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Toxicokinetics of Ag from Ag_2S NP exposure in *Tenebrio molitor* and *Porcellio scaber*: Comparing single-species tests to indoor mesocosm experiments

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ABSTRACT

Determining the potential for accumulation of Ag from Ag_2S NPs as an environmentally relevant form of AgNPs in different terrestrial organisms is an essential component of a realistic risk assessment of AgNP emissions to soils. The objectives of this study were first to determine the uptake kinetics of Ag in mealworms (*Tenebrio molitor*) and woodlice (*Porcellio scaber*) exposed to Ag_2S NPs in a mesocosm test, and second, to check if the obtained toxicokinetics could be predicted by single-species bioaccumulation tests. In the mesocosms, mealworms and woodlice were exposed together with plants and earthworms in soil columns spiked with 10 μg Ag g^{-1} dry soil as Ag_2S NPs or $AgNO_3$. The total Ag concentrations in the biota were measured after 7, 14, and 28 days of exposure. A one-compartment model was used to calculate the Ag uptake and elimination rate constants. Ag from Ag_2S NPs appeared to be taken up by the mealworms with significantly different uptake rate constants in the mesocosm compared to single-species tests (K_1 = 0.056 and 1.66 g dry soil g^{-1} dry body weight day^{-1}, respectively), and a significant difference was found for the Ag bioaccumulation factor (BAF_k = 0.79 and 0.15 g dry soil g^{-1} dry body weight, respectively). Woodlice did not accumulate Ag from Ag_2S NPs in both tests, but uptake from $AgNO_3$ was significantly slower in mesocosm than in single-species tests (K_1 = 0.037 and 0.26 g dry soil g^{-1} dry body weight day^{-1}, respectively). Our results are of high significance because they show that single-species tests may not be a good predictor for the Ag uptake in mealworms and woodlice in exposure systems having greater levels of biological complexity. Nevertheless, single-species tests could be used as a fast screening approach to assess the potential of a substance to accumulate in biota before more complex tests are conducted.

1. Introduction

A major fraction of the silver nanoparticles (AgNPs) used in everyday applications are discharged to wastewater treatment plants (WWTPs) where about 90% of these particles accumulate in sewage sludge (Courtois et al., 2019). Sewage sludge plays an important role in introducing Ag to the environment (Levard et al., 2012; Qumber et al., 2020). The concentration of Ag in sewage sludge may amount to 1–6 μg Ag g^{-1} dry matter as estimated by Gottschalk et al. (2009), while EPA (2009) reported a range of 2 to 856 μg Ag g^{-1} in sewage sludge sampled from different WWTPs. Given the high sulfide concentration and Ag affinity to bind to sulfur under both aerobic and anaerobic conditions,

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the majority of Ag in sewage sludge occurs in the form of Ag_2S (Vogt et al., 2019). For the first time in 2010, 5–20 nm Ag_2S NPs were detected in the final stage sewage sludge of a municipal WWTP (Kim et al., 2010).

The fate and stability of sulfide AgNPs (Ag_2S NPs) depend on their crystalline/amorphous properties. The presence of Ag(0) close to the edge of the Ag_2S NPs may lead to release of Ag^+ over time (Kaegi et al., 2013) and amorphous Ag_2S showed a higher degree of Ag^+ dissolution than crystalline Ag_2S (Kampe et al., 2018), although to a much slower extent than AgNPs. In recent years, more studies have been carried out under more realistic exposures either using purposely synthesized Ag_2S NPs or by aging AgNPs in sludges to simulate the Ag forms anticipated to reach the environment. These studies have looked at uptake and effects in soil organisms over different time periods in single-species exposures or in complex systems such as mesocosms. Some contradictory data have been reported for different organisms. Lower Ag accumulation from Ag_2S NPs than from other Ag forms was reported, for instance in earthworms (*Eisenia fetida*) the kinetic bioaccumulation factor (BAF_k) was 0.008 after 28 days exposure to Ag_2S NPs compared to exposure to AgNPs and $AgNO_3$ with BAF_k of 0.061 and 0.055, respectively (Baccaro et al., 2018). In the same line, the wheat *Triticum aestivum* presented lower bioaccumulation of Ag from Ag_2S NPs in exposure to 3 or 10 μg Ag g^{-1} dry soil of different Ag forms for 42 days (Lahive et al., 2021). In the opposite direction, a toxicokinetic study using four different natural soils, Talaber et al. (2020) showed that woodlice (*Porcellio scaber*), already having high Ag background concentrations (5–10 μg Ag g^{-1} dry body weight), did not accumulate more Ag during a 3-week exposure to Ag_2S NPs at 14–26 μg Ag g^{-1} dry soil. For the woodlice *P. scaber* a maximum average internal concentration of 10.6 μg Ag g^{-1} fresh weight was obtained when exposed to ≈14 μg Ag g^{-1} dry soil in OECD artificial soil spiked with sewage sludge (Kampe et al., 2018). For plants, Wang et al. (2015) reported that Ag_2S NPs can be taken up by roots and subsequently translocate throughout the leaves. Ag accumulation from Ag_2S NPs in roots of wheat (*T. aestivum*) and *Brassica rapa* was lower than pristine AgNPs and $AgNO_3$ (Khodaparast et al., 2022; Lahive et al., 2021), however Ag translocation to the shoots was faster in plants exposed to the Ag_2S NPs compared to other Ag forms (Khodaparast et al., 2022). All these studies suggest that the uptake of NPs depends on the species tested and the routes and conditions of exposure considered.

In our mesocosm experiment, exposure was conducted in soils spiked with Ag_2S NPs to simulate aged or transformed AgNPs entering the ecosystem via sewage sludge application. Plants and different invertebrates were chosen for the mesocosms, in this paper only the data from organisms living on the soil surface (woodlice and mealworms) are presented. Woodlice are well-known decomposer organisms in ecotoxicology and also as metal accumulators (Tourinho et al., 2016). Mealworms (*Tenebrio molitor* larvae) are used as food for pet animals (e.g., amphibians or reptiles) and are a good alternative source to traditional protein production (Morales-Ramos et al., 2010; Bordiean et al., 2020; Eriksson et al., 2020). Mealworms were therefore selected as a test organism based on the assumption that substances can bioaccumulate and be transferred along the food chain.

