EXTRACTION AND CHARACTERIZATION METHODS FOR MICROPLASTICS FROM ESTUARINE AND COASTAL SAMPLINGS – EXAMPLE OF THE 2019 TARA EXPEDITION

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Abstract – The Tara Microplastics 2019 mission aimed to investigate plastic pollution in rivers across different scientific fields of study: plastic chemistry, physical oceanography and marine biology. From May to November 2019, the Tara schooner collected samples from 9 of the main European rivers: Thames, Elbe, Rhine, Seine, Ebro, Rhone, Tiber, Garonne, Loire. The objectives of the present study are: i) to quantify the microplastic (MP) contamination and ii) to identify the chemical nature of microplastics. Long and tedious protocols are often necessary to extract and analyze microplastics from environmental samples. Thus, extraction methods and automated computer processing for polymer characterization were developed. Preliminary results for microplastics in the range of 500 µm – 10000 µm in Feret length from the river Tiber are presented. Most of the particles were between 800 µm and 1600 µm. The three main polymers found were polyethylene (PE), polypropylene (PP) and polystyrene (PS). These results must be compared to the results from other river to draw a consistent pattern. Their interpretation can be complex as they depend on seasons, run-offs, sampling date, and hydrodynamic features. Nevertheless, this investigation presents the advantage to apply a consistent methodological framework to very different sampling sites.

Introduction

Plastic materials are a combination of synthetic or natural polymers with additives. Thanks to their functional properties, lightness and low cost, their use is increasing worldwide since the 1950's. Because of poor waste management, a significant portion of them enters and persists in marine ecosystems.

The sources of marine plastics are diverse: they can originate from river and atmospheric transports, beach littering, and human activities at sea such as aquaculture, shipping and fishing [1]. As land-based sources are considered as the dominant input of plastics into oceans [1], [2] through rivers and run-off, studies on microplastics has recently shifted focus toward freshwater ecosystems.

Few studies have reported levels of plastic contamination of freshwater worldwide [3]. Sampling and characterization methods, studied size and reported units (for instance pieces or grams for the amount of plastics, m^2 or m^3 for the filtered water volume) vary significantly between different investigations [4]. It has been suggested that population density, levels of urbanization or industrialization, rainfall and artificial barriers play a major role in measured river-based microplastic concentrations [5].

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Concerning the identified polymers, polyethylene, polypropylene and polystyrene are predominant on surface waters due to their buoyancy and their widespread use in various industries [6].

To investigate plastic pollution in European rivers, the Tara schooner collected samples from 9 rivers: Thames, Elbe, Rhine, Seine, Ebro, Rhone, Tiber, Garonne, Loire (cf. Figure 1). Among the 19 partner laboratories, the Research Institute Dupuy de Lôme (Lorient, Fr) objectives are to quantify the microplastic contamination and to identify the chemical nature of microplastics. The study of microplastics in rivers can be more arduous than that of marine MP. Indeed, the large amount of organic and inorganic matters is a major obstacle to overcome for further analyses. Non-anthropogenic particles and biofouling could make polymer spectra identification more difficult. Although drawing a consistent pattern from the obtained results is complex - as they depend on the seasons, the run-offs on the sampling date, and the hydrodynamic features of the sampling station-, they will provide valuable data on European rivers. These data have the great advantage of having been acquired by the same methods, allowing a relevant comparison between samplings. For the specific case of the Mediterranean Sea, where plastic litter inputs are poorly understood [7], results from the Tiber river are presented. This work follows a previous investigation on plastic pollution of the Mediterranean Sea surface, and proposes some improvements to the original methodology.

