



ADVANCING TOWARDS LITTER - FREE
ATLANTIC COASTAL COMMUNITIES
BY PREVENTING AND REDUCING
MACRO AND MICRO LITTER

D.2.2.5 - Report on the potential of Pyrolysis-GC/MS and Rock-Eval® techniques to monitor small microplastics

WORK PACKAGE 2, ACTIVITY 2, TASK 4



Co-funded by
the European Union

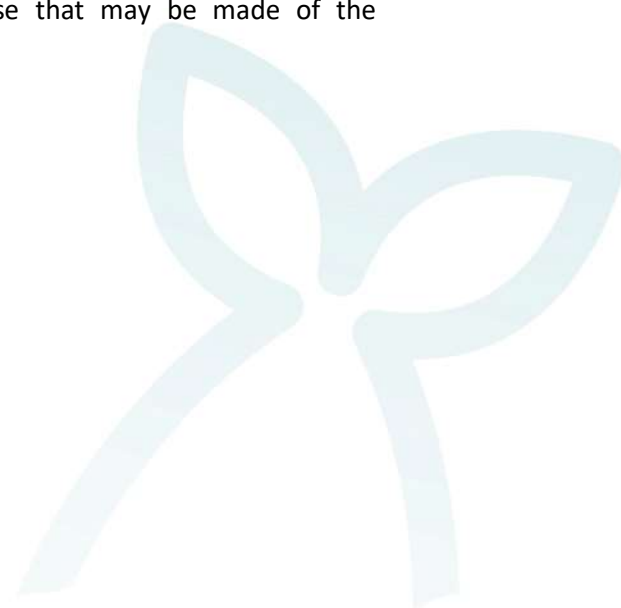




Work package	WP2
Activity and task	ACTIVITY 2, TASK 4
Date	05 / 10 / 2026
Version	2
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Summary

Despite the numerous studies already published on microplastic monitoring, there is still an urgent need to standardise and optimise methodologies in order to generate monitoring data that can be integrated into actionable outputs. In this context, the objective of the Activity 2 of Free LitterAT work package 2 is to improve and enhance capability for micro litter detection and monitoring. In particular, the task 4 includes the development and testing of methods to quantify microplastics using pyrolysis-GC/MS and the thermal Rock-Eval® techniques, to assess their potential as a complement or alternative to traditional spectroscopy techniques for the quantification of microplastics in coastline sediments.

To fulfill this task, the work conducted by CEDRE with the support of IFP Energies Nouvelles (IFPEN), was divided into three main parts. First, CEDRE and IFPEN obtained different types of sediment samples from official monitoring programs implemented on the French Atlantic Area coastline. Samples were then prepared in CEDRE laboratory using a protocol co-designed by CEDRE and IFPEN. Secondly, CEDRE worked on the development and testing of a pyrolysis-GC/MS method while IFPEN worked on the development and testing of the thermal RockEval® method, both to quantify small microplastics in sediment matrices. After assessing the performance of these two methods, they were used to quantify small microplastics contamination in the natural sediment samples obtained from official monitoring programs.

The present deliverable of the Free LitterAT project presents the final results of the study. The key results are presented below.

First, a specific sample preparation protocol based on chemical digestion with hydroxide peroxide and flotation separation using a saturated calcium chloride solution, was co-designed by CEDRE and IFPEN and successfully applied to prepare port and beach sediment samples. New features of this protocol included the preparation of 200 g of samples and the analysis of residues which are generally discarded during the preparation phase. These features allowed to reduce quantification limits and to quantify both small microplastics (>45 µm, SMP) and very small microplastics (<45 µm, VSMP), respectively.

Secondly, a pyrolysis-GC/MS method to quantify SMP was developed, validated and further tested on natural samples. Results obtained confirmed the capacity of the method to quantify small microplastics. The method allows in one analysis the quantification of microplastic quantities (in µg or mg) for each targeted polymer, providing the total microplastic concentration but also the concentration by polymer. The development and validation phase showed the method exhibits good precision, repeatability, and accuracy. In particular, the method was validated for 7 polymers (PE, PP, PS, ABS, PVC, N-6 and N-6,6) and allowed to quantify them above analytical limits in several natural samples (either port or beach sediments). Total MP concentrations ranged between 17 to 170 mg/kg DW (5 to 28 mg/kg WW) for the port sediment samples and between <LOD to 1.5 mg/kg DW in the beach samples, suggesting a higher MP



contamination in port sediments compared to beach sediments. In port sediments, polymers mainly quantified were PVC and PP with also PE, PS and N-6,6 detected. In beach sediment samples, PE, PS, PVC and N-6 were detected but only PVC was quantified above quantification limits.

Thirdly, a thermal Rock-Eval® method to quantify SMP was proposed and further tested on natural samples. Unlike the Pyrolysis-GC/MS approach, which underwent a dedicated validation procedure within the present study, the Rock-Eval® methodology relied on previously published calibration frameworks and routine instrumental quality-control procedures. Results obtained confirmed the capacity of the method to quantify small microplastics (in µg or mg). The method allows in one analysis the quantification of total concentration in microplastic. The development and validation phase showed the method exhibits good precision, repeatability, although additional validation using certified reference materials would be required to fully assess method accuracy. In addition, the Rock-Eval® analyses reveal that all studied port sediment samples are contaminated by microplastics with concentrations ranging between 6 to 63 mg/kg WW.

Port sediment samples analysed by both methods exhibit very consistent results. In addition, both methods showed that masses measured from the sieving residues (generally discarded during the sample preparation) were not negligible. This highlights the presence of very small microplastics (<45 µm) in these samples. This suggests that very small microplastics, which are frequently excluded from routine monitoring protocols, may represent a substantial proportion of the total microplastic mass present in coastal sediments. This observation raises important questions regarding the representativeness of current monitoring strategies and highlights the need to reconsider size thresholds applied in environmental assessments.

Overall, while further studies and improvements are needed, results show that tested methods have a very good potential to quantify small microplastics in sediment matrices, and they appear complementary to existing spectroscopy methods. However, the absence of calibration standards and reference materials remains a major issue, hindering the optimization of the methods and limiting their overall performance. Finally, new data have been produced on microplastic contamination in coastal sediment from the French Atlantic area, which confirms the presence of this contaminant in both port and beach sediments.



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Acknowledgements

This study, co-funded by the Interreg “Atlantic” programme as part of the Free LitterAT project, was conducted with the support of:

- CEREMA (French Public Centre for Studies and Expertise on Risks, the Environment, Mobility and Urban Planning) who kindly facilitated and organised the provision of sediment samples from the French National network for monitoring the quality of water and sediments in maritime ports (REPOM), they are coordinating,

- DDTM (Departmental Directorates for Territories and the Sea) Calvados (14), Morbihan (56) and Pyrénées-Atlantiques (64), who performed the port sediment samplings and organised samples shipment to CEDRE laboratory.



Abbreviations

ABS: Acrylonitrile-Butadiene-Styrene

ANOVA: Analysis of Variance

CEREMA: Centre for Studies and Expertise on Risks, the Environment, Mobility and Urban Planning (*Centre d'études et d'expertise sur les risques, l'environnement, la mobilité et l'aménagement*)

CaCl₂: Calcium Chloride

DDTM: Departmental Directorate for Territories and the Sea (*Direction départementale des Territoires et de la Mer*)

DW: Dry Weight

FID: Flame ionization detector

FTIR: Fourier transform infrared spectroscopy

GC/MS: Gas Chromatography/Mass Spectrometry

HC: Hydrocarbon

H₂O₂: Hydrogen Peroxide

IFPEN: IFP Energies Nouvelles

LOD: Limit of Detection

LOQ: Limit of Quantification

MINC: Mineral Carbon

MP: Microplastics

MSFD: Marine Strategy Framework Directive

MSFD TG ML: MSFD Technical Group on Marine Litter

N-6: Nylon-6

N-6,6: Nylon-6,6

OM: Organic Matter

PC: Polycarbonate

PE: Polyethylene

PET: Polyethylene Terephthalate

PMMA: Polymethyl methacrylate

PS: Polystyrene

PP: Polypropylene

PU: Polyurethane

PVC: Polyvinyl Chloride

Pyrolysis-GC/MS: Pyrolysis-Gas Chromatography/Mass Spectrometry

REPOM: National network for monitoring the quality of water and sediments in maritime ports (*Réseau national de surveillance de la qualité des eaux et des sédiments dans les ports maritimes*)

RNS-mP-P: national monitoring network for mesoplastics and large microplastics (1-5 mm) on beaches (*Réseau National de Surveillance des mésoplastiques et grands microplastiques (1-5 mm) sur les plages*)

RSD: Relative Standard Deviation

SBR: Styrene-Butadiene Rubber

SD: Standard Deviation

SMP: Small microplastics

SIM: Single Ion Monitoring

TDU: Thermal Desorption Unit
TOC: Total Organic Carbon
VSMP: Very small microplastics
WP: Work Package
WW: Wet Weight
wt: weight
4FS: Poly(4-fluorostyrene)



Context: the Free LitterAT project

The [Free LitterAT](#) project, whose full name is “Advancing towards litter-free Atlantic coastal communities by preventing and reducing macro and microlitter”, is funded by the European Interreg Atlantic program 2021-2027. Lasting 3 years, the project aims to protect biodiversity by implementing innovative approaches to prevent and reduce marine litter pollution. This project, led by CETMAR (Spain), involves 14 partners and 9 associated partners from 4 countries: Ireland, France, Spain and Portugal.

More specifically, the project aims to advance towards litter-free coastal communities by developing, testing and implementing innovative approaches for the prevention, localisation and reduction of marine litter, with a special emphasis on abandoned, lost or discarded fishing gear (also known as ALDFG) and microplastics. The main objectives of the project are:

1. Preventing marine litter by improving waste management and recycling and facilitating the implementation of SUP and PRF Directives;
2. Understanding the origin and location of litter accumulation by identifying major sources, pathways and hotspots of marine litter;
3. Reducing and removing marine litter and associated risks;
4. Promoting litter-free local communities by developing pilot actions and awareness raising activities.

The work is divided into 4 work packages (WP):

WP 1. Prevention by improving waste management and recycling: Prevent marine litter by improving waste collection, recycling and increasing awareness in Atlantic ports and coastal cities, with a specific focus on fisheries related waste, for the preservation of the oceans and marine biodiversity.

WP 2. Assessing and Monitoring micro and macro litter contamination: Develop and optimise current tools and methods for monitoring macro and micro litter and to enhance processing and analysis solutions that can facilitate and generate harmonised data that can be integrated into actionable outputs.

WP 3. Modelling approaches from micro and macro litter: from modelling outputs to sources and hotspots: Improve our understanding of the location of accumulation areas of ALDFG, macro and microplastics and their sources, supporting the environmental agencies in charge of marine litter management with powerful modelling tools.

WP 4. Reducing marine litter and associated risks: Contribute to reducing marine litter by developing and demonstrating procedures, systems and manuals to remove different types of marine litter (ALDFG, hotspots, microplastics) in different environments.

Introduction

The omnipresence of microplastics (MP) in the environment represents one of the significant ecological challenges of the 21st century. Their persistence and disruptive impact on both aquatic and terrestrial ecosystems make them a systemic threat to biodiversity and human health (Ghosh et al., 2023). While their detection is now well-documented on a global scale, accurate identification and quantification remain hindered by methodological limitations, compromising the assessment of their distribution, sources, behavior, and long-term effects (Huang et al., 2023). Despite the numerous studies already published on microplastic monitoring, there is still an urgent need to standardise and optimise methodologies in order to generate monitoring data that can be integrated into actionable outputs (Nyadjro et al., 2026).

Among the different analytical techniques available, spectroscopic techniques such as Fourier-transform infrared (FTIR) and Raman spectroscopy, provide valuable insights into the molecular signatures of polymers while allowing the acquisition of single-particle data and subsequent counting of microplastic particles. They are classically applied in the context of microplastics environmental monitoring programs. For example, these techniques are recommended for validation purposes in the context of the OSPAR Convention monitoring programme for microlitter (including microplastics) in seafloor sediments in the OSPAR Maritime Area which aims at assessing microplastics occurrence and abundance in marine sediments (subtidal and offshore) (OSPAR MicroPlastic Expert Group, 2024).

However, effectiveness of spectroscopic techniques is closely tied to the sample size, degradation state, and environmental matrix, while also requiring labor-intensive preprocessing. To overcome these constraints, advanced thermochemical methods such as pyrolysis coupled with gas chromatography and mass spectrometry (pyrolysis-GC/MS) and the Rock-Eval® technique can represent relevant alternatives (Huang et al., 2023; Dey et al., 2025; Romero et al., 2022b).

In this context, the objective of the Activity 2 of Free LitterAT work package 2 is to improve and enhance capability for microlitter detection and monitoring. In particular, the task 4 includes the development and testing of methods to quantify microplastics using pyrolysis-GC/MS and the RockEval® techniques, to assess their potential as complement or alternative of traditional spectroscopy techniques for the quantification of microplastics in coastline sediments.

To fulfill this task, the work conducted by CEDRE with the support of IFP Energies Nouvelles (IFPEN), was divided into three main parts. First, CEDRE and IFPEN obtained different types of sediment samples from official monitoring programs implemented on the French Atlantic Area coastline. Samples include port sediments collected in the context of the French National network for monitoring the quality of water and sediments in maritime ports (REPOM), coordinated by CEREMA (Public French Centre for Studies and Expertise on Risks, the Environment, Mobility and Urban Planning) and beach sediments collected on monitoring sites of the French national monitoring

program for mesoplastics and large microplastics (RNS-mP-P), coordinated by CEDRE. Samples were then prepared in CEDRE laboratory using a protocol co-designed by CEDRE and IFPEN, in relation with work conducted by Free LitterAT partners in the context of WP2-Activity 2-Task2 “Harmonised sampling guidelines to monitor microplastics in different matrices (water, sediments and biota). Secondly, CEDRE worked on the development and testing of a pyrolysis-GC/MS method while IFPEN worked on the development and testing of the Rock-Eval® technique, both to quantify small microplastics in sediment matrices. After assessing the performances of these two methods, they were used to quantify small microplastics contamination in the real sediment samples obtained from official monitoring programs. For both approaches, advantages and drawbacks were identified and the techniques and results obtained were compared with traditional spectroscopy techniques

The present deliverable of the Free LitterAT project (referred as deliverable D.2.2.5) presents the final results of the study. This report is divided into four main parts. The first part describes the samples studied and the sample preparation protocol. The second part focuses on the development and application of the pyrolysis-GC/MS method. The third part addresses the development and application of the Rock-Eval® technique and the fourth and last part discusses results obtained and the performances of tested methods in comparison with traditional spectroscopy methods.



PART 1: Samples presentation and preparation protocol

This section presents the samples considered in the study and the preparation protocol conjointly designed by CEDRE and IFPEN. It has to be noted that all samples were prepared in CEDRE laboratory to ensure consistency in the preparation and each sample analysed by both CEDRE (using pyrolysis-GC/MS) and IFPEN (using Rock-Eval®) is exactly the same to allow a complete comparison of both analytical methods. It consists in one homogenous sample prepared in CEDRE laboratory and divided into two sub-samples, one for analysis in each lab.

1. Samples considered in the study

1.1. Blanks and artificial samples

For cross-contamination assessment and validation purposes, blanks and artificial samples were prepared and analysed in the present study, as detailed below.

1.1.1. Blanks

To assess external contamination during sample preparation, two types of blanks were prepared and analysed in the same way as the rest of the samples: a procedural blank, which undergoes the same extraction steps as the samples (see section 2.2), and an atmospheric blank, where a 25 mm quartz microfibre filter (Grade 293×50, Dutscher) is exposed to ambient air each time the samples are exposed.

1.1.2. Artificial samples prepared by CEDRE (including calibration)

To assess the performance of the method, artificial samples were prepared with known concentrations of polymers by diluting in silicon dioxide (SiO₂) powder (Frontier Lab), either pure PE powder (Sigma Aldrich) or the Frontier Lab Microplastics Calibration Standard, distributed by SRA Instruments. Total mass per sample (diluent + polymers) was 2.5 mg. These samples were also used for external calibration purposes (see Part 2, section 2.2.4).

1.1.3. Samples from the WEPAL-QUASIMEME interlaboratory exercise

To assess the performance of the method, CEDRE registered to the Round 2024 of the WEPAL-QUASIMEME¹ Interlaboratory Study on the Analysis of Microplastics in Environmental Matrices (Development Exercise DE 17). This exercise aimed at evaluating the laboratory's ability to determine the polymer type of plastic particles, and the number and/or mass of plastic particles in test samples, which have been prepared specifically for this exercise by the institute organising the exercise. The test materials were prepared to enable analysis by a broad variety of analytical methods and

¹ <https://www.wur.nl/en/research/products-services/proficiency-testing-wepal-quasimeme>

techniques (visual analysis, hyperspectral imaging, FTIR, Raman and pyrolysis-mass spectrometry), and consist of water and sand materials.

This exercise involved two rounds: one with 3 water samples and the second with 3 sand samples. The 3 water samples were sent in glass flasks, containing ± 1 L of freshwater originating from a small stream, 1 working as a blank and the other two spiked with microplastics. The sand samples were sent in glass jars containing 65 g of wet sand, one working as a blank and the other two spiked with microplastics. The list of test samples included in the exercise and analysed by CEDRE is presented in Table 1 below.

Table 1: Overview of test samples sent to participants of the round 2024 of the WEPAL-QUASIMEME Interlaboratory Study on the Analysis of Microplastics in Environmental Matrices (extract from the WEPAL-QUASIMEME protocol). The type of measured data to report for each sample is detailed in the right column.

