Metal transfer to sediments, invertebrates and fish following waterborne exposure to silver nitrate or silver sulfide nanoparticles in an indoor stream mesocosm

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HIGHLIGHTS

• Mesocosm experiments allow a more realistic understanding of nanomaterial fate.
• Silver as AgNO₃ or Ag₂S NPs caused similar water and sediment contamination.
• Ag-containing particles were found in the water of both Ag-treatments.
• At day 14, Ag-treatment had no difference in Ag uptake in the snails or worms.
• Silver as AgNO₃ was more bioavailable to planarians and fish than Ag₂S NPs.

GRAPHICAL ABSTRACT

Are AgNO₃ or Ag₂S NPs bioaccumulative in freshwater riverine mesocosms?

Daily dosing

Waterborne Exposure

Water test

Food Chains

AgNO₃ uptake

Chromosomal damage

Ag₂S uptake

Particle settling to Sediment

Fate and Ecological Impact?

Bioaccumulation potential ranked
AgNO₃ > Ag₂S NPs.

Uptake kinetics, Target Organs and Body Distribution

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Ag+ is low. Consequently, there is a tendency for total Ag to deposit into depending on the exposure conditions (Naddy et al., 2007). In freshwaters, tebrates such as Daphnids, the acute toxicity of Ag+ is around 0.3 estimated 150 aging, food supplements, medical devices and biocides; contributing to the Ag-containing ENMs are produced annually, with applications in food pack- tions containing silver have been used for many years in photography and microbiology (Silver, 2003). Silver-containing engineered nanomaterials (ENMs) are also one of the most frequently reported ENMs in consumer products (Vance et al., 2015). Around one thousand tons or more of Ag-containing ENMs are produced annually, with applications in food pack- aging, food supplements, medical devices and biocides; contributing to the estimated 150–200 billion Euro economic value of nanotechnology (Laux et al., 2018). Inevitably, the manufacture and use of ENMs is contributing to their release into the environment. Early exposure models gave predicted environmental concentrations (PECs) for Ag nanoparticles (NPs) ranging from ng L−1 in surface waters to mg kg−1 in sediments (Fabrega et al., 2011). In Europe, the predicted mean concentration of Ag from ENMs was 4.15 ng L−1 and 88.2 µg kg−1 in surface waters and sediments respectively, by 2020 (Sun et al., 2016). Similar predictions were made for German watercourses, with an anticipated six-fold increase by 2050 (Giese et al., 2018). Field measurements are beginning to emerge, and in the Seine River watershed of France, measured concentrations ranged from 0.4 to 28.3 ng Ag L−1 (Wang et al., 2020). Clearly, there are concerns for Ag-containing ENMs in surface waters, and with sediment as a possible sink, the bioaccumulation hazard and effects on biota in real ecosystems is not clear.

Historically, the main environmental releases of dissolved silver were from mining and smelting, biocides and the photographic industry, with standards for total Ag in surface waters set around 0.1 µg L−1 (e.g., Canada, Purcell and Peters, 1999). Unfortunately, silver is the second most toxic metal to aquatic organisms in the periodic table (Ratte, 1999). For freshwater fish, the 96 h 50 % lethality concentration (LC50) for AgNO3 is 5–70 µg L−1 (0.05–0.65 µM) with the toxicity resulting from the ionic form of silver (Hogstrand and Wood, 1998). For freshwater invertebrates such as Daphnids, the acute toxicity of Ag+ is around 0.3–4 µg L−1, depending on the exposure conditions (Naddy et al., 2007). In freshwater, dissolved silver may preferentially bind to natural organic matter and/or form less soluble chloride complexes, such that the free ion activity of Ag+ is low. Consequently, there is a tendency for total Ag to deposit into sediments with a risk of bioaccumulation for the local biota (Hogstrand and Wood, 1998; Ratte, 1999). In freshwater, algae and invertebrates can have high bioconcentration factors (BCFs) for silver salts (from 4.8 to >105 for algae, 9 to >2200 for invertebrates, (Ratte, 1999)), with consequent concerns for dietary bioaccumulation in fish.

In general, Ag NPs are less toxic than silver salts such as AgNO3, with lethal concentrations for the former in the range ~360, ~10, and ~1360 µg L−1 for algae, crustaceans and fish, respectively (Bondarenko et al., 2013). Johari et al. (2013) reported a 96 h LC50 for Ag NPs in juvenile rainbow trout of around 2160 µg L−1. In freshwater invertebrates, such as Hydra vulgaris, and the shrimp, Parataxa australiensis, the 96 h LC50 values are around 1941 and 55 µg L−1, respectively (Lekamge et al., 2018). However, the extrapolation of toxicity data from laboratory studies to natural waters or field situations is challenging. Ag NPs may be adsorbed to organic matter in the water column, show aggregation depending on the factors from colloid chemistry (pH, ionic strength, presence of divalent ions, organic matter, etc.), or be chemically transformed (Dale et al., 2013; Lead et al., 2018; Levard et al., 2012; Zhang et al., 2019). Notably, pristine Ag NPs are transformed into much less toxic, but very persistent, Ag NPs when sulfide is present (e.g., in sediments), or in the reducing conditions of poorly oxygenated water (Dale et al., 2013; Lead et al., 2018). Indeed, pristine Ag NPs are rapidly transformed into Ag2S NPs during wastewater treat- ment processes (Kaegi et al., 2013) and Ag2S NPs are the likely form discharged to the environment. While the Ag2S form is less toxic than pristine Ag NPs (e.g., to Chironomus riparius (Lee et al., 2016); adult zebra fish, Danio rerio (Dev et al., 2015)), it is not clear if the bioaccumulation hazard remains for Ag2S NPs. In at least one study with rainbow trout, Ag2S NPs were much less bioavailable than either the metal salt or Ag NPs (Clark et al., 2019a), with similar observations made in snails and planarians (Silva et al., 2022). However, in fish at least, nanoparticles arising from the Ag2S NP exposure were also detected in the internal organs (Clark et al., 2021), indicating that some bioaccumulation of particulates occurred.

Clearly, there are a myriad of abiotic and biotic factors in natural ecosystems that may influence the fate and behaviour of ENMs (Lead et al., 2018). One useful approach has been to conduct mesocosm studies that offer some environmental realism, while also retaining the benefits of a controlled ‘laboratory’ experiment (Holden et al., 2016). Early mesocosm exposures with inert gold nanorods showed transfer of the ENMs from the water column to mainly the estuarine sediment (25 % of the dose) and biofilms (61 %), with limited accumulation in the plants and animals present (Ferry et al., 2009). Similarly, Furtado et al. (2015) found that total Ag from Ag NPs added to freshwater boreal lake mesocosms was transferred from the water column mainly to the sediments and periphyton biofilm. In wetland mesocosms, both Ag NPs and Ag2S NPs accumulated mainly in/on the surface of sediments and aquatic plant materials (Stegemeier et al., 2017). The fate of Ag ENMs in mesocosms may also depend on the
shape of the materials, and with some evidence of sulfidation of the materials in the benthic compartment (Auffan et al., 2020). However, mesocosm studies with Ag-containing ENMs have mostly been performed in static systems to simulate a lake or a pond. River mesocosms are less well understood.

