

Review

Approaches for Sampling and Sample Preparation for Microplastic Analysis in Laundry Effluents

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Abstract: The continuous growth in the production, unsustainable use, and disposal of plastics in recent decades has led to the emergence of a new type of pollutant, microplastics (MPs). In this article, the focus is on the form of MPs, which are produced by the fragmentation of textile fibres during washing processes. The problems associated with the characterisation and quantification of MPs in this type of sample are related to the wide range of concentrations, forms, and degree of degradation as well as physico-chemical and biological properties. Although the basic principles for the analysis of microplastics present in the environmental samples are known, there is the lack of standardised methods for the analysis of MPs in laundry effluents and domestic washing. Therefore, the continuous development of sophisticated analytical techniques and methodologies is required for the reliable collection and isolation, quantification, and characterisation of one of the most challenging analytes. The aim of this review is to outline the key steps of MPs analysis in laundry effluents and domestic washing, focusing on those steps that are underestimated in the current literature: sampling and sample preparation for analysis. Precisely these analytical steps, which can become the main source of analytical measurement system errors, ensure the quality of the analysis. This paper emphasises the importance of monitoring background contamination and presents guidelines to ensure quality control specifically for this type of analyte.

Keywords: water; sampling; sample preparation; density separation; digestion; textile wastewater; quality control



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1. Introduction

According to the latest standards, the term microplastics (MPs) encompasses any solid, water-insoluble plastic particle with dimension between 1 µm and 1000 µm in size. Those that are smaller than 5 mm are then referred to as large microplastics. [1]. Small pieces of floating plastic material on the ocean surface were first reported in the early 1970s, but the possible harmful effects attracted minimal attention from the scientific community [2]. It took almost 30 years to define the term microplastics as smaller fragments of plastic material whose dimensions are less than a millimetre [3].

Microplastic particles are now considered a new type of hazardous contaminant of anthropogenic origin. This type of environmental pollutant is a growing global problem for several reasons:

- (1) Plastics are characterised by a complex chemical structure and high molecular mass, which makes them less susceptible to degradation [4];
- (2) Atmospheric conditions (solar radiation, water temperature, and abrasion processes) lead to photo-induced cleavage and cross-linking of the polymer chain as well as to thermally induced degradation of the polymer chain [5], releasing potentially toxic additives (brominated flame retardants, antioxidants, light stabilisers, plasticisers, and pigments) [6,7];

- (3) Fragmentation leads to an increase in the specific surface area and hydrophobicity of MPs [8], making them a good medium for various types of pollutants, such as persistent, bio-accumulative, and toxic chemicals (PBTCs) [9–11]. These particles absorb not only persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [12] but also heavy metals [13,14], pharmaceuticals [15], and pathogenic organisms [16].

In addition, studies have shown that due to their small size, MPs are transported long distances by wind and water currents. The presence of MPs in the Antarctic is evidence of microplastics as a ubiquitous pollutant in the air [17]. The geometric parameters (size, shape, and density) of MPs have a major influence on their dry deposition and residence time in the atmosphere. Due to their elongated shape, fibre fragments have a smaller aerodynamic size than spherical particles with the same volume. This applies in particular to flat fibres, which cannot be transported over such large distances as cylindrical fibres [18]. MPs are often ingested by organisms because they mistake them for food [11,19]. Therefore, the combined effect of microplastics with adsorbed pollutants could seriously harm environment [20] but also human health [21]. In addition, fibre fragments have a more negative impact on the health of organisms due to their abundance and longer retention time after consumption by the biota. In order to reduce the use of microplastics, the European Commission adopted Regulation 2023/2055 [22], which restricts the use of microplastics intentionally added to products. Restrictions are applied for concentrations of MPs in a product limited to $\leq 0.01\%$ by weight.

Regardless of the origin of MPs, monitoring the amount of MPs present in the environment is of a great importance. MPs have spread very extensively in the environment, so they are now found in almost every part of the biosphere: from seas and oceans [23], freshwater [24,25], wastewater [26], and sediment [27] to soil [28] and air [29]. Given the confirmed presence of microplastics of all types and sizes in surface-, underground-, and wastewater [30], the question arises as to what extent drinking water is contaminated with MPs and how this affects human health [31,32]. Numerous studies provide information on the content of MPs, but the methods for sampling, isolation, purification, and identification of microplastics differ greatly. Although recommendations and principles for the analysis of microplastics in the environment have been published [1], the lack of standardised methods prevents a comprehensive interpretation and comparison of results from different research groups. The development of standardised methods for identification and classification is necessary in order to detect possible weak points in the production and life cycle of synthetic textile materials but also to carry out effective and sustainable remediation of water resources. The expression and representativeness of the results when measuring microplastics largely depend on the methodology itself. Results are usually expressed as total microplastics per unit of sample (e.g., L^{-1} in water), sometimes providing detailed classifications of size classes, colour, and shape (e.g., fibre, particle, and fragment) [21]. With increasing numbers of scientific publications on MPs contamination in environmental matrices as well as in food and drinking water, the calls for harmonised methods are becoming louder [33].

Microplastic fibres (MPFs) are the main components of microplastic pollution in the aquatic environment, which are mainly released during the entire life cycle of textiles (production, use, and disposal) [34]. It is estimated that 2×10^5 m³ effluent is discharged from WWTP per year, and with an average emission of 60 MPF L^{-1} , the total amount of MPF released would be around 18 billion particles [35]. They are also referred to as fibre fragments to distinguish them from microfibers. “Microfibers” is an established term in the field of textile technology, as it refers to fibres with a linear density of less than 1 dtex [36]. Therefore, the American Association of Textile Chemists and Colorists (AATCC) has suggested using the term “fibre fragments” instead [37].

As one of the most important manufacturing industries, the textile industry is an important component of the global economy. Globalisation processes have led to a significant increase in the production and consumption of affordable textile products. The extremely

rapid development of the textile industry is leading to the depletion of natural resources as well as a dangerous deterioration of the natural environment and the associated extreme weather events. At the same time, more and more textile waste is being produced, which is the main source of microplastics in the form of fibre fragments. Current scientific research in the field of textile technologies is focused on the search for new processes and materials for the development of advanced and multifunctional textile products that could completely transform textile technology into a sustainable system in which the generation of this type of pollutant would not occur. In addition, it is necessary to develop technologies for wastewater treatment that specifically target the removal of microplastics. For the development of such technologies, it is imperative to monitor the amount of microplastics in wastewater, especially in laundries. An imperative for the development of standardised methods is the development of a suitable methodology for the collection and preparation of samples. The development of such a method will make a significant contribution to various SDGs. In particular, the development of a standardised methodology for monitoring MPs content and the associated development of technologies for water purification will enable the conservation of marine resources (SDG 14). In addition, the MP particles present enter the food chain via drinking water (SDG 6), which can consequently affect human health (SDG 3). Due to the dispersion of MP particles through air currents, they enter global ecosystems via environmental transport (SDG 15) and can consequently contribute to climate change (SDG 13). In order to address the problem of MPs sample preparation, this article provides a critical overview of the currently known methods for the collection and preparation of composite samples with the aim of determining MPs in laundry wastewater systems, and at the same time, it offers perspectives for future developments. Finally, given the ubiquity of fibre fragments in the surrounding atmosphere, guidelines for the control of background contamination are given.

2. Textiles as a Main Source of Fibre Fragments

Generally, a distinction is made between MP particles that either enter the environment directly, such as products of daily use (e.g., facial cleansers [38], toothpaste [39]), and abrasive cleaners [40]), or are formed by the fragmentation of larger plastics into smaller fragments through photo-oxidation, mechanical, chemical, or biological action [41]. One of the most common types of MPs are fibre fragments, which, according to the International Union for Conservation of Nature (IUCN) [42], are mainly released from garments made from synthetic fibres (35%), as shown in Figure 1.