Round	Analysis Group Code	Test sample	Type of data to report
2024 - 1	DE-17	QMP016FW Bottle with 1 litre of freshwater spiked with microplastics	 number of plastic particles present in the bottle or jar (and corresponding polymer types); and/or mass of plastic particles present in the bottle or jar (and corresponding polymer types); analytical methods used
2024 - 1	DE-17	QMP017FW Bottle with 1 litre of freshwater spiked with microplastics	
2024-1	DE-17	QMP018FW Bottle with 1 litre of freshwater unspiked	
2024-2	DE-17	QMP019MS Glass jar with wet sand, spiked with microplastics	
2024-2	DE-17	QMP020MS Glass jar with wet sand, spiked with microplastics	
2024-2	DE-17	QMP021MS Glass jar with wet sand, unspiked	

1.2. Real sediment samples

1.2.1. Port sediment samples

Port sediment samples collected in the context of the French National network for monitoring the quality of water and sediments in maritime ports (in French *Le réseau national de surveillance de la qualité des eaux et des sédiments dans les ports maritimes*, REPOM), were kindly provided with the support of CEREMA, the network coordinator. In total, 10 samples were collected in 6 ports (Ports de Caen-Ouistreham, Honfleur, Lorient, Bayonne, Hendaye, Saint-Jean-de-Luz, see Figure 1 below) located on the French Atlantic Area coastline, by three Departmental Directorate for Territories and the Sea (in French *Direction départementale des territoires et de la mer*, DDTM): DDTM Calvados (14), DDTM Morbihan (56) and DDTM Pyrénées-Atlantiques (64).

Samples were collected in September 2024 by DDTM under the guidance of CEREMA. The samples were initially collected using an Eckman grab, a tool designed for precise

sediment sampling. Once retrieved, the samples were thoroughly homogenized to ensure uniformity and reduce variability in subsequent analyses. Following homogenization, the samples were carefully packaged in glass jars to preserve their integrity (ca. 1 kg). To prevent contamination or external interference, the jar lids were insulated with aluminum foil before sealing. This method ensures that the samples remain uncontaminated and suitable for laboratory analysis. Samples were then shipped to CEDRE laboratory where they were stored in a freezer at -18°C until analysis. The samples obtained are detailed in the Table 2 below.

Table 2: List of port sediment samples considered in the study

<i>Samples</i>	<i>Provider</i>	<i>Sampling location</i>	<i>Sample name</i>
1	DDTM Calvados	Port of Caen Ouistreham 37	PCO 37
2		Port of Caen Ouistreham 38	PCO 38
3		Port of Caen Ouistreham 39	PCO 39
4		Port of Honfleur	Honfleur
5	DDTM Morbihan	Port of Lorient la base	Lorient LB
6		Marina of Lorient	Lorient PL
7	DDTM Pyrénées-Atlantiques	Bayonne Commercial Port	Bayonne
8		Hendaye fishing port	Hendaye PE
9		Saint-Jean-de-Luz fishing port	Saint-Jean-de-Luz
10		Marina of Hendaye	Hendaye PL

1.2.2. Beach sediment samples

Beach sediments were sampled by CEDRE in 4 sites along the French Atlantic area coastline (see Figure 1), using an approach derived from CEDRE (CEDRE, 2018). Two of these sites (Le Stang and La Bonne Anse) are monitoring sites of the French national monitoring program for mesoplastics and large microplastics (RNS-mP-P), coordinated by CEDRE (CEDRE, 2023). The two others are located close to coastal litter accumulation sites that have been identified as potential sites to conduct pilot clean-up action in the context of Free LitterAT WP4-activity 3 (see deliverable D.4.3.5).

Sampling was conducted by collecting beach sediment at the top of the beach (fine sand), in the top 5 cm layer. Several samples were taken over 100m and mixed together before collecting a subsample of about 500 g that was stored in a glass jar and brought back to the lab. All the material used for sampling was non-plastic (either glass or metal). The samples collected are detailed in the Table 3.

Table 3: List of beach sediment samples (sand) considered in the study

<i>Samples</i>	<i>Sampling location (Municipality, department)</i>	<i>Date of sampling</i>	<i>Sample name</i>
11	Le Stang (Saint-Jean-Trolimon, 29)	April 2024	Le Stang (April)
12	Contis South (Condis, 40)	April 2024	Contis
13	Le Stang (Saint-Jean-Trolimon, 29)	July 2024	Le Stang (July)
14	Moliets (Moliets-et-Maâ, 40)	April 2024	Moliets
15	La Bonne Anse (Saint-Augustin, 17)	November 2024	La Bonne Anse

1.3. Map of sampling locations

A map with the sampling locations for both port and beach sediment samples, is presented on Figure 1, below.

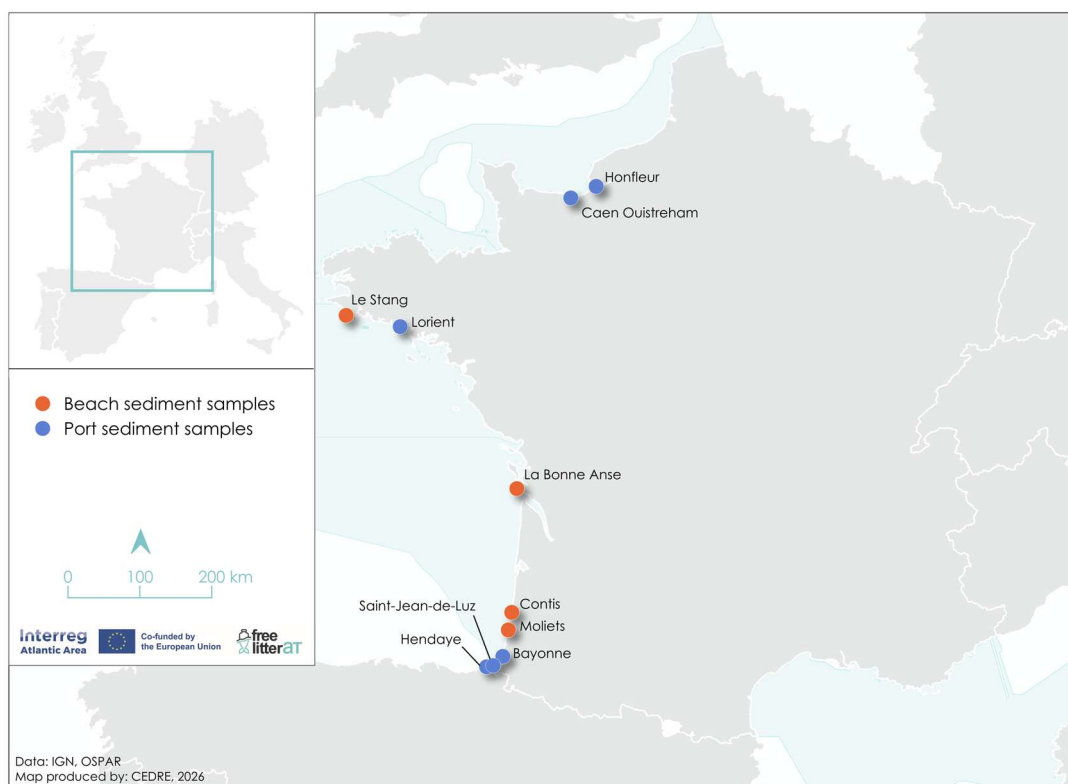


Figure 1: Sampling locations considered in the study, for either port or beach sediment samples

2. Sample preparation

A specific sample preparation protocol (Figure 2) was co-designed by CEDRE and IFPEN based on their previous experiences. The main objective of this protocol was to extract and concentrate microplastics by effectively removing organic matter (OM) and separating microplastics from the mineral matrix. The process involves a digestion with Hydrogen Peroxide (H_2O_2) 30% where organic matter is eliminated to isolate microplastics, and a flotation separation using a saturated Calcium Chloride ($CaCl_2$) solution (with a density of ca. 1.42) where microplastics are separated from the mineral matrix based on their lower density. The key innovations were two folds. First, CEDRE and IFPEN planned on upstream analysis of unprepared samples (bulk samples). Before the standard preparation steps, a part of unprepared samples is kept for analysis to investigate whether the raw signal contains usable information on microplastic content (S1 in Figure 2). Secondly, CEDRE and IFPEN planned to analyze the sieving residue. Indeed, this protocol examines the filtrates produced during the separation phases which are commonly discarded (in particular the residue obtained after the sieving step following the organic matter digestion, S2 in Figure 2). This innovative protocol aims to bridge gaps in conventional methods by broadening the analytical scope and maximizing the utility of all sample components for more complete microplastic mass estimation.

To ensure consistency in the comparison of quantification methods, a decision was taken to entrust a partner laboratory with the full responsibility of preparing the samples (CEDRE lab). This approach minimizes potential discrepancies that could arise from differences in preparation protocols between laboratories. By standardizing this critical step, the study aims to concentrate solely on the analytical comparison of the selected quantification methods (pyrolysis-GC/MS versus Rock-Eval®). This strategy ensures that the analytical results are more directly comparable, as any variability due to sample preparation has been eliminated accordingly. It enhances the reliability and accuracy of the findings, allowing the project to draw more robust conclusions about the effectiveness and precision of each quantification method.

The protocol allowed to obtain three types of subsamples as illustrated in Figure 2:

- (1) A set of samples containing 10 bulk dry and crushed port sediments (*S1 samples*).
- (2) Two samples containing residues of port sediments extraction. These residues were obtained after the elimination of the organic matter (OM) with H_2O_2 and sieving on a metallic sieve. The sieved solution containing particles $<45\ \mu m$, were filtered on quartz fiber filters ($2.2\ \mu m$) and filters were dried and crushed (*S2 samples*).
- (3) A set of samples composed by 10 powdered quartz fiber filter containing the final residue after the following steps: OM digestion with H_2O_2 , sieving, flotation separation, sieving ($>45\ \mu m$), washing, quartz fiber filter filtration ($2.2\ \mu m$), drying and crushing phases (*S3 samples*).

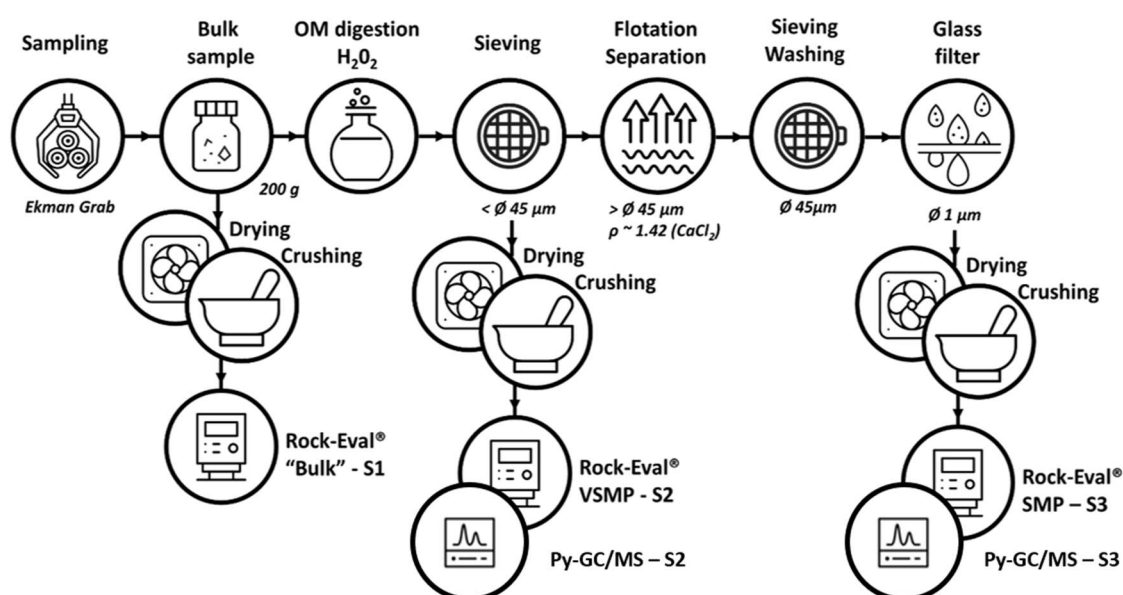


Figure 2 : Sample preparation and analytical procedures used in this study co-designed by CEDRE and IFPEN (©IFPEN) (VSMP: very small microplastics, still including matrix ($<45\ \mu m$), SMP: small microplastics ($>45\ \mu m$)).

All samples preparation were performed in CEDRE laboratory (Brest, France). The different steps of the protocol are detailed in sections below.

2.1. Prevention and quantification of external contamination in sample

The prevention and verification of external contamination is crucial in MP studies to avoid biases resulting from the ubiquitous presence of plastics in everyday objects and laboratory equipment. During the sample preparation, the following precautions were taken:

- All solvents and reagent solutions were filtered (0.2 µm) before use,
- The use of plastic utensils was minimised and replaced whenever possible by glass or metal,
- All equipment used at each preparation stage or in contact with samples were either heat-treated in an oven at 500°C for 6 hours, including quartz fibre filters, or thoroughly rinsed with filtered osmotic water and filtered ethanol,
- Operators wore cotton gowns,
- Operators limited their use of plastic gloves and washed their hands with soapy water under a strong jet of water to remove potential particles.

In addition, atmospheric, procedural and analytical blanks were analysed to assess cross-contamination (see sections 1.1.1 and 2.4).

2.2. Sample preparation

The following sections detail the digestion and extraction processes used to extract and concentrate MP in the different samples. Each type of samples was processed as detailed below and placed in 1L high beakers before subsequent preparation steps.

• *QUASIMEME-WEPAL water samples*

The QUASIMEME-WEPAL water samples (ca. 1L) were directly filtered on 25mm-diameter quartz microfibre filter (Grade 293×50, Dutscher), suitable for pyrolysis and previously heat-treated and weighed, to extract the particles. Filters were then dried, weighed with a precision balance (0.1 mg accuracy) and grinded as detailed in section 2.2.3.

• *QUASIMEME-WEPAL sediment samples*

The QUASIMEME-WEPAL sand samples were received dry and they weighed 65 g each. Received samples were directly extracted, without the digestion step, as detailed in sections 2.2.2 and 2.3.3.

• *Port sediment samples*

The sediment sample MP extraction protocol developed by CEDRE, allows the extraction of ca. 100 g of sediment. However, previous CEDRE studies suggested 100 g could be too small to allow quantification of MP considering current analytical quantification limits associated with the Pyrolysis-GC/MS method. In this context, it was decided to extract and analyse 200 g samples in the present study. Thus, for the port sediment samples, 100 g wet weight (WW) of homogenized sediment were digested and extracted in duplicate for each sample, in order to analyse 200 g WW in total per sample. For each sample, the two duplicated supernatants obtained after extraction (section 2.2.2) were filtered on the same quartz microfibre filter to combine in one sample the two 100 g extracts. As port sediment samples were rich in organic matter, it was decided to extract them whilst they were still wet in order to prevent the drying stage from affecting the

efficiency of the digestion of the organic matter. To express the results in dry weight, water contents were assessed on subsamples (see 2.3).

- **Beach sediment samples**

As beach sediment samples were mainly mineral, samples were analysed dry to allow a more homogenous analysis. Once brought back to the lab, samples were dried at 50°C to reach a constant mass. Then, they were sieved on a 1 cm mesh to remove bigger debris and particles. Then, similarly to port sediment samples, 100 g dry weight (DW) were digested and extracted in duplicate for each sample, in order to analyse 200 g DW in total per sample. For each sample, both supernatants obtained after extraction (section 2.2.2) were filtered on the same quartz microfibre filter to combine in one sample the two 100 g extracts.

2.2.1. Digestion of organic matter

The digestion process is essential for eliminating organic matter and isolating microplastics. For each sample, a 35% hydrogen peroxide (H_2O_2) solution was gradually added to the sample, approximately 10 mL every 30 minutes, with gentle stirring using a stainless-steel spatula or glass rod. The additions continued until no further reaction occurred, as indicated by the disappearance of foam. The mixture was then left at room temperature for 48 to 72 hours to ensure complete digestion. Since the reaction can be vigorous, generating intense effervescence that may cause sample loss, small, controlled additions were necessary. To minimize spillage or splashing but also to prevent external contamination (Figure 3), the beakers were covered with aluminium foil all the process. Once digestion was complete, each sample was passed through a 45 μm metallic sieve to drain excess H_2O_2 .

For two port sediment samples (PCO 38 and Lorient LB), H_2O_2 solutions filtered on 45 μm sieve were kept for analysis in order to assess the presence of particle <45 μm and the potential loss of very small microplastics during the process. To do so, H_2O_2 solutions were filtered on 25 mm quartz microfibre filter (2.2 μm , grade 293×50, Dutscher) to extract the particles. Filters were then dried, weighed with a precision balance (0.1 mg accuracy) and grinded as detailed in section 2.2.3 and kept for further analysis (S2 samples).

The remaining sediments, recovered on the 45 μm sieve, now free of organic matter, were transferred into a clean 250mL beaker for further processing.



Figure 3 : Digestion of organic matter in sediment samples ©CEDRE. Left: port sediments, right: beach sediments.

2.2.2. Extraction of MP

The method for extracting microplastics is based on density separation using a saturated salt solution of calcium chloride (CaCl_2). Classically used to extract microplastics from sediments (MSFD TG ML, 2023), the density separation technique exploits the difference in density between the plastic particles (low density) and the sediments (high density) where they are trapped. Among the different salts available, CaCl_2 was favoured for its relative high density (ca. 1.42) combined with its low cost, its relative harmlessness and reduced environmental impact compared with other salts such as zinc chloride (ZnCl_2), sodium iodide (NaI) or sodium chloride (NaCl), which prove to be unsuitable in terms of either harm, durability, or density (Maw et al., 2022).

