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Comparative analysis of microplastics detection methods applied to marine sediments: A case study in the Bay of Marseille

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ABSTRACT

An intercomparison exercise on "microplastics in sediment" was carried out by five laboratories using samples collected in the Bay of Marseille in September 2021. The results from different extraction and identification methods varied depending on the type and size classes of MPs, and was better than 80 % for the size class >300 μm and for the fragments. The variability in recovery rates can be attributed to the choice of reagents and extraction protocols. Recovery rates per laboratory were between 47 % and 113 % and the use of $ZnCl_2$ and NaI increased recovery rates by an average of 70 %. The lowest recovery rates (47 and 53 %) were attributed to the reference methods (FTIR and LDIR), conversely the highest (80 and 87 %) were attributed to identification by Nile Red. The average ranged between 23 and 53 items /50 g d.w. with decreases offshore and at greater depth.

1. Introduction

Plastic pollution is a global environmental issue that has received much attention (Jambeck et al., 2015). It impacts all environments at various scales, with significant consequences for biodiversity and ecosystem functioning (Sutherland et al., 2010), and poses a major threat to marine environments (UNEP, 2016). This pollution is characterized by both macro- and microlitter, which includes microplastics (MPs). Macrolitter comprises litter items between 25 mm and 1 m in size, mesolitter between 5 mm and 25 mm, while MPs refer to plastic items with dimensions ranging from 1 μm to 5 mm (GESAMP, 2019). MPs can be either primary or secondary: primary MPs correspond to MPs originating directly from industrial production, still unprocessed, mainly represented by pellets, whereas secondary MPs are produced by the fragmentation of larger macrolitter present in the marine environment, which has undergone erosion due to environmental factors (temperature, UV, salinity, mechanical action by waves and tides, etc.) (Peng et al., 2020). MPs are pervasive, found in all components of the marine ecosystem, including water, sediment, and biota. Their omnipresence (Gago et al., 2018) may impact marine fauna and associated food webs, as organisms can easily ingest MPs due to their small size (Giani et al., 2019; Kumar and Prasannamedha, 2021). Recent studies

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have proposed the seafloor as a major sink for MPs pollutants (Simon-Sánchez et al., 2022). However, there is a lack of solid data, mainly due to the lack of harmonization of preparation and analysis methods (Phuong et al., 2021). Although the precise effects of MPs on organisms remain poorly understood, they may pose a risk to human health (Caruso, 2019; Landrigan et al., 2020), as certain affected species are part of the human diet (Huang et al., 2020).

Given the pressure exerted by MP pollution, the importance of monitoring and studying this contamination in a stable matrix appears to be a major issue. This is why, in Europe, the occurrence of MPs in sediment has been selected as one of the indicators for environmental assessment within the framework of the EU Marine Strategy Framework Directive (MFSD) and the OSPAR convention (Bäuerlein et al., 2023). However, the use of this indicator requires efforts to harmonize MPs extraction and identification methods. The quantification of MPs can be strongly affected by the isolation method used for marine environmental samples. The recovery rate is an important factor in determining the accuracy, quality and efficiency of sample processing (Dimante-Deimantovica et al., 2022). Identification techniques also play a crucial role in avoiding false positives and negatives. Given the numerous existing techniques for extracting and identifying microplastics in sediment, it appears essential to harmonize protocols and methods, in particular to ensure the comparability of data.

Indeed, harmonization is necessary because various methods exist to quantify and characterize MPs pollution in different compartments of the marine environment, especially in sediments (Phuong et al., 2021). These methods primarily rely on chemical analyses (e.g., spectroscopy) and physical techniques (e.g., microscopy) coupled with specific extraction protocols, often involving density separation. Laboratories choose these methods based on their technical and financial capabilities. However, many methods currently used to quantify MPs are labor-intensive, time-consuming and/or require advanced and expensive equipment (Meyers et al., 2024). In addition, the lack of standardization makes it difficult to compare results and study environmental MPs contamination. As a result, MPs analysis in marine sediments has not yet reached the highest level of technological readiness (Aliani et al., 2023) and its use as an indicator in existing OSPAR and MSFD monitoring schemes has not yet been fully implemented. Several studies (Adomat et al., 2022; Bäuerlein et al., 2023; Langknecht et al., 2023; Perumal and Muthuramalingam, 2022) have shown the desire of the scientific community to improve the standardization of extraction and characterization methods for MPs in sediment. These authors also indicated the need to develop appropriate protocols adapted to specific objectives which can be oriented either for OSPAR or MSFD type monitoring, or for a fundamental research project.

One of the objectives of the JPI Oceans ANDROMEDA project was to develop technical analysis methods to quantify microplastics and establish a platform of tools for cost-effective MPs analysis. In this work, various methods for extracting and characterizing MPs in sediment were applied in a case study in the Bay of Marseille, a coastal city in the Mediterranean heavily impacted by human activities and therefore exhibiting high pollution levels. Indeed, the Mediterranean Sea is considered one of the most polluted seas in the world (Gerigny et al., 2019) due to its semi-enclosed configuration, high coastal urbanization, and associated anthropogenic activities (Sempéré et al., 2018 and references inside). It is not spared from MPs pollution (De Haan et al., 2019). Developing analysis methods to better understand this pollution is considered a priority (Maes et al., 2019), especially within the context of the MSFD. It was therefore appropriate to select the Mediterranean and the Bay of Marseille for this exercise. To do so, sediment samples were collected from different locations and depths. Sediment samples were taken on-site, cleaned, and intentionally recontaminated with a known concentration and composition of MPs in the laboratory. The spiked samples were subsequently dispatched to different partners for analysis using their specific methodologies. An intercalibration exercise was performed in view to studying the variability of results obtained using different extraction and analysis methods.

2. Materials and methods

2.1. Study area

The metropolis of Marseille is located in the south of France and ranks as one of the largest cities bordering the Mediterranean Sea (northwest coast) (Fig. 1). It is also the second-largest city in France in terms of population, exceeding 1.9 million inhabitants, and generates sewage effluent at a rate of 10,500 m^3/h (Gerigny et al., 2022; Ourgaud et al., 2022). Marseille's harbor is the largest in France, with marine, industrial and recreational activities (Sempéré et al., 2018 and references inside). The area is characterized by significant petrochemical, industrial, and summer tourism activities along the coast, resulting in considerable anthropogenic pressures. Despite these human activities, the area is also of significant ecological interest due to the presence of the Calanques National Park, designated as a Marine Protected Area (MPA). Nevertheless, this MPA is affected by tourism and several wastewater treatment plants, including one of the largest located in "Calanque de Cortiou" (Fig. 1), which discharges treated water directly into the park. The Marseille area, i.e. the Bay of Marseille in the north and the Calanques National Park in the south (sampling authorization no. 2012/BD/SC-02) was selected as a study site within this work, taking into account several factors, such as the high level of industrial and anthropogenic pressures in the region. The selected location hence enables the comparison of varying pollution levels in the same ecosystem. Consequently, this study area was chosen as it is a good candidate for taking representative samples of different environmental conditions.

