1 Supporting Information for "Implications of Pony Lake Fulvic

2 Acid for the aggregation and dissolution of oppositely charged

3 surface-coated silver nanoparticles and their ecotoxicological

4 effects on *Daphnia magna*"

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Supporting Information I: Experimental Methods

Preparation of PLFA working solution

Stock solution of 100 mg/L PLFA was prepared in 10 mM sodium nitrate (NaNO$_3$) (Sigma-Aldrich, USA) solution followed by adjusting the pH value to 7 using 0.1 N sodium hydroxide solution (VWR, Germany) and stored in a refrigerator at 4°C. For all experiments conducted in this study, the PLFA solutions were diluted to two concentrations of 5 and 30 mg/L in 10 mM NaNO$_3$ solution and their pH values were also adjusted around 7 using 0.1 N sodium hydroxide solution.

Scanning Electron Microscopy (SEM)

The colloidal stability of AgNPs was monitored in the presence of PLFA at the concentrations of 5 and 30 mg/L considering NOM concentration range in natural waters. In the case of BPEI-AgNPs, the nanoparticles were immediately aggregated at both concentrations of PLFA. Therefore, in order to characterize the morphology of the large particles, SEM analysis was performed using the BPEI-AgNPs in the presence of 15 mg/L PLFA corresponding to the medium concentration of NOM in natural waters. 1 mg/L of BPEI-Ag NP dispersions were mixed with 15 mg/L PLFA followed by centrifugation at 9390 g for 15 min (Centrifuge 5430R, Eppendorf, Germany). After supernatant was decanted, 15 µL from settled down fraction in the centrifuge tube was transferred to a silicon wafer and air-dried for overnight at a room temperature. After drying the sample was sputter coated using gold at 1×10^{-3} mbar for 30 seconds with a deposition current of 120 mA using a K675X sputter coater (EMITECH, UK) to prevent the accumulation of an electrostatic charge on the surface followed by SEM observation at an accelerating voltage of 15 kV.

Total Ag content in Daphnia magna

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41   *Daphnia magna* which were previously exposed to AgNPs were transferred into silanized

42   glass vials and allowed for the digestion as refluxing in 3 mL of concentrated trace metal grade

43   HNO$_3$ for 24 h at 70°C on a hot plate. After digestion 1 mL volume of the each digestate was

44   then diluted up to 30 mL with Milli-Q water (EMD Millipore, USA) for the quantification of

45   total Ag by ICP-MS.

46   ***ICP-MS measurements***

47   ICP-MS was used to monitor the concentration of dissolved Ag released from AgNPs and

48   total Ag content in *Daphnia magna.* External calibration with internal standardization, based on

49   Claritas PPT® Grade silver standards in 2% HNO$_3$ (SPEXCertiPrep, USA), was applied in six

50   concentration levels between 0 and 100 µg/L to measure the concentrations of Ag. Same

51   amounts of 40 µg/L Indium standard, TraceCERT® grade provided by Sigma-Aldrich (USA),

52   was added as an internal standard to all samples including blanks. Intensity of ^{107}Ag was then

53   monitored in standard mode. Other operating parameters for ICP-MS analysis are listed in Table

