Chapter

Preparation and Analysis of Standard Microplastics

Raffaella Mossotti, Giulia Dalla Fontana, Anastasia Anceschi, Enrico Gasparin and Tiziano Battistini

Abstract

Over 14 million tons of microplastic have been accumulated in water resources and they are increasing yearly. About 8% of European microplastic released into the water are from synthetic textiles. This kind of microplastic is generally in the form of microfilaments. They have a higher potential to enter the food chain due to their size and shape. Although microfilaments generate great concern, no precise guidelines for their quantification and qualification are yet available. Thus, in this chapter, the origin of microfilaments is fully investigated. After that, a novel approach for identifying and counting microplastic with fiber shape is presented. An accurate method for preparing microfilament standard suspensions is described to facilitate lab tests and have a reliable methodology for monitoring microplastic pollution.

Keywords: textiles, synthetic thread, microplastics with fiber shape, standard suspension, quantification

1. Introduction

This chapter presents a reliable method for preparing standard microfilaments to facilitate lab testing and monitoring of microplastics in different matrices. The scope is to achieve a positive impact on the quality control of all operations, from sampling to counting and identification. Using standard synthetic microfilaments as references for the validation of common experimental procedures could reduce differences between data. Furthermore, a standard synthetic fiber material would allow the monitoring of the ecotoxicological impacts of microplastics on biota and human health in line with the European Commission's Green Deal, the Circular Economy Action Plan and the proposed remedial actions supported by the U.N SDGs under Goal 14.

2. What is plastic?

Plastics are defined as synthetic organic polymers typically made from petrochemicals.

Specifically, synthetic polymer molecules consist of many monomers which react in different ways. Many simple hydrocarbons, such as ethylene and propylene, can be

transformed into polymers by adding monomers to the growing chain. The combination of these monomers creates various kinds of polymers. Other substances can be added to polymers to give the final product some desired characteristics [1].

Sometimes, the term plastic is also used to indicate blends with different synthetic polymers or other low molecular weight compounds such as additives, UV or thermal stabilizers, flame retardants, dyes, antioxidants, plasticizers, etc. [2].

Because plastics are considered chemically, physically, biologically stable and resistant materials, once in the environment, they can undergo degradation upon exposure to different factors, such as sunlight, water, and wind, and break down into tiny plastic particles known as microplastics. After fragmentation, they can be transported by wind and water due to their lightweight [3].

Thus, once in the environment, microplastics accumulate and persist. Consequently, they are ubiquitous in terrestrial, fresh water and marine environments [4]. The source of microplastics includes wastewater treatment plants, landfills, automotive tires, pre-production plastic pellets, synthetic clothing, road signs, and paint [5], (**Figure 1**).

Among all sectors, the textile one is considered one of the major sources of microplastic pollution.

Textile processes are responsible for 20% of global water pollution and the washing of synthetic garments contributes to about 35% of the global release of primary microplastics. These materials are not retained during the filtration systems of wastewater treatment plants (WWTPs) and, therefore, enter the marine ecosystem directly [6].

These microplastics occur in different forms (e.g., cylindrical, spherical, etc.) and partly escape the filtration systems of WWTPs, reaching seas and oceans directly. In this respect, the identification and classification of fiber fragments are necessary to spot any weak points in the textile production process and in the life cycle of synthetic garments. The release of microplastics can occur during the different processes and use phases, including spinning, weaving, finishing (gauzing, finishing, dyeing), packaging, wear, washing, drying, and finally, at the end of life, landfill disposal [7].

Figure 1.
Example of microplastic sources in water system (https://unsplash.com).

Figure 2.
Plastics spread in the environment (https://unsplash.com).

Hartline et al. have estimated that a WWTP plant with a 94% of removal percentage and considering 0.35 m^3 of wastewater per person a day would produce for 100,000 people about 1.02 kg of microfilaments (**Figure 2**) [8].

The term microplastics was first coined by R. Thomson in 2004 by observing micrometer-sized plastic fragments in marine sediments and then refined by Arthur et al. by setting size limits above 5 mm [9, 10]. Later in 2011, Cole et al. divided microplastics into two categories: primary ones produced at a micro size and secondary ones that only reach that size through fragmentation and degradation due to environmental biodegradation effects. In 2016, nano-sized particles were also included in the definition of microplastics GESAMP [11].

Although the definition of microplastics is still being debated, the current one follows the European Chemical Agency (ECHA), "a material composed of solid polymeric-containing particles to which additives or other substances may be added. The family of microplastics includes synthetic-based particles, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamides (PA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyacrylonitrile (PAN), polymethylacrylate (PMA), elastomers and silicone rubber with particles ranging from 1 nm to 5 mm and

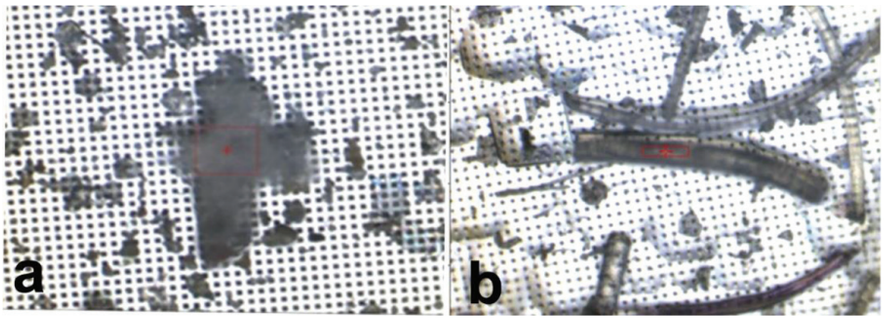

Figure 3.
Optical images obtained with an optical microscope coupled to MicroFTIR of a) polypropylene microparticle and b) polyester microfilaments.

fiber lengths ranging from 3 nm to 15 mm and a length-to-diameter ratio of > 3" [12]. The difference between particles and filaments is reported in **Figure 3**.

