RESEARCH ARTICLE

Biological transfer of silver under silver nanoparticle exposure and nitrogen transfer via a collembolan-predatory mite food-chain and ecotoxicity of silver sulfide

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HIGHLIGHTS

- AgNPs transferred and accumulated through soil animal food chain.
- AgNPs trophic transfer disturbed nutrient element N transfer.
- Ag accumulated in body tissue, but no biomagnification effects.
- Ag₂S was harmful to *F. candida* on survival and reproduction.

ARTICLE INFO

Article history:

Received July 5, 2021

Revised September 17, 2021

Accepted September 29, 2021

Keywords:

AgNPs

Ag₂S

¹⁵N

Soil animals

Food chain

GRAPHICAL ABSTRACT

ABSTRACT

The development of nanotechnology has accelerated the use of silver nanoparticles (AgNPs) in household chemicals and the accumulation of Ag in sewage treatment systems. The application of sewage sludge products to soils raises concerns over the safety of Ag in the function and biogeochemical cycles of the soil belowground ecosystem. Here, we assess the potential risk of the accumulation and transfer of Ag under AgNPs exposure and its effects on the trophic transfer of nitrogen (N) through a soil animal food chain (*Folsomia candida*–*Hypoaspis aculeifer*). The formation of stable silver sulfide (Ag₂S) was also studied via a single species test using *F. candida*. Concentrations of Ag in *F. candida* increased with increasing AgNPs concentration, as did those in the predator *H. aculeifer*, but the Ag bioaccumulation factors of both animals were < 1. *Folsomia candida* body tissue ¹⁵N abundance declined markedly compared with that of *H. aculeifer*. Silver sulfide did have adverse effects on the survival and reproduction of *F. candida*. The Ag concentrations of *F. candida* increased with increasing Ag₂S concentration in sludge-treated soils. Silver sulfide showed ecotoxicity to the collembolan, therefore ecotoxicity resulting from the transformation and fate of AgNPs in soils needs to be considered before biosolid products are applied to agricultural soils.

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1 Introduction

The development of nanotechnology has accelerated the use of nano-silver (AgNPs) products. Silver nanoparticles have anti-bacterial, plasmonic and opto-electrical properties and are widely used in household chemicals (Kampe et al., 2018),

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resulting in large amounts entering sewage treatment systems adsorbed to sludge. Wu et al. (2018) collected 56 Chinese sludge samples and found Ag concentrations of 0.64–9.92 mg kg⁻¹. Thus, the application of sewage sludges to agricultural land may lead to AgNPs accumulation in soils (Mueller and Nowack, 2008), raising safety concerns regarding AgNPs in soil ecosystems (Blaser et al., 2008; Wang et al., 2018). Negative effects of AgNPs on microbial biomass and diversity, plant growth and yield, and soil invertebrate survival and reproduction have been documented (Waalewijn-Kool et al., 2014; Wang et al., 2017; Courtois et al., 2019). Soil animals including collembolans, earthworms and enchytraeids have been studied in detail in single species tests at the molecular, individual and population levels of organization (Schlich et al., 2013; Bicho et al., 2016; Sillapawattana et al., 2016). However, multiple species ecotoxicological tests of AgNPs risks in food chains remain limited. Micro-mesocosm studies of biocenoses have greater ecological relevance than single-species tests (Kula and Römbke, 1998). A novel study has advanced our understanding of AgNPs uptake by plants and trophic transfer to herbivores, with transfer of stable-isotope-enriched ¹⁰⁹AgNPs via soybean leaves and accumulation in snails, indicating risks from AgNPs to the food chain (Dang et al., 2019). A field survey at the site of a nuclear accident at Fukushima, Japan, found that arthropods concentrated more radioactive ¹¹⁰Ag than other soil animals, and this highlights the necessity of studying the influence of AgNPs in belowground terrestrial food chains (Nakanishi et al., 2015). Predator–prey relationships are prevalent in soils and are essential to soil biogeochemical cycling and energy flow. Trophic relationships have been used as a tool to reflect the accumulation and biological transfer of elements among soil animals. For instance, predatory mites can eat collembolans as food items and their typical trophic relationships are essential for soil ecosystem functioning (e.g., organic matter decomposition and nutrient element cycles) (Pey et al., 2014). These two animal groups are prevalent in soils and several species have been adopted as standard animals for single-species ecotoxicological tests (Fountain and Hopkin, 2005; Huguier et al., 2015). Zhu et al. (2016) established a soil animal food chain comprising *Hypoaspis aculeifer* and *Folsomia candida* to study the influence of Cd at different trophic levels and its effects on nutrient element transfer. This food chain has been used in ecotoxicological studies of pollutants within trophic levels and the pollution effects on the functioning of soil ecosystems (Zhu et al., 2018). This has allowed our studies of AgNPs effects on belowground terrestrial food chains which, to our knowledge, remain unknown (Kwak and An., 2016).