To prepare 500 mL of saturated CaCl_2 solution with a specific gravity of 1.40 ± 0.01 , 565 g of CaCl_2 (technical-grade) were dissolved in 500 mL of osmosed water, with gentle heating (approximately 50°C) to facilitate homogenization. The density was checked using a densimeter (Anton Paar DMA 4500M) and adjusted, if necessary, by adding water or salt.

During extraction, two volumes of saturated CaCl_2 solution were added to the sediment sample previously placed in a 250 mL glass beaker. The beaker was placed in a 90mm-diameter crystalliser. The mixture was stirred for 2 minutes with a glass rod to remobilise the particles trapped in the sediment, then left to settle overnight. During separation, the microplastics rise and float to the surface, while the sediment sink. After around 24 hours, the supernatant was collected by overflow. To do so, additional CaCl_2 solution was poured gently over the top of the beaker to make it overflow and make it easier to recover the supernatant in the crystalliser. This extraction step was carried out 3 times, leaving 24 hours between each overflow and shaking vigorously at the start of each one to resuspend any particles that may be trapped under the sediment (Figure 4).

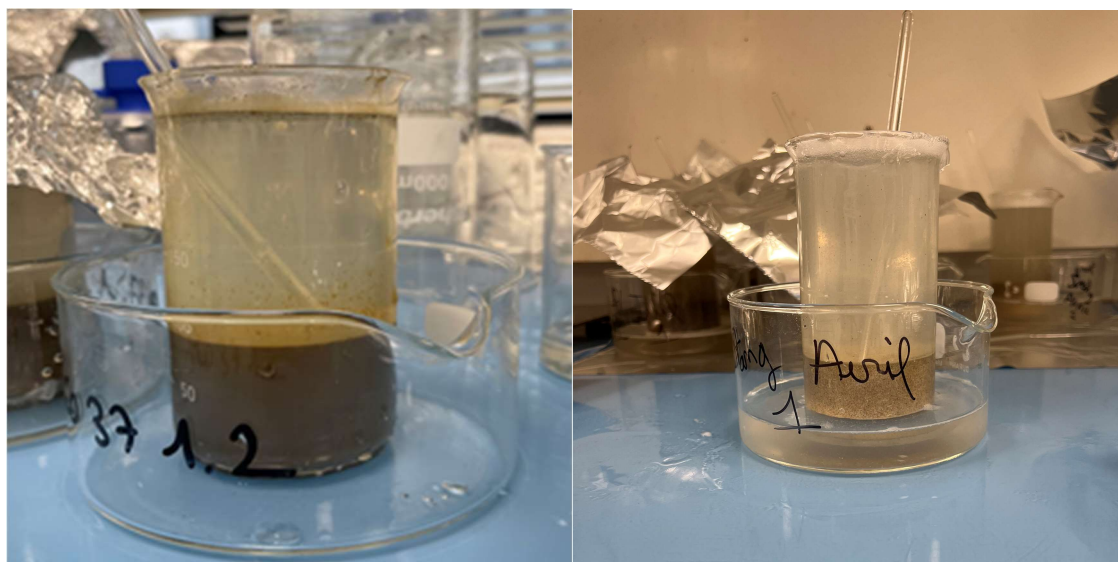


Figure 4 : MP extraction by density separation ©CEDRE. Left: before the overflow, right: during the overflow.

The supernatant collected in the crystalliser after the 3 extractions was finally filtered through a $45\mu\text{m}$ sieve, and the collected particles were rinsed with osmosed water over the sieve. The content of the sieve, entrained by the osmosis water, was then vacuum-filtered using a sinter fitted with a 25mm-diameter quartz microfibre filter, suitable for

pyrolysis and previously heat-treated and weighed (Dutscher, grade 293 x 50). For port and beach sediment samples, as indicated above, the supernatants from both sample replicates were re-united after the extraction step by filtration on the same 45µm sieve and quartz filter (S3 samples).

2.2.3. Samples drying and grinding

Finally, each filter containing sample extracts was dried in an oven at 50°C for several hours, then weighed to determine the mass of particles recovered, and finally ground using a ball mill (Retsch MM400) for 5 minutes. The ground samples were stored in small amber glass vials at room temperature until analysis. Port sediment samples S2 and S3 were divided into two glass vials, one kept for Pyrolysis-GC/MS analysis and the other for shipment to IFPEN laboratory (Rueil-Malmaison, France) for Rock-Eval® analysis.

2.3. Assessment of water content

As port sediment samples were analysed wet and they visually exhibited different level of humidity, the water content was measured for each sample. To do so, a subsample of 10 g of homogenised wet bulk sample was dried in an oven at 50°C until it reaches a constant mass. Then, it was weighed again (precision 0.01 g) to obtain the water content. The water content obtained for each bulk port sediment sample (S1) is presented in Table 4.

Table 4: Water content of each port sediment sample considered in the study

<i>Samples</i>	<i>Sample name</i>	<i>Wet mass of the subsample (g)</i>	<i>Dry mass of the subsample (g)</i>	<i>Water content (%)</i>
1	PCO 37	10.0	1.3	87
2	PCO 38	10.2	2.5	75
3	PCO 39	10.1	3.1	69
4	Honfleur	10.2	2.0	80
5	Lorient LB	10.3	3.5	66
6	Lorient PL	10.0	2.1	79
7	Bayonne	10.0	4.5	55
8	Hendaye PE	10.3	3.5	66
9	St-Jean-de-Luz	10.1	3.8	62
10	Hendaye PL	10.1	3.2	68

PART 2: Development and testing of a pyrolysis-GC/MS method

This section presents the work conducted by CEDRE on the development and testing of a pyrolysis-GC/MS method, followed by an application of the method to analyse natural port and beach sediment samples presented in Part 1. It has to be noted that once developments were completed, all analyses on natural samples were performed in one batch.

This section is divided in three sub-sections: a presentation of the pyrolysis-GC/MS technique, followed by a presentation of materials and methods used and it ends with the presentation of obtained results.

1. Presentation of the pyrolysis-GC/MS

The pyrolysis-GC/MS technique enables microplastics to be analysed by breaking down polymers at high temperature to produce volatile fragments. These fragments are separated according to their volatility by gas chromatography and identified by mass spectrometry, providing accurate identification and quantification of each polymer individually in complex matrices such as sediments (Santos et al., 2023). The result of the pyrolysis-GC/MS is a pyrogram, in which each peak corresponds to a characteristic decomposition product (also known as pyrolysates), identified by its retention time and spectral profile, with a peak area proportional to the product abundance. This method can be used to identify and quantify polymers, additives, copolymers or even secondary degradation products present in complex matrices, even without prior extraction (Santos et al., 2023, Akoueson et al., 2021).

For example, during the pyrolysis of polyethylene, the polymer chain undergoes random homolytic cleavage of the C-C bonds under the effect of heat (typically between 500 and 700°C), generating mainly free radicals. These radicals recombine or stabilise to form mainly linear alkenes (α -olefins), such as 1-hexene, 1-octene, 1-decene and other hydrocarbon fragments of varying masses. These volatile products are well separated by GC and identified by MS according to their specific spectrum, which makes it possible to assign their respective peaks in the pyrogram (Figure 5). The presence of these fragments is therefore a characteristic marker of the thermal degradation of polyethylene.

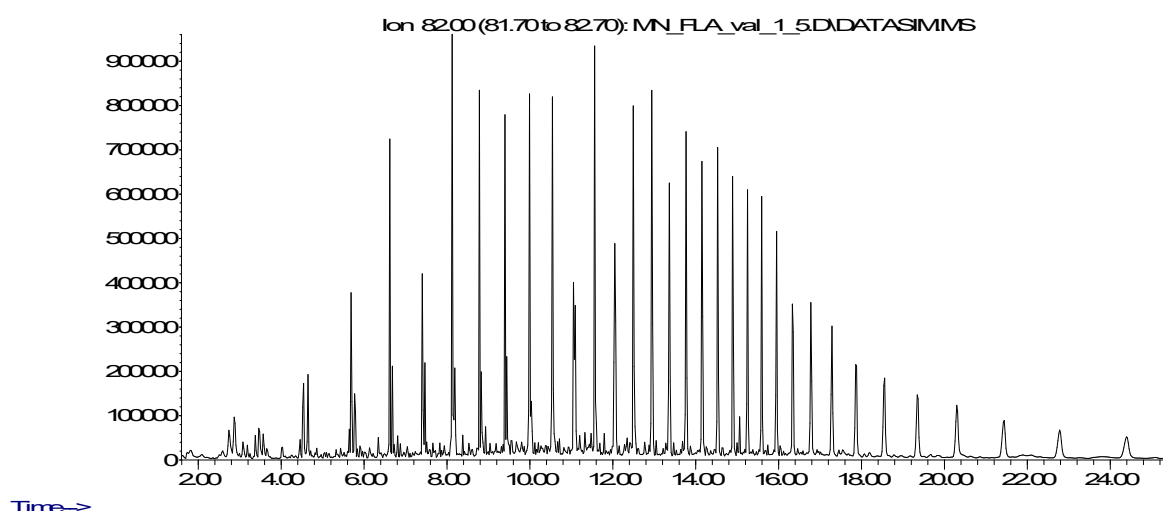


Figure 5 : Example of a polyethylene pyrogram (ion 82)

2. Materials and method

2.1. Polymers studied

12 polymers were initially considered based on the 12 polymers included in the Frontier Lab Microplastics Calibration Standard, distributed by SRA Instruments, used for both method development and calibration purposes in the present study. The twelve polymers are: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polycarbonate (PC), polymethyl methacrylate (PMMA), polyethylene Terephthalate (PET), Nylon-6 (N-6), Acrylonitrile-Butadiene-Styrene (ABS), Styrene-Butadiene Rubber (SBR), polyurethane (PU) and Nylon-6,6 (N-6,6). The polymer's chemical structures are shown in Figure 6.

$\text{-(CH}_2\text{CH}_2\text{)}_n$ Polyethylene (PE)	Polycarbonate (PC)	$\text{-(CH}_2\text{CH(CN))}_m\text{-(CH}_2\text{CH=CHCH}_2\text{)}_n\text{-(CH}_2\text{CH(Ph))}_p$ Acrylonitrile-butadiene-styrene resin (ABS)
$\text{-(CH}_2\text{CH(CH}_3\text{))}_n$ Polypropylene (PP)	Poly(methyl methacrylate) (PMMA)	$\text{-(CH}_2\text{CH=CHCH}_2\text{)}_m\text{-(CH}_2\text{CH(Ph))}_n$ styrene-butadiene rubber (SBR)
Polystyrene (PS)	Polyethylene terephthalate (PET)*	Polyurethane (PU)**
$\text{-(CH}_2\text{CH(Cl))}_n$ Polyvinyl chloride (PVC)	Nylon 6 (N6)	Nylon 66 (N66)

Figure 6: The 12 polymers included in the Frontier Lab Microplastics Calibration Standard, initially considered in the present study (©Frontier Lab)

However, it has to be noted that all calibration samples were prepared with SiO₂ as a diluent and this prevents from analysing PU as PU shows unfavourable pyrolytic behaviour for quantification when using SiO₂ as indicated by Frontier Lab (Matsueda et al., 2021).

In addition, some other polymers did not provide satisfactory results during the development and validation phase. In this context, they were not included in final quantification analysis. After the development and validation phase, the following 7 polymers were kept for quantification analysis, as presented in Table 5.

Table 5: List of polymers kept for quantification purposes in the present study

Polymers
Polyethylene
Polypropylene
Polystyrene
Acrylonitrile-Butadiene-Styrene
Polyvinyl chloride
Nylon-6
Nylon-6,6

2.2. The pyrolysis-GC/MS equipment

2.2.1. Presentation

In this study, an Agilent Technologies 7890A gas chromatograph (Figure 7), equipped with an Agilent Technologies 5975C quadrupole mass spectrometer was used with an ionisation energy of 70 eV. This system was equipped with an autosampler (GERSTEL Multipurpose Sampler MPS2 XL). Injection was carried out using a Thermal Desorption Unit (Gerstel) (Figure 7) at 320°C with a split ratio of 1/25, connected to a Cooling Injection Device (Gerstel) and equipped with a pyrolizer capable of heating the sample to 1000°C. Helium was used as the carrier gas at a constant flow rate (1 mL/min). The capillary column used was a HP 5-ms column (Agilent), measuring 30 m in length, 0.25 mm in internal diameter and 0.25 µm in film thickness.



Figure 7: Agilent Technologies 7890A gas chromatograph (Left), thermal Desorption Unit (Gerstel) used in CEDRE laboratory.

The solid sample is placed in a pyrolysis tube between two pieces of quartz wool to undergo thermal decomposition, generating monomers or dimers characteristic of the initial compounds. The tubes containing the samples are transferred from the sample holder to the pyrolyser by the Gerstel autosampler, making the analysis autonomous. An injector then introduces the sample into the system, directing the compounds towards the chromatograph consisting in a column located in an oven. It is in this column that the compounds are separated, depending on their volatility and interactions with the stationary phase. At the exit of the column, a detector coupled to a mass spectrometer is used to analyse the compounds. To do so, fragments produced by pyrolysis are ionized by electronic impact. They are then accelerated and separated according to their mass-to-charge ratio (m/z) using a shifting electric field created by four parallel metal rods. Finally, a detector measures the molecular masses of the ions and identifies the compounds present in the sample, generating a mass spectrum, or pyrogram, which shows the distribution of the ions according to their retention time in the column.

2.2.2. Pyrolysis and GC parameters

Programming the pyrolysis temperature and oven is a key stage in the development of the pyrolysis-GC/MS method. It begins with optimisation of the pyrolysis parameters, in particular the final temperature and holding time, to ensure complete degradation of the polymers into characteristic fragments. In the proposed method, the pyrolysis temperature was set at 700°C, allowing efficient fragmentation of the polymers (Hermabessiere et al., 2018). In addition, the temperature of the GC oven plays a crucial role in the separation of the fragments generated. The GC temperature program was as follows: 50 °C for 1 min, followed by a gradual rise to 320°C over 10 minutes, with a gradient of 20°C per minute.

2.2.3. Selection of Quantifier and Qualifier Ions

In the MS phase functioning in SIM (single ion monitoring) mode, for each polymer, two ions were monitored: one for quantification (quantifier ion) and the second to confirm the analyte (qualifier ion). The selection of quantifier and qualifier ions is essential to ensure accurate identification and quantification of volatile compounds. Quantifying ions are chosen on the basis of their abundance, their specificity with respect to each target polymer, and the quality of their response and repeatability. The intensity ratio between the selected ions is then compared with reference spectra to confirm the identity of the compounds analysed.

Ions selected in the present method for each targeted polymer were based on both Santos et al. (2023) and Frontier Lab application note and were optimised by CEDRE. They are presented in Table 6.

Table 6: List of quantifier and qualifier ions selected for this study (SIM mode)

Polymers	Quantifier		Qualifier
	m/z	Pyrolysate	m/z
PE	82	1-methyl-3-(1-methylethyl)-cyclopentane	83
PP	126	2,4-dimethyl-1-heptene	70
PS	208	Styrene trimer	104
ABS	170	2-Phenyl-4-phenylpent-4-enenitrile	144

SBR	104	4-phenylcyclohexene	91
PMMA	100	Methyl-methacrylate	55
PC	213	Bisphenol A	134
PVC	128	Naphtalene	166
PET	122	Benzoic acid	154
N-6	113	E-caprolactam	85
N-6,6	84	Cyclopentanone	112

2.2.4. External calibration

As previously mentioned in Part 1, section 1.1.2, external calibrations were prepared by diluting the Frontier Lab Microplastics Calibration Standard in SiO₂ powder. These calibration samples were used for PP, PS, ABS, SBR, PMMA, PC, PVC, PET, N-6, N-6,6. For PE, calibration samples were prepared by diluting pure PE powder (fine-grained powdered PE, grain size 125 µm, Sigma Aldrich) in SiO₂, allowing to extend the calibration range for this polymer. It has to be noted that, to our knowledge and except for PE, the Frontier Lab Microplastics Calibration Standard is the only standard available for the other polymers. This represents an important limitation for analytical development as it is not possible to work and optimise analytical parameters for individual polymer and calibration ranges studies are limited by polymer quantities in the standard. Quantities of polymers present in the Frontier Lab Microplastics Calibration Standard are detailed in Table 7.

Table 7: Quantities (µg) of polymers in 4 mg of Frontier Lab Microplastics Calibration Standard solution

Polymers	PE	PP	PS	ABS	SBR	PMMA	PC	PVC	PET	N-6	N-6,6
Mass of polymer in 4mg of mixture in SiO ₂ (µg)	193.0	48.2	12.5	20.3	21.0	13.8	3.5	52.1	17.9	6.5	19.0

Six levels of calibration were prepared by solid serial dilution in SiO₂ and used for method development and for quantification. This includes an analytical blank containing only SiO₂. Total mass injected per sample was 2.5 mg.

For PE, the 6 concentration levels were prepared from pure PE: 0/25/62.5/125/175/250 µg injected.

For other polymers, the calibration levels were 0 µg, Frontier Lab stock standard solution (SSS)/8, SSS/6, SSS/4, SSS/2 and the SSS itself. They were run in either duplicates or triplicates, from the lowest to the highest concentrations and they follow the analysis of an empty pyrolysis tube to ensure a clean column and detector.

Calibration curves obtained were then used to make statistical calculations as detailed below to check various parameters such as the linearity, repeatability and accuracy of the method, as well as to calculate the limits of quantification and detection for each polymer.