2.1 Textile fibers

Fibers are a class of materials consisting of a fibrous structure whose length is thousands of times higher than its diameter. Fibers are the units from which all textile materials are made. They are incredibly important to textile production as they have properties that allow them to be spun into yarn or directly made into fabric. This means they must be strong enough to hold their shape, flexible enough to be shaped into a fabric or yarn, elastic enough to stretch, and durable enough to last. Textile fibers also have to be a minimum of 5 millimeters in length as shorter ones cannot be spun together. Textile fibers are generally classified as natural or man-made. An outline is reported in **Figure 4**.

Natural fibers are further subdivided into animal (e.g., wool, mohair, cashmere, angora, silk), vegetable (e.g., cotton, flax, kapok, jute, hemp), or mineral (e.g., asbestos), as shown in **Figure 4**. Animal fibers are typically obtained from the coats or fleeces of animals, or in the case of silk, the raw material is the extruded filaments of the silkworm cocoon [13]. Vegetable fibers grow as seeds, leaves and bast fibers, whereas mineral ones are mainly asbestos fibers. In **Figure 5** an optical picture of animal (a) and vegetable (b) fibers is reported.

Wool fibers have the form of elliptical cylinders. The range diameter of around 20 μm is typical of merino wool, the most commonly used for clothes. It shows a scale

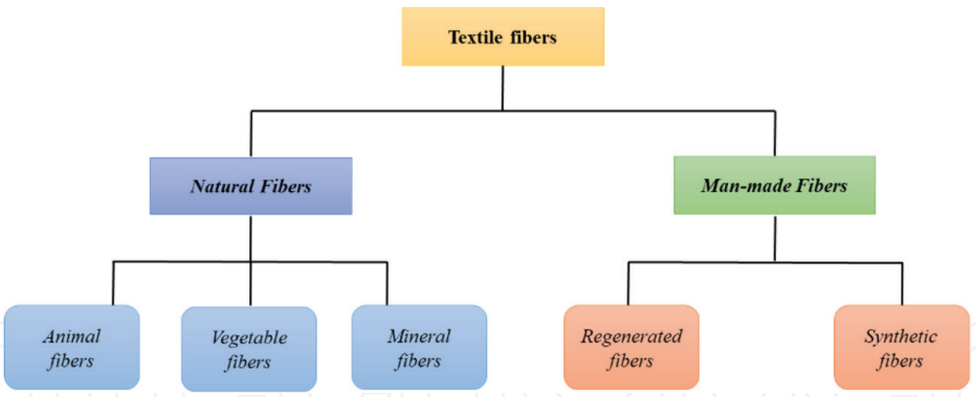

Figure 4.
Classification of textile fibers.

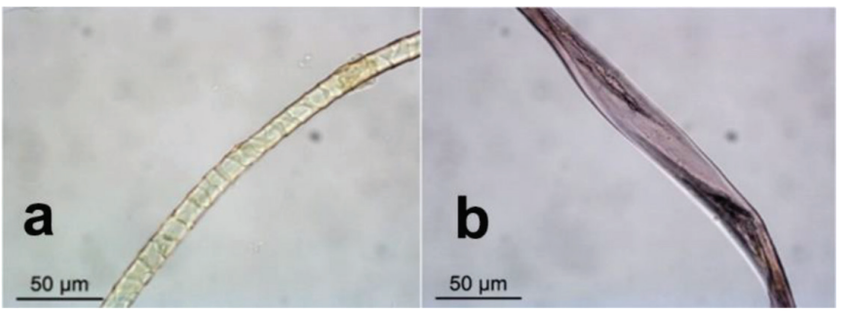

Figure 5.
Optical microscopy image of merino loose wool (a) and cotton fibers (b) obtained in transmission mode. (500 X).

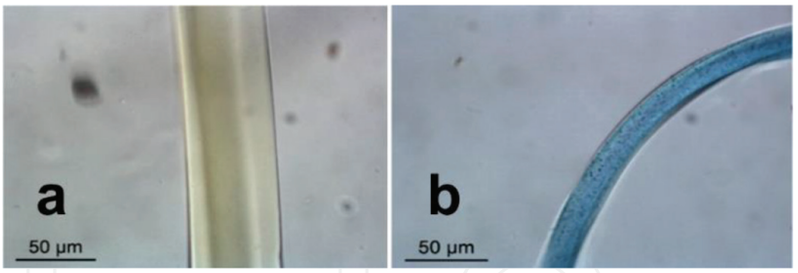

Figure 6.
Optical microscopy image of PA 6 (a) and PA 6.6 (b) fibers obtained in transmission mode (500 X).

structure with an irregular profile and a stopped one due to overlapped cuticle cells, like a tiled roof. Cotton fibers show a flat band structure with corkscrew-like twisting. The convolution frequently varies between 3.9 and 6.5 per mm and the number of reversals per mm ranges between 1.0 and 1.7. The longest cotton fiber is 2.8 cm and can be found in Scottish thread. The section of the fibers shows variable shapes such as elliptical, oval, and kidney with a well-defined central lumen parallel to the outer wall [14].

Man-made fibers are any fiber that is derived from an artificial process. The fibers made from chemical synthesis are called synthetic fibers, e.g., Polyamide 6 (PA 6), Polyamide 6.6 (PA 6.6), polyester (PET), polyacrylic (PAN), cholorofibers (PVC), and aramids (kevlar), while the fibers generated from natural polymer sources are called regenerated fibers or natural polymer fibers e.g.: Viscose, Rayon, cellulose triacetate, etc. [13]. An optical micrograph of synthetic fibers is reported in **Figure 6**.

