Supporting information

Flow Injection On-line Dilution Single Particle Inductively Coupled Plasma - Mass Spectrometry for Monitoring Silver Nanoparticles in Seawater and Marine Microorganisms

Claudio Toncelli[a,b,c], Kyriaki Mylona[a,b], Manolis Tsapakis[b], Spiros A. Pergantis[a,*]

[a] Environmental Chemical Processes Laboratory, Department of Chemistry, University of Crete, Voutes Campus, Heraklion 71003 Crete, Greece
[b] Hellenic Centre for Marine Science (HCMR), Institute of Oceanography, Gournes, Heraklion 71500, Crete, Greece
[c] Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Protection and Physiology, Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland.

Table of Contents for Supporting Information

System Cleaning. In order to clean the injection valve, loop and capillary tubing, at the beginning and the end of a working day, a cleaning solution consisting of 3% w/w nitric acid and 3% w/w hydrogen peroxide was passed through the injector and the connecting capillaries to the nebulizer at a flow of 2.0 μL min^{-1}. After ten minutes (i.e. the time needed for eluting the solution from the loop of 20 μL into the tubing), the loop was freshly re-filled with the same cleaning solution. After a total time of 20 minutes from the start of cleaning the flow injection system, the cleaning solution still present in the loop was flushed at a flow of 30 μL min^{-1}. Then two injections of seawater blank at 30 μL min^{-1} and 10 μL min^{-1}, respectively, were performed in order to be sure that no nanoparticles are present in the FI system.

Polydispersity Index calculations

The *PDi* is calculated as the ratio of the numeral average mass of NPs to the weight average mass of the NPs. The numeral average mass of the nanoparticles ($M_{mean,n}$) can be calculated as:

$$M_{mean,\,n} = \sum m_{i,\,AgNP} / n \qquad (S1)$$

Where $\sum^{m_{i,\,AgNP}}$ is the sum of all nanoparticle masses (fg) for a given injection and n is the detected spike number for the same injection. On the other hand, the weight average mass of the nanoparticle, is calculated as:

$$M_{mean,\,m} = \sum m_{i,AgNP}^{2} / \sum m_{i,\,AgNP} \qquad (S2)$$

From equations S1 and S2, the *PDi* describing the Ag NP distribution is calculated from the following expression:

$$PDi = M_{mean,\,m} / M_{mean,\,n} \qquad (S3)$$

Table S1. Physicochemical properties of AgNPs used in this study.

Experiment type	Microcosm	Mesocosm	Microcosm	Microcosm
End Capping type	BPEI	BPEI	PVP	PVP
Nominal Diameter	60nm	60 nm	60 nm	40 nm
Diameter (TEM)	57.2±6.7 nm	60.6±5.8 nm	60.8±6.6 nm	39.0±5.2 nm
Surface Area (TEM)	9.7 m^2g^{-1}	9.3 m^2g^{-1}	9.2 m^2 g^{-1}	14.2 m^2 g^{-1}
Particle Concentration in purchased suspensions	2.1E+10 NPs mL^{-1}	1.7E+10 NPs mL^{-1}	1.8E+10 particles mL^{-1}	6.8E+10 particles mL^{-1}
Hydrodynamic Diameter	98.2 nm	76.2 nm	74.7 nm	54.2 nm
Zeta Potential	46.2 mV	68.7 mV	-33.9 mV	-44.3 mV
pH of Solution	5.9	7.3	5.8	5.7

Table S-2. Typical oligotrophic eastern Mediterranean seawater composition

[a] concentrations are expressed in mg/L

Parameter	Value
pH	8.4
DOC (mmol Kg^{-1})	62 ± 5.9
Cl^-	21200[a]
Na^+	11800[a]
SO_4^{2-}	2920[a]
Mg^{2+}	1403[a]
Ca^{2+}	423[a]
K^+	463[a]
HCO_3^-	145[a]
Sr^{2+}	13[a]
Br^-	155[a]
BO_3^-	72[a]
I^-	2[a]