2.2. Sediment sampling

In September 2021, sediment samples were collected to study MPs pollution at seven different locations: one station located on the beach (Pointe Rouge Beach, hand sampling on a transect of one square meter and 3 cm depth) and six offshore stations, sampled aboard the R/V Antédon II (Ifremer) using a Reineck corer (Fig. 1). The stations were chosen to create a profile going from the coast nearby the treatment plant, out to sea, in order to follow the contamination gradient. For reasons of accessibility, and also the absence of a beach in the cove sampling zone, two points were added in the Marseille zone (st° 3 and 7). Sediment samples were collected at various depths (30, 50, 75, 100, and 150 m) along consecutive transects from Calanque de Cortiou to the open sea (Fig. 1). Two sediment samples were collected in the northern Marseille Bay: one on the beach and another at 10 m depth. Additional sediment samples were collected at 10 m depth for the intercomparison exercise with partner laboratories (referred to as negative and positive controls).

Three to five cores were collected per station to ensure that each partner had sufficient surface sediment (the first five centimeters) for three replicates. Each site was therefore sampled by coring as many times as necessary to obtain this total mass. Then, each different surface sediment sample was placed together with the other samples in a stainless-steel container (decontaminated beforehand and isolated from contamination by aluminum foil during handling on the quay). Finally, they were mixed in the boat's laboratory under clean conditions, stored on ethanol in labeled containers, and frozen at −20 °C until further analysis.

2.3. Laboratory work - preparation of the intercomparison exercise

2.3.1. Extraction of microplastics in marine sediment

To generate negative (NC) and positive (PC) control samples, the sediment was initially treated with a saturated sodium chloride solution (NaCl; 1–1.2 g.cm^{-3}, (Enders et al., 2015) to reduce potential toxic risks associated with the use of alternative extraction solutions such as zinc

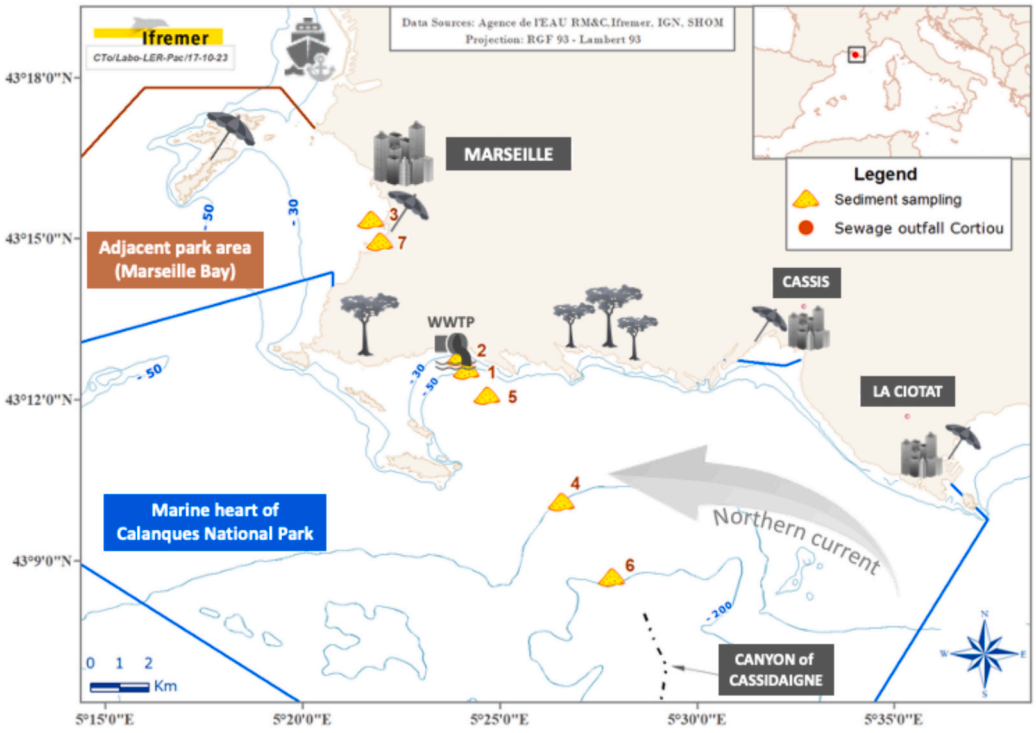

Fig. 1. Sampling sites for sediment samples in the Bay of Marseille – French waters in the Western Mediterranean Sea (Heart of Calanques National Park, projection RGF93-Lambert 83).

chloride $ZnCl_2$ (density 1.814 $g.cm^{-3}$) (Enders et al., 2015; Frias et al., 2018) for a large quantity of sediment. However, the initial results revealed certain inconsistencies and reduced effectiveness in the cleaning process, which posed challenges for the analyses. Therefore, the consortium decided to apply a second protocol described below for method comparison and enhancing sediment cleaning efficiency. This second cleaning protocol corresponds to the method currently used by Ifremer to extract MPs from sediment (Angiolillo et al., 2021). It involves a step of digesting organic matter with 30 % hydrogen peroxide (H_2O_2), followed by a density separation step using a 62.5 % $ZnCl_2$ solution. MPs with a density < 1.814 $g.cm^{-3}$ [e.g., polystyrene (PS), polypropylene (PP), polyethylene (PE) and polycarbonate (PC)], (Enders et al., 2015; Frias et al., 2018) therefore rise to the surface of the solution and separate from the denser sediment particles. The density separation extraction was done with a solution with a sediment mass ratio of at least 3:1, after which it was agitated and placed in an ultrasonic bath at ambient room temperature for 15 min (45 kHz, VWR Ultrasonic Cleaner USC-T), before being left to settle. The surface layer of the supernatant containing the MPs was collected using a pipette. The process was repeated three times, after which the cleaned sediment was dried in an oven for 48 h.

To create the NC and PC samples for each sample, 50 g d.w of the cleaned sediment were then transferred to a glass vial. Next, the NCs were sent directly to the partners and analyzed using their respective methods, after which the number of spiked MPs recovered was reported back. The PC samples were spiked with a known quantity and composition of industrial MPs provided by CARAT Gmbh (Bocholt, Germany), through Ifremer LER-PAC: five types of polymer were used (PE, polyethylene terephthalate (PET), PP, polyvinyl chloride (PVC) and PS) ranging from 500 to 1000 μm. Three particles of each polymer type were added to the cleaned sediment samples, resulting in a total of 15 items per 50 g d.w. It should be noted that that these added particles were exclusively cryomilled fragments, while in the natural environment, fibers, films, and granules may also be present. This spiked concentration of 15 items per 50 g d.w. was known only to a Ifremer operator who was not involved in the sample analysis of the PC and NC samples.