The fibers are uniaxially oriented during the melt, dry, or wet spinning process, which gives the fibers high tenacity and strength. Typically, they appear as smooth filaments, cylindrical or slightly elliptical.

Figure 6 and b shows the morphology of two nylon fibers (PA6 versus PA 6.6). [15]. They are generally semicrystalline polymers extruded and drawn in various cross-sectional shapes, which can be circular, kidney-shaped or three-lobed with smooth edges. In **Figure 6b**, the fiber shows the presence of fillers.

2.2 Why do fibers from clothes pollute?

Man-made fibers have tripled their market share from 23% in 1965 to nearly 72%. In addition, synthetic fibers have continued to grow to 75%, while cellulosic fibers, for example, have remained constant at about 6.4% [15]. Compared to natural ones, synthetic fibers do not depend on animal breeding or cultivation and are not affected by environmental factors such as seasonality and climate change.

Polyester is considered the best fiber in terms of production cost, raw material quality, and ability to improve performance and properties. Polyester fibers have reached 85% of the market share of the synthetic sector [16].

Moreover, in recent years, synthetic fibers have become the main protagonists of fast fashion (a clothing industry that produces low-quality and low-priced clothing and constantly launches new collections in a short time), generating large amounts of waste from unsold, unwanted and/or landfilled goods.

Furthermore, synthetic textiles are estimated to be responsible for a global discharge of between 0.2 and 0.5 million tons of microplastics into the oceans yearly [17]. Synthetic fragments can enter the aquatic environment during use, machine washing and drying of garments, or through leaching of waste material (pre-consumer, post-consumer) that accumulates in landfills.

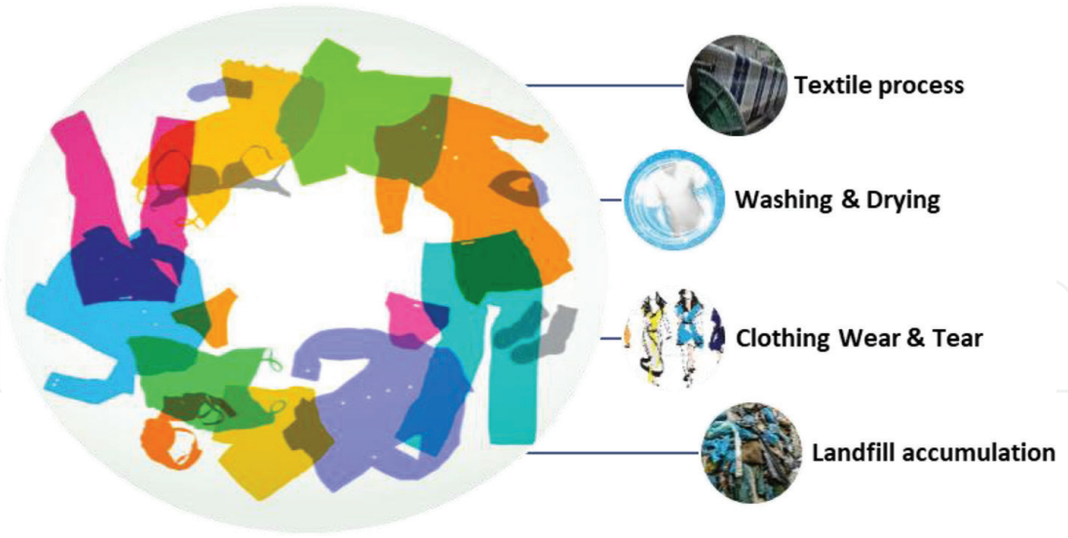

Figure 7.
Source of microplastic fibers release during textile life-cycle.

According to [18] approximately 35% of microplastics released into oceans globally originate from washing synthetic textiles, as shown by their incidence in freshwater and saline environments, near urban centers, in sewage sludge and its by-products, in wastewater treatment plant effluents, in sediments and in some biota such as invertebrates, birds, and fish.

Although wastewater from washing machines is considered the main transport route for synthetic microfilaments, air can be a possible way, too. The fibrous fragments are comparable to dispersed solid particles suspended in the air. Several researchers have pointed out that textile products, especially during manufacture, packaging, drying and use, can release microplastics. Furthermore, synthetic textiles used for upholstered furniture can release fibrous microplastics through friction and abrasion. Many works have shown that the amounts released are comparable to those produced during a washing machine cycle [19–21], as shown in **Figure 7**.

In recent years, concerns have grown about the environmental and health impacts associated with microplastic pollution. Textiles made of fibers of natural origin shed micro fragments, too. All fibers undergo a biodegradation process in water. However, natural fibers (e.g., cotton) are completely degraded in the aqueous matrix, whereas in the case of synthetic fibers, there is no complete degradation but fragmentation

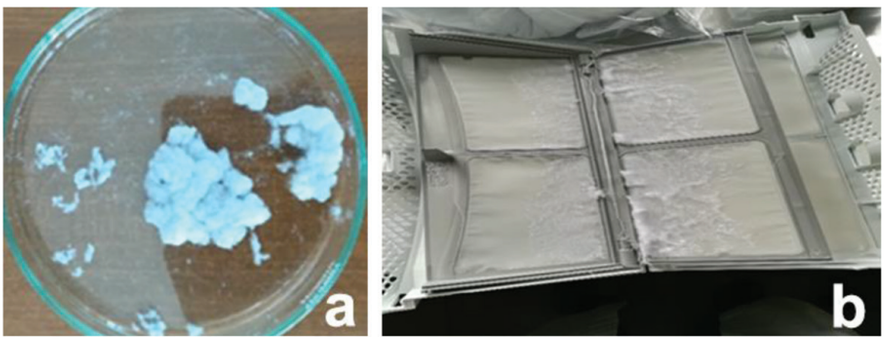

Figure 8.
Example of fiber material released from: a) the synthetic fabric during a 40-minute washing cycle (Wash & Wear) in laundry machines; b) tumble dryers (60-minute drying time).

into smaller filaments or particles that can reach nanometric dimensions. Another finding from the experimental data is that PET fibers are the most commonly found in the environment, followed by PAN, PP, and PA fibers (**Figure 8**) [22, 23].

