multi-Risk sciEnce for resilienT commUnities undeR a changiNgclimate

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AUTHORS

Claudio Lubello (UNIFI); Riccardo Gori (UNIFI); Benedetta Pagliaccia (UNIFI); Rajandrea Sethi (PoliTO); Carlo Bianco (PoliTO); Alessandro Casasso (PoliTO); Marco Coha; Monica Granetto (PoliTO); Silvia Lupato(PoliTO); Alberto Tiraferri(PoliTO)

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2. ABSTRACT

This deliverable reports the outcomes of Task T4.5.1 within Work Package 5 and addresses the growing environmental concern posed by microplastics (MPs) and other microparticles in wastewater systems. Microplastics represent an emerging global pollutant due to their persistence, small size and ability to act as vectors for hazardous chemicals. Their widespread presence in aquatic environments raises concerns for ecosystems and human health, making wastewater treatment plants (WWTPs) a critical control point for limiting their release into receiving water bodies.

The work combines large-scale field monitoring with the development of advanced analytical methodologies to improve the detection, characterization and quantification of MPs. Monitoring campaigns were conducted in three full-scale WWTPs treating municipal and industrial wastewaters, including contributions from textile and tannery districts. A harmonized sampling and analytical protocol were implemented to ensure comparability across sites. Microplastic extraction relied on multi-stage pretreatment procedures, followed by Laser Direct InfraRed (LDIR) chemical imaging for automated identification and characterization of particles in terms of polymer composition, size and morphology.

The results demonstrate that influent wastewaters contain high concentrations of microparticles, with levels and composition strongly influenced by wastewater origin and industrial activities. Treatment processes significantly reduced particle loads, with overall removal efficiencies ranging from approximately 84% to nearly complete removal in plants equipped with advanced technologies. Primary sedimentation and tertiary or membrane-based treatments were identified as key stages for particle retention. Nevertheless, small particles and fiber-like materials showed lower removal efficiencies, confirming the critical role of particle size and shape in determining treatment performance. A large fraction of retained particles accumulated in sewage sludge. Anaerobic digestion did not effectively degrade MPs and was associated with particle fragmentation, highlighting potential risks related to sludge reuse and long-term environmental persistence.

In parallel, a standardized fluorescence-based method was developed for the quantification and morphological analysis of polyester microfibers released during domestic washing processes. The method, based on intrinsic UV fluorescence and automated image analysis, demonstrated high sensitivity and reliability in real wastewater matrices. Morphological observations revealed significant fiber fragmentation during laundering, suggesting an increased potential for environmental dispersion.

Overall, the deliverable establishes an integrated framework for assessing the fate and removal of microplastics in WWTPs and advances analytical standardization for microfiber detection. The findings contribute to a deeper understanding of microparticle dynamics along the urban water cycle and support the identification of strategies aimed at reducing microplastic emissions to the environment.

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APPENDIX

4.Fate and removal of microplastics in wastewater treatment plants

4.1 Fate and removal of microplastics in wastewater treatment plants (UNIFI)

(Contributors: Claudio Lubello, Riccardo Gori, Benedetta Pagliaccia)

4.1.1 Introduction

The massive consumption of plastic products, coupled with inadequate solid waste management practices, contributed to the spread and ubiquity of microplastics (MPs) in all environmental compartments (Sun et al., 2019). MPs are typically defined as plastic debris smaller than 5 mm in size, including a wide range of particles with heterogeneous characteristics in terms of chemical composition, size, color and shape. They can be classified based on their origin into primary microplastics – intentionally produced in the form of pellets or microbeads to be used in air-blasting technology, personal care and cosmetics products, etc. (Cole et al., 2011; Magni et al., 2019; Ngo et al., 2019) – and secondary microplastics – originated from degradation/breakdown of larger plastic items via exposure to environmental stressors such as water, wind and sunlight (Eerkes-Medrano et al., 2015).

In recent years, growing research efforts have been focused on tracking MPs in the environment and assessing their potential adverse effects on ecosystems, living organisms and human health. Owing to their small size, MPs are considered bioavailable to organisms throughout the food-web, with the potential to inflict damage to biota upon ingestion (Cole et al., 2011). They could act as carrier for harmful chemicals that further increase their potential ecotoxicity (Talvitie et al., 2017b).

Wastewater treatment plants (WWTPs) are expected to play a crucial role in mitigating MPs pollution, serving as a primary barrier to prevent their massive release into receiving water bodies. Their efficiency and removal mechanisms are reasonably influenced by a complex interplay of factors – such as treatment configurations, operating conditions and particle characteristics – pointing out the need for ongoing research to optimize their performance and minimize environmental emissions. The current state-of-the-art knowledge is still affected by a high degree of variability and uncertainty in the available literature data. This variability arises not only from differences in the design of the monitored facilities but also from the diverse methodological approaches applied (e.g. sampling strategies, detection technique, particle size ranges, etc.). The lack of reference and/or harmonized protocols therefore hinder direct comparison among scientific reports, hampering the establishment of standardized assessment frameworks.

In this perspective, the activities carried out in WP4.5 – Task 4.51 – aimed to provide a comprehensive overview of the occurrence, fate and removal of MPs and other microparticles of interest across different treatment configurations. The final goal was hence the collection of a robust set of field data able to give valuable insights regarding the persistence and characteristics of MPs / microparticles across the urban water cycle and to suggest effective strategies for limiting their discharges into freshwater environments. The same data framework was therefore shared and jointly interpreted between the two work packages from complementary perspectives. In WP4.3, the impact and significance of data processing mainly concerned the contribution of WWTPs to MPs pollution in freshwater natural environments. Conversely, in WP4.5, most of the research effort was devoted to provide a comprehensive overview of the presence, fate and removal dynamics of MPs in different treatment systems. This complementary distribution of objectives ensured a coherent and integrated understanding of both environmental implications and technical performance of WWTPs with respect to MPs management. Additionally, the integrated approach ensured methodological coherence and enhanced the cross-WP analytical capacity, leading to more comprehensive and consistent project outcomes.

4.1.2 Methodologies

4.1.2.1 Description of WWTPs and sampling sites along the treatment trains

The occurrence and fate of MPs in wastewater was conducted through a series of monitoring campaigns at three WWTPs located in Tuscany, Italy. The selected WWTPs were strategically chosen to represent a wide range of treatment configurations and influent sources, including both municipal and industrial wastewater (WW). This variability allowed for a more robust assessment of the performance of different process units in removing MPs / microparticles. This choice also helped clarify the impact of specific industrial activities characteristic of the region, mainly textile and tanning facilities, on the MPs / microparticle loads and removal dynamics.

The so-called WWTPA treats municipal wastewater from the Florence urban area (Italy), conveyed to the plant through a combined sewer system. The plant has a potentiality of 600000 PE, with an average influent flow of about 200000 m^3/day. It has in use a treatment train consisting of mechanical pretreatments, a biological treatment to remove organics and nitrogen, a chemical co-precipitation of phosphorous and a secondary settling.

The so-called WWTPB is a large treatment facility receiving both domestic and industrial wastewater, with an average inflow capacity of approximately 100000 m^3/d and a design potentiality of 900000 PE. The industrial wastewater mainly comes from the textile industries located in the textile district of Prato (Tuscany, Italy) and is partially conveyed to the plant by a separate sewer system. The treatment train consists of physical pretreatments (i.e. coarse and fine screening, grit removal), primary sedimentation, equalization, biological oxidation/nitrification, coagulation–flocculation and final ozonation. The clarified effluent is partially discharged into a surface water body and partially sent to a refining section for water reclamation in the textile district.

The so-called WWTPC has a design capacity of approximately 850000 PE and treats both municipal WW and vegetable tannery WW produced in the leather-industry district of Santa Croce sull'Arno (Pisa, Tuscany). Municipal flow rate represents approximately 40 % of the total inflow but its contribution in terms of carbon and nitrogen incoming load is less than 2 %. The current configuration of the plant is characterized by two independent lines that differ in the type of WW treated. The main line (S2) is dedicated to the treatment of both tannery wastewater and part (about 67 %) of the total urban wastewater, mainly domestic, which is collected to the plant. The other line (S3) uses a membrane bioreactor (MBR)-based technology to treat the residual urban wastewater. Line S2 consists of physical pretreatments (fine screening, oil and grit removal), primary settling, biological processes (denitrification and oxidation/nitrification) and secondary settling, tertiary treatments (coagulation – flocculation) and tertiary settling. Line S3 consists of fine screening, denitrification + oxidation/nitrification, MBR and deoxygenation.

In all the above-mentioned WWTPs, average 24-h composite samples were taken through autosamplers in distinct sections along the wastewater treatment line and stored into closed stainless-steel containers equipped with a tap and an opening for the installation of a mechanical stirrer. A sub-sampling procedure was then applied to reduce the sample volume to be processed and analyzed without losing its representativeness. Depending on the expected concentration of MPs, aliquots of different volumes (Table 1), judged as sufficiently representative according to preliminary investigations (refer to DV 4.3.2 for the methodological insights), were collected from the 24-h composite samples keeping them under continuous mechanical agitation to ensure sub-sample homogeneity, transferred into glass bottles and stored at -4 °C before being processed and analyzed. All sampling campaigns were carried out in the dry season to enable data comparison among the monitored WWTPs.

Table 1: Sampling points along the water treatment line of the monitored WWTPs and related processed volume aliquots.

Plant (#)	Sampling section (#)	Volume of processed sub-samples (L)
$WWTP_A$	Municipal wastewater at the inlet section (IN)	1
	Final effluent discharged into receiving water body (OUT)	5
$WWTP_B$	Industrial wastewater from separate sewer (IN-SS)	1
	Mixed municipal and industrial wastewater at the inlet section (IN) [1]	1
	Effluent of primary settling (SED I)	1
	Effluent of secondary settling (SED II)	5
	Final effluent discharged into receiving water body (OUT)	5
	Effluent of refining section for water reclamation (REUSE)	10
$WWTP_C$	Municipal wastewater at the inlet section (IN-Mun)	1
	Industrial wastewater at the inlet section (IN-Ind)	1
	Effluent of MBR section (OUT-MBR)	5
	Final effluent discharged into receiving water body (OUT)	5

[1] Mixed municipal and textile industrial wastewater were sampled where the two fluxes are mixed (after course screening).

In the case of $WWTP_A$, grab samples of sludge of approximately 1 L each were also collected in the following sections:

- Return sludge (RET SLUDGE);
- Thickened sludge (IN DIG);
- Sludge after anaerobic digestion (OUT DIG).

The total suspended solids (TSS) and volatile suspended solids (VSS) of sludge samples, which were measured according to standard methods (APHA/AWWA/WEF, 2017), are listed in **Table 2**.

Table 2: Concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) in the sludge samples collected at $WWTP_A$.

Sludge sample (#)	TSS (g/L)	VSS (g/L)	VSS/TSS (% w/w)
RET SLUDGE	13.1	6.2	47.3
IN DIG	37.0	17.8	48.0
OUT DIG	33.0	13.5	41.0

4.1.2.2 Sample processing for MPs extraction

In the case of wastewater, sample processing for MPs extraction consisted of a multi-stage protocol including (i) sample concentration by vacuum-filtration (5 µm-mesh) and recovery of the material retained on the filter with a smaller volume of ultrapure water, (ii) Fenton reaction for organic matter digestion, (iii) density-based separation for inorganics removal and (iv) particle recovery by vacuum-filtration and subsequent filter backwashing with ethanol (EtOH). The protocol was also adapted for the analysis of sludge samples. In this case, the preliminary concentration stage was not performed due to the high expected particle concentration. In contrast, all sludge samples were diluted with ultrapure water at a Total Suspended Solids (TSS) concentration that enabled an effective Fenton reaction (i.e. 13.1 g TSS/L): for each sampling point over the treatment line, 12 g wet weight (WW) diluted sludge were hence subjected to the same pretreatment procedure described above for wastewater.

Quality Assurance and Quality Control (QA/QC) procedures – including the analysis of blank control samples – were implemented to prevent (and quantify) cross- and self-contaminations during all sampling and laboratory stages. The particle recovery rate of the applied procedure was estimated to 96 ± 4 %. For more detailed on the developed pretreatment method refer to deliverable DV 4.3.2.

4.1.2.3 Laser Direct InfraRed (LDIR)-based analysis for particle detection and characterization

A reliable and robust method based on the recently introduced Laser Direct InfraRed (LDIR) chemical imaging technique was specifically developed and fined-tuned within the project, in the framework of WP4.3 – Task 4.3.1. LDIR uses the latest quantum cascade laser technology, coupled with fast-scanning optics, to provide high-quality images and spectral data in the wavenumber range from 975 to 1800 cm–1. The MPs-dedicated LDIR workflow first provides a rapid imaging of sample area by using an IR light at a single wavenumber instead of visible cameras to locate, count and describe size and shape of particles. For each detected particle, full spectra are then acquired, while chemical identification is automatically carried out via real-time spectral matching with built-in libraries (Dong et al., 2022; N. Liu et al., 2022; Samandra et al., 2022; Scircle et al., 2020). The method therefore allows quantification and comprehensive characterization of MPs ≥ 10 µm through a fully automated workflow and with measuring times significantly reduced compared to more conventional spectroscopic techniques. Of major interest is its feasibility of acquiring a broad set of data concerning size and shape of detected particles, which could be used for modelling and predicted analyses. For more details on the analytical method applied refer to deliverable DV 4.3.2. Briefly, from a practical point of view, 20 µL-aliquots of the particle dispersion in EtOH obtained through the previously described extraction protocol were dropped on microscope reflective slides (Kevley Low-e Microscope slide) and introduced into the instrument camera for analysis. All measurements were carried out with an Agilent 8700 LDIR under the microplastic-dedicated workflow provided by Agilent Clarity software (version 1.6.83). Overall, 240 µL of the particle dispersion in EtOH (i.e. 12 depositions of 20 µL each vs. 5 mL total volume) were analyzed by LDIR-based particle analysis for each processed sub-sample. To determine such a minimum representative volume to be characterized, preliminary measurements were carried out by applying the LDIR-based particle analysis on an increasing number of depositions (of 20 µL each) and then processing collected data through statistical analysis. For more details on this, refer to section A1 of the attached Appendix.

Depending on the wastewater source, other microparticles of interest were considered in the analysis in addition to MPs. Particularly, with the aim of investigating the impact of textiles on microlitter particle production, the detection method was optimized for enabling the identification of both natural and synthetic textiles in samples collected at WWTPB. To this aim, a customized spectral library was developed by including reference spectra of single-component textile fibers in cotton, wool, acrylic, linen and viscose in addition to common plastic materials. This in-built database was used during the LDIR-based particle analysis for the automized chemical assignment of detected particles. As already documented in the literature, it is not easy to distinguish among different cellulose-based fibers (i.e. cotton, linen and in some cases viscose) due to the high similarities of their IR spectra (Peets et al., 2017): such a strong similarity was evidenced also in the LDIR spectra acquired in this study, an example of which is shown in Figure 1. Discrimination among cellulose-based textiles is made even more complicated by the fact that mixed fibers (e.g. cellulose-polyester) are often employed. Bearing in mind the above, for the chemical characterization of detected particles from WWTPB, cellulose-based textiles (i.e. cotton, linen and viscose) were considered all together under the TXT-CELL class. To give an idea of the partitioning of TXT-CELL particles, their specific chemical assignment was also reported, even if not considered sufficient reliable, especially in the case of cotton and linen.

