Water Research 261 (2024) 122055

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Py–GC–MS analysis for microplastics: Unlocking matrix challenges and sample recovery when analyzing wastewater for polypropylene and polystyrene

Jeanette Lykkemark[a], Marco Mattonai[b,c,*], Alvise Vianello[a], Alessio Gomiero[d,e], Francesca Modugno[b,e], Jes Vollertsen[a,e]

[a] Department of the Built Environment, Aalborg University, Aalborg, Denmark
[b] Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy
[c] National Interuniversity Consortium of Materials Science and Technology, Italy
[d] NORCE Norwegian Research Centre AS, Norway
[e] North Atlantic Microplastic Centre (NAMC), Norway

ARTICLE INFO

Keywords:
Microplastics
Wastewater
Sample preparation
Analytical pyrolysis
Mass spectrometry

ABSTRACT

Matrix interference and recovery when using pyrolysis gas chromatography (Py–GC–MS) to analyze wastewater for polystyrene (PS) and polypropylene (PP) microplastics (MP) was studied. Raw wastewater underwent a sample preparation train commonly applied for such matrix. The train consisted of six discrete steps to reduce the organic matter content without affecting MP in the sample. One large wastewater sample was collected, homogenized, and subdivided into 21 subsamples. Three samples were analyzed without further sample preparation. The remaining samples were divided in sets of three, and each set underwent an increasing number of steps of the procedure, up to the last set, which underwent the full treatment. The matrix effect on the determination of PS and PP was statistically evaluated by comparing in-matrix and external calibration curves at each step. Recovery of MP was assessed after each step by adding deuterated PS to the samples. A main finding was that there was no significant matrix effect for these polymers throughout the preparation train, suggesting that matrix components did not interfere with the analytical method. However, a significant loss of polymer mass was found between the steps, which may result in MPs falling below detection limits. Therefore, Py–GC–MS could be used for MP quantification before analysis by other techniques which require more extensive matrix removal. A downside of this approach is that analyzing such samples without matrix reduction will increase the need for instrumental maintenance.

1. Introduction

Microplastic (MP) research has rapidly expanded over the past decade in interdisciplinary fields related to environmental science, analytical chemistry, and toxicology. A literature survey for publications with the term “microplastics” in the title showed 8 publications in 2012, and more than 700 in 2022 (Web of Science, 2023). The growing interest in understanding the abundance and distribution of MPs is not limited to academia but also extends to public bodies, regulatory agencies, and the general public, as there is increasing concern about the potential risk MPs pose to ecosystems and human health. Recent studies have detected MPs in human blood and lungs (Leslie et al., 2022; Jenner et al., 2022), further highlighting the need for reliable analytical methods that are efficient, accurate, and precise in order to accurately evaluate the extent of human exposure to this emerging pollutant. Moreover, there are many sources of MPs, and the qualitative and quantitative determination of MPs in wastewater is crucial to evaluate the role of wastewater as a source of MP entering the environment and the related implications for aquatic ecosystems, and therefore it has also received considerable attention (Rasmussen et al., 2021; Liu et al., 2020; Simon et al., 2018; Simon et al., 2019; Liu et al., 2023). Monitoring MPs in wastewater can help evaluate the effectiveness of wastewater treatment processes, identify and track the sources of pollution, and support regulatory bodies in defining guidelines and regulations and taking the necessary

---

* Corresponding author at: Department of Chemistry and Industrial Chemistry, University of Pisa, Via Giuseppe Moruzzi 13, 56124 Pisa, Italy.
E-mail address: marco.mattonai@dcci.unipi.it (M. Mattonai).

https://doi.org/10.1016/j.watres.2024.122055
Received 4 December 2023; Received in revised form 26 June 2024; Accepted 4 July 2024

actions to keep pollutant levels below harmful levels.

However, there are currently no established quality requirements and only vague guidelines for MP detection, resulting in variations in sampling, preparation, storage, and analysis methods across different studies. Such lack of scientific consensus in various aspects of MPs research, from sampling to data interpretation, underlines the need for more comparable approaches to ensure robust and reproducible findings and consistency of the results obtained in different studies. Various institutions and organizations have promoted efforts towards shared guidelines and harmonized analytical methods to improve accuracy, reliability and data comparability when measuring MPs in environmental samples (OSPAR, 2010; European Commission, 2013; J. R. C. T. Report, 2021).

Spectroscopic methods, such as Fourier-Transformed Infrared Spectroscopy (FTIR) or Raman, are the most widely used for identifying and quantifying MPs (Xu et al., 2019). Renner et al. (2018) reported that between 2015 and 2017, 61 out of 100 peer-reviewed research articles used either FTIR or Raman, which were usually paired with microscopy (Renner et al., 2018). Both techniques have been proven successful in detecting and quantifying many common plastic types (Jenner et al., 2022; Rasmussen et al., 2021), but only when the MPs have been extracted from the matrix (Renner et al., 2018; Liu et al., 2022; Okoffo et al., 2019). Moreover, these techniques are susceptible to the influence of additives and impurities present in both the plastics and the environmental matrix (Jung et al., 2021). In recent years, thermoanalytical methods such as analytical pyrolysis coupled to Gas Chromatography-Mass Spectrometry (Py–GC–MS) for the detection of MPs have been developed (Fischer and Scholz-Böttcher, 2017; Dümichen et al., 2017; Gomiero et al., 2019). The pros and cons for FTIR and Py–GC–MS were discussed by Käppler et al. (2018). Fischer and Scholz-Böttcher (2017) state that in order to have a reliable MP analysis of environmental samples, extraction and pre-concentration of potential MPs is necessary. Additionally, they indicate that the utilization of polymer-specific indicator compounds in MP analysis using Py–GC–MS diminishes the impact of matrix effects to some extent (Fischer and Scholz-Böttcher, 2017).

As MPs have been detected in several environmental and biological matrices, preparation of the samples before analysis has become a common strategy to increase the method sensitivity and reduce the interference of the matrix on the results. Such preparation usually entails several separation, digestion, and purification steps, in which plastic particles are isolated from both the inorganic and organic components (Renner et al., 2018; Fischer and Scholz-Böttcher, 2017; Gomiero et al., 2019; Mai et al., 2018; Kirstein et al., 2021; Primpke et al., 2020; Nasa et al., 2021). Depending on the complexity of the matrix, the combination of these steps can be highly time-consuming and require significant amounts of reagents and solvents. As a result, extensive work has been directed towards enhancing and exploring sample preparation protocols for various matrices. The review by Mai et al. (2018) is just one of many discussing the methods for quantifying MP in different matrices. Based on the findings outlined there, it is evident that pre-concentration, achieved through density separation, is a widely utilized approach with diverse solution compositions and densities and with reported recovery rates ranging from 67 % to 99 % (Mai et al., 2018). Density separation is the routine method for effectively removing inorganic matter from a sample, achieved by exploiting that lighter MP particles float in a liquid of higher density (Nasa et al., 2020; Lee and Chae, 2021; Prata et al., 2019; Mattsson et al., 2022; Quinn et al., 2017). When quantifying MPs by Py–GC–MS, the removal of inorganic matter ensures that no undesired catalytic effect occurs upon pyrolysis, increasing the reproducibility of the analysis. Removal of organic matter has commonly been done with the use of hydrogen peroxide (H_2O_2), which, for example, resulted in a removal efficiency of 83 % for wastewater samples oxidized for 7 days (Okoffo et al., 2019). An attempt was made to reduce the procedure duration by heating the sample to 70 °C. However, this approach resulted in the degradation or destruction of certain MPs (Hurley et al., 2018; Sujathan et al., 2017). An effective oxidation method that also requires a shorter reaction time is the Fenton reaction, which has already been successfully employed in removing organic matter from wastewater samples and other complex matrices. However, the reaction temperature should be controlled and kept between 20 and 40 °Celsius, avoiding too harsh oxidative conditions (Okoffo et al., 2019; Hurley et al., 2018).