54   S3.

55   ***Statistical analysis***

56   Mean concentrations of dissolved Ag released from AgNPs and mean values of adverse

57   effects were compared, using an independent student's *t*-test with 95% confidence intervals.
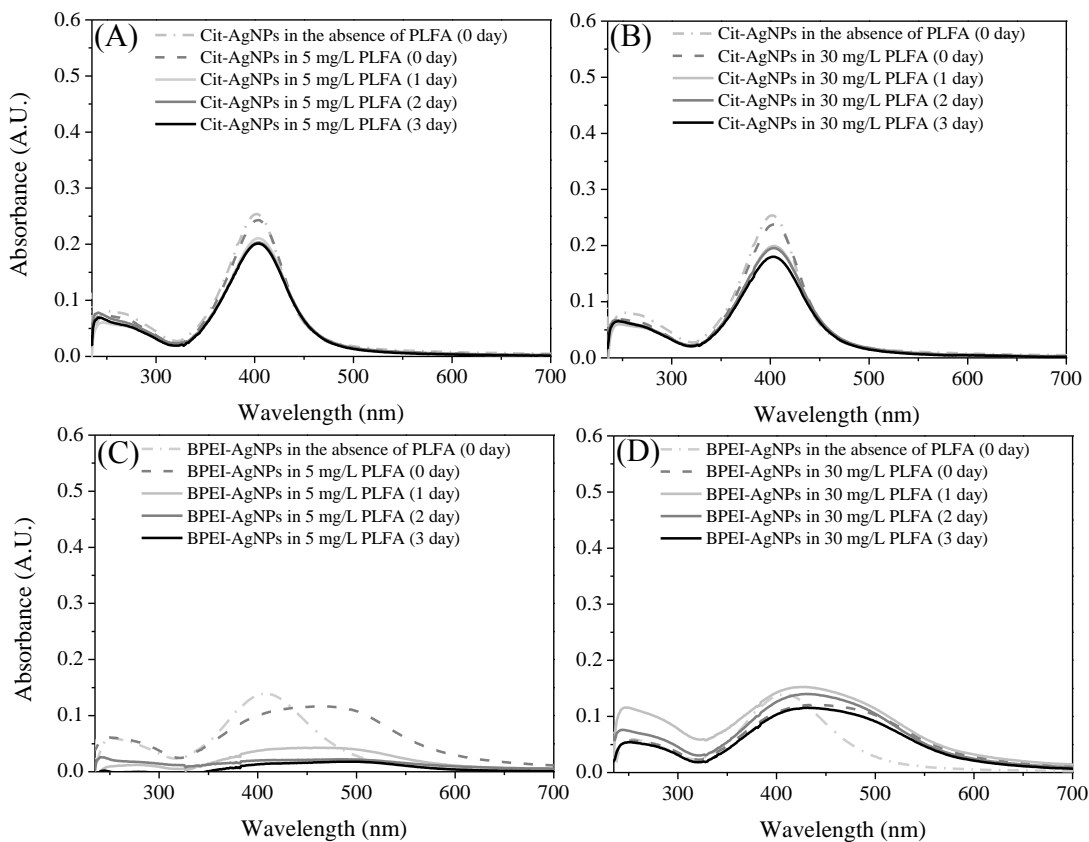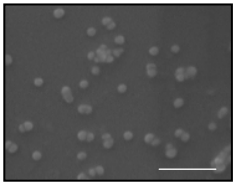
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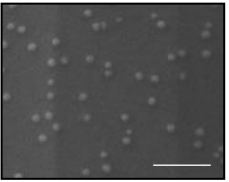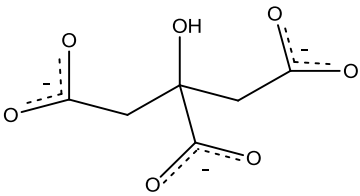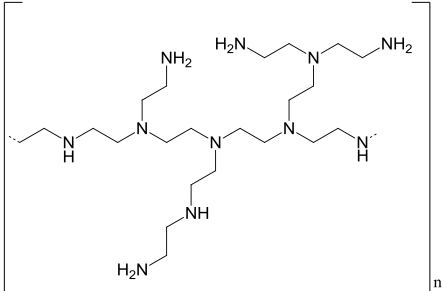**Supporting Information II: Supporting Figures and Tables**

62 **Figure S1.** UV-vis absorption spectra of 2 mg/L Cit-AgNPs (A and B) and BPEI-AgNPs (C and

63 D) incubated in 10 mM $NaNO_3$ solution in the absence and presence of 5 and 30 mg/L PLFA for

64 3 days.

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70   **Table S1.** Physicochemical properties of silver nanoparticles (AgNPs) coated with different

71   capping agents of citrate (Cit) and branched polyethylenimine (BPEI).

