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Spectroscopic evidence for adsorption of natural organic matter on microplastics

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Abstract:

The interaction of microplastics (MP) with dissolved organic matter, especially humic substances, is of great importance in understanding the behavior of microplastics in aquatic ecosystems. Surface modification by humic substances plays an essential role in transport and interaction of MP with abiotic and biotic components. Previous studies on the interaction between MP and humic substances were largely based on a model compound, humic acid (Sigma-Aldrich). In our work, we therefore investigated the interaction of natural organic matter (NOM) sampled from a German surface water with low-density polyethylene particles (LDPE). High-pressure size exclusion chromatography (HPSEC) and UV/vis absorption and fluorescence spectroscopy were used to characterize the incubation solutions after modifications due to the presence of LDPE, and Raman spectroscopy was used to characterize the incubated microplastics. While the studies of the solutions generally

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showed only very small effects, Raman spectroscopic studies allowed clear evidence of the binding of humic fractions to MP. The comparison of the incubation of NOM and a lignite fulvic acid which also was tested further showed that specific signatures of the humic substances used could be detected by Raman spectroscopy. This provides an elegant opportunity to conduct broader studies on this issue in the future.

Text

Natural organic material (NOM) and a lignite-derived fulvic acid (both less than 100 mg L⁻¹ organic carbon) were incubated with microplastic (LDPE, < 75 µm) for 3 days. Raman spectroscopic examination of the filtered and washed particles displayed an overlap of the Raman spectra of LDPE with the fluorescence emission of the humic substances used, each of which exhibited a specific spectral response. Thus, Raman spectroscopy offers a direct, simple method for the detection of humic substances adsorbed on microplastics.

Figure

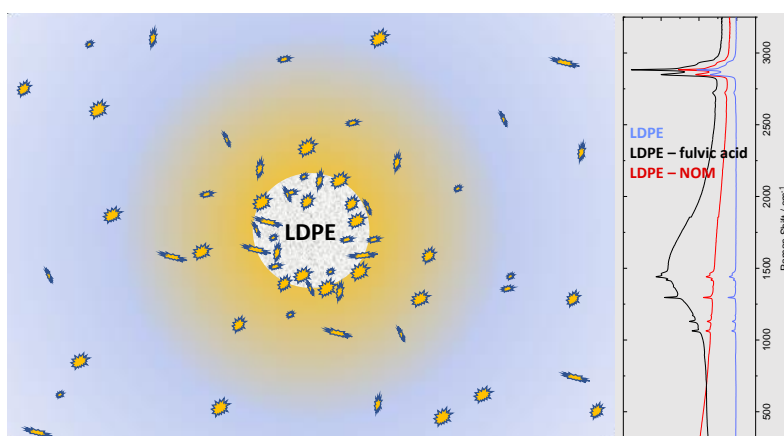
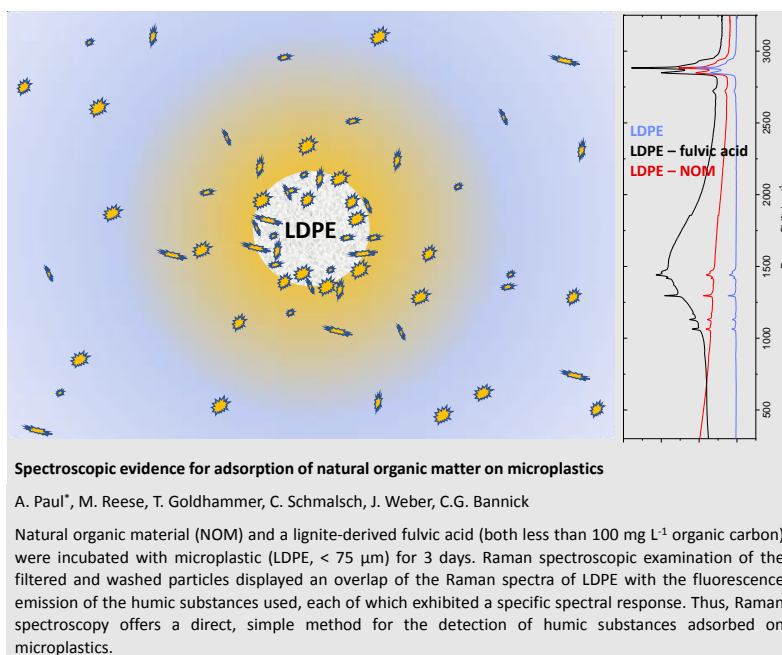


Figure with text



1. Introduction

Microplastics (MP) are plastic particles with a diameter of 1-1000 μm, whereby there is an upper limit of 5 mm for so-called “large” microplastics¹. Meanwhile a multitude of interactions of MP with both biotic and abiotic compounds of natural waters are known. Due to its small size, MP can be ingested by many organisms. According to Thompson et al.², more than 260 species are now known to become entangled in plastic waste or ingest plastics. The possible effects on animal populations are still unknown but the colonization of MP by microorganisms is well proven. Kettner et al.³ were able to determine a total of 426 eukaryote species from different trophic levels after 15 days of incubation of polyethylene (PE) on the Warnow in the Baltic Sea and a sewage treatment plant. In addition, the interaction of MP with abiotic compounds such as pollutants and pharmaceuticals has been well studied, for example the sorption of carbamazepine, 4-methylbenzylidene camphor, triclosan, and 17α-ethinyl estradiol⁴. However, despite dissolved xenobiotics, organic matter in form of dissolved organic matter (DOM) and particulate organic matter (POM), an entire class of substances is always present in all aquatic systems, which may not only adsorb onto

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MP but also modulate the interaction of MP with xenobiotics and the ingestion of MP by organisms. POM comprises materials with a diameter above 0.45 μm , and DOM, in contrast includes all compounds passing the 0.45 μm filter and with a wide spectrum from low-molecular weight compounds. DOM is often dominated by the so-called humic substances which are responsible for the typical yellow-brown color of the natural waters, which constitute 50-80% of the DOM. Humic substances are polyelectrolytic acids with molecular weights between 0.5 and 5 kDa. As a rule, three fractions are distinguished: Humic acids represent the humic substances-fraction, which is precipitated in aqueous solution at pH values <2 . Fulvic acids represent the acid-soluble fraction of humic substances. Humin contains all water-insoluble components⁵. Humic substances consist of diverse compounds and residues of different classes of organic substances, including amino acids, phenols, carbohydrates, quinonide groups, lipids, nucleic acids and degradation products of lignin. Autochthonous humic substances in aquatic systems are dominated by sugars, amines and amino acids, while allochthonous humic substances may contain more soil derived materials and degradation products of lignin⁶. Typically, the content of dissolved organic carbon (DOC) is a characteristic of humic substances. The concentration of DOC in inland waters varies between 0.5-50 mg L^{-1} . High-moor swales are the most DOC-rich sites, with concentrations up to 100 mg L^{-1} ⁷.

