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Narrowing the analytical gap for water-soluble polymers: A novel trace-analytical method and first quantitative occurrence data for polyethylene oxide in surface and wastewater

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Abstract

Water-soluble polymers (WSPs) like polyethylene oxide (PEO) have annual production volumes ranging from thousands to millions of tonnes and are used in a wide variety of applications that enable a release into the aquatic environment. Despite these facts, a lack of quantitative trace-analytical methods for WSPs prevent the comprehensive study of their environmental occurrence. Here, size exclusion chromatography was hyphenated with electrospray ionization high-resolution mass spectrometry. An all-ion fragmentation approach for the formation of diagnostic fragments independent of molecular weight, charge state, and ion species was used to quantify PEO and its derivatives in wastewater treatment plants (WWTPs) and surface water samples. Despite its inherent biodegradability, PEO concentrations found in the samples analysed ranged from <LOD-11 µg/L for surface waters (11/18 samples > 1 µg/L) and reached up to 20 µg/L (effluent) and 400 µg/L (influent) for WWTPs. A substantial shift in molecular weight ranges was observed between influent and effluent, pointing towards a molecular weight fraction between 1.3 and 4 kDa being dominant in the effluent. Due to an assumed size exclusion during sample enrichment, information on the MW-distribution of PEO is limited to MW < 55 kDa. The high concentrations widely detected for a readily biodegradable WSP such as PEO, raise strong concerns about the occurrence and fate of recalcitrant WSPs in the aquatic environment. The method presented herein may provide the tools necessary to assess the burden of these high production volume chemicals and the risk they may pose.

TOC art

Keywords

synthetic polymers, liquid polymers, liquid plastics, organic trace contaminants, micro-/nanoplastics, polyethyleneglycol (PEG)

1. Introduction

Environmental pollution by synthetic polymers has been discussed for over a decade at a scientific and social level and has become a driver for political action[1,2]. The general focus of these discussions is almost exclusively on solid polymer particles of all sizes, namely macro-, meso-, micro-, and nanoplastics. Water-soluble polymers (WSPs) do not fit within the size-based framework that has been established for discussing environmental polymer pollution, and thus are often actively excluded[3] or ignored[4,5] in such definitional approaches. Accordingly, these high production volume chemicals need to be discussed as a separate group of synthetic polymers[6,7].

Applications of WSPs in personal care products[8], pharmaceuticals[9] and other industrial sectors (e.g. wastewater treatment and oil recovery) may result in both direct and indirect emissions to the environment[10,11], which may result in considerable environmental concentrations if emission volumes are high and removal is insufficient. As a result of excluding synthetic polymers in general and thus also WSPs from registration under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) due to their alleged innocuousness, information on their production volumes can only be derived from the production volumes of their educts. Since these educts mainly serve for the production of the respective polymers or variations thereof, the production volumes of many water-soluble polymers can be estimated in the thousands to millions of tones range (e.g. poly acrylic acid (PAA) and polyethylene oxide (PEO) $>10^6$ t/a[12–14] solely in Europe). Consequently, these high production volumes in combination with the wide variety of WSP applications likely result in large amounts of WSPs being released into the environment where their fate remains largely unknown.

Despite the large production volumes and widespread applications of different synthetic polymers, environmental occurrence data is exceedingly scarce and limited to a few polymer types and chain lengths[15]. One reason for this is the inherent complexity of qualifying and quantifying WSPs in environmental matrices. Methods typically used for polymer analysis in products (e.g. Fourier transform infrared and Raman spectroscopy) likely lack the sensitivity and selectivity to deal with environmental concentrations and complex matrices and microscopic and spectrometric methods established for microplastic analysis can only be poorly transferred to non-particulate polymers[7]. Transferable methods such as pyrolysis gas chromatography-mass spectrometry (MS), are promising[16] but require further optimization and can only provide data on the total concentration of a specific polymer, losing all information on the molecular weight (MW) distribution which may play an important role in the environmental fate of WSPs. First steps towards the qualitative[17] or quantitative[7,16,18] assessment of WSPs in the aquatic environment have been taken, but data remains scarce and often narrowly focussed on certain chain lengths or directly at sources like WWTPs. Initial ecotoxicological studies already indicate a negative impact of PAA derivatives on aquatic plants and microorganisms[19]. Yet it is precisely this gap of missing occurrence data that precludes a more detailed assessment of the overall environmental impact of WSPs since it remains unknown which polymers are the most prominent, which molecular weights are predominantly present in the aquatic environment and what are environmentally relevant concentrations for lab scale experiments and risk assessment.

To address these knowledge gaps and enable more comprehensive assessment of the environmental impact of WSPs we proposed a novel analytical combination of size-exclusion chromatography hyphenated with electrospray ionization-high-resolution mass spectrometry (SEC-ESI-HRMS)[7] that utilizes the formation of diagnostic fragments independent of MW, charge state, and ion species while MW-information is retained through SEC to quantify PEO near sources but also in environmental water samples. The combination with SEC is a distinct advantage over pyrolysis-based methods in which MW information is inherently unavailable unless the sample is laboriously fractionated and the fractions are investigated independently. Herein we demonstrate the applicability of this quantification approach in two wastewater treatment plants (WWTPs) and 18 surface waters for PEO, a readily degradable but widely used polyether and derive first quantitative environmental occurrence data for this high production volume chemical.

2. Material and Methods

2.1 Chemicals and Material

PEO standards (MW: 0.98, 1.96, 3.02, 6.69, 26.1, 55.8, 180, 1000 kDa) and the used SEC column ("PSS SUPREMA" Micro Linear M) were provided by Polymer Standards and Services Mainz (DE). Methanol (analytical grade) was purchased from Carl Roth GmbH + Co. KG, Karlsruhe (DE) and Ammonium formate from Sigma Aldrich, St. Louis, Missouri (US). Ultrapure water was generated by the UV water purification system Simplicity® purchased from Merck KGaA, Darmstadt (DE). Stock solutions of the individual MWs were prepared in MeOH:H_2O 1:1 (v:v) at a concentration of 1 mg/mL. Stock solutions of high-MW PEO were placed in an ultrasonic bath until the PEO was completely dissolved. Working standards were diluted from this stock solution in MeOH:H_2O 1:1 (v:v) to the desired concentrations.