Nano-silver (0–1000 mg kg⁻¹) exposure reduced N, K and S levels in plant tissues by 0.03–0.69, 6.18–37.3, and 5%–25.6%, indicating that the AgNPs exerted negative effects on mineral nutrient uptake and assimilation (Abbas et al., 2019). Therefore, AgNPs may have effects on the transfer of nutrients within trophic levels, a topic that requires further

study. Biogeochemical cycles of nutrient elements are an essential ecological function of soil food webs, and N is a nutrient element vital to organisms. Labeled ¹⁵N has been used to determine the trophic positions of animals in food webs, and generally the average difference in ¹⁵N between trophic levels is 3.4. However, the ¹⁵N value in animals is greatly affected by pollutants and this has often been used to explore the impacts of pollutants on nutrient transfer and fractionation. Pollution stress may result in food avoidance behavior and/or increased excretion and may then influence the transfer of N from the diet to consumers. For example, the pesticide lindane elevated the ¹⁵N value in daphnia (Ek et al., 2015) and mercury (Hg)-contaminated food increased the ¹⁵N value in snowy egrets (Shaw-Allen et al., 2005). The effects of AgNPs on nutrient transfer within terrestrial soil animal food chains have been seldom studied.

In wastewater treatment plants, sewage usually undergoes sulfidation and then AgNPs are transformed to silver sulfide (Ag₂S) (Kaegi et al., 2011; Ma et al., 2014), and AgNPs tend to be sulfided and stabilized as Ag₂S (normally >94%) in the flooded soil environment, with other metal species e.g., AgNPs and AgCl are present in small amount. In agricultural soils AgNPs occur mainly in the form of Ag₂S (Li et al., 2017; He et al., 2019). Therefore, the ecotoxicity of AgNPs transformation and fate in soils must also be considered. Although the solubility and bioavailability of Ag₂S are reported to be relatively low, soil arthropods such as the collembolan *F. candida* (a vital part of soil ecosystems and representative of most soil animals) can take up the soluble fraction of Ag in pore water through their ventral tube and also by oral intake of Ag₂S-contaminated soil particles into their bodies, further risking potentially toxic effects. A dosage-related ecotoxicological test of Ag₂S in soils is therefore necessary.

Here, AgNPs and the stable form Ag₂S in soils were considered. The trophic transfer and accumulation of Ag under AgNPs exposure and the impacts of AgNPs on the transfer of N were assessed through a soil animal food chain comprising *F. candida*–*H. aculeifer*. The risks posed by Ag₂S in sewage sludge-treated soil were also assessed using the standard soil animal *F. candida*.

2 Materials and methods

2.1 Collembolan and predatory mite

The test soil animals were obtained from Aarhus University in Denmark and have been cultured in our laboratory for several years. They were reared in Petri dishes (height 10 mm, diameter 90 mm) with a layer of moist plaster of Paris mixed with activated charcoal (9:1 w/w) at 20±1°C and 75% relative humidity with a photoperiod of 16:8 h (light/dark). *Folsomia candida* individuals were fed dried baker's yeast (produced by Angel Yeast Co., Ltd., Yichang, Hubei, China; N content, 6.86 ±0.12%; [Ag], 0.05 µg kg⁻¹) and *H. aculeifer* were fed juvenile

F. candida twice a week. Distilled water was added to maintain the moisture content. 10–12 d synchronized *F. candida* individuals and 32–35 d synchronized adult *H. aculeifer* were used in the test (OECD, 2008, 2009).