The most common approach to assess the bioaccumulation kinetics of NPs, by deriving uptake and elimination rate constants, is through single-species tests (Argasinski et al., 2012). However, these tests do not account for interactions in a more complex system, such as with other organisms or with NP exposure dynamics driven by interactions between species. Mesocosm experiments can offer data from a higher tier of biological complexity, at the community level, providing meaningful information for the environmental risk assessment of NPs under more realistic exposure scenarios (Auffan et al., 2019). Mesocosm tests, however, are more labour intensive and costly, and exposures in such tests are less standardized and precise than in single-species tests. Therefore, it is essential to determine how good single-species tests can predict NP uptake kinetics in mesocosm tests (Auffan et al., 2019). Thus, this study aimed to verify the toxicokinetic profile obtained in single-species bioaccumulation tests by Khodaparast et al. (2021) and Talaber et al. (2020) with those obtained from higher tier tests conducted in indoor terrestrial mesocosms. We compared the results on the bioaccumulation of Ag from Ag_2S NPs and $AgNO_3$ from single-species and mesocosm tests and discussed the relevance of using single-species tests in the environmental risk assessment of NPs.

2. Material and methods

2.1. Materials and test organisms

Tenebrio molitor larvae were purchased from a commercial breeder in Portugal, while woodlice (*Porcellio scaber*) were collected from a compost bin in Bilthoven (The Netherlands) and shipped to Portugal. In the laboratory, the organisms were kept at 20 ± 2 °C and a 16 h:8 h light: dark photoperiod. Mealworms with a weight of 27.7 ± 6.3 mg (mean ± SD; $n = 100$) and adult woodlice with 36.1 ± 3.8 mg (mean ± SD; n = 100) were used. Lufa 2.2 (Speyer, Germany), a sandy loam soil, proposed as a reference soil for nanosafety research (Geitner et al., 2020), was used in the mesocosm and single-species tests. Its properties are, according to the supplier: nitrogen (%): 0.19 ± 0.03, organic carbon (%): 1.73 ± 0.27, pH (0.01 M $CaCl_2$): 5.6 ± 0.4, cation exchange capacity (cmolc kg^{-1}): 9.8 ± 0.5, maximum water holding capacity (WHC, %): 45.8 ± 1.9.

Polyvinylpyrrolidone coated Ag_2S NPs used in mesocosms and single-species tests were synthesized by Applied Nanoparticles (Barcelona, Spain). The particles were found stable in ultrapure water suspensions of 1 mg Ag L^{-1} for 2 days and demonstrated a low dissolution rate (<0.1%) measured according to the protocol of Avramescu et al. (2017) (Khodaparast et al., 2021).

The concentration of free Ag^+ in a solution of Ag_2S NPs (1 g L^{-1}) was 0.12% (Talaber et al., 2020). Other characteristics of the Ag_2S NPs used here have been reported by Peixoto et al. (2020), such as an average diameter of 20.4 ± 11.9 nm and Zeta-potential of −23.8 ± 4.5 mV in miliQ water. $AgNO_3$ was purchased from Sigma-Aldrich (99% purity, CAS 7761-88-8, Germany).

2.2. NP characterisation by transmission electron microscopy (TEM)

Transmission electron microscopy (TEM, JEM-2100 200 kV analytical electron microscope) analyses with hyphenated energy dipersive x-ray spectroscopy (EDX, OI Aztec 80 mm X-max) were carried out by Oxford Materials Characterisation Service (University of Oxford, UK) to determine the NP diameter and estimate the Ag:S molar ratio. One drop (20 μL) of the stock suspension of Ag_2S NPs (1320 ± 48 mg Ag L^{-1}) was drop cast on TEM grids (400 mesh holey carbon film-coated Cu, Agar Scientific) and left to dry for at least one hour before examination by TEM.

This methodology was also applied to soil porewater samples to investigate the effect of the soil environment on the NP diameter and Ag:S ratio in the soil pore water. Pore water (1 mL) was collected during the experiment (see below) by centrifuging soil samples at 14000 rpm for 5 min. A 20 μL drop from the bottom of the Eppendorf tube (pellet) was drop cast on the TEM grids.

2.3. Soil spiking and preparation

The soil was spiked with stock suspensions/solutions of either Ag_2S NPs (1320 ± 48 mg Ag L^{-1}) or $AgNO_3$ (1148 ± 41 mg Ag L^{-1}) diluted in deionized water to reach a nominal concentration of 10 μg Ag g^{-1} dry soil and 55% WHC. This concentration was chosen based on the predicted concentration of Ag engineered nanomaterials in 2050 of 0.3–0.4 μg g^{-1} for sludge-treated soil (Giese et al., 2018) on one hand, and to enable detection in biota on the other. Then the soil was mixed by hand for 5 min. The controls only received deionized water. For each treatment (Ag_2S NPs, $AgNO_3$, control), ten soil columns (20 cm length, 11 cm diameter), with a plastic mesh (1 mm) at the bottom, were filled with a

total of approx. 2 kg of soil: 1.07 kg uncontaminated Lufa 2.2 soil was added to the column up to a height of 8.25 cm, and another layer of 8.25 cm of Ag-spiked or control soil was placed on top. The preparation and sampling of the soil columns are illustrated in Fig. S1. The soil pore water was extracted (see below) from the 4 cm surface layer. During the experiment, leachates were collected in 50 mL plastic tubes attached to plastic funnels placed at the bottom of the soil columns (Santos et al., 2011). The soil columns were placed in six wine coolers, each allocating five columns, incubated at 13 °C, while the top 8 cm soil layer was exposed to room temperature (20 ± 2 °C). The light intensity was ca. ≈ 4700 Lux at the soil surface with a photoperiod of 16 h:8 h light: dark. Columns were randomised between coolers and between the 5 slots in each cooler, minimizing edge effects from light and airflow within the room.

2.4. Experimental setup in the mesocosms

Two days after introducing the spiked soil, four plants (*Triticum aestivum*, one day post emergence in spiked or control soil and then transferred to appropriate spiked or control mesocosms), six earthworms (*Lumbricus rubellus*, pre-depurated adult), ten woodlice (*P. scaber*), and ten mealworms (*T. molitor*) were added to each soil column. In addition, six bait-lamina strips were introduced into each soil column. Every day, artificial rainwater (NaCl (0.01 mM), $(NH_4)_4SO_4 \bullet H_2O$ (0.0053 mM), $NaNO_3$ (0.0059 mM), and $CaCl_2 \bullet H_2O$ (0.0039 mM); pH = 5.1) was distributed over the soil surface using a syringe (16 mL per column ~1.6 mm day^{-1}). Dry alder leaf disks (Ø 10 mm) were placed on the soil surface as food for the isopods and mealworms.

After 7, 14, and 28 days, soil columns from controls, $AgNO_3$, and Ag_2S NP exposures were destructively sampled with three replicates (columns) sampled at days 7 and 14, and four replicates at day 28. After removing the bait-lamina strips at each sampling point, plant shoots were harvested at the soil surface, while mealworms and woodlice were collected from the soil surface and, after recording their number, were kept for purging. The purging process was carried out for 24 h before animals were snap-frozen and stored at −20 °C for Ag analysis. The mealworms were kept in an empty plastic box during depuration, while the woodlice were kept in a plastic box with moist filter paper and clean food (alder leaves).

