Chapter

Round Robin Test on Microplastic Counting and Identification Method

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Abstract

The aim of this work is to verify the quality, robustness, and accuracy of a standard analytical protocol for the determination of microplastics in aqueous textile matrices. In order to reach this objective, a round robin scale identification and quantification test program was conducted. In particular, this chapter describes the round robin test, an interlaboratory comparison test on standard microfilament suspensions initiated in November 2021 by an expression of interest open call. In total, 18 laboratories expressed their interest, and 13 participants sent their results. Each of these laboratories received a set of 10 samples, accompanied by a protocol. The 10 samples consisted of three replicates per type of three different synthetic yarns and a control sample**.** The data required were the number of microplastics per sample recognized as fibers or particles, microplastic fiber lengths and diameters, and identification of the polymer using vibrational spectroscopy (μ-FTIR and/or μ-Raman). The data collected were statistically elaborated. The results highlighted that the laboratories had different recovery rates directly related to their specific procedures and equipment. Although there were issues related to the correct use of the standard method and to the behavior of operators, the method proved to be valid for the determination of microplastics in aqueous matrices.

Keywords: standard microplastic suspensions, interlaboratory test, quantification of microplastics, vibrational spectroscopy

1. Introduction

Microplastics are considered to be emerging pollutants in aquatic and terrestrial environments. Microplastics are generally defined as particles with dimensions in the range of 5 mm, and this term denotes microscopic plastic particles such as fragments, beads, or fibers [1]. They have been detected worldwide and are currently present even in remote areas such as Antarctica [2].

In recent years, a particular kind of microplastic has gained the attention of researchers and scientists after its problematic occurrence in the water environment. Indeed, among the different microplastic forms, studies have demonstrated that the

predominant shape is the fibrous or the filament form in marine and freshwater ecosystems [3, 4]. Microplastic fibers, also called microfilaments, can be produced by the fragmentation of large plastics, in particular from textile garments [5]. Many synthetic and natural microplastics are released from textiles during domestic or industrial washing processes [6]. Out of all the released microfilaments found in the environment, the synthetic ones play a crucial role as pollutants of the environment. Polyesters (PET), acrylics (PAN), and polyamides (PA) are the major contributors [7]. Thus, all the source of microplastic in water are summarized in **Figure 1**. Synthetic microplastics are released from different sources, such as personal care products, city dust, and textiles [8]. Specifically, textiles can be a source of microfilaments during their production, use, and disposal stages. The mechanical abrasion and the physical stress applied to garments in any life stages are responsible for the shedding of microplastics with a fibrous shape [9].

Furthermore, domestic filters and wastewater treatment plants (WWTPs) are sometimes unable to trap them totally. It has been estimated that significant numbers of microfilaments escape from the traps and up to 40% can enter rivers, lakes, and oceans downstream [10]. Moreover, the sludge removed from treatment plants is usually stored or landfilled, allowing microfilaments to reach the environment again.

Despite the concerns about microplastics, the consumption of synthetic textiles is constantly growing, mainly due to fast-fashion trends. For instance, more than 45 million tons of polyester garments are produced every year [10]. Consequently, the world-released microplastic keeps rising, especially in marine and freshwater ecosystems [11]. Fortunately, several scientists worldwide have been moving in this direction in recent years to identify and limit microplastic pollution.

In particular, the occurrence of microfilaments in the aqueous environment is causing increasingly colossal concern. For this reason, in order to have a clearer view of microfilament pollution, a brief overview of the potential identification method is proposed.

1.1 Overview of qualitative and quantitative identification methods

In literature, different approaches for the determination of microplastics in aqueous matrices are reported [12]. They are chosen according to the data to be obtained and their usefulness. Mainly, several methods are used to acquire microplastic data such as color, size, shape, composition, and chemical concentration expressed in terms

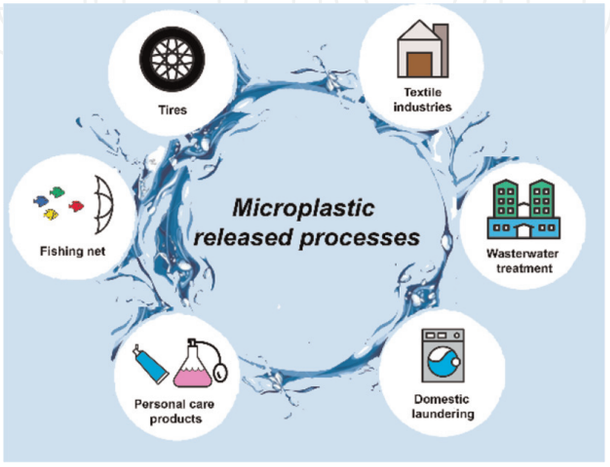

Figure 1.
Source of microplastics from textiles sources.

of number, weight, or size per unit volume or area. However, in order to obtain reliable and reproducible results, it is necessary to eliminate all possible contaminants that may interfere with the acquisition of such data.

Usually, the analysis of microplastics, as suggested by some guidelines [13], requires several steps that mainly include 3 phases: sampling, sample preparation, and determination of the type of microplastic polymer. Sample preparation is preceded by purification treatments that can be chemical or physical and are used to obtain suspensions of microplastics with reduced presence of organic and inorganic contaminants. This approach acts as a bridge between sampling and detection of microplastics as its effects influence the analytical quality of the final data in relation to specific pretreatment conditions and volumes. The identification system is applicable to any type of sample containing microplastics. An appropriate analytical approach for the identification of microplastics is shown in **Figure 2**.

2. Analytical approaches for the determination of microplastics

Microplastics are synthetic polymers of a wide variety of different shapes and colors. The choice of the analytical approach to characterize them depends on the data to be obtained in order to estimate their impact on the environment and human health. Scientific literature proposes a wide variety of techniques that provide data ranging from morphological characterization to the determination of their concentration and polymer type. The data obtained are closely related to the technique used to obtain them. Visual inspection techniques (optical and electron microscopy) are generally used for the study of morphology, color, and counting, while the study of composition is carried out by means of thermoanalytical methods, molecular spectroscopy (FT-IR and Raman), and liquid chromatography as shown in **Figure 3**.

