Canadian Journal of
Soil Science

OPEN ACCESS | Communication

Silver toxicity to the earthworm *Eisenia fetida* is inconsistent in natural and artificial soils amended with wastewater biosolids

Aboulkacem Lemtiri[a,b], **Yu Jia**[a], **Leanne Ejack**[a], **Ismail S. Akaram**[a], **Haroldo S. Dórea**[c], **Gilles Colinet**[b], **Frederic Francis**[d], **Subhasis Ghosal**[e], **Geoffrey I. Sunahara**[a], **and Joann K. Whalen**[a]

[a]Department of Natural Resource Sciences, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada; [b]Department of Biosystems Engineering, Soil-Water-Plant Exchanges, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium; [c]Departamento de Química, Universidade Federal de Sergipe Brazil, Instituto Nacional de Ciência e Tecnologia de Energia e Ambiente/INCT-E&A, 40110-040 Salvador, BA, Brazil; [d]Department of AgroBioChem, Functional and Evolutionary Entomology Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium; [e]Department of Civil Engineering, McGill University, Montreal, QC H3A 0C3, Canada

Corresponding author: **Joann K. Whalen** (email: joann.whalen@mcgill.ca)

Abstract

Silver nanoparticles (AgNPs) are present in biosolids from wastewater treatment facilities, a common soil amendment. Exposing earthworms (*Eisenia fetida*) to AgNP and $AgNO_3$ in soil with 0 and 7.5 g biosolids kg^{-1} for 28 days showed $AgNO_3$ was more lethal to earthworms in artificial soil (LC20 $\leq$ 325 mg Ag kg^{-1}) than natural soil (LC20 $\geq$ 573 mg Ag kg^{-1}). In contrast, AgNPs were more lethal in natural soil (LC20 $\leq$ 425 mg Ag kg^{-1}) than artificial soil (LC20 $\geq$ 653 mg Ag kg^{-1}). Earthworm response to silver in artificial soil may not reflect toxicity in biosolids-amended natural soils.

Key words: ecotoxicology, OECD soil, silver ions, silver nanoparticles, soil macrofauna, soil biota

Introduction

Silver nanoparticles (AgNPs) are antibacterial agents in personal care products, clothing, and cleaning equipment. Personal hygiene and washing will transfer AgNP to wastewater treatment systems. Land-spreading of wastewater-derived biosolids is a common practice that is likely to add AgNP to soil. Earthworms such as *Eisenia fetida* prefer soil containing biosolids over the same soil without biosolids based on their movement in Kaushik chambers (Bouldin et al. 2016), which suggests they will be exposed to AgNP in biosolids and on soil particles. Dissolution of AgNP produces Ag_2S with low bioavailability and Ag ions (Ag^+) that are lethal to earthworms (Shoutts-Wilson et al. 2011; Courtois et al. 2019).

Soil physicochemical properties such as pH, organic matter, and clay content influence the dissolution and sorption of AgNP in the soil matrix, affecting the AgNP exposure to earthworms. Earthworm bioassays using artificial soil recommended by the Organization for Economic Co-operation and Development (OECD) provide a standard response, but it may not represent the realistic soil physico-chemical environment of natural soil. The present study compares the effect of AgNP and Ag^+ ions (from $AgNO_3$) on *E. fetida* mortality in artificial (OECD) and natural soil (Delacour series), with and without biosolids amendment.

Materials and methods

Ag reagents and biosolids

Econix AgNPs (6.2×10^{12} particles mL^{-1} in Mill-Q Water; NanoComposix, San Diego, CA) were polyvinylpyrrolidone coated and colloidal with a particle hydrodynamic diameter of 72.4 ± 0.6 nm in deionized water by dynamic light scattering (ZetaSizer Nano ZS), and the ζ potential was -4.7 ± 0.5 mV in a $CaCO_3$ buffer. Silver nitrate ($AgNO_3$; 99.99% purity, Sigma–Aldrich) was prepared in distilled deionized water and diluted to a working stock with a nominal concentration of 10 μg mL^{-1} to prevent redox reactions. Silver solutions were kept in the dark until use within 24 h. All reagents and chemicals were obtained from commercial sources and were at least analytical grade purity.

