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Surface functionalization determines behavior of nanoplastic solutions in model aquatic environments

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Abstract :

Plastic debris are classified as a function of their size and recently a new class was proposed, the nanoplastics. Nano-sized plastics have a much greater surface area to volume ratio than larger particles, which increases their reactivity in aquatic environment, making them potentially more toxic. Only little information is available about their behavior whereas it crucially influences their toxicity. Here, we used dynamic light scattering (DLS) to explore the influence of environmental factors (fresh- and saltwater, dissolved organic matter) on the behavior (surface charge and aggregation state) of three different nano-polystyrene beads (50 nm), with (i) no surface functionalization (plain), (ii) a carboxylic or (iii) an amine functionalization. Overall, the positive amine particles were very mildly affected by changes in environmental factors with no effect of the salinity gradient (from 0 to 653 mM) and of a range 1–30 µg.L−1 and 1–10 µg.L−1 of organic matter in artificial seawater and ultrapure water, respectively. These observations are supposedly linked to a coating specificity leading to repulsive mechanisms. In contrast, the stability of the negatively charged carboxylic and plain nanobeads was lost under an increasing ionic strength, resulting in homo-aggregation (up to 10 µm). The increase in organic matter content had negligible effect on these two nanobeads. Analysis performed over several days demonstrated that nanoplastics formed evolving dynamic structures detected mainly with an increase of the homo-aggregation level. Thus, surface properties of given polymers/particles are expected to influence their fate in complex and dynamic aquatic environments.

Highlights

► The behavior of different nanopolystyrene beads was investigated by dynamic light scattering. ► Surface functionalization affects the behavior of nanopolystyrene beads. ► Carboxylate and plain nanopolystyrene beads formed microscale aggregates in seawater. ► Organic matter had negligible effect on all nanoplastics tested.► Nanoplastics formed evolving dynamic structure over time.

Keywords : Nanoplastic, Dynamic light scattering, Behavior, Aggregation, Salinity, Organic matter

1 Introduction

Owing to the exponential use of plastic items (335 million tons (MT) produced in 2016) by human societies, their mismanagement after usage is a considerable problem of this century (Galloway et al., 2017; PlasticsEurope, 2017). Every year, among the 31.9 MT of plastic wastes that are discarded in environment, between 4.8 and 12.7 MT end up in oceans (Jambeck et al., 2015; Rochman, 2018). To date, plastic debris are ubiquitous in freshwater and marine systems from rivers to oceans (Cózar et al., 2014, 2017; Lebreton et al., 2017; Woodall et al., 2014).

For about a decade, the research emphasis is laid on the small plastic debris called “microplastics (MP; < 1 mm)” originating from manufactured beads/pellets/fibers or mostly (> 80%) the weathering of bigger wastes under environmental conditions (UV light, mechanical degradation, biodegradation) (Hüffer et al., 2017; Galloway et al., 2017). However, a new class of smaller debris than MP was proposed lately, the nanoplastics (NP) (Koelmans et al., 2015) which their first report was argued in the North Atlantic Gyre (Ter Halle et al., 2017). To date, several classifications of NP were proposed : <20 µm according to the size used to classify nanoplankton (Wagner et al., 2014); <1µm owing to the colloidal nature of NP (Gigault et al., 2018); <100 nm in the narrower sense of the definition of engineered nanomaterials (Mattsson et al., 2015). This last classification is the one adopted for this present study. Similarly to MP, NP in the oceans can originate from a direct release from cosmetics (Hernandez et al., 2017), industrial activities (Dubey et al., 2015; Stephens et al., 2013; Zhang et al., 2012), drugs (Lusher et al., 2017); or from weathering of bigger waste as it was demonstrated in laboratory under biotic (Dawson et al., 2018) or abiotic conditions (Gigault et al., 2016; Lambert and Wagner, 2016).

Nanoplastics are known to have a higher surface area/volume ratio than MP. Thus, increasing interactions with persistent organic pollutants (POP) (Liu et al., 2018; Velzeboer et al., 2014) and biological membranes (Rossi et al., 2014) calling for an accurate description of NP in the context of the chemical/biological risks in aquatic systems (Koelmans et al., 2016; Paul-Pont et al., 2018). Although ecotoxicological studies reported higher detrimental effects of NP than MP (*e.g.* Jeong et al., 2016; Tallec et al., 2018), the behavior of NP (*e.g.* interactions of NP amongst themselves and other component such as organisms and macromolecules) in experimental environments and even more in freshwater and marine environments remains largely unknown. However, because the behavior drives fate and toxicity of nanoparticles (Lowry et al., 2012), it is crucial to fill the gaps limiting our knowledge in order to understand the toxicity for aquatic life (*e.g.* Paul-Pont et al., 2018), risks for the balance of ecosystems (Galloway et al., 2017; Mattsson et al., 2015) and up to human health (Wright and Kelly, 2017). Based on works from other nanomaterials, the behavior of NP may be driven by three main processes: physical transformations (homo- or hetero-aggregation); biological transformations (interaction with all components of a biological system involving oxidation and redox mechanism transforming the surface layer of particle); interaction with macromolecules (*e.g.* adsorption of polysaccharide, organic matter, protein) leading to the development of bio- (in organism) or eco-coronas (in environment) (Galloway et al., 2017; Lowry et al., 2012; Mattsson et al., 2015).

A modelling study using various scenarios showed that NP aggregation in freshwater system could decrease the risk of NP arrival in the oceans (Besseling et al., 2017). Owing to the high diversity of polymers found in oceans and thus the high diversity of their chemical structures, this model-based assumption must be compared with experimental data and *in situ* observations when methods will be developed for NP. Indeed, as reported for other nanomaterials, variations of the chemical surface of the same material affected greatly its behavior in fluids (El Badawy et al., 2010). Also, nanoparticles' behaviors are known to be impacted by environmental factors (*e.g*. pH, salinity, organic matter content) (Keller et al., 2010). Thus, the behavior of NP may be highly complex and their fate can vary from location to location. Furthermore, from our previous study in which we revealed significant toxicity of NP on oyster fertilization success and embryo-larval development, NP behavior was hypothesized as the origin of the toxicity variability of three different 50 nm plastic beads (Tallec et al., 2018). During the redaction of this present work, Cai et al. (2018) investigated the short-term influence (600 seconds) of environmental factors on behavior of NP and reported a low incidence of organic matter on the aggregation kinetics of nanopolystyrene (nano-PS) beads (plain; 100 nm) in media containing various salt (NaCl – 1-100 mM; $CaCl_2$ – 0.1-15 mM; $FeCl_3$ – 0.001-1 mM). However, the size and the surface-functionalization which display an important role in the behavior of particles (Alimi et al., 2018) were not studied and yet of great interest particularly below 100 nm (Gigault et al., 2018). Here, we performed Dynamic Light Scattering (DLS) analyses with nanopolystyrene (50 nm) exhibiting different surface functionalization (carboxyl, amine or none) employed in previous ecotoxicological studies including ours (Della Torre et al., 2014; Jeong et al., 2016; Tallec et al., 2018). The influence of several media (ultrapure water, artificial or filtered natural seawater) and environmental factors (salinity and organic matter gradients) was explored with a temporal survey on their behavior in suspension for coping with environmental variability and to anticipate further ecotoxicology testing.

