



# Sample Preparation and Analysis Methods of Microplastics

Tiziano Battistini<sup>1</sup>(✉), Masenka Mikuz<sup>2</sup>, Giulia Dalla Fontana<sup>3</sup>,  
Alessio Montarsolo<sup>3</sup>, and Raffaella Mossotti<sup>3</sup>

<sup>1</sup> Aquafil Group Ind. Env. Tech. Inn., Via Linfano 9, 38062 Arco (Trento), Italy  
tiziano.battistini@aquafil.com

<sup>2</sup> AquafilSlo, Letališka Cesta 15, 1000 Ljubljna, Slovenia

<sup>3</sup> CNR STIIMA - Intelligent Industrial System and Technologies for Advanced Manufacturing, Corso G. Pella 16, 13900 Biella, Italy

## 1 Introduction

This research study made in collaboration between Aquafil Spa and CNR STIIMA (Biella Department) is aimed at the creation of a standard method applicable to the determination of microplastics (MP) in different matrices present in the textile field such as waste water from clothes washings, water effluents or solids from industrial processes, air. A very recent dossier [1] compiled by ECHA (European Chemicals Agency) for EU commission defines “microplastic” as a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where  $\geq 1\%$  w/w of particles have all dimensions  $1 \text{ nm} \leq x \leq 5 \text{ mm}$ , or, for fibres, a length of  $3 \text{ nm} \leq x \leq 15 \text{ mm}$  and length to diameter ratio of  $>3$ . The sources of microplastics are numerous, from tires, to plastics abandoned in the environment (terrestrial and/or marine, to paints, textiles, etc.). Variations are also due to their shape and size. In the case of those released by textiles the typical morphology it is the fibrous one and their diameter and length can vary depending on the construction parameters of yarns and fabrics or on washing conditions.

Because of the environmental problem represented by microplastics, their number, shape and size are relevant parameters for assessing their impact and consequently the development of a counting technique is the only logical approach. Moreover, many of the microparticles analyzed are not of synthetic origin and therefore there is a need to identify and distinguish them from microplastics.

The method is designed to provide the nature, numerical concentration, surface area (estimated) of microplastics in an aqueous or aeriform matrix.

Depending on the matrix, it may be necessary to pre-treat the sample to concentrate the microplastics and eliminate inorganic and organic contaminants (e.g. biological) that could interfere with their identification. The method involves a preliminary observation of the sample under an optical microscope and subsequently identification of microplastics with molecular spectroscopy. The method foresees the possibility of using two different molecular spectroscopy techniques, Micro-FTIR (Fourier Transform InfraRed Spectroscopy coupled with optical microscopy) and Micro-Raman (Raman Spectroscopy coupled with optical microscopy) to identify and count plastic

particles up to a submicronic dimension. The parameters determined could be useful also for subsequent ecotoxicological studies. Moreover, the analysis of the MP will be suitable for specific textile production, textile products of the market (allowing the evaluation of MP production during the life of garments) or any other process/semi-finished/manufactured item.

The standard method describes the analysis method for a single filter. However, the errors on the qualitative and quantitative determination of the microplastics that can derive from presence of contaminants or variability between different filters imply the need to perform replications to establish accuracy and precision.

## 2 Experimental

### 2.1 Pre-screening

In case of samples of unknown origin a series of pre-screening tests are provided for the possible presence of salts or organic matter. For aqueous samples standard tests such as conductivity [2], chemical oxygen demand (COD) [3], total suspended solids (SST) [4], optical microscopy (OM) for fibre identification and evaluation of image quality [5, 6] are recommended. For what concern airborne microplastics the reference methods are those used for dust and particles emission in the work environment and in atmosphere [7].

### 2.2 Materials

Laboratory glassware is washed with demineralized water filtered on nitrate, acetate, mixed esters filters (0.45  $\mu\text{m}$  porosity). Moreover, anodisc composed of a high purity alumina (0.02–0.2  $\mu\text{m}$  porosity), silicon (5  $\mu\text{m}$ ) and gold polycarbonate membrane (0.8  $\mu\text{m}$ ) for  $\mu$ -FTIR and Raman analysis are used.