2.2 Experimental setup

2.2.1 Multiple-species toxicity test

Dried wheat leaf materials, ^{15}N -labeled (5.54 ± 0.07 atom %; N content: $2.18 \pm 0.05\%$) were used as food for *F. candida*. The steps in brief were as follows. Wheat (cultivar Yangmai 158) was cultured from seed in a container (length 62 cm, width 48 cm, height 18 cm) containing 20 kg uncontaminated soil in a phytotron for 8 weeks. $^{15}\text{NH}_4^{15}\text{NO}_3$ (1 g, 10.09 atom %; SRICI, China) solution was added to the container weekly as fertilizer (Ke and Scheu, 2008). The harvested wheat litter material was washed with distilled water, dried at 60°C and ground with a stainless-steel mill ($[\text{Ag}]$ $0.02 \mu\text{g kg}^{-1}$).

Nano-silver (AgNPs) solution was purchased from Xianfeng Nano Co., Ltd., Nanjing, China (100 mg L^{-1} , 50 nm, $[\text{Ag}]$ $>99\%$). A series of AgNPs solutions were mixed with the ^{15}N -enriched wheat to obtain concentrations of 0, 100, 200, and 400 mg kg^{-1} dry weight food, dried at 60°C , and stored in desiccators. The Ag concentrations were selected based on previous studies by Waalewijn-Kool et al. (2014) and Sillapawattana et al. (2016).

Food exposure was selected to amplify or reduce the effects of soil properties. Five hundred cleaned and gut-vomited *F. candida* individuals (synchronized for 2 d) were introduced into a Petri dish. Spiked wheat was supplied three times a week as food for *F. candida*. Any moldy food present was removed when new food was added, and distilled water was used to maintain humidity. The animals were exposed at $20 \pm 1^\circ\text{C}$, 75% relative humidity and a 16:8 h photoperiod. Each treatment was set up in triplicate. After 2 weeks, 100 individuals were collected for the determination the internal Ag concentration and ^{15}N content. The remaining individuals were starved for 2 days and then transferred into a clean Petri dish and then 10 cleaned, gut-vomited and synchronized (for 32–35 d) *H. aculeifer* were introduced into each Petri dish to form a food chain (*F. candida*–*H. aculeifer*). After 2 weeks, Ag concentrations and ^{15}N abundance in *H. aculeifer* body tissues were determined.

2.2.2 Single-species toxicity test

Uncontaminated soil samples (0–20 cm of soil profile) were taken from a forest zone in Jiangsu Province, east China ($31^\circ40'56''\text{N}$, $118^\circ52'27''\text{E}$). This soil has been subjected to ecotoxicity tests on soil animals and no apparent adverse effects were observed (Dai et al., 2018; Li et al., 2020a). The soil is a Ferri-Udic Cambosol with the following selected soil physicochemical properties: pH (H_2O) 5.10, water holding capacity (WHC) 32%, organic matter content (OM) 12.3 g kg^{-1} and cation exchange capacity (CEC) $15.2 \text{ cmol (+) kg}^{-1}$. After air-drying at room temperature and sieving (2 mm), 0.5% dry

sewage sludge (basic physicochemical properties shown in Table S1) was added in each treatment according to GB 15618–2018. A series of ground Ag_2S additions (Ag concentration $>99\%$; Alfa Aesar Chemical Co. Ltd., Haverhill, MA; 2-mm sieved) were made to the soil to give Ag_2S concentrations of 0, 100, 200, 400, and 600 mg kg^{-1} . The spiked soil samples were allowed to equilibrate for one week at 50% of WHC.

Ten cleaned and gut-vomited *F. candida* (synchronized for 10–12 d) individuals were introduced into each test vessel (5.5 cm diameter, 250 mL volume) containing 30 g spiked soil. The vessel was covered with plastic film punched with small holes. The animals were cultured in the same conditions as described above. The containers were weighed to maintain water balance twice a week. There were four replicates of each treatment. After 28 days, soil animals were extracted with a controlled temperature gradient extractor. Juveniles and adults were counted under a stereomicroscope (Leica S8 APO, Wetzlar, Germany). The body lengths were measured under a microscope (MS60, Mingmei Optoelectronic Technology Co. Ltd., Guangzhou, China). Concentrations of Ag in adult and juvenile body tissues were determined.