Then a soil core was extracted in the middle of the column and the sampled soil was divided into five sections for further analysis of the soil moisture profile, and depth distribution of total Ag concentrations (described in detail in 2.6). Sampling procedures and data from plants and earthworms will be presented in another paper, while this study only focuses on mealworms and woodlice as the soil surface-dwelling organisms.

To determine soil pH, 5 g of soil was shaken with 25 mL of 0.01 M $CaCl_2$ solution for 2 h. The pH was measured after settling the suspension for 2 h.

2.5. Single-species exposures

Data from single-species tests were obtained from Khodaparast et al. (2021) and Talaber et al. (2020). Briefly, mealworms and woodlice were exposed for 21 days in Lufa 2.2 soil spiked with the same Ag forms used in the mesocosms. For the Ag_2S NPs the Lufa 2.2 soil was spiked with 22.0 μg Ag g^{-1} soil for both organisms, while for $AgNO_3$ it was 97.0 μg Ag g^{-1} soil for mealworms and 13.0 μg Ag g^{-1} soil for woodlice. The mealworms were exposed individually, starting three days after spiking the soil while the woodlice were exposed to the soil just after it was spiked. In the single-species tests, mealworms were not depurated, while woodlice hindguts were removed before frozen. It should be noted that the use of different exposure concentrations of Ag in the mesocosm and the single-species tests did not affect the comparison as data allowed fitting the one-compartment model which assumes that exposure concentration does not affect the uptake kinetics.

2.6. Ag analysis

2.6.1. Ag concentrations in test organisms from mesocosms

At each sampling time, two mealworms per column were sampled (in total 6 mealworms per exposure time), depurated, and frozen. After freeze-drying, each sample was weighed and digested in 4 mL reverse *aqua regia* (HNO_3: HCl; 3:1 *v*/v; J.T. Baker. Trace analysis) by heating on a hotplate. After evaporating the acids to <2 mL, the sample was diluted using 1% HCl to a volume of 45 mL as described by Ribeiro et al. (2017). Three blanks and certified reference material (DOLT-5) were also analyzed. The digests were analyzed for Ag using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Nexion 350D, Perkin-Elmer Inc., Waltham, MA). The certified reference material DOLT-5 showed an average recovery of 108 ± 1.5% (± SD; n = 3). The limit of detection (LOD) was 0.5 μg Ag L^{-1}.

Woodlice were digested in another lab, using a slighly different procedure. Lyophilized single woodlice (n = 15 per exposure) were digested in a mixture of 65% HNO_3:36% HCl (1:4 *v*/v) using a microwave and analyzed according to Talaber et al. (2020). Digestion was conducted in quartz inserts at 200 °C and 800 W power, heating for 25 min, then kept at constant temperature for 15 min, and finally left for 45 min cooling to 60 °C. Digests were analyzed for Ag by flame Atomic Absorption Spectrometry (AAnalyst 100, Perkin Elmer). Samples were calibrated using Ag standards (Sigma Aldrich, Germany) before analysis and after every 15 samples to ensure the accuracy of measurements. The LOD was 1.62 μg Ag per g animal dry weight.

2.6.2. Ag in soil and soil pore water

Soil samples were digested using *aqua regia* in a microwave (Milestone) (US-EPA, 1996). The digest was diluted (acid concentration of c. 3%) and Ag concentration determined by ICP-MS (Perkin Elmer Nexion 350 D).

Three replicate 35 g moist soil samples from each spiked soil batch were saturated to 100% of WHC and incubated overnight at 20 °C, at the beginning of the test to collect soil pore water (SPW). As the presence of plants may change soil moisture content, on the last day of the test samples from the 0–4 cm soil surface were taken and kept at 4 °C for one day while checking the soil moisture content of the other samples, and then also saturated to 100% WHC and incubated overnight at 20 °C. Subsequently, the samples were centrifuged through 0.02 *g* glass wool and 0.45 um PVDF filters (soaked in 0.1 M $CuSO_4$ to minimise Ag loss (Cornelis et al., 2010)) at 2000 g for 1 h. To measure the concentration of dissolved Ag, 2 mL of the extracted SPW were centrifuged through 10 kDa PES ultra-filtration tubes (soaked in 0.1 M $CuSO_4$ to minimise Ag loss (Cornelis et al., 2010)) at 4000 g for 30 min. The extracted and ultrafiltered SPW samples were diluted with HNO_3 before measuring the Ag concentrations by ICP-MS.

2.7. Toxicokinetic models

A one-compartment model (van den Brink et al., 2019) was used to describe the Ag uptake kinetics in both the test organisms exposed in the single-species tests and the mesocosm experiment:

$$C_t = C_0 + \left(\frac{K_1}{K_2 + K_{growth}}\right) * C_{exp} * \left(1 - e^{\left(-\left(K_2 + K_{growth}\right) * t\right)}\right) \quad (1)$$

Where C_t = internal Ag concentration in the test organism at t days (μg Ag g^{-1} dry body weight); C_0 = background internal concentration at t = 0 (μg Ag g^{-1} dry body weight); K_1 = uptake rate constant (g dry soil * g^{-1} dry body weight * day^{-1}); K_2 = elimination rate constant (day^{-1}); C_{exp} = Ag exposure concentration (μg Ag * g^{-1} dry soil); and t = time (days); K_{growth} = growth rate constant calculated using an exponential model or the Von Bertalanffy model (day^{-1}). A dynamic, kinetic-based bioaccumulation factor (BAF_k) was calculated as the ratio of K_1 and K_2 (g dry soil g^{-1} dry body weight); a steady-state BAF_{ss} as the ratio of the

total Ag concentration in the animals (μg Ag * g^{-1} dry body weight) at the last day of the experiment and the total Ag concentration in the soil (μg Ag * g^{-1} dry soil).

The decreasing rate of total Ag concentration in woodlice from the control group (non-exposed animals) during the mesocosm test was estimated by the first-order decay model as follows:

$$C(t) = C_0 * e^{-K*t} \quad (2)$$

Where $C(t)$ and C_0 are the concentrations in the woodlice (μg g^{-1} dry body weight) at time t (days) and t = 0, respectively, and K is the decay rate constant (day^{-1}).

In the 21-day exposure of the single-species test, mealworms gained weight, so the K_{growth} was included in the toxicokinetics calculations to account for the possible growth dilution of Ag in the organisms (Ardestani et al., 2014). In the mesocosm test, mealworm growth was limited, while isopod weight did not change during both experiments; therefore no correction for K_{growth} was applied.

The same modelling approaches of the current study were applied to the raw data of the single-species tests to make a proper comparison between the current mesocosm study and earlier single-species tests.

