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Chemical composition of microplastics floating on the surface of the Mediterranean Sea

Kedzierski Mikaël [1, *], Palazot Maialen [1], Soccalingame Lata [1], Falcou-Préfol Mathilde [2], Gorsky Gabriel [3, 5], Galgani Francois [4], Bruzaud Stéphane [1], Pedrotti Maria Luiza [5]

[1] Université Bretagne Sud, UMR CNRS 6027, IRDL, F-56100 Lorient, France
[2] University of Antwerp, Systemic Physiological and Ecotoxicological Research (SPHERE), 2020 Antwerp, Belgium
[3] Sorbonne Universités, UMR CNRS 7093, LOV, F-06230 Villefranche sur mer, France
[4] IFREMER, LER/PAC, F-20600 Bastia, France
[5] Research Federation for the study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans-GOSEE, Paris, France

* Corresponding author : Mikaël Kedzierski, email address : mikael.kedzierski@univ-ubs.fr

Abstract :

The Mediterranean Sea is one of the most studied regions in the world in terms of microplastic (MP) contamination. However, only a few studies have analysed the chemical composition of MPs at the Mediterranean Sea surface. In this context, this study aims to describe the chemical composition as a function of particle size, mass and number concentrations of MPs collected in the surface waters of the Mediterranean Sea. The chemical composition showed a certain homogeneity at the Mediterranean Sea scale. The main polymers identified by Fourier Transform Infra-Red (FTIR) spectroscopy were poly(ethylene) (67.3 ± 2.4%), poly(propylene) (20.8 ± 2.1%) and poly(styrene) (3.0 ± 0.9%). Nevertheless, discrepancies, confirmed by the literature, were observed at a mesoscale level. Thus, in the North Tyrrhenian Sea, the proportion of poly(ethylene) was significantly lower than the average value of the Mediterranean Sea (57.9 ± 10.5%). Anthropic sources, rivers, or polymer ageing are assumed to be responsible for the variations observed.

Highlights

► Microplastic from two basins of the Mediterranean Sea were studied. ► Microplastic chemical composition was homogeneous at the Mediterranean scale. ► Anomaly areas were evidenced in terms of relative composition in PE and PP. ► Carbonyl and fouling indices on PE particles provided new insights. ► Circulation patterns were proposed to explain the observed distribution.

Keywords : Microplastic, s Mediterranean Sea, FTIR, Chemical composition, Poly(ethylene)

1. Introduction

In recent years, plastic pollution has emerged as a major environmental and health issue (Avio et al., 2017). It is defined as ubiquitous from the remote and uninhabited polar land to the heights of Mount Everest (Cózar et al., 2017; Lacerda et al., 2019; Napper et al., 2020). However, this pollution is mainly assessed in oceanic gyres such as subtropical regions or the North Pacific gyres and in milder coastal regions such as the Mediterranean Sea (Bryant et al., 2016; Cózar et al., 2015; Thiel et al., 2018). The Mediterranean Sea is a semi-enclosed sea with densely populated coasts that concentrates miscellaneous intensive marine and terrestrial activities and receives water from important river catchments (e.g. Nile, Ebro, Rhône and Po). It is therefore no coincidence that it has been reported to be one of the largest hotspots of plastic litter accumulation in the world with an estimated input of plastic of approximately 100 kt per year (Cózar et al., 2015; Jambeck et al., 2015).

These plastic particles are composed of large proportions of microplastics (MPs) ranging from millimetre-sized to micrometre-sized particles (Van Cauwenberghe et al., 2015). MPs are found to be present in every studied Mediterranean shoreline and island from 18 coastal countries (Fytianos et al., 2021). Despite an important spatial and temporal variability, highest microplastic concentrations are found to be near the more densely populated coastlines (Pedrotti et al., 2016). Statistical calculations have estimated that more than 75% of floating plastics reside in the 50 km near-shore waters (Liubartseva et al., 2018). Moreover, a segregation of plastic types with increasing distance to shore has been observed (Pedrotti et al., 2016). The detected plastic types are diverse but some are predominant on the sea surface because of their widespread use and their buoyancy: poly(ethylene) (PE), frequent in food packaging (e.g. in films and bottle caps); poly(propylene) (PP), used as packaging material and plastic parts in various industries; poly(amides) (PA) and poly(styrene) (PS) (Martellini et al., 2018). Polymer type can be identified through different techniques. Among them, Fourier Transform Infra-Red

(FTIR) spectroscopy is one of the most commonly used in microplastic studies. To assess the level of degradation (mainly due to oxidation by solar UV rays) by infrared spectroscopy, indicators such as the carbonyl index (CI) and the hydroxyl index (HI) are generally used (Julienne et al., 2019). For instance, it is well-known that the CI of PE or PP is correlated to the level of degradation in air and to a lesser extent in water (Andrady, 2017, 2011; K. Zhang et al., 2021). Thus, CI and the HI increase with residence time in the environment, but variability in natural conditions hinder the estimation of the exposure time of MPs in the field (Chen et al., 2021). However, on a larger scale, these different indices enable to compare the oxidation state, and thus the degradation state of MPs collected on the sea surface.

Despite a growing number of papers, knowledge is still lacking to fully understand the distribution and concentration and chemical composition of MPs on the surface of the Mediterranean Sea (Baini et al., 2018; Cincinelli et al., 2019; de Haan et al., 2019; Vianello et al., 2018; Wakkaf et al., 2020; Zayen et al., 2020). A major study, carried out in 2016 on an area extending from the western basin of the Mediterranean Sea to the Adriatic Sea, revealed that poly(ethylene) and poly(propylene) were the most prevalent polymer categories at the sea surface (Suaria et al., 2016). Furthermore, the different studies are uneasy to compare because of the heterogeneity of the Mediterranean Sea environment (hydrodynamic features, seasonality) and the variety of methodologies used (eg. sampling, microplastic extraction, analysis, sizes considered, concentration units) (Cincinelli et al., 2019).

In this context, where a global vision at the scale of the Mediterranean Sea was missing, the Tara Ocean Foundation, a French non-profit organisation dedicated to the study of the world's oceans, carried out microplastic samplings for 5 months in 2014 across the entire Mediterranean Sea. This expedition thus made it possible to map various areas using the same methodologies.

Therefore, this study aims to quantify and qualify microplastic pollution at the surface of the Mediterranean Sea. The first section describes sample collection and preparation, the chemical

analysis by Attenuated Total Reflectance (ATR)-FTIR spectroscopy and the statistical approach used to process the data. In the second section, the results are first described at the Mediterranean Sea scale. Then results between basins are compared to evidence spatial and temporal variations. In congruence with the current state of knowledge, different hypotheses are proposed to explain the observed tendencies. Afterwards, methodological recommendations are suggested to better investigate the microplastic pollution.

2. Material and methods

2.1. Sample collection

Microplastic samples were collected in Mediterranean Sea waters during the Tara Expedition which was conducted between June and November 2014 (Fig. 1). Sampling was conducted using a 4.4 m long manta net (mesh size: 333 µm; net opening: 16 x 60 cm), in 154 sites which were selected based on ocean colour satellite images supplied by ACRI-ST and analysed with the Mercator circulation model. Geographical coordinates and dates of analysed sampling are available at Zenodo Data Publisher http://www.zenodo.org. At each site, the manta net was towed on the sea surface for ca. 60 min behind the boat at an average speed of 2.5 knots, enabling thus the filtration of about 507 m^3 of seawater.

