Environmental Pollution xxx (2016) 1–9

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

An investigation into the effects of silver nanoparticles on natural microbial communities in two freshwater sediments☆

Shaopan Bao [a, b, 1], Han Wang [a, b, 1], Weicheng Zhang [a], Zhicai Xie [a], Tao Fang [a, *]

[a] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

A R T I C L E I N F O

Article history:
Received 31 March 2016
Received in revised form
5 June 2016
Accepted 29 June 2016
Available online xxx

Keywords:
Silver nanoparticles
Freshwater sediment
Enzyme activities
Bacterial community structure

A B S T R A C T

The expanding production and usage of commercial silver nanoparticles (AgNPs) will inevitably increase their environmental release, with sediments as a substantial sink. However, little knowledge is available about the potential impacts of AgNPs on freshwater sediment microbial communities, as well as the interactions between microbial communities and biogeochemical factors in AgNPs polluted sediment. To address these issues, two different sediments: a eutrophic freshwater sediment and an oligotrophic freshwater sediment, were exposed to 1 mg/g of either $AgNO_3$, uncoated AgNPs (35-nm and 75-nm), or polyvinylpyrrolidone coated AgNPs (PVP-AgNPs) (30–50 nm) for 45 days. High-throughput sequencing of 16S ribosomal ribonucleic acid (16S rRNA) genes using the Illumina MiSeq platform was conducted to evaluate the effects of Ag addition on bacterial community composition. Moreover, sediment microbial biomass and activity were assessed by counting cultivable bacterial number and determining enzyme activities. During the 45-day exposure, compared with no amendment control, some treatments had resulted in significant changes and alterations of sediment biomass or bacterial enzyme activities shortly. While the microbial components at phylum level were rarely affected by AgNPs addition, and as confirmed by the statistical analysis with two-factor analysis of similarities (ANOSIM), there were no significant differences on bacterial community structure across the amended treatments. Redundancy analysis further demonstrated that chemical parameters acid-volatile sulfide (AVS) and simultaneously extracted silver (SE-Ag) in sediment significantly structured the overall bacterial community in sediments spiked with various silver species. In summary, these findings suggested that the ecotoxicity of AgNPs may be attenuated by the transformation under complex environmental conditions and the self-adaption of sediment microbial communities.

1. Introduction

The rapid development of nanotechnology has aroused the wide usage of engineered commercial nanomaterials (ECNMs) in industrial and consumer products. Among the most promising nanomaterials, silver nanoparticles (AgNPs) have been incorporated into diverse commercial products such as clothing, plastics, cosmetics and food containers (Frattini et al., 2005; Sondi and Salopek-Sondi, 2004). Growth in production and application of AgNPs will inevitably increase the risk of silver exposure in environment, and the most possible exposure route of AgNPs is considered to be through wastewaters and surface waters (Baun et al., 2008; Boxall et al., 2007). Concerns are therefore being raised about their potential impacts on aquatic organisms including bacteria, invertebrates, fish and planktons.

Following their release into the aquatic environments, ECNMs were assumed to be mainly deposited in the sediments after their agglomeration and sedimentation (Baun et al., 2008; Cong et al., 2014; Ramskov et al., 2015). Sediments were deemed as good models to evaluate the toxicity of ECNMs on sediment functioning and quality (Beddow et al., 2014), and the microorganisms in sediment are pivotal in maintaining the balance of biogeochemical processes (Battin et al., 2008). Therefore, the potential interactions between ECNMs and sediment microbial communities deserve an in-depth study to better assess the impacts of ECNMs.

There has been a number of studies conducted to evaluate the

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author. Institute of Hydrobiology, Chinese Academy of Sciences, 7 Donghu South Road, Wuchang District, Wuhan 430072, China.
E-mail address: fangt@ihb.ac.cn (T. Fang).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.envpol.2016.06.071
0269-7491/

impacts of AgNPs on microbial community in estuarine sediments (Beddow et al., 2014; Bradford et al., 2009; Echavarri-Bravo et al., 2015) and activated sludge (Alito and Gunsch, 2014; Yang et al., 2014). However, the results of these studies were inconsistent and led to different conclusions, such as the antimicrobial effects of AgNPs were reduced by the exposure to saline estuary water in estuarine environment (Bradford et al., 2009), whereas in activated sludge, AgNPs significantly changed the microbial community structure and negatively affected waste removal efficacy (Alito and Gunsch, 2014; Yang et al., 2014). The reason for these discrepant results is that the environmental factors (e.g., pH, organic matter (OM), redox state, sulfide and chlorine) may alter AgNPs toxicity by promoting surface complexation and thus mediate effects on sediment biogeochemical processes (Kim et al., 2010; Pokhrel et al., 2013). Since freshwater environment has different physico-chemical characteristics comparing to those presented in estuarine sediment or activated sludge, the published studies could provide little information regarding how AgNPs will affect freshwater sediment microbial community structure. There is therefore a need to understand the impacts of AgNPs on freshwater sediment processes and microorganisms.

