

SUPPLEMENTARY INFORMATION

Comparative biokinetics of pristine and sulfidized Ag nanoparticles in two arthropod species exposed to different field soils

Iva TALABER[†], Cornelis A.M. VAN GESTEL[‡], Anita JEMEC KOKALJ^{†*}, Gregor MAROLT[§], Sara NOVAK[†], Primož ZIDAR[†], Damjana DROBNE[†]

[†] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia

[‡] Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

[§] Faculty of Chemistry and Chemical technology, University of Ljubljana, Večna pot 113, Ljubljana, Slovenia

* corresponding author, anita.jemec@bf.uni-lj.si

Table S1. Measured total Ag concentrations (average \pm SD) in the four different test soils spiked with different Ag forms *at day 0* of the biokinetic experiments with *Porcellio scaber* and *Folsomia candida*. Data are shown separately for the isopod and springtail experiments. Nominal concentration in both cases was 10 $\mu\text{g Ag/g}$ dry soil.

Soil type	Natural background of Ag in soil ($\mu\text{g Ag/g}$ dry soil)	Ag form	<i>P. scaber</i> ($\mu\text{g Ag/g}$ dry soil) (n=3)	<i>F. candida</i> ($\mu\text{g Ag/g}$ dry soil) (n=2)
<i>Lufa 2.2</i>	0.49 \pm 0.16	AgNO ₃	13.4 \pm 0.6	5.55 \pm 0.21
		3-8 nm Ag NPs	12.9 \pm 1.6	n.d.
		50 nm Ag NPs	13.8 \pm 0.4	6.48 \pm 0.124
		20 nm Ag ₂ S NPs	22.5 \pm 1.8	11.3 \pm 0.39
<i>North Wales</i>	0.95 \pm 0.36	AgNO ₃	15.3 \pm 0.88	9.39 \pm 0.33
		3-8 nm Ag NPs	12.6 \pm 1.5	n.d.
		50 nm Ag NPs	14.9 \pm 0.8	7.19 \pm 0.101
		20 nm Ag ₂ S NPs	26.2 \pm 0.8	14.6 \pm 0.39
<i>Woburn</i>	0.22 \pm 0.06	AgNO ₃	12.9 \pm 1.3	6.94 \pm 0.71
		3-8 nm Ag NPs	9.4 \pm 0.2	n.d.
		50 nm Ag NPs	n.d.	7.76 \pm 0.59
		20 nm Ag ₂ S NPs	20.4 \pm 1.6	9.93 \pm 0.48
<i>Dorset</i>	0.19 \pm 0.02	AgNO ₃	n.d.	5.42 \pm 0.79
		3-8 nm Ag NPs	10.7 \pm 0.4	n.d.
		50 nm Ag NPs	12.2 \pm 0.75	6.59 \pm 0.62
		20 nm Ag ₂ S NPs	13.8 \pm 0.57	13.5 \pm 0.230