2.8. Statistical analysis

The toxicokinetic parameters were estimated using non-linear regression in SPSS (version 24) for fitting eq. (1) to experimental data. The significance of the differences between K_1 or K_2 values for the different Ag forms within each experiment, and between single-species and mesocosm tests (for the same Ag form) were tested with a Generalized Likelihood Ratio (GLR) test ($X^2_{(1)} > 3.84$; $p < 0.05$) (Sokal and Rohlf, 2012).

One- or two-way ANOVA analyses with the Holm-Sidak Method ($p < 0.05$) were applied to compare pH values in soil or pore water and Ag concentrations in soil, pore water, and test organisms between Ag forms and/or sampling times. The ANOVA analyses were done using Sigmaplot 14.0.

3. Results and discussion

3.1. TEM coupled EDX

The results of the TEM-EDX analysis of the stock suspension showed that each Ag_2S NP particle contained at least 69 mass % Ag (Fig. S2 and Table S1). These particles were made as sulphadised AgNPs and based on the characterisation that was published for these Ag_2S NPs, they showed high stability (Khodaparast et al., 2021; Peixoto et al., 2020; Talaber et al., 2020). The TEM and EDX analysis of the extracted soil pore water showed that only 3 of the 15 detected spectra presented minimal Ag (Fig. S3 and Table S2).

3.2. Ag concentrations in soil and soil pore water

The pH_{CaCl2} of the Ag-spiked soils never differed >0.4 units compared to the control (Table S3). The background Ag concentration in the Lufa 2.2 soil was 0.049 ± 0.005 μg Ag g^{-1} dry soil (mean ± SD; n = 3). The total measured Ag concentrations in the soil spiked with $AgNO_3$ and Ag_2S NPs at the nominal concentration of 10 μg Ag g^{-1} and also total and dissolved Ag concentrations in SPW are given in Table 1. The concentrations measured in the soil did not differ much from the nominal ones in the mesocosm test and also showed to be homogeneous as evidenced from low variation between replicates. The measured concentrations were used to estimate Ag uptake and elimination rate constants in the test organisms.

At the beginning of the test (two days after spiking), the total Ag concentration in soil pore water was similar for both Ag forms at day zero (1.86 and 1.97 μg Ag L^{-1} for $AgNO_3$ and Ag_2S NPs, respectively).

Table 1
Total Ag concentrations in soil (dry weight) and total and dissolved Ag concentrations in pore water from Lufa 2.2 soil spiked at a nominal concentration of 10 μg Ag g^{-1} with $AgNO_3$ and Ag_2S NPs in the mesocosm test. Data are presented as average ± SD (n = 11 for soil samples, n = 3 for soil pore water). Statistically significant differences (one-way ANOVA followed by Holm-Sidak Method (α = 0.05)) between all treatments at each sampling time (within each column) are shown by different small letters.

Treatment	Ag concentration in soil (μg Ag g^{-1})	Total Ag concentration in soil pore water (μg Ag L^{-1})		Dissolved Ag concentration in soil pore water (μg Ag L^{-1})	
		Day 0	Day 28	Day 0	Day 28
Control	0.049 ± 0.005 a	<DL	0.325 ± 0.223 a	<DL	0.192 ± 0.076 a
$AgNO_3$	10.6 ± 1.06 b	1.86 ± 1.73 a	1.74 ± 0.224 b	2.47 ± 1.64	0.649 ± 0.178 b
Ag_2S NPs	12.8 ± 0.876 c	1.97 ± 3.11 a	0.673 ± 0.228 a	<DL	0.127 ± 0.017 a

DL The detection limit was 0.03 μg Ag L^{-1}.

After 28 days, the soil pore water of $AgNO_3$ spiked soil contained almost the same concentration of Ag, but for Ag_2S NPs, it was lower than its concentration at day 0 and significantly lower than for $AgNO_3$ at day 28. The dissolved Ag concentrations, expressed as a percentage of the total concentration in extracted soil pore water from Lufa 2.2 soil spiked with $AgNO_3$ and Ag_2S NPs during the mesocosm test and the single-species test (data from Khodaparast et al., 2021) are given in Fig. 1. The percentage of dissolved Ag for $AgNO_3$ was about 80% on day 0 and decreased to 45% during the 28-day mesocosm test. For Ag_2S NPs the dissolved Ag concentration was below the detection limit at t = 0 and reached 20% on day 28.

The specific experimental conditions in our mesocosms may explain why Ag from Ag_2S NPs was more available than $AgNO_3$. Firstly, significant detachment rates were found during saturated column tests with the same Ag_2S NPs used in this study and Lufa 2.2 soil (Norrfors et al., 2021). Daily, water was added to our mesocosms and leaching out at the bottom, while during the single-species test only a few drops of ultrapure water were added to replenish water loss. Water flowing along pore walls where Ag_2S NPs were attached may have exerted shear forces, resulting in detachment of these NPs and making them more available

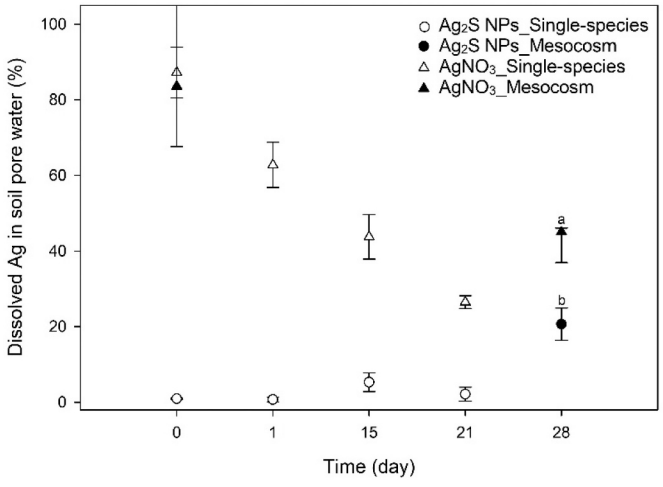

Fig. 1. The percentage of dissolved Ag (compared to the total measured Ag concentration) in pore water from Lufa 2.2 soil spiked with $AgNO_3$ and Ag_2S NPs during the single-species test (for mealworms (Khodaparast et al., 2021)) and the mesocosm test. Error bars represent standard error (n = 3, except Ag_2S NPs at day 28, n = 4). Statistically significant differences (one-way ANOVA followed by Holm-Sidak Method (α = 0.05)) between $AgNO_3$ and Ag_2S NP treatments are presented with different letters.

for uptake. Similarly, water addition resulted in significantly higher Ag concentrations in earthworms compared to a no water addition condition during 28 days of exposure to Ag_2S NPs (Baccaro et al., 2019). On the other hand, ionic Ag ($AgNO_3$ and some from Ag_2S) is always in a dynamic exchange between the solid and liquid fractions of the soil and known to sorb strongly to soil organic matter, effectively making it non-available (Cornelis et al., 2012). Similarly, watering did not influence the uptake of Ag from Ag(0) NPs and $AgNO_3$ in the earthworm *E. fetida*, while for Ag_2S NP exposed animals slightly higher bioaccumulation of Ag was observed by watering (to replenish the loss of moisture) (Baccaro et al., 2021). Ag_2S NPs have higher surface charges at most pH values compared to non-sulfidized Ag (Levard et al., 2011). Ag_2S NPs have been found less (Li et al., 2019) or more (Yecheskel et al., 2016) mobile in soil columns compared to Ag(0) NPs, but we hypothesize that the specific conditions in the mesocosm with higher water content in the soil enhanced the bioavailability of the Ag_2S NPs.