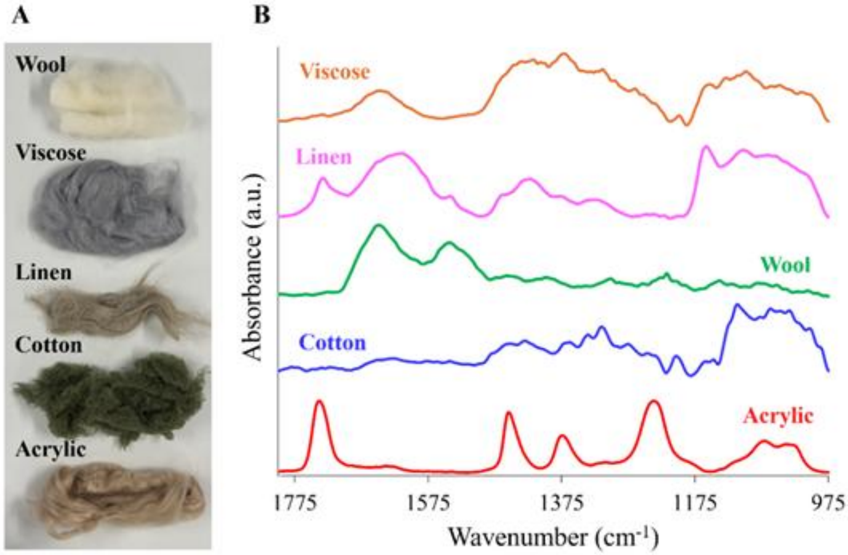

Figure 1: Textile fibers in acrylic, cotton, linen, viscose and wool (A) and examples of their LDIR spectra (B) used for the integration of a customized spectral library for particle chemical identification during LDIR-based particle analysis.

4.1.2.4 Data analysis

Only particles in the 10 µm – 5 mm size range which were chemically identified with a hit quality index (HQI) ≥ 0.80 were considered for data elaboration, where HQI is a parameter ranging between 0 and 1 describing how closely the sample spectrum matches that in the reference library.

Depending on the wastewater source, different classes of microparticles were included in the analysis in addition to MPs.

- For WWTPA and WWTPC, chemical assignments were performed by using the default spectral library, including both MPs and cellulosic particles. It is worth noting that LDIR associates with the general class "Cellulosic" a broad range of cellulose-based materials from both natural and chemically modified origin.
- In the case of WWTPB, due to the high expected impact of textiles, the customized spectral library described at section 4.2.3 was employed for chemical identification, allowing the following particle categories to be distinguished: (i) textile-derived particles (TXT) of both natural origin (i.e. cotton, linen and wool) and synthetic/artificial nature (i.e. viscose, acrylic, polyamide), (ii) cellulose-based particles (CELL) from both natural and chemically modified/artificial sources, which were in turn classified into non-textile items (nTXT-CELL) and textile-derived items (TXT-CELL), and MPs. It should be considered that some polymers excluded from the TXT class, such as PET, PES and others, may still originate from textile sources. Consequently, the actual contribution of industrial and domestic activities to textile micro-sized particle generation may be underestimated in this study. This categorization choice was made to ensure that only polymers with a clearly identifiable textile origin were included in the TXT group, as excluded materials may derive from a variety of industrial applications and/or domestic contributions (e.g. garment washing), making source attribution less certain.

The reason for including in the analysis particles from both synthetic and natural sources mainly refer to their potential ecotoxicological effects to biota: despite their origin, these micro-sized items could act as carrier for harmful chemicals absorbed from the surrounding environment and/or present as additive in the pristine products (Talvitie et al., 2017b), thus requiring targeted monitoring.

The concentrations of MPs/microparticles were expressed (net of blank) as number of items per liter in the case of wastewater samples. For sludge, the concentrations were reported on both dry weight and wet weight basis, as number of items per g TSS/g WW. The MPs/microparticle concentrations were shown as average values ± standard deviations among the sub-sample aliquots processed for each sampling site. The average removal efficiency of each monitored process unit was calculated as follows (Eq. 1):

$$Removal\ efficiency_i = \frac{C_{in,i} - C_{out,i}}{C_{in,i}} \quad (1)$$

where C_(in,i)and C_(out,i)(items/L) are the average particle concentrations entering and leaving process unit i, respectively.

Data on chemical assignments were directly used to determine the polymer distribution of detected particles. Regarding size and morphology characterization, based on the shape-related parameters aspect ratio (AR) and circularity (C) given by the LDIR-based analysis, particles were classified into fibers (AR ≥ 3 and AR ≤ 0.33), pellets (0.6 ≤ C ≤ 0.9), spheres (C > 0.9) and fragments (0.33 < AR < 3, C < 0.6) (Liu et al., 2022). Depending on the assigned morphology, a characteristic size was associated with each particle, i.e. maximum length between wide and height for fibers and diameter for the other morphologies. Chemical and geometric characterization results are presented as percentage data with respect to the total of items detected in the processed samples (Eq. 2):

$$Relative\ abundance\ of\ particle\ class\ i = \frac{n.\ items_i}{n.\ items_{TOT}} \cdot 100\% \quad (2)$$

where $n.\ items_i$ is the number of particles of each polymeric / size / morphology class and $n.\ items_{TOT}$ is the total number of particles identified in the sample under observation.

To enable a more comprehensive characterization of particles found in the processed samples, particle volume and mass were also estimated as follows. By using the numerous 2D geometrical parameters provided by the LDIR-based particle analysis, shape-dependent models available in the literature (Barchiesi et al., 2023; Simon et al., 2018) were applied to estimate the volume of each particle i detected in the processed samples (Vi, µm3). Knowing the density of particle i (ρi, g/cm3) and its volume, particle mass (mi, µg) was easily calculated: mi = Vi · ρi: in this way, numerical concentrations (items/L) were converted to a mass basis (µg items/L).

4.1.3 Results

4.1.3.1 Full-scale monitoring at $WWTP_A$

The average particle concentrations found in the analyzed raw and treated wastewater from WWTPA are listed in Table 3. Based on these values, an average MPs removal efficiency of 84 % (88 % without including cellulosic items in the dataset) was estimated for WWTPA: this value was line with that reported in the literature for plant configurations not equipped with advanced tertiary treatment technologies (Kardel et al., 2025). More remarkable discrepancies with respect to literature data were highlighted in terms of MPs concentrations. According to that reported in previous scientific papers, the MPs content in municipal wastewater typically varies from many thousands of particles per liter (Hidayaturrahman & Lee, 2019; Simon et al., 2018) to only a few particles per liter (Bayo et al., 2020; Pittura et al., 2021), decreasing by at least one order of magnitude in the final effluents. Such a high inter-plant variability is not only related to the different WWTP-related conditions – such as process units employed, nature of treated wastewater, type of sewer system, etc. – but is also largely influenced by the diverse sampling, pretreatment and analysis methods for particle detection and characterization, thus hampering direct comparisons among scientific reports (Sun et al., 2019). It can be noted that the concentration data obtained for WWTPA were lower than those emerged from the first monitoring campaign described in DV 4.3.2, while only minor differences were evidenced between the estimated removal rates. This evidence could be ascribed to the methodological improvements introduced in this project stage. In particular, the volume of the particle dispersion in EtOH analyzed by LDIR was increased by 300 % (60 µL vs. 240 µL), thus enanching the system capability to dected the actual particle concentration and their full distribution within the samples under observation. Referring exclusively to MPs, the mass concentrations predicted for the analyzed samples of IN and OUT were 2014 and 1465 µg MPs/L, respectively.

Table 3: Particle concentrations – expressed as number of items per liter – found in raw wastewater and final effluent samples collected at WWTP$_A$.

	IN (items/L)	OUT (items/L)
With CELLULOSIC	14205 ± 563	2300 ± 369
Without CELLULOSIC	10624 ± 2074	1309 ± 369

The classifications of detected particles in terms of polymer type, size and shape are presented in Figure 2. The chemical and physical characterization data aligned with those collected from the previous monitoring campaign described in deliverable DV 4.3.2: therefore, the same considerations introduced above can be applied to the interpretation of the present results, especially regarding particle sources/production paths and consistency with the current state-of-the-art knowledge.

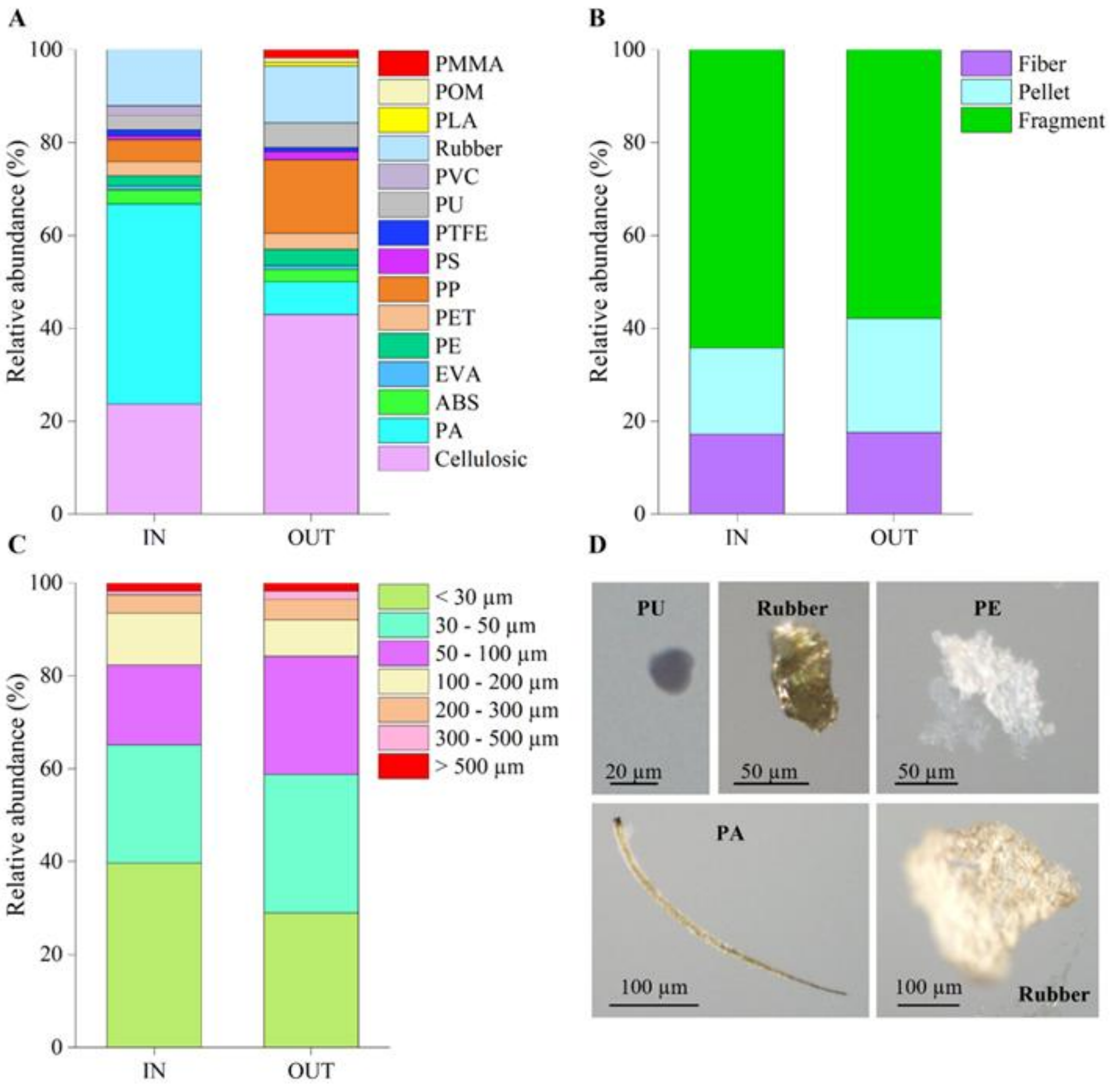

Figure 2: Characterization of particles found in the samples of raw wastewater and final effluent collected at WWTPA in terms of polymer type (A), shape (B) and size (C), and high-magnification visible images of many particles detected (D).

Cellulosic particles were largely present in the processed samples, accounting for about 24 % and 43 % of total items in IN and OUT, respectively. The relatively high content of cellulosic can be ascribed to a broad range of sources, including both natural cellulose and its artificial/semi-synthetic derivates from textiles and/or other consumer products. The abundance of cellulosic microparticles in municipal wastewater is documented in the literature. For instance, Gies et al. (2018) found a relatively high content of modified cellulose and cotton – accounting for about 59 % and 7 % of particles chemically identified, respectively – in the influent of a large WWTP in Canada receiving both municipal wastewater and stormwater from combined sewers. Rubber represented a relevant fraction in both raw and treated wastewater (approximately 12 % of total particles in both IN and OUT). Small rubber debris can largely originate from tire erosion across roads in the urban area served by the combined sewer and/or directly in the treatment site (Kole et al., 2017). By exploiting the material specification of reference spectra including in the LDIR in-built library, it was estimated that about 29 % of rubber items in raw wastewater derived from tire wear, thus highlighting their impact on the overall MPs load entering the plant. Due to their secondary production source – typically derived from abrasion/degradation mechanisms – rubber particles were largely in the form of fragments (approximately 57 % and 42 % of rubber items, in IN and OUT, respectively, Figure 3).

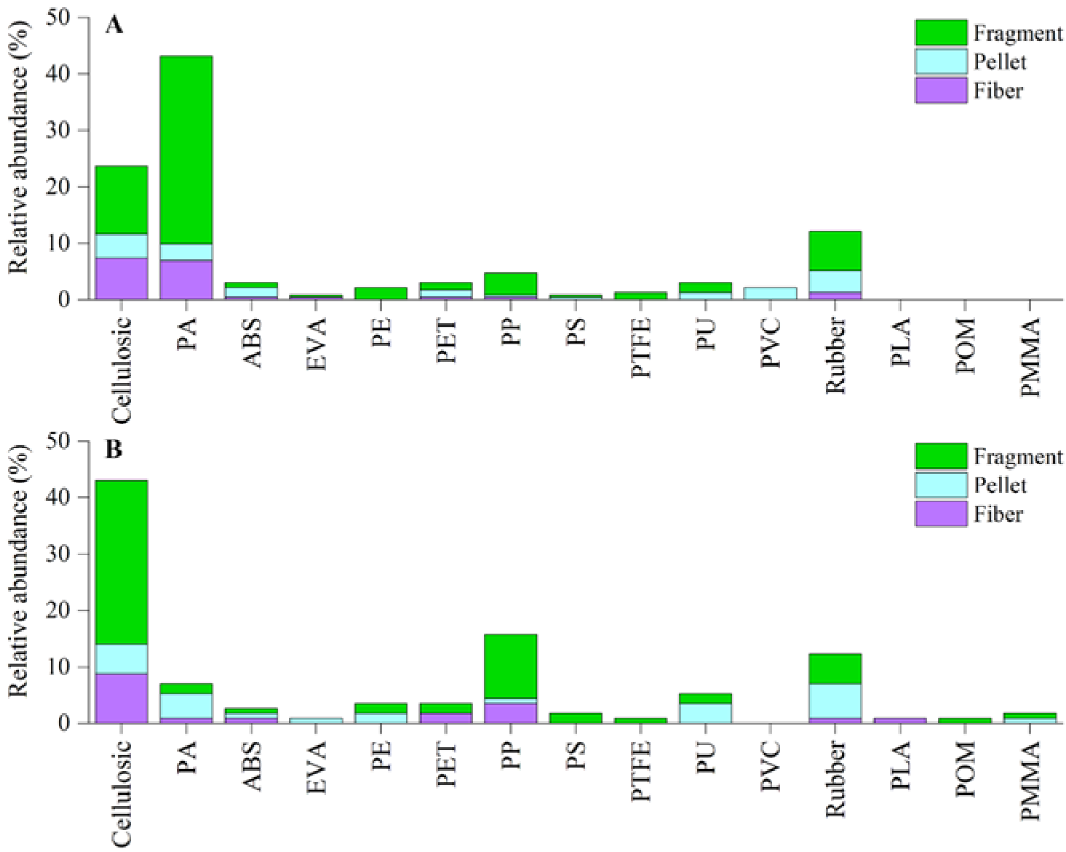

Figure 3: Chemical and shape cross-classification of particles in raw wastewater (**A**) and final effluent (**B**) collected at $WWTP_A$.

