Identification and Quantification of Microplastics in the Marine Environment Using the Laser Direct Infrared (LDIR) Technique

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Abstract :

Here, we evaluate for the first time the performances of the newly developed laser direct infrared (LDIR) technique and propose an optimization of the initial protocol for marine microplastics (MPs) analysis. Our results show that an 8 μm porosity polycarbonate filter placed on a Kevley slide enables preconcentration and efficient quantification of MPs, as well as polymer and size determination of reference plastic pellets of polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), with recoveries ranging from 80–100% and negligible blank values for particle sizes ranging from 200 to 500 μm. A spiked experiment using seawater, sediment, mussels, and fish stomach samples showed that the method responded linearly with significant slopes (R^2 ranging from 0.93–1.0; $p < 0.001$, $p < 0.01$). Overall, 11 polymer types were identified with limited handling and an analysis time of ca. 3 h for most samples and 6 h for complex samples. Application of this technique to Mediterranean marine samples (seawater, sediment, fish stomachs and mussels) indicated MP concentrations and size distribution consistent with the literature. A high predominance of PVC (sediment, fish stomachs) and PE and PP (seawater, mussels) was observed in the analyzed samples.

Graphical abstract
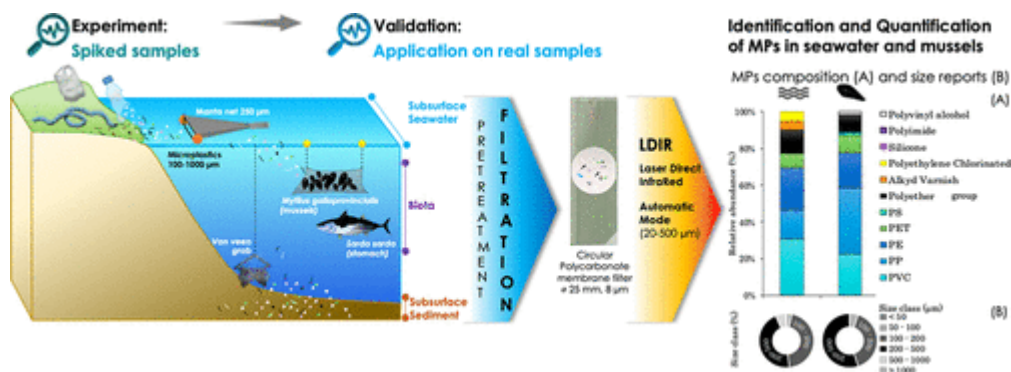
Keywords : Microplastics, LDIR, detection, seawater, sediment, marine organisms

Synopsis

Development of a reliable and time efficient method using sample preconcentration onto polycarbonate filters (8 µm) and the LDIR system for detection and characterization of MPs in environmental matrices is reported.

1. INTRODUCTION

Microplastics (MPs) coming from both industrial and domestic products have been defined as particles spanning a large spectrum of sizes (1 μm - 5 mm)[1], shapes, and chemical composition[2,3,4]. They have been found in soil, water, air, sediment, fish and snow[2–14,15]. They can be considered as a potential global threat for terrestrial and aquatic organisms, including humans[8,16–25]. Although knowledge in this domain is still very limited and there is little evidence of the impact of microplastics consumption on human health, the organic additives they contain (*e.g.,* phthalates) may contribute to cardiovascular mortality in adult men, with data also suggesting the existence of increased risk for adult women[24]. Recent MP detection techniques including vibrational spectroscopies such as micro-FTIR (Fourier Transform Infrared[26–28]; FTIR imaging[29]; FPA (Focal Plan Array); FTIR and image analysis[30,31] (automated analysis pipeline) and Raman spectroscopy[32,33] provide information about the number of plastic particles in each size range. Thermal analysis[34,35] (*e.g.,* pyrolysis followed by GC/MS detection) is a destructive technique which provides information on the chemical composition of the detected polymer, but not on particle count or size distribution[35–38]. The main drawbacks of these methods are the duration of analysis, the limited membrane filter sub-areas scanned, the destructive aspect of the methods and, for Raman, potential fluorescent artifacts coming from other components in the sample[37,39]. Hence, results obtained remain dependent on both the experimental protocol and on the operator, and are usually time consuming. There is therefore a need for an automatic method for MP detection and characterization (counting and identification) in order to avoid personal bias (sample contamination, over- and underestimations).

Among IR techniques, the recent advent of Laser Direct Infrared (Agilent, 8700 LDIR Chemical Imaging System) technology could provide rapid and automatic information on MP sample mixtures in terms of composition, diameter and thickness in a wide range of sizes without the need for visual preselection of particles for analysis, and without compromising the spectrum quality. Nevertheless, the method recommended by the manufacturer required an initial particle sorting step followed by manual deposition of the MPs in ethanol on a highly reflective glass slide (*i.e.,* Kevley slide). This approach regularly induced biases such as under- or overestimation of MPs in marine samples due to difficult handling, which may result in the

analysis of sorted particles which are not necessarily representative of the sample as a whole[6,7,40,41]. There is therefore a need for the development and optimization of a cost-effective method (featuring reliable and time efficient identification) based on hyperspectral imaging for the detection and characterization of MPs in environmental matrices. Here, we evaluate the use of a series of membrane filters (cellulose nitrate, aluminum oxide and polycarbonate) to obtain a preconcentration of MPs on filters which can then be placed on the Kevley slide as a support for LDIR transflectance measurements with minimal spectroscopic interference in the mid-infrared (1800 cm^{-1} to 975 cm^{-1}) region[7,29,42]. The efficiency of different filters compared to the use of a Kevley slide alone was tested for a panel of 5 types of polymers (with different densities[36,43]), which count among the most abundant polymer types found in the aquatic environment, be it in seawater, sediments or marine biota[44]. This abundance is related to their use in product packaging. This abundance is related to their use in product packaging which is dominated by polyethylene (PE) (29.8%) followed by polypropylene (PP) (19.3%), polyethylene terephthalate (PET) (7.4%) and polystyrene (PS) (6.7%)[43,45]. Moreover, PVC is commonly found in marine sediments due to its high density[44]. We also selected the 5 RPPs in order to study different types of polymers with different densities[36].