2.3. Method validation

The method validation was conducted following the method validation guide *Labo-Stat* by Feinberg (2009). It relies on a series of statistical tests which, when successfully validated, assess the method's ability to meet key performance criteria such as accuracy, linearity, and repeatability. In the present study and as a complement to the validation process, a test using an internal standard was conducted and CEDRE participated to an interlaboratory comparison exercise was also carried out to further evaluate the method's robustness and external reliability. Method validation statistics were performed on Excel software (Microsoft) except for ANOVA analyses that were done on MATLAB (MathWorks).

2.3.1. Analysis of calibration curves linearity

For validation purposes, 3 ranges of calibration samples were analysed in duplicate and over 3 days (1 calibration in duplicate per day).

Linearity in calibration is defined as the ability of a method to produce experimental results, referred to as responses (commonly denoted as Y), that are proportional to the concentration of the analyte being measured (X). If a linear model does not meet the criteria for linearity, it is advisable to consider an alternative model that better fits the data, such as a quadratic model.

In the present study, a linear regression was applied to each calibration curves obtained and the linearity of the response was assessed by considering the regression coefficient (R^2).

2.3.2. Analysis of variance

In the present study, a complementary goodness-of-fit test using analysis of variance (ANOVA) was conducted. This test involves performing replicated analyses (denoted j) to calculate the repeatability and reproducibility of the method. The ANOVA allows the identification of two types of variability among the measured values and their corresponding means:

- Model regression error: the difference between the observed values and those predicted by the regression model.
- Inadequacy of the linear model: the extent to which the relationship between the response and the concentration deviates from a straight line, indicating that the linear model may not adequately describe the data.

2.3.3. Maximum acceptable deviation

In analytical method validation, maximum acceptable deviations are established to evaluate various method performance parameters such as precision, accuracy, linearity, repeatability, and reproducibility. These deviations define the thresholds within which analytical results are deemed acceptable and reliable. The maximum acceptable deviations should be determined based on regulatory requirements, the specific characteristics of the analysis, and the acceptable error tolerances for the intended application. These criteria are often outlined in guidelines or standards, such as those provided by ISO. It is essential to document and justify these deviations as part of the method validation process. For natural samples, which may exhibit matrix effects due

to their complex composition, the maximum acceptable deviations are typically around $\pm 20\%$ (Feinberg, 2009).

In the present study, the relative standard deviation (RSD, expressed in %) was calculated by comparing the calibration curves modelled data and injected sample concentrations for each replicate of the 6 concentration levels. RSD obtained were then compared to the $\pm 20\%$ threshold.

2.3.4. Detection and quantification limits (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for each polymer were calculated using the standard deviation of the response (σ) and the slope (S) of the calibration curve. The LOD was defined as $3 \times (\sigma/S)$, and the LOQ as $10 \times (\sigma/S)$. This approach allows for an estimation of the method's sensitivity based on the variability of the signal at low concentrations and the response of the system. These values indicate the lowest concentrations at which the analytes can be reliably detected (LOD) and accurately quantified (LOQ) under the experimental conditions used. LOD and LOQ were expressed in μg injected and extrapolated in mg/kg (ppm).

2.3.5. Assessment of method accuracy using artificial samples

To assess the accuracy of the quantification method, artificial samples (see Part 1, section 1.1.2) made of SiO_2 diluent spiked with known concentrations of polymers were analysed. 3 samples with different 3 different concentration levels were analysed in duplicate. For each sample, ca. 2.5 mg of artificial sample, precisely weighed, were introduced in a pyrolysis tube and analysed. The 7 polymers were quantified and results obtained were compared with the known concentrations present in each sample. The relative difference between injected and recovered mass was expressed as a percentage error. This approach verifies the method's ability to provide correct measurements under controlled conditions, free from matrix effects, and serves as a key indicator of analytical performance.

2.3.6. Test of an internal standard

Internal standards are compounds added in known quantities to a sample prior to preparation or analysis, typically used to correct losses during preparation steps or injection variations by compensating for fluctuations in the injected mass and adjust detector response for more accurate quantification. It also serves as a reference to monitor system stability, ensure analytical reproducibility, and account for matrix effects in complex samples. Lauschke et al. (2021) showed that macroplastics quantification data was improved by use of internal standards.

In this study, poly(4-fluorostyrene) (4FS, Polymer Source, Inc) was tested as a potential internal standard, as it showed promising results in previous studies (Lauschke et al., 2021). To do so, 20 μg of 4FS was added to samples with different concentrations, used to prepare a PE calibration. PE calibration curves obtained with ($A_{\text{PE}}/A_{4\text{FS}} = f(C_{\text{PE}}/C_{4\text{FS}})$) where A and C are abundance and concentration, respectively) or without ($A_{\text{PE}} = f(C_{\text{PE}})$) 4FS were compared.

However, the test conducted in the current study did not lead to conclusive improvements and 4FS was therefore not further used for calibration purposes in the present study.

2.3.7. Test of the method in the context of the QUASIMEME-WEPAL interlaboratory exercise

Quantification of MP in QUASIMEME-WEPAL water and sand samples (see Part 1, section 1.1.3) was done using the method developed and validated. To do so, ca. 2.5 mg of sample, precisely weighed, were introduced in a pyrolysis tube and analysed. As indicated in section 2.1, only 7 polymers were considered for quantification: PE, PP, PS, ABS, PVC, N-6 and N-6,6. All samples were analysed in one batch. External calibration was performed by analysing serial solid dilutions of the Microplastics Calibration Standard, in duplicate, including analytical blanks prepared with only SiO₂. Results were expressed in mg of polymer/kg of water or sediment (mg/kg or ppm).

2.4. Application of the method for analysis of natural samples

Quantification of MP in natural samples was done using the method developed and validated. To do so, ca. 2.5 mg of sample, precisely weighed, were introduced in a pyrolysis tube and analysed. As indicated in section 2.1, only 7 polymers were considered for quantification in the natural samples: PE, PP, PS, ABS, PVC, N-6 and N-6,6. All samples were analysed in one batch. External calibration was performed by analysing serial solid dilutions of the Microplastics Calibration Standard, in duplicate, including analytical blanks prepared with only SiO₂. For port sediment samples that were analysed wet, results were expressed first, in mg of polymer/kg WW of sediment (mg/kg or ppm) and then in mg/kg DW considering the water content of each sample (see Part 1, section 2.3). For beach sediment samples that were dried before analysis, results were expressed directly in mg/kg DW.

3. Results

3.1. Method validation

In this section, only results obtained for the 7 polymers exhibiting satisfactory responses during the validation phases (PE, PP, PS, ABS, PVC, N-6 and N-6,6) are presented.

3.1.1. Calibration curves and linear regressions obtained

Calibration curves obtained based on the analysis of 3 series of serial solid dilution of either pure PE powder or the Microplastics Calibration Standard, for the 7 polymers that were kept after the validation phase, are shown on Figure 8.

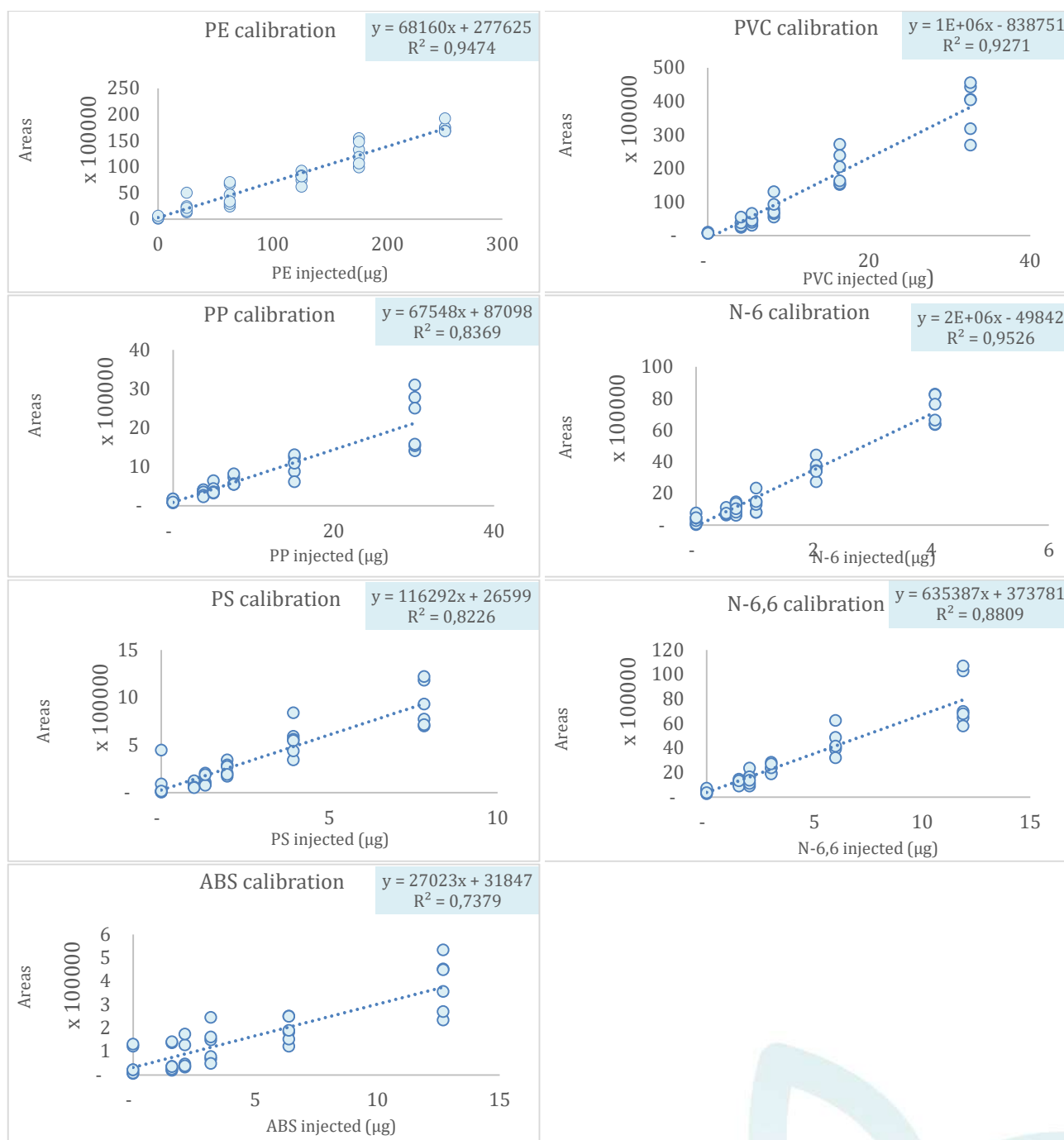


Figure 8: Calibration curves obtained for the 7 polymers considered for quantification

The validation of the calibration curves for the different polymers was carried out by evaluating the linearity of the instrumental responses as a function of the injected amounts. The determination coefficients (R^2) vary between 0.7379 and 0.9526, depending on the polymer, reflecting differing levels of linearity. The best linearities were observed for N-6 and PE, with respective R^2 values of 0.9526 and 0.9474, indicating an excellent correlation between the measured signals and the injected masses. PVC also showed good linearity ($R^2 = 0.9271$), followed by N-6,6 ($R^2 = 0.8809$) and PP ($R^2 = 0.8369$). In contrast, the results were less satisfactory for PS ($R^2 = 0.8226$) and especially for ABS, which had an R^2 of 0.7379, suggesting greater response variability or less linear instrumental behaviour. These results show that although most analysed polymers exhibit acceptable linearity ($R^2 > 0.8$), the accuracy of quantification may vary depending

on the polymer type, highlighting the need for specific calibrations and rigorous quality control to ensure measurement reliability.

3.1.2. Analysis of variance

In complement, the ANOVA allowed the assessment of the model regression error (the difference between the observed values and those predicted by the regression model) and the inadequacy of the linear model (the extent to which the relationship between the response and the concentration deviates from a straight line). The results of the ANOVA are shown in Table 8. For the 7 polymers, the regression values exceed the reference threshold of 4.35, confirming that the linear relationship is statistically significant. Values obtained to assess the inadequacy of the linear model are all below the critical value of 3.10, indicating that deviations from linearity are not significant. Overall, these results show that the linear model adequately describe the data and this model is appropriate for the 7 polymer's calibrations. It must be noted that the reference values mentioned previously result from the statistical analysis, and are specific to the set of data considered.

Table 8: Results of the analysis of variance for the 7 polymers considered

	PE	PP	PS	ABS	PVC	N-6	N-6,6	Reference value considered in the present study
Regression values	716	156	162	87.4	410	748	227	> 4.35
Inadequacy of the linear model	0.75	0.08	1.00	0.21	0.46	1.45	0.15	< 3.10

3.1.3. Maximum acceptable deviations

For each polymer, the RSD were calculated based on calibrations curves, by comparing real concentrations injected and modelled data, for the 6 replicates of the 6 concentration levels. Results obtained for the 7 polymers are shown in Figure 9. Results indicate that most data points fall within or near this interval, indicating acceptable calibration performance for most levels and polymers, with a few notable outliers. In particular, results show:

- PE and PP display relatively stable calibration across levels, although PP shows some values outside the $\pm 20\%$ range.
- PS and ABS show wider variation, especially at lower concentration levels.
- PVC and N-6 exhibit generally good repeatability, although PVC has outliers above 40%.
- N-6,6 shows tighter clustering around the 0% line, suggesting good calibration performance.

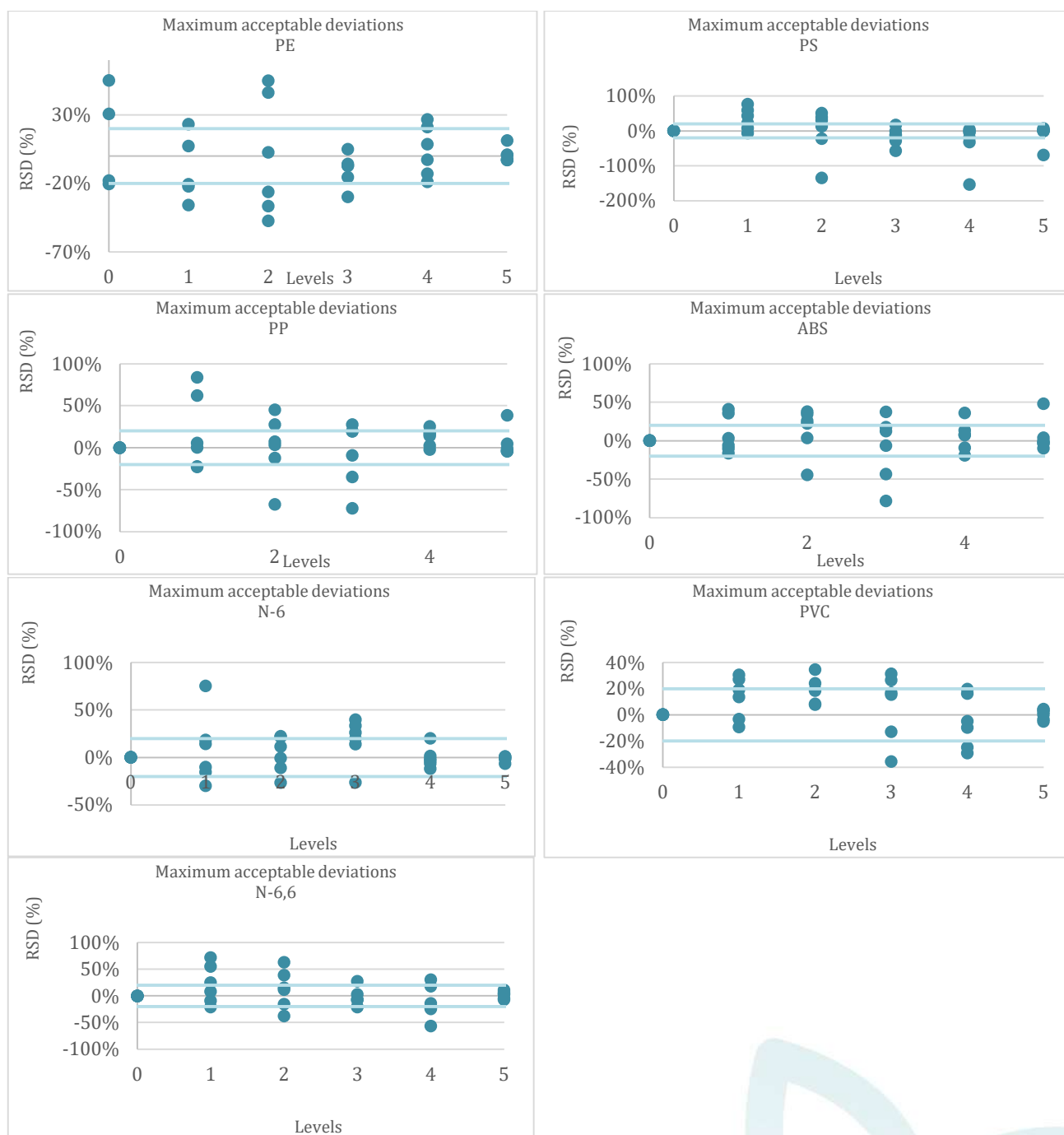


Figure 9 : RSD (%) obtained for the 6 replicates of the 6 concentration levels for the 7 polymers considered. The horizontal lines at $\pm 20\%$ indicate the maximum acceptable deviation range, which is a commonly accepted threshold for natural matrices.

3.1.4. Detection and quantification limits

LOD and LOQ obtained, expressed in μg injected in 2.5 mg of diluent, are shown in Table 9. The table also presents the lowest and the highest concentration levels analysed in calibrations for each polymer (without considering blanks). Except for PE, for which a pure standard exists, other polymers concentrations ranges are limited by the Frontier Lab Microplastics Calibration Standard own concentrations.

These results confirm both the absence of individual standards for each polymer and the fact that polymers have different concentrations in the Frontier Lab standard, is a

limiting factor as LOQ appear very close to the highest concentration level analysed for some polymer such PS and N-6.

