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Toxicokinetics of silver nanoparticles in the mealworm *Tenebrio molitor* exposed via soil or food

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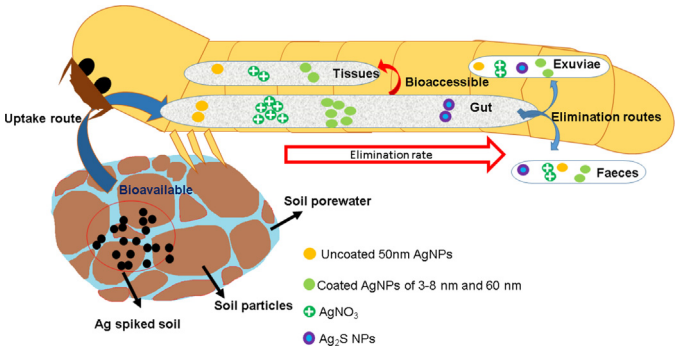
HIGHLIGHTS

- Mealworms accumulate Ag_2S NPs despite its low dissolution in soil pore water.
- Ag_2S NPs were accumulated less than pristine AgNPs and ionic Ag in mealworms.
- Mealworms exposed via soil and via food showed different Ag uptake kinetics.
- AgNPs dissolution in soil pore water influences its uptake in the mealworms.
- Mealworms eliminate Ag through the faeces and by shedding exuviae.

GRAPHICAL ABSTRACT



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ABSTRACT

Silver nanoparticles (AgNPs) may reach the soil compartment via sewage sludge or nanoagrochemical applications. Understanding how NPs interact with biological systems is crucial for an accurate hazard assessment. Therefore, this study aimed at determining the Ag toxicokinetics in the mealworm *Tenebrio molitor*, exposed via Lufa 2.2 soil or via food to different Ag forms (uncoated 50 nm AgNPs, paraffin coated 3–8 nm and PVP-stabilised 60 nm, Ag_2S NPs 20 nm, and ionic Ag). Mealworms were exposed for 21 days followed by a 21-day elimination phase (clean soil/food). A one-compartment kinetics model with inert fraction (simulating a storage compartment, where detoxified forms are located) was used to describe Ag accumulation. Fully understanding the uptake route in mealworms is difficult. For that reason several approaches were used, showing that food, soil and pore water all are valid uptake routes, but with different importance. Silver taken up from soil pore water or from soil showed to be related to Ag dissolution in soil pore water. In general, the uptake and elimination rate constants were similar for 3–8 nm and 60 nm AgNPs and for $AgNO_3$, but significantly different for the uncoated 50 nm AgNPs. Upon food exposure, uptake rate constants were similar for 50 nm AgNPs and $AgNO_3$, while those for 60 nm and 3–8 nm AgNPs and for Ag_2S NPs also grouped together. NP exposure in soil appeared more difficult to characterize, with different patterns obtained for the different NPs. But it was evident that upon soil or food exposure, particle characteristics highly affected Ag bioavailability and bioaccumulation. Although Ag_2S NPs were taken up, their elimination was faster than for other Ag forms, showing the lowest inert fraction. The significantly different elimination rate constants suggest that the mechanism of elimination may not be the same for different AgNPs either.

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1. Introduction

The production of nearly 1000 tons of silver engineered nanomaterials per year (Giese et al., 2018) raises concerns about the bioaccumulation of silver nanoparticles (AgNPs) in organisms living in exposed environments. The estimated consumption of AgNPs amounts about 360 to 450 ton year^{-1} (McGillicuddy et al., 2017), with the largest proportion being used in consumer electronics, textiles, and for medical purposes (Giese et al., 2018). A substantial proportion of the nanoparticles (NPs) used end up in the sewage system and therefore may accumulate in sewage sludge (Blaser et al., 2008). When NPs are discharged to the receiving environment, their characteristics will no longer be the same as those in the pre-exposure phase due to their interactions with and transformations by their surroundings (Svendsen et al., 2020). Thus, their behaviour will also change depending on the environmental conditions at discharge. The tendency of silver (Ag) to form complexes with organic matter, chloride or other compounds such as Mn/Fe oxides in the soil may further reduce its mobility and bioavailability. Coutris et al. (2012) reported that $AgNO_3$ may immobilize rapidly in the soil, nevertheless there are indications that AgNPs may behave like a source of bioavailable Ag following slow dissolution over time (Diez-Ortiz et al., 2015). Soil pH was reported to affect the transformation of ionic Ag, Ag-NP and AgCl-NPs in soil (Sekine et al., 2015). AgCl was found to be formed and persist in acidic conditions, but in neutral to alkaline soils sulfur-bound Ag was the dominate Ag form (Sekine et al., 2015). In addition to pH, the redox conditions also affect the silver transformation, while under anaerobic and aerobic conditions, Ag_2S and $AgCl_x^{(x-1)-}$ are expected to form, respectively (Levard et al., 2012). Because of the presence of a large proportion of sulfide and the reduced conditions during wastewater treatment, Ag_2S is expected to be formed from Ag ions or AgNPs (Kim et al., 2010), decreasing the adverse effects of silver in the environment due to its low water solubility (Coutris et al., 2012). Ag_2S NPs have high chemical stability and may still be stable following seven months incubation in neutral, alkaline and acidic soils (Sekine et al., 2015).

One of the main applications of sewage sludge is as agricultural land fertilizer (Fytili and Zabaniotou, 2008). In European countries, over 10 million tons of dry sewage sludge are produced each year, about 40% of which is applied to agricultural lands as fertilizer (Roig et al., 2012). Giese et al. (2018) predicted concentrations of AgNPs in non-sludge-amended soils for 2017 of 30 pg kg^{-1} that may increase to 10 μg kg^{-1} soil by 2050, while sludge-amended soils are predicted to contain 1130 pg kg^{-1} in 2017 and to achieve 30–40 times higher values than non-sludge-amended soils by 2050 (Giese et al., 2018). Besides studying their toxic effects, understanding AgNP bioaccumulation potential and toxicokinetics is critical for decoding their behaviour, mechanism of toxicity and environmental risk (Baalousha et al., 2016; Petersen et al., 2019; Wang, 2011). Metal based NPs don't necessarily degrade like pesticides, leading to potential long-term exposures. Toxicokinetics approaches may help with assessing the potential for bioaccumulation of different sizes of AgNPs and different forms of Ag (different coatings, NP or ionic etc.) by deriving uptake and excretion rate contants (Argasinski et al., 2012). Internal concentrations reflect the bioavailable concentration for uptake from the environment (Hug Peter et al., 2018) linked with the bioaccessible fraction associated with the internalization of metals through the cell membrane (Loureiro et al., 2018). After ingestion, the gut may work as a barrier for the uptake of NPs and its conditions may lead to the dissolution or aggregation of NPs in the lumen (Van Der Zande et al., 2020). While dissolved metals may be taken up through solute transporters in the cell membrane of gut epithelial cells, NPs are too big to be taken up in this way (Van Der Zande et al., 2020). Endocytosis is the most likely uptake mechanism for NPs in the gut, however, intracellular digestion is another possible uptake route for invertebrates (Van Der Zande et al., 2020). So, if Ag NPs are bioaccumulated, different biodynamics may be expected from those observed for Ag^+ (Croteau et al., 2011). Therefore, NP behaviour in the environment (e.g. dissolution rate, or partitioning between the soil solid phase and the soil pore water) depends on their physicochemical properties and understanding and parameterisation of these processes are crucial to accurately assess their environmental risk (Oomen et al., 2018; Sørensen et al., 2019).

To improve the available hazard data for AgNPs towards soil organisms, it is essential to assess AgNP toxicity and bioaccumulation in different terrestrial organisms (Tourinho et al., 2016). The aim of this study was to address the knowledge gap still existing for the hazard assessment of NPs in soil, and focus on the toxicokinetics of Ag in mealworms exposed to different AgNPs, exploring the roles of coating, particle size and sulface chemistry (e.g. sufidation) and their interaction with AgNP dissolution. Ag_2S NPs were used to simulate ageing of AgNPs in the waste water treatment plant. The mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidade) was chosen as a test organism. This holometabolic insect lays its eggs in moist soil and has three larval stages usually inhabiting the top 15 cm of the soil (Tashiro, 1990). The number of exuviae (exoskeltons) they release during their growth is dependent on the specimen but also on the surrounding environmental conditions (Greenberg and Ar, 1996; Morales-Ramos et al., 2010). Mealworms are commonly used as food for pet animals (e.g. frogs or snakes) and nowadays several approaches are being developed to use them as food (Eriksson et al., 2020) (protein source) in aquaculture and poultry (Morales-Ramos et al., 2010). Because of their long-term larval stage, their active feeding behaviour and commercial availability, mealworms are a suitable species for assessing exposure through both soil and food. As there is some debate in the literature as to whether exposure via soil is an important route of exposure for mealworms, and which fraction (soil and/or pore water) plays the most important role, we explore this question in detail, and some preliminary toxicity tests and approaches to unravel the uptake route are also presented. The results are presented as a one-compartment kinetics model with inert fraction (simulating a storage compartment, where detoxified forms are located) and differences in both uptake and elimination rates for the different AgNPs and forms are elucidated.

2. Materials and methods

2.1. Test organisms, soil and silver forms

Tenebrio molitor larvae were obtained from a commercial breeder and kept in the laboratory at 20 ± 2 °C with a 16:8 (light:dark) h photoperiod. Mealworms were separated according to their weight, in order to use animals weighing 22 ± 6 mg (mean ± standard deviation (SD); n = 216). Considering the test duration (six weeks), the larval stage was chosen, corresponding to this weight range, to ensure that the animals did not enter the pupa stage and that their active behaviour was similar during the test.

The sandy loam Lufa 2.2 soil (Speyer, Germany) was used in this study, presenting an organic carbon content of 1.61 ± 0.15%, pH_{CaCl2} of 5.4 ± 0.2, cation exchange capacity (CEC) of 9.7 ± 0.4 cmol$_c$ kg^{-1} and maximum water holding capacity (WHC) of 44.8 ± 2.9% (average ± SD). Oat flakes (Flocos de Aveia Salutem) were acquired from a local store. AgNPs 3–8 nm (paraffin coated) and AgNPs 60 nm (polyvinylpyrrolidone, PVP) were provided by AMEPOX (Poland), and AgNPs 50 nm (Sodium Citrate) and Ag_2S NPs (PVP) by AppNAno. $AgNO_3$ was purchased from Sigma-Aldrich (99% purity).

2.2. Nanoparticle characterization

The stability of the NPs was examined in suspensions prepared in a weak ammonium acetate solution (0.01 M) in ultrapure water (UPW, pH: 7) as per the method by Thomassen et al. (2001), by measuring the hydrodynamic size, polydispersity index (PDI), Zeta-potential (Z-potential) and dissolution at different time points. The suspension concentration used for characterization for all materials tested was 1 mg Ag.

L^{-1}. Analyses were performed using a Malvern Zetasizer (Nano ZS, equipped with a LASER of 632.8 nm and a scattering angle of 173°) and Sarstedt polystyrene cuvettes (Ref: 67.742, 10 × 4 × 45 mm) or Malvern Zetasizer (DTS0170, disposable folded capillary cells) for hydrodynamic size and Z-potential, respectively. Measurements were carried out at 20 °C following 2 min equilibration time at 2, 4, 24 and 48 h post-dilution to match the timepoints used for the dissolution study, as described below. This allowed us to study potential physicochemical changes taking place, due to for example Ostwald ripening (Yao et al., 1993) as soon as the NPs were dispersed into the respective solutions. Malvern Zetasizer Software (version 7.13) was used by applying the built-in values for the refractive indices (η) and absorption coefficients (α) for Ag and Ag_2S. The obtained results are the average of five consecutive measurements from 14 replicates per measurement.

The protocol of Avramescu et al. (2017) was followed for monitoring Ag dissolution. This protocol is commonly used in metal bioaccessibility assays (Dodd et al., 2013) and is a modification of the European Committee for Standardization (CEN) guidance EN 71-3:2019 on "Safety of toys - Part 3: Migration of certain elements" (European's Committee for Standardisation (CEN). CEN/TC 52., 2019). The dissolved Ag levels were determined in UPW suspensions of 1 mg $Ag.L^{-1}$ using three replicates for each NP and at 5 timepoints (0, 2, 4, 24 and 48 h). The timepoints were chosen to allow studying the kinetics of the NPs dissolution behaviour and to recreate the respective dissolution curves during the high activity phase following NP dispersion. Samples were picked randomly and part of each was filtered using 0.02 μm pore-diameter syringe filters (Anotop™, Whatman), while the rest was used for simultaneous hydrodynamic size and Z-potential measurements. Ag concentrations were determined using an ICP-MS (PerkinElmer, Nexion 3000) following acidification of samples with pure ICP-grade HNO_3 (Sigma Aldrich; CAS Number 7697-37-2) to a final acid concentration of 2%. The calibration curves (correlation coefficient >0.999) were made using ICP-grade standards. ^{103}Rh was used as internal standard and all samples and standards were spiked for QC purposes. Samples failing the QC thresholds were recalibrated and reanalyzed. The acquired results are presented as percentage of dissolved Ag (%dissolved Ag = ([Dissolved Ag^+] / [initial total Ag concentration]) * 100%), according to the OECD classification scheme (OECD, 2015). TEM (transmission electron microscopy) images of AgNPs 3–8 nm and AgNPs 50 nm have been reported by Ribeiro et al. (2014) and Baccaro et al. (2018), respectively. The stability of pristine AgNPs and Ag_2S NPs in different aquatic media has been also reported by Silva et al. (2020).

