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

Supplementary Information

Transformations of Silver Nanoparticles in Wastewater Effluents: Links to Ag Bioavailability

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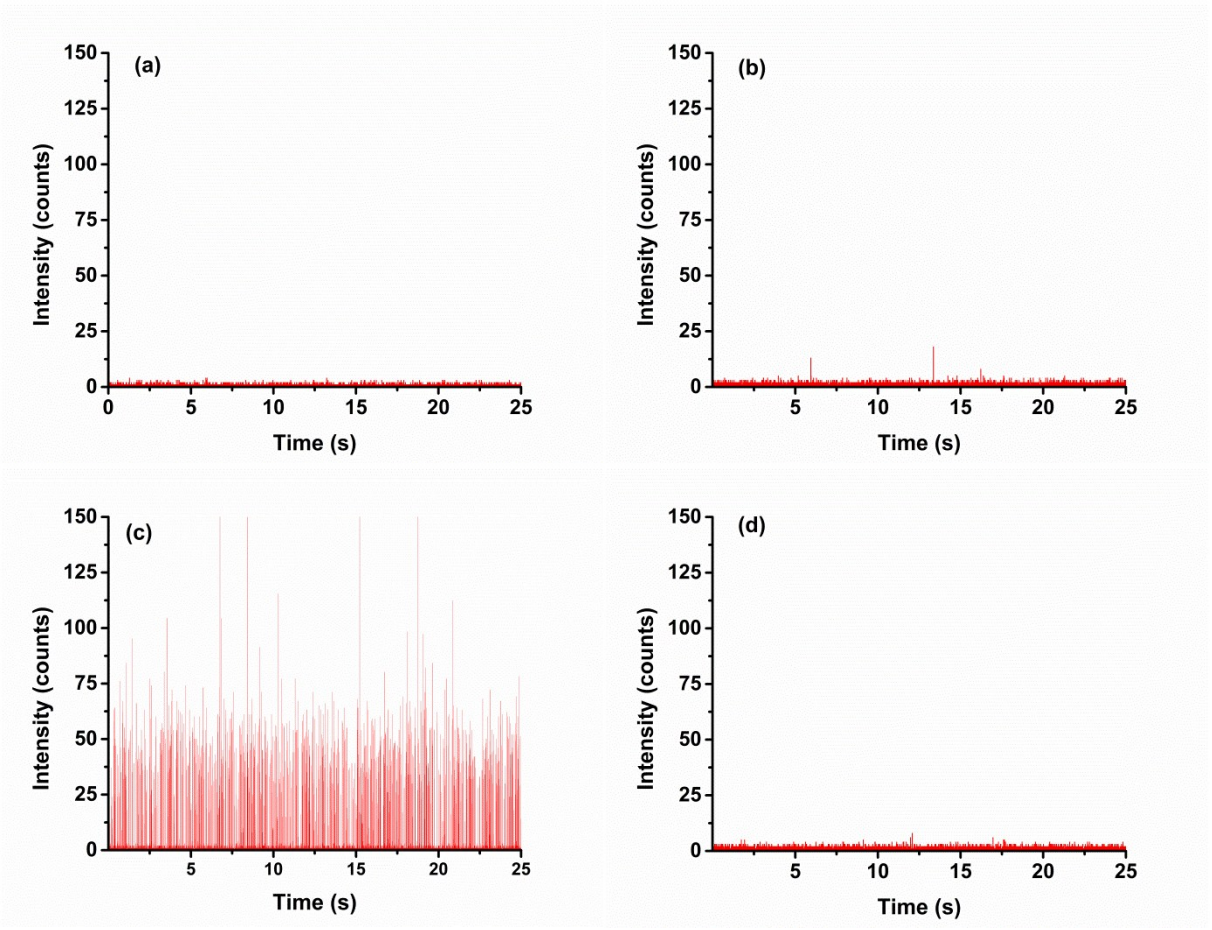

Figure S1. Time resolved signals for Ag acquired for a) Milli-Q water, b) Milli-Q water after passage through the IEC, c) Ag NP suspension (100 ng L^{-1}) after passage through IEC, and d) Milli-Q water after passage through IEC (i.e., washing) immediately following the analysis of a suspension of Ag NP (part c). Figures were obtained from Hadioui *et al*. and the original data were presented in the supplementary information section of Hadioui *et al*. (2014) [1].

