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

Aging reduces the toxicity of pristine but not sulphidised silver nanoparticles to soil bacteria

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SUPPLEMENTARY INFORMATION

12 pages, 2 figures, 2 tables

Soil properties

Table S1: Soil properties: Classification, origin, soil texture, 100% water holding capacity (WHC) in mL per 100 g soil (dry weight), soil pH measured in 0.01 M CaCl2 and pore water (PW) pH, organic matter content (OM), and cation exchange capacity (CEC). Data taken from Heggelund et al 2014 [1].

Origin	Classification	Sand %	Silt %	Clay %	100% WHC [mL]	Soil pH_{CaCl2}	PW pH_{H2O}	OM %	CEC [mval/100g]
Acidic Heath	Sandy	91.7	4.7	3.5	49.2	3.1	4.2	8.00	5.4

Nanoparticle synthesis

Synthesis details can be found for Ag-PVP in Starnes et al 2015[2] and for Ag-citr in Cobaleda-Siles et al 2017[3].

Ag_2S synthesis (manuscript in prep):

Briefly, a 1L aqueous solution of $Na_2S.9H_2O$ and Polyvinylpyrrolidone (PVP) 55kDa was heated to a specific temperature under vigorous stirring. Then a concentrated solution of the $AgNO_3$ precursor was injected at a defined $[AgNO_3]$/[PVP] ratio for desired nanoparticle size. To ensure complete reaction of the precursors the solution was stirred at the synthesis temperature for 15 min. To remove excess S^{2-} the synthesised Ag_2S nanoparticles were purified by centrifugation and resuspended in Milli-Q-water with 55kDa PVP (1 mg/mL).

Nanomaterial characterisation

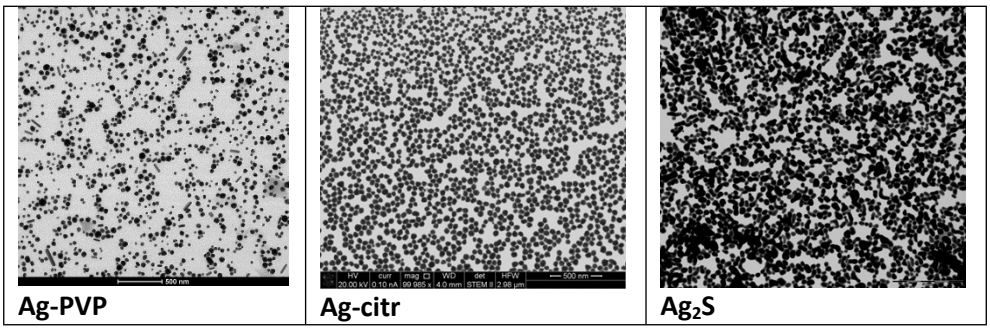

Figure S1: TEM images of tested nanoparticles. Ag-PVP image provided by Dr Stella Marinakos from Duke University. Scale bars are 500 nm.

Table S2: Nanoparticle stock characterisation. Astericesdenote information provided by the suppliers

Nano-material	Coating/stabiliser	TEM Size [nm]	NTA mean size [nm]	Zeta potential [mV]	Reference

Ag-citr	5 mM sodium citrate	49.1 ± 6.3	60.5 ± 0.6	-50.0 ± 2.3	
Ag-PVP	Polyvinylpyrrolidone	58.3 ± 12.9[2]*	88.2 ± 1.1	-11.6 ± 0.3	Starnes et al 2015[2]
Ag_2S	Polyvinylpyrrolidone	36.1 ± 9.7	84.8 ± 1.1	-25.7 ± 1.7	

ISO 10712 medium

Table S3: Nutrient concentrations in the ISO 10 721 (1995) media

Nutrients	Preculture Solution (mg/L)	Test Nutrient Solution (mg/L)
$NaNO_3$	500	500
$K_2HPO_4 \times 3H_2O$	120	120
KH_2PO_4	60	60
yeast extract	50	-
$C_6H_{12}O_6$	2000	2000
$MgSO_4 \times 7H_2O$	200	200
iron(III) citrate	0.5	0.5

