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Exploring the Role of Polystyrene Microplastics in Cu Binding in Sea Surface Waters: An Experimental Perspective for Future Research

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Abstract

The present study investigates the role of microplastics (MPs) (polystyrene (PS) microbeads) in copper (Cu) binding within the sea surface microlayer (SML) and underlying water (ULW). A mesocosm experiment was conducted, with both SML and ULW samples obtained daily, comparing mesocosms containing MPs with those free of them. The SML enrichment in dissolved Cu (Cu-D) and the Cu-complexing capacity (L_T) were found to be significantly higher in the MP-treated mesocosms, with stability values of Cu-ligand complexes (logK′) being higher in the SML of MP treatments. Significant differences in Cu-D and L_T between control and MP treatments were found in SML and ULW across treatments and over time. Cu-D was negatively correlated with transparent exopolymer particles (TEPs) in the ULW of both treatments, while L_T was positively correlated with TEPs in the SML of MP treatments. Experimental data indicate that the co-existence of TEPs and MPs favors Cu binding with organic matter in the SML, suggesting that MPs may enhance this process. The impact of MPs on dissolved Cu complexation is probably attributed to the production of organic ligands, via enhanced TEP production, without excluding direct adsorption onto biofilm-coated MPs. The present study provides insight into the role of microplastics in Cu cycling in marine surface waters, focusing on the microenvironment of the SML.

Keywords: sea surface microlayer; copper; copper-complexing ligands; microplastics; TEPs; mesocosms

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1. Introduction

The sea surface microlayer (SML) is the thin, uppermost layer of the ocean surface, which is in contact with the atmosphere [1], representing a unique ecological niche that controls numerous environmental processes. Differences in its physical, chemical, and biological properties compared to the underlying waters (ULWs) make the SML a key

global regulator of constituents' distribution, particle cycling, photochemical and microbial transformations, and air–sea gas and energy exchange, especially under changing climate conditions [2–6]. SML is found to be enriched in surface-active organics, which form a complex hydrated gelatinous structure [1,7,8], with polysaccharides and proteins identified as the major components of the SML [9].

Transparent exopolymer particles (TEPs), which are ubiquitous in the SML [10], are formed by the coagulation of dissolved polysaccharides. They are either directly produced as exudation products from phytoplanktonic microorganisms and bacterial activity and/or are abiotically formed from dissolved organic matter (DOM) precursor material, such as carbohydrates [11–13]. Due to their surface-active nature and stickiness, TEPs play an important role in aggregate formation [13–15], creating stable surface films and offering complexation sites for metals [16]. These films form rich microhabitats colonized by bacteria, thereby contributing to the biogeochemical cycling of trace metals. Therefore, the bioavailability and fate of trace metals are influenced by the characteristics of the SML and the presence of organic matter [17–22]. However, despite the constantly increasing research interest, studies on metal speciation and their interactions with organic matter still remain limited.

The present study focuses on copper (Cu), which has the strongest affinity with organic matter among transition metal ions [23], plays an essential role in marine organisms' metabolism, and forms strong organic complexes [24,25]. These characteristics influence Cu bioavailability and its association with particles and sediments in the marine systems, hence affecting marine biogeochemical cycles [26]. In seawater, Cu occurs in the form of free ions, complexes, organo-metallic compounds, or associated with colloidal or particulate organic matter [27]. The bioavailability and toxicity of Cu to organisms are highly dependent on its chemical speciation, which is also affected by water quality parameters, as shown in the Biotic Ligand Model (BLM) [28].

Particles of non-natural organic origin, including microplastics (MPs), containing potential metal-complexing sites, may accumulate in the SML [29,30]. Their presence in surface waters, including the SML [31–33], is increasing due to the extensive use of plastic materials in combination with their buoyancy characteristics. Recent studies have shown that plastic surfaces provide new habitat sites for marine microbial species and microorganisms, interfering with organic matter cycling [34–38]. It has been experimentally shown that microplastics increase the production of organic carbon and its aggregation into gel particulates [39]. The aggregates formed can either remain in the SML [40] or sink to deeper layers of the water column [41].