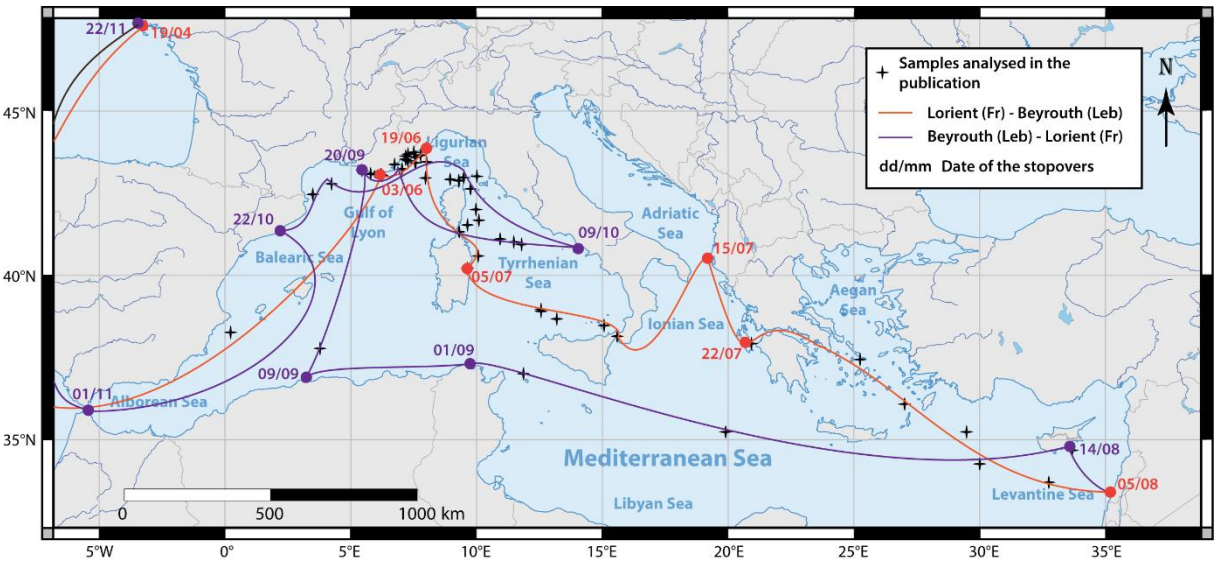

Fig. 1. Routes of the Tara Mediterranean campaign.

2.2. Laboratory preparations

2.2.1. Sample processing

In the laboratory, the samples were gently transferred into Petri dishes. Floating plastic debris were carefully removed from the other components (plankton, wood, etc.) to separate plastic particles, zooplankton and organic tissues. This process was done using a light box and a dissecting stereomicroscope to see the content of the samples with high contrast and ensure the removal of all the smallest and/or transparent plastic particles. Samples were double-checked to ensure the detection of all plastic particles, even the smallest ones. Blank control was removed from the analysis, including airborne textile fibres identified on the basis of their shape. The reminder of the sample was reserved for further zooplankton analysis.

2.2.2. Plastic analysis

The plastic particles were counted, measured and weighed. Firstly, plastic was digitally imaged with a ZooScan digital scanner (resolution: 2400 dpi). Then image post-processing was performed with the ZooProcess and plankton identifier software that enumerates and gives a set of morphological parameters for each object (Gorsky et al., 2010; Grosjean et al., 2004).

Once this step was finished, plastics samples were transferred to the Ifremer LERPAC laboratory (France) to be weighed before chemical analysis. The average length ($\bar{L}$) of the MPs per samples were calculated from the size ranges obtained by sieving. The fractions were dried in an oven at 50°C for 24 hours and weighed on an electronic balance (accuracy: 0.1 mg). The average mass ($\bar{m}$) of MPs per sample is calculated from these weighing.

A total of 15,654 particles, larger than 315 µm from 54 selected sites, were wet sieved by size class (]5-2mm],]2-1mm],]1-0.5mm],]0.5-0.315mm]), sorted and then transferred to 96-well

microplates and named with a unique identifier at the Institut de Recherche Dupuy de Lôme (IRDL, Lorient, France) (Kedzierski et al., 2019b). The preparation was performed in an area dedicated to the Tara Mediterranean Sea samples. Contamination risks were avoided during the sample preparation stage by cleaning the different parts of the apparatus, especially glassware, with distilled water, ethanol and/or acetone. The use of plastic apparatus was avoided as far as possible. If this was impossible, spectra of these materials were obtained by Fourier Transform Infrared Spectroscopy (FTIR) to check whether any potential contamination of the samples had occurred.

2.3. Statistical approach and protocol

The objective here was to determine the proportions of the different chemical categories that made up the particles identified via ZooScan as potentially MPs. Two cases are presented.

The first case consisted in determining these proportions on the scale of the entire Mediterranean Sea, as well as for the three sub-basins where data were available: the Gulf of Lion, the Tyrrhenian Sea and the eastern Mediterranean basin. For this purpose, 1,458 identifiers were randomly drawn from the database of particle identifiers (Kedzierski et al., 2019b). Of these 1,458 particle identifiers, 813 were collected from the Gulf of Lion basin, 413 from the Tyrrhenian Sea and 227 from the eastern Mediterranean basin. The interval of confidence (*IC*) of the proportion (*p*) was calculated using the following equation:

$$IC_{\frac{\alpha}{2}} = \left[p \pm u_{1-\frac{\alpha}{2}} \sqrt{\frac{p(1-p)}{n}} \right] \quad (1)$$

with $u_{1-\frac{\alpha}{2}}$ the fractal of order α of the standardized normal law. As it is classical to take a confidence level of 95%, then α=0.05 and $u_{1-\frac{\alpha}{2}} = 1.96$. Finally, *n* is the number of particles belonging to this chemical category.

In the second case, the proportions were studied manta by manta. In order to determine the number of particles to be studied for each of the mantas, the following equation (3) was used:

$$n = \frac{\frac{1}{4} + \frac{\varepsilon^2}{\left(u_{1-\frac{\alpha}{2}}\right)^2}}{\frac{\varepsilon^2}{\left(u_{1-\frac{\alpha}{2}}\right)^2} + \frac{1}{4N}} \qquad (2)$$

With N the number of the manta's particles identified by the ZooScan as potentially MPs, and ε the maximum value of the interval of confidence (in this study: 0.1). Once the different proportions determined, the interval of confidence was refined using the following equation:

$$IC_{\frac{\alpha}{2}} = \left[p \pm u_{1-\frac{\alpha}{2}} \sqrt{\frac{p(1-p)}{n} * \frac{(N-n)}{(N-1)}}\right] \quad (3)$$

The interval of confidence on the mean of results other than proportions (eg. average mass and average length) was obtained from the standard deviations (σ) using the following equation:

$$IC_{\frac{\alpha}{2}} = \left[p \pm u_{1-\frac{\alpha}{2}} \frac{\sigma}{\sqrt{n}}\right] \qquad (4)$$

Interval of confidences for data available in the literature on the chemical nature of MPs in the Mediterranean Sea were also obtained via these equations. It was thus possible to compare in the discussion our results with those of the scientific literature, taking into account the varying number of MPs analysed from one publication to another. Data were collected directly from articles, supplementary materials, or from the authors (see "Acknowledgements").

To compare the results, p-value was calculated on R using the prop.test() function which allows for the test of Equal Proportions (Newcombe, 1998a, 1998b; The R Core Team, 2019).

2.4. Fourier-transform infrared spectroscopy (FTIR)

The MPs spectra were acquired using an Attenuated Total Reflection Fourier Transform Infrared spectrometer (ATR-FTIR Vertex70v, Bruker). All spectra were recorded in absorbance

mode in the 4,000-600 cm^{-1} region with 4 cm^{-1} resolution and 16 scans. Each piece of plastic was placed onto the germanium diamond cell (ATR Golden Gate). After each analysis of MPs by ATR-FTIR, the sample holder was cleaned with ethanol. The sample chamber was also cleaned out with a vacuum cleaner after every sixty analyses.

2.5. Determination of the chemical nature

FTIR spectra had already been analysed in the course of previous studies using POSEIDON (Plastic pOllutionS ExtractIon, DetectiOn and aNalysis) software which is a free and open source software under development (Beta version) (Kedzierski et al., 2019b, 2019a). This software, was developed with R i386 3.1.2 (The R Core Team, 2019).