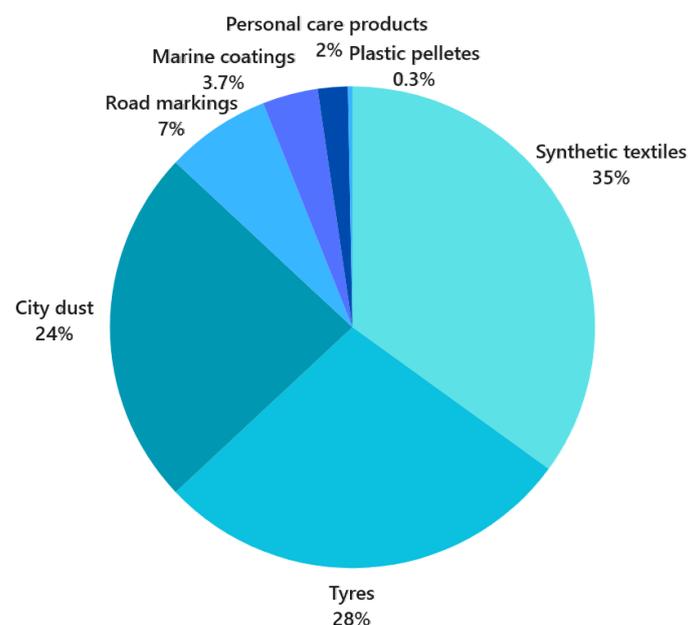


Figure 1. Main sources of microplastic in oceans [42].

The growing concern about environmental pollution has led to an exponential increase in the number of scientific publications attempting to estimate the amount of MP particles in the environment and their effects on living organisms. Figure 2 shows the number of published articles related to the analysis of microplastics in various environmental samples between 2013 and 2023. According to the data available in the “Web of Science Core Collection” database with the keywords microplastic(s), in contrast to the numerous scientific studies dealing with pollution of the marine environment and sediments, the pollution of wastewater by microplastics has so far only been studied to a limited extent. As part of wastewater, effluents from laundry and domestic washing are distinguished. Considering that synthetic textiles are one of the most important sources of MPs (Figure 1), laundry effluents samples containing fibre fragments were almost not examined at all until 2017 and still not sufficiently.

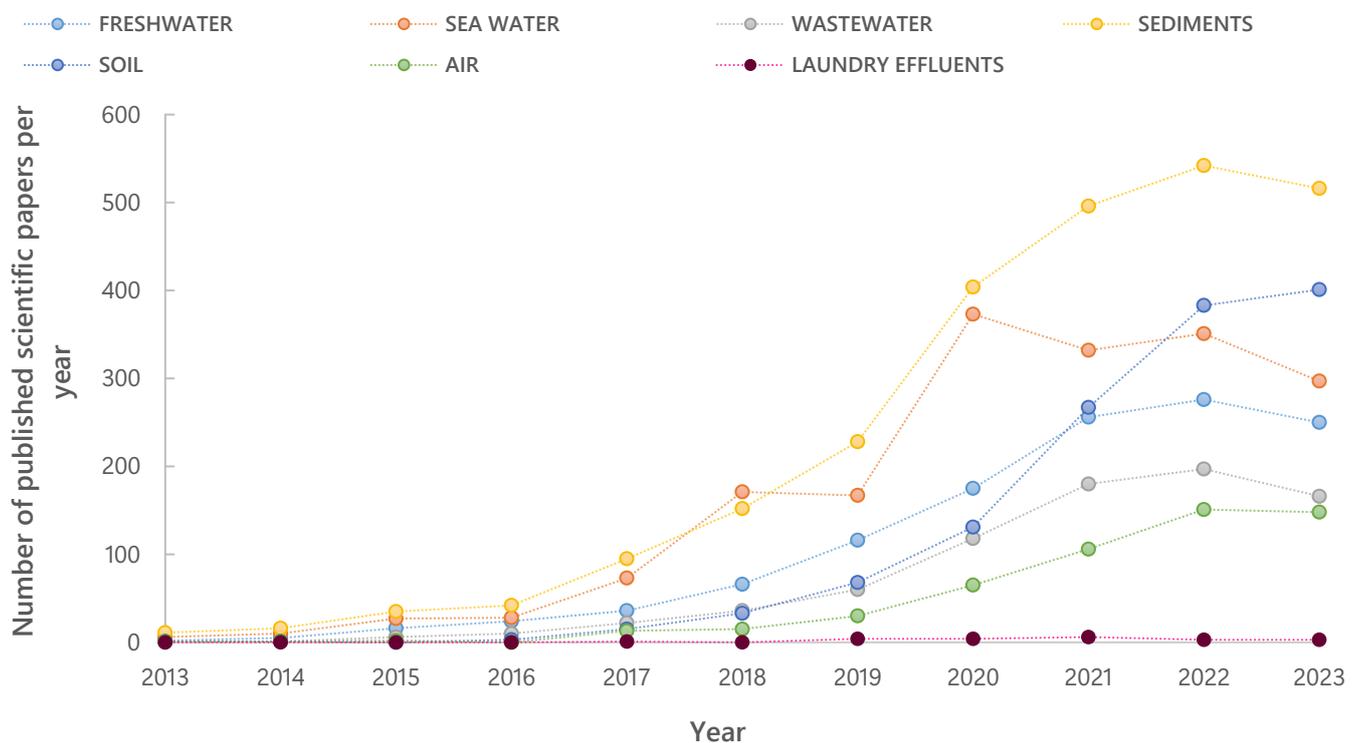


Figure 2. Publications in the field of microplastics research in different environmental samples from 2013 to 2023 (data collected from the Web of Science Core Collection).

Wastewater treatment plants (WWTPs) are one of the most important transport routes and entry points for MPs into aquatic ecosystems, with rates ranging from 0.93×10^{-6} MP per day [26] to 1.4×10^8 MP per day [43]. Although the removal efficiency of WWTP is more than 98%, large quantities of MPs enter WWTP every day, and a considerable amount of MPs pass through the filters and are discharged into natural waters [44]. The fibre fragments released from textiles can be differentiated according to their origin. Natural (cotton, wool, and silk) and man-made cellulosic fibres are found in wastewater in much higher concentrations than synthetic fibres [45]. Nevertheless, they are biodegradable, which is not a major ecological problem. This only applies to standard materials. Cellulose fibres treated with dyes or additives (organic compounds for the functionalisation of textiles, polymer films, etc.) are leached out by fibre fragmentation and enter aquatic ecosystems [46,47]. The fibre fragments are mainly released into the wastewater during the washing process, which then enters the municipal wastewater via the sewer system [48]. The estimated number of fibres released is 1.2×10^7 per wash cycle and kilogram of clothing [49].

It is assumed that most of the fibre fragments released from the surface of synthetic textile materials enter water bodies via the drainage pipes of the washing machines. During

each washing process, textile materials are exposed to chemicals and mechanical agitation. The tendency to release these particles depends firstly on the characteristics of the textile materials (construction characteristics, texture, and type of fabric, yarn, and fibres) as well as on the factors of the “Sinner’s circle” in the washing process (chemicals—use of detergent, washing temperature, duration of the process, and mechanical agitation) [50]. The detergents are often alkali-based, which in turn facilitate release of fibre fragments through migration of oligomers formed by hydrolysis [51]. Furthermore, it has been confirmed that powder detergent formulations enhance the emission of fibre fragments due to more mechanical friction between the material and the machine drum [49]. The ageing process of textiles themselves should also be taken into account, as the fibre structure is damaged by wear and UV radiation. It has been shown that older textiles release up to 25% more fibre fragments compared to newer materials [52,53].

3. Challenges in the Analysis of Microplastic Particles in Environmental Samples

Generally, environmental samples consist of the analyte (the species of interest) in a matrix most often rich in unwanted substances that may interfere with further analysis. Before analysing MPs in the laboratory, the sample must be collected, stored, transported, and processed appropriately. The process of analysing MPs in environmental samples can be roughly divided into five main steps, which are shown in Figure 3.

