Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary information

Toxicokinetics of pristine and aged silver nanoparticles in *Physa acuta*

Patrícia V. Silva[1], Cornelis A. M. van Gestel[2], Rudo A. Verweij[2], Anastasios G. Papadiamantis[3,4], Sandra F. Gonçalves[1], Iseult Lynch[3], and Susana Loureiro[1]

[1]Department of Biology & CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[2]Department of Ecological Science, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands
[3] School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, B15 2TT Birmingham, UK
[4]NovaMechanics Ltd., 1065, Nicosia, Cyprus

E-mail contact: pverissimo@ua.pt

7 pages (4 figures, 6 tables)

Table S1. Characteristics of the Ag-NPs dispersed in ultrapure water (UPW). Shown are Z-potential values (mV), polydispersity Index (PDI) and mean hydrodynamic diameter (nm) measured by Dynamic Light Scattering (DLS); Dissolved Ag concentration ($\mu g.L^{-1}$) and percentage of dissolution measured by ICP-MS of 3-8 nm, 50 nm and 60 nm Ag-NPs and Ag_2S-NPs measured in UPW stock solutions at a nominal concentration of 1 mg $Ag.L^{-1}$. All values are given as mean and standard deviation (mean ± SD).

Nanoparticle	Timepoint (h)	Z-potential (mV)	DLS (nm)	PDI	Dissolved Ag concentration ($\mu g.L^{-1}$)	% Dissolution
3-8 nm Ag-NP	0	0.03±0.52	144±19.4	0.29±0.04	65±7.06	6.50±0.71
	2	0.20±0.55	115±4.64	0.23±0.04	76.6±5.17	7.66±0.52
	4	-45.7±2.93	121±3.74	0.26±0.02	91.9±5.04	9.19±0.50
	8	-41±5.9	112±1.92	0.20±0.03	109±17.8	10.9±1.78
	24	-43.1±6.33	116±5.79	0.21±0.04	187±25.2	18.7±2.52
	48	-34.1±12.3	125±20	0.17±0.05	210±40.4	21.1±4.04
50 nm Ag-NP	0	-35.6±11.2	59±1.19	0.22±0.01	52.7±3.51	5.27±0.35
	2	-54.9±1.34	58.1±1.48	0.23±0.01	86.1±16.4	8.61±1.64
	4	-42.6±6.67	58.1±1.75	0.23±0.01	108±31	10.8±3.10
	8	-38.7±18.1	58.7±1.69	0.23±0.01	166±41.8	16.6±4.18
	24	-32±19.6	56±1.61	0.23±0.02	162±57.6	16.2±5.76
	48	-25.1±6.78	54.8±1.34	0.23±0.03	261±60.2	26.1±6.02
60 nm Ag-NP	0	1.02±43.3	n.a.	n.a.	n.a.	n.a.
	2	-20.6±29.7	996±635	0.91	1.97±0.01	28.2±0.11
	4	-6.11±53.7	1265±673	1	2.05±0.03	29.2±0.33
	8	n.a.	n.a.	n.a.	n.a.	n.a.
	24	-3.86±38.5	1406±778	0.83	1.90±0.08	27.2±0.94
	48	-24.8±21.6	1800±1497	0.93	2.05±0.06	29.3±0.73
Ag_2S-NP	0	-33.9±0.74	215±9.76	0.34	n.a.	n.a.
	2	-33.1±14	206±22.3	0.41	0.13±0.05	0.04±0.02
	4	-36.2±5.64	206±23.1	0.38	0.03±0.01	0.01±0.002
	8	n.a.	n.a.	n.a.	n.a.	n.a.
	24	-44.4±3.36	207±32.9	0.4	0.02±0.005	0.01±0.001
	48	-42.1±4.37	212±18.2	0.37	0.05±0.01	0.01±0.003

n.a. not analysed at that time point.