Recent studies show the importance of humic substances on the interaction of xenobiotics with MP in several processes. The encapsulation of polystyrene microspheres into calcite crystals occurs only after the treatment of the microspheres with humic acid⁸. Moreover, matrix properties such as pH, ionic strength, and DOM also influence sorption capacity of MP for hydrophobic organic contaminants⁹. The toxicity of nanoplastics to zooplankton is influenced by temperature, salinity, and

POM¹⁰, as tested with humic acid (Sigma-Aldrich). Dissolved organic matter further promotes the aging process of polystyrene MP under dark and ultraviolet light conditions with OH radical playing a leading role in the aging process of MP¹¹. Wu et al.⁴ investigated the possible effect of humic acid on the sorption of pollutants on polyethylene and found that the concentration of pollutants in the solution decreased as the concentration of the humic acid used increased. Moreover, no significant accumulation of humic acid on PE was observed thus indicating either humic matter acting as a sorbent for pollutants or as a coating of PE competing with the pollutant. Chen et al. 2018¹² studied the interaction of commercial humic acid (Sigma-Aldrich) with polystyrene particles by scanning electron microscopy. It turned out that the sorption of humic acid on polystyrene is dependent both on the particle size of the MP and the pH value. Furthermore, it could be shown that the sorption of the organic substance led to a change in the particle surface and consequently a modified behavior in aquatic systems must be expected. A more hydrophilic surface of MP could promote both better wetting and colonization by microorganisms. As a result, the density of the plastic would increase and thus promote transport into deeper water layers, as has been observed with PE film in seawater¹³. MP settle until they reach the often varying density of the surrounding seawater, so they remain adrift and can potentially be transported long distances by ocean currents¹⁴. Another aspect arises from the concept of “ecocorana” that form on MP by absorbed layers of large amounts of humic and fulvic acids, excreted waste products and expressed lipids and polysaccharides, proteins and macromolecules¹⁵. Once absorbed by the particles, the charge and flexibility of the humic substances tend to stabilize and disperse the particles in the water column, which could enhance their bioavailability to filter-feeding and suspending organisms.

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On search for an easy method to prove the interaction of humic substances and MP, incubation experiments with MP and humic matter at DOC concentrations below 100 mg L⁻¹ were performed and a number of spectroscopic methods and high-pressure size chromatography (HPSEC) was tested. We both examined the solutions to check for materials “taken out” and the recovered MP after incubation for materials “added-on” by the following analytical procedures: HPSEC, UV/vis absorption, fluorescence, and Raman spectroscopy. For the incubation experiments we used MP particles < 75 µm of a low-density polyethylene (LDPE), which is one of the most abundant polymers contributing to MP formation. In 2019, about 29.8% (15.1 million tons) of plastics produced in the EU were forms of polyethylene (PE) which makes PE the most widely produced plastic, followed by polypropylene¹⁶. Applications for LDPE and Linear Low-Density Polyethylene, which make up about 58.4 % of PE, are primarily as reusable bags, food packaging, and films in agriculture¹¹.

LDPE particles were incubated with natural organic matter (NOM) extracted by reverse osmosis from a humic rich surface water, Lake Großer Schwarzer See, located in Brandenburg (Germany) and with a lignite derived fulvic acid. While DOM comprises all types of dissolved organic matter, including chemically treated or synthetic material, the term NOM refers to those compounds that are representative of the natural waters from which they are taken. In contrast to NOM, isolated humic and fulvic acids have the advantage of comprising relatively homogeneous, well-characterized materials that are useful for obtaining reproducible and comparable results. On the other hand, there is a suspicion that these isolated materials are merely artificially formed because of their isolation process and therefore have limited representativeness for the properties of the natural DOC. Moreover, as could be

shown by Lie et al., different humic fractions may behave in a different way regarding their sorption to MP¹⁷.

The following questions should be clarified by our study (i) which methods are suitable to prove adsorption of NOM onto MP, (ii) is it possible to further characterize and quantify this fraction by the chosen methods?

2. Material & Methods

2.1. Materials

MP. The LDPE < 75 µm used was kindly provided by Plastics Europe. According to Peacock 2000¹⁸ the density of LDPE is between 0.90 and 0.94 g/cm³. A laser scattered light spectrometer has been used to determine the particle size distribution of the untreated LDPE (LA 960, Horiba, Retsch, Haan, Germany). The LDPE tested (n=7) has a particle size distribution of 10 µm - 68 µm, with a median of 20 µm and mean of 22 µm. Thereby 99 % of all particles fall between 10 µm and 45 µm, and 95 % of the particles are smaller than 34 µm.

To achieve a homogeneous distribution of the particles for the incubation experiments, the LDPE storage vessel was first shaken for 20 minutes in an overhead shaker at approx. 40 rpm. This allowed a homogeneous subsample to be taken and the particle surface on which the organics adsorbed to be kept as constant as possible.

Humic substances. NOM was extracted in 2003 in the shore area of the Lake Großer Schwarzer See in Brandenburg (53°09'29.2 "N 12°46'29.4 "E) by reverse osmosis according to Serkiz and Perdue¹⁹ and subsequently freeze-dried. During the extraction of the NOM, the lake water has already been filtered through a 0.45 µm filter at the

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beginning and can thus be assigned to DOC. The oligotrophic water body of the Lake Großer Schwarzer See has a brown color resulting from the fact that water with a high humic content is regularly fed into the lake from a rewetted peat bog via an overflow ditch²⁰. The NOM obtained has been comprehensively characterized in the past with similar properties as reported for Fennoscandian humic substances^{21, 22}. In addition to NOM from the Großer Schwarzer See, hereafter referred to as “SZS”, a terrestrial lignite fulvic acid (LFA, batch 060417) from a lignite formation kindly provided by WH Pharmawerk Weinböhla GmbH was used for the incubation experiments.

