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Research paper

The bioaccumulation of silver in *Eisenia andrei* exposed to silver nanoparticles and silver nitrate in soil

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ABSTRACT

The bioaccumulation potential of silver from exposure to $AgNO_3$ and Ag nanoparticles (AgNP) was investigated. In the first exposure, the bioaccumulation of Ag in *Eisenia andrei* was compared between $AgNO_3$ (1.09 mg Ag kg^{-1} dry soil) and $AgNP_A$ (20 nm, PVP-coated at 3.90 mg Ag kg^{-1} dry soil) when amended into a natural field soil. In a second experiment, $AgNP_B$ (40 nm, PVP-coated) was added to biosolids, aged for 3 d, and then mixed into the field soil (77.95 mg Ag kg^{-1} dry soil). Results demonstrated very low bioaccumulation potential for all exposure scenarios, producing bioaccumulation factors (BAF_k) of 0.89 and 0.74 for with $AgNP_A$ (20 nm) and Ag^+ (as $AgNO_3$), respectively, in soil, and 0.12 $AgNP_B$ in biosolids-amended soil. Earthworms exposed to $AgNP_B$ in the biosolids-amended soil showed reduced tissue Ag concentrations following the 21-d elimination period compared to the earthworms from the $AgNP_A$ exposure in soil. Uptake of Ag into tissue was not related to the measureable Ag^+ within test soil extracts, and evaluation of earthworm tissue via transmission electron microscopy (TEM) confirmed the presence of AgNP within exposed test organisms, despite differences in nanoparticle size, exposure concentrations and matrices between the tests. The growing evidence of accumulation of AgNP within test organisms warrant further long-term research efforts to evaluate the significance of the long-term fate and effects of AgNPs in general.

1. Introduction

Soil represents a likely environmental sink for many manufactured nanomaterials either through aerial deposition, or from the application of biosolids contaminated with nanomaterials (Gottschalk et al., 2013; Hendren et al., 2013; Keller and Lazareva, 2014; Tourinho et al., 2012; Kaegi et al., 2011; Kim et al., 2010; Klaine et al., n.d.). Silver nanoparticles (AgNP) are the most commonly manufactured nanomaterials and, due to their extensive use in everyday consumer and household products, they are expected to accumulate within biosolids as a result of the waste water treatment processes used throughout much of the developed world (Keller and Lazareva, 2014; Benn et al., 2010). Exposure studies suggest that engineered AgNP will accumulate in biosolids, but will likely be transformed to Ag_2S or coated in organics, resulting in a reduction of bioavailability and subsequent toxicity (Kaegi et al., 2011; Kim et al., 2010; Coleman et al., 2013; Brunetti et al., 2015; Kaegi et al., 2015; Lombi et al., 2013; Gitipour et al., 2013; Whitley et al., 2013; Levard et al., 2012; Lowry et al., 2012). It is unclear if the suggested transformations are sufficient to mitigate risk to the soil environment with implications for potential longer term effects (Levard et al., 2012; Stegemeier et al., 2015; Sekine et al., 2014). Many studies have examined the toxicity of AgNP to soil invertebrates (Coleman et al., 2013; Velicogna et al., 2016; van der Ploeg et al., 2014; Waalewijn-Kool et al., 2014; Schlich et al., 2013; Tsyusko et al., 2012; Coutris et al., 2012; Shoults-Wilson et al., 2011a; Shoults-Wilson et al., 2011b), and those that consider long term or sublethal effects, often find these endpoints to be much more sensitive to the nanomaterials when compared to a soluble metal form (e.g. $AgNO_3$) (Velicogna et al., 2016; Shoults-Wilson et al., 2011a; Diez-Ortiz et al., 2015a; Gardea-Torresdey et al., 2014). Recent research demonstrates that the route of exposure and uptake in oligochaetes may not be based solely on bioavailable Ag^+, highlighting the need to understand the bioavailability of AgNP at sublethal levels and the ability of earthworms to internalize and eliminate the Ag material once taken up(Diez-Ortiz et al., 2015b; Makama et al., 2015).