2.3 Chemical analysis

The extracted *F. candida* and *H. aculeifer* individuals were starved for 2 days to remove interfering substances from their gut contents. After cleaning (ultrapure water), rinsing and freeze drying (-80°C), the animals were weighed (precision $\pm 1 \mu\text{g}$, XS3DU, Mettler Toledo, Columbus, OH) and stored in a desiccator at 4°C before analysis.

2.3.1 ^{15}N isotope analysis

The weighed animals from multiple species tests were analyzed for the ^{15}N isotope using a Flash EA 2000 Series Elemental Analyzer connected via a ConFlo IV to a DeltaV Advantage isotope ratio mass spectrometer (FLASH-EA-DELTA-V, Thermo Finnigan, Waltham, MA) (Ek et al., 2015). Precision of the ^{15}N measurement was $<0.10\%$. The labeled abundance of the ^{15}N isotope is expressed directly using atom % ^{15}N .

2.3.2 Analysis of Ag in body tissues

The animals were digested with 300 μL of a mixture of HNO_3 (65%; ultrapure) and HClO_4 (70%; ultrapure, Nanjing Chemical Reagent Co., Ltd.) (7:1 v/v). Prior to analysis, 1 mL 1 mol L^{-1} HNO_3 was added to dissolve the residue (Zhang and van Gestel, 2017; Li et al., 2020a). Silver concentrations in digest solutions were determined by ICP-MS (7700x, Agilent Technologies, Santa Clara, CA). Certified reference material GBW08551 (pork liver, Institute of Geophysical and Geochemical Exploration, Langfang, Hebei, China) was included for quality control and the Ag recoveries ranged from 87.6% to 108%. The bioaccumulation factors (BAF) of *F. candida* and *H. aculeifer* were calculated using the following formula

(Scheifler et al., 2002): $BAF = C_c/C_f$, where C_c represents the Ag concentration in consumer tissues and C_f the Ag concentration in the food resource. Here, the form and concentration of AgNPs were not assessed during the intake process, thus the results are simply Ag concentrations.

2.4 Statistic analysis

All data are expressed as mean $\pm$ standard error (SE). Probit regression modeling was used to determine the lethal concentration that reduced survival by 50% (LC50) and effective concentration that reduced reproduction and growth by 50% (EC50) at the 5% protection level. Significant differences in different treatments were determined by One-way analysis of variance (ANOVA). Mean values were compared using the Tukey test at the 5% protection level. Statistical analysis was conducted using Microsoft Excel 2016 and the SPSS 21.0 software package (SPSS Inc., Chicago, IL).

3 Results

3.1 Accumulation and transfer of Ag in the food chain

No deaths of *F. candida* and *H. aculeifer* were found in this experiment. The concentrations of Ag in body tissues of *F. candida* and *H. aculeifer* increased significantly with increasing concentration in AgNPs-spiked food ($F_{4, 14} = 122$, $P < 0.001$; $F_{4, 14} = 35.2$, $P < 0.001$ respectively, Fig. 1). The food Ag concentrations (wheat spiked with AgNPs-contaminated *F. candida*) were significantly and positively correlated with the concentrations in the corresponding animals: $Y = 0.0529x + 3.30$, $R^2 = 0.972$ (*F. candida*); $Y = 0.175x + 0.689$, $R^2 = 0.983$ (*H. aculeifer*). In *F. candida* (Fig. 1A) the Ag concentrations in body tissues at 100, 200, 400 and 600 mg AgNPs kg^{-1} treatments were 15.3, 27.1, 41.8, and 54.9 times higher than in the control treatment, respectively, with corresponding values in *H. aculeifer* (Fig. 1B) of 2.93, 3.75, 5.55 and 6.19. *Folsomia candida* had higher internal Ag concentrations than *H. aculeifer* in all AgNPs treatments.

No significant difference was found in BAF values from prey *F. candida* to predator *H. aculeifer*, while the BAF values of Ag from spiked wheat to *F. candida* decreased with increasing AgNPs concentration (Table 1). The BAF value (from wheat to *F. candida*) at 600 mg AgNPs kg^{-1} treatment was 39.8% lower than that at 100 mg kg^{-1} .