Additionally, enzymatic treatment has been used to remove organic matter, as it ensures the selective digestion of organic components (e.g. cellulose or proteins) without damaging microplastics (Cole et al., 2014). Mintenig et al. (2017) employed an enzymatic-oxidative procedure for purification, whereby the process involved sequential addition of sodium dodecyl sulphate, protease, lipase, and cellulase to the sample. The efficiency of the same procedure was investigated, and further improvements were made by Löder et al. (2017). The study suggests that the universal enzymatic purification protocol (UEPP) holds significant promise as a harmonized operating procedure for routine monitoring in microplastics (MPs) studies (Löder et al., 2017).

Previous literature publications suggest that less extensive sample preparation could be used before Py–GC–MS analysis (Dümichen et al., 2017; Eisentraut et al., 2018). However, very limited information is available on the entity of matrix effects generated by matrix components. To the authors' knowledge, only a few studies have investigated matrix effects on thermoanalytical products when quantifying polymers in wastewater (Fischer and Scholz-Böttcher, 2017). The organic matter in wastewater consists of both dissolved and particulate fractions, with the majority comprising proteins, carbohydrates, and lipids (Raunkjaer et al., 1995). Such complex mixture of organic components could be challenging for detection when using thermoanalytical methods. Hence, a more in-depth knowledge of these aspects could provide valuable insights to optimize preparation strategies further. Here, we describe a systematic investigation of the effects of a sequence of widely used preparation steps for quantitative MP analysis in wastewater samples by analytical Py–GC–MS.

2. Materials and methods

2.1. Samples

A single 60 L sample of raw wastewater was taken at the inlet of the wastewater treatment plant Renseanlæg Øst, Aalborg, Denmark, right after mechanical screening of the inflow. The wastewater was brought back to the laboratory and stirred for one week to ensure maximum homogeneity. Stirring of the sample was performed at room temperature and without aeration, therefore this is not representative of a fresh raw wastewater sample. It was then split into 21 subsamples of 2 L each. To further ensure homogeneity, subsamples were obtained by first transferring 1 L of the raw wastewater into 21 2-L beakers and then adding the second liter in all the beakers. Deuterated polystyrene (PS-d8, PSS Polymer Standards Service GmbH, Germany) was used as an internal standard and spiked to the sample's aliquots (50–63 μg). Spiking was performed by directly adding the solid PS-d8 particulate to the samples. Spiking from a solution was avoided, as it would have led to loss of the internal standard during filtration. The set of 21 samples was subjected to triplicate analysis following each of the six steps of the sample preparation process, with an additional three samples being directly analyzed before undergoing any treatment.

2.2. Sample preparation

The sample preparation protocol involved a series of six sequential steps (Fig. 1). This procedure was adapted and modified from the preparation methodology described by Liu et al. (2019), Hurley et al. (2018), Löder et al. (2017), and Molazadeh et al. (2023). Sample preparation was started for all samples simultaneously to avoid differences in the outcome due to microbial growth or sample ageing. In step

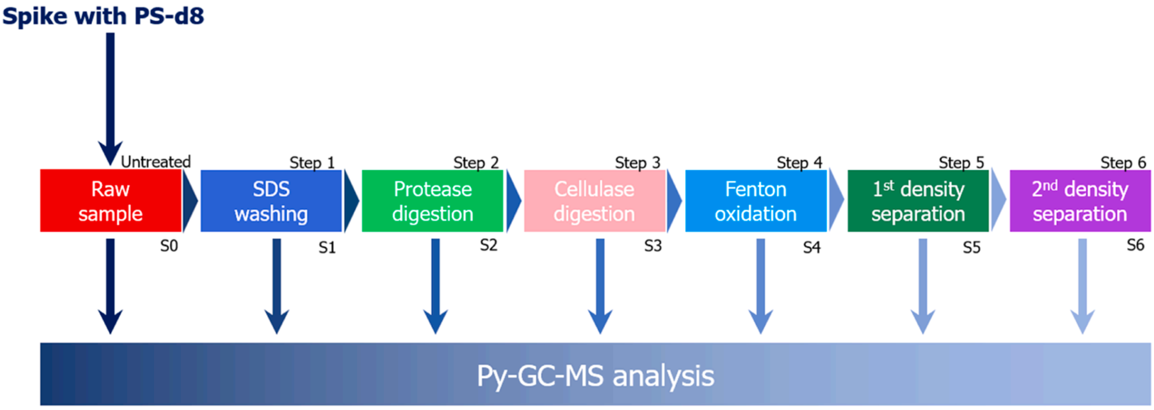

Fig. 1. Flowchart of the experimental methodology.

1, the sample (S1) was washed with a sodium dodecyl sulfate 5% w/vol solution for at least 48 h at 50 °C. For step 2, protein digestion involved the addition of 500 μL of protease solution (Protease from Bacillus sp.®, Sigma-Aldrich, USA) to 300 mL of tris buffer (pH 8.2). The sample was then incubated for at least 48 h at 50 °C. Cellulose was digested by enzymatic hydrolysis using 500 μL of cellulase (Cellulase enzyme blend®, Sigma-Aldrich, USA) and 500 μL of cellulolytic enzyme mixture (Viscozyme®L, Sigma-Aldrich; USA) in 300 mL of acetate buffer (pH 4.8), for a least 48 h at 50 °C, the cellulose digestion was implemented for Step 3. Step 4 involved a Fenton oxidation using 200 mL of Milli-Q water, 145 mL of 50 % H_2O_2, 65 mL of 0.1 M NaOH, and 62 mL of 0.1 M $FeSO_4$. The temperature was kept between 15 and 30 °C, and the sample was left overnight to ensure sufficient reaction time. In step 5, the sample (S5) was density separated using a sodium polytungstate (SPT) solution (TC-Tungsten Compounds, GmbH, Germany; $\rho = 1.8$ g cm^{-3}), where the sample was mixed by blowing in air from the bottom of the glass separatory funnel for 30 min. After settling for 24 h, a fraction of the settled material was purged, and the sample was left for further settling for 3 h. Subsequently, the supernatant was filtered on a 10 μm stainless steel filter. In the final Step 6, the sample (S6) underwent an additional density separation.

After each step, sample preparation was stopped for three of the samples. These samples were filtered using a 10 μm stainless steel filter and flushed with milli-Q water. Afterwards, they were flushed with 50 % ethanol in water (HPLC grade, Sigma-Aldrich), transferred to a 10 mL headspace vial, and dried in an evaporation bath (TurboVap® LV, Biotage) at 50 °C using a gentle nitrogen flow. Finally, the dried sample particles were re-suspended in 2–3 mL of 50 % ethanol in water, and of 25–50 μL aliquots of the suspensions were transferred to a deactivated stainless steel pyrolysis cup for analysis (see Section 2.3). To ensure a more homogeneous sampling of these suspensions, these aliquots were taken while stirring the suspension using a magnetic stir bar. In addition, samples with no PP or PS spiking at each preparation step were analyzed in triplicate, to ensure representativeness of the aliquots.

2.3. *Py–GC–MS analysis*

A volume ranging from 25 to 50 μL of the previously prepared suspensions was transferred to a pre-weighed pyrolysis cup and left to dry overnight. The sample was then weighed before adding 20 μL of a 0.1 g L^{-1} solution of PS-d8 in HPLC-grade dichloromethane (Sigma-Aldrich, USA) and weighed immediately afterwards to calculate the exact amount of said PS-d8. After adding the internal standard (IS), the sample was dried for at least 2 h. Immediately before Py–GC–MS analysis, 5 μL of a 0.2 g L^{-1} solution of deuterated anthracene (A-d10) in HPLC-grade hexane (Sigma-Aldrich, USA) was added to the sample and dried for 5 min. The cup was plugged with heated quartz wool to avoid sample loss while the sample's cup was dropped into the pyrolysis furnace.

