Identification and quantification of polystyrene microplastics in marine sediments facing a river mouth through NMR spectroscopy

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A B S T R A C T

Accurate identification and quantification of microplastic pollution in marine sediments are crucial for assessing their ecological impact. In this study, we explored the potential of Nuclear Magnetic Resonance (NMR) spectroscopy as an analytical tool for the analysis of microplastics in complex environmental matrices such as marine sediments. Two common plastic polymers, polystyrene (PS) and acrylonitrile butadiene styrene (ABS), were investigated. The marine sediments facing the Tiber River mouth (Italy) were collected according to a bathymetric gradient. Results demonstrated the successful detection and quantification of PS in all sediment samples (within a range of 12.3–64.6 μg/L), while no ABS significant signals were found. An increment trend with depth was observed in the PS signal, relatable to its physicochemical properties and the Tiber River plume hydrodynamic characteristics. The NMR's non-destructive nature and minimal sample preparation represent a promising avenue for standardizing protocols to assess the microplastic distribution and impact in marine sediments.

1. Introduction

Microplastics (MPs), synthetic solid particles made up of a polymeric matrix ranging in size between 1 μm and 5 mm (GESAMP, 2020), present a major human-health risk (Hwang et al., 2020; Ragusa et al., 2021). Their small size depends on their origin that can be primary or secondary. Primary MPs are intentionally produced in microscopic size for their use especially in cosmetics and detergent products (Boucher and Billard, 2020). Secondary ones are the result of mechanical fragmentation and secondary photo-biodegradation (Eriksen et al., 2014; Song et al., 2017). Due to their small size coupled with their capacity to adsorb pollutants, they may harm cellular activity and functionality, accumulate in tissues, and subsequently in food webs (Avio et al., 2015; Browne et al., 2013; Campos et al., 2021; Chen et al., 2021; Ding et al., 2018; Espinosa et al., 2016; Mattsson et al., 2015, 2017; Rochman et al., 2013, 2014; Sussarellu et al., 2016; Winkler et al., 2020; Yokota et al., 2017). Microplastic (MP) contamination affects all ecosystems (Campanale et al., 2020a; Cincinelli et al., 2019; Crew et al., 2020; Dris et al.,

2016, 2018). However, their presence in the marine waters represents the main subject of nearly all available studies; while knowledge relating to MP contribution by rivers to the marine environment is relatively scarce (Alfonso et al., 2021). It was estimated that over 8000 tons of MPs were transported into the Mediterranean Basin exclusively from riverine sources, in the decade 2006–2016 (Boucher and Billard, 2020).

Furthermore, there is a persisting lack of data concerning how different polymeric fragments sink at different depths and distances away from the coast according to the polymeric density, river discharge, flow regime, and sediment type (Boucher and Billard, 2020; Campanale et al., 2020b; Koelmans et al., 2019b).

The interpretation and comparison of different monitoring data are further complicated by disparate differences in sampling and extraction methodologies, as well as a different array of analytical approaches. Current identification methods are conducted either by visual sorting or using analytical tools. The visual sorting technique is done with a stereomicroscope in which MPs are divided according to their shape, color,

and state of degradation (Hidalgo-Ruz et al., 2012), loosely associated with specific plastic polymer. Further, this technique is prone to human error. In addition, results given as "number of items" (*i.e.*, fragments) do not aid in distinguishing the impact of MPs on the ecosystem (Horton et al., 2017), neither data expressed in mass (*i.e.*, g). Raman and FTIR spectroscopy are the most commonly used analytical tools in MP analyses (Frias et al., 2018; Hidalgo-Ruz et al., 2012; Mistri et al., 2020; Vermeiren et al., 2020). These techniques allow for the characterization of the polymer type for each fragment, even when they have microscopic dimensions (1–10-20 µm) (Cunningham et al., 2020). However, these techniques are time-consuming (Song et al., 2015), depending on the size and thickness of the fragments, and the obtained results are again available as number of items/surface area or volume). Moreover, organic or inorganic adherent substances, dyes, or additives could compromise the Raman and FTIR spectroscopic identification (Hidalgo-Ruz et al., 2012; Song et al., 2015).

For all of these reasons, a fast, replicable, validated protocol to ensure a greater extraction efficiency from complex environmental matrices, a chemical (rather than visual) identification of the plastic polymers, and proper weight quantification, are necessary to soundly assess the impact of different MPs (Cincinelli et al., 2019; Prata et al., 2019; Yang et al., 2021; Zhang et al., 2020). Ceccarini et al. (2018) used Nuclear Magnetic Resonance (NMR) spectroscopy to confirm the information previously obtained by pyrolysis-GC/MS on molecular weight and the oxidized fraction of polymers. Peez et al. (2019a) proposed exploiting NMR spectroscopy to overcome the limitations of commonly used analytical methods (Tirkey and Upadhyay, 2021) for MP detection. This high-resolution and widely used tool in metabolomics and pharmaceuticals (Guidi et al., 2020; Petrella et al., 2020) presents unique strengths for characterizing the chemical composition of a complex mixture. The determination is quite simple, non-destructive, and can be applied independently of the size of the plastic fragments. NMR can be used to characterize and quantify MPs by exploiting the magnetic properties of the hydrogen nucleus contained in these tiny plastic particles. When a sediment sample containing MPs, and appropriately prepared, is subjected to a proton NMR analysis, the hydrogen nuclei resonate at specific frequencies in response to the applied magnetic field and a subsequent radiofrequency pulse. By measuring these resonances, it is possible to obtain valuable information about the composition and structure of the MPs present in the sample. Additionally, the intensity of the NMR signals can be used to determine the concentration of MPs, enabling their quantification.

