

Fate of TiO₂ nanoparticles entering sewage treatment plants and bioaccumulation in fish in the receiving streams



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ARTICLE INFO

Article history:

Received 15 July 2016

Received in revised form 16 September 2016

Accepted 16 September 2016

Available online 17 September 2016

Keywords:

Nano-TiO₂

Wastewater treatment plant

Receiving streams

Fish

Eco-toxicology

ABSTRACT

Wastewater treatment plants (WWTPs) can be important point sources for the discharge of engineered nanomaterials to natural surface waters. In this study, we investigated the fate of nano-TiO₂ in two full scale WWTPs and their receiving streams in northern China (i.e., the Binhe and Minxin rivers, which flow to the Xiaohe River). High resolution transmission electron microscopy, energy dispersion X-ray spectroscopy and X-ray photoelectron spectroscopy analyses indicated that Ti in colloids present in the WWTP effluents and receiving streams originated mainly from engineered nano-TiO₂. Most of the nano-TiO₂ (74–85%) in the influent sewage was removed by the activated sludge, but significant concentrations (27–43 µg/L Ti) remained associated with colloids in the effluents. Surprisingly, the total concentration of Ti in the receiving river streams was higher (52–86 µg/L), indicating the importance of other sources such as urban runoff. These relatively high Ti concentrations are unlikely to originate from natural sources since they are significantly higher than those in the nearby Yuqiao Reservoir (<5 µg/L), representing regional background levels. Consistently, Ti levels in fish collected from the Xiaohe River (including muscle and other tissues) were significantly higher than those of fish from the reservoir. Overall, TiO₂ releases through WWTP effluents and other sources (including runoff) are contributing to the relatively high observed concentrations of suspended Ti in the Xiaohe River, which results in accumulation in edible fish tissue.

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1. Introduction

With the fast development of nanotechnology in the recent decades, engineered nanomaterials (ENMs) have been widely used in a variety of commercial products and industrial processes (Gao et al., 2013; Musarrat et al., 2010; Piccinno et al., 2012; Wei et al., 2015). Nano-sized titanium dioxide (nano-TiO₂) is one of the most widely used ENMs due to its unique ultraviolet light absorption, thermal stability, optical properties, and photocatalytic activity (Al-Kattan et al., 2014; Piccinno et al., 2012; von Goetz et al., 2013; Weir et al., 2012; Windler et al., 2012). Nano-TiO₂ can be found in paints, food additives, cosmetics and skin-care products, coating and cleaning agents, plastics, cement, medicines, and electronics. Human consumption of nano-TiO₂ as an ingredient in food products has been estimated at 1 mg kg⁻¹ body weight per day (Weir et al., 2012). Nano-TiO₂ is one of the most commonly used nanomaterials, with a global production of about 10 million tons

(Landsiedel et al., 2010; Piccinno et al., 2012). In China, the production of nano-TiO₂ was estimated to be 1800 tons in 2014 (Gao et al., 2013).

With its widespread use in household and industrial products, nano-TiO₂ is commonly present in domestic sewage, industrial effluents and surface runoff from the paints on building facades (Brar et al., 2010; Kaegi et al., 2008; Kiser et al., 2009; Weir et al., 2012). Wastewater treatment plants (WWTPs) have been shown to be an important point source for ENMs entering aquatic systems (Gottschalk et al., 2009; Nowack and Bucheli, 2007; O'Brien and Cummins 2010; Stone et al., 2010). A few studies reported that WWTPs remove the majority of nano-TiO₂ present in the influent sewage, whereas a small fraction would end up in the effluent and discharged to natural water systems (Brar et al., 2010; Kang et al., 2009; Kiser et al., 2009; Nyberg et al., 2008; Zhou et al., 2015). Several modeling efforts have been conducted to predict the environmental concentrations of nano-TiO₂ in inland surface waters in support of environmental risk assessments (Barton et al., 2015; Gottschalk et al., 2009, 2011, 2013, 2015; Johnson et al., 2011; Mueller and Nowack, 2008; Musee, 2010; O'Brien and Cummins 2010; Praetorius et al., 2012; Sun et al., 2014). However, the reliability of these simulations has not been validated with data collected from

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receiving water bodies (Gondikas et al., 2014; Neal et al., 2011). Analytical limitations associated with the measurement and characterization of nano-TiO₂ and other ENMs in such complex aquatic systems (owing to nanoparticle transformations and the interferences of many other inorganic, organic and biological materials) represent a major challenge for such risk assessment efforts.

If released to natural water systems at sufficiently high concentrations (e.g., 1–200 mg/L), nano-TiO₂ could cause adverse effects to aquatic organisms such as stunted growth, delayed metamorphosis, malformations, organ pathology, and DNA damage (Hao et al., 2009; Clemente et al., 2014). Many studies have demonstrated that nano-TiO₂ can impact *Daphnia magna*, algae, fish, and nematodes (Aruoja et al., 2009; Jacobasch et al., 2014; Wang et al., 2009; Zhu et al., 2008). Several recent studies showed that continued exposure to nano-TiO₂ for up to 13 weeks could cause some mortality, reproduction impairment, and tissue damage in both *Daphnia* and zebrafish even at 0.1 mg/L (Federici et al., 2007; Zhu et al., 2010; Wang et al., 2011).

