



Transfer of sulfidized silver from silver nanoparticles, in sewage sludge, to plants and primary consumers in agricultural soil environment

Pauline Courtois, Annette de Vaufleury, Anna Grosser, Christine Lors, Franck Vandebulcke

► To cite this version:

Pauline Courtois, Annette de Vaufleury, Anna Grosser, Christine Lors, Franck Vandebulcke. Transfer of sulfidized silver from silver nanoparticles, in sewage sludge, to plants and primary consumers in agricultural soil environment. *Science of the Total Environment*, 2021, 777, pp.145900. 10.1016/j.scitotenv.2021.145900 . hal-03499742

HAL Id: hal-03499742

<https://univ-fcomte.hal.science/hal-03499742>

Submitted on 10 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Transfer of sulfidized silver from silver nanoparticles, in sewage sludge, to plants
and primary consumers in agricultural soil environment**

Pauline Courtois^{1*}, Annette de Vaufleury², Anna Grosser³, Christine Lors¹, and Franck Vandebulcke¹

¹ Univ. Lille, IMT Lille Douai, Univ. Artois, Yncrea Hauts-de-France, ULR 4515 - LGCgE, Laboratoire de Génie Civil et géo-Environnement, F-59000 Lille, France

² University of Bourgogne Franche-Comté, Department Chrono-environnement, UMR UFC/CNRS 6249, 16 route de Gray, Besançon Cedex, 25030, France

³ Częstochowa University of Technology, Faculty of Infrastructure and Environment, Częstochowa, Poland

*Address correspondence to: Pauline COURTOIS

e-mail: pauline.courtois@univ-lille.fr

Université de Lille, Sciences et Technologies

Laboratoire de Génie Civil et géo-Environnement, LGCgE EA4515

Cité Scientifique, Bât. SN3 – 59655 Villeneuve d'Ascq - France

**Transfer of sulfidized silver from silver nanoparticles, in sewage sludge, to plants
and primary consumers in agricultural soil environment**

Pauline Courtois^{1*}, Annette de Vaufleury², Anna Grosser³, Christine Lors¹, and Franck Vandembulcke¹

¹ Univ. Lille, IMT Lille Douai, Univ. Artois, Yncrea Hauts-de-France, ULR 4515 - LGCgE, Laboratoire de Génie Civil et géo-Environnement, F-59000 Lille, France

² University of Bourgogne Franche-Comté, Department Chrono-environnement, UMR UFC/CNRS 6249, 16 route de Gray, Besançon Cedex, 25030, France

³ Częstochowa University of Technology, Faculty of Infrastructure and Environment, Częstochowa, Poland

*Address correspondence to: Pauline COURTOIS

e-mail: pauline.courtois@univ-lille.fr

Université de Lille, Sciences et Technologies

Laboratoire de Génie Civil et géo-Environnement, LGCgE EA4515

Cité Scientifique, Bât. SN3 – 59655 Villeneuve d'Ascq - France

Abstract

Consumer products containing silver nanoparticles (AgNPs) release silver (Ag) to the environment, particularly wastewater. Sewage sludge (SS), which contains numerous contaminants including Ag, is recycled by spreading on agricultural land. Although slight impacts and bioaccumulation of Ag sulfide (Ag₂S, the main species found in SS) in terrestrial organisms have been demonstrated, possible trophic transfer into plants and subsequently animal species has not been examined. Accordingly, the present study experimentally measured the transfer of Ag from AgNPs and sulfidized Ag into plants and primary consumers and compared their bioavailability. Nine plant cultivars were grown in soil mixed with SS containing Ag, which revealed that bioaccumulation of Ag by plants is species-dependent. Ryegrass (the plant species with the greatest accumulation – up to 0.2 mg kg⁻¹) was then cultivated on a larger scale to expose snails and locusts for several weeks. While locusts did not accumulate Ag after two weeks of exposure, snails exhibited Ag bioaccumulation after 5 weeks when soil was accessible. Sulfidized Ag derived from AgNPs were less available (bioaccumulation up to 2.5 mg kg⁻¹) than the Ag from the original AgNPs (bioaccumulation up to 15 mg kg⁻¹). This transfer potential of Ag could have consequences for food webs due to chronic exposure linked to SS spreading practices. This study shows that transformations of AgNPs in treatment plants attenuate but do not completely eliminate the risk of Ag to plant and animal species SS.

Keywords

Sulfidized silver, ryegrass, snails, locusts, food chain, bioaccumulation

1. Introduction

The various properties of silver nanoparticles (AgNPs) most notably biocidal action (Reidy et al., 2013), have led to their widespread use in the pharmaceutical, cosmetic, textile, electronics and optics industries, among many other applications (European commission, 2012, 2014; McGillicuddy et al., 2017). Throughout their life cycles, nanofunctionalized consumer products containing AgNPs release Ag into the environment and especially wastewater (Adam et al., 2018; Donner et al., 2015). Silver (Ag) is mostly trapped in biosolids thanks to the efficiency of wastewater treatment methods (Kaegi et al., 2011; Tiede et al., 2010). However, these biosolids, which contain between 1 and 20 mg kg⁻¹ of silver (Ag) on average (depending on the source: Gottschalk et al., 2013; U.S. EPA, 2009) and maximum concentrations up to 850 mg kg⁻¹, are often spread on agricultural fields as fertilizer (European commission, 2017; Eurostat, 2017). According to a scenario analyzed by Blaser et al. (2008) an estimated 140 tons of Ag enter agricultural soils each year due to the spreading of sewage sludge (SS) in the European Union alone.

In response to these practices, the toxicity and ecotoxicity of Ag have been widely studied. Most of these works have studied Ag ions or intact AgNPs but it is now known that AgNPs released in wastewater undergo transformations in sewers and during the biosolid treatment (Levard et al., 2012; Ma et al., 2014; Pradas del Real et al., 2016). Indeed, AgNPs and Ag⁺ ions can react with sulfur to give silver sulfide (Ag₂S) (Sekine et al., 2015). It is precisely Ag₂S that is the predominant Ag species in SS, which has different properties than AgNPs or Ag⁺ (Levard et al., 2013). Another previous study showed that the speciation of Ag after anaerobic digestion was Ag₂S and Ag linked to thiols, that is to say two sulfidized forms of silver (Courtois et al., 2020). These Ag species have received less attention, and studies using SS containing realistic amounts of sulfidized Ag are scarce (Courtois et al., 2019).

Under simplified conditions, Ag can be bioaccumulated by plants (Geisler-Lee et al., 2012, 2014; Kaveh et al., 2013; Nair and Chung, 2014; Rui et al., 2017; Song et al., 2013; Stampoulis et al., 2009; Yasur and Rani, 2013) and animal species like nematodes (Luo et al., 2016) and earthworms (Baccaro et al., 2018; Diez-Ortiz et al., 2015). Studies performed under more realistic conditions have also shown an Ag accumulation in species like woodlouse (Kampe et al., 2018) or earthworms (Lahive et al., 2017; Velicogna et al., 2017). Studies under realistic conditions have also reported that the accumulation of Ag from sulfidized Ag in plants was lower than that of Ag from AgNPs but was still significant (Doolette et al., 2015; Pradas del Real et al., 2016, 2017; Stegemeier et al., 2015; Wang et al., 2015). These preliminary results are worrying and suggest that Ag could transfer up the food chain and reach a wide range of animal species.

For the first time, the bioavailability and the toxicity of various forms of Ag to a plant species and 2 soil invertebrates will be compared in a trophic chain experiment in order to assess the transfer of sulfidized Ag (Ag₂S and Ag linked to thiols) from soil to plants and two complementary primary consumers. Small herbivorous invertebrates that are staple foods for many small carnivores and omnivores, including humans : the terrestrial snail *Cantareus aspersus* and the locust *Locusta migratoria* (Barker and Watts, 2002; Poma et al., 2017). These two species are complementary: one is a soft body invertebrate in close contact with soil, whereas the other has a cuticle and spends most of its life on plant cover. During this experiment, the Ag content in soil, plants and animals was quantified and vital parameters such as biomass and survival were monitored.

2. Materials and methods

2.1 Soil

Soil from organic agriculture was collected from the Haut de France region (GPS coordinates: 50°35'58.1"N 3°09'01.5"E) in January 2019 for the preliminary experiment and in September 2019 for the main experiment. The soil is a slightly leached to calcareous (presence of chalk granules) brown soil developed on wind-blown silts on chalky substrate, shallow to deep (soil map by DRAAF, 2013) classified as Luvisol (LV) according to WRB (World Reference Base) (Food and Agriculture Organization of the United Nations, 2015). For both experiments, the first 20 cm of soil was collected a few days before the beginning of the experiment. The soil was not dried in order to preserve the natural microbiota. To remove stones and roots and to homogenize and aerate the soil, the soil was sieved wet with a 5 mm mesh. The pH was and approximately 6.76 ± 0.01 (measured in KCl 1M according to ISO 10390:2005) and the total carbon and the dissolved organic carbon were 15.44 ± 1.34 mg g⁻¹ and 27.31 ± 1.34 mg L⁻¹. The characterized metal content of the dry soil was: 0.53 ± 0.34 mg kg⁻¹ Ag, 0.32 ± 0.06 mg kg⁻¹ cadmium (Cd), 18.68 ± 0.36 mg kg⁻¹ copper (Cu), 336.18 ± 9.83 mg kg⁻¹ manganese (Mn), 11.45 ± 0.17 mg kg⁻¹ nickel (Ni), 52.73 ± 1.61 mg kg⁻¹ lead (Pb), 49.78 ± 0.40 mg kg⁻¹ zinc (Zn) (method described in 2.7.2).

2.2 Sewage sludge

Four wastewater treatment plants (WWTPs) situated in southern Poland were pre-selected in order to monitor the content of Ag during a two-year period. Among the four facilities, the facility that produced SS with the lowest level of contamination in metals (silver, arsenic, cadmium, copper, manganese, nickel, lead) and a preferable C:N ratio was selected. According to a previous study, SS from the selected WWTP is a good source of nutrients for earthworms without inducing stress related to the presence of contaminants (Suleiman et al., 2017). The selected WWTP (Poland, GPS coordinates: 50°55'22.81 19°07'10.41) is a small-sized plant that uses activated sludge technology to support an

agricultural area (flow: 1,000 m³/d, population equivalents: 20,000). The metal content of the dry SS was as follows: 11.53 ± 1.43 mg kg⁻¹ Ag, 0.95 ± 0.22 mg kg⁻¹ arsenic (As), 1.10 ± 0.24 mg kg⁻¹ Cd, 140.62 ± 22.55 mg kg⁻¹ chromium (Cr), 145.43 ± 37.38 mg kg⁻¹ Cu, 186.78 ± 59.13 mg kg⁻¹ Mn, 19.11 ± 9.02 mg kg⁻¹ Ni, 32.21 ± 5.86 mg kg⁻¹ Pb, and 2510.36 ± 615.99 mg kg⁻¹ Zn (average based on measurements between March and December 2016, according to the method described in 2.7.2.). Before agricultural reuse, anaerobic stabilization of the SS was performed at laboratory scale.

2.3 Silver species

The AgNPs used for this study were the standard reference materials Ag NM300K from the European Commission Joint Research Centre (JRC), which have been fully characterized (Klein et al., 2011). These nanoparticles are spherical and are formulated as a colloidal dispersion with a nominal Ag content of 10.2% by weight, dispersed in 4% w/w each of polyoxyethylene glycerol trioleate and polyoxyethylene (20) sorbitan mono-laurate (Tween 20). The Tween 20, the dispersant solution of AgNPs, will be called “Dispersant” or “Dis” throughout the rest of the manuscript. The nominal size of 99% of the particles is approximately 15 nm, without coating. Transmission electron microscopy indicated a size of 17 ± 8 nm. Smaller nanoparticles of approximately 5 nm are also present (Mendes et al., 2015). The size distribution determinations by using zeta-sizer analysis resulted in about 100 nm (Klein et al., 2011). The NM300K nanoparticles were kindly provided by the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Schmallenberg, Germany). Each bottle (lot) contained approximately 2 g of NM300K diluted in dispersant corresponding to a volume of 2 mL. This solution contained 10% (w/w) Ag (0.1 g of Ag per 1 mL).

A solution of AgNO₃ was prepared by dissolving AgNO₃ powder in milliQ water, taking into account the molar mass of each atom so that the concentration obtained corresponds well to a concentration of Ag and not of AgNO₃.

These two solutions of nanoparticles and silver ions were diluted with milliQ water to obtain Ag concentrations of approximately 2 mg ml⁻¹.

2.4 Plant species

2.4.1 Preliminary experiment

Nine plant cultivars (of which two cultivars were the same species) were used in this study: turnip, cabbage, hemp, lettuce, ryegrass cultivar 1 (calao variety), ryegrass cultivar 2 (bocage variety), radish, Dutch clover and wheat. These species were chosen because they are consumed by humans or livestock, and can either be cultivated directly after sewage sludge spreading, or after a rotation of land use where the spreading of sewage sludge has taken place previous years, either are conventionally studied in the laboratory. *Brassica napus* (Emerald variety, reference 9273), *Brassica oleracea* (Proteor variety, ref. 4890), *Lactuca sativa* (Pierre Benite variety, ref. 5775) and *Raphanus sativus* ("18 days" variety, ref. 7190) seeds were obtained from Baumaux Seeds Company (<https://www.graines-baumaux.fr/>). These seeds were untreated for conventional cultivation. *Cannabis sativa* (Futura 75 variety) seeds were obtained from HEMP it (<https://hemp-it.coop/>). *Lolium perenne* variety calao seeds were from Semences de France (<https://www.semencesdefrance.com/>). *Lolium perenne* variety bocage and *Trifolium repens* seeds were from Carneau (<http://carneau.fr/>). *Triticum aestivum* L. (L., KWS Mistral variety) seeds were from Florimond Desprez (<https://www.florimond-desprez.com/>).

2.4.2 Main experiment

For this experiment, ryegrass cultivar 2 (*Lolium perenne*, bocage variety) was selected as the plant species, as explained in the results. The seeds used in the main experiment were from the same batch used for the preliminary experiment.

2.5 Invertebrate species for the main experiment

Two species at the bottom of many food chains and widely consumed by humans in several regions of the world were studied (Barker and Watts, 2002; Poma et al., 2017). The first tested species was *Cantareus aspersus* (ex *Helix aspersa*), a phytophagous and detritivorous pulmonate gastropod mollusk, known to accumulate some metallic trace elements and is even used as an indicator of habitat contamination (Baroudi et al., 2020; Beeby, 1985; Carbone and Faggio, 2019; De Vaufleury, 2015; Hopkin, 1989). This species is native to the peri-Mediterranean area and occupies a wide range of habitats worldwide (ISO 15952:2018). The snails used in this study were from the farm of laboratory Chono-Environment (University of Franche-Comté, Besançon, France) and were raised as described by Gomot de Vaufleury (2000). Juvenile individuals weighing 995.7 ± 214.6 mg ($n = 369$) were used. The second tested species was *Locusta migratoria*, a phytophagous orthopteric arthropod found almost everywhere in the world (Jarwar et al., 2019; Zhang et al., 2019), which has been less studied in this context. Few studies have shown the capacity of this member of Orthoptera to accumulate Cd and Pb (Devkota and Schmidt, 2000; Zhang et al., 2012). Sub-adult locusts were purchased online from La ferme aux insectes (Gng distribution sarl, Crevecoeur le Grand, France) (<https://www.lafermeauxinsectes.com/fr/>). Upon delivery, individuals were housed under observation and depuration for 12 h before installation in the mesocosms, that is, 21 days after the addition of snails in the mesocosms, and 7 weeks after plant seeding. The average weight of the locusts was 646.6 ± 84.0 mg ($n = 194$). These two

species are complementary: one is a soft body invertebrate in close contact with soil, whereas the other has a cuticle and spends most of its life on plant cover. The metal contents of both species were measured at the beginning of the mesocosm experiment (Table 1).

Table 1: Metals contents in animals (whole locusts and snails without shell) at the initial time point (after fasting) in mg kg⁻¹ (dry matter). <LOQ means under limit of quantification i.e. <0.4 µg L⁻¹ for Ag, <1,9 µg L⁻¹ for Pb and < 6,7 µg L⁻¹ for Sb.

	Ag	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Zn
<i>C. aspersus</i>	<LO Q	0.828 ± 0.129	0.760 ± 0.015	1.100 ± 0.084	1.828 ± 0.136	152.8 36 ± 42.23 1	0.058 ± 0.100	92.20 8 ± 14.06 6	0.640 ± 0.022	1.924 ± 0.042	<LO Q	0.013 ± 0.022	310.9 78 ± 37.82 8
<i>L. migratoria</i>	<LO Q	0.825 ± 0.122	0.045 ± 0.041	0.043 ± 0.038	1.622 ± 1.540	44.00 1 ± 2.573	0.024 ± 0.042	6.688 ± 1.600	1.357 ± 0.865	1.116 ± 0.865	0.128 ± 0.201	<LO Q	130.0 64 ± 10.12 7

2.6. Experimental scheme of the two-step mesocosm experiment

2.6.1. Anaerobic digestion of sewage sludge

A batch anaerobic digestion (AD) of SS was performed in four continuous stirred-tank bioreactors at the beginning of 2017. These bioreactors were glass vats filled with 6 L of SS maintained under mesophilic conditions at a temperature of 37 °C with constant mixing (180 rpm) using a mechanical stirrer. Details of the equipment as well as the methods of analysis of pH, volatile fatty acids, volatile solids, total solids and ammonium nitrogen were described previously (Grosser, 2017).

In one bioreactor, 6 L of SS was digested without any addition. In a second bioreactor, the SS was spiked with 240 mg of AgNPs NM300K. In a third bioreactor, the SS was spiked with the corresponding quantity of dispersant used to suspend the AgNPs, and in a fourth bioreactor, the SS was spiked with 240 mg of AgNO₃. The chosen Ag concentration (40 mg L⁻¹) in the bioreactors is justified in Supplemental information 1.

After 31 days of AD, the process had stabilized, the bioreactors were stopped, and the 4 digestates were collected and centrifuged at 12100 rcf for 15 minutes. The SS was frozen until use and was thawed slowly at 8 °C for 3 days before use.

Tiede et al. (2010) and Kaegi et al. (2011) showed that SS treatment traps approximately 90% of Ag in biosolids. In our study, approximately 216 mg of Ag (of the 240 mg initially added) should have remained in the SS. After AD, each bioreactor contained approximately 950 g of fresh SS. The Ag concentration in the fresh SS was therefore estimated to be approximately 227 mg kg⁻¹ (calculation: $[(0.9 \times 240) \times 1000] / 950$).