2.3. Acute toxicity test with $AgNO_3$

To find a suitable exposure concentration for the bioaccumulation tests, two acute toxicity tests were carried out considering exposures via soil and food using $AgNO_3$, as it was expected to be the most toxic Ag form. For the soil exposure, soil was spiked with $AgNO_3$ to final concentrations of 1, 10, 100, 1000 mg Ag kg^{-1} dry soil. Three replicates with five mealworms each were used for each concentration and control. The five organisms were weighted and transferred into each plastic container (135 mm x 85 mm) with 200 g moist soil (40% WHC). The organisms were fed with 5 pieces of oat flakes (~150 mg) twice a week, breaking them up to ensure low area and prevent mealworms from resting on the flakes and thus preventing exposure. Although acute toxicity tests usually do not include food, this methodology tried to combine an acute toxicity test with the conditions needed for the bioaccumulation test.

For the food exposure test, oat flakes were spiked at 1, 10, 100, 1000, 1500 mg Ag kg^{-1} dry food. Thirty mL of spiked suspension was added to 20 g of oat flakes in a plastic pot and mixed for a few minutes. The pot was then closed to let the oats absorb the suspension while the mixture was stirred 3 to 4 times. The size of the plastic pot was big enough to avoid overlap of oat flakes. Three replicates were used for each concentration and control. Five organisms were added to each plastic pot including 500 mg spiked food (about 17 pieces of oat flakes) on the surface of 200 g clean, non-spiked soil. The food was collected from the soil surface and replaced twice a week to be sure that enough food was always available.

Only water was added to the soil or food for the control group. Test containers were closed with lids with 5–6 small holes for aeration and incubated at 20 ± 1 °C and 16:8 (light:dark) h photoperiod.

Mealworms were monitored each week for up to 3 weeks (21 days, T21) for weight changes and mortality. Mealworm biomass gain (%) was calculated (based on number of alive mealworms) as:

$$\left(\frac{WeightT21 - WeightT0}{WeightT0}\right) * 100 \quad (1)$$

where *WeightT21* is the individual weight at day 21 and *WeightT0* the weight at the start of the experiment.

2.4. Understanding soil as an exposure route for mealworms

To understand the importance of mealworm exposure through soil, several methods were used to discriminate between pore water exposure and soil particle ingestion. Mealworms were exposed to 200 g of Lufa 2.2 soil (40% WHC) for 6 days, using 3 replicates with 10 mealworms each. To infer on the ingestion of soil particles by the mealworms, two methods were used: 1) acid digestion and 2) burning at 500 °C. For acid digestion, mealworms were digested individually in concentrated acids to find any undissolved particles, as acid digestion is unable to dissolve soil silica particles. More details can be found below, in the mealworm digestion method for Ag analysis. For the burning method, mealworms were collected from the soil and left to empty their guts for 0, 24 h, 36 h and 48 h. Mealworms not exposed to soil (from cultures) were also tested. The ash samples of depurated (up to 48 h) mealworms and non-depurated ones (T0) collected after burning were weighed and differences between mealworms soil-exposed and unexposed to soil were analysed. This experiment was also important to determine the proper time needed for mealworms to depurate their guts in the case of soil ingestion. In addition, to investigate whether depuration affects the retrieval of Ag concentrations in mealworms following exposure, organisms were exposed to 20 g soil (Lufa 2.2 soil, 40% WHC, individually in plastic pot ø 65 mm) spiked with 100 mg Ag kg^{-1} dry soil of AgNPs 3–8 nm for 21 days. Three mealworms were collected at each sampling point (1, 3, 6, 9, 15, 21 days) and frozen individually (−20 °C) with or without depuration. For depuration, the organisms were kept in an empty plastic pot for 24 h as mealworms have been reported to have <24 h gut transit time (Yang et al., 2015) and also based on the optimal depuration time determined as described above. Following acid digestion, the Ag concentration in the mealworms was determined using graphite furnace Atomic Absorption Spectrometry (AAS; PinAAcle 900Z, PerkinElmer, Singapore).

Faeces were also collected after exposure to soil and acid digested to infer the presence of soil particles. For that, 20 mealworms were exposed to 100 mg Ag kg^{-1} dry soil (3–8 nm, 50 nm or 60 nm AgNPs, $AgNO_3$, or Ag_2S NPs) in each of three replicates in a plastic pot with 450 g soil for six days followed by 24 h depuration in an emptypot. In the acid digestion procedure for faeces, the final digested solution was centrifuged (3000 rpm, 10 min) and the pellet and the supernatant were separated. The pellet was dried at 60 °C for two days and weighted to account for the amount of soil (percentage of soil in faeces = total soil dry weight * 100 / total faeces dry weight). The supernatant was measured for total Ag content in faeces by graphite furnace AAS (PinAAcle 900Z, PerkinElmer, Singapore).

To further explore the soil and/or pore water uptake, the soil was dyed at different moisture contents. Lufa 2.2 soil was coloured by mixing 28 mL of edible color (GLOBE GREEN DYE, for Confectionery) with 400 g soil. Then the soil was dried to a constant weight in an

oven at 60 °C for two days and finally, the moisture content was adjusted to 20% or 40% of the WHC or the soil was used completely dry. Mealworm intestines were dissected and monitored under a stereomicroscope after exposure to dyed soil from 2 to 48 h (2, 4, 6, 12, 24, 48 h) and the pictures recorded whereby color intensity could be detected.

2.5. Soil exposure: experimental setup for bioaccumulation test

Based on the results from the acute toxicity test (low toxicity) and the lack of information on the efficacy of exposure to Ag through soil, the concentration of 100 mg Ag kg^{-1} dry soil was chosen for soil exposures where low Ag uptake or high Ag elimination rates were expected. Although this concentration is far from the environmentally expected Ag concentration, it enabled the analysis of Ag and assessment and understanding of Ag toxicokinetics also at lower exposure concentrations. Therefore, Lufa 2.2 soil was spiked with 100 mg Ag kg^{-1} dry soil for 3–8 nm, 60 nm or 50 nm AgNPs and $AgNO_3$, and 22 and 226 mg Ag kg^{-1} of Ag_2S NPs. Different concentrations were used for Ag_2S NPs, Because of an incorrect labelling of the original stock, the lower concentration (22 mg kg^{-1}) was only about 25% of the nominal one. The higher concentration (226 mg Ag k^{-1}), was included because of the expected low bioavailability and bioaccessibility of Ag_2S NPs, also was a bit lower than anticipated because of the incorrect labelling of the original stock. All Ag forms were spiked into the soil as a suspension/solution. The soil was moistened up to 40% of its maximum WHC by adding deionised water. In the case of 3–8 nm and 60 nm AgNPs, where the concentration in the stock solution was rather low, the spiking was done in three steps with soil allowed to air-dry at 20 °C between each step. Following three days of equilibration, soil exposures started. To determine the soil pH, 5 g of soil were shaken with 25 mL of 0.01 M $CaCl_2$ solution for 2 h, with the pH measured after 2 h settling of the suspension (Tourinho et al., 2016). To measure available silver concentrations in the soil, the $CaCl_2$ suspension was filtered through a 0.45 μm membrane filter after precipitation overnight and analysed by graphite furnace AAS (PinAAcle 900Z, PerkinElmer, Singapore).

Mealworms were exposed to the spiked soil for a 21-day uptake phase and then transferred to clean soil for a 21-day elimination phase. In total 36 animals were used for each treatment, and 12 for the control. All were kept individually in plastic pots (ø 65 mm) containing about 20 g moist soil. Three replicates (with one mealworm each) were randomly collected per sampling point (1, 3, 6, 9, 15, 21 days) for both the uptake and elimination phases and frozen individually (−20 °C). The test was carried out at 20 °C and 16:8 h (light:dark) photoperiod and mealworms were fed with oat flakes (1 piece of oat which was replaced twice weekly).

2.5.1. Total and dissolved Ag in soil pore water

In a separate experiment, three replicate plastic pots were filled with 450 g spiked soil (100 mg Ag kg^{-1} dry soil) for each treatment (Ag form). At each sampling point (0, 1, 6, 15, 21 days) the soil pore water was extracted by saturating soil samples (the equivalent of 25 g dry weight) with deionised water for 24 h, followed by centrifugation through a 70 μm nylon filter (soaked in 0.1 M $CuSO_4$) at 2000 g for 1 h.

To determine the Ag dissolved during 21 days, 2 mL of the collected soil pore water was centrifuged at 3000 rpm for 1 h using a 3 kDa membrane filter. The soil pore water and its ultrafiltrate were acidified with concentrated HCl to a total concentration of 3% HCl and stored for Ag analysis by graphite furnace AAS (PinAAcle 900Z, PerkinElmer, Singapore).

The first-order decay model was used to calculate the decrease rate of total and ionic Ag in the soil pore water, as follows:

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

where C(t) = concentration in the pore water at time t (μg Ag L^{-1}), C_0 = concentration in the pore water at $t = 0$ (μg Ag L^{-1}), K = decrease rate constant (day^{-1}).

2.6. Food exposure: experimental setup for bioaccumulation tests

Oat flakes were spiked with a solution/suspension of the different Ag forms to reach a nominal concentration of 100 mg Ag kg^{-1} dry food as described above. For the control, only deionised water was added to the oats. Spiked food was air dried at 25 °C for a few days. Plastic pots (ø 65 mm) containing 20 g moist (40% of WHC) Lufa 2.2 soil were used for the test. Mealworms were exposed to contaminated food for 21 days as the uptake phase and then offered clean food for another 21 days (elimination phase). A single oat piece (~30 mg) was added on the soil surface and replaced three times a week to ensure the permanent presence of food. Three mealworms were frozen (−20 °C) at each sampling point (1, 3, 6, 9, 15 and 21 days) for both the uptake and elimination phase. For each treatment, 36 organisms were used, and another 12 for the control, and kept individually as in the soil exposure test (20 °C and 16:8 (light:dark) h photoperiod). Each mealworm was allowed to depurate the gut for 24 h in an empty plastic pot after which the animal and exuviae (if available) were frozen, dried and stored for Ag analysis. Mealworms were monitored every day for moulting, so the complete exuviae could be collected after shedding and cleaned gently with a brush and Milli-Q water.

2.7. Total Ag analysis in mealworms

Mealworms were digested with a mixture of concentrated HNO_3: HCl (3:1. v/v; PanReac AppliChem, Trace analysis) as described by Ribeiro et al. (2017). Concentrated HCl (37%) and HNO_3 (69%) were added to the dried samples for the digestion. Then the acids were allowed to evaporate to less than 2 mL and diluted with 1% HCl up to a volume of 45 mL. Blanks and reference material were analysed in triplicate at each digestion run. The digests were analysed for Ag by graphite furnace AAS (PinAAcle 900Z, PerkinElmer, Singapore). Analysis of the certified reference materials DOLT-3 and DOLT-5 showed acceptable recoveries of Ag (95% ± 12%, mean ± SD; n = 23). To find the limit of detection (LOD) for AAS measurements a blank was measured 20 times; the standard deviation was multiplied by three to get the detection limit. The LOD for graphite furnace AAS was 0.026 μg Ag L^{-1}.

Soil samples were dried at 50 °C and then digested for 7 h in a mixture (4:1. v/v) of concentrated HCl and HNO_3 (J.T. Baker; 37% and 70%, respectively) in closed Teflon containers, in an oven (Binder ED 53) at 140 °C. The digests were diluted with demineralized water up to 10 mL and analysed by flame AAS (AAnalyst 100, PerkinElmer, USA). Recovery of Ag from the DOLT-4 reference material was 90% ± 2.4% (n = 2).

Dried food (spiked oat) was digested using a microwave (Berghof speed wave) with the same mixture of concentrated acids as for the soil samples. Digestion was carried out applying a temperature ramp of 10 up to 180 °C (25 min), then held at 180 °C (25 min) and ramp 5 down to 100 °C (10 min). The digests were diluted with Milli-Q water up to a volume of 10 mL. The recovery of Ag from the reference material (DOLT-5) for the digestion of food samples was acceptable (99% ± 7%, n = 3). Flame AAS (AAnalyst 100, PerkinElmer, USA) was used to measure the total Ag content.

2.8. Toxicokinetic models

A one-compartment first order model with inert fraction (Fi) was used to describe the data. An inert fraction is the stored fraction of metal in the organism related to detoxification processes which is not excreted during the elimination phase. Fi ranged from 0 to 1, higher values show slow or slight elimination of metal from the storage compartment (Tourinho et al., 2016; Vijver et al., 2006). Eq. (3) was fitted to data from the uptake phase, while Eq. (4) was used for the elimination phase (van den Brink et al., 2019).

$$Q(t) = C_0 + \left(\frac{K_1}{K_2 + K_{growth}}\right) * C_{exp} * \left(1 - e^{-(K_2 + K_{growth}) * t}\right) \quad (3)$$

$$Q(t) = C_0 + \left(\frac{K_1}{K_2 + K_{growth}}\right) * C_{exp} * \left(Fi + (1-Fi) * \left(e^{-(K_2+K_{growth})*(t-t_c)}\right)\right) \quad (4)$$

In these equations, Q (t) = internal Ag concentration in the mealworms 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 soil or food g animal^{-1} day^{-1}); K_2 = elimination rate constant (day^{-1}); C_{exp} = Ag exposure concentration (mg Ag kg^{-1} dry soil or food); t = time (days); t_c = time at which the animals were transferred to clean soil or clean food (days); Fi = inert fraction; K_{growth} = the growth rate constant calculated using the Von Bertalanffy model (Paine et al., 2012).