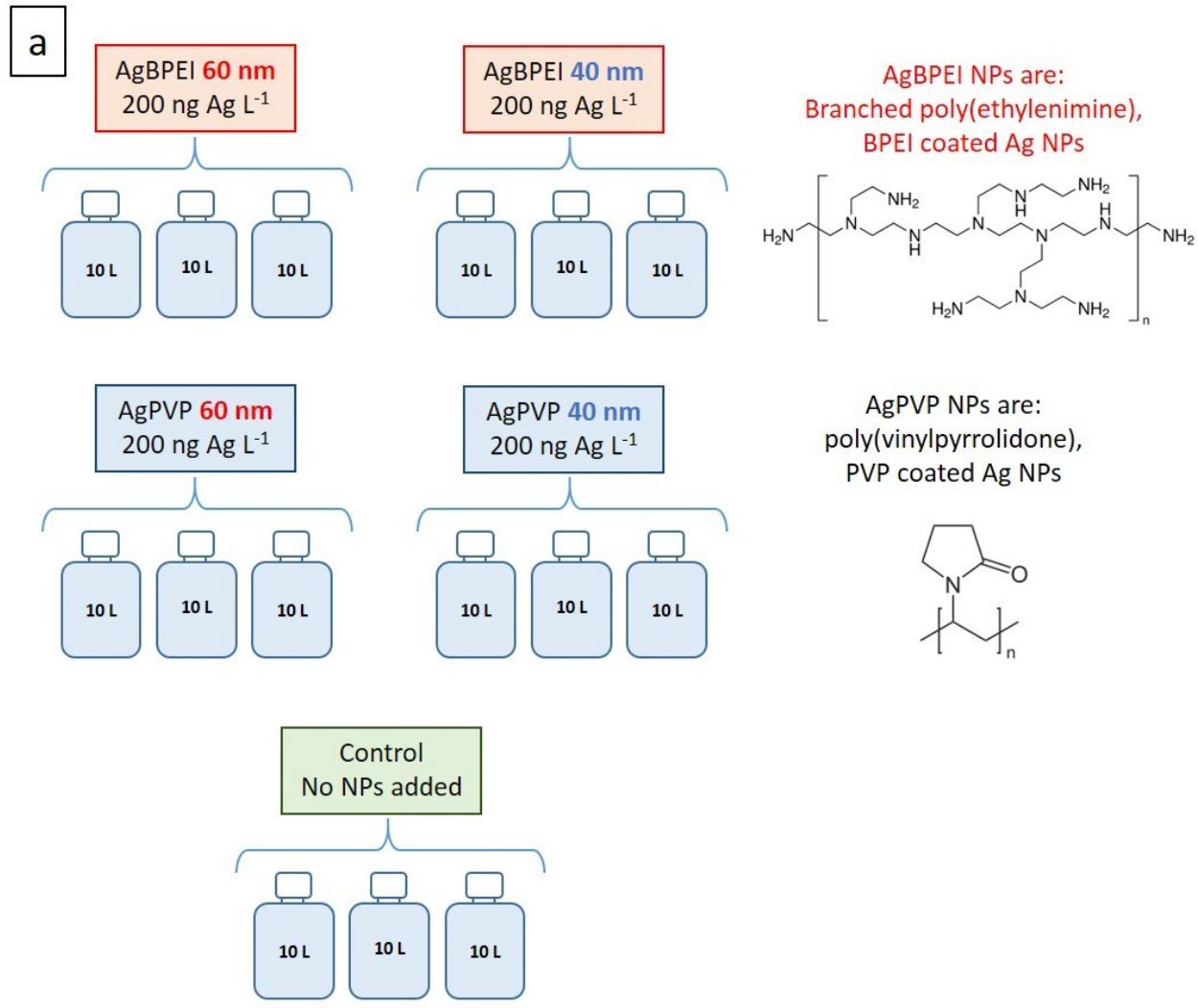

Figure S1. Schematic view of the microcosm experiment: 10 L low-density polyethylene Nalgene bottles were filled with oligotrophic seawater and a specific type (size and coating) of AgNPs. Pictures of 10 L tanks (b) and the same tanks once deployed in a seawater pool (c) are also presented. Analytical samples were taken from each of these tanks at predefined time points. Their analysis gave rise to the graphs shown in the main manuscript Figures 4 and 5.

