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Nano-TiO_2 affects Cu speciation, extracellular enzyme activity, and bacterial communities in sediments☆

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ABSTRACT

In aquatic ecosystems, titanium dioxide nanoparticles (nano-TiO_2) coexist with heavy metals and influence the existing forms and toxicities of the metal in water. However, limited information is available regarding the ecological risk of this coexistence in sediments. In this study, the effect of nano-TiO_2 on Cu speciation in sediments was investigated using sequential extraction. The microcosm approach was also employed to analyze the effects of the coexistence of nano-TiO_2 and Cu on extracellular enzyme activity and bacterial communities in sediments. Results showed that nano-TiO_2 decreased the organic matter-bound fraction of Cu and increased the corresponding residual fraction Cu. As a result, speciation of exogenous Cu in sediments changed. During the course of the 30-day experiment, the presence of nano-TiO_2 did not affect Cu-induced changes in bacterial community structure. However, the coexistence of nano-TiO_2 and Cu restrained the activity of bacterial extracellular enzymes, such as alkaline phosphatase and β-glucosidase. The degree of inhibition also varied because of the different properties of extracellular enzymes. This research highlighted the importance of understanding and predicting the effects of the coexistence of nanomaterials and other pollutants in sediments.

1. Introduction

Titanium dioxide nanoparticles (nano-TiO_2) are one of the most widely used engineered nanomaterials, with applications ranging from sunscreens (Nohynek et al., 2007), cosmetics (Auffan et al., 2010), food additives (Weir et al., 2012) to paints and photocatalysts (Li et al., 2008). However, large-scale production and use of nano-TiO_2 results in unintended yet inevitable release of this material into the environment (Windler et al., 2012). Aquatic environments are especially at risk for nano-TiO_2 contamination caused by inputs from effluents of production facilities and wastewater treatment plants (Navarro et al., 2008; Nowack and Bucheli, 2007).

Several studies demonstrated that nano-TiO_2 can cause toxic and adverse effects on aquatic organisms, including fishes (Chen et al., 2011; Ma and Diamond, 2013; Zhang et al., 2007), *Daphnia magna* (Amiano et al., 2012), algae (Cardinale et al., 2012; Li et al., 2015), and bacteria (Binh et al., 2014; Jomini et al., 2015). However, most toxic effects were found at high nano-TiO_2 levels under laboratory conditions. It also should be noticed that the actual concentration of nano-TiO_2 in natural water is very low (3 ng L^{-1} to 1.6 μg L^{-1}), and lethal concentration is difficult to reach (Gottschalk et al., 2013). Therefore, some studies focused on complex interactions between nano-TiO_2 and other trace pollutants in real aquatic environments (Fan et al., 2011; Liu et al., 2015; Yang et al., 2012, 2014; Zhu et al., 2011). Nano-TiO_2 exhibits special physicochemical characteristics, such as large specific surface areas, high interface effects, and reactivity; as such, this compound can adsorb trace pollutants in water and influence their biological behavior and toxicities. Zhu et al. (2011) reported that the presence of nano-TiO_2 (2 mg L^{-1}) increased the toxicity of the marine antifouling compound tributyltin (TBT) up to 20-fold compared with TBT alone in abalone embryos. For heavy metals, Fan et al. (2011) and Liu et al. (2015) demonstrated that, at a concentration generally considered to be safe in the environment, nano-TiO_2 remarkably enhanced the toxicity of Cu to *D. magna* by increasing Cu bioaccumulation. Most existing studies were conducted in the water phase, whereas few studies focused on the environmental risks of nano-TiO_2 in sediments.

Nano-TiO_2 enters the aquatic environment and settles from the water column, leading to their entrainment and accumulation in

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the sediment over time (Boncagni et al., 2009; Keller et al., 2010; Tong et al., 2015). Sediments are also the main sink and source of other pollutants (heavy metals and organic pollutants) and thus play a key role in the transportation and storage of potentially toxic substances (Zhang et al., 2014). Interaction between nano-TiO_2 and other pollutants are also more likely to occur in sediments than in the water phase. The mechanism through which these interactions influence the behavior, fate, and toxicity of nano-TiO_2 and other pollutants remain unknown.

Bacterial communities in sediments are taxonomically and functionally diverse and play essential roles in biogeochemical processes, including nutrient cycling, organic matter decomposition, and bioremediation of pollutants (Gao et al., 2011). Moreover, benthic bacteria serve as the foundation of food webs in sediments because they are a crucial food source to organisms in higher trophic levels (Alongi, 1994). Studies on the potential effects of nano-TiO_2 on these communities, not to mention the effects of the coexistence of nano-TiO_2 and other pollutants, remain scarce despite the ecological importance of sediment bacterial communities.

In this study, we established a series of microcosms and investigated for the first time the effect of nano-TiO_2 on Cu speciation in sediments, as well as the effects of the coexistence of nano-TiO_2 and Cu on sediment bacterial communities. Speciation of Cu in sediments was determined using Tessier five-step sequential extraction. Bacterial community composition and diversity were evaluated using terminal restriction fragment length polymorphism (T-RFLP) analysis. Changes in extracellular enzyme activity, such as alkaline phosphatase and β-glucosidase, were also analyzed. Results provide a strong evidence for the ecological risks caused by the coexistence of nano-TiO_2 with other environmental pollutants.