Liquid biosolids were from a municipal wastewater treatment plant in Calgary, Alberta. Biosolids were class A (can be applied to agricultural fields) with 76.6 g solids kg^{-1}, pH 5.8, and total Kjeldahl N of 68.5 g kg^{-1} with 23.4 g NH_4-N kg^{-1}, 42.9 g P kg^{-1}, and 9 mg Ag kg^{-1}, all on a dry weight (DW) basis (Linares et al. 2020). Samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for total Ag content after digestion with 65% HNO_3.

Soil preparation

Natural soil was a Black Chernozem silt loam soil (360 g sand kg^{-1}, 129 g clay kg^{-1}) of the Delacour series from an agricultural field within 20 km of the biosolids source in Calgary, Alberta. Land-spreading of biosolids typically occurs on fields in proximity to the municipality to minimize the logistic and cost of disposing these wet, odorous materials. Natural soil contained 86 g organic matter kg^{-1}, 68 g organic C kg^{-1}, 6.0 g total N kg^{-1} with a field bulk density of 0.8 g cm^{-3}, and pH 7.7. Soil was air-dried, sieved (<2 mm mesh), and then stored at room temperature in closed opaque containers before the experiment.

Artificial soil contained 694 g silica kg^{-1}, 200 g kaolin clay kg^{-1}, 100 g peat kg^{-1} (<2 mm mesh), and 6 g $CaCO_3$ kg^{-1}. After preparation, the artificial soil was a sandy loam with 791 g sand kg^{-1}, 142 g clay kg^{-1} with 85 g organic matter kg^{-1}, 64 g organic C kg^{-1}, pH 6.2, and a bulk density of 1.1 g cm^{-3}. Additional details of the Delacour and OECD soils are reported by Linares et al. (2020).

Earthworm toxicity testing

The 28-day earthworm bioassay measured earthworm survival in an abbreviated standard toxicity test (OECD 2004) with proportionately less soil due to the cost of AgNP. The experimental unit was a 500 mL Mason jar containing 150 g of air-dried soil (natural or artificial). We added 1.12 g of wet biosolids to the biosolids-amended soil. The reference control (0 mg Ag kg^{-1} without biosolids) and vehicle control (0 mg Ag kg^{-1} with biosolids) were moistened with deionized water to 70% water holding capacity (WHC). Soils (with and without biosolids) were spiked with $AgNO_3$ or AgNP by adding the nominal solution (0, 4, 40, 400, and 800 mg Ag kg^{-1} DW soil) and mixing with a spatula while adding deionized water to reach 70% WHC. Eight positive controls were prepared, containing 1 g of biosolids mixed with either 6.3 g of boric acid kg^{-1} DW Delacour soil or 5.0 g of boric acid kg^{-1} DW OECD soil and moistened to 70% WHC. Preliminary testing confirmed 100% earthworm mortality with these boric acid concentrations. All jars were capped with an air-tight lid and left at 20 °C for 1 week. The experimental design had 2 soil types × 2 silver sources × 5 concentrations × 2 biosolids types, replicated 4 times, plus 8 positive controls, for a total of 168 experimental units (jars) that were arranged in a completely randomized block design.

Adult *E. fetida* earthworms with fully developed clitella were cultured on moist earthworm bedding in dark culture boxes at 20 ± 2 °C in the laboratory for at least 1 month before the experiment began and were fed weekly on a grain-based diet. Ten individuals (each weighing 200–650 mg) were added per jar, which was then covered with a perforated metal lid, wrapped in an aluminium foil, and placed in a dark environmental chamber at 20 ± 2 °C and approximately 65% ambient humidity. Food (0.5 g per earthworm) and water (to maintain 70% WHC) were added weekly.