Table S2. Characteristics of the Ag-NPs dispersed in APW medium. Shown are Z-potential values (mV), PDI and mean hydrodynamic diameter (nm) measured by DLS; Dissolved Ag concentration ($\mu g.L^{-1}$) and percentage of dissolution measured by ICP-MS of 3-8 nm, 50 nm and 60 nm Ag-NPs and Ag_2S-NPs measured in APW medium at a nominal concentration of 1 mg $Ag.L^{-1}$. All values are given as mean and standard deviation (mean ± SD).

Nanoparticle	Timepoint (h)	Z-potential (mV)	DLS	PDI	Dissolved Ag concentration ($\mu g.L^{-1}$)	% Dissolution
3-8 nm Ag-NP	0	-25.4±2.41	131±8.77	0.30±0.04	80±0.28	8±0.03
	2	-0.14±0.27	137±2.57	0.20±0.02	121±15.1	12.1±1.51
	4	-23.6±0.99	153±5.33	0.22±0.01	123±11.1	12.3±1.11
	8	-21.7±0.76	162±2.77	0.23±0.01	136±8.20	13.6±0.82
	24	-24.4±1.12	242±10.5	0.36±0.03	209±15.2	20.9±1.52
	48	-22.3±2.02	270±5.61	0.40±0.03	209±15.2	20.9±1.52
50 nm Ag-NP	0	-15.8±0.79	75.6±1.52	0.27±0.01	78.9±14	7.89±1.40
	2	-6.07±8.81	187±9.09	0.31±0.03	68.9±24.2	6.89±2.42
	4	-18.2±1.07	236±5.29	0.36±0.05	71.8±27.7	7.18±2.76
	8	-18.4±0.63	254±8.36	0.42±0.02	83.9±31.4	8.39±3.14
	24	-19.8±0.89	1133±168	0.1±0.01	107±34.5	10.7±3.45
	48	-19.7±1.27	1840±503	1±0	140±21.5	14±2.15
60 nm Ag-NP	0	-20.7±4.2	79.22	0.31	n.a.	n.a.
	2	-16±2.59	154±34.7	0.30	0.16±0.04	2.3±0.60
	4	-18.4±3.71	174±48	0.32	0.12±0.01	1.65±0.08
	8	n.a.	n.a.	n.a.	n.a.	n.a.
	24	-22.4±2.15	132±14	0.24	0.08±0.01	1.12±0.18
	48	-24.8±1.44	134±9.41	0.24	0.06±0.003	0.82±0.05
Ag_2S-NP	0	-9.55±0.66	191±6.55	0.33	n.a.	n.a.
	2	-9.31±2.51	213±30.6	0.4	0.71±0.06	0.20±0.02
	4	-8.93±0.49	206±16.8	0.41	0.15±0.16	0.04±0.05
	8	n.a.	n.a.	n.a.	n.a.	n.a.
	24	-8.35±2.18	183±20.6	0.37	0.12±0.17	0.03±0.05
	48	-7.97±3.68	159±13.5	0.35	0.01±0.002	0.002±0.0005

n.a. not analysed at that time point.