2.6.2. *Experimental design of preliminary exposure*

The preliminary experiment consisted of Ag exposure of several plant species in order to observe the accumulation behaviors in each plant.

The 9 plant cultivars (of which two cultivars were the same species) were grown in a mixture of the agricultural soil and SS digested with AgNO₃. The mixture contained 19.9 kg of agricultural fresh soil (water content: 18.3 %) and 1.27 kg of thawed SS (water content 76.7 %) (i.e. 60 g of SS per kg of final mixture, justification in Sup. Inf. 2). The mixture was stirred frequently each day for 7 days and sifted immediately before distribution into boxes to ensure a relatively powdery soil facilitating seed germination. AgNO₃, like AgNPs, can react with sulfur and give Ag₂S and Ag linked to thiols molecules. In a previous experiment, we were able to verify that the Ag⁺ ions added to sewage sludge did indeed transform into Ag₂S and Ag linked to thiols molecules during anaerobic fermentation (Courtois et al., 2020).

Each plant cultivar was sown in 3 boxes to establish triplicates. Each box (17 cm x 13 cm x 5 cm) contained 740 g of fresh mixture. An identical number of seeds was sown in each of the 3 replicate boxes, but this number varied according to the characteristics of the species, specifically, plant size and acceptable plant density (Sup. Inf 3).

The plants were grown for 49 days in greenhouses (temperature maintained at 20 °C, with 16 h of light and 8 h of darkness). Watering varied according to the needs of the boxes but the triplicates of one plant cultivar received the same quantity of water.

2.6.3. Experimental design of main exposure

Lolium perenne cv. *bocage* seeds were sown in 6 different treatments (Figure 1):

- (a) 3 without SS: soil only (mesocosms named “Control”), soil soaked with dispersant (“Dis”), and soil soaked with AgNPs dispersed in dispersant (“AgNPs”), and
- (b) 3 with digested SS: SS only (“AD-control”), SS with dispersant (“AD-dis”), and SS with AgNPs (“AD-AgNPs”).

The proportions of SS and soil were identical to the preliminary exposure (60 g of fresh SS for 1 kg of mixture). The mixtures with SS contained 12.847 kg of agricultural wet soil and 820 g of thawed SS. The mixtures without SS contained 13.572 kg of agricultural wet soil and 95 mL of solution (AgNPs solution, dispersant solution or milli Q water). Thus, the amount of dispersant or AgNPs was the same in the conditions with and without SS.

The mixtures were stirred frequently each day for 7 days and then sifted immediately before distribution in boxes to ensure a relatively powdery soil facilitating seed germination. These 6 types of mixtures were used to prepare 6 mesocosms containing exactly 2.000 kg of fresh mixture (rectangle plastic boxes of 29 cm x 14 cm x 10 cm). The use of larger boxes in a much sunnier period (for natural light) was intended to avoid these two potential sources of stress observed during the preliminary experiment (see the results 3.1.).

In half of the replicates, cages allowed the animals access to soil and plants. In the other half of the replicates, a net positioned 3-4 cm above the soil limited the animals’ access to plants only.

257 An identical mass of seeds was placed in each box (1.500 ± 0.001 g of seeds per box). The
258 plants were grown for 28 days in greenhouses (temperature maintained at 20 °C with 16 h of
259 light and 8 h of darkness and approximately 50% air humidity). Daily watering with rainwater
260 was identical for all boxes.

261 After these 28 days of growth, 10 snails were added to each mesocosm. After 48 days of plant
262 growth (20 days after adding snails), 5 locusts were added to each mesocosm. The addition of
263 locusts to the mesocosms was purposely shifted in time to ensure that the snails were exposed
264 for a sufficient time: we expected that the locusts, which are known to be voracious, would
265 quickly consume the vegetation. The experiment was stopped when the quantity of plants
266 became too small to continue. In total, the snails were exposed to these conditions for 34 days
267 and cohabitated with the locusts for the last 14 of these 34 days. In each mesocosm, a small
268 opaque dark plastic flowerpot cut in half served as a shelter for the animals.

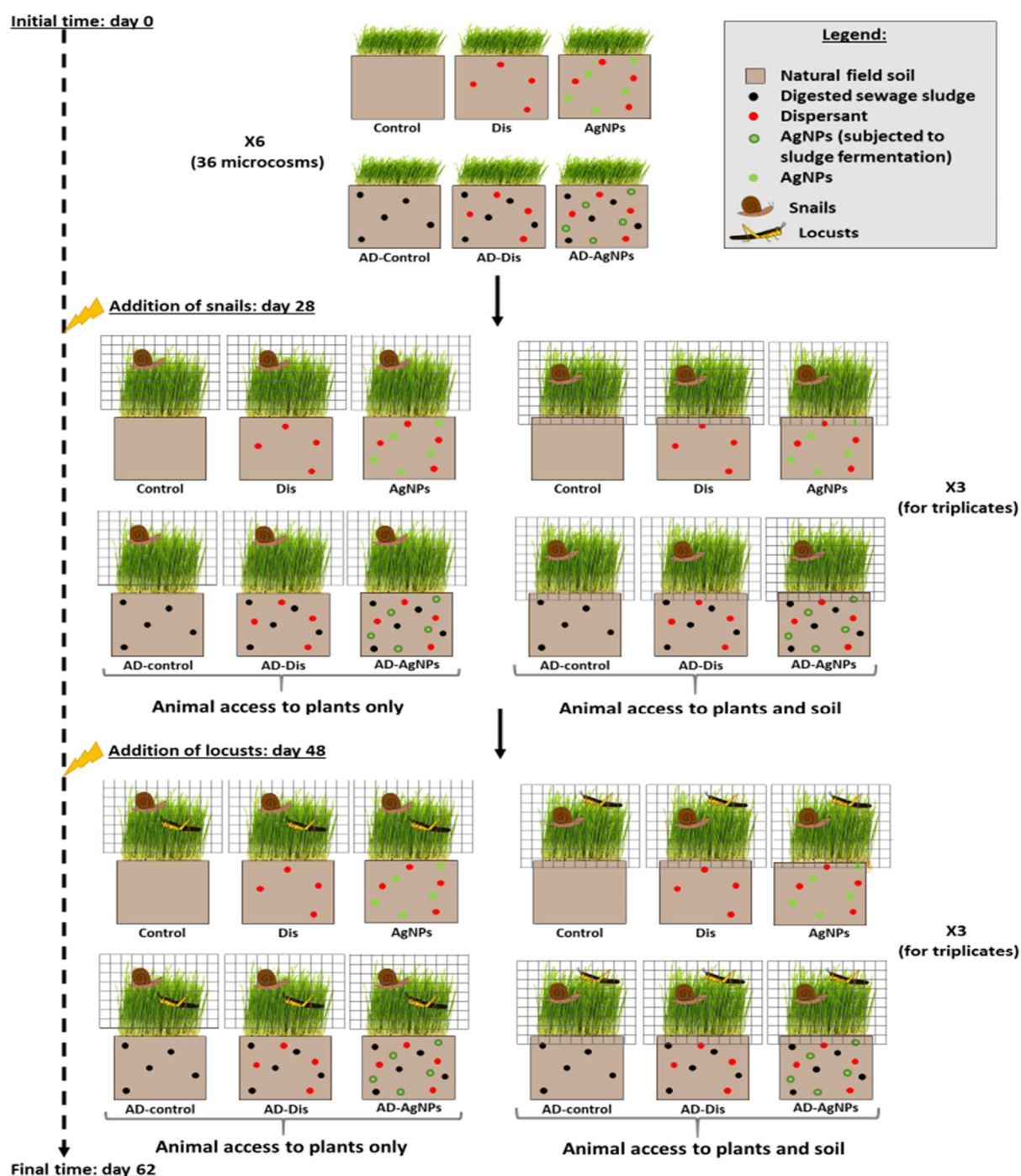


Figure 1: Scheme of the main experimental design. The “Control”, “Dis” and “AgNPs” conditions correspond to soil only, soil mixed with dispersant solution or soil mixed with AgNPs NM300K solution, respectively. The “AD-control” condition corresponds to soil mixed with sewage sludge digested without any added Ag. The “AD-dis” and “AD-AgNPs” conditions correspond to soil mixed with sewage sludge supplemented with either a dispersant solution or a solution of AgNPs NM300K before anaerobic digestion.

2.7. Analyses

2.7.1 *Life traits of plants and animals*

The appearance and size of the plant leaves were analyzed only visually, throughout the two experiments.

For animals, the following life traits were measured in the main experiment:

- The survival of the snails and locusts was observed by counting.

- Groups of 10 snails or 5 locusts were weighed before and after exposure, just after initial or final fasting. Before and after exposure, the animals were fasted 24 h in empty clean containers without food to empty the gut. Evolution of biomass (measured just after fasting) was evaluated by calculating the percentage of weight lost/gained by the group in one mesocosm.

- During the final fasting, feces of snails and locusts were collected to provide an estimate of food consumption. Feces were dried for 48 h in an oven at 60 °C and weighed. The quantity of feces produced during fasting was divided by the number of animals present in the box at the end of the experiment (which varied depending on mortality in the mesocosms). This parameter reflects the consumption (plant and/or soil) of the animals a few hours/days before the end of exposure.

2.7.2 *Metal content in sewage sludge, soil, plants and invertebrates.*

For both experiments (preliminary and main), soil samples were collected at the beginning of exposure and dried at 80 °C until total dehydration (stabilization of the weight). The dried samples were ground with a mortar and pestle.

Leaves of plants were also analyzed in both experiments. For the preliminary experiment, 3 samples of leaves of each species were collected at three time points: day 21 (T1), day 35 (T2) and day 49 (T3). For the main experiment, leaves were collected at 28 days (initial time

for snails) and at 62 days (final time) of exposure. The leaves were rinsed several times with milli Q water, wiped, dried in an oven at 40 °C until total dehydration and crushed. Invertebrates were analyzed only in the main experiment. At the beginning of the experiment, several animals were frozen at -80 °C just after the initial fasting. At the final time point, when the number of survivors allowed, 6 snails and 3 locusts per mesocosm were frozen at -80 °C (just after final fasting). The snails were removed from their shell, and then whole locusts and snails (without shells) were dried separately in an oven at 60 °C until reaching a constant weight and ground to a powder using a mortar and pestle. In addition, for 1-3 snails of each Ag condition (number depending on mortality), the shell, foot and visceral mass were separated after thawing according to the method of Gomot de Vaufléury and Pihan (2002). Some studies have shown that the digestive gland is the main site for storing metals in snails (Hopkin, 1989; Pauget et al., 2015). The viscera and foot were separately dried and ground to powder in the same way as the intact bodies. No replicates could be made for this last measurement. As little data is available in snails for the bioaccumulation and distribution of Ag between the foot and the visceral mass, these two parts were analyzed separately. It was a first attempt to see if Ag uptake and subsequent distribution in snail tissues varied depending on the sources of exposure (plant or soil and plant). Due to the small size of the NPs, it was predicted that for snails exposed to soil, dermal transfer of Ag could occur.

For the preliminary experiment, soil mineralization was performed by digesting 500 mg of soil in aqua regia (HNO₃:HCl 2:5, v:v ultratracés, Optima®, Fisher Scientific, Illkirch, France). The resulting solutions were analyzed by ICP-MS (inductively coupled plasma mass spectrometry, Thermo Scientific X Series II, Courtaboeuf, France) to quantify As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn; Ag was quantified by ICP-AES (inductively coupled plasma atomic emission spectroscopy, ICAP 6500 Radial de Thermo Scientific, Courtaboeuf, France). A certified material (loamy clay 1, CRM052, LGC Standards, Molsheim, France) was employed

the same day with both instruments for verification and gave recovery rates of 85 % to 120% for all metallic elements. For the main experiment, 300 mg of sample was digested in 7 mL of concentrated HNO₃ using a Berghof microwave digestion system (speed wave MWS-2-Microwave pressure digestion, Berghof, Eningen, Germany), and same metals were analyzed by ICP-OES (inductively coupled plasma optical emission spectrometry, Thermo Scientific, Courtaboeuf, France). The protocol used to analyze the metal contents in the sewage sludge monthly during the year 2016 was exactly the same than the one for the main experience. For mineralization of plants and animals, digestion was performed in acid medium (using HNO₃, H₂SO₄ and HCl₄) at high temperature as described by Bernard et al. (2010). Lanier et al. (2019) showed that this method was effective for the reference organisms. The obtained solution was then used to quantify As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn and Ag by ICP-OES (Agilent Technologies 5110, USA, for the preliminary experiment and Varian 720-ES, USA, for the main one). Some additional metals were quantified only in the main experiment: Sb, Mo and Hg. These metals are conventionally analyzed in foodstuffs intended for humans and animals (Directive 2002/32 of European Commission, 2002; Regulation n° 1881/2006 of European Commission, 2006) or in the context of ecotoxicological analyses. The effectiveness of the mineralization was verified and validated by mineralizing reference materials (IC-INCT-OBTL-5 from LabMix24 Germany for plants and TORT-3 and DOLT-5 from National Research Council Canada for animals).

2.7.3 Statistical analyses

The majority of the data did not follow a normal distribution, and the variances were not homogeneous (Shapiro, Lilliefors and Bartlett tests). Thus, Scheirer-Ray-Hare nonparametric tests and post-hoc tests based on ranks were used. For data following a normal

distribution with homogeneous variances, ANOVA and Tukey's post-hoc tests were used. Tests were realized in R software (R Core Team, 2008).

3. Results

3.1. Preliminary experiment

Metal content in soil

The Ag contents were slightly lower than expected. The metal contents in soil mixed with sewage sludge containing Ag sulfide were: $9.649 \pm 0.303 \text{ mg kg}^{-1}$ (dry matter) Ag, $8.666 \pm 0.268 \text{ mg kg}^{-1}$ As, $0.633 \pm 0.096 \text{ mg kg}^{-1}$ Cd, $5.439 \pm 0.168 \text{ mg kg}^{-1}$ Co, $22.228 \pm 0.572 \text{ mg kg}^{-1}$ Cr, $23.112 \pm 0.685 \text{ mg kg}^{-1}$ Cu, $358.650 \pm 15.556 \text{ mg kg}^{-1}$ Mn, $11.613 \pm 0.381 \text{ mg kg}^{-1}$ Ni, $74.376 \pm 3.264 \text{ mg kg}^{-1}$ Pb, and $88.105 \pm 3.549 \text{ mg kg}^{-1}$ Zn. These metal contents will be compared with the values in the main experiment (Table 2 in section 3.2.).

Life traits of plants

All plant cultivars grew normally on the medium except clover. For all species, some leaves turned yellow in the last week of exposure.

The yellowing of the leaves observed at the end of the preliminary experiment suggested harmful effects on the plants. Such effects might alter the Ag bioaccumulation capacity of the plants, but only at time T3, since the plants had a healthy appearance for the first 6 weeks. Although it is possible that the yellowing was caused by Ag, it is more likely due to a deficiency related to the limited amount of substrate and/or the successive drying and watering, as the intensity of the artificial lights in the greenhouse dried the soil surface very quickly.

Metal accumulation in plants

Clover could not be sampled at T1 and T2 due to a lack of biomass. Among the other plant cultivars, hemp and the two ryegrass cultivars accumulated the most Ag in their leaves (mean of 0.06 mg kg⁻¹ of dry leaves for these species) (Figure 2). The accumulation of Ag was rapid and was maintained over time because the amount of bioaccumulated Ag was statistically similar among T1, T2 and T3 for all species.

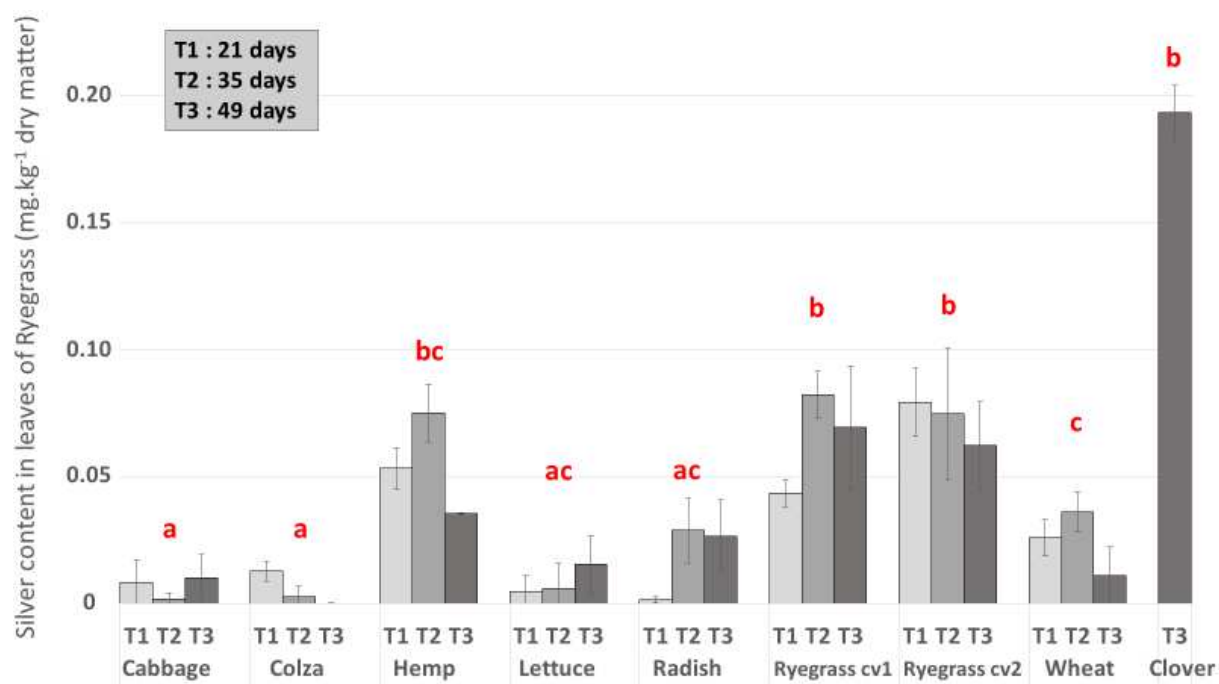


Figure 2: Silver concentration in leaves of plants in mg kg⁻¹(dry matter). Significant differences are indicated by different letters (comparison between species only, data from all time points pooled since there were no significant differences among the 3 time points for each species). T1, T2 and T3 correspond to the dates of sample collection, i.e. 21, 35 and 49 days, respectively.

Ryegrass cultivar 2 was selected for the main experiment because it had the lowest variation of Ag content over time and the highest concentration of Ag.