2. EXPERIMENTAL PROCEDURE

2. 1. Materials, reagents, and filters

Reference plastic pellets (RPPs) of polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) purchased from CARAT GmbH (Bocholt, Germany) (Table S1) were fragmented into smaller MPs using a centrifugal grinder (1 minute at 1 800 rpm; Retsch ZM1000), then recovered with stainless steel sieves (200-500 μm), pre-rinsed with 1 mL of 80% (v/v) ethanol and dried in an ISO class 6 cleanroom (temperature: 22 °C; SAS pressure:

+15 Pa; SAS brewing rate: 30 Vol h^{-1}; lab pressure: +30 Pa; brewing rate: 50 vol h^{-1}). The diameter of the three size ranges (<200, 200-500 and >500 μm) of fragmented MPs was ensured with two pre-rinsed stainless-steel sieves (200 μm and 500 μm). Each fraction was recovered on a burnt aluminum foil. The sieving was repeated twice to select particles of homogeneous diameter. Between each use the sieves were washed in an ultrasonic bath, then passed through MilliQ water and ethanol. In addition, between the handling of each size class, the samples were heated in an oven at 450°C for 6 hours. Reagents were obtained from Chem-Lab, Belgium (NaCl, ≥ 99.8%); PROLABO, France (KOH pellets and HNO$_3$ 68%). Various aqueous working solutions were prepared using ultrapure water produced on site by a Milli-Q system, Millipore (Molsheim, France) with a specific resistivity of 18.2 MΩ cm (25 °C) and a total organic carbon (TOC) content < 2 ppb. Different circular 25 mm diameter membrane filters (Table S2) were tested and provided by Whatman for Anopore (Anodisc aluminum oxide, 0.22 μm pore size, 60 μm thickness) and Nucleopore (polycarbonate: PC, 0.2 and then 8 μm pore sizes with 6-11 μm thickness), and by Sartorius GmbH Germany for cellulose nitrate filters (0.45 μm pore size, 115-145 μm thickness). The filters were deposited on Kevley slides coated with Ag/SnO$_2$ for infrared reflectance (Agilent).

2. 2. LDIR conditions

Analysis of MP shape, size and chemical composition was performed using an infrared imaging microscope Agilent Technologies 8700 LDIR instrument (Chemical Imaging System, Germany) running a fully automated (20–500 μm) method using the Clarity software (Agilent version 1.3.9) (Fig. S1). A proprietary quantum cascade laser (QCL) was used as a light source operating at high speeds, with an elevated wavelength accuracy (spectral resolution of 8 cm^{-1}) and fast-scanning optics using a full spectrum in the mid-IR range. The LDIR system uses a single-point mercury cadmium telluride (MCT) detector

(thermometrically cooled), rapid scanning optics and works under nitrogen flow (10 L min⁻¹). The particle spectra were collected in transflectance mode, when the laser light passes through the particle and reflects off the Kevley slide before passing through the particle again and generating transmission-like spectra. For very small particles (< 20 µm) which might not be automatically detected, the system may need to refocus to obtain an optimal spectrum. In this case, the per particle analysis time may be up to 8 s, in manual mode (line-profile tool) and the characteristics (*e.g.,* size information, polymer and HQI) are not listed in the Clarity results table. These results are converted into an absorbance scale and a library search must then performed for identification[46] (~400 reference spectra for 30 families of polymer types and several non-polymer compositions). The LDIR library was enriched by the spectra of the 5 RPPs. We highlight the importance of enriching the LDIR library with spectra of certified reference polymers (in our case the spectra of the 5 RPPs considering PC background signal) to ensure quality identification. Following this work, we will continue to enrich our library with certified pure or recycled plastics frequently found in the environment due to their wide use in packaging or construction. In addition, the Hit Quality Index (HQI) is a match acceptance criterion between sample spectra and database reference that must be defined to ensure reliable identification. The HQI is calculated by the first derivative algorithm and displayed in the query analysis window. In this study, a particle containing plastic polymer(s) was qualified as an MP when the HQI (spectrum quality) was ≥ 0.75 compared to the reference spectrum. The value of 0.75 was selected and adapted based on previous work[47–50] and on the limited range of wavelengths (1800 - 975 cm⁻¹) studied. If the HQI was < 0.75, the particle was classified as undetermined. In this case, the HQI of each identified MP (polymer) can be expressed according to its morphological parameters (width, height, diameter, area, thickness). Finally, HQI were individually inspected and interpreted based on the similarity of their absorption frequencies to those of chemical

bonds in known polymers. However, we punctually, noted that when the automatic mode and/or the identification did not answer correctly, the LDIR had to be switched off and on again.

2. 3. Test of suitable filter material for LDIR measurements

Before treatment, MPs were pre-rinsed in sequence in an ultrasonic bath, first in 50 mL of Milli-Q water and then in 1 mL of 80% (v/v) ethanol (one burnt glass beaker per polymer and rinsing type) before being left to dry under a fume hood in a piece of burnt aluminum foil[51,52]. Filters were also pre-cleaned before use with 100 mL of ethanol/Milli-Q water 20:80 (v/v) using a stirring table (24 hours in a burnt glass Petri dish). For recovery experiments, the 5 polymer types of ground referent plastic pellets (RPPs) (Table S1) were manually deposited at different concentrations onto each circular membrane filter (Table S2) using a binocular magnifier with a stainless-steel dissecting needle. The filters were subsequently placed on the Kevley slide, itself fixed onto a support (sample holder) and then introduced into the instrument for analysis. For comparison, the same procedure was performed directly on the Kevley slide alone. For each filter type and for each polymer type, triplicates were made. To speed the process up, the filter area was analyzed in automatic mode with method settings for the particle size range of from 100 µm to 500 µm, and thus adapted to the size range of the MPs used for spiking.