3.3. Ag uptake in mealworms

The background Ag concentration (C_0) in the mealworms was below the detection limit for the mesocosm experiment but averaged 0.36 ± 0.06 and 0.26 ± 0.09 μg g^{-1} (mean ± SD; $n = 3$) in the single-species tests for $AgNO_3$ and Ag_2S NPs, respectively (Table S4).

The Ag body concentrations in the mealworms were significantly different between Ag_2S NP and $AgNO_3$ exposures and over time (Ag body concentrations in the mealworms versus Ag forms and sampling times; two-way ANOVA with the Holm-Sidak post-hoc test, $p < 0.001$). At the end of the test, Ag concentration was two times higher in the mealworms exposed to Ag_2S NPs than $AgNO_3$ (Table S4).

For Ag_2S NP-exposed mealworms, Ag was detected in feces at days 7 and 14 at concentrations of 16.7 and 15.6 μg Ag g^{-1}, respectively; for $AgNO_3$, feces from mealworms had 15.9 and 19.6 μg Ag g^{-1}, respectively, while control mealworm feces presented levels below the detection limit at day 7 and 2.5 μg Ag g^{-1} at day 14. Ingestion of soil and soil pore water are the main exposure routes for mealworms (Khodaparast et al., 2021). The presence of Ag in the feces of mealworms exposed to both Ag forms confirms their oral uptake of Ag and the ability to excrete it.

The estimated toxicokinetic parameters are shown in Table 2, and the uptake curves are in Fig. 2. The K_1 and K_2 values for Ag_2S NPs did not differ significantly ($X^2_{(1)} < 3.84$; $p > 0.05$) from those for $AgNO_3$. In the mesocosm test, the derived Ag BAF_{ss} and BAF_k from Ag_2S NPs exposure were 1.8-fold and 2-fold higher than for $AgNO_3$ exposure, respectively (Table 2). Contrary to our findings, lower Ag bioaccumulation from Ag_2S NPs compared to $AgNO_3$ has been reported for soil invertebrates exposed in the single-species tests, for example in mealworms (Khodaparast et al. (2021), earthworms (*E. fetida*) (Baccaro et al., 2018), woodlice (*P. scaber*), and springtails (*F. candida*) (Talaber et al., 2020). Also, exposure to soil amended with sewage sludge spiked with PVP-coated AgNPs (40 nm, 77.95 μg Ag g^{-1} dry soil) resulted in a lower BAF_k (0.12 g soil g^{-1} dry body weight) in the earthworm *Eisenia andrei* compared to $AgNO_3$ (1.09 μg Ag g^{-1} dry soil; $BAF_k = 0.74$ g soil g^{-1} dry body weight) (Velicogna et al., 2017).

Based on the GLR test, the Ag uptake rate constants in mealworms from the single-species test agreed with the values obtained for the mesocosm experiments for $AgNO_3$, but not for Ag_2S NPs (Table 2). Kinetics in the mealworms were also calculated over a similar exposure period for both Ag forms to discard differences due to different exposure times. This analysis also showed no significant differences between K_1 and K_2 for the single-species and mesocosm tests for $AgNO_3$ (Table S5; $X^2_{(1)} < 3.84$; $p > 0.05$), while significant differences between tests were observed for Ag_2S NPs ($X^2_{(1)} > 3.84$; $p < 0.05$).

BAF_k values for $AgNO_3$ were similar in the single-species and mesocosm tests (0.40 and 0.39), while for the Ag_2S NPs they were higher in the mesocosm test (0.79) compared to the single-species test (0.15). It is noteworthy that as the steady-state was already reached in the single-species test, the BAF_{ss} of day 21 could be compared to the BAF_{ss} of day 28 for the mesocosm test. The BAF_{ss} based on the internal Ag concentration in the mealworms exposed to $AgNO_3$ after 28 days in the mesocosm test was similar to that in the single-species test after 21 days of exposure although the exposure concentration was 10 times higher in the latter test. The steady-state in the Ag uptake kinetics in the $AgNO_3$ exposures indicates the ability of the mealworms to eliminate Ag, although a more accurate estimation of elimination rate constants would require the inclusion of an elimination phase.

For the mealworms exposed to Ag_2S NPs, the BAF_{ss} was 5.5 times higher in the mesocosm test (28 days) compared to the single-species test (21 days) (Table 2). Based on their dissolution at day 28, although the majority was non-dissolved in the mesocosm pore water, the Ag_2S NPs were probably bioavailable to the mealworms in the particulate and also dissolved form during the mesocosm test. In a recent study, the long-term (9 months) bioaccumulation of Ag from AgNPs and $AgNO_3$ in the earthworm *E. fetida* could not be accurately predicted because of the late dissolution of Ag_2S NPs caused by watering upon long-term exposure (Baccaro et al., 2021). This could also be hypothesized for mealworms during our mesocosm test, which led to significantly different uptake patterns compared to the single-species test. Therefore, it can be concluded that the uptake kinetics in the mealworms of Ag from Ag_2S NPs determined in the single-species test do not allow estimating Ag uptake in the more complex mesocosm system. Furthermore, mealworms were not depurated during the single-species test as the amount of soil found in their gut was too low and by its short residence time may not significantly contribute to the total body Ag concentration. Considering the depuration of mealworms for the mesocosm test and the

Table 2
Uptake and elimination kinetic parameters for the bioaccumulation of Ag in mealworms (*Tenebrio molitor*) exposed to $AgNO_3$ at 97.0 and 10.6 μg Ag g^{-1} dry soil or Ag_2S NPs at 22.0 and 12.8 μg Ag g^{-1} dry soil in Lufa 2.2 soil in single-species and mesocosm tests, respectively. Corresponding 95% confidence intervals are given between brackets. Significant differences (likelihood ratio test, $X^2_{(1)} > 3.84$; $p < 0.05$) between K_1 and also K_2 values are indicated with different capital letters for the difference between single-species and mesocosm test for the same Ag form, different small letters for the difference within a mesocosm test between different Ag forms, and different Greek alphabet letters for the difference within a single-species test between different Ag forms. BAF_k is calculated as the ratio of K_1 and K_2 values, BAF_{ss} as body concentration at the end of the tests divided by soil concentration.