3.2. Main experiment

Metal contents in mesocosm mixtures

In general, there was little difference in metal concentrations among the 6 mesocosm conditions (Table 2). Zn content was higher in the conditions with SS than in those without.

Ag content was significantly higher in the conditions with AgNPs or AD-AgNPs than in Control and Disor AD-control and AD-dis, respectively. However, the Ag content was significantly lower in AD-AgNPs than in the AgNPs mesocosms (almost two times less). It is therefore important to carefully interpret the comparisons of the AgNPs and AD-AgNPs conditions.

In the preliminary experiment, the Ag concentration ($9.65 \pm 0.30 \text{ mg kg}^{-1}$ of dry matter) was intermediate between the two Ag concentrations in the main experiment.

Table 2: Metal concentration in the soil at the beginning of the experiment in the 6 mesocosm conditions (in mg kg^{-1} , in dry matter). Standard errors were indicated. Statistically significant results are indicated by stars. Red stars indicate that the silver concentrations were higher in the AgNPs and AD-AgNPs conditions than the others, although the Ag concentrations were significantly different between the AgNPs and AD-AgNPs conditions. Green stars indicate that the Zn concentrations were higher in the conditions with sludge than in the sludge-free conditions.

	Control	Dis	AgNPs	AD-control	AD-dis	AD-AgNPs
Ag	0.529 ± 0.340	0.576 ± 0.258	* 11.150 ± 0.420	2.124 ± 0.252	1.903 ± 0.810	* 6.993 ± 0.223
As	4.131 ± 0.128	4.168 ± 0.141	3.950 ± 0.059	4.374 ± 0.256	4.301 ± 0.157	3.888 ± 0.212
Cd	0.320 ± 0.061	0.308 ± 0.061	0.310 ± 0.027	0.296 ± 0.037	0.323 ± 0.029	0.304 ± 0.026
Co	4.173 ± 0.092	3.879 ± 0.042	3.789 ± 0.066	4.105 ± 0.117	4.102 ± 0.105	3.854 ± 0.188
Cr	25.675 ± 1.656	24.775 ± 0.834	24.300 ± 1.036	28.300 ± 1.089	26.550 ± 0.580	26.275 ± 1.150
Cu	18.675 ± 0.359	18.425 ± 0.050	17.475 ± 0.222	19.900 ± 0.141	19.775 ± 0.150	18.500 ± 0.424
Mn	336.175 ± 9.831	326.425 ± 22.948	307.775 ± 4.844	336.725 ± 0.974	337.250 ± 17.989	307.100 ± 15.112
Ni	11.450 ± 0.173	11.225 ± 0.050	10.950 ± 0.191	11.875 ± 0.096	11.900 ± 0.245	11.175 ± 0.275
Pb	52.725 ± 1.611	51.875 ± 0.957	51.150 ± 3.527	52.900 ± 0.173	52.333 ± 0.896	50.375 ± 2.251
Zn	49.775 ± 0.403	49.025 ± 0.450	47.200 ± 0.424	* 76.350 ± 3.753	* 72.125 ± 2.114	* 69.100 ± 1.633

Plant life traits: leaf growth

Ryegrass growth was observed in all mesocosms. From the second week, it appeared visually that the *Lolium perenne* grew much faster in the mesocosms with SS than those without SS (Sup. Inf. 4). This visual difference in biomass was maintained until the end of the experiment. Visually, there was therefore no effect of Ag on the length and the biomass of ryegrass; only the effect of SS was visible.

Animal life traits: survival and biomass

Snail and locust survival were not correlated with condition or soil access according to the statistical tests (non-parametric ANOVA) (Table 3).

Locust biomass did not vary significantly between the different treatments and the two types of access. However, for snails, there was on average a gain in mass or a very small mass loss for snails in contact with the soil. By comparison, a greater mass loss was observed for individuals who did not have access to the soil, although the differences between access to soil and plants and access to plants only were significant only in 2 of 6 conditions (AD-control and AD-AgNPs). No effect of Ag (regardless of the initial chemical form) or dispersant was observed.

Table 3: Survival and biomass gained or lost between the beginning and end of the experiment. P means ‘access to plants only’, and P+S means ‘access to plants and soil’. Standard error were indicated. Statistical tests showed no correlation between the survival or the biomass and the Ag presence.

<u>Condition</u>	<u>Access</u>	<u>Snails</u>		<u>Locusts</u>	
		<u>Survival</u> <u>(%)</u>	<u>Gain/Loss of</u> <u>weight (%)</u>	<u>Survival</u> <u>(%)</u>	<u>Gain/Loss of</u> <u>weight (%)</u>
Control	P	76.67 ± 25.17	- 0.15 ± 0.12	86.67 ± 23.09	+ 0.06 ± 0.04
	P+S	73.33 ± 11.55	+ 0.04 ± 0.07	60.00 ± 20.00	+ 0.05 ± 0.20
AD-control	P	93.33 ± 11.55	- 0.17 ± 0.08	53.33 ± 23.09	+ 0.07 ± 0.09
	P+S	83.33 ± 15.28	+ 0.29 ± 0.04	53.33 ± 23.09	+ 0.16 ± 0.08
Dis	P	93.33 ± 5.77	- 0.11 ± 0.03	86.67 ± 23.09	0.00 ± 0.09
	P+S	63.33 ± 11.55	- 0.01 ± 0.23	46.67 ± 23.09	- 0.14 ± 0.08
AD-dis	P	83.33 ± 15.28	- 0.26 ± 0.27	60.00 ± 34.64	- 0.04 ± 0.05
	P+S	86.67 ± 15.28	+ 0.10 ± 0.02	60.00 ± 20.00	+ 0.06 ± 0.08
AgNPs	P	83.33 ± 15.28	- 0.15 ± 0.04	53.33 ± 41.63	+ 0.01 ± 0.14
	P+S	83.33 ± 15.28	+ 0.04 ± 0.07	66.67 ± 23.09	+ 0.09 ± 0.09
AD-AgNPs	P	66.67 ± 23.09	- 0.16 ± 0.16	46.67 ± 23.09	+ 0.12 ± 0.21
	P+S	76.67 ± 15.28	+ 0.32 ± 0.14	60.00 ± 20.00	+ 0.03 ± 0.17
Average		80.28 ± 15.94	- 0.02 ± 0.21	61.11 ± 24.82	+ 0.04 ± 0.13

Feces production by locusts and snails was the same under all conditions (control, dispersant, AgNPs, AD-control, AD-dis, AD-AgNPs), indicating similar levels of consumption in the days preceding the end of the experiment. Feces production was greater for snails in contact with the soil than those not in contact with the soil (Figure 3), consistent with the biomass results. For locusts, feces production was not significantly linked to the soil access, form of Ag or SS presence/absence.

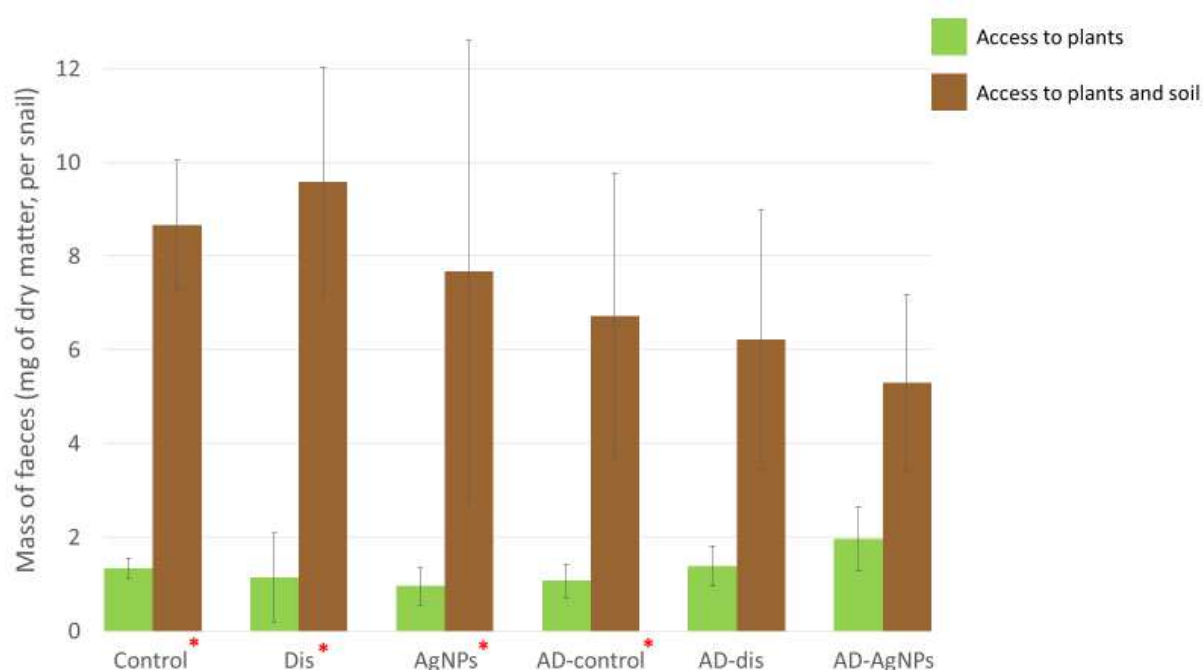


Figure 3: Quantity of faeces produced by snails during the final fasting depuration in mg of dry matter. Red stars indicate significant differences between 'access to plants' and 'access to plants and soil' within the same condition.

Metal accumulation in plants

Consistent with the results of the preliminary experiment, ryegrass accumulated Ag when cultivated in soil enriched with Ag (Figure 4 A). In the condition without SS (AgNPs), the Ag content in the aerial parts of ryegrass was very significant (mean of 0.68 mg kg⁻¹ of Ag in dry matter). By comparison, in the AD-AgNPs condition, Ag was detectable in ryegrass, but the content was lower (0.02 mg kg⁻¹ in mean) and did not differ significantly from that in the mesocosm without Ag. These results indicated that the sulfidized Ag contained in the SS was

455 less bioavailable to ryegrass than AgNPs. This conclusion was confirmed by the calculation
456 of the bioaccumulation factor (BAF) (Sup. Inf. 8): the BAF for ryegrass was 0.061 in the
457 AgNPs mesocosms but 0.003 in the AD-AgNPs mesocosms, a difference of 20-fold.

458

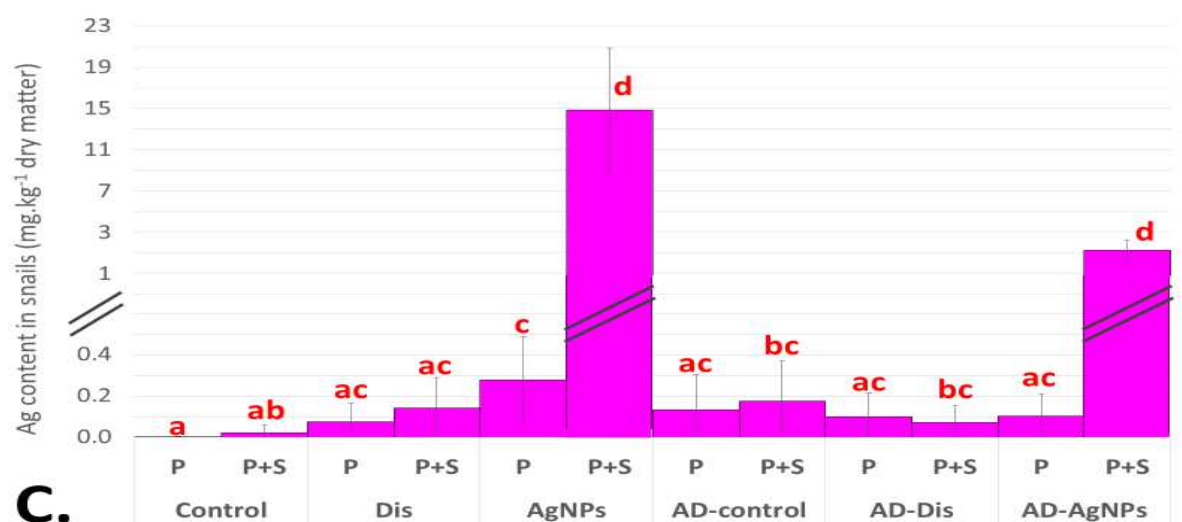
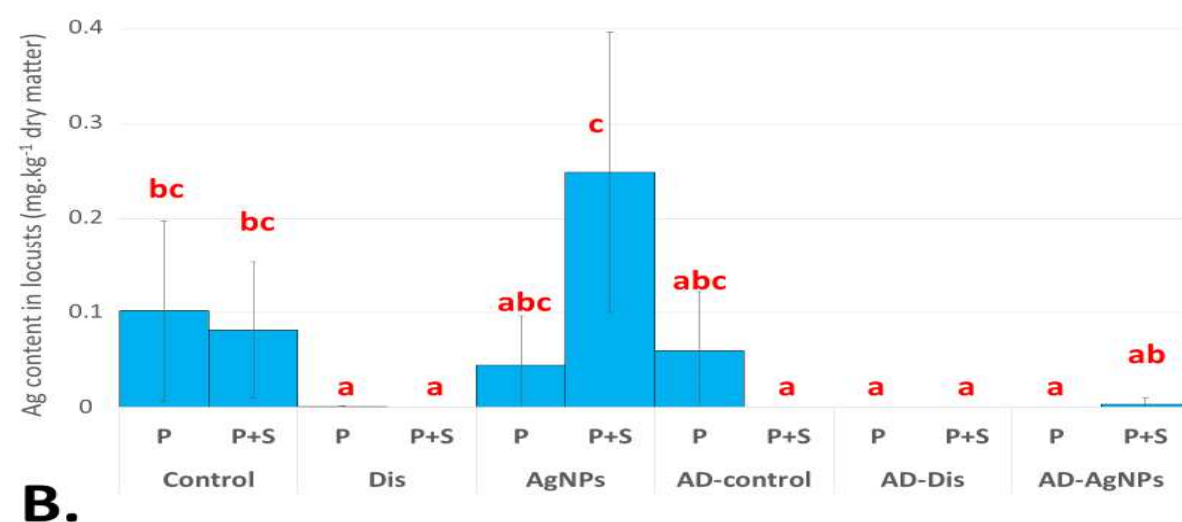
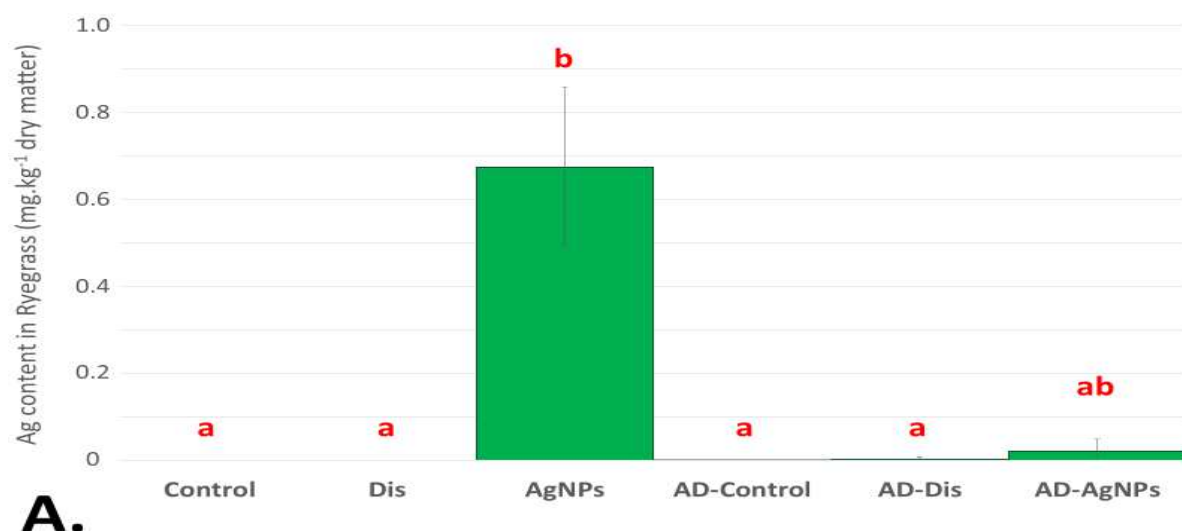


Figure 4: Silver concentration in organisms at the end of experiment. A. Silver content in the aerial part of ryegrass in mg kg⁻¹ (dry matter). Significant differences are indicated by different letters. B. Silver content in whole locusts in mg kg⁻¹(dry matter). Significant differences are indicated by different letters. C. Silver content in snails (without shell) in mg kg⁻¹(dry matter). Significant differences are indicated by different letters.

At the final time point, the accumulation of As, Cd, Co, Cr, Cu, Hg, Pb, Sb and Zn in the aerial parts of ryegrass was similar under all conditions. For Mn, Mo and Ni, the accumulation was lower when the plants were cultivated under SS conditions compared to conditions without SS, regardless of the Ag concentration in the soil (Sup. Inf. 5). In general, ryegrass rapidly accumulated metals, and between 4 and 9 weeks, the content of these metals in the plants remained relatively unchanged.

Metal accumulation in locusts.

The Ag content in whole locusts was very low (Figure 4 B), and there was no significant difference in Ag content between locusts exposed to Ag conditions and those exposed to conditions without Ag.

The accumulation of Cd, Cu, Co, Sb, As, Mo, Hg, Ni, Pb and Zn was quite similar under all conditions (Sup. Inf. 6). The few significant differences in Cr or Mn content, like the differences in the BAF for Cr (Sup. Inf. 8), cannot be attributed to Ag, SS or type of access. The BAF for Zn was lower in all mesocosms with SS (2.20 on average) than in the mesocosms without SS (3.27 on average) (Sup. Inf. 8).

Metal accumulation in snails.

In contrast to the results observed in locusts, snails significantly bioaccumulated Ag under two conditions: AgNPs with soil and plant access, which yielded the highest concentrations (15.25 ± 5.73 mg kg⁻¹ of dry matter), and AD-AgNPs with soil and plant access (Figure 4 C). The BAF for AD-AgNPs with soil and plant access was 4 times lower than that for AgNPs with soil and plant access (Sup. Inf. 8). In the other conditions with Ag

without access to soil, the Ag content in snails was not different from that of snails that were not exposed to Ag.

Moreover, Ag accumulation was higher in the feet than in the viscera of snails exposed to Ag conditions (Figure 5). This result should be considered preliminary because the mortality observed in the mesocosms did not allow replicas to be made.