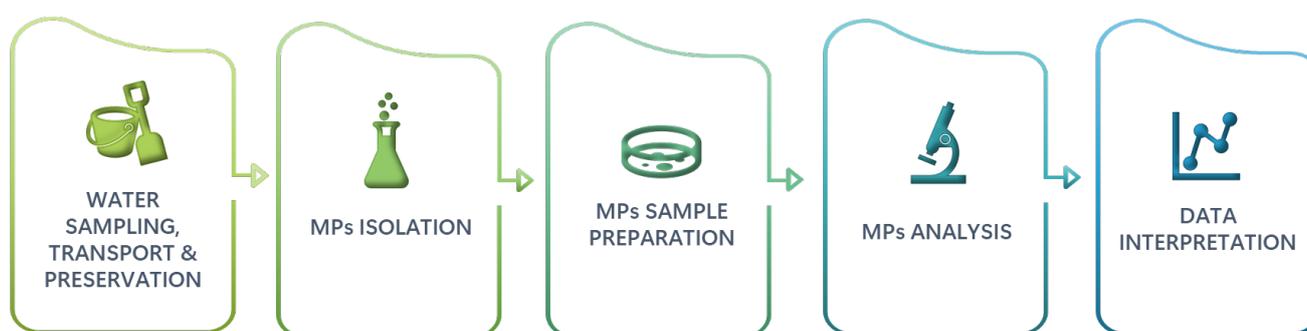


Figure 3. Diagram of the most important steps in analysing microplastics in environmental samples.

The process of sampling and isolation of MPS as well as MPs analysis and data interpretation is, overall, time-consuming. It is well known that sampling is one of the most time-consuming steps in the whole analytical procedure and depends on the water body.

The main problem regarding the methodology of sampling, isolation, and characterisation of MPs is reflected in their inconsistency, and often, the methods used have limitations in terms of accuracy and detection limits [54]. Different research groups use different sampling equipment, which differ mainly in the pore size, which ultimately determines the lower limit of the particle size. Furthermore, different analytical techniques determine the maximum particle size that can be detected. Finally, the accuracy of the analysis depends on the efficiency of the isolation methods but also on whether the processing of the sample and leads to a deterioration of the material.

Since sampling involves taking only a small portion of the total material to be analysed, the samples taken must be representative. Therefore, researchers are limited in the amount of a taken sample. Furthermore, conclusions based on the results of the most careful analysis may be invalid due to inadequate sampling. It should be borne in mind that MPs represent a group of polymeric materials of different shapes and chemical composition (including synthetic and biopolymers), which also contain various additives and adsorbed pollutants in their structure and exhibit different stages of ageing. It is not surprising that many of the sampling methods currently in use are unsuitable for analysing MP, as most methods are derived from standard water sampling methods that are not adapted to the physico-chemical properties of MPs. Therefore, we emphasise that representativity is only

a concept, and the obtained results of MPs analysis should be subject to statistical analysis in order to determine their significance.

Although plastic materials do not degrade over time in a controlled environment, improper storage of samples can lead to changes in MPs size distribution. Therefore, the collected samples must be stored in an appropriate manner to minimise possible physico-chemical and biological changes due to sample storage. The samples can be preserved by refrigeration, the use of a suitable sample container, or the addition of preservative chemicals [55]. The samples are usually stored in a refrigerator at 4 °C [56] or deep-frozen at below −20 °C [57] until they are analysed. In some cases, the identification and characterisation of the biological substance may be relevant for other aspects of the research, which is why different fixation solutions are used (70–80% ethanol [58] or 4–5% formalin [59]). Regardless of the sampling strategy, the collected wastewater samples must be further processed in the laboratory due to their heterogeneous composition (e.g., density separation or removal of organic matrices) before MPs identification can be performed.

The extraction of microplastics from any environmental matrix is the most important step, as it is influenced by the size, shape, and density of the type of matrix and MPs present. It follows that a possible differentiation of the extraction process can be based on the type of matrix to be analysed. Finally, the choice of method for analysing MPs strictly depends on the type of acquired data (mass, number, shape, size of particles, chemical composition of MP, degree of degradation and chemical composition of degradation products, surface properties, chemical composition of additives and absorbed pollutants, etc.) [1]. Moreover, depending on the chosen methods for MPs analysis but also the complexity of the sample matrix, appropriate collection and preparation techniques are selected. Thermal analysis techniques, for example, do not require a prior sample preparation step, as the pyrolysis of the matrix components and polymer materials takes place at different temperatures. In contrast, microspectroscopy requires isolation of the MPs so that interfering compounds do not completely mask the characteristic signal of the MPs. With these techniques, the MPs content can be expressed by the total number of particles, whereas thermal analysis techniques provide mass data.

Various analytical techniques are currently used for MPs identification, characterisation, and quantification, including Fourier transform infrared spectroscopy (FTIR) [60], Raman microscopy [61], scanning electron microscopy (SEM) [62] with energy-dispersive X-ray (EDX) [63], particle size distribution (PSD) [64], chromatographic techniques [65], and thermal analysis [66]. Of all available methods, IR spectroscopy, especially in combination with microscopy (μ -FTIR), currently offers the best prospects for standardisation. In addition to chemical identification, this method also provides information on the number, size, and shape distribution of the particles with a resolution of up to 10 μ m [67].

Due to the above-mentioned factors, a variety of methods and techniques have been proposed for the analysis of MPs in different environmental samples [1], making it necessary to develop an absolutely standardised methodological approach in the shortest possible time.

4. Sampling Strategies for Wastewater Samples

Water is a complex matrix with characteristic physico-chemical properties, according to which a suitable sampling method is selected. It has been shown that sediments in particular, as an inseparable part of water, serve as a sink for many anthropogenic compounds and that they contain increased amounts of MPs, especially in the vicinity of urban centres [68], which is why they must also be sampled to estimate MPs pollution.

The choice of sampling strategy is determined by the research objective and the assumed distribution of MPs in the field. Different polymeric materials are distributed differently in the water column. MPs with low density, such as PP and PE, are mainly found in the surface layers of the water [69], while plastics with a higher density (PET, PVC, PC, and PU), which have settled in the sediments and are embedded there, are not sufficiently represented in the surface water samples [70]. The spatial distribution of

microplastic particles in water is primarily influenced by the properties of the MP material, such as density, shape, size, and adsorption of chemicals but also the tendency to form agglomerates and consequently the formation of biofilms [71], as well as by the type of water. In addition, the distribution also depends on temporal-geographical factors such as wind, water currents, and waves, as turbulence can lead to resuspension.

Several parameters must be considered when selecting sampling equipment: price, ease of use, sampling location, and compatibility of analytes and cleanliness of equipment. When selecting a suitable container and sampling equipment, particular attention should be paid to the compatibility of the analyte with the materials of the sampling equipment, as the sample may be additionally contaminated during sampling. The use of worn sampling equipment (nylon nets, pump systems, etc.) made of polymer materials such as PVC, PTFE, PE, PP, and PVDF leads to the release of MPs from the equipment into the sample [72,73]. It is necessary to perform field and procedural blanks to assess the effect of cross-contamination on the final test result. The use of equipment such as metal sieves and glass bottles is recommended. However, the minimisation of possible contamination then leads to a reduction in the representativeness of the sample. It is therefore necessary to optimise the amount of the sample. When analysing the WWTP influent, where the concentration often exceeds 100 MP dm^{-3} , it is advisable to take a smaller sample volume so that the identification of plastic particles by infrared spectroscopy does not take too long [74].

When talking about sampling wastewaters, there are two main strategies: (1) bulk sampling and (2) volume-reduced sampling. Bulk sampling involves taking the entire volume of the sample without subsequent reduction, which is the most frequently used method in sampling textile wastewater. Grab samples are single water samples, which are most commonly taken with stainless steel buckets and then stored in glass containers [75]. The main advantage of this method is that, theoretically, all MPs present in the environmental matrix can be sampled without size limitation. In addition, this type of sampling is fast and can reduce the risk of contamination by reducing exposure time in the environment.

