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Comparison of methodologies for microfiber analysis across different stages of wastewater from a textile dyeing company

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ABSTRACT

Microfibers (MFs) — fibrous particles <5 mm in length, including both synthetic and natural fibers — from the textile industry pose growing environmental challenges, particularly through wastewater generated during production. This study presents a comprehensive comparison of analytical methodologies for detecting and characterizing MFs and MPs across four significant wastewater stages — underground water, inflow, outflow, and sludge — from an Italian textile dyeing factory. The novelty of this work lies in its integrated evaluation of sample-specific pretreatment strategies (no treatment, quick oxidative, and prolonged oxidative), filtration materials (stainless steel, aluminum oxide, and mixed cellulose ester membranes), and the use of two distinct micro-FTIR spectroscopy systems from different laboratories. This multifaceted approach enabled the identification of optimal workflows tailored to sample organic content and matrix complexity. Results showed that quick oxidative treatment is ideal for rapid processing, while prolonged treatment offers a more sustainable option. Single-step filtration proved to be the most practical and effective. The comparative micro-FTIR analysis revealed that polymer identification depends strongly on filter compatibility and instrument configuration. Inflow samples exhibited the highest MF concentration (825 MFs/L), and the wastewater treatment plant (WWTP) achieved a partial removal efficiency of 36.97%. The most common MF types were polyester, and cellulose-based materials (e.g., cotton, rayon and lyocell) with a notable presence of transparent and blue-fluorescent microfibers linked to fluorescent whitening agents. These findings not only highlight the variability of MFs and MPs pollution across wastewater stages but also provide a standardized analytical framework for environmental monitoring and regulatory assessment of microfiber emissions from industrial textile operations.

1. Introduction

Microplastics are widely recognized as a major environmental issue due to their broad distribution across aquatic and terrestrial ecosystems. The textile sector has been identified as a significant contributor to microplastic pollution, primarily through the release of microfibers during various stages of synthetic textile production, use, and laundering (Acharya et al., 2021). Synthetic microfibers are released in high concentrations during household fabric washing (Weis and Falco, 2022), and these fibers often pass through wastewater treatment plants and accumulate in aquatic environments, where they pose potential ecological risks (Rasheed, 2024; Balestra et al., 2024).

Fibrous microplastics, commonly referred to as synthetic microfibers, represent a specific subset of microplastics that predominantly originate from textiles. These microfibers are typically released during laundering, abrasion, or degradation of fabrics. Microfibers may be natural (e.g., cotton, wool), regenerated (e.g., rayon, lyocell, viscose), or synthetic (e.g., polyester, polyamide). However, only fibers composed of synthetic polymers or those that have undergone significant chemical modification—such as dyeing, finishing, or coating—can be classified as microplastics due to their environmental persistence and polymer content (Liu et al., 2019; Acharya et al., 2021; Gaylarde et al., 2021; Santini et al., 2022; Liu et al., 2023). According to European Commission Regulation (EU) 2023/2055, any fiber $\leq$ 5 mm in length is classified as a microplastic if it contains $\geq$ 1% synthetic polymer by weight or is fully coated with such a material. Therefore, regenerated fibers such as rayon may be considered microplastics because they are chemically treated during production. However, unlike conventional synthetic microplastics, rayon remains biodegradable under environmental conditions, which may reduce its persistence and long-term ecological impact (Zambrano et al., 2019).

Effluents from textile wet processing industries have been found to

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contain microfiber concentrations over 1000 times higher than those from municipal wastewater treatment plants (Ramasamy et al., 2022). A study by Xu et al. (2018) estimated that the wastewater treatment plant associated with the textile industry was discharging approximately 4.89 $\times$ 10^8 microfibers, including both natural and synthetic fibers, into the receiving water daily. Similarly, Zhou et al. (2020) investigated microfiber pollution in wastewater from textile mills and centralized WWTPs in a Chinese industrial park, founding that microfiber concentrations in textile wastewater could reach 54,100 MFs/L. Despite treatment processes removing over 85% of these fibers, WWTP effluent still contained an average of 537.5 MFs/L, releasing an estimated 430 billion microfibers into the environment each day.

Synthetic microfibers, derived from petroleum-based materials like polyester, polyamide, and acrylic, are highly resistant to biodegradation, making them persistent pollutants in the environment (Tyagi, 2024; Naik et al., 2024). While these microfibers have received significant attention, natural fibers, such as cotton and wool, are less studied, even if they contribute to microfiber pollution, due to the chemical treatments applied during production, which amplify their environmental impact (Pedrotti et al., 2021; Liu et al., 2021; Athey et al., 2020; Collie et al., 2024). The dyeing process further exacerbates the problem, as the use of dyes and additives increases microfiber shedding during washing, releasing additional microfibers into the environment (Suaria et al., 2020).

The environmental impact of microfiber contamination is significant, affecting aquatic ecosystems and potentially human health. Aquatic organisms, from zooplankton to larger fish, can directly or indirectly assimilate microfibers, leading to bioaccumulation within food webs and posing risks to marine biodiversity (Zhang, 2022). Microfibers have also been detected in seafood, drinking water, salt, and air, raising concerns about human exposure through ingestion or inhalation (La Porta et al., 2023; Zhang et al., 2020). Although definitive health impacts on humans are still being studied, microfibers can release hazardous additives such as phthalates and bisphenol A (BPA), which have been associated with endocrine disruption, DNA damage, and cytotoxic effects (Meeker and Ferguson, 2011; Campanale et al., 2020; Wang et al., 2024). Moreover, microfibers may act as vectors for other environmental contaminants, including dyes, flame retardants, and plasticizers, potentially compounding toxicological risks (Hartline et al., 2016; Periyasamy, 2023). While further toxicological studies are needed, these findings support growing concern about microfiber exposure and its possible implications for human health. Recent studies have emphasized the complexity of such effluents and the need for advanced treatment solutions targeting synthetic contaminants (Jioui et al., 2023; Ben Ali et al., 2025).