2.4. *Instrumental parameters*

All analyses were performed using a Py–GC–MS system composed of a micro furnace pyrolyzer EGA/Py-3030D (FrontierLab, Japan) and an autoshot sampler AS-1020E (FrontierLab, Japan) connected to an 8890 gas chromatograph and a 5977 mass spectrometric detector (Agilent Technologies, USA). All cups used for the experiments were stainless steel. Blank analysis, conducted without using any cups, was performed before every run of new samples to ensure no contamination. The instrument sensitivity was measured throughout the day and over the experimental period. The polymer mass concentrations were calculated by fitting the results onto calibration curves obtained by pyrolyzing polypropylene (PP) and polystyrene (PS) polymer standards. Standard PP was directly analyzed as solid particles, while PS was analyzed as a 0.1 g L^{-1} solution in dichloromethane, to allow reliable weighing of polymer masses below 10 μg. The parameters of the Py–GC–MS method were: pyrolysis temperature 600 °C, interface: 280 °C, GC carrier: He (1 mL min^{-1}), injection: split mode 30:1 at 280 °C. Pyrolysis products were separated using an HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA). The temperature program was: 40 °C isotherm for 6 min, 20 °C min^{-1} up to 310 °C, then isotherm for 21 min. The analytes exiting the capillary column were detected using a quadrupole MS operating in EI positive mode (m/z range 35–550), and the transfer line and ion source were at 280 °C and 230 °C, respectively.

2.5. *Quality assurance/quality control*

QA/QC was performed following the guidelines described in the study by Kirstein et al. (2021) and Chand et al. (2022) to prevent cross-contamination of the samples. All glassware and the stainless-steel filters were muffled at 500 °C for at least 3 h before use or extensively rinsed with milli-Q water prior to use. All chemical solutions were filtered through 0.7 μm glass fiber filters. Sample preparation was carried out in a fume hood to limit airborne contamination (Wesch et al., 2017). Furthermore, to secure the best recovery, the same filter(s) were used throughout the sample preparation of an individual sample.

3. Results and discussion

3.1. *Py–GC–MS profiles and calibration curves in wastewater samples*

Representative profiles of a wastewater sample before any treatment (S0) and after the full preparation process (S6) are available in the Supplementary Material. Pyrolysis markers of two polymers, polypropylene (PP) and polystyrene (PS) were detected in all samples. Untreated samples provided very complex profiles, with peaks ascribable to species of different origins. At low retention times, small aromatics and phenols were detected. These compounds can be obtained from the pyrolysis of proteins (Orsini et al., 2017) At high retention times, homologue series of both linear alkanes and alcohols were detected. These compounds are ubiquitous species, in this case, likely associated with lubricants used at the wastewater sources, in the wastewater treatment plants, and the piping systems. Finally, fatty acids and sterols were detected at high retention times, indicating the presence of microorganisms.

A two-fold decrease in the total ion current was observed after the full sample preparation. This decrease mainly involved the high background noise due to small aromatics, alkanes, alcohols, and fatty acids, allowing the peaks of polymer pyrolysis products to increase their signal-to-noise ratios.

The main matrix components bearing a hydrocarbon chain in the Py–GC–MS profiles showed linear structures, suggesting a low chance of interference in the quantitation of PP using its trimer, which has a branched structure. On the other hand, the quantitation of PS through its monomer was unreliable, as expected from previous studies, due to the presence of numerous small aromatic compounds. For this reason, PS was quantified using its dimer. Extracted ion profiles are shown in Fig. 2. External and in-matrix calibration curves were built for PP and PS. In-matrix calibration curves were built by standard addition at each step of the preparation procedure, adding polymer standards ranging from 1 to 51 μg.

PP quantitation was based on the intensity of the area of the *m/z* 126 signal at 10 min retention time. *m/z* 126 is a significant fragment in the mass spectrum of the PP pyrolysis marker 2,4-dimethylhept-1-ene, which was chosen as the quantification compound. This *m/z* value was previously used for quantitative analysis of PP by Okoffo et al. (2020), Matsueda et al. (2021) and Dierkes et al. (2019). Fischer and Scholz-Böttcher (2019) used the same indicator compound, but the signal at *m/z* 70 was used instead for the quantitation. PS pyrolysis yields three main groups of pyrolysis compounds, namely dimers, trimers, and tetramers, all considered suitable for quantification (Krauskopf et al., 2020). Styrene is also formed in the pyrolysis of PS. However, the use of styrene as quantification marker for PS is deemed challenging due to its ubiquity (Krauskopf et al., 2020). Nevertheless, some studies still utilize the styrene monomer as pyrolysis product of PS used for quantification due to its high sensitivity (Dierkes et al., 2019). 3-butene-1,3-diyldibenzene (styrene dimer) was employed by Gomiero et al. (2019) as a quantification compound, utilizing the signal corresponding to *m/z* 208, as also recommended by Krauskopf et al. (2020) due to its higher sensitivity compared to the trimer. Based on these studies, the signal at *m/z* 208 in the mass spectrum of the styrene dimer was chosen to quantify PS. Deuterated styrene (*m/z* 112) was used to quantify PS-d8, as no risk of matrix interference exists for this compound. All calibration curves exhibited a coefficient of determination (R^2) greater than 0.87. For PS and PS-d8, the R^2 values exceeded 0.97. Details of the calibration parameters for all polymers are included in the Supplementary Material. The slopes of the in-matrix calibration curves were compared to assess the presence of matrix effect and to evaluate how the different steps of sample preparation impact it. The term “matrix effect” will be used in this study to address a change in the slope of the calibration curve, i.e. a change in the instrumental response. Statistical analysis to compare the slopes was performed through ANOVA, with a confidence interval of 0.95. Hence, a significant difference was considered when the p-value < 0.05. Numeric results of the statistical tests are included in the Supplementary Material.

3.2. *Matrix effect for PP and PS*

All calibration curves for PP and PS (Fig. 3, a and b respectively), including external and in-matrix curves, had a coefficient of determination $R^2 > 0.87$, with the external calibration curves exhibiting an R^2 of 0.99. The lower R^2 value for the in-matrix calibration curves may be attributed to the comparatively small amount of PP and PS added for the in-matrix calibration, in contrast to the amount already present in the sample. Notably, the signal area at *m/z* 126 for the molecule 2,4-dimethylhept-1-ene normalized with the IS was detected in samples without PP addition, the same was observed for the indicator ion of styrene dimer for PS.

For PP and PS, the in-matrix calibration curves slopes did not exhibit any significant differences, indicating no change in instrumental response throughout the sample preparation process. When comparing the external calibration curve for PP to the in-matrix ones, no significant differences were detected between the external and the in-matrix calibration curves slopes built with raw wastewater (S0) and with the sample obtained after the final density separation of the treatment (S6). However, significant differences were seen between the external calibration curve and all the intermediate steps. As for PP, no significant