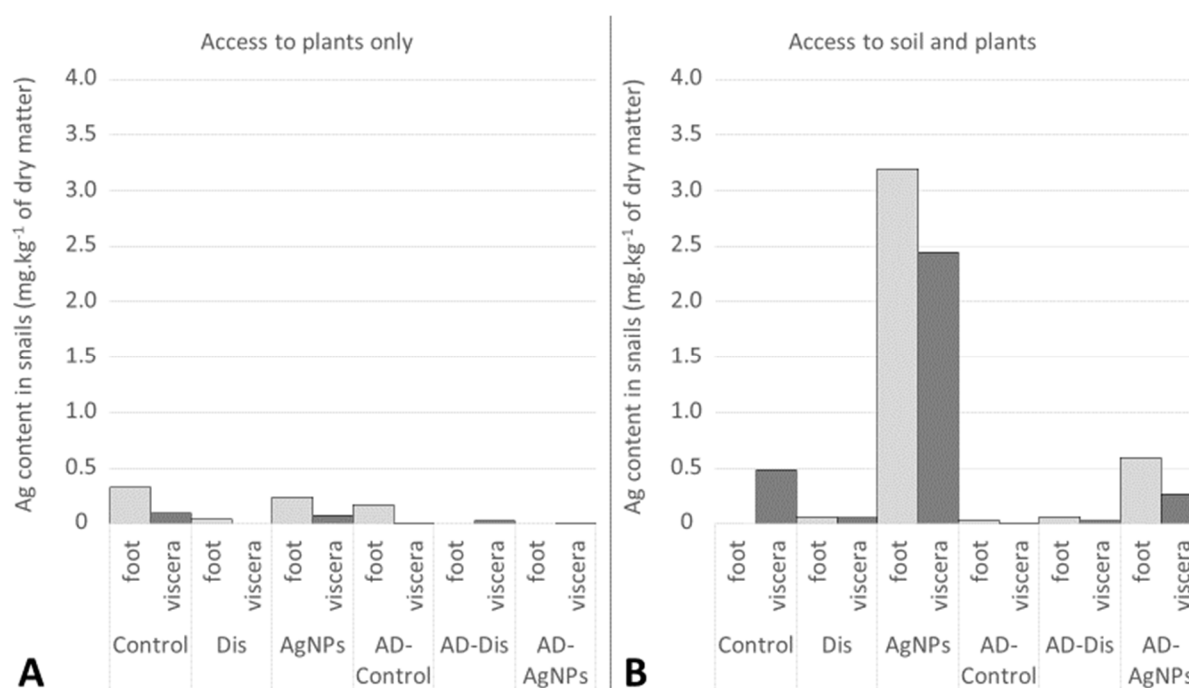


Figure 5: Silver concentration (in mg kg⁻¹ of dry matter) in snail foot and viscera exposed to (A) to plants only and (B) to soil and plants. The absence of error bars and statistical comparisons is due to the impossibility of making replicas.

For snails, the accumulation of Cu, Sb, As, Cr, Mn, Mo, Hg, Ni and Zn was similar in all conditions. However, the accumulated amount of Cd was significantly greater in snails that were in contact with soil than in snails without access to soil (Sup. Inf. 7). The average BAF for Cd was 5.99 for snails with plant+soil access but 3.33 for snails with plant access only (Sup. Inf. 8). Similar effects were observed for Co and Pb, but the differences between the two types of access were not statistically significant.

4. Discussion and conclusion

Transfer and effects of Ag on plants

The results of the preliminary experiment showed that the 9 plant cultivars studied accumulated a small amount of Ag in their aerial parts when Ag was added to the soil in sulfide form via SS. These low concentrations are consistent with the results of Pradas del Real et al. (2016), who found that Ag levels in plants were not detectable with a similar experimental design and Ag soil concentration. Ag accumulated quickly and reached a threshold value (aft 3 weeks). The level of accumulation varied and ranged from 0.005 to 0.200 mg kg⁻¹ (dry matter) depending on the plant species, implying that the magnitude of the effects of spreading of SS rich in Ag could vary by crop type. Additional analyses of the main crops for which spreading of SS is a common practice in Europe or even globally would enable informed decisions on the choice of plots to amend with SS based on plant species to be cultivated. SS very rich in Ag could even be recycled in a manner other than as a biosolid on agricultural plots, for example, in the remediation of highly polluted soil, in order to "dilute" the contaminants already present on site ; the risks of this method are still being assessed (Jaskulak et al., 2020; Kubátová et al., 2016).

The beneficial effects of SS on plant growth and biomass have been known for several years (Kumar et al., 2017) and were confirmed in the main experiment on ryegrass. Ag did not visually affect the appearance and aerial biomass of this species, consistent with the observations of Pradas del Real et al. (2016). In their study, sulfidized Ag affected the root growth of rape and wheat even with Ag concentrations around 1 mg kg⁻¹. Moreover, our results confirmed the lower bioavailability of sulfidized Ag compared with AgNPs previously reported in the literature (Doolette et al., 2015; Pradas del Real et al., 2017, 2016; Stegemeier

et al., 2015; Wang et al., 2015), since the bioaccumulation of sulfidized Ag by ryegrass was approximately 20 times lower than that of AgNPs according to the BAF calculations. Indeed, Ag₂S is very poorly soluble compared with AgNPs (Levard et al., 2013; Pradas del Real et al., 2017), which reduces the release of Ag⁺ ions that can easily penetrate plant cells, particularly since the Ag added to SS before anaerobic digestion persists for several weeks in sulfidized form in soil (according to a study using the same soil, SS from the same treatment plant, and similar anaerobic digestion with identical Ag concentrations; Courtois et al., 2020).

Transfer and effects of Ag on soil invertebrates

Similar to the results in plants, sulfidized Ag did not appear to have a strong impact on the life traits of animals. At the internal concentration measured in the present study, Ag did not affect the life traits (biomass and survival) of locusts and snails. Studies of woodlice and earthworms have yielded similar results (Kampe et al., 2018; Lahive et al., 2017; Velicogna et al., 2017; Courtois et al., 2020), although these studies used a different mode of contamination (direct exposure to soil and not via contaminated food).

While locusts did not significantly accumulate Ag, their period of exposure was shorter than that of snails. Studies have shown that for snails, 2 weeks is sufficient to observe significant bioaccumulation of Cd (Coeurdassier et al., 2002; Gimbert et al., 2006), whereas 4 weeks is required for Ag, Cu, Pb and Zn (De Vaufleury, 2015; Mariet et al., 2017). We can therefore conclude that the duration of exposure of snails in the present study (almost 5 weeks) was sufficient to allow the bioaccumulation of Ag and other metals, whereas 2 weeks of exposure of locusts may not have been sufficient to observe significant bioaccumulation of metals like Ag. Comparable data are not available for locusts because most studies recover animals from the field and therefore the duration of exposure is unknown (Devkota and Schmidt, 2000; Soliman and El-Shazly, 2017; Zhang et al., 2012). This first reassuring result

should therefore be confirmed by longer exposure of locusts. However, in addition to the short exposure time, the Ag levels in locusts may not be detectable due to the low Ag levels in plants.

When snails could get to the soil, they ate more and gained more weight. The snails were likely more comfortable in this type of mesocosm configuration, as access to the soil probably offers higher local humidity and more hiding places, resulting in higher soil consumption and contact. When Ag was present, the snails bioaccumulated more Ag when access to soil was permitted. It is possible that Ag was transferred to the snails via the pedal sole due to direct contact or was only adsorbed on the foot via mucus. The snails may also ingest more Ag by consuming soil particles to supplement their nutritious intake, as described previously by Gomot et al. (1989) and Pauget et al. (2012). Both channels of Ag accumulation are likely since Ag was found not only in the foot but also the viscera. However, studies have shown that the locations of metal accumulation do not necessarily indicate the route of contamination. Studies of *H. aspersa* (Coeurdassier et al., 2002) and *Arion ater* (Ireland, 1982) showed that regardless of the mode of Cd exposure, the bioaccumulated metal was distributed in a similar way, indicating redistribution of the metal in the organism. A similar phenomenon can be envisaged for Ag contamination. Moreover, although the production of mucus during movements when fasting must have allowed the majority of the particles adsorbed on the foot to detach from the animal, a small part of the Ag content in snails might have only been adsorbed on the foot, limiting the potential negative effect on the measured endpoints (survival, mass). Indeed, it has been shown that intake of AgNPs with food significantly affects other parameters, e.g. lipid peroxidation levels, catalase and glutathione-S-transferase activities, cell death and immunological parameters, even when the concentration of Ag in snail tissues is not detectable (Radwan et al., 2019). However, when a predator consumes the snail, this small amount of Ag still transferred via the food chain. A

comparative tomographic analysis of snails in contact with plants only and those previously in contact with soil might reveal whether part of the Ag is adsorbed on the foot or if all of it is passed into the tissues of the snail. Such an analysis would also identify the main sites of bioaccumulation of Ag in this snail species.

In the most realistic condition (AD-AgNPs with access to soil), the snails accumulated approximately 2.3 mg kg^{-1} of Ag, compared with 15 mg kg^{-1} in the same condition with AgNPs. These contents are clearly higher than those found in a study by Mariet et al. (2017) that examined the bioaccumulation of Ag in different areas of an old lead-silver mine. In Mariet et al. (2017), the Ag concentration reached 1.14 mg kg^{-1} in the digestive gland (a part of the viscera) of *Cantareus asperses* snails exposed to soil containing 14.5 mg kg^{-1} of Ag (BAF: 0.054). Several factors may explain the lower transfer observed in this previous field study, for instance, soil characteristics, metal speciation in soil, reduced contact and eating of soil due to the humus cover and the possibility of eating various plants exhibiting different capacities of Ag accumulation. Regardless, our results are reassuring in the context of land spreading of SS and are consistent with previous studies of ryegrass and earthworms (Courtois et al., 2020 ; Velicogna et al., 2017): the Ag found in SS is less bioavailable than AgNPs due to its speciation. The higher organic matter content in soil supplemented with SS may have increased this bioaccumulation gap (between snails in AgNPs P+S and in AD-AgNPs P+S). In fact, the snails under AD-AgNPs conditions probably consumed fewer soil particles to meet their nutritional needs than those under AgNPs conditions, and therefore the latter also consumed more metals. In addition, it would be interesting to compare the speciation of Ag in plants grown under AgNPs conditions and AD-AgNPs conditions. Wang et al. (2015) and Pradas del Real et al. (2017) showed that the speciation of bioaccumulated Ag varied according to plant treatment. In these studies, Ag_2S -NPs treatment resulted in bioaccumulation of Ag mainly in Ag_2S form, AgNPs treatment resulted in bioaccumulation of

Ag mainly in metallic form and linked to a thiol molecule, and AgNO₃ treatment resulted in bioaccumulation of Ag mainly linked to a thiol molecule. If Ag₂S could enter plants via the roots, the bioaccumulated Ag₂S might be less bioavailable, resulting in less bioaccumulation in animals.

In the present study, snails in contact with soil contaminated by AgNPs bioaccumulated high levels of Ag (with a mean BAF equal to 1.367). AgNPs are sometimes directly applied to crops for their nanopesticide or nanofertilizer properties (Chhipa, 2019; Khan and Rizvi, 2017). Such practices could increase the content of Ag entering the trophic network, although the absorption of sulfidized Ag by soybean roots has been shown to be greater than the absorption of AgNPs by its leaves (Dang et al., 2019). In the latter study, soybean was grown hydroponically, so it is possible that sulfidized Ag linked to organic matter in soil and / or SS would be less bioavailable.

In conclusion, the addition of Ag to agricultural soil via the spreading of contaminated SS resulted in transfer of this metal to plants, in a species-dependent manner, especially ryegrass which reached Ag concentration up to 0.2 mg kg⁻¹. Snails and locusts exposed only to ryegrass did not notably bioaccumulate the Ag unlike snails exposed also to the soil as occurs in the field. The transfer of Ag in snails was higher when AgNPs were directly applied to the soil (up to 15 mg kg⁻¹) but still reached 2.5 mg kg⁻¹ when Ag was brought by SS showing a reduced bioavailability to snails of sulfidized Ag compared to AgNPs, both indirectly due to a lower transfer to plant and directly from the soil. It is the first-ever demonstration that sulfidized Ag and to a lower extent AgNPs in soil is bioavailable to snails whereas this is not the case for locust. The experimental design used provide information of great significance either for the SS recycling and the potential risk of the release of Ag NP in the environment whether regarding the possible human exposure by eating cultivated Ag-

contaminated plants or exposure of various consumers via food web transfer of Ag. Transformations of AgNPs in treatment plants attenuate but do not completely prevent the accumulation of Ag in plant and animal species. SS spreading is a common practice, and it may be possible to make informed decisions on the choice of plots to amend with SS according to the plant species planned for cultivation. Thus, it seems important to add Ag to the list of metals controlled in SS (European directive 86/278/CEE, 1986) in order to ensure that SS containing Ag is spread primarily on plots that will receive cultivated species that accumulate little Ag. When SS is too rich in Ag, other strategies for SS recycling, could be employed, such as remediation of polluted soil.

Acknowledgments

The authors thank the “Plateforme serre cultures et terrains expérimentaux” of the University of Lille and Eric Schmitt, Nathalie Faure, Chloé Ponitzki and Cédric Glorieux who made the greenhouse experiments possible. Thanks also to Francis Douay, Régine Fuentes, Marta Jaskulak, Julie Dransart-Leclercq from LGCgE, Anna Grobelak from Czestochowa University of Technology and Nadia Crini from the Technological Platform for the Study of Old and Present Environments for their technical or intellectual help in this work. The authors also thank Dominique Dubois and Johanna Caboche for their analytical contribution.

Funding

This study was funded by the ANSES in the ETNA2 project context and by a grant from the University of Lille and the SMRE doctoral school.

The authors declare no competing financial interest.

References

- Adam, V., Caballero-Guzman, A., Nowack, B., 2018. Considering the forms of released engineered nanomaterials in probabilistic material flow analysis. *Environ. Pollut.* 243, 17–27. <https://doi.org/10.1016/j.envpol.2018.07.108>
- 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>
- Barker, G., Watts, C., 2002. Management of the invasive alien snail *Cantareus aspersus* on conservation land.
- Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020. Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecol. Indic.* 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Beeby, A., 1985. The Role of *Helix aspersa* as a Major Herbivore in the Transfer of Lead Through a Polluted Ecosystem. *J. Appl. Ecol.* 22, 267–275. <https://doi.org/10.2307/2403344>
- Bernard, F., Brulle, F., Douay, F., Lemièrre, S., Demuyne, S., Vandenbulcke, F., 2010. Metallic trace element body burdens and gene expression analysis of biomarker candidates in *Eisenia fetida*, using an “exposure/depuration” experimental scheme with field soils. *Ecotoxicol. Environ. Saf.* 73, 1034–1045. <https://doi.org/10.1016/j.ecoenv.2010.01.010>
- 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>
- Carbone, D., Faggio, C., 2019. *Helix aspersa* as sentinel of development damage for biomonitoring purpose: A validation study. *Mol. Reprod. Dev.* 86, 1283–1291. <https://doi.org/10.1002/mrd.23117>
- Chhipa, H., 2019. Applications of nanotechnology in agriculture. *Methods Microbiol.* 115–142.
- Coeurdassier, M., Vaufléury, A.G., Lovy, C., Badot, P.-M., 2002. Is the cadmium uptake from soil important in bioaccumulation and toxic effects for snails? *Ecotoxicol. Environ. Saf.* 53, 425–431. [https://doi.org/10.1016/S0147-6513\(02\)00004-0](https://doi.org/10.1016/S0147-6513(02)00004-0)
- Courtois, P., Rorat, A., Lemièrre, S., Guyoneaud, R., Attard, E., Levard, C., Vandenbulcke, F., 2019. Ecotoxicology of silver nanoparticles and their derivatives introduced in soil with or without sewage sludge: A review of effects on microorganisms, plants and animals. *Environ. Pollut.* 253, 578–598. <https://doi.org/10.1016/j.envpol.2019.07.053>
- Courtois, P., Rorat, A., Lemièrre, S., Guyoneaud, R., Attard, E., Longepierre, M., Rigal, F., Levard, C., Chaurand, P., Grosser, A., Grobelak, A., Kacprzak, M., Lors, C., Richaume, A., Vandenbulcke, F., 2020. Medium-term effects of Ag supplied directly or via sewage sludge to an agricultural soil on *Eisenia fetida* earthworm and soil microbial communities. *Chemosphere* 128761. <https://doi.org/10.1016/j.chemosphere.2020.128761>
- Dang, F., Chen, Y.-Z., Huang, Y.-N., Hintelmann, H., Si, Y.-B., Zhou, D.-M., 2019. Discerning the Sources of Silver Nanoparticle in a Terrestrial Food Chain by Stable Isotope Tracer Technique. *Environ. Sci. Technol.* 53, 3802–3810. <https://doi.org/10.1021/acs.est.8b06135>
- De Vaufléury, A., 2015. Landsnail for Ecotoxicological Assessment of Chemicals and Soil Contamination – Ecotoxicological Assessment of Chemicals and Contaminated Soils Using the Terrestrial Snail, *Helix aspersa*, at Various Stage of Its Life Cycle: A Review, in: Armon, R.H., Hänninen, O. (Eds.), *Environmental Indicators*. Springer Netherlands, Dordrecht, pp. 365–391. https://doi.org/10.1007/978-94-017-9499-2_23
- Devkota, B., Schmidt, G.H., 2000. Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agric. Ecosyst. Environ.* 78, 85–91. [https://doi.org/10.1016/S0167-8809\(99\)00110-3](https://doi.org/10.1016/S0167-8809(99)00110-3)
- Diez-Ortiz, M., Lahive, E., Kille, P., Powell, K., Morgan, A.J., Jurkschat, K., Van Gestel, C.A.M., Mosselmans, J.F.W., Svendsen, C., Spurgeon, D.J., 2015. Uptake routes and toxicokinetics of