Efforts to mitigate microfiber contamination from textile dyeing and manufacturing are essential to reduce environmental pollution. Key strategies include enhancing wastewater treatment processes, developing biodegradable fibers, and adopting sustainable design principles in textile production (Allen et al., 2024; Vassilenko et al., 2021). Research indicates that preliminary wastewater treatment processes can remove 35%–59% of microfibers, while after primary treatments achieve removal rates of 50%–98% (Sun et al., 2019). However, traditional wastewater treatment facilities often lack capability to effectively capture microfibers, allowing them to pass filtration systems and enter aquatic ecosystems. Advanced technologies such as membrane bioreactors (MBRs) and microfiltration have shown significant potential in reducing microfiber emissions from industrial effluents (Browne et al., 2011). In addition to wastewater treatment improvements, innovations in textile production processes have contributed to minimizing microfiber pollution. Sustainable dyeing practices and the use of eco-friendly dyes help reduce fabric degradation, thereby limiting the release of microfibers during manufacturing and subsequent washing cycles (Surana et al., 2024).

Wastewater treatment plant samples often contain substantial amounts of organic matter and inorganic solids, requiring specialized techniques to capture microplastics and microfibers from their complex matrices. These methods are crucial for enabling accurate quantification and identification of microplastics, with the removal of organic material being particularly critical for reliable chemical analysis (Sun et al., 2019). Hydrogen peroxide (H_2O_2) is a widely used reagent for organic matter degradation, with studies optimizing its effectiveness across various concentrations (15–35%), temperatures (20 °C to 100 °C), and reaction durations (ranging from a few hours to a week) (Al-Azzawi et al., 2020; Thomas et al., 2020; Phuong et al., 2021). The ISO 4484-2:2023 standard further provides a comprehensive framework for analyzing microplastics released by the textile industry into wastewater and solid waste. By employing advanced molecular spectroscopy techniques such as micro-FTIR and micro-Raman, the standard foresees detailed analysis of particle count, morphology, size distribution, and polymer composition. These techniques yield critical data for ecotoxicological assessments and enable consistent comparisons across textile processes and life cycle stages. Additionally, the standard outlines procedures for sample preparation, purification, and analysis, addressing the diverse physical and chemical challenges associated with sample matrices. This ensures robust and reliable microplastic evaluations, aiding efforts to mitigate their environmental impact.

Recent research has further emphasized that methodological variation—particularly in spectroscopic workflows—can significantly influence microplastic detection and characterization outcomes. Liu et al. (2023) systematically demonstrated how inconsistencies in sample preparation, particle selection (manual vs automated), filter types, and spectral interpretation protocols could lead to substantial discrepancies in the reported concentrations and polymeric composition of microplastics in wastewater. Their comparative analysis revealed that even subtle changes in imaging parameters or software tools can bias results, reinforcing the necessity of harmonized workflows. Complementing this, Primpke et al. (2020) introduced siMPle, an open-source tool for μFTIR and μRaman analysis, which achieved higher polymer identification accuracy than commercial tools and offered greater flexibility for analyzing environmental samples. These findings collectively underscore the need for robust instrumentation, standardized pretreatment protocols, and reproducible data analysis workflows in wastewater microplastic research.

The complexity of microfiber pollution necessitates effective methodologies for their quantification and characterization in wastewater. Accurate analysis is critical for understanding microfiber behavior within wastewater treatment systems and for developing strategies to mitigate their environmental impact. This study focuses on comparing methodologies for the analysis of microfibers in wastewater samples from an Italian textile dyeing factory. Specifically, it evaluates various pretreatment, filtration, and characterization techniques applied to underground water and wastewater at different treatment stages, including inflow, outflow, and sludge. By identifying the most effective methodologies, this research aims to contribute to the development of standardized approaches for microfiber analysis and to inform future pollution control strategies within the textile industry. Additionally, after determining the most effective pretreatment method, this study seeks to quantify microfiber concentrations, analyze their length distribution, and characterize microfibers released from different stages of textile dyeing operations. Furthermore, it assesses the efficiency of the existing wastewater treatment system in capturing these microfibers.

2. Materials & methods

2.1. Materials

Wastewater samples (1 L each) were collected from an Italian textile dyeing company that processes both natural and synthetic fabrics. The samples were taken from four stages of the process: underground, inflow, outflow, and sludge (Fig. 1). Prior to sampling, all equipment was thoroughly cleaned with distilled water and sterilized with ethanol

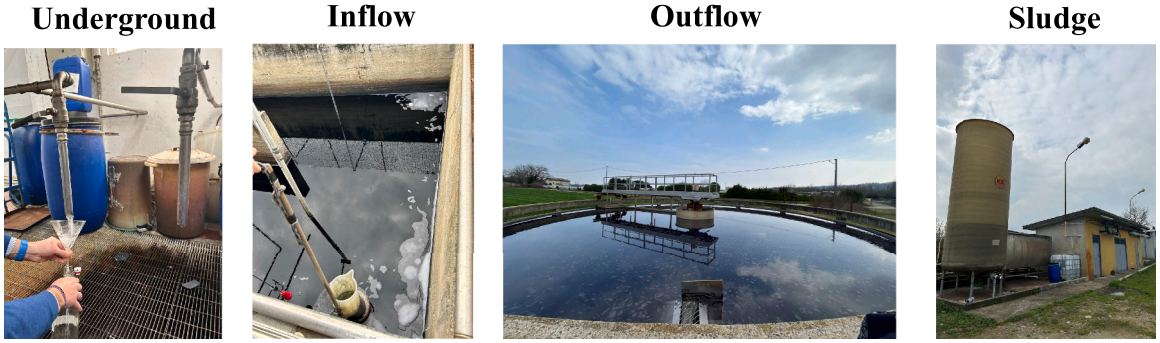


Fig. 1. Wastewater samples were taken from different stages.

to prevent contamination. Samples were collected in capped glass bottles and refrigerated to minimize bacterial growth. To reduce plastic contamination, glass and metal alternatives were used. Strict contamination control measures were implemented, including the use of nitrile gloves, orange cotton lab coats, aluminum foil covers, and thorough cleaning of all surfaces with ethanol and Milli-Q water.