Table 9: LOD and LOQ (in µg injected) for the 7 polymers considered. The table also presents the lowest and the highest concentration levels analysed in calibrations for each polymer (excluding the blank).

	PE	PP	PS	ABS	PVC	N-6	N-6,6
Lowest concentration level analysed (µg injected)	25.0	3.78	0.98	1.59	4.09	0.51	1.49
Highest concentration level analysed (µg injected)	250	30.1	7.81	12.7	32.6	4.06	11.9
LOD (µg injected)	18.37	3.87	2.43	7.14	2.24	0.48	1.52
LOQ (µg injected)	36.54	11.7	7.36	21.7	6.80	1.45	4.60

These limits were extrapolated to the analysis of 65 g of sediment, 1 L of water and 200 g of sediment corresponding respectively to sediment and water from the QUASIMEME-WEPAL exercise and the natural samples analysed in the present study. This extrapolation was done considering the injection of 2.5 mg of sample for analysis, the total mass of extracted samples including quartz fibre filter of 42 mg, and a 100% recovery rate. Extrapolated limits are presented in Table 10.

Table 10: LOD and LOQ calculated for different types of samples considered in the study

		PE	PP	PS	ABS	PVC	N-6	N-6,6
	LOD (µg injected)	18.27	3.87	2.43	2.43	2.24	0.48	1.52
	LOQ (µg injected)	36.54	11.71	7.36	7.36	6.80	1.45	4.60
200 g of sediment	LOD (mg/kg)	1.53	0.32	0.20	0.60	0.19	0.04	0.13
	LOQ (mg/kg)	3.07	0.98	0.62	1.82	0.57	0.12	0.39
65 g of sediment	LOD (mg/kg)	4.72	1.00	0.63	1.85	0.58	0.12	0.39
	LOQ (mg/kg)	9.44	3.03	1.90	5.59	1.76	0.38	1.19
1L of water	LOD (mg/kg)	0.31	0.06	0.04	0.12	0.04	0.01	0.03
	LOQ (mg/kg)	0.61	0.20	0.12	0.36	0.11	0.02	0.08

3.1.5. Assessment of method accuracy using artificial samples

Results of the assessment of the method accuracy using artificial samples are presented in Table 11. The results present the relative error between the quantified and injected mass of the 7 polymers, in the 3 samples analysed in duplicate. The relative difference between the duplicates is also presented. Results show that for polymers such as PE, PP, PS, PVC, and N-6, most deviations are below 20% except for lower concentrations where the relative error between quantified and injected reach 30 and 33% (for PS and N-6, respectively). In contrast, for N-6,6, one data point -corresponding to the highest concentration- shows a significant deviation of 65%. As for ABS, two points stand out with relative errors of 67% and 170%, respectively.

Table 11: Relative error between quantified and injected masses (in %) of the 7 polymers, in the 3 artificial samples analysed in duplicate (SD: standard deviation, RD: relative difference)

		PE	PP	PS	PVC	N-6	N-6,6	ABS
Artificial sample 1	Injected mass (μg injected)	30.3	7.56	1.96	8.18	1.02	2.98	3.19
	Quantified mass $\pm$ SD (μg injected)	23.9 $\pm$ 4.06	6.21 $\pm$ 0.21	1.37 $\pm$ 0.11	6.94 $\pm$ 1.32	0.68 $\pm$ 0.08	3.65 $\pm$ 0.45	8.59 $\pm$ 2.54
	Relative error (%)	21%	18%	30%	15%	33%	-22%	-170%
	Duplicate RD (%)	17%	3%	8%	19%	12%	12%	30%
Artificial sample 2	Injected mass (μg injected)	60.6	15.1	3.92	16.4	2.04	5.96	6.37
	Quantified mass $\pm$ SD (μg injected)	49.8 $\pm$ 2.89	11.2 $\pm$ 5.65	4.30 $\pm$ 0.08	16.3 $\pm$ 4.17	1.91 $\pm$ 0.02	5.93 $\pm$ 1.36	10.6 $\pm$ 0.03
	Relative error (%)	19%	26%	-10%	0%	6%	1%	-67%
	Duplicate RD (%)	6%	50%	2%	26%	1%	23%	0.1%
Artificial sample 3	Injected mass (μg injected)	120	30.1	7.81	32.6	4.06	11.9	12.7
	Quantified mass $\pm$ SD (μg injected)	135 $\pm$ 9.40	23.2 $\pm$ 0.50	6.42 $\pm$ 1.21	35.9 $\pm$ 0.71	3.79 $\pm$ 0.11	19.6 $\pm$ 0.59	15.3 $\pm$ 5.47
	Relative error (%)	-12%	23%	18%	-10%	7%	-65%	-21%
	Duplicate RD (%)	7%	2%	19%	2%	3%	3%	36%

3.1.6. Test of an internal standard

Internal calibration tests were conducted on a series of PE samples using poly-4-fluorostyrene as the internal standard. Despite consistent injection concentrations across all samples, the peak areas corresponding to the internal standard exhibited significant variability. This instability in the chromatographic response of poly-4fluorostyrene compromised its effectiveness as a corrector for instrumental or analytical fluctuations.

As a result, applying the internal standard ratio did not improve the quality of the calibration curves. As shown in Figure 10 and Figure 11, the use of Poly-4-fluorostyrene in this context appears ineffective introducing an additional source of variability. In this context, it was concluded that further testing and optimisation was needed to assess the relevance of using poly-4-fluorostyrene as an internal standard for MP quantification purposes using pyrolysis-GC/MS.

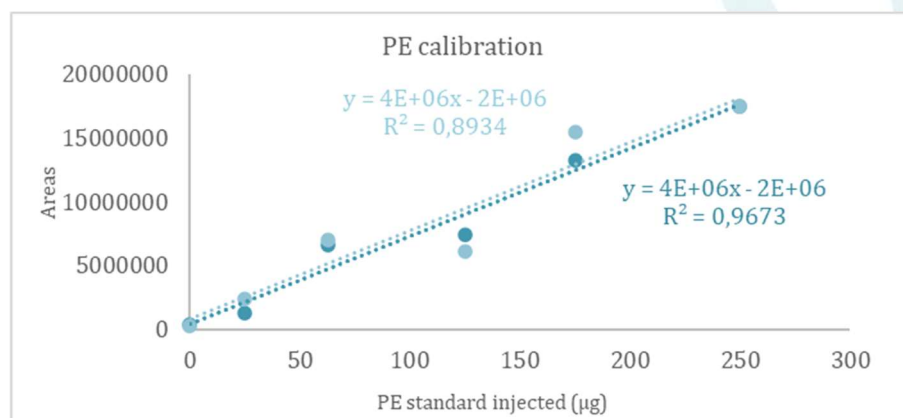


Figure 10: PE calibration curve without taking the internal standard into account

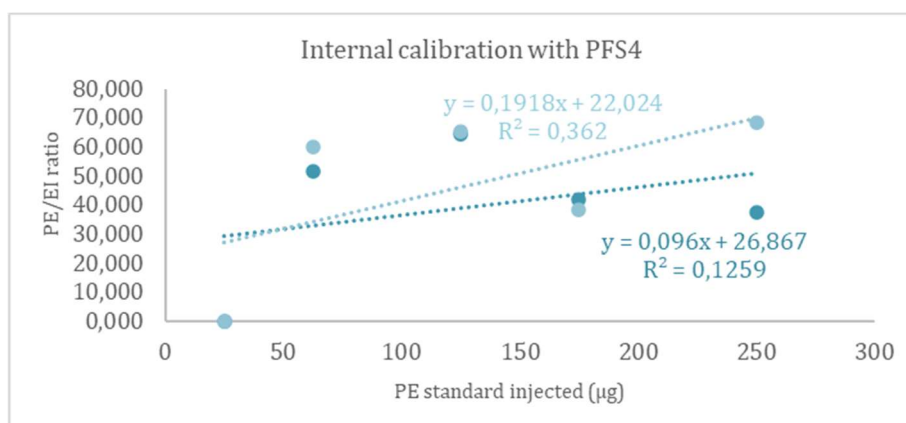


Figure 11: PE calibration normalization using the internal standard

3.1.7. Test of the method in the context of the QUASIMEME-WEPAL interlaboratory exercise

The analysis of QUASIMEME-WEPAL samples aimed at assessing the performance of the whole analytical method for both sediment and seawater samples, from sample preparation to polymer quantification. All samples were processed under the same conditions as natural samples considered in the present study, ensuring methodological consistency.

The results of analysis of QUASIMEME-WEPAL samples are shown in Table 12. As samples were known to not contain ABS, N-6 and N-6,6, which was confirmed by analysis conducted by CEDRE, only results for PE, PP, PS and PVC are shown.

Overall, results obtained in the QUASIMEME-WEPAL exercise highlight important limitations for the application of the developed method under the conditions of the interlaboratory test. All spiked concentrations appeared to be below the quantification limits of the method, both in water and sediment samples. This suggests that the target concentrations were not sufficiently above the analytical limits to enable reliable quantification. This limitation could potentially be overcome by increasing the sample mass analysed, which was restricted in the present exercise (65 g of sediment and 1 L of water). On the other hand, despite the absence or very low expected concentrations of polymers in several samples, PP, PS and PVC were detected above the LOQ in all sediment samples, while PVC was detected above the LOQ in all three water samples. These results strongly suggest contamination during sample preparation, a hypothesis supported by the procedural blank, which exhibited concentrations close to the LOQ for PP and PS and above the LOQ for PVC.

Although the QUASIMEME-WEPAL exercise highlighted limitations related to quantification limits and potential contamination during sample preparation, it provided valuable insights for further method development. Results obtained on natural sediment samples analysed in this study demonstrate that the method is capable of detecting and quantifying microplastics in environmentally contaminated matrices. However, additional optimisation is still required to improve sensitivity at very low environmental concentrations. The exercise also confirmed that contamination control

remains a critical aspect of the analytical workflow and that the systematic use of procedural and atmospheric blanks is essential.

Table 12: Average concentrations of polymers (mg/kg $\pm$ SD) obtained in the QUASIMEME-WEPAL samples (conc.=concentration)

Samples		PE		PP		PS		PVC	
		Expected conc.	CEDRE results	Expected conc.	CEDRE results	Expected conc.	CEDRE results	Expected conc.	CEDRE results
LOQ water samples (mg/kg)		0.61		0.20		0.12		0.11	
LOQ sediment samples (mg/kg)		9.44		3.03		1.90		1.76	
Water (mg/kg)	Q_16	0.2 (<LOQ)	<LOD	0	<LOD	0	<LOD	0	0.25 $\pm$ 0.01
	Q_17	0.09 (<LOQ)	<LOD	0	<LOD	0.09 (<LOQ)	<LOD	0	0.70 $\pm$ 0.03
	Q_18	0	<LOD	0	<LOD	0	<LOD	0	0.19 $\pm$ 0.02
Sediment (mg/kg)	Q_19	0	<LOD	1.37 (<LOQ)	3.9 $\pm$ 0.1	0	3.40 $\pm$ 0.00	1.37 (<LOQ)	12.4 $\pm$ 0.8
	Q_20	1.01 (<LOQ)	Detected but <LOQ	0	9.8 $\pm$ 0.1	0	6.70 $\pm$ 0.01	0	29 $\pm$ 1
	Q_21	0	<LOD	0	3.60 $\pm$ 0.03	0	3.30 $\pm$ 0.02	0	11.3 $\pm$ 0.3
Blank sediment (expressed in mg/kg)	Q_control	0	<LOD	0	3.3	0	2.8	0	13.7

3.2. Analysis of natural samples

3.2.1. Quality control

In the context of the natural sample analysis, two blanks were analysed: an atmospheric blank and a procedural blank. The results obtained are presented in the Table 13.

Table 13: Areas (counts) and calculated masses (μ injected) measured in the atmospheric blank and procedural blank analysed in the context of the natural sample analyses

		PE	PP	PS	ABS	PVC	N-6	N-6,6
LOD (μ g injected)		18.27	3.87	2.43	2.43	2.24	0.48	1.52
LOQ (μ g injected)		36.54	11.71	7.36	7.36	6.80	1.45	4.60
Areas (counts)	Blank_atm	115 633	99 857	78 958	20 695	698 547	487 956	389 564
		127 893	103 658	39 876	24 310	765 942	369 589	478 225
	Blank_pro	140 506	178 991	11 204	19 876	845 137	564 982	698 452
		171 248	155 763	22 435	28 709	710 384	432 945	589 117
Calculated masses (μ g injected)	Blank_atm	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Blank_pro	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

For both blanks, all calculated mass values are below the quantification limits. This indicates that there was no significant external contamination during the analysis of natural samples, despite the contamination detected in the QUASIMEME WEPAL sample analysis. This suggests the contamination was either a one-off event or it was detected due to the lowest quantities of sample analysed, exacerbating the effect of external contamination on the measured concentrations.

3.2.2. Port sediment samples

Results obtained for port sediment samples (S3 samples targeting SMP > 45µm) are presented in WW in Table 14 and in DW in Table 15. Results show that several polymers are quantified above the analytical limits (PP, PS, PVC and N-6,6), one is only detected but not quantified as concentrations are lower than the LOQ (PE) and two are not detected at all (ABS and N-6). Differences are observed from one port sample to another, except for PP, PVC and N-6,6 that are quantified or detected in most or all samples. Differences in total concentrations are observed between the samples and these differences are exacerbated when results are expressed in ppm DW due to the difference in water content between the samples.

Table 14: Average concentrations (±SD) for the small microplastics (>45 µm) obtained in samples S3 (mg/kg WW)

mg/kg (ppm) WW	PE	PP	PS	ABS	PVC	N-6	N-6,6	Total concentration of MP quantified
LOD (mg/kg)	1.53	0.32	0.20	0.60	0.19	0.04	0.13	/
LOQ (mg/kg)	3.07	0.98	0.62	1.82	0.57	0.12	0.39	/
PCO 37 - S3	Detected but <LOQ	1.3 ±0.3	<LOD	<LOD	21 ±1	<LOD	Detected but <LOQ	22
PCO 38 - S3	<LOD	Detected but <LOQ	<LOD	<LOD	8.0 ±0.9	<LOD	Detected but <LOQ	8
PCO 39 - S3	Detected but <LOQ	<LOD	<LOD	<LOD	5.1 ±0.8	<LOD	Detected but <LOQ	5
Honfleur - S3	Detected but <LOQ	<LOD	1.32 ±0.05	<LOD	15 ±5	<LOD	1.02 ±0.00	16
Lorient LB - S3	<LOD	1.12 ±0.04	<LOD	<LOD	25 ±2	<LOD	Detected but <LOQ	26
Lorient PL - S3	Detected but <LOQ	1.38 ±0.04	<LOD	<LOD	15.4 ±0.2	<LOD	Detected but <LOQ	17
Bayonne - S3	<LOD	Detected but <LOQ	<LOD	<LOD	5 ±1	<LOD	Detected but <LOQ	5
Hendaye PE - S3	<LOD	Detected but <LOQ	<LOD	<LOD	24.9 ±0.3	<LOD	Detected but <LOQ	25
Saint-Jean-de-Luz - S3	<LOD	1.12 ±0.04	<LOD	<LOD	24 ±1	<LOD	Detected but <LOQ	25
Hendaye PL - S3	<LOD	1.77 ±0.05	<LOD	<LOD	25.89 ±0.00	<LOD	Detected but <LOQ	28

Table 15: Average concentrations ($\pm$ SD) for the small microplastics (>45 μ m) obtained in samples S3 (mg/kg DW)

mg/kg (ppm) DW	PE	PP	PS	ABS	PVC	N-6	N-6,6	Total concentration of MP quantified
PCO 37 - S3	Detected but <LOQ	10 $\pm$ 2	<LOD	<LOD	160 $\pm$ 6	<LOD	Detected but <LOQ	170
PCO 38 - S3	<LOD	Detected but <LOQ	<LOD	<LOD	32 $\pm$ 4	<LOD	Detected but <LOQ	32
PCO 39 - S3	Detected but <LOQ	<LOD	<LOD	<LOD	17 $\pm$ 2	<LOD	Detected but <LOQ	17
Honfleur - S3	Detected but <LOQ	<LOD	10.2 $\pm$ 5.3	<LOD	112 $\pm$ 35	<LOD	7.9 $\pm$ 0.01	130
Lorient LB - S3	<LOD	5.6 $\pm$ 0.3	<LOD	<LOD	126 $\pm$ 11	<LOD	Detected but <LOQ	132
Lorient PL - S3	Detected but <LOQ	6.7 $\pm$ 0.2	<LOD	<LOD	73 $\pm$ 1	<LOD	Detected but <LOQ	80
Bayonne - S3	<LOD	Detected but <LOQ	<LOD	<LOD	39 $\pm$ 11	<LOD	Detected but <LOQ	39
Hendaye PE - S3	<LOD	Detected but <LOQ	<LOD	<LOD	73 $\pm$ 1	<LOD	Detected but <LOQ	73
Saint-Jean-de-Luz - S3	<LOD	3.0 $\pm$ 0.1	<LOD	<LOD	62 $\pm$ 3	<LOD	Detected but <LOQ	65
Hendaye PL - S3	<LOD	5.5 $\pm$ 0.1	<LOD	<LOD	81 $\pm$ 0	<LOD	Detected but <LOQ	87

In addition, results of the analysis of very small microplastics (<45 μ m) in filtered H₂O₂ solutions (S3 samples) are presented in WW and DW in Table 16 and Table 17, respectively.