2.3.2. *Quality control and quality assurance*

Sample handling was carried out in clean conditions and the operators wore cotton laboratory coats throughout the experiment. Plastic tools were avoided during sampling and storage. The lab materials used were made of stainless steel (pliers and dissecting needles were pre-rinsed with ethanol and Milli-Q water) or glass (same cleaning process). Sampling equipment was abundantly pre-rinsed in the lab with water, followed by 1 L of ethanol, and finally with 1 L of Milli-Q. Afterwards, to prevent airborne MPs contamination, the equipment was wrapped in calcined aluminium foil (450 °C for 6 h) and stored in clean stainless-steel boxes for transport to the boat and deployment at sea.

2.3.3. *Methods of microplastic characterization by different partners*

The following partners participated in this study:

- Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) – Belgium (Oostende),
- French Institute for Exploitation of the Sea (Ifremer) LER-PAC - France (La-Seyne-Sur-Mer),
- Mediterranean Institute of Oceanography (MIO) - France (Marseille),
- Spanish Institute of Oceanography (IEO-CSIC) – Spain (Madrid),
- Tallinn University of Technology (TalTech) - Estonia (Tallinn).

For reasons of neutrality and confidentiality, the methods used by the partners were numbered from 1 to 6 and are referred to as "Partner 1" (P1), "Partner 2" (P2), etc. throughout the report. Five laboratories participated in this exercise, including one which used two different evaluation techniques; it was considered that the second technique represented an additional partner. Multiple extraction/ characterization methods were applied by certain partners (Table 1).

2.3.4. *Data processing*

The intercomparison exercise included four samples:

Table 1
Extraction and characterization methods for microplastics in marine sediments.

	Identification method	Extraction method	Main equipment costs	Method automation	Handling time per week or month
Partner 1 (P1)	Visual identification using an epifluorescence stereomicroscope (Nile Red)	$H_2O_2/ZnCl_2$ density extraction	Main equipment: florescence stereomicroscope - ≈ €16,000	Manual	7 samples processed/ week
Partner 2 (P2)	Fluorescence microscopy (Nile Red) + FTIR spectroscopy	H_2O_2/NaI density extraction	Main equipment: florescence stereomicroscope - ≈ 70,000 € + FTIR	Semi-automated	10 samples processed/ week
Partner 3 (P3)	Visual identification using a "hot needle test" (polymer heat reaction)	NaI density extraction	Main equipment: stereomicroscope	Manual	9 samples processed/ month
Partner 4 (P4)	FTIR spectroscopy	NaI density extraction	Main equipment: FTIR Perkin Elmer 400 - ≈ €120,000	Manual	9 samples processed/ month
Partner 5 (P5)	Visual identification using an epifluorescence stereomicroscope (Nile Red)	NaI density extraction	Main equipment: florescence stereomicroscope	Manual	9 samples processed/ month
Partner 6 (P6)	LDIR chemical identification and FTIR spectroscopy	Saturated NaCl density extraction	Main equipment: FTIR Perkin Elmer 400 - ≈ €120,000	Semi-automated	3 samples processed/ week

- NC1, corresponding to the results of the first negative control (sediment cleaning with NaCl).
- NC2, corresponding to the results of the second negative control (sediment cleaning with $H_2O_2/ZnCl_2$).
- PC2, corresponding to the results of the second positive control, i.e. the sediment cleaned to create NC2 contaminated with CARAT plastic particles equivalent to a theoretical concentration of 15 items/50 g d.w.
- Seven marine environmental samples (in-situ samples). The PC enabled the comparison of the different MP extraction and identification methods used by the partners. The two negative controls, NC1 and NC2, were analyzed to compare the efficiency of two MPs extraction methods for sediment cleaning using different reagents.

The percentage difference between the two cleaning methods was calculated as follows: ([NC2] - [NC1])/[NC1]. A negative percentage signifies that NC2 underwent more effective cleaning compared to NC1. The higher the negative percentage, the more thorough the cleaning process. Conversely, a positive percentage indicates that NC2 contained more particles than NC1, with higher values indicating a less effective method. Excel software (Microsoft office Standard 2019 version) was used to process the data.

3. Results

The results outlined in this section were obtained from the analysis of the abovementioned samples by the various project partners. Each partner applied the MP extraction/characterization methods they generally apply for marine sediments (Table 1), based on the equipment and resources available in their respective laboratories.

3.1. Intercomparison exercise

3.1.1. Negative control

Two procedures were used to create the negative control samples (more information in the "Materials and methods" section). After the analysis of the results obtained for NC1 performed using NaCl, the partners decided to apply an alternative sediment cleaning protocol to optimize sediment cleaning. Data from NC1 was retained solely to provide recommendations in terms of the effectiveness of the two reagents for sediment cleaning. Only the results obtained from NC2 (utilizing H_2O_2 and $ZnCl_2$) were considered for further analysis.

Fig. 2 shows the total MPs contamination found by each partner in the NC2 samples, per size class, where the target concentration was 0 items/50 g d.w. Fig. 3 represents MPs concentrations found in NC2 by partners based on their typologies (granules, fibers, fragments, films and others). All the partners detected MPs in NC2, regardless of the extraction/characterization method used. The lowest number found was one

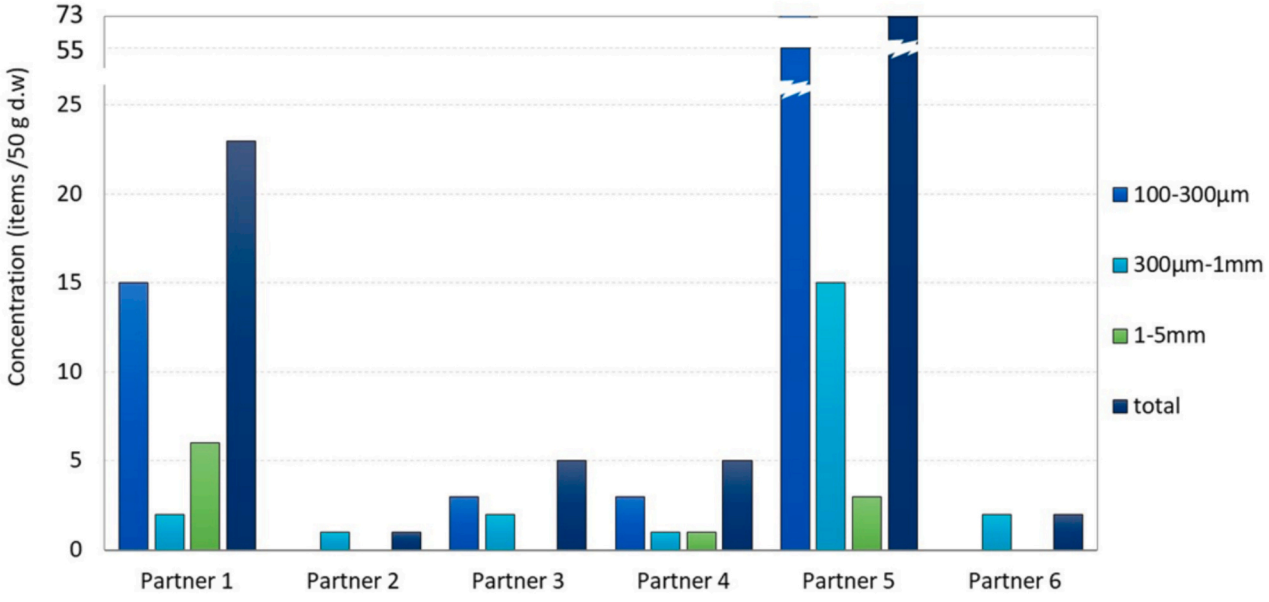

Fig. 2. Microplastics (MPs) concentration obtained by each partner in the negative control (NC2) (50 g dry weight (d.w.) of sediment), per size class.

