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Analytical methods to measure microplastics in drinking water

Review and evaluation of methods

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Contents

Abstract

Article 13(6) of EU Directive 2020/2184 on the quality of water intended for human consumption (recast) states that within 3 years of its entry into force on 12th January 2021 the European Commission shall adopt a methodology to measure microplastics in drinking water. To support the development and adoption of such a methodology, the Joint Research Centre has undertaken a review of the scientific knowledge base regarding the nature, distribution and quantities of microplastics in drinking water. From the literature and supported by in-house experience, potential instrumental techniques were identified and documented to summarise their technical capabilities, limitations and where possible also information on sample throughput as well as running and investment costs. Consideration was given to the relevant activities and progress of standardization bodies relating to microplastic analysis methods. Finally, with a view to defining the scope of a monitoring methodology, including appropriate reporting criteria, the possible descriptors for microplastics have been considered – in particular particle size, particle shape and polymers of interest – as well as suitable measurement metrics (mass/number). This information, which is summarised in this report, forms the basis for making informed and pragmatic recommendations about the key parameters to be measured and the most appropriate analytical techniques to use in the development of a methodology.

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Executive summary

Directive (EU) 2020/2184 of 16th December 2020 of the European Parliament and the Council on the quality of water intended for human consumption (recast) entered into force on 12th January 2021. This Directive is referred to as the Recast-Drinking Water Directive (DWD) and within 3 years of its entry into force, the European Commission should adopt a methodology to measure microplastics in water intended for human consumption, informally also called drinking water (DW).

To support the definition of a methodology as required by the recast-DWD, the Joint Research Centre (JRC) has been asked to undertake a review of the scientific knowledge base regarding the nature, distribution and quantities of microplastics in DW. Secondly, from the literature and supported by in-house experience, potential instrumental techniques have been identified and documented with consideration being given to summarising their technical capabilities, limitations and where possible also information on their sample throughput, running and initial investment costs. Thirdly, consideration has been given to relevant activities and progress in guidelines and method standardisation by international standardization bodies such as CEN, ISO and ASTM. Finally, with a view to establishing the scope of a monitoring methodology, including appropriate reporting criteria, a range of possible descriptors for microplastics have been considered – in particular particle size, particle shape and polymers of interest – as well as suitable measurement metrics (mass/number based).

The review showed that the number concentrations of particles and fibres in drinking water varied across five orders of magnitude. The majority of studies reported microplastics occurrence within the range 100 - 0.01 particles per litre with European studies typically showing levels below 1 particle per litre. To obtain these results, three main categories of techniques have been used: fluorescence microscopy, optical micro-spectroscopy (using Raman or Infra-Red) and thermo-analytical techniques (pyrolysis and thermal-extraction-and-desorption coupled with gas chromatography / mass spectrometry). Each of them has a particular combination of strengths and limitations in terms of the measurement metric (polymer weight or particle number), sensitivity, minimum particle size and ability to identify polymer type. Regarding harmonization of analytical methods only two partially relevant standards have been published. The first, ASTM D8332-20, is a “Standard Practice for Collection of Water Samples for the Identification of Microplastics” while the second, ISO 24187, sets out the key principles to be followed in the development of sampling, sample treatment and detection procedures. Neither of the standards adequately satisfies the needs of a methodology tailored specifically for monitoring microplastics in drinking water.

It has been concluded that no single analytical technique can yet be considered fully suited to the task of identifying and quantifying microplastics contaminants in drinking water for monitoring purposes. Consequently, the adoption of a methodology will require some degree of compromise and pragmatic choices must be made regarding the critical parameters to monitor. Key factors in determining a suitable method or combination of methods will be sensitivity and specificity but for routine widespread monitoring aspects such as comparability of measurement results obtained in different laboratories, investment costs, sample throughput and cost per sample should also be considered.

The information collated in this report is part of the JRC science-for-policy role to assist the process of making informed and pragmatic recommendations about the key parameters to be measured and subsequently to identify suitable methodologies for achieving this.

1 Introduction

Microplastics are persistent pollutants which increasingly are present in land, water and air. It is well known that they produce harmful effects on the environment and concerns arise that they potentially also may have effects on human health. Plastics as such are generally considered to have low toxicity, but the effects of microplastic particles is not yet known. In addition, microplastics can carry additives, residual monomers, biofilm and adsorbed chemicals, some of which are of toxicological concern. Following ingestion, such compounds may be released in the gastrointestinal tract and potentially taken up by the body. Because the information about exposure of humans to microplastic particles and/or associated compounds is scarce, a valid risk assessment is currently not possible[1].

There has been much debate on what needs to be done to understand and reduce the impact of microplastics with numerous international calls for action on the issue (for instance from United Nations UN,[2] World Health Organization WHO,[1] Organisation for Economic Co-operation and Development OECD[3]). The European Commission (EC) has responded in key policy documents such as the Green Deal and the European Plastics Strategy and is now driving the development of legislation though a recently published restriction under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)[4] or as part of the review of legislation such as the Drinking Water Directive (Recast-DWD).

The recast of *Directive (EU) 2020/2184 of 16th December 2020 of the European Parliament and the Council on the quality of water intended for human consumption*,[5] informally referred to as the Drinking Water Directive (DWD), requires that the European Commission shall adopt a methodology to measure microplastics in water intended for human consumption, informally called drinking water (DW), by 12th January 2024, as detailed in Article 13 Paragraph 6 of the Directive.

> *"By 12/1/2024 the Commission shall adopt delegated acts [...] in order to supplement this Directive by adopting a methodology to measure microplastics with a view to including them on the watch list [...]."*

Accordingly, the Joint Research Centre (JRC) of the European Commission, in its role of scientific support for the policy, has been asked to describe the key considerations for identifying possible analytical methods for detecting and quantifying microplastic contaminants in DW. Firstly, a review of the scientific knowledge base regarding the nature, distribution and quantities of microplastics in DW was undertaken. This serves to provide key information on the range of concentrations, sizes and types of microplastics which could be expected to be found in real-life DW samples. Secondly, from the literature and supported by in-house experience, analytical techniques have been identified and documented with consideration being given to summarising their technical capabilities, limitations and, where possible, also information on their sample throughput, running and initial investment costs. In addition, the scientific literature was screened to identify commonly used sampling approaches and sample treatment procedures for the analysis of DW. Thirdly, in addition to scientific literature, consideration has been given to any relevant activities and progress in guidelines and method standardisation by European and international standardization bodies such as European Committee for Standardization (CEN), International Organisation for Standardization (ISO) and ASTM International. Finally, with a view to establishing the scope of a monitoring methodology, including appropriate reporting criteria, a range of possible descriptors for microplastics have been considered – in particular particle size, particle shape, polymers of interest – as well as measurement metrics (mass/number) so that informed and pragmatic recommendations about the key parameters to be measured and reported will be made.

2 Reported presence of microplastics in drinking water

Evaluating the suitability of analytical methods for detecting and characterising microplastics requires an understanding of the expected levels of contamination. To this end, the scientific literature was screened for studies on the detection of such particles in relevant water samples. In particular, peer-reviewed articles specifically reporting the analysis of microplastics sampled from DW were identified and evaluated. The objective of this review was to extract key information regarding the nature of microplastics in DW, their concentration, the analytical techniques used for their detection and quantification as well as the sampling methods applied.

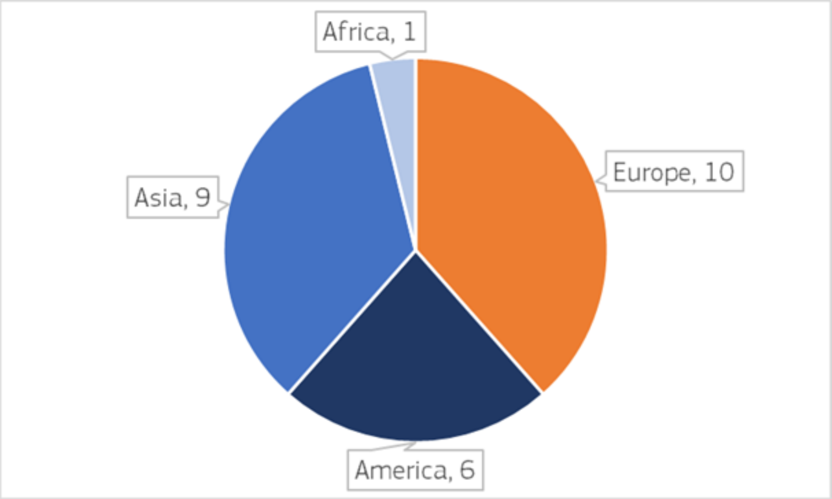
2.1 Methodology applied for the literature review

The literature search was performed in December 2021 with the bibliographic databases Scopus and Google Scholar, using the search string "drinking *and* water *and* microplastics" for titles, abstracts and key words. The search resulted in 207 articles published between 2013 and 2022. These articles were then analysed for relevance to this literature review leaving 21 articles, which reported studies of DW collected from the following sources: public and private institutions, households, residential and commercial areas,[6–20] city plumbing system,[21] hydrants[22,23] and fountains[24]; two articles analysing water sampled at various points in the water distribution network[25,26].

2.2 Sampling sites and conditions

Sampling sites, from which data are reported in the literature, are spread over four continents, namely Europe, America, Asia, and Africa (see Figure 1). While most studies focussed on the investigation of DW in single countries, Mukotaka et al.[15] and Kosuth et al.[11] collected their samples in different countries. The 11 studies that investigated DW samples from Europe, reported data from Germany (5 studies), France (2), United Kingdom (2), Denmark (1), Finland (1), Iceland (1), Italy (1), Norway (1), Slovakia (1), Sweden (1), and Switzerland (1).

Figure 1. Number of studies investigating DW samples from different geographical regions. Total number of studies is 21, of which two included samples collected from more than one region.



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The samples were taken at different points of the water supply chain, including:

- Water tap, where a consumer collects water for daily use;
- Hand-over point between the water supply system;
- Private site (building or plot);
- Public fountain/well.

The sample collection was generally carried out in one of two different ways:

a) container sampling: water was collected into containers followed by filtration off-line (14 studies);

b) at-source filtering: a filtering device was directly attached to the water supply (8 studies).

This includes one study (Yuan et al.)[6], which used both sampling procedures.

The volume sampled varied substantially between studies and depended on the type of sample collection (see Table 1). In case of at-source on-line filtering, quite large volumes of water were collected, the filtered volume being between 9 litres and 2250 litres (average: 907 litres, median: 900 litres). In case of container sampling, the volume of the water samples varied between 0.5 litres and 100 litres (average: 11 litres, median: 1 litre).

Table 1. Volumes sampled by the different studies, split up into studies with at-source filtration and container sampling. Yuan et al.[6] used both sampling procedures.

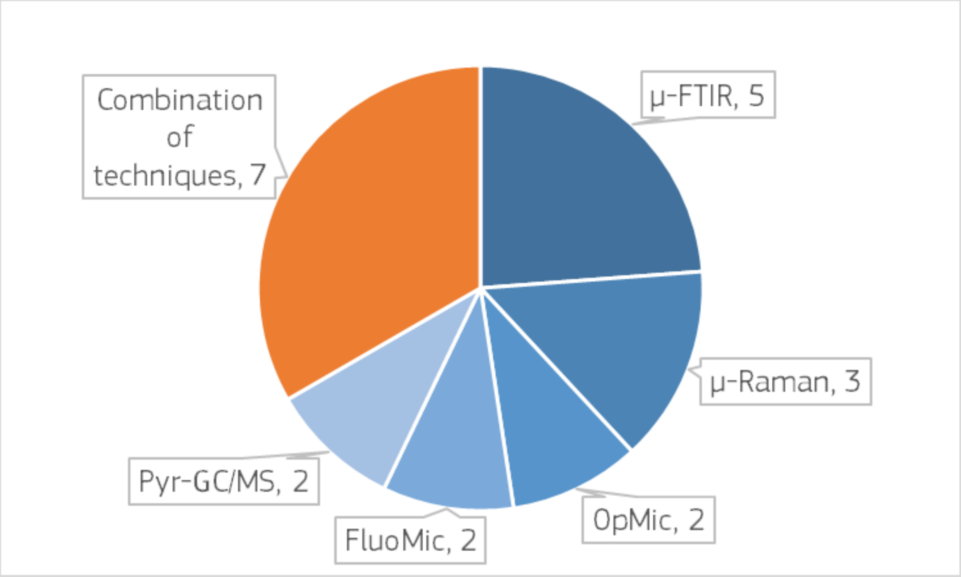
At-source filtering		Container sampling	
Author(s)	Volume [L]	Author(s)	Volume [L]
Johnson et al.[26]	2250	Chanpiwat et al.[25]	100
Mintenig et al.[10]	1850	Ásmunddóttir et al.[21]	22.5
Pittroff et al.[17]	1300	Shen et al.[16]	10
Gomiero et al.[22]	1000	Yuan et al.[6]	9
Weber et al.[18]	900	Zhang et al.[12]	4.5
Kirstein et al.[23]	750	Chu et al.[7]	1
Feld et al.[13]	50	Ferraz et al.[19]	1
Yuan et al.[6]	9	Lam et al.[20]	1
		Tong et al.[8]	1
		Shruti et al.[24]	1
		Kankanige et al.[9]	1
		Pratesi et al.[14]	0.5
		Mukotaka et al.[15]	0.5
		Kosuth et al.[11]	0.5

Source: own production

2.3 Techniques used for detection and quantification

After filtration, the nature and the quantity of plastic particles were determined using different techniques. Figure 2 gives an overview about the frequency of techniques or combination of techniques applied. Various combinations of techniques were used, including spectroscopic techniques (Fourier Transform Infrared spectro-microscopy µ-FTIR, Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy ATR-FTIR or/and Raman spectro-microscopy µ-Raman) with other optical techniques, such as optical microscopy (OpMic) or/and fluorescence microscopy (FluoMic), or Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis (SEM-EDX).