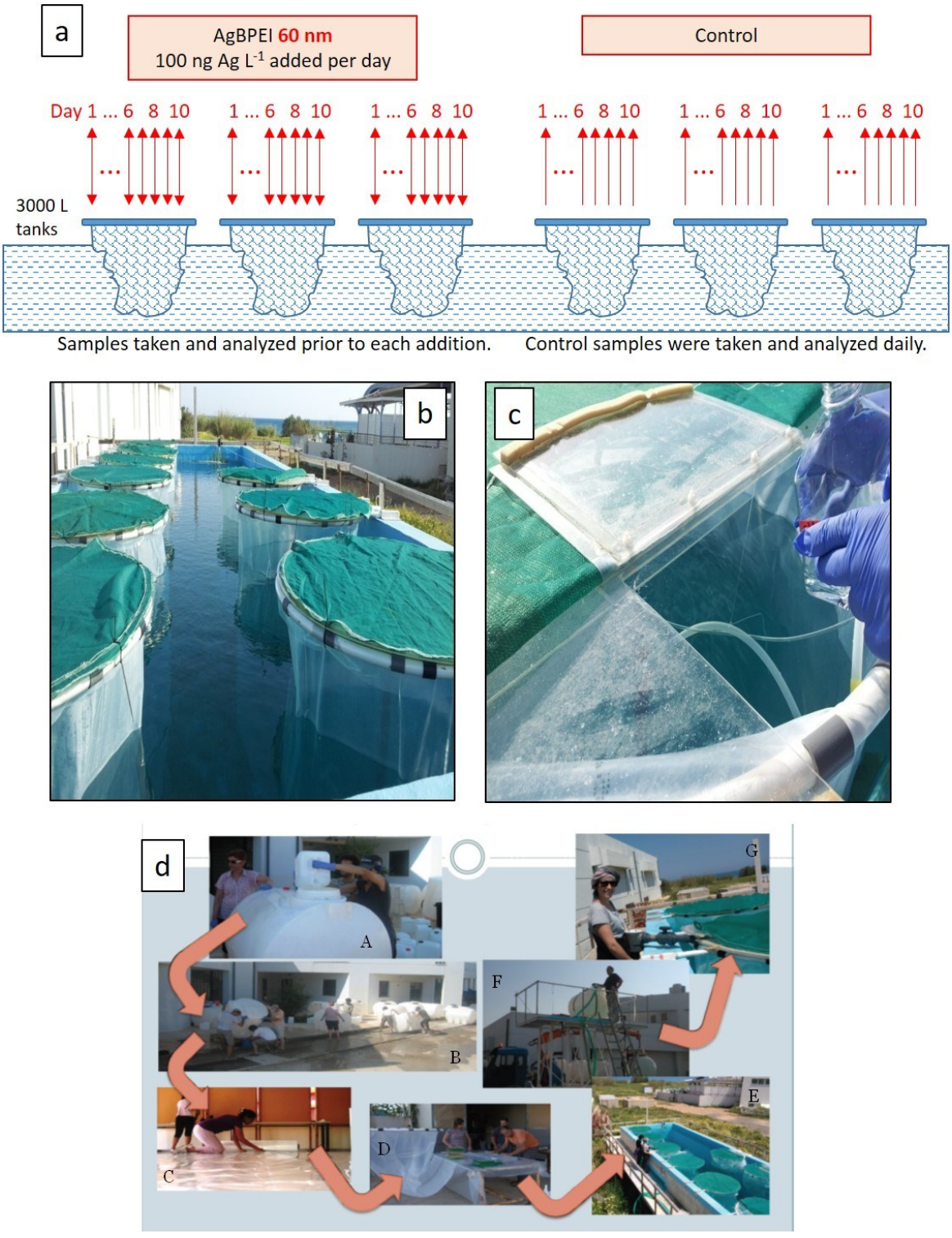

Figure S2. Schematic view of the mesocosm experiment (**Fig. S2a**). Branched poly(ethylenimine) silver nanoparticles (AgBPEI) of 60 nm nominal diameter were introduced daily into each seawater containing 3000 L tank (**Fig. S2a**) at an amount that gives an added 100 ng Ag L^{-1} per day per tank. This was continued for 10 days resulting in a maximum input concentration of 1 µg Ag L^{-1}. The tanks were in a large seawater pool supplied with a continuous flow of seawater. Mild agitation inside the tanks was regulated by a bubbler. Photographs showing the setup of the