In our previous work, Ag2S NPs, as the most relevant environmental transformation (form) of Ag NPs, was explored for its bioaccumulation potential in single species studies on: dicotyledonous crop, Khodaparast et al. (2022); wheat crop, Lahive et al. (2021); earthworms, Bacaro et al. (2019); meal worms, Khodaparast et al. (2021); planarians, Silva et al. (2022); aquatic snails, Silva et al. (2020) and trout, Clark et al. (2019a). In the present study, the same Ag2S NPs were used in indoor stream mesocosms. The fate and bioaccumulation of total Ag from the Ag2S NP exposure compared to that of AgNO3 was measured. The specific objectives were to analyse the water and sediment samples to confirm and understand the dynamics of the exposure, and to evaluate the bioavailable fractions of silver in the sediment using a serial extraction method. The mesocosms contained a range of invertebrates that occupied different niches and trophic levels (planarians, oligochaete worms, chironomids, snails, daphnids) and with rainbow trout as a ‘top’ predator in the food chain. The total Ag concentrations in the biota were determined in order to understand the bioaccumulation in the different organisms, and related changes in trace electrolytes in these organisms. Furthermore, where possible the study determined the particle number concentration in the water using single particle inductively coupled plasma mass spectrometry (spICP-MS).

2. Methodology

2.1. Experimental design for the mesocosm studies

The experiment was conducted in an indoor modular mesocosm system at the University of Aveiro, Portugal, in April 2018. The study design consisted of 36 artificial rivers made of glass (length: 2 m, width: 0.200 m and depth: 0.225 m). The rivers were arranged in triplicates, where each triplicate set was fed by water from the same sump (102 L) at a flow rate of 4 L min⁻¹. A water reservoir tank with 800 L capacity supplied the water to the system. The plumbing was designed such that each set of three rivers could operate on flow-through (i.e., single pass for flushing the system) or be recirculated. The latter mode was used for the experiments. Each river was filled with 7 kg of sediment (99% of sand with <2 mm grain size, previously burned at 500 °C for 4 h) mixed with grounded alder leaves (Alnus glutinosa) (1% w/w), to provide organic matter and serve as the food source for chironomid larvae. The sediment was carefully spread over the bottom of each river to a thickness of approx. 2 cm. A sandy sediment was chosen for several reasons. Firstly, ecological relevance since river ecosystems especially in Portugal do have areas of 100% sand (Balsinha et al., 2009), but mainly to minimise the risk of Ag2S NPs being further transformed by geochemical reactions with clays, silt and complex NOM (Dale et al., 2015), and pragmatically to enable speciation calculations of the Ag species present in the mesocosm. The organic matter was kept to 1% to minimise the risk of fungal growth in the mesocosm and to be consistent with our previous work. Each river was then filled with 35 L of “artificial pond water” (APW, Naylor et al., 1989; Vidal et al., 2014). The APW medium was prepared by filling the reservoir tank with deionised water and adding the following solutions: 1.99 mmol L⁻¹ CaCl2·2H2O, 0.499 mmol L⁻¹ MgSO4·7H2O, 0.77 mmol L⁻¹ NaHCO3, 0.08 mmol L⁻¹ KCl, 0.046 mmol L⁻¹ KH2PO4, 1 mmol L⁻¹ NaNO3, 0.107 mmol L⁻¹ Na2SiO3·9H2O, at a pH ranging from 7.81 to 7.90, at day zero. The final water volume in each unit (sump + 3 rivers) was approximately 207 L. The APW represents a moderately hard freshwater with measured concentrations (Table S1) of Na⁺ (~52 mg L⁻¹ or 2.26 mmol L⁻¹), K⁺ (~7.8 mg L⁻¹ or 0.2 mmol L⁻¹), Mg²⁺ (~14 mg L⁻¹ or 0.57 mmol L⁻¹), and Ca²⁺ (~83 mg L⁻¹ or 2.07 mmol L⁻¹). The rivers were initially filled with freshly prepared APW and left to acclimate for 2 days for equilibration of the water chemistry. After that, the following invertebrates were introduced in each river: Physa acuta (snail) (60 per river), Girardia tigrina (planarian) (50 per river), C. riparia (non-bit ing midge) (150 per river), Oncorhynchus mykiss (rainbow trout) (3 per river), Daphnia magna (water flea) (0.25% ration for the fish equating to approximately 13 Daphnia per river per day), Lumbricus variegatus (oligochaete worm) (approx. 900 mg fresh weight per river). The life stages were as follows: snails, 2 months old; chironomid larvae, 6 to 7 days old; adult planarians (length around 1.5–2 cm); adult oligochaete worms (~6–10 mg fresh weight), Daphnia (adults and juveniles from the culture to ensure enough food for the fish), fingerlings of rainbow trout (<5 g). Rainbow trout were kept in rectangular plastic chambers (dimensions: height 11.5 cm, length 16.5 cm, width 14 cm), weighted to keep them submerged, and with plastic mesh-covered apertures (1 mm mesh) on both sides of each chamber to allow free water movement, but also to prevent the invertebrates from entering the box. This was done so that the food items eaten by the fish in each river could be managed and to restrict the fish to the water column. Similarly, for the unexposed control and Ag2S NP treatments, the daphnids were kept in cylindrical plastic chambers (dimensions: height 16 cm and diameter 11.5 cm, apertures covered by 1 mm mesh). These Daphnia were restricted to their chamber in the water column and were collected to feed the fish each day. Two weeks prior to the start of the experiment, unglazed ceramic tiles (20 cm²) were incubated in the Mau river (an unpolluted area in Sever do Vogua, Portugal) to allow a natural colonization of biofilm to serve as a food source for the snails.

