Quantifying surface topography helps reveal the combined effects of microplastics UV-aging and organic matter on trace metal adsorption in seawater

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Abstract

This study explores the impact of UV-aging and organic matter (humic acid - HA) on adsorption of essential (Cu, Co, Ni) and nonessential (Cd, Pb) trace metals onto microplastics (MPs), polypropylene (PP) and polystyrene (PS), in seawater. Surface characterization using atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) revealed that UV aging significantly alters microplastic surface properties. AFM measurements show an increase in both surface roughness and surface area under UV radiation. This increase strongly correlated with enhanced metal adsorption, with uptake varying according to polymer type and aging state. Surface transformations upon prolonged MPs aging significantly altered the preferences of Cd, Co and Cu in adsorption dynamics.

Cu and Pb showed the highest adsorbed amounts, especially on aged microplastics. The influence of HA was metal-specific and dependent on the aging state of the microplastics. HA had no significant effect on Cd, Co, Ni and Pb, but modulated Cu adsorption, enhancing it on unaged MPs and reducing it on aged MPs, which was consistent with the speciation modeling.

This work is the first to assess the combined effects of organic matter and aged MPs on the adsorption of Cd, Co, and Ni in seawater, and to employ AFM for microplastic quantitative surface topography in such context.

Keywords:; atomic force microscopy; humic acid; polypropylene; polystyrene; seawater; trace metals; UV-aging

Highlights:

- Organic matter did not affect Cd, Co and Ni adsorption on unaged or aged MPs
- AFM reveals the key role of MPs surface topography in metal–MPs interactions
- UV aging increases MPs surface, boosting metal adsorption regardless of humic acid
- Cu and Pb revealed the highest adsorbed amounts, especially on aged microplastics
- Humic acid alters Cu adsorption; effects vary with plastic aging

Introduction

The pervasive presence of microplastics (MPs) in marine environments has emerged as a critical environmental concern, with growing evidence of their ecological and biogeochemical implications. MPs, defined as plastic particles between 1 μm and 5 mm (Frias and Nash, 2019), originate from the degradation of larger plastic debris or are directly introduced into the environment as primary MPs through industrial and consumer products (Andrady, 2011; Bhaumik and Chakraborty, 2024; Browne et al., 2007; Guo and Wang, 2019; Khalid et al., 2021). Their small size, high surface-area-to-volume ratio, and diverse polymeric composition make them highly interactive with surrounding chemical species. Consequently, marine MPs are not inert particles; rather, they act as dynamic vectors for different aquatic constituents, inorganic, as well as organic (Crispin and Parthasarathy, 2024; Wang et al., 2020; Zarfl and Matthies, 2010). Within both marine compartments, the water column and sediments, microplastic surfaces readily sorb diverse contaminants, including trace metals (Bhaumik and Chakraborty, 2024; De la Torre et al., 2023; Dobaradaran et al., 2018; Fajković et al., 2022; Khalid et al., 2021; Rapljenović et al., 2024a, 2025).Besides the chemical properties of the adsorbed species, these interactions are partly controlled by the physicochemical properties of the MPs, such as polymer type, crystallinity, surface charge, and degree of aging, as well as environmental conditions like pH, salinity, and the presence of dissolved organic matter (DOM) (Bhaumik and Chakraborty, 2024; Khalid et al., 2021).

Trace metals (TMs) in various marine systems are of particular concern due to their toxicity, persistence, and potential for bioaccumulation. While some metals like Cu, Ni, and Co are essential micronutrients, others such as Pb and Cd are toxic even at low concentrations. The adsorption of these metals onto MPs alters their speciation, potentially reducing their bioavailability in the water column while increasing their residence time and transport potential (Bhaumik and Chakraborty, 2024; Rodrigues et al., 2022). Moreover, MPs can facilitate the transfer of adsorbed metals through trophic levels, posing risks to marine organisms and, ultimately, human health (Desforges et al., 2015; Jinhui et al., 2019; Khalid et al., 2021; Santana et al., 2017).

The interaction between MPs and TMs is further complicated by the presence of natural organic matter (OM), particularly humic substances (HS), which are abundant in marine environments (Zigah et al., 2017). HS, composed of humic and fulvic acids, contain functional groups, such as carboxyl and phenolic hydroxyl groups, that can form stable complexes with metal ions (Lu et al., 2022; Yang and Van den Berg, 2009). These functional groups can either compete with MPs for metal binding or influence on the metal adsorption by modifying the surface properties of MPs (Luo et al., 2022). For instance, humic acid (HA) has been shown to significantly influence the adsorption dynamics of Cu, Pb, and Zn onto MPs, particularly

polypropylene (PP) fibers, by formation of metal-humic acid complexes that adsorb on MPs with a greater extent than inorganic metal species (Rapljenović et al., 2024b).

Aging processes, including photooxidation, mechanical abrasion, and biofouling, also play a pivotal role in modifying the surface chemistry of MPs. Aged MPs often exhibit increased surface and roughness, higher polarity, and the presence of oxygen-containing functional groups, all of which enhance MPs affinity for metal ions (Bhaumik and Chakraborty, 2024; Khalid et al., 2021).

Despite the growing body of research on MP–metal interactions in marine environment, significant knowledge gaps remain. Most studies have focused on pristine MPs under simplified laboratory conditions, often neglecting the complex interplay of environmental factors such as DOM and MP aging. Thus, many investigations of MP-metal interactions were performed in ultrapure water (Chen et al., 2024 and reference therein; Tenea et al., 2024; Wang et al., 2020), in model electrolyte solutions (S. Liu et al., 2022; Wang et al., 2019) or in freshwater (Q. Liu et al., 2022; Naqash et al., 2020 and references therein; Turner and Holmes, 2015). In one of the earliest studies on trace metal adsorption on microplastic in marine environment, authors found enhanced trace metal amounts accumulated on MPs, compared to local sediment (Holmes et al., 2012). Moreover, beached pellets accumulate trace metals to a significantly greater extent than the virgin ones (Holmes at al. 2012). Recent studies also confirmed that MPs in marine environment has high affinity and rapidly adsorb TMs (Brennecke et al., 2016; Gao et al., 2021; Godoy et al., 2019; Mao et al., 2020). Specifically, Gao et al. 2021 considered detailed adsorption kinetics of metals on artificially aged MPs, which has significantly enhanced capacity for metal adsorption, with the higher metal content proportional to the time of metal incubation. Recently, the influence of organic matter on trace metal adsorption on MPs in marine environment began to be considered, firstly by applying ultrapure water (Lu et al., 2022; Qiao et al., 2019), and then seawater (Rapljenović et al., 2024b). Although Wang et al. (2023) considered adsorption of only Cu and Pb on aged MPs in the presence of organic matter, their experiment was not conducted in seawater. However, the combined effects of aging and organic matter on the adsorption behaviour of trace metals in seawater remain poorly understood.

Among the various techniques used to characterize the surface properties of MPs, Atomic Force Microscopy (AFM) offers high-resolution, three-dimensional nanoscale insights into surface morphology, as well as nanomechanical characterization of different, non-living and living, structures (Eaton and West, 2010; Mišić Radić et al., 2023). Although AFM has been widely applied in studies investigating the effects of UV exposure on plastic degradation (Barish and Goddard, 2011; Galluzzi et al., 2025; Nie et al., 1999; Nowicki et al., 2000), its

usage in the context of trace metal adsorption onto microplastic surfaces remains limited, despite the critical role surface morphology plays in adsorption dynamics.

In the context of trace metal adsorption in seawater, in this study, AFM was applied for the first time to investigate the morphological changes of the microplastics' surface.. By quantifying changes in surface area and roughness over varying UV exposure durations, we aim to elucidate how aging influences the adsorption potential of MPs for trace metals, thereby contributing to a more comprehensive understanding of their environmental behaviour and role in contaminant transport. Also, the additional novelty of this work is exploring Cd, Co and Ni adsorption onto aged MPs in the presence of humic acid. By comparing adsorption behaviours in the presence and absence of HA and evaluating the role of aged versus pristine MPs, with TMs speciation modeling, we aim to clarify the metal–MP interactions under environmentally relevant conditions.