2 Materials and Methods

2.1 Nanoplastics

Three types of nanopolystyrene beads (50 nm) were used in this study: (i) without surface functionalization – PS-Plain; (ii) with carboxyl groups – PS-COOH; (iii) with amine groups – PS-NH_2. All NP were purchased from Polysciences/Bangs Laboratories and stored at 4°C prior to experiments. Polymer types were previously confirmed by Raman microspectroscopy analysis (Tallec et al., 2018). Commercial suspensions were supplied in ultrapure water (UW) with a small amount of surfactant (<0.1%; Tween-20©). All tests were performed with the same batch of particles.

2.2 Dynamic Light Scattering (DLS) analyses

For DLS analyses, commercial suspensions of NP were diluted in UW at a stock concentration of 1,000 mg.L^{-1} then at a work concentration of 100 mg.L^{-1} in the selected media according to Tallec et al. (2018). It was the optimal concentration allowing high reproducibility and sufficient detection level of particles by DLS. The need to use high particles concentration to reach a high measurement accuracy is common in the field of nanoparticles such as PS (50 mg.L^{-1}; Cai et al., 2018); iron oxide (200 mg.L^{-1}; Chekli et al., 2013); gold (20 mg.L^{-1}; Liu et al., 2012); titanium oxide (50 – 80 mg.L^{-1}; French et al., 2009; Loosli et al., 2013) and zinc oxide (100 mg.L^{-1}; Mohd Omar et al., 2014). The analysis of colloidal fraction from environmental matrices required ultrafiltration (Mintenig et al., 2018). The ultrafiltration factor used by Ter Halle et al. (2017) to detect traces of NP from the North Atlantic Gyre was 200. Therefore the concentration of our working solutions could be extrapolated inversely leading to an environmental value theoretically equivalent to 500 µg.L^{-1}.

DLS measurements were performed with a nano-Zetasizer ZS (Malvern Instruments, UK) using an angle of 173° Backscatter, a temperature of 20°C and an equilibration of 120 sec (González-Fernández et al., 2018). We used the implemented data analysis software to measure the mean size of particles/aggregates (Z-average; nm), the aggregation state (polydispersity index – PDI; Arbitrary Units (A.U.)) and the mean surface charge (ζ-potential; mV) of NP. When the PDI exceeded 0.2, particles were deemed to be aggregated. The accuracy of all measures was verified with the report quality from the implemented software and a counting rate being always higher than 100 kcps. The nanoplastic suspensions were injected in disposable fold capillary cells (DTS 1060C, Malvern Instruments, UK) with syringes to obtain a final volume of 1 mL. All measurements were performed in triplicate (13 runs and 10 sec.measure^{-1} for PDI and Z-average; 40 runs and 10 sec.measure^{-1} for ζ-potential) according to González-Fernández et al. (2018). No effect of the surfactant is expected owing to its residual concentration (< 0.0001%) in all samples (Douglas et al., 1985).

2.3 Effects of environmental conditions

2.3.1 Influence of media

All particles were tested in three different media: (i) ultrapure water (UW; pH 6.6 ± 0.2); (ii) artificial seawater (ASW; pH 8.1 ± 0.1; 30 practical salinity unit [PSU]; NaCl 450 mM, KCl 10 mM, $CaCl_2$ 9 mM, $MgCl_2$ 30 mM and $MgSO_4$ 16 mM); (iii) 2-µm filtered natural seawater from the Bay of Brest sampled in January 2018 (FSW; pH 8.2 ± 0.1; PSU 32).

2.3.2 Influence of the salinity

To investigate the influence of the salinity, UW and ASW were used to create five intermediate solutions according to Loucaide et al. (2008): 0% (0 PSU; 0 mM), 25% (7.5 PSU; 163.25 mM), 50% (15 PSU; 326.5 mM), 75% (22.5 PSU; 489.75 mM) and 100% of ASW (30 PSU; 653 mM). Solutions were made one-day prior experiment and kept in dark condition at 4°C until use.

2.3.3 Influence of the organic matter

We used humic acid as a proxy of the presence of dissolved organic matter (DOM) in freshwater and estuarine/coastal environments (Baalousha et al., 2008; Cai et al., 2018; Fabrega et al., 2009). Humic acid (OM; CAS 1415-93-6) was purchased from Sigma-Aldrich. A stock solution of 1 g.L^{-1} was prepared in UW or ASW and stirred during 24h then filtered on 0.2 µm (aPES membrane) according to Yang et al. (2013). For testing the influence of the OM concentration, work solutions were adjusted at three concentrations (1, 10 and 30 mg.L^{-1}) corresponding to realistic aquatic concentrations (Cai et al., 2018). Measurements were performed immediately after contact (T0).

NP behavior was also observed over time in UW and ASW alone and with organic matter (UW+OM and ASW+OM) at the intermediate concentration (10 mg.mL^{-1}). Measurements were performed at T0, T24h and T48h.

2.4 Statistical analyses

Statistical analyses and graphical representations were conducted using the R Software (R Core Team, 2016). Before statistical comparisons, normality and homoscedasticity were screened with the Shapiro-Wilk and Levene's methods, respectively. All analyses were operated using one-way ANOVA followed by pairwise comparisons (Tukey's method) when needed. Effects of treatment on the size average were performed only when the PDI was greater than a threshold set to 0.2 indicating the start of an aggregation. The significance threshold was set at a $p\text{-value} < 0.05$. Data are expressed as the mean ± standard deviation (SD).