Figure 2. Number of studies using various techniques for analysing the particles found. Combination of techniques explained in the text. Acronyms of techniques: see list of abbreviations (page 66).



Source: own production

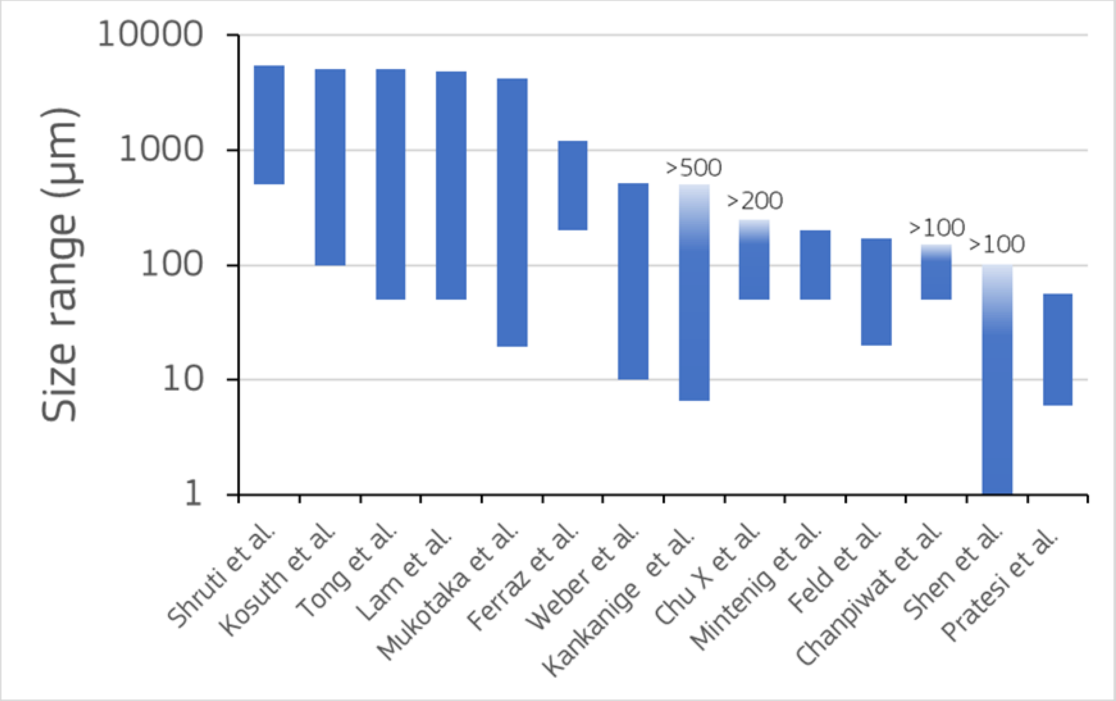
The determination of the *number* of microplastic particles was more frequently used than quantifying the *mass* of the particles. Among the number-based techniques, µ-FTIR and a combination of OpMic and µ-FTIR were most frequently used, followed by µ-Raman and FluoMic. Mass measurements were carried out in only three studies, two of which used pyrolysis gas chromatography-mass spectrometry (pyr-GC/MS) alone[21,22] and one which used both pyr-GC/MS and µ-FTIR[23].

2.4 Reported data on microplastics present in drinking water

2.4.1 Reported size and shape

Most of the authors reported the size range they applied for detecting microplastic particles. Figure 3 (logarithmic vertical scale) shows that the size ranges varied to quite some extent and that there was no obvious common approach for choosing them.

Figure 3. Particle size rages covered by the different studies[7–10,13–16,18–20,24,25,27]. Due to the wide variety of size ranges applied, vertical axis is logarithmic. Where the upper limit was reported as 'greater than', the bars have a thinned-out top end.



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The lower boundaries of the considered size ranges were between 1 and 500 µm, which mainly depended on the pore size of the filter used for filtration and/or the size limit of detection of the instrumental technique applied. The majority of studies used filters with cut-off values of 10 µm or more and consequently could provide no information about the presence of smaller microplastics. The upper size boundaries of reported particle size varied from 50 µm to 5 mm. However, some studies did not explicitly describe size ranges and in particular the upper limit but reported that the sizes of the found microplastics were above or below a specific size. Approximately half of the studies[7–9,12,13,15,16,20,21,24,25] reported the size of microplastics split up into size classes. A direct comparison of results is not possible, since each publication used different size classes in terms of number (from two to five) and in size range for each classes.

In all the reported studies, the analysed particles were collected by filtration and so the analysis data is representative of the fraction whose size is above the cut-off of the finest filter. However, the lack of harmonisation of reported size (ranges) makes it impossible to draw firm conclusions about the average size of the microplastics in DW.

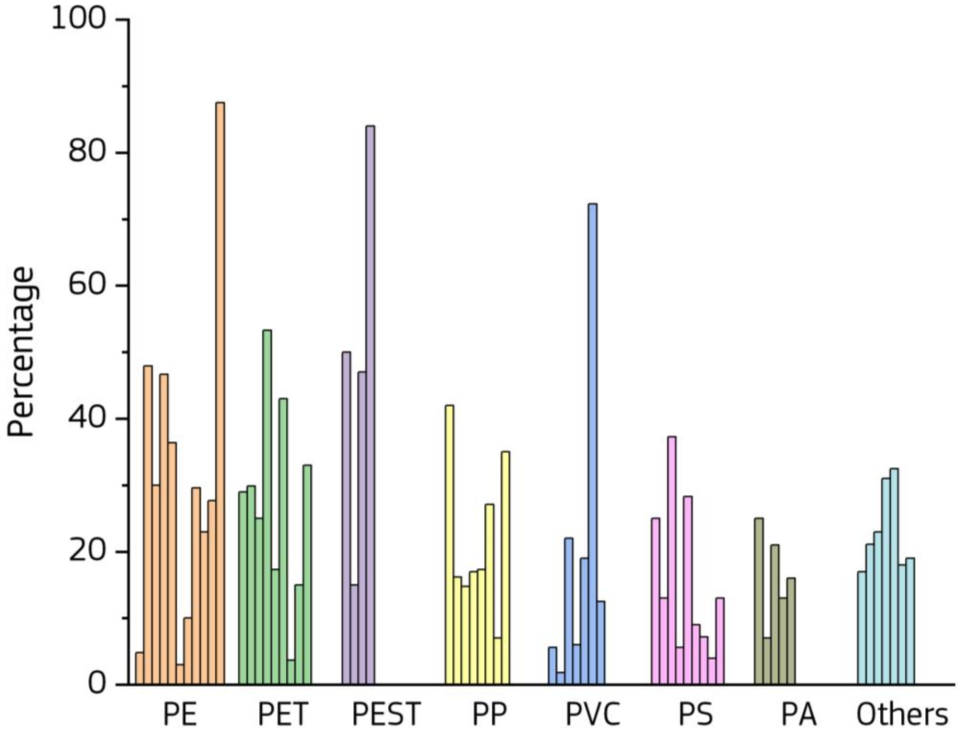
Regarding the particles' shape, over all the studies, particle counting showed the majority of microplastic particles in DW to be fibres (50%) or fragments (44%), while spherical particles (5%) and films (1%) were found infrequently.

2.4.2 Reported polymers

Several studies, using instrumental techniques able to identify the chemical composition of the microplastics, reported the type and the percentage of microplastics found in DW samples. In some cases, the studies presented the percentage of all the identified objects in the sample and in the blank, including the non-plastic particles. The main items reported in the studies that were classified as non-plastic consisted of cellulose, protein, cellophane, polybutadiene, epoxy resins, rubber and precipitated dyes. For the purpose of this analysis, the percentages of the objects were recalculated by considering only the plastic particles and excluding the other contaminants.

From Figure 4 it can be seen that polyethylene (PE), polyethylene terephthalate (PET), polyester other than PET (PEST) and polypropylene (PP) were the most frequently found polymers in DW samples. Other polymers detected were polystyrene (PS), polyvinylchloride (PVC) and polyamide (PA).

Figure 4. Percentage (particle number) of microplastics polymers found in DW samples. The columns show the percentage of specific polymer types within the total population of microplastics reported in the individual articles. For the acronyms, refer to the list of abbreviation (page 66) The sub-group “Others” includes acrylic compounds (AC), acrylonitrile butadiene styrene (ABS), copolymer polyethylene-polypropylene (co PE+PP), Styrene-Ethylene-Butadiene-Styrene (SEBS), polyacrylamide (PAM), polyurethane (PU), polyphenylene sulphide (PPS), poly(isoprene) (PI), polytetrafluoroethylene (PTFE), poly(methyl phenyl siloxane) (PMPS), polymethyl methacrylate (PMMA)



Source: own production

Evaluation of sample blanks, which is an indication of the level of background contamination, is dominated by the same polymers with PE, PP, PET and PEST making up 75% of the reported polymer contaminants.