Unlike hydrophobic organic pollutants and heavy metals that are known to accumulate in organisms, bioaccumulation of nano-TiO₂ in aquatic organisms remains controversial. Some studies report that dietary and waterborne exposure to nano-TiO₂ does not lead to TiO₂ accumulation in fish muscle although it accumulated in the gill, gut and skin of the fish (Boyle et al., 2013; Qiang et al., 2015; Zhu et al., 2010). In contrast, zebrafish has been observed to take up nano-TiO₂ from water even at low ng/mL level, and accumulate it in the liver, muscle and other tissues (Bar-Ilan et al., 2013). All these studies were conducted in labs with organisms exposed to artificially prepared and well dispersed nano-TiO₂ suspension. To our knowledge, there are no published field studies on the occurrence and accumulation of “weathered” nano-TiO₂ in wild aquatic organisms.

This study characterizes the occurrence and fate of nano-TiO₂ in WWTP influents, effluents, biosolids and receiving waters, and assesses the accumulation of nano-TiO₂ in associated fish. To characterize the nano-TiO₂ in the complex samples, a novel analytical approach involving consecutive dialysis against inorganic acids and digestion with 30% of H₂O₂ to minimize analytical interferences was developed. The treated samples were then characterized with high resolution transmission electron microscopy (HRTEM) equipped with an energy dispersion X-ray spectroscopy (EDS), and X-ray photoelectron spectroscopy. Therefore, this study provides unprecedented field data on how WWTP influence nano-TiO₂ concentrations in various environmental compartments (i.e., water, and aquatic organisms) to enhance risk assessment.

2. Materials and methods

2.1. Materials and reagents

Anatase type TiO₂ in different sizes (5–10, 60 and 100 nm in diameter, purity > 99.8%) were purchased from Aladdin Biochemical Technology Co., Ltd, Shanghai, China. Nitric acid (GR, 65%), hydrochloric acid (GR, 37%), sulfuric acid (GR, 98%), hydrofluoric acid (GR, 40%), hydrogen peroxide (GR, 30%) and sodium dodecyl benzene sulfonate were purchased from Chemical Reagent Supply Company, Tianjin, China. Ethanol (GR, 95%) and methanol (GR, 99.99%) were purchased from Concord Technology Co., Ltd, Tianjin, China.

2.2. Wastewater treatment plants and receiving streams site description

This research was conducted at two full scale WWTPs and their receiving streams in Shijiazhuang, Hebei Province, China. The effluents of the WWTPs were directly discharged in Binhe River and Minxin River, and finally flowed to Xiaohe River. Fig. 1 illustrates the sampling sites of the WWTPs, Binhe River, Minxin River and Xiaohe River. To understand the impacts of WWTPs on the receiving water system, a remote control sampling site was selected at Yuqiao Reservoir (Fig. 1),

which is the drinking water source for the residents of Tianjin, China, and is around 360 km from the WWTPs. The Yuqiao Reservoir is highly protected following strict rules and actions.

Schematics of the WWTPs including sampling locations are shown in Fig. 2A. Anaerobic-Anoxic-Oxic (AAO) biological treatment process and tertiary treatment (WWTP1 used tertiary filtration, WWTP2 used denitrification) are used in the full-scale municipal WWTPs. The source water compositions of the two WWTPs were different. About 90% of the WWTP1 influent was domestic sewage, while the WWTP2 influent consisted of about 30% of domestic sewage and 70% industrial wastewater. The daily processing capacity of WWTP1 was 250,000 tons sewage, and the effluent was discharged into Binhe River. While the daily processing capacity of WWTP2 was 600,000 tons sewage, and the effluent was discharged into Minxin River.

2.3. Water and fish sampling

Water sample collection: influents and effluents in each treatment unit of two typical WWTPs were collected in April 2015. Sampling locations are shown in Fig. 2A. At each sampling site, 600 mL of influent and effluent were collected in pre-cleaned plastic bottles. The samples collected from the anaerobic basin and aerobic basin were a mixture of water and activated sludge. They were allowed to settle for 2 h and finally the supernatant liquid was taken as effluent, while the solid precipitate was considered as biological solid sample of the basin. To further explore the fate of nano-TiO₂ in natural water environment, water samples were also taken from Binhe, Xiaohe, Minxin Rivers and Yuqiao Reservoir. Three parallel samples were collected at each sampling site. The samples were stored on ice (<4 °C) until shipping to the laboratory, then stored in a refrigerator at a temperature of –24 °C.

Biological sample collection: Crucian carp is very common in fresh water in China, thus crucian carp was selected as a representative aquatic organism in the current study. Three Crucian carp were taken from Xiaohe River and three were collected from Yuqiao Reservoir by fishing in April 2015. All biological samples were brought back to laboratories within an hour (to guarantee the fish were alive) and immediately dissected. The dissected tissues, such as gill, brain, heart, hepatopancreas, spleen, kidney, muscle, gut and skin of the fish were stored in a hermetic plastic bag and frozen until sample analysis.

