

## Supplementary information

### Multigenerational exposure of Ag materials (nano and salt) in soil – environmental hazards in *Enchytraeus crypticus* (Oligochaeta)

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**Table S1:** Soil pH (0.01 M CaCl<sub>2</sub>) measured at the start (t=0) and at the end (t=32) of each generation in a multigeneration test with *Enchytraeus crypticus* exposed in LUFA 2.2 soil spiked with AgNO<sub>3</sub> and Ag NM300K. F5 and F6 were exposed to clean soil.

pH (CaCl <sub>2</sub> )														
Generation	F0		F1		F2		F3		F4		F5		F6	
Time (days)	0	32	0	32	0	32	0	32	0	32	0	32	0	32
AgNO <sub>3</sub> (mg Ag/kg soil DW)														
0	6.3	6.1	6.1	5.9	6.1	5.8	5.9	5.4	6.0	5.8	5.9	5.9	6.0	5.6
45	5.7	6.2	5.9	6.2	6.1	5.8	5.7	6.1	5.9	6.0	6.1	6.0	6.0	5.8
60	5.6	6.1	5.7	6.3	6.0	6.1	5.8	6.0	5.8	6.2	6.1	6.1	6.1	5.9
Ag NM300K (mg Ag/kg soil DW)														
0	5.8	5.6	6.0	6.0	6.0	6.0	6.0	6.3	6.2	5.9	6.1	5.7	6.0	6.0
20	5.8	6.1	6.0	6.3	6.1	6.3	6.1	6.2	6.1	6.1	6.1	6.0	6.1	6.2
60	5.8	6.1	6.1	6.1	6.0	6.1	6.0	6.2	6.1	6.1	6.2	6.1	6.1	6.1

**Table S2:** Measured total soil concentrations and internal Ag concentrations in the organisms (AV ± SE) measured at the end of each generation, so at days 32, 64, 96, 128, 160, 194 and 224 of the multigenerational tests with *Enchytraeus crypticus* in LUFA 2.2 soil spiked with AgNO<sub>3</sub> (45 and 60 mg Ag/kg soil DW) and Ag NM300K (20 and 60 mg Ag/kg soil DW). Also shown are the generation and effect level of exposure. [% Recovery]: recovery of Ag in the spiked soils compared to nominal concentrations of AgNO<sub>3</sub> and Ag NM300K. F5 and F6 were exposed to clean soil.

Gene-ration	Time (days)	Effect level	Measured total (mg/ kg soil DW) [% recovery]	Internal conc. (mg Ag/kg body DW)	Measured total (mg/ kg soil DW) [% recovery]	Internal conc. (mg Ag/kg body DW)
				AgNO <sub>3</sub>	Ag NM300K	
<b>F0</b>	32	Control	0	0.08±0.013	0.05±0.05	0.13±0.02
		EC <sub>10</sub>	38.2±0.5 [84.9%]	7.2±2.4	20.3±0.5 [102%]	7.9±1.1
		EC <sub>50</sub>	67.7±1.5 [113%]	13.7±1.4	57±3.2 [95%]	23.7±2.7
<b>F1</b>	64	Control	0.05±0.05	0.07±0.02	0.04 ± 0.04	0.20±0.03
		EC <sub>10</sub>	33.0±0.2 [73.0%]	10.6±1.2	17.1±0.1 [85.5%]	6.6±0.9
		EC <sub>50</sub>	57.7±2.5 [91.2%]	22.6±2.9	58.3±1.6 [97.2%]	17.6±3
<b>F2</b>	96	Control	0	0.07 ± 0.02	0.09 ± 0.003	0.2 ± 0.02
		EC <sub>10</sub>	35.1±3.2 [78.0%]	17.6 ± 2.8	50.2±0.4 [251%]	15.2 ± 1.4
		EC <sub>50</sub>	63.2±3.6 [105%]	16.6 ± 4.5	47.6±1.8 [79.3%]	81.3 ± 20.9
<b>F3</b>	128	Control	0	0.12±0.03	0.13±0.04	0.00
		EC <sub>10</sub>	26.0±0.9 [57.7%]	19.9±1.7	16.9±0.4 [84.5%]	7.8 ± 0.6
		EC <sub>50</sub>	47.5±0.65 [79.1%]	21.7±2.2	53.0±0.7 [88.3%]	28.1 ± 1.8
<b>F4</b>	160	Control	0.68±0.13	0.09±0.02	0.04±0.04	0.07 ± 0.02
		EC <sub>10</sub>	50.3±5.0 [112%]	8.2±2.2	22.8±0.4 [114%]	11.4 ± 1.3
		EC <sub>50</sub>	52.4±0.09 [87.3%]	13.1±1.3	59.5±1.02 [99.1%]	35.6 ± 5.3
<b>F5</b>	192	Control	0.09±0.001	0.06 ± 0.01	0.05 ± 0.05	0.12±0.007
		0	0.13±0.3	0.09 ± 0.02	0.0	0.15±0.03
		0	0.09±0.1	0.03 ± 0.01	0.14 ± 0.13	0.41±0.07
<b>F6</b>	224	Control	0	0.05±0.003	0.05±0.05	0.05±0.009
		0	0	0.09±0.007	0.0	0.02±0.008
		0	0	0.06±0.01	0.05±0.05	0.04±0.008

**Table S3.** Detailed information regarding control performance of *Enchytraeus crypticus* at each generation of the multigenerational exposure in LUFA 2.2 soil to AgNO<sub>3</sub> and Ag NM300K, including the survival fraction, numbers of juveniles. AV±SD: Average ± Standard Deviation. CoV: coefficient of variation. F5 and F6 were exposed to clean soil.

Generation	Time (days)	Survival fraction (%)	CoV no. Adults (Survival) (%)	Number of juveniles (AV±SD)	CoV no. Juveniles (Reproduction) (%)
AgNO <sub>3</sub> experiment					
F0	32	98 ± 0.04	4	3310 ± 549	18
F1	64	93 ± 0.05	5	5250 ± 298	6
F2	96	90 ± 0.07	8	3061 ± 308	10
F3	128	89 ± 0.09	10	3211 ± 345	10
F4	160	97 ± 0.06	6	4528 ± 620	14
F5	194	91 ± 0.17	20	4564 ± 621	14
F6	224	97 ± 0.06	6	2700 ± 398	15
Ag NM300K experiment					
F0	32	93 ± 0.06	7	3909 ± 355	10
F1	64	94 ± 0.02	2	3200 ± 357	10
F2	96	93 ± 0.03	3	2920 ± 251	9
F3	128	95 ± 0.1	10	4353 ± 466	10
F4	160	96 ± 0.04	5	4460 ± 723	20
F5	194	94 ± 0.05	5	3640 ± 854	20
F6	224	93 ± 0.1	10	2473 ± 428	20