In case of soil exposure, the contribution of soil and pore water to total Ag uptake was taken into account and for that purpose Eqs. (5) and (6) were fitted to data from the uptake phase and the elimination phase, respectively (Ribeiro et al., 2017):

$$Q(t) = C_0 + \left(\frac{(C_{exp\,soil} * K_{soil}) + (C_{exp\,SPW} * K_{SPW})}{K_2 + K_{growth}}\right) * \left(1 - e^{(-(K_2+K_{growth})*t)}\right) \quad (5)$$

$$Q(t) = C_0 + \left(\frac{(C_{exp soil} * K_{soil}) + (C_{exp SPW} * K_{SPW})}{K_2 + K_{growth}}\right) * \left(Fi + (1-Fi) * e^{-((K_2+K_{growth})*(t-t_c))}\right) \quad (6)$$

In these equations, $C_{exp\ soil}$ = Ag exposure concentration in soil (mg Ag kg^{-1} dry soil); $C_{exp\ SPW}$ = Ag exposure concentration in soil pore water (mg Ag L^{-1}); K_{soil} = uptake rate constant from the soil (g soil g animal^{-1} day^{-1}); K_{SPW} = uptake rate constant from the soil pore water (L spw g animal^{-1} day^{-1}).

Eqs. (7) and (8) were used to estimate the relative contribution of soil and soil pore water uptake to the total Ag uptake in the mealworms:

Uptake from soil (%):

$$\left(\frac{C_{exp\,soil} * K_{soil}}{C_{exp\,soil} * K_{soil} + C_{exp\,SPW} * K_{SPW}}\right) * 100 \quad (7)$$

Uptake from soil pore water (%):

$$\left(\frac{C_{exp\,SPW} * K_{SPW}}{C_{exp\,soil} * K_{soil} + C_{exp\,SPW} * K_{SPW}}\right) * 100 \quad (8)$$

2.9. Statistical analysis

The stability of the NPs over time was analysed using the Kruskal-Wallis H test ($p < 0.05$), with time as the independent variable and the respective physicochemical descriptor (Z-potential, hydrodynamic diameter) as the dependent variable. The Dunn-Bonferroni post-hoc test was used to test for significant changes between the different timepoints. Two-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test ($p < 0.05$) was performed to determine statistical significance of differences between treatments and the control over time in the acute toxicity test for biomass gain. The significant differences of the total Ag concentration between depurated and non-depurated mealworms were checked with one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). The one-way ANOVA followed by the Holm-Sidak method ($p < 0.05$) was used to demonstrate the significant differences of total Ag concentrations in soil, 0.01 M $CaCl_2$ extractable, and soil pore water between the spiked soil with different Ag forms. Two-way ANOVA followed by the Holm-Sidak method ($p < 0.05$) was used to analyze statistically significant differences over time in total Ag and dissolved Ag concentrations measured in the soil pore water extracted from the spiked soil with different Ag forms and in the mealworm body concentrations for food and soil exposure during the uptake and elimination phases for the different Ag treatments. SigmaPlot 14.0 software was used for determining normality, homoscedasticity of the data and running the ANOVA.

The toxicokinetics parameters were estimated by non-linear regression in SPSS (version 23). Akaike information criteria (AIC and AICc) were used to find the best-fitting model for each data set (Akaike, 1974). A Generalised Likelihood Ratio Test (Sokal and Rohlf, 2012), was used to test for significance of the differences between K_1 or K_2 values for the different Ag forms.

3. Results

3.1. Nanoparticle characterization

NP stability over time was tested in UPW, with pH regulated to 7 using ammonium acetate. The monitored descriptors Z-potential, hydrodynamic diameter (including the PDI as a measurement quality parameter) and dissolution are presented in Fig. S1 in the Supplementary Information.

The Z-potential of the tested NPs (Fig. S1A) differed significantly over time for the Ag_2S NPs ($\chi^2_{(2)} = 34.083$, $p = 0.002$), 3–8 nm Ag NPs ($\chi^2_{(2)} = 38.093$, $p < 0.01$), 50 nm Ag NPs ($\chi^2_{(2)} = 25.952$, $p < 0.01$), and 60 nm AgNPs ($\chi^2_{(2)} = 9.929$, $p = 0.042$). Based on the Dunn-Bonferroni post-hoc test significant changes in Z-potential for the Ag_2S NPs took place between 4 and 24 h ($p = 0.01$), and from 24 to 40 h. For the 3–8 nm and 50 nm AgNPs significant changes took place during the first 4 h following dispersion ($p < 0.02$), with no significant changes observed for the remainder of the time. For the 60 nm AgNPs the Z-potential significantly changed during the first 2 h following dispersion ($p = 0.031$).

The DLS results (Fig. S1C and D) demonstrated that the hydrodynamic sizes of the AgNPs were substantially higher than the respective nominal ones (TEM size of the 3–8 nm AgNPs ranged from 3 to 8 nm (Ribeiro et al., 2017), and was 47.3 ± 5.3 nm and 20.3 ± 9.8 nm for 50 nm AgNPs and Ag_2S NPs, respectively (Baccaro et al., 2018)). For the 50 nm AgNPs, the hydrodynamic size was close to the reported nominal value. Significant changes in hydrodynamic sizes over time were found for the 3–8 nm ($\chi^2_{(2)} = 23.555$, $p < 0.001$) and 50 nm Ag NPs ($\chi^2_{(2)} = 26.477$, $p < 0.001$), but not for the Ag_2S NPs ($\chi^2_{(2)} = 2.749$, $p = 0.601$) and the 60 nm AgNPs ($\chi^2_{(2)} = 1.482$, $p = 0.686$). The PDI (Fig. S1B), denoting the quality of the hydrodynamic diameter measurements, were between 0.17 and 0.41 for the Ag_2S NPs, 3–8 nm and 50 nm AgNPs, denoting good mono-dispersity and relatively narrow size distributions. The PDI values (~1) for the 60 nm AgNPs were consistent with the substantially large sizes observed (~1000 nm) denoting strong agglomeration of the particles.

The hydrodynamic size of the 3–8 nm AgNPs initially decreased over time, which can be attributed to dissolution or potential de-agglomeration of the particles from the stock solution. These differences are further supported from the pairwise timepoint comparison, as the size difference between 0 and 2 h was significant (Kruskal-Wallis H test followed by the Dunn-Bonferroni post-hoc test, $p < 0.001$). At later timepoints, significant size drops (4 versus 8 h: $p = 0.004$) were recorded, while from 8 h onward post-suspension no significant differences ($p > 0.05$) were observed. The 50 nm AgNPs presented a small, but significant, size-drop that was observed solely between 8 and 24 h ($p = 0.007$), with all other pairwise comparisons not being significant ($p > 0.05$).

The dissolution behaviour (Fig. S1E) of the AgNPs was consistent with the results for the Z-potential and hydrodynamic diameter. Ag_2S NP dissolution was in all cases <0.02%. The 60 nm AgNPs presented consistent dissolution between 27.2%–29.3% at all timepoints. The 3–8 nm and 50 nm AgNPs showed rapidly increasing % dissolved Ag during the early post-dispersion period, reaching the expected plateau at 48 h post-dispersion. The observed % dissolved Ag in the time period

between dispersion and 48 h post-dispersion were 6.5%–21.1% and 5.27%–26.1% for the 3–8 nm and 50 nm AgNPs, respectively.

3.2. Acute toxicity test

The acute toxicity test with $AgNO_3$ showed maximum mortalities of mealworms exposed to the spiked soil and food of 34% (±41%, mean ± SD, n = 3) and 27% (±11%, mean ± SD, n = 3), respectively, after 21 days. No significant difference ($p > 0.05$) in mealworm mortality was found compared to the control for any of the soil or food concentrations tested (Fig. S2). Mealworms exposed to 1000 mg $AgNO_3$ kg^{-1} via soil showed a significant decrease (Dunnett's test, $p < 0.05$) of biomass gain from the 7th day of exposure onwards (Fig. 1). Similarly, at 1500 mg Ag kg^{-1} dry food and after 21 days the mealworms also presented a significant decrease in their biomass gain (compared to the control) (Dunnett's test, $p < 0.05$) (Fig. 1).

3.3. Soil ingestion by mealworms

The acid digestion and burning/ashing methods used did not show any evidence of soil ingestion by the mealworms (Fig. S3). However, the mealworm intestine was totally coloured after just 2 h exposure to the dyed soil moistened at 40% of its WHC (Fig. S4). When kept on completely dry soil, the change of the intestinal color was not as clear as with the moist soil. Mealworm faeces showed a notable amount of soil, amounting (±SD, n = 3) to 9.6 ± 3.19 mg soil dry weight in pooled faeces from 20 mealworms, after the exposure to $AgNO_3$ spiked soil. The results of the acid digestion of mealworm faeces are shown in Table 1. The faeces contained 51 to 71% of soil, while the concentration of Ag in the faeces was at least 60.5 mg Ag kg^{-1} faeces for AgNPs 60 nm and maximum 194 mg Ag kg^{-1} faeces for Ag_2S NPs. The total Ag concentration in the mealworms exposed to $AgNO_3$ was 21% lower (±26%, mean ± SD, n = 18) when correcting for the gut content: Fig. S5.

To check the difference between depurated and non-depurated animals, in the bioaccumulation test with the 3–8 nm AgNPs mealworms were analysed with and without their gut content. There was no significant difference in the measured total Ag concentrations in the mealworms (Tukey's post-hoc test, $p > 0.05$), except for the third day (Tukey's post-hoc test, $p < 0.05$) of the uptake phase of the bioaccumulation test (Fig. S6).

3.4. Ag concentrations in soil and food

The background Ag concentration in Lufa 2.2 soil was below the detection limit while in the case of the oat flakes it was 0.01 mg Ag kg^{-1}.

Table 1
The measured total Ag concentrations in the Lufa 2.2 soil spiked with different Ag forms and in the faeces of mealworms (*Tenebrio molitor*) (pooled 20 mealworms) exposed to this soil. Also shown is the percentage of soil in the faeces of the mealworms. Data are shown as average ± SD; n is the number of replicates used.

Ag form	Soil Ag concentration (mg Ag kg^{-1} dry soil); n = 4	Faeces Ag concentration (mg Ag kg^{-1} faeces); n = 3	Soil in faeces (%); n = 3
Control	0 ± 0	0.50 ± 0.70	51.0 ± 13.3
$AgNO_3$	86.0 ± 3.74	81.1 ± 43.6	71.1 ± 3.34
AgNPs 3–8 nm	105 ± 13.2	123 ± 51.6	71.2 ± 7.12
AgNPs 50 nm	95.0 ± 13.5	68.7 ± 17.6	64.3 ± 6.20
AgNPs 60 nm	101 ± 6.66	60.5 ± 17.6 (n = 2)	53.9 ± 26.8 (n = 2)
Ag_2S NPs	209 ± 17.5	194 ± 52.9	65.8 ± 8.9

The measured Ag concentrations in the spiked soil (total, $CaCl_2$ extractable and in pore water) and spiked food are given in Table 2. The measured concentrations were used in the toxicokinetic models to calculate Ag uptake and elimination rate constants in the mealworms.

The total and dissolved concentrations of Ag in the pore water from $AgNO_3$ spiked soil significantly decreased from day 6 onwards with decrease rates of 0.25 and 0.34 day^{-1} for total and dissolved Ag, respectively (Holm-Sidak method, $p < 0.05$) (Table 3). No significant ($p > 0.05$) changes were detected in the total and dissolved porewater Ag concentrations for the 3–8 nm AgNPs, while only dissolved Ag concentration showed a decrease rate of 0.03 per day. Total Ag concentrations in the pore water of 50 nm AgNP spiked soil displayed no significantly ($p > 0.05$) differences during 21 days, however, the dissolved Ag concentration was significantly lower after 21 days compared to the start (Holm-Sidak method, $p < 0.05$). The 60 nm AgNPs showed significantly (Holm-Sidak method, $p < 0.05$) lower total Ag concentrations in the pore water after 21 days compared to days 0 and 1, while the dissolved Ag concentration was significantly (Holm-Sidak method, $p < 0.05$) lower than day 0 at all sampling times; decrease rates were 0.10 and 0.34 day^{-1}, respectively.

The total Ag concentration of pore water in case of Ag_2S NPs after one day was significantly higher than the first and 15th day. The dissolved Ag concentrations in pore water of Ag_2S NP spiked soil showed no significant changes during the 21-day incubation period (Table 3).