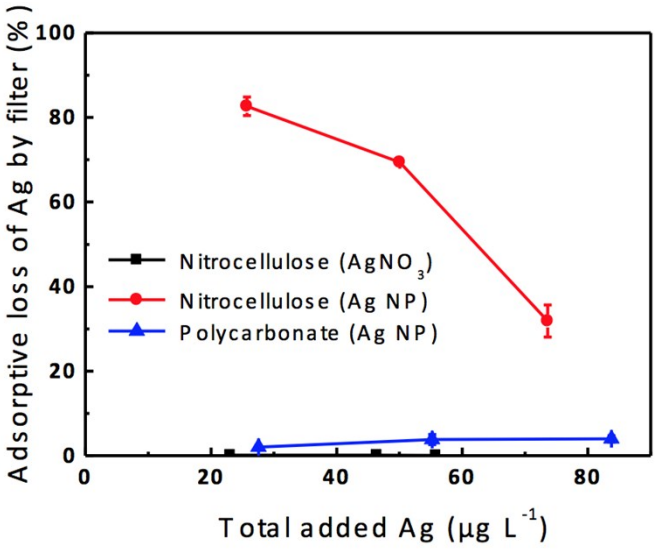

Figure S2. Total Ag retained by filter membranes (nitrocellulose or polycarbonate) of 3.0 µm pore size, after filtration of $AgNO_3$ solutions or Ag NP suspensions of various concentrations.

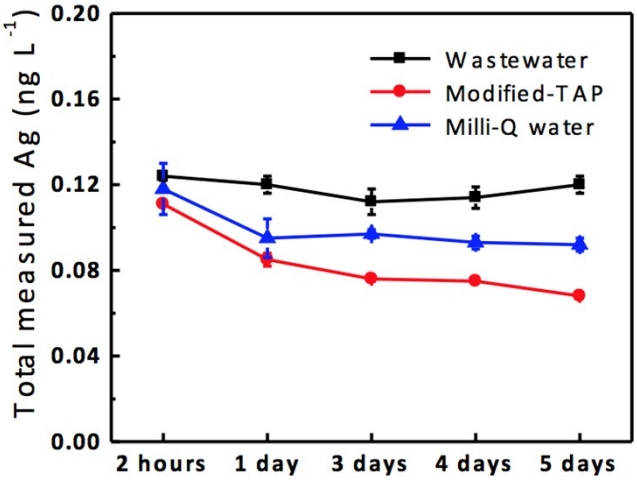

Figure S3. Total Ag measurements for a Ag NP suspension (starting concentration of 0.120 µg L^{-1}) after 2 hours, 1 day, 3 days, 4 days and 5 days of incubation in a polypropylene tube.

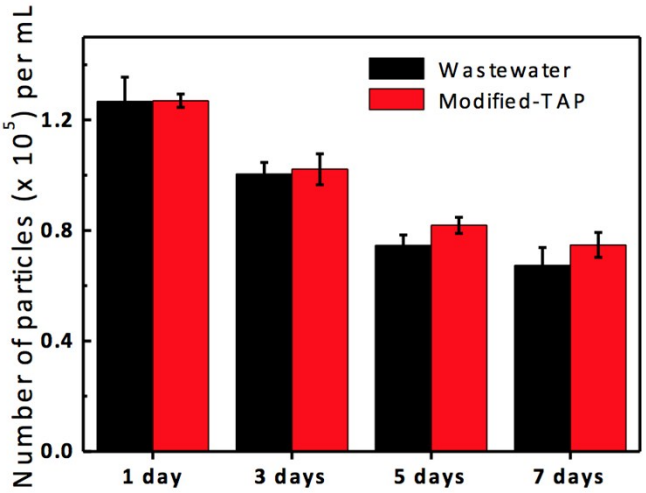

Figure S4. Particle number concentrations for a Ag NP suspension of 100 ng L^{-1} after 2 hours, 1 day, 3 days, 4 days and 5 days of incubation in wastewater and modified-TAP medium.