2.4. Analysis of Ti

All samples were digested with acid solution and the amount of Ti was determined. 25 mL of water sample was digested by adding 6 mL of nitric acid and 2 mL of hydrochloric acid. Biosolid and biological samples were freeze-dried before digestion. 50 mg of dried biosolid from the WWTPs was digested in a solution consisting of 6 mL of HNO₃, 2 mL of HCl, 1 mL of HF and 1 mL of H₂O₂. Biological samples including the tissues of the fish, such as gill, brain, heart, hepatopancreas, spleen, kidney, muscle, intestines, skin (the mass of each sample was <100 mg) were digested in a solution consisting of 6 mL of HNO₃, 2 mL of HCl and 1 mL of H₂O₂. The digestion process was performed in a microwave (MSD-8G) with a three-step program (details are provided in Table S2). The Ti⁴⁺ in the digested samples was analyzed on an inductively coupled plasma mass spectrometer (ICP-MS) using a scandium (Sc) internal standard (*m/z* = 45).

2.5. Colloid isolation

In order to make clear the compositions of the Ti-bearing suspended solids, they were separated from the wastewater samples and used for subsequent comprehensive characterization. It has been demonstrated that <5% of the TiO₂-NPs can pass through 0.45 or 0.7 μm pores after filtering water solubilized consumer products and personal care products (Weir et al., 2012). A preliminary study was conducted to assess the filtration efficiency using membranes (diameter 25 mm, glass-fiber filter)

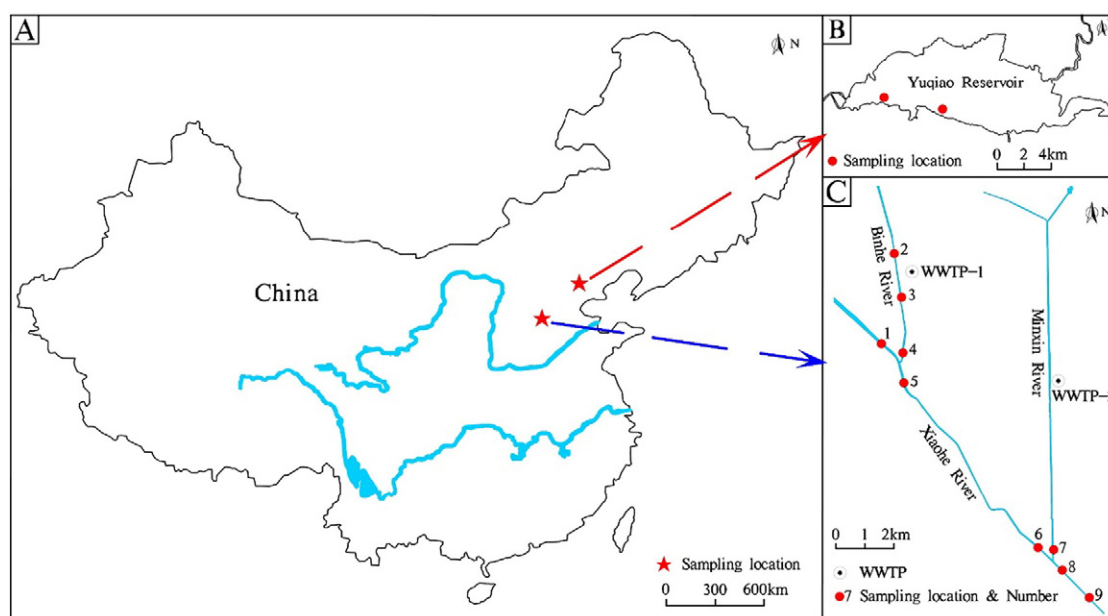


Fig. 1. Map showing the locations of (A) Yuqiao Reservoir in Tianjin and the WWTPs in Shijiazhuang; (B) sampling sites at Yuqiao reservoir in Tianjin; (C) detailed information about the sampling sites in Binhe River, Minxin River and Xiaohe River.

with 0.15, 0.45 and 1.0 μm pore size (the related information is provided in SI). The results indicated that >99% of TiO_2 nanoparticles composed of three different particle sizes (5–10 nm, 60 nm and 100 nm) could be effectively intercepted on the three membranes, and no significant difference was observed among the membranes. This suggested that filtration may promote the aggregation of nano- TiO_2 to larger particles. Thus, glass fiber filter with pore size of 0.45 μm was used to separate TiO_2 particles from water samples. Forty to fifty liters of water samples

were filtered immediately after they were delivered to laboratory. Thereafter, the glass fiber filter was put in a glass beaker with 100 mL of distilled water and sonicated (80 Hz) for 30 min to disperse the solids intercepted on the filter. The filter was taken out and the isolated colloid was loaded in a 3500 Da dialysis tube and dialyzed against HCl (remove carbonate and acid-soluble salts) and hydrofluoric acid (remove silica and other inorganics) (Leenheer, 2009; Leenheer et al., 2007; Song et al., 2010; Westerhoff et al., 2011). The isolated colloid was dialyzed