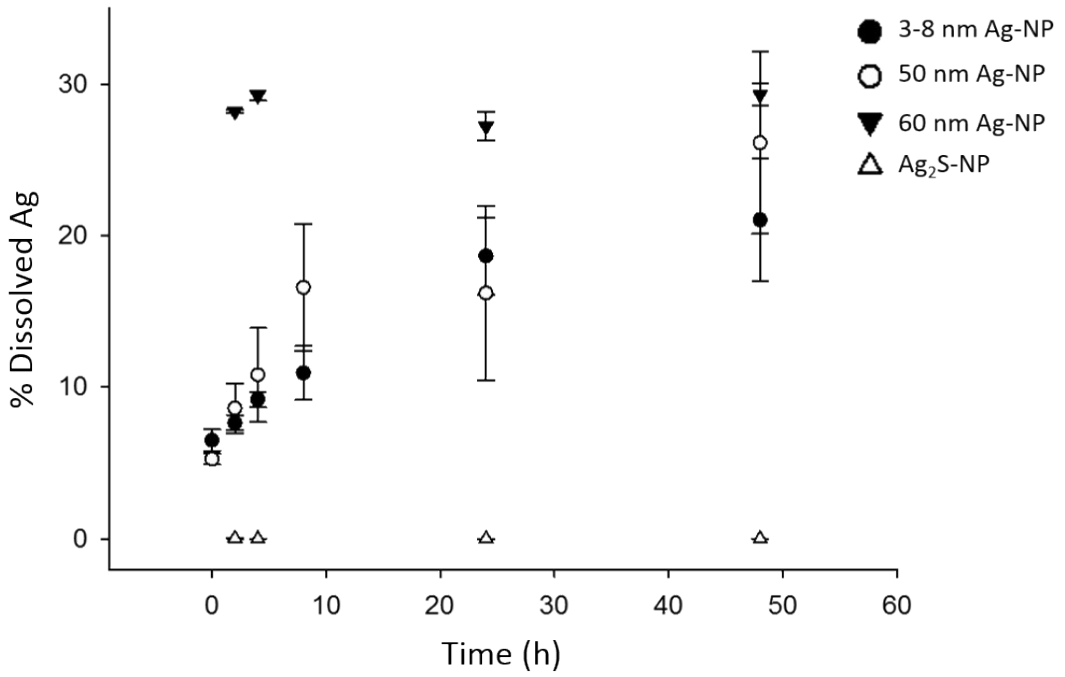

Figure S1. Percentage of dissolved Ag at 0, 2, 4, 8, 24 and 48 hours from 3-8 nm and 50 nm Ag-NPs and at 2, 4, 24 and 48 hours from 60 nm Ag-NPs and Ag_2S-NPs, measured by ICP-MS in ultrapure water (UPW) stock solutions of 1 mg $Ag.L^{-1}$. Error bars indicate the standard deviation.