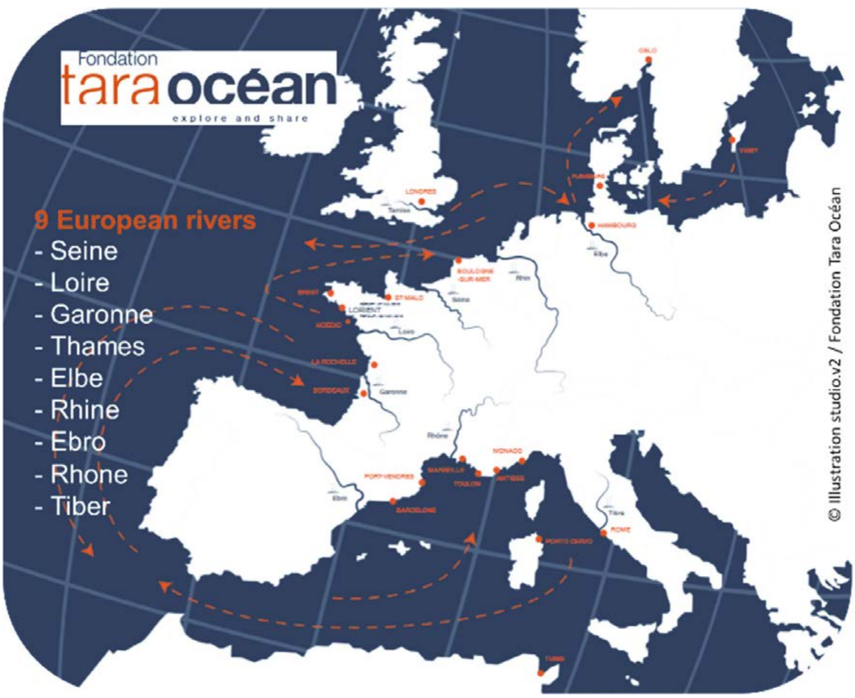

Figure 1 – Routes of the Tara Microplastics campaign in 2019.

Materials and Methods

Samplings were carried out in five sampling sites for each river (cf. Figure 2): i) at sea in front of the river mouth, ii) at the mouth with two salinity gradients, iii) downstream of the first city encountered (Roma for the Tiber), iv) upstream of this city. At each of these sites, numerous samplings were done to study plastic debris and the associated biota. To collect microplastics, a 300 µm-mesh size manta trawl was used to filter tens to hundreds of cubic meters of water (cf. Figure 2). Largest pieces of organic matter were removed (branches, leaves, algae, fishes…). Then, samples were stored in a freezer at -20 °C.

Figure 2 – Sampling sites along the Tiber River.

After defrosting in the lab, the remaining organic matter was digested with a combination of different chemical digestion processes, using acidic or alkaline solutions: a potassium hydroxide (10 % KOH) solution, a hydrogen peroxide (30 % H_2O_2) solution, the Fenton's reagent (H_2O_2 with an iron catalyst) [8]. Depending on the amount of organic matter, the process was adapted to every sample. Then, using a saturated sodium iodide (NaI) solution (density = 1.8 g/cm^3), high density inorganic matter was also removed. Filtrations through a 500 µm-mesh stainless-steel sieve were performed after each step. Afterwards, microplastics were rinsed, disposed in Petri dishes and dried at 50 °C (cf. Figure 4). For technical reasons, only microplastics superior to 500 µm to 5 mm were considered in the present study.

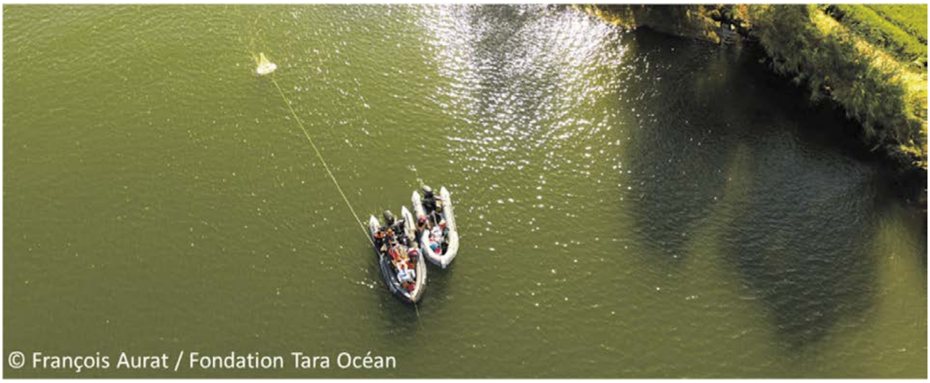

Figure 3 – In-use manta trawl during a sampling on the Tiber.

The Petri dishes were then photographed in high resolution images (Nikon D850 with an AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED lens). Particles were counted and measured using the Fiji image processing software (cf. Figure 4) [9]. Although particles below 500 µm were removed by sieving, some smaller ones could remain. They were excluded with the Fiji analysis (particles with area inferior to 500 µm-diameter circle area were not considered). Using a statistical approach, a particle random drawing enabled to limit the amount of work needed to analyze the extracted microplastics by infrared spectroscopy (ATR-FTIR). This method, developed in a previous study, allows to analyze only a statistically representative proportion of the total population of microplastics collected [10]. MP 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 4000–600 cm^{-1} region with a resolution of 4 cm^{-1} and 16 scans. All the spectra were then identified using the POSEIDON (Plastic pOllutionS ExtractIon, DetectiOn and aNalysis) software which was developed with R i386 3.1.2 [11].