Visual sorting is a method based on observing and counting microplastics with a stereomicroscope or optical microscope. This method allows for an error of over 70% for particles smaller than 50 μm and false positives for fibers larger than 200 μm [14, 15]. Environmental aqueous matrices are rich in cellulosic or fibrous protein material, which can be mistaken for degraded plastic material. Therefore, the lack of recognition of the chemical nature of the polymer leads to possible errors. In some cases, optical screening involves the use of dyes to increase the accuracy during visual inspection. For example, some authors identified microplastics such as PE

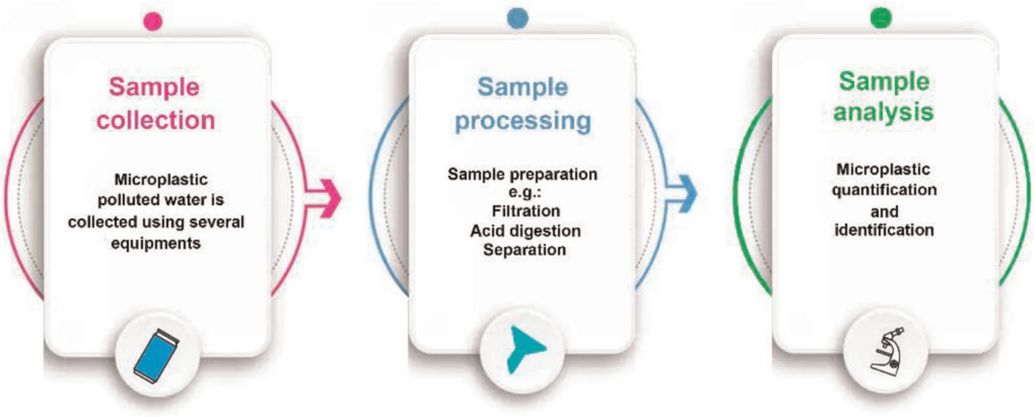

Figure 2.
Identification system for microplastics.

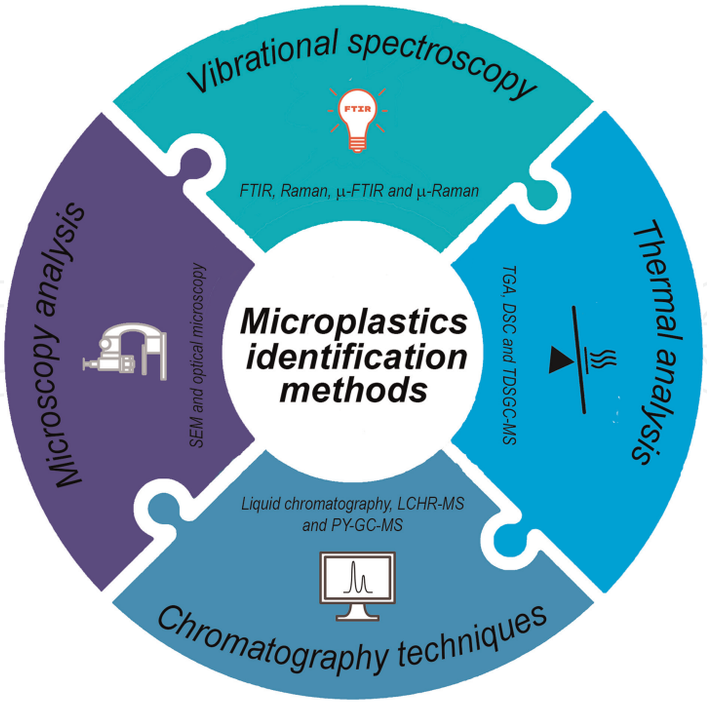

Figure 3.
Microplastic identification methods.

(polyethylene), PS (polystyrene), PP (polypropylene), and nylon 6 by using Nile Red fluorescent tagging [16, 17]. However, the coloring does not highlight polymers such as polyurethane (PU) and polycarbonate, and therefore, it limits its use.

In comparison with optical microscopy, scanning electron microscopy (SEM) can give images at high resolution of morphology, examine surface condition, and provide a qualitative determination of the chemical composition by energy dispersive x-ray spectroscopy (EDS) [18]. It is a technique coupled with SEM microscopy, generally used for the identification of organic material with high content of inorganic minerals and salts (Ca/Mg/Sr) and microplastics rich in chemical elements such as C/Cl/S/Ti.

At the same time, some authors proposed chromatography techniques coupled to high-resolution mass spectrometry (LCHRMS) for the quantification and chemical identification of microplastic (e.g., PS in natural waters). However, this technique does not provide data concerning the shape and size [9, 19].

Within visual sorting, the gravimetric method can be used to quantify microplastic particles or filaments with different sizes [20] in samples with high water volumes such as wastewater from laundry machines.

During a washing cycle of different synthetic standard fabrics or clothes at different operative conditions, the gravimetric method can be used for the determination of the mass of microfilaments released through a sieve at predeterminate porosity [6, 7, 21, 22].

At present, thermal techniques such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), sometimes coupled with chromatography-mass spectrometry (TDSGC-MS) or pyrolysis (Py-GC-MS), are used for the quantitative determination of microplastics [12, 23].

The samples are subjected to high-temperature treatments in order to produce thermal degradation, and the volatile compound products are analyzed for their polymer identification by means of a mass spectrometer. However, these techniques

have the disadvantage of being able to analyze only samples with a size >500 μm, and this prevents the determination of microplastics. The technique requires basic sample preparation, compared to others, but it is destructive and does not allow for multiple or parallel analyses. In this field, liquid chromatography can be used, too. However, it requires high quantities of sample volumes for the preliminary extraction step of the sample. Moreover, it only provides reliable results for a limited number of polymers such as PET and PE.

The drawback of all these analytical techniques is that they work for the identification of polymer nature and other additives such as, UV or thermal stabilizers, flame retardants, dyes antioxidants, plasticizers, and so on, but they do not give any information about the physical characteristics such as shape, number of microplastics, or color [24].