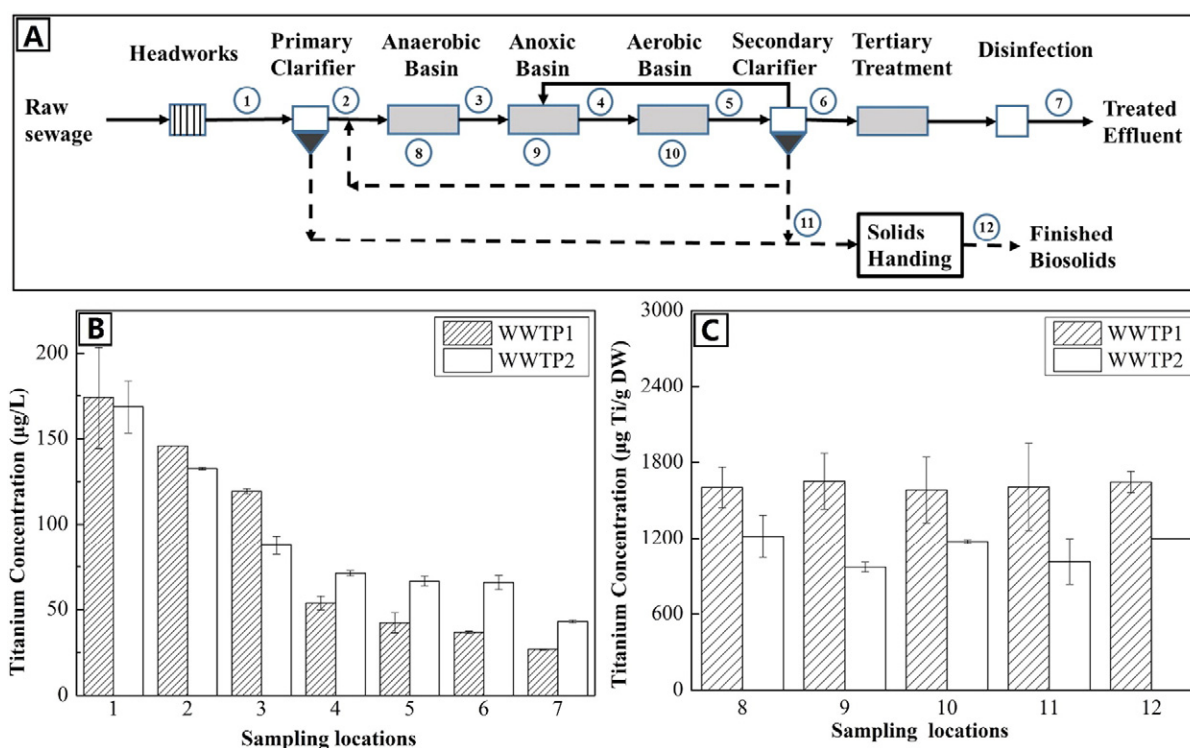


Fig. 2. Schematic of the two WWTPs (A) including sampling locations (circles): (1) influent, (2) primary effluent, (3) anaerobic basin effluent, (4) anoxic basin effluent, (5) aerobic basin effluent, (6) secondary effluent, (7) treated effluent, (8) anaerobic basin biosolids, (9) anoxic basin biosolids, (10) aerobic basin biosolids, (11) secondary biosolids, and (12) finished biosolids. Solid lines indicate direction of water flow. Dashed lines represent direction of flow of solids. The Ti^{4+} concentration in the influent, effluent (B) and biosolids (C) of the two WWTPs. WWTP1-8 refers the sample point 8 of WWTP1; WWTP2-9 refers the sample point 9 of WWTP2.

against 4 L of 0.1 M HCl until all salts were visually dissolved and the color of the permeate solution was negligible, and then dialyzed against deionized water until the solution conductivity was $<100 \mu\text{S cm}^{-1}$. It was subsequently dialyzed for at least 24 h against 4 L of 0.2 M HF to remove silica or silicates and then dialyzed repeatedly against deionized water until the permeate conductivity was $<10 \mu\text{S cm}^{-1}$. To minimize potential interferences from organic biomass and inorganic substances, the isolated solids from wastewater and river water after dialyzing were transferred to a glass beaker and digested with 30% of H_2O_2 at 90°C until foaming ceased (Carter, 1995). During this digestion process, TiO_2 remained intact and unchanged.

2.6. Characterization of Ti-bearing suspended solids

Suspended solids in WWTP effluent and receiving surface waters were characterized and analyzed for the presence of Ti. The morphology of the separated solids was obtained on a JEM-2100F high resolution transmission electron microscopy (HRTEM) equipped with an energy dispersion X-ray spectroscopy (EDS) for elemental analysis and operated at 200 kV. The solids isolated from the WWTP effluent and river water after dialysis and digestion were dispersed in ethanol solution with sonication. The suspension was dropped on copper wire, air-dried and subjected to HRTEM/EDS analysis.

The X-ray photoelectron spectroscopy (XPS, PHI 5400 ESCA System) using monochromatized $\text{Al K}\alpha$ X-ray as the excitation source was applied to study the compositions and chemical states of Ti atoms.

2.7. Quality control and assurance

Feld and trip blanks of Milli-Q water (Millipore water purifier with $18.2 \text{ M}\Omega\text{-cm}$ conductivity) were included in the sampling process. The level of Ti in the blanks was $0.04 \pm 0.002 \mu\text{g/mL}$, and the sample concentrations were corrected by subtracting the blank concentration. Three parallel samples were sampled at each sampling site and the results were reported as the mean of the replicates. All experimental containers were soaked for 12 h with 20% HNO_3 solution, and washed with distilled water and Milli-Q water before use.