2.2. Incubation experiment

Stock solutions of containing 87.4 mg L⁻¹ DOM were prepared 24 hours in advance and used after filtering (0.45 µm). Table 1 illustrates the set-up of the experiment including both the composition of the individual samples and the analytical procedures used here.

Table 1: General set-up of incubation experiments and applied analytical methods (HPSEC – high pressure size exclusion chromatography, EEM – fluorescence excitation and emission matrix, SZS – NOM from Lake Großer Schwarzer See, LFA- Lignite fulvic acid)

Experiments SZS-1 & SZS-2	LDPE	SZS A	MilliQ	LDPE MilliQ E1	SZS E2	LDPE SZS E3	LDPE SZS E4	LDPE SZS E5	LDPE SZS E6
Experiment LFA	LDPE	LFA A	MilliQ	LDPE MilliQ E1	LFA E2	LDPE LFA E3	LDPE LFA E4	LDPE LFA E5	LDPE LFA E6
Materials	Raw Materials / Stock solutions			Samples after 72 h of incubation					
pH		x	x	x	x	x	x	x	x
HPSEC		x	x	x	x	x	x	x	x
UV/Vis absorption		x	x	x	x	x	x	x	x
Fluorescence (EEM)		x	x	x	x	x	x	x	x
Raman (solids)	x			x		x			

Altogether six samples were prepared gravimetrically and incubated parallel in glass bottles. The amount in all MP containing samples was adjusted to 100 mg LDPE and added 200 mL of either MilliQ® water or DOM solution SZS or LFA. Whereas LDPE in MilliQ® water (E1) and pure humic matter (E2) were each studied as a single sample, four parallel samples containing LDPE in humic matter (E3 – E6) were prepared as replicates. Two independent incubation experiments with SZS were designed as a true analytical duplicate, whereas the third incubation, performed with LFA, primarily served to study the effect of a strongly differing humic substance on the individual peak positions of the chromatographic and spectroscopic methods. All incubations were performed at room temperature.

During incubation, all samples were shaken overhead for 72 h in 250 ml glass bottles at approximately 15 rpm. To avoid contact of the solution with the bottle cap, the bottles were sealed with a piece of aluminum foil before closing. In addition, the bottles were wrapped with aluminum foil to minimize the direct incidence of sunlight. After incubation, samples containing LDPE were filtrated using an apparatus consisting of a vacuum pump, a hose, a Witt's pot, a glass bottle, a stainless-steel

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attachment, a sinter plate, a 5 μm stainless steel filter, and a silicone seal. To avoid adhering experimental solution, the pellet was washed once with MilliQ® water and was then transferred to a snap cap jar and dried overnight at 40 °C covered with aluminum foil.

UV/vis and fluorescence spectroscopic analysis of stock solutions filtrated prior to incubation and of incubated samples was performed immediately after filtration. The remaining solutions were stored in the dark at 4°C and used for HPSEC after dilution within one week.

2.3. Methods

High-pressure size exclusion chromatography (HPSEC)

Humic matter was fractionated by a custom-designed automated size exclusion chromatography²³. Two chromatograms are displayed for each chromatographic separation (Toyopearl HW 50 S resin column, 250mm \times 20 mm, elution with 29 mmol L⁻¹ phosphate buffer at pH 6.5 and a flow rate of 1 mL min⁻¹). A first chromatogram is obtained after chromatographic separation by means of detection at 256 nm, referred to as UV detection mode. Thus, only the components of the sample that absorb in the UV are detected here. To characterize the total organic carbon content of a sample, combustion is subsequently carried out in a UV-irradiated thin-film reactor and the resulting CO₂ is detected by means of infrared spectroscopy, thus generating a second chromatogram, referred to as the OC detection mode.

UV/vis absorption spectroscopy

Absorption spectra were recorded in the spectral range 200-780 nm on a v-650 spectrometer (Jasco) with a spectral bandwidth of 0.5 nm, and a spectral resolution of

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2 nm. All samples were measured in 1 cm quartz cells using MilliQ® water as a reference. Humic water was diluted by a factor of ten gravimetrically using fresh MilliQ® water. The resulting optical density at $\lambda_{\text{exc}} = 300$ nm for the fluorescence studies was determined about 0.13 and 0.11 for SZS and LFA. Absorption spectra are summarized in the ESI in Fig. S3.

Fluorescence emission and excitation matrices (EEMs)

All humic matter containing samples were diluted by a factor of 10 gravimetrically and were measured in a quartz cuvette (d=1.0 cm) in a 90° symmetry using a Fluoromax-4 spectrofluorometer (Horiba Jobin-Yvon) equipped with a reference channel. The calibration of the instrument included both the wavelength dependence of the spectral responsivity of the detection system (emission correction) and the wavelength dependence of the photon flux (excitation correction). All fluorescence spectra presented are technical fluorescence spectra, i.e., the spectra were corrected for instrument specific contributions (emission and excitation correction) as well as dark counts from the detector (blank correction). Fluorescence emission and excitation matrices (EEMs) were registered for the excitation range $\lambda_{\text{exc}} = 265 - 550$ nm, and for an emission range of $\lambda_{\text{em}} = 280 - 600$ nm, with an increment of 5 nm each yielding 3770 data points. The integration time per datapoint was adjusted to 0.5 s as a compromise between a reasonable measurement time and spectral resolution of the EEMs. Contour plots of EEMs are summarized in the ESI in Fig. S4.

Multivariate curve resolution (MCR)

MCR of EEMs was performed by using The Unscrambler® X Vers. 10.5 (CAMO Software) as described in Haferkamp et al.²⁴. MCR iterations were initialized with the constraints of “non-negative spectra” and “non-negative concentrations”, and

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sensitivity to pure compounds was set to 100. The maximum number of iterations was set at 1000.

Raman spectroscopy

Raman spectra of dried LDPE powders were measured with a Raman spectrometer RXN1 (Kaiser Optical Instruments) using a fiber optical probe (MR/NC optic). The MR/NC probe was operated with 785 nm at 17 mW at a distance of 1 cm. Raman scattering was detected in the spectral range 150 - 3250 cm^{-1} . Intensity and dark corrected Raman spectra using a cosmic ray filter were obtained by integration over six accumulations of five seconds exposure. Under these conditions, even repeated measurement did not melt the polymer or reduced the observed fluorescence emission.