2. Materials and methods

2.1. Chemicals and solutions

Stock solutions (1 g/L) of $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Cd(NO_3)_2$, and $Pb(NO_3)_2$, perchloric and nitric acids, sodium acetate, 1,2-cyclohexanedione dioxime (nioxime), sodium hydroxide (30 %, w/w), humic acid (HA), and sodium tetraborate decahydrate salt were used in this investigation. All mentioned reagents were purchased from Merck, Darmstadt, Germany and were, except HA, of Suprapur® grade. For all reagent preparations and rinses, Milli-Q® (MQ) water (Merck Millipore) of type I reagent grade was utilized. Before usage, nitric acid (10 %, w/w) and MQ water were used to clean and rinse all bottles, cuvettes, flasks, strainers, tweezers, and other laboratory tools used for experiment setup and sampling. Standard solution of Cd(II), Co(II), Cu(II), Ni(II), and Pb(II) (concentration of individual metals, 10 mg/L) was made by diluting the commercial single metal stock solutions in 10 mL of MQ water. Standard trace metal (TM) solution was used in adsorption experiments in which the final TM concentrations were 10 µg/L.

HA stock solution (1 g/L) was made by mixing 50 mL of MQ water with 50 mg of HA standard and 50 µL of Suprapur® grade NaOH (30 %, w/w) in a flask. The mixture was then sonicated at 40 kHz with a peak ultrasonic output of 240 W for 60 minutes at room temperature in an ultrasonic bath (emmi EMAG AG, Mörfelden-Walldorf, Germany). The presence of any TMs was checked in the HA stock solution. For that purpose, HA stock solution (100 µL) and concentrated HNO_3 (200 µL) were added to 100 mL of MQ water. This solution's TM content was determined after it was exposed to UV light for 24 hours. In relation to the mass of metals

adsorbed on microparticles, recorded TMs concentrations in HA solution were insignificant (≤1 %), hence they had no discernible effect on the reported amounts of adsorbed TMs. The impact of adding HA on the pH of seawater was examined before model studies. Final concentration of HA in adsorption experiments was 1 mg/L.

Seawater used in experiments was collected from the middle Adriatic Sea eastern coast (43.7234° N, 15.8458° E), a location that was not under noticeable anthropogenic influence. The ATI Orion 320 pH meter was used to measure the pH (8.10), while the salinity (39.0) was measured with the S-10E refractometer (Atago, Tokyo, Japan). The water was then refrigerated at 4 °C. Prior to the adsorption experiments, seawater was filtered using a cellulose nitrate filter (pore size 0.45 µm, Sartorius, Göttingen, Germany) and UV-irradiated for 48 hours. This was done to destroy any organic matter and microorganisms that might compete with the additional HA for TMs. The same UV source was used both for seawater treatment and for microplastic aging: a 150 W medium-pressure mercury UV lamp (Hanau, Germany). Unlike low-pressure mercury lamps with a single dominant emission line, this lamp emits a broad polychromatic spectrum between approximately 200 and 600 nm, with major emission peaks at 254, 280, 313, 366, 405, 436, 546 and 575 nm, consistent with reported spectra for medium-pressure mercury lamps.

2.2. Microplastics (MPs)

The polystyrene (PS) fragments used for experiments were made by grinding the single-use plastic product, a PS spoon, in a cryogenic mill (6875 SamplePrep Freezer/Mill, SPEX, USA). Polypropylene (PP) preproduction pellets were purchased from TVK (Tiszaújváros, Hungary). Average length of PS fragments ranged between 2 and 6 mm, while PP pellets were on average 4.1 mm in diameter and 2.1 mm in length. Before any experiments, plastic microparticles were washed with 10 % nitric acid and rinsed with MQ water.

2.2.1. Aging of MPs

To prepare UV-aged microplastics, PP pellets (36 g) and PS fragments (20 g) were spread in a single layer in multiple Petri dishes and irradiated with the above-described UV lamp for 0, 4, 22 and 52 days, with occasional manual turning of particles. The distance between the lamp and the microplastic particles was maintained within a 25–35 cm range throughout aging. The lamp operated in 24 h-on / 24 h-off cycles, and only the effective exposure time was used to define the aging duration.

The irradiance at the microplastic surface was not measured, and therefore the total fluence received by individual particles cannot be specified as a single value. However, all particles

were aged in the same experimental setup, under identical lamp power and operating schedule, and were regularly redistributed and turned during irradiation. MPs used in adsorption experiments were subsampled randomly from these bulk-aged batches, so the analyzed microplastic particles are expected to represent an average irradiation history across the 25–35 cm distance range rather than extreme local exposure conditions.

Although the model adsorption experiment (described in section 2.3.) was designed to simulate a natural seawater environment, UV irradiation of the microplastics was conducted in air. This approach was chosen firstly due to the physical properties of the microplastics used - specifically, the low and nearly identical densities of PP and PS relative to seawater, combined with their small particle sizes. As a result, both PP pellets and PS fragments floated on the seawater surface. Consequently, UV irradiation in seawater would not have provided meaningful exposure conditions for these materials, justifying the use of air as the irradiation medium. Published results on MPs aging in different environment, *i.e*. in air *vs*. in seawater, showed that aged MPs exhibited the same surface characteristics and under UV-irradiation, however, the change was more expressed on the samples irradiated in the air than on the samples irradiated in seawater (Liqi Cai et al., 2018; Mao et al., 2020). Secondly, artificial UV-aging of microplastics in air was applied as a controlled and accelerated simulation of photooxidative weathering. Although natural aging of microplastics in marine environments occurs under more complex and variable conditions than those reproduced in the laboratory, microplastic particles can persist in seawater for much longer periods and are therefore exposed to solar irradiation over extended timescales. In the natural environment, this irradiation is less intense and less constant than the direct artificial UV treatment used here, but its cumulative effect over months to years can still result in substantial changes in polymer surface chemistry and morphology. Thus, the present UV-aging procedure was used to generate defined and reproducible weathering states, reflecting increasing degrees of photooxidative alteration rather than exact environmental exposure times. The selected aging periods of 4, 22 and 52 days should therefore be interpreted as comparative levels of surface weathering relevant to environmentally persistent microplastics.

2.2.2. Microplastic surface analysis

2.2.2.1. Microscopy

The surface morphology of unaged and aged MPs was analysed using a low vacuum scanning electron microscopy (SEM) and atomic force microscopy (AFM). Using a secondary electron detector and a Hitachi tabletop microscope TM4000Plus II running at 5 kV, SEM imaging was carried out. The AFM imaging of the microplastics was performed using a multimode scanning

probe microscope with a NanoScope IIIa controller (Bruker, Billerica, MA, USA) equipped with vertical engagement JV scanner that has a maximum z-range of about 6.0 µm Topographic images were acquired in contact mode with silicon-nitride probes (DNP-10, Bruker, nominal frequency 12–24 kHz, spring constant of 0.06 N m^{-1}), with the linear scanning rate between 0.8 and 1.5 Hz and with resolution of 512 pixels. NanoScope™ software (Bruker, NanoScope Analysis, version 3.0) was used to process and analyse the images. All AFM images are presented as raw data, except for the first-order two-dimensional flattening. A two-component adhesive was used to adhere plastic pieces to a metal plate to prepare the samples for AFM imaging.

2.2.2.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of unaged and aged microplastics were acquired on FTIR spectrometer (Bruker Tensor 27) with attenuated total reflection (ATR). To record the spectra, range of 400–4000 cm^{-1} was used. ATR-FTIR spectroscopy was used to record the band changes on the samples surface formed due to the UV light exposure. In order to gain representative spectra, several measurements were recorded for each sample.