Nanoparticle NTA characterisation

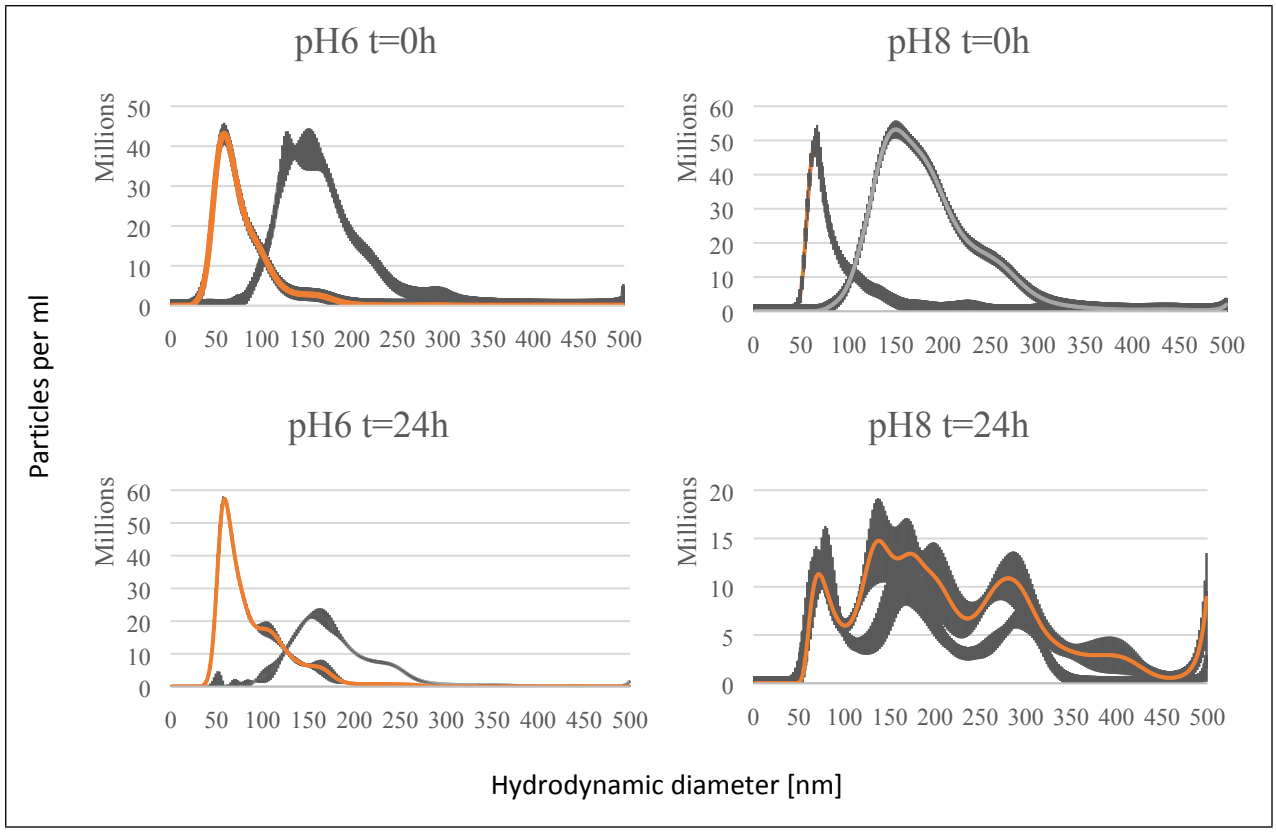

Figure S2: NTA hydrodynamic diameter [nm] ± standard deviation of Ag-citr in soil pore water extracts with different pHs (pH6 and pH8) at t=0h and t=24h, orange: particles in pore water, grey: pore water without particles.

Results statistical data analysis

Table S4: Results of GLM analysis and post hoc Tukey pairwise comparison comparing growth inhibition of *A. globiformis* by different aging treatments in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8. Different letters denote significant differences between treatments (p>0.05).

	Comparison	R^2	df	*F*-value	*p*-value	Tukey	
$AgNO_3$							
ISO	concentration	96.25	6	214.08	0.000	unaged	A
	treatment		2	1.73	0.187	aged	A
	interaction		12	1.88	0.059	dissolved	A
ISO+FA	concentration	97.93	6	367.01	0.000	unaged	C
	treatment		2	40.27	0.000	aged	B
	interaction		12	12.34	0.000	dissolved	A
pH6	concentration	98.73	5	651.14	0.000	unaged	B
	treatment		2	39.11	0.000	aged	A
	interaction		10	25.69	0.000	dissolved	A
pH8	concentration	99.01	5	718.25	0.000	unaged	C
	treatment		2	81.35	0.000	aged	A
	interaction		10	80.07	0.000	dissolved	B
Ag-PVP							
ISO	concentration	95.23	6	147.39	0.000	unaged	B
	treatment		2	15.42	0.000	aged	A
	interaction		12	9.09	0.000	dissolved	A
ISO+FA	concentration	98.28	6	445.12	0.000	unaged	B
	treatment		2	35.92	0.000	aged	B
	interaction		12	14.79	0.000	dissolved	A
pH6	concentration	97.31	5	177.99	0.000	unaged	C
	treatment		2	215.85	0.000	aged	B
	interaction		10	44.71	0.000	dissolved	A
pH8	concentration	99.36	5	737	0.000	unaged	C
	treatment		2	640.35	0.000	aged	B
	interaction		10	234.71	0.000	dissolved	A
Ag-citr							
ISO	concentration	93.46	6	117.62	0.000	unaged	A
	treatment		2	2.44	0.098	aged	A
	interaction		12	1.61	0.118	dissolved	A
ISO+FA	concentration	98.6	6	349.67	0.000	unaged	C
	treatment		2	513.32	0.000	aged	B
	interaction		12	54.94	0.000	dissolved	A
pH6	concentration	96.66	5	127.75	0.000	unaged	C
	treatment		2	178.73	0.000	aged	B
	interaction		10	42.03	0.000	dissolved	A
pH8	concentration	99.06	5	536.4	0.000	unaged	B
	treatment		2	437.84	0.000	aged	B
	interaction		10	138.95	0.000	dissolved	A