Furthermore, the concentration of MPs can be expressed in mg/g of sample. The application of this method by Peez was successful in qualitatively and quantitatively determining MPs (Peez and Imhof, 2020; Peez et al., 2019b, 2019a, 2022). In particular, Peez et al. (2019b) have demonstrated the capacity of NMR spectroscopy to detect polyethylene terephthalate (PET) added to homogeneous matrices (made by single constituents: *e.g.,* river water or sand or freshwater aquatic biofilm or a particular amphipod species), utilized as proxies for environmental samples. Nelson et al. (2019) also applied the same procedure differing only in the addition of PBAT to matrices made of different field soils. The key differences between our study and those of Peez et al. (2019b) and Nelson et al. (2019) are that they reported the validation of NMR acquisition of single polymers artificially added to simple matrices or to soil (and not to sea sediments). To our knowledge, NMR spectroscopy has not yet been tested for the identification and quantification of MP in environmental samples. In this paper, we present the first application of NMR spectroscopy for the detection and/or quantification of two types of MPs, polystyrene (PS) and acrylonitrile butadiene styrene (ABS), in marine sediments sampled at different bathymetric levels in front of the Tiber River mouth.

This river is the Italy's second most extended catchment basin and the longest in Central Italy. It receives large quantities of domestic and industrial wastewater and water from surface runoff of non-cultivated, cultivated, and urban areas (Patrolecco et al., 2015), acting as a

conveyor belt for substances from the land and atmosphere into the sea.

This study is a preliminary step toward the setting up of a standardized NMR protocol applicable to different not homogeneous environmental matrices, in order to obtain the most amount of information in the shortest time with a single analysis. In this study, special attention was paid selectively to two of the six most commonly observed polymer types, *viz.* polystyrene (PS) and acrylonitrile butadiene styrene (ABS) (Koelmans et al., 2019a), characterized by solubility in the same solvent, chloroform ($CHCl_3$) (Brandolini and Hills, 2000; García et al., 2009; Peez et al., 2019a). The choice of chloroform as a solvent capable of dissolving and thus revealing the presence of PS and ABS simultaneously is only the first step toward the discovery of a semi-universal solvent that can quantitatively dissolve more polymers at the same time.

In particular, PS microplastic polymers could have different densities and origins and can cause severe effects on organisms directly or indirectly by acting as a carrier for other persistent pollutants (Amelia et al., 2021; Avio et al., 2015; Koelmans et al., 2016; Rochman et al., 2013). The other most abundant polymers dispersed in nature, like polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polyvinyl chloride (PVC), were excluded from this study due to the fact that they are not soluble in $CHCl_3$.

2. Materials & methods

2.1. Characteristics of sampling area facing the Tiber River mouth

The Tiber River is the second Italian river by catchment area extension (17.375 km^2) and the third by length (405 km). It contributes about 20 % to the fluvial inputs in the Tyrrhenian Sea and receives large quantities of domestic wastewater, industrial wastewater, water from the surface runoff of agricultural areas as well as wastewater from urban water treatment plants (Patrolecco et al., 2015), acting as a conveyor belt for the most diverse anthropogenic substances toward the sea. About 4.5 million inhabitants, of which >80 % are from the province of Rome, impact the Tiber River along its path. The average annual discharge in Ripetta (the hydrographic station near the mouth) hits its maximum in winter and minimum in summer (Iadanza and Napolitano, 2006). In this way, during the summer, the salt wedge intrudes into the river channel and develops in a stable layered structure (Pagnotta et al., 1989). This results in a hypopycnal plume with reduced transport capacity dominated by buoyancy rather than by the power of the flow (Syvitski et al., 2005; Wright and Coleman, 1971). Some of the important characteristics regarding the Tiber River delta are: i) the delta is composed of only two arm extensions (a natural one, called "Fiumara Grande", and an artificial one, called "Canale di Traiano"); ii) the marine sediments are characterized by horizontal sediment gradation in parallel to the delta coastal configuration; iii) the sediment configuration changes gradually from the river mouth up to the open sea. The delta front extends up to 25 m deep and is characterized by slight slopes, between 0.20 % and 0.35 %. In this area, the sediment is composed of sandy and a sandy pelitic fraction, with grain sizes between 1.5 and 4.5 phi (ϕ). At the limit between the delta front and the prodelta area, the sediment is sandy pelitic type with an average size of 4.5 to 6 ϕ. Reaching beyond 25 m of depth, in the prodelta area, the slope increases to 0.62 % and the sediment is highly silty with grain sizes between 6 and 7.5 ϕ. The sandy fraction is greater on the delta front, although when proceeding offshore it decreases rapidly to <10 %. The percentage of silt is greater at the limit between the delta front and the prodelta area, while the clayey fraction increases gradually proceeding offshore constituting of 30–40 % of the total sediment fraction (Bellotti and Tortora, 1985; Bellotti et al., 1993).

2.2. Marine sediment sampling

Sediment samples were collected in July 2020 in the marine area facing the mouth of the Tiber River, between Fiumara Grande and

Canale di Traiano (Lazio, Italy). The samples were collected along four bathymetric levels (5 m, 10 m, 20 m, and 30 m), located from 300 m up to 6 km seaward from the mouth of the Tiber (Fig. 1). For each bathymetric level, four stations were identified, and three sediment collection samples were taken from each station (with a Van Veen SG-400 grab sampler) and placed in a new unused single polypropylene container. Each grab has a capacity of 400 cm^2 of mass and 5 L of volume. The samples were transported and processed at LESA (Laboratory of Experimental Ecology and Aquaculture of the University of Rome "Tor Vergata").

2.3. Quality assurance and quality control

To reduce external contamination, particular attention was paid to each phase of the protocol by applying the suggestions from Prata et al. (2019). Cotton lab coats and gloves were worn. Stainless steel glassware and equipment were carefully washed and rinsed with distilled water before and after each use to further reduce contamination by any plastic fragments adhering to the surfaces due to the electrostatic charge. Glassware, equipment, and solutions were also covered with aluminum foil during each use. The work surfaces were cleaned with 95 % ethanol and dried with absorbent paper. All procedures, from the plastic objects' fragmentation to the solubilization in chloroform, were performed under a fume hood to reduce airborne microfibers. To further reduce possible external contamination, all liquids used for cleaning or diluting solutions were pre-filtered before use (Schleicher & Schuell 5891 filter papers).