For pretreatment, 15% hydrogen peroxide (Merck KGaA, Darmstadt, Germany) was used. Filtration was performed using the following filters to evaluate their effectiveness:

- 53 μm pore size stainless steel filter (Labopolis), calcinated at 450 °C prior to use.
- 0.2 μm pore size alumina Anodisc filter (Whatman, 47 mm diameter).
- 0.2 μm pore size mixed cellulose ester membrane filters (MCE) (Advantec, 47 mm diameter).

2.2. Sample preparation and microfiber/microplastic recovery

The summary of different methodologies carried out to recover microfibers/microplastics from wastewater is given in Table 1. Each method is described in terms of pretreatment conditions, filter types used, and method-specific features relevant to its applicability. All pretreatment techniques—including no treatment, quick oxidative, and prolonged oxidative treatments—were carried out separately and tested using each of the three filter types (stainless steel, aluminum oxide, and MCE). The inclusion of prolonged oxidative treatment and aluminum oxide filters follows the ISO 4484–2 standard, ensuring compliance with international guidelines. Meanwhile, the quick oxidative treatment protocol and associated filter combinations were developed based on prior experimental work to assess processing efficiency under practical constraints.

For prolonged oxidative treatment the volume of hydrogen peroxide is required for treatment. It was calculated based on the chemical oxygen demand (COD) of the samples using the equation (ISO 4484-2 standard):

$$V = \frac{COD \times Sv}{70.06}$$

Where:

- V = Volume of 15% hydrogen peroxide (L)
- COD = Chemical oxygen demand (mg O_2/L)
- Sv = Sample volume (L)

For samples with a COD of 31 mg/L and a sample volume of 50 mL (Sv=0.05 L), the required volume of 15% hydrogen peroxide was calculated as:

$$V = \frac{31 \times 0.05}{70.06} \approx 0.023L(23mL)$$

Filtration was conducted using two approaches. The first approach involved two steps: an initial filtration using a stainless-steel filter with a 58 μm pore size (pre-calcinated at 450 °C), followed by a secondary filtration using MCE filters with a 0.2 μm pore size and aluminum oxide filters 0.2 μm for finer particle separation. The second approach consisted of a single-step filtration using only MCE filters with a 0.2 μm pore size.

2.3. Analysis of MPs/MFs and comparison of methodologies and instruments

2.3.1. Quantification and length distribution of MPs/MFs

Microscope was used to analyze and identify MPs and MFs. To quantify the amount of MPs and MFs and analyze their length distribution, an optical microscope, Leica ORTHOLUX II POL-MK (Wetzlar, Germany), equipped with and without UV light (Alonefire SV10, 365 nm), was used. This setup allowed for the visual detection and measurement of MPs/MFs, including those containing fluorescent whitening agents (FWAs). Particles were categorized into predefined size ranges to evaluate their distribution patterns and identify dominant particle sizes.

2.3.2. Efficiency assessment of the wastewater treatment plant and chemical characterization of microplastics and microfibers

The amount of MPs and MFs was quantified at each stage of the sampling process—underground water, inflow, outflow, and sludge—to assess the overall distribution of microparticles throughout the system. However, to evaluate the performance of the wastewater treatment

Table 1
Summary of different methodologies for recovering microfibers/microplastics from wastewater.

Treatment Code	Pretreatment Conditions	Filter Type	Method Highlights
No Pretreatment (Control)	No chemical treatment.	• Stainless steel (53 μm pore size) • Aluminum oxide (0.2 μm) • MCE (0.2 μm)	Used as a baseline to evaluate whether pretreatment is necessary for all sample types.
Quick Oxidative Treatment	50 mL wastewater treated with 150 mL of 15% H_2O_2 at 50 °C for 2 h, continuous agitation at 450 rpm.		Efficient for quick processing due to short-duration digestion at elevated temperatures.
Prolonged Oxidative Treatment	50 mL wastewater treated with 23 mL of 15% H_2O_2 at room temperature for 7 days.		Focuses on sustainability through extended digestion at ambient temperature.

plant (WWTP), only the concentrations of MFs in the inflow and outflow samples were compared. Treatment efficiency was then calculated as the percentage reduction in microfiber count from inflow to outflow.

Chemical characterization of the MPs and MFs was performed using two different micro-Fourier-transform infrared (μFTIR) spectroscopy systems: the Shimadzu AIM-9000 Micro-FTIR and the Thermo Scientific™ Nicolet™ iN10 Infrared Microscope, each actively used in different laboratories.

For spectral analysis using the Shimadzu AIM-9000 Micro-FTIR system, filters containing the recovered particles were placed directly into the instrument without any additional sample preparation. Spectral acquisition was carried out in transmission mode under high-resolution conditions, using an aperture size of 50 × 50 μm and collecting 40 scans per sample within the spectral range of 700–4000 cm^{-1}. The resulting spectra were initially compared against the Shimadzu LabSolution T-Polymer 2 library.

For the Thermo Scientific™ Nicolet™ iN10 infrared microscope, individual particles were carefully isolated using fine stainless-steel tweezers and transferred onto aluminum EZ-Spot Micro Mount slides. Spectral data were recorded in reflection mode using a mercury-cadmium-telluride (MCT) detector, with a 50 × 50 μm aperture, 16 scans per particle, and a spectral range of 675–4000 cm^{-1}. The collected spectra were processed using OMNIC Picta software (ThermoFisher Scientific, Massachusetts, USA) and compared against the "Synthetic Fibers by Microscope" reference library.

For both micro-FTIR analysis, a minimum spectral match confidence of 70% was used as the threshold for positive polymer identification, followed by visual comparison of characteristic absorption bands with the corresponding reference spectra to confirm material identity.

2.3.3. Evaluation and optimization of methodologies for microplastic and microfiber analysis

The methodologies utilized for recovering and analyzing MPs and MFs were systematically compared based on pretreatment, filtration, and chemical characterization techniques.

