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Influence of different types of nanomaterials on their bioaccumulation in a paddy microcosm: A comparison of TiO_2 nanoparticles and nanotubes

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ABSTRACT

We investigated the environmental fate and bioaccumulation of TiO_2 nanomaterials in a simplified paddy microcosm over a period of 17 days. Two types of TiO_2 nanomaterials, nanoparticles (TiO_2-NP) and nanotubes (TiO_2-NT), were synthesized to have a negative surface charge. Ti concentrations in the environmental media (water, soil), crops (quillworts, water dropworts), and some lower and higher trophic organisms (biofilms, algae, plant-parasitic nematodes, white butterfly larva, mud snail, ricefish) were quantified after exposure periods of 0, 7, and 17 days. The titanium levels of the two nanomaterials were the highest in biofilms during the exposure periods. Bioaccumulation factors indicated that TiO_2-NP and TiO_2-NT were largely transferred from a prey (e.g., biofilm, water dropwort) to its consumer (e.g., nematodes, mud snail). Considering the potential entries of such TiO_2 nanomaterials in organisms, their bioaccumulation throughout the food chain should be regarded with great concern in terms of the overall health of the ecosystem.

1. Introduction

Among available manufactured nanomaterials (e.g., nanoparticles (NPs), nanotubes (NTs), nanowires, and fullerene and its derivatives), titanium dioxide (TiO_2) is one of the most widely used to date. Titanium dioxide nanomaterials have been incorporated into the bulk materials of paints and coatings to produce materials with enhanced hardness and contrast ratios. There has also been a surge in the inclusion of TiO_2 nanomaterials in consumer products, including sporting goods, sunscreens, and lacquers (Peralta-Videa et al., 2011). A recent study suggested that TiO_2-NP production ranged from 7800 to 38,000 metric tons per year in the USA alone (Hendren et al., 2011), and will be approximately 2.5 million metric tons by 2025 (Robichaud et al., 2009).

According to the Korean Ministry of Environment, total amount of nanoparticles (TiO_2 and SiO_2 are 99%; total quantities of TiO_2 are similar to those of SiO_2) used in 2008 was estimated to be more than 58,732 tons in Korea (Hong, 2008). Commercially available fertilizers containing TiO_2-NPs are used extensively in greenhouses, indoor organic agriculture, and rice paddy fields without any regulation. In particular, the paddy field is a common type of farmland for rice or water dropwort (*Oenanthe javanica DC.*) where the aquatic environment supports both lower (e.g., snails and small fish such as minnows) and higher trophic organisms (e.g., predators, including humans). Although available information on the amount of production and use of TiO_2 nanomaterials in agricultural purposes is lacking, large quantities of TiO_2 nanomaterials are considered to introduce into actual rice paddies in Korea. Given the potential entry of TiO_2 nanomaterials into the environment, their bioaccumulation throughout the food chain should be regarded with great concern.

It has been shown that the uptake efficiency and effects of various NPs on the growth and metabolic functions of plants varies. For example, TiO_2-NPs absorb light, but also have the benefit of promoting the activity of Rubisco activase, an enzyme involved in carbon fixation, which accelerates spinach growth (Nair et al., 2010). TiO_2-NPs (anatase crystal phase) also have an influence on photosynthesis and plant growth rates, as their structural properties enhance nitrogen metabolism by several enzymatic activations (Yang et al., 2006). The physicochemical properties of TiO_2-NPs are thus considered to be advantageous when used with agricultural chemicals. However, in addition to non-point source pollution

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through waste water systems, there are general concerns regarding the proliferation of manufactured TiO_2-NPs in agriculture, including possible ecosystem health effects throughout the environment.

The widespread use of TiO_2 nanomaterials is of increasing toxicological concern. Ecological risk assessment of TiO_2-NPs predicts that environmental exposures of TiO_2-NPs may be sufficient to be toxic to some fish species (Hao et al., 2009; Yeo and Kang, 2009; Yeo and Kim, 2009, 2010). TiO_2-NPs have higher photocatalytic activity at their photoinducible, redox-active surface, thus potentially producing reactive oxygen species (ROS) in the presence of UV light (Yeo and Kang, 2006; Armelao et al., 2007). Such ROS can form in cells as a consequence of a myriad of stimuli ranging from abiotic and biotic stresses, production of hormonal regulators, and cell processes such as polar growth and programmed cell death (Bailey-Serres and Mittler, 2006), suggesting that ROS generation plays a crucial role in TiO_2-NP-induced cytotoxicity (Yeo and Kang, 2006). In contrast, there were no apparent adverse effects of TiO_2 nanoparticles on algae (*P. subcapitata*) (Blaise et al., 2008), *Caenorhabditis elegans* (Wang et al., 2009), *Eisenia fetida* (Hu et al., 2010), *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Chydorus sphaericus*, *Thamnocephalus platyurus*, *Chironomus riparius* (Klaine et al., 2008; Cattaneo et al., 2009; Zhu et al., 2010), or *Hydra attenuata* (Yeo and Kang, 2010).