2.4. Sample preparation

2.4.1. MPs standard solutions

The standard samples were obtained by mechanical fragmentation of commercially extruded polymers PS and ABS, previously identified by the code according to DIN 7728 and 16780 and ISO 1043/1. Commercial packaging plastics were separately grinded with a Dremel 3000® inside a petri dish until obtaining fractions of course and fine fragments. The particles were subsequently sieved to obtain fragments ranging from 1 to 1000 μm in diameter. The two standard polymer solutions were then obtained by dissolving 3 mg of fragments (weighed with an Analytical Balance Mettler Toledo, XS105 DualRange, 0.01 mg readability) in 2 mL of deuterated chloroform (CDCl$_3$) (DLM-29TC, Cambridge Isotope Laboratories, Inc.) in 10 mL glass tubes. The solvent was chosen according to previous studies (Brandolini and Hills, 2000; García et al., 2009; Peez et al., 2019a) and to selectively extract only ABS and PS, which are highly soluble in this condition with respect other polymers like PVC or PET. This consideration can be used to remove possible overlapping signals, which can interfere with the measurement of PS and ABS signals. Each solution was vortexed and then held for 30 min at 30 °C. Afterward, 600 μL of the solution was placed in a 5 mm NMR tube for subsequent analyses. These solutions were used only for identifying the NMR signal but not for quantifying the polymers.

2.4.2. Calibration curve solutions

The calibration solutions of PS were prepared from a stock solution containing 1 mg of PS in 1 mL of chloroform (CHCl$_3$; BP1145- 1, Fisher). Different aliquots (10 μg, 25 μg, 50 μg, 100 μg, and 150 μg) were left to evaporate in 10 mL glass tubes. Once completely dry, 600 μL of CDCl$_3$ containing 1 % *v*/v of TMS was added, and the spectra were acquired.

To test the accuracy of this calibration curve, seven solutions were prepared by dissolving 3 mg of PS for each solution and diluted in order to contain a mass in a range between 120 μg and 1820 μg of PS in CDCl$_3$.

2.4.3. Protocol for MPs extraction in marine sediment samples

A total of 600 mL for each of the four bathymetric levels (150 mL of sediment from each site) were analyzed in 3 replicates for a total of 12 samples. The organic digestion was performed in 2 L flasks by placing 600 mL of sediment and 200 mL of filtered hydrogen peroxide solution at 30 % (Schleicher & Schuell 5891 filter paper) (Vermeiren et al., 2020). The solution was slowly poured and stirred with a glass rod. All the flasks were left in a water bath for 24 h at room temperature.

The MPs were separated by density from the sediment by adding a zinc chloride (ZnCl$_2$) saturated solution (Bergmann et al., 2017; Coppock et al., 2017; Vermeiren et al., 2020; Zobkov and Esiukova, 2017), made by 1.7 kg of salt in 1 L of filtered H$_2$O. A ZnCl$_2$ solution was added until the solution reached 1 cm from the top of the flask. Following Coppock et al. (2017), a sequence of 5 min stirring, and 5 min rest was repeated three times; then, the solution was left to decant overnight under a fume hood at room temperature. Once settled, the supernatant was filtered with a Millipore vacuum pump equipped with a Buchner funnel (XX1004724 Millipore; HAWP04700 Millipore 0.45 μm filter). In this way only plastic fragments with dimensions >0.45 μm were retained on the filter. Additional ZnCl$_2$ solution was carefully added with a squeeze bottle against the walls (to facilitate the detachment of any plastic fragments adhering to the walls) until the solution reached
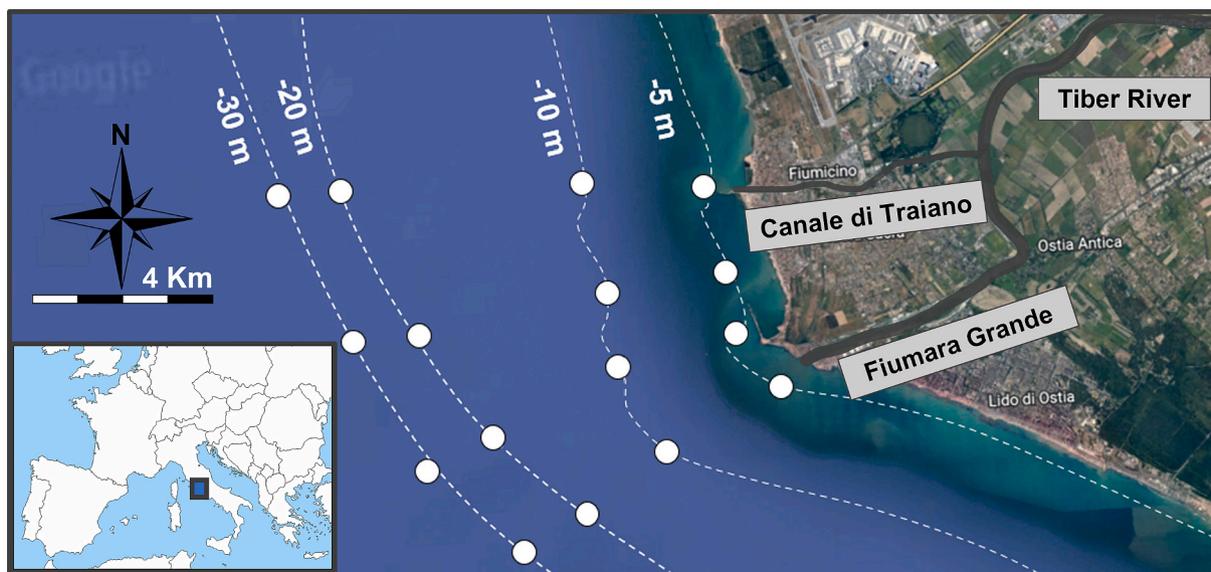

Fig. 1. Sampling sites at the mouth of the Tiber River. Maps Data: Google, ©2022 SIO, NOAA, U.S. Navy, NGA, GEBCO.

the top of the flask. The entire sequence was repeated three times to collect all supernatant material from each sample. The Buchner funnel walls were then washed with the ZnCl₂ solution to retrieve the MPs adherent to the glass surface.

