

1 SUPPORTING INFORMATION

2 Ageing, dissolution and biogenic formation of nanoparticles, how do these factors
3 affect uptake kinetics of silver nanoparticles in earthworm?

4

5 M. Baccaro¹, A. K. Undas², J. de Vriendt¹, H. J. van den Berg¹, R. Peters², N. W. van
6 den Brink¹

7 ¹Division of Toxicology, Wageningen University, 6708 WE Wageningen, The
8 Netherlands

9 ²RIKILT, Wageningen University, 6708 WB Wageningen, The Netherlands

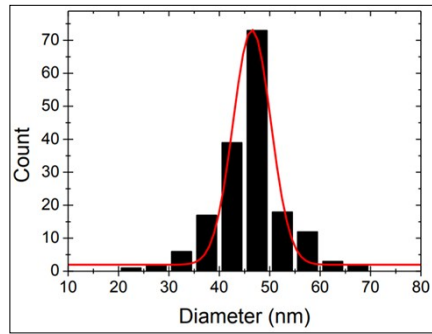
10 E-mail: marta.baccaro@wur.nl

11

12 S1 Synthesis and characterization of Ag-NP

13 Silver nitrate (AgNO₃), trisodium citrate (Na₃C₆H₅O₇), and tannic acid (C₇₆H₅₂O₄₆) were
14 purchased from Sigma Aldrich. Briefly, 100 mL volume of aqueous solution containing
15 sodium citrate (SC) (5 mM) and tannic acid (TA) (0.25 μM) was prepared and heated
16 by a heating mantle in a three-neck round-bottomed flask for 15 min under vigorous
17 stirring. After boiling had commenced, 1 mL of AgNO₃ (25 mM) was injected into this
18 solution. The solution became bright yellow. Immediately after the synthesis of Ag
19 seeds and in the same vessel, solution was diluted by extracting 19.5 mL of sample
20 and adding 16.5 mL of MilliQ water. Then, the temperature of the solution was set to
21 90 °C and 500 μL of SC (25 mM), 1.5 mL of TA (2.5 mM), and 1 mL of AgNO₃ were
22 sequentially injected (time delay ~ 1 min). By repeating and adjusting the amount of
23 Ag precursor injected, different generations of Ag-NPs of progressively larger sizes
24 were grown. Aliquots were purified by centrifugation (10000 g) in order to remove the
25 excess of TA and further redispersed in SC 2.2 mM before sample characterization

26 (Figure S1a, Table S1a). EDX on individual particle (Table S1b) shows that particles
 27 contain Ag (spectrum 11 within the particle) with a small layer of covering film
 28 (spectrum 12, close to the edge of the particle) which is similar in composition to the
 29 surrounding matrix (holey carbon film and additives). No sulphur could be detected.



30

31 Figure S1a. Ag-NPs size distribution based on TEM analysis (particle number=318)

32 Table S1a. Mean diameter by intensity distribution, polydispersity Index and Z-potential

33 measured by Dynamic Light Scattering (DLS)

	Diameter mean (nm)	Standard Deviation (nm)
Intensity	72.94	2.02
Polydispersity Index	0.137	0.024
	mV	Standard Deviation (mV)
Z-potential	-57.9	1.2

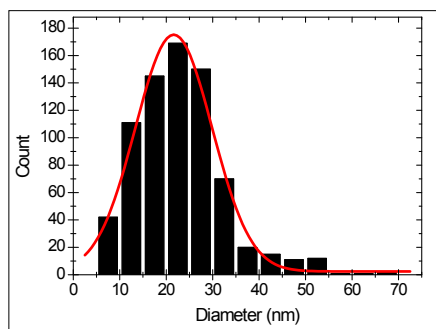
34

35 Table S1b. Particle elemental composition characterization by TEM-EDX

	Spectrum Label	Spectrum 12	Spectrum 11
	C	91.23	81.08
	O	0.89	1.97
	Si	0.22	0.35
	Ag*	3.22	10.41
*Signal from the particles is relatively small due to the reduced penetration depth.			

36 Synthesis and characterization of Ag₂S-NP

37 Briefly, a concentrated solution of AgNO₃ precursor was injected to 1 L volume of
38 aqueous solution containing Na₂S * 9H₂O and PVP 55 kDa under vigorous stirring and
39 [AgNO₃]/[PVP] ratio to get the desired size. The solution became dark grey
40 immediately and it was kept at synthesis temperature for 15 min to ensure complete
41 reaction of the precursors. Resultant Ag₂S nanoparticles were purified by
42 centrifugation (1000 g) in order to remove the excess of S²⁻ and further redispersed in
43 MilliQ water with the same PVP (1 mg/mL) before sample characterization (Figure S1b,
44 Table S1c). EDX on individual particles (Table S1d) shows clear difference between
45 mostly unreacted Ag (spectrum 16) and Ag₂S (spectrum 17) in the Ag/S ratio. This
46 indicates that not all of the Ag-NPs were converted to Ag₂S. Spectrum 18 is the
47 surrounding matrix.