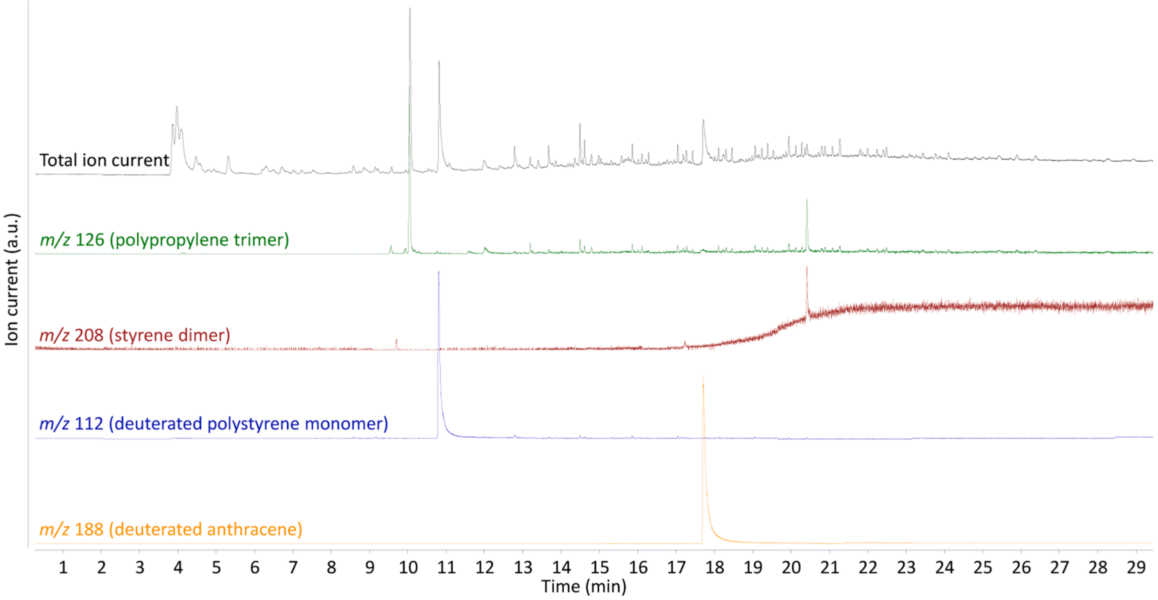

Fig. 2. Total ion chromatogram of a representative S6 wastewater sample, and extracted ion profiles for the pyrolytic markers of PP (dimer), PS (trimer), deuterated PS (monomer), and of the internal standard (deuterated anthracene).

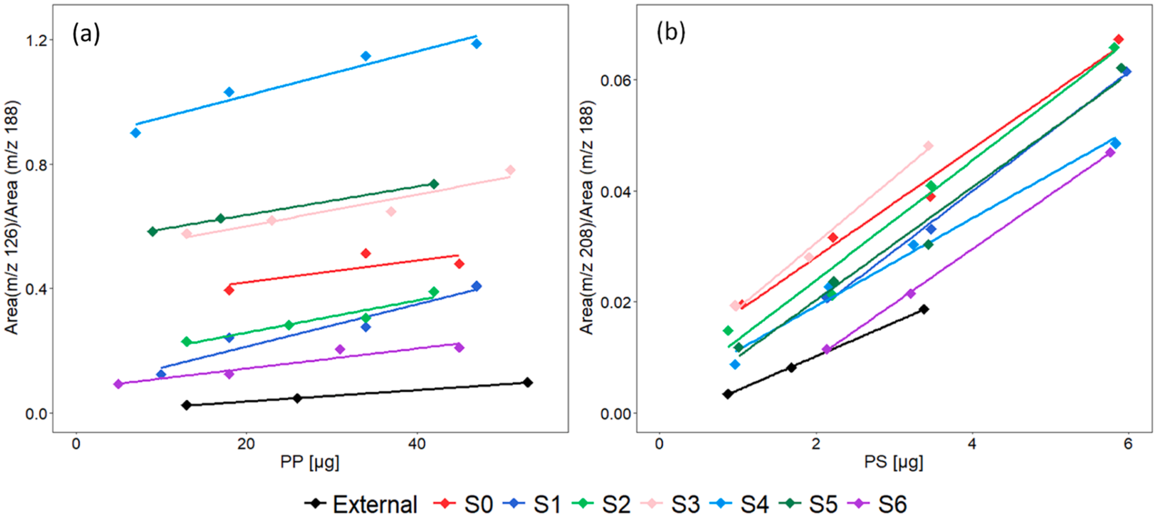

Fig. 3. External and in-matrix (S0-S6) calibration curves for PP (a) and PS (b)using normalized peak areas of marker compounds. For in-matrix calibration curves, the x-axis indicates the weight of the polymer spike. Calibration parameters are available in the Supplementary Material.

differences were found among all the in-matrix PS calibration curves slopes. Contrary to PP, the slope of the external calibration curve for PS was only significantly different from the in-matrix one made in sample S3 (cellulase digestion) and S4 (Fenton oxidation).

As stated before, matrix effect is intended in this study as a change in the instrumental response, i.e. a change in the slope of the calibration curves. The most frequent cause for such an effect in Py–GC–MS experiments is a change in the pyrolysis mechanism taking place in the furnace. A change in the mechanism, due to interactions between the polymers and the matrix, can alter the pyrolytic yields of each pyrolysis product, including the markers for PP and PS, and change the correlation between polymer amount and peak area. Therefore, the different instrumental responses observed at intermediate sample preparation steps are most likely due to the changes in composition of the matrix. As the matrix changes along the sample preparation process, its ability to interfere with the pyrolysis mechanism of PP and PS changes, and matrix effects are observed. Interestingly, negligible matrix effects were observed for both polymers in samples S0 and S6. While a negligible matrix effect for S6 was expected, the fact that S0 showed the same instrumental response of the external calibration curves is surprising. This result indicates that the untreated wastewater matrix has a lower ability of interfering with the pyrolysis mechanisms of PP and PS. This is extremely interesting, as it shows that Py–GC–MS analysis provides comparable analytical sensitivities for the quantitation of PP and PS in our experiments, despite the wastewater matrix. It also implies that the external calibration curve could be reliably used to quantify PP and PS in untreated environmental samples, not only samples that have undergone the complete preparation procedure.

While significant changes in the slopes of the calibration curves were observed only for some of the samples, consistent variability was observed for the y-intercept values. This variability did not follow a specific trend with the increase of the number of preparation steps, which was expected since real samples were used, resulting in variations in the native PP and PS content.

As for the change in slope, the changes in the values of the y-axis are due to the composition of the matrix, and how it changes along the sample preparation steps. Natural materials have the potential to introduce interfering indicator compounds during pyrolysis (Fischer and Scholz-Böttcher, 2017; Dierkes et al., 2019). Fischer and Scholz-Böttcher stated (Fischer and Scholz-Böttcher, 2017) "The use of polymer-specific indicator compounds in MP analysis makes Py–GC–MS independent of matrix residues to a certain extent". Consistent with their findings, the current study observed no matrix effect in the untreated wastewater and the final sample after preparation. It is worth noticing that indicator compounds for PS, PVC, and PE have been shown to be influenced by pyrolysis products of biopolymers. Specifically, the detection of styrene monomer from biopolymers may lead to interference, emphasizing the importance of selecting appropriate indicator compounds to mitigate matrix effects (Fischer and Scholz-Böttcher, 2017). In a different study, it has been reported that the measurement of PP and PS in environmental samples is not substantially influenced by naturally occurring substances unless there is a considerable amount of wood present (Dierkes et al., 2019). On the other hand, Matsui et al. (2020) recommend that sample preparation should be employed when dealing with matrix-rich samples to avoid interference and ensure accurate quantitation (Matsui et al., 2020). A less likely explanation is cross-contamination during sample handling and preparation. Cross-contamination was already described in previous literature results from Mintenig et al. (2017) and Simon et al. (2018), where laboratory blanks were shown to contain MPs up to average particle numbers between 151 and 2110, respectively. As the different calibration curves correspond to different sets of preparation steps, the risk of cross-contamination was different for each of them, introducing a potential explanation for the different y-intercept values.

3.3. *Quantitation of microplastics*

The native content of PP and PS in all samples was calculated using the external calibration curves. Calculation of the native content from the in-matrix curves was deemed unreliable, as these curves were built with only one of the three sub-samples at each sample preparation step, while the external ones were built with all subsamples and all replicates at all steps. Nevertheless, native PP and PS contents calculated with in-matrix calibration curves are available in the Supplementary Material for completeness.

The results are shown in Fig. 4(a) and (b) for PP and PS, respectively. The figure shows total polymer concentrations in the samples, which were calculated by the measured polymer weights by accounting for aliquoting and total original sample volume. External calibration curves have also been used for MP quantitation in many other studies described in the literature (Fischer and Scholz-Böttcher, 2017; Gomiero et al., 2019; Fischer and Scholz-Böttcher, 2019; Funck et al., 2020).

