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Effects of zinc oxide and titanium dioxide nanoparticles on green algae under visible, UVA, and UVB irradiations: No evidence of enhanced algal toxicity under UV pre-irradiation

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HIGHLIGHTS

- This study investigates effects of ZnO NPs and TiO_2 NPs on green algae under visible, UVA, and UVB irradiations.
- Both NPs negatively affect the algal growth, with no significant differences in results among the light conditions.
- It indicates that no enhanced toxicity was associated with UVA or UVB in this study.
- This was attributed to the photocatalytic activity of ZnO NPs and TiO_2 NPs in both UV and visible range.

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ABSTRACT

Some metal oxide nanoparticles are photoreactive, thus raising concerns regarding phototoxicity. This study evaluated ecotoxic effects of zinc oxide nanoparticles and titanium dioxide nanoparticles to the green algae *Pseudokirchneriella subcapitata* under visible, UVA, and UVB irradiation conditions. The nanoparticles were prepared in algal test medium, and the test units were pre-irradiated by UV light in a photoreactor. Algal assays were also conducted with visible, UVA or UVB lights only without nanoparticles. Algal growth was found to be inhibited as the nanoparticle concentration increased, and ZnO NPs caused destabilization of the cell membranes. We also noted that the inhibitory effects on the growth of algae were not enhanced under UV pre-irradiation conditions. This phenomenon was attributed to the photocatalytic activities of ZnO NPs and TiO_2 NPs in both the visible and UV regions. The toxicity of ZnO NPs was almost entirely the consequence of the dissolved free zinc ions. This study provides us with an improved understanding of toxicity of photoreactive nanoparticles as related to the effects of visible and UV lights.

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1. Introduction

The increasing use of nanoparticles in consumer products has resulted in the release of nanoparticles into multiple environmental media. Metal oxide nanoparticles are of particular concern, owing to the possible release of toxic heavy metals into the environment (Gajjar et al., 2009). Some metal oxides are known to be photoreactive substances, which can function as photosensitizers, and certain metal oxides also function as semiconductors (Baruah et al., 2009). When nanoparticles are present in aqueous environments, they are generally exposed to sunlight, which can alter the toxicity characteristics of photoreactive nanoparticles; this illustrates the real need for research investigating the manner in which light at different wavelength ranges can influences the toxicity of the nanoparticles that absorb the light.

Zinc oxide nanoparticles (ZnO NPs) (Sakthivel et al., 2003) and titanium dioxide nanoparticles (TiO_2 NPs) (Hurum et al., 2003; Hartmann et al., 2010) are photoreactive substances with visible and UV light absorption properties. ZnO NPs and TiO_2 NPs have been previously employed in a broad range of applications. They are used in sunscreen, cosmetics (Serpone et al., 2007), toothpaste, and textile (Wang et al., 2009). The effects of ZnO NPs and/or TiO_2 NPs have been previously evaluated in aquatic species including fish (Warheit et al., 2007; Zhang et al., 2007; Zhu et al., 2008, 2009a, 2010b; Bai et al., 2009; Hall et al., 2009; Johnston et al., 2010; Xiong et al., 2011), water flea (Adams et al., 2006; Hund-Rinke and Simon, 2006; Lovern and Klaper, 2006; Lovern et al., 2007; Warheit et al., 2007; Heinlaan et al., 2008; Hall et al., 2009; Lee et al., 2009; Zhu et al., 2009b, 2010a, 2010b; Blinova et al., 2010), and algae (Linkous et al., 2000; Hund-Rinke and Simon,

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2006; Franklin et al., 2007; Warheit et al., 2007; Aruoja et al., 2009; Hall et al., 2009; Hartmann et al., 2010; Hund-Rinke et al., 2010; Miller et al., 2010, 2012; Ji et al., 2011; Metzler et al., 2011; Sadiq et al., 2011). Franklin et al. (2007), Aruoja et al. (2009), and Miao et al. (2010) tested that toxicity of ZnO NPs to freshwater green algae (*Pseudokirchneriella subcapitata*) and marine diatom (*Thalassiosira pseudonana*). The algal toxicity of TiO_2 NPs was studied by microscopic studies (Hartmann et al., 2010; Metzler et al., 2011; Sadiq et al., 2011). Miller et al. (2012) evaluated phototoxicity of TiO_2 NPs to four marine phytoplanktons (*Thalassiosira pseudonanan, Skeletonema marinoi, Isochrysis galbabana*, and *Dunaliella tertiolecta*).

Most of the previous studies of nanotoxicity thus far conducted have evaluated the toxicity of nanoparticles themselves, but there has been very little study thus far conducted on the toxicity of NPs exposed to UV or sunlight in a natural environment. As the toxicity of photo-reacted NPs may differ from that of NPs themselves, research into this phenomenon is clearly overdue. There has, however, been some research conducted into the phototoxicity of NPs in human cells (Roberts et al., 2008; Gopalan et al., 2009). On the other hand, with regard to the research into phototoxicity for an ecosystem, there has been only a few studies on the toxicity of quantum dots (Kim et al., 2010) and C_{60} (Yang et al., 2010) on *Daphnia magna* under UV conditions, as well as some research into the effects of ZnO NPs and TiO_2 NPs, the photoreaction of which was elicited by simulated sunlight (Hund-Rinke and Simon, 2006; Ma et al., 2011) and fluorescent light and UV (Hong and Otaki, 2006; Miller et al., 2012).