Earthworm survival was determined after 28 days. The validity of the test was confirmed based on the 100% survival in the vehicle and reference controls, and 100% mortality in the positive controls. Living adults were rinsed and placed on moist filter paper for 48 h to void gut contents and then counted and frozen at −20 °C. Differences in earthworm survival between treatments were estimated by non-parametric probit curve fitting, using the 20% lethal concentration of AgNP or $AgNO_3$ (LC20 with 95% confidence interval (95% CI), expressed as mg Ag kg^{-1} dry soil) as an indicator of the Ag concentration that allows 80% survival compared to negative controls.

Results and discussion

Measured Ag concentrations in both soils were within ±3% of the nominal concentrations, and we recovered >95% Ag added to the soil (Table 1). In natural (Delacour) soil, AgNP had greater lethality than $AgNO_3$, but the reverse was true for artificial (OECD) soil, where $AgNO_3$ showed higher lethality (Fig. 1). Based on the 95% CI, the AgNP (no biosolids) was more lethal in the Delacour soil (LC20 in mg Ag kg^{-1} DW soil = 35 ± 76 (95% CI)) than in the OECD soil (LC20 = 777 ± 56 (95% CI); Fig. 1B). In contrast, the lethality of $AgNO_3$ (with biosolids) was lower in the Delacour soil (LC20 = 758 ± 74 (95% CI)) than the OECD soil (LC20 was close to zero; Fig. 1C). The toxicity of $AgNO_3$ with biosolids in the OECD soil could be the result of earthworms ingesting more of the soil-biosolids mixture, and thereby being exposed to Ag^+ that was not strongly bound to organo-mineral surfaces. The LC20 values ≤ 35 mg Ag kg^{-1} are more lethal than the LC50 of 144 mg Ag kg^{-1} for *E. fetida* exposed to AgNP in artificial soil reported by Garcia-Velasco et al. (2016). Earthworm survival was similar in Delacour soil and OECD soil in the AgNP and biosolids treatment, and when exposed to $AgNO_3$ and no biosolids (Figs. 1A and 1D).

The inconsistent earthworm response to Ag sources in artificial and natural soils (Fig. 1) contrasts with Shoutts-Wilson et al. (2011), who found that $AgNO_3$ was more toxic to *E. fetida* in natural soil versus artificial soil. They postulated that characteristics of the natural sandy loam soil (lower CEC, organic matter, clay content, and pH than the artificial soil) favored Ag^+ uptake in earthworms (Shoutts-Wilson et al. 2011). The natural soil in our study had a higher pH than the artificial soil, but similar organic matter content and lower clay content (Linares et al. 2020). Earthworm uptake of metal ions tends to be greater in soils with lower pH (Garcia-Velasco et al. 2016), which may make $AgNO_3$ less toxic in natural soil (pH 7.7) than artificial soil (pH 6.2), possibly contributing to the low LC20 value in the biosolid-amended OECD soil (Fig. 1C).