Firstly, the software performed two pre-processing steps: first the baseline correction and then the spectra normalization (Kedzierski et al., 2019a; Liland, 2015). The machine learning process was performed using k-nearest neighbour classification (Ripley, 1996; Venables et al., 2002). The learning database consisted in 969 spectra of MPs and other particles (natural organic materials) collected during Tara 2014 scientific campaign. During the identification step, if the entire k-nearest neighbour belongs to the same class, the spectrum was directly identified; otherwise a majority of vote was performed. If there were less than 3 votes, the spectrum was classified in the "unknown" category. In order to test the accuracy of the final classification, a two steps verification was performed involving a hierarchical cluster analysis and then principal component analysis (Kedzierski et al., 2019a). Then, the average spectrum of each subcluster generated was calculated and verified. If the average spectrum of a subcluster did not match the correct cluster, the spectrum or the group of spectra was manually identified and possibly reallocated to another class. Thus, the chemical nature of 4,723 spectra was analysed with POSEIDON. Results were presented for the dominant polymers (i.e. poly(ethylene), poly(propylene), poly(styrene)) and the rarer categories (i.e. poly(ethylene-vinyl acetate), Ethylene propylene rubber, poly(methyl methacrylate), poly(amide),

poly(urethane), poly(vinylchloride), poly(ethylene), and poly(propylene) like) have been grouped together under the category of "Other polymers".

After confirmation of the plastic nature of the particles by FTIR analysis, the number of microplastics and the mass of the particles could be confirmed. From these data, the concentrations in number (particles/km²) and mass (mg/km²) could be calculated. From these values, the average individual mass of the collected microplastics was calculated (mg).

2.6. Chemical indices

Chemical indices were calculated specifically for PE, a type of microplastic with several advantages. First of all, it is ubiquitous and can be found in most samples, which allows the indices to be followed over a large geographical area. Although it is possible that the concentration of PE may vary over time, it can be found in all seasons. Secondly, it is often present in large quantities, allowing for robust calculations. Finally, the ageing of PE samples is commonly followed in the literature with these indices in both artificial and natural conditions.

To evaluate the state of ageing of PE, two indices, the carbonyl index (CI) and the hydroxyl index (HI) were modified after Julienne et al., 2019:

$$CI = \frac{I_{1800-1700}}{I_{1480-1460}} \qquad (5)$$

$$HI = \frac{I_{3900-3200}}{I_{1480-1460}} \qquad (6)$$

A new index, called fouling index (FI), was also developed as part of this study to account for the extra band appearing on many PE spectra between 1,040 and 1,020 cm^{-1} (Kedzierski et al., 2019b, 2019a):

$$FI = \frac{I_{1040-1020}}{I_{1480-1460}} \qquad (7)$$

2.7. Other variables

From the total number of microplastics collected (N) and the percentage of PE (PE_p), the number of PE particles (N_{PE}) was calculated for each of the 54 samples studied. Then, the number of PE spectra to be analyzed (n_{PE}) was determined by randomly drawing 10% of the N_{PE} for each of the 54 manta samples. Thus, a total of 1,086 PE spectra were randomly selected for the calculation of the indices. This process was carried out 100 times in order to limit variability: the average indices were obtained from these 100 calculation processes. All the calculations were carried out with the R software i386 3.1.2 (The R Core Team, 2019). The results obtained were then compared with the help of a z-test thanks to the z.test() function available in the BSDA library of R.

2.8. Presentation of the results

All the results were expressed at several spatial scales: at the Mediterranean Sea scale, at the scale of the main sub-basins of the Mediterranean Sea (i.e. Gulf of Lyon, Tyrrhenian Sea, and Eastern Basin), and at the scale of the sampling area. An intermediate scale was also introduced to facilitate the link between the sub-basin and the sampling area scales. It groups the results obtained every 2° of latitude (eg. between 36 and 38°E), and every 10° of longitude (eg. between 0 and 10°E). Similarly, over time, the results were also presented by month. Grouping the results in this way reduces the uncertainty associated, and facilitate the identification of anomalies through statistical tests.

3. Results

3.1. Overview of the microplastic pollution in the Mediterranean sea and its sub-basins

3.1.1. Chemical composition and indices

The collected particles were mainly PE (67.3±2.4%), PP (20.8±2.1%), and PS (3.0±0.9%) (Fig. 2.a). The other identified polymers (i.e. poly(ethylene-vinyl acetate), ethylene propylene rubber, poly(methyl methacrylate), poly(amide), poly(styrene), poly(urethane), poly(vinylchloride), and poly(ethylene) and poly(propylene) like) represented 6.0±1.2% of the total sampled particles. Thus, MPs represented about 97% of the particles identified as such by the ZooScan. For the three sub-basins studied (Gulf of Lyon, Tyrrhenian Sea and eastern Mediterranean basin) the distribution of polymer types was similar to the one observed at the Mediterranean Sea scale. Nevertheless, in the Tyrrhenian Sea, the PP rate was significantly higher than the average value of the Mediterranean Sea. In the Eastern Mediterranean basin, the data showed a significantly lower rate of the category "other polymers". The proportions of the different chemical natures highlighted in this study varied significantly according to the particle size range (Fig. 2.b). Thus, the PE content of the samples was 76.1±7.0% for particles between 2 and 5 mm, and fell to only 38.3±6.4% in the 315 to 500 µm particle size range. PS showed a similar tendency, with proportions of 7.7±4.4% to 0.5±0.9% respectively. Conversely, the PP content of the samples increased as the particle size range decreased. Indeed, PP represented 10.6% of the particles for particles between 2 and 5 mm, and increased up to 49.1±6.6% in the particle size range from 315 to 500 µm. Thus, the PP/PE ratio remained relatively constant for MP belonging to the size classes between 500µm and 5 mm with values in the range of 0.2 to 0.32. It then increased sharply to a value of 1.5 for particles in the 315 µm to 500 µm size range.

For the other chemical natures, no significant variation was observed. Regarding the indices, the mean values were 0.77±0.02 for the Carbonyl Index (CI), 2.88±0.16 for the Hydroxyl Index (HI) and 0.45±0.02 Fouling Index (FI).

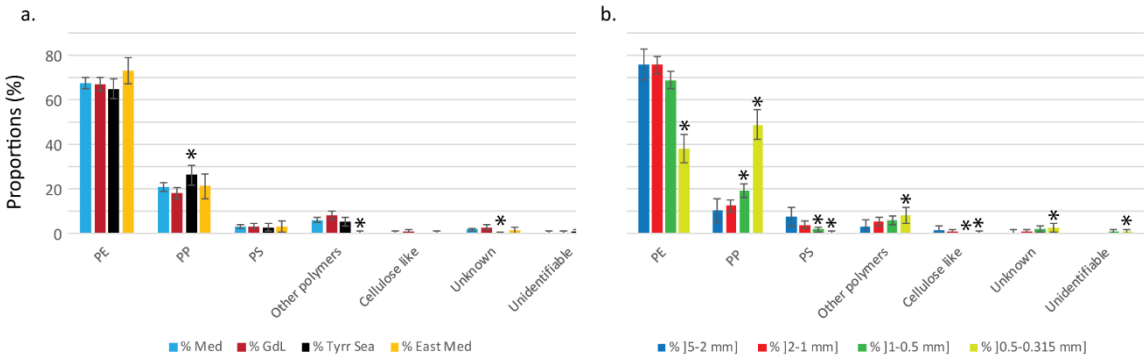

Fig. 2. Proportion of the chemical natures of the MPs collected (±Interval of confidence). a. Proportions of the different chemical natures at the Mediterranean scale (Med) as well as for the three sub-basins studied: the Gulf of Lyon (GdL), the Tyrrhenian Sea (Tyrr Sea) and the eastern Mediterranean basin (East Med) (±Interval of confidence). The chemical composition varied little from one basin to another. Only the Tyrrhenian Sea had a significantly (*) higher PP content than the Mediterranean Sea. b. Proportions of the different chemical natures according to the size range of the collected particles (±Interval of confidence). Statistically significant variations (*) of the chemical nature were evidenced depending on the size range studied.