3 Results

3.1 Influence of media

The PS-NH_2 stayed at a nanometric scale in all media: 53.3 ± 2.3 nm in UW, 52.5 ± 0.5 nm in ASW and 67.9 ± 0.8 nm in FSW (Figure 1A). A small aggregation was observed in FSW (PDI > 0.2; ANOVA, F= 110.8, p-value < 0.001). In contrast, the aggregation level of the PS-COOH and PS-Plain solutions increased significantly (ANOVA; PS-COOH: F= 135.3, p-value < 0.001; PS-Plain: F= 358.5, p-value < 0.001) following the same trend between each medium (Figure 1A). Particles stayed at a nanometric scale (PS-COOH; 63.4 ± 3.43 nm; PS-Plain: 56.0 ± 0.2 nm; PDI < 0.2) only in UW and formed microscale aggregates in ASW (PS-COOH: 1,835.0 ± 240.0 nm; PS-Plain: 2,106.7 ± 75.4 nm) and FSW (PS-COOH: 4,530.3 ± 528.0 nm; PS-Plain: 4,810.3 ± 370.2 nm).

The ζ-potential of all particles was significantly different between UW and seawater (ANOVA; PS-NH_2: F= 91.3, p-value < 0.001; PS-COOH: F =71.5; p-value < 0.001; PS-Plain: F= 51.7, p-value < 0.001), the values were buffered in seawater (Figure 1B). For the PS-NH_2, in ASW and FSW, a mean reduction of 69% of the particle surface charge was observed in comparison with UW (58.0 ± 2.5 mV). For the PS-COOH, the ζ-potential increased by 20% and 70% in ASW and FSW, respectively, as compared to UW (-40.7 ± 3.4 mV). Similarly for the PS-Plain, a significant increase of 11% was observed in ASW and 34% in FSW compared to UW (-43.1 ± 0.8 mV).

3.2 Influence of the salinity

No statistical effect (ANOVA, p-value > 0.05) of the ionic strength gradient was observed on the aggregation of PS-NH_2 particles (mean value = 51.2 ± 1.8 nm) (Figure 2A). Despite this observation, the increase of salinity caused a significant reduction (ANOVA, F= 35.7, p-value < 0.001) of the ζ-potential with values corresponding to 58.0, 39.2, 32.8, 22.5 and 27.4 mV at 0, 163.25, 326.5, 489.75 and 653 mM, respectively (Figure 2B).

A significant effect on aggregation level of the PS-COOH (ANOVA, F= 74.5, p-value < 0.001) and PS-Plain (ANOVA, F= 253.5, p-value < 0.001) suspensions was demonstrated from 489.75 mM and 326.5 mM, respectively (Figure 2A). The size of the PS-COOH suspension increased 18-fold from 0/163.25/326.5 mM (mean value = 70.8 ± 25.9 nm) to 653 mM (1,835 ± 240 nm). Close results were observed for the PS-Plain with a 38-fold increase from 0/163.25 mM (mean value = 53.8 ± 6.4 nm) to 653 mM (2,106 ± 75 nm). A significant effect in the ζ-potential was observed with an increasing trend along the gradient for both PS-COOH (ANOVA, F= 6.7; p-value < 0.001) and PS-Plain (ANOVA, F= 15; p-value < 0.001) from 326.5 mM (Figure 2B). Compared to 0 mM where PS-COOH and PS-Plain had a mean ζ-potential of -43.9 and -42.2 mV, respectively, a maximal increase of 26% and 28% was observed at the highest ionic strength (PS-COOH: -32.6 ± 3.5 mV; PS-Plain: -30.2 ± 2.1 mV).

3.3 Influence of the organic matter

The highest concentration of organic matter (30 $mg.L^{-1}$) in UW affected significantly (ANOVA, F= 1426, p-value < 0.001; PDI > 0.2) the average size of the PS-NH_2 solution leading to the formation of aggregates with a mean size of 99.4 ± 1.8 nm while no aggregation was reported for lower levels of organic matter (1 and 10 $mg.L^{-1}$; mean value = 56.1 ± 1.4 nm) (Figure 3). The addition of organic matter decreased significantly (ANOVA, F= 1497, p-value < 0.001) in a dose-response manner the ζ-potential of the PS-NH_2 in UW (1 $mg.L^{-1}$: 46.2 ± 0.7 mV; 10 $mg.L^{-1}$: 40.5 ± 0.4 mV; 30 $mg.L^{-1}$: 24.3 ± 0.4 mV) (Figure 4). In contrast, no effect (ANOVA, p-value > 0.05) of the organic matter on the PS-NH_2 (average size and ζ-potential) was observed in ASW (Figure 3 & 4).

For other NP (PS-COOH and PS-Plain), their size and surface charge were statistically similar (ANOVA, p-value > 0.05) regardless of the organic matter contents. In UW, the PS-COOH and PS-Plain suspension remained at a nanoscale without aggregation with an average size and a ζ-potential of 57.3 ± 1.5 nm/-42.5 ± 5.0 mV and 51.8 ± 1.3 nm/-43.0 ± 4.7 mV, respectively (Figures 3 & 4). In ASW and regardless of the OM concentrations, aggregation reached 1,777 ± 34 nm for the PS-COOH and 2,082 ± 206 nm for the PS-Plain with a ζ-potential of -27.9 ± 1.6 mV and -35.7 ± 3.8 mV, respectively.

3.4 Temporal stability

The PS-NH_2 remained at a nanometric scale in all media (UW and ASW with or without OM) but formed small homo-aggregates (PDI > 0.2) at T48h in UW (67.4 ± 1.6 nm; ANOVA, F= 33.1, p-value < 0.01), ASW (71.8 ± 1.3 nm; ANOVA, F= 181.5, p-value < 0.001) and ASW+OM (96.6 ± 1.6 nm; ANOVA, F: 1300, p-value < 0.001) (Figure 5). In UW+OM at T48h, the PS-NH_2 did not aggregate and displayed a size of 56.3 ± 0.3 nm (ANOVA, p-value > 0.05). Concerning the ζ-potential, no effect was recorded in ASW with or without organic matter (mean value = 23.8 ± 2.9 mV) (Figure 6). However, significant decreases in UW (-12%; ANOVA, F= 47.9, p-value < 0.001) and UW+OM (-13%; ANOVA, F= 148.2, p-value < 0.001) were observed at T48h (UW: 45.7 ± 1.3 mV; UW+OM: 40.4 ± 0.4 mV) compared to T0 (UW: 51.4 ± 0.4 mV; UW+OM: 46.7 ± 0.8 mV).