2.2 Samples

Samples were obtained from a total of 18 surface waters (from Hesse (DE) and North-Rhine-Westphalia (DE)) and two WWTPs (influent and effluent; Hessen (DE)). For sample details see Table S 1 and analysed areas in Table S 2. The location of the sampling sites is shown in Figure S 1.

2.3 Sample Extraction

Each sample was enriched by the factor 1000. The cartridges (OASIS HLB 3cc (60 mg), Waters, Milford (MA, US)) were conditioned with 3x 2 mL MeOH and washed with 4x 2 mL H_2O. After conditioning, the filtered, 200 mL aqueous sample was passed through the cartridge. Once the cartridges were dried for 20 min under an N_2 stream, the enriched substances were eluted with 4x 1.5 mL MeOH. After evaporation to dryness under a gentle stream of N_2 at 50 °C, the samples were reconstituted in 200 µL of MeOH:H_2O 1:1 (v:v) and filtered through a syringe filter (ø 0.2 µm).

2.4 SEC-HRMS measurement

All measurements were performed on an HPLC (Nexera X2 Prominence LC, LC-30AD, Kyoto (JPN)) coupled to an ESI-QTOF MS X500R (Sciex, Darmstadt (DE)) equipped with a Turbolon Spray ESI ion source. The instrument was operated in sequential window acquisition of all theoretical mass spectra (SWATH) acquisition mode. The SWATH acquisition mode is a data independent acquisition (DIA) mode that allows both to perform a survey scan and to divide a mass range selected in Q1 into defined mass windows in order to collect high-resolution fullscan composite MS/MS spectra. SWATH mode enabled the selection of broad and independent mass ranges of Q1 (m/z 200-2000) and TOF (m/z 40-300) that was impossible in many other instrumental settings. The MS/MS spectrum obtained contains the combined fragments of all detected precursor masses in the selected Q1 window which is required since no single precursor mass can be defined for WSPs like PEO. Chromatography was performed by injection of 10 µL on an SEC column (PSS SUPREMA, MicroLinear M) with isocratic elution (H_2O:MeOH 70:30 (v:v, LC MS Grade) + 5 mM ammonium formate) for 15 min. For further MS parameters please consult Table S 3.

2.5 Quality assurance and quality control

A procedural blank was prepared to determine potential contaminations from used sample preparation materials (cartridges, pipette tips, syringe filters, etc.), the mass analyser (cause by e.g.

PEG calibration standards) and other sources. For this purpose, ultra-pure water was extracted and analysed in triplicate analogously to the samples.

iLOD and iLOQ were determined analysing PEO standards of three different MW (3.02, 26.1 and 1,000 kDa concentrations were 0.5, 0.5 and 2.5 µg/mL in of MeOH:H_2O 1:1 (v:v)) near an expected iLOQ determined from the calibration and are defined as the S/N of 3 and 9 respectively.

To calculate the recovery of a single molecular weight fraction (Rec_{Fx}), ultrapure water samples were enriched according to the sample extraction in Section 2.3 (enrichment factor of 1000) and analysed according to section 2.4. Each sample was measured in triplicate. The samples were spiked with different MWs of PEO (0.98, 1.96, 3.02, 6.69, 26.1, 55.8 and 180 kDa) before and after enrichment and the recovery was calculated according to Equation E(1). The recovery was tested at a concentration of 25 µg/L, which ensured that even analytes with low recoveries and high ion suppression can be analysed.

$$Rec_{Fx}[\%] = \frac{\bar{A}_{SB} - \bar{A}_{NS}}{\bar{A}_{SA} - \bar{A}_{NS}} * 100 \qquad E(1)$$

$\bar{A}_{SB}$: Mean area (n=3) of PEO MW-sections in the samples spiked before enrichment

$\bar{A}_{SA}$: Mean area (n=3) of PEO MW-sections in the samples spiked after enrichment

$\bar{A}_{NS}$: Mean area (n=3) of PEO MW-sections in the matrix blank

Two surface water samples (River Rhine Neuenkamp (kilometer 778), River Rhine Wiesbaden-Schierstein (Harbour; kilometer 506) and one WWTP effluent (Idstein-Beuerbach (1))) were examined to investigate the influence of the matrix on the ESI-ionisation efficiency. These samples were enriched according to the sample extraction in Section 2.3 (enrichment factor of 1000) and analysed according to section 2.4 as well.

Each pre-enriched matrix was spiked with a concentration of 25 µg/mL (corresponds to 25g/L in the original sample) of PEO 3.0 kDa into the final extract and analyzed in triplicate. Matrix effects were calculated according to Equation E(2).

$$ME[\%] = \frac{\bar{A}_{SA} - \bar{A}_{NS}}{\bar{A}_{Std}} * 100 \qquad \mathrm{E(2)}$$

$\bar{A}_{Std}$: Mean area (n=3) of PEO MW-sections in a Standard

The same pre-extracted samples (spiked with 25 µg/L PEO 3.0 kDa, analyzed in triplicate) were used to calculate the repeatability.

3. Results and discussion

3.1 Fragment based quantification method

Chromatography hyphenated with mass spectrometry is often the method of choice to detect and quantify organic micropollutants in complex environmental matrices. Its application to WSPs, however, is exacerbated by the inherent complexity of synthetic polymers. Unlike most organic micropollutants, which often result in one signal of a specific mass and few isotopologues or additional ion species, polymers can consist of hundreds or even thousands of homologues as well as their respective isotopologues and ion species[20,21]. This has two detrimental consequences: (1) There is not *one* mass of interest for each polymer type, but rather hundreds or even thousands, that may vary according to the MW of the polymer, its multiple possible charge states and adducts formed. (2) Total signal intensities for WSPs are distributed over all these signals, resulting in low individual signal intensities even at high WSP concentrations. To counteract these drawbacks, we employ an all-ion fragmentation approach that utilizes the formation of MW-independent diagnostic fragments from PEO resulting in a severe reduction of masses of interest with significantly increased intensities (Figure 1). While the formed fragments are MW-independent, the ionization and fragmentation efficiencies are not (MW-dependent differences in sensitivity are shown in Figure S2), and thus must be taken into consideration during data processing.