Other analytical methods available for the identification of microplastic are vibrational spectroscopic techniques, such as mid-infrared (FTIR), near-infrared (NIR), and Raman spectroscopy. FTIR and Raman spectroscopy are complementary techniques that transform the interaction of light with the sample into a spectrum that contains all the information about the chemical structure [25]. Raman spectroscopy is sensitive to the variation of the polarization of the molecule during vibrational motion, while FTIR is affected by the variation of the dipole moment in reflection, transmission, and total attenuated reflection (ATR).

Raman is a fundamental technique for the recognition of aromatic compounds and double bonds, while FTIR is used for the identification of polar functional groups of molecules such as hydroxyl, carbonyls, carboxyl, amino groups, and so on. Both are nondestructive techniques; Raman can be performed on any type of matrix, even liquid; it requires minimal sample preparation and allows for the identification of contaminants of inorganic nature, too. The Raman and FTIR techniques can be coupled with an optical microscope and, at the same time, be applied for sorting and recognizing microplastics. μ-FTIR can be used for the identification of particles larger than 10 μm and μ-Raman for particles larger than 0.2 μm [26]. Moreover, SEM or AFM (Atomic Force Microscopy) in combination with infrared spectroscopy can be used to visualize and chemically identify microplastics [27]. Although many approaches can be applied for the quantification and identification of microplastic, there are no precise guidelines to follow in relation to microfilament identification. In this chapter, a standard protocol for identification of microplastic with fibrous shape is proposed by using μ-Raman and μ-FTIR, respectively, or in a complementary approach [26, 28].

2.1 Analytical approach for the determination of microplastic with fibrous shape

The analytical approach for the determination of microplastics are summarized in **Figure 4**. Typically, it involves three main stages. The first step is related to the sampling of microplastics coming from wastewater; the second step is related to the sample preparation and the third to the choice of an appropriate detection method to identify all their characteristics.

The standard protocol proposed is used to carry out qualitative and quantitative analyses of fibrous microplastics in textile aqueous matrices by means of analytical vibrational spectroscopy techniques (μ-FTIR and μ-Raman).

Textile wastewater is a complex matrix because it is rich in organic material, microplastics with fibrous shape, dyes, salt, fiber contaminants (natural fiber), and activated charcoal from the production or finishing processes of the fiber. In order to obtain information on the nature of the polymer constituting the microplastics,

Figure 4.
Identification system for microplastics in the textile sector.

particularly when spectroscopic techniques are used, it is necessary to reduce any interference from other substances. In this regard, the analytical protocol provides suitable information on the reduction of contaminants during the sample preparation.

The following steps have been identified:

- Preliminary identification in the sample of chemical-physical parameters such as conductivity, chemical oxygen demand, and total suspended solid (TSS);
- Optical pre-screening of samples;
- Pre-treatment according to preliminary results with oxidation/digestion, acid/basic treatment, sonication, and so on;
- Filtration of microplastic samples through filters;
- Characterization of microplastics: counting and identification by optical microscopy and molecular spectroscopy.

In addition, a protocol for the preparation of standard microfilament suspensions was also prepared to facilitate the control of the whole laboratory tests (counting, monitoring, and identification of microplastics). This aspect is an added value of the method because standard fibrous microplastics with established dimensional and structural parameters are not available in the market. For this purpose, four standard suspensions of the most commonly used synthetic fibers such as PA6, PA 6.6, PP, and PET were prepared [29].

In conformity with ISO 5725 and ASTM E691 standard procedures, the protocol was validated by means of a round robin test (RRT). However, during the preparation, it was not possible to use samples from textile wastewater obtained from production processes or washing machines as they are not homogeneous and highly variable.

In order to reduce their variability, 3 replicates of 3 standard water suspensions of PA 6, PET, and PP at different concentrations were used.

3. Round Robin test

3.1 What is a RRT?

The identification and counting of microplastics face challenges due to the complexity and heterogeneity of the materials. These challenges are also paired with the

lack of referenced certified methods and standards for microplastic analysis. In the absence of accredited procedures and standards, the robin round test (RRT) is one of the best approaches to identify and quantify microplastics.

The RRT is an advantageous approach since it is specific, pre-defined, and requires the involvement of several labs worldwide. It involves the presence of an organization that is able to supply samples and precise instructions and consecutively to evaluate the lab results [12]. Thus, RRT allows for the determination of the reproducibility of a process or analysis by means of multiple analyses performed independently. The statistical elaboration of the results provides a top-down evaluation of the variability by analyzing the outcomes of different labs [30]. Thus, the benefits and applicability of the RRT are diverse and can be strategically designed for various purposes.

In microplastic analysis, few studies carry out the RRT for the comparison of the results. For instance, Muller et al. presented the results of an international comparative study on the common analytical technique used in microplastic analysis [31]. Since there is a large discrepancy between the 17 labs involved in the study, the study pointed out the urgent necessity of a standardized method for microplastic analysis.

A novel approach to microplastic analysis was proposed by Mossotti et al., who found out an optimized protocol for the determination of microplastic with fibrous shape in water [29]. Three different synthetic filaments cut at predetermined lengths can be used as internal standards for microplastic identification. For the first time, an analytical method for microplastic identification was subjected to a RRT involving 18 labs around the world.

3.2 Aim of STANDARD METHOD prEN ISO 4484-2 PROTOCOL

The purpose of this RRT is to identify with adequate accuracy the number and type of standard microfilaments in suspension.

All the analyzed data of the samples were divided into polymer groups, counted, and then compared with their targets, thus obtaining the evaluation of the accuracy and reliability of the method.

3.3 Round Robin test design

3.3.1 General information

The RRT on prEN ISO 4484-2 was carried out from January to March 2022, and the trial was organized by Aquafil S.p.A (Italy) and CNR-STIIMA (Italy).

18 laboratories based in 17 European countries and a non-European one took part in the RRT. The study participants were from Italy, Germany, the U.K, Sweden, Spain, and South Korea. In **Figure 5**, the number of participants from each country is reported.