mesocosm experiment are in **Fig. S2b-d**. The mesocosm tanks in the seawater pool are in **Fig. S2b** whereas the addition of the AgNPs is shown in **Fig. S2c**. In **Fig. S2d** the setup of the system is shown in steps **A-G**. More specifically polypropylene tanks were used for the collection of seawater from Gouves harbor. Prior to sample collection the tanks were washed three times with a 4% HCl solution with a final de-ionized water rinse (**A, B**). Polyethylene bags used in the mesocosm experiment were cut accordingly to hold 3000 L of seawater (**C, D**) and placed in a seawater pool. Each bag had a Plexiglas cover and a net above it (**E**). Subsequently, the polyethylene bags were filled up with seawater originating from the polypropylene tanks via gravity (**F, G**).

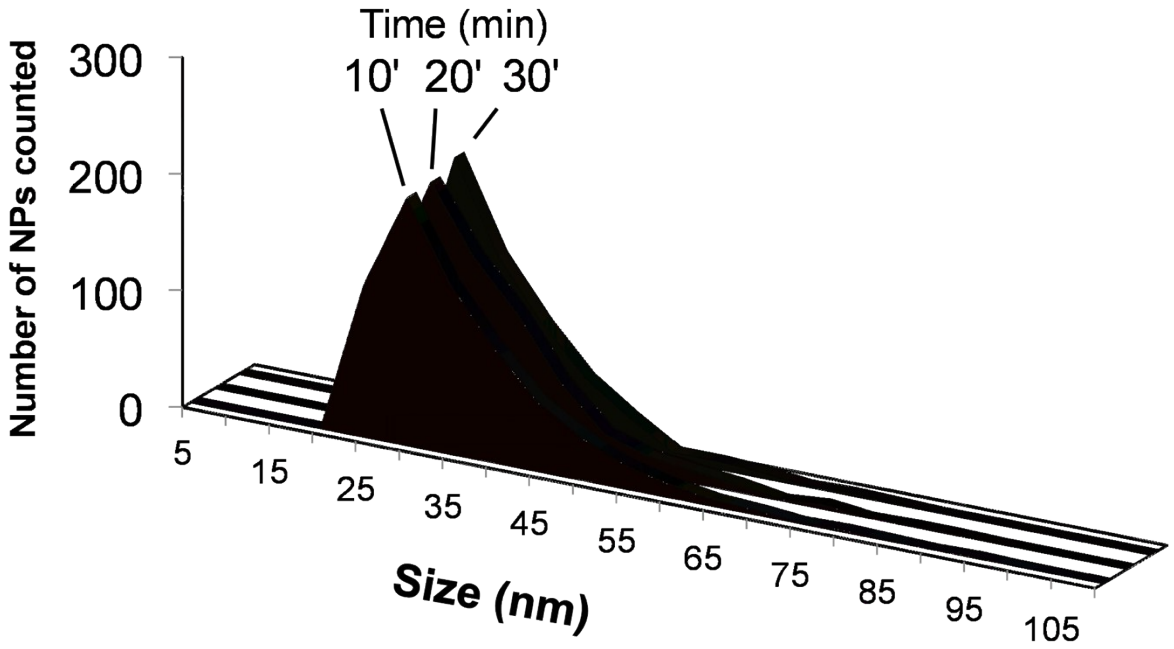

Figure S3. Histogram of size distribution for AgBPEI (nominal size 60 nm) accumulated in 0.2 micrometer filter after 9 days of exposure in seawater mesocosm experiments which underwent continuous spiking of 100 ng Ag L^{-1} per day. Profiles show NP size distributions for different ultrasonication extraction times (i.e 10, 20 and 30 min) using deionised water. Each sample corresponds to a total seawater filtered volume of 15 L, deionized water volume used during ultrasonication was 14 mL per filter.