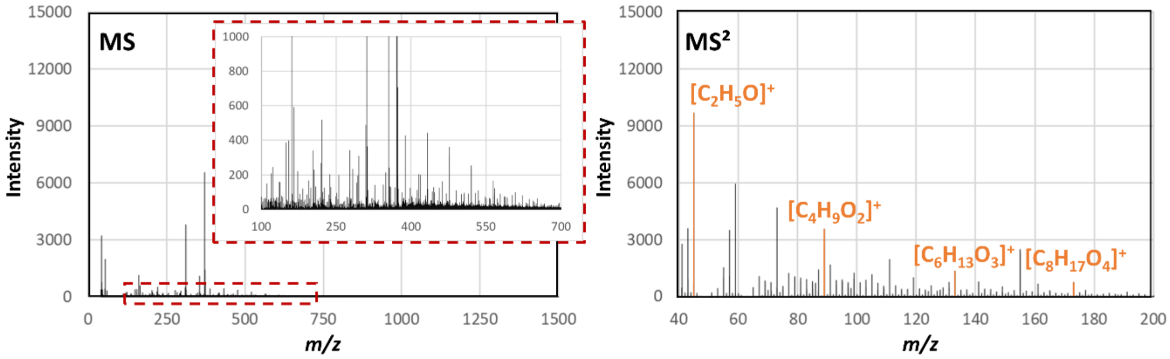

Figure 1: Averaged MS (left) and MS² (right) spectra at an elution time of 8.0 - 8.5 min (concentration maximum of PEO) of the river Rhein taken at Schierstein Harbour in Wiesbaden, Germany. While the MS spectrum shows a wide range of low intensity signals (most < 200) the MS² shows 4 signals attributed to diagnostic fragments of PEO (marked in orange) with intensities ranging from 1000 to > 9000.

After pre-concentration of samples through solid phase extraction (SPE) and analysis with SEC-ESI-HRMS in full scan and SWATH mode, quantification is performed through the combined intensity of the diagnostic fragments $[C_{2n}H_{4n+1}O_n]^+$ with n= 1, 2, 3, and 4 (m/z 45, 89, 133, and 177). The SEC elution time provides information about the MW that is otherwise lost during fragmentation and separates the polymer from low-MW interferences carrying PEO side-chains (e.g. nonylphenolethoxylates). SPE using HLB cartridges was chosen as generic enrichment method that enables high enrichment factors but not further optimized. Procedural blanks show that the used materials did not cause any significant contamination of the samples with PEO in the investigated MW-range. If the method is transferred to other polymers this has to be tested independently and the use of glass cartridges may be advised. Blank values (areas are listed in Table S 4), SPE recoveries, and response factors (influenced by ionization and fragmentation efficiencies) are all MW-specific and are thus calculated and corrected individually for MW-fractions derived from the elution time to increase the accuracy of quantification (MW calibration and corresponding response factors are shown in Figure S 3). MW specific fractions were determined by analysing different MW standards. The resulting concentrations for individual MW-fractions are then summed up and corrected by the matrix effects, which were observed to be largely independent from the MW (workflow see Figure 2). The resulting method cannot differentiate between PEO, PEO block-copolymers and PEO derivatives and transformation products (e.g. modifications at the chain end as they occur during the biodegradation of PEO) that still contain unaltered PEO units of a significant chain length forming these diagnostic fragments. The formation of specific fragments by PEO derivatives was proven exemplarily for alkylated PEO-polypropyleneoxide copolymer (Figure S4). Consequently, this method results in the sum of PEO and its derivatives quantified as PEO. For simplicity, we refer to this as PEO concentration throughout the manuscript.