The laboratories that participated in this study represent universities, research institutions, laboratory equipment suppliers, and laboratories owned by private companies, as reported in **Figure 6**.

10 samples divided into 3 sample types called Sample 1, Sample 2, and Sample 3 (**Table 1**) were prepared for each laboratory, and in addition, 3 replicates per sample were prepared. The tenth sample corresponded to the Control Sample.

Considering the RRT membership of 18 laboratories, a total of 180 samples were prepared. The preparation of the samples, in particular the cutting of the microfilaments, is described in detail elsewhere.

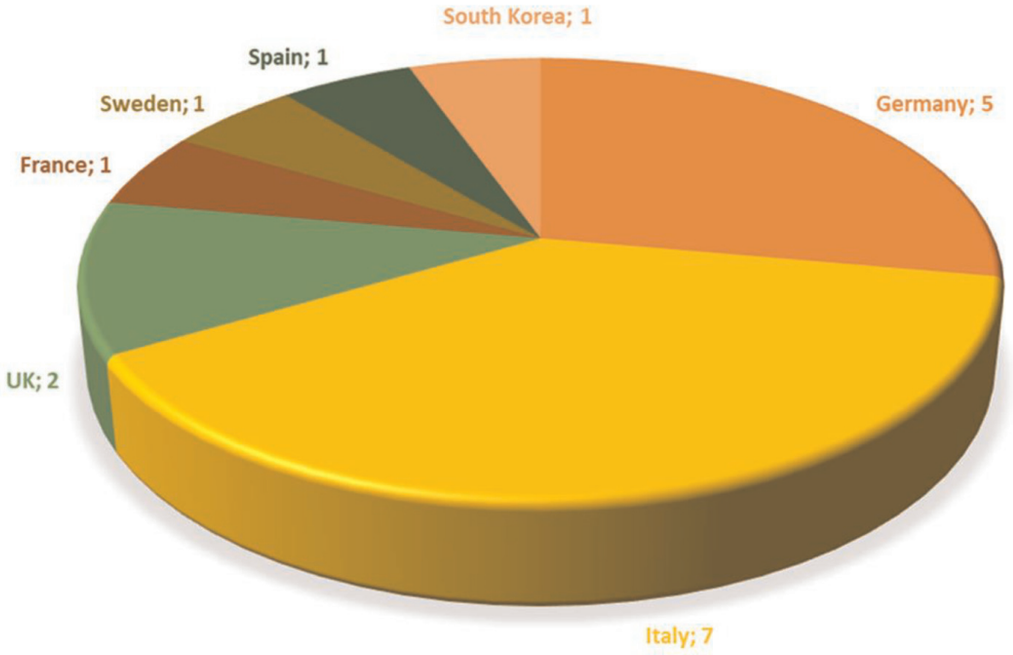

Figure 5.
Laboratories involved from different countries.

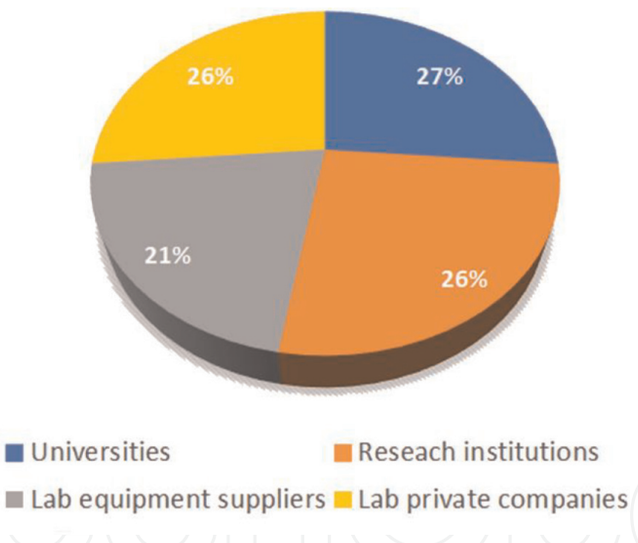

Figure 6.
The participants came from various sectors, including universities, research institutions, laboratory equipment suppliers, and laboratories owned by private companies.

After cutting, the procedure follows these steps:

a. The fibere are dispersed in 10 ml of demineralized water and 7 ml of sodium hypochlorite to remove the wool used for the microtome cutting. * ISO 1833-4:2017, Textiles-quantitative chemical analysis-Part 4: Mixture of certain protein fibers with certain other fibers.

b. The suspension was shaken in a 50 ml flask with a mechanical stirrer at 130 r.p.m. for 40 min at room temperature.

SAMPLES	STANDARD MF (microfilaments)
SAMPLE 1	MF 1 Yellow PA 6 thread (180 filaments; 3450 dtex).
SAMPLE 2	MF 2 White PP thread (80 filaments; 1300 dtex).
SAMPLE 3	MF 3 Cream PET thread (256 filaments; 2970 dtex).

Table 1.
Microfilaments used for the preparation of the standard samples.

c. The vial was washed with 50 ml aliquots of demineralized water and subsequently with a 40 ml aliquot of H_2O/ethanol (1:1) in order to recover any fibers left attached to the walls and transfer them to the bottle.

d. The sample was filled with demineralized water to a volume of 500 ml.

The described procedure is reported in **Figures** 7 and 8.

Each participating laboratory was provided with the analytical protocol (see Annex) to be followed in order to perform the determination of the microfilaments contained in the standard samples, as well as instructions for the results. For each

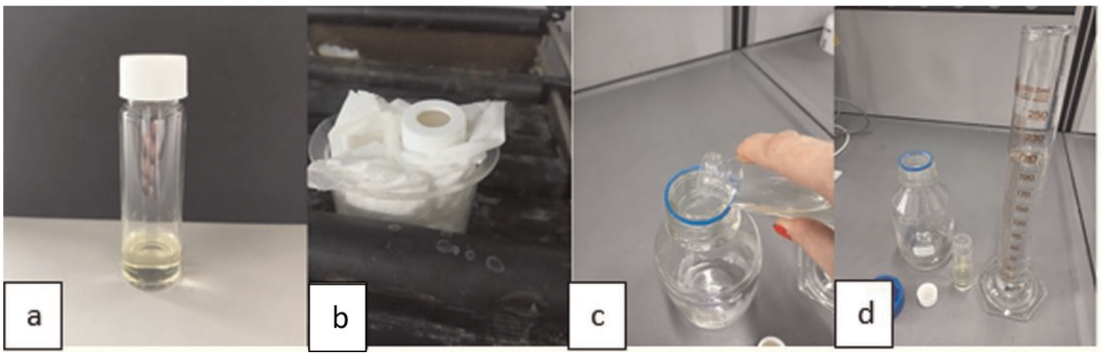

Figure 7.
Procedure for the preparation of standard sample.