Microplastics act as vectors of pollutants in seawater, including trace metals, which may exert toxic effects on marine organisms after chronic exposure [42–45]. Metal adsorption onto MPs is affected by the physical and chemical properties of environmental substrates and organic matter [46–48]. Metals can be directly adsorbed onto microplastics' surfaces [49–51] or through surface accumulations of hydrous metal oxides (Fe, Mn) and biogenic matter [52,53]. More specifically, Cu adsorption on polystyrene (PS) microplastics is evidenced by physical adsorption, electrostatic attraction, or complexation, mainly in the presence of biofilms and/or after MPs aging [53–57]. Biofilms act as complex adsorbent systems on MPs, mainly formed by extracellular polymeric substances (EPS), such as TEPs [58,59]. They can trap and accumulate substances, including trace metals, with different adsorption mechanisms and binding sites [60,61]. Therefore, biofilms may be crucial in predicting the adsorption capacity of MPs, including PS. Recent studies provide evidence that biofilms have a significant influence on the adsorption processes and the fate of trace metals, including Cu, in the aquatic environment [54].

This preliminary study attempts to explore the applicability of an electrochemical metal speciation method in investigating MPs (PS microbeads)—Cu-binding processes in marine surface waters. An 11-day mesocosm experiment was designed, during which both SML and underlying water (ULW) samples were obtained daily from mesocosms containing PS MPs and compared with those obtained from mesocosms free of them. We tested whether PS microbeads can be traced by differential pulse anodic stripping voltammetry (DPASV) as Cu-binding ligands. Moreover, under the simulated mesocosm conditions, we examined the impact of PS MPs on Cu binding in the presence of TEPs in surface waters and the SML. To the best of the authors' knowledge, the present work constitutes the first attempt at experimentally investigating the role of microplastics on metal cycling in surface waters, with special emphasis on the SML, for which pertinent data are not available.

2. Materials and Methods

2.1. Experimental Set-Up and Sampling

A mesocosm experiment was designed in order to study the effects of MPs on marine carbon dynamics in both SML and downward carbon export "POSEIDOMM project, https://cordis.europa.eu/project/id/702747 (accessed on 3 August 2025)". The experimental set-up and sampling methodology are described in detail in Galgani et al. [39]. Briefly, in three mesocosms (3 m^3 cylindrical polyethylene bags), an aqueous solution of transparent PS microbeads of 30 μm diameter (Sigma-Aldrich, nr. 84135, Luis, MO (Missouri), USA) was added, with a final concentration of 430 MPs per L (corresponding to 5.92 μg C L^{-1}), representative of current surface ocean conditions [33]. Another 3 mesocosms without MPs were considered as controls. The duration of the experiment was 11 days (day 0 to 10) from 26 May 2017 to 6 June 2017. The first sampling (day 0) was reported as the day after the mesocosms were filled, to allow for the formation of the SML. MPs were added to the mesocosms after sampling at day 0. Each mesocosm was covered by a clear PVC lid to avoid atmospheric contamination. The mesocosm experiment took place in the Creta Cosmos facility in the Hellenic Center for Marine Research (HCMR) in Crete "CRETACOSMOS | Hellenic Centre for Marine Research (HCMR) (9 October 2025)".SML sampling was performed by employing the glass plate technique [62]. A 30 × 30 cm glass plate sampler (4 mm thick) was vertically immersed into the water column through the sea surface and subsequently withdrawn at a very slow rate (~6 cm s^{-1}), with the sea surface microlayer adhering onto both surfaces of the glass plate, due to surface tension effects. Prior to sample collection, the glass plate was dipped into seawater in triplicate, and the amount of water that adhered was discarded. The procedure was repeated between 50 and 80 times to obtain the necessary volume for analysis, tracking the exact amount of dips per mesocosm. The thickness (d, μm) of the sampled SML was estimated as follows:

$$d_{SML} = V \times 10^4 / (A \times n)$$

where V is the SML volume collected, A is the sampling area of the glass plate (A = 1800 cm^2), and n is the number of dips.

The apparent thickness of the SML ranged between 37 and 72 μm, with an overall mean of 54.7 ± 9.0 μm in agreement with previous studies [63,64]. For the collection of the ULW samples (1 m depth), a 2 m long plexiglass tube with a 5 cm diameter was used [39].

SML and underlying water samples were collected on days 0, 1, 3, 5, 7, 9, and 10. The SML was always sampled prior to the underlying water. Acid-cleaned HDPE containers were used for the collection of samples subject to dissolved copper (Cu-D) and copper-complexing capacity (L_T) determinations. Samples were immediately refrigerated and

transported to the laboratory. All necessary precautions were taken, and clean techniques were applied during sampling collection to minimize contamination. Sample pretreatment and pre-concentration for Cu-D analysis were carried out inside a clean room environment (class 10,000 US Stds), applying clean handling techniques [65]. The glass plates and labware used were acid-cleaned (HCl 10%) and thoroughly rinsed with ultrapure water prior to use.