Treatment	Test	Duration (Days)	K_1 (g soil g^{-1} dry body weight day^{-1})	K_2 (day^{-1})	BAF_k (g soil g^{-1} dry body weight)	BAF_{ss} (g soil g^{-1} dry body weight)	K_{growth} (day^{-1})
$AgNO_3$	Mesocosm	28	0.07 (0.018–0.120) **A, a**	0.18 (0.021–0.33) **A, a**	0.39	0.37	0.0
	Single-species	21	0.26 (0–0.63) **A, α**	0.65 (0–2.04) **A, α**	0.40	0.35	0.25
Ag_2S NPs	Mesocosm	28	0.056 (0.028–0.084) **A, a**	0.071 (0.009–0.132) **A, a**	0.79	0.66	0.0
	Single-species	21	1.66 (−) **B, α**	11.1 (−) **B, α**	0.15	0.12	0.003

(−) very wide 95% confidence intervals.

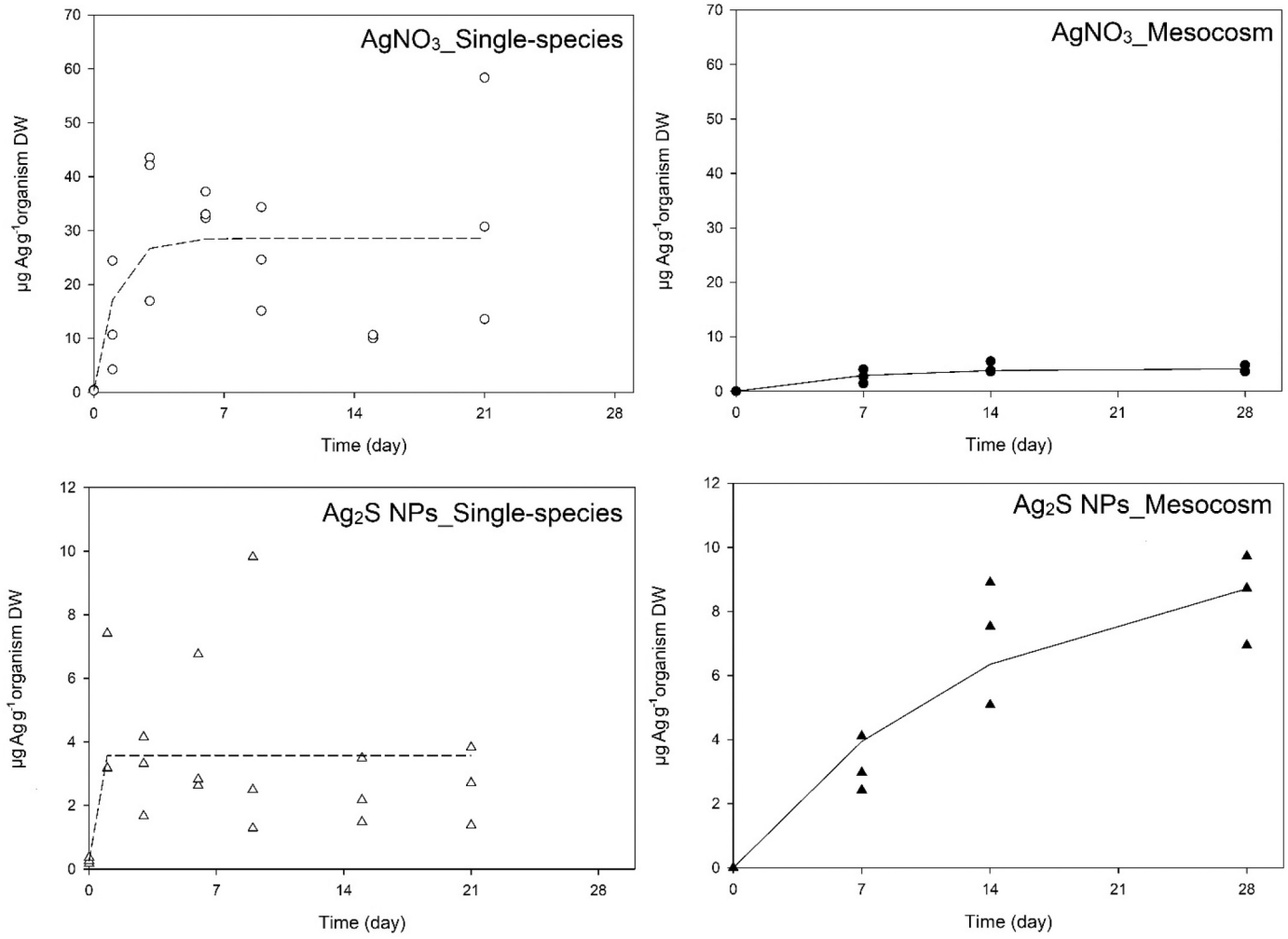

Fig. 2. The total concentration of Ag in mealworms (*Tenebrio molitor*) in single-species and mesocosm tests in Lufa 2.2 soil: Top graphs: exposure to $AgNO_3$ at 97.0 and 10.6 µg Ag g^{-1} dry soil in single-species (left) and mesocosm tests (right), respectively; Bottom graphs: Ag_2S NP exposure at 22.0 and 12.8 µg Ag g^{-1} dry soil in the single species (left) and mesocosm (right) tests, respectively. Solid and dashed lines show the fit of a one-compartment model to the Ag concentrations in the mealworms from the mesocosm and single-species test, respectively (eq. 1). See Table 2 for the kinetics parameters derived from the model fits.

higher bioaccumulation factors, the bioavailability of Ag_2S NPs was higher in the mesocosm test compared to the single species test.

3.4. Ag uptake in woodlice

The background Ag concentration (C_0) in woodlice averaged 9.35 ± 4.4 µg Ag g^{-1} dry body weight (mean ± SD; $n = 15$) for mesocosm and 7.04 ± 1.35 µg Ag g^{-1} dry body weight (mean ± SD; $n = 3$) for single-species tests. This difference between background Ag concentrations may be due to the different origins of the cultures used for the single-species and mesocosm tests. During the exposure, background Ag concentration in control isopods from the single species test did not change. In the mesocosm test, the woodlice could slowly eliminate some of the background Ag concentration due to the longer time of exposure (28 days), possibly because it concerned Ag attached to the gut cuticle or stored in the midgut epithelial cells. It is unclear which forms of Ag these woodlice were previously exposed. Elimination of Ag was not expected in woodlice; however, the decrease rate of Ag in the control group was low, 0.03 day^{-1}, which is similar to what was reported for the elimination rate constant of Ag from $AgNO_3$ and 3–8 nm and 50 nm AgNPs (Talaber et al., 2020; Tourinho et al., 2016).