2. 4. Marine sample collection and lab pretreatments

(a) *Samples to verify the linear response of the method*

For the method development, we used three solutions representing matrices of the marine environment with different characteristics. These included surface seawater, sediment, and a mixture of mussel soft parts as representative of biota. Most of the samples were collected onboard the R/V

Antedon 2 (Fig. S2, Table S3). Briefly, surface seawater samples were collected in the NW Mediterranean Sea in Marseille Bay (43.246475 °N, 5.363858 °E) in May 2021 using 5 L precombusted glass bottles, then sieved (stainless steel sieve: 2000 µm mesh size) and an aliquot < 2000 µm was transferred into 100 mL precombusted bottles. For each replicate of the experiment (3 per type of filter), 50 mL were prefiltrated in the cleanroom using precleaned filters (3 successive rinses with 50 mL of Milli-Q water, and then 1 mL of 80% ethanol). Sediment samples were collected in Marseille Bay in 2018 at 15 m depth using a stainless steel Van Veen grab sampler. The content was poured onto a pre-cleaned stainless-steel tray, and the first 2–5 cm of the sediment surface were collected in a pre-cleaned glass bottle (0.5 - 1 kg of fresh material) and stored in the dark at -20 °C. Finally, mussel samples were collected by fisherman at Lazaret Bay in La Seyne/Mer (43.085765 °N, 5.903220 °E), wrapped in combusted aluminum foil and stored in the dark at -20°C. These samples constituted working solutions representing different types of matrices that were used for the device optimization experiments.

(b) Field samples

Once the method was optimized, four additional marine sample types including sediments, sub-surface seawater, mussels and fish (Fig. S2, Table S3) were collected in the NW Mediterranean Sea and analyzed for their MP content. Subsurface seawater MP samples were collected using a Manta net (0.50 x 0.15 m opening) mounted with a 250 µm mesh size cod end and towed horizontally at the surface approximately 50 m behind the research vessel at an average speed of 2.5 knots for 20 minutes[14] (with the end of the net cable shortened so that it moved to the side of the ship automatically). After each tow the net was washed and rinsed on board, the cod end sampler was removed and rinsed as well (1L of Milli-Q water and then in 100 mL of 80% (v/v) ethanol), and the sample was transferred to pre-combusted 1 L glass bottles and stored in the dark at 4 °C while awaiting analysis the

following day. MP abundance was determined by multiplying the length of tows (tow time × vessel speed) by the size of the manta trawl mouth and is expressed in MPs km^{-2}. Mussels (*Mytilus galloprovincialis* Lamarck, 1819; 3 pools of 2 individuals, 6.9 ± 0.3 cm, 5.0 ± 0.1 g) were collected by the 'Service des Phares et Balises' (La Provence vessel) of the Interregional Directorate of the Mediterranean Sea, French Ministry of the Sea, whereas fish (*Auxis rochei* (Risso, 1810); 40.7 ± 0.5 cm, 938.8 ± 117.4 g) were collected by local fisherman in Banyuls/Mer Bay. Fish were immediately dissected in the laboratory at which time their stomachs were extracted, placed in precombusted glass jars, and stored in the dark at 4 °C until pretreatment and filtration. Marseille Bay is located at the eastern edge of the Gulf of Lion (NW Mediterranean Sea) and is influenced by strong wind regimes (mainly the northwestern Mistral wind), high solar radiation[53] and episodic intrusions from the Rhône River[54] which provide important inputs of particles[55] as well as organic contaminants and MPs[14,56–58].

(c) Pretreatment of marine samples

MP extraction from the above-mentioned matrices was performed according to previous studies[59–62] with some modifications depending on the nature of the matrix. Surface seawater samples collected by manta net were treated in the laboratory on the day following collection: they were divided into 40 mL sub-samples using a Motoda box (6 times), transferred into 150 mL precombusted glass bottles, and digested by 30 mL HNO$_3$ (68%) during 72 h at 60 °C. Biota samples (pool of 2 mussels with an average weight of 5 g wet weight (WW) per individual; individual fish stomachs (average weight: 25 g WW) were digested by 30 mL KOH (10%) during 48 to 72 h at 60 °C before separation[63]. Sediment samples (2 g DW) were not digested but were studied in their raw state[12]. According to previous work[44] we analyzed only 2 g DW of sediment because this study area (Marseille Bay) is considered to be highly anthropized and with an accumulation of plastics and waste on

the sea floor, as shown by previous results (PAE concentrations on the bottom, link with plastics)[23, 64,65]. For MP extraction, all samples were mixed with a sodium chloride saturated solution (350 g L^{-1}, 35‰) to disperse the sample by gravity floatation, then centrifuged (40 mL, 5 minutes, 3 500 rpm). The floatation process was repeated three times before transferal of the supernatant into 150 mL vials using precombusted glass Pasteur pipettes. The solution was then filtered onto various filter membranes and MPs were recovered as such on these filters.

2. 5. Numerical analysis

The recovery tests for the four different filters were evaluated using the polymer identification rate (in %). For the 5 RPPs, this value is defined as MP detected / MP added, based on an index of the comparability of the query and reference spectra, and is considered here as a measurement of MP analysis efficiency. Recoveries were not corrected for procedural blanks, since no contamination was observed for MPs ranging between 200-500 µm.

The efficiency of particle size measurement by the developed method was tested by triplicate analyses of groups of RPPs in 3 size ranges (<200, 200-500 and >500 µm). All MPs were previously sieved and deposited onto 8 µm PC filters using a binocular magnifier. Efficiency is defined here as the percentage of MPs whose size, as measured by LDIR, falls within the size range expected by sieving. Although this experiment is not a strict calibration of the size measurements by LDIR, it does give a broad estimate of the efficiency of the size measurement.

Correlations between spiked and detected MP concentrations in different marine matrices were assessed using generalized linear model (GLMs[66]). The percentage of detected MPs was calculated to obtain the recovery rates of the 5 RPPs. Significance of GLMs was set at *P*-value less than 0.05, with $* \leq 0.05$, $** \leq 0.01$, and $*** \leq 0.001$. Concentrations and percentages were corrected for initial working solution concentrations. Analyses were performed using Statistica 9.1 software (StatSoft, Inc. 2010, STATISTICA ® data analysis software system, version 9.1.). Concentration of MP polymers detected in field samples (seawater, mussel, sediment and fish stomach were expressed in relative abundance (%) and were corrected using procedural blanks (0.67 ± 0.58). The proportion of MP size classes (%) were also analyzed for each marine matrix, as defined <50, 50-100, 100-200, 200-500, 500-1000, >1000 µm. All experiment and procedural tests, blanks and marine matrix analyses were conducted in triplicate.