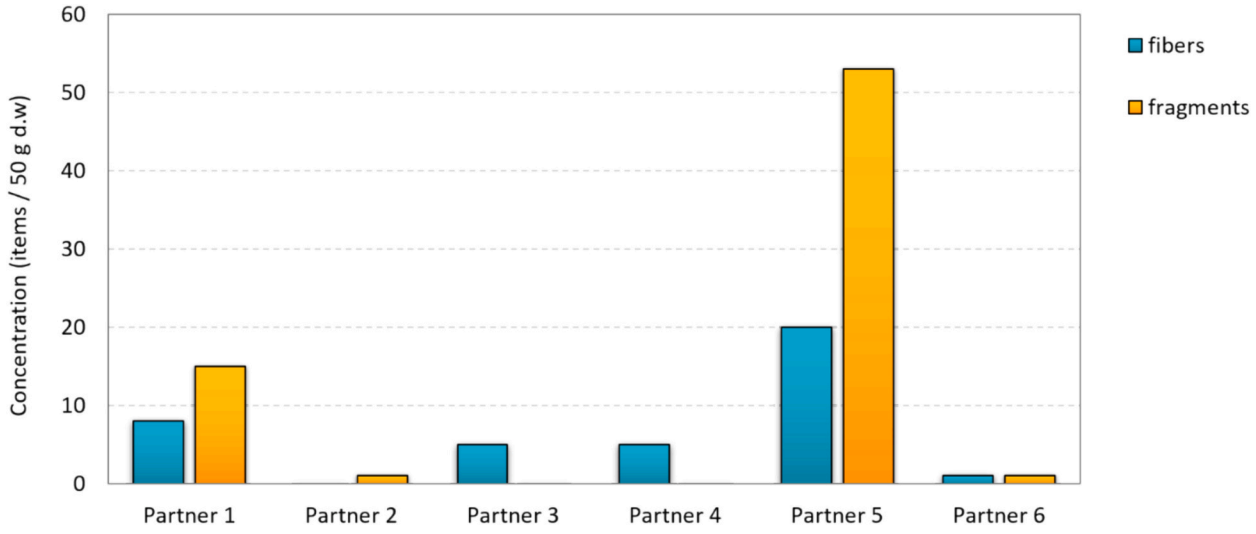

Fig. 3. Microplastics (MPs) concentration found by each partner in the negative control number 2 (NC2) regarding typology (by 50 g dry weight (d.w.) of sediments).

fragment (P2, size class 300 μm-1 mm), while the highest number was 73 items/50 g d.w. P5 found 55 particles for size class 100–300 μm, 15 particles for size class 300 μm-1 mm, and 3 particles for size class 1–4 mm, representing 75 %, 21 % and 4 % of all the contaminating plastics found, respectively. Out of the 73 particles found, 20 were fibers and 53 were fragments (27 % and 73 % of all plastics found, respectively). The MPs concentration found by P1 fell within the range of values observed by P2, P3, and P4 (low values) and P5 (extreme value). These partners also detected both fibers and fragments, indicating suboptimal cleaning for these typologies.

The comparison of results obtained for NC1 (NaCl) and NC2 (H_2O_2/$ZnCl_2$) is presented in Fig. 4, with the percentage difference between the two cleaning methods indicated (see 'Materials and methods' section). It should be noted that the extraction and identification techniques of each partner inevitably influenced the percentage of difference between the cleaning techniques.

Four partners showed negative percentages with values of −75 % (P2, Nile red and FTIR), −71 % (P3, hot needle test), −38 % (P4, FTIR), and −99 % (P6, LDIR/FTIR), indicating that less contamination was detected in NC2 compared to NC1. Two positive percentage differences appear, 130 % (P1) and 170 % (P5), which both corresponded to fluorescence identification analyses, where the particles detected were primarily fragments accompanied by a few fibers (15 fragments and 8 fibers for P1 and 53 fragments and 20 fibers for P2). It was important to characterize the particles not recovered in the NCs in order to improve the analysis of the results. To qualify these particles not recovered during cleaning, it is possible to refer to the results provided by a reference method used by one of the partners, namely the μFTIR. It appears overall that these unrecovered particles corresponded to PVC of size class 300–1000 μm.

3.1.2. *Positive control*

Only the results of the positive Control 2 (PC2) from the second sediment cleaning method are considered in this study. Due to the unique contamination typology in PC2, only fragments of size class 500–1000 μm were considered. The results are presented in Fig. 5, with the recovery rates displayed above the histograms. A recovery rate < 100 % indicated that a concentration lower than 15 items/50 g d.w was obtained by a partner (underestimation), while a recovery rate > 100 % indicated an overestimation of MP contamination. No partner achieved a recovery rate of 100 %. The variation in concentrations of fragments within the size class 500–1000 μm as found by the partners, ranged from 7 items/50 g d.w (P2, Nile red + FTIR) to 17 items/50 g d.w (P4, FTIR), with recovery rates of 47 % and 113 %, respectively. Two partners (P1 and P5) achieved recovery rates equal to or above 80 % (80 % and 87 %, respectively). Only one recovery rate was overestimated at 113 % (17

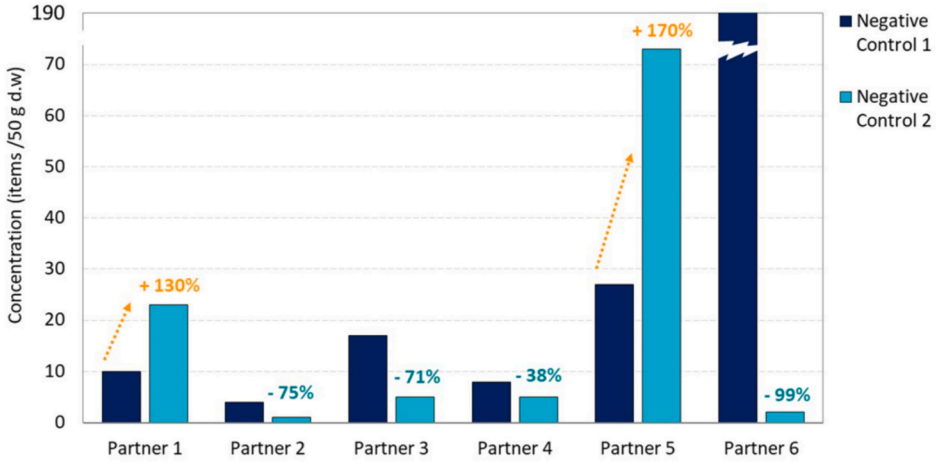

Fig. 4. Comparison of microplastics (MPs) concentrations observed by each partner in negative control NC1 (NaCl extraction) and in NC2 (H_2O_2 digestion +$ZnCl_2$ extraction) for 50 g dry weight (d.w.) of sediment per sample, with their percentage difference indicated.

