Ecotoxicology and Environmental Safety 140 (2017) 148–155

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

How do physicochemical properties influence the toxicity of silver nanoparticles on freshwater decomposers of plant litter in streams?

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ARTICLE INFO

Keywords:
Silver nanoparticles
Size and coating
Leaf litter decomposition
Microbial decomposers
Invertebrate shredders
Feeding behavior

ABSTRACT

AgNP physicochemical properties may affect AgNP toxicity, but their effects on plant litter decomposition and the species driving this key ecosystem process in freshwaters have been poorly investigated. We assessed the impacts of AgNPs with different size and surface coating (100 nm PVP (polyvinylpyrrolidone)-dispersant, 50–60 nm and 35 nm uncoated) on freshwater decomposers of leaf litter by exposing leaf associated microbial assemblages to increasing concentrations of AgNPs (up to 200 mg L^{-1}) and of $AgNO_3$ (up to 25 mg L^{-1}). We further conducted a feeding preference experiment with a common invertebrate shredder, *Limnephilus* sp., which was allowed to feed on microbially-colonized leaves previously exposed to AgNPs and $AgNO_3$. Leaf decomposition and microbial activity and diversity were inhibited when exposed to increased concentrations of 100 nm AgNPs ($\geq$25 mg L^{-1}), while microbial activity was stimulated by exposure to 35 nm AgNPs ($\geq$100 mg L^{-1}). Invertebrate shredders preferred leaves exposed to 35 nm AgNPs (25 mg L^{-1}) and avoided leaves exposed to $AgNO_3$ ($\geq$2 mg L^{-1}). Results from the characterization of AgNPs by dynamic light scattering revealed that AgNps with PVP-dispersant were more stable than the uncoated AgNPs. Our results highlight the importance of considering the physicochemical properties of NPs when assessing their toxicity to litter decomposers in freshwaters.

1. Introduction

Silver nanoparticles (AgNPs) have been increasingly used over the last decade (PEN, 2013) mainly due to their strong antimicrobial activities and other unique physiochemical properties (catalytic activity, Jiang et al., 2011; specific electronic properties, Dubey et al., 2009). AgNPs are incorporated in textiles, detergents, personal health care products, and also have several medical applications. In Europe, the production of AgNPs in 2012 was 5.5 t (Piccinno et al., 2012), and the Nanoparticle Database reported that 68 AgNP products exist among the 645 commercialized single-element NPs worldwide (Nanowerk, 2015). The growing production of AgNPs arouse the concern of their release into freshwaters, where AgNPs and ionic Ag released from these NPs might be toxic to aquatic species (Moore, 2006; Navarro et al., 2008a; Fabrega et al., 2011) compromising the ecological processes they drive (e.g., organic matter decomposition, Pradhan et al., 2011).

The decomposition of allochthonous organic matter, such as riparian plant litter, is a key process in freshwater ecosystems. This organic matter is degraded by fungi and bacteria and, subsequently, incorporated into food webs (Graça, 2001). Fungi, particularly aquatic hyphomycetes, play a key role in plant litter decomposition in streams (Baldy et al., 2002; Pascoal and Cássio, 2004) because they produce a variety of extracellular enzymes that degrade complex polysaccharides of plant cell walls, including cellulose, hemicellulose and lignin, making leaf material a more appropriate source of carbon and energy for invertebrate shredders (Suberkropp, 1998), which in turn transfer energy to higher trophic levels (Graça and Canhoto, 2006).

The few studies on the impacts of AgNPs on plant litter decomposition showed that leaf decomposition, microbial biomass and fungal reproduction are inhibited by exposure to nano and ionic Ag (Pradhan et al., 2011). These studies suggested that the increased release of nano metals to the environment might affect aquatic microbial communities with impacts on organic matter decomposition in streams. On the other hand, considerable number of studies have reported lethal toxicity of ionic and nano Ag to aquatic invertebrates mainly through waterborne exposure (Zhao and Wang, 2012; Blinova et al., 2013; Silva et al., 2014; Ali et al., 2014), but less is known on sublethal effects such as their feeding activity (Croteau et al., 2011; Zhao and Wang, 2011; Mouneyrac et al., 2014).