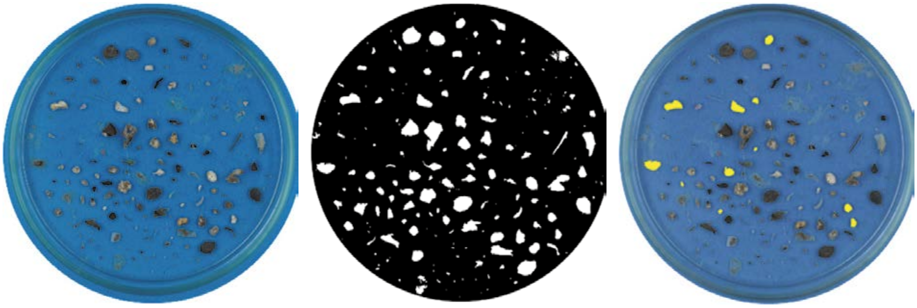

Figure 4 – From left to right: high definition photograph of a Petri dish containing the extracted microplastics; binary mask generated by Fiji after thresholding; randomly drawn particles colored in yellow.

Results and discussion

For the sampling site downstream of Roma, 1500 particles were detected. Most of them were in the size range of 800 µm to 1600 µm and showed a median value of 1066 µm in Feret length. Among them, 92 particles were randomly drawn to be analyzed by infrared spectroscopy. Their size distribution showed a shift to higher values and a significantly higher median value at 1698 µm. This gap suggests that the quantity of drawn particles was not sufficient to be representative of the whole particle population in this specific study. Further analyses are necessary to verify if this gap applies to every sample. However, the random draw process applied to the Petri dish photographs is crucial. Indeed, it allows to pick microplastics in the whole size range, and not only those that easily catch the human eye.

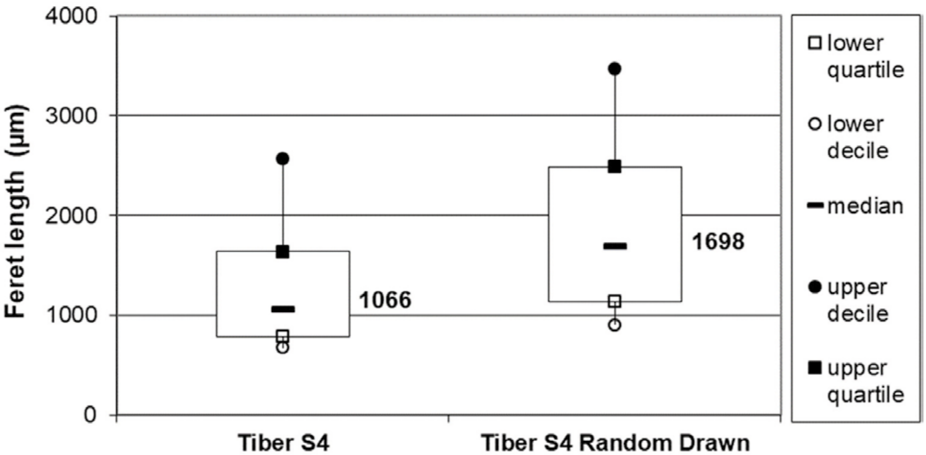

Figure 5 – Feret length of all detected particles and random drawn particles from Tiber S4 (downstream of Roma).

The preliminary results showed that polyethylene, polypropylene and polystyrene were predominant within the randomly drawn microplastics (cf. Figure 6).This is in congruence with polymers found in previous studies [3]. Indeed, their prevalence in numerous packaging products and various plastic parts is well known. Nevertheless, the unknown category is quite significant (10 %) and consists in unidentifiable particles and remaining organic matter. Thus, these results also suggest that more particles must be drawn and more efforts are necessary to increase the accuracy of the particle identification, compared to previous studies concerning the sea surface [6]. River samplings contain a high amount of organic matter and the extraction process is not sufficient to remove all organic matter.

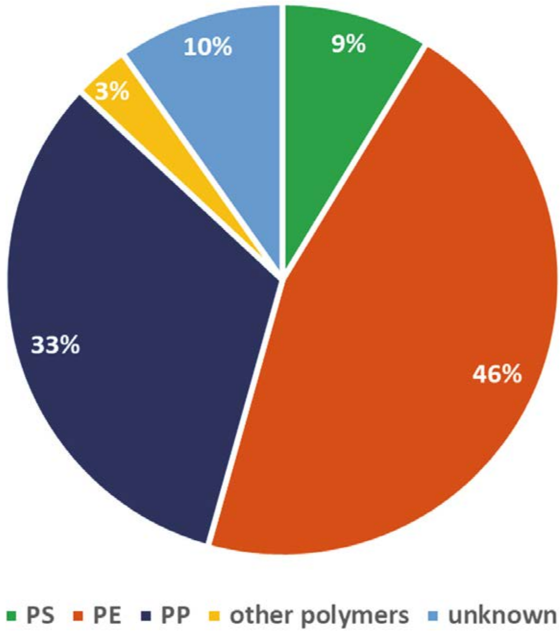

Figure 6 – Proportions of polymer types for randomly drawn microplastics from Tiber S4. PS: Polystyrene; PE: Polyethylene; PP: Polypropylene.