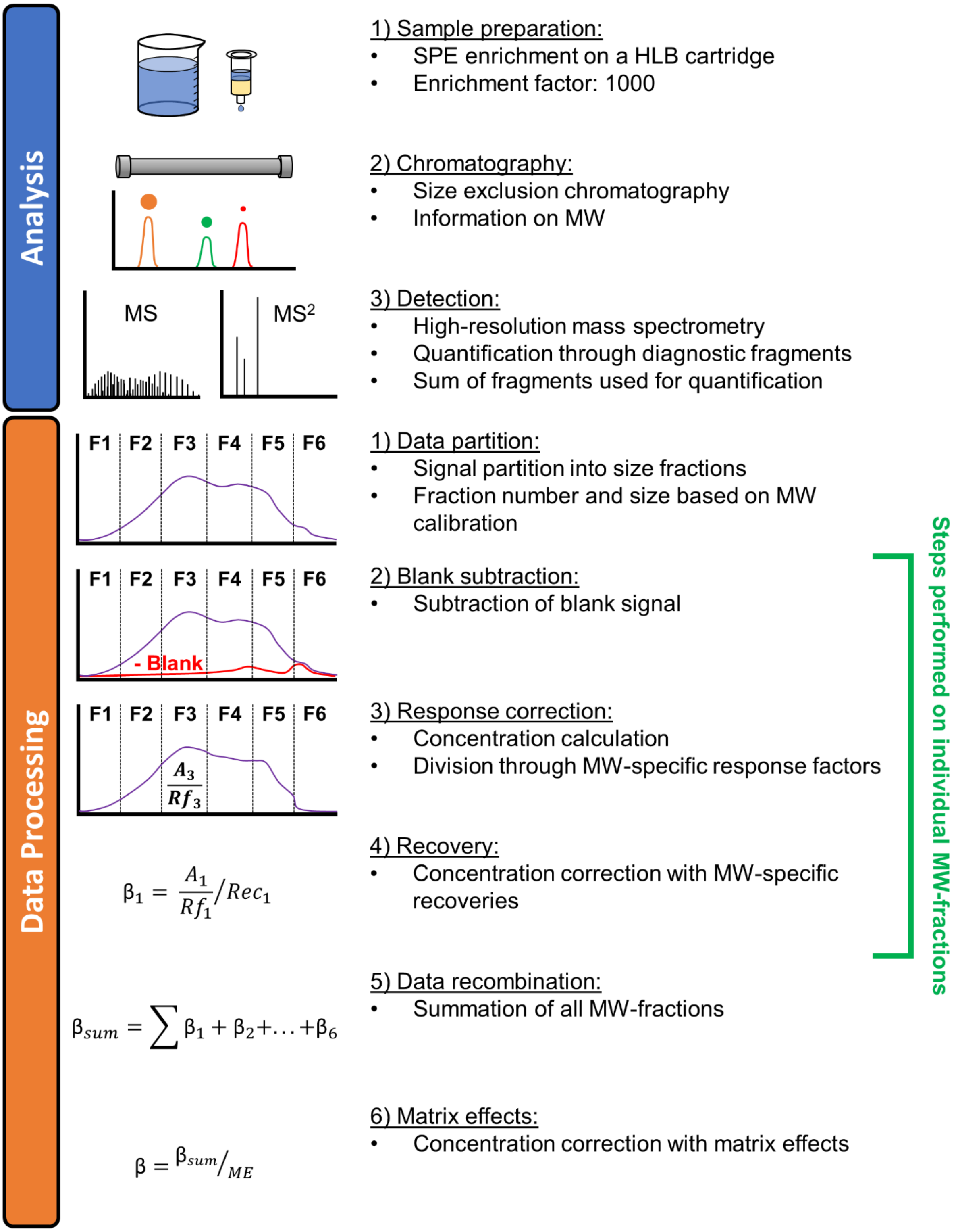

Figure 2: Analytical (3 steps) and data processing (6 steps) workflow of PEO quantification. Data processing steps with a large MW-dependency (blank subtraction, response correction, and recovery) were performed independently for individual MW-fractions.

3.2 Method performance

Method performance was validated including recovery, both, the instrumental limit of quantification (iLOQ) and detection (iLOD) (Table 1), matrix effects and repeatability.

Table 1: Recovery, instrumental limit of quantification (iLOQ) and instrumental limit of detection (iLOD) of the developed method for different PEO MWs.

	MW PEO [kDa]							
Analytical Performance Characteristic	**0.98**	**1.96**	**3.02**	**6.69**	**26.1**	**55.8**	**180**	**1,000**
iLOD [µg/mL]	N/A	N/A	0.54	N/A	1.15	N/A	N/A	5.36
iLOQ [µg/mL]	N/A	N/A	1.61	N/A	3.46	N/A	N/A	16.07
Recovery [%]	93	99	97	69	19	0	0	N/A

N/A = not available; were not used to determine this performance characteristic.

iLOD and iLOQ show a strong dependence of the sensitivity on the MW of PEO which is caused by reduced ionization and/or fragmentation efficiencies with increasing MW (calibrations are shown in Figure S 2). Consequently, an overall method LOD/LOQ cannot be given since it strongly depends on the MW-distribution of PEO in the sample. Recoveries of PEO show a district drop at 55 kDa, which limits the application range of the method. This drop may be caused by irreversible sorption of high-MW PEO to the SPE material which may be circumvented by alternative methods like freeze-drying.

Therefore, MW-dependent correction as described in Section 3.1 was deemed necessary.

Regarding matrix effects, each of the matrices examined (see section 2.5) caused ion suppression (-18% and -25% in surface waters; -49% in WWTP effluent).

Calculated matrix effects were used for MW-independent correction of the environmental samples. For surface water samples the mean matrix effect of River Rhine (sampled in Neuenkamp and Wiesbaden-Schierstein ($\overline{\mathrm{ME}}$= -21%)) was used and for WWTP samples respectively the matrix effect of the effluent (WWTP 1 (ME= -49%)). Triplicates of the same water samples spiked before enrichment were used to determine the repeatability, which was expressed as the relative standard deviation (RSD) of the measured PEO content in the three replicates (RSD: 2-14%). Corresponding peak areas are listed in Table S 5.

Signals found in the procedure blank (see Table S4) were divided into MW sections (schematically shown in Figure 2) and subtracted from any analytical results. Using the SEC, it was found that the

MWs of PEO in the procedural blank were all below 6.0 kDa and therefore only affect MW sections 1, 2 and 3.

3.3 Occurrence of PEO in wastewater treatment plants and surface water

The influents and effluents of two WWTPs were analysed for PEO, both showing a clear reduction in concentration of approximately 95% during wastewater treatment (Figure 3). Interestingly, the MW-range is shifted towards 1.3-4 kDa during water treatment for both WWTPs despite pronounced differences in their influent PEO MW-distribution. The shifted MW-range implies that 1.3-4 kDa may be the main environmental MW-fraction of PEO, at least when WWTPs are considered as dominant point source. Since MWs > 55 kDa were insufficiently enriched (Table 2), a further distribution maximum and possible concentrations would be neglected. Due to the increasing sorption tendency[22–24] with increasing MW, however, it can be assumed that PEOs >55 kDa are of minor importance for the analysis of aqueous samples. It is known that lower MW PEO is more rapidly transformed by microorganisms and that sorption increases at higher MW[22]. Thus, the observed MW-range is likely a result of higher MWs more rapidly adsorbing to sludge while lower MWs are more quickly mineralized leading to (almost) complete elimination outside the MW-range from 1.3 – 4kDa. Consequently, the emitted MW-range may strongly depend on sludge and hydraulic retention time as well as the microbiological activity in the WWTP and thus further studies are required to confirm if the observed MW-range is universally emitted from WWTPs.