Daphnids are especially sensitive to dissolved silver compared to the other invertebrates used in the mesocosms, and in order to create an Ag-contaminated food source for the fish in the mesocosms with 10 μg L⁻¹ AgNO3, the Daphnia were exposed in a separate aquarium of APW (not directly exposed within the mesocosm) at a nominal concentration of 1 μg Ag L⁻¹ (i.e., 10-fold less to give a sub-lethal dose to the Daphnia). This was lower than the nominal 10 μg L⁻¹ of total Ag used in the main mesocosm, but was necessary to avoid mortality, considering the reported LC50 at 48 h of exposure to AgNO3 of 3.38 μg L⁻¹ with food and 1.04 μg L⁻¹ without food for Daphnia (Ribeiro et al., 2014). Daphnids were fed every other day with the microalgae Raphidocelis subcapitata at concentration of 3 × 10⁵ cells ml⁻¹. The daphnids were then used as ‘contaminated food’ for the rainbow trout in the mesocosms. Trout were fed daily on a 0.25% ration of daphnids (equating to about 13 Daphnia per river). The other food chain in the mesocosm involved the snails in the mesocosm that fed on the biofilm on the ceramic tiles or growing on the sediment/glass walls of each river. The planarians may have predated on the snails, chironomids or Lumbricus and the chironomids grazed on the organic matter (ground Alder leaves) in the sediment.

The unit of replication in the study design was a group of three rivers fed by a shared sump. There were 12 sets of three rivers in all (4 sets per treatment). Groups of three rivers were randomly designated either as controls (no added silver), or exposed to 10 μg L⁻¹ of total Ag via the water as either silver nitrate (AgNO3, analytical grade from Sigma Aldrich) or as silver sulfide nanoparticles (Ag2S NPs, batch number 6, from Applied Nanoparticles, Barcelona). We have characterised Ag2S from Applied Nanoparticles many times before (e.g., Clark et al., 2019a; Clark et al., 2019b), with details in Silva et al. (2020) for the aquatic media used here, and the specific batch used in the current experiments in Peixoto et al. (2020). The Ag2S NPs (Manufacturer’s information) had a nominal size and concentration of 20 nm and 1.32 g Ag2S L⁻¹, respectively, with the latter confirmed directly before use in the experiment. The Ag2S NPs were supplied in a colloidal dispersion stabilized by 1 mg mL⁻¹ of 55 kDa polyvinylpyrrolidone (PVP). Transmission electron microscopy was conducted to confirm the primary particle diameters of the Ag2S NPs to be 20.4 ± 11.9 nm (mean ± S.D., n = 613, Peixoto et al. 2020)). Dynamic light scattering was performed in artificial pond water with 1 mg L⁻¹ dispersions of the Ag2S NPs and gave a mean (± S.D.) hydrodynamic diameter of 336 ± 26.6 nm (by intensity at pH 7.8, n = 3). The measured total Ag concentrations and particle number concentrations in the rivers are reported below.

The exposure to AgNO3 was achieved by preparing a stock solution of 13.04 mg L⁻¹ of total Ag in ultrapure water and adding 250 mL of the stock solution to the sump that fed each of the three rivers, allowing it to mix to achieve a final nominal concentration of 10 μg L⁻¹ of total Ag. For

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**References:**

Auffan et al., 2020

Balsinha et al., 2009

Clark et al., 2019a

Clark et al., 2019b

Dale et al., 2015

Peixoto et al., 2020

Vidal et al., 2014

Vidal et al., 2014

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the exposure to AgS NPs, the required volume of total Ag was added directly from the liquid stock as supplied by Applied Nanoparticles (with stock concentration being of 1.15 g Ag L\(^{-1}\)) to the sump, then also allowed to mix to achieve a final nominal concentration of 10 μg Ag L\(^{-1}\) of total Ag. Each unit of three rivers was dosed daily at 10 a.m. to ensure the exposure of 10 μg Ag L\(^{-1}\) was maintained. This concentration was selected to be sub-lethal to all of the test species (except for daphnids and AgNO\(_3\), as explained above) and to enable a physiological accumulation of Ag in the organisms, but also to facilitate measurement of Ag in the system. Each triplicate set of rivers was exposed for up to 14 days to one treatment (AgNO\(_3\) or AgS NPs), and the rivers were subjected to destructive sampling on either day 2, 7, or 14. The temperature was set at 15 ± 1 °C and a photoperiod of 16 h: 8 h dark was applied.

The rivers were measured daily for routine water chemistry (dissolved oxygen (DO), pH, temperature, total ammonia, electrolytes) and for total Ag determination. In addition to the daily water quality measurements on days 1, 6, and 13, water samples were collected hourly over the first four hours after dosing, and after 24 h before the next dosing, in order to understand the exposure profile within a single dosing period. Water and sediment samples were also collected along the length of each river for Ag determination, near the inflow, middle, and outflow of each river on the day of destructive sampling. Biota were sampled for total Ag analysis on days 2, 7, and 14 (end of the experiment), except the fish which were withdrawn from the study early for ethical reasons and sampled on days 2 and 6 only. In each sampling time and for each treatment, invertebrates were collected from 4 rivers (one river per replicate) with the help of Pasteur pipettes and placed in containers with clean APW medium for a 24 h depuration period. Afterwards, the organisms were rinsed in ultrapure deionised water, pooled in Eppendorf tubes and frozen at −80 °C until required for analysis.

### 2.2. Metal analysis of water and sediment samples

A volume of 10 mL of mesocosm water was taken from each river for total Ag determination (n = 4 per treatment at days 2, 7 and 14) and acidified with 0.5 mL of neat nitric acid (Fisher, Primar Plus Trace Metals Analysis Grade). The samples were then analysed for Ag, Cu, Zn and Fe using ICP-MS and Ca, Mg, K and Na using inductively coupled plasma optical emission spectrophotometry (ICP-OES). It was also expected that the daily dosing with Ag would result in a peak concentration of Ag that then equilibrated in the system in the subsequent 24 h before the next dosing. Therefore, water samples were collected more frequently (at 10 min, 1 h, 2 h, 4 h and 24 h after dosing) to understand the 24 h profiles of Ag concentrations in the water column at the start, middle and end of the experiment. These were analysed as above.

The total concentration of Ag, along with the elements Ca, Mg, Na, K, Zn, Cu and Fe, present in the sediments was determined at days zero, 2, 7 and 14. Approximately 30 g of the moist sediment (n = 12 samples per treatment) was oven dried to constant mass at 85 °C. All the samples of about 250 mg of dry sediment were digested using 10 mL of neat nitric acid in covered 50 mL glass beakers. The acid mixture was allowed to gently simmer at 110 °C on a hotplate for at least 2 h in the fume hood (adapted from Chen and Ma, 2001). The digests were then cooled and diluted with 2 % (v/v) nitric acid into 25 mL volumetric flasks. They were transferred to propylene tubes, tightly stoppered and stored in a dark place until required for ICP-OES or ICP-MS analysis, as appropriate.

The extractable Ag fractions in all dried sediment samples were determined by a two-step sequential extraction method at days zero, 2, 7 and 14, following Tatsi et al. (2018). The first extraction was with ultrapure deionised water (Milli-Q water, Elga, 18.2 μS) to release the water extractable fraction of Ag and followed by the dilute acid extractable fraction of Ag extracted with 0.1 M nitric acid; both at ratios 1:10 (sediment:solution) in 15 mL polypropylene centrifuge tubes. The tubes were shaken (IKA Labortecnik KS250) for 1 h, followed by centrifugation for 10 min at 4500 × g (Harrier 18/80). Each solution was decanted, then acidified with 2 % nitric acid and stored in the dark until analysis by ICP-MS.