Before being removed, the filter was covered with a 2 mL solution containing 30 % H_2O_2 for 15 min to perform a second organic digestion step (Fig. A1 in *Supplementary materials*). The hydrogen peroxide was removed by vacuum activation, and the filter was washed with distilled water following the same filtration procedure previously described. The filter was then transferred into a 10 mL glass tube, heated at 60 °C in the oven until complete dehydration had occurred, and lastly, the glass tube was filled with chloroform. After 30 min, filters were removed, and the tubes with chloroform were inserted in a nitrogen flow extractor (Heating Module Reacti-Therm III™) for about an hour at 37 °C. Once samples were wholly dried, 600 μl of CDCl₃ containing 1 % *v/v* of TMS were used to reconstitute the sample, and the resulting solution was transferred to a 5 mm NMR tube.

2.5. ¹H-NMR acquisition

¹H-NMR spectra were acquired with a Bruker Avance 700 MHz spectrometer equipped with a Triple resonance TXI probe and a SampleXpress Lite autosampler. The experiments were performed at 298 K using 1.5 s of acquisition time and a relaxation delay of 2 s. Although these delays are insufficient to allow complete relaxation of the polymer signals, we did not use the absolute value of the integrals to quantify the concentration in the samples; instead, we used a calibration curve linking the known amount of polymer in each sample to the NMR signal integral. This strategy allowed us to obtain the results in a shorter period. Eight scans were used for the standard solutions, 1024 for the sediment samples, and 128 for the curve calibration solutions. Spectra were processed using the Topspin 2.1 software. A line broadening of 5 Hz was applied to all spectra.

Using the two standard solutions, the limits of detection (LOD) and quantification (LOQ) were calculated using the following formulas:

$$LOD = \frac{2}{SN}m_p; LOQ = \frac{5}{SN}m_{p.}$$

where *SN* is the signal-to-noise *ratio* measured after 1024 scans using a solution containing m_p μg of polymer.

3. Results

3.1. MPs standard characterization

The plastic fragments obtained by mechanical trimming of commercial objects, which were used as a standard for ABS and PS, are shown in Fig. 2. They appeared heterogeneous in shape, thickness, and length (1–1000 μm), quite different from virgin standards available on the market (beads, pellets, or fibers).

The chemical structure of each polymer and their ¹H-NMR spectra in CDCl₃ are displayed in Fig. 3. The signal assignment was performed using the chemical shifts referenced in previous works (Brandolini and Hills, 2000; Peez et al., 2019a; Peez and Imhof, 2020) (Table 1).

The LOD and LOQ for each polymer were determined for each standard spectra by estimating the mass of a polymer that would give rise to an NMR signal with a 2 or 5 signal to noise (SN) after 1024 scans for the LOD and LOQ, respectively (Table 1). The aromatic region of the spectrum contains the sum of the contributions from PS and ABS. ABS shows a signal at 5.482 ppm in the NMR spectrum characteristic of hydrogens bound to sp2-carbons. This signal is distinctive of ABS with respect to PS alone, so the presence of ABS in natural sediments can be detected using this signal. However, considering that the relative composition of the three ABS components (polystyrene, polybutadiene, and polyacrylonitrile) may vary depending on the manufacturer, its quantification poses a more significant challenge. For this reason, in environmental sediments, the presence but not the quantity of this polymer can be assessed.

3.2. MPs' identification and quantification in marine sediments

The PS presence was assessed in every sample of the four isobaths (for a total of 12 samples) (Fig. 4). No signals corresponding to ABS were found in any samples.

The absence of ABS signals suggests that the concentrations were below the detection limit or that this polymer was absent within the tested samples. This translates into a mass contained in 1 L of sediment, below 5 μg for ABS standard considered in this work. However, this limit can change by considering different ABS compositions.

From the analysis of ¹H-NMR spectra of all the bathymetric samples, the presence of PS and not ABS was observed. The calibration curve for PS (Fig. 5), described in *Materials and Methods 2.4.2*, was constructed to determine the amount of this polymer in the sediment samples.
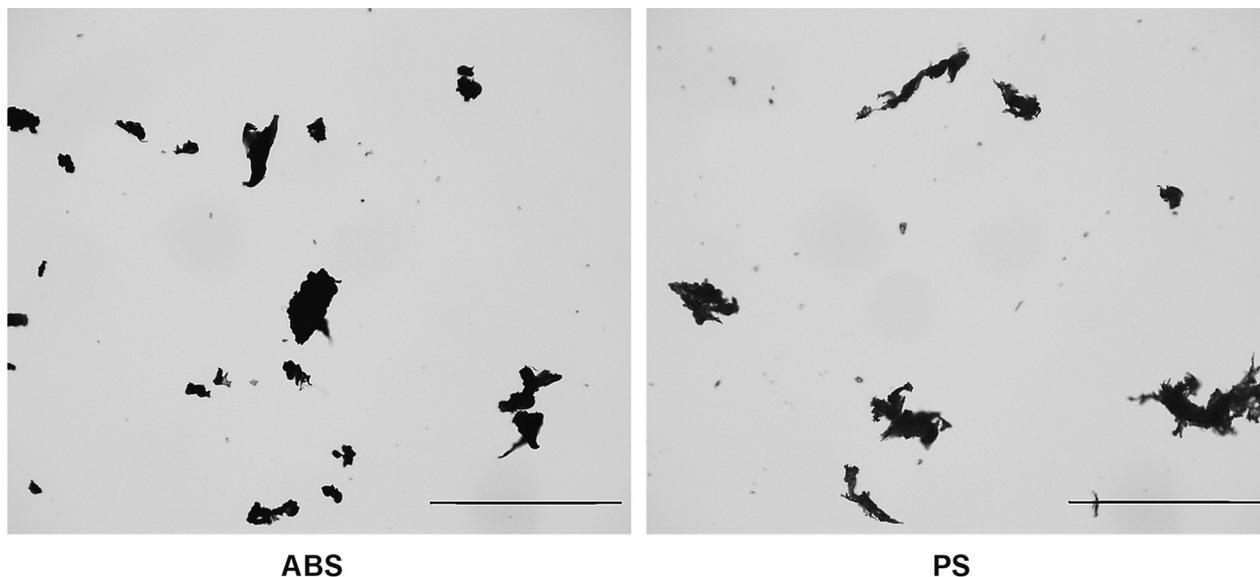