2.3 Environmental impact

In recent years, a growing concern about microplastic environmental pollution and health impacts has emerged. Several studies have shown a certain degree of chronic exposure to microplastic pollution is an integral part of contemporary life [24]. Due to their shape, microplastic can be ingested by all living organisms, from plankton to humans. Furthermore, another source of concern is the potentially toxic chemicals that they can contain, such as additives, monomers, catalysts and other by-products. Once microplastics have been released into the environment, due to their fragmentation, degradation and chemical contents, they can reach the biota and consequently enter the food chain. In addition, microplastics have characteristics such as size, shape, polymer composition and even color that can potentially be more important than their concentration in the environment to induce adverse effects, making it more challenging to identify their impact on organisms. In addition, fibrous microplastic fragments in terms of size (length and diameter) geometry, physical properties and surface characteristics may be responsible for the levels of biological interfaces with tissues and cause pathology. Small microplastics can easily penetrate cells and organs and carry a considerable content of harmful substances due to the high surface area unit they possess [24, 25].

2.4 Microplastic textiles: Related problems

Nowadays, estimating and measuring the quantities of microplastics, particularly those with fiber shapes, is challenging. Estimating the number of released microplastics is highly uncertain because of the lack of standardized sampling and measurement methods. Furthermore, the obtained data are not fully shared by the scientific community and are not validated with inter-laboratory tests. At present, the experimental and the analytical protocols under study are mainly focused on the determination of microplastic with particle shapes, leaving out fiber-shaped ones.

Indeed, microplastic textile standard methods are rarely used in the study cases. Existing methods for preparing MFs (microfilaments) are focused on cutting or cryogenically grinding synthetic filaments, resulting in a wide distribution of fiber lengths [26]. Some scientists have prepared nylon, polyethylene terephthalate (PET) and polypropylene (PP) microplastic fibers with pre-determined lengths (40, 70 or 100 μm) using a cryotome protocol. They proved that this method effectively produces tens of thousands of MFs suitable for testing [27].

Despite these promising results, the proposed analytical techniques have several drawbacks since they are limited in counting and separation.

Thus, a novel approach to counting and identifying fibrous microplastics is becoming fundamental. For this reason, a standardized analytical method and its subsequent validation must be obtained.

A possible solution to this lack could be the use of appropriate standard microfilaments. The more specific issues are microplastic cut-off size, sample type, sampling procedure, laboratory sample processing, identification techniques and reporting units. Therefore, a new routine for qualitative and quantitative microplastic analysis with fiber shape could be established to have a standardized analytical method to compare different lab results.

3. Strategy to solve the problem

Since microfilaments of standard material are not commercially available, a possible solution for the determination of microplastics could be the preparation and analysis of standard microfilaments in aqueous suspensions. This reliable method can help laboratories to monitor the quality of their analytical procedures. The advantage of such a procedure is that it is possible to produce different types of microfilaments with a narrow size distribution as well as blend them. This protocol could fill the gap in the knowledge of the identification and quantification of fibrous microplastics in textile or environmental matrices. In particular, the proposed procedure achieves the following objectives:

- The preparation of suspensions of known concentration of standard synthetic microfilaments, representative of the textile industry.
- The use of microfilament suspensions as an internal standard during the analysis of a real sample to monitor the quality of all operations and analyses.
- The preparation of suspensions of known concentration that can be used for inter-laboratory and inter-calibration tests.