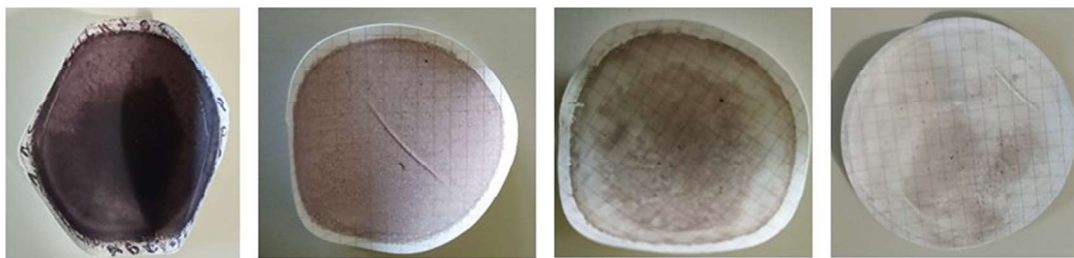
To avoid contaminations only glass caps are used. Furthermore, operator should preferably wear clothes made of natural fibres. In any case, contaminations can be taken into account performing blank tests. All reagents for preparation of the sample and cleaning of filters were RPE- for analysis.

### 2.3 Methods

#### 2.3.1 Preparation of Samples

Preparation of samples depends also by the pre-screening tests. Pre-treatments can be necessary before or after filtration of the washing effluent with a 15%  $\text{H}_2\text{O}_2$  solution for a duration between 7–30 days with the aim of eliminating any trace of organic matter without damage MP including Fig. 1 shows some examples of hydrogen peroxide pre-treatments carried out on nitrocellulose filters.

Always depending on the pre-screening in OM it will be possible to decide to perform a pre-dilution with water from the starting sample in order to produce a sub-sample to be tested with micro-FTIR/Raman spectroscopy for the identification and counting of the MP.



**Fig. 1.** Examples of nitrate/acetate filters after different pretreatments

To obtain a homogeneous distribution of MP on the filter the water sample is first homogenized it is placed in vigorous stirring (or ultrasonication) at 30–50 °C for at least one night in order to improve the hydration of any MP present. Reducing the wall effect and the possible flotation due to the surface tensions and the hydrophobic nature of the MPs. The sub-sample aliquot will then be diluted to a factor that allows an adequate particle count in the final filter.

The dilution is carried out with pre-filtered demineralised water and by washing (with recovery solution) of the equipment (pipettes, cylinders, etc.) used for the collection and dosing of the primary sample.

### 2.3.2 Filtration

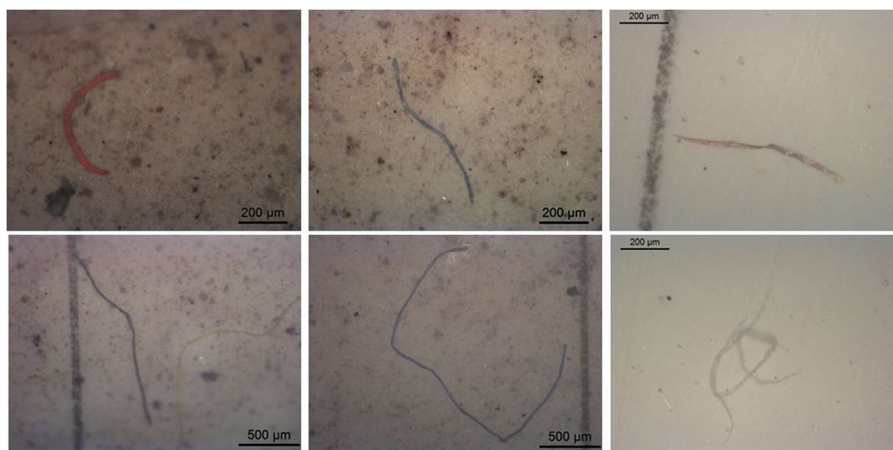
If the sample has a high presence of MP and suspended solids, even after the pre-treatment, the filtration will take place in two separate steps: filtration of the decanted supernatant, filtration of the final deposit (precipitate) with recovery by final washing operation. The subsequent analysis will be conducted on the two filters produced and the final report must consist of the sum of the results obtained. In order to be able to determine the MP in a matrix, a significant aliquot must first be transferred to a suitable filter (made with a vacuum system) to allow the subsequent quali-quantitative analysis. Consequently three different approaches must be followed depending on the physical state of the starting matrix: a powdery solid or a compound of several solid materials, an aqueous solution (liquid), an aeriform.