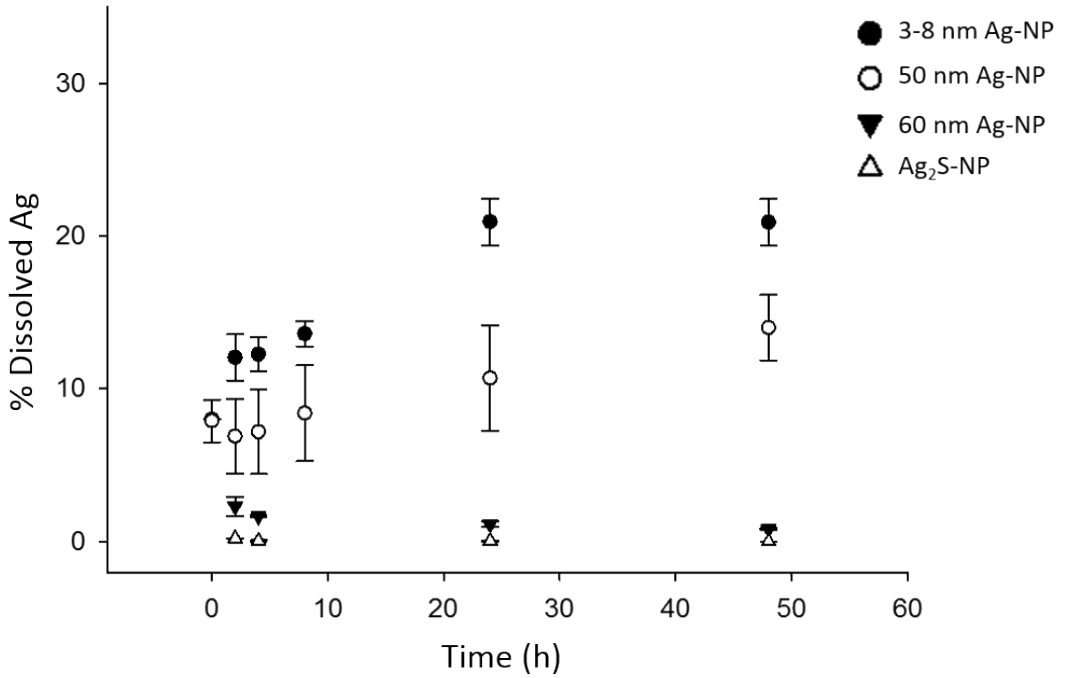

Figure S2. Percentage of dissolved Ag at 0, 2, 4, 8, 24 and 48 hours from 3-8 nm and 50 nm Ag-NPs and at 2, 4, 24 and 48 hours from 60 nm Ag-NPs and Ag_2S-NPs, measured by ICP-MS in APW medium solutions at a nominal concentration of 1 mg $Ag.L^{-1}$. Error bars indicate the standard deviation.

Table S3. Total Ag concentrations determined at day 0 and dissolved Ag concentrations (µg $Ag.L^{-1}$) determined at days 0, 1 and 2 in water samples of all exposures of the Ag-spiked water test. Values are given as mean and standard deviation (mean ± SD; *n*=3). Different capital letters in bold within a column and different small letters in italics within a line indicate statistically significant differences (one-way ANOVA followed by Holm-Sidak Method ($p<0.05$)).

Ag form	Total Ag concentrations (µg $Ag.L^{-1}$)	Dissolved Ag concentrations (µg $Ag.L^{-1}$)		
	Day 0	Day 0	Day 1	Day 2
3-8 nm Ag-NP	11.1±0.62 **A**	0.12±0.04 **A** / *a*	0.26±0.14 **A** / *a*	0.25±0.14 **A** / *a*
50 nm Ag-NP	8.28±0.30 **B**	0.03±0.02 **A** / *a*	0.27±0.16 **A** / *a*	0.32±0.29 **A** / *a*
60 nm Ag-NP	10.3±0.42 **A**	3.25±0.48 **B** / *a*	3.57±0.62 **B** / *a*	3.64±0.06 **B** / *a*
Ag_2S-NPs	16.8±0.72 **C**	0.08±0.1 **A** / *a*	0.17±0.09 **A** / *a*	0.05±0.08 **A** / *a*
$AgNO_3$	7.51±0.26 **B**	2.35±0.2 **B** / *a*	2.49±0.16 **C** / *a*	2.99±0.12 **C** / *b*

Table S4. Measured concentrations given as mean and standard deviation (mean ± SD; *n*=3) in water spiked at a nominal concentration of 10 µg $Ag.L^{-1}$ and non-spiked sediment at days 0, 3 and 7 of the uptake phase of all exposures of the Ag-spiked water and clean sediment test. Measured concentrations in water are expressed in µg $Ag.L^{-1}$ and in the sediment are in µg $Ag.kg^{-1}$. kd_{water} and ki_{sed} (day^{-1}) are the Ag concentration decrease rate constant in water and Ag concentration increase rate constant in sediment, respectively. Different capital letters in bold within a column and different small letters in italics within a line indicate statistically significant differences (one-way ANOVA followed by Holm-Sidak Method ($p<0.05$)).