Due to the relatively low concentrations of microplastics but also their uneven temporal-spatial distribution [76], the question of the representativeness of the sample arises. It is therefore recommended that instead of taking a single sample, the wastewater should be sampled repeatedly at regular intervals over a certain period of time [77–79]. The time intervals between sampling vary between 15 min [43] and 2 h [80] over a total period of 24 h, and the total volumes of composite samples can also vary.

Volume-reduced samples are those whose volume is usually reduced during sampling so that only the part of the sample that is of interest for further processing is retained, whereby the representativeness of the sample is guaranteed without the entire sample having to be transported to the laboratory. This sampling approach is rarely used in WWTP, but the contamination of natural recipient waters with MPs from the surrounding WWTP is monitored in this way [16,81]. For sludge, the samples are sieved through sieves [82], while for water, the samples are collected by filtering with nets [83].

The most important characteristic of the sampling nets is the mesh size, which varies in a wide range from 50 to 3000 μm , consequently affecting the volume of samples collected and the amount of isolated MPs. So far, the pore sizes used in different studies have not been standardised, so the amount of MPs found varies, making it impossible to compare the results (Figure 4).

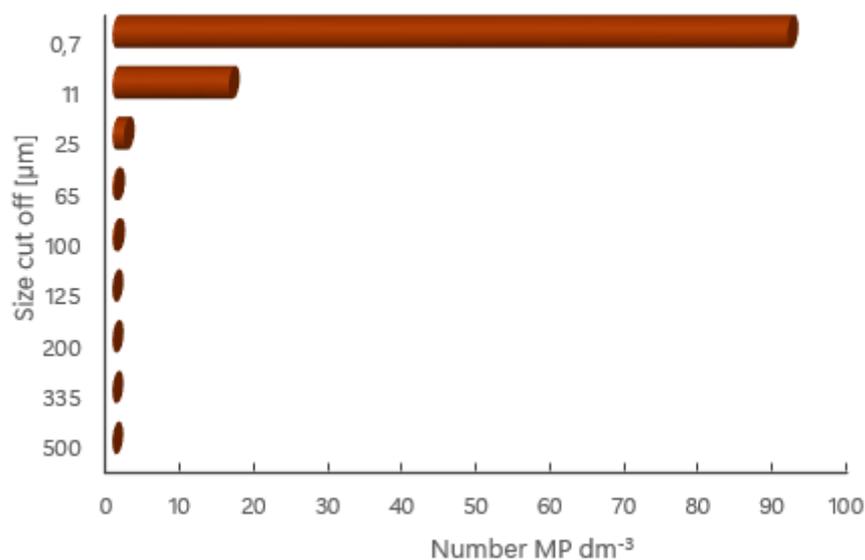


Figure 4. Correlation between the number of determined MPs in wastewater effluents and mesh size cut-off. The corresponding references from the figure bottom upwards are: [84], [85], [86], [80], [87], [88], [89], [88], [57].

An additional problem with sampling is the wide range of MP shapes [90]. Elongated fibre fragments can pass through pores with relatively small dimensions (much smaller than the maximum dimensions of an elongated shape) or remain in pores larger than their dimensions due to their irregular shape. As a result, there is an overestimation or loss of some MPs whose one dimension is significantly larger than the others, which should definitely be taken into account when interpreting the research results.

As nets and trawls are not sufficient to retain fibres from water samples, sampling is approached with a (manual or motor) pump that filters large volumes of water through a built-in filter [91], resulting in a more efficient sampling methodology of fibres compared to nets. In addition, sampling with the pump allows for more accurate monitoring of the sample volume, and it is also possible to choose a wide range of filters with different properties. However, due to the lower sampling volume, it may be more suitable for sampling in areas with higher contamination [92]. Moreover, treated water from a WWTP with a low TS can be pumped at a high flow rate over a long period of time. The extended sampling time makes it possible to estimate the time-dependent frequency of MPs in the wastewater [93]. However, the influent or activated sludge contains a large amount of suspended solids that clog the sieve, so a lower flow rate is then required. As an alternative, the use of a sampling tank is recommended. Nowadays, autosamplers are receiving a lot of attention due to their ability to fractionate the sample volume through a system of pumps and filters [94].

Self-developed filtration devices are used most rarely. The devices usually consist of an electric [95] or membrane pump [96], a unit consisting of a series of sieves [97] or filters [94] with different hole/pore sizes mounted on a substrate and located in a housing made of polymer material such as polycarbonate (PC [96]) or polyvinyl chloride (PVC [89]), through which the sample water flows, and the flow meter [96].

Laundry Effluents Sampling

The methods used to take samples for analysing MPs in wastewater from the washing process differ depending on whether they are used to analyse commercial washing machines or laboratory washing machines. In laboratory devices for washing simulation, metal cuvettes are used, the contents of which are then transferred to glass bottles (separately for each washing process [65] or assembled [98]). The MP particles themselves are then isolated by filtration with a glass fibre filter [99] or a PVDF filter [49].

In commercial washing machines, the washing process is carried out according to the manufacturer's instructions, and the collection of the released fibres is performed by directly isolating the MPs by filtering the effluent using an external, adapted filtering system. The filtration system usually consists of a drain hose connected to a peristaltic pump with a Teflon tube and a membrane made of either PTFE [100], nylon fabric [101], or stainless steel [102].

5. Sample Preparation and Isolation of Microplastic Particles

The biggest challenge in MPs analysis, especially with a complex matrix such as laundry effluents, is the selection of a suitable and efficient method for isolating particles from environmental samples. Due to their large specific surface area, plastics can serve as a backbone for the adsorption of surfactants (the main component of industrial and domestic detergents). The aggregates formed exhibit changed physico-chemical behaviour [103], therefore leading to underestimation of the fibre fragments due to the insolubility of inorganic substances and the entanglement of several fibre fragments [49].

Depending on the type, detergents differ considerably in their chemical composition. In addition to organic components such as surfactants and builders, powder detergents also contain inorganic, water-insoluble components (zeolites), which further complicates the isolation of MPs [104].

Therefore, this type of matrix requires a different approach to find the optimal method for particle extraction. This section gives a critical overview of the best-known methods of MPs extraction from different environmental matrices that could be used in the future in developing and optimising the extraction of fibre fragments from laundry effluent.

Determining the order and type of sample processing procedures used is extremely important, as it ultimately affects the results of the analysis. For samples containing high levels of mineral and organic matter, both digestion and density gradient separation methods are used, with varying orders of processing. If organic digestion is carried out first, a large amount of reagent is required, as wastewater and sediment samples contain a lot of organic material. In addition, a considerable amount of foam is formed during oxidation with H_2O_2 , which leads to loss of the sample. On the other hand, if separation by density is performed first, many impurities remain in the supernatant, leading to clogging of the filter pores when collecting MPs and unremoved organic compounds. It is therefore very important to optimise the sample processing sequence, taking into account the composition of the matrix. In the following sections, the methods for particle isolation are divided into three categories, depending on the properties by which the particles are separated:

- (1) Particle size » size-based approach;
- (2) Density of the particles » density-based approach;
- (3) Chemical composition of the particles » chemical composition-based approach.

Table S1 summarises the main advantages and disadvantages of proposed methods for MPs isolation, which can be found in Supplementary Materials.

5.1. Size-Based Approach

Particle size-based separation methods (sieving and filtration) are often used for the isolation of microplastic particles from environmental samples to reduce the volume/mass of the collected samples. Filtration is also the last step in sample preparation for analysing MPs after density gradient separation or chemical digestion and also as a method for sample concentration. Filters made of nylon [105], nitrocellulose [106], glass fibres [107], and polycarbonate and aluminium oxide [108] are used. Certain wastewaters from WWTPs and natural waters with low total solids content, including tap water and bottled water, can be treated by direct filtration to concentrate and isolate MP particles. Immediately prior to filtration, it is necessary to assess the organic and mineral content to avoid clogging the filter while minimising the potential interference of organic material in the spectroscopic characterisation of MPs. Seawater and freshwater samples differ in terms of salinity, which

in turn can influence the distribution of the microplastic particles themselves. However, differing sample preparation is not mentioned in the previous research.

