



Effect of sulfidation and dissolved organic matters on toxicity of silver nanoparticles in sediment dwelling organism, *Chironomus riparius*



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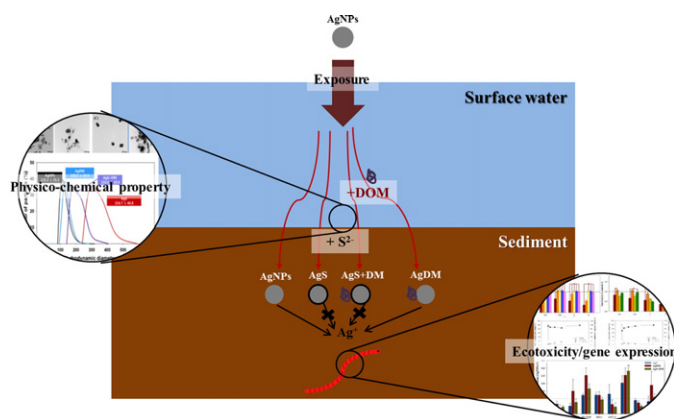
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HIGHLIGHTS

- The effect of S^{2-} and DOM on toxicity of AgNPs in *C. riparius* was investigated.
- S^{2-} treatment affected dispersion of AgNPs and reduced toxicity in *C. riparius*.
- DOM did not affect dispersion of AgNPs and modulate toxicity in *C. riparius*.
- S^{2-} affected distribution of Ag to environmental and biotic compartments.
- These results reinforce importance in S^{2-} in ecotoxicity of AgNPs in sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

The properties, fate, and toxicity of silver nanoparticles (AgNPs) are readily modified in the environment. Thus, in order to predict the environmental impact of AgNPs, the toxicity test should be conducted to assess the interactions of AgNPs with environmental matrices. Dissolved organic matter (DOM) is known to mitigate AgNPs toxicity in natural systems, and it is also known that silver binds strongly to sulfur. Little is known, however, about the effect of sulfidation and to what extent it could compete with DOM in the sediment. We therefore investigated the effect of sulfide on a sediment dwelling organism, *Chironomus riparius* using ecotoxicity endpoints. We then investigated how sulfide and a combination of sulfide and DOM affect the toxicity of AgNPs in *C. riparius*. We also monitored the concentrations of silver in the water and sediment compartments, as well as in *C. riparius* tissue, in the presence and absence of sulfide. Finally, in order to investigate how sulfide and DOM affect the release of ions from AgNPs, we also monitored released Ag⁺ in each treatment. In the presence of sulfide, AgNPs were found to be less toxic to *C. riparius* in acute and chronic endpoints than AgNPs alone, whereas DOM treatment did not modulate the toxicity of AgNPs. Sulfide treatment reduced the release of Ag⁺ from AgNPs. Water-spiked AgNPs with sulfide were found to be more slowly incorporated into both sediment and larvae as compared to the AgNP alone. Overall, the results suggest that the presence of sulfide in sediment mitigates the ecotoxicity of AgNPs in *C. riparius*.

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1. Introduction

Much concern has been raised over the increased use of silver nanoparticles (AgNPs) in various fields, due to their adverse impact to the environment. In order to assess and manage the environmental risks of nanoparticles, there is a growing need to evaluate their ecotoxicity in an environmentally relevant setting. In this context, we studied the effects of frequently occurring ligands on AgNPs toxicity in aquatic environments. It has been hypothesized that Ag^+ ions released from AgNPs may have a substantial toxic effect on the environment (Fabrega et al., 2011), and the reduction of Ag^+ by water chemistry indicators could partially mitigate AgNPs toxicity (El Badawy et al., 2010; Levard et al., 2013a; Levard et al., 2013b). In aquatic environment, water chemistry indicators and ligands such as sulfide, dissolved organic matter (DOM) and chloride, have been shown to mitigate the toxicity of AgNPs. For instance, Choi et al. (2008) and Levard et al. (2013b) demonstrated that chloride reduced the growth inhibition toxicity of *Escherichia coli* caused by AgNPs. Other studies using fish embryos, *Caenorhabditis elegans* and *Daphnia magna* also showed that humic acid from DOM or sulfide mitigated the toxicity of AgNPs owing to their strong ability to bind Ag^+ ions (Levard et al., 2013a; Poda et al., 2013). Levard et al. (2013a) demonstrated that production of Ag_2S by sulfidation of AgNPs can reduce the release of Ag^+ ions from AgNPs, hence reducing toxicity. Poda et al. (2013) showed that negatively charged carboxylates in humic acid are also capable of reducing the toxicity of AgNPs by scavenging Ag^+ ions.

Although there have been a number of studies on the sulfidation of Ag surface, little is known about its fate in the sediment compartment of aquatic environments, and even less regarding its toxicity to sediment dwelling organisms. In the aquatic environment, the sediment appears to be the ultimate repository of AgNPs (Gottschalk et al., 2009). Although appreciable levels of sulfide and DOM occur in sediment (Deonaraine and Hsu-Kim, 2009), little is known about their mitigating effects upon AgNP toxicity.