3.2 Atom % ^{15}N in different trophic levels

The abundance of ^{15}N (atom % ^{15}N) in *F. candida* body tissues declined significantly with increasing spiked AgNPs concentration in wheat ($F_{4, 14} = 12.7$, $P < 0.001$, Fig. 2). The relationship between abundance of ^{15}N and AgNPs concentration in spiked wheat was negative: $Y = -0.0005x + 1.42$, R^2

Fig. 1 Silver concentrations in body tissues of *F. candida* (A) and *H. aculeifer* (B) on plate dietary exposure test. Results expressed as mean $\pm$ standard error (SE), $n = 3$. Lowercase letters indicate the differences between different treatment means.

$= 0.783$ (*F. candida*), $Y = -0.0002x + 0.656$, $R^2 = 0.806$ (*H. aculeifer*). Atom % ^{15}N values in *F. candida* were generally twice as high as in *H. aculeifer* and declined more than in *H. aculeifer*. The atom % ^{15}N values of *F. candida* in the control were 1.10, 1.19, 1.20 and 1.25 times higher than AgNPs at 100, 200, 400 and 600 mg kg^{-1} , respectively. Significant differences in atom % ^{15}N of *H. aculeifer* bodies were found only at 600 mg AgNPs kg^{-1} , 15.9% lower than the control.

3.3 Toxic effects of Ag₂S on *Folsomia candida*

In the single species test, both survival (the number of adults) and reproduction (the number of juveniles) rates of *F. candida* decreased dramatically with increasing Ag₂S concentration in

Fig. 2 Atom % ^{15}N in body tissues of *F. candida* and *H. aculeifer* on plate dietary exposure test. Results expressed as mean $\pm$ standard error (SE), $n = 3$. Lowercase letters indicate the differences between different treatment means.

sludge-treated soils ($F_{5, 23} = 5.34$, $P < 0.005$; $F_{5, 23} = 17.3$, $P < 0.001$, respectively, Fig. 3). The adult and juvenile numbers of *F. candida* showed negative relationships with the Ag_2S concentration in sludge-treated soils: $Y = -0.0045x + 9.78$ (adult), $R^2 = 0.945$, $Y = -0.156x + 243$, $R^2 = 0.921$ (juvenile), respectively. The number of adults started to decrease at 400 mg Ag_2S kg⁻¹ (by 17.9%) compared with that at 0 mg kg⁻¹. A decreasing trend in juveniles occurred early at 100 mg kg⁻¹ treatment, decreasing by 16.7% compared with 0 mg kg⁻¹. The LC50 and EC50 values of Ag_2S on *F. candida* were 831 (697–1078) and 667 (430–46738) mg kg⁻¹. No significant differences in the number of adults and juveniles were found in control and 0 mg kg⁻¹ Ag_2S treatments.

The mean body lengths of both adults and juveniles of *F. candida* in Ag_2S -spiked sludge-treated treatments showed no significant difference with increasing Ag concentration ($F_{5, 23} = 0.53$, $P > 0.05$; $F_{5, 23} = 1.27$, $P > 0.05$ respectively, Fig. 4). The body length of juveniles in the control was significantly lower than that in the 0 Ag_2S mg kg⁻¹ treatment, only 83.9% of the latter ($F_{5, 23} = 6.23$, $P < 0.005$).

Internal Ag concentrations in body tissues of *F. candida* adults and juveniles increased greatly with increasing Ag concentration in sludge-treated soils ($F_{5, 23} = 11.5$, $P < 0.001$; $F_{5, 23} = 15.4$, $P < 0.001$ respectively, Fig. 5). The relationship between Ag concentrations of adult and juvenile *F. candida* bodies and Ag_2S concentrations in sludge-treated soils was positive: $Y = 0.002x + 0.422$, $R^2 = 0.868$ (adult), $Y = 0.0024x + 0.285$, $R^2 = 0.903$ (juveniles), respectively. In the 600 mg Ag_2S kg⁻¹ treatment the internal Ag concentrations in adult and juvenile *F. candida* were 8.35 and 10.3 times higher than in the control.

Fig. 3 Number of adults (A) and juveniles (B) in spiked soil after 28 d of exposure. Results expressed as mean $\pm$ standard error (SE), $n = 4$. Lowercase letters indicate the differences between different treatment means.