n.d. not included in experiment

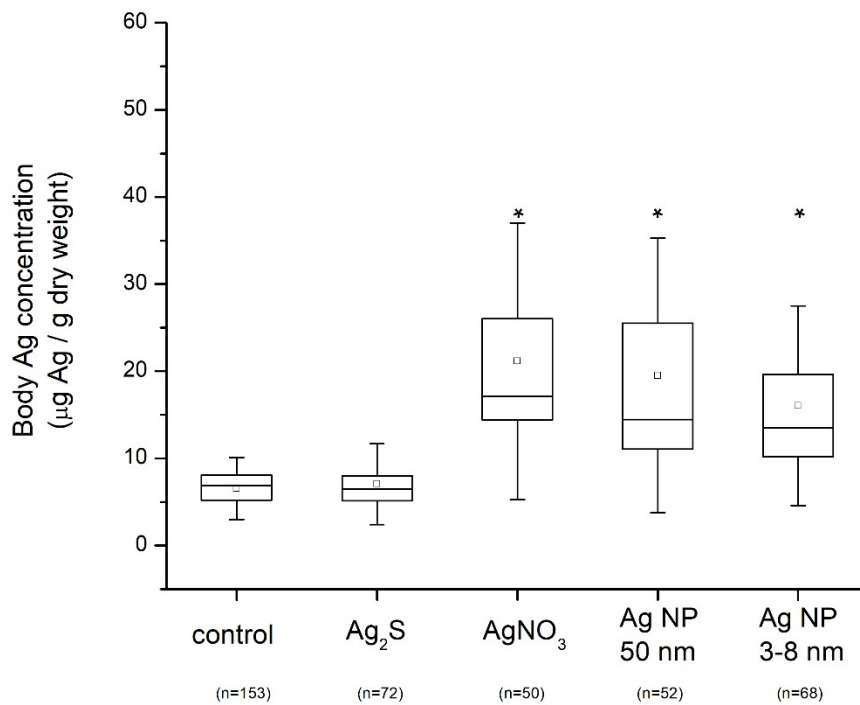


Figure S1. Ag body concentrations in *Porcellio scaber* after 3 weeks of exposures to soils spiked with different Ag forms. Shown are pooled data of the $21 \leq t \leq 42$ day time points (after transfer to clean soil)) from all four test soils. In case of controls pooled data from the $0 \leq t \leq 42$ day time points are presented. Statistically significant differences ($p < 0.05$) from the control are highlighted.

Table S2. Biokinetic parameters describing the uptake and elimination of Ag in *Porcellio scaber* exposed to different Ag forms in four different test soils*. Values refer to estimates from the one compartment model \pm SE, with corresponding p values, when >0.05 .

Soil type	Ionic Ag		50 nm Ag NPs		3-8 nm Ag NPs		20 nm Ag ₂ S NPs	
	k ₁	k ₂	k ₁	k ₂	k ₁	k ₂	k ₁	k ₂
<i>Lufa 2.2</i>	0.079 ± 0.019	0.020 ± 0.015 n.s. p=0.21	0.051 ± 0.010	0.049 ± 0.016	0.032 ± 0.008	0.026 ± 0.016 n.s. p=0.11	0 ± 0.001 n.s. p=0.92	0.000 \pm --- n.s. p=1
	recalculated 0.057 ± 0.007	Assumed 0			recalculated 0.021 \pm 0.003	Assumed 0	Assumed 0	Assumed 0
<i>Woburn</i>	0.032 ± 0.008	0.008 ± 0.015 n.s. p=0.88	/		0.017 ± 0.007	0.000 \pm --- n.s. p=1	0.000 \pm 0.001 n.s. p=0.92	0.000 \pm --- n.s. p=1
	recalculated 0.021 \pm 0.003	Assumed 0			recalculated 0.017 \pm 0.003	Assumed 0	Assumed 0	Assumed 0
<i>North Wales</i>	0.066 ± 0.015	0.002 ± 0.010 n.s. P=0,84	0.141 ± 0.026	0.031 ± 0.011	0.036 ± 0.005	0.000 ± 0.001 n.s. p=1	0.000 ± 0.003 n.s. p=0,85	0.000 \pm 0.226 n.s. p=1
	recalculated 0.064 \pm 0.005	Assumed 0			recalculated 0.036 ± 0.003	Assumed 0	Assumed 0	Assumed 0
<i>Dorset</i>	/		0.076 ± 0.031	0.025 ± 0.026 n.s. p=0.35	0.091 ± 0.015	0.003 ± 0.009 n.s. p=0.89	0.166 ± 0.094 n.s. p=0.09	0.544 ± 0.307 n.s. p=0.08
			recalculated 0.051 \pm 0.010	Assumed 0	recalculated 0.086 \pm 0.006	Assumed 0	Assumed 0	Assumed 0

*When the estimate of k₂ (elimination rate constants) was not significantly different from zero, we had to calculate k₁ again, because k₁ and k₂ are dependent. Hence, to derive a better estimate of the uptake rate constant (k₁), we applied the model again, by fixing k₂ close to zero (assigning a value of 0.00001). We chose this approach to be able to apply the same model to all datasets. The arrows indicate the route towards the newly calculated kinetics parameters.