2. 6. Quality control and quality assurance

All the experiments concerning the choice of filters, the efficiency of size measurements and spiked addition, as well as the application on real samples were performed in triplicate. Procedural blanks were performed for every 10 filters analyzed so as to evaluate contamination during processing steps. A control of the efficiency of the available reference spectrum library for all sample types with spiked microplastics was also established in this study. The handling of samples was carried out in controlled air conditions in an ISO class 6 cleanroom and operators wore cotton laboratory coats throughout the experiment. All plastic tools were avoided during sampling and storage. All lab materials used were made of stainless steel (pliers and dissecting needles were pre-rinsed with ethanol and then Milli-Q water), calcined aluminum (combusted at 450 °C for 6 h) or glass pre-rinsed with ethanol and then Milli-Q water before combustion at 450 °C for 6 h. Sampling equipment was pre-rinsed in the lab (abundantly pre-rinsed with water, then with 1 L of ethanol and finally with 1 L of Milli-Q), wrapped in calcined aluminum and stored in clean stainless-steel boxes being transported to the boat and deployed at sea. We used calcined aluminum (450 °C for 6 h) to cover all surfaces and glassware, and to store all the materials and equipment to prevent airborne MP contamination. Before LDIR analysis, to reduce contamination, filters containing MPs were placed on a Kevley slide, protected by a precombusted aluminum cover, dried at room

temperature under a fume hood (in the clean room) and stored in a Petri dish in the dark at 4°C.

3. Results and discussion

3.1. Selection of filter membranes for LDIR measurements

Table 1 presents the recoveries of MPs deposited on four different filters (placed on the Kevley slide) and analyzed by LDIR transflectance mode. MP identification rates for the 5 RPPs directly detected on the Kevley slide ranged from 85 ± 13 (PVC) to 113 ± 13% (PP). When MPs were placed on a filter material placed on the top of the Kevley slide, only 0.2 µm polycarbonate filters showed acceptable results for LDIR measurements (74-90%), although they were poorly recovered (Anodisc filters: 30-68%) or not recovered at all, as was the case on cellulose nitrate (Table 1). Despite previous investigations showing that Anodisc filters[29] were suitable for MPs analysis, especially for PE, our results indicate very low recoveries, especially for PVS and PS (Table 3).

Table 1: Recoveries of the 5 RPPs (polyethylene (PE); polypropylene (PP); polyvinyl chloride (PVC); polystyrene (PS); polyethylene terephthalate (PET)). Ten individual MPs spiked directly on the Kevley slide or on different 25 mm circular filters (deposited on the Kevley slide). Averaged percentages are given with a standard deviation based on triplicate analyses. Recoveries were defined as MP detected / MP added (in %) and were not corrected for procedural blanks. nd: not detected. RPPs are listed according to their increasing density (g ml^{-1}) (Table S1).

	KS	Anodisc	Cellulose nitrate	PC[a]	PC[b]
PP	113 ± 13	68 ± 16	nd	74 ± 13	80 ± 20
PE	85 ± 13	47 ± 33	nd	76 ± 21	87 ± 12
PS	95 ± 10	nd	42 ± 38	68 ± 27	100
PVC	85 ± 14	6 ± 10	7 ± 12	91 ± 17	100
PET	93 ± 12	30 ± 27	nd	89 ± 19	100

KS: Kevley slide glass surface; Anodisc: Aluminum oxide; PC[a]: Polycarbonate/Nuclepore, porosity 0.2 µm; PC[b]: Polycarbonate/Nuclepore, porosity 8 µm).

Anodisc filters span optical transmissions from UV to IR and can be employed for the analysis of plastic polymers that include only C-C and C-H bonds (which is the case of PP and PE), but not for polymers such as PS and PVC (*i.e.,* polymers that include C=C and C-Cl bonds as well). Indeed, PP and PE exhibit IR bands between 1470 to 1370 cm^{-1}, while PS and PVC bands range from 1300 to 600 cm^{-1} (Fig. 1)[67], where a high signal of the aluminum oxide (Anodisc filter) is observed. This issue was also well highlighted by Primpke et al., (2020). Polycarbonate filters have previously been employed for chemical imaging of both stretch and bend C-H regions[27] (2980-2780 cm^{-1} and 1480-1440 cm^{-1}, respectively), leading to average results in reflectance and good results in transmittance for PE[29]. It is worth noting that the PC membrane has a characteristic IR spectrum spanning a range from 3800 to 700 cm^{-1} with characteristic bands (aromatic stretching, υCH: 3000-2800 cm^{-1}; υC=O: 1800-1600 cm^{-1}; υC=C: 1600-1500 cm^{-1}; υC-O: 1300-900 cm^{-1}; aromatic rocking, δ-CH: 900-700 cm^{-1}) and therefore, interference with the analyzed material is to be expected, particularly for PET which exhibits identical chemical functions (C=O, C=C and C-O) as PC (Fig. 1). On the other hand, for PP, PE and PVC, which do not have these functional groups, their analysis is not impacted by the background signal of the PC filters. Therefore, as the PC membrane is always present during the sample analysis, the polymer spectra LDIR library was enriched with the 5 RPPs spectra, taking into account the PC filter background signal (Fig. 1). The polycarbonate detected in our samples was not considered in the identification of plastics, but as PC represents a minor portion of global plastic production[68], the loss of information on this polymer type remains negligible.