4 Discussion

4.1 Bioaccumulation and trophic transfer of Ag under AgNPs exposure

Multiple species studies are commonly used in higher tier tests for pollutant assessment. These multiple-species integrative tests consider fluctuations within populations and interactions between populations (Hund-Rinke and Kördel, 2003). The internal Ag concentrations in *F. candida* and *H. aculeifer* increased with increasing AgNPs in the spiked wheat, indicating that Ag could be transferred through the trophic relationship, with the AgNPs-spiked wheat ingested by *F. candida* and then transferred to the predator *H. aculeifer*, although it remains unclear which forms of Ag (Ag nanoparticles and/or dissolved Ag ions) were ingested by the two arthropods. The dissolution and release of toxic Ag^+ ions and the physical interaction of nanoparticles make the toxicity of

Fig. 4 Body length of adults and juveniles in spiked soil after 28 d of exposure. Results expressed as mean $\pm$ standard error (SE), $n = 4$. Lowercase letters indicate the differences between different treatment means.

Fig. 5 Silver concentrations in adult and juvenile body tissues in spiked soil after 28 d of exposure. Results expressed as mean $\pm$ standard error (SE), $n = 4$. Lowercase letters indicate the differences between different treatment means.

AgNPs complex. Sillapawattana et al. (2016) found that the released free silver ions and the formation of reactive species were the main causes of AgNPs toxicity in *F. candida*. A toxicity study of ZnO nanoparticles on collembolans in soil systems confirmed that Zn particles, ZnO nanoparticles, and free Zn ions all contributed to uptake and toxicity (Tourinho et al., 2013). Comparative studies on the toxicity of AgNPs and AgNO₃ indicated that the toxicity of AgNO₃ on the survival and reproduction of *F. candida* was higher than that of AgNPs, but higher internal Ag concentrations were found for AgNPs than AgNO₃ (Waalewijn-Kool et al., 2014; Mendes et al., 2015).

Schlich et al. (2013) reported similar results with a higher Ag concentration in the earthworm *Eisenia andrei* under AgNPs exposure than AgNO₃ in soil at spiked concentration of 200 mg kg⁻¹. These results indicate that nanoparticles are available to soil collembolans. Soil animals have different response patterns to AgNO₃ and AgNPs. At the cellular level, glutathione reductase, total glutathione and metallothionein showed delayed responses to AgNPs compared to AgNO₃, suggesting that AgNPs had a lower Ag⁺ release rate or a lower rate of Ag uptake. Different patterns of catalase and glutathione S-transferase indicate a specific nanoparticle effect (Mendes et al., 2015). A disorder of antioxidant systems may cause some disorders of their metabolism. Here, the BAF values of Ag (from spiked wheat to *F. candida*) decreased with increasing AgNPs in spiked wheat. The collembolans may respond by reducing their intake of Ag-contaminated food or by accelerating Ag excretion to regulate their metabolism.

Hypoaspis aculeifer is the only soil-living predator among standard organisms (OECD, 2008) but to date studies are limited of its sensitivity and applicability in the assessment of a broader range of chemicals, especially studies on its bioaccumulation of pollutants (Huguier et al., 2015). Bioaccumulation is a sensitive indication parameter that can reflect the risk of pollutants to higher trophic levels (Ardestani et al., 2014). In this experiment, feeding *F. candida* was the only way for *H. aculeifer* to ingest Ag and its capture efficiency is highly dependent on the velocity and rate of encounter with *F. candida* (Baatrup et al., 2005). Contaminated *F. candida* and/or weak *F. candida* (nutrient deficiency by avoidance of contaminated wheat) may restrict its mobility and facilitate predation by *H. aculeifer*. The BAF values (from *F. candida* to *H. aculeifer*) showed no significant difference in this experiment and were all < 1 (Table 1). The internal metal concentrations were significantly lower in *H. aculeifer* than in *F. candida*, possibly due to dilution during transfer or the pollution effects were not significant. No bioenrichment effects were manifest and too little Ag was transferred from *F. candida* to *H. aculeifer* to produce higher toxic effects to higher trophic levels. No deaths of *H. aculeifer* were found here and generally the secondary risk of AgNPs on its survival was not significant.