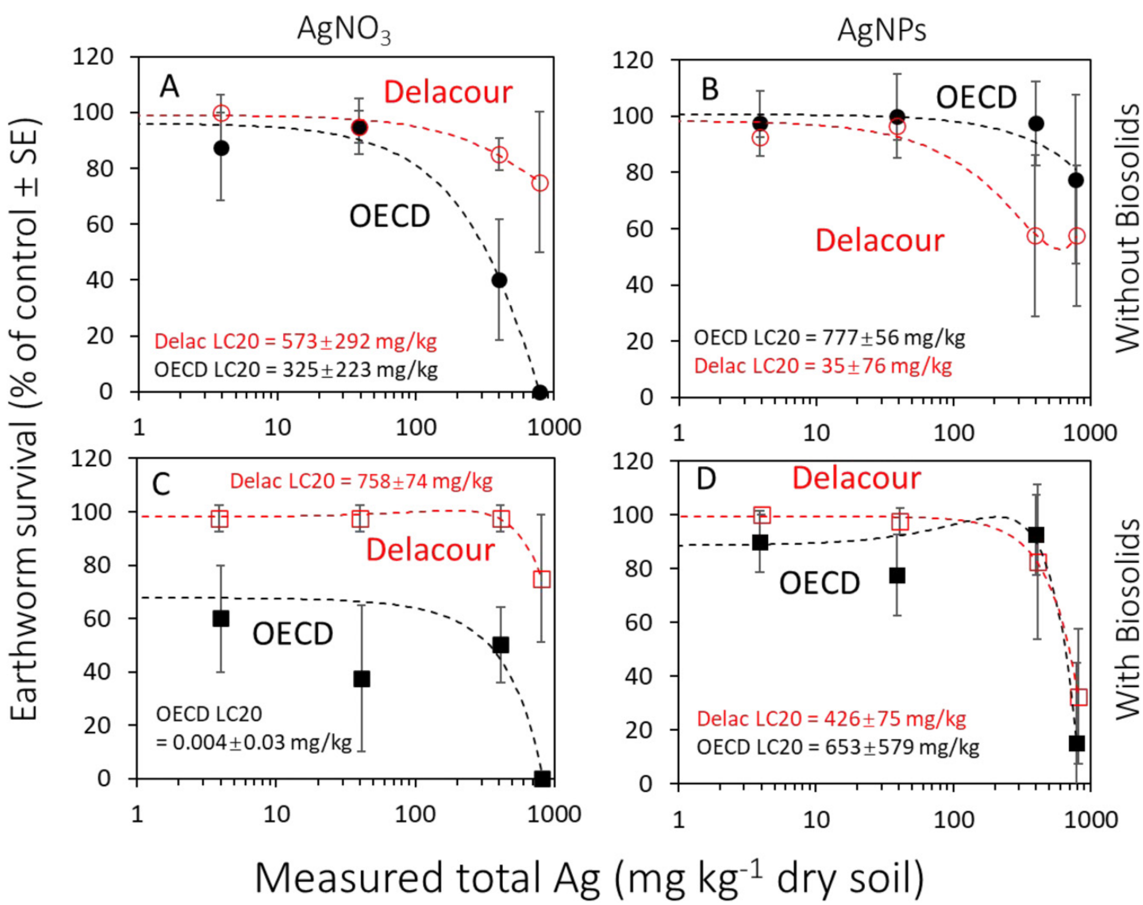
Earthworms absorb AgNP through ingestion of AgNP attached to soil colloids as well as dermal exposure, with the majority likely being through ingestion (Garcia-Velasco et al. 2016). However, it is unclear whether the toxicity of AgNP arises from its intrinsic properties, from its dissolution and release of Ag^+, or both (Shoutts-Wilson et al. 2011; Garcia-Velasco et al. 2016). In natural soil, mortality was higher with increasing concentrations of AgNP (≥400 mg Ag kg^{-1}, with an LC20 of 35 ± 76), but only in the absence of biosolids (Fig. 1). However, AgNP must dissolve to Ag^+ before toxic effects are observed, which can occur in the soil or after earthworms have absorbed AgNP into subcutaneous cells or in-

Table 1. Soil Ag concentration (mg total Ag kg^{-1} dry soil) used for the earthworm bioassay, showing the nominal and measured concentration in soils that received biosolids (+Biosolids) or had no biosolids (−Biosolids) before spiking with silver nanoparticles (AgNP) or $AgNO_3$ salt.

Nominal soil Ag (mg Ag kg^{-1} dry soil)	−Biosolids				+Biosolids			
	AgNP		$AgNO_3$		AgNP		$AgNO_3$	
	Delacour	OECD	Delacour	OECD	Delacour	OECD	Delacour	OECD
0	nd*	nd	nd	nd	0.1 ± 0.0†	nd	nd	nd
4	3.9 ± 0.0	4.0 ± 0.1	3.9 ± 0.2	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.1	3.9 ± 0.0	4.0 ± 0.0
40	39 ± 2	40 ± 1	39 ± 0.5	40 ± 1	41 ± 1.1	41 ± 0.0	40 ± 0.8	41 ± 3.0
400	395 ± 7	398 ± 9	401 ± 10	389 ± 8	408 ± 5	412 ± 11	410 ± 12	392 ± 12
800	796 ± 15	795 ± 21	785 ± 22	789 ± 20	812 ± 14	822 ± 20	803 ± 11	814 ± 27

Note: OECD, Organization for Economic Co-operation and Development.
*nd indicates not detectable at <1 ng Ag L^{-1}, using ICP-MS.
†Data are expressed as mean ± SE, $n = 4$.