$AgNO_3$ spiked soil had significantly higher porewater Ag concentrations compared to all other Ag forms except for the 60 nm AgNPs (Holm-Sidak method, $p < 0.05$). Total Ag concentrations in pore water from soil spiked with pristine AgNPs and Ag_2S NPs did not differ significantly ($p > 0.05$). Although the Ag concentration of the soil spiked with

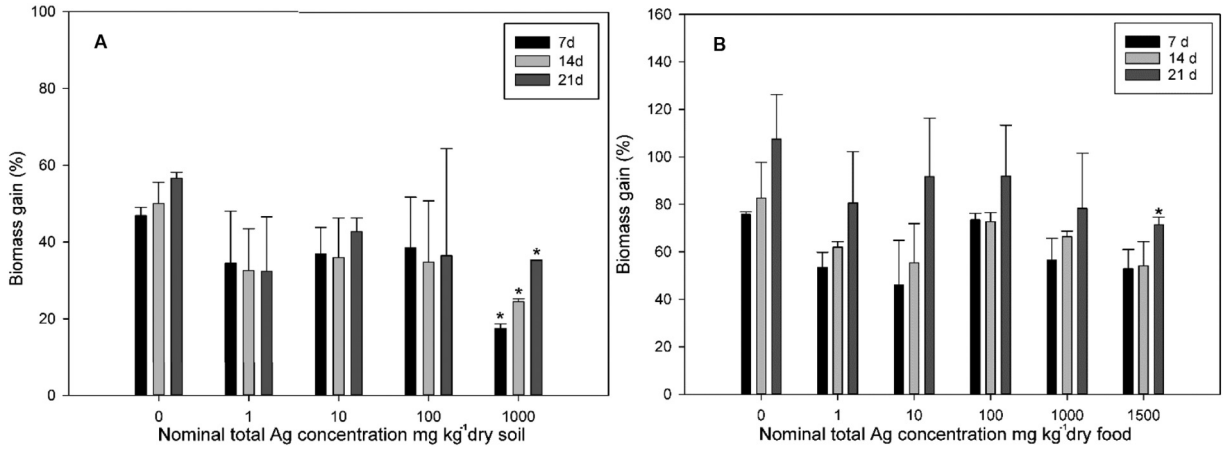

Fig. 1. Effect of $AgNO_3$ on the biomass gain (%) of the mealworm *Tenebrio molitor* following 21 days exposure in Lufa 2.2 soil (A) or food (B). * indicates significant difference compared to the control according to a two-way ANOVA, followed by Dunnett's post hoc test ($p < 0.05$). Error bars represent standard error.

Table 2
Nominal and measured total Ag concentrations and the 0.01 M $CaCl_2$-extractable Ag concentrations in the Lufa 2.2 soil, total Ag concentration in soil pore water and in food spiked with different Ag forms (nominal concentration of 100 mg Ag kg^{-1} dry food). Data are shown as average ± SD for soil; only one replicate was used for food analysis. Statistically significant differences (one-way ANOVA followed by Holm-Sidak Method ($p < 0.05$)) within each column are shown in different capital letters.

Ag form	Soil			Soil pore water	Food
	Nominal concentration (mg Ag kg^{-1} dry soil)	Measured concentration (mg Ag kg^{-1} dry soil); n = 2	$CaCl_2$-extractable concentration (mg Ag kg^{-1} dry soil); n = 2	Initial total Ag concentration (μg Ag L^{-1}); n = 3	Measured concentration (mg Ag kg^{-1} dry food),
Control	0	n.d.	n.d.	0.09 ± 0.02 A	0.01
$AgNO_3$	100	97.6 ± 15.3 A	0.40 ± 0.01 A	137 ± 49.5 D	110
AgNPs 3–8 nm	100	101 ± 1.34 A	0.49 ± 0.01 A	160 ± 24.3 D	99.9
AgNPs 50 nm	100	80.4 ± 2.83 A	0.38 ± 0.02 A	81.7 ± 18.0 D, C	75.0
AgNPs 60 nm	100	121 ± 2.05 A	0.39 ± 0.001 A	167 ± 62.6 D	104
Ag_2S NPs	22	22.0 ± 0.424 A	n. d.	14.5 ± 12.9 A, B	67.5
Ag_2S NPs	226	227 ± 3.61 A	0.001 ± 0.0 A	30.6 ± 9.48 B, C	–

Ag_2S NPs was twice as high as for the other Ag forms, it had significantly (Holm-Sidak method, $p < 0.05$) lower dissolved Ag concentrations in the pore water at all sampling times (Table 3).

3.4.1. Soil exposure toxicokinetics

The pH_{CaCl2} of the Lufa 2.2 soil (Table S1) was slightly higher when spiked with $AgNO_3$ or 60 nm AgNPs, but was hardly affected by the other Ag forms. The pH of Ag spiked soils never differed more than 0.5 units compared to the control.

Although less than 20% mortality was observed during the bioaccumulation test with the different Ag forms, the mealworms gained little weight (growth constant rate (K_{growth}), 0.09 ± 0.11 per day, mean ± SD, n = 7) during the 42 days test period. Therefore, a growth constant rate (K_{growth}) was applied to the toxicokinetics model to consider the possible dilution of the body Ag concentration in the organism by growth (Ardestani et al., 2014). At the beginning of the bioaccumulation test (T_0), the background Ag concentration (C_0) in the mealworms was 0.24 ± 0.14 μg Ag g^{-1} dry body weight (mean ± SD, n = 12).

Uptake and elimination patterns and kinetics of Ag in the mealworms exposed to different Ag forms, estimated with the first model based on the soil exposure (Eqs. (3) and (4)), are displayed in Fig. 2 and Table 4. Total Ag concentrations in mealworms exposed to $AgNO_3$ reached steady state after 6 days with a level of 34 μg Ag g^{-1} dry body weight after 21 days of the exposure period. The Ag body concentration declined after transferring the animals to the clean soil, but did not completely return to the background level.

The pristine 3–8 nm and 60 nm AgNPs almost showed the same patterns of Ag uptake and elimination in the mealworms (Fig. 2). Steady state was reached after 6 days with body concentrations of 23.8 and 22.9 μg Ag g^{-1} dry body weight, respectively after 21 days of uptake. Also elimination rate constants for the Ag taken up from the 3–8 nm and 60 nm AgNPs were similar (Table 4).

The 50 nm AgNPs were the only pristine AgNPs that pursued a different pattern, reaching steady state body Ag concentrations only after 15 days (Fig. 2). Although a lower Ag body concentration of 11.3 μg Ag g^{-1} dry body weight was reached at the end of the exposure period compared to other pristine AgNPs, it could not be eliminated as well as for the other pristine AgNPs leading to the highest inert fraction of all the treatments (Fi = 0.54).

After 21 days, the Ag concentration in mealworms exposed to Ag_2S NPs at 22 and 226 mg Ag kg^{-1} dry soil was 2.6 and 15.4 μg Ag g^{-1} dry body weight, respectively. At both exposure concentrations, elimination was faster than for the other Ag forms. At the exposure concentration of 226 mg Ag kg^{-1} dry soil, which was twice than that for the other Ag forms, the body concentration at the end of the elimination phase was 0.28 ± 0.29 μg Ag g^{-1} dry body weight (mean ± SD, n = 3), close to the background Ag concentration.

The kinetics parameters were the same for $AgNO_3$ and for the 3–8 nm and 60 nm AgNPs with K_1 = 0.27–0.32 $g_{soil}\ g^{-1}_{animal}\ day^{-1}$ and K_2 = 0.81–1.09 day^{-1} (Table 4). Uptake rate constant for the 50 nm AgNPs (K_1 = 0.06 $g_{soil}\ g^{-1}_{animal}\ day^{-1}$) however, differed significantly ($X^2_{(1)} > 3.84$; $p < 0.05$) from all other Ag forms except for Ag_2S NPs (226 mg Ag kg^{-1} dry soil), while the elimination rate constant (K_2 = 0.12 day^{-1}) was significantly different ($X^2_{(1)} > 3.84$; $p < 0.05$) compared to all Ag forms. The parameters for the Ag uptake from Ag_2S NPs (226 mg Ag kg^{-1} dry soil) were similar to all other Ag forms tested in this study (K_1 = 0.13 $g_{soil}\ g^{-1}_{animal}\ day^{-1}$, K_2 = 1.35 day^{-1}), except for the K_2 for the 50 nm AgNPs. Only for the lower Ag_2S NPs concentration

Table 3
Total Ag and dissolved Ag concentrations measured in the pore water of Lufa 2.2 soil spiked at a nominal concentration of 100 mg Ag kg^{-1} with different Ag forms at each sampling time (days 0, 1, 6, 15, 21) and calculated decrease rate constants. Data are shown as average ± SD (n = 3). Statistically significant differences (two-way ANOVA followed by Holm-Sidak Method ($p < 0.05$)) within each column (treatments) and row (time) are shown in different capital letters and small letters in italics, respectively.

	Soil Ag concentration (mg Ag kg^{-1} dry soil); n = 4	Total Ag concentrations in soil pore water (μg Ag L^{-1})					Total Ag decrease rate (day^{-1})
		Day 0	Day 1	Day 6	Day 15	Day 21	
$AgNO_3$	86.0 ± 3.74 A	321 ± 8.78 A/*a*	311 ± 33.3 A/*a*	15.8 ± 5.11 A/*b*	55.9 ± 22.6 A/*b*	110 ± 21.8 A, B/*b*	0.25
AgNPs 3–8 nm	105 ± 13.2 A	109 ± 29.3 B/*a*	160 ± 6.70 A, B/*a*	139 ± 54.5 A/*a*	134 ± 18.7 A/*a*	148 ± 37.2 A, B/*a*	0
AgNPs 50 nm	95.0 ± 13.5 A	112 ± 74.2 B/*a*	82.5 ± 2.19 B/*a*	98.3 ± 59.1 A/*a*	55.7 ± 9.23 A/*a*	62.9 ± 15.4 A, B/*a*	0.03
AgNPs 60 nm	101 ± 6.66 A	239 ± 19.9 A, B/*a*	240 ± 77.5 A, B/ *a*	89.3 ± 76.1 A/*a, b*	72.4 ± 19.2 A/*a, b*	40.9 ± 13.8 A/*b*	0.10
Ag_2S NPs	209 ± 17.5 B	86.1 ± 35.2 B/*a*	272 ± 203 A/*b*	109 ± 23.3 A/*a, b*	81.6 ± 108 A/*a*	228 ± 217 B/*a, b*	0
	Dissolved Ag concentrations in soil pore water (μg Ag L^{-1})						Ag^+ decrease rate (day^{-1})
	Day 0	Day 1	Day 6	Day 15	Day 21		
$AgNO_3$	280 ± 34.8 A/*a*	197 ± 52.0 A/*b*	35.5 ± 10.4 A/*c*	22.9 ± 3.66 A/*c*	28.9 ± 3.47 A/*c*		0.34
AgNPs 3–8 nm	18.2 ± 1.95 D/*a*	19.0 ± 1.86 D/*a*	10.5 ± 0.518 B/*a*	12.4 ± 1.51 A/*a*	12.1 ± 1.38 B/*a*		0.03
AgNPs 50 nm	45.9 ± 9.10 C/*a*	45.0 ± 4.59 C/*a*	35.1 ± 12.9 A/*a, b*	24.8 ± 5.99 A/*a, b* n = 2	24.7 ± 2.89 A, B/*b*		0.03
AgNPs 60 nm	226 ± 28.8 B/*a*	158 ± 18.4 B/*b*	31.3 ± 15.7 A/*c*	13.2 ± 1.43 A/*d*	14.1 ± 2.77 B/*d*		0.34
Ag_2S NPs	0.770 ± 0.241 E/*a*, n = 2	0.413 ± 0.206 E/*a*, n = 2	0.836 ± 0.038 C/*a*	2.17 ± 1.99 B/*a*	1.83 ± 2.30 C/*a*		0