3. Results & discussion

3.1. Incubation

After incubation, LDPE particles are colloiddally distributed in the humic matter solution, in contrast to the blank sample where the LDPE particles still floated on top of the solution (cf. Fig. S1a). The polymers of the blank sample also behaved differently during the washing process after filtration than the samples incubated with humic matter. In the latter, the water used for rinsing uniformly wetted the entire sample, while individual water droplets formed on the LDPE of the blank sample (Fig. S1b). In addition, the LDPE separated from samples SZS-E3-E6 and LFA-E3-E6 incubated with SZS and LFA, respectively, exhibited a yellowish color compared to the fresh white of the LDPE incubated with MilliQ® (see samples MilliQ-E1.) The sample designations here follow the incubation scheme as shown in Tab. 1.

There were moderate changes in the pH values of solutions after incubation in general but only minor differences between those samples containing LDPE (E3-E6) and MP-free humic samples (E2) studied here (cf. Tab. S1). While for SZS-A a decrease from pH = 9.16 to pH = 8.61 and 8.63 for SZS-E2 and SZS-E3-E6 was observed, the initial pH value of incubation experiments with LFA brought about a slight increase in the initial pH from 6.14 to values of 6.62 and 6.78 for LFA-E2 and LFA-E3-E6. Incubation of LDPE in MilliQ® water (E1) did not affect the pH values which remained close to pH = 7 for all DOC-free samples.

3.2. HPSEC

Typical chromatograms of both SZS and LFA are summarized in Fig. 1. In the organic carbon mode (OC, Fig. 1a, b), four chromatographic fractions can be separated with increasing retention time: high-molecular weight (HMW), humic matter (HM), low-molecular weight acids (LMWA), and low-molecular weight neutral and amphiphilic compounds (LMWN). HMW matter includes mainly hydrophilic biopolymers, such as polysaccharides and proteins, and metal-organic colloids according to Huber et al.¹⁶. The dominant fraction in SZS is the HM fraction, including humic acids and fulvic acids with a typical high absorbance in the UV range (Fig. 1 c, d). At longer retention times, the LMWA, which represent the sum of fractions of all mono- and diprotic low-molecular weight organic acids, can be seen as shoulder in SZS, whereas in LFA, a pronounced peak is observed. Finally, the minor peaks at retention times above 60 min represent the LMWN, e.g., sugars, alcohols, aldehydes, ketones, and amino acids.

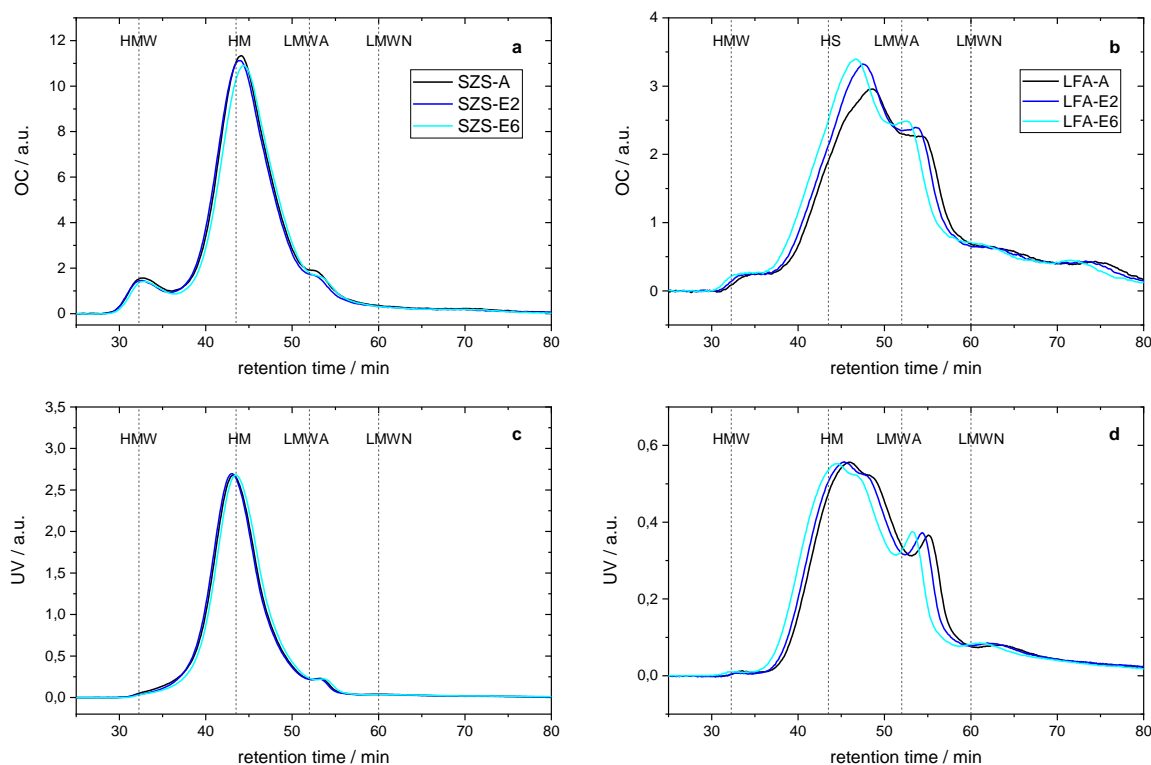


Figure 1: Upper panel - chromatograms in organic carbon mode (OC) (a - SZS, b - LFA) and lower panel - chromatograms of SZS and LFA in UV detection mode (UV) (c - SZS, d - LFA). Vertical dotted lines indicate the fractions high molecular weight (HMW), humic matter (HM), low molecular weight acids (LMWA) and low molecular weight neutrals (LMWN) with increasing retention time. Sample names according to incubation scheme in Tab. 1.

All in all, the chromatograms of the SZS samples show typical features of humic surface waters and NOM dominated by the HM fraction. There is no indication of an additional peak in samples incubated with LDPE suggesting that if LDPE particles have passed the filter into the solution, this fraction may be neglected here. Only minor differences can be seen between the incubation solution (SZS-A, LFA-A) as well as the pure incubated SZS and LFA (SZS-E2, LFA-E2) and the samples incubated with LDPE (SZS-E6, LFA-E6). However, the observed differences are

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systematic for two independent incubation experiments using SZS (SZS1 and SZS2, cf. Fig. S2a and Fig. S2b): Incubation with LDPE leads to a slight shift of the HM fraction to higher retention times and thus lower molecular weight. Furthermore, the solution incubated with LDPE shows a slightly lower concentration in the HM fraction region especially in organic carbon detection mode. Since this effect was also observed in the two incubations with SZS, it can be assumed that a systematic effect is involved. However, the observed trends cannot be quantitatively confirmed, as can be seen from the uncertainties determined based on the samples E3-E6 prepared in parallel in each incubation experiment, shown graphically in Fig. 2a - d.