A bioaccumulation test, which is conducted at or below sublethal effect levels, measures the potential for organisms to take up potentially harmful substances, and whether those materials are accumulated or eliminated (e.g., metabolized or depurated), and to what extent. This approach has been used in a limited way to examine AgNP but few published studies examine the bioaccumulation potential of AgNP to oligochaetes in a natural soil, or through biosolids amendment. There is some evidence that Ag from AgNP may be more likely to accumulate in tissues compared to Ag from $AgNO_3$ (Schlich et al., 2013; Coutris et al., 2012),

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however there is no consensus(Garcia-Alonso et al., 2011). Similar bioaccumulation factors have been reported for Ag as a result of exposure to AgNP of varying sizes (nm – μm), coatings and exposure routes as for $AgNO_3$ for some soil invertebrates(Coleman et al., 2013; Tourinho et al., 2016), while others(Schlich et al., 2013; Shoults-Wilson et al., 2011a) have found greater bioaccumulation factors and increased body burden of Ag in organisms exposed to $AgNO_3$ compared to those exposed to AgNP. More recently, Makama et al. (2016) found that the coating and charge of AgNP can influence the rate of Ag uptake in *L. rubellus* more so than the size or form of Ag.

To complement a series of toxicity tests wherein the effect of silver from different exposure scenarios were considered(Velicogna et al., 2016), a bioaccumulation test was performed to determine the uptake and elimination kinetics of Ag in *Eisenia andrei* following exposure to AgNP (20 nm) and $AgNO_3$, for which both substances were spiked directly into the test soil. In addition, a second study was conducted to examine the uptake and elimination kinetics of Ag when *E. andrei* were exposed to soils amended with biosolids contaminated with AgNP (40 nm), given that silver exposure will be primarily through this mechanism for soil invertebrates.

2. Material and methods

2.1. Test materials

A field-collected (Vulcan, Alberta) sandy loam was used for all exposure studies. The soil had a pH of 5.8 (0.01 M $CaCl_2$); organic matter content of 2.6%; and a grain size distribution of 8.6% clay, 75% sand and 16% silt. The soil was air-dried, sieved to <4 mm and homogenized, and was mixed to an optimal moisture content of 25% (i.e., 50% of the maximum water holding capacity, which was also 50%). For the first experiment with no biosolids, silver nitrate ($AgNO_3$) (CAS 7761-88-8) was purchased from Sigma Aldrich, and the AgNP (CAS 7440-22-4) were purchased as dry powder from NanoAmor (USA) as 20 nm with 0.3% polyvinylpyrrolidone (PVP) ($AgNP_A$); humic acid (CAS 1415-93-6) (Sigma-Aldrich) was also purchased for the creation of a dispersion for spiking. For the secondary test utilizing biosolids, the AgNP (CAS 7440-22-4) were purchased as from NanoComposix (USA) as 40 nm with 88% PVP (by mass) dry powder ($AgNP_B$) (manufacturer supplied size as 38.6 nm; SD = 9.8 nm (TEM)).

Initial characterization of particle size distribution for both AgNP products was determined using dynamic light scattering (DLS) (Malvern Zetasizer ZS), and transmission electron microscope (TEM) (FEI Technai G2) imaging with energy dispersive x-ray spectroscopy (EDS) (Oxford Instruments Inca Energy TEM 250 Microanalysis System). Initial characterization of $AgNP_A$ was determined in aqueous dispersions containing humic acid for addition to the soils. Characterization of $AgNP_B$ was determined in both an aqueous dispersion (for amendment of the biosolids), as well as in a biosolid extract to characterize the AgNP that was being added to the soil. Biosolids extracts were prepared by spiking biosolid "cake" (20% dry mass) with an aqueous AgNP dispersion (4.7 g L^{-1}, ultrasonication 10 min, bath) using a ratio of 1 g cake: 3 mL dispersion, to create a slurry which was sealed and aged in the dark 3d at room temperature (~20 °C). The slurry was then mixed with 15 mL of Nanopure water on a rotary mixer for 2 h, centrifuged at 2800 ×*g* for 10 min and passed through a 0.45 μm nylon syringe filter. Because the filtrates were noticeably dark with dissolved organic matter, the nanoparticulate fraction was separated from the dissolved fraction by passing 1.5-mL sub-samples through a centrifugal filter (Amicon ® Ultracel 3K, cellulose) at 4000 ×*g* for 30 min, after which the supernatant was discarded and the particles were recovered and diluted 2× with Nanopure water. In total, the following bioaccumulation tests were conducted: (i) silver exposure in soil with no biosolid amendment using either $AgNO_3$ (to represent an ionic Ag^+ exposure) and 20 nm Ag nanoparticles ($AgNP_A$); and (ii) silver exposure in a soil amended with biosolids that contained 40 nm Ag nanoparticles ($AgNP_B$).