The structure of the filter is an important parameter in filtration. For example, a PC filter with circular, flat pores is ideal for isolating the MP fragment. Together with the filter structure, the pore size of the filter determines the minimum size of the quantified particles [109]. Filters with larger pores (300–350 μm) are also used to isolate MPs of larger dimensions. However, in such filters, the passage of small particles and fibrils of different lengths is allowed, resulting in a loss of some of the information [85]. Conversely, using a filter with smaller pores (<1 mm) results in a wider range of isolated particle sizes but can lead to the accumulation of suspended particles and the clogging of the filter. The MPs are then covered with matrix residues, making them almost invisible on the filter surface [109]. In this case, a compromise must be found between the ability to retain small particles with small dimensions and the clogging of the filter. An alternative approach would be sequential filtration with progressively smaller pore sizes [110].

The choice of filter material depends primarily on the analytical technique used. In addition, the filter material must allow unhindered visual identification of the particles. Some filters are made of plastic materials (e.g., PTFE or metal-coated PC membranes), which can lead to contamination of the samples. When using such filters, it is necessary to make an assessment of contamination by analysing blanks [111]. However, if possible, the use of such filter materials should be avoided. For Raman microscopy, the use of silicon filters or PC filters coated with metal (Au or Al) is recommended. The ideal solution is to use PC membrane filters, which, unlike filters made of PTFE, silicon or cellulose, do not have their own high-intensity Raman spectrum [112]. Glass fibre filters are suitable due to their low cost and easy availability on the market. However, the recorded Raman spectra result in a lower particle contrast compared to PC filters, which is primarily due to the white colour of the filter and the high reflectance [113]. In IR spectroscopy, a distinction must be made between the selection of the filter with regard to the reflection and transmission mode of the image. Reflective surfaces are used for measurements in reflection mode, the most common of which are gold-coated PC membranes [114]. For measurements in transmission mode, the substrates must be permeable to IR radiation. Aluminium oxide filters (anodisc) or silicone filters are most commonly used for this purpose. Anodisc filters are only transparent for the range between 3600 and 1250 cm^{-1} , which partially obscures the footprint region [108]. Silicon filters, on the other hand, are permeable to the mid-IR range (4000–600 cm^{-1}) and allow the entire MIR spectrum of isolated particles to be recorded [115].

5.2. Density-Based Approach

Density separation is the most commonly used technique to concentrate and isolate MPs from samples whose matrix contains a high proportion of mineral matter. The density separation using a saturated salt solution promotes the flotation of less dense particles (MP in the supernatant), while mineral matter is retained in the form of precipitate. Finally, the MPs are separated from the supernatant by filtration and then identified and analysed.

The density of MPs varies from 0.80 to 2.30 g cm^{-3} depending on the type of polymer and the manufacturing process of plastics. The density values of the most commonly used plastics are listed in the Table 1. The density values of plastics are generally lower than those of most mineral materials (>2.6 g cm^{-3}).

One of the biggest disadvantages of this method is that it is time-consuming. The treatment itself takes 2–5 h, while the deposition time is 24–72 h [116,117]. If necessary, the process can be carried out several times to ensure that all particles of mineral substances are separated from the matrix [118,119].

Since the density values of polymers are in a wide range, the choice of ionic compound plays a crucial role. That is, salt solutions of different densities differ in their ability to separate and extract MP. Therefore, a higher-density salt solution prepared in this way will

also isolate a greater number of particles of different polymer types. The key properties of the most-used salt are presented in Table 2.

Table 1. Densities of most commonly used polymers.

Polymer Type	Polymer Density (g cm ⁻³)	Polymer Type	Polymer Density (g cm ⁻³)
LDPE	0.89–0.93	PVAL	1.19–1.31
HDPE	0.94–0.98	PFTE	2.10–2.30
PP	0.85–0.92	PVC	1.16–1.58
PS	1.04–1.10	PMMA	1.17–1.20
PA	1.12–1.15	PET	1.37–1.45
PES	1.24–2.30	PC	1.20–1.22
PAN	1.09–1.20	PU	1.20–1.26
POM	1.41–1.61	CA	1.22–1.24

Table 2. Properties of density solutions for the separation of microplastic particles from mineral matrices.

	Water Solubility at 25 °C (g dm ⁻³)	Density (g cm ⁻³)	Toxicity	Price per 100 g (EUR)	References
Sodium chloride, NaCl	360	1.0–1.2	LOW	3.4	[118,120–122]
Sodium bromide, NaBr	943	1.37–1.4	LOW	18.77	[123]
Sodium tungstate dihydrate, Na ₂ WO ₄ × 2 H ₂ O	742	1.4	LOW	58.4	[122]
Sodium polytungstate, 3 Na ₂ WO ₄ × 9 WO ₃ × H ₂ O	3100	1.4	LOW	332	[124]
Calcium chloride, CaCl ₂	811	1.30–1.35	LOW	43.2	[28,125]
Zinc chloride, ZnCl ₂	4320	1.5–1.8	HIGH	17.24	[126–128]
Zinc bromide, ZnBr ₂	4470	1.71	HIGH	34.4	[122]
Sodium iodide, NaI	1842	1.6–1.8	HIGH	108	[118,125,126,129]

Of the salts shown, sodium chloride (NaCl) and sodium tungstate dihydrate (Na₂WO₄ × 2 H₂O) are inexpensive but non-toxic and environmentally friendly. However, by using saturated solutions of these salts, it is possible to isolate only low-density MP, while high-density MPs such as PVC, PET, PC, or PU remain in the sediment [116,120]. In addition, NaCl easily crystallises on the walls of the glassware, which can lead to material loss.

The optimum density of solutions for isolating particles of higher densities is 1.6–1.8 g cm⁻³, which applies to solutions of zinc chloride and sodium iodide. However, these substances can be expensive and hard to handle. The use of zinc chloride is considered the most effective and cheapest method, but it is very dangerous. ZnCl₂ is a toxic and corrosive substance, mostly used in combination with acid, which can affect the chemical and morphological structure of the particles [130]. However, these disadvantages can be overcome by careful use, continuous reuse, and proper waste disposal (Table S2 in Supplementary Materials). The use of calcium chloride has proven to be a safer alternative. However, it is not suitable for samples with a high content of organic substances, as a flocculation occurs in the presence of Ca²⁺ ions [28]. NaI solutions separate oleophobic fibres with high efficiency (93%) [125]. However, NaI reacts with cellulose filters, creating a black film and making visual identification difficult. However, the use of NaI is recommended because it is environmentally friendly and can be recycled several times [131], which in turn lowers the price, under the condition, however, that it is not used in combination with a cellulose filter.

The distribution of the particles in the environmental samples is extremely heterogeneous. To ensure the representativeness of the sample, it is therefore desirable to take as

large a sample as possible. Increasing the sample volume also increases the amount of reagents required, hence the high cost of the analytical procedures. In addition, highly soluble salts such as ZnCl_2 and NaI require larger amounts of reagent to obtain saturated solutions compared to NaCl (Table 2 and Figure 5). Sodium iodide (NaI) solution, which is quite expensive, is often used at low concentrations. A more favourable approach is an adapted gradient separation method involving a fluidisation step in a sodium chloride (NaCl) solution, which reduces the sample mass by up to 80%, and then, the flotation process is carried out in a significantly reduced volume of salt solutions used for the separation of the heavier polymers [126,132].