It is interesting to note that additional experiments suggest that the 8 µm PC filter slightly improved the recoveries compared to the 0.2 µm filter, with yields of 80-100% (Table 1). One possible explanation may be related to the porosity of the filter, which could modify its hold and rigidity of the filter. This difference in structure is observable to the naked eye once the filter is placed on the Kevley slide. Indeed, unlike an 8 µm filter, a 0.2 µm filter placed on the Kevley slide does not remain flat but undulates and remains opaque white (not

transparent), which can create interferences in the analysis of particles deposited on these finer filters (for which a flat surface and a homogeneous thickness are necessary). Therefore, the 8 μm porosity PC filter was selected as being the most appropriate support for MP detection by LDIR and maintained for the remainder of the study. It is important to note that although the Kevley slide gives satisfactory results similar to those of the PC filter (itself deposited onto the Kevley slide), the PC filter has the advantage of being able to preconcentrate the particles before the LDIR analysis. Finally, 3 squares (3 × 7 mm) were measured on a clean PC filter to obtain a background signal.
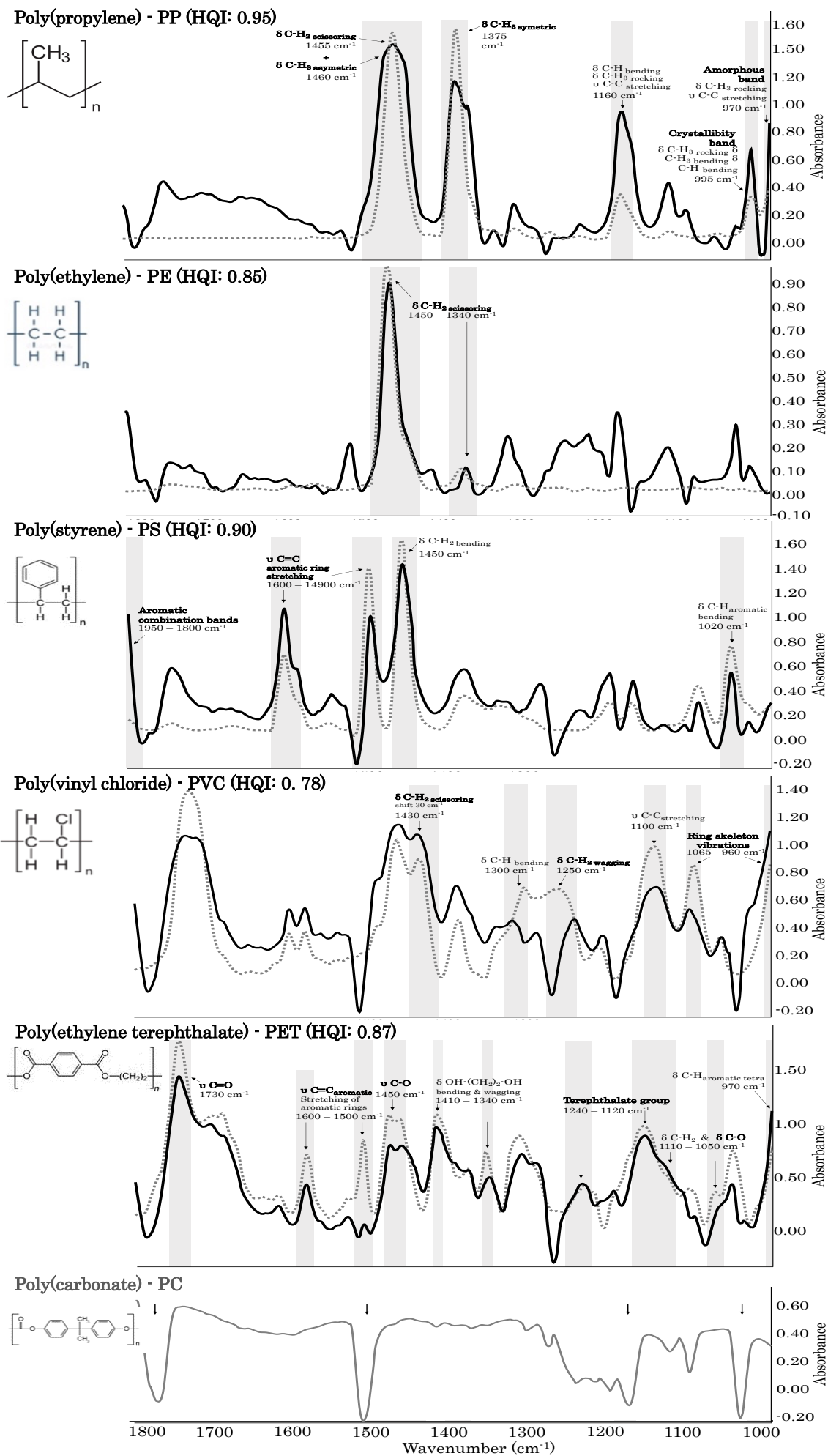
Figure 1: List of important vibration modes and mode assignments for the LDIR spectra of the 5 RPPs studied, listed according to their increasing density (g ml⁻¹) (Table S1). Absorption bands listed are representative of vibrations critical for polymer identification (in bold: the main ones; *pers. comm.*: Anne Bonhommé; Jung et al., 2018). If the Hit Quality Index (HQI) (spectrum quality) was ≥ 0.75 compared to the reference spectrum, the particle was classified as 'identified'. Dotted line: polymer spectra from the LDIR library and solid line: spectra of the analyzed RPPs, in grey: polycarbonate spectrum.

3.2. Comparison of the size of spiked MPs and LDIR measurements

The results show an effective size measurement by the LDIR system with averages of 85 ± 11%, 88 ± 10% and 98 ± 3%, for the three size range groups (<200, 200-500 and >500 μm), respectively (Fig. 2). It should be noted that the correspondence between sieving and size measured by LDIR is less optimal for MPs smaller than 200 μm. The analysis of the efficiency of size measurement does not seem to be dependent on the nature of the polymer, except for both PP and PE < 200 μm, which present the lowest efficiency (69-78%).

