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Trophic transfer of nano-TiO_2 in a paddy microcosm: A comparison of single-dose versus sequential multi-dose exposures☆

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ABSTRACT

In the present study, replicated paddy microcosm systems were used to investigate the environmental fate and trophic transfer of titanium nanoparticles (NPs) over a period of 14 days. Most TiO_2 NPs immediately settled down in the sediment, and high accumulations of nano TiO_2 in the sandy loam sediment and biofilm were observed. The test organisms (quillworts, water dropworts, duckweeds, biofilms, river snails, and Chinese muddy loaches) and environmental media (freshwater, sandy loam sediment) were exposed to sequential low doses (2 mg/L at 1 h, 4 days, and 9 days) or a single high-dose (6 mg/L) of TiO_2 NPs. The bioconcentration factors (BCFs) of nano-TiO_2 in biofilms, quillworts, duckweeds, and Chinese muddy loaches were higher in the sequential multi-dose group than in the single-dose group. Chinese muddy loaches showed higher bioaccumulation factors (BAFs) over their prey than river snails. The difference in the carbon isotope ratios between Chinese muddy loaches and river snails was less than 2‰, and an approximately 4‰ difference in the stable nitrogen isotope ratio was observed in the two aquatic predators from their major prey (e.g., biofilms or particulate organic matter). The trophic levels between biofilms and river snails and between biofilms and Chinese muddy loaches were 2.8 and 2.4 levels, respectively. These results indicate that these two predators consumed biofilm and other alternative preys at a higher level than biofilm. Although the trophic transfer rates of TiO_2 are generally low, relatively higher biomagnification factors (BMFs) were found in Chinese muddy loaches (0.04–0.05) than in river snails (0.01–0.02). These results suggest that TiO_2 NPs show greater movement in the sediment than in the water and that TiO_2 NPs can be retained through aquatic food chains more after a sequential low-dose exposure than after a single high-dose exposure.

1. Introduction

Nano-titanium dioxide (TiO_2) has become a part of daily life; it is used in products such as toothpastes, sunblock, cosmetics, and lamps with photocatalytic functions (Rahman et al., 2002; Wolf et al., 2003; NIOSH, 2011). In particular, nano-TiO_2 has been used broadly in the agricultural sector (Nair et al., 2010) because it promotes photosynthesis by enhancing acyclic photophosphorylation activities (Hong et al., 2005), increasing the germination rate and dry weight (Zheng et al., 2005), and catalyzing Rubisco activase to heighten the carbon fixation capacity of the Calvin circuit of photosynthesis (Gao et al., 2006, 2008; Linglan et al., 2008; Xuming et al., 2008). In addition, anatase crystal-type nano-TiO_2 is considered advantageous for nitrogen metabolism in plants, accelerating the conversion of inorganic nitrogen into organic nitrogen in proteins and chlorophyll (Yang et al., 2006).

The worldwide use of nano-TiO_2 in agriculture and its potential entry to the ecosystem are of increasing concern (Nam et al.,

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2014). Ecological risk assessment of nano-TiO_2 predicts that environmental exposures of nano-TiO_2 could be sufficient to affect various ecological receptors, including cyanobacteria (Cherchi and Gu, 2010), zebrafish (*Danio rerio*) (Griffitt et al., 2008; Zhu et al., 2008, 2009; Yeo and Kang, 2009; Yeo and Kim, 2009, 2010; Park et al., 2014) and carp (*Cyprinus carpio*) (Hao et al., 2009; Wang et al., 2009). Titanium dioxide is widely known as a prominent photocatalysts, and the TiO_2 NPs as anatase and rutile crystal types has high photocatalytic activity at its photo-inducible, redox-active surface; thus it could potentially produce 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, such as 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 nano-TiO_2-induced cytotoxicity (Yeo and Kang, 2006, 2012). The toxicity of TiO_2 nanoparticles (NPs) is also related to genotoxicity and mutagenicity in mammalian model species (Koedrith et al., 2014). In Korea, TiO_2 NPs are used extensively in greenhouses, indoor organic agriculture, and rice paddy fields without any regulation (Lee, 2010; Seo, 2010; Seo et al., 2011; Cho et al., 2013). The paddy field is a common type of farmland for rice and water dropwort (*Oenanthe javanica* DC) in which the aquatic environment supports both lower (e.g., river snails and small fish such as minnows) and higher trophic organisms (e.g., predators, including humans). Given the potential entry of TiO_2 NPs into the environment, their bioaccumulation throughout the food chain should be regarded with great concern (Yeo and Nam, 2013).

Reliable ecological risk assessments of nano-TiO_2 are based on not only concentrations in the environment but also its environmental fate and dynamics through food chains (Zhu et al., 2010a, 2010b). Some researchers have addressed the introduction of NPs and their movement in the ecosystem. Ferry et al. (2009) simulated the ebb and flow of a coastal ecosystem to investigate how gold NPs spread into organisms. The environmental fate of silver NPs leached from consumer products was also simulated in a pilot estuarine mesocosm (Cleveland et al., 2012). In our previous study, the dynamics of nano-TiO_2 were shown in environmental media (e.g., sediment, water) and its bioaccumulation into organisms in a microcosm were modeled on the Korean style of rice paddy agriculture (Yeo and Nam, 2013). Realistic designs such as microcosms and mesocosms are thus being used to verify the environmental fate of NPs.

