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Accumulation and effects of sediment-associated silver nanoparticles to sediment-dwelling invertebrates

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ABSTRACT

Sediment is increasingly recognized as the major sink for contaminants including nanoparticles (NPs). Thus, sediment-living organisms are especially susceptible to NP exposure. Studies of the fate and effects of NPs in the sediment matrix are still in their infancy, and data from such studies are in high demand. Here, we examine the effects of exposure to sediment mixed with either aqueous Ag (administered as $AgNO_3$) or Ag NPs (13 nm, citrate-capped) at a nominal exposure concentration of 100 μg Ag/g dry weight sediment on four benthic invertebrates: two clones of the gastropod *Potamopyrgus antipodarum* (clones A and B) and two polychaete species (*Capitella teleta*, *Capitella* sp. S). Our results show that both species sensitivity and Ag form (aqueous Ag, Ag NPs) play a role in bioaccumulation and effects. Following two weeks of exposure, both clones of *P. antipodarum* were found to be insensitive towards both Ag forms (generally low Ag accumulation, no toxicity). In contrast, the two *Capitella* species varied widely with respect to Ag uptake and observed toxicity. *Capitella* sp. S was adversely affected by both aqueous Ag (mortality) and Ag NPs (growth), whereas *C. teleta* was not affected by either Ag form. For neither polychaete species was the observed toxicity directly related to bioaccumulation. Therefore, future nano-ecotoxicological research should focus on understanding differences in uptake and handling mechanisms among species and the relationship between bioaccumulation and toxicity.

1. Introduction

Silver (Ag) nanoparticles (NPs) are widely used in various fields, such as medical science, domestic appliances, food and textile industries, and are incorporated into products, such as clothing, paints, cosmetics, health care items, refrigerators and washing machines (Benn and Westerhoff, 2008; Wijnhoven et al., 2009). Following usage, NPs can be discharged via waste water effluents, e.g., as a result of leaching of Ag during washing of Ag NP-containing clothing (Benn and Westerhoff, 2008; Kaegi et al., 2011). This raises concern about the likelihood of risk of Ag NPs released into aquatic systems, as NP production is steadily growing (Maynard, 2006), and environmental release is unavoidable (Oliver et al., 2014; Wang et al., 2014a,b). At present NP monitoring programs are lacking, however, the predicted environmental concentration of Ag NPs released into the environment from use in consumer products is about 0.01 μg/L (Tiede et al., 2009), and estimated Ag concentrations are 0.3 μg/L in freshwaters and 0.02 μg/kg in soil (Mueller and Nowack, 2008). Once released into the aquatic environment, the properties and forms of Ag NPs will influence their stability, persistence and fate in environmental matrices. Ag NPs may bind to ligands, react with dissolved and particulate organic matter (OM), aggregate/agglomerate, dissolve to release free Ag^+ ions or remain as individual NPs (Levard et al., 2012; Lowry et al., 2012). Sediment is increasingly recognized as the major environmental sink for NPs (Baun et al., 2008; Klaine et al., 2008) and sediment-dwelling organisms are thus highly relevant for assessing the fate and effects of NPs (Wang et al., 2014a,b). However, there are relatively few nano-ecotoxicology studies that have examined bioaccumulation and effects of NPs in sediment-dwellers.

Abbreviations: AAS, atomic absorption spectrometry; Ag, silver; BAF, bioaccumulation factor; BB, weight-specific body-burden; DL, detection limit; DLS, dynamic light scattering; dw, dry weight; NP, nanoparticle; PdI, polydispersivity index; SD, standard deviation.

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Sediment-dwellers can potentially be exposed to and accumulate sediment-associated contaminants, such as Ag NPs, through 1) ingestion of sediment and other food items and subsequent accumulation over intestinal epithelia, and from 2) porewater and overlying water followed by diffusion over the outer body surface. For instance, sediment has been found to be an important pathway for Ag uptake in the polychaetes *Arenicola marina* and *Nereis diversicolor* (i.e., more than 70% and 20–54% of the Ag body-burden was related to sediment ingestion, respectively; Casado-Martinez et al., 2009; Rainbow et al., 2009), but whether the same applies for Ag NPs is not known. In general, deposit-feeders meet their nutritional requirements from the organic fraction of ingested sediment, which is known to be a relatively poor food source (i.e., estuarine sediments typically contain less than 5% organic matter) (Lopez and Levinton, 1987). Therefore, these organisms have evolved feeding strategies involving ingestion of large volumes of sediment, selective feeding on smaller-sized particles, and/or complex digestive systems optimized to extract organics from sediment (e.g., Lopez and Levinton, 1987; Taghon et al., 1978). This feeding mode is likely to increase the likelihood of accumulating sediment-associated contaminants, including Ag NPs.

In the few published studies focusing on Ag NP exposure via sediment, both Ag added to sediment as aqueous Ag (i.e., dissolved form) and Ag NPs are reported to be available for uptake by benthic organisms, such as the polychaete *N. diversicolor* (Cong et al., 2011; García-Alonso et al., 2011) and the mollusk *Macoma balthica* (Dai et al., 2013). In neither of these two studies were any of the test organisms found to be severely affected at the organism-level by either Ag form, whereas for *N. diversicolor* both genotoxicity and cytotoxicity was increased for worms exposed to Ag NPs compared to aqueous Ag (Cong et al., 2011). In the published literature, discrepancies exist with regard to the extent to which Ag NP toxicity is primarily the result of release of Ag ions from the Ag NPs (Navarro et al., 2008; Völker et al., 2015) or is particle-specific (Croteau et al., 2011; Fabrega et al., 2009; Khan et al., 2012; Zhao et al., 2010). To date the mechanisms of uptake and toxicity of aqueous Ag and Ag NP forms, especially in sediment, remains to be determined.