3.2. Spatial and temporal variations

3.2.1. Chemical nature

The PE proportions changed little with latitude and remained close to the Mediterranean Sea average (Fig. 3.a). However, it was significantly lower between 40 and 42°N latitude (i.e. 57.9±10.5%), corresponding to samples from the northern Tyrrhenian Sea. The CI was significantly lower between 34 and 36°N (i.e. 0.65±0.07), matching samplings from the eastern Mediterranean Sea basin. For the other latitudes, no significant difference from the Mediterranean average could be found. The HI also showed little variation and none that was statistically significant. Lastly, the fouling index was characterized by larger variations, and significantly larger values between 36 and 40°N (i.e. 0.63±0.09 and 0.61±0.11). Finally, these

four variables all showed the same general pattern suggesting an increase that was more or less marked from 34 to 38°N latitude, then a decrease from 38 to 42°N latitude and finally an increase from 42 to 44°N latitude.

Although not significant compared to the average of the Mediterranean Sea, the percentage of PE in the samples tended to increase with increasing longitude (Fig. 3.b). However, the variability also tended to increase at the same time. Thus, this trend did not result into statistically significant variations. The CI tended to decrease between 0 and 30°E longitude and was significantly lower (i.e. 0.61±0.08) between 20 and 30°E. A similar pattern was identifiable for the HI, with a significantly lower mean value between 20 and 30°E longitude (i.e. 2.1±0.48). Finally, in the case of the FI, a different trend was observed with an increase between 0 and 30°E longitude, with a significantly different value between 10 and 20°E longitude (i.e. 0.51±0.05). Then, between 20 and 40°E longitude, the FI decreased.

Over the sampling period, the monthly averages did not differ significantly in terms of percentage of PE. The CI was characterized by variations whose general pattern suggested a seasonal signal. Indeed, the CI decreased from June to August, reaching for this last month a significantly lower value (i.e. 0.68±0.06). Then from August to October, the CI values increased again. The same pattern was visible for the HI with a decrease from June to August and an increase from August to October. For this index the mean value in August was significantly lower with 2.29±0.45. On the contrary, the FI study showed an inverse tendency with an increase from June to August and a decrease from August to October: the FI value of July and August were significantly higher (0.56±0.06 and 0.53±0.07, respectively), while that of October was significantly lower (0.36±0.01).

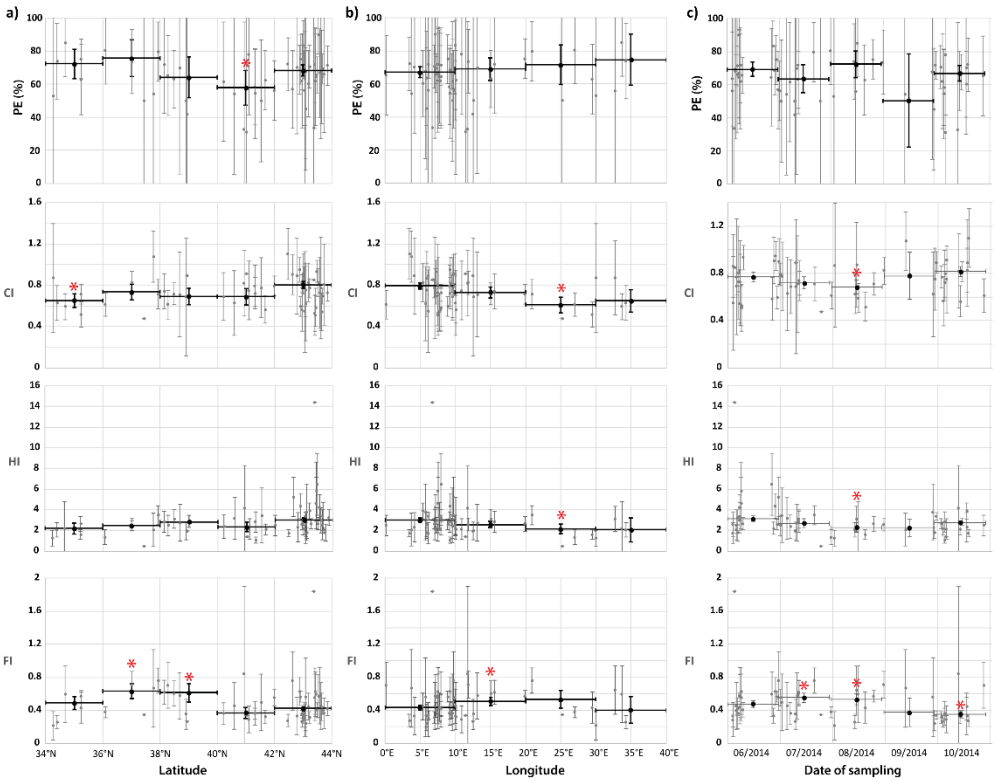

Fig. 3. Evolution of the percentage of PE and the associated indices (CI: carbonyl index; HI: Hydroxyl index; FI: Fouling index) as a function of latitude, longitude and sampling period (±Interval of confidence). a. Evolution of the four variables as a function of latitude. b. Evolution of the four variables as a function of longitude. c. Evolution of the four variables as a function of the sampling date.

3.2.2. Particle size

The mean size of the collected microplastics was stable according to the latitude of collection, except between 36 and 38°N with a mean value of 667±80 µm. This value was significantly lower than the mean value of the Mediterranean Sea (Fig. 4.a). In contrast, between 34-36°N, 38-40°N, 40°-42°N, and 42°-44°N the mean sizes were respectively 871±98µm, 800±109 µm, 883±104 µm, 765±31 µm, which were not significantly different from the Mediterranean Sea mean value.

In terms of longitude, the mean was stable and no significant variation could be found for the longitude ranges between 0-10°E (772±31 µm), 10-20°E (840±68 µm), 20-30°E (700±100 µm), 30-40°E (780±150 µm) (Fig. 4.b). Concerning the sampling date, June samplings showed a significantly smaller mean size (730±39 µm) whereas no significant variation in mean size could be demonstrated for the following months (July: 789±76 µm; August: 837±85 µm; September: 654±176 µm; October: 823±50 µm) (Fig. 4.c).

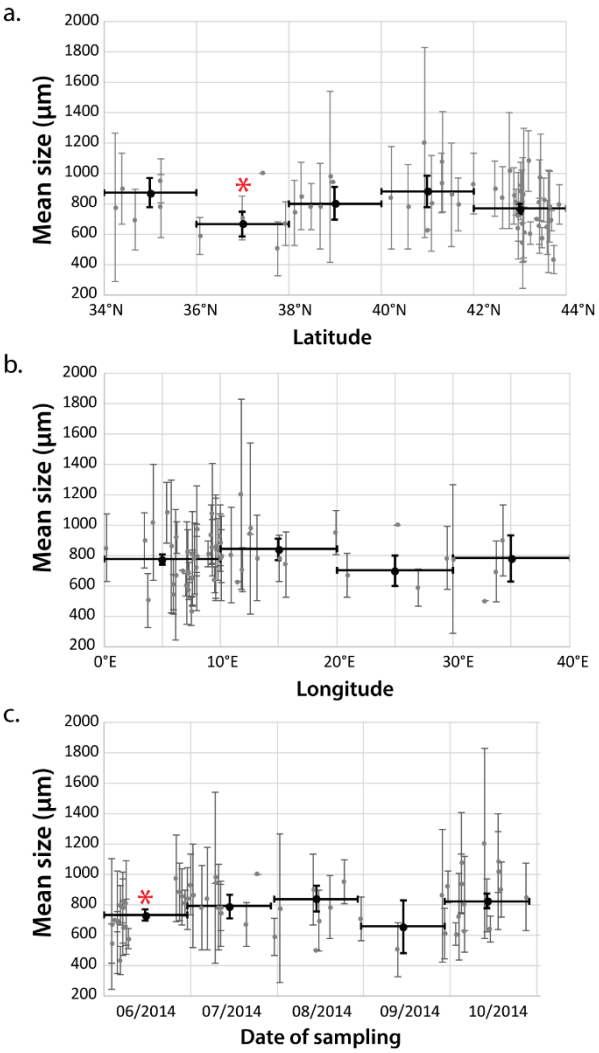

Fig. 4. Evolution of the microplastic mean size as a function of latitude (a.), longitude (b.) and sampling date (c.) (±Interval of confidence). The mean size was globally homogeneous and little statistically significant change (*) could be demonstrated through space and time.