Table S5: Results of GLM analysis and post hoc Tukey pairwise comparison comparing growth inhibition of *A. globiformis* in different media treatments under unaged (UA) and aged (A) conditions in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8.

	Comparison	R2	df	F-value	p-value
$AgNO_3$					
ISO v ISO+FA	concentration	95.81	6	129.03	0.000
UA	treatment		1	0.06	0.813
	interaction		6	0.38	0.889
ISO v ISO+FA	concentration	96.81	6	169.65	0.000
A	treatment		1	12.18	0.001
	interaction		6	0.94	0.482
pH6 v pH8	concentration	96.13	5	135.03	0.000
UA	treatment		1	13.79	0.001
	interaction		5	6.60	0.000
pH6 v pH8	concentration	88.8	5	39.25	0.000
A	treatment		1	9.89	0.004
	interaction		5	5.13	0.002
Ag-PVP					
ISO v ISO+FA	concentration	96.93	6	175.61	0.000
UA	treatment		1	0.11	0.741
	interaction		6	3.29	0.012
ISO v ISO+FA	concentration	95.11	6	99.54	0.000
A	treatment		1	18.18	0.000
	interaction		6	8.25	0.000
pH6 v pH8	concentration	99.49	5	1108.73	0.000
UA	treatment		1	8.93	0.006
	interaction		5	66.66	0.000
pH6 v pH8	concentration	97.92	5	265.18	0.000
A	treatment		1	4.43	0.044
	interaction		5	16.83	0.000
Ag-citr					
ISO v ISO+FA	concentration	98.96	6	531.23	0.000
UA	treatment		1	0.52	0.477
	interaction		6	9.59	0.000
ISO v ISO+FA	concentration	95.7	6	119.1	0.000
A	treatment		1	8.29	0.007
	interaction		6	6.02	0.000
pH6 v pH8	concentration	99.35	5	850.37	0.000
UA	treatment		1	50.64	0.000
	interaction		5	52.19	0.000
pH6 v pH8	concentration	98.37	5	314.03	0.000
A	treatment		1	65.07	0.000
	interaction		5	37.53	0.000

Table S6: Results of GLM analysis and post hoc Tukey pairwise comparison comparing growth inhibition of *P. putida* by different aging treatments in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8. Different letters denote significant differences between treatments (p>0.05).

	Comparison	R2	df	F-value	p-value	Tukey	
$AgNO_3$							
ISO	concentration	99.91	6	8720.21	0.000	unaged	A
	treatment		2	1.92	0.157	aged	A
	interaction		12	2.68	0.007	dissolved	A
ISO+FA	concentration	99.66	6	2447.53	0.000	unaged	B
	treatment		2	9.32	0.000	aged	A
	interaction		12	3.13	0.002	dissolved	A
pH6	concentration	99.88	5	6634.1	0.000	unaged	B
	treatment		2	281.88	0.000	aged	A
	interaction		10	297.33	0.000	dissolved	A
pH8	concentration	98.77	5	721.6	0.000	unaged	A
	treatment		2	2.79	0.072	aged	A
	interaction		10	0.84	0.592	dissolved	A
Ag-PVP							
ISO	concentration	99.41	6	1429.16	0.000	unaged	A
	treatment		2	1.62	0.207	aged	A
	interaction		12	3.57	0.001	dissolved	A
ISO+FA	concentration	98.47	6	198.82	0.000	unaged	A
	treatment		2	250.16	0.000	aged	B
	interaction		12	140.39	0.000	dissolved	C
pH6	concentration	99.86	5	1805.55	0.000	unaged	A
	treatment		2	5490.81	0.000	aged	B
	interaction		10	1381.66	0.000	dissolved	C
pH8	concentration	98.18	5	168.73	0.000	unaged	C
	treatment		2	88.65	0.000	aged	B
	interaction		10	145.01	0.000	dissolved	A
Ag-citr							
ISO	concentration	98.91	6	491.83	0.000	unaged	B
	treatment		2	248.42	0.000	aged	B
	interaction		12	105.50	0.000	dissolved	A
ISO+FA	concentration	97.75	6	124.46	0.000	unaged	C
	treatment		2	47.57	0.000	aged	B
	interaction		12	112.01	0.000	dissolved	A
pH6	concentration	99.59	5	658.68	0.000	unaged	C
	treatment		2	1195.66	0.000	aged	B
	interaction		10	460.46	0.000	dissolved	A
pH8	concentration	76.27	5	8.7	0.000	unaged	A
	treatment		2	23.73	0.000	aged	B
	interaction		10	6.39	0.000	dissolved	A