The results shown in Fig. 4 suggest a loss of mass for both polymers across preparation steps. ANOVA was conducted with a confidence interval of 95 % to determine if there was a significant difference in mass between the steps. The analysis revealed a significant difference in the

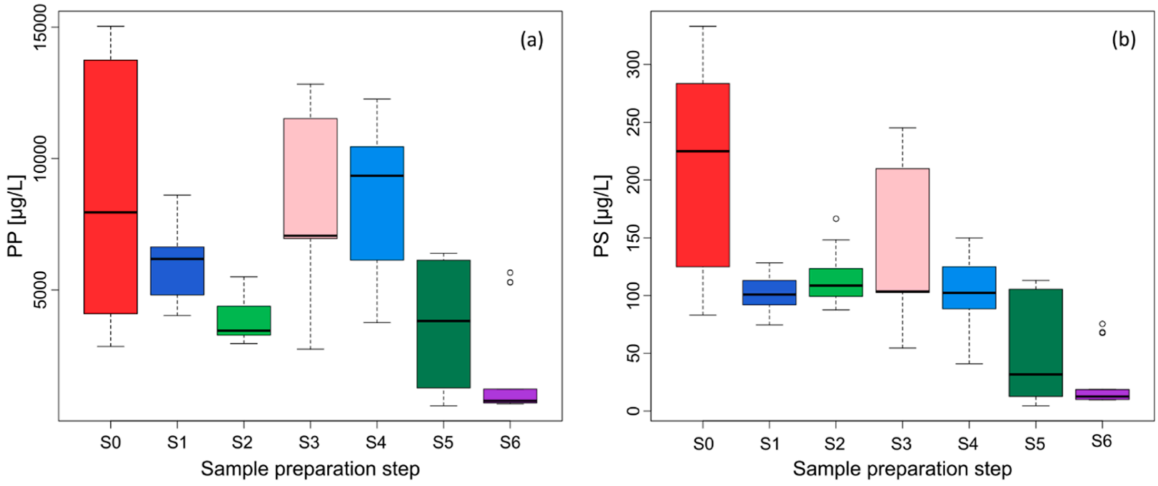

Fig. 4. Polymer concentrations of PP (a) and PS (b) at the different preparation steps, calculated from polymer masses measured by Py–GC–MS by accounting for aliquots and original sample volume.

determined PP mass during sample preparation, with observed differences between S0 and S2, S5, and S6. Similarly, for PS, significant differences in determined polymer mass were observed between untreated wastewater and samples analyzed after the other steps, except S3. Notably, a higher concentration of PP was detected in samples S3 and S4 compared to samples S1 and S2, which underwent fewer preparation steps. A similar observation was made for PS, with a high concentration detected only in sample S3 compared to samples S1 and S2. The heterogeneity of the initial sample led to variations of native amounts of the polymers among different samples, making calculation of the recovery impossible, however an overall decreasing trend is noticeable. In addition, the possibility of higher recoveries at specific preparation steps due to laboratory handling cannot be ruled out which could also explain the non-monotonous decreasing trend of polymer masses as the number of preparation steps increases.

Nevertheless, the results showed an overall significant decrease in the content of both polymers in the samples, especially when comparing the untreated samples with the fully treated ones. This suggests that sample preparation can lead to partial loss of the polymers. However, it should be noted that the trends in Fig. 4 are non-monotonous, and that the calculation of polymer concentrations from the external calibration curves does not account for the innate variability between samples, as well as for the presence of matrix effects that has been observed in samples S1 through S5 for PP, and in samples S3 and S4 for PS. When matrix effect is present, the external calibration curve will provide an over- or underestimation of the native polymer concentrations compared to the in-matrix curves. Therefore, a rigorous evaluation of polymer recovery should rely only on PS-d8, which was added in all samples in known amounts. This will be addressed in Section 3.4.

The loss of polymer during sample preparations aligns with the findings of Simon et al. (2018), who reported recoveries of 77.7 ± 11.6 % for PS microbeads and only 57.6 ± 25.1 % for HDPE particles. These results suggest distinct behaviors between beads and naturally occurring particles. Furthermore, a study by Halbach et al. (2021) showed recoveries of $<$ 50 % when investigating density separation using Py–GC–MS. It is noteworthy that this was evaluating only one step, whereas our study involves multiple steps.

The risk of underestimating MP concentrations due to losses during preparation has been a subject of recent discussion in the research field. In a meta-analysis conducted by Way et al. (2022), recovery rates of MP were examined based on published data up until January 2021. The analysis revealed MP recovery rates in wastewater effluent and sludge of 76 and 89 % for high-density polymers and low-density polymers, respectively. Additionally, a weighted mean calculation estimated that MP research may be underestimating the actual MP abundance by approximately 14 % (Way et al., 2022). It is important to note that this meta-analysis focused on particle numbers rather than weight. Still, it provides an indication that sample preparation procedures carry a risk of losing MP particles, which corroborates the observed loss of PP and PS in this study.

One step in the preparation procedure is the density separation, and another study investigated recovery rates specifically in wastewater effluent and sludge, reporting recovery rates ranging from 57 % to 98 % (Liu et al., 2022). Based on their experiments, the researchers also concluded that density separation methods for MP may not effectively recover all types of polymers, leading to underestimation of both MP quantity and polymer types. Kang et al. (2020), in their review, highlighted that higher-density separation solutions generally result in higher recovery rates (Kang et al., 2020). Therefore, the utilization of a sodium polytungstate solution at 1.8 g cm^{-3} in this study had the potential to yield a higher recovery rate. Previous studies have also demonstrated a favorable recovery rate (84.5 ± 3.3 %) following enzymatic purification using PE as a representative polymer model (Löder et al., 2017).

3.4. Matrix effect for PS-d8

All calibration curves for PS-d8 exhibited a coefficient of determination $R^2 > 0.97$ (Fig. 5). The impact of the matrix effect appeared to be more pronounced when quantifying PS-d8, a clear pattern did not emerge upon solely considering the in-matrix calibration curves. For instance, a significant difference between slopes was observed between S1 (SDS washing) and the subsequent enzyme treatment steps samples S2 and S3 (protease and cellulase digestion). However, in the last three steps of the treatment, no significant differences were observed, which was expected since the matrix would be more similar in the latter steps after removal of proteins and cellulose. Additionally, there was a matrix difference between the protease step (S2) and both the first (S5) and second density separation (S6); however, a significant difference was only found when comparing cellulase treatment (S3) to the first density separation (S5), but no matrix effect was observed when comparing cellulase treatment (S3) and the second density separation (S6). This could be explained by further removal of inorganic matter that interfered with PS-d8 and was not visible when the sample was less clean.

There was no evidence of a matrix effect when comparing the external calibration curve to untreated wastewater (S0), the first density separation (S5), and the second density separation (S6). Thus, the sample preparation steps appeared to be unnecessary. However, it is

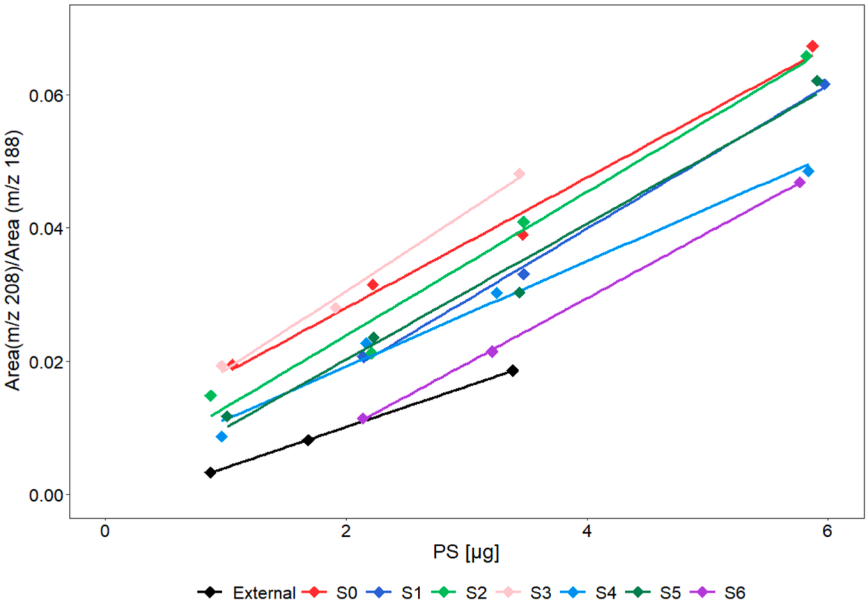

Fig. 5. External and in-matrix (S0-S6) calibration curves for PS-d8 using normalized peak areas of marker compounds. For in-matrix calibration curves, the x-axis indicates the weight of the polymer spike. Calibration parameters are available in the Supplementary Material.

noteworthy that continuing the sample preparation through to the final step did not produce a matrix effect and may enhance the sensitivity of the quantification.