Comparing existing data is challenging because of the dearth of available information on the environmental dynamics of TiO_2 NPs (Zhu et al., 2010a, 2010b; Yeo and Nam, 2013), despite their toxic effects across various ecological receptors (Wang et al., 2009; Yeo and Kim, 2009, 2010; Lim et al., 2013). Moreover, little information is available regarding the quantitative trophic transfer of TiO_2 NPs in aquatic organisms, so it is unclear whether TiO_2 NPs can be transferred during predation and whether biomagnification can occur through food chains. Interpreting such quantitative trophic transfer of TiO_2 NPs warrants more comprehensive experimental systems testing stable isotopes via food chains.

We created several microcosm environments based on a Korean rice paddy model. Stable isotope compositions ($\delta^{13}C$, $\delta^{15}N$) were quantified from environmental media (freshwater, sandy loam sediment) and various trophic level organisms (aquatic plants, biofilms, river snails, and pond loaches) to evaluate the relationships between trophic transfer and biomagnification rates in the artificially designed microcosm environments. In addition, nano-TiO_2 exposure regimes (a single dose versus sequential multi-dose exposures) were tested to determine their effects on bioconcentration, bioaccumulation, and biomagnification through the simplified food chains.

2. Materials and methods

2.1. Properties of nano-TiO_2

For nano-titanium dioxide (TiO_2 NPs), the P25-type TiO_2 were purchased from Sigma–Aldrich (USA). The crystallinity of the particles was measured using X-ray diffraction (XRD; D8 Advance, Bruker, Germany) with a nickel filter and Cu*K* radiation (30 kV, 30 mA) within a 5–80° range in the 2θ value at a scan rate of 10°/min. The size and shape of the particles were analyzed using a field-emission scanning electron microscope (FE-SEM; LEO SUPRA 55, Carl Zeiss, Germany). The surface composition was identified using energy-dispersive X-ray spectroscopy (EDS) with field emission-transmission electron microscopy (FE-TEM; JEM-2100F, JEOL, USA) and GENESIS 2000 software. A particle size analyzer (ELS-Z2; Otsukael, Japan) was used to analyze the surface charge and mobility of the particles, the changes in particle size, and the zeta-potential. The assay samples were diluted in distilled water at 25 °C with a refractive index and viscosity of 1.3328 and 0.8878 cP, respectively.

2.2. Establishment of the microcosm and nano-TiO_2 exposure conditions

2.2.1. Microcosm design

Experimental microcosm systems (Fig. 1) were prepared according to a previously validated design by Yeo and Nam (2013) with some modifications. Simplified paddy microcosms mimicking a local paddy field with environmental compartments and organisms (freshwater, sediment, biofilms, quillworts, duckweeds, water dropworts, river snails, and Chinese muddy loaches) were maintained in a greenhouse at the Engineering College of Kyung Hee University, Korea. Sediment (top 10 cm), composed mainly of sandy loam, was collected from a local paddy field in Seocheon-Dong (37°14 39.77′ N, 127°04 30.08′ E), Youngin, Gyunggi Province. The sediment was analyzed following the method of the U.S. Department of Agriculture (USDA, 1993). The results indicated that the soil texture was sandy loam (pH 6.75, ORP 22.4 mV, EC 48.33 μS cm^{-1}, TOC 48.33 mg L^{-1}, sand 63.33%, silt 25%, clay 11.67%). After analysis, the sediments were sieved through a 3-mm mesh sieve, homogenized, and dispensed into sediment trays. The water dropwort was planted after filling the microcosm container (20,350 cm^3; 37 cm width × 22 cm length × 25 cm height) with 10–10.5 cm of sediment and was filled with 10 cm of freshwater (7–7.2 L) and maintained until the beginning of the exposure experiments (Fig. 1). The microcosm systems were then maintained at 20 ± 2 °C for 30 days prior to the start of the experiments. No food was added during the stabilization period.

A constant water level was maintained, including compensation for water loss by evaporation, during the microcosm's stabilization period by installing a water tank (width 26 × depth 18.5 × height 13.8 cm) and a pump (10 W). The system automatically allowed water to drain from the upper tank to serve as a reservoir throughout the exposure periods. Because circulatory systems were used to introduce the TiO_2 nanomaterials into the paddy microcosm experiment (Fig. 1), the TiO_2 nanomaterials were examined in the upper tank of water. Each sediment tray contained 7.7 kg of sediment.

After the circulatory system became stable, river snails (*Cipangopaludina chinensis*, 6–7 river snails/the microcosm container) and Chinese muddy loaches (*Misgurnus mizolepis*; 6 fish/the microcosm container) were placed in the simplified microcosm systems. River snails and Chinese muddy loaches are often found in