2.2. Soil amendment, no biosolids

The 20 nm Ag product ($AgNP_A$) did not readily disperse in water and was therefore added to a 2% humic acid (HA) solution prior to addition to the test soil; the methodology is described elsewhere(Velicogna et al., 2016). Briefly, 1 g of HA was added to 2 L of deionized (DI) water with sufficient 0.02 M NaOH to adjust the pH to 9.5 (±0.5), and mixed on a magnetic stirrer/hotplate at 50 °C until the pH reached 7 (±0.5); the solution was then filtered through a P5 paper filter (Fisher Scientific) to remove any undissolved HA particulates. The $AgNP_A$ were then added to the HA solution to create a 6.3 mg L^{-1} dispersion, sufficient to spike soils to the desired test concentration, and sonicated in a bath for 1 h. The HA-$AgNP_A$ suspension was then added to air-dried test soil, along with a sufficient volume of DI water to bring the soil to its optimal moisture content. For the $AgNO_3$ exposure, the $AgNO_3$ was dissolved in DI water, then added to test soil, and mixed with additional DI water to make up the optimal moisture content. The Ag spiked soil pH was measured at 5.88 and 6.76 (0.01 M $CaCl_2$) for the $AgNO_3$ and $AgNP_A$ treatments respectively. The test concentrations were selected based on prior sublethal effect tests with *E. andrei* that had investigated effects on reproduction (Velicogna et al., 2016); the final soil concentrations were 3.90 ($n = 3$, SD = 0.19) and 1.09 ($n = 4$, SD = 0.17) mg Ag kg^{-1} dry soil for the $AgNO_3$ and $AgNP_A$ treatments, respectively. The concentrations were selected to avoid toxic concentrations (i.e., below sublethal levels).

2.3. Biosolids-soil amendment

The $AgNP_B$ (40 nm) powder was prepared as a 10 g L^{-1} aqueous dispersion with nanopure™ water (>18 Ω cm Barnstead™ 4 cartridge water filtration system) that was added directly to the container as received from the supplier (to reduce loss of material from transfer). The total Ag concentration was verified by ICP-MS analysis of a diluted subsample (prepared in 2% HNO_3). The biosolids were obtained from the St. Hyacinth (Quebec) water treatment facility as a 20% solids (by mass) cake. The biosolids had a pH of 7.97, and organic matter content of 54%, a sulfate content of 3.6×10^{-3} M, and a total Ag content of 7.32 mg kg^{-1}. The biosolids were frozen upon collection and stored at −20 °C until use. One week prior to the test start, a sufficient mass of biosolids was weighed into a polypropylene container, sealed and stored at room temperature (~20 °C) in the dark for 3 d to thaw; the mass was sufficient to amend the soil at a rate of 10 g biosolids kg^{-1} dry soil, as per the Ontario Ministry of Environment Guidelines for the Utilization of Biosolids and Other Wastes on Agricultural Land (Ontario Ministry of Environment (MOE) and Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), 1996). After 3 d, the biosolids were spiked with the $AgNP_B$ dispersion and equilibrated for an additional 3 d under air-tight conditions, at ~20 °C in the dark; the subsequent redox potential of the biosolids measured was 4.97 to 4.96 mV. The spiked biosolids were then mixed with the air-dried test soil, and Nanopure™ water was added to bring the soil up to the optimal moisture content. The biosolid amended test soil had a pH of 6.4 (0.01 M $CaCl_2$). A higher test concentration was used for the biosolids experiment as transformation of the Ag within the biosolids (e.g., through sulfidation and other mechanisms) was expected to reduce the bioavailability of Ag in general; the resultant test concentration was 77.95 mg Ag kg^{-1} dry soil ($n = 3$, SD = 2.78).

2.4. Bioaccumulation experiments

Bioaccumulation tests were conducted following the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals: Bioaccumulation in Terrestrial Oligochaetes