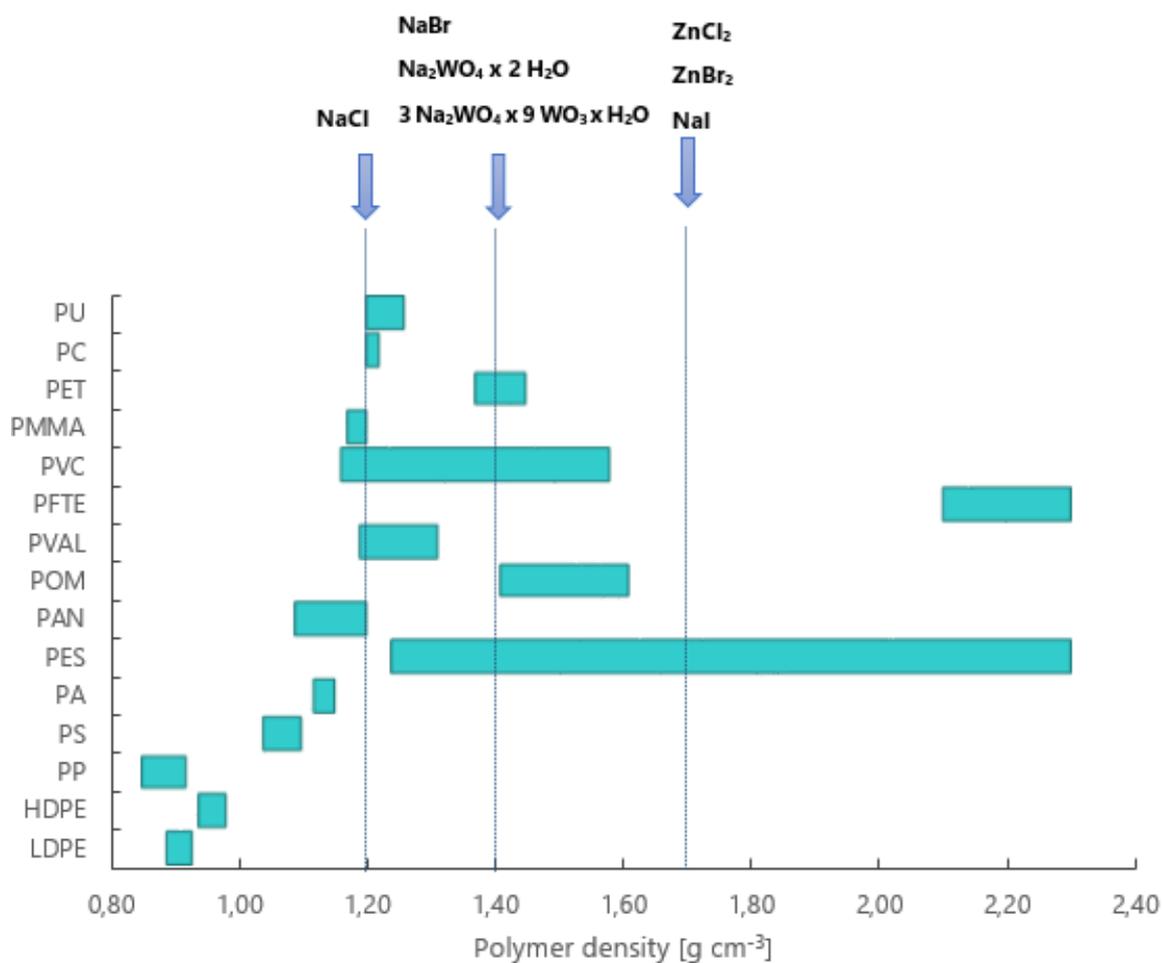


Figure 5. Diagram illustrating the possibility of using different salt solutions to extract different types of MPs.

In addition, the most suitable reagent is selected taking into account the structure and shape of the MP. Salt solutions with a higher density separate MP fibres with a higher density more effectively. However, they are ineffective for particles or fragments floating in low-density solutions due to their larger specific surface area [133].

The density gradient separation method may seem like a simple approach, but the efficiency of extraction can be affected by a number of factors:

- (1) Plastics contain various additives in their structure, which in turn affect their density and subsequent separation and significantly reduce buoyancy [123,134];
- (2) Some fractions of microplastic particles, such as PET/PVC, LDPE/PP, and HDPE/PP, cannot be separated and require additional processing, as their density ranges overlap;
- (3) The efficiency of MPs isolation is inversely proportional to particle size; the smallest proportion of isolated particles are those with smaller dimensions [135];

- (4) Due to their hydrophobic nature, the particles often coalesce into agglomerates to which various pollutants and microorganisms are adsorbed and form biofilms [41]. Agglomerates exhibit altered specific density, which in turn alters their distribution along the water column. As a result, incomplete isolation of the particles occurs, leading to an underestimation of the amount of MP particles present in the sample [136]. The separation is then carried out over several cycles and with an extended flotation time [116] but also in combination with peroxide digestion [133].

Each density separation protocol has potential advantages and disadvantages with respect to the analyte and the associated matrix. Ultimately, the most suitable technique is selected through a trade-off between particle yield, purchase price, and environmental impact.

5.3. Chemical Composition-Based Approach

Although density separation has been shown to be effective for the removal of inorganic substances, studies have shown that it is not sufficient for the efficient extraction of MPs for samples with a high organic content [137]. This is because the density of most microplastic particles ($\rho = 0.9\text{--}1.6 \text{ g cm}^{-3}$) is approximately equal to the density of the organic species, so they will float on the surface of the saturated salt solution together with the separated MP. Organic compounds interfere with the isolation by influencing the physical behaviour of the polymer particles, especially during density separation. At the same time, the remaining matrix components lead to false-positive results and thus hinder the detection and characterisation of the MPs [138].

This section presents the most commonly used digestion protocols. Taking into account all possible digestion methods, the favoured method should be the one compatible with the composition of the matrix. In addition, for samples with a complex matrix, it is necessary to perform several digestions to completely remove the organic species present. Although the order of application of extraction methods has no influence on the efficiency of MPs isolation, pre-digestion of laundry wastewater samples can lead to a higher yield if MPs are trapped in formed aggregates with detergent components.

To obtain the highest extraction yield, the following factors must be optimised:

- (1) Type of reagent (acids, bases, and oxidising agents or enzymes);
- (2) Reagent concentration;
- (3) Processing temperature (room temperature or elevated temperature);
- (4) Duration of digestion (from a few hours to days);
- (5) Successive treatments.

In addition to effectively destroying the organic substance, the agents used must leave the polymer particles unchanged in terms of weight, volume, shape, and colour [139]. It is always possible that the surface structure and morphology of the particles are damaged during digestion by mechanical friction [140], degraded by the use of an aggressive chemical reagent [141], and damaged by heating [142]. As the size range of MPs varies, particles with smaller dimensions and a larger specific surface area are more susceptible to degradation. In addition, the fact that microplastics in environmental samples have typically been exposed to the abrasive influence of atmospheric conditions and photodegradation over a long period of time before potential sampling and isolation is likely to make them less chemically resistant than the MPs standards used to assess the degradation effects of treatment [139]. Therefore, the data obtained for the digested samples should be interpreted with caution.

In order to test the efficiency and the potential effects of the methodology used to isolate MPs, a digestion experiment can be carried out under controlled conditions. This is performed by preparing a blank sample consisting of a sample matrix to which known amounts of fibres or granules are added [142]. Polymer resistance is tested by exposing the blank to a digestion mixture. The structural integrity and chemical composition of the polymer are assessed using optical and spectroscopic analysis techniques [141,143]. In any case, one should bear in mind that any method that involves the use of aggressive reagents at high temperatures over a long period of time will damage the MPs. The following

text presents the main advantages and disadvantages of digestion reagents in relation to efficiency for selected matrices and preservation of MPs morphology.