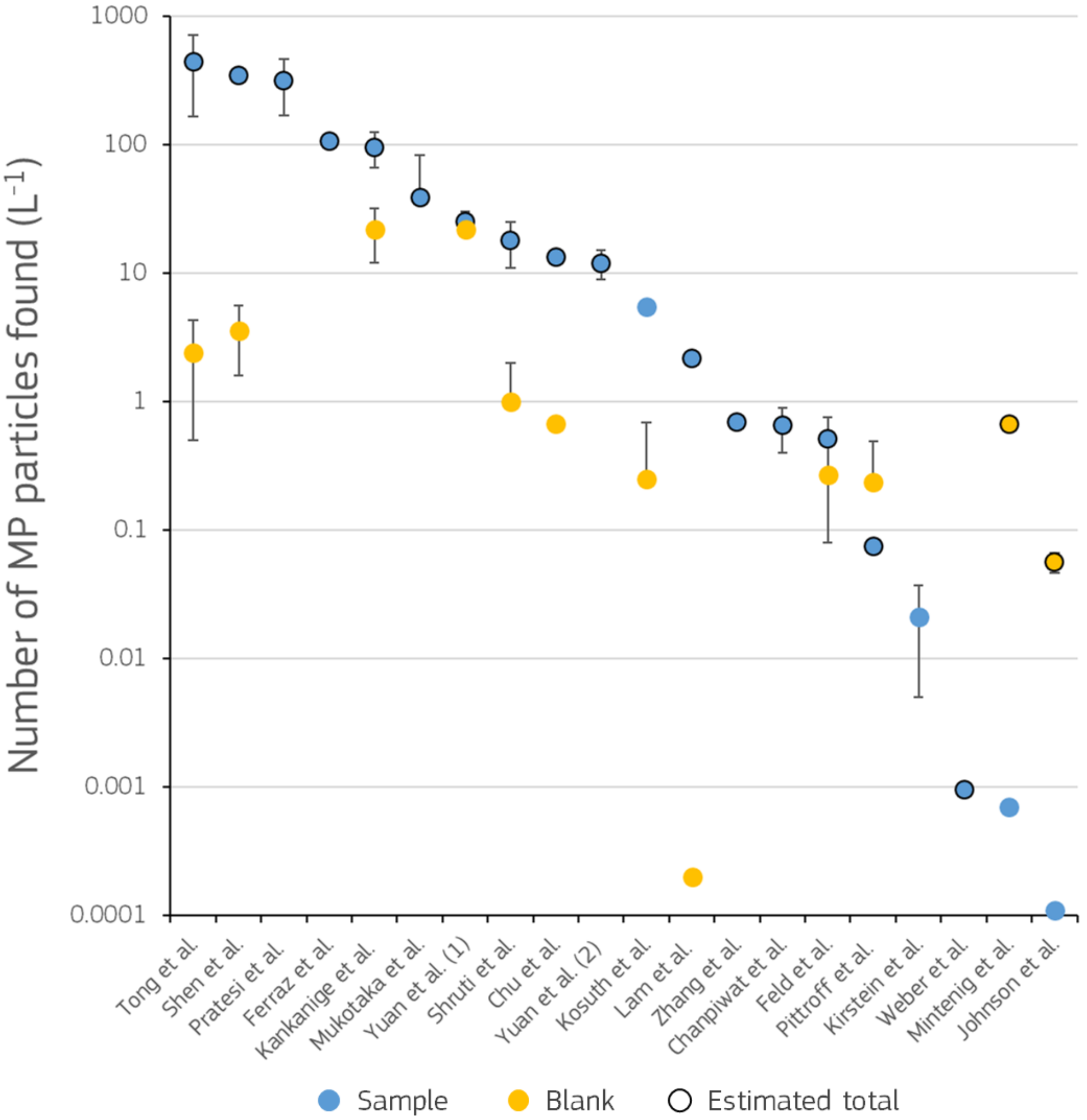
Some studies which had found particulates of PE, PVC or PA in DW also noted that these polymers were commonly used in the water pipes and fittings of the supply network being investigated. As none of the studies was able to identify conclusively the origin of the particles, it remains uncertain if the construction materials of water distribution network may be contributing to the presence of microplastics or if these were originally in the source water and avoided capture when passing though in the water treatment plant.

2.4.3 Reported number quantities

For the reporting of the number of microplastic particles, different units were used such as particles per litre, particles per cubic metre or particles per sample. To enable the comparison of the data produced in different studies, all number-based results were recalculated (normalised) to obtain data expressed as particles per litre. In the case where authors reported the number of microplastic particles separately for different size ranges or/and different polymers, all sub-results for one sample were summed. For studies reporting the number of particles per sampling site or at different times during the year, the average of the single results was calculated.

Figure 5 shows a comparison of the number-based study results (average of all study samples and blank values), normalised as described above. The amount of microplastic particles found in DW vary between 0.0001 and 440 particles per litre, which represents a difference of about 6 orders of magnitude between the highest and the lowest value. Such a huge variation between studies may be caused by differences in the sampling location (country and site of sampling, see paragraph 2.2), but also by procedural differences. The latter comprise the volume and type of sampling ('container sampling' or 'at-source filtering') and/or the applied detection technique. In addition, the quality assurance measures applied by the laboratory as well as variations in the measured background level and the approach on how to deal with it (whether or not subtracting it from the measurement results) might have been factors impacting the results.

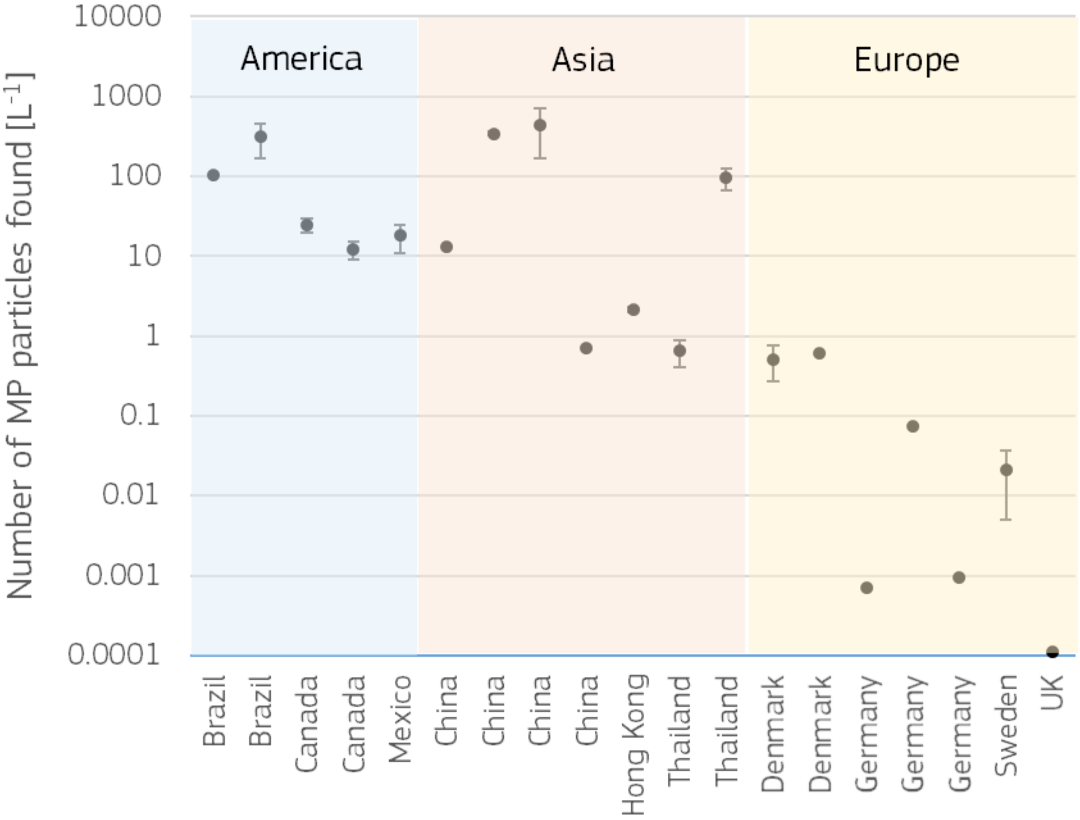
Figure 5. Number of microplastic (MP on vertical axis) particles found in samples (as mean) and blanks (where indicated in the study description); an estimated total (open circles) was calculated in cases where the study authors stated having subtracted the blank from the sample result and the blank value was reported. Due to the wide variety of size ranges applied, vertical axis is logarithmic. For Yuan et al., the data for the two different sampling procedures are shown separately: (1) container sampling; (2) at-source filtering.



Source: own production

The data from the studies focussing on specific countries (see Figure 6) suggest that on average the measured levels of microplastics present in DW seem to be lower in Europe (0.000-0.6 particles per litre) than in America (12-316 particles per litre) and Asia (0.7-440 particles per litre). For Europe, less than one microplastic particle per litre of DW was found – a result which was associated with higher sampling volumes (50-2250 litres) and sample collection by at-source filtering. Only one other study (Yuan et al.[6], Canada) applied the same type of sampling, while all other author groups used container collection which risks of higher background contamination (see also below).

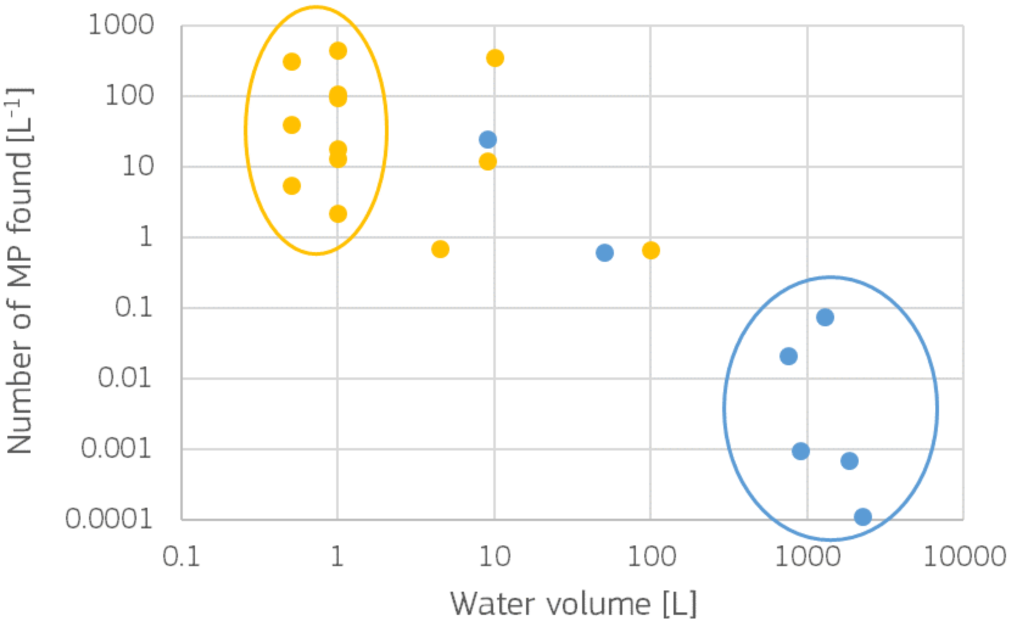
Figure 6. Measured microplastic (MP on vertical axis) number concentration (including error bars) by country and region as reported by the studies focussing on one country. Due to the wide variety of size ranges applied, vertical axis is logarithmic.



Source: own production

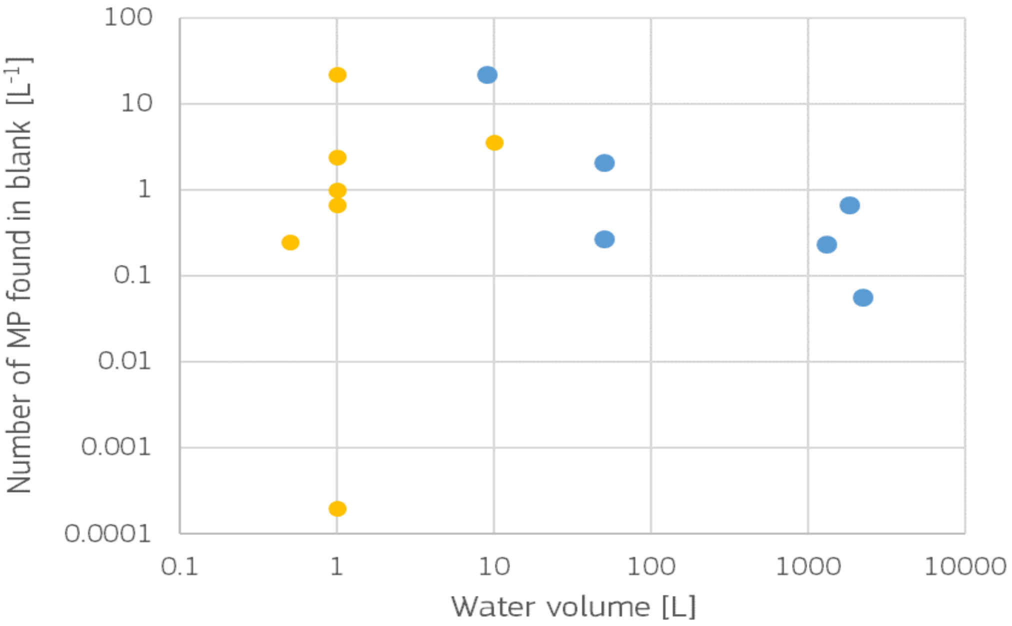
When plotting the number of microplastic particles found as a function of the volume of water sampled and the type of sampling (Figure 7), it looks like there is some sort of correlation. While a direct impact of the water volume seems unlikely, an influence of the sampling procedure is much more plausible, because at-source filtering is carried out in a closed system, while during container sampling the sample is – to some extent – exposed to ambient air (which may carry a certain load of microplastics). Accordingly, the measurement results for at-source filtering (blue dots in Figure 7) tend to be lower than for container sampling (yellow dots) across all studies.

Figure 7. Number concentration of microplastic particles (MP on vertical axis) reported in relation to the water volume filtrated and the applied sampling procedure. Container sampling (yellow dots); at-source filtering (blue dots). Due to the wide variety of size ranges applied, vertical axis is logarithmic.



Source: own production

Figure 8. Number concentration of microplastic particles (MP on vertical axis) found in blank samples (background level) in relation to the water volume filtrated and the applied sampling procedure. Container sampling (yellow dots); at-source filtering (blue dots). Due to the wide variety of size ranges applied, vertical axis is logarithmic.