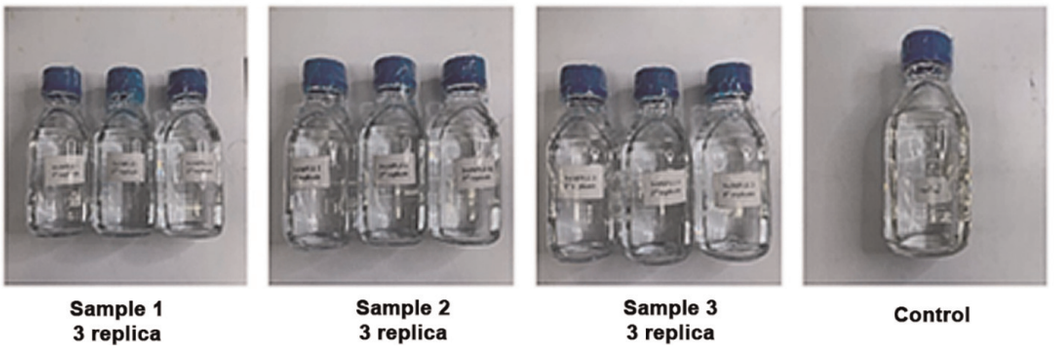

Figure 8.
A total of 10 samples were prepared for each lab.

sample, the number of total microplastics present in the sample, polymer type, and physical characteristics (microfilament lengths) had to be reported. In addition, the presence of contaminants had to be described.

4. Data collection and analysis

4.1 Working conditions

Table 2 shows the working conditions used by the laboratories when available. As shown in **Table 2**, the main drawbacks reported by laboratories are:

- Two labs used LDIR equipment (laser direct infrared imaging).
- One lab used Sterlitech silver filters.
- One lab decided to use only one filter per replicate, which made it difficult to filtrate the water sample and subsequently to count the microfilaments.

Lab.	Instrument	Filter material	Filter dimensions
Lab1	Micro-FTIR iN 10 Thermo	Silicon	Diameter 13 mm
			Pore size 1 μm
Lab2	Micro-FTIR: reflection	Cellulose acetate nitrate	Diameter 47 mm
			Pore size: unknown
Lab3	Micro-Raman (Horiba XploRA Plus)	Silicon	Diameter 13 mm; pore size 5–6 μm
Lab4	Micro-FTIR: transmission (iN 10 Thermo)	Silicon	Diameter 13; pore size 5–6 μm
Lab6	Micro-FTIR (iN 10 Thermo)	Allumina	Diameter 47 mm
			Pore size 0.2 μm
Lab7	Micro-FTIR (LUMOS II, Bruker, USA)	Silicon	(1) Diameter 10 mm; pore size 17 μm
	Micro-Raman for second filtration (XploRA PLUS, Horiba, France)		(2) Diameter 10 mm; pore size 5 μm
Lab9	Micro-FTIR (micro-ATR) (PerkinElmer Spotlight 400)	Sterlitech silver	Diameter 25 mm; pore size 3 μm
Lab10	8700 LDIR	Au-coated polycarbonate	Diameter: Unknown; pore size 0.8 μm
Lab11	8700 LDIR	Polycarbonate	Diameter: Unknown; pore size 8 μm
Lab12	8700 LDIR	Metal	Diameter 47 mm; pore size 20 μm
Lab13	Micro-Raman (XploRA Plus Microspectrometer, Horiba Scientific).	Esters of Cellulose	Diameter 47 mm: pore size 0.45 μm
Lab16	Micro-FTIR (PerkinElmer Spotlight 400)	Sterlitech silver	Diameter 11 mm; pore size 3 μm
Lab17	Micro-Raman	Silicon	Diameter 13 mm; pore size 1 μm

Table 2.
Laboratory working conditions.

- One lab decided to carry out two filtration steps: the former with a 17 μm silicon filter and the latter with a 5 μm silicon filter. The former was analyzed by μ-FTIR and the latter by μ-Raman.

- One lab had difficulty in the identification and counting of the microfilaments in some replicates due to the presence of yellowish mush (probably wool not completely degraded during treatment with hypochlorite solution).

- One lab highlighted the presence of silicates and iron probably due to contamination of demineralized water.

5. Results

All the laboratories processed the data as follows:

- Total count per target polymer type,

- Normalization of the data n° detected /n° target,

- Total count and normalization of nontarget material data.

5.1 Data analysis and visualization

After the collection of the data from each participant, the results were statistically elaborated. Actually, graphical descriptive analysis was used to compare the data obtained from the laboratories. They were not always homogenous as sometimes diverse working conditions were applied, and in few cases, some data were missing or incomplete.

5.2 Available data

Table 3 shows all the missing data in red. Eight laboratories could not perform the study due to several drawbacks, such as instrumental breakdown or unavailability of filtering systems.

5.3 Rate calculation

It was required to report all the microplastic materials found in each replicate. Since many microplastics not belonging to standard microfilaments were found in the suspensions, the recovery rate was evaluated by dividing the microplastics found in each sample by the number of standard microfilaments. This number was interpreted as the fraction of the total standard microfilaments over their theoretical quantity.

5.4 Recovery rates

Figure 9 shows the fraction of standard microfilaments found in each sample (Sample 1, Sample 2, and Sample 3) and for each replicate [1–3] obtained from all the different laboratories.