4.2 Effects of AgNPs on trophic transfer of nitrogen

A soil food net is essential for the transfer of substances and energy and N is an important major nutrient element whose transfer can directly reflect changes in the functioning of food webs. ¹⁵N content is now considered to be a promising bioindicator of the influence of pollution on the biogeochemical cycles of nutrient elements and the provision of soil ecosystem functions due to the important relationship between concentrations of contaminant in consumers and their ¹⁵N values (Ek et al., 2015). Here the relationship between ¹⁵N and AgNPs concentrations in spiked wheat was significantly negative. Considering the feeding habits of *F. candida* and *H. aculeifer*, the only labeled N resources available to them were spiked wheat and *F. candida*,

Table 1 BAF values for the transfer of Ag to *F. candida* and *H. aculeifer*.

Exposure treatment	BAF	
	<i>F. candida</i>	<i>H. aculeifer</i>
AgNPs 100	0.093 ^a	0.23 ^a
AgNPs 200	0.083 ^{ab}	0.23 ^a
AgNPs 400	0.063 ^{bc}	0.22 ^a
AgNPs 600	0.056 ^c	0.19 ^a

Note: Different letters in the upper right corner indicate the differences between the mean values of different treatments.

respectively. Silver (although it is not clear in which form) was transferred from *F. candida* to *H. aculeifer* and decreased the N transfer through them at higher concentrations. Collembolans have selective feeding which may avoid polluted food or strengthen their detoxification system to discharge pollutants (Fountain and Hopkin, 2005). A previous study found that the Collembola may alter their excretion rates for detoxification thus affecting stable isotope fractionation (Staadén et al., 2010). Consequently, the inter ^{15}N of *F. candida* decreased, as did that in *H. aculeifer*.

4.3 Toxic effects of Ag_2S on *F. candida*

Soil organisms have different contact models with trace metals in soils. For annelid worms such as earthworms and enchytraeids they may be through oral and dermal routes; arthropods such as collembolans may make contact through ventral tubes and oral routes (Lock and Janssen, 2003; Fountain and Hopkin, 2005). In the case of most trace metals, dissolved or free ions in pore water are the main uptake source and are toxic to collembolans through the ventral tubes. However, results for some undissolved pollutants and several food-exposure tests all indicate that oral uptake is also an important additional route of exposure in collembolans (Lock and Janssen, 2003; Ju et al., 2019; Li et al., 2021). Generally, Ag_2S in soils will not readily dissolve in pore water and soil ingestion seems to be the main route of Ag_2S exposure to collembolans. Adverse effects may be due to the dissolution of Ag_2S particles in the collembolan gut. Here, Ag_2S had significant toxic effects on the numbers of adults and juveniles of *F. candida*. The decline in reproduction may be due to energy diversion to self-detoxification processes (Hopkin, 1990). No significant difference was found in the body length, indicating that body length was not sensitive to Ag toxicity. Li et al. (2020b) reported similar results with body length not sensitive in contrast to survival and reproduction in a field soil pollution assessment. Adverse impacts of Ag_2S were also observed in soil microbial communities, terrestrial soil isopods and the provision of ecosystem services over longer exposure times or at higher concentrations (Kraas et al., 2017; Kampe et al., 2018; Schlich et al., 2018). Given the Ag concentrations in natural field soils (Kramer et al., 2002), the risk of Ag_2S seems quite low. However, our study was a

simulated amplification test to demonstrate the ecotoxicity of AgNPs transformation and fate in soils, and the long-term effects of AgNPs and their fate in soils require further study.

5 Conclusions

This study has demonstrated the potential risk of accumulation and transfer of Ag under AgNPs exposure and has used quantitative data via tagging N isotope tracer techniques to reveal the pollution effects of AgNPs on the trophic transfer of N through a soil animal food chain (*F. candida*–*H. aculeifer*). Silver under AgNPs exposure was transferred and accumulated from *F. candida* to *H. aculeifer* and disturbed the transfer of the major nutrient N, but no biomagnification effect was found using the Ag BAF values. Silver sulfide had adverse effects on the numbers of adult and juvenile *F. candida* and the inter Ag concentration in animal bodies increased as Ag_2S increased in sludge-treated soils. The ecotoxicity of the transformation and fate of AgNPs in soils should therefore be a matter of public concern.

Acknowledgment

This research was funded by the National Natural Science Foundation of China (41977136).

Electronic supplementary material

Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s42832-021-0125-z> and is accessible for authorized users.

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