3.2.3. Concentrations

The mean concentration in number was stable for the different latitude ranges and the only significantly lower value (i.e. 50,438±25,130 particles/km²) was between 40 and 42°N latitude (Fig. 5.a). The study of these variables as a function of longitude did not reveal a clear trend (Fig. 5.b). Thus, the mean concentration in number showed little variation except between 30 and 40°E, which was significantly lower (i.e. 35.278±31.433 particles/km²). Similarly, for the mean concentration in mass, the only significantly different value was between 30 and 40°E, with a mean value of 10.0±5.4 g/km². Finally, in the case of the mean concentration in number and in mass as a function of the sampling periods, the variations could suggest a seasonal signal (Fig. 5.c). Thus, the mean concentration in number decreased from June to September, with a significantly lower mean value in September (i.e. 25,800±24,300 particles/km²). Then, in October, the mean value increased. A similar pattern could be described for the mean contration in mass with a decrease from June to September and an increase in October. Two mean values appeared significantly lower, those of July (23.3±12.5 g/km²) and especially September (8.8±7.3 g/km²).

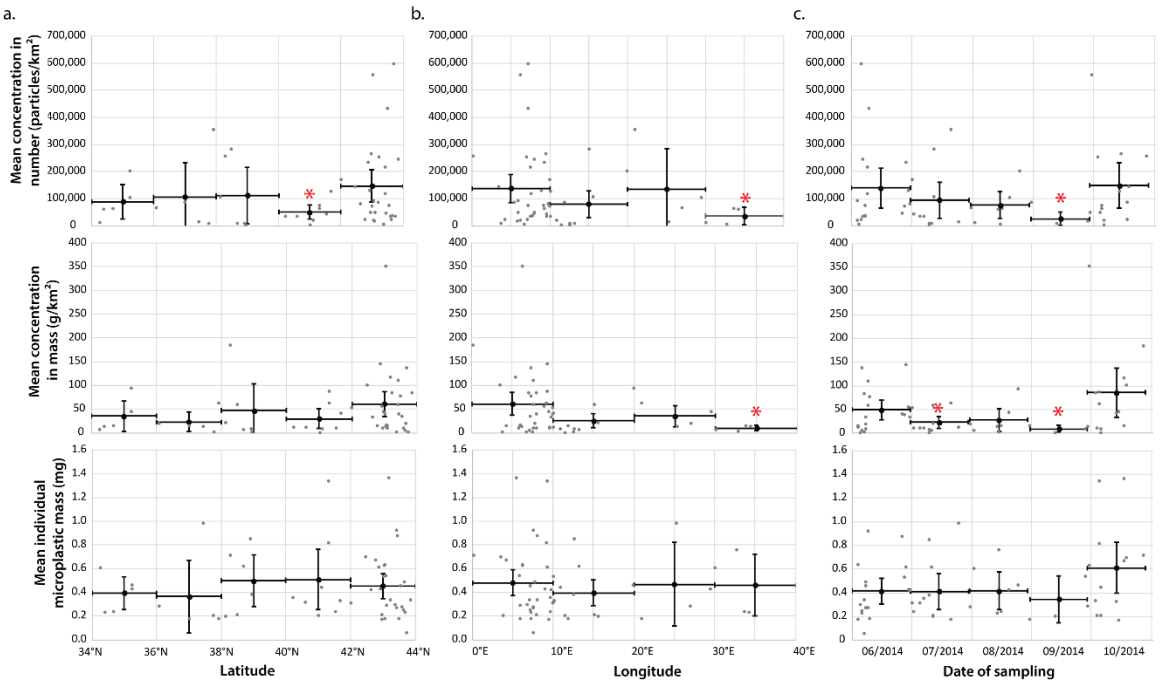

Fig. 5. Evolution of the mean concentrations in number and in mass, and mean individual microplastic mass as a function of latitude (a.), longitude (b.) and sampling date (c.) (±Interval of confidence).

3.2.4. Seasonality

In order to test the hypothesis of a seasonal fluctuation of the variables, it was possible to rely on the comparison of samples taken at different periods in the same geographical area (Tab. 1). For this purpose, the results obtained in the areas of the Gulf of Lyon in June and September-October, as well as in the Tyrrhenian Sea in June-July and October were compared. This comparison for these two areas did not reveal any significant difference between the sampling periods for most values (e.g. mean size, concentration in number, percentage of PE, CI). In contrast, the concentration in mass and the mean individual showed larger discrepancies. However, the large variability in sample mass did not allow to highlight a statistically significant difference. The only significant differences were observed for HI (Tyrrhenian Sea) and FI (Tyrrhenian Sea, Gulf of Lyon).

Table. 1. Results of the different sampling periods in the Gulf of Lyon and the Tyrrhenian Sea (±Interval of confidence; *: significant value).

	Gulf of Lyon		Tyrrhenian Sea	
Date of sampling	Jun.	Sept.-Oct	Jun.-Jul.	Oct.
Number of sampling	14	8	13	7
Mean size (µm)	698±14	839±23	847±10	867±52
Concentration in number (particles/km²)	141,878±92,512	148,167±126,955	93,651±48,624	82,051±73,477

Concentration in mass (g/km²)	43.7±22.9	88.4±80.1	35.3±21.1	57.8±42.7
Average individual microplastic mass (mg)	0.4±0.1	0.6±0.3	0.4±0.1	1.8±2.7
PE (%)	69.3±4.9	69.1±5.9	66.5±6.5	63.6±11.5
CI	0.8±0.02	0.9±0.02	0.8±0.02	0.8±0.03
HI	3.0±0.34	2.9±0.1	3.2±0.12*	2.5±0.22*
FI	0.4±0.04*	0.3±0.01*	0.4±0.02*	0.4±0.04*

3.3. Potential interactions between variables and particle size

3.3.1. Concentration

The manta by manta study of the mean concentration in number (Fig. 6.a) and in mass (Fig. 6.b) as a function of the microplastic mean size did not reveal any significant variation except above 1,000 µm with a marked lower value of 58,043±36,010 particles/km². On the other hand, quite logically, the mean individual microplastic mass increased strongly when the mean size increased (Fig. 6.c). Thus, the extreme values were significantly different from the average mass value calculated at the Mediterranean Sea scale. At the Mediterranean scale, an average density of microplastics of about 0.9 could be calculated from these data. This value is consistent with that of PE and PP particles (Kedzierski et al., 2017).