- silver nanoparticles and silver ions in the earthworm *Lumbricus rubellus*. *Environ. Toxicol. Chem.* 34, 2263–2270. <https://doi.org/10.1002/etc.3036>
- Directive 2002/32 of European Commission, 2002. Directive 2002/32/CE du Parlement Européen et du Conseil du 7 mai 2002 sur les substances indésirables dans les aliments pour animaux. ResearchGate.
- Directive européenne 86/278/CEE, 1986. Directive du Conseil du 12 juin 1986 relative à la protection de l'environnement et notamment des sols, lors de l'utilisation des boues d'épuration en agriculture (86/278/CEE).
- Donner, E., Scheckel, K., Sekine, R., Popelka-Filcoff, R.S., Bennett, J.W., Brunetti, G., Naidu, R., McGrath, S.P., Lombi, E., 2015. Non-labile silver species in biosolids remain stable throughout 50 years of weathering and ageing. *Environ. Pollut.* 205, 78–86. <https://doi.org/10.1016/j.envpol.2015.05.017>
- Doolette, C.L., McLaughlin, M.J., Kirby, J.K., Navarro, D.A., 2015. Bioavailability of silver and silver sulfide nanoparticles to lettuce (*Lactuca sativa*): Effect of agricultural amendments on plant uptake. *J. Hazard. Mater.* 300, 788–795. <https://doi.org/10.1016/j.jhazmat.2015.08.012>
- DRAAF, N.-P. de C., 2013. Inventaire, Gestion et Conservation des Sols dans le Nord - Pas de Calais [WWW Document]. URL http://draaf.hauts-de-france.agriculture.gouv.fr/IMG/pdf/Demarche_IGCS_dans_le_Nord-Pas_de_Calais_v2013-04-06_cle014cb8.pdf (accessed 1.8.20).
- European commission, 2017. Report from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the committee of the regions on implementation of free trade agreements.
- European commission, 2014. Nanosilver: Safety, health and environmental effects and role in antimicrobial resistance. *Mater. Today* 18, 122–123. <https://doi.org/10.1016/j.mattod.2015.02.014>
- European commission, 2012. COMMISSION STAFF WORKING PAPER Types and uses of nanomaterials, including safety aspects Accompanying the Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee on the Second Regulatory Review on Nanomaterials, CELEX1.
- Eurostat, 2017. Sewage sludge disposal from urban wastewater treatment, by treatment method, 2017 (% of total dry mass) - Statistics Explained.
- Food and Agriculture Organization of the United Nations, 2015. World reference base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. FAO, Rome.
- Geisler-Lee, J., Brooks, M., Gerfen, J., Wang, Q., Fotis, C., Sparer, A., Ma, X., Berg, R., Geisler, M., 2014. Reproductive Toxicity and Life History Study of Silver Nanoparticle Effect, Uptake and Transport in *Arabidopsis thaliana*. *Nanomaterials* 4, 301–318. <https://doi.org/10.3390/nano4020301>
- Geisler-Lee, J., Wang, Q., Yao, Y., Zhang, W., Geisler, M., Li, K., Huang, Y., Chen, Y., Kolmakov, A., Ma, X., 2012. Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology* 7, 323–337. <https://doi.org/10.3109/17435390.2012.658094>
- Gimbert, F., de Vaufléury, A., Douay, F., Scheifler, R., Coeurdassier, M., Badot, P.-M., 2006. Modelling chronic exposure to contaminated soil: A toxicokinetic approach with the terrestrial snail *Helix aspersa*. *Environ. Int.* 32, 866–875. <https://doi.org/10.1016/j.envint.2006.05.006>
- Gomot, A., Gomot, L., Boukraa, S., Bruckert, S., 1989. Influence of soil on the growth of the land snail *Helix aspersa*. An experimental study of the absorption route for the stimulating factors. *J. Molluscan Stud.* 55, 1–7. <https://doi.org/10.1093/mollus/55.1.1-a>
- Gomot de Vaufléury, A., 2000. Standardized Growth Toxicity Testing (Cu, Zn, Pb, and Pentachlorophenol) with *Helix aspersa*. *Ecotoxicol. Environ. Saf.* 46, 41–50. <https://doi.org/10.1006/eesa.1999.1872>
- Gomot de Vaufléury, A., Pihan, F., 2002. Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: Metal bioavailability and bioaccumulation. *Environ. Toxicol. Chem.* 21, 820–827. <https://doi.org/10.1002/etc.5620210419>

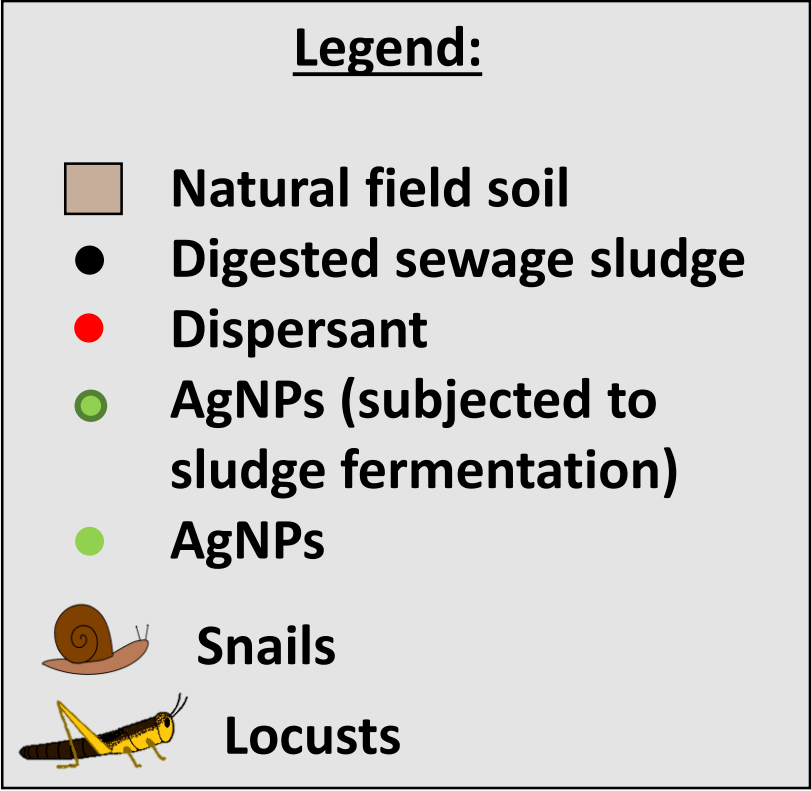
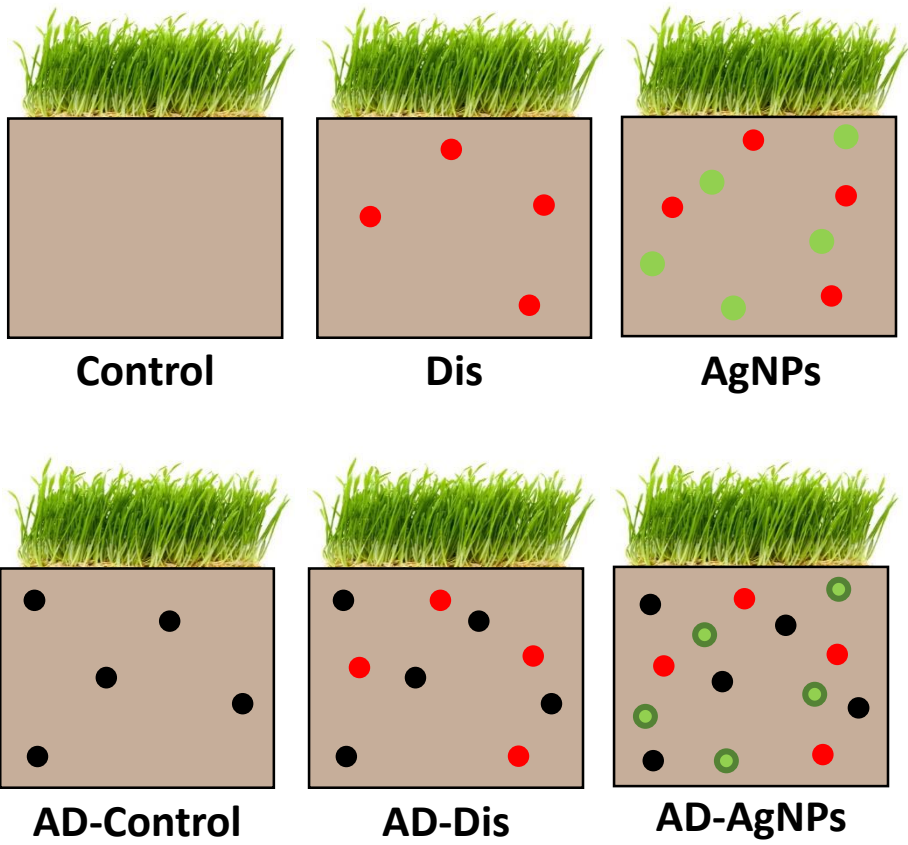
- Gottschalk, F., Sun, T., Nowack, B., 2013. Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environ. Pollut.* 181, 287–300. <https://doi.org/10.1016/j.envpol.2013.06.003>
- Grosser, A., 2017. The influence of decreased hydraulic retention time on the performance and stability of co-digestion of sewage sludge with grease trap sludge and organic fraction of municipal waste. *J. Environ. Manage., Environmental management as a pillar for sustainable development* 203, 1143–1157. <https://doi.org/10.1016/j.jenvman.2017.04.085>
- Hopkin, S.P., 1989. Ecophysiology of metals in terrestrial invertebrates. *Ecophysiol. Met. Terr. Invertebr.*
- Ireland, M.P., 1982. Sites of water, zinc and calcium uptake and distribution of these metals after cadmium administration in *Arion ater* (Gastropoda: Pulmonata). *Comp. Biochem. Physiol. A Physiol.* 73, 217–221. [https://doi.org/10.1016/0300-9629\(82\)90058-5](https://doi.org/10.1016/0300-9629(82)90058-5)
- ISO 15952, 2018. ISO 15952:2018 _ Qualité du sol — Effets des polluants vis-à-vis des escargots juvéniles (Helicidae) — Détermination des effets sur la croissance par contamination du sol.
- Jarwar, A.R., Hao, K., Bitume, E.V., Ullah, H., Cui, D., Nong, X., Wang, G., Tu, X., Zhang, Z., 2019. Comparative Transcriptomic Analysis Reveals Molecular Profiles of Central Nervous System in Maternal Diapause Induction of *Locusta migratoria*. *G3 Genes Genomes Genet.* 9, 3287–3296. <https://doi.org/10.1534/g3.119.400475>
- Jaskulak, M., Grobelak, A., Vandenbulcke, F., 2020. Effects of sewage sludge supplementation on heavy metal accumulation and the expression of ABC transporters in *Sinapis alba* L. during assisted phytoremediation of contaminated sites. *Ecotoxicol. Environ. Saf.* 197, 110606. <https://doi.org/10.1016/j.ecoenv.2020.110606>
- Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., Siegrist, H., 2011. Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. *Environ. Sci. Technol.* 45, 3902–3908. <https://doi.org/10.1021/es1041892>
- 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*: Silver nanoparticles in sewage sludge. *Environ. Toxicol. Chem.* 37, 1606–1613. <https://doi.org/10.1002/etc.4102>
- Kaveh, R., Li, Y.-S., Ranjbar, S., Tehrani, R., Brueck, C.L., Van Aken, B., 2013. Changes in *Arabidopsis thaliana* Gene Expression in Response to Silver Nanoparticles and Silver Ions. *Environ. Sci. Technol.* 47, 10637–10644. <https://doi.org/10.1021/es402209w>
- Khan, M.R., Rizvi, T.F., 2017. Application of Nanofertilizer and Nanopesticides for Improvements in Crop Production and Protection, in: Ghorbanpour, M., Manika, K., Varma, A. (Eds.), *Nanoscience and Plant–Soil Systems, Soil Biology*. Springer International Publishing, Cham, pp. 405–427. https://doi.org/10.1007/978-3-319-46835-8_15
- Klein, C.L., Stahlmecke, B., Romazanov, J., Kuhlbusch, T.A.J., Van Doren, E., De Temmerman, P.-J., Mast, J., Wick, P., Krug, H., Locoro, G., Hund-Rinke, K., Kördel, W., Friedrichs, S., Maier, G., Werner, J., Linsinger, T., Gawlik, B.M., Comero, S., Institute for Health and Consumer Protection, European Commission, Joint Research Centre, Institute for Environment and Sustainability, Institute for Reference Materials and Measurements, 2011. NM-Series of representative manufactured nanomaterials: NM-300 silver characterisation, stability, homogeneity. Publications Office, Luxembourg.
- Kubátová, P., Hejzman, M., Száková, J., Vondráčková, S., Tlustoš, P., 2016. Effects of Sewage Sludge Application on Biomass Production and Concentrations of Cd, Pb and Zn in Shoots of *Salix* and *Populus* Clones: Improvement of Phytoremediation Efficiency in Contaminated Soils. *BioEnergy Res.* 9, 809–819. <https://doi.org/10.1007/s12155-016-9727-1>
- Kumar, V., Chopra, A.K., Kumar, A., 2017. A Review on Sewage Sludge (Biosolids) a Resource for Sustainable Agriculture. *Arch. Agric. Environ. Sci.* 2, 340–347. <https://doi.org/10.26832/24566632.2017.020417>
- Lahive, E., Matzke, M., Durenkamp, M., Lawlor, A.J., Thacker, S.A., Pereira, M.G., Spurgeon, D.J., Unrine, J.M., Svendsen, C., Lofts, S., 2017. Sewage sludge treated with metal nanomaterials inhibits earthworm reproduction more strongly than sludge treated with metal metals in bulk/salt forms. *Environ. Sci. Nano* 4, 78–88. <https://doi.org/10.1039/C6EN00280C>

- Lanier, C., Bernard, F., Dumez, S., Leclercq-Dransart, J., Lemièrre, S., Vandenbulcke, F., Nesslany, F., Platel, A., Devred, I., Hayet, A., Cuny, D., Deram, A., 2019. Combined toxic effects and DNA damage to two plant species exposed to binary metal mixtures (Cd/Pb). *Ecotoxicol. Environ. Saf.* 167, 278–287. <https://doi.org/10.1016/j.ecoenv.2018.10.010>
- Levard, C., Hotze, E.M., Colman, B.P., Dale, A.L., Truong, L., Yang, X.Y., Bone, A.J., Brown, G.E., Tanguay, R.L., Di Giulio, R.T., Bernhardt, E.S., Meyer, J.N., Wiesner, M.R., Lowry, G.V., 2013. Sulfidation of Silver Nanoparticles: Natural Antidote to Their Toxicity. *Environ. Sci. Technol.* 47, 13440–13448. <https://doi.org/10.1021/es403527n>
- 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>
- Luo, X., Xu, S., Yang, Y., Li, L., Chen, S., Xu, A., Wu, L., 2016. Insights into the Ecotoxicity of Silver Nanoparticles Transferred from *Escherichia coli* to *Caenorhabditis elegans*. *Sci. Rep.* 6, 36465. <https://doi.org/10.1038/srep36465>
- Ma, R., Levard, C., Judy, J.D., Unrine, J.M., Durenkamp, M., Martin, B., Jefferson, B., Lowry, G.V., 2014. Fate of Zinc Oxide and Silver Nanoparticles in a Pilot Wastewater Treatment Plant and in Processed Biosolids. *Environ. Sci. Technol.* 48, 104–112. <https://doi.org/10.1021/es403646x>
- Mariet, A.-L., Pauget, B., de Vaufléury, A., Bégeot, C., Walter-Simonnet, A.-V., Gimbert, F., 2017. Using bioindicators to assess the environmental risk of past mining activities in the Vosges Mountains (France). *Ecol. Indic.* 75, 17–26. <https://doi.org/10.1016/j.ecolind.2016.11.042>
- 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>
- Mendes, L.A., Maria, V.L., Scott-Fordsmand, J.J., Amorim, M.J.B., 2015. Ag Nanoparticles (Ag NM300K) in the Terrestrial Environment: Effects at Population and Cellular Level in *Folsomia candida* (Collembola). *Int. J. Environ. Res. Public Health* 12, 12530–12542. <https://doi.org/10.3390/ijerph121012530>
- Nair, P.M.G., Chung, I.M., 2014. Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. *Environ. Sci. Pollut. Res.* 21, 8858–8869. <https://doi.org/10.1007/s11356-014-2822-y>
- Pauget, B., Faure, O., Conord, C., Crini, N., de Vaufléury, A., 2015. In situ assessment of phyto and zooavailability of trace elements: A complementary approach to chemical extraction procedures. *Sci. Total Environ.* 521–522, 400–410. <https://doi.org/10.1016/j.scitotenv.2015.03.075>
- Pauget, B., Gimbert, F., Scheifler, R., Coeurdassier, M., De Vaufléury, A., 2012. Soil parameters are key factors to predict metal bioavailability to snails based on chemical extractant data. *Sci. Total Environ.* 431, 413–425. <https://doi.org/10.1016/j.scitotenv.2012.05.048>
- Poma, G., Cuykx, M., Amato, E., Calaprice, C., Focant, J.F., Covaci, A., 2017. Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. *Food Chem. Toxicol.* 100, 70–79. <https://doi.org/10.1016/j.fct.2016.12.006>
- Pradas del Real, A.E., Castillo-Michel, H., Kaegi, R., Sinnet, B., Magnin, V., Findling, N., Villanova, J., Carrière, M., Santaella, C., Fernández-Martínez, A., Levard, C., Sarret, G., 2016. Fate of Ag-NPs in Sewage Sludge after Application on Agricultural Soils. *Environ. Sci. Technol.* 50, 1759–1768. <https://doi.org/10.1021/acs.est.5b04550>
- Pradas del Real, A.E., Vidal, V., Carrière, M., Castillo-Michel, H., Levard, C., Chaurand, P., Sarret, G., 2017. Silver Nanoparticles and Wheat Roots: A Complex Interplay. *Environ. Sci. Technol.* 51, 5774–5782. <https://doi.org/10.1021/acs.est.7b00422>
- R Core Team, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Radwan, M.A., El-Gendy, K.S., Gad, A.F., Khamis, A.E., Eshra, E.H., 2019. Responses of oxidative stress, genotoxicity and immunotoxicity as biomarkers in *Theba pisana* snails dietary exposed to silver nanoparticles. *Chem. Ecol.* 35, 613–630. <https://doi.org/10.1080/02757540.2019.1631816>