ABS   **PS**

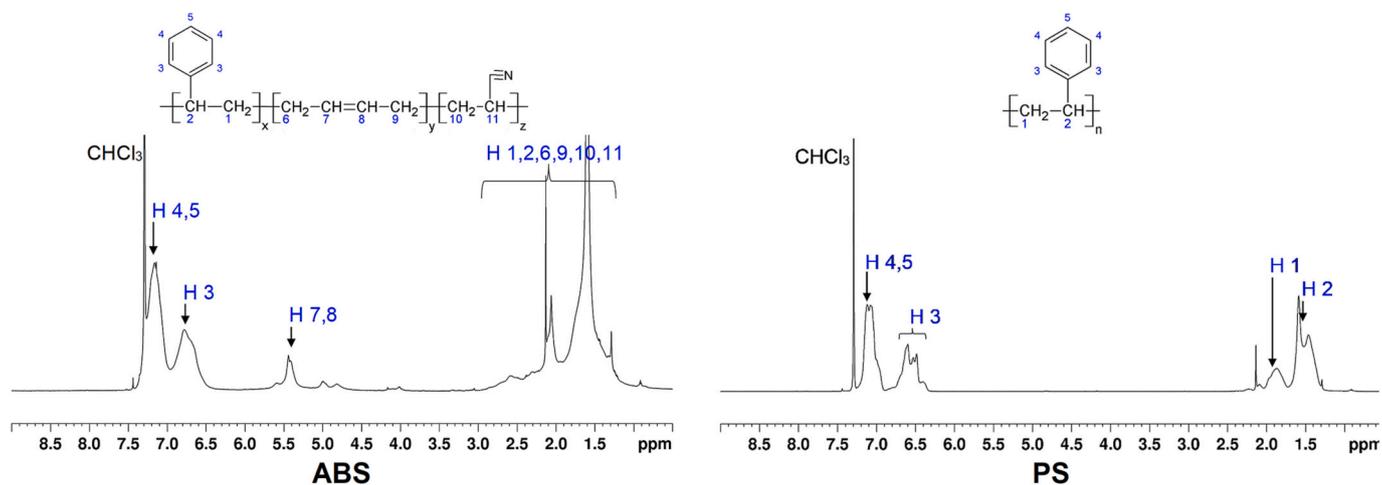**Fig. 2.** Images of plastic fragments produced in the laboratory (bar = 1 mm).

Fig. 3. Chemical structure and ^{1}H-NMR spectra in CDCl$_3$ at 298 K of the two polymers: ABS (A) and PS (B). The signals for the various hydrogens of the two molecules are indicated in the spectra with arrows.

Table 1
Assigned chemical shifts (δ) of the two polymers. Signals suitable for quantification and or identification in natural sediments are indicated in bold. LOD and LOQ values are also reported.

Polymer	δ [ppm]	Assignment	LOD [μg]	LOQ [μg]
ABS	2.900–1.200	H1,2,6,9,10,11	2.9	7.2
	5.482	**H7,8**		
	6.756	H3		
	7.164	H4,5		
PS	1.878	H1	0.6	1.4
	1.463	H2		
	6.400–6.800	**H3**		
	7.112	H4,5		

To test the accuracy of this calibration curve, seven independent model samples were prepared as described in section *2.4.2* of *Materials and Methods*. The results in Table 2, show an average accuracy of 99 ± 13 %.

The NMR measurements on marine sediments revealed a PS's average value of 27 ± 16 μg per liter of sediment.

The PS mass values demonstrated a high variability among replicates at the different isobaths, evidenced by the high standard error (Fig. 6). However, a clear incremental trend of PS presence toward deeper sediments is discernable when considering the means. In addition, the presence of paramagnetic metals that can interfere with the NMR quantification can be probably excluded in the marine sediment samples

because the same, in the final stage of preparation, were dissolved in chloroform. In such a solvent, the solubility of metals is drastically reduced. If there were traces of these metals, they would cause significant line broadening of polymer signals and give a false negative result.

