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# Dietary transfer of zinc oxide particles from algae (*Scenedesmus obliquus*) to daphnia (*Ceriodaphnia dubia*)

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ABSTRACT

The rapid increase in production and usage of ZnO particles in recent years has instigated the concerns regarding their plausible effects on the environment. Current study explores the trophic transfer potential of ZnO particles of different sizes (50, 100 nm and bulk particles) from algae (*Scenedesmus obliquus*) to daphnia (*Ceriodaphnia dubia*) and the contribution of $ZnO_{(ions)}$ (effect of dissolved Zn ions that remain in test medium after separation NPs) to the overall toxicity of $ZnO_{(total)}$ (impact of both particle and dissolved Zn ions). Toxicity and uptake of $ZnO_{(total)}$ and $ZnO_{(ions)}$ in algae were found to be dependent on the concentration and particle size. Feeding of Zn accumulated algae (517 ± 28, 354.7 ± 61 and 291 ± 20 μg/g dry wt.) post-exposure to 61 μM of $ZnO_{(total)}$ of 50, 100 nm and bulk ZnO particles caused a significant decrease in the survival (15–20%) of daphnia. A significant amount of Zn accumulation was observed in daphnia even after the 48 h depuration period. Biomagnification factor was found to be nearly 1 for all the sizes of ZnO particles tested. For 50 nm ZnO, the BMF was higher when compared to other two sizes, reaching the mean value of 1.06 ± 0.01 at 61 μM. Further analysis revealed that the dietary uptake of different sizes of ZnO particles caused ultra-structural damages and degradation of internal organs in daphnia.

## 1. Introduction

Zinc oxide (ZnO) nanoparticles are well known for their high photocatalytic activity and biocompatibility when compared to other inorganic nanoparticles (NPs). ZnO NPs are being used in sunscreens (UV protection), cosmetics, ointments (antimicrobial), (Osmond and Mccall, 2010; Ma et al., 2013) paints (Burnett and Wang, 2011), plastics and electronic semi-conductors (Hsu et al., 2013). Among the metal oxide nanoparticles produced annually, ZnO NP stands to be the third highest globally produced nanoparticle after nano $SiO_2$ and nano $TiO_2$ (Piccinno et al., 2012). There is an increase in the annual consumption of ZnO NPs based products and these NPs can be released into the aquatic environment (Gottschalk et al., 2013) and could pose potential toxic risks to the aquatic organisms (Renzi and Guerranti, 2015; Sajid et al., 2015; Skjolding et al., 2016). ZnO NPs are reported to be toxic to freshwater organisms such as algae (Chen et al., 2012a, 2012b; Lee and An, 2013; Pendashte et al., 2013; Suman et al., 2015; Bhuvaneshwari et al., 2015) and daphnia (Adam et al., 2014; Xiao et al., 2015; Bhuvaneshwari et al., 2016; Azevedo et al., 2016; Bacchetta et al., 2017). Microalgae being the primary producers, constitute the basis of the aquatic food web and toxicity evaluation of nanomaterials at this level is essential (Bhuvaneshwari et al., 2015). An aquatic crustacean (*Daphnia*) was selected as a test organism at higher trophic level owing to its small size, short life-cycle, easy handling and ability to filter feed (Koivisto and Ketola, 1995).

Once the NPs enter into the aquatic system, its stability, mobility and reactivity alters dynamically (Nowack and Bucheli, 2007). The toxicity of metal oxide nanoparticles on any aquatic organism is dependent mainly on surface area, particle size, chemical composition, dissolution and the physicochemical characteristics of medium (Li et al., 2011; Lowry et al., 2010; Beaudrie et al., 2015). ZnO NPs have been reported to release $Zn^{2+}$ ions in the experimental medium (Ma et al., 2014; Dong et al., 2016; Wang et al., 2016; Bacchetta et al., 2016). Several studies confirm the toxicity of released Zn ions on aquatic organisms (Seo et al., 2014; Adam et al., 2014), while few others emphasize that the toxic effects could not be solely ascribed to dissolved ions (Merdzan et al., 2014; Xiao et al., 2015). In contrast to the reports of Li et al. (2017) and Manzo et al. (2011) stating the particle effect on aquatic organisms, few studies have highlighted that both the particles and ions are likely to contribute to the toxicity of ZnO NP (Poynton et al., 2010). Adam et al. (2014) claimed that faster dissolution of Zn ions from ZnO NPs attributed maximum to chronic toxicity in *Daphnia magna*. As inferred by others (Lopes et al., 2014; Bhuvaneshwari et al., 2016), ZnO particles do play a significant role in