Conclusion

Manta trawl samplings, microplastic extraction and infrared polymer identification were carried out using similar protocols in 9 European rivers. Thus, this investigation presents the advantage to apply a consistent methodological framework to very different sampling sites.

Preliminary results showed that the extraction and characterization of plastic particles collected from rivers were significantly more arduous than marine plastics. Indeed, high amounts of organic and inorganic matter were found, making the extraction steps necessary to isolate the microplastics. Besides, these steps are often long and tedious and are potential sources of human mistakes, contamination or loss of microplastics. Regarding MP characterization, automated and statistical approaches were developed successfully but need more optimization efforts. Despite these experimental issues, these new data are precious to quantify the microplastic input from European rivers. The Tiber was investigated for the first time with these methods. Obtained results will help to understand microplastic concentration and chemical nature in the Mediterranean sea, especially in the Tyrrhenian sea [6]. For a complete understanding of the obtained results, the influence of the seasons and the hydrodynamic conditions must be understood. Moreover, temporal monitoring campaigns are crucial.

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References

[1] P. J. Kershaw and C. M. Rochman, "Sources, fate and effects of microplastics in the marine environment: part two of a global assessment," GESAMP, 2016. [Online]. Available: http://www.gesamp.org/site/assets/files/1275/sources-fate-and-effects-of-microplastics-in-the-marine-environment-part-2-of-a-global-assessment-en.pdf

[2] M. Sweet, M. Stelfox, and J. Lamb, "Plastics and shallow water coral reefs - Synthesis of the scince for policy-makers," United Nations Environment Program,2019. Available: [Online]. https://wedocs.unep.org/bitstream/handle/20.500.11822/27646/plastic_corals.pdf?sequence=1&isAllowed=y

[3] M. Kedzierski, M. L. Pedrotti, S. Bruzaud, and L. Frère, *Plastic pollution in the ocean: what we know and what we don't know about*. 2018. doi: 10.13140/RG.2.2.36720.92160.

[4] R. Dris *et al.*, "Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles," *Environ. Chem.*, vol. 12, no. 5, p. 539, 2015, doi: 10.1071/EN14172.

[5] L. C. M. Lebreton, J. van der Zwet, J.-W. Damsteeg, B. Slat, A. Andrady, and J. Reisser, "River plastic emissions to the world's oceans," *Nat. Commun.*, vol. 8, no. 1, Art. no. 1, Jun. 2017, doi: 10.1038/ncomms15611.

[6] M. Kedzierski *et al.*, "Chemical composition of microplastics floating on the surface of the Mediterranean Sea," *Mar. Pollut. Bull.*, vol. 174, p. 113284, Jan. 2022, doi: 10.1016/j.marpolbul.2021.113284.

[7] A. Cincinelli, T. Martellini, C. Guerranti, C. Scopetani, D. Chelazzi, and T. Giarrizzo, "A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea," *TrAC Trends Anal. Chem.*, vol. 110, pp. 321–326, Jan. 2019, doi: 10.1016/j.trac.2018.10.026.

[8] R. R. Hurley, A. L. Lusher, M. Olsen, and L. Nizzetto, "Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices," *Environ. Sci. Technol.*, vol. 52, no. 13, pp. 7409–7417, Jul. 2018, doi: 10.1021/acs.est.8b01517.

[9] J. Schindelin *et al.*, "Fiji: an open-source platform for biological-image analysis," *Nat. Methods*, vol. 9, no. 7, Art. no. 7, Jul. 2012, doi: 10.1038/nmeth.2019.

[10] M. Kedzierski *et al.*, “Microplastics in Mediterranean Sea: A protocol to robustly assess contamination characteristics,” *PLOS ONE*, vol. 14, no. 2, p. e0212088, Feb. 2019, doi: 10.1371/journal.pone.0212088.

[11] M. Kedzierski, M. Falcou-Préfol, M. E. Kerros, M. Henry, M. L. Pedrotti, and S. Bruzaud, “A machine learning algorithm for high throughput identification of FTIR spectra: Application on microplastics collected in the Mediterranean Sea,” *Chemosphere*, vol. 234, pp. 242–251, Nov. 2019, doi: 10.1016/j.chemosphere.2019.05.113.