2.2. Analyses

2.2.1. Copper-Complexing Capacity (L_T)

Copper-complexing capacity (L_T) values were determined in all 6 mesocosms studied, in the surface microlayer (SML) and underlying water (ULW). The determination was performed through differential pulse anodic stripping voltammetry (DPASV) by direct titration of unfiltered samples, using the technique of standard additions (a) to the sample at its natural pH and (b) to a sample aliquot acidified at pH < 2 and UV irradiated overnight [27,66,67]. A sample volume of 25 mL was added to the cell, which was previously rinsed with the sample in order to minimize the effects of potential adsorption onto the cell walls. The equilibration time after each metal addition was 15 min (which was proven to be adequate for the equilibration of Cu ions; i.e., the peak height did not change after this period of time) [27,68], which was also confirmed in a subset of samples. The voltametric conditions were 300 s N_2 gas purge, 180 s deposition time, and −0.6 V deposition potential, with Cu ion additions ranging up to 4×10^{-7} M. Ligand concentrations and corresponding conditional stability constants were calculated by applying the linear transformation plot, assuming 1:1 metal to ligand complexes [69,70]. The line slope determines the L_T concentrations (slope = $1/L_T$), and the intercept on the y-axis represents the value of the conditional stability constant (K') (intercept = $1/K'L_T$). L_T (expressed in metal ion equivalents) is the concentration of metal ions added to the seawater sample prior to their measurement as "free" metal ions (including hydrated metal ions and inorganic complexes, being labile for the applied electrochemical method and experimental conditions). The "inert fraction", in terms of the DPASV method applied, refers to the fraction not dissociating or not released from the particles under the selected measurement timescale (stirring rate applied during the accumulation time). The quantity of the electro-inactivated metal is a measure of the amount of ligands (L) in the sample as a result of multiple interactions [27].

Electrochemical measurements were carried out using an Autolab III (Eco-Chemie, Utrecht, The Netherlands) voltammetric instrument connected to a three-electrode cell (VA 663, Metrohm stand, Herisau, Switzerland) with a static mercury drop electrode (SMDE) as the working electrode. The reference electrode was an Ag/AgCl (3 M KCl). A carbon rod electrode served as the auxiliary electrode.

2.2.2. Dissolved Copper (Cu-D)

For the determination of dissolved Cu concentrations (Cu-D), samples collected during the mesocosm experiments were initially filtered through 0.22 μm polycarbonate filters, acidified at pH < 2, and UV-irradiated (400 W) overnight. They were subsequently pre-concentrated using the Toyopearl AF Chelate 650 M resin, according to the method described by Willie et al. [71] and Milne et al. [72]. Measurement of Cu-D in the pre-concentrated samples was performed through an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Thermo-Elemental X-series II (Thermo Electron Corporation, Massachusetts, USA), equipped with a collision/reaction cell (CCT) operated in hydrogen/helium (H_2/He) mode. A concentric nebulizer coupled with a Peltier-cooled spray chamber maintained at 4 °C was used for sample introduction. Argon was used as the plasma gas (13 L/min), with an auxiliary gas flow of 0.8 L/min and a nebulizer gas flow of

0.88 L/min. The RF power was set at 1400 W. The collision/reaction cell was operated with a H_2/He gas mixture at a flow rate of 6.2 mL/min to reduce polyatomic interferences. The instrument operated in peak hopping mode with a dwell time of 25 ms per isotope. Each measurement was performed with 3 replicates, 25 sweeps per replicate, and one point per peak. CCT tuning was carried out using a 7% H_2/He gas mixture to optimize the operating parameters of the Thermo Scientific X Series II ICP-MS. The instrument demonstrated the following performance metrics: oxide ratio (CeO^+/Ce^+) < 3% and doubly charged ion ratio (Ba^{2+}/Ba^+) < 2%. The instrument also exhibited high sensitivity, with signal intensities exceeding 50,000 counts/sec for cobalt (^{59}Co) and 280,000 counts/sec for indium (^{115}In), using a 10 ppb in 2% HNO_3 standard solution containing Ce, Ba, In, and Co. Signal stability was maintained with a relative standard deviation (RSD) of <2.5% over a period of at least 3 min for cobalt (^{59}Co) and indium (^{115}In). The mass calibration error for ^{115}In and ^{59}Co was less than 0.1 amu. The instrument was calibrated using the external calibration method with five concentration points. The standard solutions were prepared from a concentrated solution specifically intended for use in ICP-MS (1000 mg/L Cu Certipur®), which was obtained from Merck (Darmstadt, Germany). The quality of the curve fitting was evaluated using the coefficient of determination ($r^2 = 0.9998$).