Although the proliferation of nanomaterials has the potential to impact on several ecological receptors and food webs (Klaine et al., 2008), little is known about the environmental fate and bioaccumulation of TiO_2 nanomaterials (Griffitt et al., 2008). In an attempt to remedy this knowledge gap, the present study used replicated paddy microcosm systems to investigate the environmental fate of TiO_2 nanomaterials. Microcosm compartments include environmental media (freshwater, sediment), plants (quillworts, water dropworts), and lower (biofilms, algae, plant-parasitic nematodes, white butterfly larva) and higher trophic (mud snail, ricefish) organisms. The objectives of this paper are two-fold. First, we determined whether the shape of TiO_2 nanomaterials (NPs vs. NTs) can influence their uptake in the various aforementioned species (plants, lower and higher trophic organisms). Second, we investigated whether bioaccumulation of TiO_2-NPs and TiO_2-NTs could occur in the simplified paddy microcosm over a period of 17 days. Performing this microcosm approach on the environmental fate and trophic transfer of TiO_2 nanomaterials can be used to develop ecological model species used in risk assessment and bioremediation strategies.

2. Material and methods

2.1. Microcosm design

Experimental microcosm systems, shown in Fig. 1, were prepared according to a previously validated design by Ferry et al. (2009) with some modifications. Simplified paddy microcosms mimicking a local paddy field with environmental compartments and organisms (e.g., freshwater, sediment, biofilms, quillworts, water dropworts, algae, plant-parasitic nematodes, white butterfly larva, mud snail, and ricefish) were maintained in a greenhouse at the Engineering College of Kyung Hee University, Korea. Sediment (top 4 cm), composed mainly of clay, was collected from a local paddy field on Seocheon-Dong (37°14 39.77′ N, 127°04 30.08′ E), Youngin, Kyunggi Province, as a reference site. The sediments were then sieved through a 3-mm mesh sieve, homogenized, and dispensed into sediment trays. Three replicate types (control, TiO_2-NP exposed, TiO_2-NT exposed) of sediment trays were placed into each tank and elevated 7 cm from the upper tank (Fig. 1b). The depth of the soil was 5–5.5 cm, and the root of the water dropwort (4.5–5 cm of length) planted in the paddy microcosm was filled with 2–2.2 L of freshwater. Microcosm systems containing each environmental compartment and organism were then operated and maintained for 30 days at 20 ± 2 °C prior to the start of this experiment.

After water dropwort had grown to 25–30 cm, an electrical pump was used to deliver water to the upper tank. The system was automatically controlled to allow water to drain from the upper tank to serve as a reservoir throughout the exposure periods. Since these circulatory systems were used to introduce TiO_2 nanomaterials into the paddy microcosm experiment (Fig. 1), we examined the TiO_2 nanomaterials in the upper of the water. Each sediment tray contained 2.3 kg of sediment. The flow was driven by submersible pumps set to timers. Tanks were monitored continuously (8 times per day; driving for 2 h and resting for 1 h) for several water quality parameters, including temperature and pH. Evaporated water in the modulated microcosm was automatically maintained by the water circulatory systems. Water filtration systems with a 40 μm mesh were used to protect small living organisms. After the circulatory system became stable, mud snails and ricefish were placed in the simplified microcosm systems. Mud snails and ricefish are often found in paddy fields in Korea. Mud snails (*Cipangopaludina chinensis*) consume water dropwort (5–6 mud snails/6075 cm^3; 27 cm width × 15 cm length × 15 cm height), while ricefish (*Oryzias sinensis*, 5 fishes/6075 cm^3) feed on micro-algae (*Spirogyra* spp.). The Micro-algae (*Spirogyra* spp.) was commonly suspended in the water of the paddy microcosm with approximately 10–100 μm in width, which was collected by a filter paper. The aggregated biofilm was frequently found in the surface of the walls, which was scratched to collect biofilms. These experimental paddy microcosm setups were exposed to TiO_2 nanomaterials for 17 days.