Source: own production

It can be assumed that contamination during sample processing – even though varying quite substantially across studies (see Figure 8) – is generally independent from the sampled volume. Under this assumption, it would seem likely that concentration values determined from the analysis of small-volume samples are dominated by the presence of microplastic contaminants introduced during processing/analysis rather than the number of microplastics originally in the DW. To reduce this effect, sampling volumes should be as high as practically feasible. As it will be noted later (see section 4), this volume range (or even a higher one) is already a recommendation in the ASTM standard D8332-20[28] on sampling water for microplastics analysis (1500 L).

Consequently, the low level of microplastics expected in European DW suggests that to reduce analytical uncertainty and the impact of background contamination during sample processing (see below), a high sample volume should be analysed, probably around 1000 litres.

2.4.4 Reported mass quantities

The three studies[21–23] in which mass-based measurements were carried out reported plastic contents of the analysed water of 0.52 ng/L, 6.1 ng/L and 7.2 µg/L. The range of variation in the measurement results is less pronounced than in the case of number concentrations, but with four orders of magnitude it is still very high.

2.4.5 Reported background/contamination levels

The level of background contamination was reported for 13 out of 21 studies that produced number-based results. The (normalised) levels of contamination were reported to be between 0.0002 and 22 particles per litre (see Figure 5, yellow dots); the majority (70%) of author groups stated to have a background between 0.1 and 10 particles per litre.

For mass-based results, only one out of three studies reported a blank value, even though all author groups stated that they analysed (procedural or field) blanks. Kirstein et al.[23] calculated estimates of mass values from particle number-size data. The estimated mass in the blanks ranged from 1-784 ng depending on the water supply line sampled. All other author groups, which produced mass-based results, reported their limits of detection (LOD) for the different polymers covered by their method being 1 µg[21], 0.5-1.1 µg[22] and 1.2-5.2 µg[23], respectively.

2.5 Key points from literature reports of microplastics in drinking water

The examination of the literature has provided a valuable insight into the current knowledge about the occurrence of microplastics in DW as well as the sampling and analytical requirements that will have to be considered in establishing a methodology which, within the limitations of current technology, can best satisfy the needs of monitoring.

- With the exception of a few studies in China, Thailand and Brazil particle number concentrations are low with results being at most a few tens of particles per litre. In particular, studies in European countries resulted in levels, which were a further 1-3 orders of magnitude lower than in the other countries/regions on a particle-per-litre basis (below 1 particle per litre).
- Most studies used microscopy and spectro-microscopy-based methods and thus report microplastic particle numbers. Only 10% of reports provided particle mass by thermo-analytical techniques.

- Particle counting techniques used were equally divided between the ones using chemically specific spectroscopy, which could positively identify a particle as being a polymer, (µ-FTIR 28% and µ-Raman 21% of the studies) as well as non-chemically specific techniques (OpMic 7% and FluoMic 17%).
- Particle counting studies using larger water volumes (>50 litres) and filtration directly from the water supply (at-source filtering) generally reported lower calculated particle concentrations (<1 particle/litre), with values generally being close to background levels.
- Sampling volumes should be as high as practically feasible, probably around 1000 litres, to reduce the analytical uncertainty and the impact of background contamination during sample processing.
- It was found that seven common synthetic polymers (PE, PET, PEST, PP, PS, PVC and PA) make up close to 90% of the microplastics found in DW.
- The classification of microplastics shapes showed that predominantly fibres and fragments were found.
- Although the majority of studies used techniques based on microscopy rather than thermal analysis, very little information on particles size distributions of the polymer particles was available. Most studies reported only total particle numbers or particle numbers across a few broad size bins. Similarly, there was no information regarding the size distribution of different polymer types.

3 Analytical techniques tested in inter-laboratory comparisons or validation exercises

The scientific literature contains many publications, which report the analyses of microplastics in a wide range of different matrices using methodologies relying on the three most common classes of techniques (optical microscopy, vibrational spectroscopy and thermo-analytical techniques). Unfortunately, only a few of these studies have undertaken a systematic evaluation or validation of the techniques despite the importance of such activities in achieving reproducible and reliable data being highlighted by the scientific community.[29] Such studies provide an important insight into the capabilities and limitations of analytical procedures and the related instrumental techniques themselves. In the literature, 6 inter-laboratory studies[30–35] were identified which evaluated techniques for microplastics in aqueous media but only the three examples described below (paragraphs 3.1, 3.2 and 3.3) were judged to be particularly relevant to the monitoring of microplastics in DW.

There are a number of other such initiatives planned in the future within ongoing projects, such as those in the Horizon 2020 research cluster CUSP[36] comprising five microplastic projects. Unfortunately, the outcomes from these initiatives may not be available before the conclusion of the projects (4 projects will end 31st March 2025 and one 31st March 2026) and therefore beyond the time frame set by the DWD for the identification of a methodology.

3.1 JRC/BAM inter-laboratory comparison study on PET in water

In 2021, an inter-laboratory comparison (ILC) study[32] on sample containing PET particles in (clean) water was carried out by the JRC in collaboration with the German Federal Institute for Materials Research and Testing (BAM) with nearly 100 participating laboratories from 28 countries. It was left to the participants to choose the analysis method, including whether to identify the polymer of the particles found and whether to determine particle number or/and mass. In addition to the measurement results, information was collected on the practices applied for sample treatment, measurement conditions, instrument settings, data analysis strategies and so on, including strategies to prevent contamination during sample processing. 98 datasets could be used for evaluation and provided a comprehensive overview of the state of the art of microplastic analysis in water and in particular the quantification of the PET particles flake-shaped fragments present in the samples.[37]

The study participants used a variety of different analysis techniques. For identification and quantification of the PET particles present in the samples, predominantly µ-FTIR was used (37 out of 62 laboratories) followed by µ-Raman (15) and, for mass measurements, pyr-GC/MS (9). Simpler techniques (OpMic, FluoMic, gravimetric analysis) were employed as well, but only to determine the number of 'plastic' particles (without verifying that it is indeed PET) or all particles. In addition, emerging or less frequently described techniques, such as LDIR, NMR and HPLC, the latter only being applicable for hydrolysable polymers like PET, were used.

The measurement results in this study scattered almost evenly over the range of reported values of about three orders of magnitude. This applies to both, number- and mass-based results as well as across the different analysis techniques employed.

While the majority of µ-FTIR and µ-Raman users obtained PET number concentrations that fell within the indicative range of expected results, no conclusions about the effect of measurement parameters on polymer identification performance could be drawn, as the identity of the material (PET) was known to the participants. In the case of the thermo-analytical techniques, the few results that were submitted indicate that GC/MS after pyrolysis, with or without an intermediate sorption-desorption step, is a suitable technique for the identification and the quantification of PET in aqueous matrices. However, the analysis of methodological details (contamination prevention, sample treatment, measurement conditions, instrument set-up, parameter settings etc.) showed that a huge variety of conditions were applied and thus there is ample room for harmonisation. This correlates with statements made elsewhere[33,38–40] about the further need for harmonisation and standardisation in the field.

Relevance to DWD methodologies: Even thought this study was not specifically designed for the investigation of microplastics in DW, it shows that even with an 'easy' sample, containing clean water as matrix without any intentionally added non-polymeric materials and for which the identity of particles (PET) was known, the scatter of measurement results was still very high. This low inter-laboratory reproducibility was largely independent of the analysis technique applied and was also observed when only individual techniques such as µ-FTIR, µ-Raman or pyr-GC/MS were considered. This is a severe problem for the comparability of measurement results generated by different laboratories. However, specific aspects of the general laboratory practices, sample treatment, measurement conditions or data analysis contributing to this outcome of the study could not be identified and, thus, no recommendations could be provided for future harmonisation.

3.2 Southern California Coastal Water Research Project (SCCWRP) inter-laboratory comparison study

This study was undertaken by the Southern California Coastal Water Research Project (SCCWRP)[30,41] to support California Senate Bill 1422, which requires the development of State-approved standardized methods for quantifying and characterizing microplastics in DW. Accordingly, an inter-laboratory microplastic method evaluation study was carried out with 22 participating laboratories from six countries, to evaluate the performance of the commonly used techniques: optical microscopy, Fourier-transform infrared spectro-microscopy (µ-FTIR), and Raman spectro-microscopy (µ-Raman). The study did not contemplate the use of the pyrolysis techniques for the analysis of the samples. An important additional aspect of the study was that the participants also provided information regarding the time required in both sample treatment and analysis.

Three samples of simulated clean water (1 µm filtered deionized water) spiked with microplastic particles and a laboratory blank were sent to each laboratory with a prescribed standard operating procedure for particle extraction, quantification, and characterization. The samples contained known amounts of microparticles over four size fractions (1–20 µm, 20–212 µm, 212–500 µm, >500 µm), four polymer types (PE, PS, PVC, and PET), three shapes (fragments, spheres and fibres) and six colours (clear, white, green, blue, red, and orange). The total particle number per sample was 609 (±132) of which 249 (±60) were in the range above 20 µm. The samples also included around 80 false positives (natural hair, fibres, and shells) as example of particles which can be mistaken for microplastics. Particles were collected via filtering/sieving and the subsequent analysis included optical microscopy, which was used to count all particles above 20 µm with characterisation of size, shape and colour. Chemical characterisation by µ-FTIR or µ-Raman was done on a sub-sample of the collected particles.

Among the participating laboratories, mean particle recovery determined using stereomicroscopy was 76% ± 10% (standard error, SE). For particles in the three largest size fractions, mean recovery was 92% ± 12% (standard deviation, SD). On average, laboratory contamination from blank samples was 91 particles (± 141 particles SD). µ-FTIR and µ-Raman accurately identified microplastics by polymer type for 95% and 91% of analysed particles, respectively. Per particle, µ-FTIR required the longest time for analysis (12 min ± 9 min SD). Participants demonstrated good recovery for particles greater than 50 µm and excellent chemical identification for particles greater than 20 µm in size with notable increases in accuracy and precision being possible following training and further method refinement. This work has formed the basis for the methods being applied for microplastics monitoring in DW in the State of California.

Relevance to DWD methodologies: This study was specifically designed to evaluate methods applicable for the monitoring of DW and has utilised sample spikes, which were heterogeneous in composition, density, colour, size and shapes. Furthermore, the inclusion of non-plastic materials which could result in possible false positives was an additional and relevant complicating factor which would be expected to occur in real-life samples. The number and size ranges of the microplastics used were realistic considering what could be expected to be recovered by filtration from larger volume sampling of DW.

Method performance was highly dependent on particle size, with good recovery for particles >50 µm. Both µ-FTIR and µ-Raman were effective at identifying microplastic particles and differentiating them from non-plastics but there were performance differences based on particle size. µ-FTIR could only identify particle polymer types in size fractions above 20 µm whereas µ-Raman could do so for particles in size fractions as low as 3 µm which were the smallest size particles in the trial. This size issue could be of concern for monitoring DW systems since water treatment plants has shown to be effective only in filtering particles above 20 µm[42] and the primary target size relevant to health may be particles smaller than this size.

It was concluded that both µ-Raman and µ-FTIR were accurate (>90%) in determining the nature and number of particles in the size fraction above 20 µm. When applied to particles in range 1-20 µm the method using µ-FTIR showed an important decrease in accuracy and recovery to around 30%. In contrast, the method applying µ-Raman could maintain a good level of accuracy down to 1 µm. An important additional outcome of the study was information about the realistic time required for sample analysis based on the experience of the participant laboratories. It was found that average analysis time per sample was 10 hours for µ-FTIR and 19 hours for µ-Raman. It was noted that time and time-related costs for all stages of microplastic sample analysis are currently barriers to routine, effective monitoring. Overcoming barriers will require new approaches for automation and incorporation of tiered monitoring that uses less expensive methodologies for screening level questions.