The method detection limit (DL), calculated as the standard deviation of a low-level sample multiplied by three, was equal to 1.1 nM. Analytical blank tests were performed daily. Accuracy and precision were assessed through the use of the certified reference material (CRM) for coastal water CASS-5 (National Research Council of Canada) and acidified seawater samples of the inter-laboratory exercise 'Quality Assurance of Information for Marine Environmental Monitoring in Europe' (QUASIMEME). The results obtained were in good agreement with the CRM-certified values (CASS-5: measured value 5.6 ± 0.93 nM, n = 5; certified value 6.0 ± 0.44 nM), while for QUASIMEME tests, acceptable Z-scores ($-2 < Z < 2$) were received (lab code Q122).

2.3. SML Enrichment Factors (EF)

The SML is generally considered to be enriched in several compounds relatively to ULW, including trace metals [2,73]. However, the enrichment mechanism is rather complex, being affected by the presence of organic matter and suspended particulate matter, the binding affinities of metals, as well as physicochemical parameters [74,75]. The degree of enrichment of a specific chemical substance in the SML is calculated using the Enrichment Factor (EF) as follows:

$$EF = C_{SML}/C_{ULW}$$

where C_{SML} is the concentration of the chemical substance in the SML and C_{ULW} its corresponding concentration in the ULW. EF values > 1 indicate enrichment in the SML, while EF values ≤ 1 indicate depletion.

2.4. Statistical Analysis

Temporal and treatment variations between the control and microplastic treatments in the SML and ULW were analyzed using a repeated-measures two-way ANOVA. The fixed factor considered is the treatment (microplastics addition/control), and the random effect is time (days). Sample replicates within the treatments have been assumed to have equal variabilities of differences. A value of $p < 0.05$ was considered to indicate significant differences. A sample *t*-test was used for differences in EF values from unity. Spearman's correlation coefficient was used to assess a possible linear association between two parameters. The statistical package SPSS v. 20.0 was applied.

3. Results and Discussion

In the simulated conditions of high PS MPs in the mesocosm experiment, their role in Cu binding with organic matter, in both SML and ULW environments, was investigated. Background natural conditions at the beginning of the experiment (day 0) were similar in all mesocosms, with no significant differences between controls (no MPs addition) and treatments (mesocosms amended with MPs). Differences started evolving during the bloom phases, which manifested in all mesocosms, but with clear and significant differences between the plastic and the non-plastic conditions [39]. At the beginning of the experiments (days 1 to 3), a clear development of a phytoplanktonic bloom occurred, which declined smoothly until day 10 [39,40]. TEP production was mostly related to the development of *Synechococcus*, a known TEP producer [39]. In SML, TEPs started to increase on day 3, and in the presence of MPs, they considerably accumulated in SML until day 7 (TEPs data from Galgani et al. [40]; Figure 1A). TEPs in ULW showed an increase from day 3 to 5, being more pronounced in MP-treated mesocosms (TEPs data from Galgani et al. [39]; Figure 1B). EFs for TEPs ($mm^2\ L^{-1}$) were found to be significantly higher (repeated two-way ANOVA) (Table 1) in the MP-treated mesocosms than in the controls (Figure 1C) [40]. These results point out that MPs' presence enhances the formation of TEP aggregates [39,40]. Aided by the presence of MPs, TEPs also enhance the SML accumulation of organic matter and, in particular, of extrapolymeric gels (Figure 1A–C; Table 1).