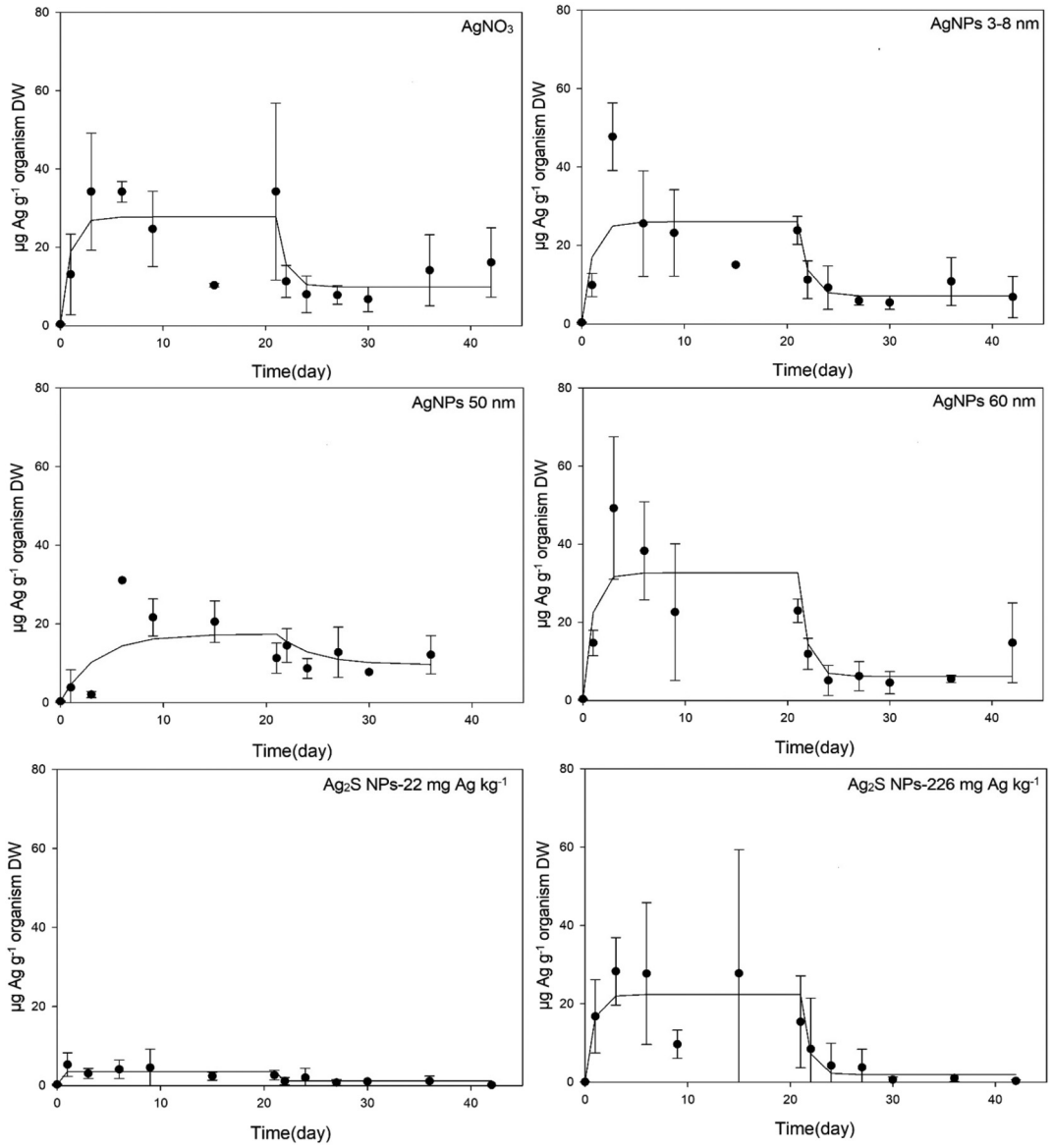

Fig. 2. Uptake and elimination kinetics of Ag in mealworms (*Tenebrio molitor*) exposed to 100 mg Ag kg^{-1} dry soil in Lufa 2.2 soil spiked with 3–8 nm, 60 nm, or 50 nm AgNPs or with $AgNO_3$ and to 22 and 226 mg Ag kg^{-1} dry soil in soil spiked with Ag_2S NPs. Points show mean values, error bars represent standard deviations (n = 3). Lines show the fit of a one-compartment model to the Ag concentrations in the mealworms (Eqs. (3) and (4)).

Table 4
Uptake and elimination kinetic parameters for Ag in mealworms (*Tenebrio molitor*) exposed to 100 mg Ag kg^{-1} dry soil of different Ag forms and 22 or 226 mg Ag kg^{-1} dry soil of Ag_2S NPs. Model 1 describes results of a standard one-compartment model assuming uptake from soil, model 2 considered uptake through soil and soil pore water (SPW). K_1 is the uptake rate constant, K_2 the elimination rate constant, K_{soil} the uptake rate constant from the soil, K_{SPW} uptake rate constant from the soil pore water, and Fi the inert fraction. Corresponding 95% confidence intervals are given between brackets. Significant differences ($X^2_{(1)} > 3.84$; $p < 0.05$) between K_1 and K_2 values for the different Ag forms, as shown by a likelihood ratio test, are indicated with different letters within each column.

Model	Ag form	K_1 ($g_{soil}\ g^{-1}_{animal}\ day^{-1}$)	K_2 (day^{-1})	K_{soil} ($g_{soil}\ g^{-1}_{animal}\ day^{-1}$)	K_{SPW} ($L_{spw}\ g^{-1}_{animal}\ day^{-1}$)	Fi
Model 1	$AgNO_3$	0.32 (0–0.635) A	0.81 (0–1.99) A			0.35 (0.13–0.565)
	AgNPs 3–8 nm	0.27 (0.042–0.491) A	1.00 (0.087–1.91) A			0.26 (0.075–0.453)
	AgNPs 50 nm	0.06 (0.009–0.115) B	0.12 (0–0.395) B			0.54 (0.133–0.953)
	AgNPs 60 nm	0.31 (0.048–0.572) A	1.09 (0.066–2.11) A			0.18 (0–0.373)
	Ag_2S NPs 22	1.89 (–)	12.6 (–)			0.28 (–)
	Ag_2S NPs 226	0.13 (0–0.312) A, B	1.35 (0–3.22) A			0.08 (0–0.391)
Model 2	$AgNO_3$		0.811 (0–2.16)	0.12 (–)	0.14 (–)	0.35 (0.119–0.575)
	AgNPs 3–8 nm		1.00 (0.03–1.97)	0.15 (–)	0.07 (–)	0.26 (0.046–0.460)
	AgNPs 50 nm		0.12 (0–0.402)	0.03 (–)	0.03 (–)	0.54 (0.123–0.963)
	AgNPs 60 nm		1.09 (0.04–2.14)	0.12 (–)	0.13 (–)	0.18 (0–0.380)
	Ag_2S NPs 22		10.7 (–)	0.55 (–)	1.61 (–)	0.28 (0–0.674)
	Ag_2S NPs 226		1.35 (0–4.69)	0.12 (–)	0.10 (–)	0.08 (0–0.405)

(–) It was not possible to calculate the 95% confidence intervals. No common letter showed a significant difference between kinetics.

Table 5
The relative contribution of uptake from soil and soil pore water (SPW) to the total uptake of Ag in mealworms exposed to different Ag forms in Lufa 2.2 soil. K_{soil} is uptake rate constant from the soil, K_{SPW} uptake rate constant from the soil pore water, $C_{exp\ soil}$ Ag exposure concentration in soil, and $C_{exp\ SPW}$ Ag exposure concentration in soil pore water.

Ag form	K_{soil} ($g_{soil}\ g_{animal}^{-1}\ day^{-1}$)	K_{SPW} ($L_{spw}\ g_{animal}^{-1}\ day^{-1}$)	C_{exp} soil (μg Ag g^{-1} soil)	C_{exp} SPW (μg Ag L spw^{-1})	Uptake from soil (%)	Uptake from SPW (%)
$AgNO_3$	0.12	0.14	97.6	137	38	62
AgNPs 3–8 nm	0.15	0.07	101	161	57	43
AgNPs 50 nm	0.03	0.03	80.4	81.7	50	50
AgNPs 60 nm	0.12	0.13	121	167	40	60
Ag_2S NPs 226	0.12	0.10	227	30.6	90	10

(22 mg Ag kg^{-1} dry soil), K_1 and K_2 values were much higher but not significantly ($X^2_{(1)} < 3.84$; $p > 0.05$) different due to a large variation. Moreover, the lowest Fi value of all Ag forms was found for the higher Ag_2S NPs concentration (226 mg Ag kg^{-1} dry soil) (Table 4).

When accounting for both exposure routes (model 2, Table 4), $AgNO_3$ showed the highest uptake rate constant from soil pore water (K_{spw}) of all Ag forms. Calculating the relative contribution (Table 5) of exposure routes revealed also higher Ag uptake from soil pore water (62%) than soil in mealworms exposed to $AgNO_3$. Of the pristine AgNPs, 60 nm AgNPs had higher K_{spw} and also higher Ag uptake from pore water than soil, similar to $AgNO_3$. The uptake of Ag from the 50 nm AgNPs showed equal contributions from the soil and soil pore water, while for AgNPs 3–8 nm Ag uptake was highest from the soil (57%). For Ag_2S NPs, 90% of the Ag uptake was from the soil (Table 5).

The statistical comparison of the raw experimental data (two-way ANOVA with the Holm-Sidak post-hoc test) demonstrated that significant difference exists for the combined effect of the uptake and elimination phases ($p < 0.001$) and when we consider the different treatments ($p < 0.001$). The Holm-Sidak post-hoc test demonstrated that significant difference exists between the Ag_2S (22 mg Ag kg^{-1} dry soil) with all the other Ag treatments ($p = 0.02$ for the 50 nm Ag NPs and $p < 0.001$ for the rest), while no other statistical significant difference ($p > 0.05$) was observed during any other pairwise comparison.

During the uptake phase (0–21 days), statistical significant difference was observed (two-way ANOVA and Holm-Sidak post-hoc test) for both the combined effect of the different treatments over time ($p = 0.011$) and the pairwise treatments comparisons ($p < 0.001$). The pairwise description of the various treatments using the Hom-Sidak post-hoc test demonstrated significant differences between the Ag_2S (22 mg Ag kg^{-1} dry soil) with the other Ag treatments ($p < 0.001$ for all), with the exception of the 50 nm AgNPs treatment ($p = 0.412$). From the remaining pairwise comparisons, significant difference was observed in the case of the 50 nm AgNPs with the 60 nm AgNPS ($p = 0.002$) and the $AgNO_3$ ($p = 0.014$) treatments.

During the elimination period (22–42 days), the statistical analysis of two-way ANOVA with the Holm-Sidak post-hoc test demonstrated no significant differences for the combined effect of the different treatments over time ($p = 0.808$), but there is significant differences between the individual treatments ($p < 0.001$). The pairwise comparison of the different treatments using the Holm-Sidak post-hoc test, demonstrated that no significant difference ($p = 0.998$) exists between the Ag_2S (22 mg Ag kg^{-1} dry soil) and the Ag_2S (226 mg Ag kg^{-1} dry soil) treatments, but the Ag_2S (22 mg Ag kg^{-1} dry soil) is significantly different with all other treatments (3–8 nm AgNPs: $p = 0.06$, 50 nm AgNPs: $p < 0.001$, 60 nm AgNPs: $p = 0.019$, $AgNO_3$: $p < 0.001$). The Ag_2S (226 mg Ag kg^{-1} dry soil) differs significantly with the 50 nm AgNPs ($p < 0.001$) and the $AgNO_3$ ($p < 0.001$) treatments. No other statistical significant differences ($p > 0.05$) were observed between the remaining pairwise comparisons.

3.5. Food exposure toxicokinetics

The Ag concentration in mealworms fed with $AgNO_3$ spiked food reached steady state after 3 days of exposure and was 10.0 μg Ag g^{-1} dry body weight after 21 days (Fig. 3). After feeding with clean food, Ag body concentration decreased slowly (Fi = 0.57) (Table 6).

The 3–8 nm and 60 nm AgNPs followed a similar pattern to reach steady state body Ag concentrations in the mealworms after one day of exposure to spiked food (Fig. 3). The Ag concentrations in mealworms exposed to 3–8 nm and 60 nm AgNPs were 10.8 and 10.0 μg Ag g^{-1} dry body weight, respectively after 21 days of exposure. The elimination patterns of 3–8 nm and 60 nm AgNPs were also comparable with a considerable fraction of the Ag retained at the end of the depuration period (Fi = 0.69–0.72). The uptake of Ag from the 50 nm AgNPs reached steady state after 3 days (Fig. 3), but showed a higher elimination than for the other pristine AgNPs (Table 6). In mealworms exposed to Ag_2S NPs, steady state Ag concentration was reached after one day of exposure, but elimination was faster than for all other Ag forms with the body concentrations decreasing to the background level (Fig. 3, Table 6).

For $AgNO_3$ and the 50 nm AgNPs, the K_1 and K_2 values were similar and much lower than for the other Ag forms (Table 6). The estimated K_1 values for Ag uptake in the mealworms exposed to food spiked with 3–8 nm and 60 nm AgNPs and Ag_2S NPs were equal at 1.96–2.18 $g_{food}\ g_{animal}^{-1}\ day^{-1}$ (Table 6) and so were the K_2 values (13.5–21.7 day^{-1}). The Fi value for $AgNO_3$ (0.57) was similar to that for the 3–8 nm and 60 nm AgNPs (0.69–0.72), while the Fi for Ag_2S NPs was the lowest among all Ag forms (Table 6). The Fi value for AgNPs 50 nm (0.31) was different to that for the other pristine AgNPs.

When comparing the combined uptake and elimination patterns, no significant differences could be observed either between the different Ag treatments (two-way ANOVA, $p = 0.098$) or for the combined effect of the over time period of the different treatments ($p = 0.353$). Similarly, no statistically significant differences were observed during the uptake phase (days 1–21), with p values for the individual treatments and the differences of the treatments over time being 0.876 and 0.465, respectively. During the elimination phase, statistically significant differences were observed between the different treatments ($p < 0.001$). Based on the Holm-Sidak post-hoc test, Ag elimination in Ag_2S NP exposures differed significantly from all other treatments (3–8 nm AgNPs: $p < 0.001$; 50 nm AgNPs: $p = 0.003$; 60 nm AgNPs: $p < 0.001$; $AgNO_3$: $p < 0.001$). The 3–8 nm AgNPs elimination phase did not significantly ($p > 0.05$) differ with other than the Ag_2S NPs treatments, while the 50 nm AgNPs (besides Ag_2S NPs) differed significantly with the 60 nm AgNPs ($p = 0.005$) and $AgNO_3$ ($p = 0.014$). The 60 nm AgNPs and $AgNO_3$ elimination differed significantly from the Ag_2S and 50 nm AgNPs as presented above, which means that these can be grouped together considering the elimination rate constants.

Total Ag concentrations in the exuviae recovered from exposed mealworms are reported in Table 6. Exuviae from mealworms exposed to Ag_2S NPs had the highest Ag amount (71.8% of the body burden), followed by those from $AgNO_3$ (6.4%), and AgNP (1.2–6.2%) exposures.

