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The effect of chronic silver nanoparticles on aquatic system in microcosms☆

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ABSTRACT

Silver nanoparticles (AgNPs) inevitably discharge into aquatic environments due to their abundant use in antibacterial products. It was reported that in laboratory conditions, AgNPs display dose-dependent toxicity to aquatic organisms, such as bacteria, algae, macrophytes, snails and fishes. However, AgNPs could behave differently in natural complex environments. In the present study, a series of microcosms were established to investigate the distribution and toxicity of AgNPs at approximately 500 μg L^{-1} in aquatic systems. As a comparison, the distribution and toxicity of the same concentration of $AgNO_3$ were also determined. The results showed that the surface layer of sediment was the main sink of Ag element for both AgNPs and $AgNO_3$. Both aquatic plant (*Hydrilla verticillata*) and animals (*Gambusia affinis* and *Radix spp*) significantly accumulated Ag. With short-term treatment, phytoplankton biomass was affected by $AgNO_3$ but not by AgNPs. Chlorophyll content of *H. verticillata* increased with both AgNPs and $AgNO_3$ short-term exposure. However, the biomass of phytoplankton, aquatic plant and animals was not significantly different between control and samples treated with AgNPs or $AgNO_3$ for 90 d. The communities, diversity and richness of microbes were not significantly affected by AgNPs and $AgNO_3$; in contrast, the nitrification rate and its related microbe (*Nitrospira*) abundance significantly decreased. AgNPs and $AgNO_3$ may affect the nitrogen cycle and affect the environment and, since they might be also transferred to food web, they represent a risk for health.

1. Introduction

Because of excellent antibacterial properties, silver nanoparticles (AgNPs) are one of the most widely used nanomaterials in consumer products (Fabrega et al., 2011). More than 400 consumer products contain AgNPs (Schlich et al., 2016). With production, transportation, utilization and disposal, AgNPs are easily discharged into environments (Benn et al., 2010; Benn and Westerhoff, 2008), for example into the aquatic environment that is one of the most important sinks of AgNPs in the nature (Lin et al., 2010). In Europe and US, levels can reach up to 1–5.8 mg kg^{-1} AgNPs in sludge of wastewater treatment plant (WWTP) (Gottschalk et al., 2009). However, the measurements of AgNPs concentrations in environments are still scarce. To the best of our knowledge only one study has been performed in British WWTP in 2010 showing that it contains ~3.3 μg L^{-1} Ag including ~12 ng L^{-1} AgNPs in sewage (Johnson et al., 2014).

Previous studies documented that AgNPs may have adverse effects on various aquatic organisms in different trophic levels, such as bacteria (Choi and Hu, 2008; Fabrega et al., 2009; Ivask et al., 2014; Yuan et al., 2013), algae (Gonzalez et al., 2015; Kwak et al., 2016; Navarro et al., 2008; Oukarroum et al., 2011), macrophytes (Jiang et al., 2012, 2014, 2017; Oukarroum et al., 2013; Van Koetsem et al., 2015; Zou et al., 2016), invertebrates (Ali et al., 2014; Cong

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et al., 2014; Griffitt et al., 2008; Zhao and Wang, 2011) and fishes (George et al., 2011; Kwok et al., 2012; Osborne et al., 2015; Wu et al., 2010). However, most of these studies were performed with pure cultures at high AgNPs concentration and for a short time exposure, thus the toxicity of AgNPs in these studies may not represent the one in natural complex environments. Nonetheless, there are reports on the distribution and toxicity of AgNPs in real or mimicked natural aquatic environments. In aquatic microcosms, biotic and abiotic factors affected aggregation, dissolution, transformation and toxicity of AgNPs after 24 h exposure (Bone et al., 2012; Unrine et al., 2012). In a 20 d experiment, with mesocosms in a boreal lake, the sediment and periphyton were the two biggest sinks of AgNPs (Furtado et al., 2015) while in natural estuary, the surface layer of sediment (upper sediment) was the sink of AgNPs but these particles at up to 1 mg L^{-1} did not change bacterial diversity (Bradford et al., 2009). In a natural estuary, photosynthesis of the phytoplankton community was significantly reduced when the AgNPs concentration was higher than 0.5 mg L^{-1} after a 24 h exposure (Baptista et al., 2015). A mesocosm experiment to mimic a fresh water wetland system suggested that for one pulse AgNPs exposure (1 mg kg^{-1}), there was no significant effect of AgNPs on sediment microbial communities in long-term (300 d), but in short-term (30 and 60 d) exposure, AgNPs could decrease cell density and increase alpha diversity (Moore et al., 2016). Species associated with the nitrogen cycle are sensitive to AgNPs. It was shown that in activated sludge, the denitrification was inhibited by 0.5 mg L^{-1} AgNPs after 12 h exposure (Chen et al., 2014). After 7 d exposure, 40 mg L^{-1} AgNPs significantly decreased the number of ammonia-oxidizing bacteria and thereby ammonia oxidation activity (Yang et al., 2014). Hitherto, the distribution of AgNPs and their effects to different components of aquatic ecosystem (such as water column, sediment, microbe, macrophyte, animal…) are still not well-known. Therefore, the main goals of this study were: 1) to analyze the distribution of AgNPs in aquatic microcosms that included the water column, sediment, a macrophyte and animals; 2) to analyze if chronic AgNPs can affect biological components in aquatic ecosystem. To answer these issues, we set up aquatic microcosms that contain sediment, lake water, one aquatic macrophyte (*Hydrilla verticillata*) and aquatic animals (*Gambusia affinis* and *Radix spp.*). *H. verticillata* and *Radix spp*. are native and very common in China while *G. affinis* is an introduced species, but now rather common in south of China and has been already used in ecotoxicology studies (Hou et al., 2011; Xie et al., 2010). *Radix spp.* and *G. affinis* represent two habitat types, demersal and surficial, respectively (see review in Pyke, 2005).