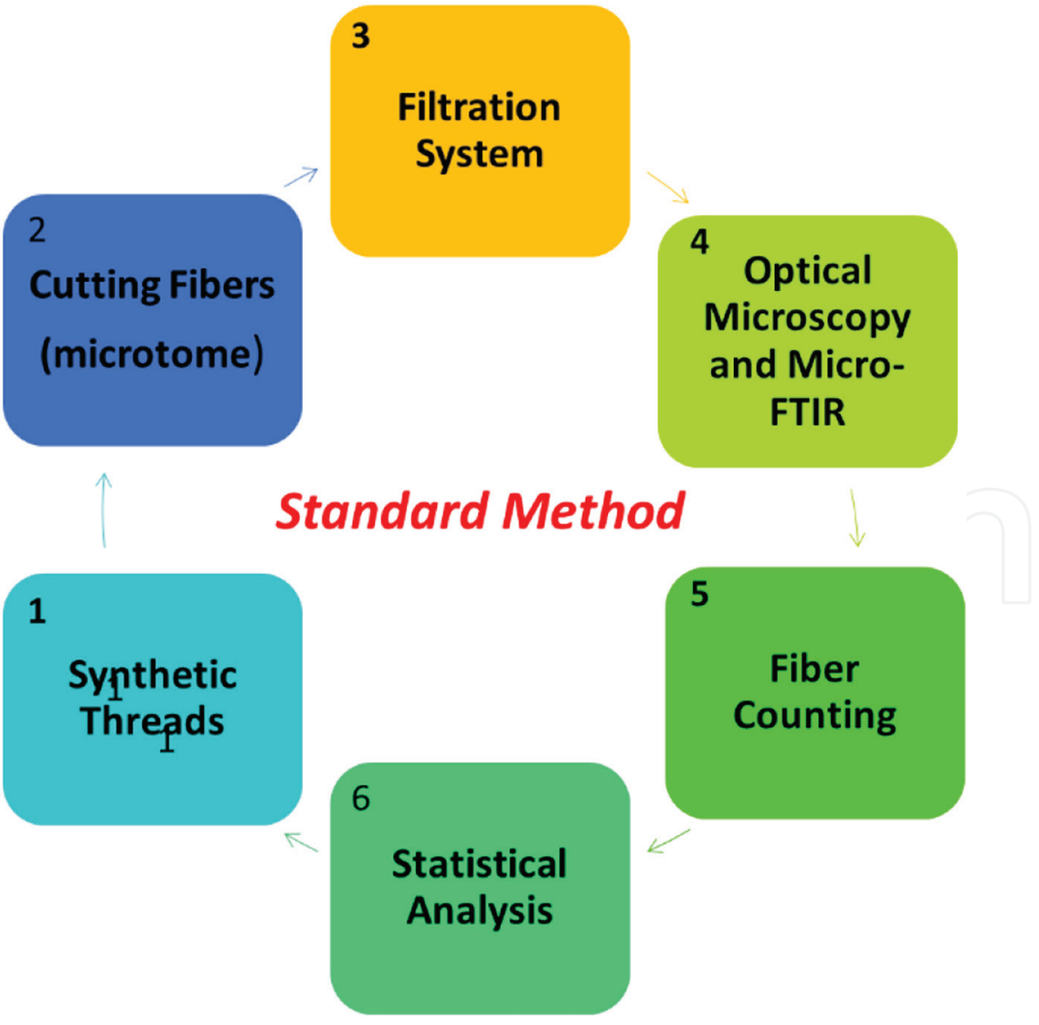

Figure 9.
Schematic diagram of the standard method steps.

- Identification, counting and analysis of fibrous microplastics in aqueous and non-textile aqueous matrices (**Figure 9**).

Mossotti et al. [28] developed a user-friendly method to prepare microfilament standard suspensions that can facilitate lab tests. Specifically, different synthetic threads of PA 6, PA 6.6, PET, and PP, which are shown in **Figure 10**, were used for the preparation of standard suspensions. They are commercial materials supplied by Aquafil S.p.A with a known number of filaments.

The parameters associated with all the yarns are: 1) Yellow PA 6 (180 filaments; 3450 dtex). 2) Blue PA 6.6 (68 filaments; 200 dtex). 3) Cream PET (256 filaments; 2970 dtex). 4) Orange PP (72 filaments; 70 dtex). An example of synthetic thread is shown in **Figure 11**.

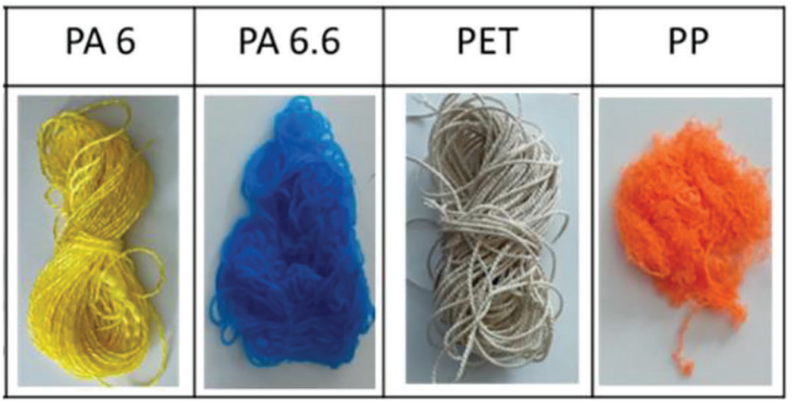

Figure 10.
Image of synthetic threads used for the preparation of the standard solution.

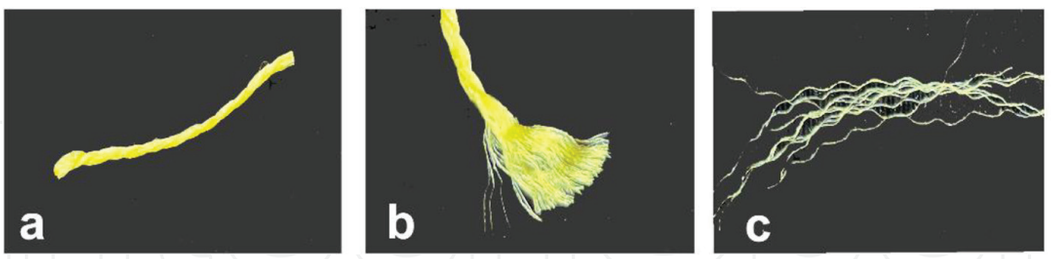

Figure 11.
a) Yarn; b) filaments; c) single filaments.

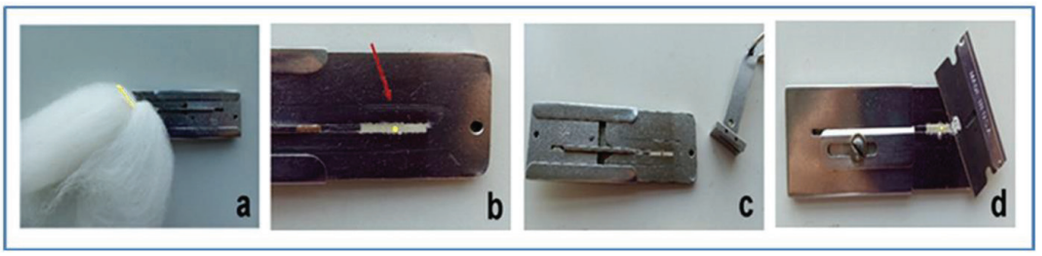

Figure 12.
a) Standard fibers and wool placed in a microtome slide; b) the protruding fringe removed by razor blade b) the fiber length chosen using a suitable pusher d) the cut fibers measure about 200 μm.