### 2.3. Silver and electrolyte determination in biota

Elemental analysis in biota followed the method of Clark et al. (2019a). For each replicate stream, typically three animals were pooled from that stream to get enough biomass to analyse (in mg dry weight): snails, 2–9 mg; planarians, 2–10 mg; Lumbriculus 30–80 mg; chironomid larvae, 6–18 animals to obtain a dry mass of 2–6 mg; Daphnia, 15–30 animals to obtain a dry mass of 3–5 mg. Tissues were freeze-dried (Labfroz freeze drier), then weighed and digested in Eppendorf tubes (n = 2 to 4 samples) with 0.5 mL of neat nitric acid for 2 h in a water bath set at 65 °C. After cooling, the digests were diluted to 1.5 mL using ultrapure deionised water and stored in the dark until subsequent analysis by ICP-MS for total Ag. Following metal analysis by ICP-MS, the digest samples were diluted 1:2 with 2 % (v/v) nitric acid and analysed by ICP-OES for Na, K, Ca, Mg, Fe, Cu and Zn. The certified reference tissue DORM-4 (National Research Council Canada) was also analysed as above for total silver (80.9 ± 15.2 as mean percentage recovery ± S.D., n = 20, with percentage coefficient of variation = 18.8%).

### 2.4. Single particle ICP-MS for particle size and particle number concentration

Water was sampled on days 1, 2, 6, 7, 8, 10, 13 or 14 to determine if the total Ag measured in the water column included particulate forms of Ag. A 10 mL volume of each river water was taken and stored at 4 °C until analysis. Analysis was performed according to Clark et al. (2021), with minor modifications. The particulate content of the water was measured using an iCAP RQ ICP-MS (Thermo Fisher) with the same settings as previously reported (Clark et al., 2021) in time resolved analysis mode. Each sample was analysed for 60 s by measuring the Ag\(^{107}\) m/z ratio with a 3 ms dwell time. The sample wash out time was 60 s, and between samples a wash solution of 4 % HCl and 2 % HNO\(_3\) was used. The sample flow rate was calculated by aspirating deionised ultrapure water and determining the difference gravimetrically over 2 min (n = 5). The transport efficiency was calculated using a well characterised 60 nm Au NP standard (BBI solution, UK). The instrument was calibrated using dissolved standards ranging from 0.5 to 4 μg L\(^{-1}\). All solution and suspension preparation, and ICP-MS analyses, were conducted in a laboratory managed under an ISO 9001 certified Quality Management system. Each sample (standards and unknowns) produced 20,000 data points that were used to calculate the particle number concentration using a bespoke Excel sheet that has been previously assessed (Clark et al., 2019c), and were standardised to the number of particles L\(^{-1}\).

### 2.5. Statistics

Unless stated otherwise, all data are shown as mean ± standard error of the mean (S.E.M). Following descriptive statistics, the Kolmogorov-Smirnov test was used to assess the normality of the distribution of the data. Independent Student’s t-tests and one-way analysis of variance (ANOVA, Tukey post hoc test) were used to check for significant differences among responses from the treatments. Data for fish tissue were statistically analysed as described in Clark et al. (2019a). Briefly, after checking for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe), data were analysed by one-way ANOVA (e.g., treatment effects), or two-way ANOVA (e.g., treatment by time effects). Where data were not parametric, the Kruskal-Wallis test was applied. Figures were prepared using SigmaPlot 14.5 and statistical analyses were carried out using IBM SPSS Statistics 22.

### 3. Results

#### 3.1. Confirming the exposure – profiles of total Ag in the water column

The total silver concentration was measured in the water column during the experiment (Fig. 1). In the control rivers, the total Ag concentration remained below the procedural detection limit (<0.155 μg L\(^{-1}\)). The total Ag
from the AgNO₃ treatment showed a progressive increase in the water column over the first seven days of dosing and reached a steady plateau of around 13 μg L⁻¹ for the remainder of the experiment. In contrast, the total Ag from the Ag₂S NPs treatment continued to rise until day 11, peaking at around 28 μg L⁻¹, and then decreasing to around 12 μg L⁻¹ by the end of the study. However, the grand mean values for the total Ag concentrations measured in the water column (after extraction in nitric acid) were close to the nominal exposure concentration (10 μg L⁻¹), with values of around 12–13 μg L⁻¹ in both silver treatments at days 7 and 14, suggesting that the water was reaching a steady-state concentration (Fig. 1C).

This was also confirmed by the daily profiles of total Ag in the water column after dosing (Fig. S1). On the first day of the experiment, the silver dose, regardless of treatment, was quickly removed from the water column. The latter observation is presumably due to adsorption of dissolved Ag and/or the Ag-containing particles to the glass of the mesocosm and to the sediments. The nominal dose was not achieved on the first day with total Ag concentrations peaking at around 5 μg L⁻¹ (Fig. S1A), and then gradually dissipating over the remainder of the 24 h so that only around 1 μg L⁻¹ remained by the next dosing period. However, by day 6 the dosing profile for total Ag achieved much steadier concentrations, with the AgNO₃ treatment being close to nominal, but the Ag₂S NPs treatment showing a spike in concentration in the first five hours after dosing, but then declining to around 12 μg L⁻¹ over the next 24 h (Fig. S1B). Ultimately, by day 13, the daily profile after dosing was steadier and maintaining close to the nominal concentrations, suggesting the water was reaching an equilibrium with the other compartments in the mesocosm (Fig. S1C).

The presence of silver-containing particles in the water column was measured using spICP-MS (Fig. 1B). There were no silver particles detected in the controls. The silver nitrate reported particulate Ag that was most likely derived from the complexation of Ag with the chloride in the APW to form sparingly soluble AgCl particles. For the Ag₂S NPs treatment, particles were detected, but initially at a lower particle number concentration than for the AgNO₃ treatment. For both Ag treatments, the particle number concentration increased over time and this observation is consistent with the repeated dosing procedure used to maintain the exposure. The electrolyte composition of the APW in the rivers was monitored (Table S1), but there were no statistically significant differences between treatments and a consistent water chemistry was observed throughout.