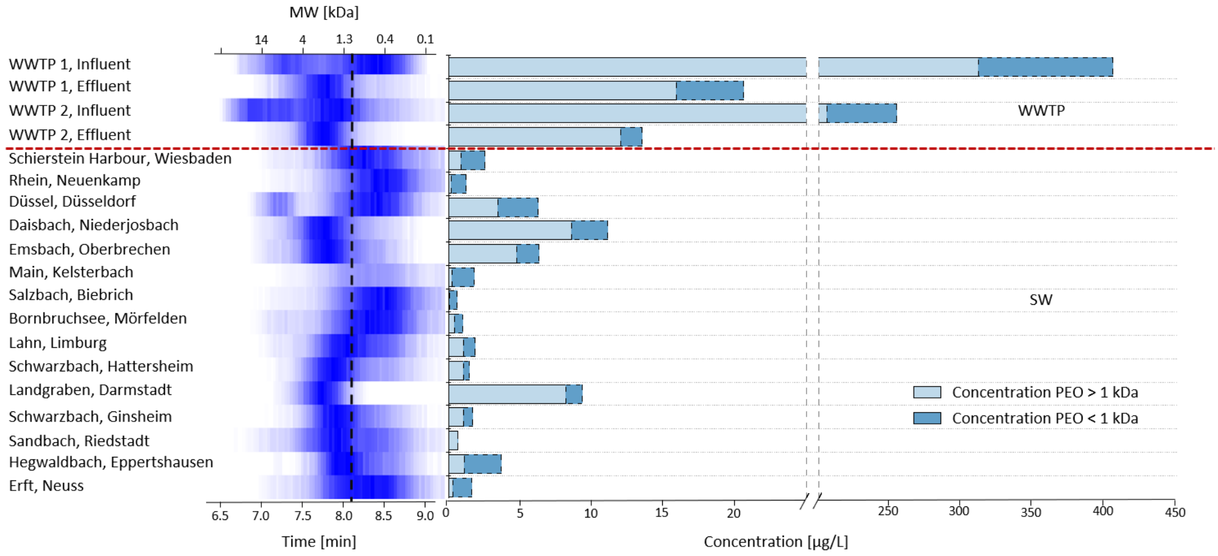

Figure 3: MW distributions (left) and concentration (right) of PEO in waste water treatment plants (WWTP) and surface water (SW). Results from Karbach (Karbach), Waterside (Biebrich) and Pfänderbach (Schwarzenacker) are not shown since their concentration were below iLOD. Concentrations of PEOs MW < 1 kDa (indicated by dashed black line (left) and light blue area (right) were outside the validation range and are thus only semiquantitative. The samples were analysed as single determinations. An approximate error for PEO concentrations in the dominant MW-range can be estimated using the relative standard deviation of triplicate determinations of spiked samplesduring the validation (2-14 %).

PEO is known to be readily biodegradable. Nevertheless, the exceedingly high influent concentrations (about 254 and 405 µg/L) comparable to other surfactants like LAS (linear alkylbenzenesulfonate)[25], resulted in effluent concentrations up to 20 µg/L despite 95% reduction during water treatment. These are remarkable effluent concentrations for a readily biodegradable organic micropollutant[22,26,27,22]. To investigate if these effluent concentrations resulted in elevated surface water concentrations 18 surface waters were analysed. Concentrations of PEO in the surface waters investigated vary from below the iLOD (3 samples; not shown in Figure 3) to approx. 11 µg/L. In total, 11/18 surface waters investigated exceeded a concentration of 1 µg/L and one sample even exceeded 10 µg/L. The mean and median concentrations of PEO in surface waters were 3.4 µg/L and 1.8 µg/L, respectively. Surface waters sampled downstream of a WWTP (e.g. Daisbach (Niederjosbach) and Landgraben (Darmstadt)) showed a similar molar mass distribution of PEO as the two effluent samples investigated. In most surface waters a lower MW-range of PEO (< 3,0 kDa) was observed, which may be the result of ongoing degradation in the aquatic environment. Biodegradation of PEO occurs under both aerobic[28–30] and anaerobic[31–33] conditions and results mainly in oxidation of the terminal hydroxy group to the corresponding aldehyde, which is converted into carboxylic acid derivatives after further oxidation followed by a stepwise chain degradation[20,34,35]. Thus, resulting intermediates are likely also detectable by the method used herein until mineralization is achieved. Depending on the MW, biodegradation (> 90% removal of dissolved organic carbon) in freshwater takes between 20 d (MW < 14,000 Da) to 65 d (MW >14,000 Da) [20,36]. In addition to MW, crucial for the biodegradation of PEO is a syntrophic association of different bacteria (e.g.: *Flavobacterium sp. and Pseudomonas sp.*) and the medium present (e.g.: freshwater or saltwater)[20,21,29,34]. Regarding the MW distributions detected, one exception is the Düssel (Düsseldorf), in which a second MW maximum at approx. 7,000 Da was observed. As this river passes through an urban centre, further significant sources cannot be excluded. Such discrepancies in the observed MW distribution may hint at additional sources and could be a further advantage of analytical methods that preserve the MW information unlike thermoanalytical approaches that rely on thermolysis of an aliquot of the sample. However further studies regarding the occurrence and especially the MW distributions of PEO specifically and WSPs in general are required to confirm this hypothesis.

These results show that PEO is a widespread environmental water contaminant that occurs in the µg/L range. The environmental occurrence of PEO, however, seems to be focussed on a rather narrow MW range compared to its applications that can reach up to hundreds of thousands of Da or more. Transferring the method successfully to other WSPs depends primarily on whether the respective polymer forms diagnostic fragments. In addition, further investigations are needed regarding the response factors and the fragmentation behavior of block co-polymers, derivatives and transformation products.

Due to the high production volumes and numerous applications of WSPs being directly correlated with their emission into the aquatic environment, their presence and distribution in the water cycle seems likely but has rarely been demonstrated so far. The presented study shows that even the readily biodegradable PEO occurs in surface waters in the µg/L range, which raises questions about the environmental occurrence and impact of other WSPs that have similar production volumes but are not biodegradable. Currently, there are only a few studies dealing with the concentrations of WSPs in the water cycle (see Table 2) and most of them are focussed on sources like WWTPs and thus provide only little insight into the occurrence in the environment.

Table 2: Overview of WSP concentrations in different matrices.

