

## Supplementary Information

### **Toxicokinetics of Ag (nano)materials in the soil model *Enchytraeus crypticus* (Oligochaeta) – impact of aging and concentration**

Fátima C.F. Santos<sup>1</sup>, Paula S. Tourinho<sup>1</sup>, Janeck J. Scott-Fordsmand<sup>2</sup>, Cornelis A.M. van Gestel<sup>3</sup> and Mónica J.B. Amorim<sup>1\*</sup>

<sup>1</sup>Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup>Department of Bioscience, Aarhus University, Silkeborg, Denmark

<sup>3</sup>Department of Ecological Science. Faculty of Science, Vrije Universiteit Amsterdam De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

\* corresponding author:

Mónica J.B. Amorim

Department of Biology & CESAM, University of Aveiro

Campus de Santiago, 3810-193 Aveiro, Portugal

E-mail: [mjamorim@ua.pt](mailto:mjamorim@ua.pt)

**Table S1:** Total and 0.01 M CaCl<sub>2</sub>-extractable concentrations measured at days 0, 1, and 14 (mean ± SE) of the bioaccumulation tests with *Enchytraeus crypticus* in LUFA 2.2 soil spiked with AgNO<sub>3</sub> and Ag NM300K. Also included are nominal concentrations of Ag, aging period, and pH (0.01 M CaCl<sub>2</sub>) at days 0 and 28. [% recovery]: % recovery compared to nominal Ag concentration. LOD: Limit of Detection for soil and for 0.01M CaCl<sub>2</sub>-extractable concentrations (0.003 mg Ag/kg soil DW).

Ag	Aging (days)	Nominal (mg Ag/kg soil DW)	pH		Measured total (mg Ag/kg soil DW) [% recovery]			CaCl <sub>2</sub> -extractable (mg Ag/kg soil DW)		
			day 0	day 28	day 0	day 1	day 14	day 0	day 1	day 14
AgNO <sub>3</sub>	3	0	5.3	5.7	-	<LOD	<LOD	-	<LOD	<LOD
	3	5	5.5	5.7	-	4.1 ± 0.13 [83%]	4.2 ± 0.18 [84%]	-	0.04 ± 0	0.005 ± 0
	3	45	5.4	5.7	41.4 ± 2.9 [92%]	33.8 ± 2.1 [75%]	39.7 ± 0.36 [88%]	0.13 ± 0.03	0.2 ± 0.02	0.08 ± 0.05
	14	0	5.6	5.6	0.078 ± 0.08	-	0.85 ± 0.8	0.003 ± 0.002	-	0.01 ± 0.002
	14	5	5.6	5.6	4.7 ± 0.22 [94%]	4.5 ± 0.36 [91%]	4.3 ± 0.12 [87%]	0.003 ± 0.002	0.005 ± 0	0.007 ± 0
	14	45	5.6	5.6	37.9 ± 2.6 [84%]	39.3 ± 0.8 [87%]	35.4 ± 2.9 [79%]	0.10 ± 0.03	0.15 ± 0.02	0.02 ± 0.01
NM300K	3	0	5.9	5.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	6	5.7	5.8	4.4 ± 0.06 [74%]	4.9 ± 0.3 [82%]	4.2 ± 0.3 [70%]	<LOD	0.003 ± 0.002	<LOD
	3	60	5.6	5.8	55.5 ± 3.5 [92%]	49.9 ± 3.4 [83%]	44.9 [74%]	0.17 ± 0.03	0.11 ± 0.008	0.215 ± 0
	14	0	5.9	5.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	14	6	5.9	5.8	-	4.7 ± 0.38 [79%]	4.5 ± 0.3 [75%]	-	<LOD	0.005 ± 0.08
	14	60	5.8	5.8	53.5 ± 4.02 [89%]	49.4 ± 6.7 [82%]	44.8 ± 1.6 [75%]	0.17 ± 0.04	0.2 ± 0.12	0.1 ± 0.03

**Table S2:** Summary of literature review of toxicokinetic studies with Silver (Ag) salts and Ag nanomaterials (NMs), in enchytraeids and earthworms exposed in different conditions.