As for PP and PS, similar observations were made for PS-d8, where no matrix effect was detected between the external calibration curve and untreated wastewater (S0), as well as the second density separation (S6). This confirms the overall conclusion that sample preparation may be unnecessary for the quantification of PP, PS, and PS-d8 when the matrix is wastewater. Additionally, the external calibration curves can be used for quantification and remain valid even after undergoing the sample preparation of this study.

The results of MP quantitation showed that a significantly lower amount of PP and PS was detected in the fully treated samples than in untreated ones. As the PS-d8 was added to the samples before preparation, its quantitation was used to estimate the polymer recovery. However, it is important to note that the quantity of PS-d8 added prior to analysis exceeded the amount present in the subsample within the pyrolysis cup. Hence, these results can only provide an indication of the recovery, and unfortunately, reanalysis of the samples was not feasible due to the destructive nature of the technique. The findings revealed a decline in polymer mass with an increasing number of preparation steps (Fig. 6). Recoveries as low as 40 % were observed for the samples after all steps. As mentioned in Section 3.3, Simon et al. (2018) reported a recovery rate of 57.6 ± 25.1 % for irregularly shaped particles which used a similar sample preparation. As mentioned earlier, the recovery for PS-d8 serves as an indication of the tendency, and the numerical values of the recoveries maintain the same overall trend when replicates are analyzed. Another study by Horton et al. (2021) found a discrepancy in the recovery between spiked samples in wastewater sludges and effluents. The recovery was notably lower in wastewater sludges at 52.4 ±

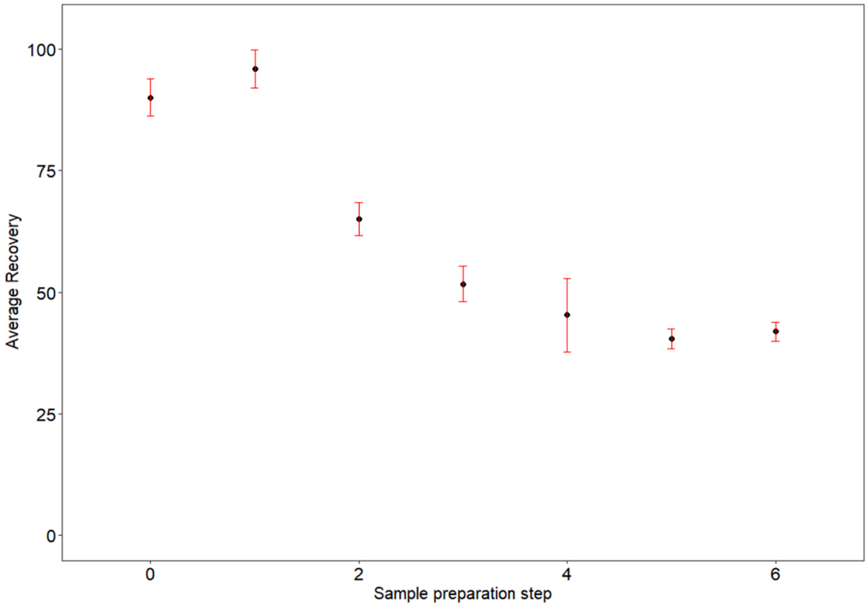

Fig. 6. PS-d8 recoveries evaluated with in-matrix calibration as a function of the number of preparation steps.

14.1 %, compared to 101 ± 26.8 % in effluent. This highlights the considerable challenges of achieving high recovery in complex matrices, as they found that the matrix interacts with the polymers leading to polymer loss. Therefore, the addition of PS-d8 to the environmental samples before preparation is the most suitable way to evaluate the recovery (Horton et al., 2021). Additionally, a slight improvement in recovery was observed after the SDS washing step, which can be explained by SDS's ability to disintegrate organic matter and thereby separate entrapped MPs (Löder et al., 2017). The high recovery observed in the initial two steps implies that the sample preparation process may be redundant for the thermal degradation method.

It is important to notice that the trend in estimated polymer recovery for PS-d8 is consistent with those observed in the previous Section for PP and PS. This suggests that the recoveries for PP and PS calculated in this study provide a reliable estimation, even with the risk of matrix interference or cross-contamination during handling. Although the samples only provide an indication of recovery, the observed trend strongly indicates a loss of microplastic particles during sample preparation. From the literature, a higher recovery rate was expected (Way et al., 2022; Liu et al., 2022; Kang et al., 2020).

3.5. *Effect of matrix on instrumental performances*

As discussed, the negligible effect of the matrix on the analytical sensitivity for the quantitation of PP and PS suggests that Py–GC–MS could analyze wastewater samples without extensive preparation. However, the contamination of the instrument must also be considered when analyzing untreated samples. The analysis of a complex environmental matrix by Py–GC–MS could generate a high amount of non-informative pyrolysis products, which could, in time, reduce instrumental performances and increase the frequency of instrument maintenance.

With the aim of evaluating the effect of matrix pyrolysis products on instrumental performances, the signal intensity of the internal standard A-d10 was monitored throughout the series of analyses (Fig. 7). A decrease in the signal intensity was noticed as the number of analyses increased. The decline in signal intensity can be linked to the presence of samples with complex matrix, which, in turn, may raise instrument maintenance requirements. Therefore, while sample preparation led to partial loss of MP without significant increase in analytical sensitivity, it, conversely, improved instrumental performance by reducing the need for maintenance and consequently enhancing the response factors of compounds of interest in the mass spectrometric detector. After analyzing 26 samples, regular maintenance, such as cleaning the pyrolysis needle, was conducted, which is indicated with the red line in Fig. 7. After maintenance, instrumental sensitivity was only partially restored. Additional, more drastic maintenance such as replacing the GC column or the quartz pyrolysis tube would have most likely led to a more significant restoration of the instrumental performances.

4. Conclusions

The absence of a significant matrix effect for PP and PS throughout the samples and the sample preparation used in this study indicates that the matrix components did not significantly affect the pyrolysis process or the sensitivity of the analytical method. In addition, the quantitation of PP and PS at the different steps of the preparation revealed a significant loss of polymer mass, as the complex sample preparation process increased the likelihood of MPs falling below the instrumental detection limits. A more in-depth investigation of this aspect could be obtained by replicating the experiments using filtered, deionized water containing known amounts of PP and PS. These experiments could also be performed to separate the contribution to polymer loss due to the sample preparation steps, and due to the nature of the wastewater matrix. Furthermore, the analysis of untreated samples increased the need for instrumental maintenance, as the response factors of the mass spectrometer were found to rapidly decrease as the number of analyses of matrix-rich samples increased.