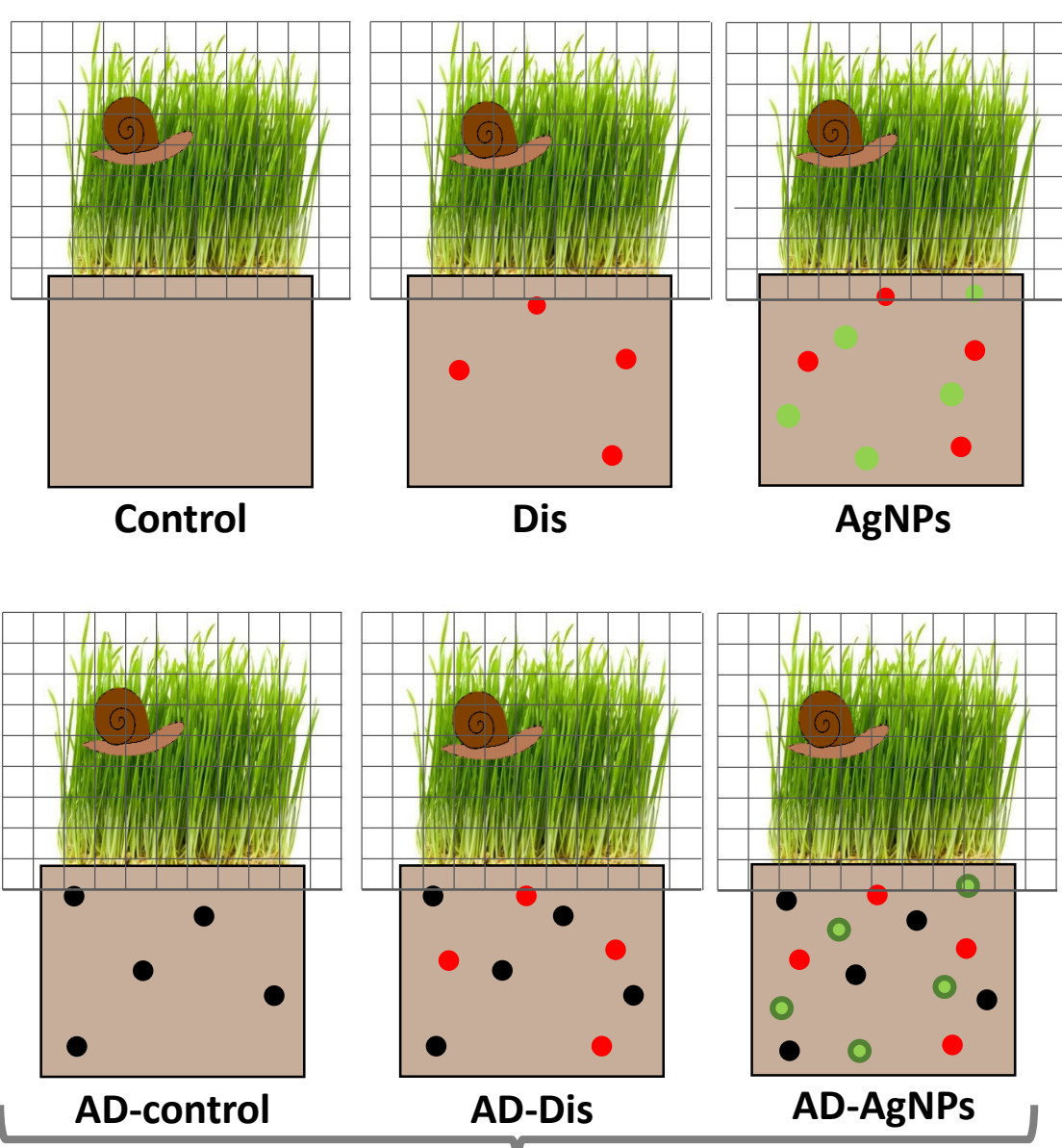
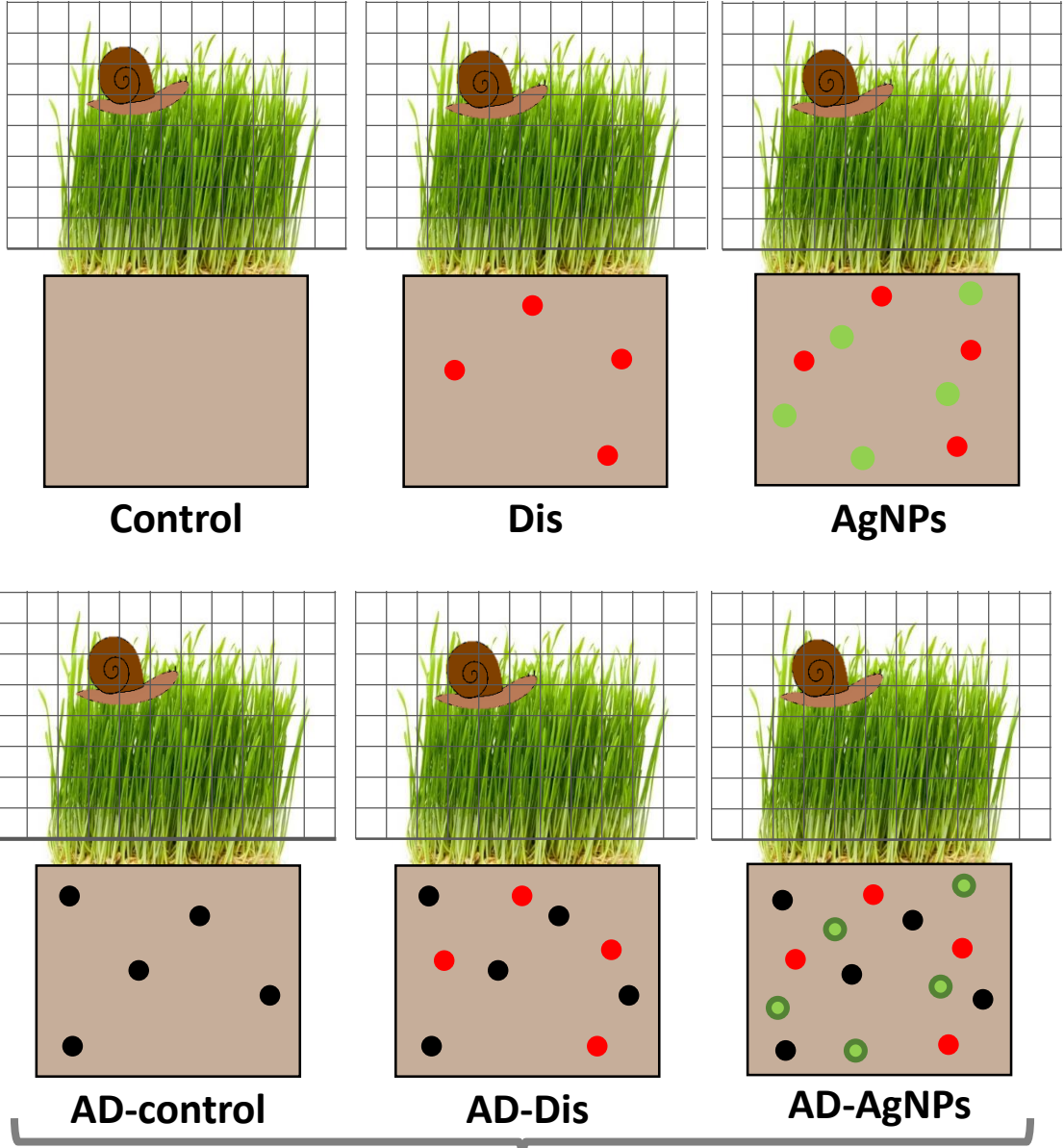
- Regulation n° 1881/2006 of European Commission, 2006. Règlement (CE) N°1881/2006 de la commission du 19 décembre 2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires.
- Reidy, B., Haase, A., Luch, A., Dawson, K., Lynch, I., 2013. Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. *Materials* 6, 2295–2350. <https://doi.org/10.3390/ma6062295>
- Rui, M., Ma, C., Tang, X., Yang, J., Jiang, F., Pan, Y., Xiang, Z., Hao, Y., Rui, Y., Cao, W., Xing, B., 2017. Phytotoxicity of Silver Nanoparticles to Peanut (*Arachis hypogaea* L.): Physiological Responses and Food Safety. *ACS Sustain. Chem. Eng.* 5, 6557–6567. <https://doi.org/10.1021/acssuschemeng.7b00736>
- 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>
- Soliman, M., El-Shazly, M., 2017. Bioaccumulation of Heavy Metals by Grasshoppers and a Mantid Along a Pollution Gradient. *Ecol. Balk.* 9, 7–21.
- Song, U., Jun, H., Waldman, B., Roh, J., Kim, Y., Yi, J., Lee, E.J., 2013. Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*). *Ecotoxicol. Environ. Saf.* 93, 60–67. <https://doi.org/10.1016/j.ecoenv.2013.03.033>
- Stampoulis, D., Sinha, S.K., White, J.C., 2009. Assay-Dependent Phytotoxicity of Nanoparticles to Plants. *Environ. Sci. Technol.* 43, 9473–9479. <https://doi.org/10.1021/es901695c>
- Stegemeier, J.P., Schwab, F., Colman, B.P., Webb, S.M., Newville, M., Lanzirrotti, A., Winkler, C., Wiesner, M.R., Lowry, G.V., 2015. Speciation Matters: Bioavailability of Silver and Silver Sulfide Nanoparticles to Alfalfa (*Medicago sativa*). *Environ. Sci. Technol.* 49, 8451–8460. <https://doi.org/10.1021/acs.est.5b01147>
- Suleiman, H., Rorat, A., Grobelak, A., Grosser, A., Milczarek, M., Płytycz, B., Kacprzak, M., Vandenbulcke, F., 2017. Determination of the performance of vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses in three earthworm species: *Eisenia fetida*, *Eisenia andrei* and *Dendrobaena veneta*. *Bioresour. Technol.* 241, 103–112. <https://doi.org/10.1016/j.biortech.2017.05.104>
- Tiede, K., Boxall, A.B.A., Wang, X., Gore, D., Tiede, D., Baxter, M., David, H., Tear, S.P., Lewis, J., 2010. Application of hydrodynamic chromatography-ICP-MS to investigate the fate of silver nanoparticles in activated sludge. *J. Anal. At. Spectrom.* 25, 1149. <https://doi.org/10.1039/b926029c>
- U.S. EPA, U.S.E.P.A., 2009. Targeted National Sewage Sludge Survey Statistical Analysis Report.
- 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>
- Wang, P., Menzies, N.W., Lombi, E., Sekine, R., Blamey, F.P.C., Hernandez-Soriano, M.C., Cheng, M., Kappen, P., Peijnenburg, W.J.G.M., Tang, C., Kopittke, P.M., 2015. Silver sulfide nanoparticles (Ag₂S-NPs) are taken up by plants and are phytotoxic. *Nanotoxicology* 9, 1041–1049. <https://doi.org/10.3109/17435390.2014.999139>
- Yasur, J., Rani, P.U., 2013. Environmental effects of nanosilver: impact on castor seed germination, seedling growth, and plant physiology. *Environ. Sci. Pollut. Res.* 20, 8636–8648. <https://doi.org/10.1007/s11356-013-1798-3>
- Zhang, X., Jie, D., Liu, J., Zhang, Jianqin, Zhang, T., Zhang, Jianzhen, Ma, E., 2019. Aryl hydrocarbon receptor regulates the expression of LmGSTd7 and is associated with chlorpyrifos susceptibility in *Locusta migratoria*. *Pest Manag. Sci.* 75, 2916–2924. <https://doi.org/10.1002/ps.5600>
- Zhang, Z., Song, X., Wang, Q., Lu, X., 2012. Cd and Pb Contents in Soil, Plants, and Grasshoppers along a Pollution Gradient in Huludao City, Northeast China. *Biol. Trace Elem. Res.* 145, 403–410. <https://doi.org/10.1007/s12011-011-9199-2>

Initial time: day 0

X6
(36 microcosms)



⚡ Addition of snails: day 28

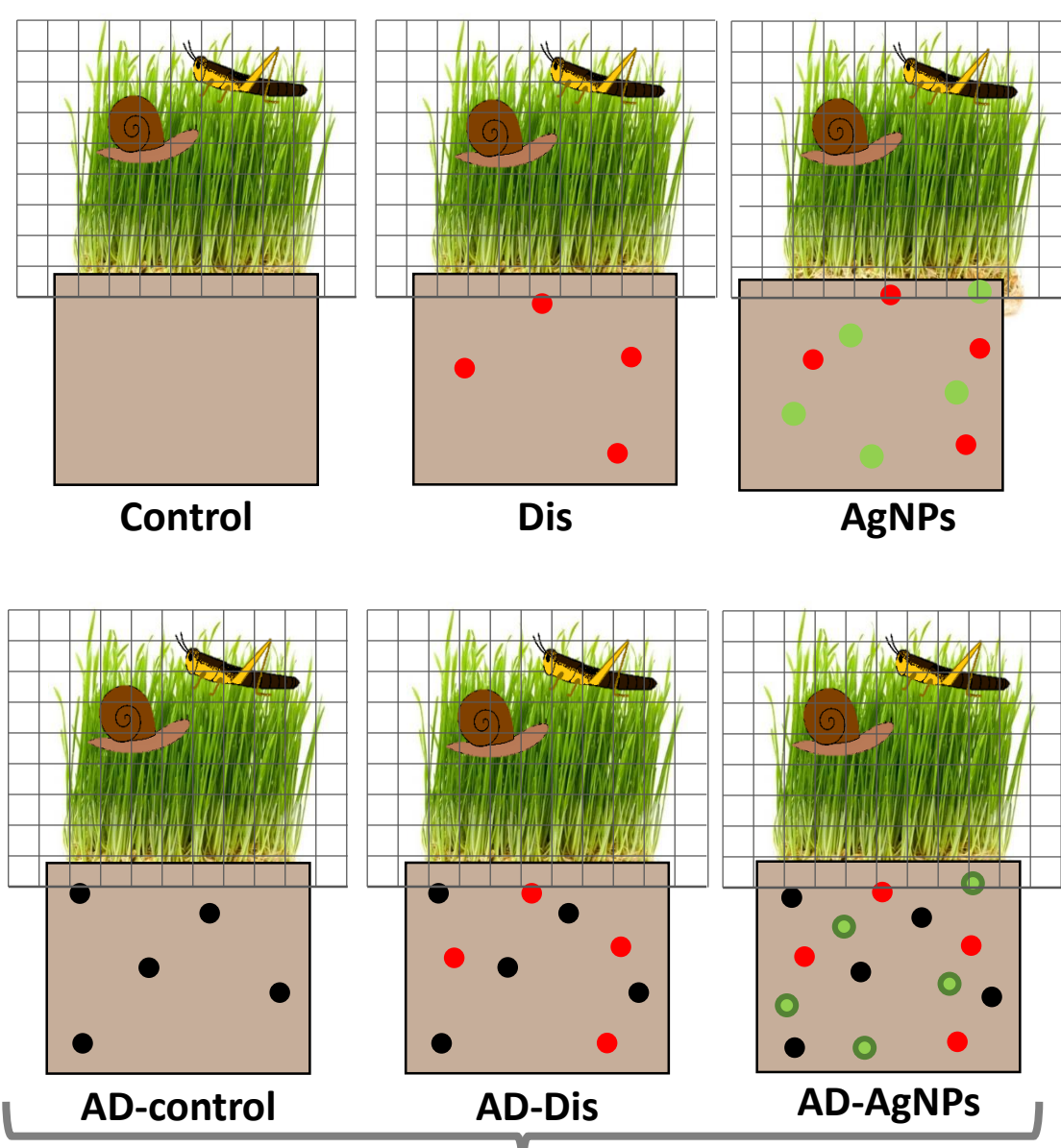
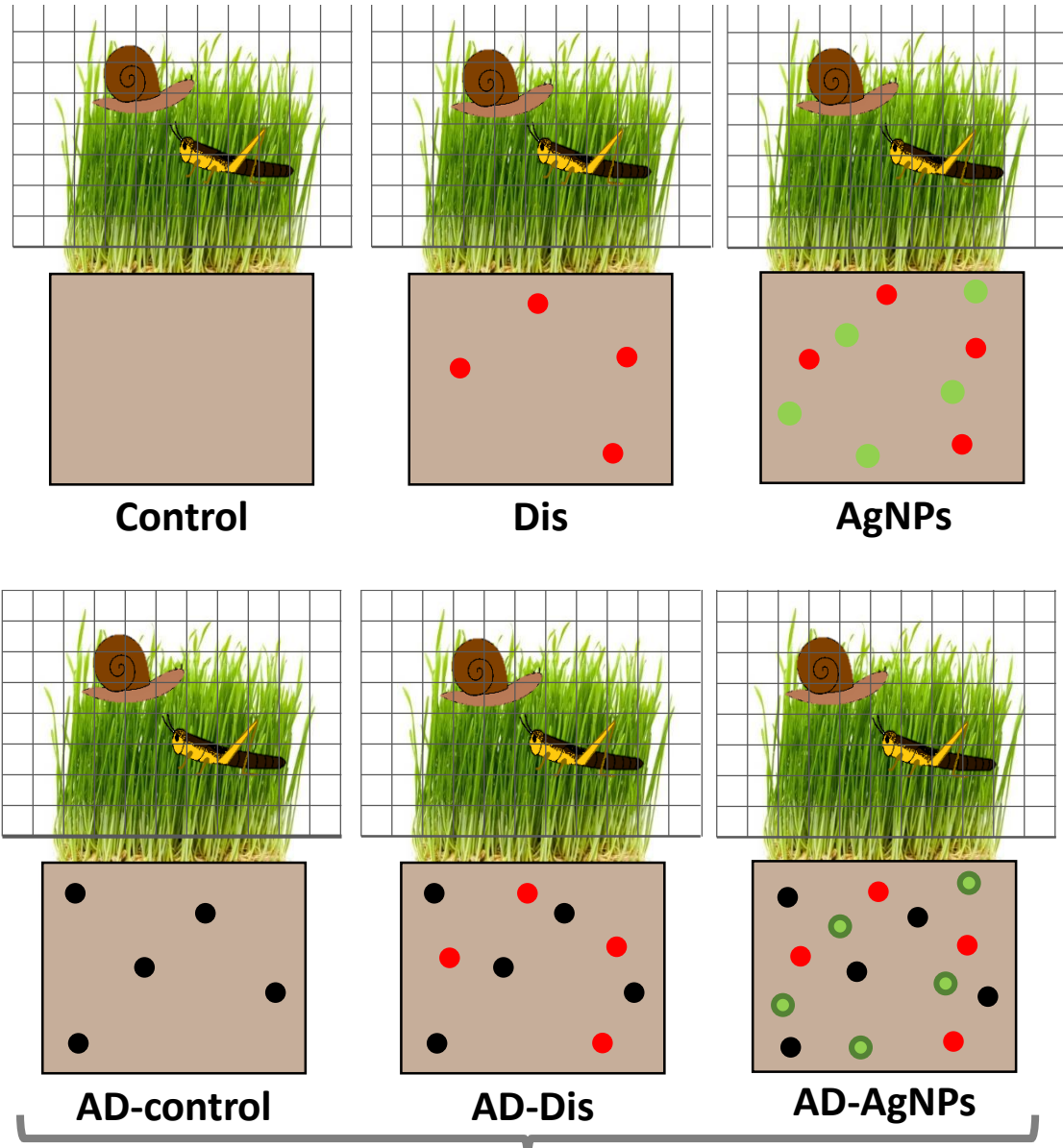