Large quantities of polyamide (PA) particles were found in the processed samples, especially in raw wastewater (about 43 % of total items in IN), and mainly attributed to synthetic textiles: in areas with minor industrial inputs, their presence into wastewater can be therefore largely attributed to microfiber release during laundry processes in households (Hernandez et al., 2017). To be noticed that cellulosic and PA fibers – for which a textile origin could be reasonably predicted – together accounted for about 14 % and 10 % of total items in IN and OUT, respectively (Figure 3). The other polymers found – including polypropylene (PP), polyethylene terephthalate (PET), polyethylene (PE), polytetrafluoroethylene (PTFE), polyurethane (PU), polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC), polylactic acid (PLA), polyoxymethylene (POM), ethylene vinyl acetate (EVA) and polymethyl methacrylate (PMMA) –

can be associated with multiple sources such as single-use plastics, packaging, personal care products, consumer products, synthetic textiles, mechanical components, piping systems, foam, paints, etc. The wide variety of polymers identified in the processed samples therefore reflects the diverse and multifaceted origins of MPs in municipal wastewater. This heterogeneity highlights the complexity of the MPs release pathways, which may involve a broad spectrum of domestic and urban sources contributing to the overall pollution load.

Regarding size distribution, it was found that 82 % and 84 % of particles detected in both raw and treated wastewater samples, respectively, had a characteristic size lower than 100 µm, with a relevant fraction of them – approximately 40 % and 29 % of total items in IN and OUT, respectively – smaller than 30 µm. The relatively high abundance of the smallest size classes recorded in this monitoring campaign aligned with previous scientific reports (Simon et al., 2018). In terms of morphology, fragments – comprising about 64 % and 58 % of total particles, in IN and OUT, respectively – represented the prevalent category, followed by pellets and fibers. No speres were found, thus suggesting the secondary origin of detected micro-sized debris. The results in terms of geometrical description of identified particles are consistent with the existing literature framework, which highlights that fragments – primarily resulting from the degradation and/or mechanical breakdown of larger plastic debris – tend to be one of the most prevalent type of MPs in wastewater (Bayo et al., 2020; Magni et al., 2019; Sun et al., 2019). Typically, pellets were associated with the smallest size classes – most of them measuring less than 30 µm (77 % and 71 % of total pellets in IN and OUT, respectively) – while fibers were mostly longer than 100 µm (68 % and 60 % of total fibers in IN and OUT, respectively). In contrast, fragments exhibited a broader size distribution, consistent with their secondary origin (Figure 4).

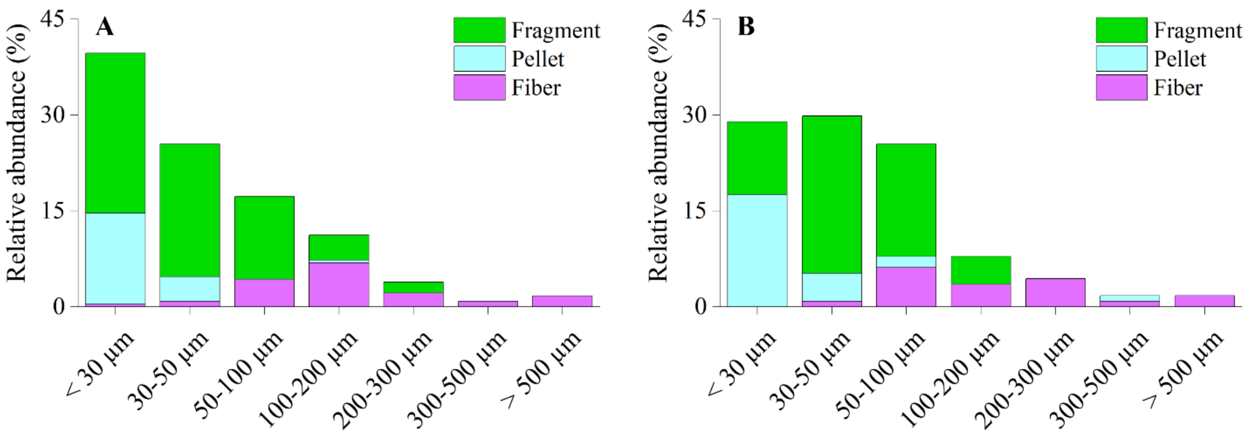

Figure 4: Size and shape cross-classification of particles in raw wastewater (**A**) and final effluent (**B**) collected at $WWTP_A$.

Further insights into the potential removal of specific classes of particles are given below. Referring to chemical composition, the lowest removal was observed for PP particles (approximately 46 %) and reasonably ascribed to their low density (0.83 – 0.92 g/cm3, Sun et al., 2019): this property would limit their tendency to settle during sedimentation processes, hence promoting their persistence and transport through subsequent treatment stages. However, a linear correlation was not observed between polymer density and removal efficiency (Figure 5A): this finding would suggest that the mechanisms governing MPs removal in wastewater treatment processes are influenced by a complex interaction of parameters, among which particle shape and size are two of the main determining factors. These physical characteristics affect settling behaviour, interaction with sludge flocs and tendence of particles to be retained or bypass the various treatment stages, thus playing a more significant role than density alone. Considering the size and shape cross-classification (Figure 4), lower removal rates were observed for fibers (especially those in the 50 – 100 µm and 200 – 300 µm size ranges), pellets < 50 µm and fragments measuring from 30 µm to 200 µm (Figure 5B). As widely reported in the literature, smaller particles can more easily escape the treatment processes, which are generally more effective at capturing larger plastic debris that tends to settle by gravity and/or become trapped in sludge flocs (Dris et al., 2018; Talvitie et al., 2017b).

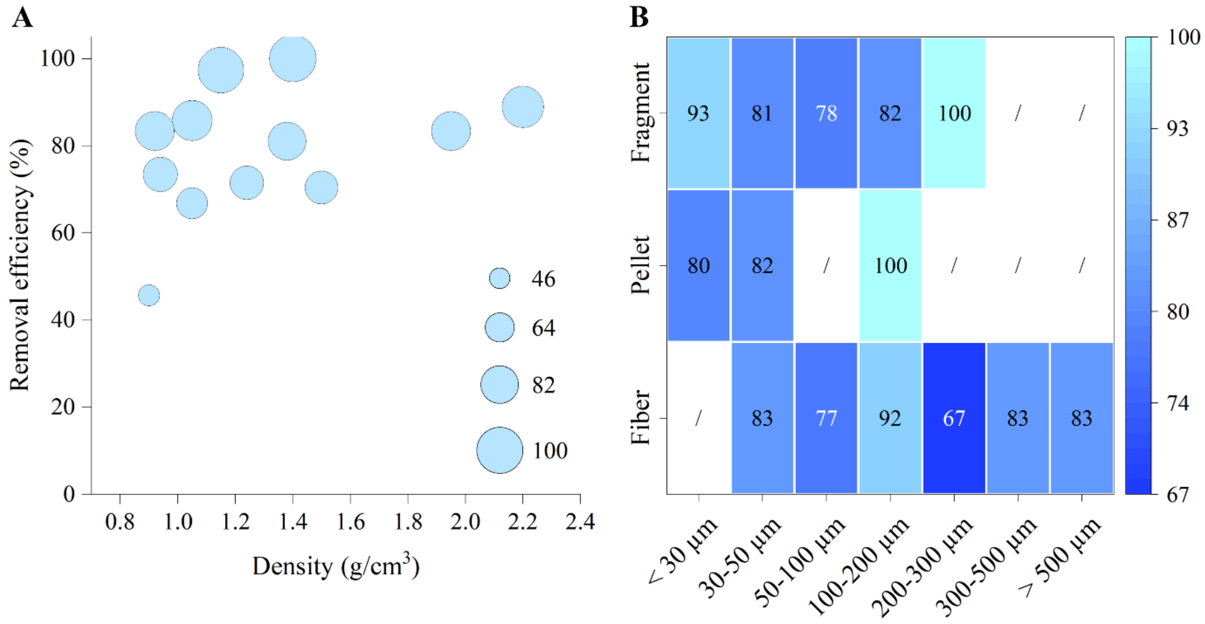

Figure 5: Particle removal efficiency (%) as a function of both polymer density (**A**) and particle size vs. shape cross-classification (**B**) estimated from analysis of raw and treated wastewater samples collected at $WWTP_A$.

The particle concentrations found in the samples collected along the sludge treatment line are listed in Table 4. The collected data confirmed that most particles removed in the water treatment line are retained into sewage sludge, in line with that reported in the literature (Egea-Corbacho et al., 2023; Tang & Hadibarata, 2021).

Table 4: Particle concentrations – expressed on both a wet weight and a dry weight basis – found in the sludge samples collected at $WWTP_A$.

	RET SLUDGE (items/g WW)	**IN DIG (items/g WW)**	**OUT DIG (items/g WW)**
With CELLULOSIC	1173 ± 54	2526 ± 796	3339 ± 1397
Without CELLULOSIC	1039 ± 207	2100 ± 376	2721 ± 1142
	RET SLUDGE (items/g TSS)	**IN DIG (items/g TSS)**	**OUT DIG (items/g TSS)**
With CELLULOSIC	89562 ± 4124	68265 ± 21521	101182 ± 42321
Without CELLULOSIC	79294 ± 15834	56763 ± 10168	89099 ± 25233

Comparing RET SLUDGE and IN DIG, it could be predicted that the sludge thickening stage overall contributed to an increase in the particle concentration expressed as number of items per g wet weight. No particle removal was exerted by anaerobic digestion (AD); conversely, an increase in the MPs concentrations – on both dry weight and wet weight basis – was observed in the digestated sludge. These findings suggested that potential modifications might occur on MPs during sludge treatments, in agreement with that reported in other scientific reports (Chand et al., 2024; Pittura et al., 2021). Moreover, a contribution, even minor, from the in-situ production of MPs, reasonably resulting from the equipment employed (e.g. sludge piping systems, electromechanical components, etc.), could be expected. Referring to anaerobic digestion, in line with the present study, Chand et al. (2024) found that the MPs concentrations

– expressed as number of items per kg wet weight – increased by 5 % in the digester outlet sludge of a large WWTP in Sweden. This evidence could be mainly attributable to the fact that organic matter degrades during anaerobic digestion, being largely converted into biogas. In contrast, MPs, due to their chemical nature, are less prone to biodegradation under anaerobic conditions. This different degradation extent could lead to a relative enrichment of MPs in the digestated sludge, manifesting as an apparent increase in concentration when normalized to dry weight. In addition, mechanical and thermal stresses within the digester could cause fragmentation of particles, increasing their numerical abundance. Surface modifications – including biofilm formation – can also alter the properties of MPs, affecting their behavior and distribution in the sludge matrix. Consequently, all factors presented above may potentially contribute to the relative enrichment of MPs in digestated sludge, raising concerns about their persistence and potential environmental impacts when applied to agricultural soils.

The polymeric, dimensional, and morphological distributions of particles within the analyzed sludge samples (Figure 6) were generally consistent with those detected in both raw and treated wastewater, reasonably reflecting the selective retention, transport and partitioning processes that occur throughout the wastewater and sludge treatment lines.

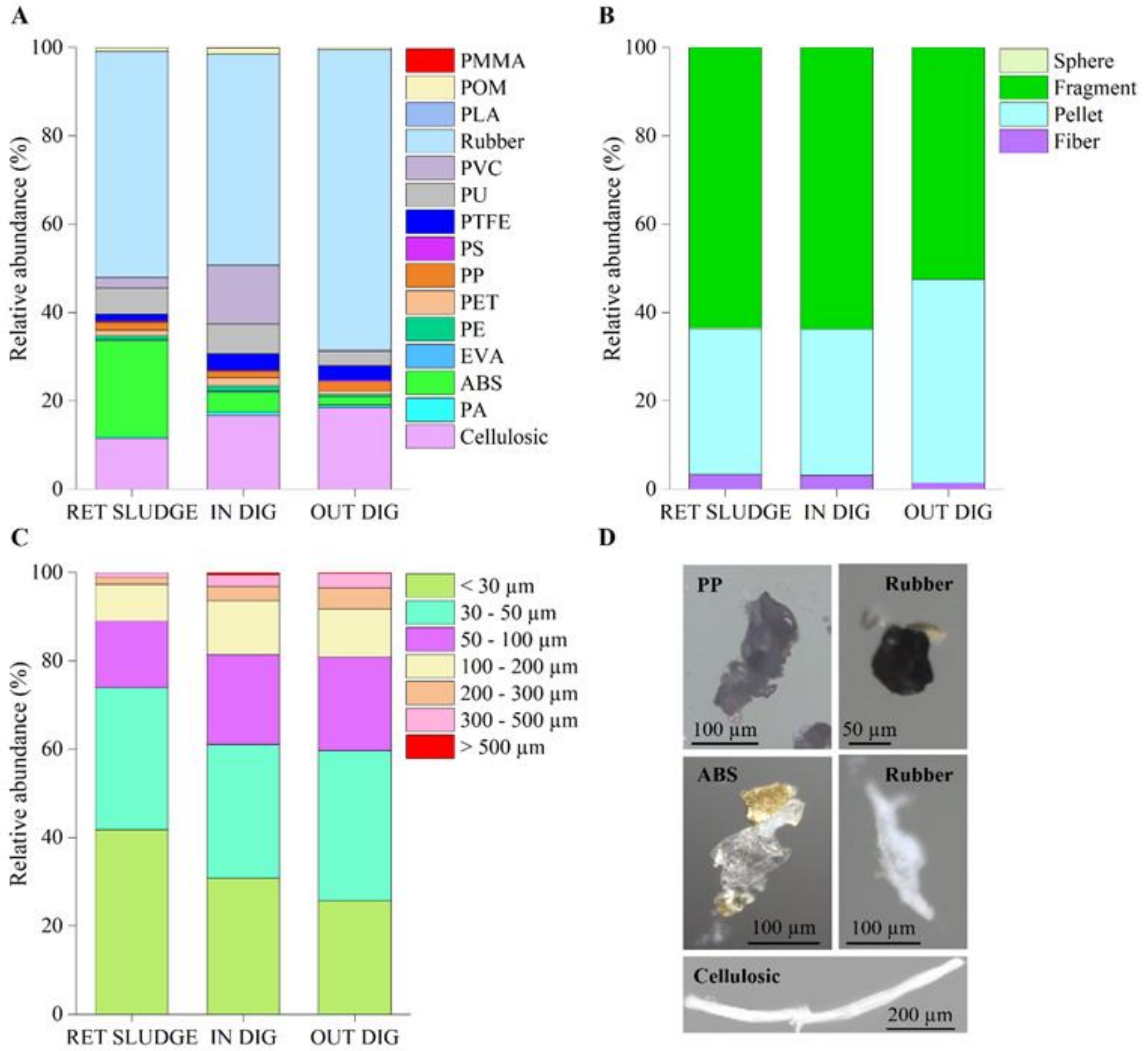

Figure 6: Characterization of particles found in the sludge samples collected at $WWTP_A$ in terms of polymer type (**A**), size (**B**) and shape (**C**), and high-magnification visible images of many particles detected (**D**).