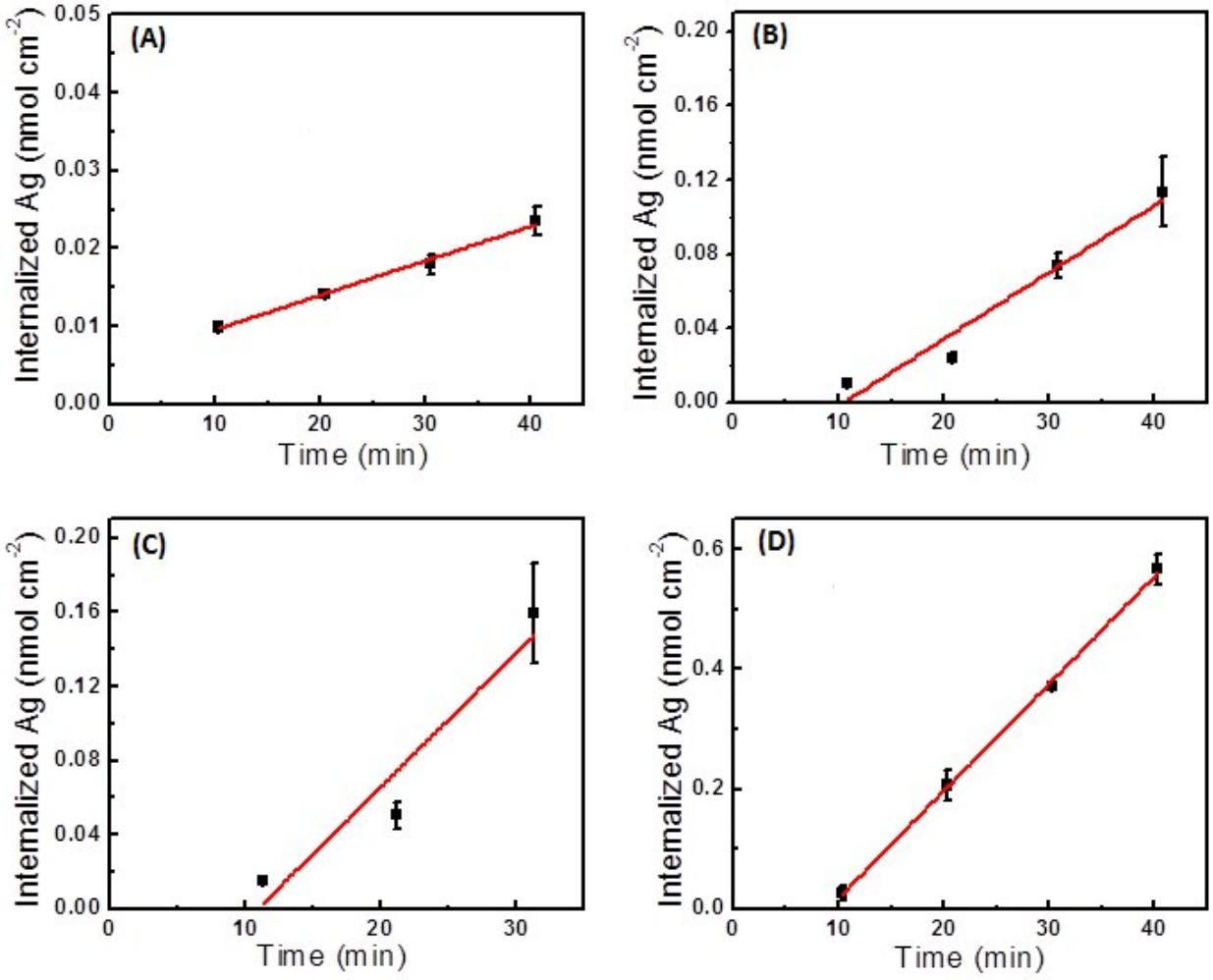

Figure S5. Measured Ag biouptake of algal cells (*C. reinhardtii*) upon exposure to $AgNO_3$ of A) 0.2 μmol L^{-1}, B) 0.5 μmol L^{-1}, C) 1.0 μmol L^{-1} and D) 1.3 μmol L^{-1} in modified-TAP (control) media as a function of time.