The PS-COOH and PS-Plain suspensions stayed at a nanoscale in UW (+/- OM) despite the apparition of small aggregates (PDI > 0.2) at 48h for the PS-COOH in UW (68.2 ± 1.7 nm; ANOVA, F= 50.1, p-value < 0.001) and UW+OM (77.4 ± 4.6 nm; ANOVA, F= 38.9, p-value < 0.001) and at 24h for the PS-Plain only in UW (63.3 ± 5.0 nm; ANOVA, F= 16.6, p-value < 0.01) (Figure 5). In both ASW (ANOVA, F= 42, p-value < 0.001) and ASW+OM (ANOVA, F= 50.6, p-value < 0.001), the PS-COOH formed aggregates with an average size close to 2,000 nm at 24h and 4,000 nm at 48h (Figure 5). The size of the PS-Plain's aggregates in ASW was 1,884 ± 223 nm but exceeded the size limit of the zetasizer (10 µm) at T24h and T48h, thus no statistical analysis was performed even if a clear trend is obvious with bigger aggregates at T24h and T48h compared to T0. In ASW+OM, the PS-Plain formed bigger aggregates (ANOVA, F= 209.7, p-value < 0.001) at T24h (8,343 ± 228.3 nm) compared to T0 (3,034 ± 187 nm) and T48h (4,637 ± 480 nm). For PS-COOH, the ζ-potential increased significantly in UW (+32%; ANOVA, F= 23.73, p-value < 0.01) and UW+OM (+27%; ANOVA, F= 18.04, p-value < 0.01) at T48h compared to T0 (Figure 6). A significant increase of the ζ-potential was observed over the time for the PS-Plain in UW (+55%; ANOVA, F= 108.9, p-value < 0.001). In UW+OM, no change was observed at T0 (-24.3 ± 0.4 mV) and T48h (-29.9 ± 0.4 mV) but a significantly lower value (ANOVA, F= 8.1, p-value < 0.05) was recorded at T24h (-45%; -48.8 ± 1.3 mV) (Figure 6). From 0 to 48h, no effect on the ζ-potential (ANOVA, p-value > 0.05) was observed in ASW with or without organic matter for the PS-COOH and PS-Plain (Figure 6).

4 Discussion

In ultrapure water, all NP displayed a great stability and stayed at a nanometric scale over time because they presented a high positive or negative charge maintaining electrostatic repulsive forces limiting or reducing aggregation processes (El Badawy et al., 2010; Lin et al., 2010). In contrast, modifications of NP features (aggregation and ζ-potential) were observed in the presence of salts. The behavior of the PS-COOH and PS-Plain suspensions was drastically affected in both artificial and natural seawater through the formation of microscale homo-aggregates. In contrast, the PS-NH_2 displayed high stability and stayed dispersed. Because particles were tested exactly in the same media, the observed behavior must be related to the surface functionalization as previously reported for other engineered nanomaterials (ENMs) (Liu et al., 2012). Consistent with our study, fast homo-aggregation of plain and carboxylate nano-polystyrene beads (25 and 50 nm) in seawater was previously demonstrated with aggregates larger than 1 µm (Della Torre et al., 2014; Tallec et al., 2018; Wegner et al., 2012). This large aggregation could have strong outcomes in aquatic environments because when the size of aggregates exceeds 1 µm, nanoparticles loss their Brownian behavior in favor of sedimentation processes (Klaine et al., 2008). Results differed from recent studies using carboxylate and plain nanopolystyrene of 100 nm where no homo-aggregation was observed under an increasing ionic strength (Cai et al., 2018; González-Fernández et al., 2018). This difference can be explained by the specific features of the particles (*e.g.* size, surface chemistry and heterogeneity) (Alimi et al., 2018). Homo-aggregation in seawater is one of the most frequent behavior observed for nanomaterials (Christian et al., 2008). It is due to interactions between the negative surface charge of NP and cationic elements naturally present in seawater such as calcium or sodium ions (El Badawy et al., 2010). Hence, the ζ-potential of the PS-COOH and PS-Plain became less negative in seawater decreasing the NP stability (Cai et al., 2018; Lin et al., 2010). Indeed, under the presence of salts, attractive forces (including van der Waals forces) and particles sticking efficiency increased according to the Derjaguin-Landau-Verwey-Overbeek (DLVO) model resulting in homo-aggregation (Alimi et al., 2018; Liu et al., 2012). Thus, the difference of salinity between artificial (30 PSU) and natural (32 PSU) seawater can explained the observed variation of aggregation level of the PS-COOH and PS-Plain. The ionic strength increased from ASW to FSW, as well as the screening of the repulsive electrostatic interactions. In the presence of an excess of added salt, the particles diffuse ion double layer reduces and possible specific ions condensation might occur and decrease the particles net surface charge density. Moreover, in natural seawater, other molecules such as extracellular polymeric substances (EPS) produced by bacteria can also intensify aggregation in comparison to an artificial and controlled medium (Summers et al., 2018). Regarding the PS-NH_2, its strong stability in all media was presumably due to a positive coating characterized by a low effect of the ionic strength (particles and homo-aggregates stayed at a nanometric scale) in comparison to the two other particles tested. Despite a decrease in the ζ-potential when particles are suspended in seawater, it appears sufficiently high to ensure repulsive mechanism. Overall, the nature of the surface groups and the particles interface has a stronger influence than the nature of the core polymer on the particles aggregation (Liu et al., 2012). To thoroughly test this hypothesis, particles of different nature (PE, PP, PS, non-plastic material) presenting the same coating should be compared in controlled experimental solutions.

Humic substances (HA) – used as proxy of dissolved organic matter (DOM) – had negligible effect on the behavior of all particles in comparison to the presence of salts. Usually, organic matter tends to stabilize nanoparticles by increasing steric surface repulsive forces. The presence of cations can overcome and disrupt this stabilization by several mechanisms (*e.g.* bridging, electrical repulsion compression) promoting aggregation (Zhang et al., 2009). In agreement with our study, divalent cations (Mg^{2+} and Ca^{2+}) triggered homo-aggregation of carbon nanotubes despite the presence of organic matter (reviewed by Christian et al., 2008). Only for the PS-Plain, HA had a stabilizing impact in seawater causing a partial disaggregation over the temporal experiment (between T24h and T48h) presumably due to an increase in the steric repulsion level. In contrast to previous studies using nanomaterials (Baalousha, 2009; Loosli et al., 2013), no shift in the ζ-potential of NP was observed, explaining the negligible effect of the DOM. This fact is possibly linked to the concentration of HA used in the present study. At a concentration of 100 $mg.L^{-1}$, HA caused a shift in the ζ-potential of iron oxide nanoparticles whilst no effect was perceived at 10 $mg.L^{-1}$ (Baalousha, 2009) which is consistent with our results at the same concentration. In ultrapure water, an adsorption of HA on the PS-NH_2 is hypothesized with a decrease of the ζ-potential along the increasing gradient of HA doses. Indeed, the anionic groups of HA interact easily with the positive surface charge of the PS-NH_2. This adsorption reduced the net charge density and resulted in a small aggregation at the highest HA dose, even though the PS-NH_2 mean size distribution always stayed below 100 nm. Overall, DOM have limited effects on NP behavior in our experimental design but a recent study demonstrated that 25 nm NP can in reverse affect the assembling of DOM with suspected consequences on carbon flux in oceans according to the environmental concentration of NP (Chen et al., 2018).