At the end of the mesocosm experiment, at day 28, the total body Ag concentration was significantly higher in $AgNO_3$ than in Ag_2S NP exposed woodlice (Table S4, Holm-Sidak, $p < 0.001$). The estimated toxicokinetic parameters are shown in Table 3 and the corresponding uptake curves are shown in Fig. 3. The Ag uptake rate constant K_1 in $AgNO_3$ exposed woodlice was significantly higher in the single-species compared to the mesocosm test ($X^2_{(1)} > 3.84$; $p < 0.05$). Due to the very low elimination rate of $AgNO_3$ in the mesocosms, the BAF_k value was very high.

Ingestion of soil particles is the main exposure route for woodlice, with metal adsorption to the surface cuticle and pleopode uptake (drinking) being negligible compared to oral uptake as reported for *P. scaber* by Vijver et al. (2005). Moreover, woodlice are coprophagous (König and Varma, 2006), so they may have eaten their feces or mealworm feces during the mesocosm test. Exposure of organisms to NPs via coprophagy merits further research because for example in mealworms the Ag concentration in feces was similar to that in soil. However, there is no information regarding the forms and transformations of NPs by passing through the animal's body, which may affect its bioavailability (Svendsen et al., 2020).

In the mesocosm and single-species test, the Ag body concentrations of woodlice increased upon exposure to $AgNO_3$ but did not during the 21-day and 28-day exposure to Ag_2S NPs, so no curves were fitted to these data (Fig. 4). The non-elevated Ag body concentration from Ag_2S NP exposures in the single-species test of Talaber et al. (2020) (used here as a comparison) and also in the mesocosm test seemed to be similar. Nevertheless, the significant decrease in total Ag concentration in the

Table 3
Uptake and elimination kinetic parameters for the bioaccumulation of Ag in woodlice (*Porcellio scaber*) exposed to $AgNO_3$ at 13.0 and 10.6 μg Ag g^{-1} dry Lufa 2.2 soil in single-species and mesocosm tests, respectively. Corresponding 95% confidence intervals are given between brackets. Significant differences (likelihood ratio test, $X^2_{(1)} > 3.84$; $p < 0.05$) between K_1 and K_2 values for the single-species and mesocosm test are indicated with different capital letters.

Treatment	Test	Duration (days)	K_1 (g soil g^{-1} dry body weight day^{-1})	K_2 (day^{-1})	BAF_k (g soil g^{-1} dry body weight)	BAF_{ss} (g soil g^{-1} dry body weight)
$AgNO_3$	Mesocosm	28	0.037 (–) **A**	$1*10^{-10}$ (0–0) **A**	(*)	2.25
	Single-species	21	0.26 (0–0.562) **B**	0.23 (0–0.545) **B**	1.1	1.57

(–) very wide 95% confidence intervals; (*) too high value.

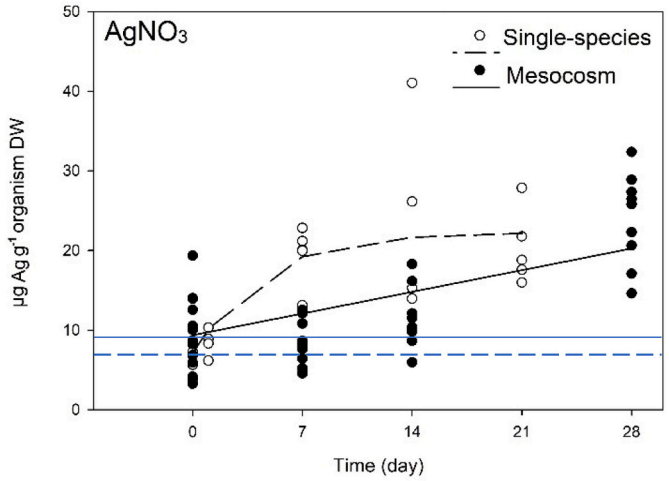

Fig. 3. The total concentration of Ag in woodlice (*Porcellio scaber*) exposed to Ag NO_3 at 13.0 and 10.6 μg Ag g^{-1} dry Lufa 2.2 soil, in single-species and mesocosm tests, respectively. Lines show the fit of a one-compartment model to the Ag concentrations in the woodlice (eqs. 1). The horizontal blue lines show the average Ag background concentrations in woodlice for each test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

control isopod group of the mesocosm test may suggest a slight uptake of Ag from Ag_2S NPs in the mesocosm test.

For isopods, Kampe et al. (2018) reported that after 14 days of exposure to Ag_2S NPs, 71% of measured Ag was in the isopod hindgut which included the gut content. It should be mentioned that there was a difference in the methodology applied to prepare the woodlice for analysis between the mesocosm test and the study by Talaber et al. (2020). In our study, woodlice were purged for 24 h, producing a similar result as the one of Talaber et al. (2020) by removing the isopod hindgut before freezing. In both studies, the midgut or hepatopancreas remained inside woodlice, where most stored metals are located, including Ag. Therefore, these two methodologies are comparable, not affecting the validation of the comparison between these two tests.

Estimated kinetics in woodlice from the single-species test could not predict the uptake of Ag from $AgNO_3$ in the mesocosm test. In the latter there was not much accumulation of Ag in the woodlice during the first 14 days of exposure to $AgNO_3$ followed by a strong increase of internal Ag concentration on day 28. This was different from the single-species test where fast uptake occurred during the first week reaching a plateau. Woodlice are metal accumulators (Ghemari et al., 2019) with slow elimination of Ag (Talaber et al., 2020; Tourinho et al., 2016). This in fact agrees with the increased accumulation of Ag after 28 days of exposure in the mesocosms. In addition, woodlice were exposed to the soil immediately after spiking in the single-species test, while in case of the mesocosm test the spiked soil was incubated for two days, possibly allowing for immobilization of Ag ions in the soil (Coutris et al., 2012). This may explain the difference in the bioavailability of the $AgNO_3$ during the first weeks between the single species and mesocosm tests.

Bioaccumulation in invertebrates is species-specific and dependent on life-history, morphology, and physiology of the animal (Mortensen et al., 2018; van der Zande et al., 2020). The Ag concentrations were significantly higher in the woodlice compared to the mealworms, and also kinetics differed between these species. This difference may be due to different routes of exposure, with soil pore water being slightly more important than soil exposure for mealworms (Khodaparast et al., 2021), while woodlice are exposed mainly through soil ingestion. In addition, the organisms also differ in the way they deal with Ag, with woodlice accumulating silver in granules in the S-cells of the hepatopancreas (Tourinho et al., 2016). Mealworms are mostly on the soil surface but woodlice dig into the soil leading to different exposure conditions. In the soil, the woodlice could also feed on the plant roots or be exposed to the rhizophora and plant exudates where the bioavailability of Ag may be different compared to the soil surface. This implies that different soil invertebrates need to be investigated in order to provide a more integrated view on the bioaccumulation potential of nanomaterials.