2. Material and methods

2.1. Preparation of nano-TiO_2 suspension

Aeroxide P25 nano-TiO_2 particles were purchased from Acros Organics (Belgium). A Brunauer–Emmett–Teller (BET) specific surface area of 55.29 $m^2\ g^{-1}$ was measured using Nova 2200e BET surface area analyzer (Quantachrome, FL, USA). Stock suspension of nano-TiO_2 (1 g L^{-1}) was prepared by adding 1 g of P25 nano-TiO_2 particles to 1000 mL of sterile Milli-Q water. The mixture was placed in ultrasonic bath (100 W, 40 kHz) for at least 30 min to break large agglomerates and homogenize the dispersion. The nano-TiO_2 stock solution was further sonicated for 30 min before use. Nano-TiO_2 particles in water were visualized using a transmission electron microscope (TEM, JEM-2100F, JEOL, Japan) operated at 100 keV (Fig. S1, Supplementary material). Hydrodynamic diameter and zeta potential (ζ-potential) were measured via dynamic light scattering by using Zetasizer (Zetasizer Nano Series, Malvern Instruments, UK). In the suspension, nano-TiO_2 exists as large agglomerates formed from aggregated primary particles. The average hydrodynamic diameter and ζ-potential were 827.1 ± 8.63 nm and −7.20 ± 0.17 mV, respectively.

2.2. Environmental sample collection

Surface sediments (0–3 cm) were collected from an inner lake of Miyun Reservoir in Beijing, China (40°29′7″N, 116°51′59″E). This site was selected because it is far from the main population and industrial centers. Sediments (12 kg) were collected and placed in six sterile polyethylene bags. Lake water sample (30 L) was obtained from this station by using three acid-washed polyethylene buckets. The sediment and lake water samples were transported into the laboratory under refrigeration. The sediments were sieved to 2 mm to homogenize the sample and remove rocks. The physical and chemical properties of the samples were then analyzed. Grain sizes of the sediment are mainly distributed within 23–158 μm. The contents of N, C, S, and H are 0.34% ± 0.02%, 4.04% ± 0.18%, 0.25% ± 0.02%, and 0.94% ± 0.04%, respectively. The concentrations of the trace metals in the sediment samples were as follows (mg/kg): Cd, 0.20 ± 0.05; Cr, 121.91 ± 4.26; Cu, 41.78 ± 2.32; Ni, 40.84 ± 1.16; and Zn, 77.05 ± 2.30.

2.3. Experimental design

Microcosms were established using 12 polypropylene containers (1.1 L). Each container contained 200 g of sediment sample (sieved to 2 mm) and 500 mL of overlying lake water. The height of the sediments in the container was approximately 8 mm. To simulate the actual exposure route, we added 5 mL of 1 g L^{-1} nano-TiO_2 or 100 mg L^{-1} $Cu(NO_3)_2$ stocks to the overlying water to achieve the target exposure concentration. The following four treatments (three replicates per treatment) were established: 10 mg L^{-1} nano-TiO_2 alone in overlying water, 1 mg L^{-1} Cu alone, 10 mg L^{-1} nano-TiO_2 + 1 mg L^{-1} Cu, and without nano-TiO_2 and Cu as control. To homogeneously distribute nano-TiO_2 and Cu, we added the corresponding stock solution, stirred the overlying water slowly for 5 s, and then thoroughly mixed with the sediment. All microcosms were placed in a climate box for 30 days at 20 °C under a 12 h light: 12 h dark cycle (visible light: 9.18 ± 0.16 W/m^2; UVA: 0.46 ± 0.04 W/m^2). Four sediment samples were randomly collected from each microcosm, and the samples were pooled (total wet weight approximately 6 g) before addition of corresponding doses and denoted as “day 0” subsamples. After 1, 4, 10, 15, 20, and 30 days of exposure, the sediment of each microcosm was subsampled in the same manner as that in day 0. Moreover, extracellular enzyme activity assays were conducted on the day the subsamples were collected. A portion of the subsamples was stored at 4 °C for metal analysis, and the remaining samples were stored at −80 °C for subsequent DNA extraction to characterize bacterial community structure and diversity. Water samples (2 mL) were also collected at each sampling days to monitor the concentrations of nano-TiO_2 and Cu in overlying water (the target exposure concentration is denoted as the concentration at day 0). In addition, salinity (0.27‰), pH (7.91), and temperature (21.3 °C) in the water were monitored during the course of the experiment and were stable throughout the experiment. Potential was measured in superficial sediments, and the mean value was −70.76 mV. All containers and equipment used in the experimental setups and in sampling were thoroughly washed with acid (14% HNO_3, 24 h) and then sterilized (120 °C, 30 min) to avoid any contamination.

2.4. Metal analysis

Wet sediments were used to determine the natural concentrations of Cu in the samples. To be able to report the results on a dry weight basis, we measured the moisture content (60 °C, 24 h) of the sediment subsamples prior to digestion. For the analysis of total Cu and total Ti, the sediment subsamples (0.25 g dry weight equivalent) were digested with acid mixture (HNO_3-HF-$HClO_4$) by using the method proposed by Belzile et al. (1989). To determine Cu speciation in the sediment, sediment subsample (1 g dry weight equivalent) were extracted by Tessier sequential extraction procedure (Tessier et al., 1979; Fan et al., 2002). This extraction method divides Cu into five phase fractions: exchangeable fraction, carbonate-bound fraction, Fe-Mn oxide-bound fraction, organic matter-bound fraction, and residual fraction. Cu concentrations were measured by inductively coupled plasma-optical emission