In this study, the effects of ZnO NPs and TiO_2 NPs on the green microalga *P. subcapitata* were evaluated under visible light, UVA (315–400 nm), and UVB (280–315 nm) irradiation exposure conditions. Visible light condition was used as a control compared to UV conditions because algae cannot survive under dark condition. Since UV lights sterilize the algae, nanoparticles were pre-irradiated by UV before exposure to algae. Algae were selected as the test species owing to their importance as a primary producer in the aquatic environment. The other objective of this study was to obtain a better understanding of the toxicity difference of photoreactive ZnO NPs and TiO_2 NPs as it relates to photoreactive toxicity under visible and UV light.

2. Materials and methods

2.1. Nanoparticle characterization

The ZnO NPs (Sigma–Aldrich, St. Louis, MO, USA) and TiO_2 NPs (P25, Evonik Degussa, Germany) were supplied in the form of a white powder. ZnO NPs and TiO_2 NPs sizes were <100 nm and 21 nm, respectively. The particle morphology was characterized using field emission transmission electron microscopy (FE-TEM), recorded on a JEM-2200FS (JEOL Ltd., Japan). BET surface area of particles was measured by particle size analyzer (UPA-150, microtrac, USA). The particle size distribution and the hydrodynamic diameters of nanoparticle aggregates with zeta potentials in the algal medium were recorded using an electrophoretic light scattering spectrophotometer (ELS-8000, Otuska Electronics Co., Japan). Absorbance measurements were conducted using a UV/vis spectrophotometer (Biochrom, Libra S32 PC, England). The UV absorption spectra of the ZnO NPs and TiO_2 NPs were measured as a function of irradiation time (ranging from 0 to 60 min) at concentration of 10 mg L^{-1} in test algae medium.

2.2. Preparation of NP suspension

In the preparation of the aqueous suspensions, ZnO NPs and TiO_2 NPs were suspended in OECD algae culture medium (pH 7.7) (OECD, 2006). Stock solution of ZnO NPs and TiO_2 NPs (100 mg L^{-1}) dispersed in algae medium was sonicated for 10 min at 40 KHz in a water bath sonicator (Hwashin Instrument Co, LTD., Korea). The ZnO NPs concentrations were prepared as 0, 0.05, 0.1, 0.2, and 0.3 mg ZnO NP L^{-1}. The TiO_2 NPs concentrations were prepared as 0, 0.5, 1, 3, and 10 mg TiO_2 NP L^{-1}. The 24-well microplates (ID 17 mm × height 17 mm, volume 3.8 mL/well) were used as test units. Each well was filled with 1.8 mL of test solution. Based on the result of measurement for the exposure concentration of each of the ZnO NPs and TiO_2 NPs, there were no changes in pH under the test conditions adopted (pH 7.8 ± 0.1 of ZnO NPs and pH 7.7 ± 0.2 of TiO_2 NPs).

2.3. Phototoxicity experiment

The test solutions were placed in a photoreactor (model LZC-4, Luzchem Research Inc., Ottawa, ON, Canada) equipped with 16 UV lamps at a spectral distribution of 316-400 nm (for UVA) or 281–315 nm (for UVB), then irradiated for 20 min. The light intensity was measured at 8.20 (for UVA) or 5.68 (for UVB) mW cm^{-2}. UV doses (1-min irradiation) were 0.49 (for UVA) or 0.20 (for UVB) J cm^{-2} (Dose (J cm^{-2}) = intensity (mW cm^{-2}) × time (s) × 10^{-3}). Light intensity was assessed using a spectroradiometer (SPR 4001, Luzchem Research Inc., Ottawa, ON, Canada) in the carousel position. Following irradiation with UV on the algal medium, the algae were inoculated in test units for the bioassay. For the irradiation with visible light, the assay was conducted routinely under laboratory fluorescent lamps without UV irradiation. The absorbance spectra of the visible, UVA, and UVB lamps are provided in Fig. 1. Visible light was used as a control. To assess a possible effect of UV pre-irradiation to algae, the toxicity test without nanoparticles under UV pre-irradiation conditions was conducted in the same manner as the nanoparticle toxicity test under UV pre-irradiation conditions.

2.4. Algal assay

P. subcapitata was provided by the National Institute of Environmental Research (NIER, Incheon, Korea), and cultured in our laboratory for several months prior to testing. The growth inhibition test with *P. subcapitata* was conducted in the algal medium according to the modified OECD guidelines for the testing of chemicals No. 201 (OECD, 2006), and Blaise and Vasseur (2005) without EDTA. The algae were inoculated at an initial density of 1×10^4 - cells mL^{-1} onto 3.8-mL 24-well microplates containing 1.8 mL of exposure solution, in triplicate. In the case of TiO_2 NPs, shading effect due to nanoparticles was tested by the method of Hund-Rinke and Simon (2006). The microplates were placed on the algal growth chambers, and shaken continuously at 100 rpm under continuous fluorescent illumination (approximately 10,000 lux), then incubated further at 24 ± 1 °C. Algal cell densities exposed to ZnO NPs and TiO_2 NPs were measured by absorbance and chlorophyll fluorescence intensity, respectively. Absorbance was measured using a spectrophotometer (GENESYS 20, Thermo Spectronic, Rochester, NY, USA) at 685 nm. Fluorescence of chlorophyll was measured according to Aruoja et al. (2009). 50 μL of algae sample was added to 200 μL of ethanol in 96-well microplate, and then the plate was shaken for 3 h in the dark. Fluorescence intensity was measured using a microplate reader (SpectraMax M2&M2e, Molecular Devices, Sunnyvale, CA) at excitation wavelength of 420 nm and emission wavelength of 671 nm. The morphology of *P. subcapitata* was evaluated using a microscope (Oylmpus BX51, Olympus Cooperation, Tokyo, Japan) equipped with a high-resolution adaptor (Cytoviva Inc., Alabama, USA). The IC50 values (the inhibitory concentration causing a 50% inhibition of growth rate) were calculated at 24 h intervals for 72 h.

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