	Sample 1			Sample 2			Sample 3			Control
	R1	R2	R3	R1	R2	R3	R1	R2	R3	
Lab. 1										
Lab. 2										
Lab. 3										
Lab. 4										
Lab. 7										
Lab. 9										
Lab. 10										
Lab. 11										
Lab. 13										
Lab. 17										

Table 3.
Data collected from each laboratory. Red (missing data).

Only the relative fraction of the target material identified was calculated, and the absolute values were not reported.

If no evident problems of contamination occurred, the fraction of standard microfilaments was just above 0.5. However, some significant differences were found between the laboratory data. Lab 1 did not obtain accurate data for Sample 3 because during the filtration of the 1st replicate of the 3rd sample, the filter broke. The data of Lab 2 were not processable for each reference sample due to the incorrect acquisition mode of the spectra of the microplastics collected on the chosen filter. Moreover, the use of an automatic analysis system and the lack of a good reference spectral library could have amplified the mistakes. Lab 4 did not find any microplastic fibers in Sample 1 for all the replicates due to problems of working conditions during micro-FTIR automatic analysis identification, as indicated by the lab itself, for example,. selection of brightness and contrast. In fact, in this case, Lab 4 obtained data only for PP particle contaminants and not for microplastic fibers. This drawback determined a lower fraction target for all other samples Labs 6, 11 (replicate 1 of sample 2), and 16 had a recovery rate above 1, which means that they found a number of standard microfilaments higher than the present quantity. Laboratory 10 performed only one replicate for each sample and did not report anything different from the target material. Laboratory 17 did not carry out the analysis on two of the three samples and reported only the data of replicate 3 of Sample 2. The lack of results did not depend on the quality of the standard samples.

Labs 3, 7, 9, 11, and 13 reached good recovery rates for all the three samples analyzed. Labs 7 and 9 reached a recovery rate of 80–90%. The results highlighted that the labs that followed all the steps in the standard protocol reached the target fraction, confirming the quality and reproducibility of the method.

Moreover, the analysis of the results highlighted that the μ-FTIR and μ-Raman techniques also allow the identification, counting, and monitoring of the pollutants and their sources of contamination.

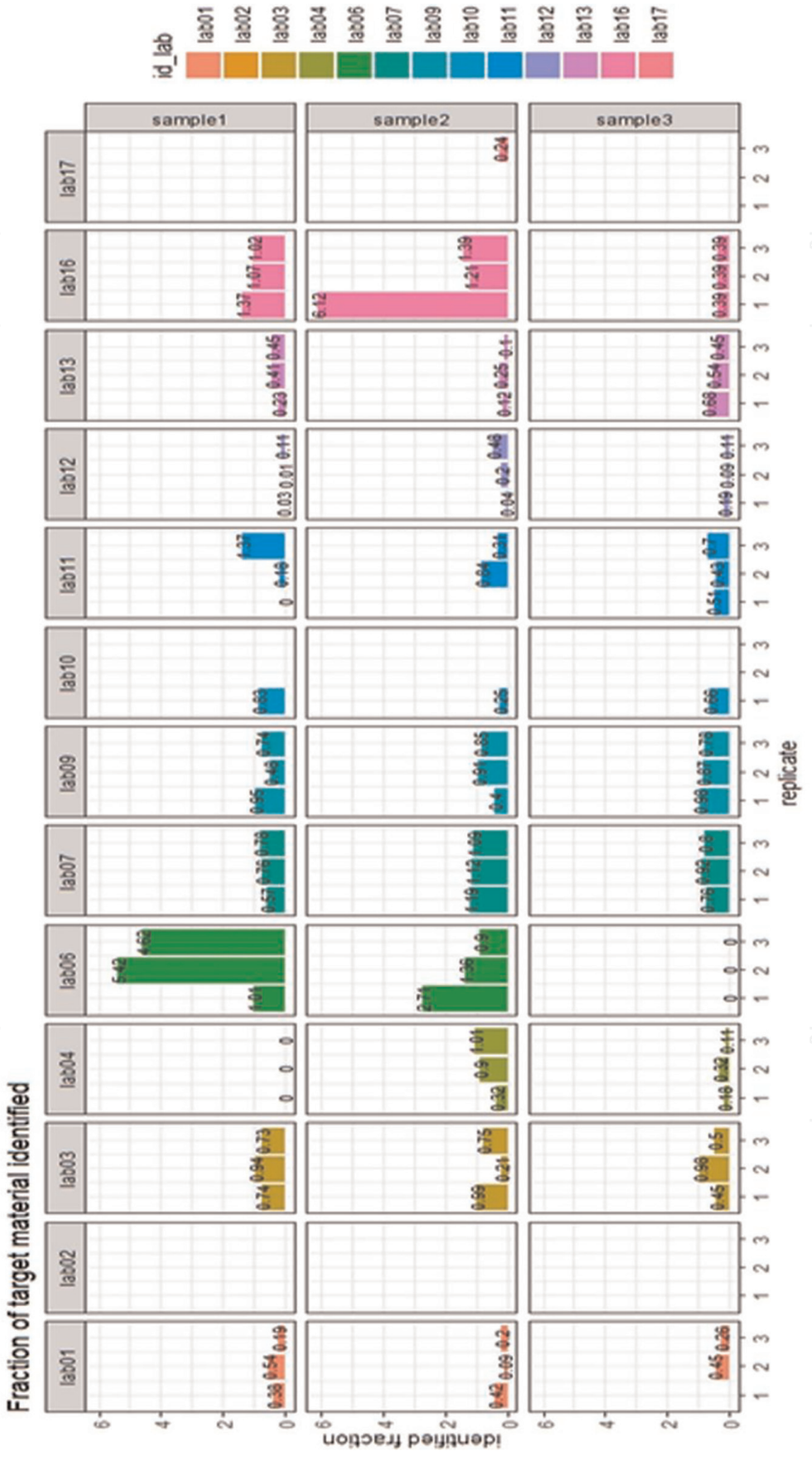

Figure 9.
Fraction of target material identified in the samples.

