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ANALYSIS OF μ PLASTICS POLLUTION IN THE MEDITERRANEAN SEA USING μ FT-IR ANALYSIS

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Introduction

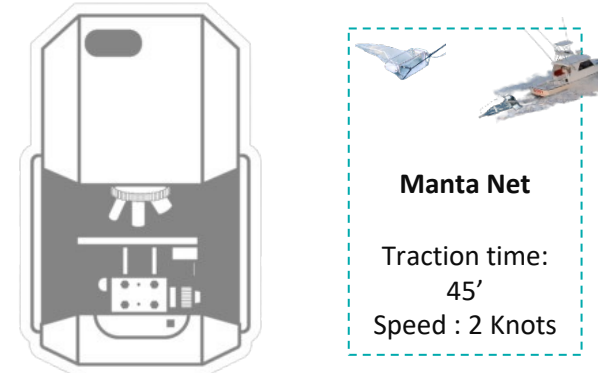
Pollution of marine environments by microplastics is a hot topic for society and science. There is a need of data for thoroughly characterizing the pollution, detecting hot-spots and developing mitigation measures. However, scientific expeditions to collect samples are costly and time-consuming.

SEAPlastics, an initiative by AgroParisTech's students, addresses this by collecting samples from Mediterranean hotspots and organizing public awareness events in ports of France, Spain and Italy.

AgroParisTech supports this effort in analyzing the collected samples and quantifying the pollution levels.

However, there is no stabilized and rapid analytical method allowing their rapid identification and quantification. Currently, visual inspection, optical spectroscopy methods such as μ FT-IR and RAMAN imaging are mainly used. (1) In the case of small microplastics (< 1 mm) and the presence of adhering biofilms, visual inspection leads to overcount or undercounting due to false positives or negatives, while manual spectroscopy methods become cumbersome and reliable quick monitoring is difficult. This is why our aim is to develop a semi-automatic μ FT-IR routine to analyze environmental samples.

Moreover, there is a need for standardization in the sample collection, preparation and data collection and units to permit intralaboratories comparisons.



Objectives

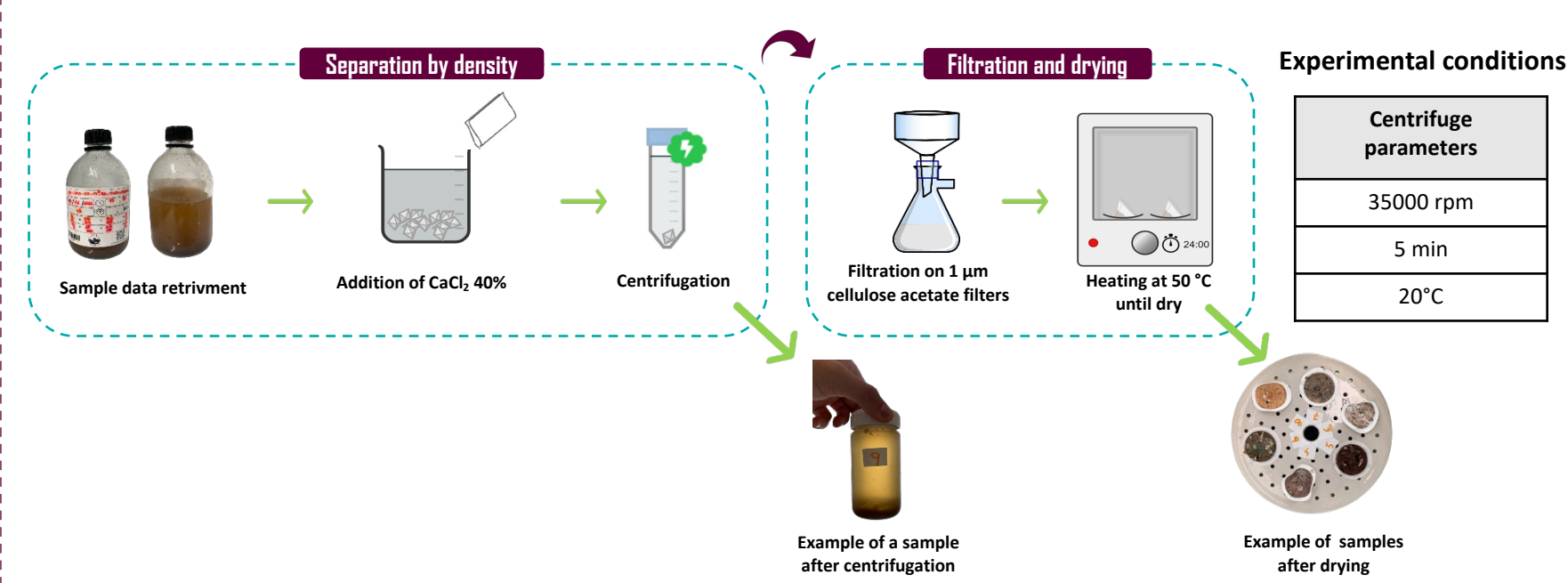
- Development of SEAPlastics' samples preparation
- Microplastic particle counting
- Development of the methodology to analyse the samples with μ FT-IR
- Development of a semi-automatic identification routine
- Data treatment of the samples