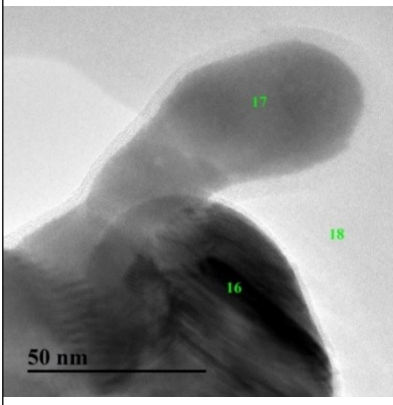
48

49 Figure S1b. Ag-NPs size distribution based on TEM analysis (particle number=759)

50 Table S1c. Mean diameter by intensity distribution, polydispersity Index and Z-potential
51 measured by Dynamic Light Scattering (DLS)

	Diameter mean (nm)	Standard Deviation (nm)
Intensity	302.8	1.1
Polydispersity Index	0.85	0.03
	mV	Standard Deviation (mV)
Z-potential	-2.19	0.14

52 Table S1d. Particle elemental composition characterization by TEM-EDX

	Spectrum Label	Spectrum 16	Spectrum 17	Spectrum 18
	C	72.81	87.6	95.55
	O	2.82	0	2.35
	Si	0.99	0.5	0
	S	0.22	2.36	0
	Ag*	15.69	5.42	0
	Ag/S ratio	72	2.3	n/a

*Ag signal from the particles is relatively small due to the reduced penetration depth.

53

54

55 S2 Soil characterization

56 Table S2. Characterization of natural soil

Parameter	Value	Unit
Median granular size	115	µm
Total nitrogen	1380	mg N kg ⁻¹
Potassium	29	mg K kg ⁻¹
pH (in water)	5.2	-
Organic matter	5.4	%
CaCO ₃	0.2	%

57

58 S3 Statistical analysis

59 Table S3a. Post hoc Tukey multiple comparison of test between particulate (≥ 20nm)

60 and total Ag concentrations of all time points in soil treated with Ag-NPs, AgNO₃ and

61 Ag₂S-NPs following one way ANOVA (F (5, 18) = 19.26)). Positive confidence interval

62 indicates that concentrations are higher in first factor, and vice versa.

	Mean Diff.	99.90 % CI of diff.	P Value
Particulate Ag-NP vs. Particulate AgNO ₃	-0.09551	-0.782 to 0.591	0.9930
Particulate Ag-NP vs. Particulate Ag ₂ S-NP	-0.451	-1.137 to 0.2355	0.1472

Particulate AgNO ₃ vs. Particulate Ag ₂ S-NP	-0.3555	-1.042 to 0.331	0.3538
Particulate Ag-NP vs. Total Ag-NP	-0.7678	-1.454 to -0.08138	0.0037
Particulate AgNO ₃ vs. Total AgNO ₃	-0.6875	-1.374 to -0.000996	0.0099
Particulate Ag ₂ S-NP vs. Total Ag ₂ S	0.07815	-0.6083 to 0.7646	0.9972
Total Ag-NP vs. Total AgNO ₃	-0.01512	-0.7016 to 0.6713	>0.9999
Total Ag-NP vs. Particulate Ag ₂ S NP	0.3169	-0.3696 to 1.003	0.4733
Total Ag-NP vs. Total Ag ₂ S	0.395	-0.2914 to 1.081	0.2521

63

64 Table S3b. Post hoc Tukey multiple comparison test between concentrations of
65 different forms of Ag in worms exposed to Ag-NPs, AgNO₃ or Ag₂S-NPs
66 (concentrations at 28 days of exposure) following one way ANOVA (F (5, 17) = 194.6).