The evaluation focused on identifying the most effective approach for maximizing recovery efficiency and ensuring precise analysis. After determining the suitable pretreatment method for each type of wastewater during the initial experiments, subsequent steps were carried out using the selected methodology. Prior to hydrogen peroxide treatment, samples were filtered using a 1 μm PTFE membrane (Omnipore, 47 mm diameter) to remove any potential contamination. To ensure the reliability and accuracy of the analytical results, procedural blanks were incorporated throughout the study. These blanks were prepared using ultrapure water and subjected to the same handling, filtration, and digestion processes as actual samples—including exposure to all reagents, laboratory glassware, filters, and instruments. The purpose was to detect any contamination introduced during sample processing.

A total of 600 mL of wastewater from each stage was analyzed, with the volume evenly divided into three replicates. For underground water, no pretreatment was required due to the negligible organic content. For inflow and outflow wastewater, the quick oxidative treatment method was employed to effectively reduce organic matter within a shorter processing duration, ensuring efficient sample preparation. Sludge samples also underwent the quick oxidative treatment method, but to further enhance the analysis, a density separation step using a 1:1 NaCl solution was performed. This additional step successfully separated floating and sinking particles, improving the clarity and quality of both microscopic analysis and micro-FTIR characterization.

For the filtration process, a single-step filtration was determined to be the optimal method. Although two-step filtration, which involves an initial filtration with a stainless-steel filter followed by finer filtration, is often recommended for challenging samples to reduce clogging and improve analysis quality, both approaches produced equally clean samples in this study due to the effectiveness of the pretreatment process. To save time and streamline the workflow, the single-step filtration method was selected as the preferred approach.

3. Results and discussion

3.1. Comparison of methodologies

3.1.1. Pretreatment methods

The selection of pretreatment methodology depended on the type of sample and its organic load, as summarized in Fig. 2.

I. **Underground water:** Due to the negligible organic matter present in underground water, no pretreatment was required, making this the most sustainable and efficient option for this sample type. The filters remained clean, and no difficulties were encountered during the analysis phase.

II. **Inflow and outflow wastewater:** Although no pretreatment method eliminated chemical usage, the presence of organic matter resulted in dirty appearances on the filters, especially those with lower pore sizes (e.g., aluminum oxide and MCE filters). This affected visual analysis and micro-FTIR characterization. Two oxidative pretreatment strategies were employed:
   - Quick oxidative treatment: 50 mL of wastewater was treated with 150 mL of 15% H_2O_2 at 50 °C for 2 h under continuous agitation (450 rpm).
   - Prolonged oxidative treatment: 50 mL of wastewater was treated with 23 mL of 15% H_2O_2 at room temperature for 7 days, in accordance with ISO 4484–2.

Both methods effectively reduced organic content, enabling clear filter surfaces and accurate identification of microfibers during micro-FTIR characterization. The choice between these methods depends on the choice to prioritize time efficiency with the quick method or sustainability with the prolonged method.

III. **Sludge:** No pretreatment method caused severe clogging on filters, especially for smaller pore sizes. The accumulated organic layer obscured microfibers and hampered subsequent analysis, making it unsuitable for sludge samples. Two oxidative pretreatment methods can be utilized to remove organic content. Additional treatments [e.g., density separation (NaCl solution) or ultrasonic bath] were required to improve filter cleanliness and enable accurate microscopic and micro-FTIR analysis.

No pretreatment is only suitable for low-organic-content samples (e.g., underground water) but caused severe limitations for inflow, outflow, and sludge samples. Quick oxidative treatment is ideal for time-sensitive applications, as it efficiently reduces organic content in a shorter duration. However, prolonged oxidative treatment offers a more sustainable alternative by eliminating the need for elevated temperatures, ensuring cleaner filters and improved analysis quality (Fig. 2).

3.1.2. Filter performance

The filtration process was optimized by evaluating the performance of three filter types: aluminum oxide (porosity –f– 0.2 μm), MCE (f 0.2 μm), and stainless steel (f 58 μm). The first approach was two-step filtration, which involves an initial filtration with a stainless-steel filter followed by finer filtration, and the second one is one step filtration with finer pore size. The single-step filtration method was selected as the preferred approach because after treatment filters were clean for analysis.

Stainless steel filters were highly durable and provided the fastest filtration for larger particles, but their wide pore size (58 μm) resulted in significant losses of smaller microfibers, making them unsuitable for finer particle analysis. Additionally, calcination of the stainless-steel filters caused warping, further complicating the analysis process. While the aluminum oxide filter (0.2 μm) offered high-resolution

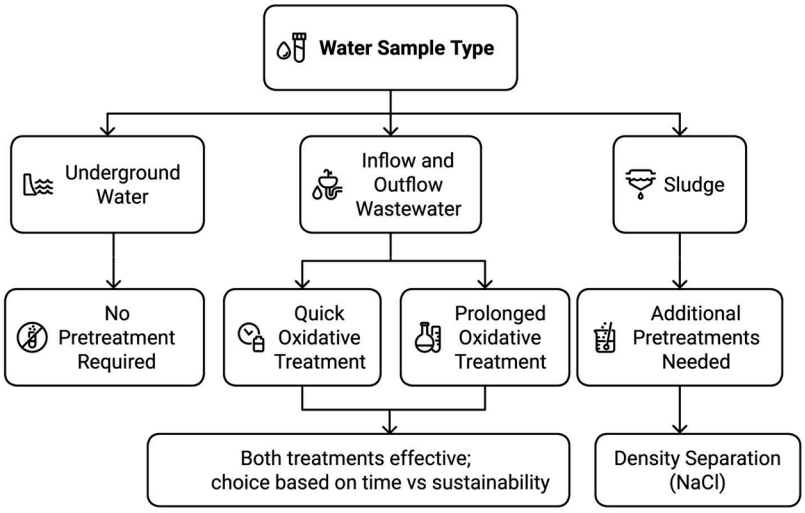


Fig. 2. Decision flowchart for selecting pretreatment methods based on water sample type and organic content.

filtration suitable for detailed analysis in transmission mode micro-FTIR, it was delicate, prone to breakage, and required longer filtration times, which made it less practical for routine use. On the other hand, the MCE filter (0.2 μm) was identified as balanced option due to its cost-effectiveness, and suitability for filtration. However, MCE filters may not be ideal for microplastic/microfiber analysis due to potential chemical interference and incompatibility with certain spectroscopic techniques.