Methodology and experimentation

a) Development of the protocol for the removal of inorganic and organic material

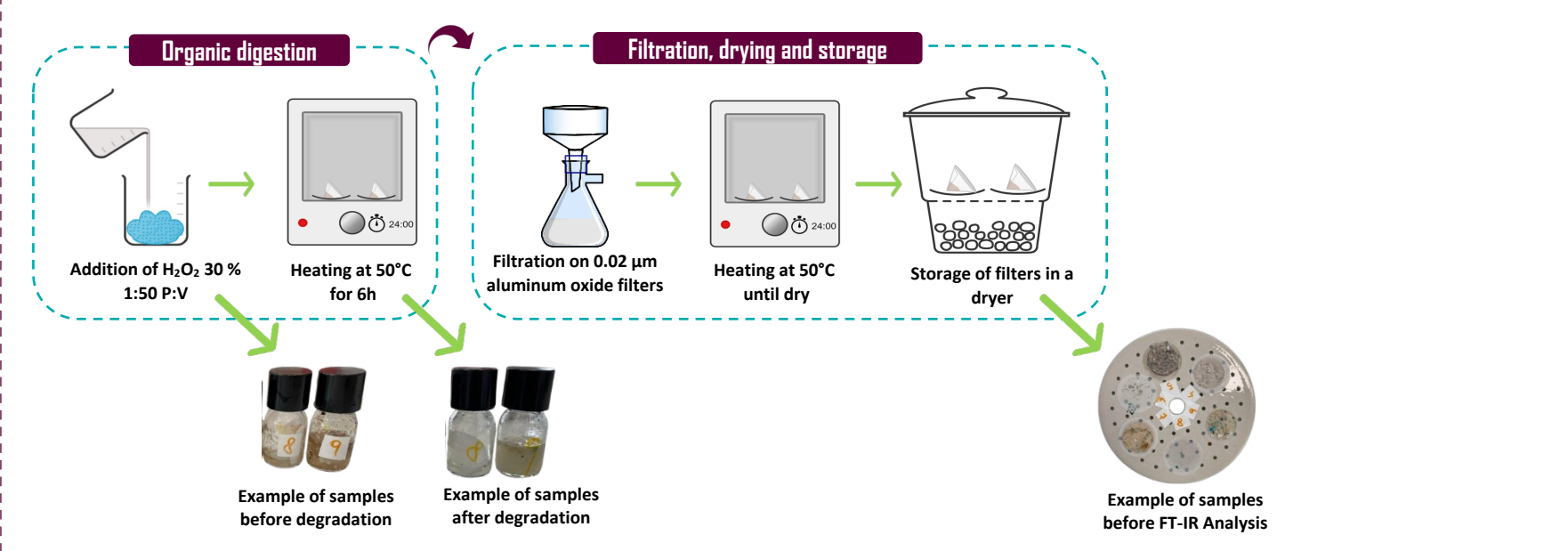
Inorganic matter removal

- 1) choice of the separation method of possible inorganic contaminants present within the sample
- 2) choice of operational conditions



Organic matter removal

- 1) choice of the reagent to use to remove or lessen the organic contaminants present within the sample
- 2) choice of operational conditions



Lastly the dry sample have been weighted and the data saved in an excel file

b) Optimization of the acquisition parameters for μ FT-IR

Test have been conducted using the reference polymer (PP). Fifty points were chosen on the surface of different particles and the variables, resolution and number of scans were varied in order to find the optimal collection parameters.