Table S3. Biokinetic parameters describing the uptake and elimination of Ag in *Folsomia candida* exposed to different Ag forms in different test soils*. Values refer to estimates from the one compartment model \pm SE, with corresponding p values, when >0.05 .

Soil type	Ionic Ag		50 nm Ag NPs		20 nm Ag ₂ S NPs	
	k ₁	k ₂	k ₁	k ₂	k ₁	k ₂
Lufa 2.2	0.063 \pm 0.011	0.100 \pm 0.025	0.312 \pm 0.179 n.s. p=0.09	1.039 \pm 0.603 n.s. p=0.09	0.016 \pm 0.006	0.676 \pm 0.256
Lufa 2.2			Assumed 0	Assumed 0		
Woburn	0.152 \pm 0.021	0.047 \pm 0.012	0.203 \pm 0.069	0.263 \pm 0.099	0.016 \pm 0.003	0.148 \pm 0.036
North Wales	0.094 \pm 0.049 n.s. p=0.06	0.569 \pm 0.300 n.s. p=0.06	0.017 \pm 0.005	0.046 \pm 0.033 n.s. p=0.16	0.015 \pm 0.008 n.s. p=0.07	0.609 \pm 0.341 n.s. p=0.08
North Wales	Assumed 0	Assumed 0	recalculated 0.010 \pm 0.003	Assumed 0		
Dorset	0.276 \pm 0.048	0.097 \pm 0.023	0.381 \pm 0.059	0.033 \pm 0.014	0.164 \pm 0.073	1.514 \pm 0.732

*When the estimate of k₂ (elimination rate constants) was not significantly different from zero, we had to calculate k₁ again, because k₁ and k₂ are dependent. Hence, to derive a better estimate of the uptake rate constant (k₁), we applied the model again, by fixing k₂ close to zero (assigning a value of 0.00001). We chose this approach to be able to apply the same model to all datasets. The arrows indicate the route towards the newly calculated kinetics parameters.

Porcellio scaber

Ionic Ag: N Wales = Lufa > Woburn (Dorset not tested)
 3-8 nm AgNPs: Dorset > N Wales > Lufa = Woburn
 50 nm AgNPs: N Wales > Dorset = Lufa (Woburn not tested)
 Ag₂S NPs: k₁ = 0 in all soils

Folsomia candida

Ionic Ag: Dorset > Woburn > Lufa k₁ = 0 in N Wales
 50 nm AgNPs: Dorset > Woburn > N Wales k₁ = 0 in Lufa
 Ag₂S NPs: Dorset > Woburn = Lufa k₁ = 0 in N Wales

Figure S2. Overview of variation in the uptake rate constant (k₁) in the isopod *Porcellio scaber* and the springtail *Folsomia candida* exposed to different Ag forms across four different test soils. > significantly higher at p<0.05, = no significant difference at p<0.05.

Model with a stored fraction

In case of isopods, we also applied a model with a parameter representing a stored fraction (stored fraction model) as described by Van den Brink et al. (2019), since a significant proportion of the Ag was not eliminated.

Uptake ($t < t_e$)

$$C = C_0 + SF * C_{exp} * k_1 * t + (1 - SF) * C_{exp} * \left(\frac{k_1}{k_2}\right) * (1 - e^{(-k_2 * t)})$$

Elimination ($t > t_e$)

$$C = C_0 + SF * C_{exp} * k_1 * t_e + (1 - SF) * C_{exp} * \left(\frac{k_1}{k_2}\right) * (1 - e^{-k_2 * t_e}) * e^{(-k_2 * (t - t_e))}$$

Where C = silver concentration in the organism at time t ($\mu\text{g Ag/g dry body weight}$), C_{exp} = silver exposure concentration ($\mu\text{g Ag/g dry soil}$), k_1 =uptake rate constant ($\text{g dry soil/g dry body weights/day}$), k_2 = elimination rate constant (day^{-1}), SF = stored fraction, t = time (day), t_e = time at which animals were transferred to clean soil and C_0 = Ag concentration in animals at time 0.