These results highlight that the main advantages of sample preparation are the pre-concentration of MP particles and the reduced need for instrumental maintenance. Therefore, sample preparation should focus mainly on concentrating the analytes in small sample volumes, limiting MP losses as much as possible, rather than on ensuring quantitative removal of the matrix using many steps. This, in turn, suggests that Py–GC–MS can be employed for detecting and quantifying MPs prior to analysis with other techniques, such as FTIR and Raman spectroscopies, which require more extensive matrix removal. This makes it a valuable tool for monitoring and screening MPs in wastewater. Imaging spectroscopic techniques can provide valuable information about the size and number of MP particles, which cannot be obtained through the thermal degradation method alone, contributing to a more comprehensive and complementary characterization of the samples.

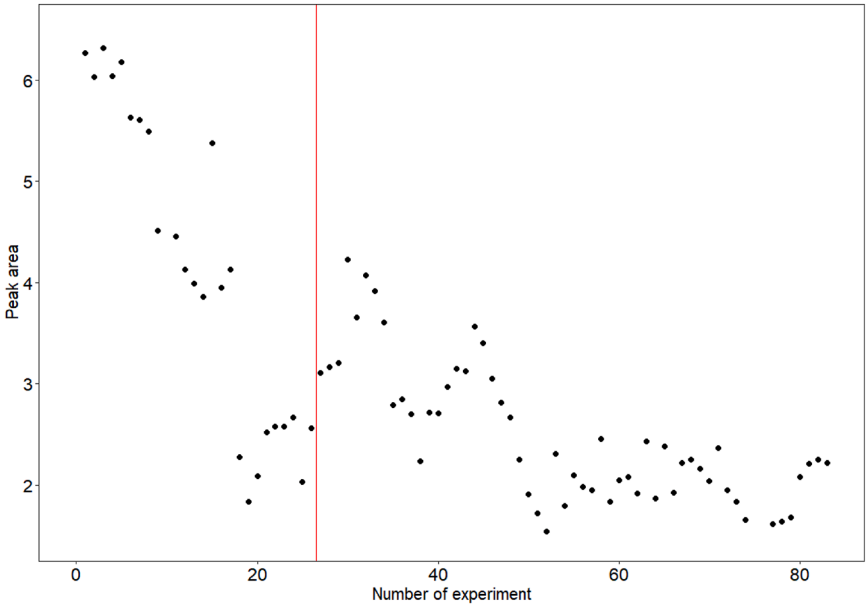

Fig. 7. Area of A-d10 (million au) over the number of experiments conducted. The red line shows maintenance.

Without the application of sample preparation, such imaging techniques would not be feasible, limiting the insights into the morphology and abundance of MP particles.

Future studies should be aimed at investigating polymer recovery and matrix effect when dealing with other environmental matrices, such as soil, seawater, and biota. The influence of sample matrix should also be evaluated on other types of polymers, including polycondensation polymers, which are expected to be more reactive and sensitive towards matrix components.

CRediT authorship contribution statement

Jeanette Lykkemark: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Mattonai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alvise Vianello:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Alessio Gomiero:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. **Francesca Modugno:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Jes Vollertsen:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

Francesca Modugno reports financial support was provided by North Atlantic Microplastic Center (NO). Marco Mattonai reports financial support was provided by Frontier Laboratories Research Fund 2023 (JP). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The project was supported by the North Atlantic Microplastic Centre (www.namc.no) Pillar 1 “Sensitive Analytical Methods”. The Authors also wish to thank the Center for Instrument Sharing of the University of Pisa for providing the Py-GC-MS instrumentation, and the Frontier Laboratories Research Fund 2023 (recipient: Marco Mattonai) for financial support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122055.

References

Chand, R., Kohansal, K., Toor, S., Pedersen, T.H., Vollertsen, J., 2022. Microplastics degradation through hydrothermal liquefaction of wastewater treatment sludge. J. Clean. Prod. 335, 130383 https://doi.org/10.1016/J.JCLEPRO.2022.130383.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4 https://doi.org/10.1038/srep04528.

Dierkes, G., Lauschke, T., Becher, S., Schumacher, H., Földi, C., Ternes, T., 2019. Quantification of microplastics in environmental samples via pressurized liquid extraction and pyrolysis-gas chromatography. Anal. Bioanal. Chem. 411 (26), 6959–6968. https://doi.org/10.1007/s00216-019-02066-9.

Dümichen, E., Eisentraut, P., Bannick, C.G., Barthel, A.K., Senz, R., Braun, U., 2017. Fast identification of microplastics in complex environmental samples by a thermal degradation method. Chemosphere 174, 572–584. https://doi.org/10.1016/j.chemosphere.2017.02.010.

Eisentraut, P., et al., 2018. Two birds with one stone?fast and simultaneous analysis of microplastics: microparticles derived from thermoplastics and tire wear. Environ. Sci. Technol. 5, 608–613.

European Commission, 2013. “MSDF Guidance on Monitoring Marine Litter,”. doi:10.2788/99475.

Fischer, M., Scholz-Böttcher, B.M., 2017. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. Environ. Sci. Technol. 51 (9), 5052–5060. https://doi.org/10.1021/acs.est.6b06362.

Fischer, M., Scholz-Böttcher, B.M., 2019. Microplastics analysis in environmental samples – recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal. Methods 11 (18), 2489–2497. https://doi.org/10.1039/C9AY00600A.

Funck, M., Yildirim, A., Nickel, C., Schram, J., Schmidt, T.C., Tuerk, J., 2020. Identification of microplastics in wastewater after cascade filtration using Pyrolysis-GC–MS. MethodsX 7. https://doi.org/10.1016/j.mex.2019.100778.

Gomiero, A., Øysæd, K.B., Agustsson, T., van Hoytema, N., Van Thiel, T., Grati, F., 2019. First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. Chemosphere 227, 705–714. https://doi.org/10.1016/j.chemosphere.2019.04.096.

Halbach, M., Baensch, C., Dirksen, S., Scholz-Böttcher, B.M., 2021. Microplastic extraction from sediments established?-A critical evaluation from a trace recovery experiment with a custom-made density separator. Anal. Methods 13 (44), 5299–5308. https://doi.org/10.1039/d1ay00983d.

Horton, A.A., et al., 2021. Semi-automated analysis of microplastics in complex wastewater samples. Environ. Pollut. 268 https://doi.org/10.1016/j.envpol.2020.115841.

Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ. Sci. Technol. 52 (13), 7409–7417. https://doi.org/10.1021/acs.est.8b01517.

JRCT Report, 2021. “Current status of the quantification of microplastics in water,”. doi:10.2760/6228.

Jenner, L.C., Rotchell, J.M., Bennett, R.T., Cowen, M., Tentzeris, V., Sadofsky, L.R., 2022. Detection of microplastics in human lung tissue using μFTIR spectroscopy. Sci. Total Environ. 831, 154907 https://doi.org/10.1016/J.SCITOTENV.2022.154907.

Jung, S., Cho, S.H., Kim, K.H., Kwon, E.E., 2021. Progress in quantitative analysis of microplastics in the environment: a review. Chem. Eng. J. 422 (no. May), 130154 https://doi.org/10.1016/j.cej.2021.130154.

Kang, P., Ji, B., Zhao, Y., Wei, T., 2020. How can we trace microplastics in wastewater treatment plants: a review of the current knowledge on their analysis approaches. Sci. Total Environ. 745, 140943 https://doi.org/10.1016/j.scitotenv.2020.140943.

Käppler, A., et al., 2018. Comparison of μ-ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. Anal. Bioanal. Chem. 410, 5313–5327.

Kirstein, I.V., et al., 2021. Drinking plastics? – Quantification and qualification of microplastics in drinking water distribution systems by μFTIR and Py-GCMS. Water Res. 188, 116519 https://doi.org/10.1016/J.WATRES.2020.116519.

Krauskopf, L.M., Hemmerich, H., Dsikowitzky, L., Schwarzbauer, J., 2020. Critical aspects on off-line pyrolysis-based quantification of microplastic in environmental samples. J. Anal. Appl. Pyrolysis 152 (no. April), 104830. https://doi.org/10.1016/j.jaap.2020.104830.