	Cit-AgNP	BPEI-AgNP
Shape	Sphere	Sphere
Particle surface	Citrate	Branched polyethylenimine
Ag mass concentration (mg/mL)	1.06 ± 0.01	1.00 ± 0.01
Dispersion solution	2 mM of aqueous citrate solution	Milli-Q water
Core size (nm)[a]	23.5 ± 2.5	22.1 ± 1.2
D_h (nm)[b]	23.9 ± 0.1	64.6 ± 5.3
PdI[b]	0.11 ± 0.01	0.25 ± 0.02
ZP (mV)[b]	$- 34.9 \pm 3.0$	16.7 ± 3.1
pH	7.00 ± 0.10	7.04 ± 0.01
SEM image[c]		
Structure of capping agent		

72   [a] Core size defined as a size of Ag core without citrate or BPEI coating was determined from
73   SEM images and was analyzed from 30 individual particles using ImageJ software.

74   [b] Hydrodynamic diameter (D_h), polydispersity index (PdI), and zeta potential (ZP) were
75   analyzed by a Malvern Zetasizer Nano ZS using 10 mg/L of AgNPs dispersions in Milli-Q water.

76   [c] Morphology of AgNPs was observed by FEI Quanta 250 FEG Scanning Electron Microscopy
77   (SEM) (Scale bar = 200 nm).

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81 **Table S2.** Elemental composition (% (w/w) content of a dry and ash-free sample) of Pony Lake

82 Fulvic Acid (PLFA).[1]

	C	H	O	N	S	P
PLFA	52.5	5.4	31.4	6.5	3.0	0.6

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86 **Table S3.** Operating parameters for ICP-MS analysis.

Parameters	Setting
Forward power (W)	1550
Nebulizer gas flow (L/min)	1.1
Sweeps	10
Replicates	5
Dwell time (ms)	30

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Table S4. Mean hydrodynamic diameters (D_h) and standard deviations (nm) as well as PdI[a] values of Cit- and BPEI-AgNPs dispersed in 10 mM NaNO$_3$ solution in both the absence and presence of PLFA. Mean values and standard deviations were calculated for 3 replicates.

| Day | DLS[b] | 2 mg/L Cit-AgNPs | | | 2 mg/L BPEI-AgNPs | | |
		without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA	without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA
0	D_h	52.1 ± 3.0	58.9 ± 6.6	50.0 ± 1.1	42.5 ± 0.4	4880.0 ± 3355.9	1456.0 ± 329.5
	PdI	0.11 ± 0.00	0.36 ± 0.00	0.31 ± 0.04	0.25 ± 0.01	1.00 ± 0.00	0.92 ± 0.06
1	D_h	45.0 ± 10.3	62.0 ± 2.6	57.8 ± 4.2	41.9 ± 12.6	1373.0 ± 304.1	191.0 ± 23.4
	PdI	0.22 ± 0.03	0.34 ± 0.00	0.18 ± 0.03	0.20 ± 0.00	0.96 ± 0.06	0.33 ± 0.05
2	D_h	130.1 ± 90.1	56.4 ± 4.6	58.6 ± 12.9	46.5 ± 13.0	1541.5 ± 65.8	181.7 ± 10.6
	PdI	0.70 ± 0.12	0.33 ± 0.05	0.20 ± 0.03	0.30 ± 0.01	0.96 ± 0.01	0.33 ± 0.03
3	D_h	3127.5 ± 193.0	59.6 ± 5.2	81.8 ± 11.9	157.4 ± 131.1	1583.0 ± 340.8	242.0 ± 92.5
	PdI	1.00 ± 0.00	0.35 ± 0.00	0.18 ± 0.01	0.30 ± 0.01	0.97 ± 0.05	0.36 ± 0.05

[a] Polydispersity index

[b] Dynamic light scattering

Table S5. Mean zeta potential values and standard deviations (mV) of Cit-AgNPs and BPEI-AgNPs dispersed in 10 mM NaNO$_3$ solution in both the absence and presence of PLFA. Mean values and standard deviations were calculated for 3 replicates.