The temporal analysis demonstrated that NP formed evolving dynamic structure. Indeed, an increase of the homo-aggregation level is reported over time for all NP tested in ASW and ASW+OM. Hence, it is highly plausible that NP will not be find individually in oceans. Owing to the high aggregation level observed here, if NP have a negative surface charge (acquired naturally or after weathering in rivers) as observed in marine environment (Fotopoulou and Karapanagioti, 2012), the risk of an intake in oceans from freshwater appears therefore low. However, certain events can influence the retention of plastic particles in rivers such as flooding leading to a washout and a huge export of plastic debris to marine systems as recently described in rivers around urban area of the United Kingdom (70% of microplastics were exported by the flooding) (Hurley et al., 2018). Every year, between 1.15 and 2.41 MT of plastic debris go into oceans from rivers (Lebreton et al., 2017). Thus, in future models or experiments, it will be crucial to consider the behavior of NP with a temporal and seasonal aspect as reported with silver nanoparticles (Ellis et al., 2018).

In the context of increasing number of studies using NP to highlight their toxicity on freshwater (Besseling et al., 2014; Cui et al., 2017; Mattsson et al., 2017) and marine (Canesi et al., 2016; Tallec et al., 2018) organisms, this study revealed that it is unavoidable to properly characterize NP behavior in the experimental systems. Indeed, the risk assessment is completely modified if particles stayed individual or formed aggregates in the medium (Lowry et al., 2012). The presence of aggregates for PS-COOH and PS-Plain in seawater would be part of their lower toxicity observed in Tallec et al. (2018) compared to PS-NH_2 for which we showed in the present study strong stability at nanometric scale. Then, because the PS-Plain formed microscale aggregates in seawater but not in freshwater, the low toxicity of the PS-Plain observed in our previous study on oyster planktonic-stages (Tallec et al., 2018) is far from comparable to the severe outcomes in the freshwater crustacean *Daphnia galeata* (Cui et al., 2017). Likewise, for a given biological model, various results may be observed according to the medium tested. For instance, the toxicity of the PS-NH_2 appeared stronger on the survival of the rotifer *Brachionus plicatilis* in artificial seawater (EC_{50} = 2.75 ± 0.67 $\mu g.mL^{-1}$) than in natural seawater (EC_{50} = 6.62 ± 0.87 $\mu g.mL^{-1}$) (Manfra et al., 2017). Colloids, organic matter, macromolecules (*e.g.* EPS, proteins) or compounds released by organisms in the water, at their epithelium interface or inside the organism, are likely to interact with nanoparticles (corona formation) and change their behavior and bio-availability and consequently potential harmful effects (Lowry et al., 2012). Because, this corona can be specific according to the surface functionalization (Lundqvist et al., 2008), an examination of the influence of natural compounds may be one priority for the ecotoxicological community. For instance, changes in the toxicity of various nanomaterials such as polystyrene (Nasser & Lynch, 2016), titanium dioxide (Yang et al., 2012), silver (Fabrega et al., 2009), multiwalled carbon nanotubes (Edgington et al., 2010) were reported under co-exposures with humic acids or macromolecules.

We conclude that the surface functionalization is deemed to be a major parameter determining the fate of 50 nm NP in our experimental design and potentially in aquatic environments. The data presented here, complemented by previous studies including the recent publication of Cai et al. (2018) with 100 nm PS-Plain, emphasizes the need for a thorough characterization of NP considering at least size and coating, in relevant environments in order to better design experiments, understand end-points and define toxicity thresholds for this new threat. More experiments are required to understand effect of other environmental conditions on NP behavior especially the weathering of particles which can affect their properties as previously observed with MP (Rummel et al., 2017). These results provide new experimental data to consider in the assessment of NP fate in future modeling studies.

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6 Authors Contributions

KT, AH, IPP, GB, CG-F designed experiments. KT, OB conducted experiments. DLS data were analyzed by KT and OB. Data were interpreted by OB, KT, AH, IPP, PS and GB. KT wrote the initial draft in concertation with AH and IPP. All authors read and contributed to the final manuscript.

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8 Figure Legend

Figure 1. Size average (nm; A) and ζ-potential (mV; B) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) in three media: ultrapure water (UW; white), artificial seawater (ASW; light grey) and 2-µm filtered natural seawater (FSW; dark grey). DLS analysis were replicated 3 times and data