Referring to RET SLUDGE, numerical balances were found to be coherent across most particle classes in terms of size, shape and identified chemical species (Figure 7). Specifically, the estimated concentrations – based on particle removal in the water treatment line – showed relatively good agreement with the concentrations measured in the sludge. More remarkable discrepancies between predicted and observed values were evidenced for PA, rubber and ABS in terms of polymer types and for the largest particles (> 500 µm). These differences may be ascribed to a combination of factors, including sampling and analysis uncertainties, as well as different partitioning behaviors of particles between the water and sludge treatment lines. Such variability could result from differences in the particle density, surface properties and/or interaction with organic matter, all of which are factors that could influence the fate of particles during wastewater treatment.

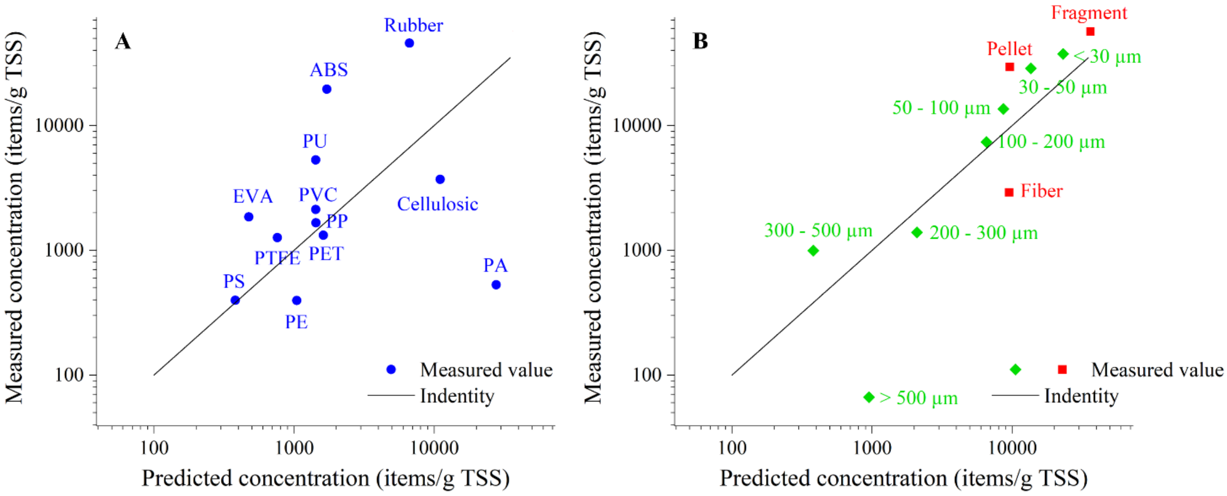

Figure 7: Correlation between estimated and measured particle concentrations in RET SLUDGE from $WWTP_A$. Estimated values were derived from particle removal in the wastewater treatment line, while measured concentrations are those directly observed in the sludge samples. Correlations are shown for particle classes in terms of polymer type (**A**), shape and size (**B**).

The physical characterization data helped understanding the fate of MPs in the AD stage. As previously explained, AD did not exert MPs removal but could have a major effect on the size and shape distributions of particles. This hypothesis would be corroborated by the fact that particles > 500 µm – all associated with a fiber-like morphology – decreased during AD while significant percentage increases were observed for all others size classes (Figure 8). This could suggest the occurrence of fragmentation phenomena induced by the mechanical and thermal stressors in the digester unit, as mentioned above.

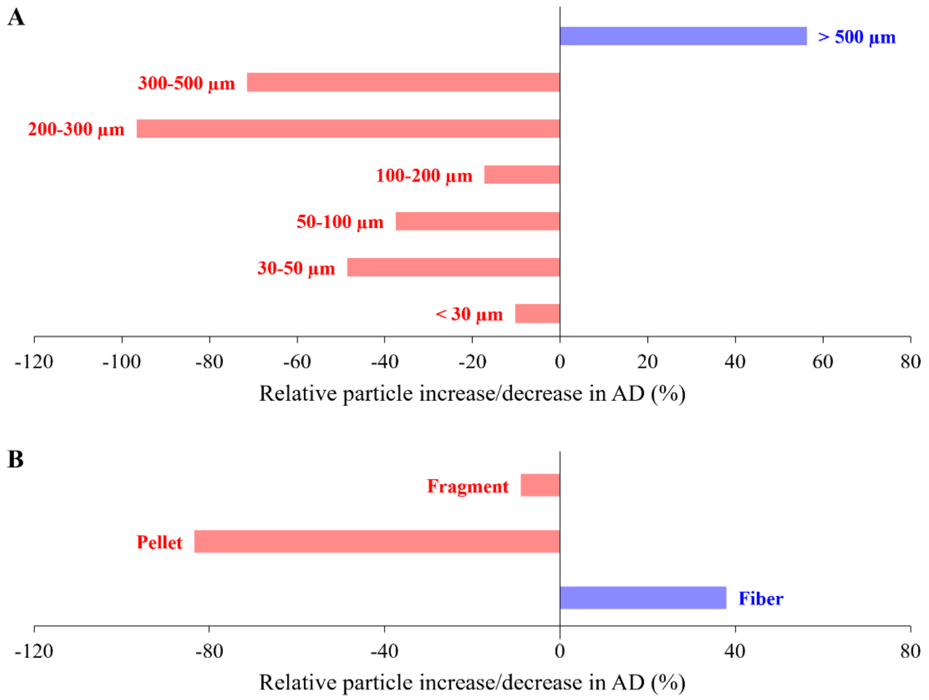

Figure 8: Percentage increase (in red) and decrease (in blue) in the particle concentrations during anaerobic digestion, classified based on their size (**A**) and shape (**B**). These percentages were calculated from the average particle concentrations (on wet weight basis) of IN DIG and OUT DIG samples collected at $WWTP_A$.

4.1.3.2 Full-scale monitoring at $WWTP_B$

Figure 9 summarized the main results of the monitoring campaign conducted at WWTPB in terms of particle concentration and removal. The average particle concentration progressively decreased along the wastewater treatment train from about 76576 (IN) to 2706 items/L (OUT). Particles were largely removed during primary settling (approximately 80 %). The effectiveness of preliminary and primary treatments in removing micro-sized particles, including MPs, is confirmed by literature data. Talvitie et al. (2017b) collected and analyzed 24-h composite samples from a large WWTP in Finland observing that the mechanical and chemical pretreatment stage (i.e. screening, grit removal and chemically enhanced primary sedimentation) exerted an average microlitter removal of about 98 %. Pittura et al. (2021) studied the occurrence and fate of MPs in a large WWTP in Italy founding that about 47 % of them were removed in the primary settling. Murphy et al. (2016) carried out a monitoring campaign in a secondary WWTP in Scotland discovering that about 61 % of MPs present in the grit and grease effluent were removed in the primary clarifier. Biological processes typically promote particle incorporation into sludge flocs and their subsequent separation in the secondary clarifier, showing removal efficiency up to 80 % according to literature data (Talvitie et al., 2017b). With respect to that reported in other scientific reports, secondary treatments in the monitored WWTP only marginally contributed to the overall particle removal, indicating a limited additional purification during this phase. Tertiary treatments, including coagulation-flocculation and final ozonation, further enhanced the particle removal, eliminating about 79 % of the remaining particles from the secondary settling effluent. Overall, the monitored WWTP exhibited an average particle removal efficiency of about 96.5 %. This efficiency further increased, reaching about 99.1 %, following the implementation of advanced treatments in the refining section for water reclamation. The trend observed in terms of percentage removals agreed with literature data, which, among the examined papers, range from 64.4 % (Liu et al., 2019) to 99.8 % (Simon et al., 2018) for WWTPs having tertiary treatment configurations (for more details on the literature survey, refer to deliverable DV 4.3.2).

MPs accounted for a fraction of the total microparticles detected, ranging from 41 % and 59 %, and their concentrations decreased from 42767 to 400 items/L along the treatment train. The average MPs mass concentrations predicted at the inlet and outlet sections of the plant were 1552, 35430 1405 and 2 µg MPs/L for IN-SS, IN, OUT and REUSE, respectively.

The relative abundance of cellulosic particles (CELL) varied from 0 % (RESUSE) to 40 % (IN), corresponding to concentrations up to 30342 items/L. As previously mentioned, the CELL category comprises a wide range of materials of both natural origin and artificial or semi-synthetic sources, such as fibers from textiles (e.g. cotton, linen and viscose), naturally occurring cellulose and various industrially processed cellulose-based materials. The concentrations of textile-derived items (TXT) – including those from both natural and synthetic/semi-synthetic sources – ranged from 3620 to 18783 items/L depending on the sampling section. Consistently with the origin of the industrial wastewater collected to WWTPB, approximately 56 % of the particles detected in IN-SS had a textile source (3620 ± 361 items/L): indeed, textile processing operations are known to largely release fibers during washing, dyeing and finishing phases (Gambino et al., 2025). These values were in line with those reported in the literature for textile industrial discharges. Akyildiz et al. (2022) found microfiber concentrations ranging from 893 to 4452 items/L in the wastewater from a textile company, which decreased down to 310 – 2404 items/L in the final effluent of the on-site treatment system consisting of physical-chemical process units (percentage removal = 38 – 65 %). Xu et al. (2018) detected average abundances of microfibers of 334.1 ± 24.3 items/L and 16.3 ±1.2 items/L in the inlet and outlet sections, respectively, of a textile industry WWTP, evidencing a removal efficiency of the treatment train in use (i.e. primary settling, biological processes and tertiary treatments) of about 95.1 %. The TXT relative content decreased in the mixed municipal and industrial wastewater (IN) down to 25 %: this would indicate that larger quantities of non-textile particles originated from domestic sources and urban runoff reasonably enter the plant through the combined sewer system serving the close urban areas with limited industrial inputs. Regardless of their percentage relative contents, TXT / MPs / CELL particles had higher concentrations in the mixed municipal and industrial wastewater (IN) than in the textile-industrial influent (IN-SS). This evidence seems to contradict the common belief that industrial discharges from textile-related facilities are a primary source of MPs / microfibers. However, many considerations can be made to explain this pattern. These data would first highlight the high impact of household contributions to TXT production: in particular, domestic laundry activities are a well-documented source of fiber release during garment washing (Hernandez et al., 2017). The temporal variability in industrial operations may also contribute to the lower MPs concentrations observed in IN-SS compared to IN. Industrial MPs / microparticle emissions could fluctuate according to production cycles, maintenance activities or changes in textile processing steps. While a 24-h composite sample minimizes short-term (within-day) variability, it does not account for differences occurring across days, weeks or production phases. If sampling takes place during a period of reduced production, equipment cleaning and/or low fiber-intensive processing, the collected samples may underestimate typical MPs / microparticle emissions. Furthermore, industrial wastewater streams could be high in volume but diluted in microparticle concentration since many textile processes, such as fabric dyeing, typically used large quantities of water (Gambino et al., 2025). Taking all the above into account, when industrial streams are mixed with municipal wastewater, the combined MPs concentration could hence appear higher compared to the industrial component alone. Considering the volumetric partitioning between the industrial and urban discharges conveyed to WWTPB through the combined sewer system, average MPs, CELL and TXT concentrations of about 51495, 36538 and 22079 items/L, respectively, could be predicted for the municipal wastewater fraction of IN.

The percentage removals of the MPs, CELL and TXT classes reflected the trend observed for total particles: primary sedimentation exerted the highest contribution, with average removal efficiencies of about 79.0 %, 92.4 %, and 53.8 %, respectively, while the refining section for water reclamation enabled an almost complete removal of CELL and decreased the final content of MPs and TXT by more than 98 %.

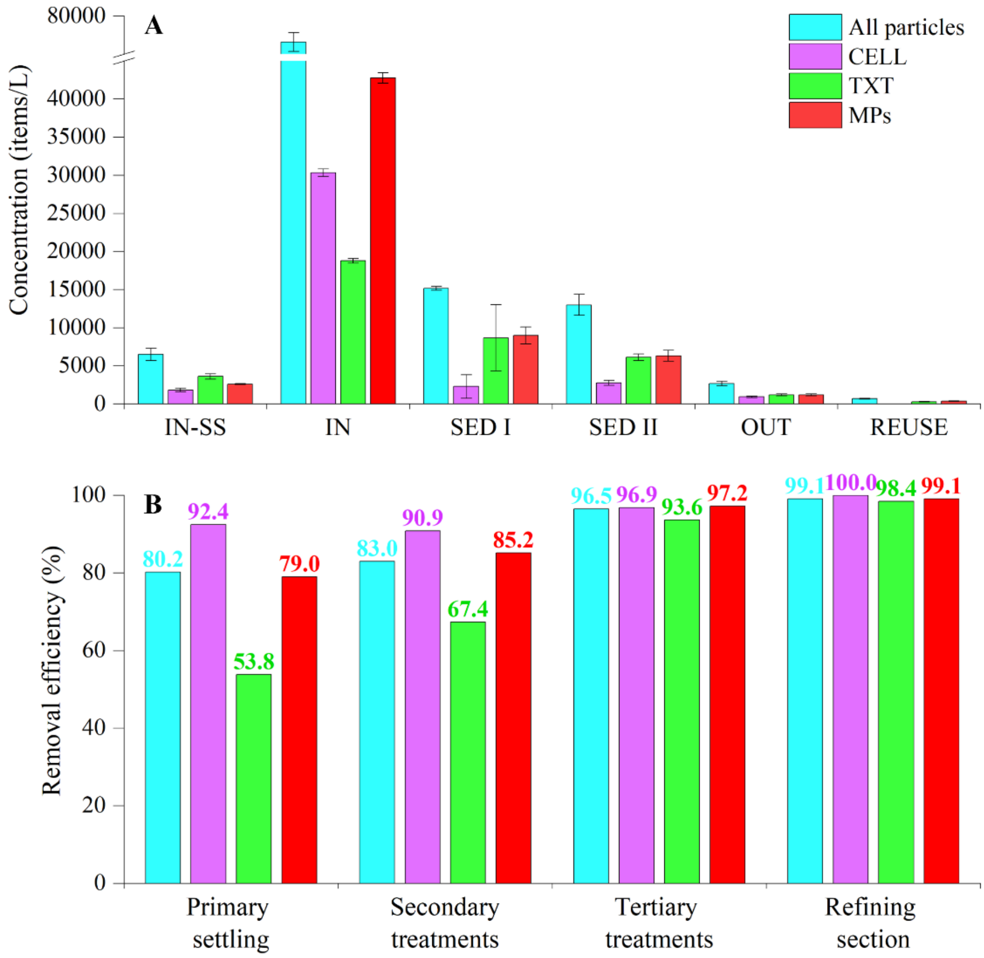

Figure 9: Particle concentration – expressed as number of items per liter – found in the samples collected at $WWTP_B$ (**A**) and their cumulative percentage removal along the wastewater treatment train (**B**).