La Nasa, J., Biale, G., Fabbri, D., Modugno, F., 2020. A review on challenges and developments of analytical pyrolysis and other thermoanalytical techniques for the quali-quantitative determination of microplastics. J. Anal. Appl. Pyrolysis 149 (no. March), 104841. https://doi.org/10.1016/j.jaap.2020.104841.

La Nasa, J., Biale, G., Mattonai, M., Modugno, F., 2021. Microwave-assisted solvent extraction and double-shot analytical pyrolysis for the quali-quantitation of plasticizers and microplastics in beach sand samples. J. Hazard. Mater. 401 https://doi.org/10.1016/j.jhazmat.2020.123287.

Lee, J., Chae, K.J., 2021. A systematic protocol of microplastics analysis from their identification to quantification in water environment: a comprehensive review. J. Hazard. Mater. 403, 124049 https://doi.org/10.1016/j.jhazmat.2020.124049.

Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199 https://doi.org/10.1016/J.ENVINT.2022.107199.

Liu, F., Nord, N.B., Bester, K., Vollertsen, J., 2020. Microplastics removal from treated wastewater by a biofilter. Water 12 (4). https://doi.org/10.3390/W12041085 (Switzerland).

Liu, F., Olesen, K.B., Borregaard, A.R., Vollertsen, J., 2019. Microplastics in urban and highway stormwater retention ponds. Sci. Total Environ. 671, 992–1000. https://doi.org/10.1016/j.scitotenv.2019.03.416.

Liu, Y., et al., 2023. Exploration of occurrence and sources of microplastics (>10 μm) in Danish marine waters. Sci. Total Environ. 865 https://doi.org/10.1016/j.scitotenv.2022.161255.

Liu, Y., Wang, B., Pileggi, V., Chang, S., 2022. Methods to recover and characterize microplastics in wastewater treatment plants. Case Stud. Chem. Environ. Eng. 5 (no. January), 100183 https://doi.org/10.1016/j.cscee.2022.100183.

Löder, M.G.J., et al., “Enzymatic Purification of Microplastics in Environmental Samples,” 2017, doi:10.1021/acs.est.7b03055.

Mai, L., Bao, L.J., Shi, L., Wong, C.S., Zeng, E.Y., 2018. A review of methods for measuring microplastics in aquatic environments. Environ. Sci. Pollut. Res. 25 (12), 11319–11332. https://doi.org/10.1007/s11356-018-1692-0.

Matsueda, M., et al., 2021. Preparation and test of a reference mixture of eleven polymers with deactivated inorganic diluent for microplastics analysis by pyrolysis-GC–MS,". J. Anal. Appl. Pyrolysis 154 (no. January), 104993. https://doi.org/10.1016/j.jaap.2020.104993.

Matsui, K., et al., 2020. Identification algorithm for polymer mixtures based on Py-GC/MS and its application for microplastic analysis in environmental samples. J. Anal. Appl. Pyrolysis 149 (no. February), 104834. https://doi.org/10.1016/j.jaap.2020.104834.

Mattsson, K., Ekstrand, E., Granberg, M., Hassellöv, M., Magnusson, K., 2022. "Comparison of pre treatment methods and heavy density liquids to optimize microplastic extraction from natural marine sediments," no. 0123456789, pp. 1–9.

Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. "Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging," vol. 108, pp. 365–372, , doi:10.1016/j.watres.2016.11.015.

Molazadeh, M., Liu, F., Simon-sánchez, L., Vollersten, J., 2022. Buoyant microplastics in freshwater sediments–how do they get there ? Sci. Total Environ. 860, 2023. https://doi.org/10.1016/j.scitotenv.2022.160489.

Okoffo, E.D., et al., 2020. Identification and quantification of selected plastics in biosolids by pressurized liquid extraction combined with double-shot pyrolysis gas chromatography–mass spectrometry. Sci. Total Environ. 715 (no. January), 136924 https://doi.org/10.1016/j.scitotenv.2020.136924.

Okoffo, E.D., O'Brien, S., O'Brien, J.W., Tscharke, B.J., Thomas, K.V., 2019. Wastewater treatment plants as a source of plastics in the environment: a review of occurrence, methods for identification, quantification and fate. Environ. Sci. (Camb.) 5 (11), 1908–1931. https://doi.org/10.1039/c9ew00428a.

Orsini, S., Parlanti, F., Bonaduce, I., 2017. Analytical pyrolysis of proteins in samples from artistic and archaeological objects. J. Anal. Appl. Pyrolysis 124, 643–657. https://doi.org/10.1016/j.jaap.2016.12.017.

OSPAR, 2010. Guideline for Monitoring Marine litter on the Beachs in the OSPAR Maritime Area, 1. OSPAR Commission, p. 84.

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-santos, T., 2019. "Trends in Analytical Chemistry Methods for sampling and detection of microplastics in water and sediment : a critical review Density separation," vol. 110, pp. 150–159. doi:10.1016/j.trac.2018.10.029.

Primpke, S., Fischer, M., Lorenz, C., Gerdts, G., Scholz-Böttcher, B.M., 2020. Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics. Anal. Bioanal. Chem. 412 (30), 8283–8298. https://doi.org/10.1007/s00216-020-02979-w.

Quinn, B., Murphy, F., Ewins, C., 2017. Validation of density separation for the rapid recovery of microplastics from sediment. Anal. Methods 1491–1498. https://doi.org/10.1039/c6ay02542k.

Rasmussen, L.A., Iordachescu, L., Tumlin, S., Vollertsen, J., 2021. A complete mass balance for plastics in a wastewater treatment plant–macroplastics contributes more than microplastics. Water Res. 201 https://doi.org/10.1016/j.watres.2021.117307.

Raunkjaer, K., Hvitved-Jacobsen, T., Nielsen, P.H., Transformation of organic matter in a gravity sewer. 1995.

Renner, G., Schmidt, T.C., Schram, J., 2018. ScienceDirect Analytical methodologies for monitoring micro (nano) plastics : which are fit for purpose ? Curr. Opin. Environ. Sci. Health 1, 55–61. https://doi.org/10.1016/j.coesh.2017.11.001.

Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Res. 142, 1–9. https://doi.org/10.1016/j.watres.2018.05.019.

Simon, M., Vianello, A., Vollertsen, J., 2019. Removal of >10 µm microplastic particles from treated wastewater by a disc filter. Water 11 (9). https://doi.org/10.3390/w11091935 (Switzerland).

Sujathan, S., Kniggendorf, A., Kumar, A., Roth, B., Rosenwinkel, K.H., Nogueira, R., 2017. Heat and bleach : a cost-efficient method for extracting microplastics from return activated sludge. Arch. Environ. Contam. Toxicol. 73 (4), 641–648. https://doi.org/10.1007/s00244-017-0415-8.

Way, C., Hudson, M.D., Williams, I.D., Langley, G.J., 2022. Evidence of underestimation in microplastic research: a meta-analysis of recovery rate studies. Sci. Total Environ. 805, 150227 https://doi.org/10.1016/j.scitotenv.2021.150227.

Web of Science, "Web of science." Accessed: Apr. 23, 2023. [Online]. Available: https://www-webofscience-com.zorac.aub.aau.dk/wos/woscc/analyze-results/3a71adb3-a9f5-4d68-bd19-4aec334828a9-849562e8.

Wesch, C., Elert, A.M., Wörner, M., Braun, U., Klein, R., Paulus, M., 2017. Assuring quality in microplastic monitoring: about the value of clean-air devices as essentials for verified data. Sci. Rep. 7 (1) https://doi.org/10.1038/s41598-017-05838-4.

Xu, J., Thomas, K.V., Luo, Z., Gowen, A.A., 2019. Trends in analytical chemistry FTIR and Raman imaging for microplastics analysis : state of the art, challenges and prospects. Trends Anal. Chem. 119, 115629 https://doi.org/10.1016/j.trac.2019.115629.