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Shifts in the metabolic function of a benthic estuarine microbial community following a single pulse exposure to silver nanoparticles

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ABSTRACT

The increasing use of silver nanoparticles (AgNPs) as a biocidal agent and their potential accumulation in sediments may threaten non-target natural environmental bacterial communities. In this study a microcosm approach was established to investigate the effects of well characterized OECD AgNPs (NM-300) on the function of the bacterial community inhabiting marine estuarine sediments (salinity 31‰). The results showed that a single pulse of NM-300 AgNPs (1 mg L^{-1}) that led to sediment concentrations below 6 mg Ag kg^{-1} dry weight inhibited the bacterial utilization of environmentally relevant carbon substrates. As a result, the functional diversity changed, but recovered after 120 h under the experimental conditions. This microcosm study suggests that AgNPs under environmentally relevant experimental conditions can negatively affect bacterial function and provides an insight into the understanding of the bacterial community response and resilience to AgNPs exposure, important for informing relevant regulatory measures.

1. Introduction

The rise of nanotechnology has led to an increase in the manufacturing and use of novel materials that, at the nano-scale, exhibit different characteristics to their respective bulk materials. AgNPs are an example, and, owing to their antimicrobial properties, are being incorporated into a wide variety of applications, such as health and personal care products, and also in disinfection and anti-biofouling agents, similar, for example to chlorination. However, silver is a toxic and persistent metal that can be bio-accumulated at different trophic levels (Boisson et al., 1998; Croteau et al., 2011; López-Serrano et al., 2014). As much of the AgNPs and derived forms of silver are disposed of through domestic waste water (Blaser et al., 2008), the question arises whether accumulation in the receiving estuarine environment could negatively affect the functioning of resident bacterial communities that play an important role in biogeochemical processes, such as nutrient recycling and bioremediation (Gao et al., 2011).

Toxicity data of AgNPs have been generated using a wide variety of particle types and target organisms (e.g. different bacterial species) making comparison across multiple studies difficult. In addition, environmental conditions can affect the physicochemical characteristics of the AgNPs, and, as a result their toxicity. Thus there is an urgent need for studies that model environmentally relevant and realistic conditions (Epstein et al., 2014; Stone et al., 2014). While research in the marine environment relating to the ecotoxicity of engineered nanomaterials (ENMs) in general and AgNPs in particular has gradually increased over the last decade (Baker et al., 2014), studies relating to the effects and fate of AgNPs in marine sediments are still scarce. It is particularly important to fill this knowledge gap, because estuarine sediment is one of the environmental compartments where silver is likely to accumulate (Luoma et al., 1995). For this reason, the present study established a series of microcosms with the aim of characterizing the environmental hazard of AgNPs on the functioning of the benthic bacterial community inhabiting marine estuarine sediments. The effects of AgNPs on the degradation of environmentally relevant substrates and on the bacterial functional diversity were assessed with the Biolog EcoPlate™. Living bacterial community structure and

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bacterial abundance were examined with the phospholipid fatty acid (PLFA) analysis. This is important for further understanding the potential risks of AgNPs on the ecosystem services provided by bacteria as for example nutrient recycling. The conditions in the microcosms were typically marine (salinity 31‰) as under high concentrations of chloride, silver is potentially more mobile than in fresh water (Luoma et al., 1995) and exhibit higher antibacterial activity (Gupta et al., 1998; Levard et al., 2013). The AgNPs used were NM-300, a reference nanomaterial accepted by the OECD Working Party on Manufactured Nanomaterials (WPMN) international testing programme (Klein et al., 2011).

2. Materials and methods

2.1. Silver nanoparticles

The silver nanoparticles used in the exposures were the NM-300 Silver Ø < 20 nm purchased from LGC Standards (JRCNM03000a) and supplied in an aqueous suspension. The concentration of AgNPs in the suspension was 10% (w/w) of the total weight. The stabilizing agents were polyoxyethylene glycerol trioleate and Tween 20 (all at 4% w/w) (Klein et al., 2011) and 7% NH_4NO_3 (Kermanizadeh et al., 2012). The actual size of the NM-300 AgNPs used in the present study was analysed with transmission electron microscopy (Jeol 1200 TEM; FENAC, University of Birmingham) and the surface charge or zeta potential assessed with a Zetasizer Nano (Malvern instrument). The ionic silver content of fresh AgNPs stock suspensions was measured in Milli-Q water with an ion selective electrode (ISE, Nico 2000 Ltd).

2.2. Working suspensions

Working suspensions for dosing the microcosms were prepared at an initial concentration of AgNPs of 360 mg L^{-1} (within the previously shown range of stability) in sterile Milli-Q water following the manufacturer's protocol (Klein et al., 2011), and as previously reported in the literature for other AgNP dosing experiments (Bradford et al., 2009; Kermanizadeh et al., 2012). The following modifications were made in order to ensure optimal particle dispersion in the working suspension: AgNP suspensions were vigorously shaken for 4 min and ultrasonicated twice for 15 min in an ultrasonic bath (Grant XUB25) containing ice.