4. Discussion

We demonstrated the capacity of NMR to detect and quantify ABS and PS polymers in marine sediments. We obtained qualitative and quantitative information for two different polymer types by processing environmental multiphasic matrices of marine sediments with a single analysis: PS signal was detected in all 12 sediment samples, and in contrast no ABS signals were detected. We would like to emphasize that the peculiar nature of the matrix of marine sediments facing a river mouth doesn't allow the application of some important analytical steps necessary for its validation and optimization, *i.e.,* blind samples. Peez et al. (2019a) and Nelson et al. (2019) used blind samples on monophasic matrices, represented by either river water, sand, freshwater aquatic biofilm, amphipod species, or on field soils, in which the polymers under investigation (PET or PBAT) were absent. The reason why we couldn't apply blind samples in our study, in which we tried to detect more than one polymer, was because it would be necessary to obtain environmental matrices without either of the two polymers (PS and ABS) that we were investigating. However, this was not feasible due to the fact that PS was present in all the samples. We have evaluated a potential solution to overcome the lack of blind samples for purifying
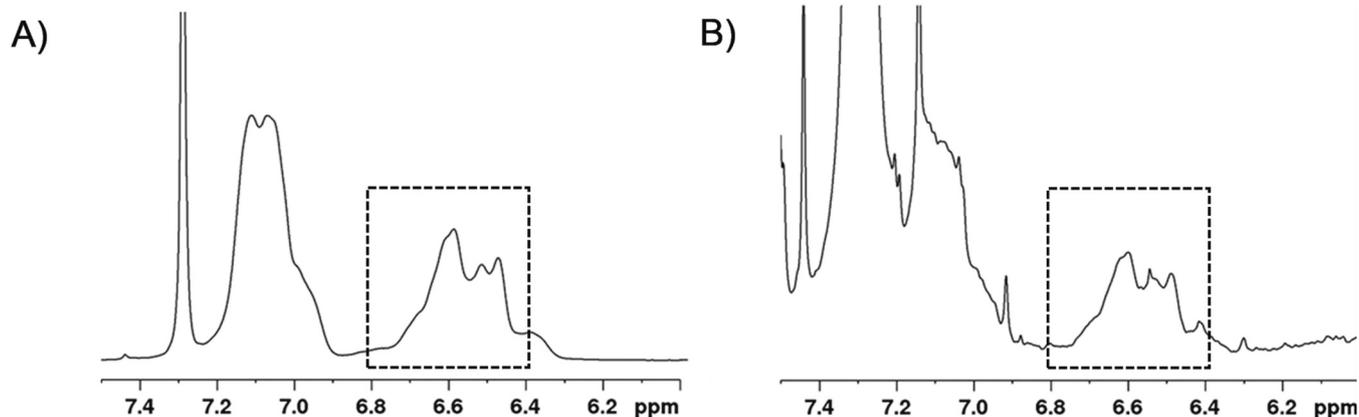

Fig. 4. ^{1}H-NMR spectra of a standard solution of PS (A) compared to the ^{1}H-NMR spectra of processed marine sediment at 30 m containing PS (B). The PS characteristic signals are marked in the dotter box.
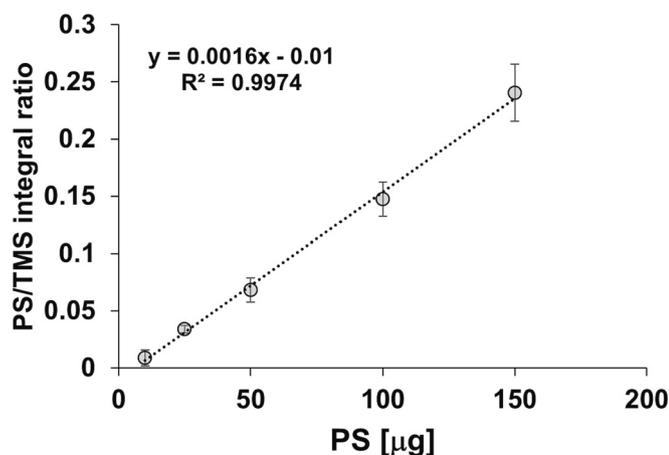
Fig. 5. Calibration line for PS in CDCl₃.

Table 2

The integral values measured between 6.4 and 6.8 ppm, the calculated mass from the equation of calibration curve reported in Fig. 5 and the nominal mass weighted for seven independent model samples tested to assess the accuracy of the PS calibration curve.

Integral value	Calculated mass [μg]	Nominal mass [μg]	Accuracy %
0.1682	109	120	91
0.3020	190	200	95
0.2660	168	190	89
1.0950	673	630	107
1.2508	768	630	122
3.0402	1858	1810	103
2.5562	1563	1820	86

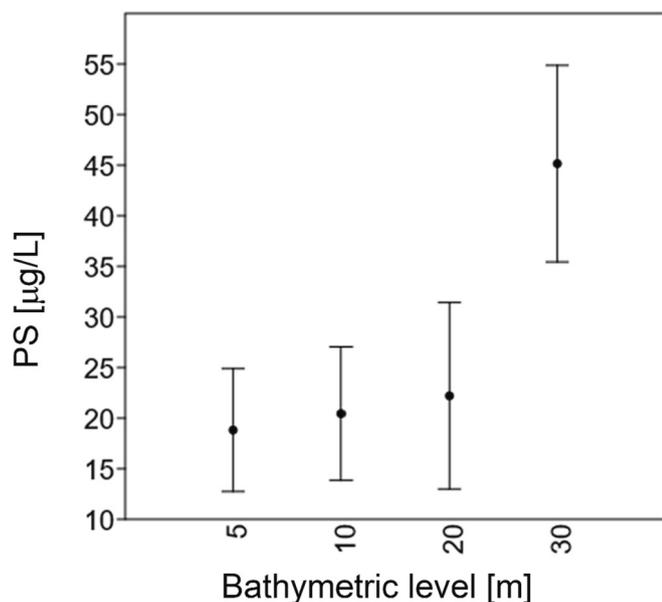**Fig. 6.** Whisker plot with error bars of PS [μg/L] found in each bathymetric level.

the sediment samples by first conducting chemical digestion and density separation. However, these processes change the sediment characteristics and then make this 'purified' matrix useless for blind samples. The other potential solution we considered was the creation of an 'artificial' environmental matrix (with all the different biotic and abiotic components), but this solution is quite airy, taking into consideration that matrices such as marine sediments are highly heterogeneous and dynamic. Marine sediment facing river mouths, in fact, can vary according to biotic and abiotic characteristics of the river, the site, the distance from the coast, the depth, meteorologic profile (seasonally and daily) as well as hydrodynamic and bioturbation conditions in the sea site. Moreover, MPs do not have a homogeneous distribution in the environment. Therefore, each sampling site facing a river mouth would require standardized environmental parameters (bathymetry/distance-from-the-coast/season) for each specific blind sample. Even this solution was rejected *a priori* because it was rather contrived and unrealistic. Hence, given the omni-present background noise represented by marine sediments, we decided to focus on the specific chemical shift of PS in the spectrum and use it to quantify this polymer in the sediments after building up the calibration curve. In conclusion, our preliminary study, based on an ecological (*viz.* integrated) approach, obtained an efficient quantification of polystyrene polymer which, however, could be applied to different types of marine sediment.