WSP	Concentration	Matrix	Reference
PEO	WWTP 1: 405/20 µg/L WWTP 2: 254/13 µg/L	2 WWTPs (influent/effluent)	This study
PEO	<LOD-11 µg/L	18 surface water samples	This study
PEO	~1.5-7.4 µg/L	Total of 33 WWTP effluents	[18]
PEO	1.7-31 mg/kg dry weight	5 WWTP sludges	[15]
Alcohol ethoxylates (C_{10}-C_{18} EO_n)	1-98 mg/kg dry weight	5 WWTP sludges	[15]
Poly-(N-vinylcaprolactam)	70 µg/L	WWTP effluent	[16]
PVP	7 mg/L	WWTP effluent	[37]
PEO	1.6-8.8 mg/kg	Sediment	[38]
Alcohol ethoxylates (C_{12}-C_{18} $EO_{n=2,3,6,8}$)	0.007-0.085 mg/kg	Sediment	[38]

The study of PEO effluent concentrations conducted by Freeling et al. shows results that are generally lower as the effluent concentrations analysed herein[18]. Given that Freeling et al. selected a range of PEO chain lengths and defined one mass of interest for each of these chain lengths in their approach this discrepancy can be explained by an underestimation of the total PEO concentration caused by a reduced analyte spectrum. Nevertheless, both studies found effluent concentrations within the same order of magnitude, consequently giving further validity to each other's results.

First studies have already indicated potential negative effects of WSPs like PAA and polyvinylpyrrolidone (PVP) and their transformation and degradation products[39–42]. As such, degradation products of PEO formed after ozonation can be formaldehyde and formic ester. Even if these are readily degradable and unstable in (waste)water, both are nevertheless classified as hazardous and corrosive[13–15,42,43]. Furthermore, various transformation products e.g. low MW Polyacrylamide (PAM) with variable functional groups exhibit the potential to cross cell membranes[44,45]. Recent studies show an ecotoxicological impact of PAA and various acrylate copolymers, although at very high test concentrations of 100 mg/L[19]. However, even if PAA and PVP do not exhibit any acute toxicity (no acute effect up to 50 mg/L on *Daphnia magna*.), initial studies already indicate a chronic toxicity. In standardised toxicity tests, Modellini et al. showed chronic impairments on reproduction and growth of *Daphnia magna* at 5-10 mg/L WSP (PAA, PVOH, PVP and PEO).[46] Considering a study by Antić et al., where a concentration of 7 mg/L of PVP was found in a WWTP effluent, direct adverse effects on the environment caused by the WSPs studied by Modellini et al. cannot be excluded.[37] However, not only the direct but also the indirect effects of WSPs can impact the environment negatively. The ability of certain WSPs, especially cationic ones, to bind heavy metals and organic micropollutants may increase the mobility and bioavailability of these toxins.[47,48]

For WSPs not only knowledge on environmental concentrations is required to derive a realistic ecotoxicological impact assessment but also on the MW most common in the environment. Including the MW in these assessments is important since high and low MW WSPs may lead to significantly different results. The polyacrylic acid-based WSPs studied by Rozman et al. are significantly more recalcitrant compared to PEO, but are produced and used on the same scale[12–14,19,21,49,50]. Accordingly, higher environmental concentrations would be assumed for these WSPs, either in the aqueous phase or adsorbed to particulate matter. Depending on the environment, (bio)degradation may be hampered or incomplete. For example, Eubeler et al. already demonstrated a reduced degradability of PEO in salt water with marine inoculum compared to freshwater. Especially for higher molecular weight compounds, this may result in higher WSP concentrations[36].

Since WSPs not only differ in their chemical nature, but also in molecular weight distribution and in some cases even in the degree of chemical modification, the analytical assessment of their occurrence and fate in the environment is extremely challenging. This includes not only the wastewater cycle, but also soil, sludge and particulate matter. Further investigations should therefore include these compartments, and all stages of the water cycle. Due to the application of WSPs for waste and drinking water treatment, potential residues cannot be excluded. Nevertheless, it is not only the anthropogenic influenced water cycle that is affected. Studies by Wang et al., in which PEO was already found in freshly fallen snow, show how invasively WSPs have already entered the natural water cycle[51].

4 Conclusion

So far (semi-)quantitative environmental occurrence data for WSPs is largely missing, which impacts the ability to assess any potential risk associated to them. The SEC-ESI-HRMS approach utilizing diagnostic fragment formation (fragments independent of molecular weight (MW), charge state, and ion species) within selected PEO MW-fractions for identification and quantification of PEO and its derivatives in surface water and WWTP influent and effluent demonstrated that even for the benign and readily biodegradable PEO environmental concentrations in the µg/L range were observed despite a concentration reduction of 95% during wastewater treatment. Moreover, these concentrations are not the result of highly invasive events but result from everyday applications in every household or the production of consumer products and pharmaceuticals. Concentrations for other recalcitrant WSPs like PVP or PAA can at this point only be speculated on, but may be considerably higher than for PEO. Using size exclusion chromatography, it is possible to assess the MW range present in the samples analyzed. Despite pronounced differences in the MW distribution in the influent, a shift of the MW range for both WWTPs to 1.3-14 kDa in the effluent was observed, suggesting WWTPs as the main input source for PEO. This may impact studies on the environmental behavior, fate, and impact of PEO and in extension of other WSPs of a similar behavior is observed. If the observed MW is confirmed as dominant in the aquatic environment follow-up studies on these aspects should focus on PEO of this mass range first to ensure a high environmental relevance.

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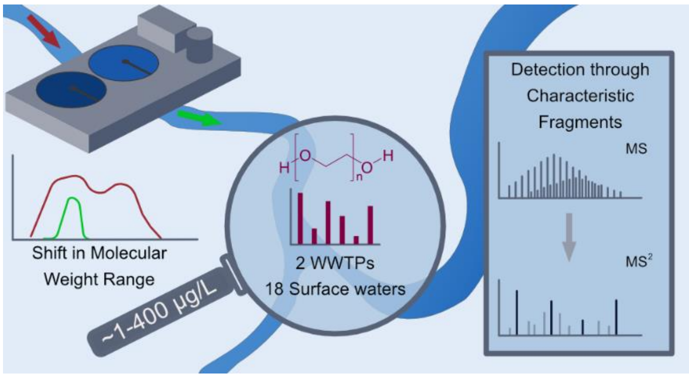

Figure 1: For Table of contents only.

Competing of Interest

The authors declare that they have no competing financial or personnel interests.