2.3. Adsorption Experiment

In order to set up the adsorption experiment, 4 g of pre-weighted PP pellets (unaged and aged for 4 d and 52 d) and 680 mL of filtered and UV-irradiated seawater were added to 2 L FEP (fluorinated ethylene propylene, NALGENE® Thermo Fisher Scientific, Waltham, MA, USA) bottles. This ensured that all bottles contained the same concentration of MPs. For each polymer and aging level, two bottles were prepared: one serving as a control without organic matter, and the other was supplemented with 1 mg/L of humic acid. 10 µg/L of each TM was then added into each bottle. At 30 rpm, the FEP bottles were continuously shaken on a stirrer. Figure S1.1 in Supplementary material displays the experiment's flow chart. The use of inert FEP bottles ensured the integrity of the adsorption experiment by preventing any loss or contamination of metal ions.

The experiments lasted 14 days in total. Each bottle's microplastics were sampled at predetermined intervals, with samplings conducted at 0 h (microplastic samples taken before immersing into solution) and after 1 h, 3 h, 24 h, 72 h, 7 d, and 14 d. About 0.5 g of microparticles were separated for the sample process and put into quartz glass cuvettes. To ensure a constant plastic-to-solution ratio, 50 mL of solution was also removed with every plastic sample. The microplastic samples were treated with 4 mL of nitric acid and 1 mL of perchloric acid (both Suprapur® grade) as part of an acid-leaching process. Cuvettes were shaken periodically during 24 h. Using a micropipette, the leachate was quantitatively

transferred to a 100 mL glass conical flask, diluted with MQ water, and then transferred to FEP bottles, which were exposed to UV light for 24 hours. Described process made sure that all of the metals were leached from the plastic surfaces and where humic acid was present in solution, were unbound from organic matter. Acid control (blank) solutions were prepared, as well, from acid-leachate of MPs (previously washed with 10 % HNO_3 and rinsed with MQ water). Every day throughout the trial, the room temperature was recorded, and it varied slightly between 19.0 °C and 21.0 °C.

2.4. Trace metal analysis

All laboratory equipment used for trace metal sampling and analysis was cleaned with 10 % nitric acid (analytical grade, Fisher Scientific, Loughborough, UK) and then rinsed high purity MQ water (18.2 MΩ cm at 25 ◦C).

The mass fractions of TMs (Cd, Co, Cu, Ni, and Pb) adsorbed on plastic MPs, as their concentration in obtained leachate solutions were determined by triple quadrupole mass spectrometer with inductively coupled plasma (ICP-QQQ Agilent 8900, Agilent Technologies, USA) with indium (10 ppb) as an internal standard. For measurement purposes, the sample (leachate solution) was diluted 2x with ultra-pure water (MQ). Quantification was performed by external calibration with standards (0, 0.1, 1, 10 and 100 ppb) prepared in 2 % HNO_3. Analysis of the certified reference material GEMS (Global environment monitoring system for freshwater of the United Nations Environment Program (UNEP)) with the recoveries was showed in Table S1.1.

2.5. Speciation Modeling

Using Visual MINTEQ 3.1 software, TMs speciation in seawater (Table 1) was calculated according to the composition of major and trace elements. The software's NICA-Donnan humic model (NDHM) was used to estimate TM interactions with organic materials. Humic acid (HA) parameters given by the software were used and DOC parameters were set to the ratio of active DOM to DOC by a factor of 2, according to Sipler and Bronk (2015).

Table 1.

2.6. Statistical analysis

Data processing and selected visualizations were performed in Python (v3.12; *pandas*, *seaborn* and *matplotlib*). All inferential and model-based analyses were conducted in R (v4.5.2), including (i) multivariate analysis of joint metal-adsorption patterns (redundancy analysis, RDA), (ii) univariate factorial analyses for individual metals, and (iii) non-linear modelling of adsorption kinetics. The dataset comprised adsorption amounts of Cd, Co, Cu, Ni

and Pb (ng/g) measured on plastic samples categorized by polymer type (PP, PS), UV exposure (0, 4, 52 days), humic acid (HA; 0 or 1 mg/L), and adsorption time (t; h).

2.6.1. Multivariate analysis

To analyse joint patterns in metal adsorption across treatments, we used redundancy analysis (RDA; Legendre and Legendre, 2012). The analysis is based on the late adsorption phase, defined here as all measurements taken at times ≥ 24 h, where adsorption kinetics are close to steady state for most metal–plastic combinations. Before RDA, metal concentrations were column-wise centred and scaled to unit variance so that all metals contribute equally regardless of their absolute concentration ranges.

RDA was carried out with the model *Y ~ Type * UV + HA* using the function *rda* (R package *vegan*; (Oksanen et al., 2025). This model includes main effects of plastic type (*Type*), UV ageing (*UV*) and humic acid (*HA*), as well as the *Type: UV* interaction, allowing the effect of UV ageing on metal adsorption to differ between PP and PS. The choice of the interaction model (*Y ~ Type * UV + HA*) over the additive model (*Y ~ Type + UV + HA*) was based on permutation tests comparing alternative RDA models (Table S4.1) and on adjusted explained variance (Table S4.2): the interaction model showed higher adjusted R^2 (R^2_{adj} = 0.69 vs 0.59) and a significantly better fit than the additive model (permutation test of nested models: F = 7.13, p = 0.0006). Further extending the model to include interactions with HA (*Y ~ Type * UV * HA*) did not improve model performance (R^2_{adj} = 0.66 and p = 0.99), so *Y ~ Type * UV + HA* was retained for all subsequent RDA analyses.

The proportion of total variance explained by the constrained component (R^2) and its adjusted version (R^2_{adj}) was estimated using *RsquareAdj*. The significance of the global model, individual terms (*Type, UV, HA, Type:UV*) and canonical axes (RDA_1–RDA_5) was assessed with permutation tests (*anova.cca*), using 9,999 unrestricted permutations of residuals under the reduced model. Results are reported as F-ratios, p-values and variance components per term.

The assumption of homogeneous multivariate dispersion across treatment levels was evaluated using *betadisper* and *permutest* (package *vegan*) to distinguish differences in multivariate centroids (location) from potential differences in within-group variability (beta-dispersion), particularly among UV treatments. For graphical interpretation of the RDA results (biplots with site scores, species scores and centroids), we used *ggplot2* (Wickham, 2016).

2.6.2. Univariate analysis

To complement the multivariate RDA, we analysed each metal separately using univariate linear models. For each metal (Pb, Cu, Ni, Cd, Co), we restricted the analysis to the late

adsorption phase (measurements at times ≥ 24 h), consistent with the multivariate analysis, where adsorption kinetics were close to steady state. For a given metal, adsorption was modelled as a function of plastic type, UV ageing and humic acid: *Y1 ~ Type * UV + HA*, with all predictors treated as fixed factors. Tests of effects were obtained using the Anova function in the *car* package in R (Fow and Weisberg, 2019). To account for heteroscedasticity among treatment combinations, we used heteroscedasticity-robust tests with the *HC3* covariance estimator (*white.adjust = "hc3"*). Reported F-ratios and p-values are based on Type III sums of squares under sum-to-zero contrasts (*contr.sum*). The strength of each effect was quantified using partial η^2, computed from the same linear model with the function *eta_squared* (package *effectsize*; (Ben-Shachar et al., 2020). Effect sizes are interpreted using conventional benchmarks (≈ 0.01 small, ≈ 0.06 medium, ≈ 0.14 large).

In the *post hoc* analysis, we obtained model-based estimated marginal means (EMMs) for each *Type* × *UV* combination using the *emmeans* package (Lenth, 2025), averaging over HA. Pairwise comparisons among these means were conducted with Holm-adjusted p-values, and treatment groups were summarised using a compact letter display (*cld*), where combinations sharing a letter are not significantly different at $\alpha = 0.05$ (package *multcomp*; (Hothorn et al., 2008)). The *emmip* function was used to graphically display the *Type * UV* interaction patterns implied by the fitted models and the analysis.

2.6.3. Adsorption kinetics modeling

Multivariate and univariate analyses were based on quasi steady-state adsorption values (t ≥ 24 h), allowing comparison of final adsorption capacity among treatments. To use the full time series and quantify both the approach to equilibrium (adsorption rate) and the equilibrium capacity, adsorption kinetics were modelled separately for each Metal × Type × UV × HA combination.