Table 16: Average concentrations ($\pm$ SD) for very small microplastics (<45 μ m) obtained in samples S2 (mg/kg WW)

mg/kg (ppm) WW	PE	PP	PS	ABS	PVC	N-6	N-6,6	Total concentration of MP quantified
LOD (mg/kg)	1.53	0.32	0.20	0.60	0.19	0.04	0.13	/
LOQ (mg/kg)	3.07	0.98	0.62	1.82	0.57	0.12	0.39	/
Lorient LB - S2	<LOD	<LOD	<LOD	<LOD	3.6 $\pm$ 0.2	<LOD	Detected but <LOQ	3.6
PCO 38 - S2	<LOD	<LOD	<LOD	<LOD	10.4 $\pm$ 0.7	<LOD	Detected but <LOQ	10.4

Table 17: Average concentrations ($\pm$ SD) for very small microplastics (<45 μ m) obtained in samples S2 (mg/kg DW)

mg/kg (ppm) DW	PE	PP	PS	ABS	PVC	N-6	N-6,6	Total concentration of MP quantified
Lorient LB - S2	<LOD	<LOD	<LOD	<LOD	9.7 $\pm$ 0.4	<LOD	Detected but <LOQ	9.7
PCO 38 - S2	<LOD	<LOD	<LOD	<LOD	13.9 $\pm$ 0.9	<LOD	Detected but <LOQ	13.9

Results show that only PVC is quantified in these samples with concentrations of 3.6 ± 0.2 and 10.4 ± 0.7 mg/kg WW found in Lorient LB-S2 and PCO 38-S2, respectively. N-6,6 is also detected in the two samples but below the LOQ. All other concentrations are below the LOD for the two samples.

3.2.3. Beach sediment samples

Results obtained for beach sediment samples are presented in Table 18. Except for PVC that was detected above the LOQ in one sample (Le Stang, April), all polymers are below LOQ and most of them are also below LOD, indicating a sample contamination below the analytical limits of the method.

Table 18: Average concentrations ($\pm$ SD) obtained for beach sediment samples (mg/kg or ppm DW)

mg/kg (ppm) DW	PE	PP	PS	ABS	PVC	N-6	N-6,6	Total concentration of MP quantified
Limit of detection (LOD)	1.53	0.32	0.20	0.60	0.19	0.04	0.13	/
Limit of quantification (LOQ)	3.07	0.98	0.62	1.82	0.57	0.12	0.39	/
Le Stang (April)	<LOD	<LOD	<LOD	<LOD	1.49 ± 0.08	<LOD	<LOD	1.49
Contis	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Le Stang (July)	Detected but <LOQ	<LOD	<LOD	<LOD	Detected but <LOQ	<LOD	<LOD	<LOQ
Moliets	<LOD	<LOD	<LOD	<LOD	Detected but <LOQ	<LOD	<LOD	<LOQ
La Bonne Anse	<LOD	<LOD	Detected but <LOQ	<LOD	Detected but <LOQ	Detected but <LOQ	<LOD	<LOQ



PART 3: Development and testing of a Rock-Eval® method

This section presents the work conducted by IFPEN on the development and testing of a Rock-Eval® method, followed by an application of the method to analyse the natural samples presented in Part 1. This method has already been the subject of scientific publications by the research group involved in this project, covering the construction of regression lines (Romero-Sarmiento et al., 2022b), the estimation of LODs and LOQs (Romero-Sarmiento et al., 2025), and the implementation of the technique on real samples (Romero-Sarmiento et al., 2024).

This section is divided in three subsections: a presentation of the Rock-Eval® technique and associated equipment, followed by a presentation of materials and methods applied and it ends with the presentation of obtained results.

1. Presentation of the Rock-Eval® technique

The Rock-Eval® device is an open-system pyrolysis and oxidation instrument that has been primarily developed to characterize the thermal properties of the sedimentary organic matter using different standardized analytical methods. This device was mainly used to investigate the potential of source rocks for petroleum system explorations (e.g., Espitalié et al., 1977; Espitalié et al., 1986; Lafargue et al., 1998; among others). It has been also used to quantify the total organic carbon (TOC) and the mineral carbon (MINC) contents of any sedimentary rock. Since the 2000s, this technique has been increasingly tested in other geoscience applications including: (1) the characterization of organic matter in soils (e.g., Disnar et al., 2003; Sebag et al., 2006); (2) the study of past climate changes and global carbon cycle (e.g., Baudin et al., 2007), (3) the determination of the origin of geothermal scales (Romero-Sarmiento et al., 2022a) and recently (4) the quantification of microplastics in sediments displaying different mineral matrix (Romero-Sarmiento et al., 2022b). To illustrate this methodology, Figure 12 summarizes the Rock-Eval® parameters recently proposed to characterize polymer samples (Romero-Sarmiento et al., 2022b). The Total HC_{polymer} provides the quantity of hydrocarbon compounds released during the total pyrolysis of the polymer.

The Total CO_{polymer} provides the quantity of the carbon monoxide released during both the total pyrolysis and oxidation of the polymer. The Total CO₂polymer provides the quantity of the carbon dioxide released during both the total pyrolysis and oxidation of the polymer. Tpeak_{polymer} parameter corresponds to the temperature at the FID signal reaches its maximum. PC_{polymer} and RC_{polymer} parameters represent the total amount of carbon content quantified during the pyrolysis and the oxidation, respectively. The TOC_{polymer} corresponds here to the sum of both PC_{polymer} and RC_{polymer}. Concerning the units, the Total HC_{polymer}, CO_{polymer}, CO₂polymer are expressed in mg/g whereas PC_{polymer}, RC_{polymer} and TOC_{polymer} are expressed in wt.%. Finally, the Tpeak_{polymer} is expressed in °C (Romero-Sarmiento et al., 2022b; Figure 12).

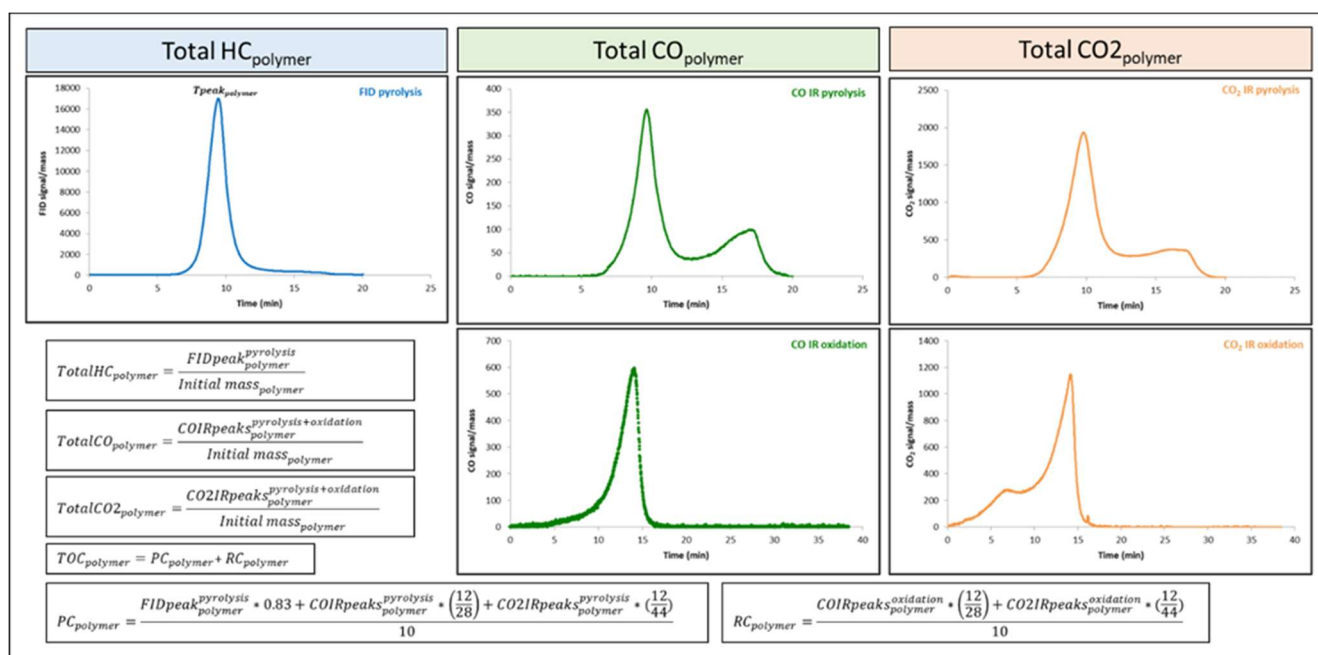


Figure 12 : Pyrolysis and oxidation thermograms showing the Rock-Eval® parameters that are now used to characterize polymer samples (modified from Romero-Sarmiento et al., 2022b)

Total HC_{polymer} (mg/g), PC_{polymer} (%), and TOC_{polymer} (%) parameters are used to quantify the polymer content (Figure 12). The linear regression between these proposed Rock-Eval® parameters and the polymer content (%) established in Romero-Sarmiento et al. (2022b) provides a screening framework to quick convert Rock-Eval® results (Total HC, PC, and TOC) obtained from environmental samples into quantified values of the polymer content (Figure 13).

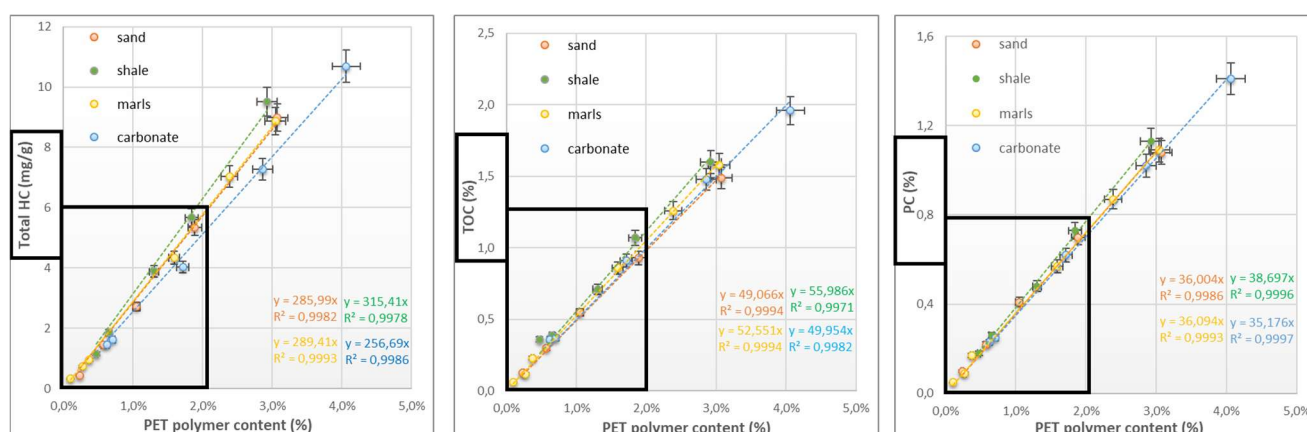


Figure 13 : Rock-Eval® parameters (Total HC, TOC, PC) versus the PET polymer content showing linear regressions that can be used to quantify the polymer content in artificial samples composed by different mineral matrices (e.g., sand, shale, marl, and carbonate, modified from Romero-Sarmiento et al., 2022b)

For a given sample after preparation procedures (treated sample), the assumption is based on that all (or a relative proportion of) organic carbon content is considered as the polymer/plastic content. It was also demonstrated that the estimations of these three parameters suffer almost no influence of the mineral matrix (Figure 13; Romero-Sarmiento et al., 2022b). The aim of this work is to evaluate the applicability of this workflow for quantifying microplastic contents in real natural sediment samples.

2. Methods: the Rock-Eval® device

A Rock-Eval® 6 device operating at IFP Energies Nouvelles (France) was used to characterize the three sets of port samples (S1, S2 and S3, as detailed in Part 1, section 2) following the analytical procedure to characterize polymer and filter samples proposed in Romero-Sarmiento et al., (2022b; 2024). This open-system instrument is equipped with two ovens dedicated and programmed for pyrolysis and combustion processes, respectively. Inside the Rock-Eval® device, these ovens are connected to two individual detectors to quantify in real-time organic and inorganic compounds released during both pyrolysis and oxidation stages. As the artificial thermal degradation of the investigated sample proceeds, total hydrocarbon (HC) compounds released during the pyrolysis step are monitored by a Flame Ionization Detector (FID) whereas the total CO₂ and CO compounds released during both pyrolysis and oxidation steps are monitored by an infra-red (IR) detector (Espitalié et al., 1986; Lafargue et al., 1998; Behar et al., 2001).

In this work, a modified Rock-Eval® Basic/Bulk-Rock method described in Disnar et al. (2003) was used to analyse 60 mg of each bulk and treated port sediments (*S1 and S2 samples*). This programmed method, that has been widely used to analyse soil samples, is characterized by a starting pyrolysis step at 200°C for 3 minutes and then the pyrolysis temperature increases from 200°C to 650°C at 25°C/min (Disnar et al., 2003). The oxidation step of the pyrolysis residue starts at 200°C for 1 minute and then the oxidation temperature increases from 200°C to 850°C at 25°C/min.

To avoid the formation of silica gel in the Rock-Eval® crucibles as thermal degradation proceeds, *S3 samples* (filters) were analysed using the Rock-Eval® pyrolysis program only, starting at 200°C for 3 minutes and then the pyrolysis temperature increases from 200°C to 650°C at 25°C/min.

The quality control and the calibration of the Rock-Eval® device is classically performed using the reference standard IFPEN-160000 to validate HC, CO, and CO₂ signals and calculated Rock-Eval® parameters. About 80 mg of this certified standard is regularly running every 10 analyses. Rock-Eval® thermograms are visualized using Geoworks-Geochemical software provided by Vinci Technologies (France). Rock-Eval® parameters previously defined to characterize polymer families (e.g., *Total HC*, *Total CO*, *Total CO₂*, *Tpeak*, *PC*, *RC*, *TOC*) were then calculated following procedures already published in Romero-Sarmiento et al., (2022b; 2024). Furthermore, Rock-Eval® FID signal deconvolutions were integrated using Python code accordingly.

3. Results of port sediment samples analysis

Table 19 summarizes the Rock-Eval® parameters calculated in this study for the ten bulk port sediments (*S1 samples*) and for the two treated port sediments after the elimination of the organic matter with H₂O₂ (*S2 samples*). Figure 14 also illustrates the obtained Rock-Eval® thermograms and corresponding *Total HC*, *Total CO* and *Total CO₂* parameters (*S1 samples*).

Table 19 : Rock-Eval® parameters calculated in this study for the 10 bulk port sediments and the 2 treated port sediments (S1 & S2 samples)

Sample	Tpeak (°C)	TOC (%)	PC (%)	RC (%)	Total HC (mg/g)	Total CO (mg/g)	Total CO ₂ (mg/g)
10 bulk drying and crushing port sediments (S1 samples)							
PCO 37 (S1)	463	6.3	2.3	3.9	23	15	165
PCO 38 (S1)	459	2.9	1.0	1.9	10	6	196
PCO 39 (S1)	398	2.8	1.6	1.3	15	5	195
Honfleur (S1)	463	5.1	2.2	2.9	22	7	204
Lorient LB (S1)	437	4.2	1.8	2.4	18	7	155
Lorient PL (S1)	452	5.3	2.1	3.2	20	12	145
Bayonne (S1)	482	3.1	1.1	2.1	9	6	118
Hendaye PE (S1)	393	5.6	1.9	3.7	17	13	171
Saint-Jean-de-Luz (S1)	453	4.0	1.0	3.0	8	13	131
Hendaye PL (S1)	455	5.4	1.4	4.0	12	15	181
2 treated port sediments after the elimination of the organic matter with H ₂ O ₂ (S2 samples)							
PCO 38 (S2)	454	5.4	2.8	2.5	22	7	172
Lorient LB (S2)	465	4.0	2.3	1.7	19	8	119

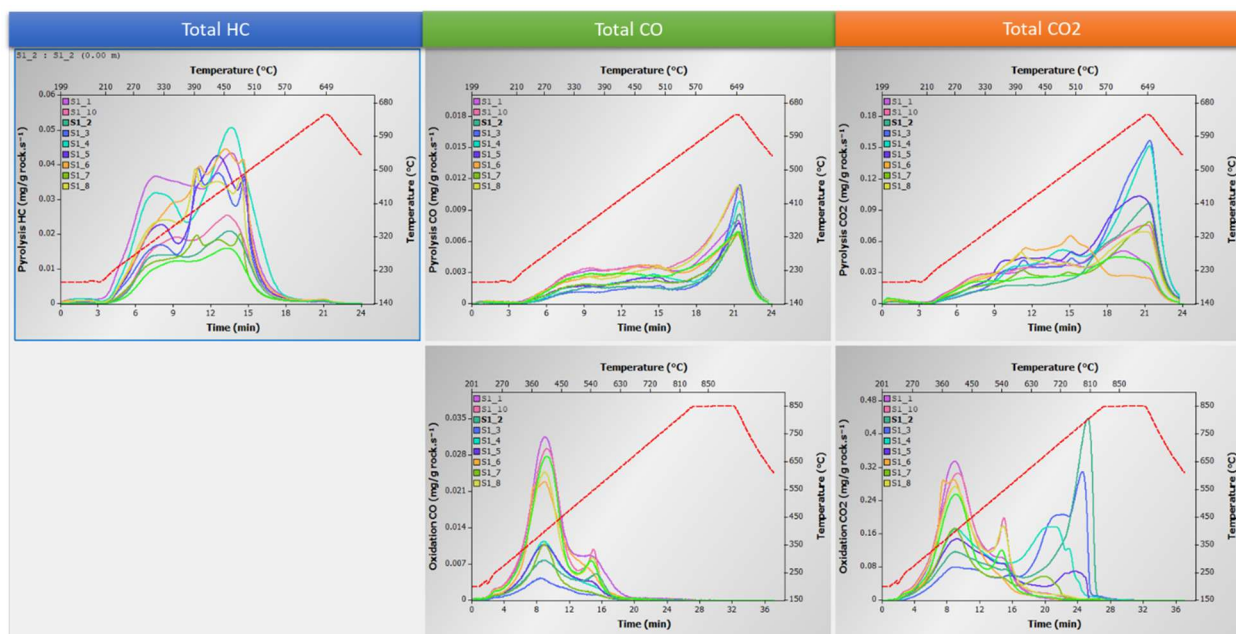


Figure 14 : Rock-Eval® thermograms and corresponding Total HC, Total CO and Total CO₂ parameters showing the thermal degradation of 10 bulk port sediments (S1 samples)

Based on calculated Rock-Eval® parameters (Table 19), a predefined Rock-Eval® signal database for pure polymers (e.g., PET, PP, PE) as well as the corresponding linear regression coefficients for the parameters *Total HC*, *TOC*, and *PC* (e.g., Figure 14 ; Romero-Sarmiento et al., 2022b) were used in this study to quantify the total relative

mass content of microplastics for the two treated port sediment samples only (S2 samples).