Although plastics particles in the environment span a wide range of sizes (from mega-, macro-, meso-, micro-, to nanoplastics)[69], in aquatic ecosystems including marine organisms, MP particles typically range from 150 to 500 μm[70], while the reported range for those found in marine sediments is from 5 to 1000 μm[71]. An examination of the literature indicates that the selected sizes of particles in the MPs spiking experiments are realistic with respect to natural MPs concentrations, and show that the use of PC filters followed by LDIR detection provides an accurate MP size determination. It must to be mentioned, however, that the relative abundance of nano-sized plastic particles is suspected to be much greater in the environment[72–76], but at this stage their detection and quantification remains highly challenging.
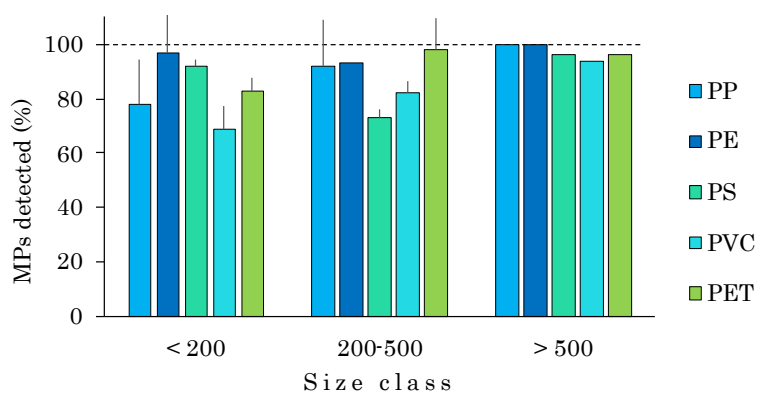

Figure 2: Detection of 5 RPPs (average ± sd of MPs detection, in %) by the LDIR method developed in this study for 3 size ranges (n = 9, size in max. length: < 200 μm; 200-500 μm and > 500 μm). The particles were manually deposited in 20 mL of Milli-Q water before being filtered onto a polycarbonate

filter (pore size 8 μm). PP: polypropylene; PE: polyethylene; PS: polystyrene; PVC: polyvinyl chloride; PET: polyethylene terephthalate, are listed according to their increasing density (g ml^{-1}) (Table S1). Column without standard deviation (sd) means that the percentage of detected particles in each of the triplicates is identical (sd=0).

3.3. Linear response to MPs spiked in different marine matrices

To assess the consistency of our method with MP levels found in different types of marine matrices, such as filtered seawater and mineral-rich and organic-rich media, spike experiments were conducted on seawater, sediment samples and extracts of mussels freshly collected in Marseille Bay. Different spike concentrations (0, 2, 5, 10, 20 and 30 MPs) for the 5 RPPs (PP, PE, PS, PVC, PET) were manually deposited on the supernatant solution (of each working solution) and concentrated on the selected 8 μm PC membrane filter, which was then placed onto the Kevley slide. Different MPs quantities were used for spiking, in agreement with the usual MP concentration in the environmental samples. MP detected sizes ranged from 44 to 1937 μm. The filters containing MPs at different concentrations were completely and automatically analyzed *via* a series of defined squares (3 x 7 mm) with the same detection method (autoscan, 75% sensitivity). Linear regressions between spiked and detected MP concentrations are reported in Figure 3 and Table S4. Independently of the initial concentration and the type of marine sample, the method responded linearly to increasing MP concentrations with very significant slopes (R^2 ranging from 0.93 to 1.0, with highly significant slopes: $p < 0.001$ or $p < 0.01$) (Fig. 3; Table S4). The linear response to increasing concentrations is lowest for PS (R^2 = 0.93) in seawater, indicating that the developed method responds linearly in a wide range of concentrations.

Averaged recoveries of the 5 RPPs spiked into marine samples (Table S4) are not significantly different from those measured for the selected filter material (PC [a, b]), except for PVC (>140 %) in all marine matrices and for PP,

PE and PET in mussels, which had lower values (69 ± 17; 78 ± 7; 81 ± 22%, respectively) (Table S4). One explanation for the high PVC recoveries observed in the three matrices might be the manual MP spiking for low concentrations samples; indeed, this is a sensitive task prone to a certain degree of incertitude. In mussel samples, the lower recoveries might be explained by the presence of dissolved organic matter, which can interfere in the infrared absorption region. According to the statistical data of the spike experiments, the concentration of samples on PC filters (8 µm) allows a reliable characterization (quantification and qualification) of different polymers in different marine samples, including complex matrices.

These results suggest that positioning a polycarbonate membrane filter on a Kevley slide constitutes a suitable technique for MP detection when using LDIR, allowing for preconcentration, reasonable handling and appropriate IR transmittance properties for polymer identification and size determination. Indeed, working on filters enables the use of large sample volumes which are more representative of the heterogeneous distribution of MPs in the environment. Automation was implemented for different environmental matrices.