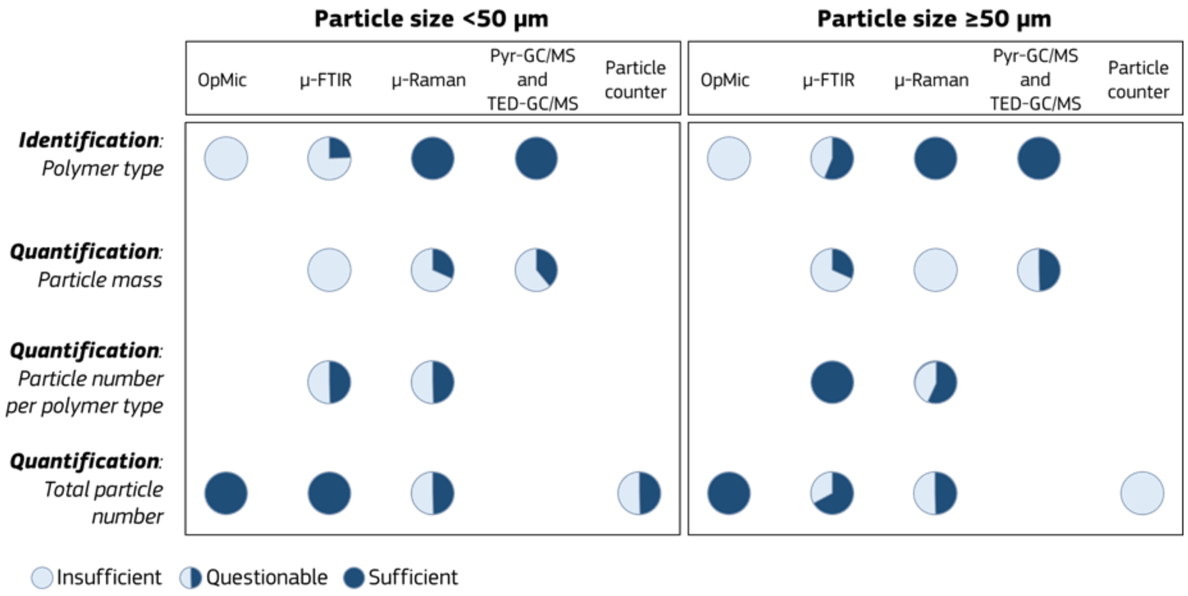
3.3 German Centre for Water Technology study for microplastics analysis in water samples

This comparative study by Müller et al.[31] from the German Centre for Water Technology (Technologie Zentrum Wasser, TZW) served as a first step to assess the suitability and comparability of the most frequently used analytical techniques in microplastic research - µ-FTIR, µ-Raman and thermal decomposition techniques. Samples comprised up to five different types of microplastic particles (PMMA, PVC, PET, PE, PS) with diameters ranging from 8 µm to 140 µm suspended in ultrapure water. The participants were supplied with two distinct sample types to examine – one with a large-size fraction (≥50 µm) and moderate particle number (≈100-900 particles) of each type of polymer and one with a small-size fraction (<50 µm) and large number of particles (2000-11000) from three specific polymer types (PMMA, PS, PE). In comparison with the SCCWRP study (section 3.2), the samples did not contain any non-plastic materials, which could give a false positive response. No specific instructions were given to the participants and no factors were evaluated except the analytical techniques. Results from 17 laboratories from eight different countries were compared. Microscopy, Fourier-transform infrared spectro-microscopy (μ-FTIR), Raman spectro-microscopy (μ-Raman), thermal-extraction-and-desorption or pyrolysis combined with gas chromatography coupled to mass spectrometry (TED-GC/MS and Pyr-GC/MS, respectively), scanning electron microscopy and the use of a particle counter were compared for total particle number, polymer type, number of particles and/or particle mass for each polymer type.

Relevance to DWD methodologies: The organizers of the study have evaluated the results based on three features. First, identification of the polymer type, which describes the ability of a method to correctly determine the polymer type of a particle. Second, quantification of mass, which describes the relative deviation of a result from the theoretical mass content of microplastic particles in the sample. Third, quantification of total particle number and detailed particle number per polymer type.

The overall outcome is summarised in Figure 9.

Figure 9 Comparison of techniques performance. Results of technique comparison for identification of polymer type, quantification of particle mass, quantification of particle number per polymer type and quantification of total particle number. The average evaluation of each technique is indicated in the figure as insufficient (light blue), sufficient (dark blue) and questionable (both colours in different percentage). For acronyms see the relative list (page 66).



Source: own production

Identification of polymer type worked best for μ-Raman and pyr-GC/MS for both size fractions but is questionable for μ-FTIR. Optical microscopy was effective in determining particle number in both size fractions but was not capable of distinguishing polymer types. The quantification of polymer mass for identified polymer types was evaluated as questionable for pyr-GC/MS and TED-GC/MS while the other techniques clearly failed to determine the correct polymer mass. Quantification of particle number per identified polymer type was evaluated successful for μ-FTIR in the large size fraction (≥50 μm) and questionable in the small size fraction (<50 μm). In the case of μ-Raman it was evaluated to be questionable for both size fractions. The quantification of total particle numbers with μ-FTIR was judged sufficient in both sizes with better overall performance being found for the small fraction. μ-Raman yielded questionable results in both size fractions. Finally, probably the most important result for DW, the quantification of particle numbers and type showed μ-FTIR to be acceptable only for the larger size fraction while for the smaller fraction it was judged questionable. Surprisingly, considering the good performance in identifying particle polymer type, µ-Raman was judged to be questionable in both size ranges but this may be influenced by effects in the trial which are not related to the intrinsic capabilities of the technique.

Overall, the suitability of a method strongly relates to the research question. µ-Raman for instance is a highly sensitive, but comparatively slow analytical technique, which usually requires an extrapolation of measured particles as only a subarea of the filter area is analysed. µ-FTIR on the other hand might discriminate against small particles (< 20 µm) but is capable of measuring more particles in the same time period and thus may not require any or a smaller extrapolation factor. This could be one reason for the rather good results of µ-FTIR for total particle numbers and only mediocre results for µ-Raman for both size fractions.

4 Current activities in international standardisation

It is widely acknowledged that harmonization and standardization of analytical methods is a key requirement for the field of microplastics and there are currently a number of activities by standardization bodies or similar entities around the globe. In the following sections, the current status on these activities is summarised.

4.1 ISO activities relating to microplastics

For the requirements of the DWD it would be advantageous to have the option of recommending a method or a set of methods which have already been through a rigorous international standardization procedure.

In recent years, efforts have been made to develop international standards in the area of microplastics and a number of projects were put on the work programme of the International Organization for Standardization (ISO). A first output of this work was a report entitled "Plastics – Environmental aspects – State of knowledge and methodology" (ISO/TR 21960:2020[43]) and a subsequent standard on "Principles for the analysis of microplastics present in the environment" (ISO 24187:2023)[44].

Besides such general guidelines, three projects[45–47] have recently been included in the ISO work programme that are relevant for the analysis of microplastics in drinking water (working titles as of February 2024):

> Water quality — Analysis of microplastic in water — Part 1: General and sampling for waters with low content of suspended solids including drinking water (ISO 16094-1)[45]
>
> Water quality — Analysis of microplastic in water — Part 2: Vibrational spectroscopy methods for waters with low content of suspended solids including drinking water (ISO 16094-2)[46]
>
> Water quality — Analysis of microplastic in water — Part 3: Thermo-analytical methods for waters with low content of suspended solids including drinking water (ISO 16094-3)[47]

These standards are expected to be published by mid-2025 as part of a series of documents that may be extended by additional standards if needed.

Already earlier, ISO started a series of standards on the collection of water samples from all kinds of sources, of which some are of importance to drinking water:

> ISO 5667-1:2023 Water quality – Sampling – Part 1: Guidance on the design of sampling programmes and sampling techniques[48]
>
> ISO 5667-3:2018 Water quality – Sampling – Preservation and handling of water samples[49]
>
> ISO 5667-5:2006 Water quality – Sampling – Guidance on sampling of drinking water from treatment works and piped distribution systems[50]

It is planned to amend this series of standards by a new document focussing specifically on microplastics:

> Water quality — Sampling — Part 27: Guidance on sampling for microplastics in water (ISO 5667-27, working title as of February 2024)[51]

A number of other ISO standards have been issued or are in preparation, which focus on the collection and analysis of microplastic fibres that are shed from textiles and released into the wash water during laundering and therefore have less relevance for the determination of microplastics in DW.

4.2 CEN activities relating to microplastics

At the European level, the European Committee for Standardization (CEN) is following selected projects run at ISO to be prepared to incorporate, where relevant, published ISO standards into the European scheme of standards via the so-called Vienna agreement ([a]).

In this context, a number of ISO standards relevant to microplastics have been transposed into European standards:

EN ISO 24187:2023

EN ISO 5667-1:2023

EN ISO 5667-3:2018

Likewise, the methodological draft standards of ISO mentioned in paragraph 4.1 have been taken up into the CEN work programme:

prEN ISO 16094-1

prEN ISO 16094-2

prEN ISO 16094-3

4.3 ASTM activities relating to microplastics

At this time, there are no ASTM standards or test guideline specifically designed for the detection and quantification of microplastics in DW but there is relevant documentation available regarding the sampling and sample treatment of a range of water types for subsequent analysis for microplastics, *i.e.*

ASTM D8332-20: Standard Practice for Collection Of Water Samples With High, Medium, Or Low Suspended Solids For Identification And Quantification Of Microplastic Particles And Fibers[28]

ASTM D8333-20: Standard Practice for Preparation of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers Using Raman Spectroscopy, IR Spectroscopy, or Pyrolysis-GC/MS[52]

These standards provide for the collection of water samples and the sample treatment for the determination and characterisation of microplastic particles and fibres. The procedures have been designed for the collection of samples from DW, surface waters, influent and effluent (secondary and tertiary) of wastewater treatment plants, and marine waters. While the procedures are not limited to these particular types of water, the applicability to other aqueous matrices must be demonstrated.

([a]) AGREEMENT ON TECHNICAL CO-OPERATION BETWEEN ISO AND CEN (Vienna Agreement), https://boss.cen.eu/media/CEN/ref/vienna_agreement.pdf

The sample collection procedure D8332-20 describes in detail the use of a filter cascade to collect particulates from DW using an apparatus which is open to atmosphere and thus risks significant contamination from the surrounding environment. For DW applications where microplastics levels are normally very low, a closed filtration cascade is preferable to reduce contact with the local atmosphere to minimise airborne contamination.

The sample preparation procedure D8333-20 covers the sample preparation of collected water samples by an oxidation with hydrogen peroxide and enzymatic digestion to remove interfering organic constituents. Such steps can be useful if the water to be investigated is known to have a high load of such compounds, but should normally not be necessary for DW.

An additional ASTM standard describes the preparation of reference samples that can be used to support the evaluation of microplastic detection and imaging techniques for a variety of matrices including DW:

> ASTM D8402-23: Standard Practice for Development of Microplastic Reference Samples for Calibration and Proficiency Evaluation in All Types of Water Matrices with High to Low Levels of Suspended Solids[53]

4.4 National standardization bodies in Europe activities relating to microplastics

The German national standardization organization (DIN) has issued Technical Specifications for the analysis of microplastics in food:

> DIN/TS 10068:2022-09 Food - Determination of microplastics - Analytical methods[54]

This document explicitly excludes the application to tap water from its scope and thus has limited relevance for DW analysis. DIN has also provide a German translation of the ISO standard 24187, with title: Grundsätze für die Analyse von Mikroplastik in der Umwelt (DIN EN ISO 24187:2024-04).[55]

The French national standardisation organisation (AFNOR) is developing a standard method for analysing microplastics in water with low solids content such as domestic DW, groundwater or commercially available bottled water (title translated into English):

> Water quality – Analysis of microplastics in water for human consumption and groundwater - Part 1: Method using vibrational spectroscopy (PR NF T90-600)[56]

This method uses vibrational spectroscopy to identify and count particulates and forms the basis of the previously mentioned ISO project 16094-2 (see above). The timeline of the AFNOR project is not known. It is understood that the eventual availability of an equivalent ISO standard would supersede the French standard.

4.5 VAMAS activities relating to microplastics

Versailles Project on Advanced Materials and Standards (VAMAS) supports world trade in products dependent on advanced materials technologies, through International collaborative projects aimed at providing the technical basis for harmonized measurements, testing, specifications, and standards. VAMAS created a technical working area (TWA) on Micro and Nano Plastics in the Environment.[57] In December 2022, the TWA launched an inter-laboratory comparison study, which had some relevance to DW, by using a well-defined mass of microplastics embedded in pressed pills. At this time, however, there has not been made public any output from this activity.

4.6 Current status of standardized methods applicable to the DWD

At this time, none of the principle international standardisation bodies has published relevant standards which may be used directly or which can be adapted to the need of establishing a methodology for implementing article 13(6) of the DWD. As detailed previously in section 4.1, ISO initiated three projects relevant to DW in mid-2022. However, with a planned duration of three years, these may not deliver before mid-2025. Moreover, if ILC activities are undertaken as part of these projects, the duration may be further extended.