The optimum corresponded to a sufficient resolution to recognize the polymer but also suitable for reduced analysis times.

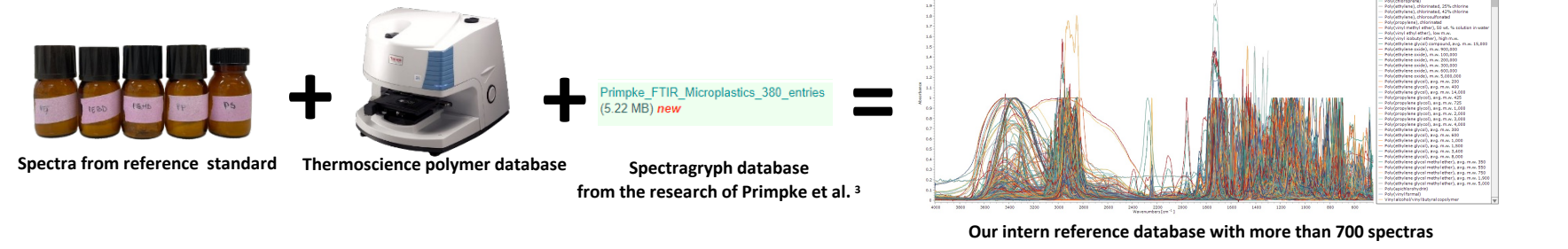
In the end a resolution equal to 4 cm⁻¹ and a number of scan equal to 16 (5 seconds) has been chosen.

c) Creation of an internal database containing the spectra of virgin plastics plus plastics recovered and analyzed by other researchers

The following virgin plastics polymers : PET, PEBD, PEHD, PP and PS, were chosen and analyzed to produce a first library which was added with Thermoscience and Primpk et al. databases to test precision and reproducibility to produce an accepted match factor of the analyzes

We found the precision to be : 94.11 ± 5.12 equal to the spectral similarity and the reproducibility equal to 100%, meaning that the same molecule was robustly recognized.

The match factor was created as the precision minus three times the standard deviation following the empirical rule, and was equal to 79, so every spectra that had a matching factor ≥ 79 was accepted.



d) Semiautomatic data collection and treatment

For the study of the polymeric composition of the sample, we used Spectragraph for the automatic correction, identification and data formatting.

This program has built in a snippet called 'Automation' that gives the user the possibility to register some action in order to be executed automatically.

- 1) Choice of the file to analyze
- 2) Removal of spikes
- 3) Smoothing of spectra
- 4) Correction of baseline to 0
- 5) Change spectrum name to user determined name
- 6) Saving the data in a pre-chosen folder.

The corrections made are:

- a. An adaptive baseline correction (15% coarseness, 0 offset);
 - b. A smoothing of the spectra by the Savitsky-Golay equation (interval of 10 and order polynomial 3);
 - c. Removal of spikes by point interpolation (2 pixels);
- Lastly the snippet 'Identify' is open, the previously mentioned sample spectra are opened and compared with our database and the matched molecule are saved in a excel file.

In excel different Macros have been coded to treat the data.

The first have been called 'matching factor':

sample ID	ref. ID	name	method	search name	rating
E 1 pinc	--	E 1 pincs plastique bibli0263.spa	FTIR	Search1	88.16
PP (base	--	PP (baseline corrected)	FTIR		88.10
E 1 pinc	--	E 1 pincs plastique bibli0264.spa	FTIR	Search1	88.46
PP (base	--	PP (baseline corrected)	FTIR		88.46
E 1 pinc	--	E 1 pincs plastique bibli0266.spa	FTIR	Search1	88.87
Cellulos	--	Cellulose acetate trimellitate	FTIR		88.01