Exposure route	Ag form	Measured concentration at day 0 (µg $Ag.L^{-1}$ and µg $Ag.kg^{-1}$)*	Measured concentration at day 3 (µg $Ag.L^{-1}$ and µg $Ag.kg^{-1}$)	Measured concentration at day 7 (µg $Ag.L^{-1}$ and µg $Ag.kg^{-1}$)	kd_{water} or ki_{sed} (day^{-1})
Water	3-8 nm	10±0.08 **A** / *a*	3.31±1.46 **A** / *b*	2.47±0.04 **A** / *b*	-0.29
	50 nm	8.82±0.43 **A** / *a*	3.17±1.01 **A** / *b*	0.35±0.26 **A** / *c*	-0.36
	60 nm	9.43±0.23 **A** / *a*	1.91±0.39** **A** / *b*	0.9±1.25 **A** / *b*	-0.49
	Ag_2S-NPs	12.2±0.74 **B** / *a*	3.58±1.66 **A** / *b*	0.75±0.39 **A** / *c*	-0.41
	$AgNO_3$	8.84±0.58 **A** / *a*	1.42±0.16 **A** / *b*	0.31±0.13 **A** / *c*	-0.60
Sediment	3-8 nm	10.1±2.95 *a*	15.3±2.32 **A** / *a*	16.9±4.08 **A** / *a*	0.08
	50 nm	10.1±2.95 *a*	12.8±1.38 **A** / *a*	18.3±1.40 **A, B** / *b*	0.09
	60 nm	10.1±2.95 *a*	22.2±5.17 **A** / *b*	25.6±4.40 **B** / *b*	0.14
	Ag_2S-NPs	10.1±2.95 *a*	24.3±2.93 **A** / *b*	20.8±1.02 **A, B** / *b*	0.12
	$AgNO_3$	10.1±2.95 *a*	32.8±20.7 **A** / *a*	18.5±1.92 **A, B** / *a*	0.12

* background concentration in sediments, therefore, was considered the same concentration at day 0 for sediments of all treatments.
** *n*=2

Table S5. Total Ag concentrations determined at day 0 and dissolved Ag concentrations (µg $Ag.L^{-1}$) determined at days 0, 1, 2 and 7 in water samples of all exposures of the Ag-spiked water and clean sediment test. Values are given as mean and standard deviation (mean ± SD; *n*=3). Different capital letters in bold within a column and different small letters in italics within a line indicate statistically significant differences (one-way ANOVA followed by Holm-Sidak Method ($p<0.05$)).

Ag form	Total Ag concentrations (µg $Ag.L^{-1}$)	Dissolved Ag concentrations (µg $Ag.L^{-1}$)			
	Day 0	Day 0	Day 1	Day 2	Day 7
3-8 nm	11.9±0.31** **A, B**	0±0 **A** / *a*	0.15±0.11 **A** / *a*	0.14±0.07 **A**/ *a*	0.05±0.01 **A** / *a*
50 nm	11.2±2.48 **A**	0±0 **A** / *a*	0.34±0.33 **A** / *b*	0.38±0.13 **B** / *b*	0±0 **A** / *a*
60 nm	11.1* **A**	0.02±0.02 **A** / *a*	1.19±0.82 **A** / *b*	0.66±0.11 **C** / *c*	0±0 **A** / *a*
Ag_2S-NPs	16.8±1.41 **B**	0±0 **A** / *a*	0.06±0.03 **A** / *b*	0.07±0.03 **A** / *b*	0±0 **A** / *a*
$AgNO_3$	9.35±0.13** **A**	0±0 **A** / *a*	0.79±0.61 **A** / *b*	0.54±0.03 **B, C** / *c*	0.01±0.01 **A** / *a*

* denotes a single measurement and ** denotes two measurements.

Table S6. Measured concentrations (mg Ag.kg^{-1}) given as mean and standard deviation (mean ± SD, *n*=3) in sediment spiked at a nominal concentration of 10 mg Ag.kg^{-1} at days 0, 3 and 7 of the uptake phase of all exposures of the Ag-spiked sediment test. Different capital letters in bold within a column and different small letters in italics within a line indicate statistically significant differences (one-way ANOVA followed by Holm-Sidak Method ($p<0.05$)).