4.1. Methodological consideration

4.1.1. Sampling design and procedure

The Mountford and Morales Maqueda (2019) model demonstrated that plastic fragments with negative buoyancy accumulate within the bathymetric contours of the deepest seabed. For this reason, the sampling sites were identified according to a spatial distribution between the two branches of the river and a bathymetric gradient. This approach is similar to that of Montuori et al. (2016) and the bathymetric pattern used by Fagherazzi et al. (2015), which were cohesive with the dynamics of river mouth deposition processes, rather than being purely based on the distance from the shoreline (Expósito et al., 2021; Graca et al., 2017; Willis et al., 2017).

After collection, the samples were stored at 4 °C, as recommended in past studies (Hidalgo-Ruz et al., 2012; Phuong et al., 2021; Prata et al., 2019). Considering that our research did not set a limit for the size of the plastic fragments, the sampled sediments were not subjected to a preliminary sieving or volume reduction (Phuong et al., 2021). In fact, for each bathymetric level, there were four sites from which 150 mL each were taken for the three replicas. Therefore, each replica was composed of a total representative volume of 600 mL: 150 mL for four sites of the same bathymetric level. Repeating this sampling pattern will help obtaining spatial and temporal data on MP amount variations and confirm whether the trend depended on fluvial influence rather than marine hydrodynamic activities (Simon-Sánchez et al., 2022).

4.1.2. Extraction protocol

The degradation of the organic matter should effectively remove biofilms and other substances while simultaneously avoid affecting MPs' structure. Enzymatic reagents, whether alkaline or acidic, are generally not considered suitable for organic matter digestion in sediment samples because they are relatively expensive and are either too aggressive or ineffective (Hurley et al., 2018; Lusher et al., 2020; Nuelle et al., 2014). Most studies use 30 % hydrogen peroxide with different time exposures (Adomat and Grischek, 2021), which does not affect the structure of MPs. The experimental confirmations by Hurley et al. (2018) verified that most of the polymers investigated (except polyamide) were not affected by 30 % H_2O_2 at 60 °C nor 70 °C, while other studies reported occasional discoloration and size reduction of polyamide, polycarbonate, and polypropylene (Nuelle et al., 2014) after treatments with 30 % H_2O_2 at room temperature for seven days. Since the exposure time strongly affects both the degradation efficiency and the effect of H_2O_2 on the structure of MPs (Hurley et al., 2018), we used 30 % hydrogen peroxide for 24 h at room temperature. We applied it directly to the sediment matrix in the same reagent:sediment volume *ratio*, used in Vermeiren et al. (2020), considering it to be the best compromise for removing organic material, especially in rich sediments (Hanvey et al., 2017).

Our digestion protocol was pondered upon within the pretext of

assuming that each sediment matrix could be different depending on the different sites (Pfeiffer and Fischer, 2020), and that marine sediments facing a river mouth are particularly rich in organic matter. As far as we know, this is the first study to apply a 2-step organic digestion with 30 % hydrogen peroxide. Although the first step was similar to other studies, what sets this study apart was the inclusion of the second organic digestion in which hydrogen peroxide was poured directly on the filter while still inserted in the funnel channel (using relatively little reagent volume for a short exposure time). The second digestion conducted directly on the filter facilitated the protocol procedure by avoiding sample loss, external contamination, over usage of reagents, and reduced exposure time. The ^{1}H-NMR spectra resulting from the second organic digestion resulted in an evident reduction of the matrix effect and, therefore, a much cleaner outline (Fig. A1 in *Supplementary materials*).

Biofilms and other compounds adhering to the fragments can change the MP volume:density *ratio* and prevent proper separation from the sediment (Löder and Gerdts, 2015). The decision to use $ZnCl_2$ instead of sodium chloride was based on the fact that zinc chloride is particularly efficient for marine sediment extraction of main plastic polymers with different physical properties and size classes, even though it is corrosive and toxic to the environment (Imhof et al., 2012). Furthermore, $ZnCl_2$ is inexpensive and can be reused after filtration (Prata et al., 2019). Recognizably, sodium chloride, even if cheaper and with a lower environmental impact (Graca et al., 2017; Maes et al., 2017; Woodall et al., 2014), may not offer an efficient solution for separating polymers with different densities or with densities altered by the adhesion of other substances. Zinc chloride is also particularly efficient in extracting higher density polymers (*i.e.,* PVC and PET). This allows the applicability of the extraction method also to the investigation of other types of polymers.

4.1.3. *^{1}H NMR application*

Assuming that characterizing plastic fragments by size, shape, and color allows us to infer their origin (Prata et al., 2019), quantitative data is needed to assess the impacts of MPs on organisms. In many papers, the concentrations of MPs used for ecotoxicology assays are significantly higher than those detected in the environment, up to an average of six orders of magnitude greater than the estimated amount from field sampling (Ockenden et al., 2021), often resulting in extreme toxicological observations (Silva et al., 2018). Quantitative data on MP abundance are extremely helpful in extrapolating the impact of MP contamination on biota.

In addition, ecotoxicological studies and methodological validation studies use commercial plastic uniform standards whose shape (*viz.* spheres or filaments) are not always representative of the secondary MP fragments detectable in environmental matrices (Peez et al., 2019a; Tirkey and Upadhyay, 2021).

Our results demonstrated the applicability of the NMR technique (already proposed by Peez et al. (2019a) on environmental monophasic matrices) to detect PS in environmental multiphasic matrices. The advantages of NMR with respect to other analytical spectroscopic instruments are multifold, as previously stated by Peez et al. (2019a). This technique can obtain qualitative and quantitative data *via* quick and efficient analyses. The MP contents are expressed in mg/g of sample. There are no requirements regarding the assessment of the plastic fragments' size, shape, nor neither it is necessary to conduct subsampling. Moreover, detection and quantification limits are extremely low, in the order of μg/L. However, an imaging of the plastic fragments' shape, color, and size, prior to the final passage into the solvent, enables comparison to be made between our dataset and pre-existing morphological data (Tirkey and Upadhyay, 2021).