Several hypotheses on the calculation method of the plastic mass concentration were here evaluated as follow:

- 1) We first consider a treatment efficiency of approximately 30% for the elimination of organic matter (OM) with H_2O_2 (70% residual OM),
- 2) Second, we consider a treatment efficiency of approximately 50% of elimination of organic matter with H_2O_2 (50% residual OM), and
- 3) Finally, as the thermal degradation proceeds, we also consider that the entire hydrocarbon (HC) signal detected during the pyrolysis temperature interval (Total HC parameter) above 390°C corresponds to the thermal domain of microplastics (Figure 14, Figure 15 & Table 20).

From each investigated port sediment sample, three estimates of microplastic mass concentration were calculated accordingly. All calculated values are expressed in ppm (mg/kg) of microplastics. Figure 16 shows that the two investigated port sediment samples contain very small microplastic (VSMP, <45µm) mean concentrations around 1336 ppm (PCO 38) and 705 ppm (Lorient LB), respectively.

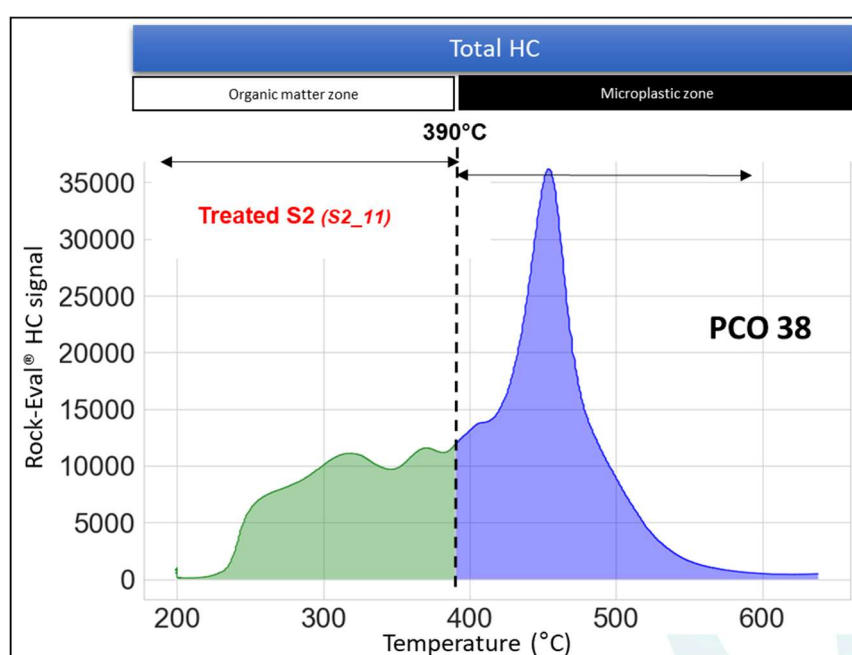


Figure 15 : An example of two domains identified by pyrolysis temperature intervals (organic matter and microplastic zones) for the treated port sediment S2_11 (PCO 38)

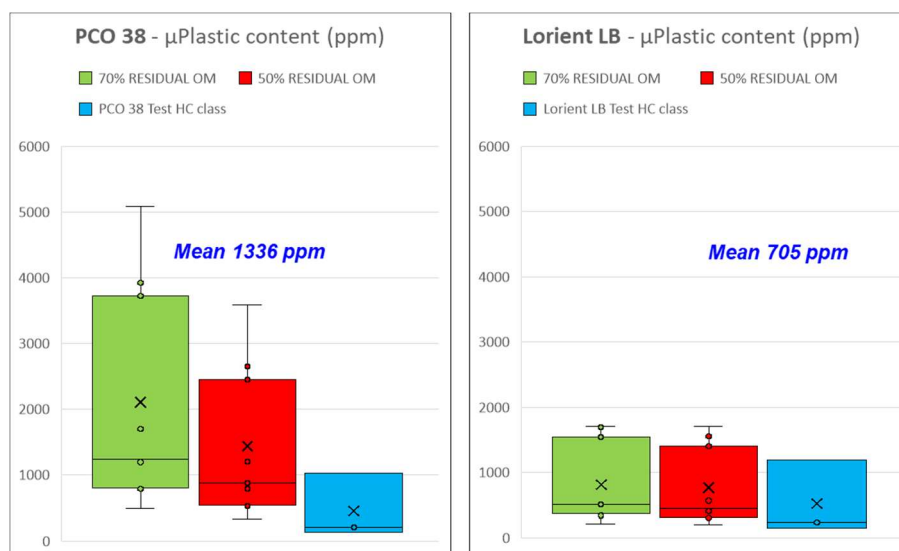


Figure 16 : Estimates of mass concentration of microplastics (ppm) in port sediment samples calculated using Rock-Eval® parameters proposed by Romero-Sarmiento et al. (2022b; 2024)

To quantify the small microplastic (SMP) content, Table 20 summarizes the Rock-Eval® parameters calculated in this study for the 10 powdered silica membranes containing the final residue (*S3 samples – MP content on filters*). Figure 17A illustrates the obtained Rock-Eval® *Total HC* thermograms (*S3 samples*). Figure 17B also shows a comparison between the bulk, treated and filter samples for the corresponding PCO 38 port sediment, suggesting that the quantification of the total relative mass content of small microplastics is more accurate for the 10 powdered silica membranes (*S3 samples*).

Table 20 : Rock-Eval® parameters calculated in this study for the 10 powdered silica membranes containing the final residue (S3 samples). Mass quantification of MP content in samples

Sample	Tpeak (°C)	Total HC (mg/g)	OM zone calculated from Total HC signal (%)	Polymer zone calculated from Total HC signal (%)	MP content on filters (mg)	Total mass sample (g)	MP content in sample (ppm)
PCO 37 (S3)	476	40	20	79	5.3	201.4	27
PCO 38 (S3)	479	24	18	81	2.1	200.2	11
PCO 39 (S3)	465	15	35	64	1.1	200.9	6
Honfleur (S3)	449	20	23	77	2.1	202.4	10
Lorient LB (S3)	475	28	23	76	4.5	169.8	26
Lorient PL (S3)	477	63	36	64	7.1	206.1	34
Bayonne (S3)	477	26	33	67	1.7	201.7	9
Hendaye PE (S3)	476	102	46	54	12.7	200.8	63
Saint-Jean-de-Luz (S3)	467	57	43	57	5.2	201.6	26
Hendaye PL (S3)	470	42	31	69	3.9	206.6	19

Obtained results indicate that the investigated port sediment samples contain SMP masses on filters varying between **1.1 and 12.7 mg** (Table 20). Furthermore, regarding *T_{peak}* parameters, these ten port samples are probably dominated by PP and N-6 polymers. Small quantities of PET and PE polymers could be also present. These observations should be confirmed by μ -FTIR or Py-GC/MS measurements.

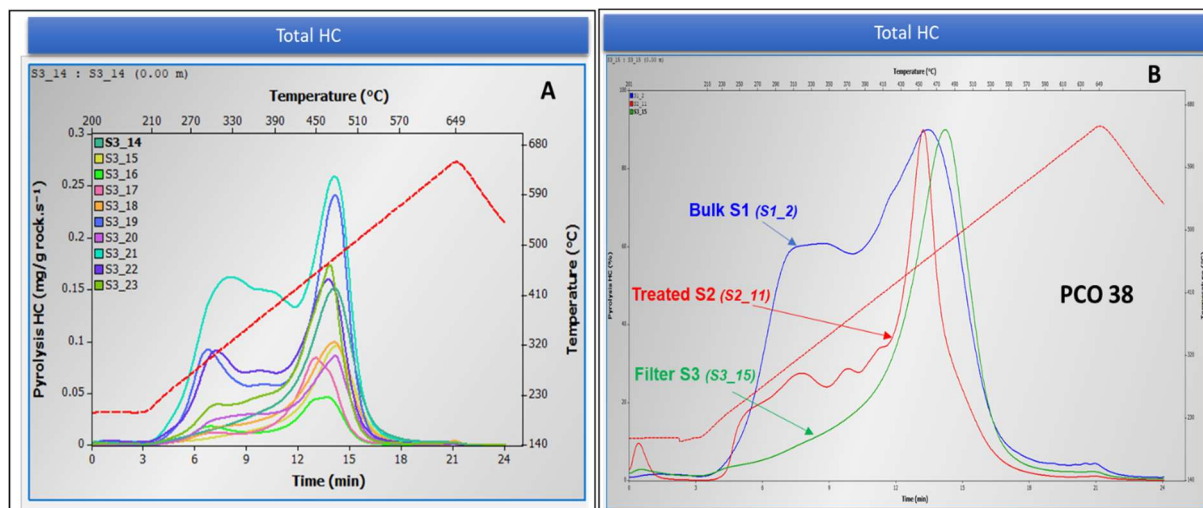


Figure 17 : (A) Rock-Eval® Total HC thermograms obtained for the 10 powdered silica membranes containing the final residue (S3 samples). (B) A comparison between the bulk, treated filtrate (< 45 μ m) and filter (>45 μ m) samples for the corresponding PCO 38 port sediment



Part 4: Discussion

This part discusses results obtained. First, it addresses the sample preparation protocol proposed, it discusses the potential of the pyrolysis-GC/MS and the Rock-Eval® then it presents a comparative analysis of results obtained with both methods and it ends with a comparative assessment of the tested methods with traditional spectroscopy techniques.

1. Samples preparation

For this study, a specific sample preparation protocol was co-designed by CEDRE and IFPEN based on their previous experiences. The main objective of this protocol was to extract and concentrate microplastics by effectively removing organic matter and separating microplastics from the mineral matrix, using chemical digestion and a flotation separation. The key innovations were three folds. First, CEDRE and IFPEN planned on upstream analysis of unprepared samples (bulk samples). Before the standard preparation steps, a part of unprepared samples was kept for analysis to investigate whether the raw signal contains usable information on microplastic content (S1 samples). Secondly, CEDRE and IFPEN planned to analyse the filtrate residues. This protocol allows to examine the sieving residues produced during the separation phases which are commonly discarded (S2 samples) allowing a quantification of both small microplastics ($>45\text{ }\mu\text{m}$) and very small microplastics ($<45\text{ }\mu\text{m}$) for a selection of samples. This innovative protocol aims to bridge gaps in conventional methods by broadening the analytical scope and maximizing the utility of all sample components for more complete microplastic mass estimation. Thirdly, based on previous works conducted by CEDRE that suggested 100 g could be too small to allow quantification of MP considering current Pyrolysis-GC/MS quantification limits, the protocol was adapted to allow the analysis of 200 g of sediment samples. To do so, two samples of 100g were extracted before being combined during the last steps of the preparation.

To ensure consistency in the comparison of quantification methods, a decision was taken to entrust a partner laboratory with the full responsibility of preparing the samples (CEDRE lab). This approach minimized potential discrepancies that could arise from differences in preparation protocols between laboratories, allowing a more robust analytical comparison of the tested quantification methods (pyrolysis-GC/MS versus Rock-Eval®).

The preparation protocol proposed was successfully implemented to prepare the different samples considered in the study. Results obtained showed that MP were detected in the three types of samples (S1, S2 and S3). In particular, very small microplastics were detected with both pyrolysis-GC/MS and Rock-Eval®, confirming that a part of the microplastic contamination is lost during the preparation steps, in particular particles that are smaller than the sieving mesh size (here: $45\mu\text{m}$). This phenomenon can result in important underestimation of the sample contamination. In this context, further studies are needed to better assess MP losses during preparation steps. It is important to mention that currently, these studies are hindered by the limited commercial availability of microplastic standards. This situation limits the capacity to produce artificial samples containing a mix of representative polymers in sufficient

concentrations, that are needed to conduct testing and optimization of the different sample preparation steps.

Results confirm that the analysis of larger quantities of sediment, though involving a double extraction that increases the preparation time, appears particularly relevant. Indeed, even with these large quantities of sample analysed, most samples exhibited concentrations below analytical limits when using the pyrolysis-GC/MS technique.

However, it is also important to mention that contaminations were detected during the preparation of one batch of samples (sediment samples of the QUASIMEME WEPAL exercise), confirming that the multi-steps preparation method implemented for sediment is prone to external contamination and that prevention measures including the preparation of atmospheric and procedural blanks are absolutely necessary when preparing these types of samples. This observation also supports the analysis of larger quantities of samples, in order to lower the effect of contamination on the final result.

2. Potential of the pyrolysis-GC/MS technique

Gas chromatography coupled with pyrolysis and mass spectrometry is an advanced technique that combines thermal pyrolysis with gas chromatography and mass spectrometry for detailed analysis of microplastics. Pyrolysis breaks down complex materials into their simpler constituents, in the absence of oxygen, by heating the sample to a high temperature (up to 800°C). This technique is particularly effective for analysing plastics, as it breaks down polymers into volatile compounds, which can then be identified and quantified. It has been particularly used in previous studies for analysing SMP in complex environmental matrices such as sediments, water, or soils, while providing detailed chemical characterization of polymers.

In the present study, a pyrolysis-GC/MS method to quantify SMP has been developed, validated and further tested on natural samples. Results obtained confirmed the capacity of the method to quantify small microplastics. The method allows in one analysis the quantification of SMP quantities (in µg or mg) for each targeted polymer, providing the total MP concentration but also the concentration by polymer. The development and validation phase showed the method exhibits good precision, repeatability, and accuracy.

However, on the 11 polymers targeted, the method was validated for only seven polymers (PE, PP, PS, ABS, PVC, N-6 and N-6,6), as performances were not satisfactory for other polymers at the tested concentrations, which were low in most cases and limited by the polymer concentrations in the standard solution used. Indeed, to our knowledge and except for PE, the Frontier Lab Microplastics Calibration Standard used in the present study is the only standard available for other polymers. This represents an important limitation for analytical development as it strongly limits the possibility to optimise analytical parameters for each polymer individually.

Results also showed that the method was able to quantify several polymers above analytical limits in several natural samples (either port or beach sediments). Total concentrations ranged between 17 to 170 mg/kg DW (5 to 28 mg/kg WW) for the port sediment samples and between <LOD to 1.5 mg/kg DW in the beach samples, suggesting a higher MP contamination in port sediments compared to beach sediments. In port

sediments, polymers mainly quantified were PVC and PP with also PE, PS and N-6,6 detected. In beach sediment samples, PE, PS, PVC and N-6 were detected but only PVC was quantified.

However, some polymers were only detected but not quantified, suggesting the method LOD/LOQ are currently too high. This was the case for natural samples but also for samples from the QUASIMEME WEPAL exercise that exhibited concentrations below the Pyrolysis-GC/MS analytical limits, considering the quantities available (65 g). Indeed, in the proposed method, only a small fraction of the sample is analysed (ca. 1/10) and the filter importantly dilutes the samples. These two factors lead to increased analytical limits. Further work is needed to assess if the analytical limits can be optimised by either lowering them or increasing the quantities injected to ensure that environmental concentrations can be quantified accurately

In addition, though the method exhibits rather good performances, it must be noted that recovery rates were only assessed on artificial samples prepared by diluting the standard solution in the diluent and directly analysed, as it was not possible to assess yields on the whole analytical process (including the sample preparation) due to the limited availability of standards. In the present study, tests were also performed with an internal standard (poly(4-fluorostyrene)). Though, it did not lead to improvement in the calibration performance, the identification and testing of potential internal standard should be continued, as this could contribute to correct losses that either occur during preparation or analytical steps and improve the overall method performance.

Overall, though results obtained are promising, the present study shows that further work is needed to progress on the development of performant pyrolysis-GC/MS to quantify small microplastic. However, this action is currently importantly hindered by the lack of commercial standards and certified reference materials. These products are urgently needed to develop performant analytical methods that will allow an accurate quantification of MP in environmental matrices and meet the requirements of environmental monitoring.

3. Potential of the thermal Rock-Eval® technique

Rock-Eval® thermal analysis is a technique initially developed for petroleum geochemistry but that has recently been adapted for the study of microplastics in environmental samples. This method involves programmed pyrolysis and oxidation of the sample under controlled temperature conditions to detect and quantify organic carbon, including synthetic polymers. Rock-Eval® is particularly used for identifying the thermal degradation properties of different plastic materials and distinguishing them from natural organic matter.

In the present study, a Rock-Eval® method to quantify SMP was proposed and further tested on natural samples. Results obtained confirmed the capacity of the method to quantify small microplastics. The method allows in one analysis the quantification of SMP quantities (in µg or mg) providing the total concentration in MP. The development and validation phase showed the method exhibit good precision, repeatability, and accuracy. In addition, the Rock-Eval® analyses reveal that all studied natural samples are contaminated by microplastics.