However, with this model, none of the parameter estimates were significantly different from zero (Table S4), probably due to limitations of our relatively small and scattered dataset, as significance of parameter estimates generally decreases with decreased degrees of freedom (Van den Brink, 2019). Additionally, the individual variability in Ag body concentrations was high, leading to very large standard errors of the modelled estimates, and hence the 95 confidence intervals are too broad to be able to estimate the biokinetic parameter with the desired level of confidence. The problem of variability might not be solved by increasing the sample size, because considerable scatter in body burden data in soil invertebrates is common, due to variability between individuals in their interaction with contaminated medium and its ingestion (Ardestani et al., 2014).

Table S4. Biokinetic parameters describing the uptake and elimination of Ag in *Porcellio scaber* exposed to different Ag forms in different test soils, obtained with the stored fraction model. Values refer to estimates from the model \pm SE, with corresponding 95% CI in brackets.

Soil type	Ionic Ag			50 nm Ag NPs			3-8 nm Ag NPs			20 nm Ag ₂ S NPs		
	k ₁	k ₂	SF	k ₁	k ₂	SF	k ₁	k ₂	SF	k ₁	k ₂	SF
<i>Lufa 2.2</i>	5 $\pm 6E5$ n.s. p=1	12 $\pm 15E5$ n.s. p=1	0.008 ± 180 n.s. p=1	0.061 ± 0.073 n.s. p=0.13	0.133 ± 0.372 n.s. p=0.84	0.256 ± 0.224 n.s. p=0.14	0.048 ± 0.052 n.s. p=0.39	0.083 ± 0.406 n.s. p=0.85	0.384 ± 0.421 n.s. p=0.40	0.000 \pm n.s. p=1	0.000 \pm n.s. p=1	0.000 \pm n.s. p=1
<i>Woburn</i>	0.038 ± 0.065 n.s. p=0,25	0.066 ± 1.187 n.s. p=1	0.599 ± 1.847 n.s. p=1	/			0.015 ± 0.012 n.s. p=.68	0.000 $\pm 2E5$ n.s. p=1	1 $\pm 1E6$ n.s. p=1	0.000 ± 0.003 n.s. p=1	0.000 \pm n.s. p=1	1 \pm n.s. p=1
<i>North Wales</i>	0.109 ± 0.243 n.s. P=0,65	0.346 $\pm 1,881$ n.s. P=0,85	0.559 ± 1.224 n.s. p=0,45	0.203 ± 0.118 n.s. p=0.23	0.152 ± 0.183 n.s. p=0.76	0.277 ± 0.121 n.s. p=0.05	0.034 ± 0.013 n.s. p=0.11	0.000 $\pm 10E5$ n.s. p=1	1 $\pm 5E5$ n.s. p=1	0.001 \pm n.s. p=1	0.004 \pm n.s. p=1	1 ± 43.45 n.s. p=1
<i>Dorset</i>	/			4,935 \pm n.s. p=1	13 \pm n.s. p=1	0.009 \pm n.s. p=1	0.100 ± 0.069 n.s. p<0.05	0.006 ± 0.826 n.s. p=0.97	0 ± 59.1 n.s. p=1	0.162 ± 0.208 n.s. p=0.68	0.624 ± 0.826 n.s. p=0.64	0.002 ± 0.026 n.s. p=0.87