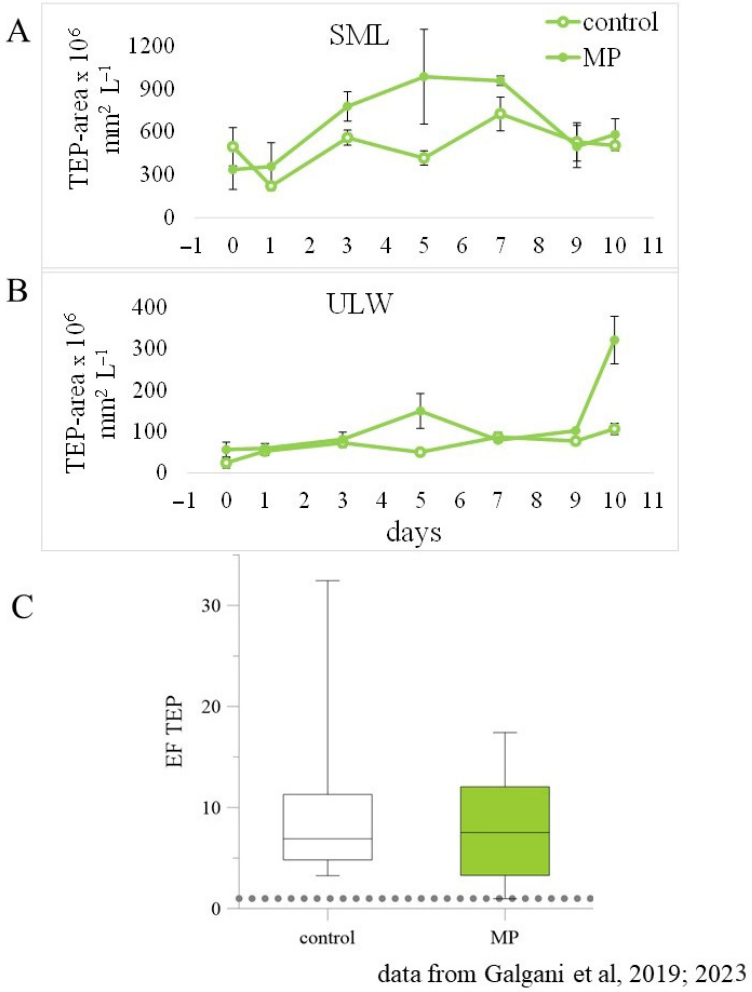

Figure 1. Mean daily values and standard errors of (**A**) TEP-area ($mm^2\ L^{-1}$) in SML; (**B**) TEP area ($mm^2\ L^{-1}$) in ULW of controls (three mesocosms) and MP treatments (three mesocosms), respectively. (**C**) Enrichment factors (EFs) of the TEP area. The gray dashed line indicates EF = 1. Repeated-measures two-way ANOVA has proven significant differences between EFs [39,40].

Table 1. Results of repeated two-way ANOVA for significant differences in treatment factor (MPs/plastic-free), time factor (1–10 days), and treatment×time interaction. Replicates within treatments are assumed to have an equal variability of differences.

Parameter		Treatments [a]		Time [b]		Treatments × Time	
		ANOVA F	p [c]	ANOVA F	p	ANOVA F	p
Cu-D	SML	F(1, 28) = 30.12	<0.001	F(6, 28) = 7374	<0.001	F(6,28) = 673.95	<0.001
Cu-D	ULW	F(1, 28) = 1824	<0.001	F(6, 28) = 5602	<0.001	F(6, 28) = 201.18	<0.001
EF Cu-D		F(2, 28) = 207.50	<0.001	F(6, 28) = 630.20	<0.001	F(6, 28) = 117.70	<0.001
L_T	SML	F(1, 28) = 17.47	0.001	F(6, 28) = 20.02	<0.001	F(6, 28) = 25.27	<0.001
L_T	ULW	F(1, 28) = 50.97	0.001	F(6, 28) = 12.96	0.007	F(6, 28) = 12.10	0.008
EF L_T		F(2, 28) = 64.00	<0.001	F(6, 28) = 48.25	<0.001	F(6, 28) = 42.25	<0.001
TEP [d]	SML	F(1, 28) = 4.20	0.049	F(6, 28) = 3.75	0.007	F(6, 28) = 1.45	0.231
TEP	ULW	F(1, 28) = 21.60	<0.001	F(6, 28) = 13.48	<0.001	F(6, 28) = 6.56	<0.001
EF TEP		F(1, 28) = 2.60	0.118	F(6, 28) = 6.32	<0.001	F(6, 28) = 3.60	0.009

[a] Fixed factor (control/ MP). [b] Random effect. [c] Significant differences are accepted for $p < 0.05$. [d] Data from Galgani et al. (2019; 2023) [39,40].

