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Individual and binary toxicity of anatase and rutile nanoparticles towards *Ceriodaphnia dubia*

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ABSTRACT

Increasing usage of engineered nanoparticles, especially Titanium dioxide (TiO_2) in various commercial products has necessitated their toxicity evaluation and risk assessment, especially in the aquatic ecosystem. In the present study, a comprehensive toxicity assessment of anatase and rutile NPs (individual as well as a binary mixture) has been carried out in a freshwater matrix on *Ceriodaphnia dubia* under different irradiation conditions viz., visible and UV-A. Anatase and rutile NPs produced an LC_{50} of about 37.04 and 48 mg/L, respectively, under visible irradiation. However, lesser LC_{50} values of about 22.56 (anatase) and 23.76 (rutile) mg/L were noted under UV-A irradiation. A toxic unit (TU) approach was followed to determine the concentrations of binary mixtures of anatase and rutile. The binary mixture resulted in an antagonistic and additive effect under visible and UV-A irradiation, respectively. Among the two different modeling approaches used in the study, Marking-Dawson model was noted to be a more appropriate model than Abbott model for the toxicity evaluation of binary mixtures. The agglomeration of NPs played a significant role in the induction of antagonistic and additive effects by the mixture based on the irradiation applied. TEM and zeta potential analysis confirmed the surface interactions between anatase and rutile NPs in the mixture. Maximum uptake was noticed at 0.25 total TU of the binary mixture under visible irradiation and 1 TU of anatase NPs for UV-A irradiation. Individual NPs showed highest uptake under UV-A than visible irradiation. In contrast, binary mixture showed a difference in the uptake pattern based on the type of irradiation exposed.

1. Introduction

Engineered nanoparticles production and utilization are projected to reach about a trillion dollar (US) business in the upcoming future (Nel et al., 2006). In a survey conducted by Piccinno et al. (2012) on the industrial usage of NPs, titanium dioxide nanoparticles (TiO_2 NPs) were noted to be the most produced NPs in Europe and worldwide with a production of about 550 and 3000 tons per year, respectively. Among the overall production, TiO_2 NPs have been widely used for cosmetic purposes, including sunscreens (70–80%) than other applications such as coatings and plastics (20%), paints (10–30%), cement (1%) and other uses (10%). The total global production of TiO_2 NPs was estimated to reach about 2.5 million metric tons per year around 2025, as predicted by Robichaud et al. (2009).

The crystalline nature of TiO_2 NPs can be used to differentiate them into three various forms namely, anatase, rutile, and brookite. Among these three forms, anatase and rutile (tetragonal in structure) were signified as the most active TiO_2 forms than the brookite, an orthorhombic form. Thus, anatase and rutile forms have been widely used for both industrial and commercial purposes (Cho et al., 2013). Based on the type of application and function it serves, anatase or rutile NPs were selected for a particular purpose. Due to the higher occurrence in nature, rutile NPs have been utilized as a white pigment in paints, plastics, food products (Winkler, 2003; Weir et al., 2012), as UV absorbers in sunscreens and cosmetics (Mueller and Nowack, 2008). While, anatase NPs have been used in various environmental oriented applications such as semiconductor catalysts in water treatment (Velhal et al., 2012; Lazar et al., 2012), and reactors in the photodegradation of wastes and pollutants in industries (Montazer and Seifollahzadeh, 2011; Mahlambi et al., 2015). It was also frequently used in self-cleaning products, solar cells, light emitting diodes, sunscreens (U.S. EPA, 2010), disinfectants (Kühn et al., 2003), and in cancer treatment (Fujishima et al., 2000; Fujiwara et al., 2015). Photocatalytic property (reactive

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oxygen species generation) of anatase and higher refractive index of rutile NPs make their usage inimitable.

Massive handling of TiO_2 NPs led to their inadvertent discharge into the environment, especially the aquatic bodies (Kaegi et al., 2008). Therefore, risk assessment of TiO_2 NPs towards various aquatic organisms becomes indispensable. Freshwater crustaceans like daphnids, in particular, play a significant role in the maintenance of aquatic system since it serves as a vital food source in the aquatic food chain (Lampert and Sommer, 2007). Due to the ease of availability of daphnids, they have been widely used in ecotoxicological studies of nanoparticles. Literature available on the toxicity on crustaceans of TiO_2 NPs concerning their crystalline form has not been well studied so far. Although an enormous toxicity experiments were carried out on daphnids for the anatase (Dabrunz et al., 2011; Hund-Rinke and Simon, 2006) and P25 (Jacobasch et al., 2014; Li et al., 2011) NPs, the data remains inconclusive. On the other hand, only a handful of information is present for rutile NPs (Bang et al., 2011; Marcone et al., 2012). In our previous toxicity study towards *Chlorella* sp. due to anatase and rutile NPs, algal cells followed a different cellular/mechanistic pathway (anatase-nucleus specific; rutile-organelle specific) due to the variance in crystallinity in response to NPs (Iswarya et al., 2015). Campos et al. (2013) analyzed the effect of various forms of TiO_2 NPs on microalgae, *Chlorella vulgaris*, and their further consequences on *Daphnia magna*. They observed that the aggregation of TiO_2 NPs with the microalgae led to the depletion of food available to the daphnia and in turn induced impairment in their growth and reproduction.