Adsorption kinetics were modelled using the pseudo-second-order (PSO) equation, which is widely applied to adsorption time courses and often provides a good empirical description when uptake is strongly influenced by surface interactions and progressive site saturation:

$$dq_t / dt = k_2 \cdot (q_e - q_t)^2$$

where q_t is the adsorbed amount at time t, q_e is the equilibrium adsorption capacity, and k_2 is the PSO rate constant.

Models were fitted in R using nonlinear least squares with *nlsLM* (package *minpack.lm*; (Elzhov et al., 2023)). The initial adsorbed amount q_0 was taken from measurements at $t=0$, and parameters were constrained to $q_e \geq 0$ and $k_2 \geq 0$. Parameter estimates and standard errors were obtained from the model summary. Fit quality was evaluated using RMSE and normalized

RMSE (nRMSE), computed by scaling RMSE to the observed data range ($y_{max}-y_{min}$). When the PSO model failed to converge, the corresponding time series was fitted with an intercept-only (constant) model.

3. Results

3.1. Characterisation of microplastics

In order to thoroughly characterize the surface of microplastics before (*i.e*. unaged) and after the certain period of UV-radiation, different microscopical (AFM and SEM) and FTIR techniques were applied.

3.1.1. Polypropylene (PP)

Figure 1 (a-c) shows AFM images of unexposed (a) and exposed (b-c) PP to UV-radiation for 4 and 22 days. Due to huge roughness of microplastics that was UV-irradiated for 52 days, AFM analysis of such samples couldn't be performed (Figure S2). Unaged PP (Fig. 1 (a)) had relatively smooth surface with average roughness of 141.8 ± 29.5 nm (Table S2). Upon UV irradiation, PP developed surface cracking (Fig. 1 (b-c)), resulting in a significant increase in surface roughness and area, *e.g*. for the 22 days of UV-radiation, microplastic average roughness increased for almost 9 times and surface area for 47 % (Table S2). The depth of the cracks increases with the time of exposure and maximal depth of cracks are given in the Table S3. Since the maximal scanning range in z-direction was ~6 μm and the cracks of the polypropylene that was UV-irradiated for 52 days were even higher, the precise depth of cracks for that particular samples couldn't be determined.

Figure 1.

Therefore, SEM technique was used to characterise the surface of 52-day UV-aged PP, Figure 1 (c) and Figure S3. Since SEM images were acquired on larger surface area than AFM images (*i.e*. 400 μm x 300 μm *vs*. 50 μm x 50 μm, respectively), SEM image of aged PP more clearly illustrate the extent surface cracking, although it doesn't provide information on crack depth and surface area.

In addition to microscopic characterisation, FTIR spectra were also recorded to see if new functional groups were formed on the aged PP.

Typical FTIR spectra of unaged PP, Figure 1 (e), contain several dominant peaks in the wavenumber range from 3000 to 2800 cm^{-1} that corresponds to C-H stretching in alkyl groups (~2950 cm^{-1} and ~2916 cm^{-1} are asymmetric, while ~2876 cm^{-1} and ~2837 cm^{-1} are symmetric C-H stretching in -CH_3 and-CH_2 group, respectively) and also contain two peaks of higher intensity at ~1457 cm^{-1} and ~1375 cm^{-1} that are result of C-H bending vibrations in -CH_3 group (symmetric and asymmetric, respectively). Other minor peaks are at ~1164 cm^{-1} (stretching C-

C, rocking CH_3, bending C-H), ~996 cm^{-1} (rocking -CH_3, wagging -CH_2), ~976 cm^{-1} (stretching C-C) and ~842 cm^{-1} (rocking CH_2) (Andreassen, 1999; Bogdanova et al., 2023; Campanale et al., 2023; Fang et al., 2012). Aged PP, Fig. 1 (e), beside mentioned characteristic PP peaks encompass additional peaks at ~1720-1750 cm^{-1} that corresponds to carbonyl (C=O) stretching and broad peak in the range from ~3580 to 3300 cm^{-1} that is result of hydroxyl (O-H) stretching (Cai et al., 2018; Campanale et al., 2023). Additionally, a group of broad overlapping peaks in the range 1330-1020 cm^{-1} are also present in FTIR spectra of aged PP, confirming the presence of oxygen-containing groups in the UV-irradiated PP (Bogdanova et al., 2023).

3.1.2. Polystyrene (PS)

Unlikely PP that forms cracks on certain sites when exposed to UV-radiation, entire PS surface generates pores (Figure 2) and become rough (Mao et al., 2020; Nowicki et al., 2000). Recent studies also reported the loss of material from PS surface under UV-irradiation (Galluzzi et al., 2025; Z. Liu et al., 2022). Our results showed that average roughness changed from 152.4 nm to 359.4 nm for unaged PS and one exposed for 52 days to UV-radiation, respectively, while average specific surface increases for 48 % (Table S2) for the same exposed period.

Figure 2.

The FTIR spectra of PS fragments before and after UV-exposure are shown in Figure 2 (e). Characteristic absorption peaks of PS benzene ring are in the range from ~3080 to 3080 cm^{-1}, corresponding to C-H aromatic stretching (Fang et al., 2012; Godoy et al., 2019), and three absorption peaks at the wavenumbers of ~1600, 1492, and 1452 cm^{-1} representing C=C stretching (Fang et al., 2012). The absorption peaks at the wavenumbers 750, 694 and 533 cm^{-1} correspond to C-H out-of-plane bending vibration absorption (Fang et al., 2012; Zolotarev, 2017). FTIR spectra of aged PS (Fig. 2 (e)) show expressed peak at 1711 cm^{-1} that is result of carbonyl stretching (Godoy et al., 2019), and broad peak in the range from ~3550 to 3250 cm^{-1} as a result of hydroxyl (O-H) stretching. As well, broad peak in the range from ~1290 to 1210 cm^{-1} corresponds to C-O stretching probably from aromatic ethers formed in aged PP.

Described microscopic analysis results on significant increase in surface area, *i.e*. adsorption capacities, of both, PP and PS, as well as detected formation of new oxygen-containing surface functional groups under the UV-radiation are valuable for the characterising the trace metals adsorption on MPs surface.

3.2. Adsorption of trace metals (TMs) on microplastics

The adsorption of trace metals (Cd, Co, Cu, Ni and Pb) on PP and PS microplastics, both unaged and UV-irradiated (aged) for different period (4 and 52 days), were investigated in the presence and absence of humic acid (1 mg/L) during a 14-day adsorption period. Measured mass fractions of adsorbed trace metals (expressed in ng of metals per g of MPs) are given in Tables S3.1 (for PP) and S3.2 (for PS).

3.2.1. Adsorption of TMs on PP

Figure 3 shows adsorption behaviour of the investigated trace metals onto unaged and aged (4 and 52 days UV-irradiated) PP, under conditions of both with and without HA. The order of adsorbed TM mass fractions on unaged PP was Cu > Pb > Ni > Co ~ Cd, while on 52 days UV-aged PP was Pb > Cu > Ni ~ Cd > Co, regardless the presence of HA. Pb and Cu showed similar adsorption pattern. Relatively low amounts of these two metals on the unaged PP were almost constant throughout adsorption experiment, while their mass fractions on aged PP (4- and 52-day UV-irradiated) gradually increased with the time of experiment, reaching the equilibrium only in the case of the 52-day UV-irradiated PP after approximately 72 h of adsorption experiment. The major difference between Pb and Cu is that the HA presence influenced Cu adsorption, while Pb amounts remained almost unaffected. The presence of HA increased Cu adsorption on unaged PP, whereas with aged PP (both 4- and 52-day), HA presence led to a decrease in Cu amount on plastics.

Adsorption experiments showed that the amounts of Cd, Co, and Ni adsorbed on PP were generally similar regardless of the presence or absence of HA in the seawater, (Fig. 3). Considering different aging degree of PP, results showed that for Cd, Co and Ni amounts of adsorbed metals on unaged PP and 4-day UV-irradiated PP were similar. With the 52 days UV-irradiated PP, mass fractions of Cd, Co and Ni increased for almost one order of magnitude (Table S3.1). Like Pb and Cu, with 52-day UV-irradiated PP pellets, Ni also showed gradual increase with the time of experiment, reaching the equilibrium at 72 h of the experiment. For Cd and Co, adsorption curves indicate that equilibrium is not achieved for 14 days.