Sediment microbial biomass and enzyme activity are sensitive indicators which can be used to evaluate the sediment quality (Zhang et al., 2016). Therefore, estimating the impacts of AgNPs on sediment microbial biomass and enzyme activity will also help us know further about the ecotoxicity of AgNPs. In addition, sediment microbial biomass and enzyme activity always have a good correlation with the relative abundance of functional microbial groups (Wu et al., 2016), estimating them would promote the understanding about the shift of sediment bacterial community when spiked with AgNPs. However, knowledge about the ecological effects of AgNPs on freshwater sediment microbial biomass and enzyme activity is still lacking, except that they were once determined in Colman et al. (2012) study, in which natural streamwater and sediment were incubated with a commercial AgNPs for approximately 10 days.

The current study aims at (1) investigating the impacts of AgNPs of different sizes and coatings on bacterial biomass and enzyme activity in freshwater sediments, (2) estimating the effect of AgNPs on microbial community diversity and structure, and (3) identifying the dominant environmental parameters associated with bacterial community in AgNPs spiked sediment. AgNPs of various sizes and coatings were used because the environmental fate and biological effects of AgNPs were greatly impacted by their own properties (e.g., size, shape, surface charge and coating) (Kuhn et al., 2014; Shang et al., 2014). Besides, two kinds of freshwater sediments sampled from eutrophic and oligotrophic lakes were employed to compare the impacts of AgNPs in different environmental conditions. Moreover, high-throughput sequencing was adopted to assess the structure and dynamics of microbial community in sediment. Along with the determination of bacterial community structure, sediment microbial biomass and activity were estimated by counting cultivable bacterial number and determining enzyme activities. To our knowledge, this is the first study to synchronously detect and quantify the bacterial community, bacterial biomass and enzyme activities in freshwater sediment spiked with AgNPs of different sizes and coatings.

2. Material and methods

2.1. Chemicals

Three types of commercially available AgNPs were chosen in this study. Two uncoated AgNPs were obtained in the form of dry powder: 35 nm AgNPs (NanoAmor, Houston, TX, USA) and <100 nm AgNPs (Sigma–Aldrich, St. Louis, MO, USA). As the average size of <100 nm AgNPs was confirmed to be 75 ± 26 nm in an earlier study (Kim et al., 2012), it was represented as 75-nm AgNPs throughout this paper. Similarly, one PVP coated AgNPs was also existing in the powdered form: 30–50 nm PVP-AgNPs (w/0.2 wt% PVP, NanoAmor, Houston, TX, USA). Silver nitrate (purity 99.8%) obtained from Sinopharm Group Co., Ltd (Beijing, China) was included to provide a reference for dissolved silver. All the purchased chemicals were at least of analytical grade.

2.2. Preparation and characterization of nanoparticles

Stock solutions of 1 mg/mL 35-nm AgNPs, 75-nm AgNPs and PVP-AgNPs were prepared by dispersing the nanoparticles in deionized water (DI water, 18.2 MΩ/cm generated by a Sartorius Arium basic laboratory water purification system, Gottingen, Germany) with sonication (50 W/L, 40 kHz) for 20 min. The silver nitrate solution was prepared analogously but without sonication. Test nanoparticle solutions were prepared immediately prior to sediment spiking.

The primary morphology and size of the particles were evaluated using transmission electron microscopy (TEM, JEM2100 (HR), JEOL, Japan) operating at 200 kV. Surface charge of AgNPs in three types of test media (DI water, DL pore water and LSL pore water) was measured after 24 h of incubation using zeta potential analyzer (Zetasizer Nano-ZS, Malvern, UK). Pore water was obtained by centrifuging according to the method of USEPA (USEPA, 2001).

2.3. Sediment and water samples

Two sediments with different properties were used in this study. Surface sediments and associated water were collected from Donghu Lake and Lushui Lake, respectively. Donghu Lake (DL) is one of the largest urban lake in China with a total surface area of 32 km^2 located in Wuhan City, and it is a eutrophic lake with a mean depth of 2.5 m (Xie et al., 2003). While the Lushui Lake (LSL) located in Chibi City, China, is an oligotrophic lake with an area of 57 km^2 and with an average depth of 10 m, serves as a drinking water reservoir (Zhu et al., 2015). The surface sediment samples (0–11 cm) were obtained with a Petersen Grab sampler, and the top few centimeters of the sediment were scraped off. The natural sediment was wet-sieved through <2 mm sieve using DI water. Overlying water was removed and the sediments were then stored at 4 °C until sediment spiking. Before use, dry weight (dw)/wet weight (ww) and organic matter content (OM) was measured following the method as described by Steffan et al. (1988). Sediments used in the present study had negligible background levels of silver (2 ± 0.6 ng Ag/g sediment in DL and 6 ± 2.0 ng Ag/g sediment in LSL based on ICP-MS, data not shown) compared with the spiked silver concentration. The associated water used in the microcosm experiment was taken from the same site by an organic glass hydrophore, and the lake water was filtered through Whatman GF/C filter paper to remove suspended dust particles.

2.4. Experimental design

For each Ag form (35-nm AgNPs, 75-nm AgNPs, PVP-AgNPs and $AgNO_3$), a nominal Ag exposure concentration of 1 mg Ag/g dw sediment was selected. One concentration was selected because our focus was on species comparison, and the specific exposure concentration was determined based on previous studies, which added similar levels of AgNPs to examine the silver transformations and the detrimental biological effects of AgNPs (Hashimoto et al., 2015; Shoults-Wilson et al., 2011; Whitley et al., 2013). The Ag-spiked sediments were prepared following Cong et al. (2011). In