Example of an excel spreadsheet

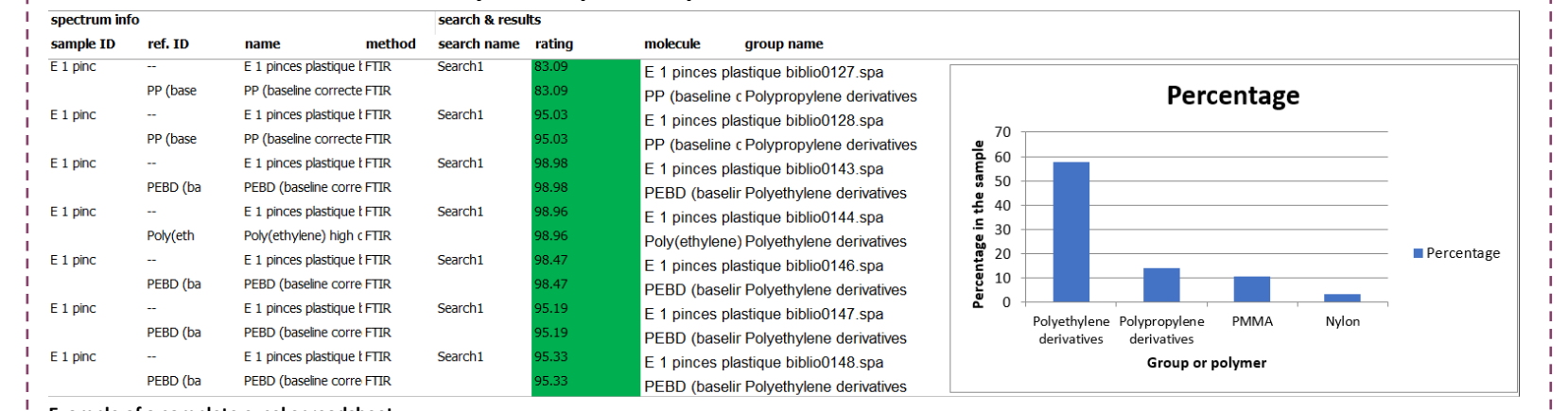
A visual feedback of acceptance of specific spectra was used. Green is accepted and red is not. Following the match factor of ≥ 79 .

The second one called 'accepted spectra': it copied the spectra having a matching factor ≥ 79 in the next column.

The third one called 'grouping': it was used to group molecule in the same 'polymeric family'. For example: PET, PEBD ad PEHD were classified as polyethylene derivatives.

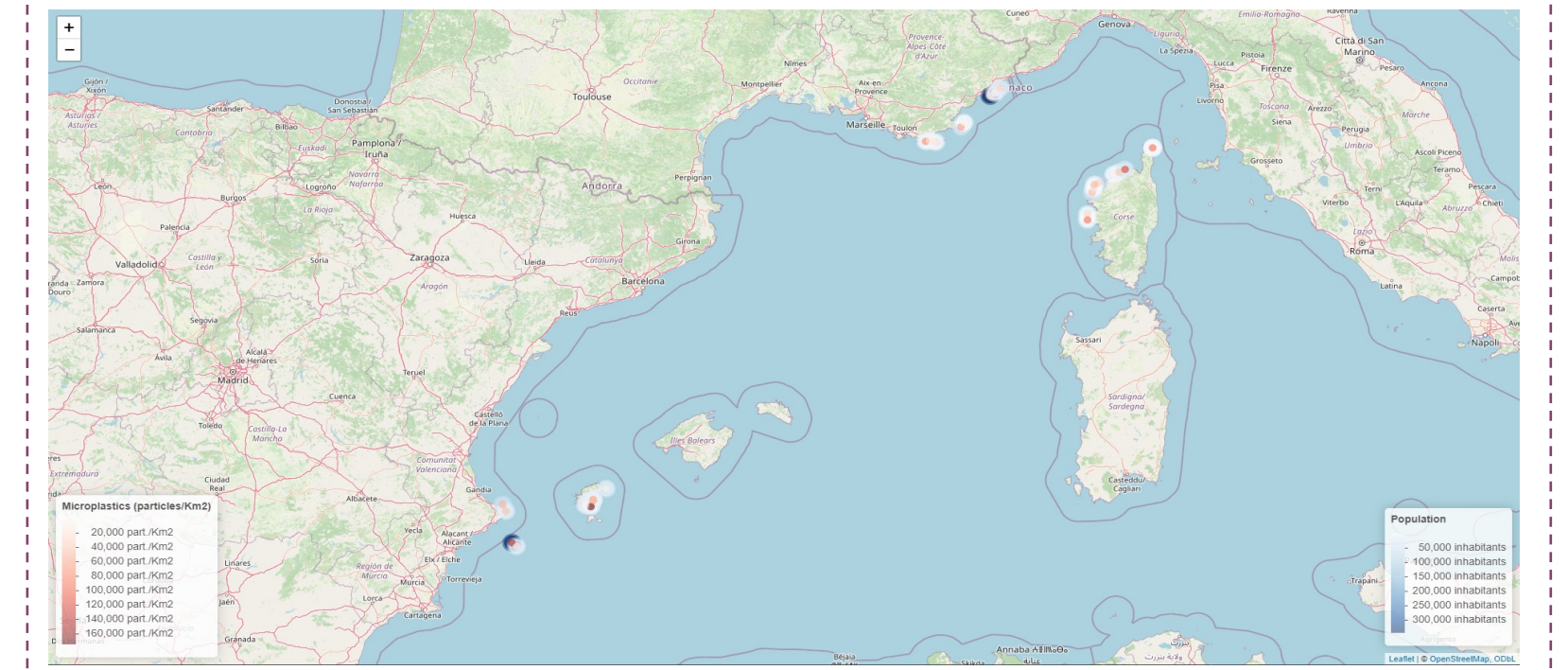
Lastly the one called 'frequency histogram': created a frequency histogram of the solely accepted spectra.