### 3.2. Total Ag concentrations in the sediment

The sediments were analysed for total Ag concentrations following a strong acid digestion (Fig. 2A). The control sediments at the beginning of the experiment had a natural Ag background of around 50 μg Ag kg⁻¹ dry weight (dw) and this showed a decreasing trend over time (not statistically significant), suggesting some redistribution of the background Ag to other compartments in the mesocosm. In contrast to the control, both the Ag treatments showed a progressive increase in total Ag concentrations within the sediment, which were significantly higher than at day 2 (Fig. 2A). One concern for a flowing river where the dosing is arriving at the inflow is that the Ag might tend to accumulate in the sediments at the top of each river (i.e., immediately below the inflow), rather than throughout the sediment along its length. Sediment was collected from the upper, middle and lower parts of each river. Overall, there was no evidence of a position effect with the sediment from all regions of each river showing similar amounts of silver accumulation at each time point (Fig. 2A).

The available fractions of Ag in the sediments were assessed using a serial extraction method using water, dilute (mild) acid and strong acid for the total metal (Fig. 2B, C and D). Only a small fraction of the silver measured, regardless of the exposure, was readily extractable in water, indicating that most of the silver was adsorbed onto or bound to the grains of the sediment and not exchangeable into the ‘pore water’ of the sediment layer. However, around 28 % in the AgNO₃ exposure and 12 % in the Ag₂S NP exposure was extractable with dilute acid, suggesting that there was a labile pool of Ag in the sediment (Fig. 2C and D).

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Fig. 1. Total measured silver concentrations (μg L⁻¹) and particle number concentrations (×10⁶ μL⁻¹) found in the water column of the rivers from the AgNO₃ and Ag₂S NP treatments, respectively. Data are mean ± S.E.M., n = 4–12 water samples. Note, control values were all below the procedural limit of detection (<0.155 μg L⁻¹). In Panel (A) and (C), there was no statistically significant difference between treatments (Kruskal-Wallis or t-test, p > 0.05) at day 2, 7 and 14, respectively. Also, in Panel (A) there were no significant time-dependent changes in the AgNO₃ concentrations. In Panel (C) *p* refer to statistically significant differences (t-test, p < 0.05) in measured silver concentrations by treatment on that day of sampling, relative to sampling on day 2.
3.3. Total silver concentrations in biota

Some of the organisms were very small, or because of matrix effects in the tissue, the samples were sometimes pooled within stream replicate to get enough biomass for a reliable metal determination. In general, for the AgNO3 treatment, all the organisms accumulated total Ag relative to the equivalent control. In most cases, the Ag accumulation from the AgNO3 treatment was greater than from the Ag2S NP treatment (Table 1). Amongst the sediment dwelling organisms, L. variegatus showed a steady accumulation of Ag from the Ag2S NP exposure over time, which was not different from the AgNO3 treatment by the end of the experiment (Table 1). However, the planarians and the chironomid larvae (Table 1) showed a much clearer response, where both organisms showed a statistically significant time-dependent uptake of Ag from the AgNO3 and Ag2S NP treatment, respectively. For the planarians, it made no difference if the animals were depurated before determining their bodily Ag concentration (data not shown), suggesting that the silver was incorporated into the internal tissues (i.e., not incidental Ag in the gut lumen) and/or irreversibly bound to their epithelial surfaces. For the freshwater snail, P. acuta, the shell was separated from the soft body in order to understand if there was any adsorption of Ag to the former. The shell did show some apparent Ag accumulation in both Ag-treatments (Fig. S2). However, the concentration of Ag in/on the shell was trivial compared to the concentration in the tissue itself, with the latter being an order of magnitude higher than the Ag
deposition on the shell. Importantly, the time course of the changes in the soft tissue concentrations of total Ag was different to those in the shell (Fig. S2), and not consistent with the notion of a biogenic redistribution from the tissue to the shell. For the shell, ‘depuration’ had no effect (Fig. S2). However, for the soft tissue, the non-depurated animals showed more total Ag than the depurated ones by day 14, suggesting that around half or more of the apparent accumulation was most likely in the gut contents as rapidly excreted compartment, although some desorption from the mucus on the surface of the foot is also possible.

For the organisms that live primarily in the water column (Daphnia and fish) the Ag accumulation was somewhat different to the other invertebrates. For Daphnia (Table 1), the animals survived well in the mesocosm for the control and Ag2S NP treatments and showed steady clear Ag accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation in Daphnia was inside the tissue is unclear, as the animals tend to ingest particulates from the water column. These Daphnia, used to feed the fish, were not depurated before the whole body Ag determination. For the AgNO3 treatments, the Daphnia did not survive in the mesocosms beyond day 2, and so there was no data for days 7 or 14. However, a separate trial was conducted in APW alone to explore AgNO3 toxicity to Daphnia (Fig. S2), and not consistent with the notion of a biogenic redistribution from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment. However, how much of this apparent accumulation from the Ag2S NP treatment.

Table 1

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Time (days)</th>
<th>Control (μg Ag g⁻¹)</th>
<th>AgNO3 (μg Ag g⁻¹)</th>
<th>Ag2S NPs (μg Ag g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-biting midge</td>
<td>2</td>
<td>0.01 ± 0.002</td>
<td>12.5 ± 3.72</td>
<td>3.42 ± 0.90</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.11 ± 0.02</td>
<td>33.7 ± 8.11</td>
<td>11.7 ± 2.66</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Water flea (Daphnia</td>
<td>2</td>
<td>0.88 ± 0.74</td>
<td>9.99 ± 1.07</td>
<td>51.8 ± 10.9</td>
</tr>
<tr>
<td>magna)</td>
<td>7</td>
<td>0.05 ± 0.02</td>
<td>ND</td>
<td>40.3 ± 23.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.39 ± 0.13</td>
<td>ND</td>
<td>52.0 ± 7.99</td>
</tr>
<tr>
<td>Planarian (Girardia</td>
<td>2</td>
<td>0.03 ± 0.02</td>
<td>1.35 ± 0.74</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>eigrina)</td>
<td>7</td>
<td>0.03 ± 0.01</td>
<td>16.4 ± 7.46</td>
<td>1.17 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.08 ± 0.02</td>
<td>62.0 ± 10.3</td>
<td>5.65 ± 2.80</td>
</tr>
<tr>
<td>Oligochaete worm</td>
<td>2</td>
<td>0.16 ± 0.02</td>
<td>2.37 ± 0.88</td>
<td>1.43 ± 0.19</td>
</tr>
<tr>
<td>(Lumbriculus variegatus)</td>
<td>7</td>
<td>0.21 ± 0.02</td>
<td>5.24 ± 1.63</td>
<td>1.91 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.28 ± 0.02</td>
<td>4.45 ± 1.99</td>
<td>4.00 ± 1.14</td>
</tr>
<tr>
<td>Snail (Physa acuta)</td>
<td>2</td>
<td>0.34 ± 0.05</td>
<td>109 ± 6.72</td>
<td>28.4 ± 7.24</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.54 ± 0.03</td>
<td>120 ± 13.7</td>
<td>53.0 ± 13.7</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.21 ± 0.23</td>
<td>59.2 ± 5.82</td>
<td>41.3 ± 11.3</td>
</tr>
</tbody>
</table>