5 Microplastic definitions, descriptors and metrics

The DWD does not provide a definition of microplastics. However, the specification of a measurement methodology must inevitably contain at least a description of what it has to be measured and descriptors (properties) to be reported as output from any monitoring program. In addition, it will be necessary to establish a metric for the reporting. Such a metric would ideally be the most appropriate to risk assessment but may, in practice, be primarily a consequence of the analytical technique applied for detection and quantification.

The scientific community has still to reliably demonstrate the existence of any specific hazards posed to humans by ingestion of microplastics. Consequently, it is not yet known exactly what are the most relevant properties to be reported as an outcome of the measurement procedure used in a monitoring program for DW. Despite the limited knowledge about effects and risks it will be necessary to consider the key descriptors and the following sections will outline key points from existing definitions.

5.1 Microplastic definitions

While it is very challenging to develop a comprehensive definition for microplastics, it is desirable to maintain consistency across EU legislation. Where possible and appropriate, it would be logical to give priority to the use or adaption of descriptors and terminology available in existing EU legislation or foreseen legislation which is at an advanced stage of development. Where this is not available or not appropriate, alignment with other national or international legislation or standardisation documents should be considered.

5.1.1 REACH restriction

The definition of a microplastic, which has been developed for the REACH restriction[4], is probably the most advanced and comprehensive in use at this time and contains many elements, which may be used directly or suitably adapted for the implementation of the DWD. The most important aspects of this definition are quoted below.

Microplastic means particles containing solid polymer, to which additives or other substances may have been added, and where the particles fulfil the following:[4]

> All *dimensions* of the particles are equal to or less than 5 mm or the length of the particles is equal to or less than 15 mm and their length to diameter ratio is greater than 3.
>
> Particles *containing solid polymer* means either (i) particles of any composition with a continuous solid polymer surface coating of any thickness or (ii) particles of any composition with a solid polymer content of ≥ 1% by weight.
>
> *Polymer* means a substance consisting of molecules characterised by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. ([b])

([b]) see no. 5 in article 3 of the REACH Regulation (EC) No. 1907/2006.

It is to be noted that the definition of the REACH restriction does not specify a lower size dimension but stipulates that single molecules are not particles. Instead, the regulation sets a pragmatic size of 0.1 µm (0.3 µm for fibres) to be the lower size limit of microplastics for the purpose of enforcing the restriction under the condition that a quantification cannot be done by available analytical methods or accompanying documentation. Another aspect of the proposed legislation is the exclusion of certain specific categories of polymers:

> *Natural polymers* – polymers that are the result of a polymerisation process that has taken place in nature and where these polymers have not been chemically modified.
>
> *Biodegradable polymers* – polymers that are (bio)degradable in accordance with tests specified in the text of the restriction.
>
> *Water soluble polymers* – polymers that have a solubility in water greater than 2 g/L as determined according the tests specified in the text of the restriction (referring to OECD Test Guidelines 105[58] and 120[59]).
>
> *Inorganic polymers* – polymers that do not contain carbon atoms in their chemical structure.

5.1.2 Marine Strategy Framework Directive (MSFD)

The Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC)[60] sets "Qualitative descriptors for determining good environmental status" (Annex I of the Directive). Descriptor no. 10 is that "Properties and quantities of marine litter do not cause harm to the coastal and marine environment". The annex in Commission Decision 2017/848[61] (Descriptor 10) sets criteria among which is micro-sized litter: "Micro-litter (particles < 5 mm), classified in the categories 'artificial polymer materials' and 'other'". The term 'marine litter' has been further described for the purpose of the MSFD as "any persistent, manufactured or processed solid material that is discarded, disposed of, or abandoned in the marine and coastal environment" in the MSFD Task Group 10 Report of April 2010 (JRC58104[62]).

In the context of the MSFD, microplastics, even though not explicitly referred to as such, are considered as a specific type of litter in the form of solid and persistent particles below a size of 5 mm (largest dimension) made of artificial polymer materials. No lower size limit is considered, but for monitoring the lowest size is normally determined by the sampling procedure, including capturing device (usually some sort of net). The European MFSD Working Group on Good Environmental Status, in collaboration with JRC, has published a "Guidance on Monitoring of Marine Litter in European Seas" (JRC83985)[63] which, for generic microlitter, recommends using an upper size limit of 5 mm for the largest dimension of the particles even though this is not explicitly stated in Commission Decision 2017/848[61]. Additionally, it divides plastic litter into four dimension classes based on biological relevance and analytical limitations: macroplastics (>25 mm), mesoplastics (5 to 25 mm), large microplastics (1 to 5 mm), and small microplastics (20 µm to 1 mm). With reference to the two classes of microplastics the authors rationalize separating them into two sub-fractions (small and large) due to the relative ease of separating and quantifying visually recognizable 1-5 mm particles compared to the more technically challenging aspects of particles between 20 µm and 1 mm in size. The upper limit of 5 mm is also consistent with the upper limit of micro-litter (including microplastics) specified in the more recent Commission Decision (EU) 2017/848[61] used for the implementation of the MSFD[60] of the European Parliament and of the Council of 17 June 2008.

Regarding the measurement of micro-litter (including microplastics), the Commission Decision 2017/848 stipulates that it shall "be monitored in the surface layer of the water column" and that the units of measurement shall be “in number of items and weight in grams (g) per square metre (m²) for surface layer of the water column [...]”.

5.1.3 ISO

ISO has recently started to provide definitions relating to microplastics (see ISO/TR 21960:2020[43]):

> *Microplastic* – any solid plastic particle insoluble in water with any dimension between 1 µm and 1000 µm.
>
> *Large microplastic* – any solid plastic particle insoluble in water with any dimension between 1 mm and 5 mm.
>
> *Nanoplastic* – plastic particles smaller than 1 µm.

This way, ISO has chosen to split up ‘microplastics’ into two size classes (1 µm – 1 mm and 1 mm – 5 mm) instead of the more commonly used single range with an upper boundary of 5 mm.[64] For the ISO definition of ‘microplastic’ it is explicitly mentioned that rubber is not included and that the defined dimension is related to the longest dimension of the particle (but strangely not for ‘large microplastics’ and ‘nanoplastics’ even though it can be assumed that the same considerations should apply). Like this, there are a number of differences between the ISO terminology and that of some other pre-existing practices by national (NOAA)[64], international bodies such as the United Nations[65] and the European Commission[61] and the REACH restriction (see paragraph 5.1.1).

Further related terms are defined with reference to the general ISO standard on Plastics Vocabulary (ISO 472:2013)[66] in the same document as follows:

> *Polymer* – chemical compound or mixture of compounds consisting of repeating structural units created through polymerization and in practice above 10,000 Dalton. Polymers comprise both plastics and elastomers.
>
> *Plastic* – material which contains as an essential ingredient a high polymer and which, at some stage in its processing into finished products, can be shaped by flow. Plastics consist mainly polymers and minor contents of additives. Plastics comprise both thermoplastic and thermoset materials (but not elastomers like rubber).
>
> *Thermoplastic* – plastic that has thermoplastic properties.
>
> *Thermoset* – plastic which, when cured by heat or other means, changes into a substantially infusible and insoluble product.
>
> *Elastomer* – macromolecular material which returns rapidly to its initial dimensions and shape after substantial deformation by a weak stress and release of the stress (examples of elastomers are rubber and synthetic rubber).

By defining ‘large microplastics’, ‘microplastics’ and ‘nanoplastics’ as *plastic* particles, these definitions exclude elastomeric polymers and consequently would not consider particulates of rubber resulting from vehicle tyre wear – a major component in the total quantity of man-made polymer particles released to the environment.

5.1.4 Legislative by State of California

For the purpose of testing DW for microplastics in the United States - State of Southern California – the following definition was adopted[67]:

> *"Microplastics in Drinking Water' are defined as solid polymeric materials to which chemical additives or other substances may have been added, which are particles which have at least three dimensions that are greater than 1 nm and less than 5,000 micrometers (µm). Polymers that are derived in nature that have not been chemically modified (other than by hydrolysis) are excluded."*

In this context, 'polymeric material' means either (i) a particle of any composition with a continuous polymer surface coating of any thickness, or (ii) a particle of any composition with a synthetic polymer content of greater than or equal to 1% by mass. 'Particle' means a minute piece of matter with defined physical boundaries; a defined physical boundary is an interface. 'Polymer' means a substance consisting of molecules characterized by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. A polymer comprises the following: (a) a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant.

5.2 Descriptors for microplastics

Microplastics monitoring under the DWD should allow the production of inter-comparable data about the microplastics effectively present in DW in the EU Member States. To achieve this, relevant descriptors of microplastics in DW must be established. The following sub-sections outline the most commonly cited descriptors for microplastics with consideration being given to their possible relevance to any interactions with biological systems.

5.2.1 Polymer type

At this time, there is no clear evidence about whether the type of polymer, which a microplastic particle is composed of, has an influence on any adverse biological response. However, knowledge of the polymer type(s) of microplastic particles present in DW may have relevance in identifying the origin of microplastic contamination in the supply chain and verifying effects of any remedial action. In addition, some types of plastic tend to contain additives more harmful than plastics.

Considering the results of the literature review regarding the types of polymers most often found in DW (see paragraph 2.4.2), the following types seem to be of highest relevance: polyethylene (PE), polyethylene terephthalate (PET), polyester other than PET (PEST) and polypropylene (PP).

Biodegradability

In the definition of microplastics developed for the REACH restriction (see paragraph 5.1.1), biodegradable plastics are specifically excluded as the objective of this action is to reduce the release to the environment of persistent polymers, whose lifetime is measured in years or decades rather than in months as in the case of biodegradable polymers. In the DWD the exclusion of such materials in monitoring is probably not justified as their ability to break-down more rapidly does not necessarily reduce the risk of provoking undesirable biological responses when ingested. In fact, there is little information to indicate whether man-made biodegradable polymer particles are more or less harmful than persistent plastics when ingested nor is their *in-vivo* degradation behaviour known. In the absence of relevant toxicological data the application of the precautionary principle would justify also reporting such polymers once identified in DW.

Solubility

Solubility, although a factor considered in relation to the REACH restriction, is probably not a relevant factor for microplastics in DW. Water soluble polymers, assuming the normal practice of sampling by filtration, should not normally be recovered from the DW supply network, however it should be noted that such polymers may interact with other chemical species in the water to form insoluble composite particles. In the case that such particulates are identified they should be included in the reported data.

5.2.2 Particle size

The size range which defines a microplastic is first to be considered in terms of the upper size limit and in this it would be advantageous to harmonise it with existing legislation and/or commonly accepted scientific conventions. On this basis, it would be reasonable to consider microplastics in DW to be the particles that have all dimensions less than 5 mm or 15 mm if the length-to-diameter ratio is above 3, as applied by the relevant REACH restriction (see paragraph 5.1.1).

In contrast to the relative simplicity of establishing a suitable upper size, there remains much discussion regarding a lower size limit. Although the term 'micro' would intrinsically imply a limitation of the size range to 1 – 1000 µm, it is increasingly being accepted that 'microplastics' should also cover particles up to 5 mm as well as sub-micron sized particles or even nanoparticles. Although the presence of secondary microplastics (*i.e.* microplastics originated from the degradation of bigger plastic items) with sizes down to the low micron range has been amply demonstrated, the limitations of analytical techniques and the complexity of reliably extracting smaller particles from real-life sample matrices has impeded progress with smaller sub-micron or nano-plastics. As the issue of these plastic pollutants has become more studied and technology has advanced, a growing body of evidence suggests the presence of sub-micron plastics in environmental samples, which presumably derive from degradation of larger particles. It would therefore be a logical precautionary measure to consider microplastics to extend below 1 µm. Furthermore, as it is not yet clearly known how particle size influences biological responses *in vivo*, there is no clear evidence which can help to establish any lower size limit on a health risk basis. It would therefore be reasonable to maintain harmony with the REACH restriction (see paragraph 5.1.1) and not impose any lower size limitation on what constitutes a microplastic in the context of the DWD.