are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

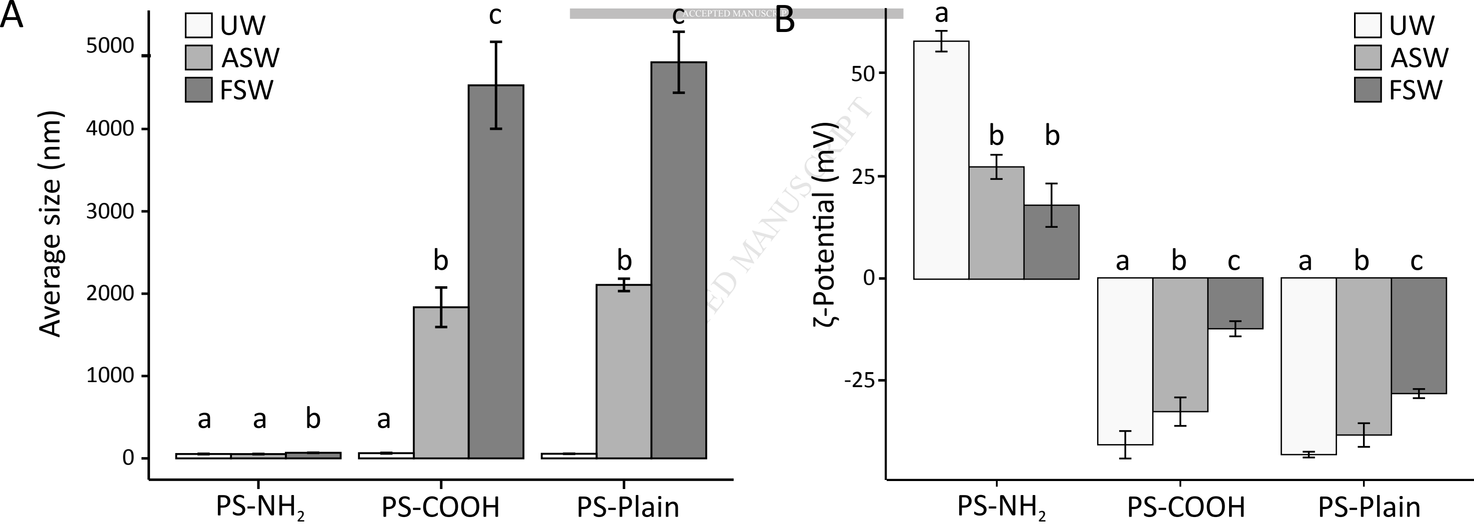
Figure 2. Average size (nm; A) and ζ-potential (mV; B) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) along a salinity gradient (0, 163.25, 326.5, 489.75, 653 mM). DLS analysis were replicated 3 times and data are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

Figure 3. Average size (nm) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) in two media (ultapure water – UW or artificial seawater – ASW) with three different doses of organic matter: 1 (white), 10 (light grey) and 30 (black) $mg.L^{-1}$. DLS analysis were replicated 3 times and data are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

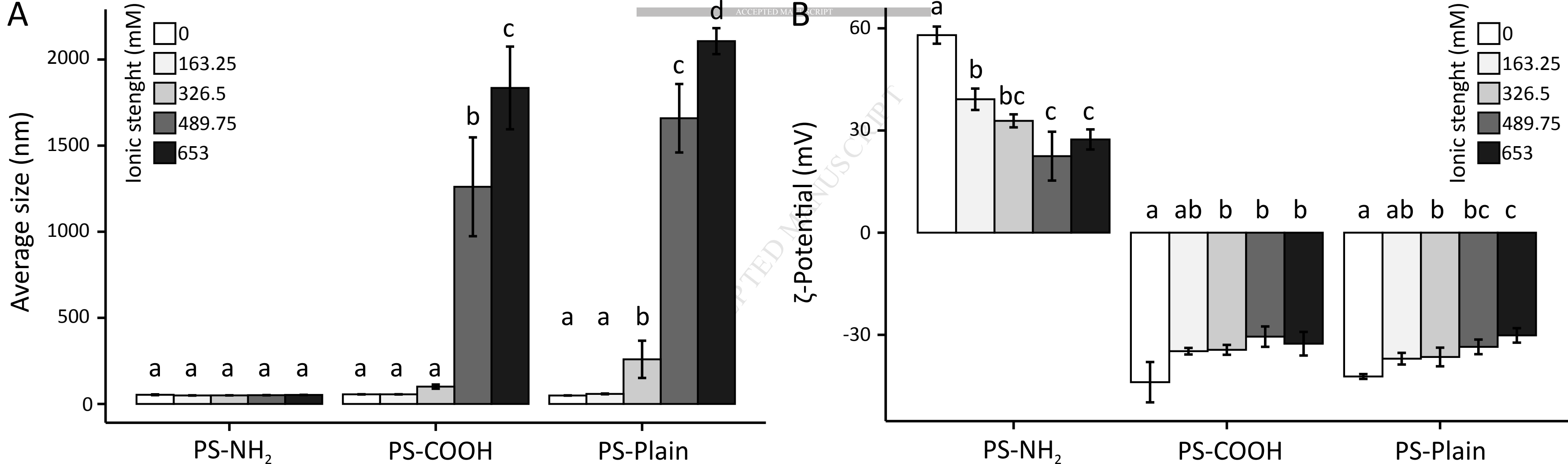
Figure 4. ζ-potential (mV) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) in two media (ultapure water – UW or artificial seawater – ASW) according to three different doses of organic matter: 1 (white), 10 (light grey) and 30 (black) $mg.L^{-1}$. DLS analysis were replicated 3 times and data are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

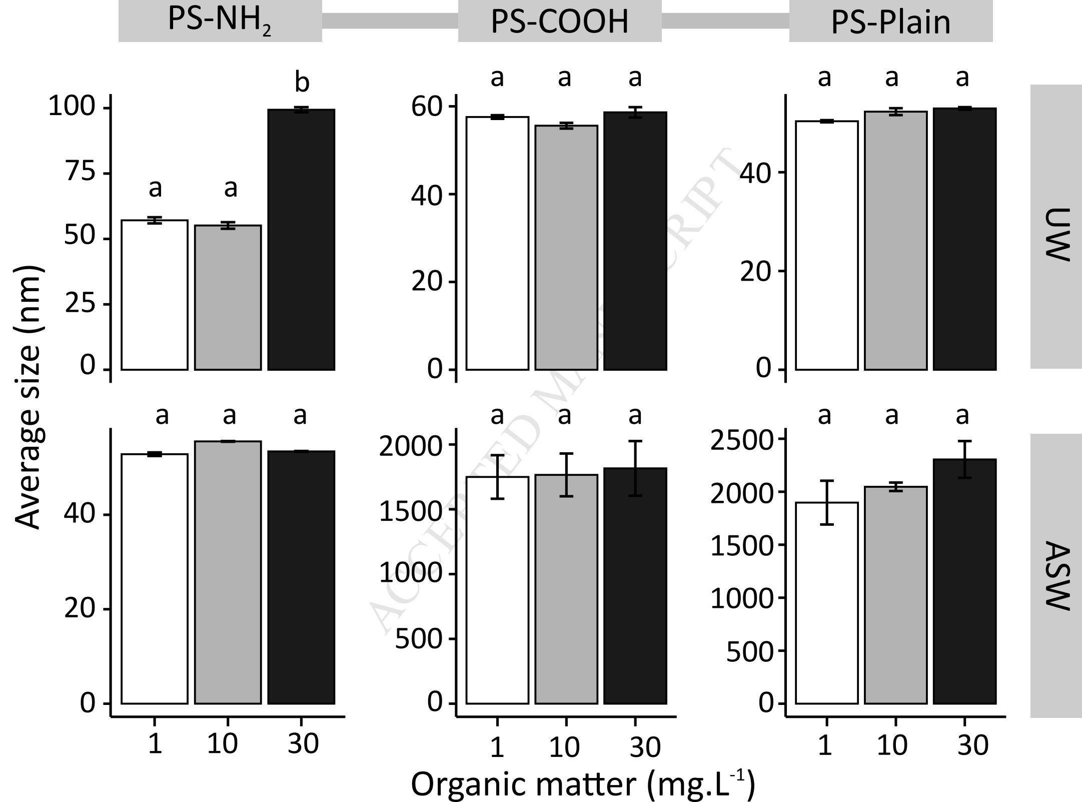
Figure 5. Average size (nm) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) over time (T0, T24h and T48h) in two media (ultapure water – UW and artificial seawater – ASW) with or without organic matter (OM, 10 $mg.L^{-1}$). DLS analysis were replicated 3 times and data are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