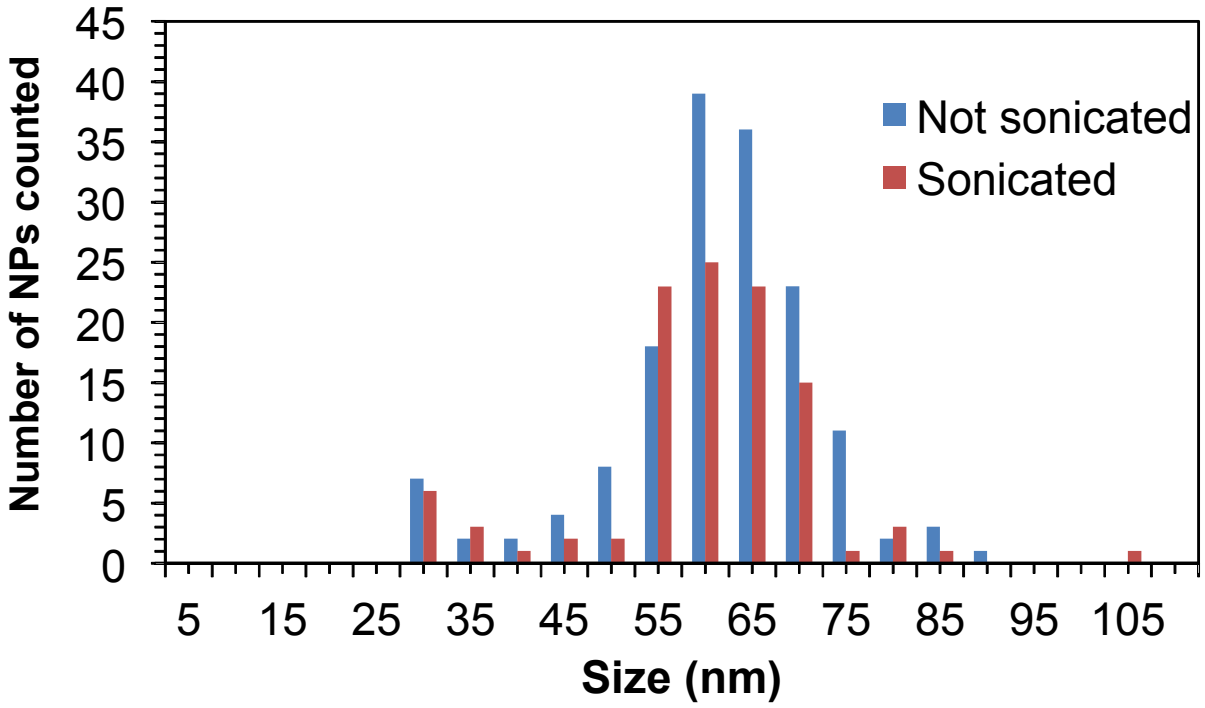

Figure S4. Size histograms of standard AgBPEI (nominal size of 60 nm) at a concentration of 200 ng Ag L^{-1} before and after ten minutes of sonication in de-ionized water