In the case of solid samples, if powdery type, a preliminary dispersion in a known volume of demineralised water or in a dispersing solution consisting of a surfactant in demineralised and filtered water will be carried out. If it is a non-powdered solid sample, it is first necessary to proceed with a suitable disintegration treatment (for example ultrasound and/or acid/basic/oxidant digestion). The filtration of a volume of the suspension will be carried out on a filter of suitable material, porosity and shape (as a function of the spectroscopic technique used). For the analysis of aqueous liquids proceed with filtration and related purification, washing and recovery procedures on 0.45 micron filters of cellulose esters.

In the case of aeriform samples, air will be sampled following the specific reference standards for the collection of dust in the air (work environment and/or emissions) using suitable filters for the subsequent analysis.

### 2.3.3 MPs Characterization

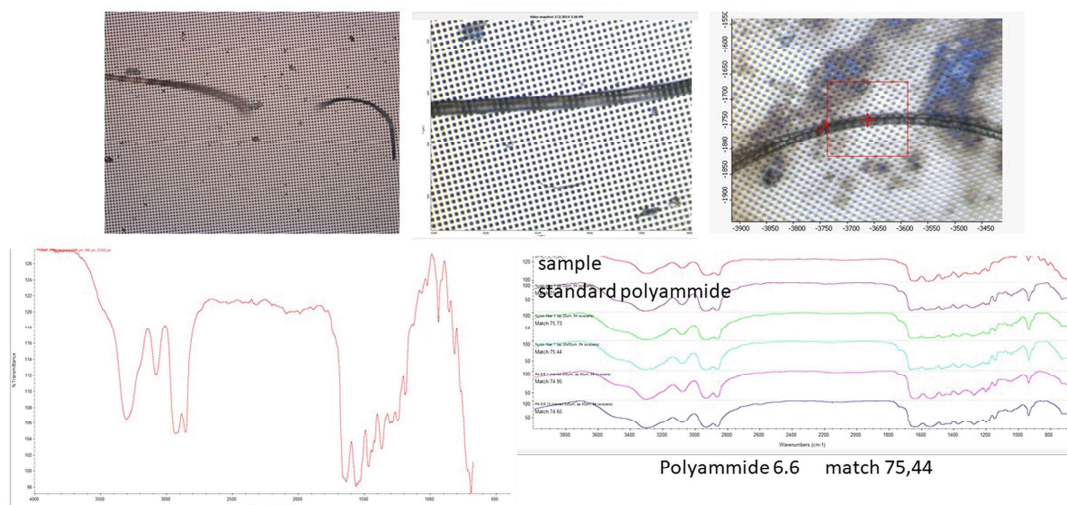
The microplastics are preventively observed and measured (length and diameter) with an optical microscope in reflected light at 50X and 100X magnifications on a filter that allows to detect the position of the particles for subsequent analyses and to exclude from the count any fibers (e.g. natural), contaminating particles and purification level of organic substances. Figure 2 shows some examples of microplastics in fibrous form, but it is possible find also particles or other forms.