Figure 3.

3.2.2. Adsorption of TMs on PS

The adsorption capacities of unaged and aged polystyrene for the investigated trace metals, in the presence and the absence of HA, are shown in Figure 4. The adsorption curves were characterized by pronounced oscillations throughout the experiment. These fluctuations may

452 be attributed to experimental variability arising from the heterogeneous size distribution of PS
453 fragments sampled at each time point. Although each sampling involved approximately 0.5 g
454 of PS, the total available surface area for metal adsorption likely varied between samples taken
455 in each sampling time since PS fragments were not uniform in size and shape. This
456 inconsistency in surface area could have influenced the observed adsorption capacities
457 (Rapljenović et al., 2024a).

458 Figure 4.

459 Like with PP, Pb and Cu also exhibited the highest adsorbed mass fractions on PS MPs.
460 Generally, the order of TM adsorption on unaged PS was Cu > Pb > Ni > Co > Cd, while on
461 52-day UV-aged PS was Pb ~ Cu > Ni > Cd > Co, regardless the HA concentration. On unaged
462 PS, Cu exhibited a higher adsorption affinity than Pb, even at the initial stage of the experiment.
463 Moreover, the amount of adsorbed Cu was constant during the adsorption experiment, while
464 Pb adsorbed fraction gradually increased. The presence of HA didn't noticeably modify the
465 adsorption curve of Pb, while one of Cu was slightly changed.

466 With aged PS (4 and 52 days UV-irradiated) Cu also showed slightly higher adsorbed values
467 than Pb, Fig. 6. In the presence of 4-day UV-aged PS, both copper and lead exhibited an initial
468 increase in adsorption onto the PS surface within the first 72 hours, regardless of the presence
469 of HA. However, by 168 hours a slight decrease in their surface abundance was observed,
470 indicating partial desorption (Godoy et al., 2019). Mass fractions of Pb and Cu adsorbed on 4-
471 day UV-aged PS were the highest at 336 h of contact time. Given that these peak values
472 occurred following desorption phase and considering possible experimental uncertainties
473 discussed earlier, these results should be interpreted with caution. Regarding the presence of
474 HA, Pb adsorption curve at 4-day UV-aged PS was almost the same, with and without HA
475 (considering experimental error), while for Cu slight decrease in adsorption was observed with
476 HA. With 52-day UV-aged PS, without HA, the adsorption curves for Pb and Cu were also
477 similar, *i.e*. metal adsorption increased with contact time and reached maximum values at 24
478 h, and afterwards they decreased. Since the UV-irradiated PS for 52 days had the maximal
479 available surface, without HA the maximum adsorbed amounts of trace metals were obtained
480 at shorter contact time. In the presence of HA, both metals reached the adsorption equilibrium
481 later, at 72 h (comparing to the analogous experiment without HA).

482 Following Cu and Pb, Ni showed the highest adsorption values on PS fragments (Fig. 4).
483 Generally, Ni exhibited higher affinity for PS compared to PP, both aged and unaged, indicating
484 higher affinity of Ni^{2+} ions, (the primary species of Ni under experimental conditions, Table 1)
485 toward PS in comparison with PP. Without HA, contrary to results on PP where Ni showed the

same behaviour on unaged and on 4 days UV-irradiated PP, adsorbed Ni on PS increased in order: unaged PS < 4-day UV-aged PS < 52-day UV-aged PS. Ni reached its maximum adsorbed value on unaged PS (without HA) after 72 h of contact time, before subsequently decreasing. The dynamic of Ni adsorption on 4-day UV-aged PS (without HA) was similar to unaged PS. Ni adsorption on 52-day UV-aged PS (without HA) distinguished from the 4-day UV-aged not only in the higher adsorption mass fractions, but as well in the faster adsorption, *i.e*. the maximum Ni adsorbed value on 52-day UV-aged PS was reached at 24 h of contact time. In the presence of HA, Ni adsorption curves on PS significantly oscillated. Cd and Co were metals with the lowest adsorption affinity on PS (Fig. 4), and the trends of their curves, regardless the HA presence, were very similar to Ni.

3.3. Results of statistical analysis

3.3.1. Multivariate analysis

Multivariate analysis of the late adsorption phase (t ≥ 24 h) showed that the RDA model explained a large proportion of the variation in standardized metal adsorption (R^2 = 0.73, adjusted R^2 = 0.69; Fig. 5). The dominant pattern in the ordination was aligned with the first canonical axis, which primarily reflects the UV-ageing gradient (Fig. 5A). The common orientation of the metal vectors along this gradient shows that adsorption of all metals generally increased with increasing UV ageing, consistent with the idea that weathered plastic surfaces provide more favourable binding sites for dissolved metals.

The second canonical axis mainly captured differences between the two plastic types, as shown by the separation of PP and PS in Fig. 5B. This indicates that polymer identity modified the UV-driven adsorption response in a metal-specific way: Cd was more strongly associated with PP, whereas Ni and, to a lesser extent, Cu were more strongly associated with PS; Pb and Co showed only weak differences between plastic types. In contrast, HA had little influence on the overall multimetal adsorption pattern, as indicated by the limited separation of HA levels in Fig. 5B. From an environmental perspective, these results suggest that plastic weathering is the primary factor increasing the capacity of microplastics to bind dissolved metals, while polymer type acts mainly as a secondary modifier of this response.

A more detailed description of the RDA results, together with the full statistical outputs for model selection, permutation tests, eigenvalues and dispersion analyses, is provided in the Supplementary Information (S4: Multivariate analysis).

Figure 5.

3.3.2. Univariate analysis

Three-way ANOVA (Table S5.1) and the corresponding effect-size heatmap (partial η^2; Figure 6 (a)) show that UV ageing has the largest effects for all metals, confirming it as the dominant driver of late-phase adsorption (Fig. S1.1), in agreement with the RDA results. Ni and Cu display strong main effects of plastic type together with clear *Type:UV* interactions, whereas Cd shows the strongest *Type:UV* effect overall, indicating a particularly pronounced, type-specific response at high UV level (52d). In contrast, Pb and Co exhibit only moderate type-related effects and weak or absent *Type:UV* interactions, consistent with their limited PP–PS contrast in the RDA. Humic acid effects are uniformly small across metals, with at most a borderline effect for Cu.

Figure 6.

Figure 6B shows that Pb adsorption increases mainly along the UV ageing gradient. PS already rises at moderate ageing (4 d), whereas PP reaches its highest values only under strong ageing (52 d). Consistent with this pattern, the three-way ANOVA indicates a dominant *UV* effect and a significant *Type:UV* interaction (Table S5.1). Post hoc comparisons confirm the stepwise UV-driven increase and show that small PP–PS difference is evident only at the intermediate UV level (4 d), while the polymers do not differ at UV0 or UV52 (Table S5.2). Humic acid shows no detectable effect (Table S5.1).

Cu follows a UV-driven increase similar to Pb, but with a much stronger polymer type signature. PS shows a pronounced rise already at moderate ageing (UV4), whereas PP remains low at UV4 and increases sharply under strong ageing (UV52). At UV52, both polymers converge to similarly high Cu adsorption (Figure 6B; Table S5.3). This pattern is consistent with significant main effects of *UV* and *Type* together with a substantial *Type:UV* interaction (Table S5.1). Humic acid shows at most a marginal tendency towards lower Cu adsorption (Table S5.1).

Ni stands out by showing consistently higher adsorption on PS than on PP under UV ageing, with the polymer contrast becoming clear at UV4 and remaining evident at UV52 (Figure 6; Table S5.4). Within polymers, PP shows a marked increase only at UV52 (UV0 ≈ UV4 < UV52), while PS increases more gradually across the UV gradient, reaching the highest adsorption at UV52. These patterns align with strong main effects of *UV* and *Type* and a smaller but significant *Type:UV* interaction, while humic acid shows no detectable influence (Table S5.1)

A clear threshold pattern emerges for Cd: adsorption stays near baseline across almost all treatments, but spikes only for strongly UV-aged PP (UV52), producing the most pronounced type-specific UV response among the metals (Figure 6, Table S5.1). *Post hoc* comparisons identify PP–UV52 as the only group that differs from all others, while the remaining treatments are statistically indistinguishable (Table S5.5). Humic acid shows no detectable influence (Table S5.1).