The end result for every sample is presented below:



Example of a complete excel spreadsheet

In R studio an Heatmap with the amount of microplastic found in a given area with regards its stable population has been created to see if there was a correlation.

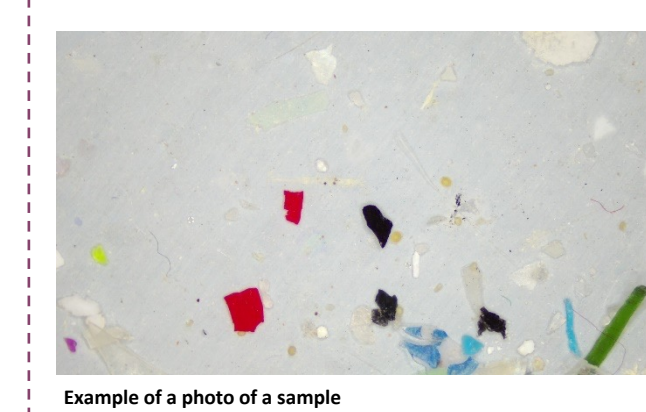


Heatmap of the sampled points by SEAPlastics

e) Counting of particles with microscopy

For the count of the particles present in the sample we used a stereo microscope.

Nine photos of each sample have been taken and carefully analyzed.



Example of a photo of a sample

Results

Microplastic quantity

Thanks to the metadata collected by SEAPlastics it was possible to quantify the amount of plastic present in the every studied spot in the Mediterranean Sea.

This was done by:

- 1) Calculating the sampled surface, equal to the velocity in km multiplied by time and width.
- 2) Calculating the net weight of the microplastic on the sample, equal to gross weight minus the filter weight.
- 3) Extrapolation of the amount of microplastic present in a km² (considering the sample representative of the normal distribution), equal to net weight divided by the sampled surface.

In order to correlated with previously collected data by other researcher we chose to add also the g/ km³ and kg/km³.

This was done by considering the sampled volume, equal to height of the water column entering the manta filter multiplied by the result of 1).

Weight (g)	Weight (g/Km ²)	Weight (Kg/Km ²)	Weight (g/Km ³)	Weight (Kg/Km ³)	
0.125	74.99	0.07	29997.60	30.00	Weight of Anodisc filter (g)
0.065	39.00	0.04	15598.75	15.60	0.12
0.085	51.00	0.05	20398.37	20.40	Conversion from knots to Km/h
0.115	68.99	0.07	27597.79	27.60	1 knot = 1.852 Km/h
0.075	45.00	0.04	17998.56	18.00	Length of sampling in Km
0.225	134.99	0.13	53995.68	54.00	2.778 Km
0.525	314.97	0.31	125989.92	125.99	Sampled area in Km ²
0.195	116.99	0.12	46796.26	46.80	1.67E-03 Km ³
0.155	92.99	0.09	37197.02	37.20	Sampled volume in Km ³
0.155	92.99	0.09	37197.02	37.20	4.17E-06 Km ³

Example of the Excel spreadsheet

Lastly the number of particles per km² and km³ have been calculated thanks to the number of particle counted using the stereoscopic microscope.