3. Results and discussion

3.1. Characterization of Ti-containing suspended solids in WWTP effluent and receiving surface waters

Fig. 3 shows the HRTEM/EDS and XPS images of the isolated Ti-containing colloids from the WWTP1 effluent (WWTP1-7, Fig. 2) and receiving surface water (RW-8, Fig. 1). The Ti-containing suspended solids in the WWTP1 effluent (Fig. 3A) and Xiaohe River surface water (Fig. 3B) were aggregates of spherical nanoparticles with diameter about 20–50 nm. The crystalline lattices could be clearly observed at a magnification scale of 5 nm (Fig. 3C and D). The lattice spacing of 0.32 and 0.48 nm correspond to the (110) and (002) facets of TiO_2 (Wang et al., 2012; Yaghoubi et al., 2015; Zheng et al., 2009), respectively. The EDS of the clusters (Fig. 3E and F) indicated the presence of primarily Ti, O, Cu and C, with trace amounts of other elements. Among them, Cu and C were mainly from the HRTEM grid.

The Ti/Al ratio has been successfully used to distinguish sunscreen nano- TiO_2 from natural Ti-bearing nanoparticles Austria in water (Gondikas et al., 2014). In natural particles, the abundance of Al is much higher than that of Ti, and the Ti/Al ratio was approximately 20–50 for engineered nano- TiO_2 particles whereas this ratio was only 0.1 for natural soils (Gondikas et al., 2014). The Ti/Al ratio of background surface soils from Shijiazhuang (the related information is provided in SI) was determined to be 0.09. In the current study, the Ti/Al ratio of the Ti-containing solids from the WWTP1 effluent and the receiving water were about 92 and 27, respectively, which were much higher

(0.09). The results suggest that the Ti in the two samples were mainly from engineered products.

Fig. 3G and H show the XPS results of the Ti-based solids extracted from the WWTPs effluent and Xiaohe River water. The binding energies (BE) of Ti 2p_{3/2} were 458.01 and 458.23 eV, which were consistent with the typical values reported for Ti (IV) (Biesinger et al., 2010; Zaki et al., 2013). In addition, the doublet splitting of Ti 2p was about 5.7 eV, which was in line with that reported in the literatures for TiO_2 (Biesinger et al., 2010; Savio et al., 2013).

About 90% of the WWTP1 influent was domestic sewage. Apparently, the major source of titanium in the domestic sewage as well as in suspended solids in the treated effluent and the Xiaohe River was likely TiO_2 originating from food additives, consumer products, household products, personal care products, and textiles (Weir et al., 2012; Windler et al., 2012). This inference is supported by HRTEM, EDS and XPS analyses and the observed Ti/Al ratios, as well as previous studies. For example, the titanium present in the effluent of a full scale WWTP in the USA was in the form of nano- TiO_x (Westerhoff et al., 2011). Another study confirmed that the Ti found in surface sediments from Xiamen Bay were solely TiO_x composed of several spherical particles (Luo et al., 2011).

3.2. Removal of titanium in wastewater treatment plants

Fig. 2B shows the Ti concentration in the influent and effluent samples from both WWTPs. Table 1 compares the measured environmental concentrations (MEC) of total Ti in WWTP effluents and waste sludge from the literature (including the results of this study). The total Ti concentration in the influents of the two WWTPs in this study was about $170 \mu\text{g/L}$, which is lower than that in WWTPs surveyed in Arizona, USA ($181\text{--}1233 \mu\text{g/L}$) (Westerhoff et al., 2011), but higher than that measured in UK ($30.5 \pm 11.8 \mu\text{g/L}$) (Johnson et al., 2011). However, the total Ti concentration in the effluents of WWTPs in this study (26 and $43 \mu\text{g/L}$) are higher than those reported for WWTPs in the USA ($<25 \mu\text{g/L}$ (Kiser et al., 2009; Westerhoff et al., 2011)), Canada ($1.0 \pm 0.6 \mu\text{g/L}$ (Khosravi et al., 2012)) and UK ($3.2 \mu\text{g/L}$ (Johnson et al., 2011)). The lower Ti concentrations reported for the UK and Canada WWTP influents and effluents were likely due to the analytical method used, because the influents and effluents were filtered through 0.22 or $0.45 \mu\text{m}$ membrane filters before Ti measurement.

Approximately 85% (WWTP1) and 74% (WWTP2) of Ti in the original influent were removed by a series of wastewater treatment processes, suggesting that most of the nano- TiO_2 could be effectively removed by the treatment trains. The secondary processing unit (from anaerobic basin to secondary clarifier) contributed to 74% (WWTP1) and 53% (WWTP2) of the total removal, indicating that the secondary processing (i.e., the biological treatment units) played an important role in the removal of nano- TiO_2 . The higher removal efficiency in WWTP1 could be explained by differences in tertiary treatment. WWTP1 used tertiary filtration, which could remove smaller particles more effectively; while WWTP2 used denitrification.