Due to the efficiency of the pretreatment process, both single-step and two-step filtration approaches produced equally clean samples. However, the single-step filtration was chosen as the optimal method.

Based on performance, availability, and analytical compatibility, the single-step filtration using aluminum oxide or MCE filters was selected for continued use, with final choice depending on the analytical instrument (micro-FTIR compatibility) and sample matrix complexity.

3.1.3. Micro-FTIR analysis

Micro-FTIR analysis played a critical role in the chemical characterization of MPs and MFs by identifying their polymeric composition. Two systems, the Shimadzu AIM-9000 and the Thermo Scientific™ Nicolet™ iN10 micro-FTIR, were compared in terms of sample preparation, analytical workflow, spectral resolution, and identification accuracy.

The Shimadzu AIM-9000 offered the advantage of direct filter analysis without requiring additional sample preparation steps, such as particle collection and transfer. Filters were placed directly into the instrument, streamlining the analysis process and saving significant time. However, this system required compatible filters for optimal results, with aluminum oxide filters specifically used in transmission mode. While MCE filters have been applied in FTIR transmission mode, they may introduce interference, affecting spectral quality. The spectra obtained using MCE filters were of lower quality than those from aluminum oxide filters, likely due to chemical interference and analytical limitations.

In contrast, the Thermo Scientific™ Nicolet™ iN10 system required manual isolation of particles from the filters using tweezers, followed by transfer to aluminum EZ-Spot slides. While this process was labor-intensive and prone to sample loss during handling, it provided superior FTIR spectra with minimal background interference. The system also required manual selection of analysis areas on the slides, further extending the processing time. Despite these challenges, the high precision and clarity of the spectra made this system ideal for detailed polymer identification, especially for complex or mixed-material samples.

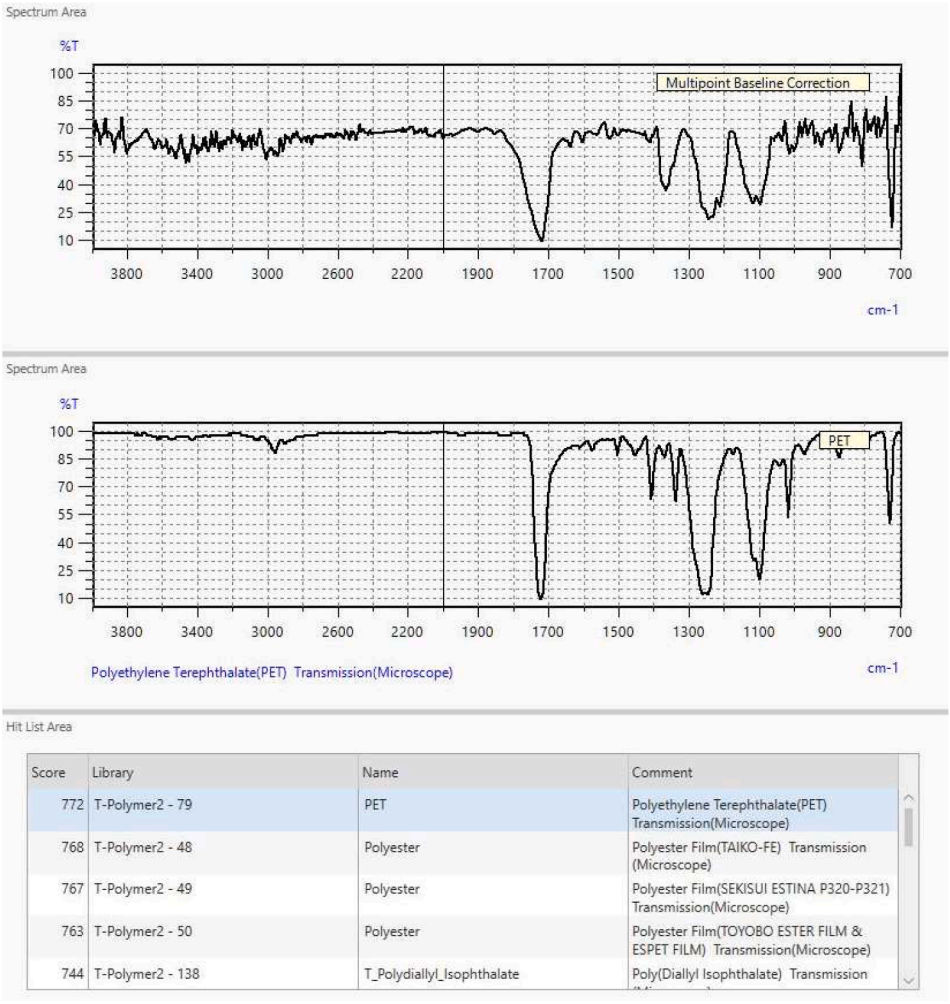
Representative FTIR spectra from both systems are shown in Fig. 3. The spectrum in Fig. 3a illustrates a microfiber analyzed using the Shimadzu AIM-9000 in transmission mode. The upper spectrum displays the sample signal after multipoint baseline correction, while the lower spectrum shows the best library match for polyethylene terephthalate (PET) from the database. A match score of 772/1000 confirmed the identity of the polymer. Slight baseline noise is visible in the sample spectrum, likely due to filter background interference, but it did not compromise identification. In Fig. 3b, the spectrum shows a microfiber analyzed using the Thermo Scientific™ Nicolet™ iN10 in reflection mode. The sample spectrum (red) is compared with the best match (purple) from the library. The top match yielded a confidence score of 87.90/100, confirming identification as polyester. Minimal baseline noise was observed, indicating cleaner spectral output due to isolated particle analysis and reduced background interference.