Chemical characterization of particles found in the processed samples is displayed in Figure 10. Regarding MPs, a large variety of polymers were detected in the monitored treatment sections, including PE, PET, PU, PTFE, PA, PVC, PS, PMMA, POM, ABS and acrylic, thus highlighting the diverse and complex range of urban and industrial sources contributing to the MPs load in the WWTP, in line with that described for WWTPA. As evidenced for raw and treated wastewater from WWTPA, Rubber particles accounted for a relevant fraction in the processed samples (except for SED I and REUSE), with relative abundances ranging from 18 % to 29 %. Tire erosion was identified as a major source of rubber-made MPs in urban wastewater (explaining up to 53 % of total rubber items found in IN): as previously mentioned, these particles can be reasonably generated across roads/highways in the urban area served by the combined sewer system, and hence collected to the plant through the urban runoff, and/or produced in-situ by vehicle traffic in the WWTP.

Among the potential sources of CELL identified above, textile materials were found to be primary contributors to the discharge of cellulose-based microparticles in wastewater. In IN-SS, cellulosic particles of textile origin (TXT-CELL) – specifically cotton, linen and viscose – constituted approximately 76 % of the total cellulosic load, reflecting the compositional characteristics of the treated industrial wastewater. This proportion decreased at the subsequent treatment stages where TXT-CELL represented from 44 % to 67 % of the total cellulosic items detected (Table 5).

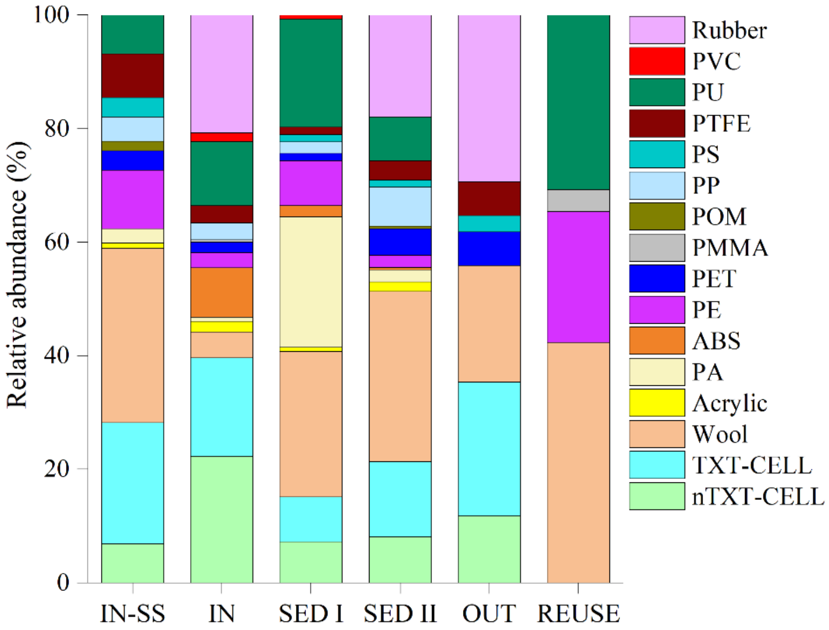

Figure 10: Chemical characterization of particles detected at different sampling points over the wastewater treatment train of $WWTP_B$.

Table 5: Relative abundance of cellulosic microparticles of textile origin in the samples collected at $WWTP_B$.

	IN-SS	IN	SED I	SED II	OUT	REUSE
CELL (% on total particles)	28%	40%	15%	21%	35%	0%
TXT-CELL (% on total CELL)	76%	44%	52%	62%	67%	/
Cotton (% on TXT-CELL)	72%	28%	75%	19%	25%	/
Linen (% on TXT-CELL)	20%	70%	25%	77%	63%	/
Viscose (% on TXT-CELL)	8%	2%	0%	3%	13%	/

A more detailed characterization of the textile-derived particles found in the processed samples from WWTPB is given in Figure 11. Natural textiles – including cotton, linen and wool – were typically the prevalent materials, together accounting for about 91 % and 88 % of total TXT in IN-SS and IN, respectively. Examples of textile fibers in cotton, linen and wool are shown in Figure 12. Referring to the average mass concentrations of TXT in IN-SS, it was estimated that the mass flows of natural TXT microparticles reaching WWTPB with industrial wastewater would be significantly higher compared to those related to synthetic textiles (Table 6): this pattern seemed to be consistent whit the characteristic of the local textile district, which is characterized by numerous facilities processing and recovering natural fibers.

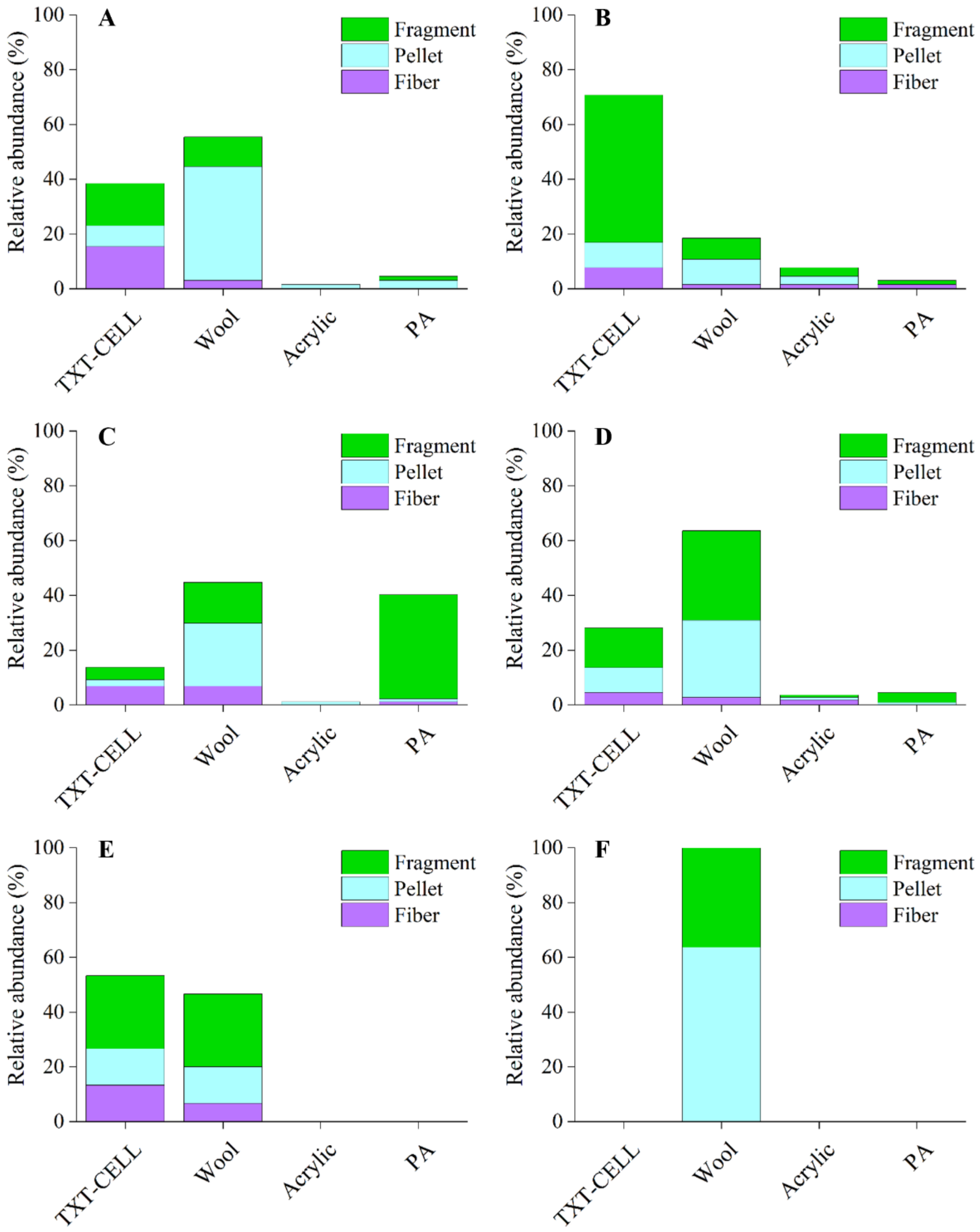

Figure 11: Chemical composition and shape cross-classification of textile particles (TXT) found in the samples collected from $WWTP_B$: IN-SS (**A**), IN (**B**), SED I (**C**), SED II (**D**), OUT (**E**) and REUSE (**F**). Relative abundances are reported as percentage on total TXT items in IN-SS, IN, SED I, SED II, OUT and REUSE, respectively.

However, it is worth noting that the actual contribution of synthetic textiles could be underestimated in this work since only polymers with a certain textile origin were considered for data elaboration. In this regard, further implementation of the analytical tool could enhance the system capability to identify a broader range of TXT, also including a wide range of mixed fibers (e.g. cotton-polyester, wool-polyester, wool-polyamide, etc.) that have a large application in the textile market. The persistence of natural textiles along the wastewater treatment train indicated their low degradation rates under the sewer/plant conditions. Indeed, harmful chemicals such as flame retardants added to non-microplastic fibers during processing and dyeing could prolong their degradation time (Xu et al., 2018). Moreover, like MPs, natural fibers may also absorb pollutants from surrounding environment that could increase their potential ecotoxicity. Bearing in mind the above, both synthetic and natural textile particles could exert negative impacts on the environment, thus reinforcing the need for comprehensive assessment and study of all microparticle types. Surprisingly, TXT particles were not exclusively present in the form of fibers but exhibited a broader distribution of shapes (Figure 11). This diversity likely reflects the combined effects of multiple phenomena such as mechanical fragmentation during washing and transport in sewer pipes, detachment of polymer-based finishes or coatings applied to fabrics and chemical/physical degradation caused by detergents, softeners, bleaching agents and elevated temperatures used in domestic and industrial laundering that could weaken the fiber structure. All these mechanisms would determine fiber breakdown and splitting, thus generating particles of more irregular morphologies.

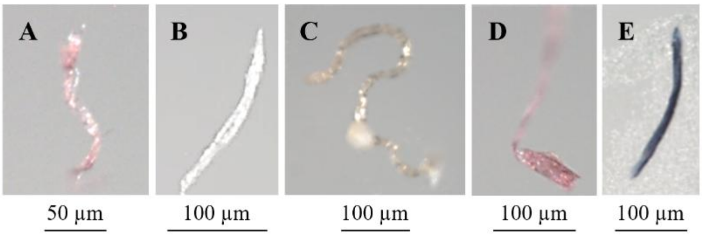

Figure 12: Example of textile fibers in cotton (**A**, **B**), wool (**C**) and linen (**D**, **E**) found in the wastewater samples collected at $WWTP_B$.

Table 6: Estimated mass concentrations / fluxes of textile microparticles conveyed to $WWTP_B$ with the industrial-textile wastewater.

	Cotton / Linen / Viscose	Wool	Acrylic	PA
Average mass concentration (µg TXT/L)	1407	978	< 0.01	6
Average mass load (tons TXT/y)	9.8	6.8	< 0.001	0.04

A diverse range of particle sizes and morphologies was identified across the samples analyzed (Figure 13). Most particles measured less than 100 µm, comprising between 57 % and 100 % of total items depending on the sampling site, with a significant fraction of them (23 – 81 %) smaller than 30 µm. Notably, in REUSE, only particles < 50 µm were detected, indicating a selective removal of larger particles by the treatment system. Morphological analysis revealed that fragments and pellets were the most frequently observed particle types, accounting for 31 – 70 % and 21 – 65 % of total items, respectively, with variability across sampling locations. In line with that evidenced for WWTPA, pellets were generally associated to the smallest size classes (< 100 µm), with a high incidence of particles < 30 µm which represented from 56 % of 94 % of pellets founds in the analyzed samples (Figure 14). In contrast, fragments exhibited a wider size distribution, suggesting a secondary origin linked to the degradation and/or mechanical abrasion of larger debris (Figure 14). Fibers, which were less abundant in all processed samples, represented between 9 % and 16 % of the detected particles. Consistently, fibers were primarily associated with the largest size fractions (from 33 % to 89 % of them longer than 100 µm), even if a non-negligible number of shorter fibers was also identified (Figure 14). It is worth noting that the highest relative abundance of fibers was observed in IN-SS: consistently with its industrial origin, most of the fibers found in IN-SS had a textile nature (63 %) – predominantly composed of natural materials such as cotton and wool – further confirming the influence of textile processing activities on the physical profiles of microparticles present in the industrial wastewater.

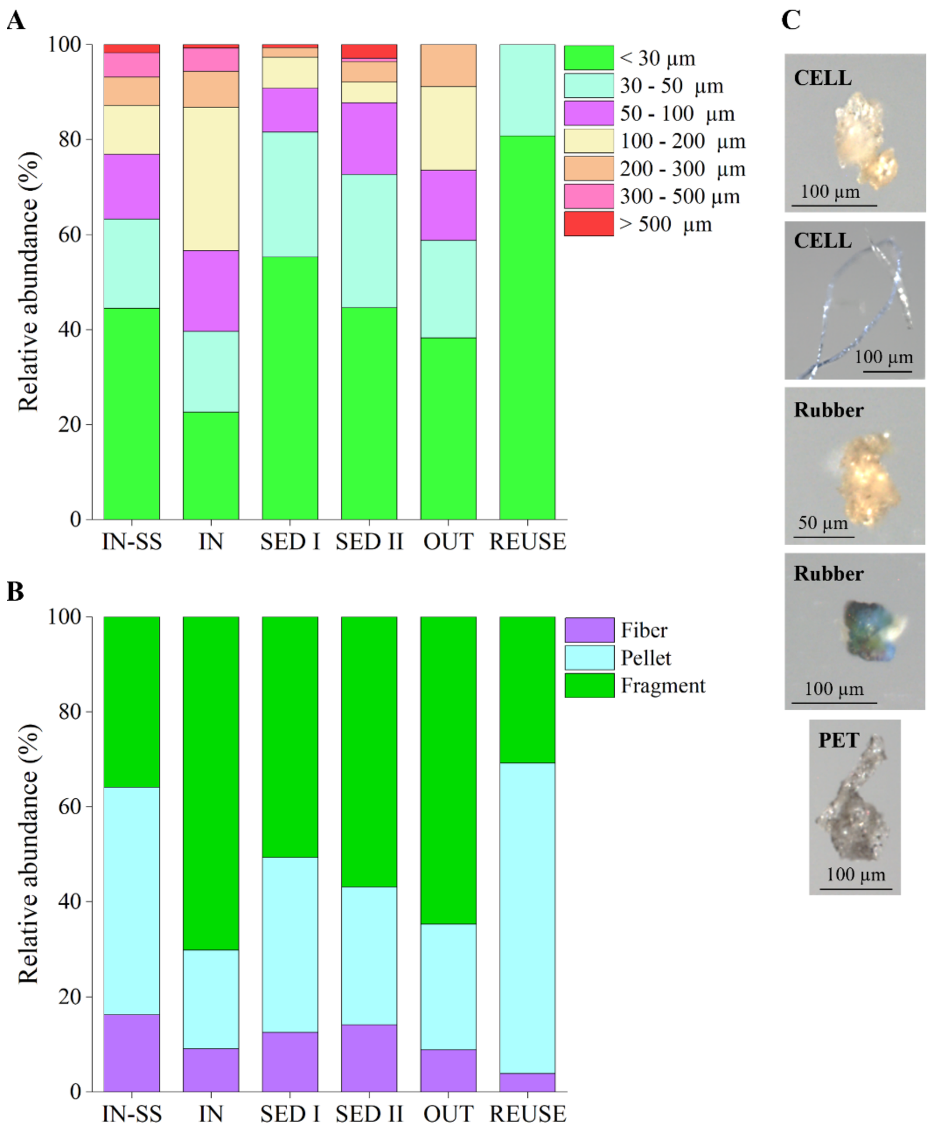

Figure 13: Characterization of particles found in the samples collected along the water treatment line of $WWTP_B$ in terms of size (**A**) and shape (**B**), and high-magnification visible images of many particles detected (**C**).