Fig. 2C shows the Ti concentration in the biosolid samples of the two WWTPs, and Table 1 compares the total Ti concentration in biosolid samples from different countries. The total Ti concentration in the two WWTP biosolids was $1583\text{--}1653 \mu\text{g Ti/g DW}$ (WWTP1) and $971\text{--}1216 \mu\text{g Ti/g DW}$ (WWTP2), respectively, indicating that biosolids were the main repository of the influent nano- TiO_2 . This is in agreement with the results reported previously showing that the removed Ti accumulated in plant solids, including primary settling tank sludge, activated sludge biomass and secondary clarifier solids (Kiser et al., 2009). Nano- TiO_2 can be removed effectively by its incorporation and/or co-aggregation with the settling flocs (Limbach et al., 2008). Compared with the WWTPs in other countries (Table 1), the concentration of total Ti in the two WWTP biosolids were much lower than that reported in the USA ($1000\text{--}6000 \mu\text{g Ti/g DW}$) (Kiser et al., 2009), the UK (379--

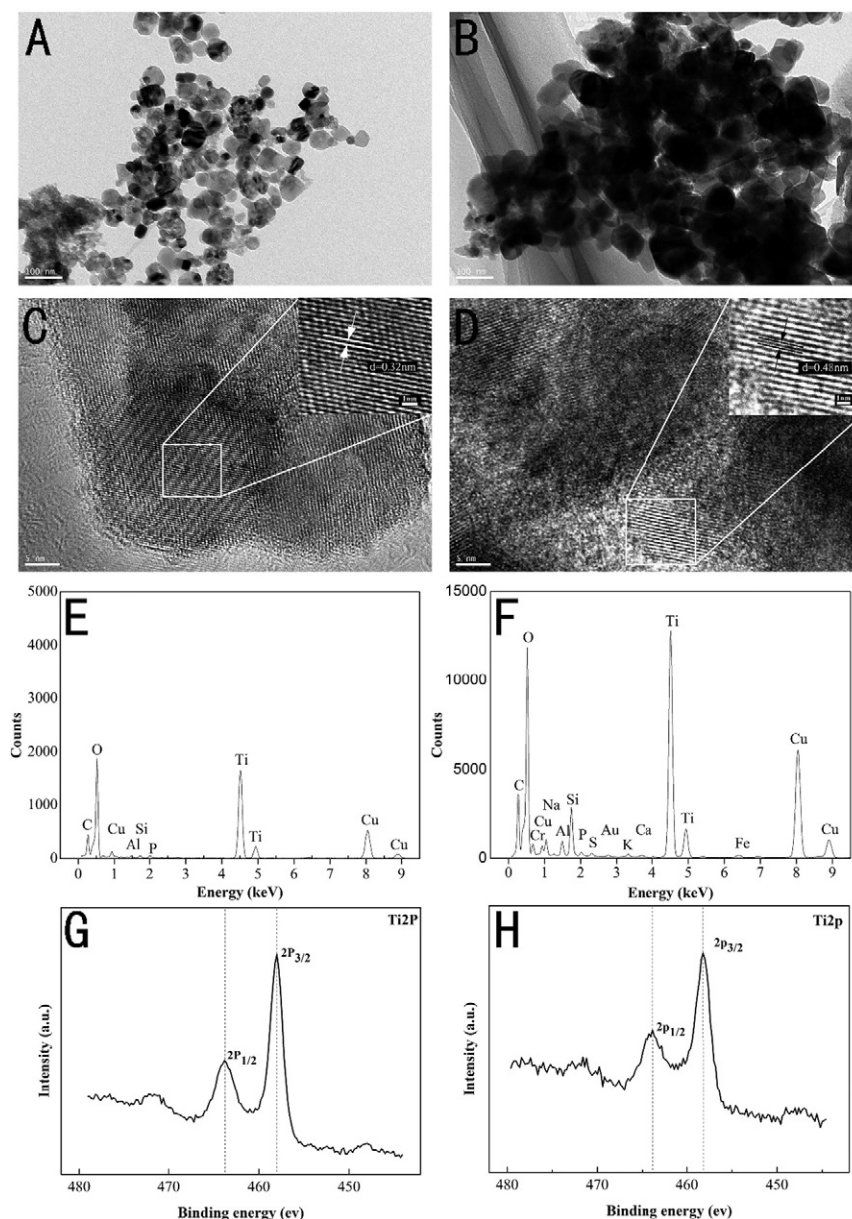


Fig. 3. HRTEM (A, C) images, EDS (E) and Ti 2p XPS (G) spectra of clusters from WWTP1 effluent (WWTP1-7, Fig. 2); HRTEM (B, D) images, EDS (F) and Ti 2p XPS (H) spectra of clusters from the receiving surface water (RW-8, Fig. 1).

Table 1

Measured concentrations of total Ti in surface waters, WWTP effluents and sludges (including the results of this study).