	Mean Diff.	99.90 % CI of diff.	P value
Particulate Ag-NP vs. Total Ag-NP	-6.301	-7.682 to -4.92	<0.0001
Particulate Ag-NP vs. Particulate AgNO ₃	0.2992	-1.082 to 1.68	0.9803
Particulate Ag-NP vs. Total AgNO ₃	-6.628	-8.01 to -5.247	<0.0001
Particulate Ag-NP vs. Particulate Ag ₂ S-NP	2.084	0.703 to 3.465	0.0018
Particulate Ag-NP vs. Total Ag ₂ S-NP	1.759	0.378 to 3.14	0.0086
Total Ag-NP vs. Particulate AgNO ₃	6.600	5.321 to 7.879	<0.0001
Total Ag-NP vs. Total AgNO ₃	-0.3275	-1.606 to 0.9512	0.9599
Total Ag-NP vs. Particulate Ag ₂ S-NP	8.385	7.106 to 9.6644	<0.0001
Total Ag-NP vs. Total Ag ₂ S-NP	8.060	6.781 to 9.339	<0.0001
Particulate AgNO ₃ vs. Total AgNO ₃	-6.928	-8.206 to -5.649	<0.0001
Particulate AgNO ₃ vs. Particulate Ag ₂ S-NP	1.785	0.5063 to 3.064	0.0039
Particulate AgNO ₃ vs. Total Ag ₂ S-NP	1.460	0.1813 to 2.739	0.0203
Total AgNO ₃ vs. Particulate Ag ₂ S-NP	8.713	7.434 to 9.991	<0.0001
Total AgNO ₃ vs. Total Ag ₂ S-NP	7.109	7.109 to 9.666	<0.0001
Particulate Ag ₂ S-NP vs. Total Ag ₂ S-NP	-0.325	-1.604 to 0.9537	0.9611

67

68 S4 Kinetic of uptake and elimination

69 Table S4. Concentrations of total Ag and particulate Ag (≥ 20 nm) measured in
 70 earthworms at different time points during uptake phase and depuration phase (mg Ag
 71 kg^{-1} wet body weight; n=4; mean \pm standard deviation).

		Uptake phase (days)			
Ag form		7	14	21	28
Ag-NPs	Particulate Ag	0.23 \pm 0.14	0.37 \pm 0.50	2.71 \pm 2.03	2.26 \pm 1.02
	Total Ag	3.47 \pm 0.28	5.64 \pm 1.53	10.54 \pm 1.98	8.53 \pm 0.77
Ag ₂ S-NPs	Particulate Ag	0.06 \pm 0.03	0.12 \pm 0.03	0.12 \pm 0.06	0.08 \pm 0.03
	Total Ag	0.19 \pm 0.06	0.29 \pm 0.05	0.27 \pm 0.02	0.50 \pm 0.12
AgNO ₃	Particulate Ag	0.10 \pm 0.06	0.31 \pm 0.26	0.78 \pm 0.58	1.96 \pm 0.61
	Total Ag	3.08 \pm 0.34	5.52 \pm 0.77	10.20 \pm 3.03	8.89 \pm 0.56

		Depuration phase (days)			
Ag form		35	42	49	56
Ag-NPs	Particulate Ag	0.24 \pm 0.09	0.43 \pm 0.09	0.54 \pm 0.48	0.95 \pm 0.17
	Total Ag	4.91 \pm 2.68	4.56 \pm 0.59	6.35 \pm 0.90	2.91 \pm 0.15
Ag ₂ S-NPs	Particulate Ag	0.09 \pm 0.05	0.04 \pm 0.02	0.04 \pm 0.02	0.06 \pm 0.02
	Total Ag	0.14 \pm 0.04	0.16 \pm 0.01	0.12 \pm 0.03	0.15 \pm 0.02
AgNO ₃	Particulate Ag	0.23 \pm 0.17	0.07 \pm 0.03	0.41 \pm 0.13	0.54 \pm 0.18
	Total Ag	3.75 \pm 1.68	2.36 \pm 0.41	6.28 \pm 0.98	3.45 \pm 0.64

72

73 S5 Potential dissolution and formation of particulate Ag during TMAH incubation

74 In order to assess any dissolution of nanoparticles during extraction by TMAH, three
 75 different amount of Ag-NP and Ag₂S-NPs were incubated with a solution of TMAH 20%
 76 overnight (final concentration 38 ng Ag L⁻¹, 100 ng Ag L⁻¹, 380 ng Ag L⁻¹). Analysis by
 77 spICP-MS showed a recovery of 102% and 97% for Ag-NP and Ag₂S-NP, respectively.
 78 Additionally, to assess potential spontaneous formation of nano-Ag during extraction
 79 step by TMAH, three different amounts of AgNO₃ (45 μ L, 90 μ L, 135 μ L of solution of 1
 80 mg mL⁻¹) were added to \approx 0.4 g of clean earthworms tissue and incubated following the
 81 same TMAH extraction procedure of the samples. SpICP-MS performed on these

82 samples measured 0.04 %, 0.02 % 0.05 % nano-Ag compared to the total Ag content
83 indicating negligible formation of particulate Ag during incubation.