All synthetic threads were subjected to microtome cutting at a length of 200 μm according to IWTO-8-97. For the cutting step, synthetic fibers were blended with wool, as shown in **Figure 12**.

The wool is added to the synthetic yarn to fill the microtome slot completely and consequently have the correct number of synthetic filaments. The wool fibers are then removed using a hypochlorite solution. This treatment successfully eliminates the wool fiber without altering the structure of the synthetic yarns. The effect of hypochlorite on the synthetic yarn is checked using FTIR analysis.

As shown in **Figure 13** the oxidative treatment does not modify the chemical structure of the synthetic yarns since no significant changes can be seen in the absorption bands.

The presence of wool fibers can be observed using an optical microscope (OM), as shown in **Figure 14**.

The wool fibers can be easily recognized using MO analysis, as shown in **Figure 15**. Indeed, they have an irregular diameter and a surface structure consisting of overlapping scales. On the contrary, synthetic fibers typically have a wider diameter and a regular shape with a homogenous and smooth surface.

Figure 15 shows an example of wool fibers used during the cutting stage.

After the hypochlorite treatment, the synthetic fibers were placed in an Erlenmeyer flask. For each polymeric yarn, three suspensions at 300, 500 and 900 ml were prepared and then filtered using silicon filters. The microfilaments collected on the filters were counted and the average value and standard deviation of 5 replicas were calculated.

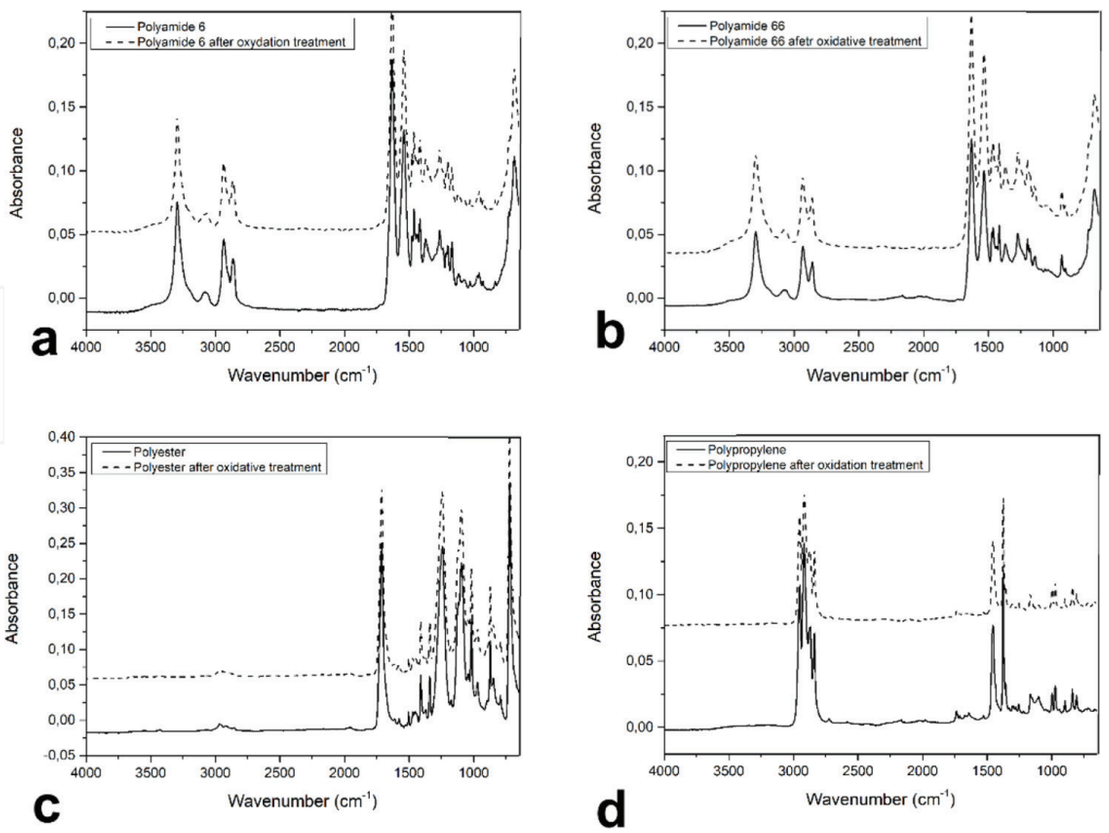

Figure 13.
Spectra of the synthetic fiber before (solid line) and after hypochlorite treatment (dotted line) of a) PA 6; b) PA 66; c) PET; d) f PP. No significant differences can be seen.

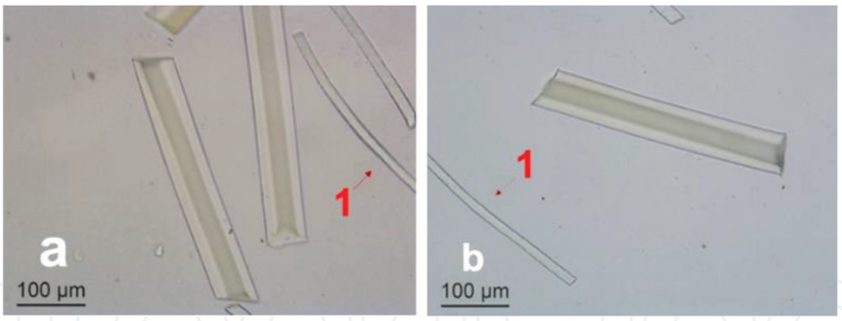

Figure 14.
Optical microscopy images (200x) of synthetic fibers (e.g., PA 6) and wool (1) cut with a microtome to 200 µm.