3.4. MP occurrence in field samples

The predetermined protocol was applied (with triplicate analyses) to freshly collected marine samples collected in the NW Mediterranean Sea. For all the samples, our results indicate a diversity of polymer nature with particle sizes ranging from 11 µm to 2 172 µm. The concentrations of MPs measured in the surface seawater samples averaged 805 417 ± 381 881 MPs km^{-2}, 4 ± 2 MPs g^{-1} WW in mussels *Mytilus galloprovincialis*, 9 ± 1 MPs g^{-1} WW in the fish stomach samples, and 2 531 ± 1 565 MPs kg^{-1} WW of MPs in marine sediments (Table 2). It should be noted that our results do not bear close comparison to other studies due to different sample collection procedures or methodological variations, as well as to the different digestion protocols, all of which can affect particle detections[77]. However, the Manta net MPs concentrations are consistent with those previously reported in the same geographical area[78,79] (max. 578 000 MP km^{-2}; av. 112 000 MP km^{-2}, range 6 000 - 1 162 000 MPs km^{-2}, respectively) and are comparable with those found in the Northeast Atlantic Ocean[80], as well as being in the same order of magnitude as those generally reported for European waters[81,82] or in Jiaozhou Bay, China[83]. Similar conclusions can be made for *Mytilus galloprovincialis* and for sediments samples, and have already been reported in other coastal areas, *i.e.*, the Mediterranean[84], the Atlantic area[11,12] or the coastal Pacific Ocean[62,85,86]. MPs concentrations of fish stomach samples are slightly higher than those reported in small semi pelagic fish (*Boops boops*) in the Balearic Islands (Spain, Mediterranean Sea)[87] and in demersal fish from Hong-Kong[88], or are considerably higher by 2 orders of magnitude than the level observed by Zhu et al. (2019) in pelagic and demersal fishes from the Maowei Sea[89]. These differences might be explained by the fact that their feeding habits are not similar.
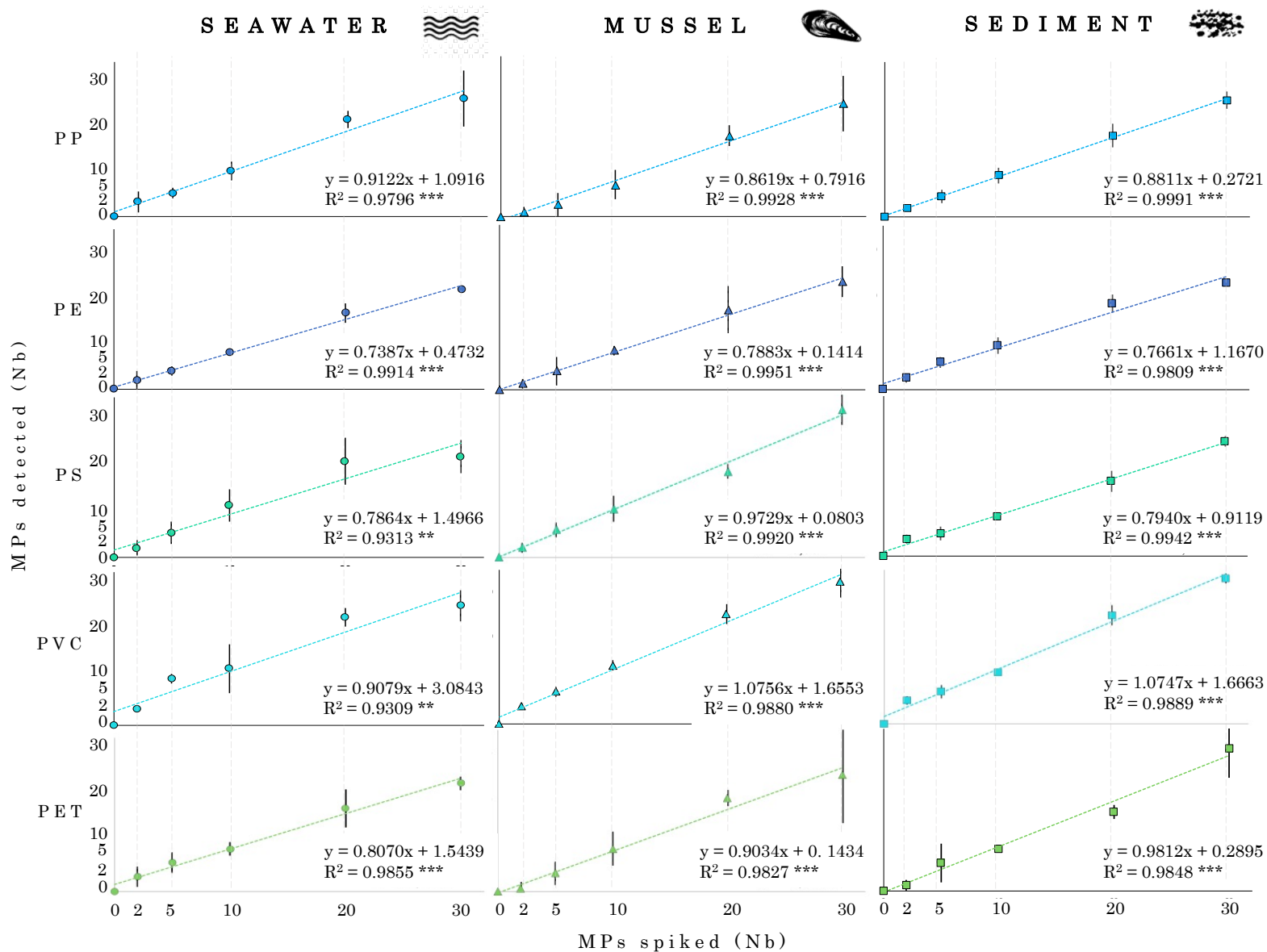
Figure 3. Concentrations of LDIR detected MPs (Nb) used for the linear regressions (with curve equations, R^2 and p values) corrected by the initial concentrations (without spiked MPs). Concentrations were not corrected for procedural blanks, since no contamination was observed. All marine compartment analyses were conducted in triplicate. Averaged recoveries of five RPPs in seawater, sediment, and mussel (*Mytilus galloprovincialis*) samples were determined from the different spiking concentrations (2, 5, 10, 20, and 30 particles, respectively) and are given with a standard deviation. Spike experiments were conducted on surface seawater samples collected in May 2021, on sediment collected in October 2018, in Marseille Bay (NW Mediterranean Sea), and on mussel samples collected in La Seyne/Mer Bay in May 2021 (Figure S1, Table S3). Slopes and y intercepts are very highly significant (***$p < 0.001$) or highly significant (**$p < 0.01$). PP, polypropylene; PE, polyethylene; PS, polystyrene; PVC, polyvinyl chloride; PET, polyethylene terephthalate, listed according to their increasing density (g mL−1; Table S1).

Table 2: MPs concentrations (average ± sd) detected by LDIR (N=3 for each matrix) in samples of marine environmental matrices (seawater (manta net); mussels (pools of 2 individuals); sediment; fish stomachs) were corrected for the procedural blanks (0.67 ± 0.58). Abundances are expressed in the most frequent units. MP detection was conducted on surface seawater (manta net) samples collected in May 2021, in Marseille Bay (northwestern Mediterranean Sea), on sediment collected using a stainless steel Van Veen grab sampler and on mussel and fish samples collected by fisherman in La Seyne/Mer Bay, in May 2021 and in Banyuls/Mer, in October 2018, respectively (Fig. S1, Table S3). MPs: Microplastics identified; mussel: *Mytilus galloprovincialis*; fish: *Auxis rochei*; ind.: individual; WW: wet weight.