In contrast to SZS, the chromatograms of LFA show broader chromatographic fractions with the HM fraction shifted to clearly higher retention times, indicating the presence of much lower molecular weight material. Likewise, the LMWA fraction is very distinct here. All in all, this is also to be expected based on the material type fulvic acid. What is unexpected, however, are the changes in the chromatograms of the incubated samples (LFA-E2, LFA-E6), which differed significantly from the initial solution (LFA-A). Both the pure LFA solution (LFA-E2) and the solution incubated with LDPE (LFA-E6) show a clear shift in the overall chromatogram towards shorter retention times compared to the stock solution (LFA-A), indicating an increase in molecular weight. The effect is most pronounced for the sample incubated with LDPE, LFA-E6. One assumption here would be that due to the process of incubation, the mechanical stress, which was of course even higher in the LDPE-containing sample, led to post-dissolution of previously undissolved sample components.

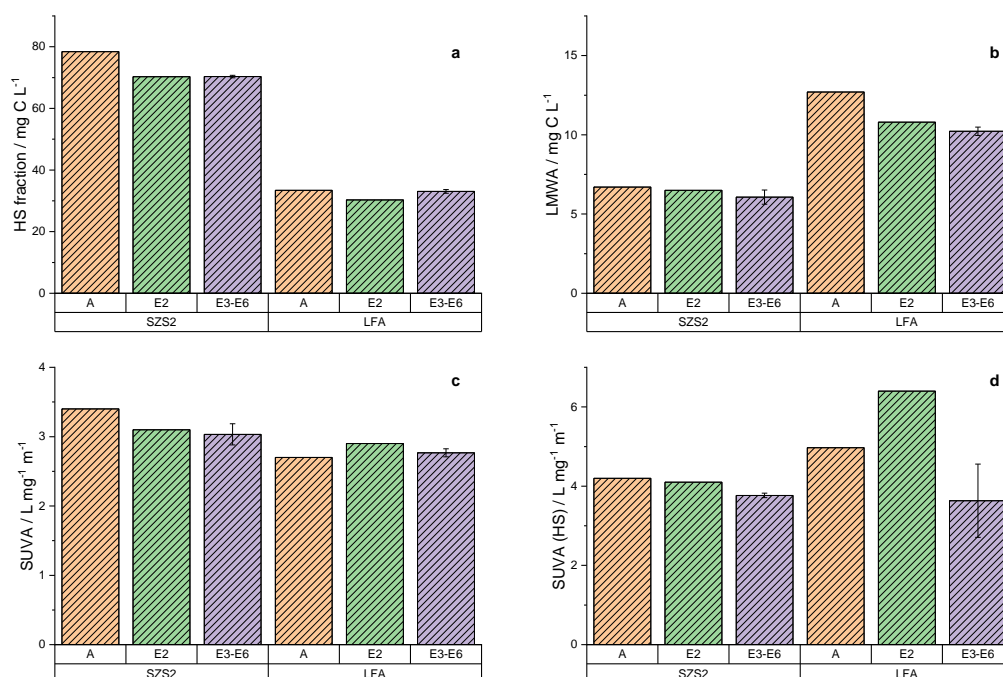


Figure 2: Comparison of HPSEC derived fractions and specific UV absorption (SUVA) for the incubation experiments SZS2 and LFA. A summary of these parameters is provided in Tab. S2.

Our main interest in the HPSEC investigation of the NOM solutions was to see if this method could be used to draw conclusions about potential adsorption of certain fractions of organic carbon onto LDPE particles based on the changes in the chromatograms. Besides adsorption on surfaces, there are additional possibilities of DOC loss in solution: the first is photochemical destruction and the second is microbial degradation, as well as the combination of both^{25, 26}. As can be seen from figure 2a – b, no strong effects were observed with the HM or LMWA fractions nor with a parameter displayed in Fig. 2c - d, the specific UV absorbance at 254 nm relative to DOC (SUVA) or to HM (SUVA(HS)).

In experiment SZS-2, the proportion of the HM fraction in stock solution SZS-A seems to be higher than in incubated solutions SZS-E2 and SZS-E3-E6 giving almost identical results (Fig. 2a). Incubation of LFA showed no change in the HM fraction for LFA-E3-E6 and only a small loss for LFA-E2. The HM fraction of SZS is about twice that of LFA, which seems plausible because the latter is a fulvic acid. As indicated by the small standard deviation, this parameter is determined very accurately and does not show large variations between samples.

The reduction of the LMWA fraction is marginal and the apparent decrease in LFA-E3-E6, as can be seen from the chromatograms in Fig. 1, is due only to a shift of fractions to higher molecular weights and not to a decrease in the LMWA fraction. Since microbial degradation preferentially reduces low molecular weight substances and no such specific degradation was detected and the samples were packed light-tight, these mechanisms fail as an explanation of possible changes in the chromatograms.

A particularly interesting parameter derived from HPSEC is SUVA. High SUVA values are indicative of aromatic compounds or conjugated double bonds. However, a ratio is also subject to a larger uncertainty, as can be seen in Fig. 2c. All observed differences are within the range of variation given by the standard deviation of triplicate samples. Only if we relate the calculation of SUVA to the organic carbon content of the HM fraction instead of total DOC (Fig. 2d), a clear effect can be deduced: for both SZS-E3-E6 and LFA-E3-E6 there is a reduction in SUVA(HS) compared to the incubated control samples SZS-E2 and LFA-E2. So, this indicates that especially highly aromatic material of NOM solutions has been adsorbed to LDPE: In the case of LFA-E2, there is also a sharp increase in SUVA of the humic

substances, which again would suggest the possibility that higher molecular weight material was post-dissolved here. This material is obviously missing in the sample LFA-E3-E6. At the same time the HPSEC analysis makes it clear that only a very small amount of the humic matters could have been adsorbed onto the LDPE. Our observation is in good agreement with the results of Li et al. that the binding affinity of DOM to MP of polystyrene could be mainly ascribed to aromatic, hydrophobic interactions¹⁵.