Chironomus riparius is a sediment-dwelling, detritus-feeding organism, and its larvae are widely used in sediment toxicity experiments, being a recommended sediment toxicity species of the OECD (Meregalli et al., 2000; OECD, 2004). The *C. riparius* larva undergoes four instars, which utilize any available food source within their range, consuming sand and silt, as well as algae and detritus (Rasmussen, 1984; Naylor and Rodrigues, 1995). Although, larvae are especially vulnerable to chemicals and toxicants that have settled to the sediment surface, limited studies are available on the toxicity of chemicals, particularly AgNPs, in the larval stage of invertebrates. Chan and Chiu (2015) reported that the exposure of marine benthic invertebrate larvae (the barnacle *Balanus amphitrite*, the slipper-limpet *Crepidula onyx*, and the polychaete *Hydroides elegans*) to coated AgNPs affect their growth, development, and settlement. It was also reported that early larval stages of the marine annelid worm *Platynereis dumerilii* are more vulnerable to AgNPs exposure than are its adult stages (García-Alonso et al., 2014).

In this study, we investigated the effect of both sulfide and DOM on the toxicity of AgNPs in the larvae of *C. riparius*. 1) Acute and chronic toxicity tests of AgNPs were first conducted with or without sulfide and DOM. 2) In order to understand the mechanism of the reduction of AgNP toxicity by sulfide, the dissolution of AgNPs and oxidative stress genes expression were investigated. 3) Finally, to mirror field conditions more accurately, further chronic toxicity test was conducted using field sediments collected from an urban polluted stream containing high levels of sulfide.

2. Materials and methods

2.1. Test organisms and media

Chironomus riparius were obtained from the Toxicological Research Center of the Korea Institute of Chemical Technology and have been

reared in our laboratory for more than 10 years. The larvae were reared on an artificial diet of fish food flakes (Tetramin; Tetrawerke) in glass chambers containing dechlorinated tap water and acid-washed sand, with aeration at 20 ± 1 °C under a 16:8-h light:dark photoperiod. All experiments were conducted in US Environmental Protection Agency moderately hard water (EPA-MHW) (USEPA, 1993).

2.2. Preparation of Ag exposure media, DOM and sulfide

AgNPs (described by the vendor as having a size < 100 nm; Sigma-Aldrich Chemical, St. Louis, MO) were homogeneously dispersed in deionized water by sonication (Branson-5210 sonicator; Branson) for 13 h at maximum power, stirring for 7 days, then filtered through a cellulose membrane (pore size 100 nm, Advantec; Toyo Toshi Kaisha) to remove NP aggregates. The preparation method was optimized to ensure that aged AgNPs were obtained. The final concentrations of Ag in the aged-AgNPs solution were estimated using multitype inductively coupled plasma emission spectrometer (ICPE-9000, Shimadzu, Tokyo, Japan).

Suwannee River Natural Organic Matter (NOM, 1R101N) was purchased from the International Humic Substances Society (IHSS). Stock solutions were prepared at 0.5 g/L in EPA-MHW and dissolved overnight using an end-over-end rotator at room temperature then filtered with an Advantac 0.45 μm mixed cellulose ester membrane (Toyo Roshi Kaisha, Ottawa, Tokyo). The NOM concentration was obtained by measuring dissolved organic carbon (DOC) (TOC-5050A, Shimadzu Instruments, MD). The inorganic carbon was removed from the samples by adding 6 N HCl then purging with N_2 gas for 5 min. The Suwannee River NOM contained 43.7% organic carbon; therefore, 0.437 mg/L DOM was converted to 1 mg/L NOM. All experimental results were presented as DOM concentrations.

Reduced sulfide solutions were prepared at preset S^{2-} concentrations (0, 0.1, 0.5, 1, and 1.5 mg/L) of Na_2S solution in EPA-MHW. AgNPs, DOM and reduced sulfide solutions were stored at 4 °C.

2.3. Characterization of AgNPs with or without sulfide and DOM

The particle shape and elemental composition of the AgNPs were determined using a transmission electron microscope (TEM; JEM-3010, JEOL Ltd., JAPAN) and an energy dispersive spectrometer (EDS; Oxford). The hydrodynamic size distribution of the particles was determined by dynamic light scattering (ELSZ-1000, Otsuka electronics, Japan).

All the analyses were conducted using four different treatments, namely, AgNPs alone (denoted as AgNPs), AgNPs with sulfide (denoted as AgS), AgNPs with DOM (denoted as AgDM) and AgNPs with sulfide and DOM (denoted as AgS + DM).

2.4. Acute toxicity test in AgNPs with or without sulfide and DOM

2.4.1. Mortality test

A mortality test was conducted using a modified OECD guideline (TG 235) (OECD, 2011). Ten fourth-instar larvae were exposed to each of the four controls (water, sulfide, DOM, sulfide + DOM) as well as the four AgNPs treatments (0.2, 0.5, 1 and 2 mg/L), the four AgNPs + sulfide treatments (0.1, 0.5, 1 and 1.5 mg/L), the single AgNPs + DOM treatment (4.37 mg/L) and the single AgNPs + sulfide + DOM treatment (0.1 mg/L and 4.37 mg/L) in 100 mL USEPA-MHW. Mortality was determined after 24 h of exposure.

2.5. Chronic toxicity test in AgNPs with or without sulfide and DOM

2.5.1. Reproduction and development tests

For the chronic toxicity test, a modified OECD guideline (TG 219) was used (OECD, 2004). 0.5 mg/L of AgNPs was spiked with the EPA-MHW in either the AgNPs, AgS (0.1 mg/L of S^{2-}) or the AgDM (4.37 mg/L of DOM) or the AgS + DM treatment conditions. Adult

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