2.3. Microcosm set up

The microcosms were established with intertidal sandy sediments and water samples collected from Cramond, Firth of Forth estuary (Scotland, United Kingdom, coordinates 55° 58′ 59.19″N, 3° 17′ 50.11″W). To minimize sediment exposure time to air during low tide, the bacterial communities inhabiting sediments in the present study were collected at the extreme lower eulittoral during springtides and therefore were mostly subtidal. Oxic sediments (depth 0–10 cm) were sampled with a hand-held corer that enabled the collection of the sediments with minimal perturbation and placed in cylindrical 5 L polypropylene (PP) containers (Ø 19.5 cm). All the containers for sampling and preparing the microcosms were cleaned with Decon Neutracon and 10% v/v HNO_3 and sterilized at 121 °C for 15 min. The initial nominal concentration of AgNPs in water was 1 mg L^{-1} as reported in previous studies (Bradford et al., 2009; Colman et al., 2012, 2014). To achieve this concentration 3 L of well mixed estuarine water were dosed with 8.33 ml of the initial AgNPs working suspension (360 mg L^{-1}), and then gently poured on to the surface of the sediments in each microcosm, and sediments were subsequently contaminated through precipitation of the AgNPs contained in the overlaying water. As the aqueous matrix of the AgNPs contained stabilizing agents it was necessary to investigate the effects of these separately. The dispersant or carrier control treatment was prepared similarly to the AgNPs treatment with the appropriate concentrations of sterile dispersant (JRCNM03001a). This dispersant treatment contained the same stabilizing agents as described earlier, but without AgNPs. Three different treatments (4 replicates per treatment) were established, the AgNPs treatment containing dispersant (T1), the dispersant alone or carrier control (T2), and the negative control treatment or unamended treatment (TC), the latter consisting of tanks dosed with sterile Milli-Q water instead of AgNPs solution. Continuous aeration at the surface of the sediments and along the water column with minimal sediment disturbance was achieved using a bubble-wall system to mimic aeration provided by the tide. The microcosm exposures were run at a temperature of 10 °C as samples were collected in the winter (January, 2013). Salinity (31‰), temperature, pH (7.9) and dissolved oxygen (7.8 mg L^{-1}) in the water were monitored during the course of the experiment and were stable throughout. The redox potential was measured in the first millimetres (<5 mm) of the superficial sediments and the mean value was 230 ± 14 mV. The organic content in sediments was <1%.

2.3.1. Sample collection and preservation for chemical and biological analysis

Water and sediment samples, collected at different time points (0, 24, 72 and 120 h), were preserved at −70 °C for biological analysis and at −20 °C for chemical analysis. Water samples were collected in 50 ml sterile and metal-free plastic tubes. Sediment samples were collected from the superficial (aerobic) sediments (<3 mm depth) with sterile plastic cores (5 mm diameter). The sampling areas in the sediments were delimited with an empty sterile corer in order to avoid repeatedly sampling the same spot. Three samples were taken from each tank and pooled together (total wet weight approx. 10 g). Samples collected for community-level physiological profiling (CLPP) with the Biolog Ecoplates™ were preserved at 4 °C and processed within 12 h.

2.3.2. Chemical analysis

2.3.2.1. Silver analysis. Water and sediment samples were acid-digested (USEPA-3005A, 1992; USEPA-3050B, 1996). The concentration of total silver was analysed by Atomic Absorption Spectroscopy (AAS) with a Perkin Elmer AAnalyst TM spectrometer (DL: 30 ppb). The adequacy of the digestion method was confirmed with soil reference material (Sigma Aldrich Metals in Soil, number SQC-001); the recovery of silver was between 95 and 99.6% of the expected values and always within the accepted concentrations.

2.3.2.2. Inorganic nitrogen and chemical oxygen demand (COD). The concentration of inorganic nitrogen species (ammonium, nitrite and nitrate) and COD in the overlaying water were analysed during the course of the experiment. Details of the methods are provided in the Supplementary information (SI).

2.3.3. Biological analysis of sediment samples

2.3.3.1. Community-level physiological profiles (CLPP). The Biolog EcoPlate™ (Biolog Inc., Hayward, CA) contains 31 different environmentally relevant carbon sources that are distributed in triplicate in a 96 well plate. Each well contains a single carbon substrate together with a tetrazolium dye to indicate the positive utilization (respiration) of the respective carbon source by formation of a purple coloured formazan product. Bacterial cell extraction from sediments was carried out by the addition of 10 ml of sterile water (75% sea water and 25% distilled water) to the wet sediments (2 g), vortexing and vigorous shaking (5 min). The sediment suspension