3.3. Absorption & Fluorescence

UV/vis absorption spectra of humic matter solutions as used for incubation experiments and obtained after incubation are provided in Fig. S3. Absorption spectra of each cuvette prepared for fluorescence measurement were recorded before and after the EEM took place. Practically no absorption changes were observed after the EEM measurement and hence no sample modification by the irradiation during the fluorescence measurement is assumed.

Typical EEMs derived from filtrated humic matter SZS1, SZS2 and LFA after 3 days of incubation with LDPE are depicted in Fig. 3. The complete collection of EEMs as used for analysis are provided in Fig. S4.

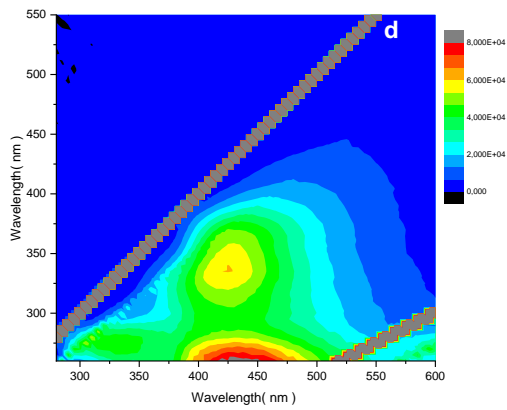
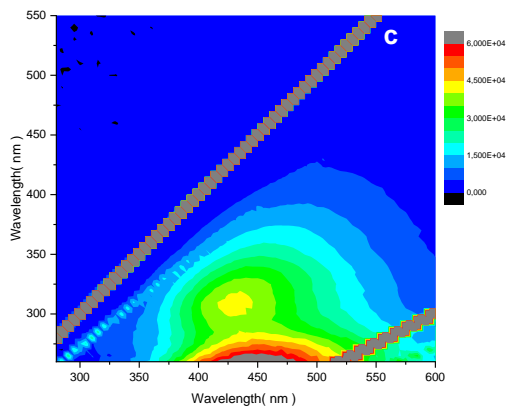
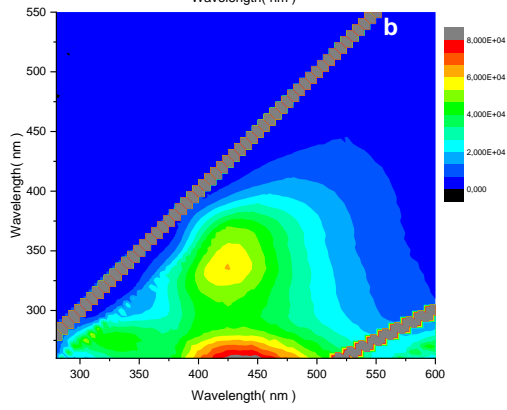
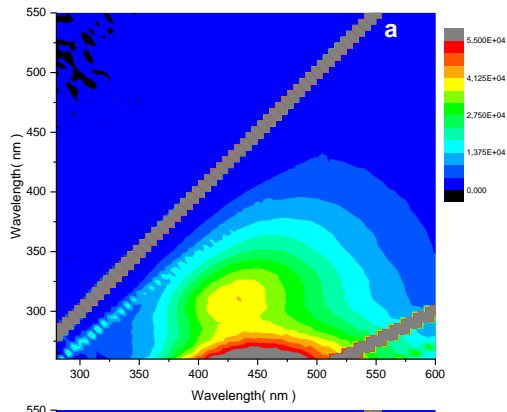


Figure 3: Fluorescence excitation emission (EEM) contour plots of humic matter after three days of incubation with LDPE: a – SZS-E3, b – LFA-E3 and without LDPE: c – SZS-E2, d – LFA-E2.

EEMs are in general characterized by two unwanted side effects occurring together with the fluorescence emission. Due to the continuous scan of both a defined excitation and emission array, a strong and narrow peak of the scattered light (Rayleigh scattering) moving diagonally in the EEM can be observed, which is also found in the second order at the double wavelength, i.e., for example at 300 nm excitation a second Rayleigh peak occurs at 600 nm. In addition, especially in the short wavelength excitation region the Raman scattering of water can be seen with a shift of about 3400 cm^{-1} to the excitation. As the intensity of the Raman scattering is strongly dependent on the frequency of excitation, it is mostly observed in the UV part of the EEM, and in contrast to the Rayleigh scattering, which is separated from most fluorophores, it is sometimes found in the same spectral region as the fluorescence emission of natural organic matter.

Both manual inspection of EEMs (cf. Fig. 3 and Fig. S4) and the spectral position and intensity of fluorescence yielded quite uniform results when LDPE incubated samples are compared with dissolved humic matter without MP after incubation (cf. Tab. S3). There were no discernible changes in either the location of the peaks or the fluorescence intensities of the peaks following incubation with LDPE. Likewise, no evidence of altered Rayleigh scattering intensities was observed, indicating complete removal of the LDPE particles during the preceding filtration.