For Co, the dominant pattern is a UV-driven increase with only modest separation between polymers. In PP, adsorption stays low at UV0–UV4 and rises mainly under strong ageing (UV52), whereas in PS it increases more gradually across the UV gradient (Figure 6). This is consistent with the ANOVA showing a strong UV effect and a smaller but significant Type effect (Table S5.1). Post hoc comparisons indicate at most a modest polymer contrast at UV4 (PS > PP), with no difference between polymers at UV0 or UV52 (Table S5.6).

3.3.3 Adsorption kinetics modeling

To capture adsorption over the full experimental time course rather than only the late phase (t ≥ 24 h), kinetic models were fitted for each Metal × Type × UV × HA combination to estimate equilibrium adsorption capacity (q_e) and the pseudo-second-order rate constant (k_2) (full model outputs are provided in Table S6.1 and Figure S6.1). Across all treatments, the clearest pattern was variation in q_e, which separated metals according to their overall adsorption capacity: Pb and Cu showed the highest equilibrium capacities, Ni was intermediate, and Cd and Co remained low (Figure S6.2). In environmental terms, this indicates that Pb and Cu are the metals most strongly retained by the tested plastics once adsorption approaches equilibrium.

The strongest treatment effect was again UV ageing, which shifted Pb and Cu towards markedly higher q_e values, especially at UV4 and UV52 relative to UV0 (Figure S6.1). This is consistent with the multivariate and univariate analyses, and supports the conclusion that plastic weathering increases the capacity of microplastics to bind dissolved metals. For Cu, humic acid was associated with somewhat lower adsorption capacity q_e under UV-aged conditions (Figure S6.1), suggesting that dissolved organic matter may partly reduce Cu uptake. In contrast, estimates of k_2 were often more uncertain than q_e and were therefore less informative for environmental interpretation.

A more detailed description of the kinetic fits, parameter uncertainty and model diagnostics is provided in the Supplementary Information (S6: Adsorption kinetic modeling).

4. Discussion

In this study we investigated the adsorption characteristics of trace metals Cd, Co, Cu, Ni and Pb onto microplastics (MPs) in seawater, with a specific focus on the influence of humic acid and MP aging. Two different types of MPs were used: polypropylene (PP) pellets and polystyrene fragments (PS), as these two polymers are frequently found in seawater (Gündoğdu, 2022; Kolda et al., 2024). Adsorption experiments included environmentally relevant concentrations of trace metals (Cd, Co, Cu, Ni and Pb, 10 µg/L) (Rapljenović et al., 2024a; Turner, 1995), microplastics and HA (1 mg/mL) (Boggs et al., 1985; Rodrigues et al., 2009).

590 Given that the size and surface morphology of microplastics are critical factors influencing their
591 interactions with metal ions, it is essential to provide a detailed characterization of the
592 microplastic surfaces, particularly those subjected to UV-induced aging. For that purpose,
593 researchers usually apply microscopic techniques like SEM, while AFM, to the best of our
594 knowledge, has not yet been applied to research examining the relationship between the
595 surface properties of MPs and their adsorption capacity toward trace metals. AFM is a powerful
596 tool for nanoscale surface characterization of microplastics undergoing UV-ageing (Barish and
597 Goddard, 2011; Galluzzi et al., 2025; Nie et al., 1999; Nowicki et al., 2000). In this work, we
598 used AFM for characterisation of the MPs surface that, beside just visual representation of
599 changing the surface, also offered quantitative determination of surface area and its
600 roughness. It should be noted that the irradiance at the microplastic surface was not quantified
601 during UV aging. Nevertheless, MPs were aged in a single-layer geometry under constant
602 lamp power, with particles regularly turned and randomly subsampled for adsorption
603 experiments. Under these conditions, the microplastic samples analysed here should be
604 viewed as representative of average irradiation exposure within the 25–35 cm distance range,
605 and the observed trends in surface roughness, functional group formation and metal
606 adsorption are interpreted in this comparative sense rather than in terms of exact fluence
607 thresholds. Our results (section 3.1) showed that, while PP formed cracks and grooves (Cai et
608 al., 2018; Luo et al., 2022; Zhu et al., 2020), entire PS surface generates pores and become
609 rough (Mao et al., 2020; Nowicki et al., 2000) under UV-exposure. AFM analyses of both PP
610 and PS (before and after UV-irradiation) showed significant increase of surface area and
611 roughness with the time of exposure (section 3.1, Table 2).

612 Table 2.

613 Thus, surface area of aged PP increased for 9.7 % (4 days UV-aged) and 47.2 % (22 days UV-
614 aged), while for 52 days UV-aged PP it was even higher (Fig. S2.1, Table S2.1), although it
615 couldn't be precisely measured due to the huge roughness. Namely, as mentioned in section
616 3.1.1, AFM imaging of the 52 day UV-aged PP sample couldn't be performed since it roughness
617 was higher than 6 μm (Fig. S2.1), what was above the maximal scanning range in z-direction.
618 Increment of the PS surface area was 6.6 %, 34.8 % and 47.8 % for 4-, 22- and 52-days UV-
619 aged PS (Table 2), respectively. As a result, the adsorption ability of both UV-aged PP and PS,
620 particularly the 52-day aged, was significantly enhanced (Chang et al., 2024; Han et al., 2021).
621 Moreover, for 52-day UV-aged MPs metal amount at 72 h of adsorption increased from at least
622 3 (Ni) to about 20 times (Pb) on PP plastics, and from about 2 (Co and Ni) to 8 times (Pb) on
623 PS, in comparison with unaged plastics (Tables S3.1 and S3.2). An increase in the amount of
624 adsorbed metal follows the increase in the MPs surface, which is more pronounced in the case
625 of PP, as shown by the AFM results (Table 2). Unlike SEM, which is largely limited to qualitative

observations, AFM provides quantitative evidence of surface evolution, showing that a ~47.8% increase in surface area and ~135.8% increase in roughness for PS after 52 days of UV exposure (Table 2) corresponds with the pronounced Pb adsorption increase of up to 8 times, directly linking measurable surface rising to enhanced metal uptake. Other researcher groups have also reported increased metal adsorption on aged MPs compared to unaged ones, on PP (Han et al., 2021; Tang et al., 2021; Wang et al., 2023) and on PS (Chen et al., 2022; Mao et al., 2020; Wang et al., 2023; Yang et al., 2021). Different increase in adsorbed metal amounts among published and this study are due to different time of exposure, as well as different conditions and time of adsorption experiment, therefore, they are difficult to compare.

Figure 7 present a detailed comparison of trace metal adsorption onto PP and PS microplastics, unaged, and UV-aged for 4 and 52 days, under seawater conditions, in the presence and absence of HA. Although the range of adsorption amounts is wide because the box plots were created using all of the recorded metal adsorption data gathered during the experiment, they show the trend of metal behaviour with regard to the kind of plastic, the degree of plastic aging, and the presence of HA. This trend is also visible by inspection of boxplots of metal adsorption in the late adsorption phase ($t \geq 24$ h; Fig. S4.1). Generally, UV treatment of the plastics resulted in enhanced adsorption of all investigated trace metals, irrespective of the presence of HA (Figs. 3, 4, 7). This observation aligns with AFM data, which revealed increased surface roughness and area following UV exposure, and was confirmed by multivariate analysis, Figure 5 (a).

Figure 7.