In spite of the precautions that were undertaken to avoid Ag losses, extrapolation of the Ag biouptake to the y-axis (0 min) still yielded non-zero values in some cases. For this reason, and in agreement with common literature practice, results were analyzed using the metal uptake fluxes (slopes of the biouptake curves). The assumption here is that adsorption to the cell wall is relatively rapid as compared to uptake (which is generally recognized as the rate-limiting step, [2]). As discussed in the main section, it is nonetheless possible some of the Ag remaining after the cysteine wash was simply Ag NP that were associated with the cell surface. Nonetheless, based upon the relative magnitudes of the y-intercept and the biouptake flux, most of the Ag was assumed to be crossing the biological membrane.

Table S1. Determinations of metal concentrations, pH and total organic carbon (TOC) in a filtered (3 μm, SSWP, Millipore) wastewater effluent sample collected at a wastewater treatment plant (WWTP) in Montreal, QC, Canada. Metal measurements were performed using semiquantitative analysis mode by ICP-MS. NM=not measured, ND= not detected

Metal	**Concentration** $\mu g\ L^{-1}$	**Metal**	**Concentration** $\mu g\ L^{-1}$	**pH**	**TOC** $mg\ L^{-1}$
Be	ND	Ag	0.002	7.7-7.8	16.2
B	40	Cd	ND		
Na	NM	Cs	0.014		
Mg	3327	Ba	2.06		
Al	1.0	La	0.001		
P	768	Ce	0.002		
K	4180	Pr	ND		
Ca	7656	Nd	0.004		
V	0.38	Sm	0.002		
Cr	ND	Eu	0.001		
Mn	2.1	Gd	0.017		
Fe	28.6	Dy	0.002		
Co	0.50	Ho	ND		
Ni	0.54	Er	0.001		
Cu	5.7	Tm	ND		
Zn	3.8	Yb	0.001		
Ga	0.007	Lu	ND		
As	0.26	Tl	0.020		
Se	0.83	Pb	0.12		
Rb	1.9	Th	0.030		
Sr	74.6	U	0.053		

Table S2. Protocol for the preparation of modified-TAP medium. MQW refers to Milli-Q water.

	Solution	Chemical/solution	Quantity/volume	Medium	pH
Step 1	Modified-Bei	$(NH_4)_2SO_4$ $Ca(NO_3)_2 * 4H_2O$ $MgSO_4 * 7H_2O$	2.31 gr 0.40 gr 0.50 gr	500 mL MQW	-
Step 2	Modified-Tris	Tris	29.04 gr	300 mL MQW	pH adjusted to 7
Step 3	Modified-TAP	Modified-Bei Modified-Tris	20.0 mL 5.0 mL	Complete to 800 mL with MQW	-

Table S3. Raw data for total dissolved Ag measured using SP-ICP-MS before and after coupling to an ion-exchange column (IEC), as well as the estimated fractions of strongly- and weakly-bound (or free) dissolved Ag forms. M-TAP, AE, and WW refer to modified-TAP medium, algal exudates and wastewater effluent, respectively. The determinations of dissolved Ag by SP-ICP-MS were made using technical triplicates (n = 3, mean ± standard deviation). Measurements were made following different equilibration times in the media (Time).

	Total dissolved Ag as measured by SP-ICP-MS			Estimated* dissolved forms	
Medium	**Time**	**Without IEC**	**With IEC**	**Strongly-bound Ag**	**Free and labile Ag complexes**
	day	*ng L^{-1}*	*ng L^{-1}*	*%*	*%*
M-TAP	1	34.2 ± 1.4	9.0 ± 1.1	26.3	73.7
M-TAP	2	34.2 ± 1.7	9.7 ± 0.6	28.5	71.5
M-TAP	7	53.4 ± 3.3	13.2 ± 2.6	24.7	75.3
M-TAP with AE	1	111.5 ± 3.0	86.3 ± 6.3	77.4	22.6
M-TAP with AE	2	117.0 ± 1.0	105.7 ± 1.0	90.3	9.7
M-TAP with AE	7	166.5 ± 9.9	159.3 ± 16.4	95.7	4.3
WW	1	79.4 ± 2.4	65.6 ± 2.7	82.6	17.4
WW	2	95.3 ± 2.7	95.9 ± 4.7	100	0.0
WW	7	180.0 ± 2.2	177.0 ± 6.9	98.4	1.6

* The estimations were made based upon the assumption that the interactions of nanoparticles and/or strongly-bound Ag complexes will be minimum or none, whereas free and/or labile (weakly-bound) Ag complexes will be reacting with the ion-exchange resin.