Acids break down organic substances such as proteins, carbohydrates, and fats quickly and efficiently. Acid treatment of the sample is most commonly carried out with nitric acid (HNO_3) [142] or perchloric acid (HClO_4) [144]. Acid mixture of HNO_3 and HClO_4 [145,146] has exceptional oxidising and corrosive properties. It is also possible to use other acids such as the peroxosulphic acid (H_2SO_5 [128]), but they result in lower efficiency. Acid treatment has the advantage of removing inorganic particles such as carbonates, which is common in laundry detergents.

The most common plastic materials on the market (PE, PP, PET, PVC, and PS) have proven to be resistant to a 5% HCl solution [146]. However, processing with strong acids (HNO_3 or HClO_4) affects the morphology of the MP, as these polymers are sensitive to pH changes and are not resistant to strong acids. Some materials (PA, PU, and PS) are completely degraded [139,147,148], while PET, PVC, and PMMA partially dissolve and agglutinate together [147,149]. The sensitivity of MPs to strong acids prevents the applicability of sample treatment with acids. In addition, processing takes quite a long time, and various undesirable reaction intermediates can be released during digestion, which can interfere with the spectroscopic identification of MP particles.

Alkaline digestion (usually with NaOH or KOH solutions) achieves an efficiency of up to 90% [150]. Results may vary depending on the type and concentration of reagent used and the incubation temperature. Alkaline treatment is effective for the hydrolysis and degradation of proteins and is therefore often used to remove MPs from biological samples [151]. Temperature plays an important role in alkaline treatment. For example, the use of 10 M NaOH at 60 °C leads to more efficient digestion [145]. In general, when choosing a reagent for alkaline treatment, KOH solution is preferred, which acts faster than NaOH and requires the use of a smaller amount of reagent.

Although alkaline digestion is effective in protein degradation, it is not as effective when water samples contain a large amount of enzymes. However, alkaline treatment has been shown to partially degrade certain polymer species such as CA, PVC, and PET [149], which make up the majority of fibres found in environmental samples. This effect can be reduced by using lower concentrations of NaOH (1M) but with the appearance of a “peeling” effect on the surface of PET due to its hydrolysis [152], which increases the possibility of the migration of oligomers formed by hydrolysis and further degradation and fragmentation of the polymer [51].

Oxidising agents degrade fat, calcareous substances, cellulose, lignin, tannin, humic substances, and chitin, which ensures highly efficient digestion of various matrices of environmental samples [153]. Oxidative digestion protocols mostly use hydrogen peroxide (H_2O_2) with a different concentration range (15–35%), temperature (25–70 °C), and reaction time (several hours to several weeks) [154].

However, the overall efficiency of H_2O_2 as an oxidising agent is questioned. Temperature regulation plays an important role in the digestion. For example, if the sample is incubated with 35% H_2O_2 (at room temperature) for 7 days, only 25% of the organics are removed [150], while increasing the temperature to 70 °C with a lower concentration of H_2O_2 increases the efficiency and shortens the reaction time while preserving the chemical structure of the MPs [155]. Studies have shown that peroxide digestion does not affect the chemical structure of various polymer types (PVC, PET, PA 6.6, ABS, PC, PU, PP, LDPE, LLDPE, and HPDE) [126]. However, there may be a change in physical properties (colour, shape, and particle size), but such effects are not an obstacle to the identification of microplastics by spectroscopic methods [128,142,147,152,156]. Although it has been shown that the use of H_2O_2 has no damaging effect on particles, the effects on plastics previously exposed to atmospheric conditions [125] have not yet been fully investigated. The main disadvantage of peroxide digestion is that at elevated temperatures and high concentrations (30%), foam forms, and the polymer suspension floats on top of the foam. Consequently, the polymer material sticks to the surface of the container, resulting in the

loss of the MPs [126,148]. Sodium hypochlorite (NaClO) is also used, whose efficiency (77%) is much lower than that of hydrogen peroxide (97%) [143].

The decomposition process by wet peroxide oxidation in the presence of Fe ions as a catalyst (better known as Fenton's reagent) has proven to be the optimal alternative. Fe(III)/Fe(II) ions catalyse the decomposition of hydrogen peroxide with the formation of radical species, which decompose organic material. Fenton's reagent provides high digestion efficiency with minimal effect on microplastics tested for a wide range of different matrices [152]. The effects of Fenton's reagent are controlled by pH (~3.0) and reaction temperature (<40 °C). For matrices rich in organic material, the temperature of the reaction mixture rises above 70 °C due to the exothermic reaction. In this case, it is advisable to use Fenton's reagent in lower concentrations or to carry out the reaction in waste baths [153]. Optimising the sample preparation also involves regulating the processing time because if the sample is exposed for a long time at an elevated temperature, the degree of solubility of the polymer increases, especially in the case of PA and PET [154]. In addition, compared to the previously described methods of sample preparation with the aim of extracting microplastic particles, Fenton's reagent offers a significant reduction in the time required to prepare the sample (up to 10 min) [157].

Enzymatic digestion is a promising method for removing organic matter, especially cellulose, proteins, and fats, which are commonly found in soil and sediments [158] but also in laundry effluents. In contrast to the chemical digestion methods mentioned above, sample preparation with enzymes takes place at a neutral pH value and moderate temperature, which ensures the preservation of the chemical and morphological structure of the MPs [159]. In addition, a digestion efficiency of over 97% can be achieved with a short incubation time (2 h) [150].

As enzymatic treatments have no significant effect on the structure of particles, it would be an ideal method for sample preparation. However, for samples with a larger mass, the efficiency of enzymatic digestion is relatively low, and the financial outlay is high [152]. To date, enzymatic treatments have been used exclusively for the isolation of plastic particles from biological samples [159,160]. In the context of wastewater and sediments, the complete removal of organic matter requires the use of different enzyme mixtures (cellulase, chitinase, lipase, protease, and proteinase) [96], which limits the applicability of such protocols. Therefore, a combination of enzymatic and chemical digestion is recommended for the implementation of enzymatic digestion for the processing of environmental samples, which leads to an increase in speed and a reduction in the economic costs of the applied protocols [161].

5.4. Advanced Techniques for the Isolation of Microplastic Particles

There are other, less common methods for isolating microplastic particles that have arisen from a desire to improve on the shortcomings of conventional methods. However, the limitations of these methods include the availability of specialised equipment, the processing time, the inability to characterise a specific polymer type and the change in surface charge after using these techniques. Advanced MPs isolation techniques include elutriation, pressurised liquid extraction, magnetic separation, electrostatic separation, and oil extraction. The oil extraction protocol (OEP) is presented in this section as the most important representative with numerous advantages.

The density separation of MPs, which is based precisely on the inherent density of the particles, does not lead to complete isolation of all types of plastics. The observed effect is a consequence of the change in their density due to the presence of additives [134] and the ageing of polymeric materials [162] as well as the adsorption of pollutants [41]. The MPs isolation protocol by oil extraction has proven to be one of the ways to overcome this problem. Crichton et al. showed that the efficiency of this extraction is higher than the separation in NaI and ZnCl₂ gradient with up to 93% [125]. During extraction, the lipophilic long-chain fatty acids present in the oils bind the hydrocarbon component of the synthetic polymers and form a micelle-like supramolecular unit. Even with high-density

polymers, the clusters formed have a lower overall density than water, making them easy to isolate. The MPs are extracted into the oil phase, while the minerals remain in the aqueous layer. The oil layer is filtered, and the filters are treated with alcohol to remove any oil residues that could interfere with further characterisation of the isolated MP particles.

OEP is a relatively inexpensive technique that requires only a minimal amount of reagents and simple laboratory equipment. It is also compatible with vibrational spectroscopy techniques and allows the identification of isolated polymer species. However, OEP has several limitations that lead to underestimation of the amount of MPs in the sample:

- (1) Individual particles with a partially hydrophilic character may remain in the aqueous phase;
- (2) For samples with a high content of organic substances, it is necessary to carry out a digestion beforehand;
- (3) When mixing a sample containing surface-active substances (e.g., wastewater from laundry), partial emulsification of the oil may occur;
- (4) Some particles may remain in the oil during the washing process.