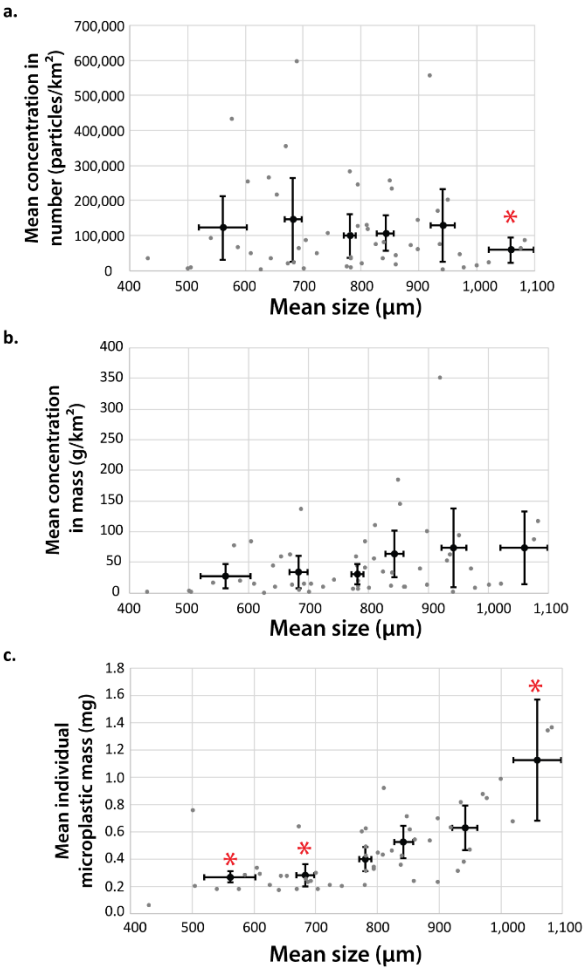

Fig. 6. Evolution of the concentrations in number and in mass, and average individual mass (±Interval of confidence) as a function of average size of the microplastics (±Interval of confidence). a. Evolution of the concentrations in number as a function of average size of the microplastics. Few significant differences can be identified (*). b. Evolution of the concentrations in mass as a function of average size of the microplastics. No statistical difference could be demonstrated. c. Evolution of the average individual mass as a function of average size of the microplastics. A clear increase of the mass of microplastics when the size of these increases was highlighted.

3.3.2. PE indices

The different indices calculated from the PE spectra showed different size-dependent evolutions. The CI (Fig. 7.a) did not show significant variation with size in contrast to the HI

(Fig. 7.b) and FI (Fig. 7.c) which both showed clearly an increase as the microplastic size class increased. Thus, HI and FI increased from 2.1±0.5 and 0.32±0.06 for the [315-500] µm size range to 4.5±0.9 and 0.59±0.11 for the [2,000-5,000] µm size range respectively. The values obtained were statistically different from the average values calculated at the Mediterranean Sea scale (HI: 2.88±0.02 and FI: 0.45±0.02).

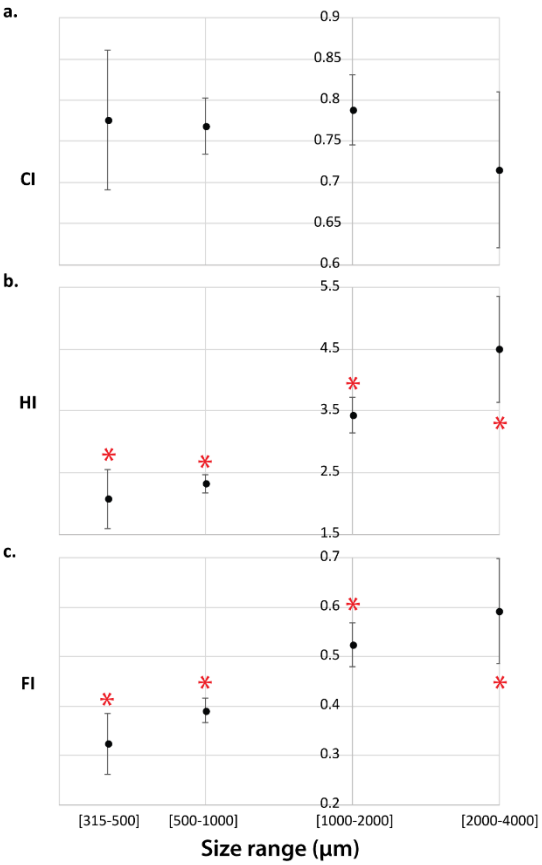

Fig. 7. Evolution of the CI (a.), the HI (b.) and the FI (c.) indices calculated from the PE spectra as a function of the size range (±Interval of confidence). No significant variations of CI (*) were found. The HI and FI, show a particular pattern where each size class is significantly different (*) from the Mediterranean mean values.

4. Discussion

4.1. Chemical composition of MPs on the surface of the Mediterranean Sea

4.1.1. Comparison with previous work and limitations

Few studies have analysed more than a few hundred MPs in the Mediterranean. Among them, Baini et al. (Tuscany, Italy) and Zeri et al. (Adriatic Sea) have shown chemical composition results very similar to those of our study (Baini et al., 2018; Zeri et al., 2018): PE rates of about 66.0±5.2% and 66.5±2.6% respectively. On the contrary, other studies have shown different results, with notably lower PE rates: 41.2±3.3% in the south of the Adriatic Sea (Suaria et al., 2016), 60.0±1.9% in the north of this sub-basin (Vianello et al., 2018), 55.0±1.7% and 43.0±4.6% in the western basin of the Mediterranean Sea (de Haan et al., 2019; Suaria et al., 2016). The results of these two publications differ significantly from those of our publication. In contrast, two other studies have evidenced high percentages of PE in the range of 76±3.9 to 79±5.4% on the southern coasts of the Mediterranean Sea. Nevertheless, these values are consistent with those found by the present study (73.1±5.8%) in the eastern Mediterranean Sea basin. Finally, it is interesting to note that the PE rate in the Mediterranean Sea is on average higher than in the Atlantic ocean (<60%) (Enders et al., 2015).

However, the comparison of the data obtained between our study and these publications presents various limitations (Baini et al., 2018; de Haan et al., 2019; Suaria et al., 2016; Vianello et al., 2018; Zeri et al., 2018). The first is undoubtedly linked to a scale effect. Indeed, the smaller the sampling in terms of number of sampling points, the more sensitive it will be to local variations. Thus, it may be more prone to deviate from the average value for the Mediterranean Sea. However, the lack of data leads to the comparison of studies carried out at different spatial scales, which necessarily raise the question of the relevance of the differences highlighted. The use of statistical calculations considering the number of particles analysed (i.e. calculation of the interval of confidence, proportion test) allows to partially compensate this bias of geographical scale.

Furthermore, the lack of standardised sampling methods is a hindrance when it comes to comparing MPs studies with each other (Cincinelli et al., 2019). The influence of the net mesh size is assumed to be crucial in MPs size measured. For example, sampling in the Seine River with an 80µm net yields on average 30 times more MPs (in numbers) than sampling with a 333µm net (Gasperi et al., 2014). As it can differ between publications, discrepancies in MP concentrations, but also in PE proportions, could be induced by this parameter. Moreover, as observed in the present study and in previous ones, the PP proportions tends to increase with decreasing size range in the Mediterranean Sea (Baini et al., 2018). Thus, the polymer proportions measured could be partially dependent on the sampling methods.

4.1.2. 4.1.2. PP/PE ratio and the size range

First of all, the evolution of the PP/PE ratio according to the size range of the MPs should be discussed. An increase in the PP rate with decreasing particle size has been demonstrated in the Mediterranean Sea by Baini et al. (Baini et al., 2018). This tendency was observed to be steadier and stronger than in our study but in both cases the general pattern remains the same. The same pattern was also found on plastic samples from different oceans and could be related according to the authors to different degradation behaviours of PP and PE in sea water (Serranti et al., 2018). In fact, this trend could be explained by a higher ageing sensitivity of PP than PE, enabling PP to fragment into small dimensions faster (Gewert et al., 2015). This ratio therefore seems to be an interesting data to characterise plastic pollution, and may be an indicator of a segregation phenomenon undergone by plastic pollution on the ocean surface.

4.1.3. PE indices

Although indices are widely used to characterise the state of atmospheric weathering of PE, they should be taken with greater caution when working on PE aged in the marine environment.

In the present results, the CI was homogeneous at the Mediterranean Sea scale. The weathering state of polymers is assumed to be homogeneous at the surface of the Mediterranean Sea: highly degraded PE (i.e. with high CI values) tending either to fragment into particles smaller than 300µm or to sediment. It is also quite possible that in the marine environment a threshold value exists for CI (Andrady, 2017).