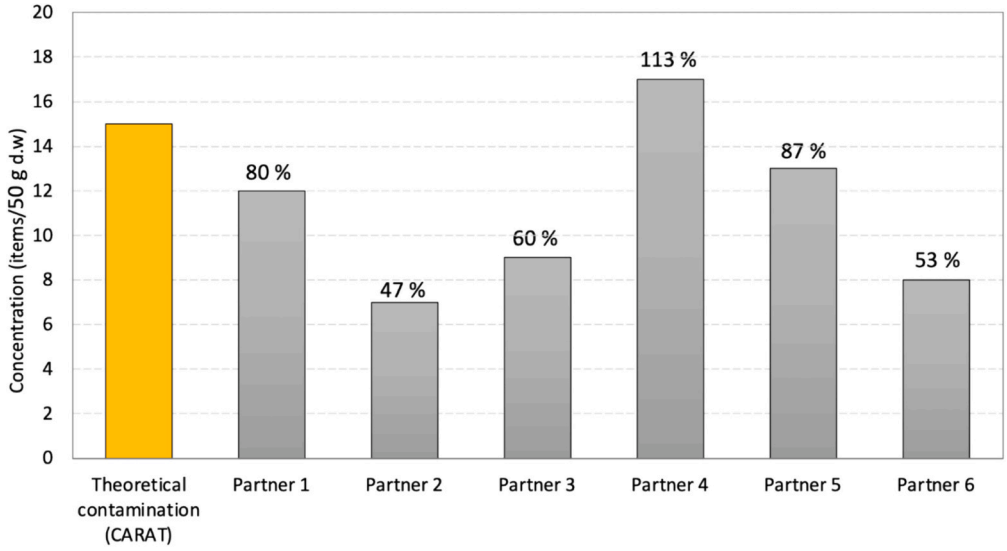

Fig. 5. Recovery rates of microplastic fragments of size class 500–1000 μm found by each partner in positive control PC2 (spiked concentration in items/50 g d.w of sediments).

items instead of 15), although this was close to the target concentration. P6 obtained a recovery rate of 53 % (P6, FTIR/LDIR).

3.2. Environmental samples

The samples taken from each station were analyzed by each partner using the same MPs extraction/characterization methods as employed in the intercomparison exercise. Fig. 6 presents the MPs concentrations found by the partners for each station, the mean concentration per station is represented by the curve. The MPs concentrations exhibit considerable variability, both spatially across different stations and between the different partners. The total concentrations obtained range from 0 (P6, station 2) to 205 items/50 g d.w (P3, station 1). To facilitate the interpretation of the results, two "extreme" concentrations were removed: the concentration obtained by P3 at station 1 (205 items/50 g d.w), and by P4, also at station 1 (139 items/50 g d.w). The average concentrations per station varied from 23 to 53 items/50 g d.w, and from 19 to 84 items/50 g d.w when extreme values were not removed. The analysis of the average curve indicated high MPs contamination values at station 1 (30 m depth) and station 3 (75 m depth), followed by a decrease in MPs concentration, potentially linked to station depth or distance from the coast. For P1 (Nile red) and P2 (combination of Nile red and μ-FTIR), concentrations were higher at station 3 (75 m) (59 items/50 g d.w and 74 items/50 g d.w, respectively) while they were higher at station 1 (30 m) for P3 (hot needle test) and P4 (FTIR) (205 items/50 g d.w and 139 items/50 g d.w, respectively).

4. Discussion

The aim of this intercomparison exercise was to study the variability of results obtained by different extraction and analysis methods, first on negative and positive control samples, then on in-situ environmental samples. The question asked was: Can similar environmental signals be found regardless of the analysis and identification method?

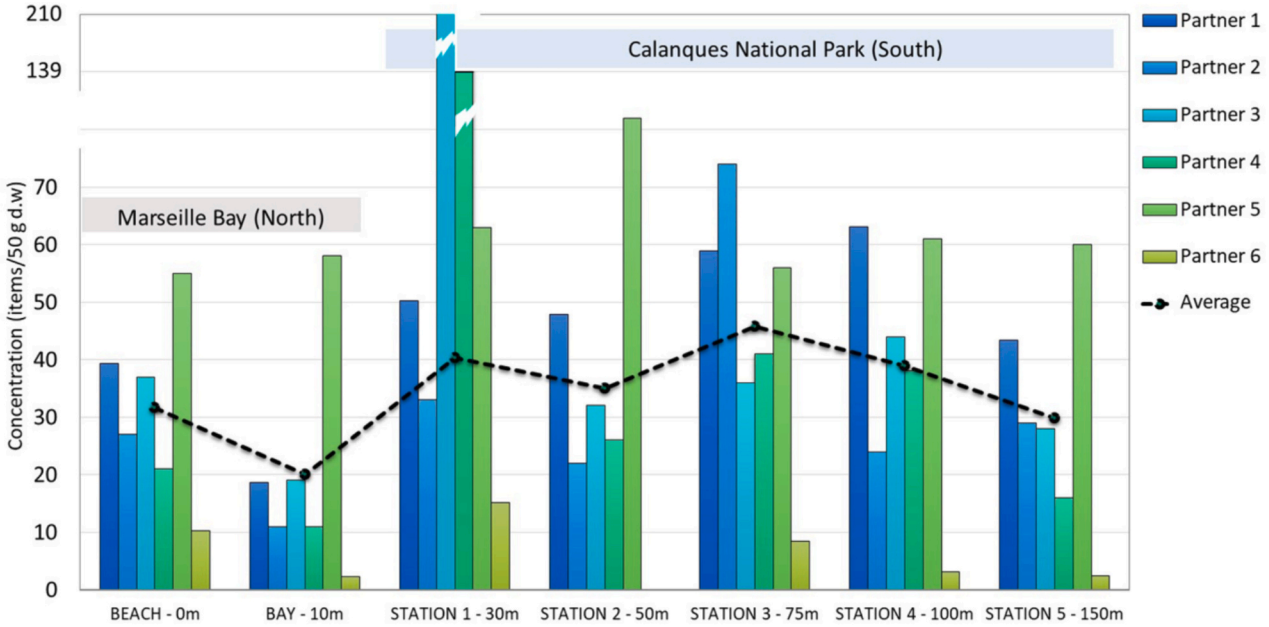

Fig. 6. Microplastics (MPs) concentration detected by each partner in the environmental samples, from the Marseille area, for all size classes and typologies.