While both instruments identified major polymers they exhibited differences in signal clarity, throughput, and filter compatibility. Table 2 summarizes these differences. The dependent variables used in the comparison were analysis time per sample, signal-to-noise ratio, and rate of successful polymer identification (i.e., percentage of yielding a confident match). The Shimadzu AIM-9000 was efficient for routine analyses with minimal preparation, while the Thermo Scientific™ Nicolet™ iN10 was better suited for research requiring high-resolution spectral data on microparticles properly collected from the filter.

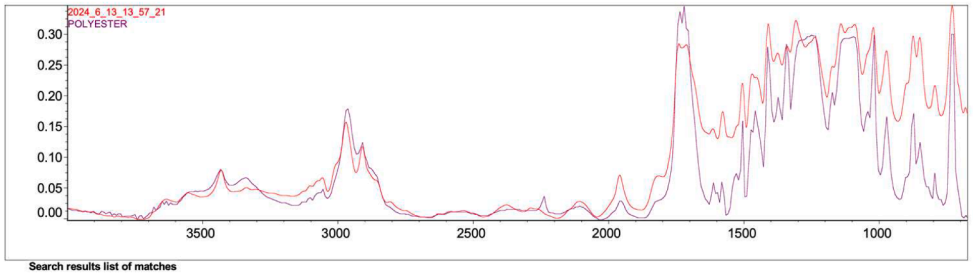
3.2. MPs/MFs analysis: quantity, composition, and dimensions

In the inflow, outflow, and sludge samples, only microfibers were observed. In contrast, the underground water sample contained a mixture of fibrous and non-fibrous particles, including microfibers, fragments, and pellets. Among the detected microparticles, microfibers accounted for 90%, fragments made up 9%, and pellets constituted 1% (Fig. 4).

MPs and MFs from all samples were measured individually, with microfiber lengths ranging from 100 μm to 4 mm (Fig. 5a). In the underground water sample, 60% of the detected microfibers were shorter than 0.5 mm, while 24% had lengths between 0.5 mm and 1 mm. Interestingly, no microfibers longer than 2 mm were observed in this sample. In the inflow, outflow, and sludge samples, the length distribution shifted, with most microfibers falling within the 0.5 mm to 1 mm range: 63% for inflow, 52% for outflow, and 33% for sludge. For these samples, microfibers shorter than 0.5 mm constituted the second largest category, highlighting the predominance of shorter microfibers across all samples. Microfibers longer than 2 mm were present in inflow,



(a)



Search results list of matches

	Index	Match	Compound Name	Library Name
1	275	87.90	POLYESTER	Synthetic Fibers by Microscope
2	242	87.02	POLYESTER	Synthetic Fibers by Microscope
3	236	82.81	POLYESTER	Synthetic Fibers by Microscope
4	241	79.66	POLYESTER	Synthetic Fibers by Microscope
5	293	79.46	POLYESTER	Synthetic Fibers by Microscope
6	298	78.91	POLYESTER	Synthetic Fibers by Microscope
7	297	78.83	POLYESTER	Synthetic Fibers by Microscope
8	228	78.72	POLYESTER	Synthetic Fibers by Microscope
9	216	77.44	POLYESTER	Synthetic Fibers by Microscope
10	238	76.39	POLYESTER	Synthetic Fibers by Microscope

(b)

Fig. 3. Representative micro-FTIR spectra and match outputs from **(a)** Shimadzu AIM-9000 Micro-FTIR, showing wavelength (cm^{-1}) vs. transmittance (%); and **(b)** Thermo Scientific™ Nicolet™ iN10, showing wavelength (cm^{-1}) vs. absorbance. Spectra were used for polymer identification of microfiber samples.

outflow, and sludge, in smaller proportions, demonstrating the variability in microfiber sizes across the sample types.

This distribution indicates that while underground water contains predominantly short microfibers, wastewater samples (inflow and outflow) and sludge show a wider range of microfiber lengths, likely due to the diverse sources and processing stages involved.

Figs. 5b and 5c illustrate the color and fluorescence distribution of MFs and MPs identified across samples. Most particles were transparent, followed by black and white (Fig. 5b). Transparent microplastics are often attributed to synthetic fibers like polyester and nylon, which are

Table 2
Comparative overview of the Shimadzu AIM-9000 and Thermo Scientific™ Nicolet™ iN10 Micro-FTIR systems used for polymer identification of microplastics and microfibers.

Metric	Shimadzu AIM-9000	Thermo Nicolet™ iN10
Sample preparation time	No preparation needed (direct filter analysis)	Requires manual particle transfer (slower workflow); risk of particle loss
Average spectral match score	Moderate (typically >70%)	High (typically >85%)
Filter compatibility	Requires suitable filters (e.g., Anodisc)	Compatible with EZ-Spot slides for isolated particles
Background interference	Moderate (dependent on filter material)	Low (minimal interference from isolated particles)
Best suited for	High-throughput screening and routine analysis	High-resolution polymer identification; complex samples

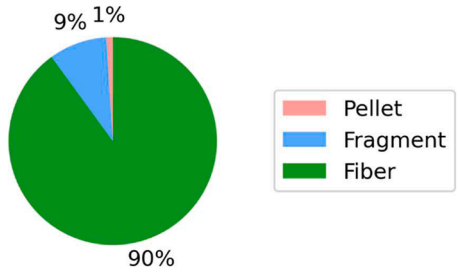


Fig. 4. Classification of microplastic types in underground water.

initially produced without pigments or dyes to be colored later in the manufacturing process (Ji, 2023). Fibers contribute significantly to transparent microplastics, as pigments can degrade or wash away during laundering or through environmental exposure (Chen et al., 2022). Environmental factors such as UV radiation, mechanical abrasion, and chemical weathering further strip pigments from fibers, leading to the dominance of transparent microplastics in aquatic ecosystems (Medyńska-Juraszek and Jadhav, 2022; Santana et al., 2021). These less visible particles pose a heightened risk of ingestion by marine organisms, emphasizing their ecological impact (Ingraffia et al., 2022).