4. Conclusions

The Ag toxicokinetics in mealworms and woodlice from mesocosms may not be reliably predicted from single-species test data. In case of mealworms, the estimation of Ag uptake rate constants from $AgNO_3$ in the single-species and mesocosm tests were similar regardless of exposure time, but for Ag_2S NPs it differed and bioaccumulation of Ag from Ag_2S NPs was higher in the mesocosm compared to the single-species test. For woodlice, uptake rate constants of Ag from $AgNO_3$ in single-species and mesocosm tests were different. Mealworms quickly reached a steady-state as they seem to be a good regulators, but woodlice kept on accumulating Ag with time, so proper assessment of the uptake kinetics requires longer exposure times with low expoure concentration for these accumulators. Indoor mesocosm experiments provide a more realistic accumulation assessment of Ag_2S NPs, with a holistic approach, by including different organisms and complex exposure conditions. However, single species tests will remain useful as a fast screening tool to assess the accumulation potential of substances including nano-, macro or advanced materials before other more complex tests are conducted.

CRediT authorship contribution statement

Zahra Khodaparast: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Cornelis A.M. van Gestel:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Ana Rita R. Silva:** Methodology, Writing – review & editing. **Geert Cornelis:** Conceptualization, Methodology, Resources, Writing – review & editing. **Elma Lahive:** Conceptualization, Methodology, Resources, Writing – review & editing. **Amaia Green Etxabe:** Methodology, Writing – review & editing. **Claus Svendsen:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **Marta Baccaro:** Methodology, Investigation, Writing – review & editing. **Nico van den Brink:** Conceptualization, Methodology,

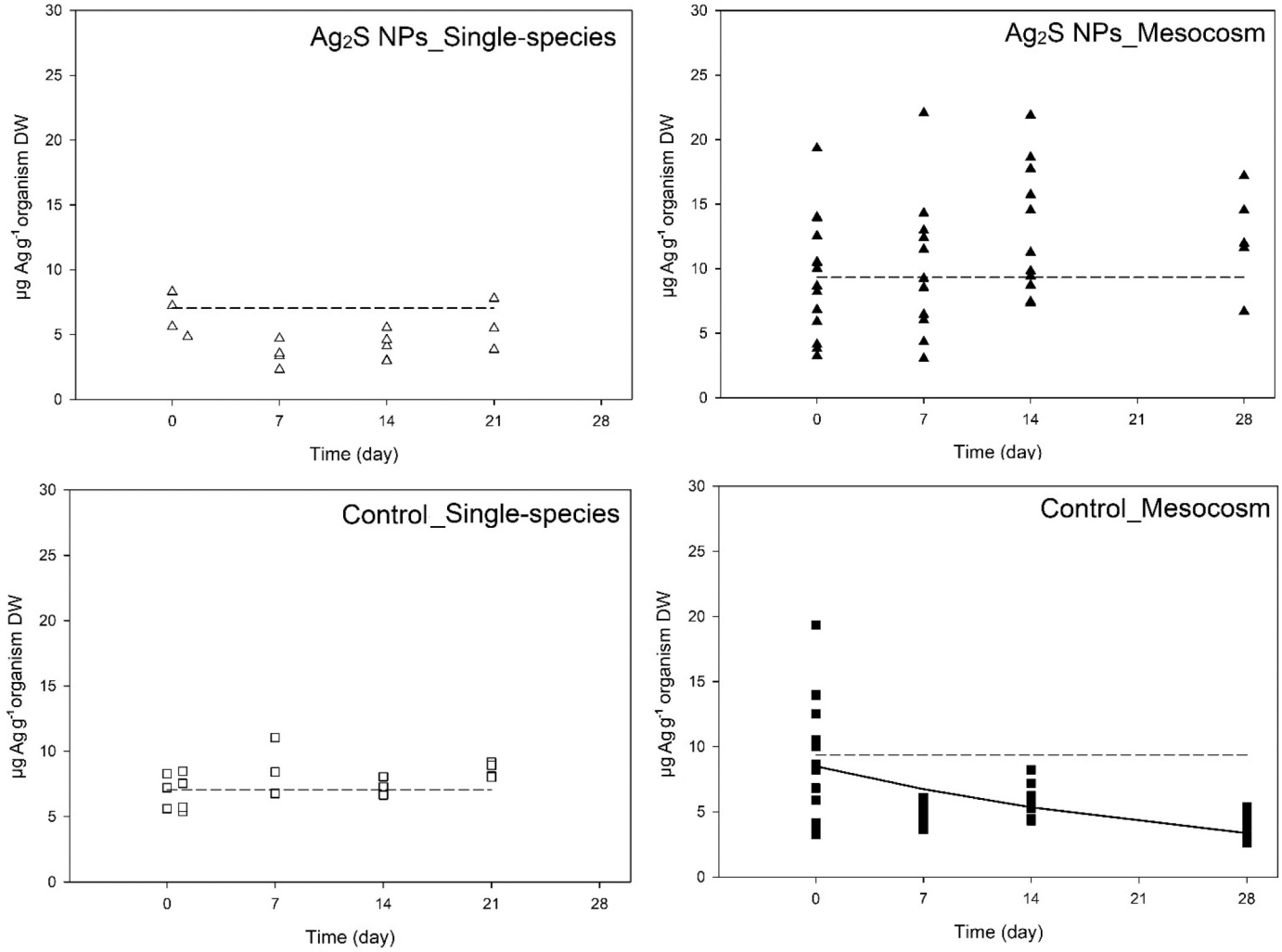

Fig. 4. The total concentration of Ag in woodlice (*Porcellio scaber*) Ag_2S NPs at 22.0 and 12.8 µg Ag g^{-1} dry Lufa 2.2 soil in single-species (left top) and mesocosm (right top) tests, respectively. The horizontal dashed lines show each test's average Ag background concentrations in woodlice. Also, given are the total Ag concentration in woodlice that were not exposed to Ag (Control) during the single-species (left bottom) and mesocosm tests (right bottom). The solid line shows the decrease of the Ag concentrations in the woodlice (eq. 2).

Resources, Writing – review & editing. **Neja Medvešček:** Methodology, Investigation. **Sara Novak:** Methodology, Writing – review & editing. **Anita Jemec Kokalj:** Methodology, Writing – review & editing. **Damjana Drobne:** Conceptualization, Methodology, Resources, Writing – review & editing. **Kerstin Jurkschat:** Conceptualization, Methodology, Resources, Writing – review & editing. **Susana Loureiro:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.impact.2023.100454.

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