Country	Location	Sources	Filter	Concentration of Ti			Reference
				Average	Range	Unit	
UK	Mid-Wales	Surface water	<0.45 μm	2.1	0.55–6.48	$\mu\text{g/L}$	Neal et al., 2011
Austria	Vienna	Old Danube Lake	>0.2 μm	–	0.49–4.0	$\mu\text{g/L}$	Gondikas et al., 2014
France	–	Seine River	N	48.7	–	$\mu\text{g/L}$	Geertsens et al., 2014
China	Shijiazhuang	Surface river water	N	67.4	52–86	$\mu\text{g/L}$	This study
America	Arizona	WWTP effluent	<0.7 μm	–	5–15	$\mu\text{g/L}$	Kiser et al., 2009
America	Arizona	WWTP effluent	N	–	1–25	$\mu\text{g/L}$	Westerhoff et al., 2011b
UK	–	WWTP effluent	<0.45 μm	–	3.2–47	$\mu\text{g/L}$	Johnson et al., 2011b
Canada	Peterborough	WWTP effluent	<0.22 μm	1.0 \pm 0.6	–	$\mu\text{g/L}$	Khosravi et al., 2012
China	Shijiazhuang	WWTP effluent	N	35.0	26.9–43.1	$\mu\text{g/L}$	This study
America	Arizona	WWTP sludge	<0.7 μm	–	1000–6000	$\mu\text{g/g}$	Kiser et al., 2009
UK	–	WWTP sludge	<0.45 μm	538	379–676	$\mu\text{g/g}$	Johnson et al., 2011b
Canada	Peterborough	WWTP sludge	<0.22 μm	175.3 \pm 1.9	–	$\mu\text{g/g}$	Khosravi et al., 2012
China	Shijiazhuang	WWTP sludge	N	1367	971–1650	$\mu\text{g/g}$	This study

N: no filter was used. –: not available.

676 $\mu\text{g Ti/g DW}$) (Johnson et al., 2011) and Canada (175 $\mu\text{g Ti/g DW}$) (Khosravi et al., 2012).

3.3. Concentrations of nano-TiO₂ in receiving waters

Fig. 4A shows the Ti concentration in the receiving waters, which was in the range of 52–86 $\mu\text{g/L}$. This is significantly higher than that in the Yuqiao Reservoir (representing regional background), which was <5 $\mu\text{g/L}$. Nevertheless, total Ti concentrations in the receiving waters were higher than that in the WWTP effluents (27–43 $\mu\text{g/L}$), implying the presence of other important sources. Although the contribution (if any) from factories located upstream that discharge directly to the Xiaohe River is unknown, urban runoff could be an important contributor of Ti in river water. For example, TiO₂ particles are used in large quantities in exterior paintings, and these particles can leach from building facades during rainfall and enter urban runoff, resulting in Ti concentrations as high as 600 $\mu\text{g/L}$ (Kaegi et al., 2008). Thus, Ti concentrations in some urban runoff could be higher than observed in these WWTP effluents.

There are very few studies about the occurrence of nano-TiO₂ in natural water systems. The concentration of Ti in the old Danube Recreational Lake, which receives nano-TiO₂ from sunscreens due to bathing and swimming, was reported to range from 0.49 to 4 $\mu\text{g/L}$ (Gondikas et al., 2014). Similarly, the Ti concentration in the <0.45 μm fraction in surface waters of the UK averaged 2.1 $\mu\text{g/L}$ with a range of 0.55–6.48 $\mu\text{g/L}$ (Neal et al., 2011). The fraction remaining on the 0.45 μm filter was not determined in their study. Since >99% of nano-TiO₂ would be intercepted by 0.45 μm membrane, the level of nano-TiO₂ in UK river water could be underestimated. The total Ti concentration in the Seine River at Paris was reported at 48.7 $\mu\text{g/L}$ (Geerts et al., 2014), which is lower than observed in our study. Note that nano-TiO₂ could cause adverse effects to zebrafish even at low $\mu\text{g/L}$ levels (Bar-Ilan et al., 2013). The observed relatively high levels of Ti suggest that more attention should be paid to the potential ecological risk of nano-TiO₂ discharges to the Xiaohe River.

3.4. Ti accumulation in crucian carp from the Xiaohe River

The concentration of Ti in the tissues of crucian carp from the Xiaohe River was significantly higher than that from Yuqiao Reservoir (Fig. 4B). The highest concentration was found in the gut, skin and gill, while the lowest was detected in the brain and muscle of the fish. Previous investigations of the accumulation of nano-TiO₂ in fish (e.g., zebra fish, goldfish and common carp) reported nano-TiO₂ enrichment in fish

intestines, skin and gills (Qiang et al., 2015; Ates et al., 2013). However, no significant accumulation of Ti was detected in fish brain, blood and muscle, which was attributed to significant aggregation in fish intestines, which results in larger particles that cannot easily penetrate these tissues (Qiang et al., 2015). Nevertheless, the Ti concentration in the muscle of fish collected from Binhe River was much higher than that from Yuqiao Reservoir, indicating that Ti can accumulate in fish muscle.