The main contaminants identified in analyzed samples were polypropylene (particles), natural fibers deriving from the storage system, sample preparation, and laboratory environment.

In particular, polypropylene particles were found by all the labs as a result of the containers and caps used in sample preparation. The presence of cotton fibers was identified, but it was not possible to understand whether it depended on demineralized water or dissolved wool tops.

However, when the nature of contamination was different, the source was the result of incorrect procedure. For example, one lab carried out additional sample manipulation before IR analysis, thus increasing the risk of microplastic loss and contamination (**Figure 10**).

Finally, a few participants did not perform the dimensional analysis of the microplastics identified in all the samples as suggested in the standard protocol. However, some dimensional data showed that the identified microplastic fibers were classified in the length range between 100 μm and 500 μm and diameters between 40 μm and 66 μm, confirming the control data.

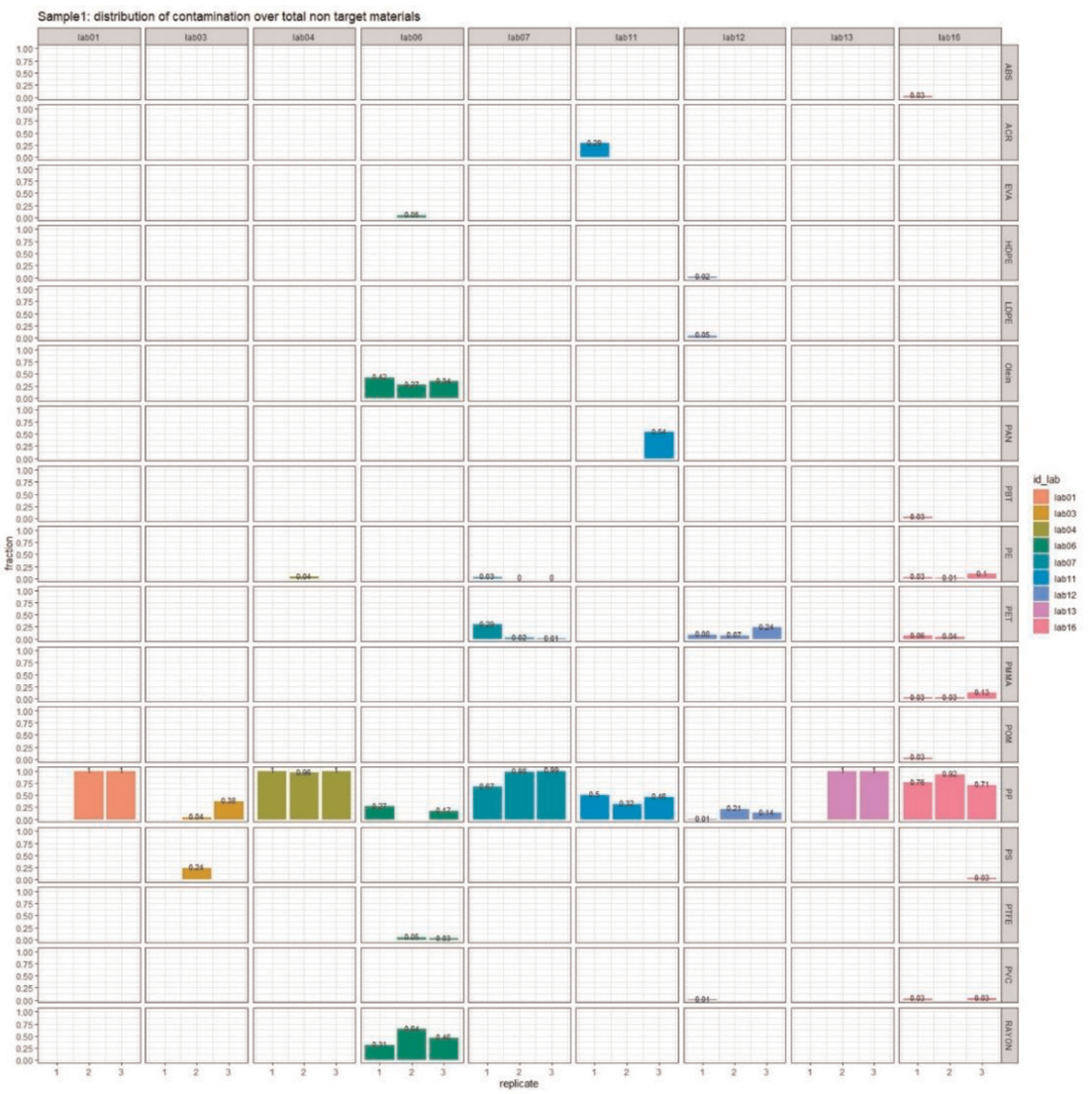

Figure 10.
Fraction of contaminant material identified in the laboratories.

6. Conclusion

This chapter has proven that the standard protocol 4484–2 allows for the determination of microplastics in aqueous textile matrices. Indeed, the round robin test was successfully carried out for an interlaboratory comparison test on the standard microfilament suspension. 18 laboratories expressed their interest, and 13 participants had sent their results. The data obtained were the number of microplastics for each sample recognized as microfilaments or particles, and identification of the polymer using vibrational spectroscopy (μ-FTIR and/or μ -Raman) had been performed. Moreover, some microfilament sizes were also indicated. The data collected were statistically elaborated by using the graphical descriptive analysis. The RRT statistical analysis has shown that the proposed protocol can be applied for the identification and counting of microfilaments in water suspension. It can be easily applied in routine analysis in order to verify the correctness of the microplastic identification procedure. Indeed, several laboratories reached a high value of recovery rate (85%), confirming the data obtained by Mossotti et al. [29]. Thus, the proposed protocol can be a suitable tool to evaluate the recovery quality of the single real sample as well as the presence of environmental contaminations.

Acknowledgements

The authors are thankful for the active support of this study by the participating laboratories that analyzed the standard samples provided, following analytical protocol, filled tables and written a test report.

Conflict of interest

The authors declare no conflict of interest.