Table S7: Results of F-test for ISO variants and GLM analysis and post hoc Tukey pairwise comparison for soil pore waters comparing growth inhibition of *P. putida* in different media treatments under unaged (UA) and aged (A) conditions in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8. n.d.: not determined due to F-test constraints.

		R2	df	F-value	p-value
$AgNO_3$					
ISO v ISO+FA UA				4.217	0.018
ISO v ISO+FA A				1.852	0.169
pH6 v pH8 UA	concentration	99.78	5	1851.92	0.000
	treatment		1	1529.03	0.000
	interaction		5	617.36	0.000
pH6 v pH8 A	concentration	99.91	5	5325.97	0.000
	treatment		1	1016.57	0.000
	interaction		5	1122.80	0.000
Ag-PVP					
ISO v ISO+FA UA				n.d.	n.d.
ISO v ISO+FA A				n.d.	n.d.
pH6 v pH8 UA	concentration	99.92	5	5896.52	0.000
	treatment		1	3259.02	0.000
	interaction		5	1549.55	0.000
pH6 v pH8 A	concentration	93.72	5	80.93	0.000
	treatment		1	3.58	0.068
	interaction		5	7.97	0.000
Ag-citr					
ISO v ISO+FA UA				n.d.	n.d.
ISO v ISO+FA A				n.d.	n.d.
pH6 v pH8 UA	concentration	99.68	5	935.97	0.000
	treatment		1	951.58	0.000
	interaction		5	765.67	0.000
pH6 v pH8 A	concentration	78.55	5	14.77	0.000
	treatment		1	14.8	0.001
	interaction		5	4.87	0.002

Table S8: Results of F-test comparing growth inhibition of Ag-PVP and Ag-citr in the same media under unaged (UA) and aged (A) conditions in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8. n.d.: not determined due to F-test constraints.

		A. globiformis		*P. putida*	
		F-value	**p-value**	**F-value**	**p-value**
ISO	UA	3.901	0.023	309.32	0.000
	A	8.686	0.001	317.12	0.000
ISO+FA	UA	0.139	0.935	766.83	0.000
	A	21.17	0.000	n.d.	n.d.
pH6	UA	14.35	0.000	1071.87	0.000
	A	1.529	0.241	n.d.	n.d.
pH8	UA	1.529	0.468	n.d.	n.d.
	A	0.241	0.708	n.d.	n.d.

Table S9: Results of GLM analysis and post hoc Tukey pairwise comparison comparing growth inhibition of *A. globiformis* and *P. putida* by different aging treatments in ISO standard testmedium, ISO medium with added fulvic acid (FA) and soil pore water extracts adjusted to pH6 and pH8. Different letters denote significant differences between treatments (p>0.05).

	R2	df	F-value	p-value	Tukey	
A. globiformis						
ISO	85.99	2	18.42	0.003	unaged	B
					aged	A
					dissolved	A
ISO+FA	72.84	2	8.05	0.020	unaged	B
					aged	AB
					dissolved	A
pH6	89.97	2	40.35	0.000	unaged	A
					aged	B
					dissolved	B
pH8	95.63	2	65.7	0.000	unaged	A
					aged	C
					dissolved	B
P. putida						
ISO	61.62	2	4.82	0.057	unaged	A
					aged	A
					dissolved	A
ISO+FA	53.17	2	3.41	0.103	unaged	A
					aged	A
					dissolved	A
pH6	95.15	2	58.8	0.000	unaged	A
					aged	A
					dissolved	B
pH8	94.4	2	50.56	0.000	unaged	B
					aged	A
					dissolved	B

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