



Impact of silver nanoparticles on benthic prokaryotes in heavy metal-contaminated estuarine sediments in a tropical environment



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ABSTRACT

Little knowledge is available about the potential impact of commercial silver nanoparticles (Ag-NPs) on estuarine microbial communities. The Hugli river estuary, India, is susceptible to heavy metals pollution through boat traffic, and there is the potential for Ag-NP exposure via effluent discharged from ongoing municipal and industrial activities located in close proximity. This study investigated the effects of commercial Ag-NPs on native microbial communities in estuarine sediments collected from five stations, using terminal restriction fragment length polymorphism (T-RFLP) technique. An increase in the number of bacteria in consortium in sediments was observed following exposure to Ag-NPs. In general microbial communities may be resistant in estuarine systems to the antimicrobial effects of commercial Ag-NPs, but key microorganisms, such as *Pelobacter propionicus*, disappeared following exposure to Ag-NPs. In conclusion, the T-RFLP analysis indicated that Ag-NPs have the potential to shape estuarine sediment bacterial community structure.

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1. Introduction

Nanoparticles (NPs) can be defined as particles with novel and distinctive physicochemical properties with at least one dimension within the range of 1–100 nm (Masciangioli and Zhang, 2003). Ionic silver and silver nanoparticles (Ag-NPs) are used as a broad spectrum antimicrobial agent in consumer products including medical equipment, clothing, pharmaceuticals, food storage containers, children's toys, cosmetics, optical devices and household appliances (Kim et al., 2007).

The most probable environmental exposure route of Ag-NPs is predicted to be through wastewaters and surface waters (Boxall et al., 2007). For instance, it has been observed that Ag-NPs can be readily released from commercially available sock fabrics previously impregnated with Ag-NPs, a treatment used to, for example, protect fabrics against odour-producing bacteria (Benn and Westerhoff, 2008). In fact, up to 1360 µg Ag-NPs could be absorbed in 1 g of sock fabric which then could leach as much as 650 µg of silver in 500 ml of distilled water during a 24-h washing cycle. Other potential sources of Ag-NPs in the aquatic environment have been identified to come from the use or degradation of many commercial products, e.g., shampoo, soap, toothpaste, paints, clothing,

cosmetics, paints, washing powders, drinks, plastic food container, wound dressings, and biocidal coatings (Benn and Westerhoff, 2008; Chopra, 2007). The release of Ag-NPs into aquatic systems has been found to have particularly high intrinsic toxicities to microbial populations (Bradford et al., 2009; Sondi and Salopek-Sondi, 2004). The increasing environmental release of Ag-NPs raises concerns about the potential effect on sediment functioning and consequently on its capacity to fulfil essential ecosystem services.

Sediment microorganisms are key players of many critical functions, such as biogeochemical cycling (e.g., carbon, nitrogen, sulphur and phosphorous cycles), plant productivity or climate regulation (Battin et al., 2008). Microbial communities are known to be sensitive ecological indicators of sediment response to environmental perturbations (Lozada et al., 2014) and can be good models to investigate Ag-NPs effect on sediment functioning and quality (Beddow et al., 2014).

Previous studies have shown that Ag-NPs can induce a decrease in microbial activity and a shift in bacterial community structure in sediments (Bradford et al., 2009). However, these pioneer studies were performed only with homogeneous dispersions of Ag-NPs (Beddow et al., 2014; Bradford et al., 2009) and usually with a single model sediment (Bradford et al., 2009). Since extrapolation of results from one type of Ag-NPs to commercial products containing Ag-NPs, and one contaminated sediment to another is difficult

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because of the great heterogeneity of sediments in terms of composition, structure and reactivity, studies conducted under more realistic concentrations and covering commercial Ag-NPs and a wide range of sediments are required.

The present study focused on the Hugli estuary, India, as a model system from which to measure the effect of Ag-NPs on estuarine microbial communities, due to its susceptibility to pollution (Antizar-Ladislao et al., 2011; Bhattacharya et al., 2015). The overall aim of the study was to determine the effects of one type of commercial Ag-NPs solution on the community structure and activity of microbial communities in estuarine sediments exposed to historic heavy metal contamination. The commercial Ag-NP nanoparticles used for this study were coated with TiO₂ solution in glycerol and used as received.

2. Materials and methods

2.1. Site description

The Hugli, which supports the world's largest magnificent mangrove block, the Sundarban (area 9620 km²), is a typical and unique ecosystem of the Indian subcontinent and has been recognized as a UNESCO World Heritage Site (Fig. 1). This is one of the most sensitive but complex ecosystems in the world and suffers from environmental degradation due to rapid human settlement, tourism and port activities, operation of excessive number of mechanized boats, deforestation, and increasing agricultural and aquaculture practices. The ongoing degradation is also related to huge siltation, flooding, storm runoff, atmospheric deposition, and other stresses resulting in changes in water quality, depletion of fishery resources, choking of river mouth and inlets, and overall loss of biodiversity as evident in recent years (Bhattacharya et al., 2003; Sarkar et al., 2007). Recent studies on the pollution status of the Hugli River and adjacent Sundarban wetland have revealed the presence of both inorganic and organic pollutants in sediments which have changed the estuary's geochemistry and affected the coastal environment (Sarkar et al., 2012; Antizar-Ladislao et al., 2011).