References

(1) Sharma, S.; Chatterjee, S. Microplastic pollution, a threat to marine ecosystem and human health: a short review, Environmental science and pollution research international. 2017, 24, pp. 21530–21547.

(2) Triebskorn, R.; Braunbeck, T.; Grummt, T.; Hanslik, L.; Huppertsberg, S.; Jekel, M.; Knepper, T. P.; Krais, S.; Müller, Y. K.; Pittroff, M.; Ruhl, A. S.; Schmieg, H.; Schür, C.; Strobel, C.; Wagner, M.; Zumbülte, N.; Köhler, H.-R. Relevance of nano- and microplastics for freshwater ecosystems: A critical review, TrAC Trends in Analytical Chemistry. 2019, 110, pp. 375–392.

(3) Hartmann, N. B.; Hüffer, T.; Thompson, R. C.; Hassellöv, M.; Verschoor, A.; Daugaard, A. E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; Herrling, M. P.; Hess, M. C.; Ivleva, N. P.; Lusher, A. L.; Wagner, M. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris, Environmental science & technology. 2019, 53, pp. 1039–1047.

(4) Alimi, O. S.; Farner Budarz, J.; Hernandez, L. M.; Tufenkji, N. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport, Environmental science & technology. 2018, 52, pp. 1704–1724.

(5) Betts, K. Why small plastic particles may pose a big problem in the oceans, Environmental science & technology. 2008, 42, p. 8995.

(6) Arp, H. P. H.; Knutsen, H. Could We Spare a Moment of the Spotlight for Persistent, Water-Soluble Polymers?, Environmental science & technology. 2020, 54, pp. 3–5.

(7) Huppertsberg, S.; Zahn, D.; Pauelsen, F.; Reemtsma, T.; Knepper, T. P. Making waves: Water-soluble polymers in the aquatic environment: An overlooked class of synthetic polymers?, Water research. 2020, 181, p. 115931.

(8) Koltzenburg, S.; Maskos, M.; Nuyken, O. Polymere: Synthese, Eigenschaften und Anwendungen; Springer Berlin Heidelberg: Berlin, Heidelberg, 2014.

(9) Harris, J. M.; Zalipsky, S. Poly(ethylene glycol); American Chemical Society: Washington, DC, 1997.

(10) Raffa, P.; Broekhuis, A. A.; Picchioni, F. Polymeric surfactants for enhanced oil recovery: A review, Journal of Petroleum Science and Engineering. 2016, 145, pp. 723–733.

(11) Sarika, R.; Kalogerakis, N.; Mantzavinos, D. Treatment of olive mill effluents Part II. Complete removal of solids by direct flocculation with poly-electrolytes, Environment international. 2005, 31, pp. 297–304.

(12) European Chemicals Agency - Acrylic acid. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15803.

(13) European Chemicals Agency - Polyethylene glycol. https://echa.europa.eu/de/substance-information/-/substanceinfo/100.123.919.

(14) European Chemicals Agency - Ethylene oxide. https://echa.europa.eu/de/substance-information/-/substanceinfo/100.000.773?_disssubsinfo_WAR_disssubsinfoportlet_backURL=https%3A%2F%2Fecha.europa.eu%2Fde%2Fhome%3Fp_p_id%3Ddisssimplesearchhomepage_WAR_disssearchportlet%26p_p_lifecycle%3D0%26p_p_state%3Dnormal%26p_p_mode%3Dview%26_disssimplesearchhomepage_WAR_disssearchportlet_sessionCriteriaId%3D.

(15) Petrović, M.; Barceló, D. Determination of anionic and nonionic surfactants, their degradation products, and endocrine-disrupting compounds in sewage sludge by liquid chromatography/mass spectrometry, Analytical chemistry. 2000, 72, pp. 4560–4567.

(16) Vidovic, N.; Krauskopf, L.-M.; Jovancicevic, I.; Antic, V.; Schwarzbauer, J. Determination of the water-soluble polymer poly(N-vinylcaprolactam) in wastewater effluents by continuous-flow off-line pyrolysis-GC/MS, Discov Water. 2022, 2.

(17) Mairinger, T.; Loos, M.; Hollender, J. Characterization of water-soluble synthetic polymeric substances in wastewater using LC-HRMS/MS, Water research. 2021, 190, p. 116745.

(18) Freeling, F.; Alygizakis, N. A.; Ohe, P. C. von der; Slobodnik, J.; Oswald, P.; Aalizadeh, R.; Cirka, L.; Thomaidis, N. S.; Scheurer, M. Occurrence and potential environmental risk of surfactants and their transformation products discharged by wastewater treatment plants, The Science of the total environment. 2019, 681, pp. 475–487.

(19) Rozman, U.; Kalčíková, G. The first comprehensive study evaluating the ecotoxicity and biodegradability of water-soluble polymers used in personal care products and cosmetics, Ecotoxicology and environmental safety. 2021, 228, p. 113016.

(20) Bernhard, M.; Eubeler, J. P.; Zok, S.; Knepper, T. P. Aerobic biodegradation of polyethylene glycols of different molecular weights in wastewater and seawater, Water research. 2008, 42, pp. 4791–4801.

(21) Trimpin, S.; Eichhorn, P.; Räder, H.; Müllen, K.; Knepper, T. Recalcitrance of poly(vinylpyrrolidone): evidence through matrix-assisted laser desorption–ionization time-of-flight mass spectrometry, Journal of Chromatography A. 2001, 938, pp. 67–77.

(22) Droge, S. T. J.; Hermens, J. L. M. Nonlinear sorption of three alcohol ethoxylates to marine sediment: a combined Langmuir and linear sorption process?, Environmental science & technology. 2007, 41, pp. 3192–3198.

(23) Podoll, R. T.; Irwin, K. C.; Brendlinger, S. Sorption of water-soluble oligomers on sediments, Environmental science & technology. 1987, 21, pp. 562–568.

(24) A. Nadler, M. Malik, J. Letey Desorption of Polyacrylamide and Polysaccharide Polyners from Soil Materials, Soil Technology. 1992, pp. 91–95.