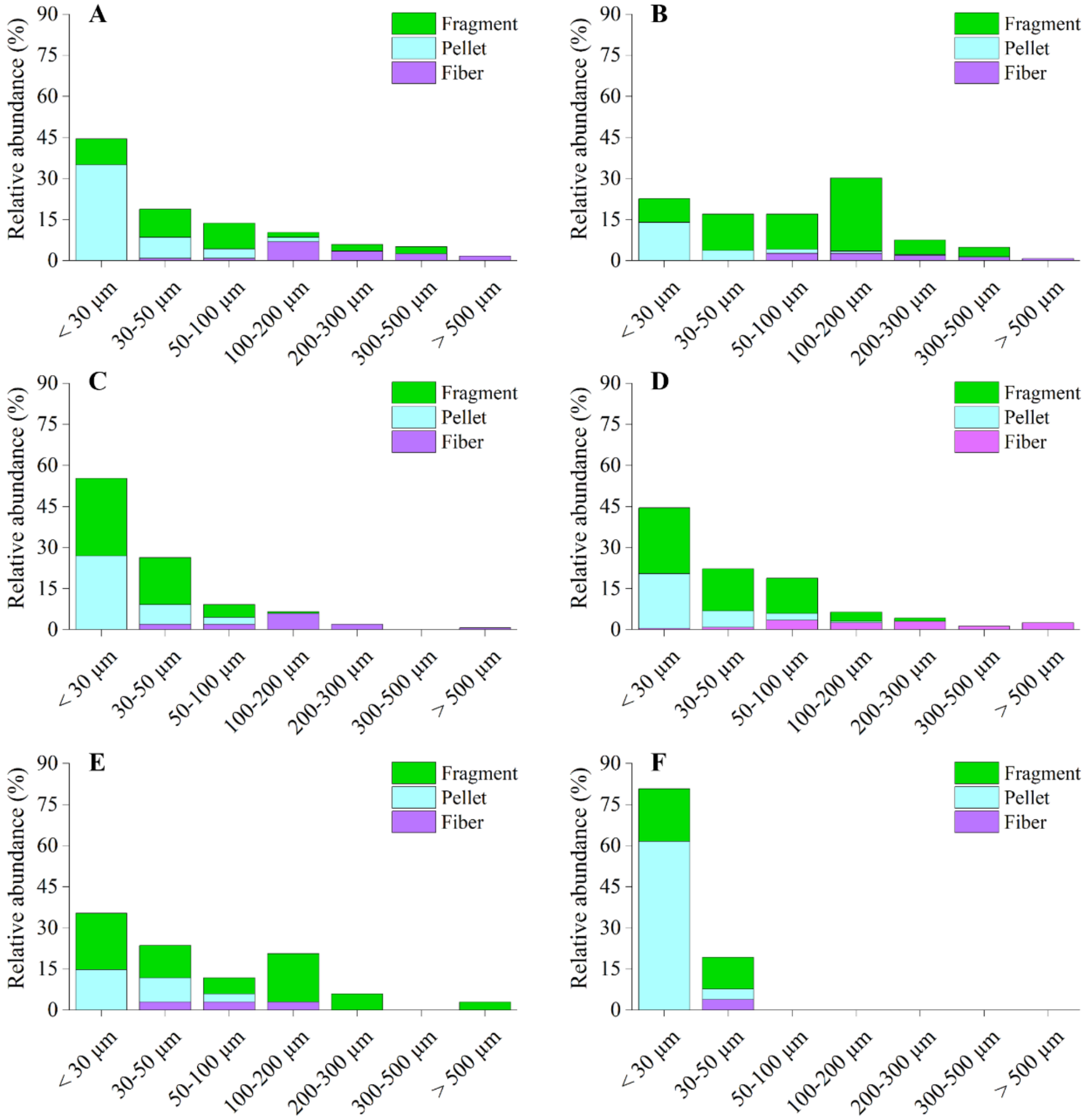

Figure 14: Size and shape cross-classification of particles found in the samples collected at $WWTP_B$: IN-SS (**A**), IN (**B**), SED I (**C**), SED II (**D**), OUT (**E**) and REUSE (**F**).

The significant role of particle size and shape in determining removal efficiency within WWTPs was highlighted also in this monitoring campaign (Figure 15). Primary settling largely influenced the distribution of particle dimensions and morphologies in wastewater collected to WWTPB. Lower removal efficiencies were suggested for particles in the form of fragments smaller than 30 µm (35 %), fibers ranging from 100 to 200 µm (56 %) and pellets < 50 µm (62 %). Secondary treatment processes demonstrated a higher efficiency in eliminating fibers (63 %) compared to fragments (23 – 27 %) and pellets (16 – 36 %). This trend may be attributed to the elongated geometry of fibers, which likely facilitates their entrapment within suspended solids and microbial flocs during the biological phase, enhancing their removal through subsequent settling processes (Sun et al., 2019). Tertiary treatments, including coagulation-flocculation and ozonation, further improved the particle removal, particularly targeting the longest fibers and largest pellets. In the refining section for water reuse, an almost complete removal of particles larger than 50 µm was achieved. However, this stage was less efficient at capturing smaller particles, especially fibers in the 30 – 50 µm size range. Due to their slender and flexible nature, fine fiber-like particles can align with the flow and pass longitudinally through filter meshes, reducing the effectiveness of filtration systems.

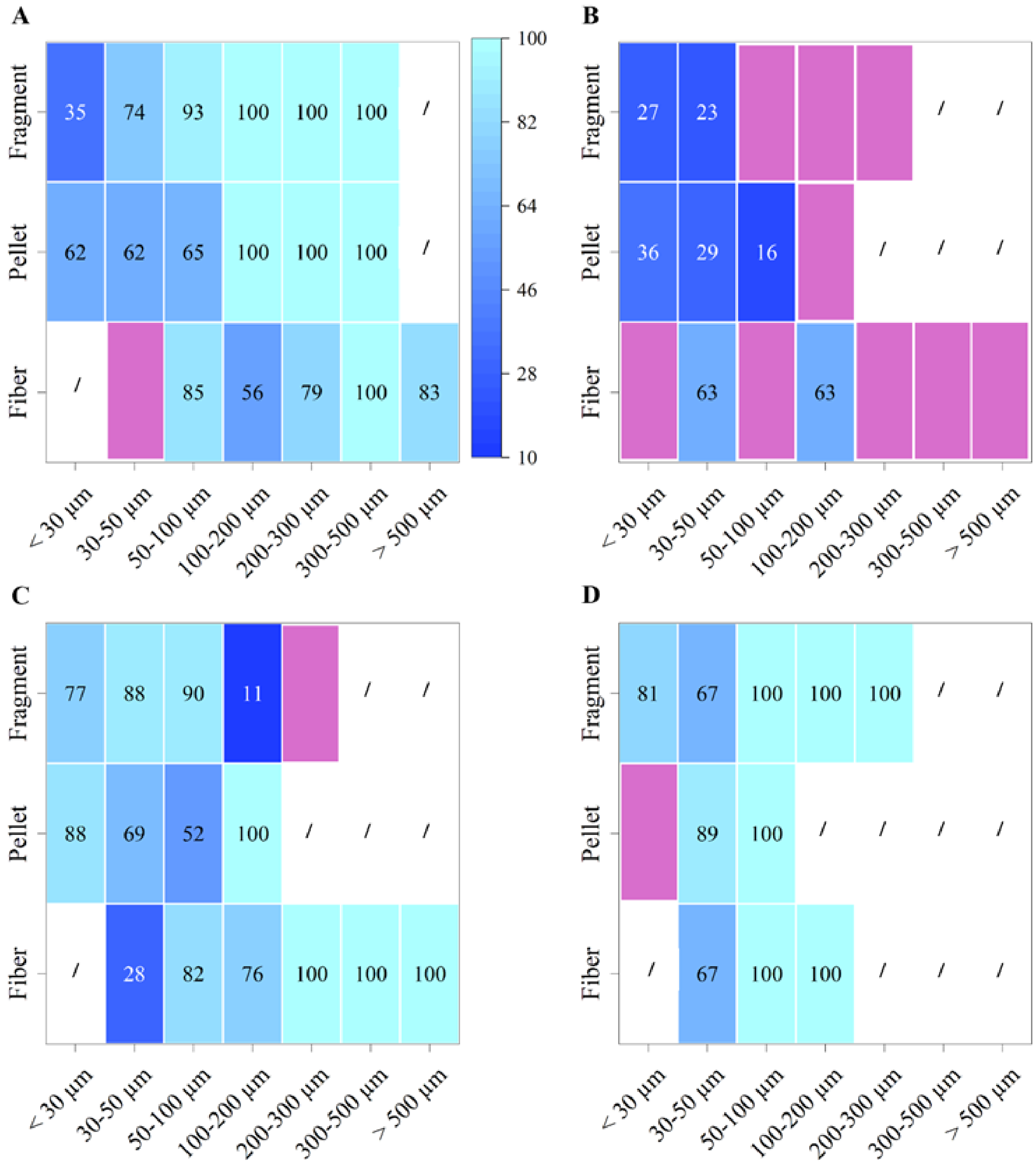

Figure 15: Heatmap describing the removal efficiencies promoted by the monitored treatment units in $WWTP_B$ – primary sedimentation (**A**), biological process and secondary settling (**B**), tertiary treatments (**C**) and refining section (**D**) – as a function of particle shape and size. The violet squares indicate an increase in the particle content in the effluent of the considered process unit, ascribable to *in-situ* production and/or to uncertainties in the particle determination/characterization.

4.1.3.3 Full-scale monitoring at $WWTP_C$

The particle concentrations detected in the processed samples from WWTPC are listed in Table 7. The data collected indicated significantly higher MPs contents in both municipal and industrial wastewater compared to the other monitored WWTPs. A relevant factor that could contribute to this difference is the absence of septic tanks in the urban area served by WWTPC, unlike the municipalities associated with WWTPA and WWTPB: the lack of this pretreatment stage may result in a higher load of MPs entering the treatement facility. MPs concentrations in the MBR effluent were higher than expected, suggesting a potential decline in its treatment performance. This anomaly was likely related to the operating conditions of the membranes, which were at the end of their service life at the time of sampling. The reduced integrity and/or efficiency of the aged membranes could have allowed a higher amount of particles to pass through the system. Moreover, the membrane degradation could also contribute to increase the MPs content in the treated effluent. Consistently, line S2 exerted a slightly higher removal efficiency compared to line S3 (99.6 vs. 98.7 %). Overall, the plant – including both water treatment lines – provided an average removal efficiency of 99.8 % which was higher than that observed for WWTPA and WWTPB. The trend observed in terms of percentage removals among the monitored WWTPs reflected the level of technological advancement of the treatment configurations in use, with the membrane-related processes strongly enhancing the plant effectiveness in particle removal, in line with the literature (Talvitie et al., 2017a). The average MPs mass concentrations estimated in the analyzed samples of IN-Mun, IN-Ind, OUT-MBR and OUT were 6986, 40836, 284 and 18 µg/L, respectively.

Table 7: Particle concentration – expressed as number of items per liter – found in the samples collected along the wastewater treatment line of $WWTP_C$.

	IN-Mun (items/L)	IN-Ind (items/L)	OUT-MBR (items/L)	OUT (items/L)
With CELLULOSIC	171185	235644 ± 20563	2286	1024
Without CELLULOSIC	167078	189441 ± 13375	1648	341

The overall characterization of particles found in the processed samples is presented in Figure 16.

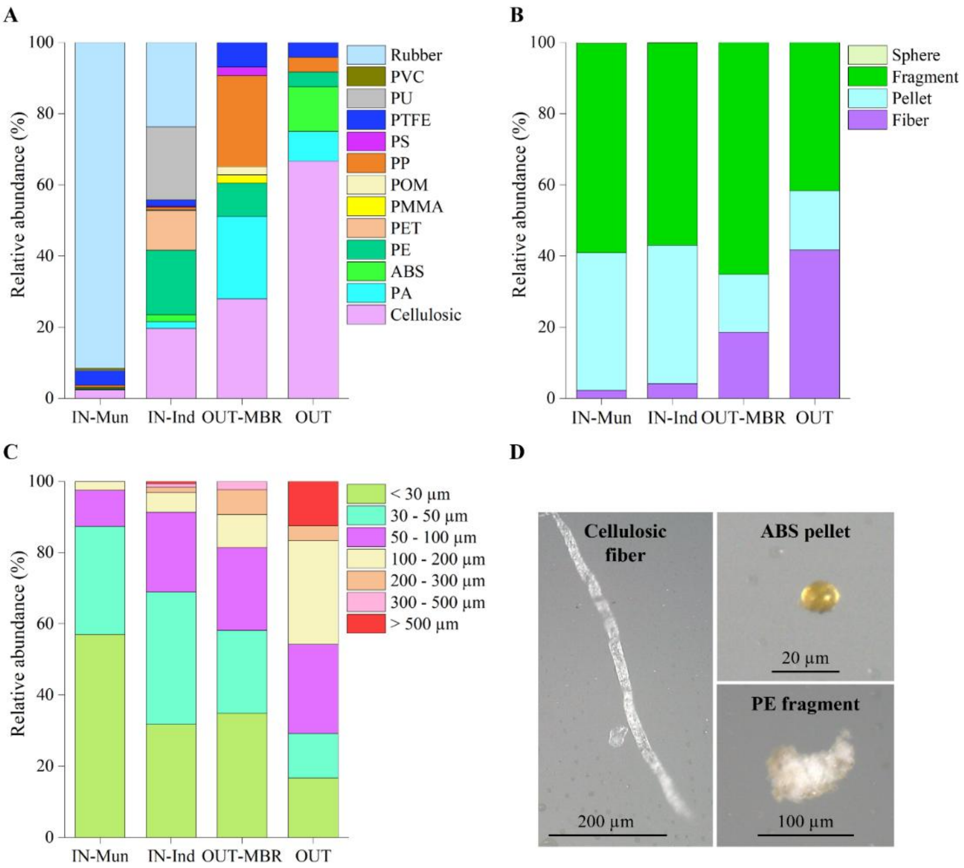

Figure 16: Characterization of particles found in the samples collected along the water treatment line of $WWTP_C$ in terms of polymer type (**A**), shape (**B**) and size (**C**), and high-magnification visible images of many particles detected (**D**).

Rubber particles – accounting for about 92 % and 24 % of total items in IN-Mun and IN-Ind, respectively – were almost completely removed over both treatment lines.

As highlighted above, the high rubber concentrations in both municipal and industrial wastewater could be largely associated with a tire-derived source (about 39 % and 31 % of rubber items in IN-Mun and IN-Ind, respectively). Cellulosic particles represented about 2 % and 20 % of total items in IN-Mun and IN-Ind, respectively: as previously detailed, this polymeric class comprises a wide spectrum of materials – including natural cellulose and its chemically modified derivates – that can be reasonably released in both industrial and municipal wastewater via multiple paths. The specific profile of MPs in tannery wastewater was likely influenced by a variety of factors related to the industrial processes themselves – such as production scale, type of processed leather and chemical formulations employed – which may contribute to both abundance and variability of MPs contamination. A broader range of polymers was observed in IN-Ind that in IN-Mun: in addition to rubber, the significant contents of PE, PU and PET – accounting for about 18 %, 21 % and 11 % of total items in IN-Ind, respectively – could be explained considering that these polymers could be commonly found in packaging, equipment, piping systems, etc., thus being potentially released in the form of micro-sized particles during processing and finishing stages of the industrial processes.