The observed blue fluorescence in the majority of particles, as shown in Fig. 5c, is likely attributed to the presence of fluorescent whitening agents commonly used in textiles. FWAs are applied during various stages of textile processing, including dyeing, finishing, and even laundering, to enhance the brightness and vivid appearance of fabrics (Yan et al., 2024; Balestra et al., 2022). These agents work by absorbing UV radiation and re-emitting it at longer wavelengths, typically in the blue spectrum, which creates a visually striking and brighter effect (Hernández et al., 2017; Medyńska-Juraszek and Jadhav, 2022). This fluorescence property is widely used in clothing, linens, and upholstery to achieve a clean and vibrant appearance. However, FWAs are not only persistent in aquatic environments but can leach from fabrics during washing, raising environmental concerns about their potential impact on aquatic ecosystems (Santana et al., 2021).

Fig. 5d illustrates the material composition of microplastics, and microfibers identified across the four sampling points, with cellulose-based materials (e.g., cotton, rayon, and lyocell) and polyester consistently present in all samples. All results presented were obtained using methods optimized for high analytical accuracy. It is important to note that while cellulose-based fibers were frequently detected, their specific origin—whether natural (e.g., plant-derived) or anthropogenic (e.g.,

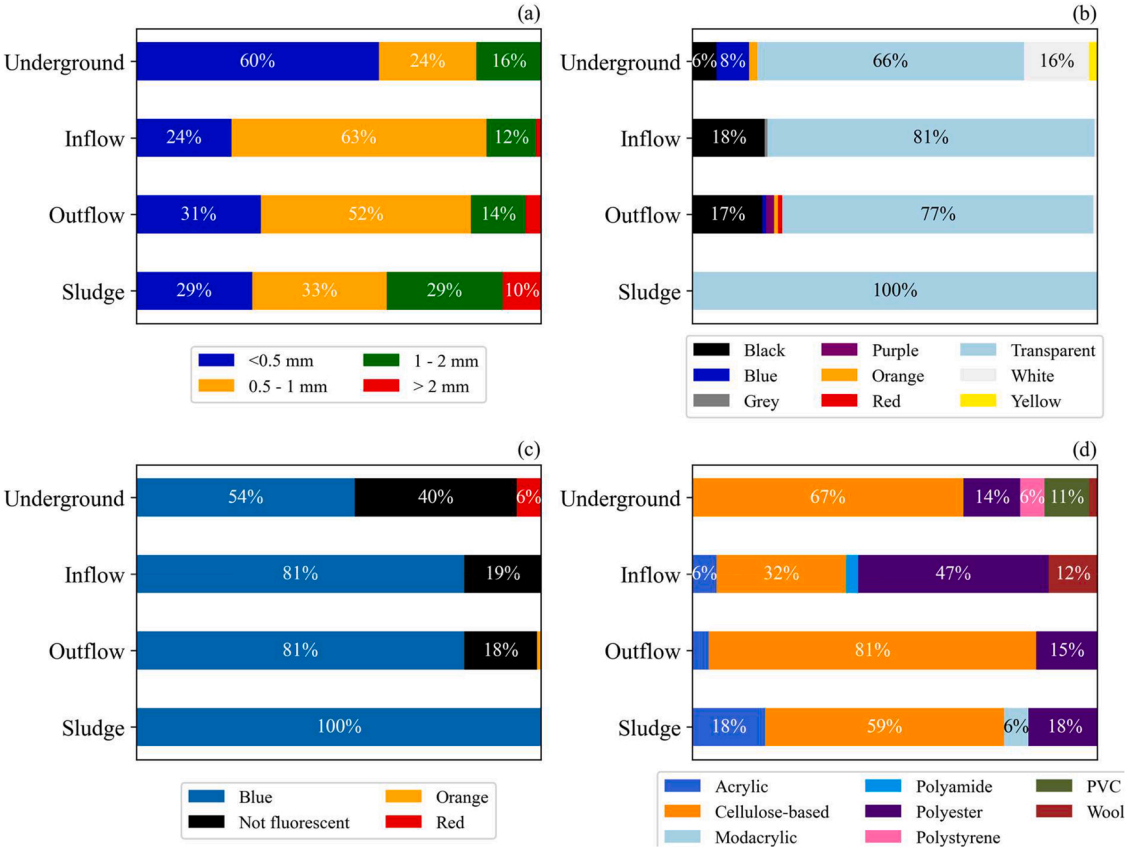


Fig. 5. **(a)** Distribution of MFs/MPs based on length; **(b)** Distribution of MFs/MPs according to color; **(c)** Distribution of MFs/MPs according to fluorescent color; **(d)** Material composition of MFs/MPs.

textile-derived)—cannot be conclusively determined based solely on FTIR spectra. This uncertainty is particularly relevant for underground water sample, where both environmental and anthropogenic inputs are possible.

The microfiber composition varied between sample types. In underground water, cellulose-based microfibers constituted the dominant group (67%), followed by polyester (14%), PVC (11%), polystyrene (6%), and wool (3%).The presence of non-fibrous MPs (PVC, PS) indicates likely contamination from non-textile sources. In inflow wastewater, synthetic microfibers were most prevalent, with polyester (47%) and cellulose-based microfibers (32%) likely reflecting discharges from textile production processes. The outflow showed a similar pattern, dominated by cellulose-based materials (81%) and polyester (15%) indicating partial retention of microfibers during treatment. In sludge samples, with significant amounts of cellulose-based materials (59%), polyester (18%), acrylic (18%), and modacrylic (6%). This suggests that the sludge acts as a sink for both natural and synthetic microfibers, particularly those with higher densities or a greater tendency to agglomerate.

This composition pattern is consistent with usage trends in the textile industry, where cellulose-based materials are favored for its comfort and breathability, and polyester for its durability and cost-efficiency (Boucher and Friot, 2017). Kanhai et al. (2017) reported that polyester accounted for nearly 50% of microfibers in marine environments, highlighting its environmental persistence. The frequent detection of rayon, a semi-synthetic fiber derived from cellulose, aligns with its widespread use in blended fabrics (Haque et al., 2024). Meanwhile, the presence of PVC and polystyrene in underground water points to potential contamination from non-textile sources, such as industrial runoff or plastic waste streams (Kumar et al., 2023).