Figure 6. ζ-potential (mV) of three 50 nm nanoplastics (PS-NH_2; PS-COOH; PS-Plain) over time (T0, T24h and T48h) in two media (ultapure water – UW and artificial seawater – ASW) with or without organic matter (OM, 10 $mg.L^{-1}$). DLS analysis were replicated 3 times and data are given as mean ± SD. Multiple pairwise comparisons were performed using the Tukey's HSD method; homogeneous groups share the same letter.

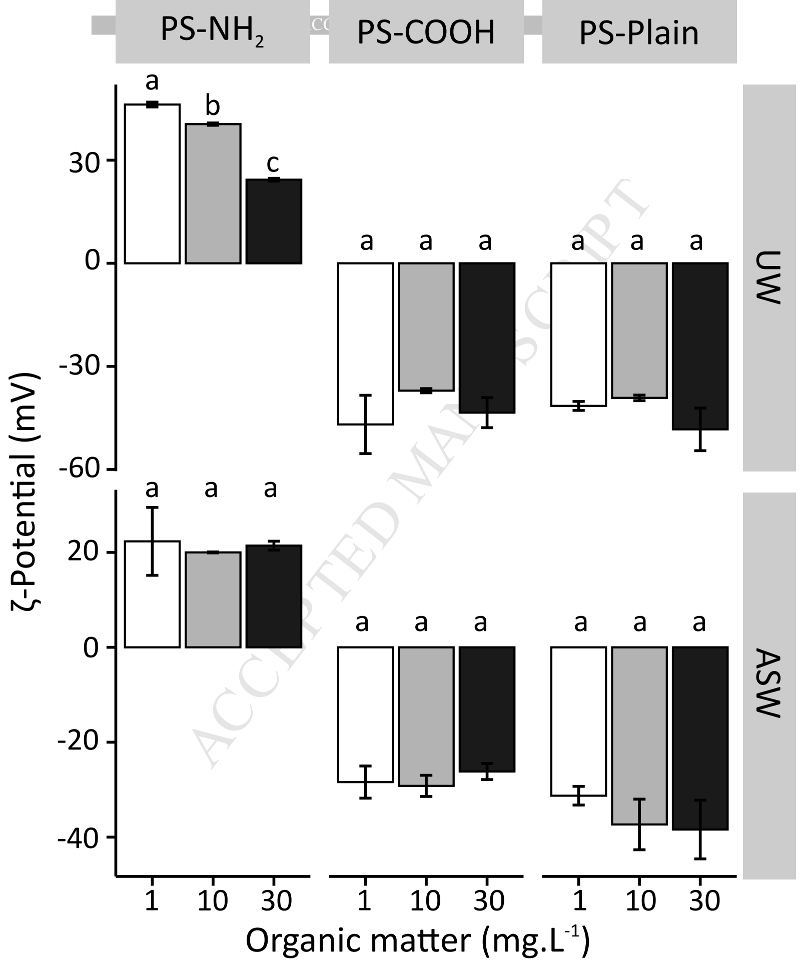


A
UW
ASW
FSW
Average size (nm)
5000
4000
3000
2000
1000
0
a
a
b
a
b
c
b
c
PS-NH$_2$
PS-COOH
PS-Plain
B
UW
ASW
FSW
ζ-Potential (mV)
50
25
0
-25
a
b
b
a
b
c
a
b
c
PS-NH$_2$
PS-COOH
PS-Plain