Annex

A. STANDARD PROTOCOL (prEN ISO 4484-2)

prEN ISO 4484-2 protocol comprises:

- A.1 Filtration equipment
- A.2 Filters and their cleaning procedure
- A.3 Filtration procedure
- A.4 Results: counting, identification, and length range of microplastic with fibrous shape.
- A.4.1 Note
- A.4.2 Calculations

- A.4.3 Identification of microplastic with fiber by μ-FTIR and μ-Raman
- A.4.4 Measure of microplastic fiber lengths
- A.4.5 Test Report

A.1 Filtration equipment

The collection of microparticles with fibrous shapes is performed on one or more filters with porosity lower than the minimum diameter of the fibers used for the standard.

Equipment:

- Erlenmeyer flask containing standard solution
- Feeding funnel filter system
- Filters
- Vacuum pump
    - Filter materials: silicon, alumina, PC gold coated, cellulose acetate nitrate, or any other material with a circular or square shape.
    - The filter diameter is a choice of the lab depending on the filtration apparatus and on the spectrometer capability/availability.
    - However, in prEN ISO 4484-2 method, filter size and porosity are as follows:
    - In the case of μ-FTIR (reflection and transmission) analysis, the possible filters that can be used have a pore size not exceeding 5 μm.
    - In the case of μ Raman filters, the pore sizes that can usually be used are 0.45 μm, 0.8 μm, 1 μm, and 5 μm.
    - Vacuum pump (vacuum system filtration of 47 mm, 25 mm, 13 mm diameter, or any other diameter depending on the used filter diameter).

A.2 Cleaning procedure

- Store the filters in glass (not plastic) Petri dishes to reduce contamination from the dish itself.
- Keep filters covered whenever possible before observing the results.
- All filters must be new or clean before being used.

- Before filtration, check the filters by an optical microscope to verify that there are no interfering particles on the surface that could come from the packaging, from their handling, or from the production process itself.

- Cleaning depends on the type of filters. A physical procedure such as an ultrasonic bath or a chemical treatment by simple immersion in solvent (pure ethanol RPE) for 10 min can be used.

A.3 Filtration procedure

- Shake the bottle vigorously before filtration (possible clumps may have formed).

- Gradually filter all the stock suspension contained in the 500 ml glass bottles (50 ml at a time) through one or more filters.

- Shake the bottle each time before pouring the suspension to make sure that all the microplastic fibers on the bottle walls are removed and filtered.

- After filtering the entire stock suspension, wash the sample bottle including the cap and the funnel walls with a few ml of water/ethanol 1:1 using a glass Pasteur pipette in order to recover the possible microparticles adhering to the glass.

- Carry out the final recovery wash (of the filtering system, the gasket, and the flask containing the stock solution) with a 1:1 solution of water/ethanol and filter through Filter 2. (see below).

- The washing operations need to be repeated at least three times, each time using an aliquot of 50 ml of water/ethanol 1:1. Use an aliquot of the last wash with a glass Pasteur pipette to wash thoroughly the filter funnel, and gasket.

- One or more filters can be used to collect all the microparticles derived from all the filtering and washing procedures for each solution. Normally, we suggest to use:

- One or more filters where microparticles are collected from the filtration of the sample and from the solution of the first rinse of the flask and of the filtering system.

- One or more filter where microparticles are collected from the filtration of the subsequent rinses with the washing solution and from the solution of the subsequent thorough rinsing of the Erlenmeyer flask and all the components of the filtering system.

A.4 Results

For each replicate, the number of particles at a given volume is given by the sum of the particles collected on all the filters used for that sample (main solution and washing water).

A.4.1 Note

For more accurate results, we suggest counting the number of fibers on each section of the filter to avoid losing the count of overlapping fibers.

Zoom in and break the image into square sections to cover the entire filter, and then, manually count the microparticles in each of the sections and the number of fibers on each section of the filter.

The same procedure can be used to identify the chemical composition of microfilaments. As an alternative to manual counting, it is possible to perform automatic counting of microplastics with the software of the optical instrument.

A.4.2 Calculation

$$\text{N°filaments } (1)/\text{L} = \frac{\text{N°microfilaments} \times 1000}{\text{Solution volume}} \quad (1)$$

where N° filaments means number of filaments obtained by automatic or manual counting as sum of filaments counted on filters.

NOTE: samples have been prepared with an initial volume of 500 ml.

Tables (Test Report) show how the data shall be entered for each sample analyzed:

- Name of the sample
- Number of replicates
- N° of total synthetic filaments (sum of fibers counted on FILTERS), from the filtration of the whole solution, washing phase of the flask, and the filtration system with water and ethanol 1:1.
- N° of total synthetic filaments (sum of fibers counted on FILTERS), from the filtration of the whole solution, washing phase of the flask, and the filtration system with water and ethanol 1:1.

A.4.3 Identification of Fibers by μ-FTIR and μ-Raman

The operative conditions of identification and analysis of microfilaments can be optimized both with μ-FTIR and μ-Raman instrument (full mapping and/or particle by particle). FTIR can be carried out using different kinds of detectors (single detector, line array dector, focal plane array (FPA) detector).

A.4.4 Measure of microplastic fibers lengths

Moreover, the measurements of the microplastic fiber lengths and diameters can be carried out with

- Optical microscope in reflected light at 50× and 100× magnifications on a filter
- Optical microscope coupled with FTIR or Raman spectroscopy

- (*) Please define the dimensional aspect of the length in classes as in **Table 4** and also refer the diameter in microns
- 5000–1000 μm,
- <1000–500 μm,
- <500–100 μm,
- <100–50 μm,
- <50–10 μm,
- <10–5 μm,
- <5–1 μm. (only for Raman, if present),
- <1–0.1 μm (only for Raman, if present),
- (**) In this area, also report, if present, any microparticle not fibrous shaped or any presence of nonsythetic fibers

Sample 1	Replicates	N° of total synthetic filament	Identification synthetic component	Range dimensional classes (*)	Others (**)	N° filaments/L
	1°					
	2°					
	3°					
	Average					

Table 4.
Example of test report to have for each sample.

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