Species	Exposure time (days)	Test Soil	Main soil characteristics	Test Substance (nominal conc) (mg/kg soil DW)	Abiotic factors	K <sub>a</sub> (kg soil/kg animal/day)	K <sub>e</sub> (per day)	C <sub>exp</sub> (mg /kg soil)		Reference
								Soil	CaCl <sub>2</sub>	
<i>Enchytraeus crypticus</i>	10	Quartz sand, pre-treated to obtain an inert matrix	pH=6.0; test medium: Ca (NO <sub>3</sub> ) <sub>2</sub> , MgSO <sub>4</sub> 7H <sub>2</sub> O, NaNO <sub>3</sub> , KNO <sub>3</sub> in deionized H <sub>2</sub> O	AgNP-PVP (1.56-50 mg/L)	20±1°C	45.8±8.01	0.071±0.069	-	-	1
				AgNP-Cit (1.56-50 mg/L)		3.62±1.46	0.231±0.151	-	-	
				AgNO <sub>3</sub> (0.5-16 mg/L)		49.5±18.5	0.259±0.190	-	-	
<i>Enchytraeus crypticus</i>	20	LUFA 2.2	pH=5.5; OC=3.5 %; CEC=9.7 cmolc/kg	40 mg/kg Ag NPs	20°C+ 75 % Humidity	0.22	0.49	39.5	-	2
<i>Eisenia fetida</i>	56	Uncontaminated soil;	pH=5.2; OM=5.4 %	Ag-NPs (15 mg/kg)	20±1°C + 47% WHC	0.061 ± 0.019	0.040 ± 0.013	9.0 ± 1.4	40.5 ± 5.4	3
				Ag <sub>2</sub> S-NPs (15 mg/kg)		0.008 ± 0.002	0.064 ± 0.020	3.7 ± 1.1	n.d.	
				AgNO <sub>3</sub> (15 mg/kg)		0.055 ± 0.007	0.044 ± 0.018	9.3 ± 0.6	37.9 ± 5.4	
<i>Eisenia andrei</i>	42	Soil amendment, no biosolids	pH=5.88 pH=6.76	AgNO <sub>3</sub>	20±3°C	0.22 (0.09–0.35)	0.31 (0.09–0.52)	3.90±0.19	-	4
				Ag NPs (20 nm)		1.51 (0–4.69)	1.54 (0–4.95)	1.09±0.17	-	
				Ag NPs (40 nm)		-	-	77.95±2.78	-	
<i>Folsomia candida</i>	28	LUFA 2.2	pH= 5.5; OC=2.09%; CEC=10.0 meq/100g; WHC=46.5%	AgNO <sub>3</sub> (30 mg/kg)	20°C+ 50% WHC	0.177 (0.119–0.234)	0.036 (0.003–0.69)	29.2 ± 3.11	0.021 ± 0.002	5
				AgNO <sub>3</sub> (60mg/kg)		0.090 (0.062–0.119)	0.014 (0.014–0.42)	53.3 ± 1.47	0.048 ± 0.004	
				Ag NPs		0.106 (0.075–0.138)	0.095 (0.051–0.138)	168 ± 3.02	0.138	
<i>Lumbricus rubellus</i>	7	LUFA 2.2 loam sand	pH=5.5±1.1; OC=2.1±0.4 w/w %; CEC=10±0.5 mEq 100 g <sup>-1</sup>	100 mg/kg Ag NPs	12±1°C+ 45% WHC	0.002 (4x10 <sup>-4</sup> –0.004)	0.026 (0–0.055)	84.9±33.8	0.03±0.01	6
				500 mg/kg Ag NPs		0.015 (0.002–0.033)	0.160 (0–0.350)	431±115	0.26±0.02	
				20 mg/kg AgNO <sub>3</sub>		0.03 (0.02–0.04)	0.031 (0.01–0.05)	23.5±2.48	0.024±0.001	
				100 mg/kg AgNO <sub>3</sub>		0.008 (0.004–0.011)	0.022 (0.009–0.035)	101±4.42	0.044±0.001	
<i>Lumbricus rubellus</i>	28	Field soil, Netherlands	pH=5.0; OM=4.3%	1.5 Ag NPs (NM300K)	1°C+ 61 %	-	-	1.2±0.03	<3	7
				15.4 Ag NPs (NM300K)		-	-	10.5±0.2	<3	

				154 Ag NPs (NM300K)		-	-	118±4	96±6	
				15.4 mg/kg AgNO <sub>3</sub>		-	-	11.5±0.1	16±7	
<i>Tenebrio molitor</i>	42	LUFA 2.2	pH=5.4; OC=1.61%; CEC=9.7 cmolc/kg	100 mg Ag/kg AgNO <sub>3</sub>	20°C +	0.32 (0–0.635)	0.81 (0–1.99)	96.7±15.3	0.40±0.01	8
				100 mg Ag/kg Ag NPs 3–8 nm	40% WHC	0.27 (0.042–0.491)	1.00 (0.087–1.91)	101±1.34	0.49±0.01	
				100 mg/kg Ag NPs 50 nm		0.06 (0.009–0.115)	0.12 (0–0.395)	80.4±2.83	0.38±0.02	
				100 mg Ag/kg Ag NPs 60 nm		0.31 (0.048–0.572)	1.09 (0.066–2.11)	121±2.05	0.39±0.01	
				22 mg Ag-kg Ag <sub>2</sub> S NPs		1.89 (-)	12.6 (-)	22.0±0.424	-	
				22 mg Ag-kg Ag <sub>2</sub> S NPs		0.13 (0–0.312)	1.35 (0–3.22)	227±3.61	0.001±0.0	

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