The analysis of particle shape and size in the processed samples revealed distinct patterns, suggesting that wastewater treatment exhibited a degree of selectivity toward certain geometrical properties. Among the different morphological categories, fragments emerged as the dominant form across all sampling sections, comprising between 42 % and 65 % of the total detected particles. In contrast, the relative content of fibers considerably varied, ranging from 2 % in IN-Mun to 42 % in OUT. In line with that observed for the other monitoring campaigns, the majority of particles detected had a characteristic size < 100 µm: most pellets – from 42 % to 86 % depending on the sampling point – measured less than 30 µm, while fibers were usually associated with largest size classes and, particularly, from 39 % to 80 % were longer than 100 µm (Figure 17).

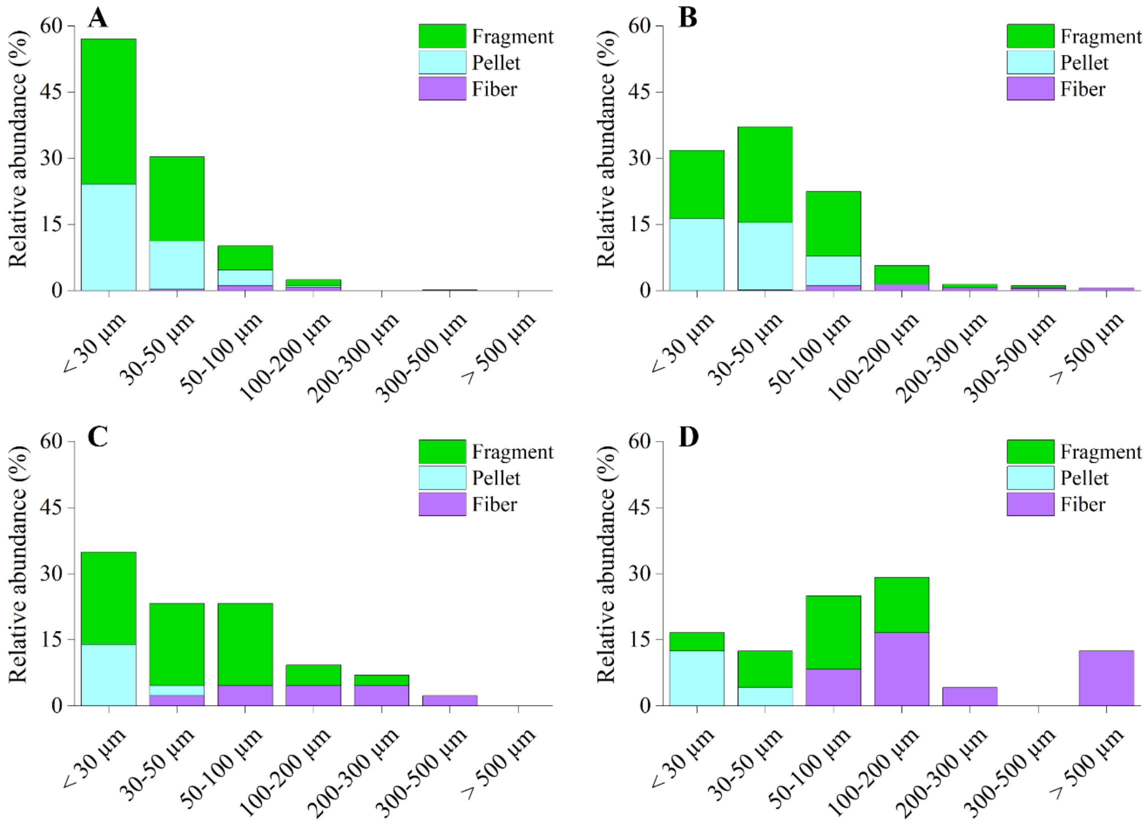

Figure 17: Size and shape cross-classification of particles found in the samples collected from WWTP$_C$: IN-Mun (**A**), IN-Ind (**B**), OUT-MBR (**C**) and OUT (**D**).

More details on the removal efficiencies towards distinct classes of particles in terms of polymer type, size and shape are provided in Figure 18. It is worth noting that the advanced technologies employed in this plant allowed a more efficient removal of particles belonging to the smallest size classes with respect to the other analyzed WWTPs. In contrast, the treatment configuration of WWTPC was less effective in trapping fibers, typically associated with the largest size classes: specifically, it was estimated that lines S2 and S3 exerted average removal efficiencies toward fibers of approximately 94 % and 89 %, respectively, while reducing the content of pellets and fragments by more than 98 %. As described in the previous section, fibers would be indeed less prone to be retained by membrane- and filtration-based systems due to their morphological features. In terms of chemical nature of targeted particles, Line S3 exhibited lower removal efficiencies towards PE and PP (51 % and 32 %, respectively) while Line S2 resulted more effective towards all polymeric classes. The decrease in the removal rate for low-density polymers such as PE and PP was in line with the evidence from the other monitoring campaigns.

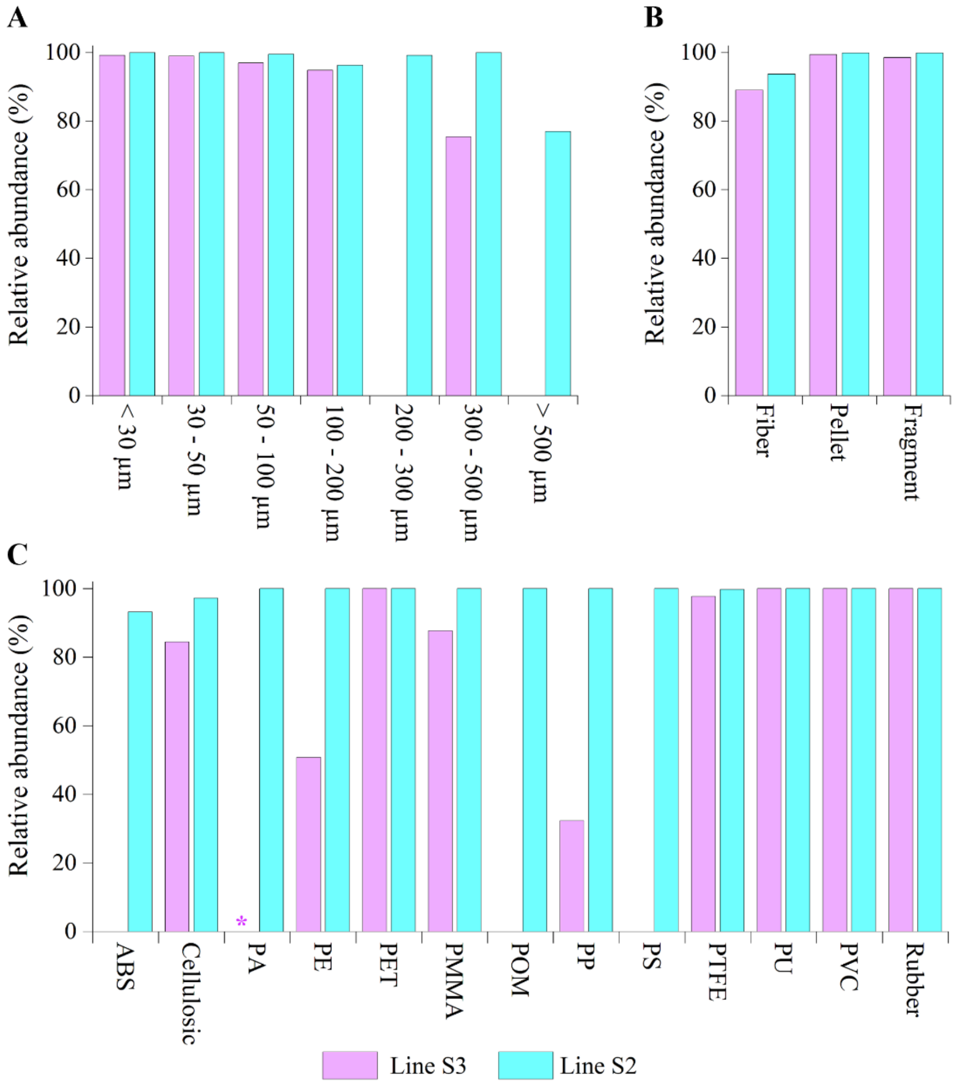

Figure 18: Removal efficiencies exerted by WWTP$_C$ towards distinct classes of particles in terms of size (**A**), shape (**B**) and chemical composition (**C**). The symbol * in the case of PA indicates that in Line S3 an increase in the PA content in the effluent was observed.

4.1.4 Scientific products and dissemination

Pagliaccia, B., Ascolese, M., Vannini, E., Carretti, E., Lubello, C., Gori, R. (2025). Methodologic insights aimed to set-up an innovative Laser Direct InfraRed (LDIR)-based method for the detection and characterization of microplastics in wastewaters, Science of The Total Environment 967, 178817. https://doi.org/10.1016/j.scitotenv.2025.178817

Pagliaccia, B., Ascolese, M., Lubello, C., Dugheri, S., Caffaz, S., Fibbi, D., Gori, R., Insights on the fate and removal of microplastics and microparticles in wastewater treatment plants, ECOMONDO, 3–6/11/2025, Rimini (Italy). Oral presentation.

Pagliaccia, B., Ascolese, M., Lubello, C., Dugheri, S., Fibbi, D., Gori, R., Insights on the fate and removal of microplastics in wastewater treatment plants: Unveiling the impact of textile industry, Symposium on Microplastics in the Environment and Water, 18–19/09/2025, Singapore. Oral presentation.

Pagliaccia, B., Ascolese, M., Vannini, E., Fibbi, D., Carretti, E., Lubello, C., Gori, R., Development of an innovative Laser Direct InfraRed (LDIR)-based methodology for monitoring microplastics in wastewater treatment plants, XII International Symposium on Environmental Engineering (SIDISA 2024), 1–4/10/2024, Palermo (Italy). Oral presentation.

Pagliaccia, B., Ascolese, M., Lubello, C., Dugheri, S., Fibbi, D., Gori, R., Insights on the fate and removal of microplastics in wastewater treatment plants: Unveiling the impact of textile industry, Symposium on Microplastics in the Environment and Water, 18–19/09/2025, Singapore. Oral presentation.

4.2 A standardized fluorescence method for the quantification and morphological analysis of polyester microfibres in washing machine wastewater (PoliTO)

(Contributors: Rajandrea Sethi, Carlo Bianco, Alessandro Casasso, Marco Coha, Monica Granetto, Silvia Lupato, Alberto Tiraferri)

4.2.1 Introduction

Microplastics are ubiquitous contaminants, originating from the degradation of larger plastics and the direct release of as-it-is produced plastic particles. Household synthetic microfibres alone contribute up to 35% of aquatic microplastic emissions (De Falco et al., 2019), and accurate detection and quantification are challenging due to their small size, complex matrices, and polymeric diversity.

Several techniques are nowadays affirmed for microplastics detection and characterisation. FTIR and Raman spectroscopy are widely used for polymer identification and size characterization (Käppler et al., 2018; Primpke et al., 2020). Pyrolysis-GC–MS provides compositional analysis but is destructive and time-consuming (Dümichen et al., 2017). Microscopy (SEM, optical) reveals morphology but almost no chemical information, whereas hyperspectral imaging and LIBS enable high-throughput classification (Araujo et al., 2018; Zhang et al., 2022). TOC analysis may overestimate microplastics concentrations due to the presence of naturally derived organic matter (Lenz et al., 2016), while gravimetric methods, though simple, are highly error-prone due to possible contaminations (Sheriff et al., 2024, Sheikhi et al., 2024). Consequently, optical and fluorescence-based approaches are increasingly favoured in microplastics and microfibres detection. For example, fluorescence techniques, including Nile Red staining, allow visualization and image-based quantification (Maes et al., 2017; Shim et al., 2016; Erni-Cassola et al., 2017), also with machine learning and excitation–emission matrices enabling automated analysis (Windsor et al., 2019; Xu et al., 2021). Additionally, other recent studies have further highlighted the potential of fluorescence-based methods and the need for improved detection protocols specifically tailored to microfibres (Balestra & Bellopede, 2025).

Considering the current state-of-art, the lack in validated detection methods specific for microfibres have motivated the search for intrinsic fluorescence-based and dye staining alternatives among the scientific community. For instance, Lupato et al. (2025) standardized a protocol based on polyester fleece microfibres' intrinsic fluorescent emission at 365 nm for microfibres detection, avoiding the need for staining and reducing preparation time.

4.2.2 Materials and Methods

The standardized fluorescence-based methodology was developed and validated using polyester microfibres. The procedure involves three main steps: (i) preparation of microfibre suspensions, (ii) filtration to retain fibres on glass fibre filters, and (iii) quantification through UV-induced fluorescence imaging and automated image analysis. A Cary Eclipse spectrofluorometer identified the optimal excitation wavelength (365 nm). The imaging setup consisted of a Sony α7 camera equipped with a 100 mm macro lens and a 365 nm UV lamp (Figure 19); ImageJ software was used for automated area computation.

Synthetic (laboratory-made) suspensions were prepared by dispersing known masses of polyester microfibres (1 mg/L to 1 µg/L) in deionized water. Wastewater samples were collected from washing machine cycles (40°C, 70 min, 1400 rpm) using polyester fleece garments and the washing setup reported in Figure 20 (Sheikhi et al, 2025). The outlet filters of the setup were bypassed to ensure the collection of all the microfibres released from the fleece during washing process. Filters (Whatman GF/F, 0.2 µm pore size) were used for fibre collection via vacuum filtration and subsequent fluorescence imaging, due to their unresponsiveness under 365 nm enlightening. Quantification was based on the correlation between the fluorescent area and known microfibre mass (Area [mm²] = 589 × M [mg]).

Figure 19: Photographical setup for integral filter picture acquisition under UV light

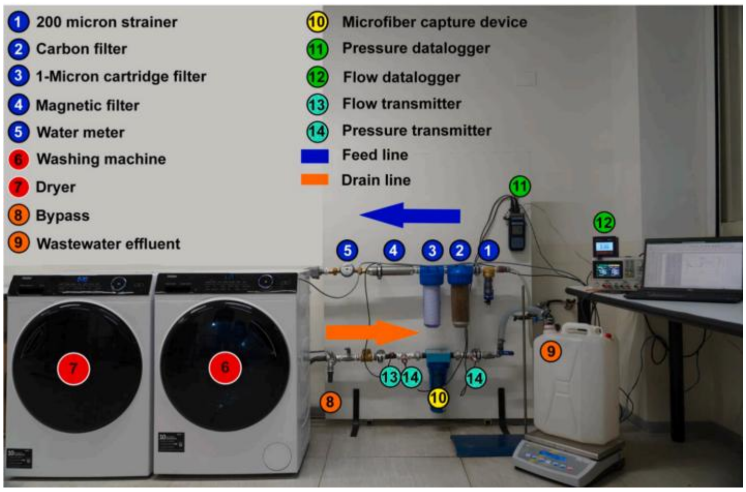

Figure 20: Washing machine and dryer experimental setup from the study about MFs release and filters removal efficiency of Sheikhi et al (2025)

4.2.3 Results

Figure 21 shows an example of results of the pictures acquisition. It can be observed as fibres invisible under visible light become clearly detectable under UV illumination. At sufficiently low concentrations, ImageJ software identifies fluorescent MFs as distinct regions of interest (ROIs), enabling fibre counting and both individual and total area quantification to be correlated to fibres mass concentration.