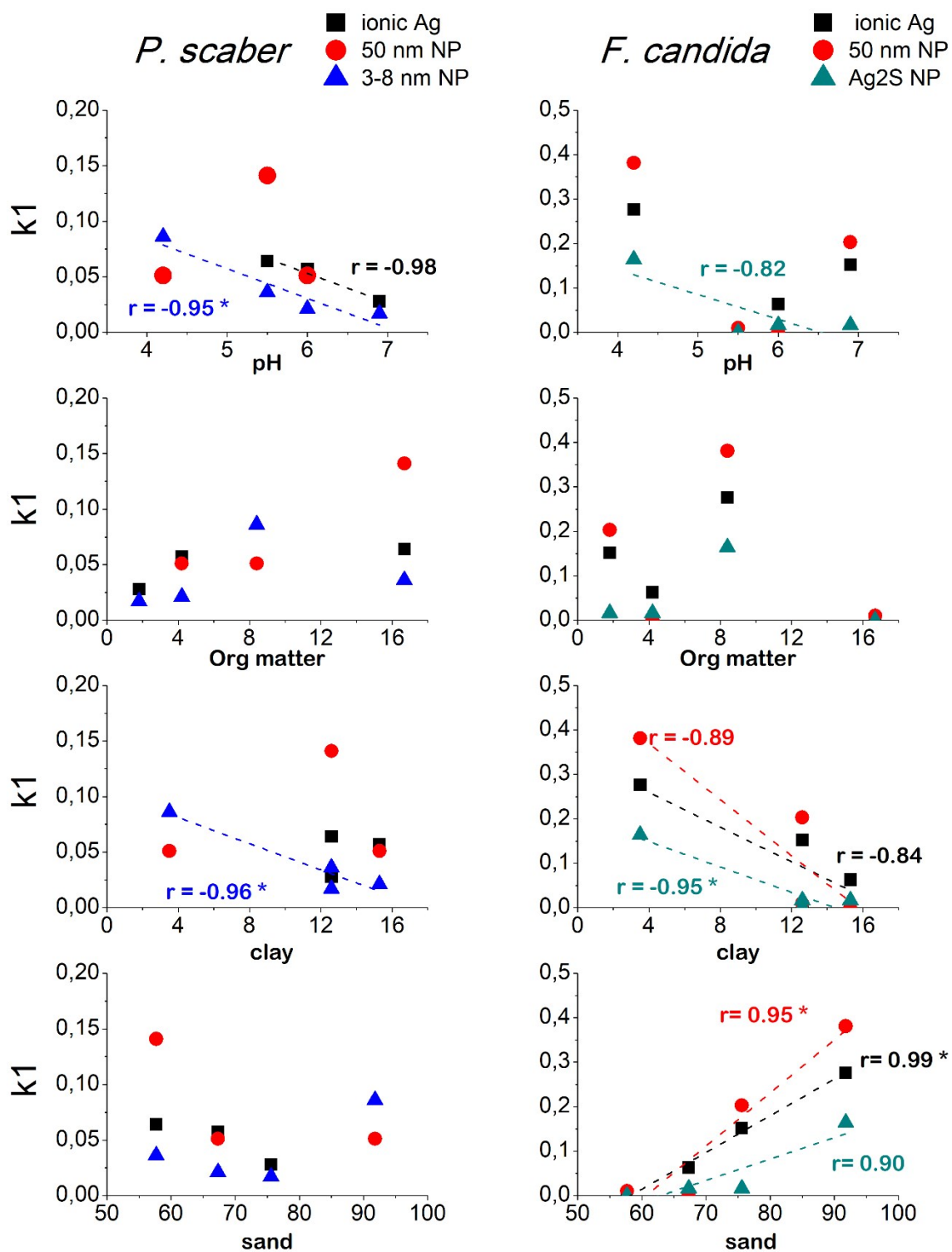


Figure S3. Relationship between k_1 values for the uptake of Ag in *Porcellio scaber* (left) or *Folsomia candida* (right) and soil properties following exposure to different Ag forms in four different test soils. Due to small sample size, linear fits are shown only in case of a relatively strong correlation. (r = Pearson correlation coefficient, * significant at $p < 0.05$)