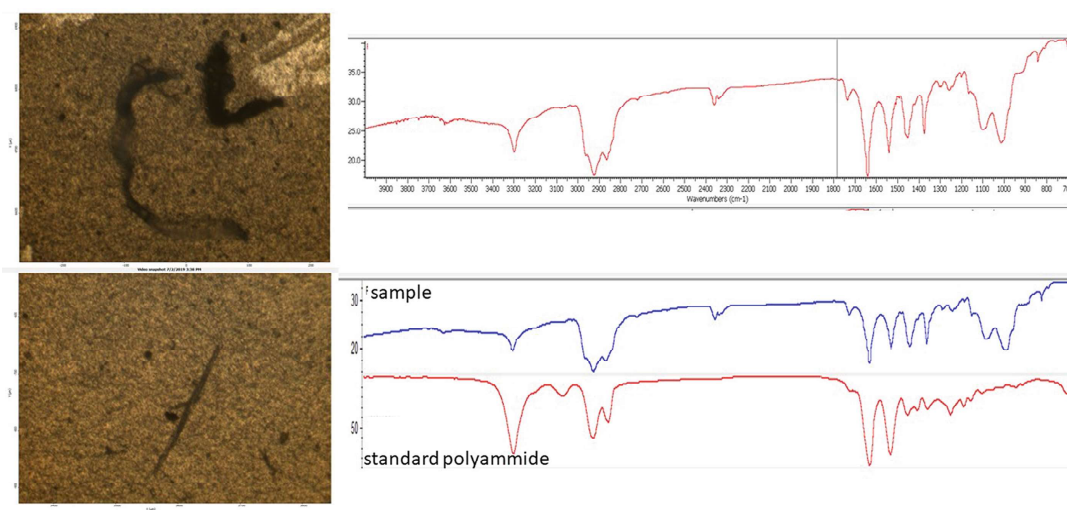
**Fig. 2.** Examples of microplastics observed in OM

Then a known volume of the previously treated aqueous dispersion will be filtered on a suitable filter (e.g. silicon filter) for identification and counting of microplastics present with spectroscopic techniques. For the identification and quantification of microplastics two molecular spectroscopic techniques can be used: micro-FTIR (for microplastics up to the size of 5–10  $\mu\text{m}$ ) or micro-Raman (for microplastics up to the size of 0.2–0.5  $\mu\text{m}$ ). In the case of micro-FTIR, to identify materials (microplastics) as small as 10 microns speed, resolution, and analytical power are increased using a liquid nitrogen cooled MCT detector. The image analysis software measures particles size, sets best fit aperture, collects spectra and background and compare spectrum with libraries. Particle analysis is simplified obtaining material identification, size, percentage of distribution and chemical image of particles within an area. Depending on the analysis performed, the most suitable filters can be selected. For transmission mode alumina or silicon filters are employed (Fig. 3), while in reflection mode gold coated filters can be used (Fig. 4). For attenuated total reflection (ATR) mode cellulose nitrate/acetate filters (porosity not higher than 5  $\mu\text{m}$ ). One of the advantages of  $\mu$ -FTIR and/or Raman identification of microplastics, is to avoid over estimations due to the possible presence of non-plastic material in the sample, such as cellulose fibres.

The image analysis aims to acquire maps and mosaics on specific areas of the sample, identify the particles to be analyzed by size and shape and to determine other characteristics such as area and length. It can be both manual or automatic (Fig. 5). In this last one, the visual image consists of more than 200 video captures combined into a