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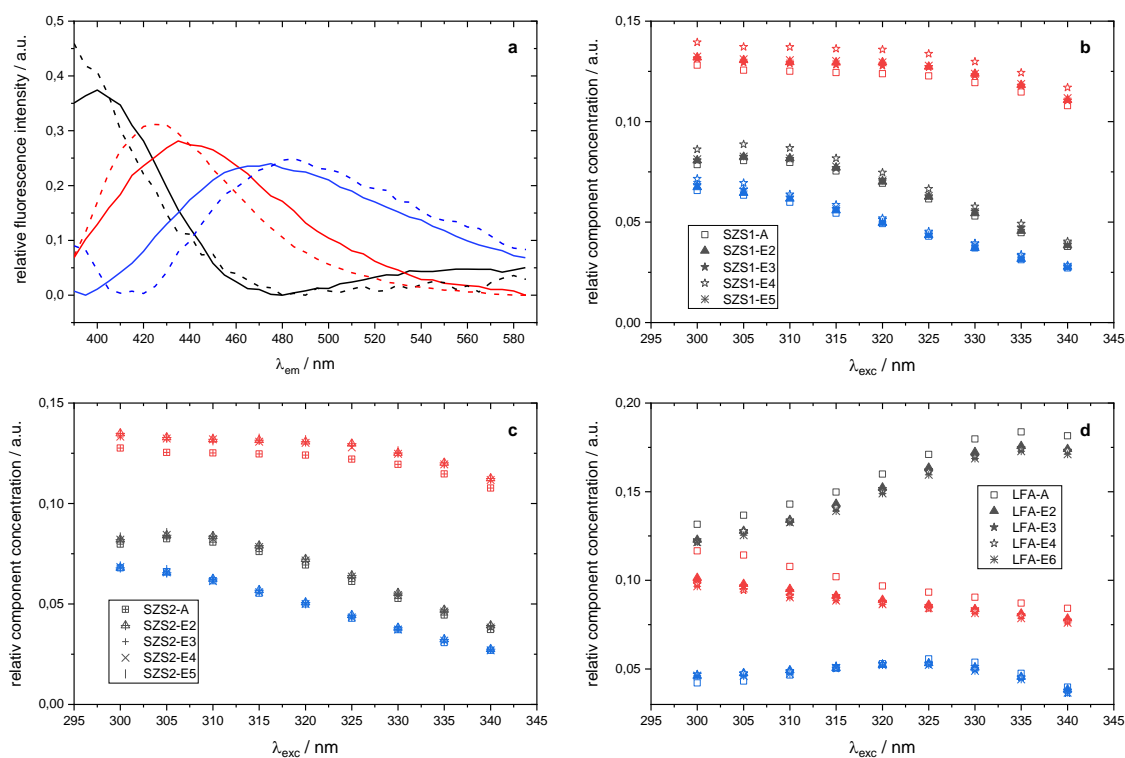


Figure 4: Results of Multivariate curve resolution (MCR) of EEMs: a) Component spectra: C1 – black, C2 – red and C3 – blue. SZS – full lines, LFA – dashed lines. b) excitation profiles of samples SZS1, c) excitation profiles of samples SZS2 and d) excitation profiles of samples LFA.

To test for minor spectral changes in the EEMs beyond manual inspection, the range of $\lambda_{\text{exc}} = 300 - 340$ nm and $\lambda_{\text{em}} = 395 - 585$ nm was examined by MCR (cf. Fig. S5). Like parallel factor analysis (PARAFAC)²⁷, this multivariate approach aims to extract information on the pure compounds that occur superimposed in a mixture²⁸. MCR analysis performed without an “initial guess” resulted in models with three spectral components (C1, C2, and C3) for the two humic samples SZS and LFA, each analyzed separately. The maxima of the determined components corresponding to fluorescence emission maxima are C1 = 305/400 nm, C2 = 325/435 nm and C3 = 300/475 nm for SZS (Fig. 4a). The corresponding fluorescence excitation profiles of

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the samples from experiments SZS1 and SZS2 in figures 4b and 4c support the previous assumption that no spectral shifts in fluorescence emission occur due to incubation with LDPE.

Similarly, for LFA, the components C1 = 335/395 nm, C2 = 300/425 nm, and C3 = 325/485 nm were determined (Fig. 4a). In contrast to the incubations with SZS, differences between the stock solution LFA-A and the incubated samples were observed here, especially for C2. A similar effect was already observed with HPSEC. However, as with SZS, no evidence of spectral change as a result of incubation with LDPE could be noted.

Incubation of humic acid (Sigma-Aldrich)¹² with polystyrene MP revealed two fluorescent compounds (C1: 320/510 nm, C2: 430/520 nm) by PARAFAC analysis. Whereas MP exhibited no effect on EEM compounds, nanoplastics, i.e., particles below 1 μm diameter, brought about a strong increase of both fluorescent compounds. The authors argue that MP polymers initially contact with the aromatic structure of DOM via π - π conjugation, and then are entrapped in the DOM by the carboxyl groups and C=O bonds, constituting a highly conjugated co-polymer with an increased electron density. This induces the fluorescence intensity increase in DOM. In general, MP characteristics such as crystallinity, weathering, and surface morphology affect sorption capacity¹³. While interaction with aromatic structures seems realistic for PS, the presence of extended π -systems is not expected for “fresh” LDPE. LDPE is a good electrical insulator, but charges easily electrostatically. Next to hydrophobic interaction, it is hence possible that an interaction with likewise charged DOC molecules can occur in this way. Moreover, next to electrostatic interaction, or π - π interactions, hydrophobic interaction, hydrogen bonding, and Van

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der Waals forces may be possible candidates for the explanation of LDPE – humic matter interaction¹³.

3.4. Raman spectroscopy

As it was not possible to find a clear proof of the removal of humic matter from incubation of LDPE with DOC, we screened the LDPE for adherent material because the changed floating behavior indicates surface modification. Therefore, Raman spectra on dry powder samples were measured including the raw LDPE as used for incubation, and dried LDPE after incubation with humic matter (SZS-E3, LFA-E3) as well as LDPE with MilliQ® (MilliQ-E1).

Fig. 5a provides an overview on Raman spectra in the spectral range 250 – 3250 cm⁻¹. All spectra show a series of narrow Raman bands typical for LDPE as provided in Tab. S4. In contrast to high-density PE, LDPE is characterized by spectral features such as an unresolved band near 1454 cm⁻¹ due to anisotropic parts, broad features near 1308 cm⁻¹ and 1084 cm⁻¹ attributed to amorphous parts, and a broad band at 897 cm⁻¹ which is characteristic of short branches within polymers²⁹ (Fig. 5b). LDPE incubated with MilliQ® water exhibits exactly the Raman spectrum as the raw material (Fig. 4a). However, when LDPE is incubated with dissolved humic matter broad Raman features occur, which we assume are due to fluorescence of organic matter adsorbed to the surface of the particles. In case of LFA, this fluorescence is observed in the form of a broad band with a maximum at about 1500 cm⁻¹. In case of SZS, however, an exponentially decreasing background occurs (Fig. 5a). This difference could be explained by the fact that the fulvic acid is the product of a chemical selection isolating only a narrow fraction of molecules whereas SZS presents a collection of many slightly different molecules over a much wider range of

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properties. Two independent incubations with SZS (SZS1, SZS2) yielded in principle the same spectral shape, but about twice as much fluorescence background for SZS1 compared to SZS2, indicating a high uncertainty when it comes to quantify the adsorbed amount of NOM. No possible explanation is deduced neither in the HPSEC nor in the fluorescence data reported in the previous sections indicating that Raman spectroscopy is the most sensitive of all methods used here.