The increase in adsorbed metal levels on 4-day UV-aged PP plastics was less noticeable for Cd, Co, and Ni compared to unaged, (Figs. 3, 5-7). It was probably due to the relatively modest increase in surface area (9.7 %, Table 2) of 4-day UV-aged PP and the inherently low affinity of these metal ions for unaged PP (Ahechti et al., 2020; Gao et al., 2019; Godoy et al., 2019). Speciation modeling (Table 1) indicated that Cd, Co, and Ni predominantly existed in the seawater as free ions or labile inorganic species, even in the presence of HA, with less than 1 % forming complexes with HA. Consequently, HA had no notable effect (Figs. 5 (b), 7) on the adsorption of these three metals onto both polymers, as confirmed by statistical analysis (section 3.3). Free Ni^{2+} ions consistently favoured PS over PP (Figs. 6, 7), contrary to the results obtained by Godoy et al. 2019, however, they performed adsorption experiments in MQ-water, while in this work seawater was used and it is well known that salinity influences on metal adsorption on MPs (Barus et al. 2021, Wang et al. 2023). For unaged or slightly aged plastics univariate analysis indicated that Co preferably adsorbed onto PS compared to PP. However, this preference was absent in more extensively aged microplastics (Figs. 6, 7).

Conversely, Cd showed no affinity toward either unaged or slightly aged MPs, but exhibited a marked preference for strongly aged PP over PS (Figs. 6 and 7).

Copper and lead exhibited significantly higher adsorption amounts on both types of MPs, with adsorption curves that were similar (Figs. 3, 4, 7). Main difference is that the presence of HA considerably changed the adsorbed Cu amounts (Fig. 5), whereas the Pb ones were relatively constant. Three-way factorial ANOVA for individual metals (Table S5.1) showed that HA presence had no effect on adsorption amounts of Pb, while the p-value for Cu was borderline. While statistical analysis did not show significant HA influence on Cu adsorption (i.e. it showed only borderline influence), Figs. 3, 7 and Table S3.1 show that Cu exhibits increased adsorption on unaged PP in the presence of HA, and its adsorption was decreased on UV-aged PP (both 4- and 52-day UV-aged) in comparison to the experiment carried out without HA. A similar trend was observed for PS, with the distinction that Cu adsorption was higher on 4-day UV-aged PS than on 52-day UV-aged PS (Figs. 4 and 7, Table S3.2). The fact that the statistical analysis did not detect HA as a significant factor affecting metal adsorption for Cu is probably due to the relatively low concentration of HA used in this work (1 mg/L). In other studies (Lu et al., 2022; Wang et al. 2023), significantly higher concentrations of HA were usually used, which had a stronger effect on Cu adsorption.

Speciation modeling (Table 1) indicated that approximately 78 % of copper in the HA-containing system existed as Cu–HA complexes. According to Volkov and Polyakov 2023), the charge of metal–HA complexes is not definitively characterized and varies depending on pH, ionic strength, and the structural properties of HA, particularly the type and abundance of functional groups. UV-induced photooxidation of MP surfaces leads to the formation of carbonyl (-C=O), hydroxyl (-OH), and carboxyl (-COOH) groups (Choi et al., 2021), with the latter two typically dissociated at seawater pH, imparting a negative surface charge to MPs (Godoy et al., 2020). Therefore, it is plausible that the Cu–HA complex in this study possessed a neutral or negative charge. The increased adsorption of Cu–HA complex on unaged PP suggests favourable electrostatic or hydrophobic interactions between the MP surface and the organic complex, resulting in a higher adsorbed fraction of complex compared to Cu inorganic species (free Cu^{2+} ions and $CuCO_3(aq)$), which predominated in the absence of HA (Table 1). In contrast, aged PP and PS surfaces, having a more pronounced negative charge due to dissociated hydroxyl and carboxyl groups (Wang et al., 2023; Godoy et al., 2020), exhibited reduced affinity for Cu–HA complexes, while maintaining relatively higher affinity for free Cu^{2+} ions. The reduced adsorption of Cu in the form of organic complexes (Cu–HA) on 52-day UV-aged PS compared to 4-day UV-aged PS, was likely due to the increased negative surface charge induced by prolonged UV exposure. Unlike Cu, speciation analysis showed that only 16 % of lead in seawater was in the form of Pb-HA complex (Table 1), while the dominant Pb

species were free ionic Pb^{2+} and its inorganic labile complexes. Pb adsorption profiles for unaged UV-aged MPs (both PP and PS) were similar with and without HA (Figs. 3, 5, 7). indicating that the presence of only 16 % of Pb in the form of organic complex was not significant, as confirmed univariate analysis (Table S5.1, Figs 5 (b) and 6).

Godoy et al. (2019) studied adsorption behaviour of trace metals onto several type of unaged microplastics, among them PP and PS, in MQ water and natural waters. In general, they found that uptakes of metals (including Cd, Co, Cu, Ni, and Pb) by MPs in MQ water increased with time until the achievement of equilibrium after approximately 120 h (5 days), while in this work equilibrium was achieved earlier (72 h for most metals). The same authors also reported that Pb had the highest adsorbed amounts on both PP and PS, following by Cu, and then other three metals (Cd, Co and Ni) that were present in one order of magnitude lower values, as in this work. Variations in experimental conditions, such as metal concentrations, MP particle size, pH, salinity, *etc*., can significantly influence adsorption outcomes (Wang et al., 2023). Despite these differences, a consistent trend has been observed across multiple studies: Pb typically exhibits the highest adsorbed amounts on MPs (PP and/or PS), followed by Cu, while Cd, Co, and Ni show comparatively lower adsorption levels (Ahechti et al., 2020; Gao et al., 2019; Mao et al., 2020). In this work, copper and lead exhibited the highest adsorption affinities on both PP and PS, and this preference became even more pronounced after prolonged UV aging through increased reactive surface and the formation of oxygen-containing functional groups. Pb adsorption is likely favoured mainly by electrostatic interaction, since among investigated metals Pb^{2+} has highest electronegativity and the smallest hydrated ionic radius (Chen et al., 2024; Nightingale, 1959; Zhou et al. 2020), properties that have repeatedly been linked with its strong adsorption on microplastics (Barus et al. 2021; Chen et al 2024; Zhou et al. 2020). Cu also showed high adsorption because, in addition to electrostatic attraction, it can form stronger surface complexes with oxygen-containing groups created during photooxidation (Zou et al., 2020), which explains its consistently high uptake on both unaged and aged MPs. However, as explained above, the electrostatic repulsions between Cu-humic acid complex and negatively charged aged MPs surface decrease copper adsorption amounts on aged MPs. By contrast to Pb and Cu, other three trace metals (Ni, Co and Cd) were less strongly retained because their electrostatic interactions with aged PP and PS were weaker due to smaller electronegativity of their ions and larger hydrated ionic radii (Nightingale, 1959). On that way, they were more susceptible to competition from seawater cations such as Na^{+}, Ca^{2+} and Mg^{2+}, which are known to suppress trace-metal adsorption on solid surfaces (Hong et al 2021).

Despite being ubiquitous in natural waters and may play an important role on metal adsorption on microplastics, organic matter was absent from the model adsorption experiments until

recently. Some studies (Godoy et al., 2019; Wang et al., 2023) highlighted the importance of OM influence on trace metal adsorption., Rapljenović et al. (2024b) showed that in the presence of 1 mg/L HA, Cu and Pb exhibited enhanced adsorption onto unaged PP pellets after 14 days of incubation. Also, they found that adsorption of Cd, Co, and Ni on unaged PP was minimally affected by HA concentration, which is consistent with the findings in this work. Up to our knowledge, combined impact of UV-aged MPs and humic acid on adsorption of Cd, Co and Ni have not been investigated so far. For Pb and Cu, Wang et al. (2023) reported that increasing concentrations (5 to 80 mg/L) of fulvic acid (about one order of magnitude higher concentrations than ones found in marine environment and in this work) led to elevated adsorption of these two metals on both unaged and UV-aged MPs. These results underscore the variable influence of organic matter on metal adsorption, which may be attributed not only to differences in the type of organic matter (e.g. HA vs. FA) but also to variations in experimental conditions such as pH, salinity, metal/organic matter concentrations and degree of the age of plastics. Overall, the effect of humic acid detected in this work was not uniform across metals because HA changed dissolved metal speciation to very different extents under our seawater conditions. Speciation modeling showed that at 1 mg/L HA, Cu was predominantly present as Cu–HA complexes (~78%), whereas only a portion of Pb (~15%) was HA bounded, while Cd, Co and Ni remained overwhelmingly as free ions or inorganic complexes. Thus, for Cd, Co and Ni, adsorption on MPs was controlled mainly by the affinity of their inorganic species for the polymer surface and by competition with major seawater cations, which explains the negligible HA effect. In contrast, Cu adsorption was sensitive to the presence of HA because HA substantially changed Cu speciation in solution, shifting Cu from mainly inorganic forms to predominantly Cu–HA complexes. The direction of that response depended on microplastic aging: on unaged MPs, Cu–HA complexes may adsorb through favourable electrostatic or hydrophobic association, whereas on UV-aged MPs the more oxidized and negatively charged surface becomes less favourable for adsorption of Cu-HA complexes. Generally, HA did not simply enhance or suppress adsorption, rather, its influence emerged only where metal speciation and surface chemistry jointly made HA-mediated binding competitive with direct adsorption of inorganic species. Abdurahman et al. (2020) found that HA itself can adsorb on MPs surface through hydrophobic π – π interactions), what might influence trace metals adsorption (especially Cu and Pb). However, since HA is negatively charged at pH value of the seawater, it is likely that it would adsorb less on negatively charged aged MPs.