Additionally, the exercise allowed studying the contamination of MPs in the Marseille area (Bay of Marseille and Calanques National Park), but these results are given for information only. To analyze the results obtained and discuss the effectiveness of the different methods used, it was important to consider various stages of the exercise and analysis: i) the sediment cleaning method (NaCl vs. $ZnCl_2$); ii) the MPs extraction methods applied; and iii) the identification methods used.

4.1. Sediment cleaning method

Changing the sediment cleaning method allowed evaluating the efficiency of different protocols for the density-based extraction of MPs. The difference in concentrations between the two NC (Fig. 4) analyses suggests that the H_2O_2/ $ZnCl_2$ cleaning was more effective than NaCl in extracting MPs from the sediment. Partners P2, P3, P4, and P6 observed an improvement (by 70 % on average) in sediment cleaning compared to the polymers found in NC1. Moreover, the remaining particles found in NC1 were made of polymers with relatively high density (1.38 $g.cm^{-3}$ or higher), including mainly PET and PVC, which cannot be effectively removed by a NaCl solution (maximum density of 1.2 $g.cm^{-3}$) (Frias et al., 2018). In contrast, partners P1 and P5 noticed a decrease in the efficiency of the sediment cleaning method as evidenced by the higher concentration of MPs observed in NC2 (positive percentage difference). The remaining polymers may originate from suboptimal cleaning of polymers present in the environmental sediment, e.g., those with high densities (such as PVC), from contamination in the laboratory, and, more notably, from false positives. The presence of high-density polymers is unlikely to have been the reason as the other partners did not detect any. Laboratory contamination remains a potential bias. A common characteristic among these partners is the use of a fluorochrome identification technique. Both partners P1 and P5 used an identification method involving Nile red with just one fluorescence filter for MPs imaging, which may have led to false positives and an overestimation of the number of plastic particles found in NC2. The particles found in samples analyzed by P1 and P5 were mostly fragments and fibers ranging between 100 and 300 μm. Organic debris, such as chitin-based particles and natural fibers, can potentially be colored using Nile red staining, leading to misidentifications (Stanton et al., 2019). However, this issue can be overcome by applying different fluorescence filters during imaging, which may improve the ability of the Nile red-based methods to distinguish plastics from non-plastics (De Witte et al., 2022; Meyers et al., 2022). This is one of the potential recommendations to improve the Nile red technique, discussed later. To conclude, the concentrations of MPs found in NC2 by P2, P3, P4, and P6 were low, confirming an improvement in cleaning with H_2O_2/$ZnCl_2$ (and/or NaI), and its relevance for this type of intercomparison exercise. To mitigate potential health risks, it is recommended to decontaminate small quantities of sediment separately and then combine them for preparing the intercomparison exercise samples. Although $ZnCl_2$ was found to be more effective in extracting MPs of all densities from sediment, the solution is more toxic compared to NaCl, and should therefore be handled with appropriate safety precautions, and be disposed of correctly. In addition, the extraction process is more costly compared to that using NaCl (Frias et al., 2018).

While density separation is a commonly employed technique for isolating MPs from sediment, alternative methods to clean sediment can also be considered, e.g., subjecting sediment samples to high temperatures for an extended period of time. This method entails heating the sediment at temperatures around 200 °C for several hours to remove any plastics present in the sediment matrix. This technique has been shown to be effective in various studies and offers a promising avenue for enhancing the efficiency of MP analysis (Grause et al., 2022).

4.2. Presence of fibers in the sediment

Except for P2, all the partners found fibers in NC2. These fibers may have resulted from either a lack of efficiency in the cleaning technique, and/or through laboratory contamination during analysis, or from identification errors (false positives). Despite achieving more efficient cleaning with $ZnCl_2$, especially for plastic fragments, the presence of fibers in NC2 indicates that even with high-density extraction solutions ($ZnCl_2$ or NaI, depending on the partners), the removal of fibers was suboptimal. This is an important factor to consider in all studies focusing on fibers, as it may lead to underestimating this type of particle. While fibers may also result from contamination by operators during the preparation of PC and NC, it is important to note that these operators were equipped with appropriate protective gear, including cotton lab coats, among other precautions. Moreover, the use of tools such as laminar flow extractor hoods, fume hoods, or similar systems for laboratory manipulations helped control MPs contamination. Moreover, the blanks were not or only minimally contaminated during the preparation of PC and NC. For instance, in the case of P1, only one fiber of size class 1–5 mm was found, indicating that operator contamination was low. Conducting a similar study in a controlled environment, e.g., a 'clean' or 'white' room, could be beneficial by minimizing potential contamination biases during sample preparation. It is noteworthy that the identification of fibers may be influenced by the identification method chosen. For example, the Nile red-based identification techniques used in our study (P1 and P5) showed a higher number of identified microfibers. This may have been a consequence of the reactivity of natural fibers, such as cotton, to Nile red, resulting in an overestimation of MPs present due to false positives, as demonstrated by Galvão et al. (2023). On the other hand, Prata et al. (2019) indicated no fluorescent staining of cotton fibers. A multi-filter approach for Nile red analysis may help reduce the likelihood of obtaining false positives due to the co-staining of organic fibers.

4.3. Treatment of samples with H_2O_2

To eliminate the organic matter present in the sediment, an H_2O_2 treatment was applied by P1 and P2. Digesting organic matter facilitates identification analyses by removing non-plastic particles that could otherwise be identified as MPs and consequently lead to false positives, e.g., when using a Nile red-based method. Additionally, for FTIR-based methods, not removing organic material may considerably increase total analysis time. Both partners obtained very different recovery rates (P1, 80 %, and P2, 47 %). Here, the use of H_2O_2 did not seem to improve the recovery rates compared to other partners. However, it is important to note that the PC sediment underwent two H_2O_2 cleanings: the first H_2O_2 cleaning during the preparation of NC and PC, and the second sediment cleaning in the analysis method to recover the MPs. The first cleaning potentially already improved particle identification quality. An H_2O_2 treatment step is strongly recommended in the procedure of MPs extraction from sediment as several studies have demonstrated how organic matter can hinder the extraction/identification of MPs (Dyachenko et al., 2017; Tagg et al., 2015) and that matrices with high organic matter content, such as biosolids, wastewater effluents, and riverbed sediments, require chemical digestion protocols such as oxidative, acidic, alkaline, or enzymatic digestion. However, the use of powerful chemical reagents can inadvertently alter the characteristics of the MPs analyzed (Schwaferts et al., 2019). H_2O_2 proved to be the most effective chemical for cleaning, and the least aggressive for polymers (Phuong et al., 2021), as it ensures the efficient digestion of organic matter without deteriorating polymers when used at temperatures below 45 °C. If these conditions are met, it is a suitable and effective pretreatment for density-based extraction. The recovery rate can however also decrease due to an excessive number of digestion steps, e.g., more than five sample treatment steps involving transfers (Dimante-Deimantovica et al., 2022). In this study, a maximum of two treatment steps were performed, it therefore seems unlikely that they accounted for the low recovery rate.