Data are mean ± S.E.M (n = 2–4). Different letters indicate significant differences amongst the treatments by organism type, within the respective day of sampling (rows). For each organism type, asterisk (*) symbols refer to statistical difference in measured silver for each treatment type with time. Absence of asterisk (*) symbols refer to no differences in measured silver, for each treatment type, with time. #, for L. variegatus exposure to Ag2S NPs, day 14 is significantly different (p < 0.05) only with respect to day 2. All statistics were determined using t-test, 1-way or 2-way ANOVA with p < 0.05 level of significance. ND - not determined as no animals were found at the sampling time point.

showed a clearer trend wherein the carcass of animals from the AgNO3 treatment accumulated more Ag than those of the Ag2S NP treatment, and with the least in the unexposed controls (Fig. 3E). The presence of more Ag in the carcass than the skeletal muscle also suggests that the nervous tissue, bone, spleen, kidney (i.e., carcass tissues other than the skeletal muscle) were also accumulating silver, with Ag from AgNO3 being more bioavailable than Ag from Ag2S NPs.

3.4. Trace metal and electrolyte composition of biota

Silver is well-known for its effects on sodium homeostasis in animals and for its interactions with other trace metals in biota. Consequently, a suite of trace elements and electrolytes were measured in the biota including Ca, Mg, Na, K, Cu, Zn and Fe (Tables S2 and S3). There were some time-dependent increases in some of the electrolytes in the organisms, perhaps reflecting growth and nutritional health in the organisms. However, there were few treatment-dependent effects and where these occurred, the changes were within the physiological scope of the animals (Tables S2 and S3). Crucially, overt electrolyte depletion and ionoregulatory stress were not observed in any of the organisms.

4. Discussion

4.1. Total Ag in the water column and partitioning to the sediment

The total silver concentration in the water column in the control mesocosm remained below the procedural detection limit (c<0.155 μg L⁻¹, Fig. 1) and with only a trace amount of around 50 μg Ag kg⁻¹ dw or less in the sediments (Fig. 2A). Some background of Ag is unavoidable in aquatic sediments, even from pristine river systems, and the values here are in the microgram range similar to previous reports (e.g., sediment cores from the upper reaches of the River Lot in France, around 310 μg kg⁻¹ dw, [Lanceleur et al., 2011]). For both Ag treatments, the exposure was progressive with daily dosing of Ag into the water column (Fig. 1) that inevitably also contaminated the sediments (Fig. 2). For AgNO3, this is expected to be a dynamic process where dissolved Ag species are formed in the water column, which then adsorb to surfaces of the particulate minerals (e.g., sand) in the sediment, and/or bind to –SH groups and other ligands in any organic matter present, such as humic acids (Adams and Kramer, 1999; Chen et al., 2012; Herrin et al., 2001). This is partly reflected in the daily dosing where peak concentration in the water column (Fig. S1; Fig. 1A) dissipated, presumably through mixing, and by transfer into the
Fig. 3. Total measured silver concentration (μg Ag g⁻¹) in dry tissue of rainbow trout (*Oncorhynchus mykiss*) within (A) intestine, (B) liver, (C) gill, (D) muscle and (E) the remaining carcass, at sampling days 0, 2 and 6, in the mesocosm experiment with AgNO₃ and Ag₂S NPs. Data are mean ± S.E.M (n = 4–5). Different letters indicate statistically significant differences amongst the treatments, within the respective day of sampling (ANOVA, p < 0.05). Absence of letters indicates no statistically significant difference in measured silver concentrations amongst the treatments, within the respective day of sampling. Asterisk “*” refers to statistically significant difference in measured silver concentrations by treatment type, with time (day 2 and day 6). Absence of asterisk symbols indicates no statistically significant differences in measured silver concentrations, for each treatment type, with time (t-test, p > 0.05).
sediment compartment of the mesocosm (Fig. 2). Speciation calculations for AgNO3 in APW (Visual MINTEQ version 3.1 https://vminteq.lwr.kth. se/) indicated that the following species were present: 7.19 % of Ag+, 64.96 % of AgCl (aq), 27.62 % of AgCl2−, 0.19 % of AgCl3−, and 0.04 % of AgSO4. At a nominal total Ag concentration of 10 μg L−1 in the water column, the free Ag+ ion concentration would be <1 μg L−1 and most of the Ag would be readily adsorbed to the sediment or glass of the mesocosm each day. Of course, the effect is cumulative, and after the first seven days of dosing the total Ag concentration in the water column reached a steady plateau of around 12–14 μg L−1 which remained for the rest of the experiment for the AgNO3 (and Ag2S NP) treatment (Fig. 1).

The speciation calculations indicated that two thirds of the total Ag was likely present as the AgCl species. Notably, the AgNO3 exposure resulted in Ag-containing particles in the water column (Fig. 1B) and these are likely sparingly soluble AgCl particles which are known to form in the presence of chloride (see discussion in Bradford et al., 2009). The APW had a reasonable ionic strength (millimolar) and is not unlike physiological saline in that regard; where the formation of nanoscale AgCl particles from AgNO3 is also routinely observed (Besinis et al., 2014; Clark et al., 2019c). Crucially, total Ag concentrations in the water or sediment from the AgNO3 compared to Ag2S NP exposures were similar (Figs. 1 and 2). This suggests that their fate in the abiotic compartments of the mesocosms were the same, and ultimately were likely driven by particle settling from the water column to the sediment, regardless of the original form of the test substance (Dale et al., 2015; Zhao et al., 2021).

Notwithstanding particle formation, dynamic changes in total Ag concentrations in the water and sediment with repeated dosing of the mesocosm are expected, and similar observations were made by Bradford et al. (2009) for marine mesocosms with AgNO3 and Ag NPs, where peak concentrations in the water eventually plateaued and Ag was adsorbed to the sediments. In the present study, the partitioning of total Ag in the sediment was also measured (Fig. 2A). For both forms of Ag added to the mesocosm, the total Ag concentration in the sediment remained within the sediment, as relatively small amounts of total Ag were being readily extracted by water or dilute acid (Fig. 2B, C and D). The sediment was very sandy with negligible organic matter to bind the Ag, and so a small labile pool of total Ag may be expected.