5. Conclusion

This study provides a comprehensive investigation into the adsorption behaviour of Cd, Cu, Co, Ni, and Pb onto unaged and UV-aged microplastics (polypropylene and polystyrene) in

seawater, in the absence and presence of humic acid (HA). Surface characterization by AFM, FTIR and SEM showed that UV aging strongly modifies microplastic surfaces. AFM measurements showed a pronounced increase in surface roughness and area with UV exposure, with surface area increasing by up to ~47% for PP and roughness increasing nearly ninefold after 22 days of irradiation. These structural changes directly corresponded to higher adsorption capacities, confirming that surface topography plays a key role in controlling metal–microplastic interactions.

UV-aging was the dominant factor controlling metal adsorption. Compared with unaged plastics, 52-day UV-aged PP showed increases in adsorbed metal amounts ranging from about threefold (Ni) to nearly twentyfold (Pb), while adsorption on PS increased up to eightfold (Pb). Across all treatments, Cu and Pb consistently exhibited the highest adsorption capacities, followed by Ni, whereas Cd and Co showed the lowest affinity. Polymer type also influenced adsorption patterns: PS generally showed higher adsorption for Ni and Cu under moderate aging, while strongly aged PP showed a pronounced increase in Cd adsorption.

The influence of humic acid was metal-specific and depended on microplastic aging. HA had negligible effects on Cd, Co, Ni and Pb adsorption, consistent with speciation modelling indicating that these metals remained predominantly as inorganic species in seawater. In contrast, Cu adsorption was modified by HA: the presence of HA enhanced Cu adsorption on unaged MPs but reduced it on aged MPs. This behaviour reflects the dominance of Cu–HA complexes (~78% of total Cu), which interact differently with pristine versus oxidized microplastic surfaces.

Overall, the results showed that microplastic weathering substantially increases their capacity to bind trace metals in marine environments, while the influence of dissolved organic matter depends strongly on metal speciation. By integrating quantitative AFM surface characterization with adsorption experiments and speciation modeling, this study provides new insight into how aging of microplastics interacts with trace metals in seawater. These findings contribute to a better understanding of the role of microplastics as vectors for metal transport and help improve assessments of their environmental impact and ecological risk.

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CRediT authorship contribution statement

Petra Vukosav: Writing - original draft, Conceptualization, Formal analysis, Methodology, Investigation, Visualization. **Ana Rapljenović**: Writing - review & editing, Methodology, Investigation**. Sunčana Geček**: Writing - review & editing, Formal analysis, Software. **Ivan Vukosav**: Formal analysis, Software. **Vlado Cuculić**: Writing – review & editing, Formal analysis, Methodology, Conceptualization, Visualization, Funding acquisition, Project administration, Resources, Supervision.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used InstaText and ChatGPT (OpenAI) to support language editing and clarity (e.g., improving readability and refining phrasing). These tools were not used to generate or interpret data, or to draw scientific conclusions. After using these tools, the authors carefully reviewed and edited all AI-assisted output and take full responsibility for the content of the published article.

Figure captions:

Figure 1. (a-c) AFM images of unaged PP (a, “0 d UV”) and PP exposed to UV radiation for 4 days (b, “4 d UV”) and 22 days (c, “22 d UV”). Images were acquired using contact mode in air and presented as height with scan sizes 50 µm x 50 µm. Vertical profile along indicated line show PP heights. (d) SEM images of 52 days UV-aged PP microplastics with 300 x magnification. (e) FTIR spectra of PP before (blue) and after (orange) 52 days of UV irradiation.

Figure 2. (a-d) AFM images of unaged (a, “0 d UV”) and UV-irradiated (b-d) PS. Polystyrene was exposed to UV radiation for: 4 days (b, “4 d UV”), 22 days (c, “22 d UV”) and 52 days (d, “52 d UV”). Images were acquired using contact mode in air and presented as height with scan sizes 50 µm x 50 µm. Vertical profile along indicated line show polystyrene heights. (e) FTIR spectra of PS fragments before and after 52 days of UV exposure.

Figure 3. Adsorption of trace metals on PP (expressed as ng of metal per g of PP) in seawater in the absence and presence of humic acid (HA, 1 mg/L). Three types of PP pellets were used: unaged (not UV-irradiated, “0 d UV”), 4 days UV-aged (“4 d UV”) and 52 days UV-aged (“52 d UV”). Concentration of each trace metal in adsorption experiments was 10 µg/L. Standard deviation (s.d.) bars represent s.d. of trace metals analysis on ICP-MS.

Figure 4. Adsorption of trace metals on PS (expressed as ng of metal per g of PS) in seawater in the absence and presence of humic acid (HA, 1 mg/L). Three types of PS fragments were used: unaged (not UV-irradiated, “0 d UV”), 4 days UV-aged (“4 d UV”) and 52 days UV-aged (“52 d UV”). Concentration of each metal in adsorption experiments was 10 µg/L. Standard deviation bars represent s.d. of trace metals analysis on ICP-MS.

Figure 5. RDA ordination plots (RDA1–RDA2) of standardized metal adsorption in the late adsorption phase ($t \geq 24$ h) for the model Y ~ Type * UV + HA. In (a), samples are coloured by UV ageing and shaped by plastic type; in (b), samples are coloured by plastic type and shaped by HA level. Dashed ellipses indicate 68% confidence regions. Yellow arrows represent metal vectors, and labelled points show the centroids of the factor levels. RDA1 is mainly associated with UV ageing, whereas RDA2 separates plastic type. The full base RDA biplot is shown in Fig. S4.2.

Figure 6. (a) Heatmap of effect sizes (partial η^2) from three-way ANOVA for individual metals in the late adsorption phase ($t \geq 24$ h). Cell colours indicate the effect sizes of Type, UV ageing, HA, and the Type: UV interaction; symbols indicate significance levels (Table S5.1). (b) Interaction plots of model-based estimated marginal means (±95% CI) for adsorption as a function of UV ageing and plastic type, averaged over HA. Letters denote groups that are not

856 significantly different according to Holm-adjusted pairwise comparisons of the Type × UV
857 combinations (α = 0.05).

858 **Figure 7**. Box plot analysis of adsorbed trace metal amounts (ng/g) on different microplastics
859 (PP - red, and PS - black box), with and without presence of humic acid (HA) (1 mg/L). Unaged
860 plastics (0 d), UV-aged for 4 days (4 d) and for 52 days (52 d). The central line within each box
861 denotes the sample median. The box spans from the first quartile (Q1; 25th percentile) to the
862 third quartile (Q3; 75th percentile), so it represents 50 % of the observed values. The whiskers
863 extend from the box to the most extreme values that are not classified as outliers according to
864 the Tukey rule (1.5×IQR criterion, where IQR stands for interquartile range). Outliers were
865 displayed according to the Tukey rule (points beyond Q1 − 1.5×IQR or Q3 + 1.5×IQR).

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