Fig. 1. Survival of earthworms (*Eisenia fetida*) incubated in soils with different measured Ag concentrations (mg Ag kg^{-1} DW soil, given in Table 1) following 28-day exposure to $AgNO_3$ (panels (A) and (C)) or silver nanoparticles (AgNP, panels (B) and (D)) in Delacour natural field soil (red symbols; ○, □) and artificial Organization for Economic Co-operation and Development (OECD) soil (black symbols; •, ■). Earthworm survival (*n* replicates, each containing 10 earthworms) was measured without biosolid (panels (A) and (B)) or with biosolid amendments (panels (C) and (D)). All data points represent mean ± the 95% confidence interval ($n = 4$ replicates).



gested AgNP (Shoutts-Wilson et al. 2011). Courtois et al. (2021) found that Ag accumulation was similar in earthworms exposed to AgNP versus $AgNO_3$, but $AgNO_3$ (via Ag^+) was more toxic and more readily absorbed through dermal routes. As we do not know the Ag speciation in the soil or earthworm tissue, it is not possible to know how much Ag^+ was released into soil pore water through AgNP dissolution, nor how much dermal exposure occurred. Still, it is reassuring that measured LC20 values for earthworm mortality were generally in the 10^1 to 10^3 mg Ag kg^{-1} range, which are up to five orders of magnitude greater than the no-hazard level of 0.05 mg Ag kg^{-1} soil (Kulikova 2021). It seems unlikely that earthworms will be impacted by AgNP accumulation in natural soils, given that measured values are from 0.01 to 7.4 μg AgNP kg^{-1} soil and the projected input of AgNP from wastewater, biosolids, agrochemicals, and other sources is 1.2×10^{-3} to 9.7 μg AgNP kg^{-1} $year^{-1}$, according to a global analysis (Kulikova 2021).

Our findings are affected by the test procedure because earthworms were crowded in jars due to limited soil mass (150 g in this study vs. 500 g in the OECD bioassay). This could increase earthworm competition for food, causing them to ingest more soil containing AgNP or Ag, particularly when biosolids were added. Still, AgNP toxicity to earthworms is probably underestimated in the bioassay. For example, over a full year (52 weeks) of aging, earthworm toxicity in AgNP-spiked soils was up to 40 times greater than the toxicity response after 1 week of exposure (Diez-Ortiz et al. 2015). The toxicity of ionic Ag^+ decreased while the toxicity of AgNP increased with aging, suggesting a "convergence" of Ag^+ and AgNP toxicity based on equilibrium in the ionic and exchangeable Ag^+ pools, relative to the reduced, sorbed, and precipitated Ag forms (Diez-Ortiz et al. 2015). Aging the soil-biosolids mixture for up to 1 year after AgNP spiking may be a more rational method of evaluating AgNP toxicity to earthworms in biosolids-amended soil.

Conclusions

$AgNO_3$ lethality in earthworms tended to be higher in artificial OECD soil than in natural soil, with and without biosolids. The opposite was true for AgNP, which was more lethal in natural soil than in artificial soil, although only in the absence of biosolids. These discrepancies indicate that we must consider the variable properties of artificial versus natural soils (organic matter, cation exchange, soil colloids-clay, humic acid, texture, pH, etc.) and the multiple reactions that alter AgNP and Ag^+ bioavailability in short-term soil ecotoxicology experiments. Using artificial soil to test the potential toxicity to earthworms of Ag-containing biosolids may lead to inaccurate conclusions that affect guidelines related to amending agricultural soils with biosolids from wastewater treatment facilities.

Acknowledgements

Technical assistance was provided by Hicham Benslim and Hélène Lalande. We gratefully acknowledge the help of Flavio Piccapietra with the ICP-MS analysis. We thank the City of Calgary (Alberta, Canada) for supplying the biosolids.