4. Discussion

The acute toxicity test showed that the mealworm *Tenebrio molitor* is tolerant to silver, exposed either via spiked soil or spiked food and no increased mortality was seen even at very high concentrations of $AgNO_3$. The high tolerance to silver brought questions about whether or not this

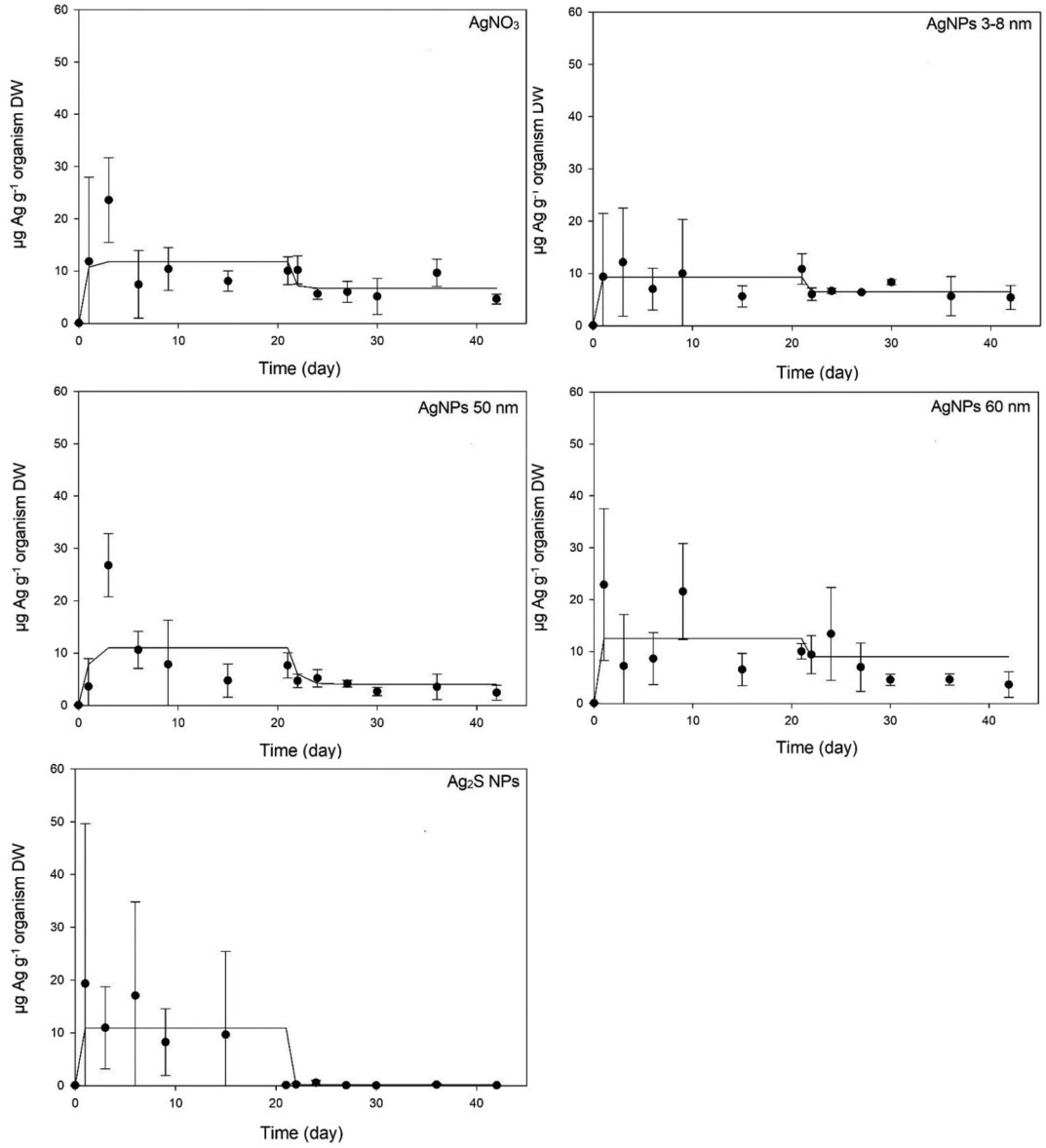

Fig. 3. Uptake and elimination kinetics of Ag in mealworms (*Tenebrio molitor*) exposed to 100 mg Ag kg^{-1} dry food spiked with different Ag forms. Points show mean values, error bars represent standard deviations (n = 3). Lines show the fit of a one-compartment model to the Ag concentrations measured in the mealworms (Eqs. (3) and (4)).

terrestrial organism is exposed in its larval stage. Bioaccumulation tests may provide an answer to that, along with other approaches that can help unravel exposure routes through soil, soil pore water and food. These tests were run with soil or food spiked with different silver forms, including different pristine AgNPs, ionic Ag ($AgNO_3$) and Ag_2S NPs to simulate the ageing of AgNPs in wastewater treatment plants.

Vijver et al. (2003) performed different tests (including visual inspection of soil particles in the dissected organisms and in digests of organisms, and also ashing of the lyophilized animals at 500 °C) and concluded that mealworms do not ingest soil. Our results contradict these findings, which were only possible by using different complementary methods. The first results obtained with the acid digestion and

Table 6
Uptake and elimination kinetic parameters for different Ag forms in mealworms (*Tenebrio molitor*) exposed to 100 mg Ag kg^{-1} food. K_1 is the uptake rate constant, K_2 the elimination rate constant and Fi the inert fraction. 95% confidence intervals are given in brackets. Statistically significant differences ($X^2_{(1)} > 3.84$; $p < 0.05$) in K_1 and K_2 values between the Ag forms, as determined by a likelihood ratio test, are shown by different letters within each column. The average amount of Ag (±SD) in the exuviae of mealworms exposed to 100 mg Ag kg^{-1} in food spiked with different Ag forms are given in the last column.

Ag form	K_1 ($g_{food}\ g^{-1}_{animal}\ day^{-1}$)	K_2 (day^{-1})	Fi	Ag in exuviae (%)
$AgNO_3$	0.26 (0–0.974) A	2.37 (0–9.36) A	0.57 (0.231–0.906)	6.4 ± 4.6 (n = 4)
AgNPs 3–8 nm	2.0 (–)	21.7 (–)	0.69 (–)	6.2 (n = 1)
AgNPs 50 nm	0.18 (0–0.479) A	1.24 (0–3.40) A	0.31 (0–0.654)	4.1 ± 3.1 (n = 6)
AgNPs 60 nm	1.96 (–)	16.3 (–)	0.72 (–)	1.2 (n = 1)
Ag_2S NPs	2.18 (–)	13.5 (–)	0.01 (–)	71.8 ± 32.8 (n = 3)

No common letter showed a significant difference between kinetics.
(–) It was not possible to calculate the 95% confidence intervals.

burning were in agreement with the study of Vijver et al. (2003) and almost no soil particles were obtained from mealworms exposed to soil. Burning/ashing showed no difference between the mealworms exposed and non-exposed to soil. However, adding the soil dyeing method showed that mealworm guts were totally coloured, which was confirmed by the acid digestion of faeces. In spite of this finding, the Ag concentrations in depurated and non-depurated mealworms following exposure to 3–8 nm AgNPs showed no differences. Therefore, we can conclude that soil may have a low residence time in mealworms and the soil present in the gut by itself does not make a significant contribution to the final Ag concentration in the mealworms. This may suggest that the amount of ingested soil is small and not contributing much to the Ag body concentrations during the bioaccumulation test. Pore water ingestion may be the more likely route, as dermal uptake seems to be unlikely due to the hard wax-coated cuticle (water-impermeable integument) (Vijver et al., 2003). Water plays a crucial role in the growth and development of *T. molitor* larvae and it is therefore the more plausible exposure route in soil (Murray, 1968).

4.1. Soil exposure

The uptake and elimination parameters (K_1, K_2 and Fi) obtained from the 3–8 nm and 60 nm AgNPs and ionic Ag exposures were almost the same, and show the ability of the mealworms to eliminate Ag after being transferred to clean soil and detoxify this metal primarily by excretion (Ardestani and Van Gestel, 2013). On the other hand, Waalewijn-Kool et al. (2014) exposed *Folsomia candida* to the same 3–8 nm AgNPs and reported a significantly higher bioaccumulation factor for $AgNO_3$ exposure, which was due to the higher elimination rate constant for nanoparticulate than for ionic Ag.

Moreover, AgNP characteristics affected their bioavailability and their bioaccumulation in the mealworms. The uptake (K_1) and elimination (K_2) rate constants for the citrate-capped 50 nm AgNPs were significantly lower than those for other AgNPs (paraffin and PVP coated, and Ag_2S) and ionic Ag, while Fi was higher. This remarkable difference may suggest a different behaviour of the 50 nm AgNPs and also different Ag handling by the mealworms. The significant differences in the toxicokinetics between the 50 nm and 60 nm AgNPs may be due to the low stability of 60 nm AgNPs compared to 50 nm AgNPs as was shown by monitoring the NP stability in the stock solutions in water. This was also supported by the higher dissolution of Ag in the soil pore water for 60 nm AgNPs when compared to the 50 nm AgNPs, despite the fact that the PVP-coated NPs should be more sterically stable. The 50 nm AgNPs were stabilised with sodium citrate while the 60 nm AgNPs were coated with PVP and the behaviour of AgNPs is related to their size, shape, size distribution, and coating. Some types of coatings, like citrate-based, are of low molecular weight and protect the NPs by electrostatic repulsion, but a hydrophilic high molecular weight polymer like polyvinylpyrrolidone (PVP) imparts steric stabilization (Burkowska-But et al., 2014). Coating has an impact on dissolution and agglomeration of NPs and depends on the media in which they are released (Topuz and van Gestel, 2017). Besides, AgNPs' bacterial efficacy is often related to their coating and, consequently, dissolution rate. Burkowska-But et al. (2014) showed that PVP-coated AgNPs had a substantially higher antibacterial efficacy than citrate-coated AgNPs (Burkowska-But et al., 2014), which agrees with the lower dissolution rate of citrate coated 50 nm AgNPs when compared to the one reported for the 60 nm AgNPs. Thus, the significantly lower uptake rate constant of the 50 nm AgNPs is most likely related to its characteristics. Confirming the findings for the mealworms, a study with differently coated 25 nm AgNPs in *Enchytraeus crypticus* showed similar uptake of PVP-coated AgNPs and ionic Ag while the uptake of citrate-coated AgNPs was much lower (Topuz and van Gestel, 2015). Studying the effect of different coatings (bovine serum albumin, chitosan and PVP) and sizes (20, 35 and 50 nm) on AgNP bioaccumulation in *Lumbricus rubellus* revealed that only for PVP-coated particles, size had an influence on Ag uptake but not for the other coatings. Therefore, the effect of the size of AgNPs (20–50 nm) on its uptake seems to be limited compared with the impact of the type of coating (Makama et al., 2016).

The toxicokinetics of Ag_2S NPs were different at the high and low concentrations tested (22 and 226 mg Ag kg^{-1} dry soil). While for the lower concentration of Ag_2S NPs the estimated uptake rate constant (K_1) was higher than that for the other Ag forms, the inert fraction was similar leading to a higher elimination constant rate (K_2). The K_1 for the higher concentration of Ag_2S NPs used was lower than that for the lower Ag_2S NPs concentration, but similar to what was observed for the other Ag forms. Hence, Ag_2S NPs could be taken up by mealworms even though they were expected not to be bioaccessible compared to the other Ag forms as they had a very low $CaCl_2$-extractable Ag concentration (0.001 mg Ag kg^{-1} soil) (Table 2). This observed low bioaccessibility for Ag_2S NPs revealed that the uptake of AgNPs is more related to the surface speciation than to their release of Ag ions, so the sulphadised form was taken up but was also eliminated more rapidly resulting in low overall internalization. Moreover, this was confirmed by the fact that Ag_2S NPs could be taken up by mealworms, but they did eliminate them faster than the other Ag forms with the lowest Fi at 226 mg Ag kg^{-1} dry soil (0.08, corresponding to 8% of the amount accumulated). The elimination rate constants in the mealworm were similar for all different Ag forms except for the 50 nm AgNPs; such a K_2 similarity for different Ag forms has been reported before for the isopod *Porcellionides pruinosus* (Tourinho et al., 2016). However, mealworms can eliminate silver more efficiently than isopods, which are able to accumulate silver as granules in the S-cells of the hepatopancreas (Tourinho et al., 2016). No significant difference between uptake and elimination rate constants of ionic Ag and 50 nm AgNPs was seen in the earthworm *Eisenia fetida* in the study of Baccaro et al. (2018). Moreover, Baccaro et al. (2018) reported a remarkably lower uptake of Ag from Ag_2S NPs in earthworms compared to ionic Ag, which is in contrast to what was found for the mealworms. In addition, a low bioavailability of Ag_2S NPs was also observed in the soil arthropod *F. candida* (Collembola) and no uptake in the isopods *Porcellio scaber* (Talaber et al., 2020). Nevertheless, in the study by Kampe et al. (2018) Ag_2S NPs showed to be bioavailable for *P. scaber* exposed to AgNP spiked sludge amended soil. To compare the bioaccumulation of pristine and aged silver NPs, Velicogna et al. (2017) exposed *Eisenia andrei* to AgNP and $AgNO_3$ spiked soil and to soil amended with spiked biosolid-AgNPs. The bioaccumulation factors were 0.89 and 0.74 for AgNPs and ionic Ag, respectively and 0.12 for biosolid-AgNP amended soil (Velicogna et al., 2017), which is in agreement with our study.