Another concern is the behavior of AgNPs in water that is known to

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http://dx.doi.org/10.1016/j.ecoenv.2017.02.039
Received 31 October 2016; Received in revised form 17 February 2017; Accepted 24 February 2017
Available online 08 March 2017

be influenced either by physical and chemical water parameters, such as temperature, ionic strength and pH (Walters et al., 2013; Siripattanakul-Ratpukdi and Fürhacker, 2014) or by physical and chemical characteristics of NPs, such as particle hydrophobicity, concentration and size (Navarro et al., 2008b; Sharma et al., 2014; Zhang et al., 2016). The toxic effects of AgNPs have shown high inter-study variability, even contradictory effects, which may be partly explained by differences in particle size and specific surface area, NP shape and aggregation, composition of test media among others (Siripattanakul-Ratpukdi and Fürhacker, 2014). There is evidence that AgNPs with smaller size tend to have higher toxicity (Scown et al., 2010; Angel et al., 2013; Silva et al., 2014). However, AgNPs with large diameter (10 nm) shortened the lifespan of *Caenorhabditis elegans*, causing lethal damage, whereas the small size (2 nm) of AgNP only affected nematode fertility (Contreras et al., 2014). Also, the presence of coating greatly influences the fate, stability and toxicity of AgNPs (Zhao and Wang, 2012; Sharma et al., 2014). In freshwater algae and daphnids the citrate-coated AgNPs were more toxic than the PVP (polyvinylpyrrolidone) -coated AgNPs despite undergoing much greater aggregation (Angel et al., 2013), indicating that the type of coating affects toxicity while aggregation does not.

The aim of this study was to assess the impacts of particle size and coating of AgNPs on freshwater microbial decomposers of plant litter. To that end, microbial communities associated with leaf litter were exposed for 28 days to 4 concentrations of AgNPs with 3 different sizes (100 nm PVP (polyvinylpyrrolidone)-dispersant, 50–60 nm and 35 nm uncoated). The toxicity of $AgNO_3$ was compared to that of Ag ions released from AgNPs to determine if the observed toxicity was due to Ag dissolution from AgNPs or was particle specific. In addition to clarify how particle size and concentration of AgNPs could affect the feeding behavior of organisms that depend on the activity of microbial decomposers, a feeding preference experiment with an invertebrate shredder was performed.

We expected that i) the coated AgNPs would show higher physical and chemical stability than the non-coated AgNPs, so they would be less reactive and toxic, ii) toxicity would increase with decreasing AgNP size and increasing concentrations of AgNPs and $AgNO_3$, and iii) invertebrate shredders would avoid to feed on leaves previously exposed to AgNP or $AgNO_3$. Information on the comparative toxicity across different sizes, coating and concentrations of AgNPs will help us to better evaluate the impacts of AgNPs on freshwater ecosystems.

2. Material and methods

2.1. Experimental design

Leaves of *Quercus robur* L. (oak), collected in September 2011, were air dried and kept at room temperature. The leaves were leached in deionized water for 2 days and cut into 12 mm diameter disks. Sets of 60 leaf disks were placed into fine-mesh bags (0.5 mm pore size) and immersed in a low-order stream located in NW Portugal (Algeriz Stream, 41° 350′N 8°220′W), to allow microbial colonization.

During leaf immersion, conductivity (34 μS cm^{-1}), pH (6.4) and dissolved oxygen concentration (98% saturation) were measured in situ using a Multiline F/set 3 no. 400327 (WTW). Stream water samples were collected and transported (4 °C) to the laboratory, and analyzed (HACH DR/2000 spectrophotometer, Loveland, CO) to determine the concentrations of nitrate (0.02 mg $N\text{-}NO_3^-$ L^{-1}), nitrite (0.0035 mg $N\text{-}NO_2^-$ L^{-1}), ammonia (< 0.01 mg $N\text{-}NH_3$ L^{-1}) and phosphate (0.01 mg PO_4^{3-}-P L^{-1}).