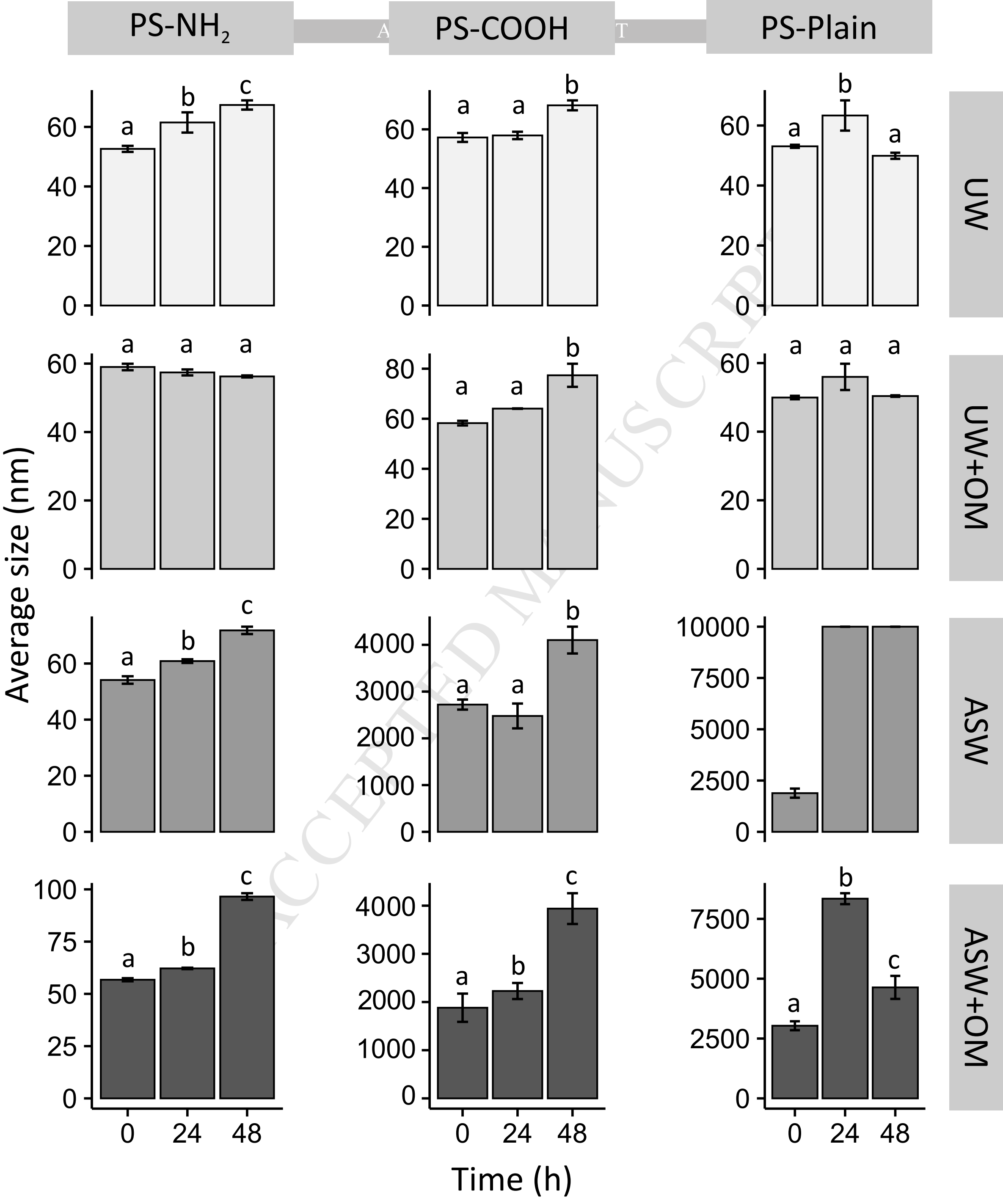

A
B
ACCEPTED MANUSCRIPT
Average size (nm)
Ionic stenght (mM)
0
163.25
326.5
489.75
653
PS-NH$_2$
PS-COOH
PS-Plain
ζ-Potential (mV)



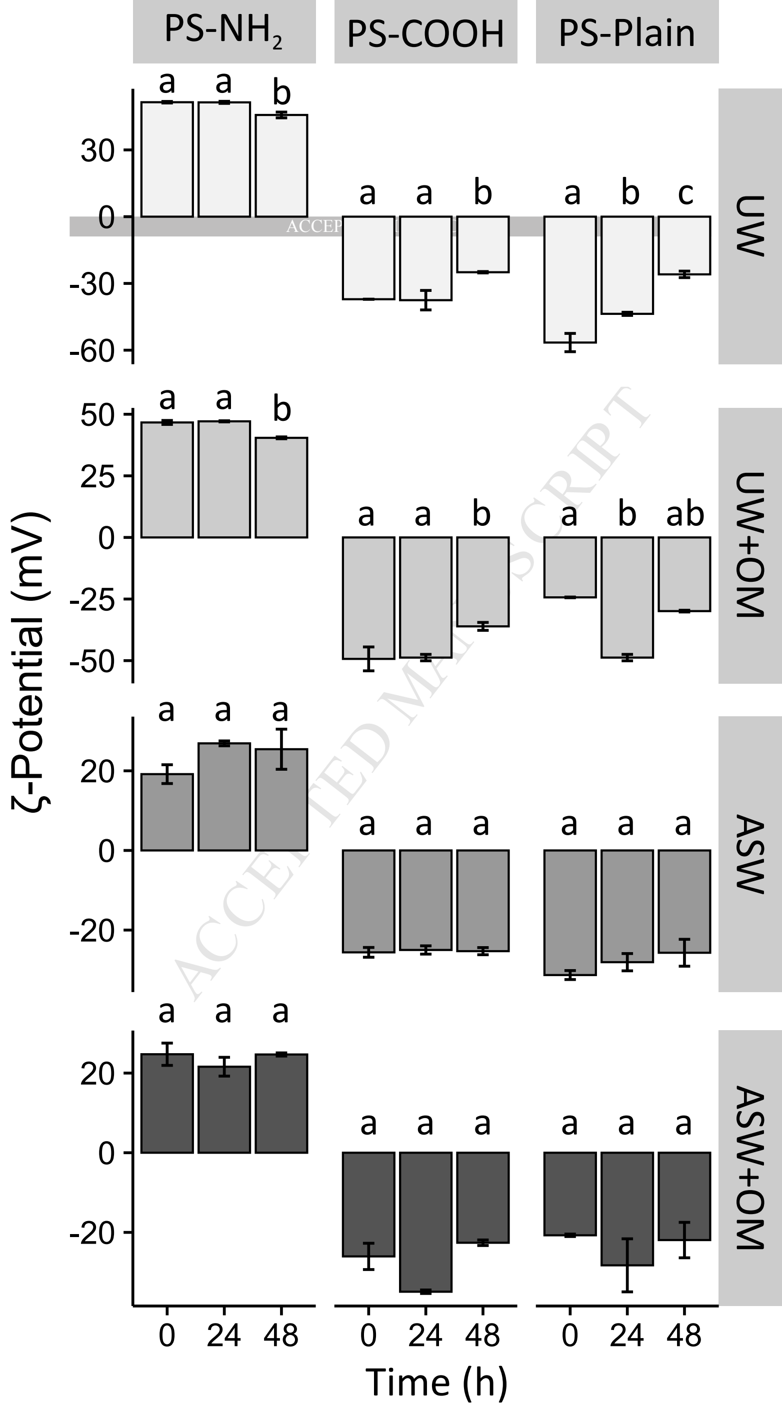
PS-NH$_2$
PS-COOH
PS-Plain
UW
ASW
Average size (nm)
Organic matter (mg.L^{-1})
a
b
1
10
30
0
25
50
75
100
20
40
60
500
1000
1500
2000
2500



PS-NH2
PS-COOH
PS-Plain
UW
ASW
ζ-Potential (mV)
Organic matter (mg.L-1)
30
0
-30
-60
20
0
-20
-40
1
10
30
a
b
c
a



PS-NH2
PS-COOH
PS-Plain
UW
UW+OM
ASW
ASW+OM
Average size (nm)
Time (h)
0
24
48



PS-NH$_2$
PS-COOH
PS-Plain
UW
UW+OM
ASW
ASW+OM
ζ-Potential (mV)
Time (h)
0
24
48
30
0
-30
-60
50
25
-25
-50
20
-20
a
b
c
ab