As mentioned above (see paragraph 5.1.1) the REACH restriction, instead of specifying a lower size dimension, sets a pragmatic size of 0.1 µm (*i.e.* 100 nm) to be the lower size limit of microplastics for the purpose of enforcing the restriction. This lower size has been proposed as it is currently not possible to characterise or identify smaller microplastics by existing analytical techniques or accompanying documentation. It should be noted that although current state-of-the-art technology could potentially identify man-made polymer particles of size as low as 100 nm[68–70] the measurement procedures and equipment required are extremely specialised and with complexity and costs that cannot be realistic options for routine monitoring by water suppliers and official control laboratories.

In the case of the DWD, the same approach of specifying pragmatic fit-for-purpose lower limits for implementation could be adopted as well. However, in this case, the limits must not be based on considerations of what is technically possible with state-of-the-art instrumentation and one-off samples. Instead, it must consider practical sampling procedures and analytical techniques which: i) are widely available commercially, ii) have known demonstrated capabilities and iii) permit levels of throughput and costs per sample which are acceptable for widespread monitoring. On this basis, it would not be reasonable to consider a lower limit of 100 nm for reporting, but rather a more realistic lower size of around 20 µm, which would be compatible with the detection and throughput limitations of analytical techniques based on optical microscopy techniques.[71]

It should be noted though that particle size is not an intrinsic limitation with the mass based thermo-analytical techniques, but rather with the sampling process where large volumes of water have to pass through fine filters for the collection of microplastics. If smaller particles are to be retained for analysis, then a finer filter must be used and this, firstly, limits the speed of filtration and, secondly, the volume which may be processed due to rapid clogging of the filter.

5.2.3 Shape

The shape of microplastic particulates is a potentially relevant parameter, which could have an influence on the interaction with living systems. At this time, no widely accepted way of categorising particle shape is known but a simple, minimum requirement could be to classify as particles or fibres based on their aspect ratio as follows:

- Particles – particulates of any shape, including pseudo spherical or irregularly shaped fragments or flakes; the ratio between maximum length and width values must be ≤3 to distinguish them from fibres.
- Fibres – particulates, of which the length to diameter ratio is >3.

Such a categorisation would be practical only in the case of using optical microscopy-based methods, such as fluorescence microscopy, µ-FTIR or µ-Raman.

5.2.4 Colour

In an environmental context, the colour of a particle may influence the tendency for fauna to ingest the particles through aspects of feeding instincts. However, in the context of monitoring microplastics in DW, reporting the colour of the particles may be of limited value. Still, it may have a relevance in helping tracing the origins of particulates.

5.2.5 Surface chemistry

Surface chemistry is likely to be a factor influencing the behaviour, stability, fate and biological activity of microplastic particles, but at this time a link to hazard or risk from DW has not been established. Currently, the technology and knowledge required to obtain relevant data about particle surface chemistry would add complexity, analysis time and cost which cannot be easily justified for monitoring of DW.

5.3 Metrics for reporting

The quantity of microplastics present in a sample of DW may be reported in two principal ways depending on the analytical techniques used for particle detection and quantification. In the case of the spectroscopy-based techniques, the particles are analysed one-by-one and consequently microplastic content would be reported as number of particles identified as microplastic per unit volume of sampled water. In the case of the thermo-analytical techniques, the result obtained is the mass of detected polymers present in a sample and consequently the reported data would be the mass per unit volume of sampled water.

As noted previously, the current knowledge about possible effects of microplastics on human health is extremely limited and consequently it is not yet known which would be the scientifically most relevant metric to be used for reporting. In case the effects on biological systems, including humans, depend on the particulate nature of microplastics, their number and size seem important and thus a number-based metric should be chosen. In case the effects of such particles rather derive from substances that a microplastic particle contains (besides the polymer itself, also additives and potential residual monomers) and which can be released once ingested or taken up into the organism, the mass metric may become more relevant. For biological effects that are triggered by agents adsorbed on the particles' surface, the surface-area appear to be the appropriate metric. Thornton-Hampton et al. (2022)[72] analysed the issue by linking the measurement metric and the toxicological effect/mechanism. They recommend to determine both number and mass concertation, but also highlight that, depending on the specific effect mechanism, other metrics (*e.g.* volume, surface area) may be more informative. However, in the absence of relevant knowledge about the determinants for effects on biological systems, the reporting metric should be determined by the analytical technique(s) used.

It should be noted though that some degree of mathematical interconversion from a number-based to a mass-based metric is theoretically possible using assumptions about particle shape and density. However, the reliability of such procedures could not be successfully proven so far.[31]

Considerations on the analytical techniques that can be used for detection and quantification of microplastics in DW are described below (section 7).

5.4 Descriptors and metrics suitable for microplastics in drinking water

Due to the lack of knowledge about the risks of microplastic to human health and about which of their properties would be decisive, the descriptors and metrics for monitoring of microplastics in DW, at present, have to be set largely according to practical considerations. Considering the existing (legal) definitions the following aspects are deemed relevant for DW monitoring:

- The metric for reporting (number or mass of particles) will depend on the technique chosen for quantification of the microplastics in DW.
- An upper size limit of 5 mm would align with existing legislative provisions in Europe (REACH, MSFD) and elsewhere (United States - State Southern California) as well as commonly used practices reported in the literature.
- A lower size limit for monitoring purposes, defined by practical considerations (pore size of filter used and limitations of analytical techniques), would probably be around 20 µm.
- Ideally, the type of polymer should be determined in order to support identification of the origin of microplastic contamination in the supply chain and verification of remedial actions. In case of need, the focus of analysis could be put on those polymers that have been found predominantly in DW so far.
- In terms of shape, it seems sufficient to distinguish two types of particulates according to the length to diameter ratio: particles ≤3 and fibres >3. However, determination of shape is only possible if microscopy-based methods (*e.g.* fluorescence microscopy, µ-FTIR, µ-Raman) are used for particle identification.

Other properties, like colour, surface chemistry and the polymers' biodegradability seem to be of less relevance for DW or/and require complex analysis technique(s).

6 Sampling and sample treatment

The levels of microplastics in DW are expected to be very low (see paragraph 2.5) and cannot be detected directly in the water by any existing analytical technique. To overcome this problem, larger volumes of water must be collected and concentrated (*e.g.* by filtering) to have enough material available for analysis, thus ensuring valid measurement values.

In practice, any method for monitoring microplastics in water will comprise various steps: sample collection (sampling), sample treatment (including filtration), determination (detection, identification and quantification) and data processing. At this time, there have been few reports specifically relating to sampling or/and sample treatment of DW. The following sub-sections summarise the key aspects of sampling and sample treatment which are judged to be most relevant to DW.

6.1 Sampling requirements of drinking water

At present, the low levels of microplastics occurring in DW cannot be detected directly by any existing analytical technique. Consequently, normal practice requires that an appropriate volume of water would be passed through filters to collect and concentrate all suspended particulate material above a size which is defined by the cut-off of the filter. Because of the very low expected concentration of microplastics in DW, collecting large (≥ 1 m^3) sample volumes seems to be the only reliable way to extract enough microplastic particles to stay above the quantification limit of existing methods, as reported in published literature.[18,73] In addition, to mitigate the usually high measurement uncertainty (see above, *e.g.* paragraphs 2.4 and 3.1) and the omnipresent risk of contamination from plastic particles present in the surrounding environment during sampling or processing (see above and in particular paragraph 2.4.5), large sampling volumes are likewise advisable. Consequently, the ASTM standard D8332-20[28] published in 2020 describes procedures to recover microplastics from a range of different types of water and, for DW, recommends a minimum sample volume of 1.5 m^3.

The filtration devices applied in the literature for this volume range are all in-house constructed flow through solutions – with the common feature being the use of stainless steel filters. This material has the advantage to be chemically and mechanically resistant, plus it supports the re-generation and multiple use of the filters.

Annex A summarises the sample treatment strategies applied by various groups[6,7,9–11,14–26,74–89] in the analysis of DW (tap water and bottled water) highlighting the relationship between sampled volumes, applied filter pore sizes, filter materials, sample treatment protocols, and expected types of contaminants. The published papers considered in this summary include the ones already cited in Section 2 plus a number of articles that were considered relevant in terms of approaches used for sample collection, treatment and analysis. The collected information shows that in all of the cases, where the sample volume exceeds 10 L, the primary particle collection and final analysis were performed on separate filters. Though the transfer from one filter to the other might lead to particle loss and thus affect recovery, it allows the use of filter materials that are compatible on the one hand with the conditions of sampling (in particular of pressure) and sample treatment that would be harsh and on the other hand with optical properties suitable for the analysis by OpMic, FluoMic, SEM, µ-FTIR or µ-Raman.

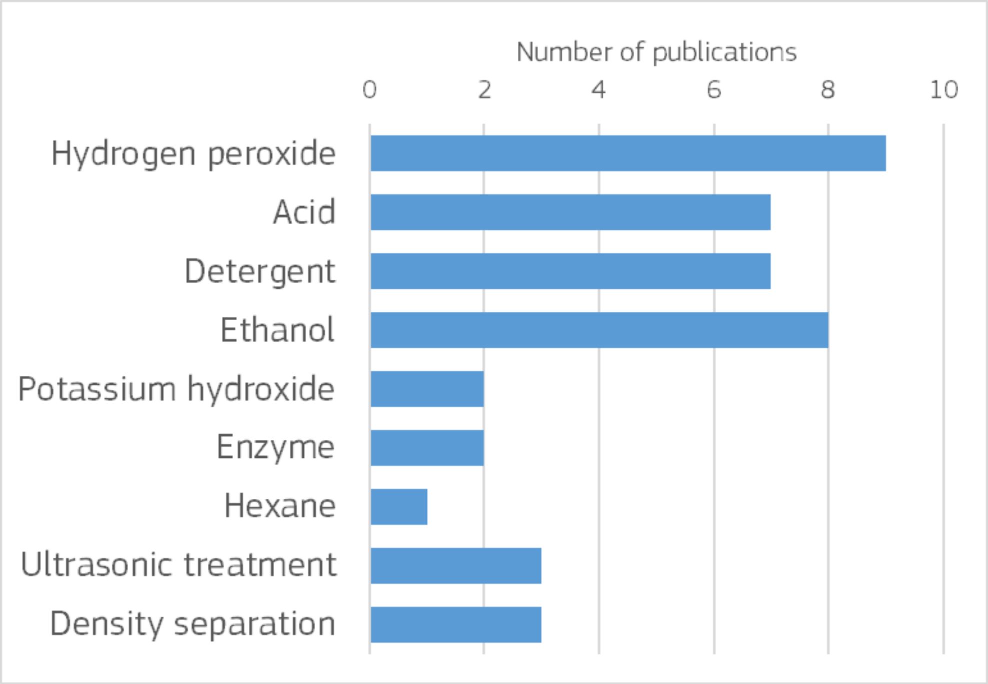
Literature data confirms that methods using larger sample volumes (thus not only collecting a higher number of microplastics but also more matrix particles) involve in general more sample treatment steps before proceeding with the particles' detection and quantification.

6.2 Sample treatment

Sample treatment serves to concentrate the targeted particles and may be needed to reduce or remove compounds present in the samples that may interfere with the detection and/or quantification of the target analytes, *i.e.* the microplastic particles. The selected procedure mainly depends on the type of matrix, the sampled volume and the technique(s) used for detection and quantification.

Among processes collecting lower sample volumes (<10 L), sample treatment often consists only of filtration or staining and filtration, but the analysis of microplastics in samples collected from very large (>1000 L) volumes may not be possible without pre-treatment to remove or reduce the amount of non-plastic particles. This is in line with the conclusions of the article of Koelmans et al.[73] which foresaw different sample volumes and suggested various treatment strategies depending on sample types. Figure 10 shows the most commonly applied procedures for DW sample treatment.