4.4. Recovery rates

Recovery rates are affected by the extraction methods employed, but it is important to consider that the identification methods used also impact these rates. The results of the PC were limited to only fragments of size 500–1000 μm to facilitate the comparison of extraction/characterization methods between the different partners.

Two of the partners (P1 and P5) achieved high recovery rates (80 % and 87 %) using a Nile red-based identification technique. This identification method appeared to be effective for characterizing MPs fragments larger than 500 μm although misidentifications were noted for particles below 300 μm. Approximately 20 % of the particles spiked into the PC were not recovered by those two partners. This underestimation could be due to various factors, such as a suboptimal extraction technique, a loss of MPs during different treatment phases, or counting and identification errors (Cadiou et al., 2020). However, the NC demonstrated a higher abundance of plastic fragments compared to other partners, therefore part of the recovery obtained might have been due to background contamination.

Nile red-based techniques may also result in an overestimation of the number of plastic particles through false negatives The application of Nile red may not always be perfectly homogeneous, and as a result, particle adsorption may not be uniform, possibly generating false negatives (Galvão et al., 2023). Conversely, Nile red stains on filters can also be mistaken for MPs. These issues could be resolved by using multiple fluorescence filters, which increases the accuracy of Nile red-based methods by reducing the rate of false negatives and positives (Meyers et al., 2022).

An overestimation was, however, also found (P4, 113 %) using FTIR identification. Although this recovery rate approximated the reference contamination, it shows that analysis efficiency is not only dependent on the analysis method used. This overestimation observed could have been due to remaining MPs in the cleaned sediment, contamination during analysis, fragmentation of MPs, identification errors due to environmental degradation of the plastics impacting their spectra, and counting errors (Cadiou et al., 2020). This observation was also confirmed by the results obtained by P6 (53 %), who used a method combining LDIR and FTIR. As the identification focused on fragments of size class 300–1000 μm for NC and 500–1000 μm for PC, i.e. relatively large MPs, the chance of false positives was low. P2 used a Nile red-based approach combined with FTIR-analysis but achieved a recovery rate of only 47 %. However, MPs below 300 μm in size were detected, suggesting fragmentation of the larger spiked MPs, which may help explain the lower recovery results.

The variability in recovery rates can therefore potentially be attributed to: 1) the choice of density reagent used (P2, $ZnCl_2$, and P6, double NaCl), 2) to the number of MPs chosen for artificial contamination (the loss of two particles on a small number (15) of spiked plastics will have a greater impact than the loss of two particles on a high number of spiked plastics (e.g., 50)), and 3) to the different extraction manipulation steps. These results could arise, in particular, from material loss and associated MPs loss during sample manipulation. This loss can be limited by processing smaller quantities of sediment and the solutions used, thus reducing potential spillage, e.g., during digestion; and by recovering all the supernatant during density extraction. The extraction step is thus crucial and can lead to significant biases in recovery results, which should be considered when comparing results between multiple studies. We advise including recommendations for extraction methods and density separation solutions used in MPs monitoring guidelines to ensure statistically comparable results over time. The “hot-needle test” method obtained an average recovery rate of 60 %, but this technique may under- or overestimate plastic particles due to identification errors, for example, due to differences in melt temperature between polymers or due to natural materials with phase transition behaviors similar to those of plastics (Lusher et al., 2020).

The results of this intercomparison exercise showed multi-factor variability, making their interpretation complex: the FTIR method remains a reliable reference method, but the results can be impacted by the digestion and extraction techniques used prior to the identification step. Despite significant investment costs, reference methods based on FTIR or RAMAN analysis are often the preferred method for MPs analysis in research projects. Nile Red-based methods appeared to be effective in identifying fragments larger than 300 μm, although this comes at a risk of obtaining false positives if only one fluorescence filter is used for imaging. While further research and development are required to prove the effectiveness of Nile red-based techniques on plastic typologies other than fragments and with smaller size classes, these methods appeared cost-effective in terms of time and money (Meyers et al., 2024), especially for monitoring MPs contamination in the framework of directives and conventions like OSPAR, MFSD, etc.

The high variability in recovery rates between methods (47–113 % based on the PC analysis) was also observed in earlier comparison exercises on MPs analysis. In a proficiency test from Quasimeme (van Mourik et al., 2021), MPs were introduced into tablets that had to be dissolved in water and were subsequently analyzed by 29 participants. Although the dissolution of these tablets in filtered water limited matrix interference, very high relative standard deviations were obtained for the recovery results of different polymers, varying from 57 to 91 % (van Mourik et al., 2021). This work clearly demonstrated that for more complex matrices such as marine sediments, proficiency testing schemes aimed at promoting method and quality control harmonization are needed to reduce variability and increase data comparability. This is a prerequisite for the accurate and precise monitoring of MPs in marine sediments.

4.5. Marine environmental samples and microplastics in Marseille Bay

MPs in the environment do not exhibit a uniform distribution (Tarafdar et al., 2022). The in-situ findings demonstrate noticeable spatial variation, stemming from both differences between stations and the methods employed for extraction and identification. This spatial variability is visually apparent in the graphical representation. More specifically, as one moves away from the coastline into the open sea within the initial few kilometers (approximately 500 to 2000 m from the shoreline), the concentration of MPs rises, peaking at stations located at depths of 30 and 75 m. Subsequently, concentrations begin to decline towards the open sea. It appears that the different analysis techniques manage to capture this environmental signal, differentiating slightly and highly contaminated areas, despite differences in absolute numbers. Thus, the sampling technique used enabled obtaining coherent results. Despite environmental variations between locations, no discernible trend or correlation was deduced in relation to depth and distance from the coast, likely due to the limited data set available.

In contrast, the contamination levels of the Bay of Marseille (beach and bay) appear to be lower than those in the “Calanque de Cortiou” (stations 1, 2 and 3). The Bay of Marseille is a heavily urbanized area, subject to major pressures (port area, tourism, presence of a wastewater treatment plant), although strict regulations within the park limit contamination inputs. However, this calanque serves as a discharge zone for treated wastewater, located at the heart of the “Massif des Calanques”. This is a significant anthropogenic bias that could contribute to MPs contamination (Kay et al., 2018). Furthermore, this suggests that MPs smaller than 100 μm, which were not identified in this exercise, could also be discharged in these effluents. More samples should be collected from this area and analyzed to statistically assess MPs contamination.

However, the primary objective of this article was not to characterize the contamination of the Marseille area, nor even to directly compare the methods used with sophisticated analytical techniques, but rather to carry out an inter-comparison exercise to verify and compare the different extraction and identification methods. The main point was above all that despite the lack of precision of techniques such as Nile Red

identification and even the hot needle test, compared to other reference methods, they could be validated in terms of demonstrating their capacity to capture an environmental signal and comprehensive trend, and by their potential for carrying out large-scale analyses at reasonable costs.