Despite the disadvantages, the combination of the non-toxicity of the oil, independence from the specific MPs density, and high efficiency makes this approach more favourable than density-based separation methods.

6. Internal Quality Control

In the development of a standardised protocol for sampling, extraction, and identification of microplastics, quality assurance (QA/QC) plays a key role in assessing the sources of variability in the experimental data. Fibre fragments are one of the most common forms of microplastics, and due to their low density and size, they can be easily transported by air [163]. Samples can always be contaminated with fibres, whether through abrasion from synthetic clothing, improperly cleaned laboratory equipment, or poor sealing of samples with the ambient air. Without any precautions against possible contamination, the objectivity of the analysis results becomes highly questionable.

One of the most common and reliable methods of internal quality control is the use of certified reference materials (CRMs) and reference materials (RMs). Today, various CRMs and RMs are being developed that differ in appearance and chemical structure. For example, there are currently RMs in the form of spheres or foils [164,165], but at the time of writing, none have been found in fibrillar form that would be most suitable for assessing water pollution from textile industry effluents or laundry effluents.

One of the most important steps to monitor and minimise contamination of samples is to perform blanks during sampling and sample processing. Blanks are samples that have undergone all manipulations and contain all reagents that a typical sample has, but they do not contain the analyte. They are used to determine in which step of the MPs analysis that sample contamination occurs. A field blank is collected by subjecting containers filled with Milli-Q or filtered DI water to the same collection procedures as the samples. For example, the container is opened at the same time as the container used for sampling, or the Milli-Q water is passed through the set of sampling sieves or nets used to assess contamination from the sampling equipment. The collected blanks are transported to the laboratory and analysed in the same way as the real samples to assess the contribution of contaminating materials. The amount of MPs is determined after vacuum filtration of the contents of the sample container [166].

Procedural (laboratory) blanks are performed to assess contamination during sample processing with MP particles present in the air. During processing, the blank is subjected to the same processing steps as the other samples (filtration, digestion, and density gradient separation) [167]. As with field blanks, the container is opened when the sample is exposed to air and closed when the sample is not exposed to air. Additional blanks for general contamination monitoring in the laboratory are performed by placing moistened filters in glass Petri dishes on common laboratory surfaces. The moistened filter paper is later analysed using microscopic or spectroscopic methods, just like environmental samples [168,169].

When processing the test results, the results of the blanks can be subtracted from those of the sample. If the particles found in the blank samples match those of the sample, subtraction is performed according to their shape and colour for the samples processed in the same batch. Since the particles obtained by monitoring the blank sample do not always match the particles of the sample, it is not possible to perform this procedure. Then, the number of particles in the blank sample is used as indicated in the information, and all samples containing a lower number of MPs than those found in the blank sample are considered undetectable [170]. Unfortunately, due to high heterogeneity of the samples containing MPs with different physico-chemical properties, it is almost impossible to compare blank controls. To ensure the accuracy of the experiments, the results of the MPs analysis and the blank samples should therefore be statistically analysed.

Mitigation of Cross-Contamination

Potential cross-contamination is further minimised by a series of prescribed recommendations to avoid contamination during sample collection and processing. In laboratory practices, it is common to use glassware and metalware [171], wear cotton clothing [172], handle samples in clean rooms with controlled air circulation, clean work areas and tools with 70% ethanol and wipe with cotton cloths [173], and clean equipment with acid followed by ultrapure water [168]. Sample bottles and used nets and sieves should be rinsed with water before sampling, and all working solutions should be filtered [174]. Plastic equipment used in the laboratory should be replaced with non-plastic material, or contact with plastic equipment should be avoided [175,176]. To ensure that the filter media are not contaminated, a visual inspection with an optical microscope is usually not carried out immediately before filtration [177].

It has been shown that fibre contamination is reduced when processing takes place under clean air conditions such as clean air flow chambers [178], laminar flow cabinets [168,177], or fume hoods [146]. In cases where it is not possible to ensure clean air conditions, it has been shown that covering the samples during processing and analysis can significantly reduce contamination [179]. Special attention should also be paid to the protective materials used to ensure that the sample is not contaminated [150]. Some researchers even exclude fibre fragments from the results and consider all fibres as artefacts of sample processing [178,180]. However, this approach is no longer appropriate, especially when analysing laundry effluent, as it distorts the actual results of contamination and thus affects the interpretation of the impact of microplastic pollution [171].

7. Conclusions

Sensitive and sophisticated methods for the identification, characterisation, and quantification of MPs are a prerequisite for monitoring MPs pollution. Research shows that it is extremely difficult to develop a standardised method for monitoring MP particles, which cover a wide range of concentrations and are of very different chemical composition, shape, size, density, colour, and degree of degradation. It is very likely that in the future, there will not be only one standardised method but several. Depending on the purpose of the research (chemical characterisation of the polymer; size distribution of the particles; qualitative information: shape, morphology, and degree of degradation of the particles; chemical characterisation of bound additives and contaminants), the most suitable (combination) method will be used for the analysis. Although microspectroscopy techniques are favoured, thermal analysis techniques must also be considered, which not only allow for monitoring of MPs content by mass but also do not require time-consuming sample preparation.

The main problem in the quantification and characterisation of MPs is the lack of CRMs and RMs to ensure traceability of the method, especially in the context of fibre fragments. In addition, fibre fragments are ubiquitous and can be found in the atmosphere, which very often leads to cross-contamination of the sample. For this reason, none of the currently available methods for analysing MPs can provide sufficiently reproducible results.

In the absence of such methods, field and procedural blanks and statistical analyses are usually used, which ultimately do not provide satisfactory results.

In order to approach the characterisation and quantification of particles with sophisticated analytical techniques, it is also necessary to collect and prepare the samples appropriately, which is also an aim of this work. The laundry effluents sample presents a particular challenge, as it contains a high content of inorganic and organic substances. The only correct way to collect such a heterogeneous sample is a composite sampling, which is also time-consuming. In addition, fibre fragments have excellent adsorption properties due to their large specific surface area, so they often bind detergent components and form aggregates. Therefore, this sample requires the use of a combination of several separation techniques in multiple steps to isolate particles. When selecting the optimal methods and reagents for the isolation of microplastic particles, it is particularly important that the structural integrity of the particles is not damaged during the treatment of the sample. This means that the particles must remain unchanged in terms of weight, volume, shape, and colour. Density separation is most commonly used to isolate microplastic particles from samples with a high content of suspended solids. For this purpose, solutions of different salts (NaCl, NaBr, NaI, ZnCl₂, ZnBr₂, etc.) are used, which differ from each other in terms of solubility, density, toxicity, and price and are selected with regard to the intrinsic density of the particles. The density separation technique is ineffective for the removal of organic species from detergent suspensions (surfactants, builders, enzymes, etc.). Various reagents (e.g., acids, bases, oxidising agents, etc.) and advanced extraction technologies (oil extraction protocol, microwave extraction, etc.) can be used to remove the organic matrix. The most promising technique is use of the Fenton reagent, which achieves an organic matrix removal efficiency of >85% under appropriately optimised reaction conditions. In combination with density separation, this enables an almost complete extraction of the MP particles and thus a more accurate MPs analysis. Finally, the filtration step follows, in which the particles are isolated for further analysis. An additional problem arises from the pronounced linearity of one dimension of the fibre fragment, which leads to the particles being ingratiated in the pores of the filter and consequently underestimated. Nowadays, there is a growing concern about the generation of textile waste and thus the release of fibre fragments into the environment. In order to address the development of technologies for their effective and sustainable removal, standardised methods for their monitoring need to be developed, to which this work ultimately aims to contribute.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su16083401/s1>, Table S1: Summation of the main advantages and disadvantages of proposed methods for MPs isolation; Table S2: Health and safety aspects for the salts used in density separation method.

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