For HI and FI, it is likely that the observed seasonal variations may be related to variations in the plastisphere. At this stage, it is difficult to interpret these temporal variations from a biological point of view. However, as the spectrometric approach is now relatively common for the chemical analysis of microplastics, the acquisition of plastisphere spectra would be relevant. The decrease in FI and HI with decreasing PE microparticle size, can be interpreted as a sign of a more limited implantation of the plastisphere on the surface of small microplastics. This phenomenon could be explained by a greater susceptibility of small microplastics to sedimentation.

4.2. Variations observed at smaller geographical scale

4.2.1. A truly heterogeneous sea?

Despite these methodological differences, could the differences observed between publications be explained by a heterogeneous chemical nature of MPs floating on the surface of the Mediterranean Sea? If our study tends to highlight differences in polyethylene levels, the analysis of previous scientific publications also seems to show some similar trends.

In the end, the average chemical compositions observed in the Mediterranean Sea can be summarised in four main patterns (P1-4) illustrating the small heterogeneities occurring in this basin (Tab. 2).

Tab. 2. Proposal of four main patterns to describe the state of contamination of the Mediterranean Sea by MPs.

Pattern	Area	Proportions (this study and other publications)	Example other than the current study
P1	North Tyrrhenian Sea	PE: ≤65% PP: >25% Others: 5-15%	(Baini et al., 2018)
P2	North of the Mediterranean Sea	PE: 65-70% PP: 15-20% Others: 5-15%	(Vianello et al., 2018)
P3	South of the Mediterranean Sea	PE: >70% PP: 15-25% Others: <10%	(Wakkaf et al., 2020; Zayen et al., 2020)
P4	P4: Centre of the western basin / maritime traffic convergence areas	PE: <60% PP: 5-25% Others: >20%	(de Haan et al., 2019; Suaria et al., 2016)

Based on this reflection, it is possible to question this heterogeneity and its origin. To this end, some hypotheses can be proposed to explain these differences. Some of them are presented through three cases: the North Tyrrhenian Sea, the South Mediterranean Sea and the case of the pattern 4.

4.2.2. The case of the North Tyrrhenian Sea

In the Northern Tyrrhenian Sea, the PE and PP proportions were respectively abnormally low and high (P1; Fig. 8) compared to other sampled areas in the Northern Mediterranean Sea (P2; Fig. 8). This lower proportions of PE in the North Tyrrhenian Sea could imply the existence of local sources introducing MP pollution with low levels of PE (i.e. <65% of PE). These MPs can come from four main sources: rivers, cities, maritime traffic and sea currents (Liubartseva et al., 2018; Soto-Navarro et al., 2020) (Fig. 8). There are currently no data for the Italian rivers flowing into the Tyrrhenian Sea. Based on data further away from this geographical framework, it appears that the composition of MP pollution in European rivers is generally poor in PE and rich in PP and PS. Indeed, PE proportions have been reported to be quite low in samples collected on the surface of lakes tributary of the Po (SAL; Fig. 8) and in the delta of the Rhône river (RR; Fig. 8) with levels of about 40% to 45% respectively (Constant et al., 2020; Imhof et al., 2013; Sighicelli et al., 2018). Other publications on north European rivers (without connection to the Mediterranean Sea) also have highlighted relatively low percentages of PE (<50%) (Mani et al., 2015). Furthermore, the relatively high average size and mass of the MPs collected in the north of the Tyrrhenian Sea can possibly be interpreted as an indication of a source of microplastic relatively close to the sampling areas.

Cities such as Rome, Naples or Livorno are other potential important sources of MPs (Liubartseva et al., 2018; Soto-Navarro et al., 2020). However, data are very scarce in the case of Mediterranean cities and, more generally, in the case of this kind of proximal sources that therefore need to be better studied (de Haan et al., 2019). In other locations around the world, MPs observed in cities (i.e. water and soils) are heterogeneous and have rather low PE contents (Wu et al., 2020; Yan et al., 2019; L. Zhang et al., 2021).

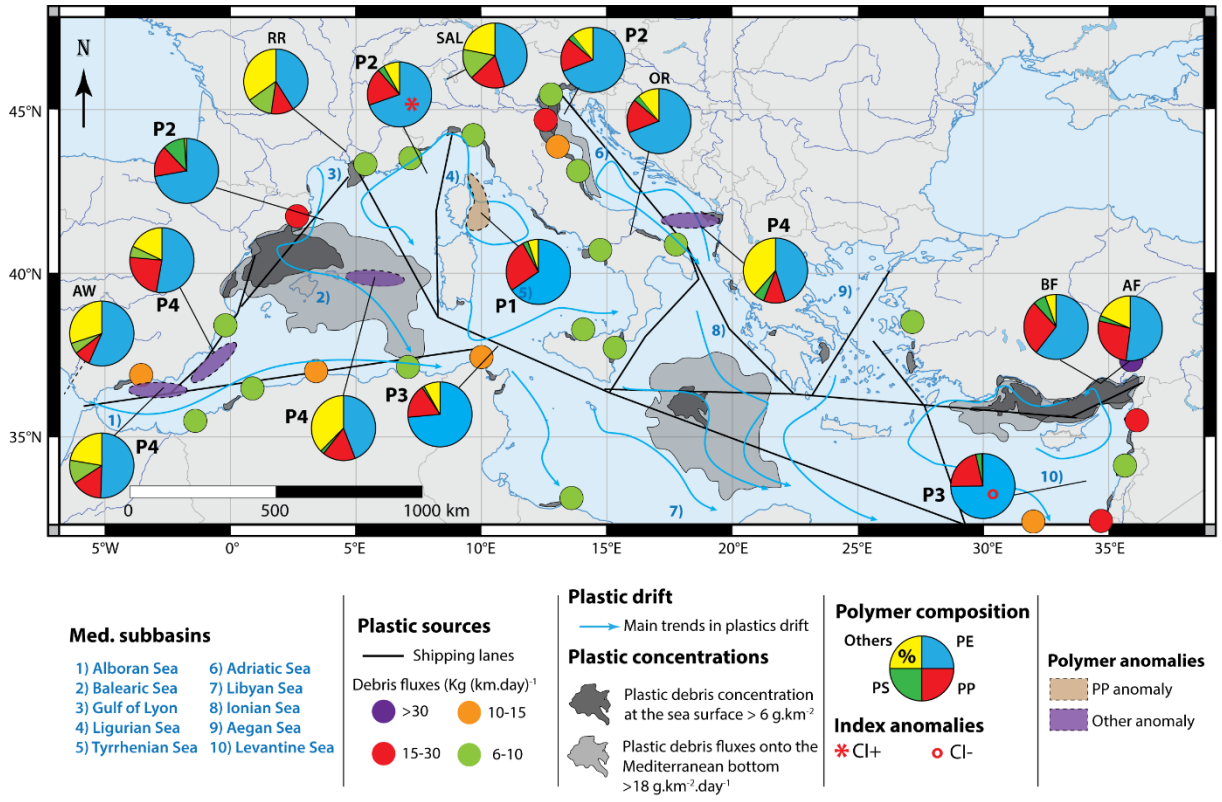

Fig. 8. Synthesis map of the sources, drift, concentration and composition of MPs in the Mediterranean Sea. Data on sources, concentrations and drifts of MPs are based on numerical modelling (Liubartseva et al., 2018). The data on chemical types are taken from the current study and various other publications (listed hereafter). Patterns – P1 to P4 (Baini et al., 2018; de Haan et al., 2019; Suaria et al., 2016; Vianello et al., 2018; Wakkaf et al., 2020; Zayen et al., 2020). Atlantic – AW: Atlantic waters (Enders et al., 2015). Rivers – RR: Rhône river (Constant et al., 2020); OR: Ofanto river (Campanale et al., 2020); SAL: Subalpine lakes (Sighicelli et al., 2018). Flooding – BF: Before flooding; AF: After flooding (Gündoğdu et al., 2018).