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inducing toxicity and feeding impairment in daphnia as compared to its ionic form. Xiao et al. (2015) reported the effect of particle rather than dissolved Zn ions in toxicity and accumulation process. In contrast, Bacchetta et al. (2016) and Bacchetta et al. (2017) reported that both particles and ions have a similar toxic effect on daphnia. Odobasic (2012) reported that the effect of Zn ions measured using metal salts such as $ZnCl_2$ and $ZnSO_4$ depends on the metal speciation (or) physicochemical characteristics of metal salts on toxicity. The toxicity exerted by those metal salts is quite different from the toxicity induced by dissolved Zn ion and ZnO NPs, owing to the release of various anionic metal ions ($Cl^-$ and $SO_2^{-4}$) along with the Zn ions. Determining the contribution of $ZnO_{(ion)}$ and $ZnO_{(total)}$ in effectively inducing the overall toxicity, is still a critical step in assessing the toxicity of ZnO NPs.

Previous studies on toxicity of ZnO NPs mainly emphasize the effect of Zn ions on individual test species like algae and daphnia. However, there is no literature on the toxicity of $ZnO_{(total)}$ along with the effects of dissolved Zn ions from NPs through dietary transfer from algae to daphnia. Most of the studies elucidate that the exposure of metals in its nano form to aquatic organisms are taken up from both water (waterborne exposure) and through diet (dietary exposure) (Buffet et al., 2011; Shaw et al., 2012; Khan et al., 2013). Nanoparticles transfer up through the food chain upon ingestion of contaminated prey by the predators, which is the primary concern in maintaining a well balanced ecological niche. Once the nanoparticles are readily internalized or accumulated in prey, it can get retained in the gut or absorbed over the epithelia and subsequently transferred to the higher trophic level organism. Nanoparticles associated with a food source (dietary exposure) have been shown to affect organism's health at higher trophic levels (Lee et al., 2015; Chen et al., 2015).

In this study, we hypothesize that the dietary transfer of ZnO particles of different sizes from freshwater algae (*Scenedesmus obliquus*) to daphnia (*Ceriodaphnia dubia*) would induce trophic toxicity. In our previous study, the internalization of ZnO particles individually in both algae and daphnia were observed through waterborne exposure (Bhuvaneshwari et al., 2015, 2016). Objectives of the present study are: (i) To study the exposure of $ZnO_{(total)}$ suspension and dissolved Zn ions (that remained in the test medium, after separation from the NPs) to algal cells and assessment of $ZnO_{(total)}$ and $ZnO_{(ion)}$ uptake in algal cells which were further used as dietary feed for daphnia to measure its trophic transfer potential; (ii) Quantification of accumulated Zn in daphnia through dietary exposure using Atomic Absorption Spectroscopy (AAS); (iii) The bio-magnification (BMF) potential of ZnO particles from algae to daphnia in natural lake water medium was measured, and further the ultra-structural altercations in daphnids was confirmed using transmission electron microscopy. The observed results are expected to provide a conducive report on the trophic transfer potential of $ZnO_{(total)}$ and $ZnO_{(ion)}$ through the dietary exposure to facilitate a better understanding of environmental implications of NPs in the freshwater ecosystem.