	Ag form	Measured concentration at day 0 (mg Ag.kg^{-1})	Measured concentration at day 3 (mg Ag.kg^{-1})	Measured concentration at day 7 (mg Ag.kg^{-1})
Sediment	3-8 nm	12.2±1.07 **A** / *a*	8.30±0.13 **A, B** / *b*	8.99±0.43 **A, B** / *b*
	50 nm	8.13±1.25 **B** / *a*	7.20±0.20 **A** / *a*	8.65±0.57 **A** / *a*
	60 nm	9.55±0.82 **A, B** / *a*	9.42±0.92 **B** / *a*	11.7±2.04 **B** / *a*
	Ag_2S-NPs	9.89±0.21 **A, B** / *a, b*	9.52±0.54 **B** / *a*	11±0.72 **A, B** / *b*
	$AgNO_3$	8.26±1.05 **B** / *a*	8.51±0.67 **A, B** / *a*	8.97±0.23 **A, B** / *a*

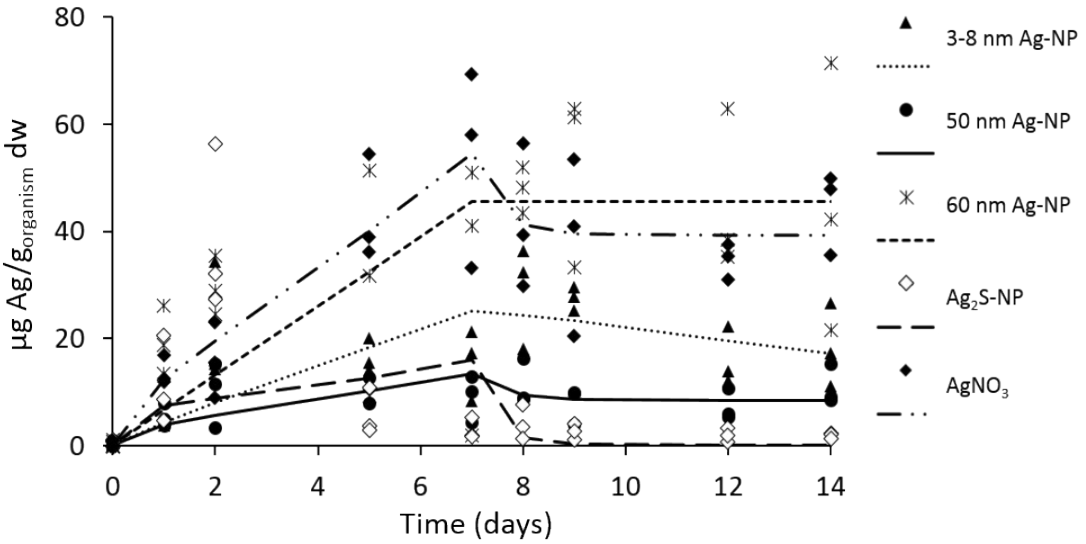

Figure S3. Uptake and elimination kinetics of 3-8 nm, 50 nm and 60 nm Ag-NPs, Ag_2S-NPs and $AgNO_3$ in the freshwater snail *Physa acuta* exposed for 7 days to water spiked at a nominal concentration of 10 µg Ag.L^{-1} and then transferred to clean water for 7 days, in the Ag-spiked water and clean sediment test. Lines represent the fit of a one-compartment model to the data, which represent Ag concentrations measured in individual snail soft bodies. **Data was modelled considering the sediment as the single exposure route.**