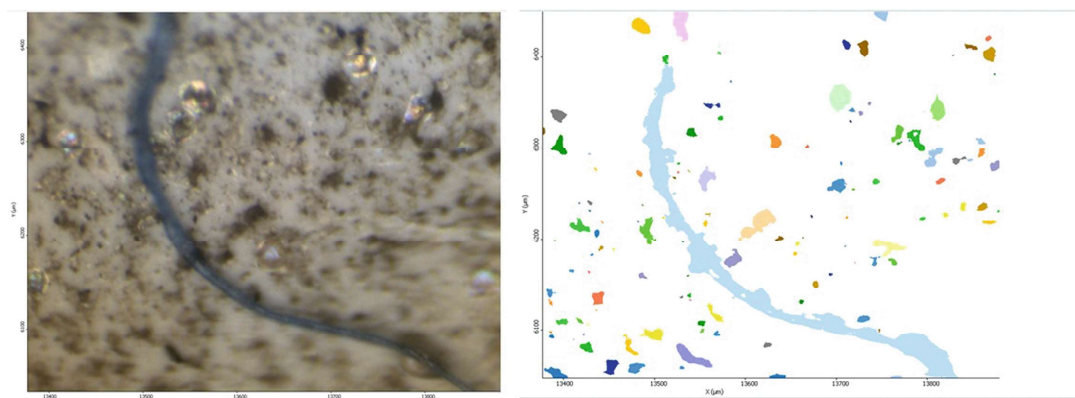
**Fig. 3.** Examples of microplastics and spectrum on silicon filter in transmission mode



**Fig. 4.** Examples of microplastics and spectrum on polycarbonate filter in reflection mode

mosaic covering approximately  $1 \text{ cm}^2$ . After a region from the video image is selected, the software identifies the target particles and proceeds to produce spectra for each particle/microfiber. These spectra are then searched against a spectral library, and a report catalogs the number of particles/fibers in the inspection area and classified according to their morphology and size, providing additional information on the sample such as: percentage distribution, morphology, number and size, mapping, section area estimation of microparticles and microfibres.

The other analytical technique used is micro-Raman spectroscopy, that exploiting sub-micron wavelength lasers as its light source is capable of resolving particles down to  $1 \mu$  and less. The other advantages compared to micro-FTIR are the ease of sampling, the use of white-light microscopes that facilitate easy viewing of particles and the



**Fig. 5.** Examples of automatic image analysis

non sensitivity to the presence of water. The major drawbacks are possible fluorescence in the samples (intrinsic or caused to impurities) that can disturb the acquisition of Raman spectra and the long time of acquisition due to the low Raman energy emission. However, the interference of fluorescence can be circumscribed with the chose of appropriate purification step before analysis and selection of acquisition parameters such as laser wavelength, laser power, photo bleaching, increasing exposition time in order to degrade the contaminants at the focal point. Moreover, the time consuming can be reduced with an automated procedure for the recognition of particles and automated measurements.

No limitations on the material of filters are reported. In general, depending on the different kind of spectroscopy used, the dimension of filters can vary in terms of shape (circular, square) and dimension (13, 25, 47 mm of diameter or 10 mm side) [8].

### 2.3.4 Optical, Micro-FTIR and/or Raman Test Report

The test report will consists of a summary table indicating: morphological and dimensional classes of microplastics, types of polymers found, estimated area (expressed in  $\text{mm}^2/\text{unit}$  of sample analyzed) per each polymer type, estimated weight (expressed in  $\text{mg}/\text{unit}$  of sample analyzed) per each polymer type. Eventually test report can include also photographs of the samples, optical images, particle mapping images, spectra, summary charts of the different classes identified (morphological, dimensional, polymer types) (Fig. 6). Moreover, the classification of MP analyses into size classes was carried out following recommendation of TR21960 ISO This classification is based on a numerical model and the “historic definition” of MP. The following size classes are proposed: 5,000–1,000  $\mu\text{m}$ , <1,000–500  $\mu\text{m}$ , <500–100  $\mu\text{m}$ , <100–50  $\mu\text{m}$ , <50–10  $\mu\text{m}$ , <10–5  $\mu\text{m}$ , <5–1  $\mu\text{m}$ . The maximum dimension of a particle or film fragment or the length of a fibre defines the size.