(25) Barceló, D.; Knepper, T. P.; Wilson, C. L., Eds. Analysis and fate of surfactants in the aquatic environment, 1st ed.; Elsevier: Amsterdam, 2003.

(26) Vogelsang, C.; Grung, M.; Jantsch, T. G.; Tollefsen, K. E.; Liltved, H. Occurrence and removal of selected organic micropollutants at mechanical, chemical and advanced wastewater treatment plants in Norway, Water research. 2006, 40, pp. 3559–3570.

(27) Yang, W.; Zhou, H.; Cicek, N. Treatment of Organic Micropollutants in Water and Wastewater by UV-Based Processes: A Literature Review, Critical Reviews in Environmental Science and Technology. 2014, 44, pp. 1443–1476.

(28) Kawai, F. Microbial degradation of polyethers, Applied Microbiology and Biotechnology. 2002, 58, pp. 30–38.

(29) Kawai, F.; Schink, B. The Biochemistry of Degradation of Polyethers, Critical Reviews in Biotechnology. 1987, 6, pp. 273–307.

(30) Kawai, F.; Moriya, F. Bacterial assimilation of polytetramethylene glycol, Journal of Fermentation and Bioengineering. 1991, 71, pp. 1–5.

(31) Dwyer, D. F.; Tiedje, J. M. Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia, Applied and Environmental Microbiology. 1983, 46, pp. 185–190.

(32) Frings, J.; Schramm, E.; Schink, B. Enzymes Involved in Anaerobic Polyethylene Glycol Degradation by Pelobacter venetianus and Bacteroides Strain PG1, Applied and Environmental Microbiology. 1992, 58, pp. 2164–2167.

(33) Schink, B.; Stieb, M. Fermentative degradation of polyethylene glycol by a strictly anaerobic, gram-negative, nonsporeforming bacterium, Pelobacter venetianus sp. nov, Applied and Environmental Microbiology. 1983, 45, pp. 1905–1913.

(34) Gu, J.-D. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances, International Biodeterioration & Biodegradation. 2003, 52, pp. 69–91.

(35) Kawai, F.; Yamanaka, H. Biodegradation of polyethylene glycol by symbiotic mixed culture (obligate mutualism), Arch. Microbiol. 1986, 146, pp. 125–129.

(36) Eubeler, J. P.; Bernhard, M.; Knepper, T. P. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups, TrAC Trends in Analytical Chemistry. 2010, 29, pp. 84–100.

(37) Antić, V. V.; Antić, M. P.; Kronimus, A.; Oing, K.; Schwarzbauer, J. Quantitative determination of poly(vinylpyrrolidone) by continuous-flow off-line pyrolysis-GC/MS, Journal of Analytical and Applied Pyrolysis. 2011, 90, pp. 93–99.

(38) Traverso-Soto, J. M.; Lara-Martín, P. A.; León, V. M.; González-Mazo, E. Analysis of alcohol polyethoxylates and polyethylene glycols in marine sediments, Talanta. 2013, 110, pp. 171–179.

(39) Cumming, J.; Hawker, D. W.; Chapman, H.; Nugent, K. Sorption of Polymeric Quaternary Ammonium Compounds to Humic Acid, Water Air Soil Pollut. 2011, 214, pp. 5–11.

(40) Muir, M. M.; Kosteretz, K. G.; Lech, J. J. Localization, depuration, bioaccumulation and impairment of ion regulation associated with cationic polymer exposure in rainbow trout (Oncorhynchus mykiss), Xenobiotica; the fate of foreign compounds in biological systems. 1997, 27, pp. 1005–1014.

(41) Park, S.-H.; Wei, S.; Mizaikoff, B.; Taylor, A. E.; Favero, C.; Huang, C.-H. Degradation of amine-based water treatment polymers during chloramination as N-nitrosodimethylamine (NDMA) precursors, Environmental science & technology. 2009, 43, pp. 1360–1366.

(42) Suzuki, J. Study on ozone treatment of water-soluble polymers. I. Ozone degradation of polyethylene glycol in water, J. Appl. Polym. Sci. 1976, 20, pp. 93–103.

(43) European Chemicals Agency - Formaldehyde. https://echa.europa.eu/de/substance-information/-/substanceinfo/100.000.002.

(44) Plastic soup foundation. THE FORGOTTEN SYNTHETIC POLYMERS AND THEIR ENVIRONMENTAL AND HUMAN HEALTH CONCERNS. A perspective-review on water-soluble, liquid, semi-solid & biodegradable polymers and engineered nanoplastics.

(45) Xiong, B.; Loss, R. D.; Shields, D.; Pawlik, T.; Hochreiter, R.; Zydney, A. L.; Kumar, M. Polyacrylamide degradation and its implications in environmental systems, npj Clean Water. 2018, 1.

(46) Mondellini, S.; Schott, M.; Löder, M. G. J.; Agarwal, S.; Greiner, A.; Laforsch, C. Beyond microplastics: Water soluble synthetic polymers exert sublethal adverse effects in the freshwater cladoceran Daphnia magna, The Science of the total environment. 2022, 847, p. 157608.

(47) Rivas, B. Water-soluble polymer–metal ion interactions, Progress in Polymer Science. 2003, 28, pp. 173–208.

(48) Julinová, M.; Vaňharová, L.; Jurča, M. Water-soluble polymeric xenobiotics - Polyvinyl alcohol and polyvinylpyrrolidon - And potential solutions to environmental issues: A brief review, Journal of environmental management. 2018, 228, pp. 213–222.

(49) Hayashi, T.; Mukouyama, M.; Sakano, K.; Tani, Y. Degradation of a sodium acrylate oligomer by an Arthrobacter sp, Applied and Environmental Microbiology. 1993, 59, pp. 1555–1559.

(50) European Chemicals Agency - 1-vinyl-2-pyrrolidone. https://echa.europa.eu/de/substance-information/-/substanceinfo/100.001.637.

(51) Wang, Z.; Saadé, N. K.; Ariya, P. A. Advances in Ultra-Trace Analytical Capability for Micro/Nanoplastics and Water-Soluble Polymers in the Environment: Fresh Falling Urban Snow, Environmental pollution (Barking, Essex : 1987). 2021, 276, p. 116698.