4.2.3. The case of the Levantine Sea

Although not significant, the PE proportions observed in our study are on average higher in the Levantine Sea region than in the rest of the Mediterranean. Statistically different from the average value for the Mediterranean Sea, two other studies have evidenced high percentages of poly(ethylene) (P3; Fig. 8), supporting the idea that the PE proportions seems higher in the

South than in the North of the Mediterranean (Wakkaf et al., 2020; Zayen et al., 2020). Although the literature on MPs and its chemical nature is excessively limited regarding major rivers of the Mediterranean Sea, this North-South difference could be explained by the rivers. This is particularly true for the Nile, since only one study has been conducted to date. This lack of knowledge has been identified as a major handicap in understanding the plastic pollution in the Mediterranean Sea (Guerranti et al., 2020; Khan et al., 2020; Martellini et al., 2018). Yet, according to modelling studies, the Nile is supposed to be one of the major MP contributor and to strongly influence the chemical nature of MPs in the Mediterranean Sea (Lebreton et al., 2012; Liubartseva et al., 2018; Soto-Navarro et al., 2020). By widening the geographical framework, it is possible to note that high PE percentages of around of 76.9% have been reported for the Ofanto river (OR, Fig. 8) in southern Italy (Campanale et al., 2020). In addition, a recent study carried out on the surface of the Bizerte lagoon (Tunisia) has shown very high percentages of PE (79%) (Wakkaf et al., 2020). Thus, unlike the Tyrrhenian Sea, it is possible that the southern rivers of the Mediterranean Sea tend to contribute to high percentages of PE resulting in local anomalies. Furthermore, even though no seasonal variation was detected in the southern Mediterranean Sea during our sampling campaign, PE proportions have been reported to be high during periods of lower river flows (BF; Fig. 8) whereas they tend to decrease during intense rainfalls (AF; Fig. 8) (Gündoğdu et al., 2018). The same trend was observed in southern India where marked seasonality exists (Veerasingam et al., 2016). This alternating water regime is observed throughout the southern Mediterranean Sea (Djellouli-Tabet, 2010; Varis et al., 2019), implying that the Levantine Sea would be fed by rivers with high PE levels during the summer period. If this hypothesis is correct, then there should be a stronger seasonal signal in the southern Mediterranean Sea. Thus, the difference between the north and the south should be more pronounced in summer than in winter. As the Tyrrhenian Sea and the Gulf of Lyon, all located in the northern part of the Mediterranean Sea, have shown

a possible seasonality, the sampling campaign may have missed a seasonality phenomenon in its southern part.

4.2.4. Pattern 4 (P4)

The P4 pattern was not found in our results, but appears in some regions such as the central western basin (Fig. 8) (Suaria et al., 2016). Driven by winds and sea currents, MPs from the Gulf of Lyon and the Balearic Sea then travel about 900 km to arrive off the Maghreb coasts (Liubartseva et al., 2018). Two main sources probably supply the centre of the western basin in MPs: plastic pollution present in the north of the basin on the one hand and the maritime traffic on the other hand. As they move away from land-based sources, it is likely that microplastics tend to settle to the ocean floor or fragment below 300µm. Then, microplastic pollution probably tends to become more sensitive to plastic inputs from marine sources. Marine sources consist, among other things, of inputs of very dense polymers such as boat paint (Suaria et al., 2016). Thus, high levels of dense polymers (22%) were observed in this area (Suaria et al., 2016). It is therefore quite possible that the very important maritime traffics in this region of the Mediterranean, combined with a low contribution via land sources, could be the origin of P4 (Fig. 8).

It is also possible that the importance of maritime traffic can also explain the presence of the P4 in the southern Adriatic Sea (PA, paint) and in the Alboran Sea (polyacrylic ship paint) (de Haan et al., 2019; Suaria et al., 2016).

4.3. Methodological contributions and recommendations

Some methodological aspects are discussed to temper the results observed in the present study. First, sampling in the same area at different dates was a relevant strategy to discriminate between variations of seasonal origin and those of spatial origin. For example, this strategy has

shown that the difference between the northern Tyrrhenian Sea and the rest of the Mediterranean Sea was not seasonal, but geographical. Nevertheless, in the southern basin, some doubts still exist about a potential seasonal phenomenon. Thus, in future work, conducting periodic samplings in the southern region would fill the blanks in seasonality assumptions.

Second, another approach that proved useful for this work was to seek to have a large number of sampling points, and to analyse only a fraction of the microplastics collected. This strategy kept the analysis effort at an acceptable level while obtaining more global results, less sensitive to the significant variability of the sampling points. Coupled with statistical tests, this approach made it possible to easily identify, for each of the parameters studied, the significantly different areas.

Spatially homogeneous sampling is also desirable. In the context of this study, the smaller number of sampling points in the eastern basin, mainly due to the geopolitical context of the south-east Mediterranean Sea at the time of the sampling campaign, implied a greater uncertainty in the results. It would therefore be desirable in the future to increase the research effort in the eastern Mediterranean Sea in order to reduce this uncertainty. In the context of our study, this has several consequences:

- The average of the variables calculated for the eastern basin have a greater sensitivity to outliers.
- The averages calculated at the Mediterranean Sea scale are necessarily closer to those of the western basin. The eastern basin is therefore more likely to have significantly different values from the Mediterranean Sea.

However, the results obtained in this study allow us to temper these potential limitations. Indeed, the comparison of the basins showed that statistically significant variations are very

small (e.g. case of PP for the Tyrrhenian Sea). This is due, on the one hand, to a real chemical homogeneity of the microplastic pollution of the Mediterranean Sea, which is reflected by relatively close average values at the scale of the basins, and, on the other hand, to the difference in the number of microplastics analysed for each of the basins in our calculations. Thus, the lower number of microplastics analysed in the eastern basin results, on the one hand, in a greater interval of confidence than for the other basins studied, and on the other hand, in statistical tests that are less sensitive to the variations measured. This implies that by increasing the research effort in this part of the Mediterranean Sea, the uncertainty could be sufficiently reduced to show a significant difference between the western and eastern part of the Mediterranean Sea.

Finally, it is interesting to rely on proportion tests to compare the percentage of the different chemical nature presented in previously published articles. Indeed, this type of test uses the proportion and the number of particles analysed as input data to determine whether these values are statistically different or not. This approach allows for an effective comparison of the results obtained between two studies while taking into account the variability in analytical effort that may exist between publications. The results will show whether there is a statistical difference between the proportions. In the case of a significant difference, however, the test will not, be able to determine whether this is related to a difference in protocol (apart from the number of particles analysed, which is considered in the test) or to a real difference in terms of plastic pollution. However, the use of this type of test implies that the input data (proportion and number of microplastics analysed) are included in the publications, which is unfortunately not always the case.

5. Conclusion

The objective of this work was to evaluate the chemical nature of the microplastic pollution at the surface of the Mediterranean Sea sampled by Manta net during the Tara Mediterranean expedition undertaken during the warm season (June-November) 2014.

Microplastic from 54 sites was analyzed by FTIR spectroscopy and size, concentrations in mass and in number were measured. The main polymers identified in this work were PE 67,3+/-2,4%; PP 20,8+/-2,1% and PS 3+/-0,9%. The results point towards a certain homogeneity of the chemical nature of microplastics in the Mediterranean during the sampling period. However, differences, confirmed by the literature, were observed at a mesoscale level. In particular, the proportions of PE and PP were low in the North Thyrrenian sea compared to the other subbasins. However, new studies involving more targeted geographical or temporal sampling or numerical modeling especially of the less explored areas such as the Alboran Sea, the Levantine Sea (both offshore and nearshore) and the Adriatic Sea will confirm, invalidate or refine some of the hypotheses made here.

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