Hugli River Estuary (HRE) (87°55'01"N to 88°48'04"N latitude and 21°29'02"E to 22°09'00"E longitude), the first deltaic offshoot of the River Ganges, is a well-mixed estuary because of its shallow depth (average ~6 m) and drains a catchment of 6 × 10⁴ km². The estuary gets semidiurnal tides with maximum range of 5.5 m at spring and minimum 1.8 m at neap. This globally significant estuary provides perpetual supply of water to the plains of West Bengal for multipurpose activities (such as, irrigation, navigation of small ships and fishing boats, human and industry consumption together with fishing) and thus supporting the lives of millions of people. A significant ecological change is pronounced in this area due to huge discharges of untreated or semi treated domestic and municipal sewage as well as effluents from multifarious industries carried by rivers as well as contaminated mud disposal from harbour dredging.

2.2. Sediment and water sampling

To monitor the spatial distribution of heavy metals as well as the impact that an accidental release of Ag-NPs may have on the associated microbial populations, surface sediments were collected during winter season (December 2012–January 2013) using a grab sampler during low tide from five key stations along the stretch of Hugli river namely, Barrackpore (S1; 22°45'51"N 88°20'40"E), Babughat (S2; 22°49'32"N 88°21'39"E), Budge Budge (S3; 22°33'58"N 88°11'16"E), Nurpur (S4; 22°12'40"N 88°40'16"E) and Diamond Harbour (S5; 22°11'13"N 88°11'24"E). The stations were

selected considering the sediment dispersal patterns along the drainage network systems (Fig. 1; Table 1). Sediment samples were collected in triplicate from the top 0 to 10 cm of the surface at each sampling site, using a pre-cleaned (acid-washed) PVC spatula, pooled and thoroughly mixed. Care was taken to ensure that there was minimal disturbance of the surface water. Immediately after collection, the samples were placed in sterilized plastic bags in the ice box and transported to the laboratory. For the determination of sediment quality parameters and metal analysis, samples were oven dried at 50 °C, gently disaggregated, and individually transferred into pre-cleaned inert polypropylene bags and stored in deep freeze prior to analysis. The five pooled samples were each divided into two aliquots: one un-sieved, for the determination of sediment quality parameters, and another one sieved through 63 µm metallic sieves, for metal analysis. For the assessment of the impact of an accidental release of Ag-NP on the dynamics of bacteria in the sediments, sediment samples and water (to facilitate sediment slurry) were collected from the same sites and transported to the laboratory at room temperature for bacterial characterization and simulation studies.

2.3. Physical and chemical analysis of sediments

The sediment samples were characterized for particle size, pH, and organic carbon (C_{org}). Particle size was determined by sieving the dried samples in a Ro-Tap Shaker (Krumbein and Pettijohn, 1938) manufactured by W.S. Tyler Company, Cleveland, Ohio and statistical computation of textural parameters was done by using the formulae of Folk and Ward (1957). The determination of pH was conducted with a digital pH metre 101E Deluxe Model (EI Products, India) using a combination glass electrode manufactured by M.S. Electronics (India) Pvt. Ltd. Organic carbon (C_{org}) content of the soil was determined following a rapid titration method (Walkey and Black, 1934) after oxidizing with potassium dichromate (K₂Cr₂O₇) in presence of sulphuric acid (H₂SO₄).

For the determination of trace metals in sediments, sediments were finely grounded (agate pestle and mortar). Triplicate samples of 0.25 g were oven dried (105 °C), ashed (450 °C) and digested in concentrated HNO₃ (Aristar, VWR) for "pseudo-total" digestion using a microwave (CEM Mars 5) following the adapted USEPA method 3051 (Yafa et al., 2004). Pseudo-total digestion was used since the main interest was in anthropogenic rather than mineral-bound elements. Following digestion the samples were evaporated to near-dryness on a hot plate, added to a 25 ml volumetric flask (three washes from beaker were also added) and diluted with dilute nitric acid (2% v/v, VWR, Aristar). This was filtered (Ashless Whatman 45) into a polypropylene centrifuge tube (Fisher) and stored at 4 °C prior to analysis with Inductively coupled plasma – mass spectrometry (ICP-MS). The following trace elements were detected using ICP-MS analysis: Ag, Pb, Cd, Cr, Cu, As and Ni.

A peat reference material (NIMT/UOE/FM/001) was similarly processed along with the sediment samples (Yafa et al., 2004). Reagent blanks were carried on in parallel with all sample analysis. In all cases blank signals were lower than 0.5% of sample signals. Silver is known for being "sticky" and cause carry-over between samples in ICP-MS analysis. To reduce high background levels of Ag in the ICP-MS, ¹⁰⁷Ag was monitored during warm-up. An alkaline wash solution (10 mg/L EDTA (as acid), 0.8 mg/L Triton X-100, 60 mg/L HN₄OH and 80 mg/L H₂O₂) was inserted to remove any prior Ag from the instrument system. The system was also rinsed with the alkaline wash solution after each sample (30 s), before the nitric acid rinse (2%, 30 s). Background levels were closely monitored during analysis to ensure that Ag counts returned to original levels and any carry-over was minimized. The expressed concentrations of each element in the samples represent the mean

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