## 2. Materials and methods

### 2.1. Test organism and experimental setup

The freshwater algae and daphnia cultures were collected from the lake which is located between 12°151′ and 13°151′ Northern Latitude and between 78°201′ and 79°501′ Eastern Longitude (Pakrashi et al., 2012) in VIT University, Vellore, India. The detailed procedure for isolation and culturing of both algae and daphnia are represented in a Supplementary material file in Sections 1.1 and 1.2. The isolated algal and crustacean species were identified to be *Scenedesmus obliquus* and *Ceriodaphnia dubia*. The algal culture was allowed to grow in BG 11 medium in a 16 h/8 h (light/dark) rhythm with the light intensity of 3000 lx using fluorescent lights (TL-D Super 80 Linear fluorescent, Philips, India) at 23 °C. Daphnids (*Ceriodaphnia dubia*) were cultured in sterilized lake water and maintained in the sterile environmental chamber under the photoperiod of 16 h/8 h (light/dark) at 20 °C. The daphnids were fed with algal cells in a concentration of $5 \times 10^5$ cells/ml (0.5 OD-optical density at 610 nm). Neonates (*Ceriodaphnia dubia*) of < 24 h old were used for all the toxicity studies. Lake water (collected from VIT Lake) was used as an experimental medium for all toxicity studies to mimic the real environmental matrix. The collected lake water was filtered through Whatman No. 1 to remove the biological substances followed by 0.22 μm filtration and sterilization. The physicochemical characterization of lake water medium is represented in Supplementary material, Table S1.

### 2.2. Characterization of ZnO particles

ZnO nanoparticles of 50 nm (surface area > 10 $m^2$ $g^{-1}$) and 100 nm (surface area 15–25 $m^2$ $g^{-1}$) was purchased from Sigma-Aldrich. Bulk form of ZnO (size < 5 μm) particles was also procured from Sigma-Aldrich. The morphology, size and shape of procured nano (50 nm, 100 nm) and bulk ZnO particles were measured using transmission electron microscopy (TEM, Philips, CM12, Netherlands). ZnO particles of different sizes was dispersed in methanol and sonicated for 5 min using ultrasound probe sonicator at 130 W (Sonics, USA). The uniformly dispersed particles were coated on 3 mm copper grid and visualized under electron microscopy. The effective diameter of ZnO particles in natural lake water medium was measured using dynamic light scattering techniques (DLS, 90 Plus Particle Size Analyzer, Brookhaven Instruments Corp., USA). The stock concentration of 100 mg/L of ZnO particles was prepared in Milli-Q water and subjected to sonication using 130 W probe sonicator for 15 min at 20 kHz. The dispersed ZnO particles were further diluted to 12, 30 and 61 μM in natural lake water medium and the effective diameter was measured at 30 min, 24 and 48 h using DLS. The nominal concentrations are represented in Supplementary material (Table S2).

### 2.3. Dissolution of ZnO particles in lake water medium

The dissolution ($Zn^{2+}$) kinetics of different sizes of ZnO particles in lake water medium was quantified according to our previous study (Bhuvaneshwari et al., 2016). Briefly, different concentrations of ZnO particles (12, 30 and 61 μM) were prepared in natural lake water medium and incubated at different time intervals for 30 min, 24 and 48 h respectively. The actual Zn concentration (9.8, 24.5 and 49.1 μM) present in ZnO particles are represented in x axis of dissolution graph (Fig. 3). Only lake water (devoid of ZnO particles) was used as a control group. After the interaction period, samples were centrifuged at 12,000 rpm at 4 °C for 30 min twice and the supernatant was filtered through 0.1 μm filter followed by 3 kDa membrane filter. The dissolved $Zn^{2+}$ ions remaining in the lake water suspensions after the separation from the NPs were measured using AAS at a wavelength of 345 nm.

### 2.4. Acute toxicity and uptake of ZnO particles on Scenedesmus obliquus

Algal toxicity assays were performed according to the OECD guidelines 201 with slight modifications (OECD, 1984). At the exponential growth stage, algal cells were harvested by centrifuging at 7000 rpm for 10 min at 4 °C. The algal pellet was suspended in sterilized lake water matrix and the final concentration of 20 ml ($5 \times 10^5$ cells/ml) was achieved. To study the effective concentration ($EC_{50}$) of ZnO particles on algal cells, the range of exposure concentrations such as 0.61, 12.3, 24.69, 49.2, 98.4, 196.8, 393.6, and 787.2 μM of 50 nm, 100 nm and bulk ZnO particles were used. Algal cells ($5 \times 10^5$ cells/ml) of 20 ml were interacted with above mentioned ZnO particle concentrations for 48 h at 24 °C in the algal chamber. The un-interacted algal cells in lake water medium (devoid of ZnO particles) were considered as control group. The intact algal cells (without any changes in size and morphology) exposed to ZnO particles was measured using the hemocytometer. The percentage loss in viability of treated cells were

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