| Day | 2 mg/L Cit-AgNPs | | | 2 mg/L BPEI-AgNPs | | |
	without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA	without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA
0	-21.2 ± 6.2	-22.0 ± 4.0	-22.2 ± 1.8	17.4 ± 6.1	-25.3 ± 5.1	-19.4 ± 0.8
1	-21.6 ± 3.6	-23.0 ± 1.5	-18.3 ± 0.6	17.5 ± 7.5	-22.5 ± 2.0	-24.4 ± 3.0
2	-20.5 ± 5.8	-17.3 ± 1.8	-16.5 ± 0.1	21.4 ± 4.5	-17.8 ± 1.7	-23.6 ± 0.8
3	-13.9 ± 9.2	-18.5 ± 0.7	-18.8 ± 0.6	12.2 ± 11.7	-19.4 ± 2.4	-24.4 ± 5.2

109 **Table S6.** Mean concentrations and standard deviations (µg/L) of dissolved Ag released from

110 Cit-AgNPs and BPEI-AgNPs dispersed in 10 mM NaNO$_3$ solution in both the absence and

111 presence of PLFA. AgNPs were separated from dissolved fraction using the ultrafiltration

112 membranes with the MWCO of 50 kDa. Mean concentrations and standard deviations were

113 calculated for 3 replicates.

Day	100 µg/L Cit-AgNPs			100 µg/L BPEI-AgNPs		
	without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA	without PLFA	with 5 mg/L PLFA	with 30 mg/L PLFA
0	1.1 ± 0.6	0.2 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.2
1	4.8 ± 0.6	1.1 ± 0.0	2.9 ± 0.2	2.5 ± 0.4	0.8 ± 0.0	2.4 ± 0.1
2	4.4 ± 0.8^{Ac}	1.3 ± 0.3	4.1 ± 0.8	2.3 ± 0.7^{Ab}	1.4 ± 0.1	3.7 ± 0.4
3	6.2 ± 0.9	2.5 ± 0.2	7.0 ± 1.2	2.4 ± 0.7	1.8 ± 0.3	5.0 ± 1.1
4	5.4 ± 0.6	3.1 ± 0.2	7.4 ± 0.2	3.2 ± 0.6	2.2 ± 0.5	7.2 ± 0.7
5	6.4 ± 1.5	3.7 ± 0.3^{Ac}	7.9 ± 0.7^{Bc}	3.9 ± 0.8	2.3 ± 0.1^{Ab}	7.8 ± 0.4^{Bb}
6	7.5 ± 1.6	3.2 ± 0.4	9.5 ± 0.4	4.1 ± 0.6	2.4 ± 0.3	7.1 ± 0.6

114 $^{Ac \text{ and } Bc, \; Ab \text{ and } Bb}$ *t*-test was performed to compare mean concentrations of dissolved Ag between
115 AgNPs samples. Ac, Bc and Ab, Bb denote significant differences for Cit-AgNPs and BPEI-
116 AgNPs samples, respectively ($p < 0.05$). The same letters in each AgNPs sample group denote
117 no significant differences.

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127 **Table S7.** Mean concentrations and standard deviations (µg/L) of dissolved Ag released from

128 Cit-AgNPs and BPEI-AgNPs in the presence of PLFA at 6 days. AgNPs were separated from

129 dissolved fraction using the ultrafiltration membranes with the MWCO of 3 kDa and 50 kDa.

130 Mean concentrations and standard deviations were calculated for 3 replicates.

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MWCO of ultrafiltration membrane	100 µg/L Cit-AgNPs		100 µg/L BPEI-AgNPs	
	5 mg/L PLFA	30 mg/L PLFA	5 mg/L PLFA	30 mg/L PLFA
3 kDa	0.6 ± 0.3^{Ac}	$< LOQ^{\dagger}$	0.2 ± 0.3^{Ab}	$< LOQ^{\dagger}$
50 kDa	3.2 ± 0.4^{Bc}	9.5 ± 0.4	2.4 ± 0.3^{Bb}	7.1 ± 0.6

135 †LOQ (limit of quantification) for ICP-MS was 0.1 µg/L for Ag. [Ac and Bc, Ab and Bb] t-test was
136 performed to compare mean concentrations of dissolved Ag between AgNPs samples using
137 different ultrafiltration membranes. Ac, Bc and Ab, Bb denote significant differences for Cit-
138 AgNPs and BPEI-AgNPs samples, respectively ($p < 0.05$).

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141 **REFERENCES**

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