Table S4. Mean particle sizes as measured by SP-ICP-MS for 400 ng L^{-1} and 100 ng L^{-1} of Ag NPs (starting concentrations) in a wastewater (WW) effluent, modified-TAP (M-TAP) and Milli-Q water after 1, 3, 5 and 7 days of exposure.

	C_0 = 400 ng L^{-1}, Ag NP			**C_0 = 100 ng L^{-1}, Ag NP**		
Exposure time	**WW**	**M-TAP**	**Milli-Q water**	**WW**	**M-TAP**	**Milli-Q water**
day	*nm*	*nm*	*nm*	*nm*	*nm*	*nm*
1	51.6 ± 0.1	50.3 ± 3.7	50.5 ± 1.6	45.6 ± 0.2	40.1 ± 4.2	43.1 ± 0.6
3	48.6 ± 0.1	45.8 ± 5.5	48.5 ± 1.4	43.0 ± 0.7	37.0 ± 3.9	41.9 ± 0.3
5	51.8 ± 0.4	48.7 ± 3.2	48.8 ± 2.6	44.5 ± 0.3	39.9 ± 3.2	44.5 ± 0.7
7	51.0 ± 0.3	46.7 ± 3.3	48.6 ± 2.2	43.2 ± 0.5	37.9 ± 0.9	43.5 ± 0.5

Table S5. Instrumental and sample size detection limits observed for 100 ng L^{-1} Ag NPs in Milli-Q water, modified-TAP (M-TAP) and a wastewater effluent (WW). The instrumental size detection limit represents the technical detection limit of the instrument under applied conditions, whereas the sample size detection limit refers to the size of the smallest particle detected in the given medium. The measurements were performed in triplicates (n = 3, mean ± standard deviation).

	Milli-Q water	M-TAP	WW
Instrumental size detection [nm]	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.1
Sample size detection [nm]	16.6 ± 0.1	20.9 ± 0.1	21.9 ± 1.73

Table S6. Biouptake fluxes for algal cells (*C. reinhardtii*) exposed to $AgNO_3$ and Ag NP in modified-TAP (control) and wastewater effluent media. *NS* refers to fluxes that were not significantly different ($p<0.05$) from zero.

Medium	**Ag source**	**Total Ag**	**Biouptake flux**
		µmol L^{-1}	*pmol cm^{-2} min^{-1}*
Modified-TAP	$AgNO_3$	0.2	0.44 ± 0.03
Modified-TAP	$AgNO_3$	0.4	3.60 ± 0.52
Modified-TAP	$AgNO_3$	0.5	7.24 ± 2.05
Modified-TAP	$AgNO_3$	1.0	17.89 ± 0.39
Wastewater effluent	$AgNO_3$	0.2	*NS*
Wastewater effluent	$AgNO_3$	0.5	*NS*
Wastewater effluent	$AgNO_3$	1.0	1.05 ± 0.03
Wastewater effluent	$AgNO_3$	1.3	1.04 ± 0.16
Wastewater effluent	Ag NP	0.5	*NS*
Wastewater effluent	Ag NP	0.8	*NS*
Wastewater effluent	Ag NP	1.3	4.34 ± 0.46
Wastewater effluent	Ag NP	2.0	2.82 ± 0.36

References

1. Hadioui, M., C. Peyrot, and K.J. Wilkinson, *Improvements to single particle ICPMS by the online coupling of ion exchange resins.* Analytical chemistry, 2014. **86**(10): p. 4668-4674.
2. Wilkinson, K.J. and J. Buffle, *Critical Evaluation of Physicochemical Parameters and Processes for Modelling the Biological Uptake of Trace Metals in Environmental (Aquatic) Systems*, in *Physicochemical Kinetics and Transport at Biointerfaces*. 2004, John Wiley & Sons, Ltd. p. 445-533.