Although other organisms such as plant-parasitic nematodes (*Meloidogyne* sp.), quillworts (*Isoetes japonica*), algae (*Spirogyra* spp.), white butterfly larva (*Artogeia rapae* larva), and biofilms were not used in the paddy microcosm systems, these organisms were introduced naturally during the exposure period. These organisms could have entered into the paddy microcosm from the collected soil used in the paddy microcosm, or from the greenhouse air. All experiments were conducted under the supervision and approval of the Institutional Animal Care and Use Committee at Kyung Hee University.

2.2. The preparation and characterization of TiO_2-NPs and TiO_2-NTs

TiO_2-NPs and TiO_2-NTs were kindly donated by Dr. Misook Kang, Department of Chemistry, Youngnam University, Korea. The TiO_2-NPs and TiO_2-NTs were prepared according to the procedure described by Chen et al. (2002) and Chae and Kang (2011). Briefly, a comprehensive chemical and structural analysis was made of a new type of TiO_2-NT, which were synthesized via the reaction of TiO_2 particles with NaOH aqueous solution. Our methodology involved the Ti ion hydroxocomplex formation (hydrolysis) using the alkaline solutions (e.g. sodium hydroxide). The resulting trititanate nanotubes were multi-walled scroll nanotubes with an intershell spacing of about 1.0 nm and an average diameter of about 9 nm. TiO_2-NP and TiO_2-NT powders were subjected to X-ray diffraction (model PW 1830; Philips, Amsterdam, The Netherlands) with nickel-filtered Cu*K* radiation (30 kV, 30 mA) at 2θ angles from 5° to 80°, with a scan speed of 10° min^{-1} and a time constant of 1 s. High-resolution transmission electron microscopy (TEM) images of the nanometer-sized samples were obtained using a JEOL 2000EX transmission electron microscope operated at 200 kV. A zeta potential assay was used to analyze the surface charges and mobility. The surface charges of the TiO_2-NPs and TiO_2-NTs were determined according to their weight ratios as found using an electrophoretic light scattering spectrophotometer (ELS Z2, Otsuka electronics, Osaka, Japan), with 90° and 20° scattering angles, respectively, at 25 °C. We have characterized the TiO_2 nanomaterials (NP and NT) after drying from their solutions in the mesocosms. Their similar size and morphology were found as demonstrated with XRD patterns and TEM images when compared to their powder (Fig. 3a, b). A part of TiO_2 nanomaterials has been aggregated, which is generally observed under TEM images. The XRD method was adopted since this analytical technique reveals information about the crystal structure, chemical composition and physical properties of TiO_2 nanomaterials, which is widely used for X-ray crystallography. TEM is a microscopy technique, capable of imaging at a higher resolution than light microscopes even as small as a single column of atoms.

2.3. Exposure conditions of TiO_2-NP and TiO_2-NT

The concentrations of the TiO_2-NP and TiO_2-NT stock solutions were 4000 μg/L. These stocks were diluted with 2.2 L of freshwater to a final concentration of 1818.2 μg/L in the microcosm environment, which is the initial concentration in the microcosm (Table 1 in detail). This concentration was chosen because it would be resistant to test organisms investigated, even though this particular concentration as applied would be a concern. The water tank was covered with aluminum foil to protect it from light after ultrasonic dispersion of the nanomaterials for 10 min.

2.4. Bioconcentration and bioaccumulation factors of TiO_2-NP and TiO_2-NT in living organisms

Living organisms were collected after each exposure period (0, 7, 17 days) (Table 1). The samples analyzed were thoroughly washed with nanopure water and then dried to constant weight at 70 °C in an oven overnight. Water samples were evaporated to dryness and then analyzed for TiO_2. Only the surficial layer of sediments (<1 cm) out of the full depth of sediment (5–5.5 cm) to evaluate the biologically relevant sediment exposure of TiO_2 nanomaterials. In addition, Ti concentrations in the sediment (0, 7, 14, 17 days) and water (0, 4, 6, 7, 11, 14, 17 days) were monitored in the mesocosm and were also analyzed using ICP-MS (Fig. 2). TiO_2 was measured after microwave digestion (Q15, Questron, USA) of the samples with 10 mL nitric acid (HNO_3) and 2 mL 10% hydrogen peroxide (H_2O_2) (USEPA, 1994). Ti