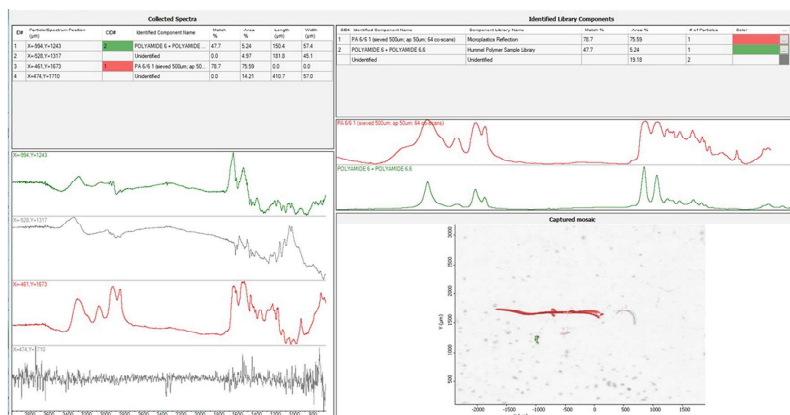


Fig. 6. Examples of one report from IR software

Figure 7 shows an example of classification of MP of water effluent textile industrial carried out by optical microscopy. In this case the length of microfibrils is between 1000–100  $\mu\text{m}$ .

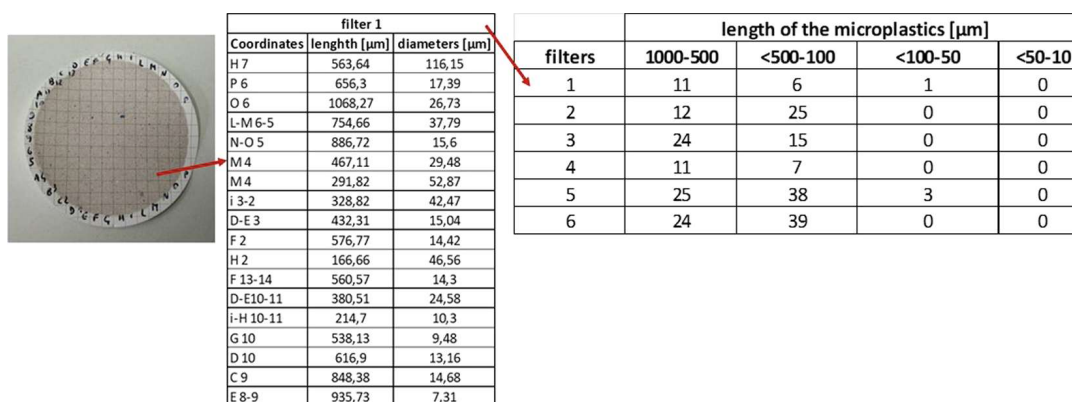


Fig. 7.

### 3 Conclusions

In this work, made in collaboration between Aquafil Spa and CNR STIMA, a draft of a standard method to identify and quantify microplastics deriving from waste water from clothes washings, water effluents or solids from industrial processes and air is described. Different starting matrices and sample pre-treatments are considered and optical microscopy and molecular spectroscopy (micro-FTIR and micro-Raman) are used in combination as analytical methods. The work is in progress and a first version has already been presented to UNI/CT 046/GL 12 “Sustainability” and it will be soon evaluated by a CEN (European Committee for Standardization). Once set up, it can be foreseen that this method could be expanded and made suitable for the analysis of microplastics of different origins.

## References

1. ECHA: European Chemical Agency: Proposal for a restriction (20/03/2019). <https://echa.europa.eu/documents/10162/0724031f-e356-ed1d-2c7c-346ab7adb59b>. Accessed 02 July 2019
2. Agency for environmental protection and technical services: APAT 2030, manual 29 (2003)
3. Agency for environmental protection and technical services: APAT 5135, manual 117 (2014)
4. Agency for environmental protection and technical services: APAT 2090B, manual 29 (2003)
5. Regulation (EU) No 1007/2011 of the European Parliament and of the Council of 27 September 2011 on textile fibre names and related labelling and marking of the fibre composition of textile products
6. UNI CEN ISO/TR 11827: Textiles - Composition testing - Identification of fibres (2012)
7. UNI EN 689 (1997), UNI EN 13284-1 (2017)
8. Hartmann, N.B., et al.: Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environ. Sci. Technol.* **53**, 1039–1047 (2019)