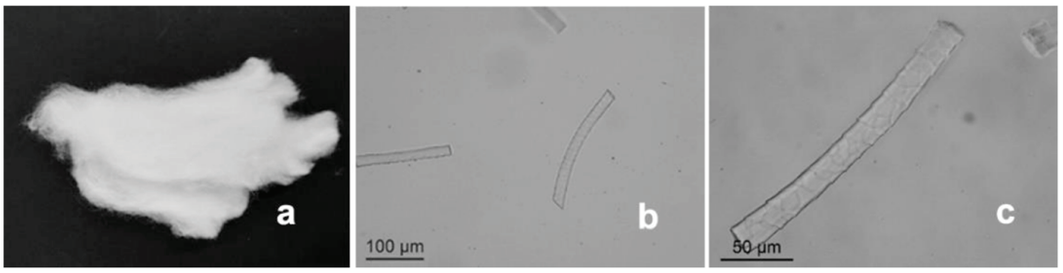

Figure 15.
a) Example of wool fine fiber used for the sample cutting stage; b) optical microscopy image of wool at 200X; c) optical microscopy image of wool at 500X. Average diameter: 16,2 µm.

An optical microscope associated with a micro-FITR was used to count the microfilaments on the filters. This technique has several advantages:

It is fast, non-destructive, reproducible, and able to collect IR signals at a high spatial resolution. Furthermore, the coupling of a MicroFTIR with an OM opens the possibility of visualization and mapping samples across the entire surface exposed.

The MicroFTIR has become an increasingly popular instrument for characterizing samples with very small dimensions which are difficult to be chemically analyzed using the conventional FTIR.

Indeed, the microscopic component provides information about morphology, size, color, and shape. On the other hand, the spectroscopic component provides information about the specific chemical bonds by capturing the absorption spectrum of the microplastic, thus performing qualitative analysis. Finally, the possibility of developing an automated spectroscopic analysis procedure is more efficient and labor-saving than other analytical methods. In MicroFTIR mapping mode, it is possible to collect spectra in different sampling points that are measured and integrated and then used to map the distribution, as shown in **Figure 16** [29].

This technology also allows the determination of the presence of contaminants inside the sample. For instance, some cellulosic fibers were found in the control water sample (hypochlorite, wool and demineralized water). Through OM analysis, the typical ribbon shape was recognized and MicroFTIR identified the characteristic absorption bands related to cellulosic fibers, as shown in **Figure 17**.

All the collected data were statistically elaborated using a logit regression analysis to study the relationship between the concentration and probability of detection of an individual microfilament, as well as the impact of the type of polymer used as shown in **Figure 18** [30]. It is as well used to investigate the relationship between a binary response variable and some other explanatory ones.

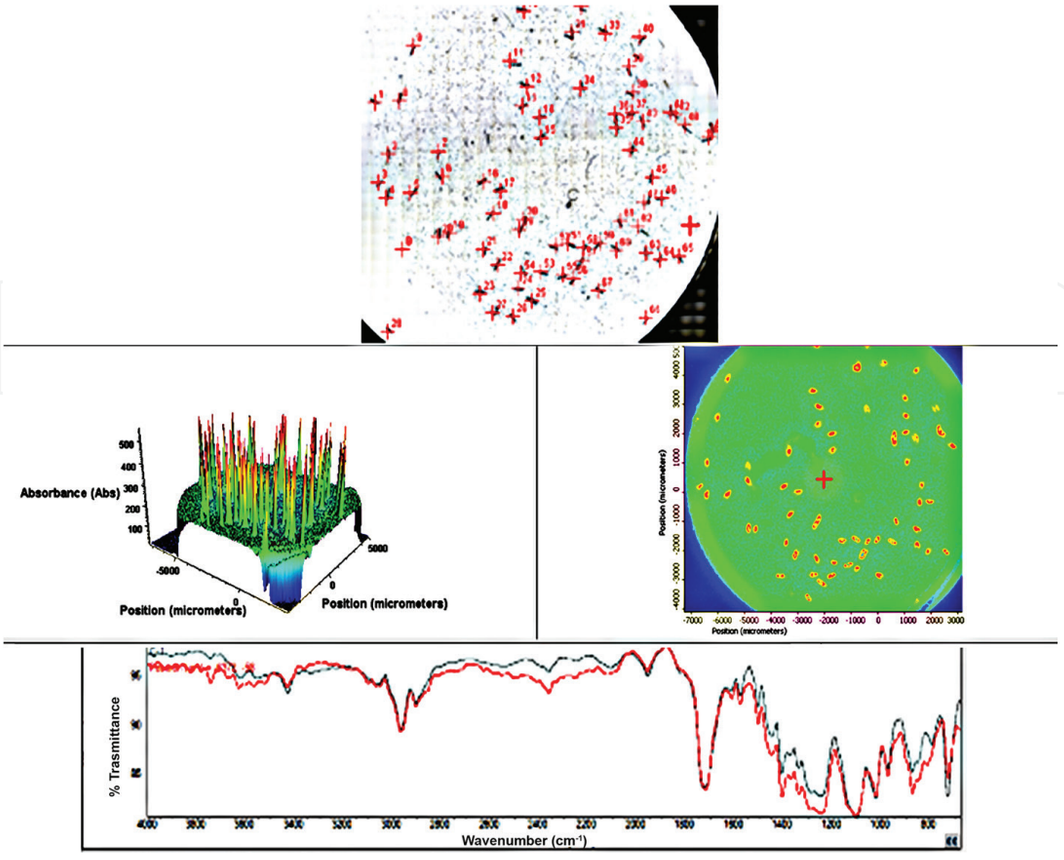

Figure 16.
Counting and chemical mapping of the microfilaments (PET) collected on a silicon filter using MicroFTIR.