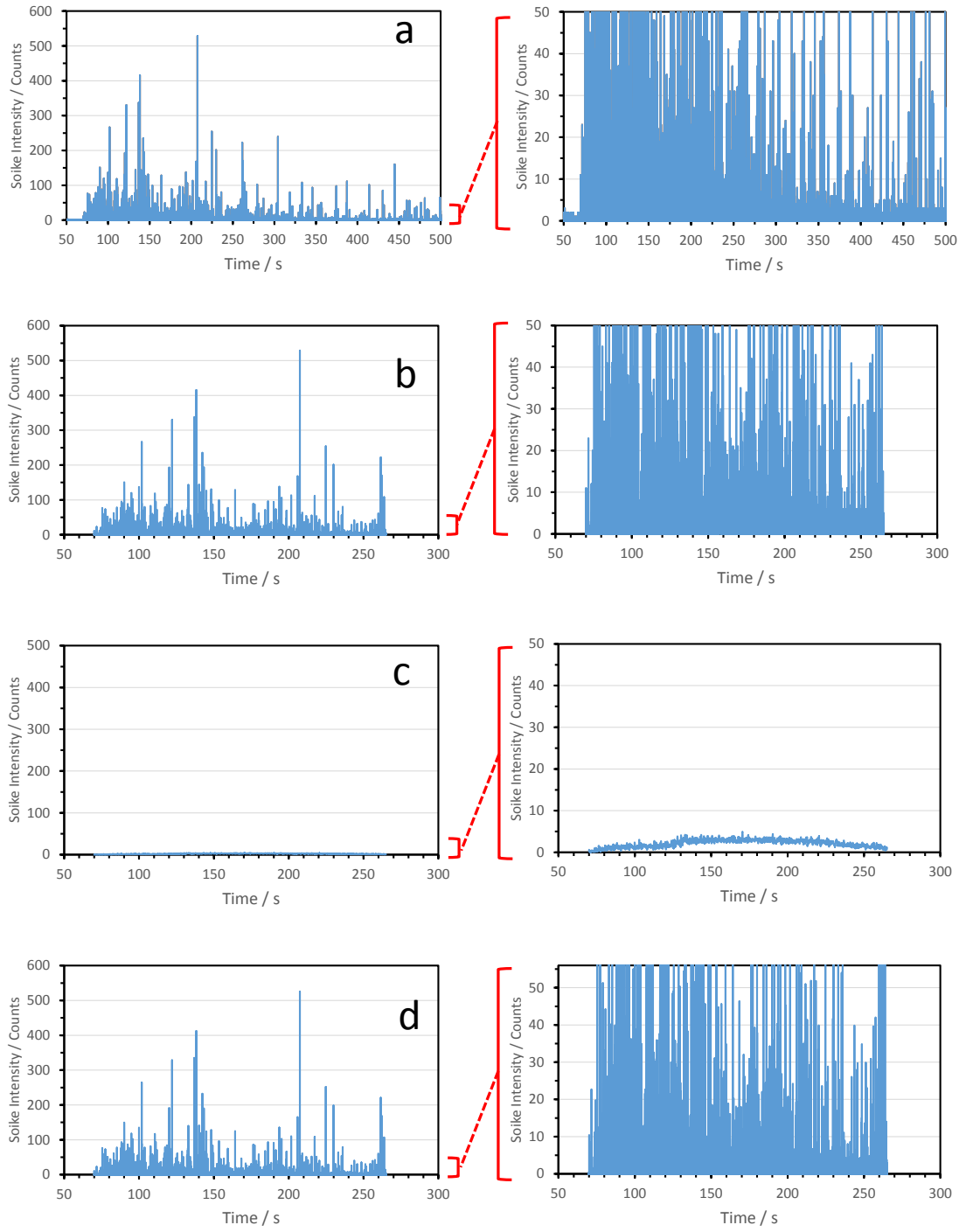

Figure S5. Single particle ICP-MS profiles for (a) AgBPEI NPs pre-concentrated on 0.2 µm filter after seven days into a mesocosm experiment after 10 min of ultrasonication of the filter in deionized water, (b) enlargement of (a) in the flow injection time range from 70 to 265 s where the continuous signal corresponding to dissolved Ag signal is present together with the Ag NP spike signals, (c) dissolved Ag continuous signal after removal of spike signals, and (d) enlargement of (a) where the dissolved Ag signal has been subtracted and thus spike signals are only present.

Dissolved Ag determination

The area under the deconvoluted dissolved Ag continuous signal (shown in Fig. S5c) is used to determine the dissolved Ag concentration in the examined filter extract size fraction. This is achieved by injecting a dissolved Ag standard of specific concentration ($C_{diss.Ag\ Std.}$), i.e. single point calibration, via the 20 μL loop. The responses obtained from this standard ($R_{diss.Ag\ Std.}$) and the subsequent analysis of the filter extract ($R_{diss.Ag\ filter\ ext.}$) are used to calculate the concentration of dissolved Ag in the filter extract. The dissolved Ag concentration in ng Ag per L of seawater ($C_{diss.Ag\ seawater}$), is obtained by dividing the total amount of dissolved Ag extracted from the filter by the total volume of filtered seawater.

$$\frac{R_{diss.\ Ag\ Std.}}{R_{diss.Ag\ filter\ ext.}} x\ C_{diss.\ Ag\ Std.} = C_{diss.\ Ag\ filter\ ext.}$$

$$\frac{C_{diss.Ag\ filter}\ x\ V_{filter\ extract}}{V_{extracted\ seawater}} = C_{diss.\ Ag\ seawater}$$