4.6. Continuity of intercomparison work

Studying MPs pollution in the marine environment presents a complex challenge, as the outcomes are influenced by numerous factors spanning from the initial sampling process to laboratory analysis methods and the interpretation of statistical analyses. Regardless of the objectives, sampling is often not sufficient to provide robust data for making comparative assessments, examining trends, or to be certain of the quantities encountered (Morgado et al., 2023; Underwood et al., 2017). The NC and PC results display considerable variability which, as explained above, can be attributed to laboratory manipulations, problems with detection tools, potential background noise, and uncertainties in the analyses. For example, it is difficult to quantify in the PC, the proportion of MPs to be attributed to artificial contamination that would correspond to an environmental signal or background noise. All these uncertainties about how to approach and process samples and interpret MPs analysis results clearly demonstrate the need to harmonize sampling techniques as well as extraction and analysis methods, through intercalibration or intercomparison exercises, even when the results are difficult to interpret. The use of intercomparison exercises on a small scale, such as is the case here, or larger scale proficiency tests, e.g., Quasimeme (van Mourik et al., 2021) is therefore an essential step in the implementation of monitoring within the framework of Regional Sea Conventions or the EU Marine Strategy Framework Directive. Multiple laboratories are involved in large scale monitoring at the regional level, often applying different analysis techniques which change over time. The development of appropriate quality control measures is essential to ensure consistent regional monitoring. This involves the inclusion of positive and negative control samples as well as participation in proficiency tests and intercomparison exercises. Moreover, stipulating only the importance of using a PC or an NC is insufficient as detailed guidelines on how to prepare them are also needed for harmonization purposes. Essential specifications include the preparation procedure for blank samples and the selection of MPs to be spiked in the PC. They must also be environmentally relevant in terms of shape, size, type of polymer and degree of weathering.

5. Conclusion

To integrate microplastics analysis into monitoring programs, it is essential to ensure that comparable data can be generated across different monitoring laboratories. In this intercomparison exercise, six different methods from five different laboratories were applied to analyze a negative control sample, a positive control sample and a series of homogenized samples of the Mediterranean Sea, in French coastal waters. Our conclusions and recommendations are summarized in Fig. 7.

Matrix relevant NC samples for MPs in sediment can be prepared by pre-cleaning natural sediments. The use of NaCl as a density separation salt is, however, not effective for removing higher density particles such as PVC and PET. This highlights the necessity of employing higher density salt solutions, such as $ZnCl_2$ or NaI, or exploring alternative techniques in addition to density separation. The study revealed high variability between methods, with PC recoveries varying from 47 to 113 %. This clearly demonstrated the limited comparability between MPs data when multiple laboratories are involved. One major concern lies in the risk of misidentifications or due to potential losses during sampling preparation. This underscores the urgent need for increased quality assurance in MPs monitoring, including the use of positive control samples and the participation in intercalibration exercises and proficiency testing schemes.

Despite the methodological differences, all the partners observed the same trend, indicating that MPs contamination in the Calanques

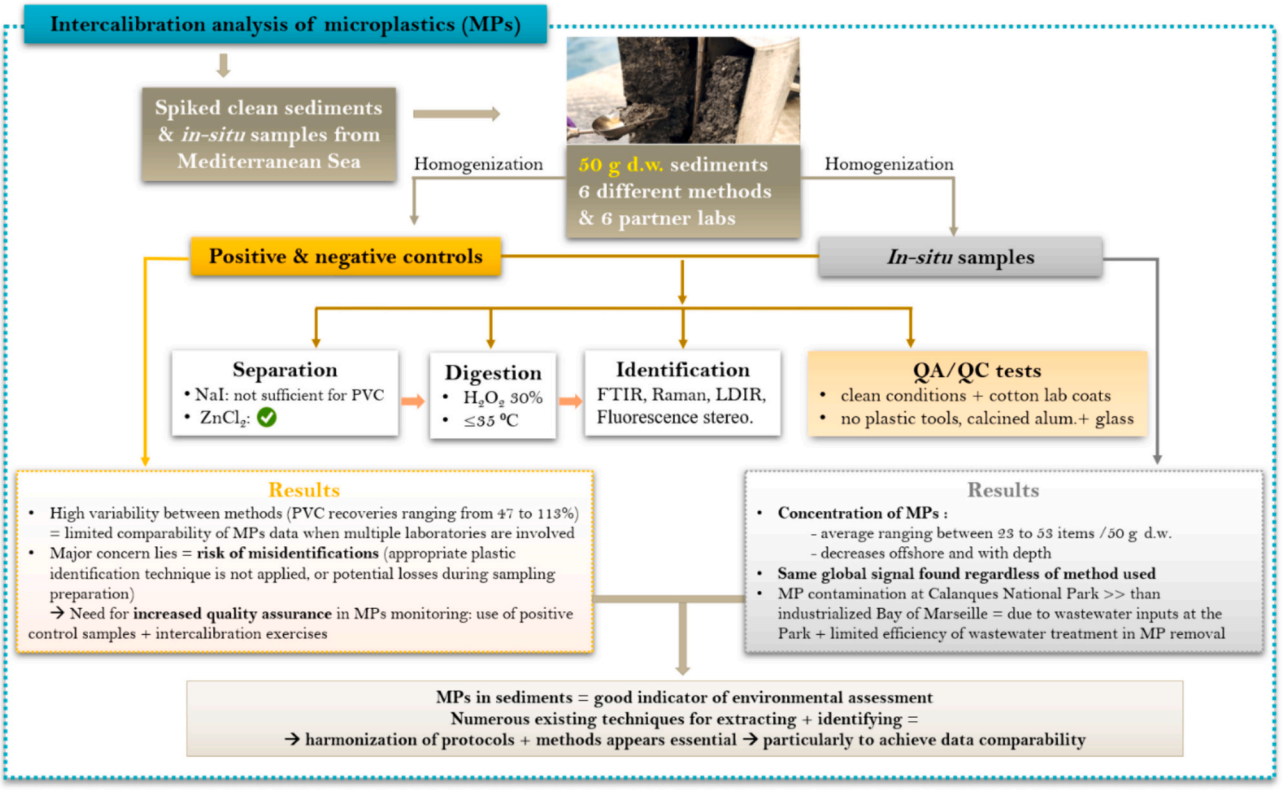

Fig. 7. Recommendations and conclusions for a reliable intercomparison analysis of MPs in marine sediments (positive/negative controls and in-situ samples).

National Park was higher than in the industrialized Bay of Marseille. This disparity can be explained by the wastewater discharges at the Calanques National Park, highlighting the limited efficiency of wastewater treatment in removing MPs.

CRediT authorship contribution statement

Olivia Gerigny: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gustavo Blanco:** Writing – review & editing, Formal analysis. **Urmas Lips:** Writing – review & editing, Investigation. **Natalja Buhhalko:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Leelou Chouteau:** Formal analysis. **Elise Georges:** Writing – original draft, Investigation. **Nelle Meyers:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **David Vanavermaete:** Investigation. **François Galgani:** Funding acquisition, Conceptualization. **Melanie Ourgaud:** Writing – review & editing, Writing – original draft, Formal analysis. **Laure Papillon:** Laboratory sample processing. **Richard Sempéré:** Funding acquisition. **Bavo De Witte:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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