Microplastic identity

The identification routine found plastics with a density higher than the seawater.

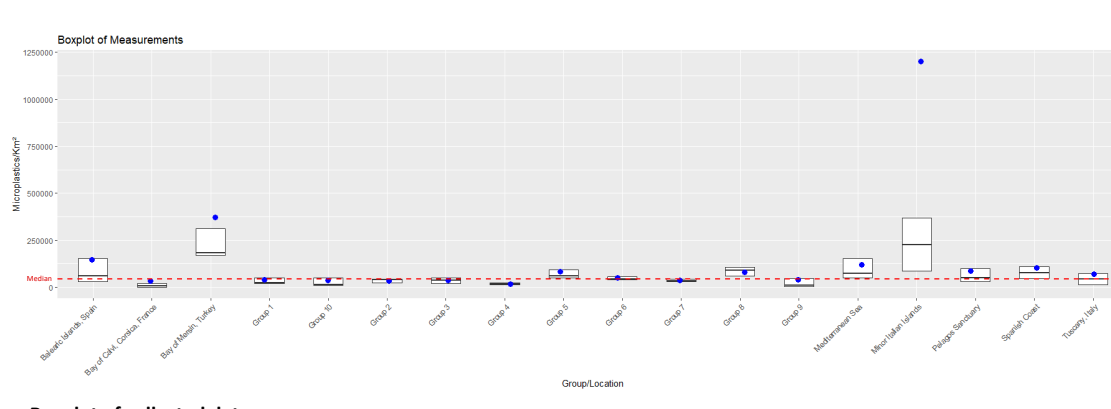
This can be explained by different reasons:

- 1) incorporation into organic matrices (algae, microalgae, etc.) with lower density,
- 2) incorporation into inorganic matrices with lower density,
- 3) sea currents...

The most sampled microplastics were composed of polyethylene derivatives (min 10%, MAX 84%) and polypropylene derivatives (min 1%, Max 30%). Other polymers where PMMA and Nylon.

Microplastic counting

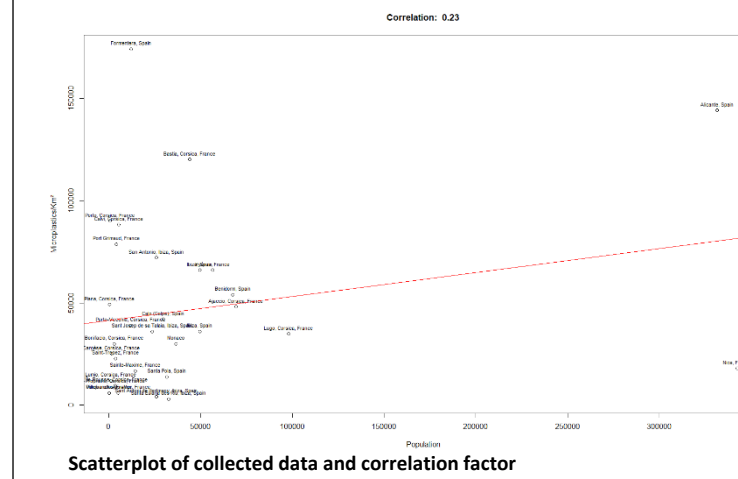
After counting on the stereo microscope was compared with data from other researchers. It was in the same range.



Scatterplot of collected data

Heatmap correlation

The correlation between the microplastic pollution and the inhabitant of the collection sites was evaluated.