Cu-D in SML ranged from 4.5 to 14 nM (mean 8.0 nM) in controls and from 4.3 to 15 nM (8.0 nM) in MP treatments (Figure 2A), from 2.3 to 10 nM (5.7 nM) in ULW, and from 2.8 to 7.9 nM (4.6 nM) in controls and MP treatments, respectively (Figure 2C). Significant differences (repeated two-way ANOVA) in the Cu-D between control and MP treatments were found in SML and ULW, both across treatments and over time (Table 1). In the SML, Cu-D concentrations rise from day 0 to 3 in both treatments (Figure 2A). After day 3, there is a gradual decrease in Cu-D concentrations in the MP treatments. In ULW, Cu-D concentrations show a continuous decrease from day 1 to 7 in both treatments (Figure 2C). Moreover, in ULW, concentrations of Cu-D are negatively correlated with those of TEPs both in controls ($r = -0.786$, $p = 0.036$, $n = 7$) and MP treatments ($r = -0.679$, $p = 0.094$, $n = 7$), implying that TEPs are implicated in processes of Cu-D adsorption onto the particulate phase. An SML enrichment in Cu-D was observed in both treatments with EFs (2.0 ± 0.9), being significantly higher (repeated two-way ANOVA) in the MP-treated mesocosms with respect to controls and over time (Figure 2E, Table 1), implying a more effective retention of Cu-D in the SML in the presence of MPs.

Mean values of L_T in SML were 279 nM in control (range 174–441 nM) and 338 nM in MP treatments (247–447 nM) (Figure 2B), while the corresponding values in ULW were 493 nM in controls (446–527 nM) and 413 nM in MP treatments (375–466 nM) (Figure 2D). Significant differences (repeated two-way ANOVA) in L_T were found between control and MP treatments in SML and ULW, both across treatments and over time (Table 1). As shown in Figure 2B,D, L_T elevated values were found in the SML of MP treatments with respect to controls, whereas in the ULW, the reverse trend was recorded. As a consequence, EFs for L_T were found to be significantly higher (repeated two-way ANOVA) in the MP-treated mesocosms with respect to controls and over time (Table 1). Nevertheless, the SML was not found to be enriched in L_T relative to ULW (EFs < 1) in MP treatments (Figure 2F). L_T concentrations were positively correlated with TEPs in the SML of MP treatments ($r = 0.821$, $p = 0.023$, $n = 7$). Values of $\log K'$, expressing the stability of Cu-ligand complexes, were calculated to be higher in the SML of MP treatments (range $\log K'$ 7.6–9.0) than controls (6.8–8.6), with the difference indicating potentially different features of the organic matter present in each case. However, in the ULW of MP treatments and controls, stability constants were similar (range $\log K'$ 6.6–7.5 in controls, 6.8–7.3 in MPs) (Table 2). The elevated L_T and $\log K'$ values in SML in the presence of MPs should be evaluated in parallel to the fact that these parameters correspond to unfiltered samples, taking into

account complexation processes related both to the dissolved phase and to Cu adsorption on particles.