We can further see that the incubation of LDPE particles with humic substances has no effect on the peak positions of typical Raman bands of the LDPE. In Fig. 5c, 2849 and 2934 cm^{-1} denote the maxima of the symmetric and unsymmetric vibration of CH_2 groups which are abundant in PE indicating only a very thin film covering the surface of the particles. Incubation of LDPE in MilliQ® only yielded comparable spectra as the blank powder used for incubation thus indicating that indeed the humic matter causes the observed background of the Raman spectra. It should be noted that typically, superposition of fluorescence is an unwanted interfering site effect in Raman spectroscopy of most organic compounds, and efforts are being undertaken to eliminate them³⁰. Due to the much higher quantum yield of organic substance fluorescence ($\Phi_F = 0.3 - 1.5\%$ at UV excitation)³¹, compared to the Raman effect, which is several orders of magnitude weaker, we have the lucky case to detect here at the same time the strong vibrations of LDPE and the weak fluorescence spectrum of small traces of humic substance together in one Raman spectrum. This is certainly due to the fact that at the excitation wavelength of 785 nm, the fluorescence emission has a very weak intensity due to the very low absorption coefficients of HS in this spectral range.

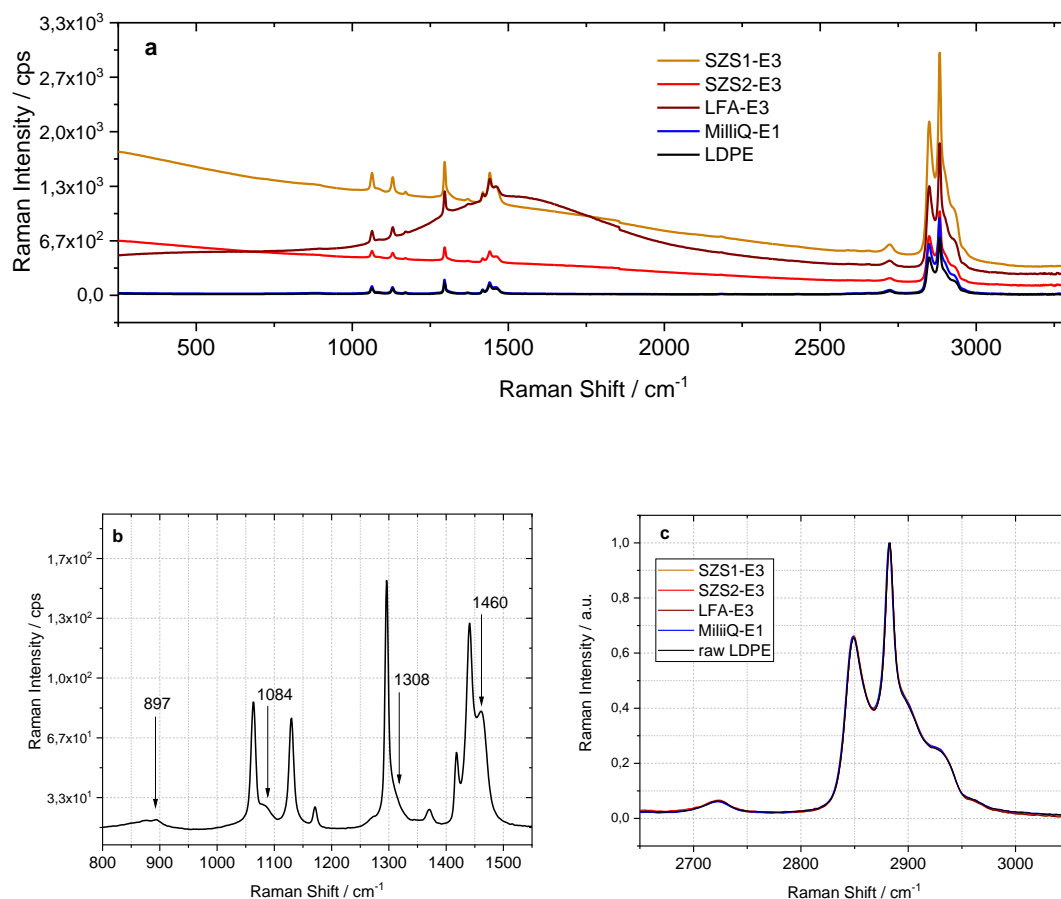


Figure 5: Raman spectra of LDPE incubated with NOM (SZS1, SZS2), Fulvic acid (LFA), MilliQ® and the raw material: a - full spectra, b - fingerprint region of raw LDPE with assignment of specific spectral features, c - region of C-H stretching vibrations after linear baseline correction and subsequent normalization to the Raman vibration at 2934 cm⁻¹.

4. Conclusions

After only three days of incubation of LDPE particles in a solution rich in humic substance (87 mg DOC L⁻¹), the appearance and hydrophobicity of the LDPE changes. Analysis by means of HPSEC indirectly showed that parts of the aromatic humic substances fraction apparently interacted with the LDPE used. Nevertheless, it is difficult to make quantitative statements about the adsorbed material and the fraction

of adsorbed material can only be determined indirectly. UV/vis absorption and fluorescence spectroscopic methods were not sensitive enough to make this effect clearly visible.

In contrast, Raman spectroscopic examination with NIR excitation at 785 nm of the incubated particles gave a clear indication of the presence of humic substances via the fluorescence detected in parallel with Raman scattering. In this context, it can be additionally emphasized that different humic substances can be clearly distinguished via the spectral shape of the detected fluorescence. Furthermore, intensity differences of a duplicate experiment gave indications for a possible quantitative approach. It is assumed that the successive independent experiments SZS1 and SZS2 coincidentally contained different proportions of the adsorbed humic substances fraction, which led to the observed differences.

In the future, a targeted variation of both the DOC concentration and the humic substances used must be undertaken to validate the observed effect. However, our study shows that Raman spectroscopy provides a direct, simple method for the detection of MP adsorbed humic substances. Raman spectroscopy does not require the laborious dilutions and chromatographic studies or time-consuming measurements and analysis of EEMs. It should also be emphasized that extremely high DOC concentrations were not used here, but only 3-4 times the concentrations of typical humic-rich waters. Furthermore, the chosen NOM SZS represents an environmentally relevant sample that is typical for surface waters rich in humic substances.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ESI provided as a separate document

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