Article information

History dates

Received: 13 May 2023
Accepted: 20 February 2024
Accepted manuscript online: 24 February 2024
Version of record online: 25 March 2024

Copyright

Data availability

Data are available on request to the corresponding author.

Author information

Author ORCIDs

Haroldo S. Dórea https://orcid.org/0000-0001-6943-3582
Gilles Colinet https://orcid.org/0000-0002-1850-5504
Frederic Francis https://orcid.org/0000-0001-7731-0849
Joann K. Whalen https://orcid.org/0000-0001-8774-0594

Author contributions

Conceptualization: SG, GIS, JKW
Data curation: JKW
Formal analysis: AL, YJ, LE, SG
Funding acquisition: JKW
Investigation: ISA, HSD, GIS, JKW
Methodology: ISA, HSD, GIS, JKW
Project administration: JKW
Resources: SG
Supervision: GC, FF, GIS, JKW
Validation: LE, GIS, JKW
Visualization: YJ, LE, GIS
Writing – original draft: AL
Writing – review & editing: YJ, LE, ISA, HSD, GC, FF, SG, GIS, JKW

Competing interests

The authors declare there are no competing interests.

Funding information

Financial support for this project was provided by the Canadian Municipal Water Consortium, a member of the Canadian Water Research Network, through grant MW2013-3.

References

Bouldin, J.L., Klaky, J.W.P., and Green, V.S. 2016. Earthworm preference bioassays to evaluate land management practices. Bull. Environ. Contam. Toxicol. **96**: 767–772. doi:10.1007/s00128-016-1744-4. PMID: 26873732.

Courtois, P., Rorat, A., Lemiere, S., Guyoneaud, R., Attard, E., Levard, C., and 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. Poll. **253**: 578–598. doi:10.1016/j.envpol.2019.07.053.

Courtois, P., Rorat, A., Lemiere, S., Levard, C., Chaurand, P., Grobelak, A., et al. 2021. Accumulation, speciation and localization of silver nanoparticles in the earthworm *Eisenia fetida*. Environ. Sci. Pollut. Res. **28**: 3756–3765. doi:10.1007/s11356-020-08548-z.

Diez-Ortiz, M., Lahive, E., George, S., Ter Schure, A., Van Gestel, C.A.M., Jurkschat, K., et al. 2015. Short-term soil bioassays may not reveal the full toxicity potential for nanomaterials; bioavailability and toxicity of silver ions ($AgNO_3$) and silver nanoparticles to earthworm

Eisenia fetida in long-term aged soils. Environ. Poll. **203**: 191–198. doi:10.1016/j.envpol.2015.03.033.

Garcia-Velasco, N., Gandariasbeitia, M., Irizar, A., and Soto, M. 2016. Uptake route and resulting toxicity of silver nanoparticles in *Eisenia fetida* earthworm exposed through standard OECD tests. Ecotoxicology, **25**: 1543–1555. doi:10.1007/s10646-016-1710-2. PMID: 27614742.

Kulikova, N.A. 2021. Silver nanoparticles in soil: input, transformation, and toxicity. Eurasian Soil Sci. **54**: 352–365. doi:10.1134/S1064229321030091.

Linares, M.G., Jia, Y., Sunahara, G.I., and Whalen, J.K. 2020. Barley (*Hordeum vulgare*) seedling growth declines with increasing exposure to silver nanoparticles in biosolid-amended soils. Can. J. Soil Sci. **100**: 189–197. doi:10.1139/cjss-2019-0135.

Organization for Economic Co-operation and Development (OECD). 2004. Test 222, earthworm reproduction test (*Eisenia fetida/Eisenia andrei*). OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

Shoults-Wilson, W.A., Reinsch, B.C., Tsyusko, O.V., Bertsch, P.M., Lowry, G.V., and Unrine, J.M. 2011. Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. Soil Sci. Soc. Am. J. **75**: 365–377. doi:10.2136/sssaj2010.0127nps.