X3
(for triplicates)

Animal access to plants only

Animal access to plants and soil

⚡ Addition of locusts: day 48



X3
(for triplicates)

Animal access to plants only

Animal access to plants and soil

Final time: day 62

Silver content in leaves of Ryegrass (mg.kg⁻¹ dry matter)

T1 : 21 days
T2 : 35 days
T3 : 49 days

0.20

0.15

0.10

0.05

0

a

a

bc

ac

ac

b

b

c

b

T1 T2 T3
Cabbage

T1 T2 T3
Colza

T1 T2 T3
Hemp

T1 T2 T3
Lettuce

T1 T2 T3
Radish

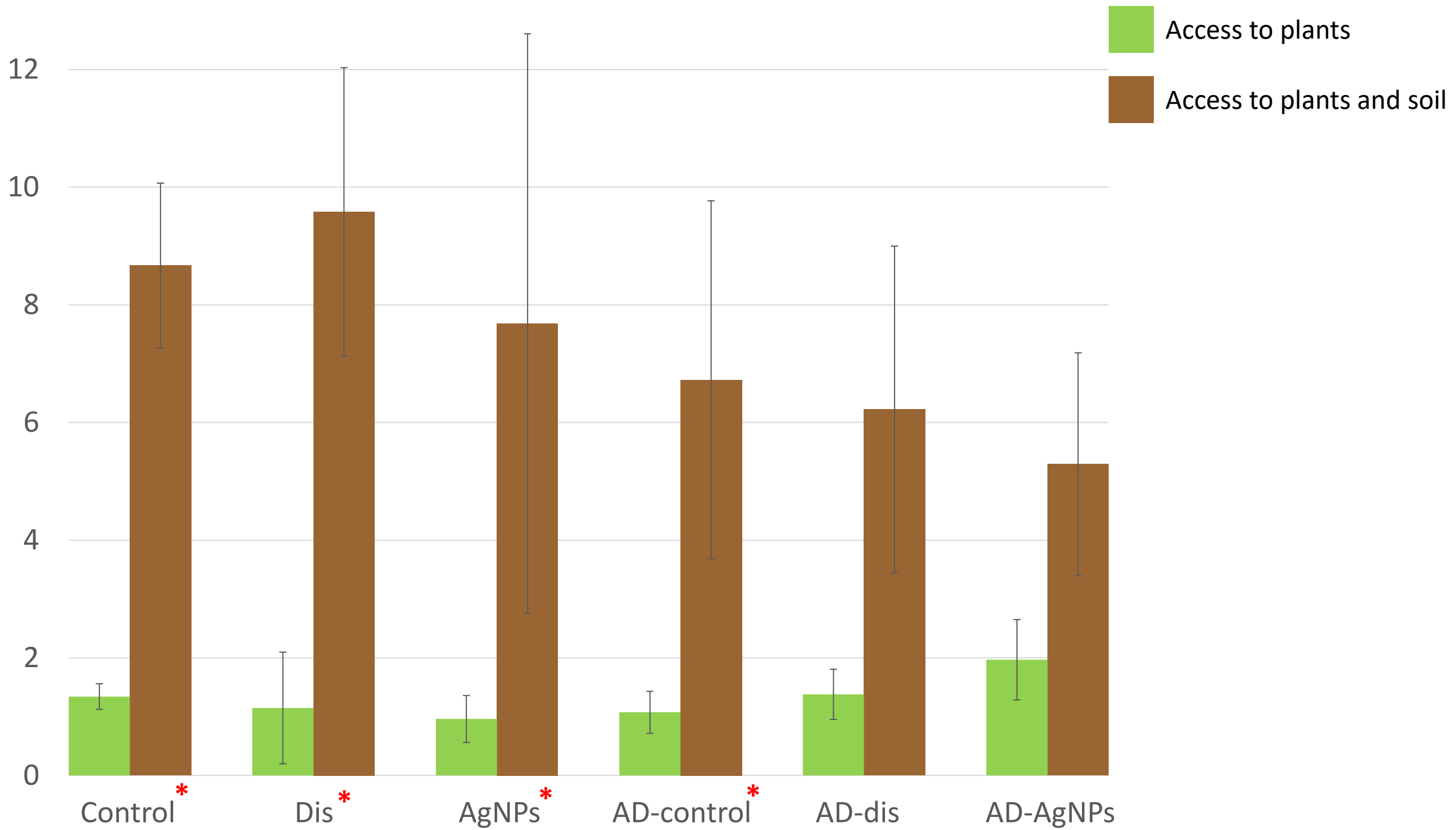
T1 T2 T3
Ryegrass cv1

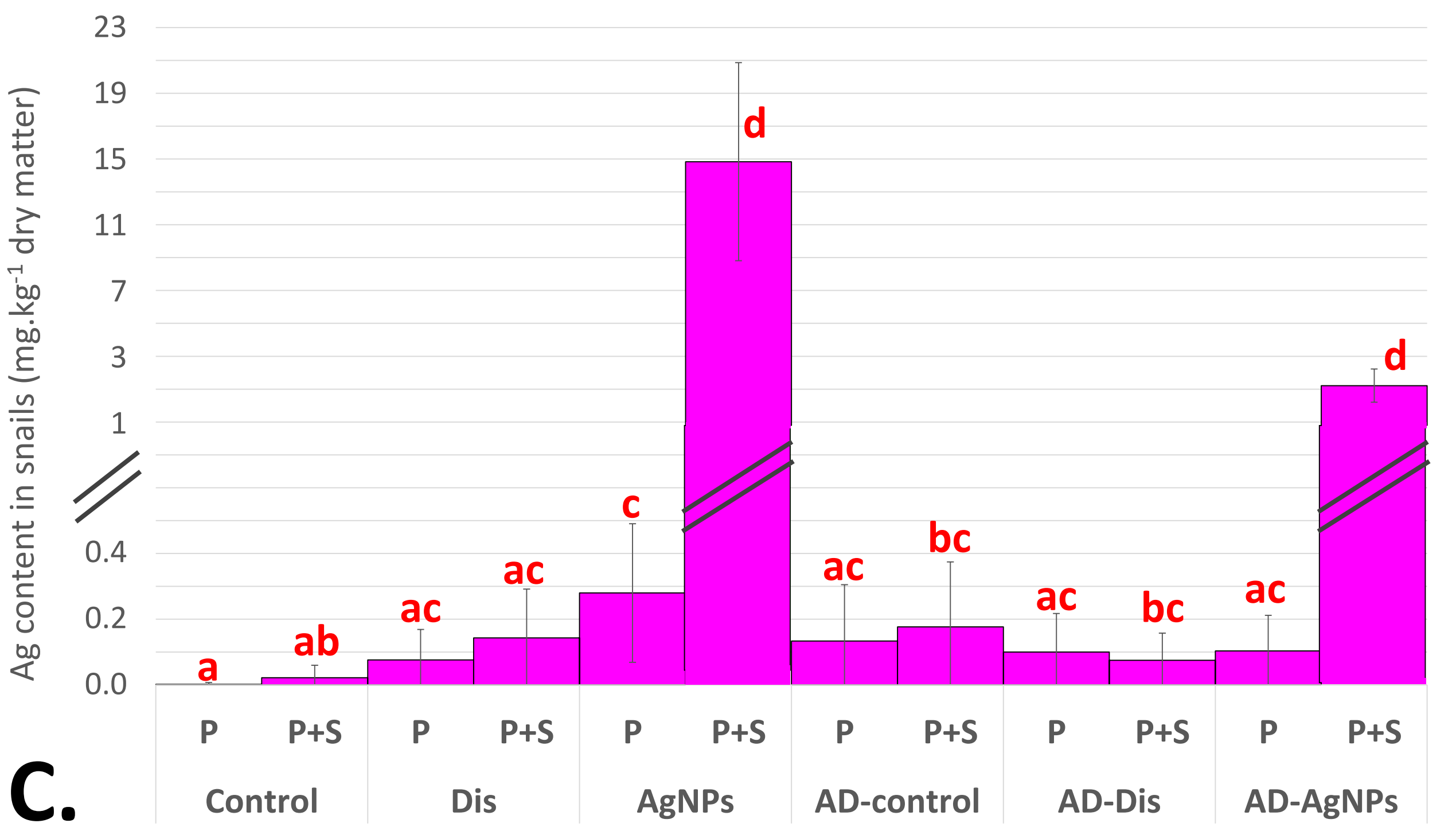
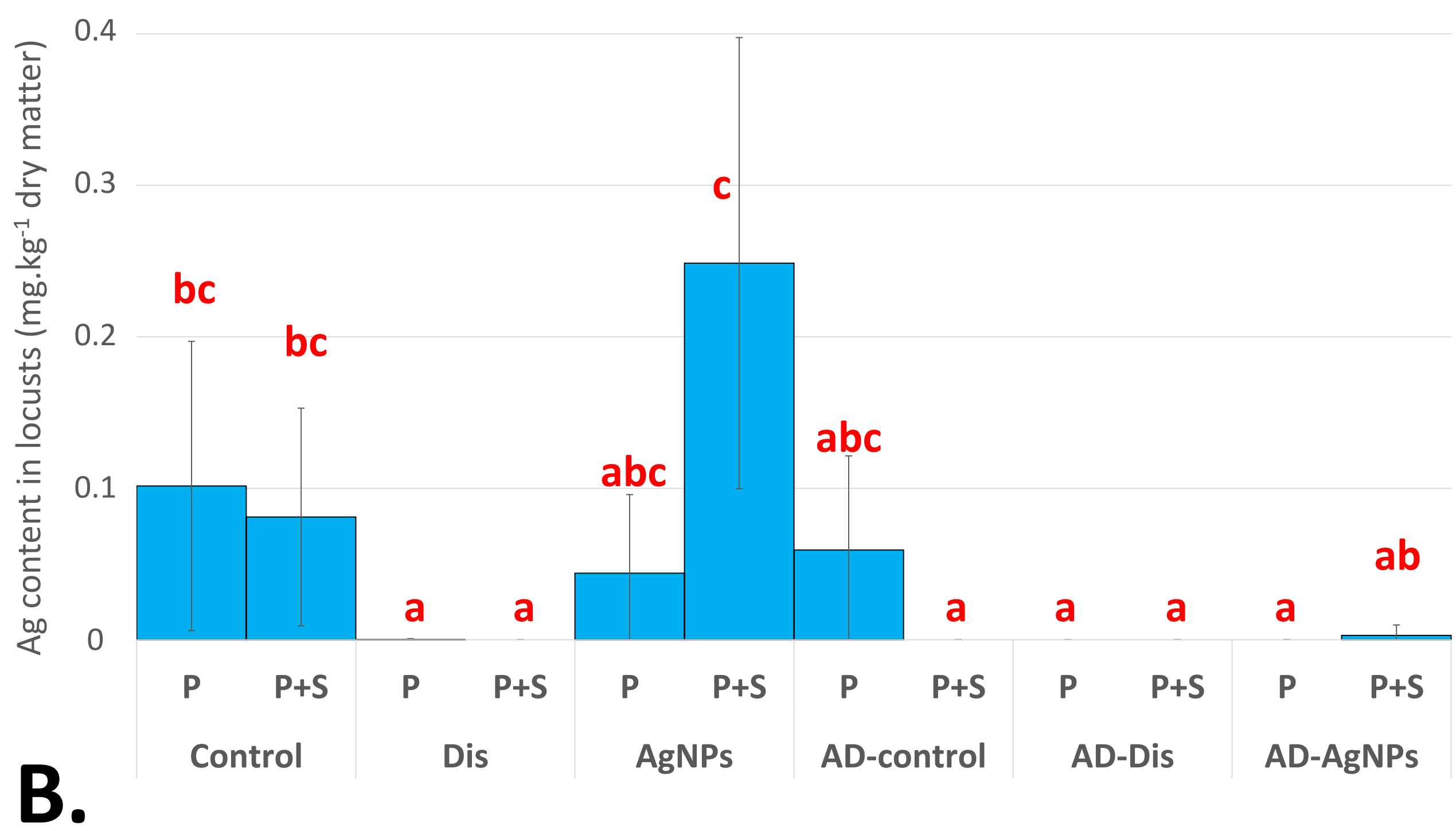
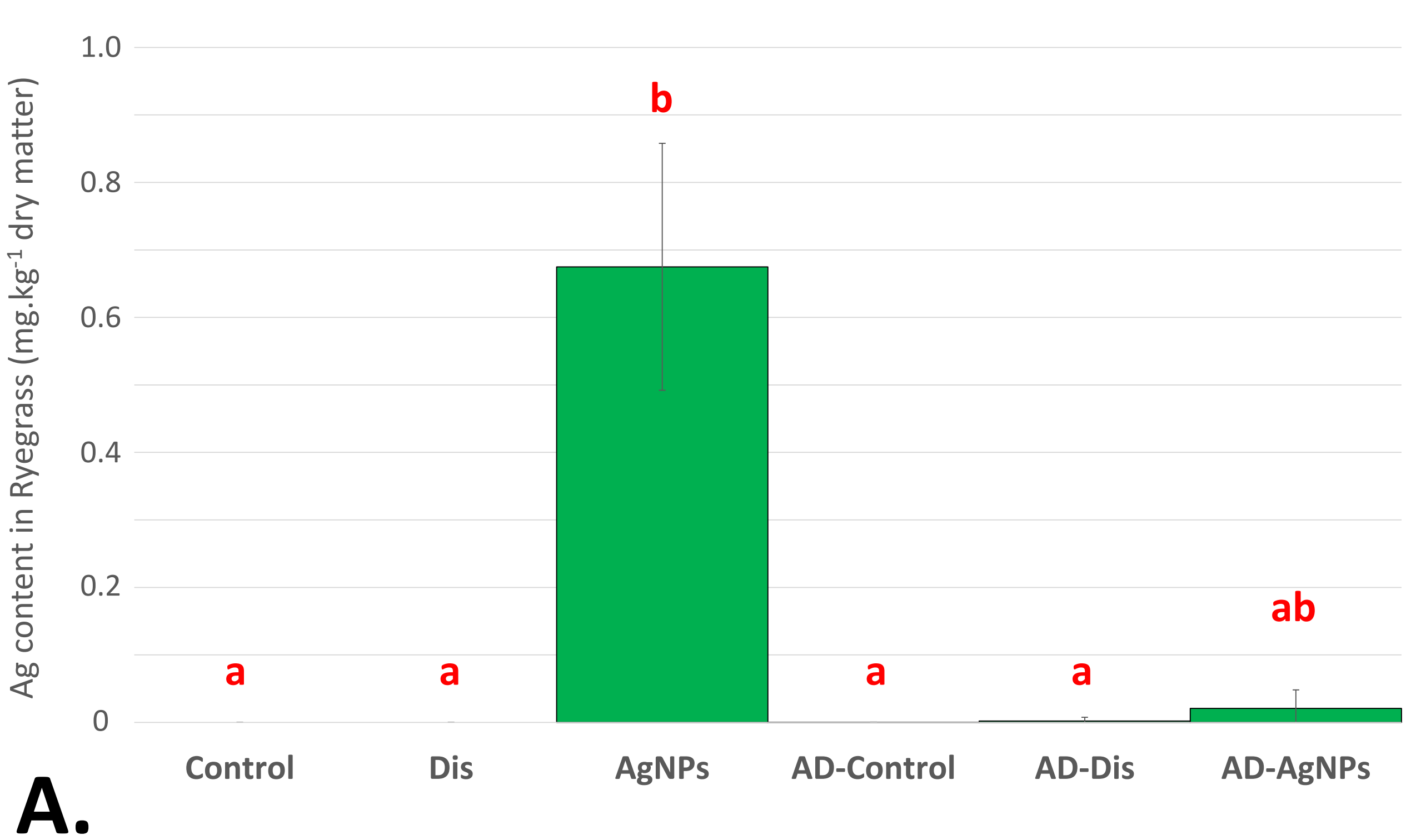
T1 T2 T3
Ryegrass cv2

T1 T2 T3
Wheat

T3
Clover

Mass of faeces (mg of dry matter, per snail)