After 10 days of stream immersion, leaf bags were returned to the laboratory for microcosm experiments. Leaf disks were rinsed with deionized water and placed in 150 mL Erlenmeyer flasks with 80 mL of sterile stream water. The information on the stream water used in the microcosms experiment can be found in Pradhan et al. (2015b).

To test for AgNPs and $AgNO_3$ toxicity, the microcosms were supplemented with increasing concentrations of AgNPs of different sizes (35 nm, 50–60 nm and 100 nm) or ionic metal as follows: 25, 50, 100 and 200 mg L^{-1} of AgNPs; and 2, 5, 15 and 25 mg L^{-1} of $AgNO_3$ ($AgNO_3$, > 99%, Sigma-Aldrich, St. Louis, MO). All treatments were done in triplicates and additional microcosms not supplemented with AgNPs were used as control. The microcosms were kept under shaking (120 rpm) at 16 °C and solutions were renewed every 7 days until the end of the experiment. After 28 days, leaf disks were freeze-dried to determine leaf mass loss, fungal biomass and diversity as described below.

2.2. Preparation and characterization of AgNPs

AgNPs tested were: AgNP spherical particles with a particle size < 100 nm, containing PVP (polyvinylpyrrolidone) as dispersant, specific surface area of 5 m^2/g, and purity of 99.5% based on trace metal analysis (CAT no. 7440-22-4, from Sigma-Aldrich, St. Louis, MO); AgNP spherical particles with a particle size 35 nm, specific surface area of 30–50 m^2/g and purity of 99.5% based on trace metal analysis (NM-0023-HP-0010, IoLiTec Ionic Liquids Technologies, GmbH, Germany); and AgNP spherical particles with a particle size 50–60 nm, specific surface area of ~12 m^2/g, and purity of 99.9% based on trace metal analysis (NM-0038-HP-0010, IoLiTec Ionic Liquids Technologies, GmbH, Germany).

The stock suspensions of each AgNPs size (35 nm, 50–60 nm and < 100 nm) was prepared according to Pradhan et al. (2012). The hydrodynamic diameters of the AgNPs in the stock suspensions, in the medium freshly prepared and after 28 days of exposure were measured by dynamic light scattering (DLS) using a Zetasizer (Nano ZS, Malvern Instruments Ltd., Worcestershire, UK). The zeta-potential of the AgNP suspensions was measured using the Zetasizer.

2.3. Metal analysis

Total Ag concentration in the medium and leaf disks was determined (CACTI, University of Vigo, Spain), after acid digestion, by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 4300 DV, U.S.). Dissolved Ag from the AgNP suspensions was determined after ultrafiltration (30 min at 3220 g, Megafuge 1.0R, Thermo Scientific Inc., Waltham, MA) using Ultracel 3k Centrifugal Filter Devices (Amicon Millipore) with a molecular cutoff of 3 kDa (pore size < 2 nm) and the Ag concentrations in the filtrate were measured for total Ag.

2.4. Leaf decomposition

To determine leaf mass loss, freeze-dried (Christ alpha 2–4; B. Braun, Melsungen, Germany) leaf disks from each replicate before and after stream colonization, and after microcosm exposure were weighed to the nearest 0.001 mg.

2.5. Activity of plant litter degrading enzymes

The extracellular enzymes analyzed were β-glucosidase involved in the last step of cellulose degradation; and phenol oxidase involved in the break down of plant fibers such as lignin. The activity of the extracellular enzymes was measured at 2 intermediate concentrations of AgNPs (25 and 100 mg L^{-1}) and $AgNO_3$ (2 and 25 mg L^{-1}).

The enzyme β-glucosidase (EC 3.2.1.21) was analyzed using fluorescent (MUF, methylumbelliferone)-linked artificial substrate (MUF-β-D-glucopyranoside, Sigma). Colonized leaf disks (2 disks per microcosm) were incubated at saturating concentrations of the substrate (0.3 mM) for 1 h in the dark at 16 °C immediately after retrieval from the microcosms. Blanks and standards of MUF (0–100 μM) were also incubated. At the end of the incubation, glycine buffer (pH 10.4) was added (1:1 vol/vol), and the fluorescence was measured at 455 nm