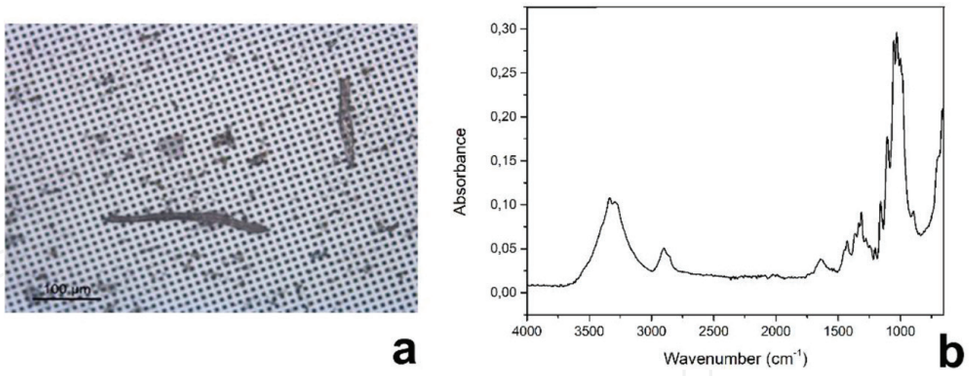

Figure 17.
a) Optical image and b) spectra of cellulosic contaminants fibers collected in a control water sample.

It was chosen because of the binary nature of the data, in which a dependent variable has two possible values expressed as identification or non-identification for each individual microfilament in the suspension. Let Y_{ij}, i = 1,..., n, j = 1,... m, denote the response, that is the number of detected microfilaments for the i-th sample and j-th replication. Let K be the theoretical number of microfilaments in the sample, that is the number of independent trials that can be performed on it. Then Y_{ij} is distributed as a binomial random variable of size K and probability of identification p_i. The logit model used explicitly the relationship between the probability of detection of the single microfilaments, p_i, and the covariates by modeling:

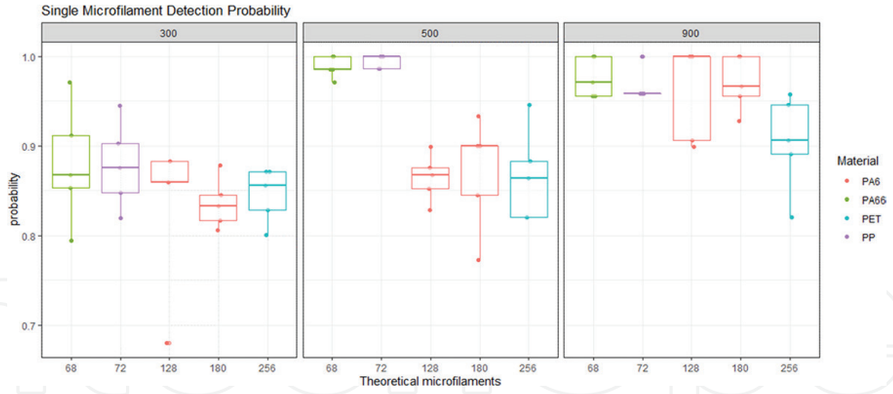

Figure 18.
Boxplot of the fraction of counted versus theoretical burrs in relation to material and solution volume.

$$\text{logit}\left(E\left(Z_{ijk}|,X_{1,ij}|,X_{2,ij}\right)\right)=\log\left(p_i/\left(1-p_i\right)\right)=\beta_o+\beta_1\,X_{1,ij}+\beta_{M(i)} \qquad (1)$$

where Z_{ijk}, k = 1,…,K is a Bernoulli random variable representing the detection of the k-th microfilament in the i-th sample and j-th replication, $X_{1,ij}$ the concentration used and $\beta_{M(i)}$ the parameter representing the material's effect used for the i-th sample.

This statistical elaboration underlines that there is a strict relationship between the concentration of the microfilaments detection probability. Indeed, increasing the number of microfilaments there is a reduction of the detection probability.

The results of statistical analysis show that:

- the fraction of microplastics detected is not the same for all materials;
- the fraction of microplastics increases with the amount of solution;
- the greater the number of theoretical microfilaments, the lower the probability of detecting all filaments;
- the type of material influences the fraction of microplastics detected;
- the probability of detecting microfilaments is greater than 95% when the microfilament concentration is less than 200 N° microfilaments/L.
- Thus, if the microfilament concentration is too high, overlapping microfilaments may occur, resulting in a loss of material identification and counting. Therefore, it is necessary to proceed with the division into several aliquots and filtration through several filters.

4. Conclusions

This chapter has tackled the problem of microplastic release from textiles by trying to identify a suitable protocol for the preparation of standard microfilaments. Indeed, there is a growing concern about the microfilament from textiles released in

the environment. Since the average textile consumption is increasing, the number of synthetic microfilaments released in particular in water is rapidly enhanced. Thus, the necessity to have a reliable method for the identification and quantification of microplastic released by textiles are becoming mandatory. For this reason, in this chapter, it has been proposed not only a complete overview of the problem of the microplastics related to the textile sector but also a novel approach for the quantification and identification of them. Therefore, this chapter describes a protocol in which some standards of different synthetic fibers have been prepared in order to introduce them in a real sample. Actually, it describes the preparation of standard suspensions with a 76–853 N filaments/L concentration range using polymer threads cut at predetermined lengths of 200 mm following IWTO-8-97 and dispersed in three solutions of 300, 500, 900 ml to obtain three different concentrations. Afterward, the solutions were filtered through a silicon filter, and the collected microfilaments were counted with optical microscopy coupled with a MicroFTIR instrument. Five replicates were carried out for each sample and the data were statistically analyzed using a logit method. The probability of detecting the microfilaments is higher than 95% when the concentration of microfilaments/L is lower than 200. Thus, these microfilaments can actually work as an internal standard and the micro-FITR can be a suitable tool for the correct identification and quantification of microplastics.

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