4.2. Bioaccumulation of total Ag from AgNO3 versus Ag2S NP exposure in the organisms

The extractable Ag in the sediments and that in the water column is likely to be bioavailable to the organisms in the mesocosm. The oligochaete worm, L. variegatus (Table 1), showed a steady accumulation of Ag from both the Ag2S NP and AgNO3 exposures, and with no difference between the Ag treatments by the end of the experiment. Bioaccumulation studies on Ag metal salts with L. variegatus are sparse, and often do not include AgNO3. One study on natural sediments spiked with silver sulfide indicated a trend (no statistical analysis) of increased total Ag accumulation in the worms compared to unexposed controls (Hirsch, 1998). The apparent uptake of Ag from Ag2S NPs has not been previously reported for L. variegatus. However, Ag (form unknown in the tissues) from exposure to AgNO3 or Ag NPs with different coatings, has been reported to be taken up by the oligochaete worm, with the uptake rates being faster than the elimination rates, implying that the Ag was bioaccumulative in the organism (Khan et al., 2015), as also observed here (Table 1). Similar observations were made by Coleman et al. (2013), who also confirmed the presence of Ag-containing particles in the oligochaete worm by spICP-MS and spectral imaging approaches.

The planarians and the chironomid larvae (Table 1) showed much higher Ag concentrations than the oligochaete worms, with a clearer time-dependent Ag accumulation from both the AgNO3 and Ag2S NPs treatment, respectively. There are only a few reports on the bioaccumulation of metals in planarians, and only one recent study on Ag from the same laboratory that conducted the mesocosm experiment reported here (Silva et al., 2022). Nonetheless, for Cd, the planarian, Dugesia japonica showed metal accumulation with the induction of metallothionein inferring metal chelation inside the tissue (Wu et al., 2011). For AgNO3, a recent dietary exposure using contaminated prey items (freshwater snails) showed that the planarian, G. tigrina, could accumulate total Ag from food (Silva et al., 2022). Crucially, the total Ag accumulation from eating AgNO3-exposed prey items was twice that of Ag2S NP treatment (Silva et al., 2022), indicating that the former was more bioavailable. Similar observations were made here with much higher total Ag concentrations in the planarians from the AgNO3 treatment than from the Ag2S NP treatment. Notably, in the present study, planarians that were depurated had the same Ag concentrations as animals that were not depurated (data not shown, regardless of type of Ag exposure), suggesting biological incorporation of the Ag into the tissue, as observed for Cd by Wu et al. (2011), Silva et al. (2022) also reported minimal differences in Ag body concentrations between depurated and non-depurated planarians exposed to different forms of Ag in the food.

Chironomids are known to accumulate metal from metal salts, depending on the type of metal, and age/body size of the animals (Krantzberg, 1989). However, while there have been studies on the toxicity of AgNO3 and Ag NPs to C. riparius (e.g., Park et al., 2015), the small size of the animals has hampered reports on Ag accumulation. One food chain study incidentally reported Ag concentrations in adult Chironomus spp., between 38 and 45 μg g−1 for AgNO3 and Ag NP exposures respectively, and although no unexposed controls were mentioned (Yoo-iam et al., 2014), the Ag accumulation reported is of the same magnitude as in the present study (Table 1).

For the freshwater snail, P. acuta, bioaccumulation of total Ag from both the AgNO3 and Ag2S NP exposures was observed (Table 1), but with less Ag accumulated from the Ag2S NP treatment. There was also some evidence of depuration (data not shown) suggesting a fraction of the apparent total Ag was likely in the gut lumen rather than in the tissues. Both AgNO3 and Ag NPs are not toxic to P. acuta at the concentrations tested in the APW used here, although it is known that the toxicity extent is also life stage and/or size dependent (Gonçalves et al., 2017). However, there are few detailed reports of Ag accumulation in this species of aquatic snail. At the same concentrations used in the present study, Silva et al. (2022) demonstrated that P. acuta could accumulate around 40 μg g−1 dw of total Ag from AgNO3 exposure over seven days, compared to 59 μg g−1 dw over 14 days in the present study (Table 1). Crucially, Silva et al. (2022) showed that there was more total Ag accumulation from the metal salt (AgNO3) than from the Ag2S NP treatment in laboratory studies, partly because the snails were not able to excrete Ag (form unknown inside the tissue) after the AgNO3 exposure (Silva et al., 2022). P. acuta also showed higher accumulation of Ag from AgNO3 compared to Ag2S NP waterborne exposures (Silva et al., 2020). The metal salt was therefore more bioaccumulative than the nano form by P. acuta, and this was also the case in the mesocosm (Table 1). There could be species differences in the handling of Ag amongst snails. In the freshwater snail, Lymnaea stagnalis, the uptake of total Ag from either AgNO3 or Ag NPs was measured from the water, with uptake rates greater than the elimination rates, leading to a net bioaccumulation for both substances (Croteau et al., 2011). Notably, L. stagnalis accumulated Ag from both food or water exposures (Croteau et al., 2011), and a combination of uptake routes is also likely in the mesocosm study here for P. acuta.

Similar to the snails, AgNO3 exposure caused appreciable Ag accumulation in D. magna (Table 1). At day 2 there was a five-fold greater total Ag in animals from the Ag2S NP treatment than from AgNO3 (Table 1) and this could simply reflect that Daphnia are filter feeders and would be removing particulates from the water column. There have been a number of previous studies on the bioaccumulation of either dissolved silver or Ag NPs on D. magna (Khan et al., 2015; Kim et al., 2016; Ribeiro et al., 2017), but not on Ag2S particles. Daphnia can accumulate around 10–30 μg g−1 dw of Ag from sublethal exposure via the water to AgNO3 or Ag NPs over 48 h (Ribeiro et al., 2017), a similar magnitude to the present study (Table 1). Ribeiro et al. (2017) also showed more total Ag accumulation from the Ag NP treatment than that for AgNO3, with the food being an important aspect. Unfortunately, not enough Daphnia remained in the mesocosm to collect for total metal measurement at days 7 and 14 for
the AgNO₃ treatment, mainly because these are a favourite food of trout. In the current study, the Ag accumulated by *D. magna* also represented a dietary hazard to the trout in the mesocosm, because the fish were fed on contaminated *Daphnia* from the appropriate treatments.

The target organs for freshwater-adapted rainbow trout exposed to AgNO₃ via the food (Clark et al., 2019b), or the water (Hogstrand et al., 1996), are relatively well-known and include the gills, gut and liver. In the mesocosm study, AgNO₃ but not Ag₂S NPs, caused total Ag concentrations to increase in the tissues (Fig. 3). This is consistent with our previous findings on in vivo (dietary) exposures in trout where total Ag (form unknown in the tissue) from the AgNO₃ exposure was more bioavailable than that of Ag₂S NPs (Clark et al., 2019b). Subsequently, Clark et al. (2021) identified that dietary exposure to either the metal salt or Ag₂S NPs resulted in Ag-containing particles being present in the internal organs, as measured by spICP-MS. The total Ag concentrations in the target organs of around 1 μg g⁻¹ dw or a few hundred ng g⁻¹ dw (Fig. 3) are typical of previous reports (e.g., AgNO₃, Hogstrand et al., 2003; AgNO₃ and Ag₂S NPs; Clark et al., 2019b; Clark et al., 2021). Interestingly, when silver is presented as a silver sulfide metal salt in the diet of trout, can be appreciable accumulation of total Ag in the liver, albeit after several months of exposure (Galvez and Wood, 1999). This at least demonstrates that Ag₂S as a chemical substance can be bioavailable to the fish, and this previous findings show this only to a very limited extent when the Ag₂S is presented in the nano form (Clark et al., 2019b). This was also the case in the mesocosm where there were very limited increases of total Ag concentrations in the tissues from Ag₂S NP treatment, and with the AgNO₃ exposure resulting in much more Ag accumulation than that from the nano form (Fig. 3).