Its main advantages include high sensitivity, minimal sample preparation, and suitability for analysing microplastics in complex matrices such as sediments and soils. However, it does not provide specific molecular identification of polymers, requiring complementary techniques such as micro-FTIR or Raman spectroscopy for a more detailed analysis. This technique is fully quantitative in terms of total carbon (including synthetic), but does not provide information on particle size, number, or polymer type. It is well-suited for global plastic content estimation in complex matrices (Romero-Sarmiento et al., 2022b).

The use of Rock-Eval® parameters, as explored in this and prior studies (e.g., Romero-Sarmiento et al., 2024), could offer an avenue to refine signal processing of the bulk sample and extract the microplastic signal with minimal preprocessing. This research avenue should be explored in future works, enhancing the overall practicality and speed of the method, and making it more suitable for large-scale applications.

4. Comparison of results obtained with both methods

In this study, a batch of identical samples were analysed in parallel with the two tested methods. Overall, results were very consistent between the pyrolysis-GC/MS and the Rock-Eval® methods as shown on Figure 18.

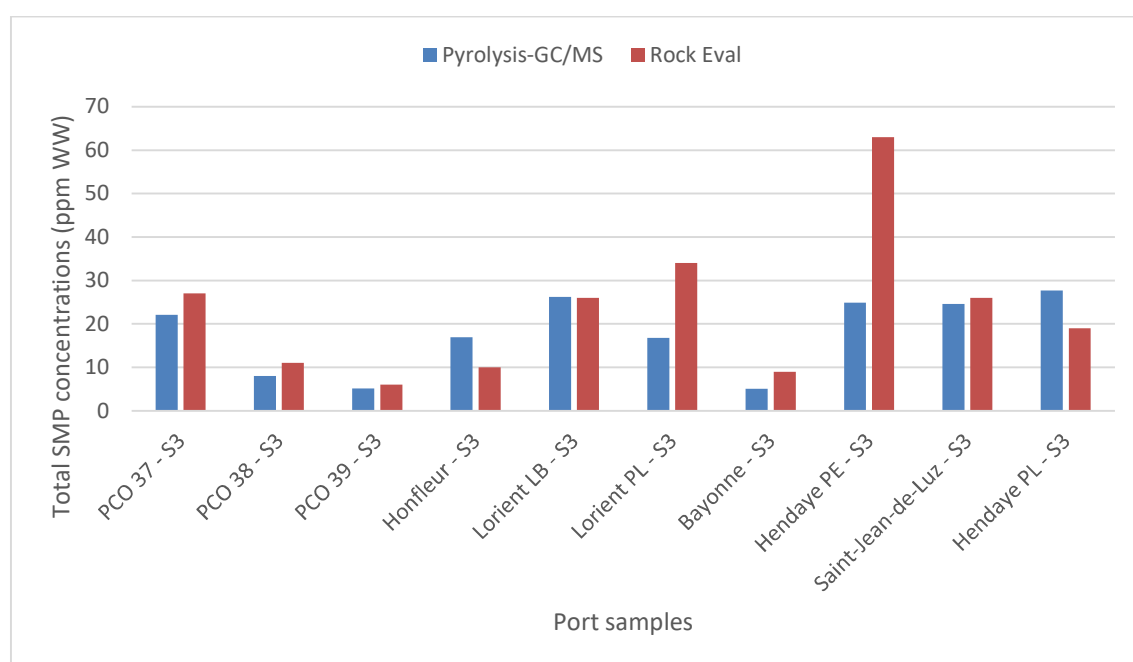


Figure 18: Total small microplastic (>45 µm) concentrations (mg/kg WW) obtained with both analytical methods for port sediments.

The SMP concentrations calculated by the pyrolysis-GC/MS range between **5 to 28 mg/kg WW** (keeping in mind that only 7 polymers were analysed) whereas concentration calculated by the Rock-Eval® range between **6 to 63 mg/kg WW** for the ten samples. These values align well with those typically observed in sedimentary compartments such as beaches and the coastal shelf (e.g., Rohais et al., 2024 and references therein).

In addition, both methods showed that masses measured from the sieving residue samples were not negligible. Results obtained by pyrolysis-GC/MS for two port sediments for which both S2 and S3 samples were analysed are presented on Figure 19.

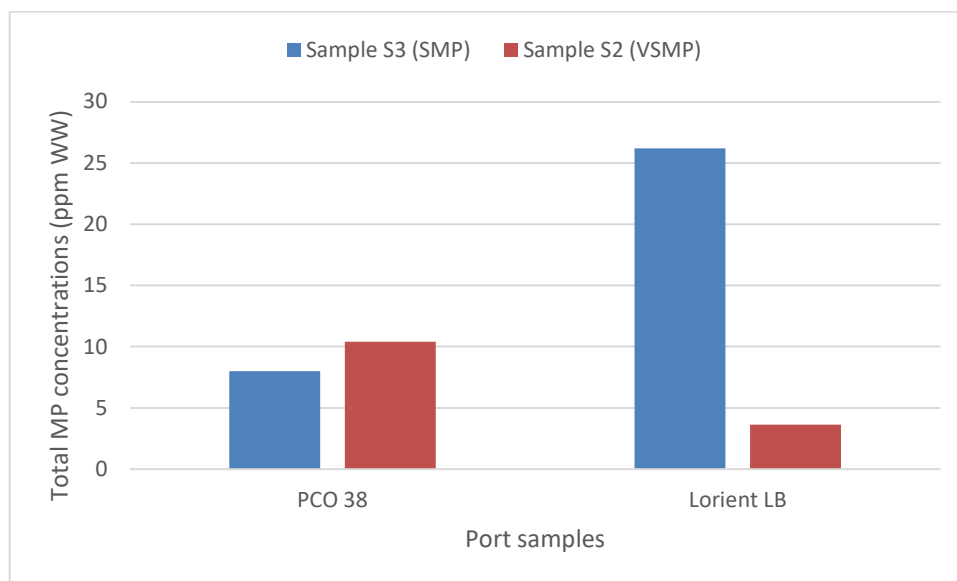


Figure 19: Total small microplastics (SMP, > 45µm) and very small microplastics (VSMP, <45µm) concentrations (mg/kg WW) obtained with pyrolysis-GC/MS for the two port sediments for which both S2 and S3 samples were analysed

With the Rock-Eval®, the masses estimated from the sieving residue samples (S2) were found to be 2 (PCO 38) to 3 (Lorient LB) times greater than the masses recovered on the filters. This discrepancy highlights the dominance of very small microplastics (<45 µm) in these samples, indicating that this fraction constitutes a significant proportion of the total microplastic load.

In this case study, neglecting microplastics <45 µm could lead to an underestimation of microplastic concentrations in sedimentary matrices. These findings emphasize the need to include smaller size fractions in monitoring protocols to ensure a more comprehensive assessment of microplastic pollution.

5. Comparison with traditional techniques

Obtained results demonstrated that the thermal pyrolysis-GC/MS and Rock-Eval® techniques can be used as additional approaches to calibrate or complete the classical spectroscopy methods used for quantifying microplastics like micro-FTIR and micro-Raman.

Micro-FTIR is one of the most commonly used methods for analysing MP. It enables the identification and characterization of various types of polymers in different environmental matrices by measuring the absorption of infrared radiation by the sample. Each polymer produces a unique infrared spectrum, similar to a fingerprint, making it a powerful tool for determining the molecular composition of microplastics. Among its key advantages, FTIR provides a reliable analysis of polymer types and, with micro-FTIR, can analyse particles as small as 20 µm. However, its spatial resolution is limited for particles smaller than 10 µm. In terms of data output, micro-FTIR provides information on particle size and number through imaging or mapping modes, and allows

for specific polymer identification. It is partially quantitative, particularly for estimating particle counts per polymer type (Huang et al., 2023).

Raman spectroscopy is based on analysing photon scattering during their interaction with molecular vibrations, which allows for the chemical composition of microplastics to be determined. It stands out for its high resolution, capable of analysing particles smaller than 1 μm , and its effectiveness in identifying transparent polymers or those with low infrared absorption, such as PVC and PET. However, this technique is sensitive to the fluorescence of certain materials, which can interfere with the analysis and limit its effectiveness in some cases. Raman spectroscopy provides highly detailed data on particle size and count, as well as polymer type. It is fully quantitative in configurations that allow automated particle detection and classification (Araujo et al., 2018).

In the present study, both tested methods, the pyrolysis-GC/MS and Rock-Eval[®], provide quantitative results in terms of mass, offering accurate measurements of total polymer quantities (in μg or mg). In addition, pyrolysis-GC/MS provides quantification by polymer based on a precise identification using molecular markers. Both methods are not affected by particle colours as it can be the case with dark particles for micro-FTIR or fluorescent particles for micro-Raman.

Both methods were also able to detect and quantify very small microplastics (<45 μm) whereas methods such as $\mu\text{-FTIR}$ are generally limited by an optimistic limit of detection of 10-25 μm . While the present study demonstrates that even the smallest microplastic fractions can have substantial environmental relevance and should be integrated into future monitoring and quantification strategies, it also highlights the need to keep this in mind when selecting analytical approaches.

Both methods have a rapid analytical step (about 40 minutes for the pyrolysis-GC/MS) and can deliver results rapidly (in less than 4 days for 10 samples once samples are prepared for both methods), whereas spectroscopy methods that imply a mapping and an analysis of each particle, require an extensive analysis time (that can be automated) in case of highly contaminated samples.

However, the two tested methods are destructive and require a time-intensive pre-processing, which poses a significant challenge. Though, this is a common limitation across many microplastic measurement techniques, including abundance-based methods (micro-FTIR and micro-Raman), this pre-processing is particularly important for pyrolysis-based methods as organic matters present in the sample can generate interferences (Bouzid et al., 2022).

Overall, the two tested method appear particularly relevant to quantify small microplastics in coastline sediments. The main drawback is they do not provide data on particle size and number as spectroscopy does and, in that sense, they do not align with the requirements of existing microplastic monitoring programmes (such as the OSPAR or the MSFD programmes for marine sediments; OSPAR, 2024; MSFD TG ML, 2023). In this context and considering the potential of the tested methods, it is recommended to consider the possibility to use these methods as an alternative or complement of traditional spectroscopy methods, in the context of existing monitoring programmes.

References

- Akoueson, F., Chbib, C., Monchy, S., Paul-Pont, I., Doyen, P., Dehaut, A and Duflos, G. (2021). Identification and quantification of plastic additives using pyrolysis-GC/MS: A review. *Science of The Total Environment*, Volume 773, 15 June 2021, 145073.
- Araujo, C. F., Nolasco, M. M., Ribeiro, A. M. P. and Ribeiro-Claro, P. J. A. (2018). Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Research*, vol. 142, p. 426-440.
- Behar F., Beaumont V., De B. and Penteado H.L. (2001) Rock-Eval 6 Technology: Performances and Developments, *Oil & Gas Science and Technology* 56, 111-134.
- Baudin, F., Combourieu-Nebout, N., Zahn, R. (2007). Organic signatures of rapid climatic changes in Western Mediterranean during North Atlantic cold events of the Last Glacial. *Bulletin Société Géologique de France* 178, 3–13.
- Bouزيد, N., Anquetil, C., Dris, R., Gasperi, J., Tassin, B. and Derenne, S. (2022). Quantification of Microplastics by Pyrolysis Coupled with Gas Chromatography and Mass Spectrometry in Sediments: Challenges and Implications. *Microplastics*, 1, 229–239.
- CEDRE (2018). Développement d'un protocole de suivi des microparticules sur le littoral de France métropolitaine dans le cadre du programme de surveillance DCSMM. Rapport R.17.63.C.
- CEDRE (2023). French national monitoring network for mesoplastics and large microplastics on beaches. 2023 Campaign report. Rapport R.24.44.C.
- Dey, S., Sen, K., Chandra Saha, N. and Saha (2025). Analytical approaches for quantifying and characterizing microplastics: Environmental impacts and bioaccumulation in aquatic Systems. *Green Analytical Chemistry*, 12, 100191.
- Disnar, J.R., Guillet, B., Keravis, D., Di-Giovanni, C., Sebag, D. (2003). Soil organic matter (SOM) characterization by Rock-Eval pyrolysis: scope and limitations. *Organic Geochemistry* 34, 327-343.
- Espitalié, J., Laporte, J.L., Madec, M., Marquis, F., Leplat, P., et al. (1977). Méthode rapide de caractérisation des roches mères, de leur potentiel pétrolier et de leur degré d'évolution. *Oil and Gas Science and Technology* 32, 23-42.
- Espitalié, J., Deroo, G., Marquis, F. (1986). La pyrolyse Rock-Eval et ses applications. *Revue de l'Institut Français du Pétrole* 41, 73-89.
- Feinberg, M. (2009). Labo-stat: guide de validation des méthodes d'analyse. *Technique et Documentation*.
- Ghosh, S., Kumar Sinha, J., Ghosh, S., Vashisth, K., Han, S. and Bhaskar, R. (2023). Microplastics as an Emerging Threat to the Global Environment and Human Health. *Sustainability*, 15(14), 10821.
- Hermabessiere, L., Himber, C., Boricaud, B., Kazour, M., Amara, R., Cassone, A., Laurentie, M., Paul-Pont, I., Soudant, P., Dehaut, A., Duflos, G. (2028). Optimization,

performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. *Analytical And Bioanalytical Chemistry*, 410 (25), 6663-6676.

Huang, Z., Hu, B. and Wang, H. (2023). Analytical methods for microplastics in the environment: a review. *Environ Chem Lett*, vol. 21, no 1, p. 383 401.

Lafargue, E., Marquis, F., Pillot, D. (1998). Rock-Eval 6 applications in hydrocarbon exploration, production and soils contamination studies. *Oil and Gas Science and Technology* 53, 421-437.

Lauschke, T., Dierkes, G., Schweyen, P. and Ternes, T. (2021). Evaluation of poly(styrene-d5) and poly(4-fluorostyrene) as internal standards for microplastics quantification by thermoanalytical methods. *Journal of Analytical and Applied Pyrolysis*. Volume 159, 105310.

Matsueda, M., Mattonai, M., Iwai, I., Atsushi Watanabe, A., Norio Teramae, N., Robberson, W., Ohtani, H., Kim, Y.-M., Watanabe, C. (2021). Preparation and test of a reference mixture of eleven polymers with deactivated inorganic diluent for microplastics analysis by pyrolysis-GC–MS. *Journal of Analytical and Applied Pyrolysis*, 154, 104993.

Maw, M. M., Boontanon, N., Fujii, S. et Boontanon, S. K. (2022). Rapid and efficient removal of organic matter from sewage sludge for extraction of microplastics. *Science of The Total Environment*, vol. 853, p. 158642.

MSFD Technical Group on Marine Litter. (2023). Guidance on the Monitoring of Marine Litter in European Seas An update to improve the harmonised monitoring of marine litter under. Luxembourg: EUR 31539 EN, Publications Office of the European Union. doi:10.2760/59137

Nyadjro, E.S., Cebrian, J., Cox, T.E., Wang, Z., Lau, Y.H., Konefal, A.M., Turnage, G., Offner, T., Gilpin, R., ... and Webster, J.A.B. (2026). Gaps and Pathways Towards Standardized, FAIR Microplastics Data Harmonization: A Systematic Review. *Microplastics*, 5(1), 11.

OSPAR MicroPlastic Expert Group (MPEG) (2024). CEMP Guidelines for the monitoring of microlitter (including microplastics) in seafloor sediments for the OSPAR maritime area OSPAR Agreement 2024-06.

Rohais, S., Armitage, J. J., Romero-Sarmiento, M.-F., Pierson, J.-L., Teles, V., Bauer, D., Cassar, C., Sebag, D., Klopffer, M.-H., Pelerin, M. (2024). A source-to-sink perspective of an anthropogenic marker: A first assessment of microplastics concentration, pathways, and accumulation across the environment. *Earth-Science Reviews* 254, 104822.

Romero-Sarmiento, M.-F., Ravelojaona, H., Maubec, N. (2022a). Corrosion inhibitors and lubricants characterization using the Rock-Eval® Shale Play™ method: case studies to determine the origin of geothermal scales. *Geothermics* 101, 102357.

Romero-Sarmiento, M.-F., Ravelojaona, H., Pillot, D., Rohais, S. (2022b). Polymer quantification using the Rock-Eval® device for identification of plastics in sediments. *Science of the Total Environment* 807, 151068.

Romero-Sarmiento, M.-F., Rohais, S., Dreillard, M. (2024). Quantification of textile microfibers from laundry wastewater using the Rock-Eval® device: Difference between natural and synthetic microfiber origin. *Science of The Total Environment* 956, 177335.

Romero-Sarmiento, M.-F., Bauer, D., Rohais, S. (2025). Detection and Quantification Limits for Polyethylene Particles Combining the Thermal Rock-Eval® Method with a Mathematical Extrapolation Procedure *Microplastics* 4 (4), 71. <https://doi.org/10.3390/microplastics4040071>

Santos, L.H.M.L.M., Insa, S., Arxé, M., Buttiglieri, G., Rodríguez-Mozaz, S. and Barceló, D. (2023). Analysis of microplastics in the environment: Identification and quantification of trace levels of common types of plastic polymers using pyrolysis-GC/MS. *MethodsX*, vol. 10, p. 102143.

Sebag, D., Verrecchia, E.P., Cécillon, L., Adate, T., Albrecht, R., Aubert, M., Bureau, F., Cailleau, G., Copard, Y., Decaens, T., Disnar, J.-R., Hetényi, M., Nyilas, T., Trombino, L., 2016. Dynamics of soil organic matter based on new Rock-Eval® indices. *Geoderma* 284, 185-203.