3.3. Efficiency assessment of the wastewater treatment plant

The amount of microplastics/microfibers released from each sample is summarized in Table 3. A total of 18 microparticles were detected in the procedural blanks, indicating potential contamination from laboratory materials or the processing environment. This baseline count was subtracted from all sample results to correct for procedural contamination and ensure accurate quantification of MPs and MFs.

The calculation for wastewater treatment plant efficiency is as follows:

$$\text{Efficiency}(\%) = \frac{\text{InflowMFs} - \text{OutflowMFs} \times 100}{\text{InflowMFs}}$$

The microfiber concentrations were 135 MFs/L for underground water, 825 MFs/L for inflow wastewater, 520 MFs/L for outflow wastewater, and 455 MFs/L for sludge. The efficiency of the wastewater treatment plant, calculated based on the difference between inflow and outflow microfiber concentrations, was determined to be 36.97%.

The boxplot provides a clear visualization of microplastic concentrations across different sample types, highlighting distinct trends and variability (Fig. 6). Underground water exhibited low microplastic and microfiber concentrations with minimal variability. In contrast, inflow wastewater showed significantly higher concentrations and greater variability, emphasizing its role as a primary source of pollution. Outflow wastewater demonstrated reduced microfiber concentrations compared to inflow, reflecting the partial removal efficiency of the treatment process, which aligns with reported removal efficiencies of 35%–59% for microplastics during the preliminary phase in wastewater treatment plants (Sun et al., 2019). Sludge, however, exhibited higher variability and elevated median concentrations, likely due to heterogeneity in organic content and particle aggregation, making it a significant sink for microplastics/microfibers.

4. Conclusion

This study presents a comprehensive comparison of analytical methodologies for the detection and characterization of microfibers and microplastics across four wastewater stages — underground water, inflow, outflow, and sludge — at a textile dyeing facility. By integrating oxidative pretreatment techniques, the use of different filters, and two distinct micro-FTIR spectroscopic instruments, key workflow optimizations and analytical trade-offs were identified for microparticle analysis in different wastewater matrices. Inflow wastewater exhibited the highest microfiber concentration (825 MFs/L), while the wastewater treatment plant demonstrated a partial removal efficiency of 36.97%. Polyester and cellulose-based materials (e.g., cotton, rayon and lyocell) were the most frequently identified fiber types. The predominance of transparent and fluorescent microfibers — likely associated with textile processing and the use of fluorescent whitening agents — suggests a need for more sustainable production practices, while the partial microfiber removal efficiency highlights the importance of improving wastewater treatment technologies.

Among the pretreatment strategies tested (no treatment, quick oxidative, and prolonged oxidative), quick oxidative treatment enabled faster sample processing, whereas prolonged oxidative treatment, aligned with ISO 4484–2, offered a low-energy alternative. Single-step filtration using MCE or aluminum oxide filters was found to be the most practical and analytically compatible method, with filter selection dependent on the spectroscopic instrument used. Instrument comparison revealed that the Shimadzu AIM-9000 enabled rapid, direct-filter analysis, while the Thermo Scientific Nicolet iN10 delivered superior spectral resolution through isolated particle analysis.

Several limitations were identified, including a practical size detection limit of approximately 100 μm, which was set as the minimum microfiber/microplastic length measured in this study to ensure reliable optical and spectroscopic analysis. As a result, microparticles smaller than 100 μm were not evaluated. Additional limitations include classification uncertainty among spectrally similar cellulose-based materials (e.g., cotton, rayon and lyocell) and potential material loss during manual particle handling for Nicolet iN10 analysis. Future research should aim to enhance particle detection below 100 μm through micro-

Table 3
The amount of microplastics and microfibers.

Sample Type	Replicate 1	Replicate 2	Replicate 3	Average Count	MPs and MFs/L
Underground Water	31	27	23	27	135
Inflow Wastewater	159	172	164	165	825
Outflow Wastewater	97	111	103	104	520
Sludge	84	114	75	91	455

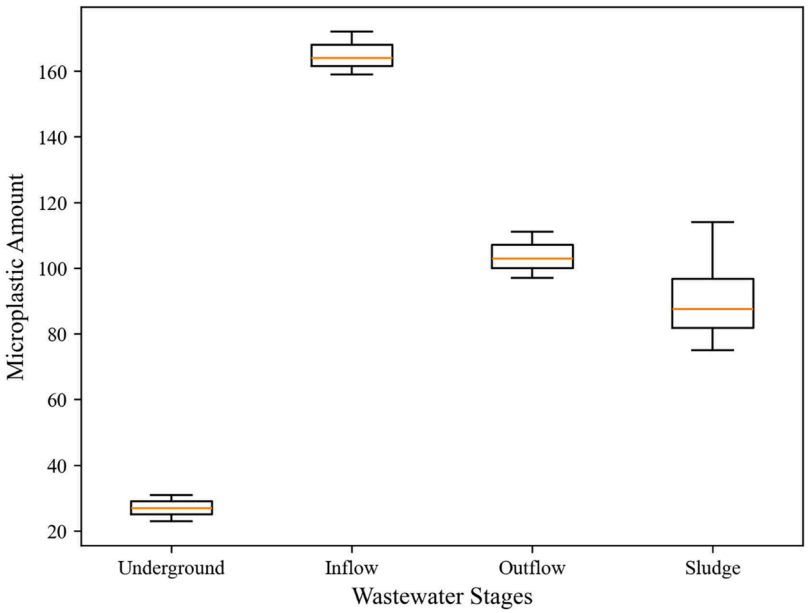


Fig. 6. Boxplot of microplastic amounts.

FTIR and micro-Raman spectroscopy or advanced imaging techniques, reduce operator-related variability through automated analysis workflows, and contribute to the development of standardized microfiber monitoring protocols to support global comparability and regulatory frameworks.

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CRediT authorship contribution statement

Sinem Hazal Akyıldız: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Valentina Balestra:** Writing – review & editing, Conceptualization. **Lia Drudi:** Writing – review & editing, Visualization. **Paola Marini:** Writing – review & editing, Supervision. **Rossana Bellopede:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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