Matrix	Site	Sampling	Abundance	Unit
Seawater	Marseille Bay	05-2021	805 417 (± 381 881)	MPs km^{-2}
			2 (± 1)	MPs m^{-3}
Mussel	Marseille Bay	05-2021	4 (± 2)	MPs g^{-1} WW
			10 (± 6)	MPs ind.$^{-1}$
Sediment	Marseille Bay	05-2021	2 531 (± 1 565)	MPs kg^{-1} WW
Fish stomach	Banyuls/mer	10-2018	220 (± 21)	MPs ind.$^{-1}$
			9 (± 1)	MPs g^{-1} WW

Among the 11 different polymers detected for all samples, PP, PE, PS, PVC, PET and the polyether group represented more than 92% of total MPs (Fig. 4.A). PVC represented 50 ± 25% of total MPs and was the predominant polymer in sediment and fish stomachs (74%), whereas PE and PP (~47%) combined were together the most abundant polymers in both seawater and mussel samples. Overall, the five polymer types with the highest production volumes found in this study include PP, PE, PS, PVC and PET, which represent up to 85% of total MPs. The Alkyd varnish group (mainly used in paint and resin products) was frequently found in seawater and fish stomachs, representing 5% of the total MPs. This polymer group has previously been reported in seawater samples from the South China Sea and southeastern coast of Korea[90–92]. In addition, our study reports the presence of the polyether group in marine samples, representing 10 ± 5% of the total MPs. We can note that the LDIR library indicates the detection of polyether and alkyl varnish compounds without additional precision as to the specific nature of the compound. Further laboratory studies should allow to better characterize the exact nature of these groups of compounds. The size distribution of the MP particles analyzed *via* the LDIR system varied depending on polymer and sample types (Fig. 4.B). The detected MPs can be grouped into 6 main size classes: <50 µm; 50-100 µm; 100-200 µm; 200-500 µm; 500-1000µm; >1000µm. The size range of all MPs detected ranged between 11 and 2 172 µm, as mentioned above. MPs sizes in seawater and mussels were predominantly in the 100-200 and 200-500 µm size ranges and were more widely distributed for fish stomach and sediment samples, with the 50-100 µm size range being the main size class in fish stomachs (Fig. 4.B). Some similarities (nature of polymer and size distribution) can be noticed between relative MPs abundance in seawater and mussels, which is consistent with a previous study that found that mussels can be considered as a bio-indicator for MPs occurrence in seawater[9].

The main advantage of the LDIR system (in automatic mode) is to characterize both the composition of the particles and their size. Resulting data concerning width, height, diameter, and particle area are shown to have promising and powerful prospects. Furthermore, the method is reliable and efficient, and providing a considerable reduction in analysis time. In conclusion, while further improvements are possible and need to be investigated or implemented, in particular through the enrichment of the library by many reference plastics (spectra of pure or recycled plastics) susceptible to be found in terrestrial and aquatic environments, we see a great potential for experimental MP assessments using this technique. This method of direct identification of MPs at the marine compartmental scale appears to be a promising and indispensable tool for the improved refining of estimations of MPs present on the global scale. These future possibilities, although not discussed in this report, will be the subject of another study.
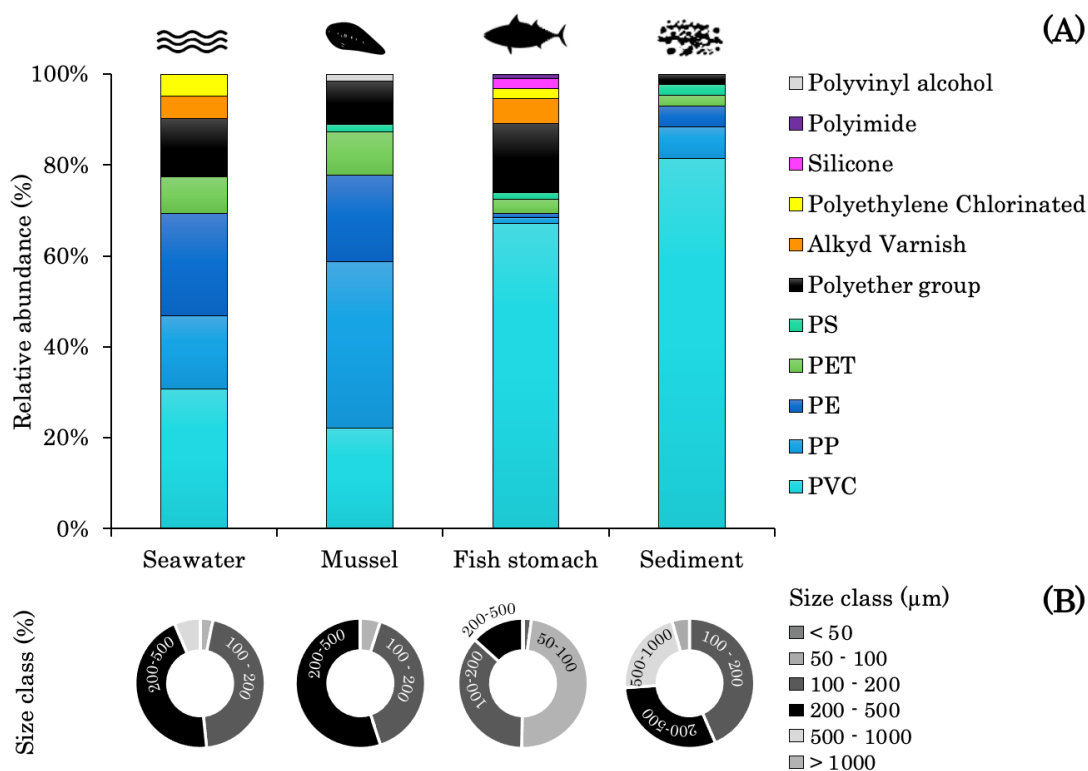
Figure 4: (A) Relative abundance (%) of MPs polymers detected in seawater, mussel (*Mytilus galloprovincialis*), sediment and fish stomach (*Auxis rochei*) samples collected in the coastal NW Mediterranean Sea and analyzed using the LDIR system. Detected concentrations were corrected from procedural blanks (0.67 ± 0.58 particles per filter). All marine matrix analyses were run in triplicate. (B) Proportion of 6 size classes (%) for each sample type. PVC: polyvinyl chloride; PP: polypropylene; PE: polyethylene; PET: polyethylene terephthalate; PS: polystyrene.

SUPPORTING INFORMATION

Additional data and details on the experimental procedure (materials, reagents and filters), marine sample collection and linear response to MPs spiked are available free of charge *via* the Internet at http://pubs.acs.org/

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