A number of prior reports strongly indicated that titanium dioxide nanoparticles caused severe toxicity towards freshwater microalgae species. However, information on their fate, behavior, and mechanism of uptake (pathways) based on crystallinity, shape, and other properties of materials is still lacking. Ji et al. (2011) stated that TiO_2 NP toxicity varied with respect to the crystalline structure of TiO_2 NPs. The two allotropic forms of TiO_2 NPs *viz*., anatase and rutile have different surface properties and reactivity. Prior studies have demonstrated that anatase phases are more cytotoxic than those of rutile phases (Hirakawa et al., 2004). Braydich-Stolle et al. (2009) have observed that rutile TiO_2 NPs were capable of initiating apoptosis through the formation of ROS, whereas pure anatase TiO_2 NPs caused cell necrosis and membrane leakage in cells. Most of the previous toxicity studies on microalgae dealt with the anatase phase and P25 form of TiO_2 (Chen et al., 2012; Clement et al., 2013; Dalai et al., 2013; Lee and An, 2013; Wang et al., 2008) and only a handful studies are available with the rutile phase (Ji et al., 2011). Therefore, it is pertinent to study the other crystalline phase, i.e., rutile TiO_2 NPs, which is also commonly employed in commercial products/applications (Winkler, 2003; Yu et al., 2013).

As UV-C radiation is a shorter and higher energized radiation than UV-A and UV-B, the photocatalytic action of TiO_2 NPs was reported to be enhanced significantly under UV-C irradiation than other UV radiations (Termtanun, 2013). Due to its greater photolytic activity and energy, UV-C irradiation is being widely used for water disinfection and in the photodegradation studies, along with TiO_2 NPs (Bushnaq et al., 2004). Since, UV-C radiation gets absorbed by the earth's atmosphere, not much attention was given in evaluating its effects on the environment and the transformations caused by it (Holzinger and Lütz, 2006; Basti et al., 2009). McGivney (2007) studied the combined effect of UV-C, vacuum UV, and TiO_2 on freshwater algae, *Pseudokirchneriella subcapitata*, and marine algae, *Tetraselmis suecica*, in a ballast water treatment system. They noticed that UV-C/TiO_2 exerted a higher mortality compared to UV irradiation alone. Most of the algal toxicity studies on TiO_2 NPs till date were carried out under different irradiation conditions such as visible light and UV light especially, UV A and UV B (Lee and An, 2013; Ji et al., 2011). To the best of our knowledge, there are limited reports highlighting the photocatalytic effects of UV-C on the toxicity of TiO_2 NPs. Vileno et al. (2007) studied the effect of TiO_2 NPs on the stiffness of human skin fibroblasts in the presence of UV-A and UV-C. Hence, it is crucial to evaluate the risk of TiO_2 NPs in the presence of UV-C on *Chlorella* sp.

In the natural ecosystem, various toxicants are expected to be present in the mixed form rather than as individuals. The toxicity assessment of a single toxicant alone does not adequately reflect the actual impact in the aquatic environment. The mode of action may vary for individual toxicants in the mixture; they may mask the effect of each other. It is indispensable to study the effect of their mixture in addition to the individual toxicants to adequately assess the environmental toxicity of different forms of toxicants (Jak et al., 1996). Zou et al. (2014) studied the toxicity of silver (Ag) NPs in the presence of TiO_2 NPs on a ciliated protozoan, *Tetrahymena pyriformis*. Increased ecotoxicity was noted due to the coexistence of TiO_2 NPs and Ag NPs. This elucidates that the level of toxicity increases in the presence of a mixture of nanoparticles rather than the individual forms (Utgikar et al., 2004) and provides an understanding of the complex interaction between different substances.

The present investigation is the first of its nature to evaluate the combined toxicity of anatase and rutile NPs towards freshwater microalgae in a freshwater matrix. It may be hypothesized that there are inherent differences in the toxic effects of the two different crystal phases of titania NPs (anatase and rutile). Their binary combination would be more toxic than the respective individual phases. The aim of the present study was to elucidate the toxic effects of the two crystalline phases of titania nanoparticles i.e., anatase and rutile, as well as their binary mixture towards freshwater algae, *Chlorella* sp. at environmentally relevant low concentration levels (0.25, 0.5 and 1 mg/L) in the lake water matrix under UV-C irradiation.

2. Materials and methods

2.1. Chemicals

Dry titanium(IV) dioxide (TiO_2) nanopowder (anatase, <25 nm, CAS No: 1317-70-0, 99.7% trace metal basis; and rutile, <100 nm (~10 nm Diam. × 40 nm L), CAS No: 1317-80-2, 99.5% trace metals basis) were purchased from Sigma–Aldrich, Missouri, USA, and their supplier information was summarized in Table 1. BG-11 broth was purchased from Himedia Labs Pvt., Ltd. (Mumbai, India). *N*, *N*-dimethylformamide was procured from SD fine chemicals

Table 1
Information about the physicochemical parameters of two different types of TiO_2 NPs has been represented in the table as per the supplier.

	Anatase NPs	Rutile NPs
Assay	99.7% trace metal basis	99.5% trace metals basis
Form	Nanopowder	Nanopowder
Particle size	<25 nm	<100 nm (~10 nm Diam. × 40 nm L)
CAS No	1317–70–0	1317–80–2
Surface area	Spec. surface area 45–55 m^2/g	Spec. surface area 130–190 m^2/g
Density	3.9 g/mL at 25 °C	4.17 g/mL at 25 °C (lit.)
Bulk density	0.04–0.06 g/mL	0.06–0.10 g/mL