Previous studies showing no nano-TiO₂ accumulation in fish muscle were conducted in the laboratory with exposure periods shorter than one month (Bar-Ilan et al., 2013; Fouqueray et al., 2013; Jovanovic et al., 2015; Qiang et al., 2015; Yang et al., 2014). A recent 8-month exposure study to nano-TiO₂ reported that Ti accumulated in zebrafish heart and brain in a time-dependent manner (Chen et al., 2011). Here, fish were exposed to high TiO₂ levels under natural conditions (e.g., Binhe River) for a relatively long term (possibly the entire fish life span). Long term exposure to nano-TiO₂ can cause oxidative stress, which may increase the permeability of fish epithelium cells (Handy et al., 2008) and enhance penetration into muscular tissue. Note that whereas TiO₂ in the Xiaohe River could be in form of bulk and/or aggregated nano-particles, aggregates (as large as 1124 ± 331 nm) can enter the blood stream through the intestines (Al-Jubory and Handy, 2013) and possibly also through damaged tissue, facilitating Ti uptake and accumulation in fish muscle. In a 21-day experiment with zebrafish exposed to nano-TiO₂ at 100 $\mu\text{g/L}$, Ti was accumulated in zebrafish liver and brain at 70–145, and 120–150 $\mu\text{g/g DW}$ (Fang et al., 2016), respectively, which were similar to the Ti tissue concentration measured in the present study (Fig. 4B). Whereas we did not observe here obvious pathological effects in Xiaohe River fish, another study of zebrafish exposed for up to 13 weeks at 100 $\mu\text{g/L}$ showed significant reproduction impairment (Wang et al., 2011). This suggests that long term exposure to TiO₂ in river water may pose some ecological risks.

4. Conclusions

The growing use of nano-TiO₂ in a variety of commercial products is increasing the likelihood of incidental or accidental releases that reach natural waters. Colloidal titanium found in the effluent from two WWTPs and the receiving streams was predominantly in the form of nano-TiO₂ agglomerates. Although a majority of nano-TiO₂ reaching WWTPs was removed by the active sludge process, relatively high Ti levels (27–43 $\mu\text{g/L}$) remained in the effluent at concentrations significantly higher than natural background levels (<5 $\mu\text{g/L}$). However, total Ti concentrations in the receiving waters (52–86 $\mu\text{g/L}$) were

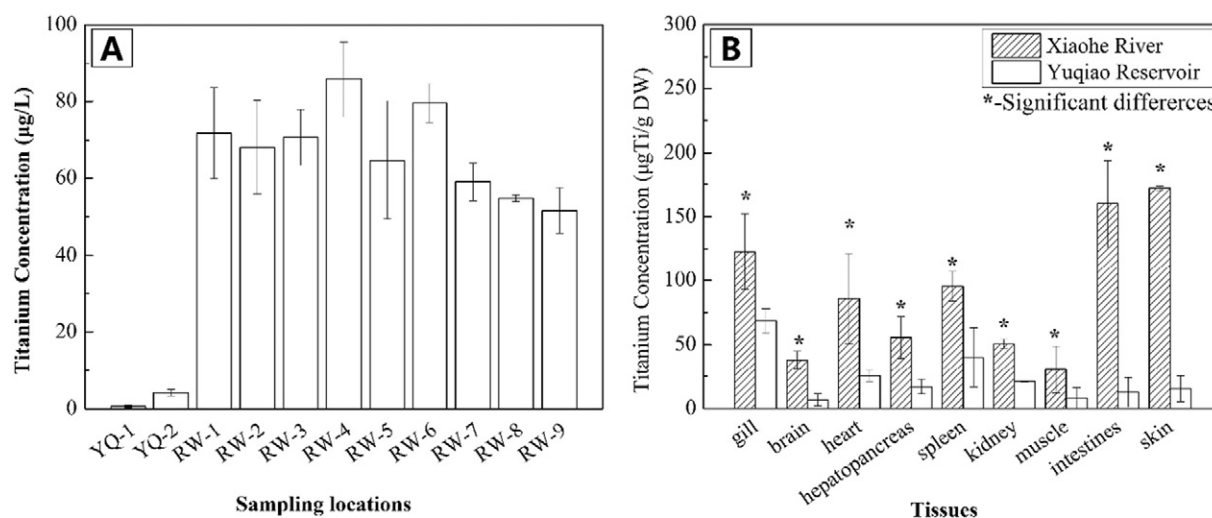


Fig. 4. Comparison of Ti concentration (A) in the receiving water and a remote control site, and (B) that in the tissues of crucian carp from Yuqiao Reservoir, Tianjin and Xiaohe River, Shijiazhuang. Sampling points are identified in Fig. 1. YQ refers to Yuqiao Reservoir; RW refers to receiving water. * represents significant difference.

surprisingly higher than in the WWTP effluents, indicating significant contribution from other sources such as urban runoff containing leachate from exterior paintings. Accumulation of Ti in various fish tissues, including edible tissue (muscle) was also observed. This underscores the need for further assessment of the long-term risk to aquatic ecosystems as well as effective approaches to mitigate unintended nano-TiO₂ releases.

Acknowledgments

We acknowledge financial support from Ministry of Science and Technology (2014CB932001), the Natural Science Foundation of China (NSFC 21325730, 21277077), Ministry of Education (20130031130005), Ministry of Environmental Protection (2012ZX07501-003-04) and the Ministry of Education Innovation Team (IRT 13024). Partial funding was provided by the NSF ERC on Nanotechnology-Enabled Water Treatment (EEC-1449500).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.impact.2016.09.002>.

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