4.3. Evidence of Ag transfer in the mesocosm and/or biomagnification

A central question for the environmental risk assessment of silver is whether or not Ag is transferred from the water to sediments and then to the biota to cause a bioaccumulation potential, or even a biomagnification risk to the food web. It is clear that both AgNO₃ and Ag₂S NPs in the water column resulted in contamination of the sediments due to dissolved metal partitioning into the abiobiotic compartments and/or particle settling (discussed above). This occurred at a similar magnitude for both the AgNO₃ and Ag₂S NP exposures, with at least a 100-fold enrichment factor for transfer of total Ag from water to sediment (compare Figs 1 and 2). This high level of enrichment is to be expected. For example, dissolved Ag releases into the river Seine resulted in sediment enrichment factors of 100–300 above the natural background level (Ayrault et al., 2010). Similar arguments have been presented for the fate and behaviour of Ag NPs (Lead et al., 2018) and these processes have been modelled for mesocosms containing Ag₂S NPs where nearly all of the microgram amounts of silver dosed into the water would incorporate into the sediments over about ten days (Dale et al., 2013).

Crucially, a fraction of the Ag present in the sediment was water or dilute acid extractable (Fig. 2B, C and D), and there were some modest increases in Ag concentrations in the biota (Table 1). First consider the AgNO₃ treatment, where, with some 200 μg kg⁻¹ of total Ag in the sediment, the values in the biota were around 4, 60, 35, and 60 μg g⁻¹ dw (or mg kg⁻¹ dw) for the oligochaete worms, planarians, chironomid larvae and snails, respectively. On a concentration basis, the organisms in direct contact with sediment therefore contained around ×1000 higher concentrations of silver compared to the control organisms. This apparent biomagnification from the sediment to the benthic organisms is typical for dissolved Ag and can even be much higher. Lancelleur et al. (2011) reported a biomagnification estimate of 10 × 10⁶ for silver into shellfish. Indoor microcosm studies estimated bioconcentration factors from the water to invertebrate biota for dissolved silver of around ×1000, but estimates were much lower from field measurements (Ratte, 1999). The situation for Ag₂S NPs was different. The measured concentrations for total Ag from the Ag₂S NP exposures in the benthic invertebrates were very modest, or broadly similar to the control animals in most cases (Table 1). Taking this into account, the animals in the Ag₂S NP treatments were probably not showing biomagnification relative to the sediments, beyond that already present in the controls. The molecular mechanisms relating to the apparent lack of biomagnification of Ag₂S NPs requires further investigation, and in part, might be due to the low bioavailability of the ENM at epithelia (Clark et al., 2019b). In terms of differences in Ag accumulation by habitat or ecological niche, only the fish and *Daphnia* were mainly in the water column, and the fish generally had lower tissue concentrations (Fig. 3) than the benthic invertebrates (Table 1), perhaps reflecting that the fish were denied access to the sediment in the study design.

The *Daphnia* fed to the fish, and the fish themselves, had negligible contact with the sediment and so bioconcentration factors relative to the water are of more interest here. For the AgNO₃ treatment, the *Daphnia* achieved around 50 μg g⁻¹ dw and the trout maximally around 7.5 μg g⁻¹ dw in the liver, with the latter consistent with reports in other fish species (Carassius auratus; Ribeiro et al., 2022). At the nominal water concentration of 10 μg L⁻¹ (or 10 ng mL⁻¹), this represents a ‘transfer factor’ of around ×5000 for *Daphnia* and ×750 for the fish. A precise bioconcentration factor (BCF) would require a demonstration that both water and biota concentrations were in steady-state, and this is difficult to prove with only a 2-week exposure for the *Daphnia*, and 6 days for the fish. Nonetheless, the calculation shows, that for AgNO₃, there is bioaccumulation of total Ag in the prey items, but this does not lead to biomagnification at the next trophic level (i.e., in predatory fish). For the Ag₂S NP treatment, the *Daphnia* achieved around 59 μg g⁻¹ dw, but the trout liver showed <1 μg g⁻¹ dw and was similar to controls. At the nominal water concentration of 10 μg L⁻¹, this represents a ‘transfer factor’ of around ×5900 for *Daphnia*, but with no evidence of biomagnification of Ag from the Ag₂S NP exposure to the next trophic level (fish). Silva et al. (2022) reached an identical conclusion for planarian predating on snails with the same substances used here. A recent study by Xiao et al. (2022) showed the biomagnification factors for dietary Ag transfer from *Daphnia* (prey items) to zebrafish were all <0.2 for AgNO₃ and Ag₂S NP exposures, indicating negligible biomagnification in the food web, that was partly explained by lower bioavailability of Ag in all its forms, possibly due to sulfidation in the intestine of the fish.

4.4. Conclusions and regulatory perspective

Additions of either AgNO₃ or Ag₂S NPs resulted in a progressive increase in the particle number concentrations in the water column, with the formation of Ag-containing particles from the metal salt. Thus, both exposures ultimately presented a particulate hazard in the water. Irrespective of the form, the total Ag dosed into the water column rapidly became associated with sediment and bioaccumulation in the biota. While these environmental standards would also protect ecosystems from exposure to Ag₂S NPs, one might argue that they would also ‘over regulate’ the nano form as the latter is much less bioaccumulative. Further research is needed to set any adjustment of the environmental.
standards for nano forms of silver, and to define the relative importance of waterborne and dietary sources of Ag$_2$S NPs in real ecosystems.

CRediT authorship contribution statement

All of the authors actively contributed to this study, either by directly preparing and characterising the nanomaterials used in the work, conducting the freshwater mesocosm experiment at the University of Aveiro led by the team headed by Professor Susana Loureiro, and/or by the subsequent chemical analysis of biota, sediment or water samples from the experiments. All of the authors contributed to the writing of the manuscript, with the editing and data analysis of the submitted paper led by Professor Richard Handy and his team at Plymouth University.

Data availability

Please contact the corresponding author with requests regarding the data.

Declaration of competing interest

The authors have no known financial or other conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157912.

References