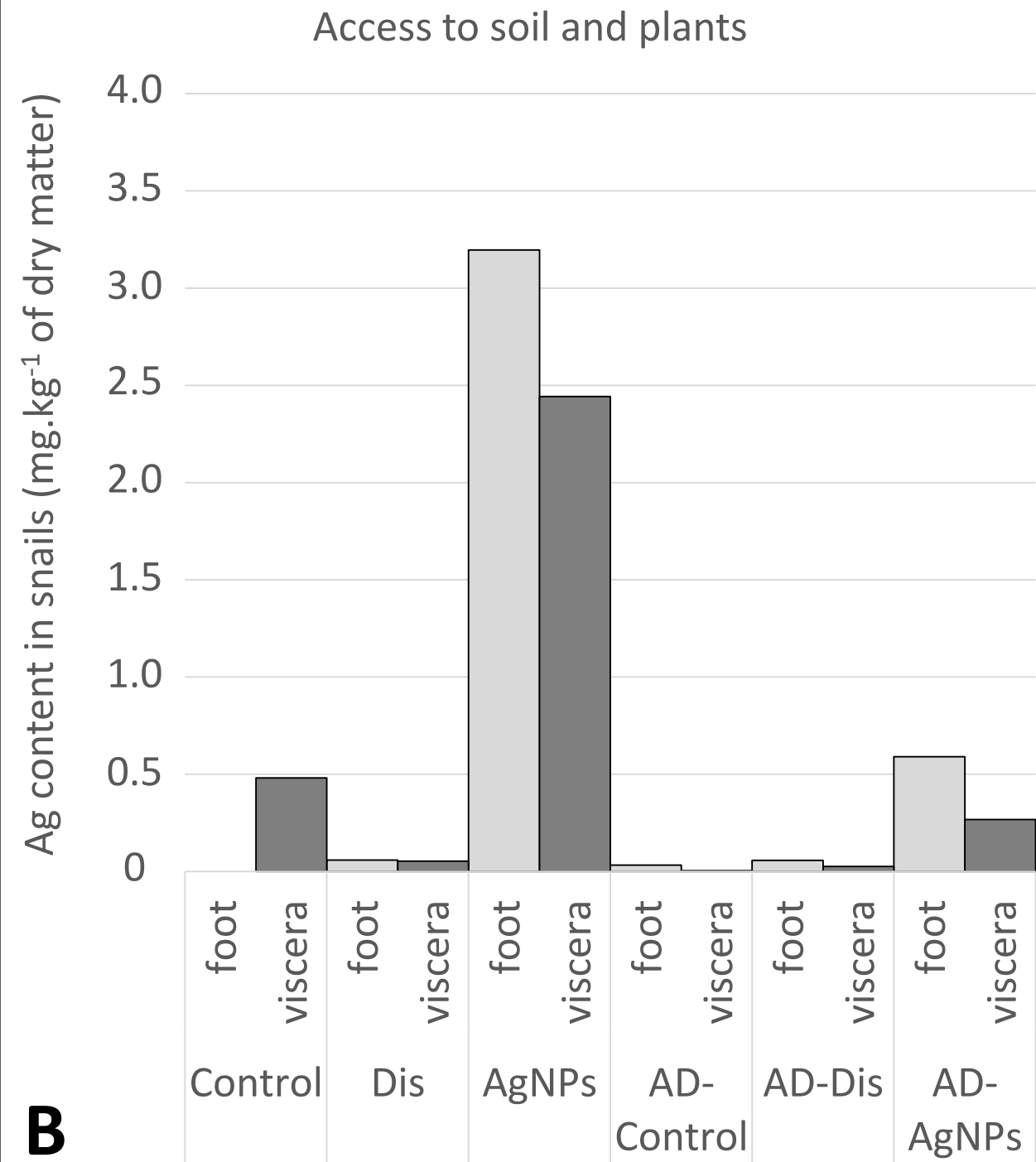
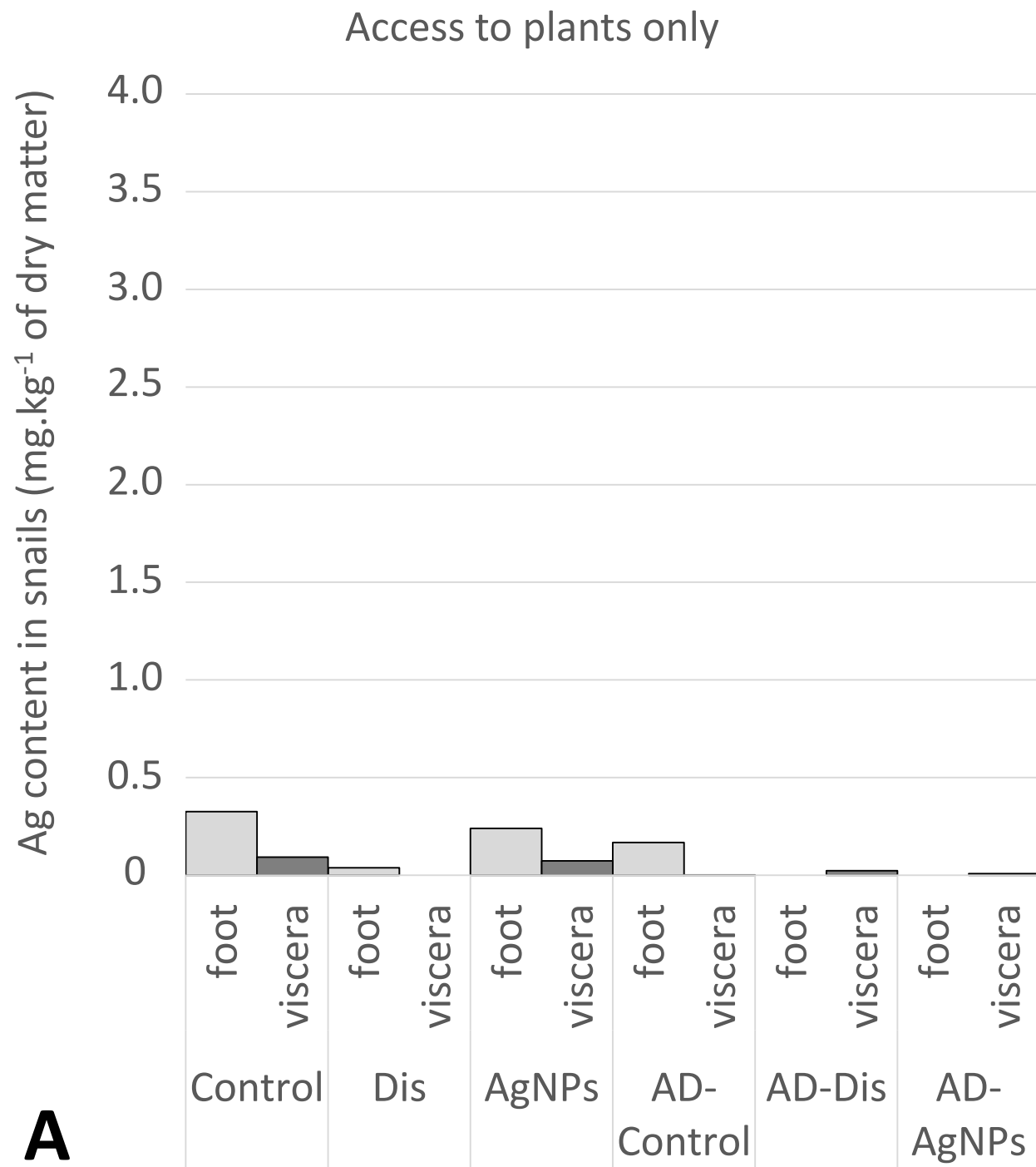


Table 1: Metals contents in animals (whole locusts and snails without shell) at the initial time point (after fasting) in mg kg⁻¹ (dry matter). <LOQ means under limit of quantification i.e. <0.4 µg L⁻¹ for Ag, <1,9 µg L⁻¹ for Pb and < 6,7 µg L⁻¹ for Sb.

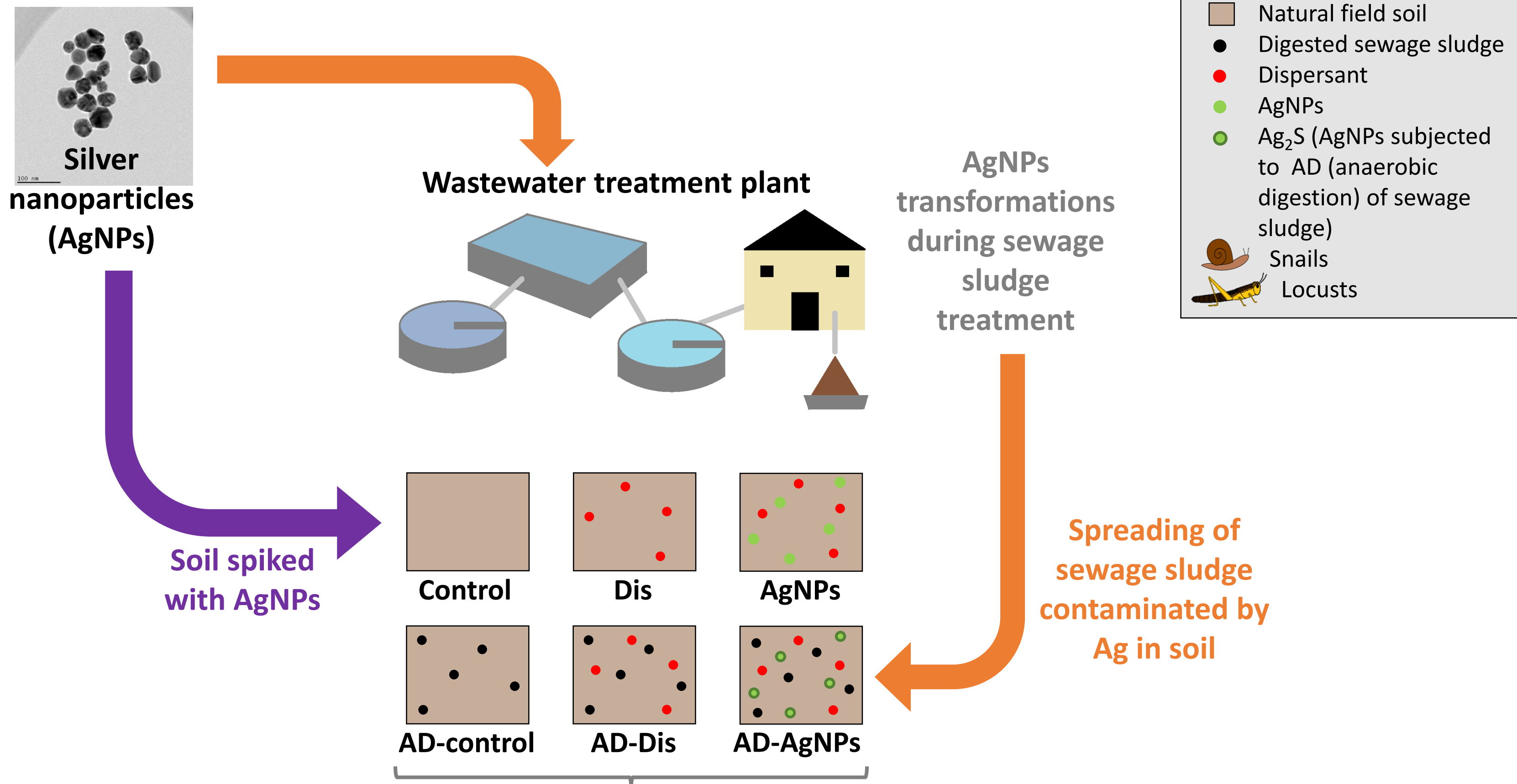
	Ag	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Zn
<i>C. aspersus</i>	<LO	0.828	0.760	1.100	1.828	152.8	0.058	92.20	0.640	1.924	<LO	0.013	310.9
	Q	±	±	±	±	36 ±	±	8 ±	±	±	Q	±	78 ±
		0.129	0.015	0.084	0.136	42.23 1	0.100	14.06 6	0.022	0.042		0.022	37.82 8
<i>L. migratoria</i>	<LO	0.825	0.045	0.043	1.622	44.00	0.024	6.688	1.357	1.116	0.128	<LO	130.0
	Q	±	±	±	±	1 ±	±	±	±	±	±	Q	64 ±
		0.122	0.041	0.038	1.540	2.573	0.042	1.600	0.865	0.865	0.201		10.12 7

Table 2: Metal concentration in the soil at the beginning of the experiment in the 6 mesocosm conditions (in mg kg⁻¹, in dry matter). Standard errors were indicated. Statistically significant results are indicated by stars. Red stars indicate that the silver concentrations were higher in the AgNPs and AD-AgNPs conditions than the others, although the Ag concentrations were significantly different between the AgNPs and AD-AgNPs conditions. Green stars indicate that the Zn concentrations were higher in the conditions with sludge than in the sludge-free conditions.

	Control	Dis	AgNPs	AD-control	AD-dis	AD-AgNPs
Ag	0.529 ± 0.340	0.576 ± 0.258	*11.150 ± 0.420	2.124 ± 0.252	1.903 ± 0.810	*6.993 ± 0.223
As	4.131 ± 0.128	4.168 ± 0.141	3.950 ± 0.059	4.374 ± 0.256	4.301 ± 0.157	3.888 ± 0.212
Cd	0.320 ± 0.061	0.308 ± 0.061	0.310 ± 0.027	0.296 ± 0.037	0.323 ± 0.029	0.304 ± 0.026
Co	4.173 ± 0.092	3.879 ± 0.042	3.789 ± 0.066	4.105 ± 0.117	4.102 ± 0.105	3.854 ± 0.188
Cr	25.675 ± 1.656	24.775 ± 0.834	24.300 ± 1.036	28.300 ± 1.089	26.550 ± 0.580	26.275 ± 1.150
Cu	18.675 ± 0.359	18.425 ± 0.050	17.475 ± 0.222	19.900 ± 0.141	19.775 ± 0.150	18.500 ± 0.424
Mn	336.175 ± 9.831	326.425 ± 22.948	307.775 ± 4.844	336.725 ± 0.974	337.250 ± 17.989	307.100 ± 15.112
Ni	11.450 ± 0.173	11.225 ± 0.050	10.950 ± 0.191	11.875 ± 0.096	11.900 ± 0.245	11.175 ± 0.275
Pb	52.725 ± 1.611	51.875 ± 0.957	51.150 ± 3.527	52.900 ± 0.173	52.333 ± 0.896	50.375 ± 2.251
Zn	49.775 ± 0.403	49.025 ± 0.450	47.200 ± 0.424	*76.350 ± 3.753	*72.125 ± 2.114	*69.100 ± 1.633

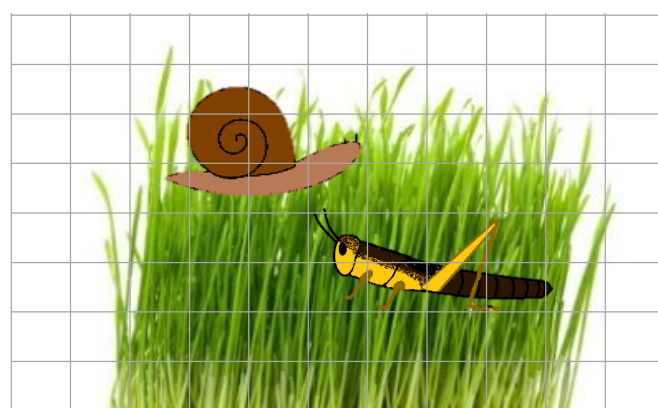
Table 3: Survival and biomass gained or lost between the beginning and end of the experiment. P means ‘access to plants only’, and P+S means ‘access to plants and soil’. Standard error were indicated. Statistical tests showed no correlation between the survival or the biomass and the Ag presence.

<u>Condition</u>	<u>Access</u>	<u>Snails</u>		<u>Locusts</u>	
		<u>Survival</u> <u>(%)</u>	<u>Gain/Loss of</u> <u>weight (%)</u>	<u>Survival</u> <u>(%)</u>	<u>Gain/Loss of</u> <u>weight (%)</u>
Control	P	76.67 ± 25.17	- 0.15 ± 0.12	86.67 ± 23.09	+ 0.06 ± 0.04
	P+S	73.33 ± 11.55	+ 0.04 ± 0.07	60.00 ± 20.00	+ 0.05 ± 0.20
AD-control	P	93.33 ± 11.55	- 0.17 ± 0.08	53.33 ± 23.09	+ 0.07 ± 0.09
	P+S	83.33 ± 15.28	+ 0.29 ± 0.04	53.33 ± 23.09	+ 0.16 ± 0.08
Dis	P	93.33 ± 5.77	- 0.11 ± 0.03	86.67 ± 23.09	0.00 ± 0.09
	P+S	63.33 ± 11.55	- 0.01 ± 0.23	46.67 ± 23.09	- 0.14 ± 0.08
AD-dis	P	83.33 ± 15.28	- 0.26 ± 0.27	60.00 ± 34.64	- 0.04 ± 0.05
	P+S	86.67 ± 15.28	+ 0.10 ± 0.02	60.00 ± 20.00	+ 0.06 ± 0.08
AgNPs	P	83.33 ± 15.28	- 0.15 ± 0.04	53.33 ± 41.63	+ 0.01 ± 0.14
	P+S	83.33 ± 15.28	+ 0.04 ± 0.07	66.67 ± 23.09	+ 0.09 ± 0.09
AD-AgNPs	P	66.67 ± 23.09	- 0.16 ± 0.16	46.67 ± 23.09	+ 0.12 ± 0.21
	P+S	76.67 ± 15.28	+ 0.32 ± 0.14	60.00 ± 20.00	+ 0.03 ± 0.17
Average		80.28 ± 15.94	- 0.02 ± 0.21	61.11 ± 24.82	+ 0.04 ± 0.13



RESULTS OF TRANSFER

Ryegrass:
significant bioaccumulation

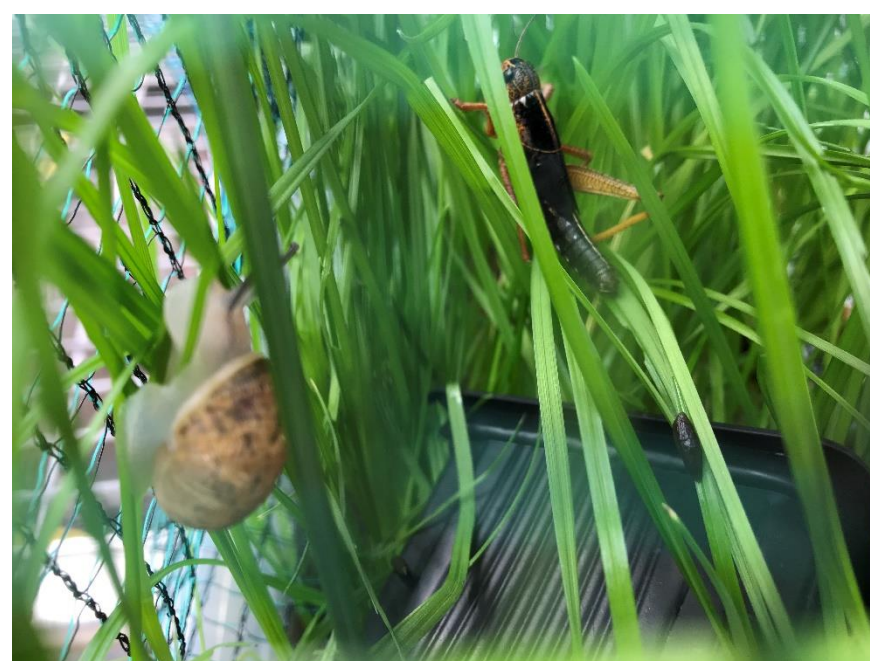


Exposure to
plants only

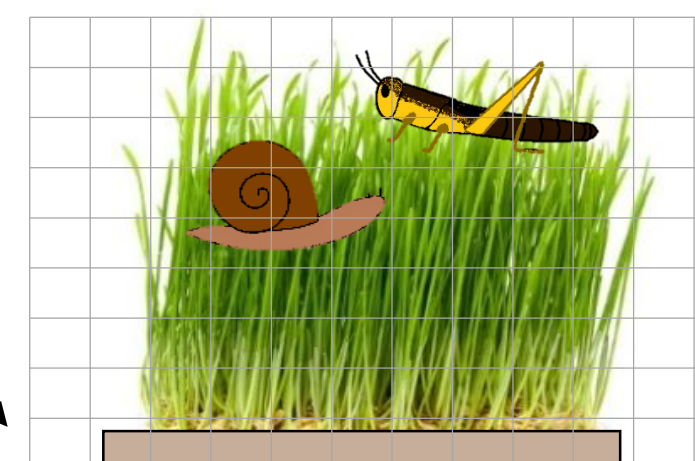
Locust: no Ag

Snail: Ag bioaccumulation ++
IF access to the soil

Sulfidized Ag is transferred to
the food chain
but at lower levels than AgNPs



Ryegrass:
weak but detectable bioaccumulation



Exposure to
plants and soil

Locust: no Ag

Snail: Ag bioaccumulation +
IF access to the soil