The present study was designed to examine the bioavailability and toxicity of a single dose of aqueous Ag and Ag NPs mixed into sediment to four sediment-dwelling organisms: two clones of the gastropod *Potamopyrgus antipodarum* (clones A and B; Jacobsen and Forbes, 1997; Jensen et al., 2001) and two polychaetes belonging to the *Capitella capitata* species complex (*Capitella teleta* and *Capitella* sp. S; Blake et al., 2009; Gamenick et al., 1998). A common trait for the selected model organisms is that they may be valuable indicators of exposure to sediment-associated NPs, as they live in intimate contact with sediment that serves as both their habitat and food source. Bioavailability was assessed by measuring Ag concentration in soft tissues, and effects were assessed as changes in survival and growth following two weeks of exposure. The organisms were chosen for study, because of their abundance in estuarine and freshwater habitats (Hall et al., 2003; Méndez et al., 1997), high sediment processing rates (Cammen, 1980; Lopez and Levinton, 1987), importance as food sources for higher trophic levels (e.g., fish and birds; Dorgelo, 1987) and their important influence on the fate of sediment-associated contaminants (e.g., Madsen et al., 1997).

2. Materials and methods

2.1. General

2.1.1. Water

All experimental water used for rearing and exposure of *Potamopyrgus* spp. was moderately hard synthetic freshwater (96 mg $NaHCO_3$, 4 mg KCl, 60 mg $MgSO_4$ and 60 mg $CaSO_4{\cdot}2H_2O$ per liter) prepared in Milli-Q water (18 Millipore $\Omega\,cm^{-1}$, Millipore) with pH of 7.4 after 24 h of aeration, according to the United States Environmental Protection Agency guideline (US EPA, 2002). *Capitella* spp. were reared and exposed in seawater (<200 μm, salinity of 31‰) and salinity was kept stable by addition of Milli-Q water as necessary. Both freshwater and seawater were aerated for at least 24 h before use.

2.1.2. Sediment

Sediment for all experiments and cultures was collected from Isefjorden, Denmark (55°40'27"N, 11°48'53"E). The top few centimeters of the sediment surface were scraped off, and transported to Roskilde University. The clean, natural sediment was wet-sieved using deionized water (to <250 μm for stock cultures and to <125 μm for experiments). After settling, the overlying water was replaced with either moderately hard freshwater or seawater (Section 2.1.1), and sediment and water were mixed and allowed to settle (repeated three times to saturate the sediment with the overlying test water). Overlying water was removed and the sediment kept frozen at −20 °C until sediment spiking. The sieved experimental sediment had an organic matter content of 6.1% ± 0.8% ($n = 3$, determined as weight loss on ignition at 550 °C for 6 h), and carbon and nitrogen contents of 4.6% ± 0.3% and 0.5% ± 0.1%, respectively ($n = 3$, both determined by elemental analysis of samples of known dw using a Carlo Erba model EA 1110CHNS element analyzer, CE Instrument).

2.2. Test organism culturing

C. teleta (previously *Capitella* sp. I) originated from Setauket Harbor, New York, USA and were identified by J. Grassle (Blake et al., 2009), whereas *Capitella* sp. S ("small") originated from the North Sea, Germany (i.e., near the island of Sylt) (Gamenick et al., 1998). The field strains of *P. antipodarum* were collected from the freshwater lake Buresø, Denmark (clone A) and at an inlet to Isefjorden, Salvadparken, Denmark (clone B), respectively. These four deposit-feeders mainly reside in the upper few cm of the sediment surface, where they feed on benthic diatoms and other living and non-living organic matter associated with ingested sediment particles. Like most deposit feeders, these species ingest several times their own body weight in sediment daily, making them susceptible to exposure to sediment-associated contaminants. All species were maintained in separate stock cultures (<250 μm sediment overlaid by aerated water) in the laboratory under constant conditions (17 °C, light:dark cycle of 12:12 h) for a minimum of one year before use. For each species, individuals were taken from the established laboratory stock cultures, and transferred to new aquaria (10 L, one aquarium per species) containing field-collected sediment (ca. 3 cm layer, <250 μm, pre-frozen) and continuously aerated water. The new worm and snail stock cultures were maintained for three months under constant conditions (17 °C, light:dark cycle of 12 h:12 h) prior to the start of the experiment. During this period, supplemental food (mixture of ground commercial fish food (Tetramin, Tetra Werke), dried baby cereal (Milpo, Milupa A/S) and dried spinach in equal ratios by weight) was added once a week during this period, and approximately one quarter of the overlying water was renewed once a month.

2.3. Characterization of Ag NPs

Citrate-stabilized Ag NPs with a nominal size of 10–15 nm were synthesized by the Joint Research Centre (JRC, Ispra, Italy) as a suspension of 0.5 mM. The Ag NPs were synthesised by the $NaBH_4$ reduction of $AgNO_3$ dissolved in a solution of sodium citrate. All reagents used were of analytical grade or better and purchased from Sigma–Aldrich.The synthesis procedure was as follows: 250 ml of