In addition to the variation in crystalline structure, the type of irradiation i.e. whether UV (A, B, C) or visible light was used also influences the toxicity of TiO_2 NPs. Amiano et al. (2012) and Clemente et al. (2014) revealed the importance of irradiation while testing the toxicity of TiO_2 NPs due to variation in the photocatalytic activity upon irradiation. Photocatalytic differences, in turn, have an impact upon their toxicity under different irradiation condition. Hence, exploring the differences in toxicity of TiO_2 NPs in relation with crystallinity and irradiation becomes pertinent.

It is very natural for the aquatic system to get consistently contaminated with a mixture of nanoparticles from various anthropogenic sources rather than individual NP types. Most of the ecotoxicological studies conducted so far have addressed the impact of individual NPs alone upon various aquatic organisms, which contradicts the actual environmental scenario. Jak et al. (1996) emphasized the necessity to evaluate the difference in toxicity of individual NPs with that of a NP mixture. Toxicity of the mixture may/may not differ from that of the individuals. Recent studies on NP toxicity pointed out the emergence of works that assess the NP toxicity studies for a NP mixture (Tong et al., 2015; Li et al., 2015). Yu et al. (2016) examined the dual effect of CeO_2/TiO_2 NPs and CeO_2/ZnO NPs on *Nitrosomonas europaea* bacteria. They had noticed antagonistic and synergistic cytotoxic effects on exposure to the binary combinations of TiO_2 and ZnO NPs, respectively, when co-exposed with CeO_2 NPs. In some cases, both additive and antagonistic effects were observed at certain combinations/concentrations of a binary mixture of anatase and rutile NPs towards the freshwater algae, *Chlorella* sp. (Iswarya et al., 2015). Hai-zhou et al. (2012) evaluated the individual and binary toxic effect of CuO and ZnO NPs towards *Daphnia magna.* A binary mixture of NPs showed higher mortality than individual NPs, which indicated the higher toxicity of binary NPs.

The foremost objective of the present study was to investigate the toxic response of a freshwater crustacean, *Ceriodaphnia dubia*, upon exposure to different crystalline phases of TiO_2 NPs, such as anatase and rutile NPs, both individual as well as a mixture in a freshwater matrix. A toxic unit (TU) approach was employed to determine the concentrations of binary mixtures (detailed in Section 2.6), which have been extensively used in most of the ecotoxicological studies of chemical mixtures (Cao et al., 2007; Naddy et al., 2015; Hu et al., 2014). Since TiO_2 NPs are photocatalytic in nature, the impact of irradiation on their toxicity was evaluated by exposing the individual as well as binary NPs under two different, environmentally relevant irradiation conditions such as UV-A and visible light. Furthermore, two modeling approaches such as Marking–Dawson and Abbott's modeling have been followed to differentiate the mode of the binary mixture into antagonistic, additive or synergistic (detailed in Section 2.7). Stability analysis (Dynamic Light Scattering, DLS) evaluated the aggregation potential of NPs in sterile lake water. Besides, interactions between anatase and rutile NPs in the mixture have been assessed with various techniques (zeta potential and TEM). NP uptake by Daphnia has been evaluated to examine their potential risk in the aquatic system.

2. Materials and methods

2.1. TiO_2 nanoparticles and preparation

Two different crystalline phases of TiO_2 nanoparticles viz., anatase with a reported size of <25 nm (CAS No: 1317-70-0, 99.7% trace metal basis) and rutile with a reported size of <100 nm (~10 nm Diam. × 40 nm L, CAS No: 1317-80-2, 99.5% trace metals basis) were procured from Sigma-Aldrich, Missouri, USA in powder form.

Stock suspensions (200 mg/L, i.e. 2.504 mM) of both anatase and rutile NPs were prepared in deionized water. Then, they were sonicated for about 15 min with the help of an ultrasonicator (130 W, 20 kHz, Sonics, USA) to ensure uniform dispersions of nanoparticles, which were utilized for further experiments.

2.2. Preliminary characterization of pristine nanoparticles

Electron microscopic analysis was performed to determine the primary size and shape of anatase and rutile nanoparticles using high-resolution transmission electron microscope (HRTEM, FEI TecnaiG2 T20 S-Twin). The average size of individual nanoparticles was calculated by measuring about 100 nanoparticles randomly from the TEM images, which was analyzed with the help of an imaging software, ImageJ.

The initial hydrodynamic size of TiO_2 nanoparticles (anatase and rutile) was analyzed in deionized water by dynamic light scattering (DLS) analysis method with the help of 90 Plus Particle Size Analyzer (Brookhaven Instruments Corp., USA).

2.3. Isolation and identification of Ceriodaphnia dubia

Freshwater crustaceans were isolated from the VIT Lake (situated on the VIT University premises), Vellore, Tamil Nadu, India, by following the protocol described by Pakrashi et al. (2013). Freshwater collected from the VIT Lake was initially checked for the presence of daphnids. Daphnids, which were predominant in the lake water were separated using a Pasteur pipette (5 mL) and added to the beakers containing sterile lake water (prepared as described in Section 2.4). Then, they were regularly subcultured under specific conditions to confirm their species uniformity. Isolated daphnids were further evaluated under a phase contrast microscope (100×, Zeiss Axiostar, USA) and identified as *Ceriodaphnia dubia* by morphological identification.

Daphnia cultures were well maintained in the sterile lake water at $26 \pm 2\,^{\circ}C$ under specific photoperiod conditions (16 h light:8 h dark) using an ambient fluorescence light (TL-D Super 80 linear fluorescent tubes, Philips LifeMax, 18W, intensity of 1250 lux) as a