2. Materials and methods

2.1. Silver nanoparticles (AgNPs)

The polyvinylpyrrolidone-coated-AgNPs (PVP-AgNPs) were characterized in previous study (Jiang et al., 2014, 2017). The stock suspension (1000 mg L^{-1}) was kept in the dark 4 °C and the average diameter of the AgNPs checked by transmission electron microscopy (TEM) was ~20 nm. The stock suspension contained ~0.15% free Ag^+ (of total Ag). The AgNPs hydrodynamic diameter distribution was determined by dynamic light scattering (DLS, Zetasizer, Malven Nano ZS90 ZEN 3600) and Zeta-potential also determined at the concentration of 5 mg L^{-1} AgNPs diluted with distilled water.

2.2. Microcosms and treatments

Fifteen microcosms were 20 × 20 × 30 cm (length, width and height) glass tanks. Each tank contained 3.5 kg air-dried sediment, 10 L lake water from nearby Donghu Lake and five *H. verticillata* fragments (~10 cm long, ~0.4 g for each). The number of fragments was chosen in order to get optimal coverage (>90% of the surface area was covered by the plant) in one month. The small tanks were located in large cement tanks (~400 L) containing running water to keep temperature constant during summer. After approximately one month, five snails (*Radix spp.*) were introduced into each microcosm and then ten days later two fish (*G. affinis*) were added into each microcosm, the number of snails and fish was chosen according to previous experiments (Cao et al., 2014; Olsen et al., 2015). After one week acclimation for the aquatic animals, 5 mg AgNPs (AgNPs-treatment) or Ag^+ (represented as $AgNO_3$) ($AgNO_3$-treatment) were added to microcosm (containing ~10 L lake water) to make the initial concentration about 500 μg L^{-1} in water column (five replicates); other five microcosms were untreated and defined as controls. Total exposure duration was 90 d. Distilled water was added to keep the microcosm volumes constant every 2 or 3 d. The sediment was taken from nearby Donghu Lake, which contains 370 mg kg^{-1} total nitrogen (TN) and 10 mg kg^{-1} total phosphorus (TP) as previously reported (Zhao et al., 2016). Other microcosm parameters such as the content of chloride (Cl^-, determined by ion chromatography (Dionex ICS-900, Thermo, US)), sulfur (S^{2-}, determined by spectrophotometry (TU-1810PC, Purkinje General, China)) and total organic carbon (TOC, determined by a TOC analyzer (Vario TOC, Elementar, Germany)), temperature, dissolved O_2 (determined by an optical oxygen electrode (YSI Pro ODO, Yellow Spring Instruments, USA)) and pH (determined by a pH electrode (Metrohm 6.0238.000, Herisau, Switzerland)) are shown in supplementary materials (Table S1). The initial number and weight of *H. verticillata*, *Radix spp*. and *G. affinis* are shown in supplementary materials (Table S2). During the time course of the experiment, four fish died in two $AgNO_3$-treated microcosms and were left within the systems, while the other 26 fish survived in the other 13 microcosms. We calculate based on a method from Ramseyer (2002) that these dead fish supply 0.14 mg L^{-1} N.

2.3. AgNPs dynamic, Ag element distribution and mass balance in microcosms

To estimate the dynamic of particle size and stability of AgNPs in microcosms, 5 mg L^{-1} AgNPs was prepared in Donghu Lake water, and the hydrodynamic diameter and Zeta-potential was monitored by the Zetasizer at 0, 1 and 2 d, since after 3 d exposure AgNPs was very low in water. As a control, the lake water without AgNPs was also monitored by the Zetasizer. After addition of AgNPs or Ag^+ (under $AgNO_3$), one mL water was taken from each microcosm at 0, 1, 2, 3, 7, 60 and 90 d to measure Ag content in the water column by a Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (X Series 2, Thermo-Fisher, Germany) after digestion with 1 mL 10% HNO_3 (Unrine et al., 2012). After 90 d exposure, five sediment-cylinders (1 cm diameter) were collected from each microcosm and dried in air. The sediment-cylinders were separated to upper (0–0.5 cm), middle (0.5–1 cm) and lower (>1 cm) layers, and then combined and grinded, giving a total of 45 sediment samples. For each microcosm, 200 mg dry weight upper, middle and lower sediment, 200 mg dry weight *H. verticillata*, all *Radix spp*. (~300 mg dry weight) and all *G. affinis* (~40 mg dry weight) were collected to be digested with 2 mL H_2O_2 and 5 mL 65% HNO_3 at 180 °C for 2 h in a Microwave Sample Preparation System (Milestone, Sorisole, Italy). Then, the digestion was diluted by 1% HNO_3 and the Ag content detected by a ICP-MS. Partition of Ag in the microcosms was estimated by calculating the mass balance at the end of the experiment in five components: 1) water column, 2) macrophyte, 3) fish, 4) snail, 5) different layers of the sediment. Ag mass in all partitions was corrected by subtracting the average concentration found in the control microcosms. The total Ag recovered (%) in each

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