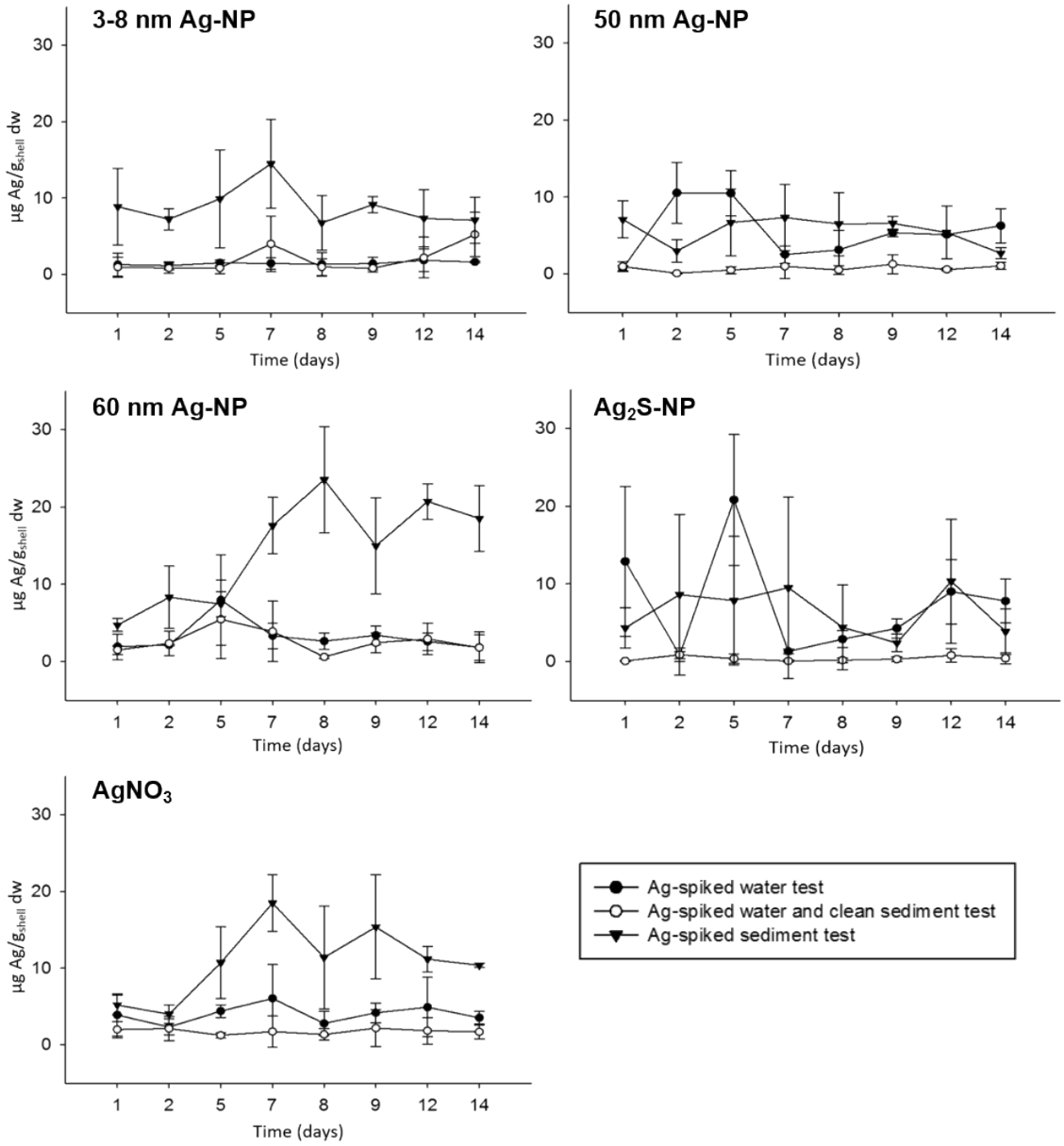

Figure S4. Silver concentrations (µg $Ag.g^{-1}$ dw) given as mean and standard deviation (mean ± SD, *n*=3) measured in the shell of *Physa acuta* exposed to 3-8 nm, 50 nm and 60 nm Ag-NPs, Ag_2S-NPs and $AgNO_3$ during the uptake and elimination phases of the Ag-spiked water test, Ag-spiked water and clean sediment test and Ag-spiked sediment test. Error bars indicate standard deviation.