Vijver et al. (2003) reported that feeding on soil organic matter rather than soil pore water is the main exposure route for metals in mealworms (Vijver et al., 2003). Our results, however, show that the soil pore water was slightly more important than soil for the total uptake in the mealworms, for the Ag forms that showed significant dissolution resulting in high concentrations of ionic Ag in the soil pore water. In this regard, $AgNO_3$ and the 60 nm AgNPs, with 60–62% of Ag uptake from the soil pore water, showed nearly 90% of the total Ag was present as ionic Ag in the soil pore water. The 50 nm AgNPs displayed an equal uptake of Ag from the soil and soil pore water with about 50% of ionic Ag in soil pore water. The 3–8 nm AgNPs and Ag_2S NPs were taken up more from the soil with 43% and 10% Ag uptake from the soil pore water and with 17% and 0.6% of ionic Ag in the soil pore water. Therefore, mealworms were indeed exposed to the soil and soil pore water and what made them so tolerant to Ag during these life stages could be their ability to eliminate Ag by different mechanisms such as shedding the exuvia (although no exuvia was collected in the soil exposure) or through the faeces.

The results suggest that there is a NPs stability phenomenon driving the uptake and elimination phases, as the insoluble Ag_2S are in all cases significantly different with the other treatments. This is also supported by the faster kinetics observed for the Ag_2S NPs compared to the rest of the treatment that presented significantly higher dissolution (see Section 3.1 and Fig. S1E), while the differences observed between the

Ag_2S treatments can be attributed to the substantially higher variation observed in the raw data for the Ag_2S (226 mg Ag kg^{-1} dry soil) treatment. Furthermore, the 50 nm AgNPs uptake results are also partly supported by the kinetics results, as they were found to be significantly different from the 60 nm AgNPs and $AgNO_3$, which present the higher ionic content, but not with the both the Ag_2S and 3–8 nm AgNPs treatments.

4.2. Food exposure

The toxicokinetics parameters K_1 and K_2 for 3–8 nm and 60 nm AgNPs were similar, while the K_1 and K_2 values for mealworms fed on food spiked with 50 nm AgNPs and $AgNO_3$ were also similar. The K_1 and K_2 values for the Ag_2S NPs were seemed to be similar to those of the 3–8 nm and 60 nm AgNPs and gave the lowest Fi (0.01). However, the statistical analysis of the mealworms body concentration showed that Ag taken up from the Ag_2S NPs was eliminated significantly different compared to other Ag forms. The latter suggests that the Ag_2S NPs could be eliminated completely and may be differently handled by the mealworms compared to the other silver forms. Metals have a high tendency to adsorb to organic matter and their desorption during gut passage depends strongly on the binding affinity to the organic matter (Tourinho et al., 2016), and also to the gut conditions such as pH or enzymes present. Ag_2S NPs may not or only slightly bind to the food as they are stable. As a consequence, they may have detached from the food and be absorbed faster during gut passage but also eliminated faster compared to pristine AgNPs. Truzzi et al. (2019) reported that Cd, Ni, and As were taken up by *T. molitor* larvae feeding on metal-contaminated substrates but not accumulated (BAF close to 1), while Hg was bioaccumulated.

These results of the statistical comparison of the raw experimental data seem to be consistent with the long-term dissolution results (48 h timepoint where the dissolution curves start reaching the expected plateau) presented in Fig. S1E in the Supplementary Information and further support the observations during the soil exposure experiments presented earlier, where a NPs-stability effect was observed. The 60 nm AgNPs present, in absolute numbers, higher dissolution (29.2 ± 0.33%) and being closer to the ionic $AgNO_3$, followed by the 50 nm AgNPs (26.1 ± 6.02%), the 3–8 nm AgNPs dissolution (48 h dissolution: 21.1 ± 4.04%) being closer to the mean (19.1 ± 1.4%) of all the NP treatments and the more stable Ag_2S NPs presenting the lowest dissolution (0.01 ± 0.003%), as expected due to it being in practice insoluble (Sekine et al., 2015). Further study is needed to verify these results, by extending the dissolution monitoring period to the entire 42-day uptake and elimination periods.

Coleoptera have a short straight and tubular foregut, while the midgut is the main site of digestion. The hindgut has a permeable cuticle to prevent loss of useful substances and starts with the ileum and then continues into the colon, rectum and anus (Sarwade and Bhawane, 2013). In *T. molitor*, the lifetime of the midgut epithelium cells is about 4 days after which they are discharged into the gut lumen and excreted through the faeces (Lindqvist and Block, 1995). If any metal taken up could not be eliminated by excretion, it may be stored by the organism to prevent further toxic effects. Pedersen et al. (2007) reported the presence of Cd-binding proteins without high cysteine contents in mealworms, although the mechanism of detoxification of metals and the role of this protein is not clear yet. Bednarska and Świątek (2016) separated the cellular components of mealworms into three fractions (S1 or cytosolic fraction including organelles, heat-sensitive and heat-stable proteins, S2 or cellular debris and the G or metal-rich granule fraction) to estimate the risk of food chain transfer of stored metals. About 30% of Cd and Zn was found to be in the S1 fraction, which has the possibility to be transferred to higher trophic levels in a food web. For predators with a low gut pH, the metals stored in granules in the mealworms could also become bioavailable (Bednarska and Świątek, 2016).

The results presented also highlighted an extra route of excretion of Ag by moulting, although it seems that the amount of Ag eliminated in exuviae depends on the Ag form/speciation and its behaviour inside the organism. Lindqvist and Block (1995) found that the main amount of discharged Cd during the moulting of mealworms was from the gut epithelium. Metals present in other tissues could not be eliminated by moulting, except for the part bound to the integument (Lindqvist and Block, 1995). Because of the low concentration of cadmium and zinc in mealworm exuviae, Vijver et al. (2003) reported that moulting may not be an important elimination route for these metals. Discharge through moulting was reported also for CeO_2 NP elimination from *Daphnia pulex* (Auffan et al., 2013), and for the elimination of zinc, cadmium and copper from chironomids (Diptera, Chironomidae) (Timmermans and Walker, 1989).

Among the different Ag forms, Ag_2S NPs had the highest concentration in the mealworm exuviae, suggesting they were mainly attached to the gut epithelium and did not reach other tissues, which was confirmed by the low Ag body concentrations found. But some caution has to be taken considering this result due to the low number of exuvia collected.

The estimated kinetics in the mealworms exposed via the soil and via the food showed a lower inert fraction upon soil exposure for all different Ag forms except for the 50 nm AgNPs. Maybe because of the different type and content of organic matter in the soil compared to the food, the ingestion time and uptake mechanisms were different resulting in different Ag storage levels in the mealworms. Tourinho et al. (2016), exposing isopods to the same 3–8 nm AgNPs via soil and via food, found higher uptake rate constants from soil exposure and similar elimination rate constants for both exposures. This is in contrast with our results, probably because isopods avoided contaminated food or because of the higher ability of mealworms to eliminate Ag when compared to isopods.

5. Conclusions

The larvae of *Tenebrio molitor* present several routes of Ag uptake, including food and soil. In the latter case, the water fraction is most important (i.e. soil pore water). Ingested soil particles are excreted through feaces, not contributing much to Ag accumulation.

The bioavailability and bioaccumulation of AgNPs are affected by their characteristics (surface coating, speciation) and related stability in the receiving environment, as Ag uptake and elimination rate constants differed for differently coated AgNPs of similar size. Ag_2S NPs, which are the most environmentally relevant and the most stable AgNP form, could be taken up by the mealworms but were also eliminated faster than the other Ag forms for both exposure routes. Therefore, bioaccumulation of different NPs, including aged forms, should be considered in the risk assessment of NPs.

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. **Anastasios G. Papadiamantis:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Sandra F. Gonçalves:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Iseult Lynch:** Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Susana Loureiro:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

There are no conflicts 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.2021.146071.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705.

Ardestani, M.M., Van Gestel, C.A.M., 2013. Dynamic bioavailability of copper in soil estimated by uptake and elimination kinetics in the springtail Folsomia candida. Ecotoxicology 22, 308–318. https://doi.org/10.1007/s10646-012-1027-8.

Ardestani, M.M., Van Straalen, N.M., Van Gestel, C.A.M., 2014. Uptake and elimination kinetics of metals in soil invertebrates: a review. Environ. Pollut. 193, 277–295. https://doi.org/10.1016/j.envpol.2014.06.026.

Argasinski, K., Bednarska, A., Laskowski, R., 2012. The toxicokinetics cell demography model to explain metal kinetics in terrestrial invertebrates. Ecotoxicology 21, 2186–2194. https://doi.org/10.1007/s10646-012-0972-6.

Auffan, M., Bertin, D., Chaurand, P., Pailles, C., Dominici, C., Rose, J., Bottero, J.Y., Thiery, A., 2013. Role of molting on the biodistribution of CeO2 nanoparticles within Daphnia pulex. Water Res. 47, 3921–3930. https://doi.org/10.1016/j.watres.2012.11.063.

Avramescu, M.L., Rasmussen, P.E., Chénier, M., Gardner, H.D., 2017. Influence of pH, particle size and crystal form on dissolution behaviour of engineered nanomaterials. Environ. Sci. Pollut. Res. 24, 1553–1564. https://doi.org/10.1007/s11356-016-7932-2.

Baalousha, M., Cornelis, G., Kuhlbusch, T.A.J., Lynch, I., Nickel, C., Peijnenburg, W., Van Den Brink, N.W., 2016. Modeling nanomaterial fate and uptake in the environment: current knowledge and future trends. Environ. Sci. Nano 3, 323–345. https://doi.org/10.1039/c5en00207a.

Baccaro, M., Undas, A.K., De Vriendt, J., Van Den Berg, J.H.J., Peters, R.J.B., Van Den Brink, N.W., 2018. Ageing, dissolution and biogenic formation of nanoparticles : how do these factors affect the uptake kinetics of silver nanoparticles in earthworms? Environ. Sci. Nano 5, 1107–1116. https://doi.org/10.1039/C7EN01212H.

Bednarska, A.J., Świątek, Z., 2016. Subcellular partitioning of cadmium and zinc in mealworm beetle (Tenebrio molitor) larvae exposed to metal-contaminated flour. Ecotoxicol. Environ. Saf. 133, 82–89. https://doi.org/10.1016/j.ecoenv.2016.06.033.

Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbühler, K., 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci. Total Environ. 390, 396–409. https://doi.org/10.1016/j.scitotenv.2007.10.010.

Burkowska-But, A., Sionkowski, G., Walczak, M., 2014. Influence of stabilizers on the antimicrobial properties of silver nanoparticles introduced into natural water. J. Environ. Sci. 26, 542–549. https://doi.org/10.1016/S1001-0742(13)60451-9.

Coutris, C., Joner, E.J., Oughton, D.H., 2012. Aging and soil organic matter content affect the fate of silver nanoparticles in soil. Sci. Total Environ. 420, 327–333. https://doi.org/10.1016/j.scitotenv.2012.01.027.

Croteau, M.-N., Misra, S.K., Luoma, S.N., Valsami-Jones, E., 2011. Silver bioaccumulation dynamics in a freshwater invertebrate after aqueous and dietary exposures to nanosized and ionic Ag. Environ. Sci. Technol. 45, 6600–6607. https://doi.org/10.1021/es200880c.

Diez-Ortiz, M., Lahive, E., George, S., Ter Schure, A., Van Gestel, C.A.M., Jurkschat, K., Svendsen, C., Spurgeon, D.J., 2015. Short-term soil bioassays may not reveal the full toxicity potential for nanomaterials; bioavailability and toxicity of silver ions ($AgNO_3$) and silver nanoparticles to earthworm Eisenia fetida in long-term aged soils. Environ. Pollut. 203, 191–198. https://doi.org/10.1016/j.envpol.2015.03.033.

Dodd, M., Rasmussen, P.E., Chénier, M., 2013. Comparison of two in vitro extraction protocols for assessing metals' bioaccessibility using dust and soil reference materials. Hum. Ecol. Risk. Assess. 19, 1014–1027. https://doi.org/10.1080/10807039.2012.719381.

Eriksson, T., Andere, A.A., Kelstrup, H., Emery, V.J., Picard, C.J., 2020. The yellow mealworm (Tenebrio molitor) genome: a resource for the emerging insects as food and feed industry. J. Insects as Food Feed 6, 445–455. https://doi.org/10.3920/JIFF2019.0057.

European"s Committee for Standardisation (CEN). CEN/TC 52, 2019. Safety of Toys - Part 3: Migration of Certain Elements. Guidance EN 71-3.

Fytili, D., Zabaniotou, A., 2008. Utilization of sewage sludge in EU application of old and new methods-a review. Renew. Sust. Energ. Rev. 12, 116–140. https://doi.org/10.1016/j.rser.2006.05.014.

Giese, B., Klaessig, F., Park, B., Kaegi, R., Steinfeldt, M., Wigger, H., Von Gleich, A., Gottschalk, F., 2018. Risks, release and concentrations of engineered nanomaterial in the environment. Sci. Rep. 8, 1–18. https://doi.org/10.1038/s41598-018-19275-4.

Greenberg, S., Ar, A., 1996. Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle Tenebrio molitor. J. Insect Physiol. 42, 991–996. https://doi.org/10.1016/S0022-1910(96)00071-6.

Hug Peter, D., Sardy, S., Diaz Rodriguez, J., Castella, E., Slaveykova, V.I., 2018. Modeling whole body trace metal concentrations in aquatic invertebrate communities: a trait-based approach. Environ. Pollut. 233, 419–428. https://doi.org/10.1016/j.envpol.2017.10.044.