Taking into consideration that each polymer has its own specific solvent, we are currently carrying out the setting up of a procedure in which the same sample is solubilized with different solvents in order to analyze the presence of all the polymers detectable with HNMR, with

one analysis. The results obtained by Peez et al. (2019a) and Nelson et al. (2019) that individuated and quantified PET and PBAT, respectively, in artificial matrices strongly encourage and sustain this approach.

4.2. *Microplastic in marine sediments facing the Tiber River mouth*

This preliminary study was aimed at testing the NMR applicability for PS detection in environmental matrices, therefore the technique was applied to a representative sediment pool of four bathymetric levels. Further samplings are planned with a greater number of sampling sites to better evaluate the distribution and quantification of PS in the marine sediments facing the mouth of the Tiber River.

We are aware that the results obtained cannot soundly represent the current distribution of PS in marine sediments, but they are consistent with some relevant attributions. Our results seem to reflect the great heterogeneity in distribution and quantity of PS expected in estuarine environments (Koelmans et al., 2019a; Martellini et al., 2018; Peng et al., 2017; Vianello et al., 2013), strongly affected by the anthropogenic pressures on the river catchment area (Jambeck et al., 2015), by coastal hydrodynamics and local microclimates. Furthermore, the results we obtained from detecting PS signal in all 12 sediment samples are consistent with observations from Crosti et al. (2018) on high levels of this polymer found in floating macro waste in the Tiber River. Additionally, from our results there appears to be a tendency for PS to accumulate at the deepest bathymetric level (down to −30 m) that we can presume to depend on hydrodynamic characteristics of the plume of the Tiber and PS chemical and physical properties. In the summer, the Tiber plume spreads slowly and can prevent the deposition of fine particles in the proximal area to the mouth, extending the discharge toward the open sea (Fagherazzi et al., 2015). This results in a hypopycnal plume with reduced transport capacity dominated by buoyancy rather than flow (Wright and Coleman, 1971; Syvitski et al., 2005). PS is a styrene polymer with a density of 1.050 g/cm^3 and negative buoyancy. However, the buoyancy becomes positive (due to the varying density) in expanded PS. However, it should be taken into consideration that the fragmentation of plastics, especially in rivers, is mainly mechanical (Delorme et al., 2021). Consequentially, once mechanically fragmented, PS loses air and the airgaps fill with water, increasing the effective density and leading to the reacquisition of negative buoyancy. Therefore, PS can be easily influenced by the buoyancy of the plume and by the activity of waves (Wright, 1977). As a consequence, PS could be transported in suspension up to the Tiber delta front, where hydrodynamism does not involve resuspension (Bellotti and Tortora, 1985) and accumulate in the deepest bathymetric levels, in accordance with the Mountford and Morales Maqueda (2019) model.

We detected PS in all the bathymetric samples, within the range of 12.3–64.6 μg/L. PS is sometimes more represented in marine sediments than ABS (Expósito et al., 2021; Nuelle et al., 2014), although sometimes the contrary is also true (Mistri et al., 2020). The heterogeneity of these results among others suggests that many factors (biotic and abiotic characteristics of the site, the distance from the coast, the depth, the seasons, as well as hydrodynamic and bioturbation conditions in the sea site) play intricate roles in the dispersion and transport of MPs into the sea. In addition, our environmental matrix showed the absence of any signals of ABS at 5.482 ppm. This means that if present, ABS, in the composition of our standard, should be in a concentration below the detection limit of 5 μg/L, but this limit can be different if we consider other ABS compositions. However, in the case of co-presence of ABS, detected by the signal at 5.482 ppm, and PS, in order to accurately assess the mass of PS with respect to ABS in natural sediments, deconvolution procedures of their signals between 6.5 and 7.5 ppm will have to be developed to discriminate the relative contribution of the two polymers.

The Tiber River receives large quantities of wastewater from four urban water treatment plants (Patrolecco et al., 2015) along its entire waterway. Furthermore, industries in this area are fragmented and made

up of many small and medium-sized enterprises (SMEs), whose data on MPs use/production/release are not attainable, to our knowledge. Consequently, this yields impractical or unrealistic expectations for finding potentially released polymer types into the sea facing the river mouth based on the corresponding SMEs on the Tiber watercourse. Therefore, it is probable that other types of textiles or industrial MPs (such as polyethylene terephthalate, polyvinyl chloride, polypropylene, polyethylene, polyamides, polyester, and nylon) could be present in our sediment samples. However, they were not the object of our investigation because they do not dissolve in chloroform.

5. Conclusions

In conclusion, in this work we describe a preliminary study on using NMR spectroscopy to detect and quantify PS and ABS microplastics in marine sediments facing the Tiber River mouth (Central Italy). The study successfully applied NMR and detected PS in all sediment samples analyzed, while no signals for ABS were found. The advantages of NMR include its efficiency, non-destructiveness, and ability to quantify MPs in μg per liter of sediment. The study's results highlight the heterogeneity in distribution and quantity of PS in estuarine environments; however, further sampling and analyses should be carried out to confirm and better understand PS distribution and its impact in coastal marine sediments. Moreover, further experiments are underway to use different solvents on the same sediment sample for the contemporaneous detection of other types of polymers. Overall, the study demonstrates the potential of NMR spectroscopy as a valuable tool for characterizing and quantifying MPs in environmental matrices.

CRediT authorship contribution statement

Giulia Papini: Investigation, Methodology, Writing – original draft
Greta Petrella: NMR Formal analysis, Writing – review & editing
Daniel Oscar Cicero: NMR Supervision, Writing – review & editing
Clara Boglione: Conceptualization, Supervision, Writing – review & editing
Arnold Rakaj: Conceptualization, Supervision, Resources, Writing – review & editing

Declaration of competing interest

The authors 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

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.marpolbul.2023.115784. These data include the Google map of the most important areas described in this article.

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