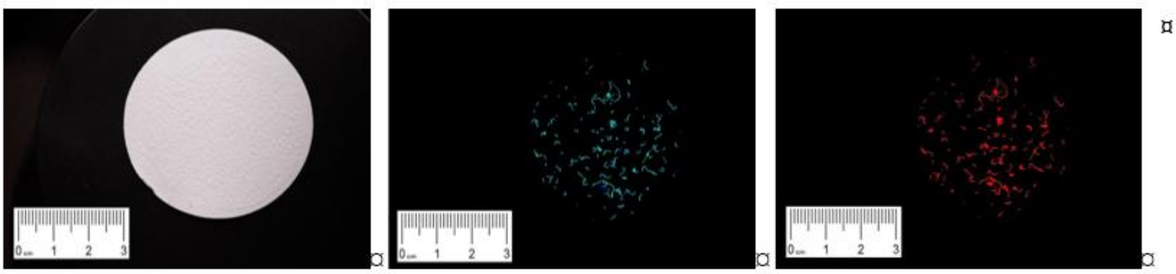

Figure 21: Filters pictures under visible light (left), UV light (centre) and after ROIs identification (right)

The fluorescence-based method demonstrated high linearity ($R^2 = 0.987$) across the range 1–125 µg, with a limit of detection (LOD) of 1 µg and limit of quantification (LOQ) of 2.5 µg (Figure 22 a). Validation using washing machine wastewater samples confirmed excellent agreement with gravimetric analyses, confirming its reliability for real-world applications.

Morphological analysis revealed substantial differences between synthetic and washing machine-derived fibres. Synthetic fibres displayed uniform lengths (mean 2.31 mm), whereas fibres from washing cycles were shorter and more fragmented (mean 1.13 mm), indicating mechanical degradation during laundering (Figure 22 b). This fragmentation increases fibre count and potentially enhances environmental dispersal. The strong correlation between total fluorescent area and fibre length (Figure 22 c) further confirmed the method's capability for combined quantitative and morphological assessment.

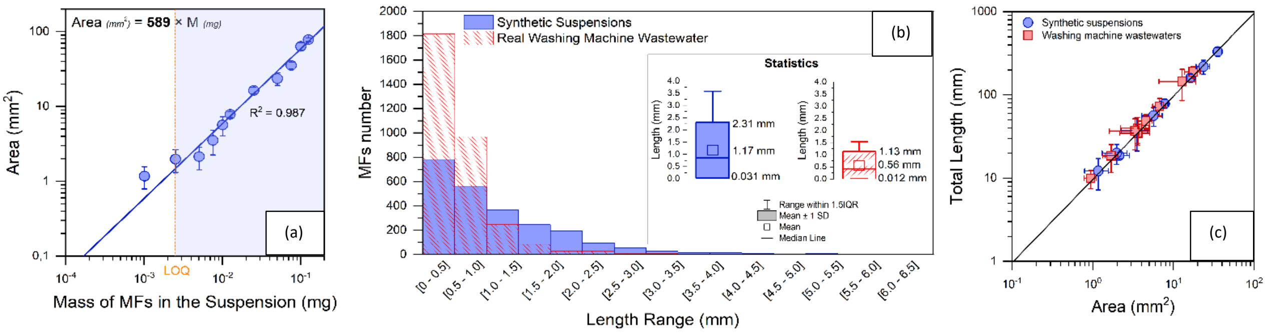

Figure 22: Calibration curve for polyester MFs, considering both synthetic and real samples (a), MFs size statistics (b), and correlation between length and area (c)

4.2.4 Scientific products and dissemination

Lupato, S., Granetto, M., Tiraferri, A., Sethi, R. (2025). Sensitive quantification and morphological analysis of microfibres in laundry wastewater: Standardization and validation of a fluorescence-based method. Journal of Hazardous Materials, 495, 138947. https://doi.org/10.1016/j.jhazmat.2025.138947

5. Conclusions

The approaches, methodologies and modelling tools developed and implemented in T4.5.1 in the overall duration of the project have set up a framework for the detection and assessment of MPs fate in Wastewater Treatment Plants, providing also useful insights in terms of MPs detection through a fluorescence-based method.

Concerning MPs fate in WWTPs, the large-scale monitoring carried out in the framework of this research enabled a deeper understanding of the occurrence, fate and removal of MPs and other microparticles of interest across different wastewater treatment configurations. The main findings can be summarized as follows.

i. The microparticle concentrations as well as their distribution in terms of polymer type, size and shape depend on the wastewater source and vary along the treatment train.

Among the monitored WWTPs, average MPs / microparticle concentrations of many thousand particles per liter were observed in raw wastewater: the level of contamination was found to be dependent on a plurality of factors mainly related to the characteristics of the urban / industrial area served by the plant, type of sewer system, etc. The MPs / microparticle concentrations strongly decreased over the treatment train, reaching values on the order of a few hundred particles per liter in the case of advanced tertiary treatment configurations. A large variety of polymers were found in the processed samples, highlighting the complex and diverse range of urban and industrial contributions to the MPs / microparticle load in wastewater. In the presence of combined sewers, relatively high quantities of rubber particles, largely originated from tire erosion, typically entered the plant with urban wastewater. Textiles were found to have a large impact on the discharge of MPs / microfibers in wastewater. Both natural and synthetic/semi-synthetic textile materials were identified, with a prevalence of cellulosic fabrics and wool. The persistence of natural microfibers along the wastewater treatment train highlighted the need for their large-scale monitoring. Similarly to the synthetic ones, they could contain harmful chemicals, added during processing, dyeing and finishing stages, and/or adsorb toxic compounds from the surrounding environment, that could prolong their degradation time and increase their ecotoxicological potential. Despite many site-specific differences, common trends were highlighted in terms of size and shape distributions. Most particles had a characteristic size lower than 100 µm, largely occurring as irregular fragments derived from secondary degradation processes.

ii. The microparticle removal efficiencies exerted by WWTPs is largely influenced by the level of technological advancement of treatment configuration in use.

Even in the absence of dedicated process units, WWTPs proved to be effective in removing MPs / microparticles, trapping most of them into sludge flocs. Depending on the treatment train in use, average removal efficiencies ranging from ranging from 84 % to more than 99 % were recorded. The implementation of advanced technologies for wastewater treatment – such as membrane-related processes, filtration systems, etc. – would hence enhance the potential for MPs/microparticle removal, preventing their massive discharge into receiving water bodies.

iii. Microparticle shape and size largely affect their removal along the wastewater treatment train, with polymer density also playing a major role.

The microparticle removal was found to be governed by a complex interplay of factors, with size and shape exerting a major role. Even in the presence of tertiary treatment configurations, WWTPs typically appeared less effective in removing microfibers: thanks to their morphology, they can more easily align with the flow direction to pass longitudinally through filter meshes, thus reducing the effectiveness of filtration units. The removal of particles belonging to the smallest size classes could be increased through the implementation of membrane-related processes. Low-density particles – such as those in PP and PE – were generally more persistent along the treatment trains: this behaviour could be ascribed to their limited settling capacity in the sedimentation units, which favors their transport to the subsequent treatment stages.

iv. Most microparticles removed during wastewater treatment are retained in sludge.

The consistency between the measured and predicted concentrations confirmed that sludge retains most particles removed during wastewater treatment. It was found that anaerobic digestion influence size and shape distribution of MPs in sludge, likely promoting particle degradation / fragmentation due to the mechanical and thermal stressors in the digester. These findings raise concerns regarding the environmental risks associated with the sludge application in agriculture. Therefore, careful assessment and monitoring of MPs/microparticles in sludge is essential to ensure sustainable resource recovery practices.

The main outcome delivered as final output of the project is therefore a multi-site dataset that considerably extends and strengthens the current state-of-the-art knowledge on the occurrence, fate and removal of MPs / microparticles across different wastewater treatment configurations. The analysis of wastewater from different catchments revealed the impact of specific urban and industrial sources on MPs distribution and load, pointing out the need for integrated source- and end-of-pipe control strategies. These insights contribute to improving both operational practices in WWTPs and broader policies aimed at mitigating MPs emissions into the environment, representing essential input for the development of risk-based management strategies, performance benchmarks and best-practice guidelines for MPs control in wastewater.

Monitoring results could be used as input data for mass-balance and fate-modelling studies, supporting both risk assessment and policy development on microplastic management. By way of examples, they could be exploited to (i) predict MPs removal dynamics in settling units, (ii) estimate MPs mass loads released at the river-basin scale, and (iii) trace MPs partitioning into sludge and their potential transfer to terrestrial ecosystems following land application. The analytical approach can also be extended to other environmental matrices (e.g. surface waters, sediments, soils, and reclaimed water), enabling an integrated understanding of MPs fluxes along the urban water cycle.

Overall, by offering a scalable methodological approach and operationally relevant insights, this work would help bridge the gap between research and practice, suggesting evidence-based decision-making in the fields of water treatment and environmental protection, which could contribute to mitigate plastic pollution within the urban-industrial-water continuum.

On the other hand, referring to the application/validation of fluorescence method, the main conclusions are summarized below.

Fluorescence-based detection represents a promising approach for the rapid, cost-effective, and accurate quantification of microfibres in laundry wastewater. The method standardized by Lupato et al. (2025) provides both mass-based and morphological data with minimal instrumentation and operational costs. Its scalability and sensitivity make it suitable for environmental monitoring and textile industry quality control. Future developments may expand its application to multi-polymer systems using differential excitation wavelengths or dye-assisted labelling for selective detection.

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Appendix

Section A1: Identification of the minimum sub-sample volume to be analyzed by LDIR

To understand the minimum number of depositions (and therefore the cumulative volume) of particle dispersion in EtOH to be analyzed, the following statistical method was applied. A total of 12 depositions of particle dispersion in EtOH of 20 µL each were characterized by LDIR-based particle analysis. The number of items in each deposition (N_i with i=1,….,12) was hence collected. All the possible combinations of sums of i=1,…,12 elements of N_i values were calculated in MATLAB environment and then converted to concentrations (expressed as number of items per mL EtOH). Data thus obtained were then statistically analyzed, collecting all relevant information regarding 25th percentiles (Q1), 50th percentiles (Q2), 75th percentiles (Q3) and mean, and represented as box charts. The trends of Q1, Q2, Q3 and mean values as a function of the number of depositions analyzed were hence studied. Examples of the statistical analysis carried out to identify the minimum number of deposition of the particle dispersion in EtOH to analyzed are presented in **Figures A1** and **A2** for $WWTP_A$ and $WWTP_B$, respectively. To be noticed that the concentration values reported in the graphs – expressed as number of items per mL EtOH – are those directly measured in the EtOH depositions and therefore do not reflect the effective particle concentration in the pristine sample: indeed, no concentration factors were considered and no blank substraction was applied. As clearly visible from **Figures A1B** and **A2B**, the values of 25th and 75th percentiles (Q1 and Q3, respectively) tended to the mean value as the analized volume of EtOH dispersion increased: particularly, in both examples proposed, for a number of depositions ≥ 9 (i.e. volume ≥ 180 µL) the relative error was ≤ 1%. Similar data were obtained for all samples under observation, thus suggesting that the anlaysis of a number of depositions of particle dispersion in EtOH ≥ 9 would enable a robust data elaboration. To further increase the representativeness without loosing the time-effectiveness of the measurement workflow, for all the experimental campaigns described in this chapter, a total of 12 depositions of 20 µL each (i.e. 240 µL vs. 5000 µL total volume) were analyzed by LDIR-based particle analyiss.

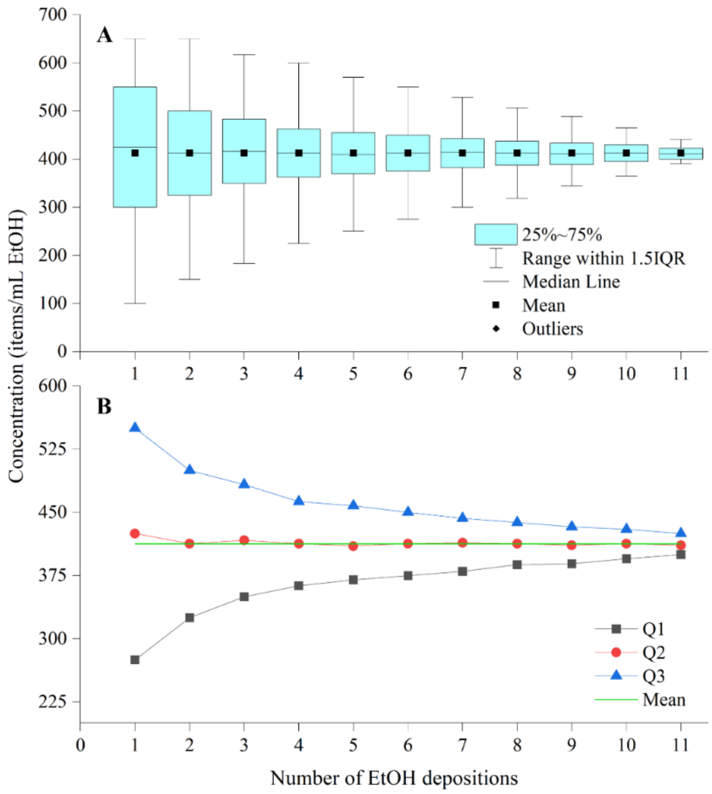

Figure A1: Box plots showing all possible particle concentrations for a processed sub-sample of raw wastewater (1 L) from $WWTP_A$ for an increasing number of depositions of the particle dispersion in EtOH analyzed (**A**) and corresponding trends for the 25th, 50th, 75th percentiles (Q1, Q2, Q3, respectively) and average values (**B**).

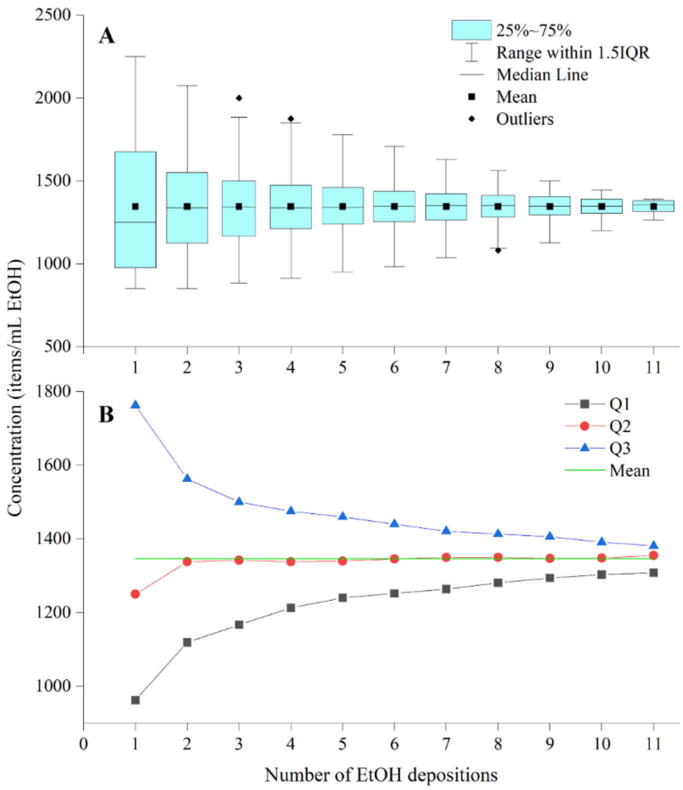

Figure A2: Box plots showing all possible particle concentrations for a processed sub-sample of raw wastewater (1 L) from $WWTP_B$ for an increasing number of depositions of the particle dispersion in EtOH analyzed (**A**) and corresponding trends for the 25th, 50th, 75th percentiles (Q1, Q2, Q3, respectively) and average values (**B**).