Scatterplot of collected data and correlation factor

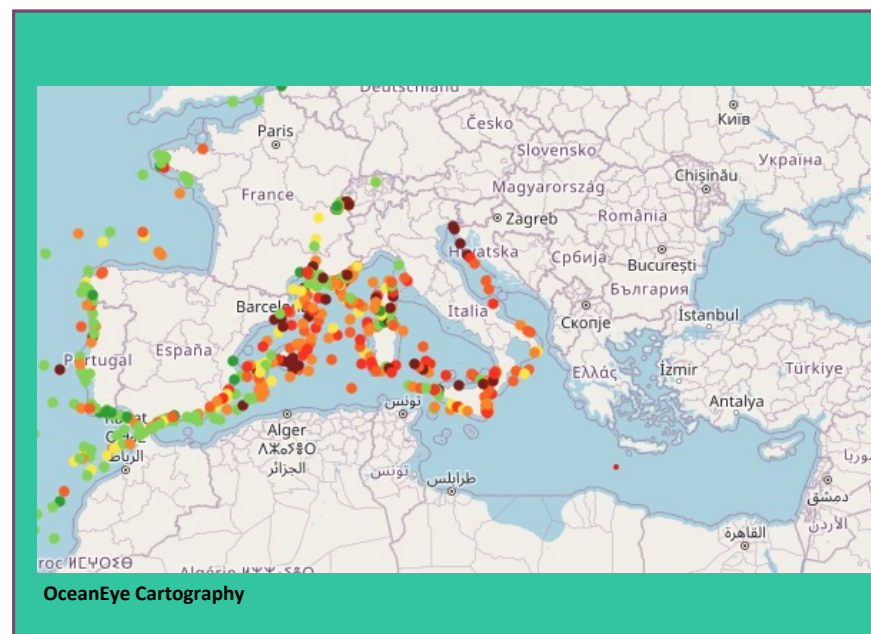
With regards of the scatter of the dataset, no correlation could be evidenced. This should be further studied using more measurements distributed over the whole year. The collection period for SEAPlastics was exclusively during the summer season.

High Data Dispersion

In our investigation of the data dispersity, we observed significant variations in measurements from triplicate samples provided by SEAPlastics. For instance, in nearby geographical areas we studied, values were recorded as 2.28·10⁴, 7.86·10⁴, and 1.68·10⁵. A similar dispersion pattern was noted in data obtained from OceanEye with values of 3.31·10⁵, 7.27·10⁵, and 6.37·10⁶.

While continuous monitoring of pollution over time is crucial, it also highlights the need for caution. Factors such as sampling techniques, laboratory procedures, and the timing of sample collection can significantly impact data scattering.

Conclusions



The Mediterranean Sea is currently grappling with the status of being one of the most heavily polluted seas across the globe. Although commendable efforts have been invested in recent years to assess and address pollution levels in the Western Mediterranean regions (encompassing the Alboran Sea, Balearic Sea, Tyrrhenian Sea, Ionian Sea, and Adriatic Sea) by SEAPlastics, OceanEye, Expédition MED ..., the same level of scrutiny has not been extended to the Eastern Mediterranean. The enclosed nature of the Mediterranean, fostering prolonged residence times for microplastics, underscores the need for a comprehensive study covering the entirety of the sea. Investigation holds the potential to unravel nuanced insights, identifying areas with the most significant impact and pinpointing crucial zones that demand vigilant monitoring. By gaining a comprehensive understanding of these dynamics, there lies an opportunity to strategically focus on and mitigate marine pollution, ultimately contributing to the preservation of the Mediterranean's ecological integrity. FT-IR has proven to be an excellent technique capable of great resolution and automation, which makes it a great candidate for contributing to the monitoring of microplastics in the marine environment

Perspectives

- ❖ μ FT-IR hyperspectral imaging coupled with image analysis can be used as a better recognition decision tool.
- ❖ Automatic image analysis routine for count, shape, volume of particles.
- ❖ In-depth evaluation of Raman spectroscopy, for the study of particles between 10 and 1 μ m.
- ❖ Use of smaller pore size filters to investigate the lower size microplastics fragments.
- ❖ Harmonizing purification techniques among studies.

REFERENCES

S. Domenek, et al. Analysis of Microplastics in Drinking Water and Other Clean Water Samples with Micro-Raman and Micro-Infrared Spectroscopy. Minimum Requirements and Best Practice Guidelines. Anal. Bioanal. Chem. 2021.

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