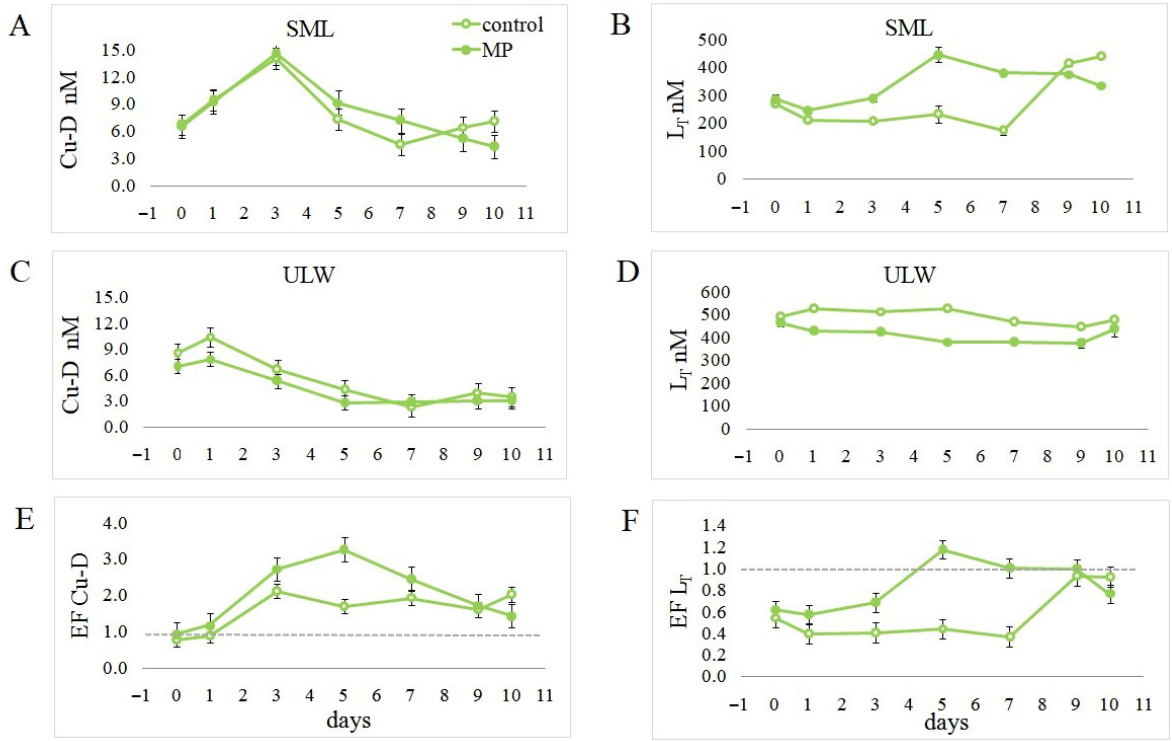

Figure 2. Mean daily values and standard error deviations of (**A**,**C**) dissolved Cu (Cu-D) (nM); (**B**,**D**) Cu-complexing ligands capacity (L_T) (nM) in SML and ULW of controls (three mesocosms) and MP treatments (three mesocosms), respectively. (**E**,**F**) enrichment factors (EFs) of Cu-D and L_T, calculated using the mean daily concentrations. The gray dashed lines indicate EF = 1. For EF > 1, the SML is considered enriched relative to the ULW. The repeated-measures two-way ANOVA has proven significant differences between EFs.

Table 2. Mean conditional stability constant values (logK′) and standard errors in SML and ULW of controls and MP treatments.

Day	logK′			
	SML		ULW	
	Control	MP	Control	MP
0	8.4 ± 0.1	8.2 ± 0.1	6.7 ± 0.1	6.9 ± 0.1
1	6.8 ± 0.1	7.6 ± 0.1	6.8 ± 0.1	6.8 ± 0.1
3	8.6 ± 0.2	8.3 ± 0.2	6.6 ± 0.1	7.0 ± 0.1
5	8.1 ± 0.2	9.0 ± 0.2	7.0 ± 0.1	7.0 ± 0.01
7	7.8 ± 0.1	8.2 ± 0.2	7.3 ± 0.2	6.8 ± 0.1
9	7.2 ± 0.3	8.0 ± 0.2	7.5 ± 0.1	7.0 ± 0.04
10	8.0 ± 0.4	7.6 ± 0.1	7.0 ± 0.1	7.3 ± 0.3

Although the maintenance of Cu in the dissolved phase (Cu-D) occurred in both treatments (MPs and plastic-free), presumably through organic complexation, it appears stronger in the SML of the MPs mesocosms. The data, in fact, suggest that the presence of MPs favors the production and/or transport of Cu ligands to SML, where stable Cu complexes are formed either in the dissolved or in the particulate phase. All mesocosms had a continuous airlift system that provided sufficient movement to mimic natural waters, including SML enrichment dynamics, and maintained a homogeneous distribution of the PS MPs in the MPs treatments. As the observed TEP concentrations were higher in the

presence of MPs, they may have contributed to the higher Cu complexation and higher amount of Cu ligands present in those treatments.