Kampe, S., Kaegi, R., Schlich, K., Wasmuth, C., Hollert, H., Schlechtriem, C., 2018. Silver nanoparticles in sewage sludge: Bioavailability of sulfidized silver to the terrestrial isopod Porcellio scaber. Environ. Toxicol. Chem. 37, 1606–1613. https://doi.org/10.1002/etc.4102.

Kim, B., Park, C.-S., Murayama, M., Michael, F., Hochella, J., 2010. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. Environ. Sci. Technol. 44, 7509–7514.

Levard, C., Hotze, E.M., Lowry, G.V., Brown, G.E., 2012. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environ. Sci. Technol. 46, 6900–6914. https://doi.org/10.1021/es2037405.

Lindqvist, L., Block, M., 1995. Excretion of cadmium during moulting and metamorphosis in *Tenebrio molitor* (Coleoptera; Tenebrionidae). Comp. Biochem. Physiol. 111, 325–328.

Loureiro, S., Tourinho, P.S., Cornelis, G., Van Den Brink, N.W., Díez-Ortiz, M., Vázquez-Campos, S., Pomar-Portillo, V., Svendsen, C., Van Gestel, C.A.M., 2018. In: Duarte, A.C., Cachada, A., Rocha-Santos, T.B.T.-S.P. (Eds.), Chapter 7 - nanomaterials as soil pollutants. Academic Press, pp. 161–190 https://doi.org/10.1016/B978-0-12-849873-6.00007-8.

Makama, S., Piella, J., Undas, A., Dimmers, W.J., Peters, R., Puntes, V.F., van den Brink, N.W., 2016. Properties of silver nanoparticles influencing their uptake in and toxicity to the earthworm Lumbricus rubellus following exposure in soil. Environ. Pollut. 218, 870–878. https://doi.org/10.1016/j.envpol.2016.08.016.

McGillicuddy, E., Murray, I., Kavanagh, S., Morrison, L., Fogarty, A., Cormican, M., Dockery, P., Prendergast, M., Rowan, N., Morris, D., 2017. Silver nanoparticles in the environment: sources, detection and ecotoxicology. Sci. Total Environ. 575, 231–246. https://doi.org/10.1016/j.scitotenv.2016.10.041.

Morales-Ramos, J. a, Rojas, M.G., Shapiro-Ilan, D.I., Tedders, W.L., 2010. Developmental plasticity in *Tenebrio molitor* (Coleoptera: Tenebrionidae): analysis of instar variation in number and development time under different diets. J. Entomol. Sci. 45, 75–90. https://doi.org/10.18474/0749-8004-45.2.75.

Murray, D.R.P., 1968. The importance of water in the normal growth of larvae of Tenebrio molitor. Ent. Exp. Appl. 11, 149–168.

OECD, 2015. Series on the Safety of Manufactured Nanomaterials No. 62. Organisation for Economic Co-operation and Development, Paris.

Oomen, A.G., Steinhäuser, K.G., Bleeker, E.A.J., van Broekhuizen, F., Sips, A., Dekkers, S., Wijnhoven, S.W.P., Sayre, P.G., 2018. Risk assessment frameworks for nanomaterials: scope, link to regulations, applicability, and outline for future directions in view of needed increase in efficiency. NanoImpact 9, 1–13. https://doi.org/10.1016/j.impact.2017.09.001.

Paine, C.E.T., Marthews, T.R., Vogt, D.R., Purves, D., Rees, M., Hector, A., Turnbull, L.A., 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods Ecol. Evol. 3, 245–256. https://doi.org/10.1111/j.2041-210X.2011.00155.x.

Pedersen, S.A., Kristiansen, E., Andersen, R.A., Zachariassen, K.E., 2007. Isolation and preliminary characterization of a Cd-binding protein from Tenebrio molitor (Coleoptera). Comp. Biochem. Physiol. Part C 145, 457–463. https://doi.org/10.1016/j.cbpc.2007.02.003.

Petersen, E.J., Mortimer, M., Burgess, R.M., Handy, R., Hanna, S., Ho, K.T., Johnson, M., Loureiro, S., Selck, H., Scott-fordsmand, J.J., Spurgeon, D., Unrine, J., Van Den Brink, N., Wang, Y., White, J., Holden, P., 2019. Strategies for robust and accurate experimental approaches to quantify nanomaterial bioaccumulation across a broad range of organisms. Environ. Sci. Nano 6, 1619–1656.

Ribeiro, F., Gallego-Urrea, J.A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A.M.V.M., Loureiro, S., 2014. Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, *Daphnia magna* and *Danio rerio*. Sci. Total Environ. 466–467, 232–241. https://doi.org/10.1016/j.scitotenv.2013.06.101.

Ribeiro, F., Van Gestel, C.A.M., Pavlaki, M.D., Azevedo, S., Soares, A.M.V.M., Loureiro, S., 2017. Bioaccumulation of silver in Daphnia magna: waterborne and dietary exposure to nanoparticles and dissolved silver. Sci. Total Environ. 574, 1633–1639. https://doi.org/10.1016/j.scitotenv.2016.08.204.

Roig, N., Sierra, J., Martí, E., Nadal, M., Schuhmacher, M., Domingo, J.L., 2012. Long-term amendment of Spanish soils with sewage sludge: effects on soil functioning. Agric. Ecosyst. Environ. 158, 41–48. https://doi.org/10.1016/j.agee.2012.05.016.

Sarwade, A.B., Bhawane, G.P., 2013. Anatomical and histological structure of digestive tract of adult Platynotus belli. F (Coleoptera: Tenebrionidae). Biol. Forum – An Int. J. 5, 47–55.

Sekine, R., Brunetti, G., Donner, E., Khaksar, M., Vasilev, K., Jämting, Å.K., Scheckel, K.G., Kappen, P., Zhang, H., Lombi, E., 2015. Speciation and lability of Ag-, AgCl-, and Ag2S-nanoparticles in soil determined by X-ray absorption spectroscopy and diffusive gradients in thin films. Environ. Sci. Technol. 49, 897–905. https://doi.org/10.1021/es504229h.

Silva, P.V., van Gestel, C.A.M., Verweij, R.A., Papadiamantis, A.G., Gonçalves, S.F., Lynch, I., Loureiro, S., 2020. Toxicokinetics of pristine and aged silver nanoparticles in Physa acuta. Environ. Sci. Nano https://doi.org/10.1039/d0en00946f.

Sokal, R.R., Rohlf, F.J., 2012. In: W.H. Freeman and Company (Ed.), Biometry the Principles and Practice of Statistics in Biological Research, forth ed. W.H. Freeman and Company, New York, USA https://doi.org/10.2307/2412280.

Sørensen, S.N., Baun, A., Burkard, M., Dal Maso, M., Foss Hansen, S., Harrison, S., Hjorth, R., Lofts, S., Matzke, M., Nowack, B., Peijnenburg, W., Poikkimäki, M., Quik, J.T.K., Schirmer, K., Verschoor, A., Wigger, H., Spurgeon, D.J., 2019. Evaluating environmental risk assessment models for nanomaterials according to requirements along the product innovation Stage-Gate process. Environ. Sci. Nano 6, 505–518. https://doi.org/10.1039/c8en00933c.

Svendsen, C., Walker, L.A., Matzke, M., Lahive, E., Harrison, S., Crossley, A., Park, B., Lofts, S., Lynch, I., Vázquez-Campos, S., Kaegi, R., Gogos, A., Asbach, C., Cornelis, G., von der Kammer, F., van den Brink, N.W., Mays, C., Spurgeon, D.J., 2020. Key principles and operational practices for improved nanotechnology environmental exposure assessment. Nat. Nanotechnol. 15, 731–742. https://doi.org/10.1038/s41565-020-0742-1.

Talaber, I., Van Gestel, C.A.M., Jemec Kokalj, A., Marolt, G., Novak, S., Zidar, P., Drobne, D., 2020. Comparative biokinetics of pristine and sulfidized Ag nanoparticles in two arthropod species exposed to different field soils. Environ. Sci. Nano 7, 2735–2746. https://doi.org/10.1039/D0EN00291G.

Tashiro, H., 1990. Chapter 40: insecta: coleoptera scarabaeidae larvae. In: Dindal, D.L. (Ed.), Soil Biology Guide. A Wiley-Interscience Publication, pp. 1191–1209 https://doi.org/10.1093/ae/38.3.181a.

Thomassen, Y., Ellingsen, D.G., Hetland, S., Sand, G., 2001. Chemical speciation and sequential extraction of Mn in workroom aerosols: analytical methodology and results from a field study in Mn alloy plants. J. Environ. Monit. 3, 555–559. https://doi.org/10.1039/b104479f.

Timmermans, K.R., Walker, P.A., 1989. The fate of trace metals during the metamorphosis of chironomids (diptera, chironomidae). Environ. Pollut. 62, 73–85. https://doi.org/10.1016/0269-7491(89)90097-3.

Topuz, E., van Gestel, C.A.M., 2015. Toxicokinetics and toxicodynamics of differently coated silver nanoparticles and silver nitrate in Enchytraeus crypticus upon aqueous exposure in an inert sand medium. Environ. Toxicol. Chem. 34, 2816–2823. https://doi.org/10.1002/etc.3123.

Topuz, E., van Gestel, C.A.M., 2017. The effect of soil properties on the toxicity and bioaccumulation of Ag nanoparticles and Ag ions in Enchytraeus crypticus. Ecotoxicol. Environ. Saf. 144, 330–337. https://doi.org/10.1016/j.ecoenv.2017.06.037.

Tourinho, P.S., van Gestel, C.A.M., Morgan, A.J., Kille, P., Svendsen, C., Jurkschat, K., Mosselmans, J.F.W., Soares, A.M.V.M., Loureiro, S., 2016. Toxicokinetics of Ag in the terrestrial isopod Porcellionides pruinosus exposed to Ag NPs and AgNO3 via soil and food. Ecotoxicology 25, 267–287. https://doi.org/10.1007/s10646-015-1585-7.

Truzzi, C., Illuminati, S., Girolametti, F., Antonucci, M., Scarponi, G., Ruschioni, S., Riolo, P., Annibaldi, A., 2019. Influence of Feeding Substrates on the Presence of Toxic Metals (Cd, Pb, Ni, As, Hg) in Larvae of Tenebrio molitor: Risk Assessment for Human Consumption. Int. J. Environ. Res. Public Health 16, 4815. https://doi.org/10.3390/ijerph16234815.

van den Brink, N.W., Jemec Kokalj, A., Silva, P.V., Lahive, E., Norrfors, K., Baccaro, M., Khodaparast, Z., Loureiro, S., Drobne, D., Cornelis, G., Lofts, S., Handy, R.D., Svendsen, C., Spurgeon, D., van Gestel, C.A.M., 2019. Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms. Environ. Sci. Nano 6, 1985–2001. https://doi.org/10.1039/c8en01122b.

Van Der Zande, M., Jemec Kokalj, A., Spurgeon, D.J.D.J., Loureiro, S., Silva, P.V.P.V., Khodaparast, Z., Drobne, D., Clark, N.J.N.J., Van Den Brink, N.W., Baccaro, M., Van Gestel, C.A.M., Bouwmeester, H., Handy, R.D., 2020. The gut barrier and the fate of engineered nanomaterials: a view from comparative physiology. Environ. Sci. Nano 00, 1–25. https://doi.org/10.1039/d0en00174k.

Velicogna, J.R., Schwertfeger, D.M., Jesmer, A.H., Scroggins, R.P., Princz, J.I., 2017. The bioaccumulation of silver in Eisenia andrei exposed to silver nanoparticles and silver nitrate in soil. NanoImpact 6, 11–18. https://doi.org/10.1016/j.impact.2017.03.001.

Vijver, M., Jager, T., Posthuma, L., Peijnenburg, W., 2003. Metal uptake from soils and soil – sediment mixtures by larvae of *Tenebrio molitor* (L.) (Coleoptera). Ecotoxicol. Environ. Saf. 54, 277–289. https://doi.org/10.1016/S0147-6513(02)00027-1.

Vijver, M.G., Vink, J.P.M., Jager, T., van Straalen, N.M., Wolterbeek, H.T., van Gestel, C.A.M., 2006. Kinetics of Zn and Cd accumulation in the isopod Porcellio scaber exposed to contaminated soil and/or food. Soil Biol. Biochem. 38, 1554–1563. https://doi.org/10.1016/j.soilbio.2005.11.006.

Waalewijn-Kool, P.L., Klein, K., Fornies, R.M., van Gestel, C.A.M., 2014. Bioaccumulation and toxicity of silver nanoparticles and silver nitrate to the soil arthropod Folsomia candida. Ecotoxicology 23, 1629–1637. https://doi.org/10.1007/s10646-014-1302-y.

Wang, W.-X., 2011. Incorporating exposure into aquatic toxicological studies: an imperative. Aquat. Toxicol. 105S, 9–15. https://doi.org/10.1016/j.aquatox.2011.05.016.

Yang, Y., Yang, J., Wu, W.M., Zhao, J., Song, Y., Gao, L., Yang, R., Jiang, L., 2015. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. Role of gut microorganisms. Environ. Sci. Technol. 49, 12087–12093. https://doi.org/10.1021/acs.est.5b02663.

Yao, J.H., Elder, K.R., Guo, H., Grant, M., 1993. Theory and simulation of Ostwald ripening. Phys. Rev. B 47, 14110–14125. https://doi.org/10.1103/PhysRevB.47.14110.