Galgani et al. [40] have shown that DOM accumulates in the SML of both treatments, but with notable differences in the nature of the organic matter; degraded and reformed compounds may be favored in the SML of MP treatments. Differences in the DOM nature may partly explain the observed differences in the EFs of Cu-D between MPs and control treatments. Metal complexation mechanisms involve not only DOM but also the colloidal phase, covering a wide range within the particle continuum [76–79]. A significant fraction of dissolved trace metals is associated with colloidal organic matter, notably polysaccharides, which constitute the dominant substances of TEPs [13]. During the initial step of TEP formation, DOM and colloidal particles coagulate, successively forming TEP precursors and subsequently TEPs [12]. Upon TEP formation, their association with metals occurs, preferably via adsorption processes [79]. The mechanism of the TEP–metals association (complexation and/or adsorption), controlled by hydrodynamic constraints, varies and depends on the relative distribution of dissolved and colloidal metals, the gradient of their residence time, and the rate of TEP formation. Previous studies suggest that TEPs' association with metals is controlled by a cycle of complexation/adsorption processes, with adsorption being the dominant process, as it occurs immediately after DOM coagulation [79,80]. The MPs–metal association mechanisms include (i) electrostatic interaction and surface complexation, with metals interacting with charged MPs via Coulombic forces (i.e., Van der Waals) [81]; (ii) π-π interactions and/or hydrogen bonding, involving the overlapping of π-orbitals between aromatic or conjugated systems in MPs [81]; and (iii) physical sorption mainly in the presence of natural organic matter and biofilms and/or after MPs aging [52,76]. PS MPs with phenyl groups interact through π–π interactions and van der Waals forces [57]. The biofilm-coating microplastics' surface, a specific niche for microbial organisms, may also act as a substrate providing binding sites for metals (i.e., Cu) [54,82,83]. Biofilms are mainly formed by extracellular polymeric substances (EPS), such as TEPs, on MPs [58,59]. Hence, metal–MP interactions, due to different surface groups and/or organic coatings on MPs, are expected. Cu adsorption on PS MPs has been recorded [55,56,82] and found to be elevated in the case of biofilm-covered MPs [23,54]. The micron-scale PS MPs can form aggregates more easily, possessing more functional groups for chemical association with trace metals, with a higher intensity in the micron-scale biofilm-covered MPs [23]. Recent studies provide evidence that electrostatic interaction on colloidal PS MPs [56] and ion exchange among hydroxyl and amine groups of EPS on biofilms [82] are possible mechanisms for the adsorption of Cu on PS MPs.

The decreasing trend characterizing Cu-D concentrations, towards the end of the experiment, for both SML and ULW layers, together with the negative correlation of Cu-D with TEPs in both controls and MP treatments in ULW, indicates the potential adhesion of Cu-D forms onto the colloidal and/or particulate phases. To that end, the decrease in Cu-D EFs with time in MP treatments further implies Cu-D binding onto TEPs in the SML. In addition, L_T values, referring to both dissolved and particulate phases in the SML of MP treatments, are positively correlated with TEPs, supporting the mechanism of Cu adsorption onto the particulate phase. Therefore, the transition from the dissolved to the particulate phase is realized through the shift from complexation in the dissolved phase to particle adsorption processes [79].

The exact impact of MPs on Cu complexation mechanisms remains unclear. The results of the present study, combined with previous ones from the same mesocosm experiment, provide indications that the presence of PS MPs affects the Cu cycling in surface waters and, in particular, in SML. The presence of MPs may affect Cu speciation indirectly via

the production of organic ligands and TEPs, without excluding direct adsorption onto biofilm-coated MPs.

4. Conclusions

Results from the present study provide evidence that the MP is a factor that strengthens Cu binding, highlighting its importance in metal cycling, especially with the expected increase in its concentration in marine waters in the near future. Cu uptake by marine organisms is primarily dependent on the concentration of labile inorganic Cu forms, whereas organically complexed Cu is also accessible to marine microorganisms [84,85]. The interference of MPs on the Cu-binding process in surface waters, as shown in the present study, affects the natural pathways of Cu assimilation and underscores the need for further research. More work is needed to clarify the mechanisms of MPs–metal binding, either by enhancing the production of natural organic–metal ligands or by direct metal binding onto their surfaces through the microbial biofilm. Furthermore, enhanced Cu binding by MPs in the surface waters and the SML may have significant ecological implications, altering the bioavailability and toxicity of Cu, potentially impacting marine life and ecosystems. MPs can act as vectors for Cu and seem to facilitate the enrichment of the air–sea interface in Cu, a unique ecological niche for many marine organisms and processes. This may lead to increased exposure and may induce potentially toxic effects on marine biota (i.e., phytoplankton and zooplankton) and organisms that inhabit or utilize the SML, by affecting their photosynthesis, growth, and production. Future experiments should include different water types (i.e., freshwater and sterile water), different types of MPs and particle sizes, as well as environmentally aged MPs, as aging is known to have an important role in the adsorption of metals on MPs [55]. An ecological and toxicological risk assessment should focus on quantifying MPs–metal binding effects, investigating the long-term impacts on different marine organisms, and exploring the potential for MPs–metal transport to the marine waters.

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