Figure 10 Sample treatment procedures mentioned in the 37 publications examined and summarised in Annex A

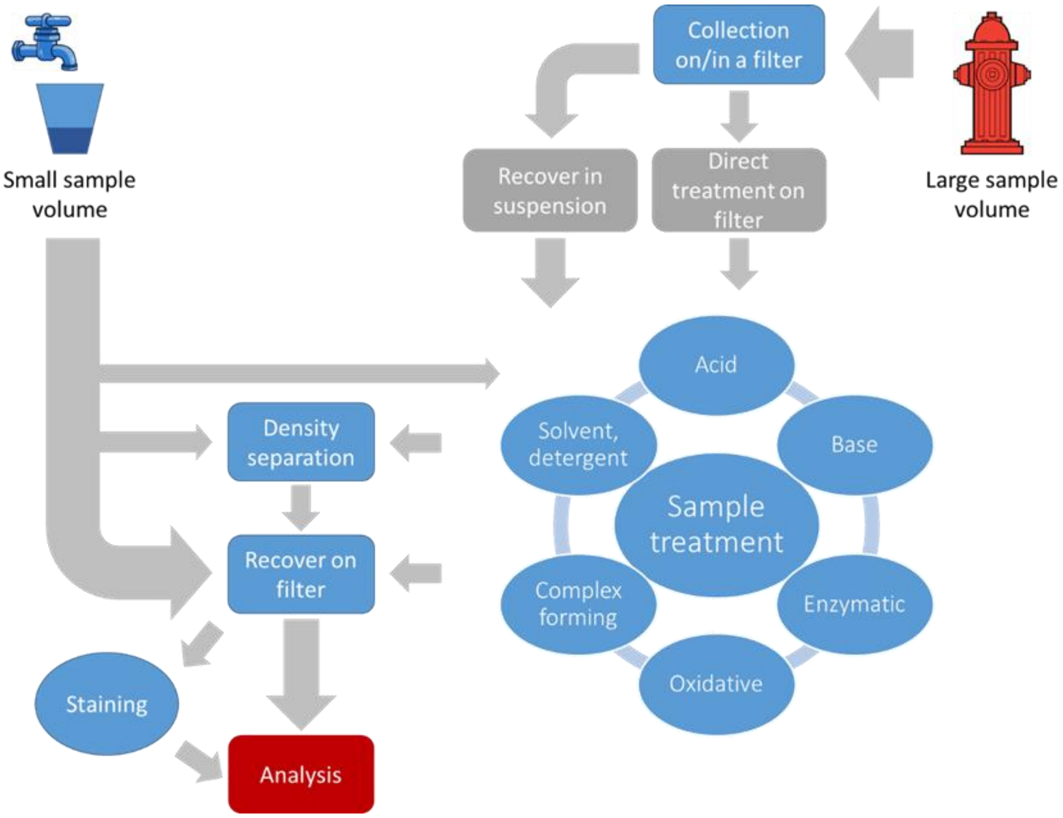


Source: own production

Among the techniques available for microplastic analysis, number counting approaches are expected to be more sensitive to non-plastic matrix constituents, while for example pyr-GC/MS is less strongly influenced. For the latter, however, some organic matrix components that produce similar decomposition products as polymers might become a source of error during data evaluation. Inorganic particulates, such as sand, do not have considerable effects on the results. However, microscopic analysis (by µ-FTIR, µ-Raman, FluoMic) can be difficult if filters are overloaded with particles and result in very long (and thus impractical) measurement times. In addition, particles covered by other materials might be impossible to be identified spectroscopically or the formation of aggregates might bias size measurements.

Various sample treatment steps can be applied to decrease the amount of non-plastic particles. The liquid sample can be treated directly or the particulate material collected on a filter is treated *in situ* or after being re-suspended in a lower volume (Figure 11). The first step in the treatment of collected particulates is typically the removal of organic materials and minerals, sometimes followed by a density separation step.[90] Finally, the resulting particles are collected on a filter that is compatible with the detection/quantification technique. Designing the sample treatment procedure(s) requires careful consideration of the sample matrix.[91,92] Freshwater samples or samples from the inlet of water treatment plants contain higher amount of organic materials (including algae, bacteria) and minerals (*e.g.* sand) compared to DW from underground sources, tap water or bottled water. For more complex samples, the application of multistep sample treatment is beneficial, while for 'clean' or low volume samples only one step might be sufficient or not necessary at all. In contrast, applying multiple treatment steps might result in microplastic particle loss and thus reduced recovery.

Figure 11 Schematic drawing on sample treatment strategies (arrow width symbolises the popularity of the strategy among the analysed literature sources, see Annex A)



Source: own production

Treated drinking water, mineral water and bottled water have usually low concentration of organic contaminants, but may contain a high concentration of minerals such as calcium and magnesium carbonate, other calcium and magnesium salts, iron oxides and precipitates from flocculation processes. These components are often suggested to be removed by treatment with inorganic acids (hydrochloric acid HCl, sulphuric acid H_2SO_4).[8,10,16,18,79] However, as acid treatment is known to affect the recovery of some polymer types (such as PA and PET), more gentle solutions, like the application of organic acids (acetic acid, citric acid) or ethylenediaminetetraacetic acid (EDTA) were also suggested.[13,74,83] In case a treatment step needs to be used that may have an impact on the recovery of some polymer, the latter could be excluded from the reporting.

The most frequently applied digestion procedure, described in the literature for water samples, was oxidative treatment with hydrogen peroxide (H_2O_2), in some cases in the presence of ferrous ions (Fenton's reagent). The applied H_2O_2 concentration varied substantially from low percentages up to a concentration of 35%; temperatures and treatment times were also very diverse.[93] With all variables, a highest applicable temperature of 60 °C seems to be accepted by most authors. The iron catalyst added to H_2O_2 might improve digestion efficiency and decrease treatment times, providing good recovery for the majority of polymer types, but might attack some others, such as cellulose acetate (CA).[94]

Enzyme digestion is specific to the organic substance(s) to be decomposed and is considered to be the gentlest among the various types of digestion, preserving polymers and particle integrity. However, it is linked with higher cost than other treatment options, especially if performed with highly purified enzymes.

Other (non-digestive) sample treatment steps, included treatment with organic solvents such as ethanol (EtOH) or hexane and various detergent solutions[10,13,16,18,26] as well as sonication and density separation.[17,19,23,25] These are not supposed to cause a chemical reaction (decomposition) of the non-plastic components, but rather to separate them based on hydrophobicity, density or due to physical forces. Mild solvents can remove adsorbed layers of contaminants from the surface of the particles thus for example decreasing the possibility to conceal the signal of polymers by fluorescent contaminants in Raman spectroscopy. Detergent solutions can help to reduce particle attachment to the surface of filtration apparatus/funnel, to supress particle aggregation and to create a better distribution of particles on the filter surface. EtOH is also applied in some cases to reduce foaming during filtration in the presence of detergents.

Sonication is a physical treatment that is applied to remove contaminants adsorbed to the particle surface, to disassemble particle aggregates and to detach particles from the filter surface, especially from woven mesh filters where particles might get trapped in the filter structure. The aid for detachment from a filter surface is particularly useful, for example, if particles need to be transferred to another filter (see above). However, sonication might also affect particle size and shape by destroying larger particles and generating smaller ones. In fact, sonication is also a procedure that is used to generate microplastic particles from larger plastic objects. As an alternative to ultrasonic treatment, the application of (filtered) compressed air was described to facilitate the removal of collected particles from the filtration device.[10]

When it is desired to mechanically separate plastic particles, which are typically low-density materials, from higher density inorganic particles (like sand), density separation is commonly used. In this procedure, the mixture of particles is placed in a liquid of intermediate density – prepared with a salt solution – which is then allowed to stand untouched until the less dense plastic material floats and separates out from the more dense inorganic material which sinks. This is usually performed in a cylindrical shaped vessel equipped with a central valve that – after closing – physically divides the denser inorganic material, which sediments to the bottom, from the lighter (microplastic) particles that floats to the surface.

Because 'microplastics' does not refer to a single material, but covers a group of polymers with a range of chemical and physical properties and includes particles in a wide size range, the sample treatment and evaluation of recovery after it is not a simple task. Chemicals used for digestion of matrix particles will attack different plastics to a different extent, and might shift also the size distribution of the particle population by consuming smaller particles - that have higher surface area - faster. Therefore, careful evaluation of recovery practices would be needed, but recovery values are rarely published. However, some reported values look to be encouraging (>80%) regarding the sampling of larger volumes. Lower (<80%) recovery values might be attributed to incomplete particle recovery from the stainless steel cartridge or to the use of too harsh sample treatment procedures, such as the application of chemicals like hexane or concentrated HCl.[18,74]

In practice, there is no single generic method or combination of methods which can be universally recommended for treating solids collected from DW as the sample composition will depend on its origins and treatment prior to reaching the collection point. The preceding discussion highlight some of the steps which can be taken but the decision of which, if any, should be applied must be determined by the analyst based on knowledge about the sample and prior experience.

7 Instrumental techniques to detect and quantify microplastics

In practice, water samples may contain only low concentrations of microplastics besides particles of natural organic matter or minerals. This mixture of materials means that any relevant analytical technique must be able to unambiguously distinguish the man-made polymers from the other particulate material.

The scientific literature reporting the analysis of microplastics in various matrices is extremely broad and many instrumental techniques have been reported as having been applied to characterise a range of microplastics properties. However, for the specific application of monitoring microplastics in DW – considering specificity, sensitivity, availability, validation status, cost and throughput - the viable options can be reduced to three, which are briefly described below.

The first and generally simplest technique, fluorescence microscopy (see paragraph 7.1), relies on preferentially staining microplastics with a dye which i) absorbs well to hydrophobic materials such as plastics and ii) can be seen to fluoresce when illuminated by an appropriate light source. Fluorescence microscopy can provide information on number, size distribution and shape of the stained particles.

The second category of techniques is based on spectro-microscopy (see paragraph 7.2), which combine optical microscopy to visualise particles with Infra-Red spectroscopy or Raman spectroscopy as a means to identify the composition of individual particles. By these techniques normally particle-by-particle analysis is performed in combination with optical imaging and they can directly provide information on particle number, size distribution, shape and chemical composition.

The third category covers thermo-analytical techniques (see paragraph 7.3) based on gas-chromatography mass-spectrometry (GC/MS) which permit the identification and mass-based quantification of plastic materials but cannot directly provide any information on particle size, morphology and number.

Annex B reports a summary of the key aspects of these instrumental techniques for a better comparison of principle, price, analysis time and information that can be obtained from a single analysis.

It should be added that the scientific literature contains reports on the use of a small number of other techniques such as scanning electron microscopy (SEM, combined with X-rays, fluorescence or cathode-luminescence),[95] single particle extinction and scattering (SPES)[96,97] and chromatographic techniques, like high-performance liquid chromatography (HPLC).[98–102] However, none of these techniques will be considered further as they are either unable to specifically identify polymers (SEM, SPES) or are limited to specific plastic compositions (HPLC) and are therefore not suited for a generalised application.

7.1 Fluorescence microscopy

Fluorescence microscopy (FluoMic) is a rapid screening technique of microplastics with more accuracy than simple visual inspection by optical microscopy, without the need of an expensive apparatus.[103,104] FluoMic exploits the peculiar properties of fluorescent molecules (fluorophores) which have the capacity to absorb light energy and emit light at longer wavelength after few nanoseconds.[105,106] The microscope apparatus is built with an excitation light source and a system of filters able to remove the signal coming from the source itself and to select the desired range of emission wavelengths. In this way it is possible to discharge the signals coming from non-fluorescent objects or materials that emit at different wavelengths.[105,106]

In the field of microplastic investigation, FluoMic has gained consideration since Andrady[107] suggested the use of the Nile Red (NR) dye to stain particles coming from environmental samples. NR is a hydrophobic, water insoluble dye previously used to visualize hydrophobic organic components, such as lipids or vesicles.[103] Due to its hydrophobic properties, this molecule tends to preferentially adsorb on the surface of plastic particles thanks to van der Walls interactions or dipole interaction in the case of polar polymers.[103,104,108] The preferential absorption of the dye on plastic with respect to more hydrophilic materials (for instance, mineral particles) means that when illuminated with an appropriate light source the resulting fluorescence permits discrimination between plastic and non-plastic particles.

In the last 5 years, a series of other molecules have been also tested to stain the plastics or the organic residues present in the samples, with different results. The list includes molecules that are water soluble (*i.e.* Eosin B, Rose Bengal, Acridine Orange, Basic Blue 24, Crystal violet, Lactophenol Blue, Neutral Red, Safranin T, Tryphan blue, Rhodamine B, Fluorescein isothiocyanate, methylene blue, Rhodamine 6G) or insoluble (*i.e.* NR and NR derivatives, oil red ENG, Hostasol Yellow 3G, fluorescein).[94,103,104,108–110] More recently, also pyrene, 1-pyrenebutyric acid N-hydroxysuccinimidyl ester (PBN) and a new probe based on perylene-diimide,[111–113] as well as white light emitting stanene-oxyboride (SnOB) nanosheets were evaluated.[114] However, from the different studies NR emerges presenting advantages with respect to other dyes, in terms of higher affinity for microplastic particles, reduced staining time, and lower toxicity.[94,104,108,109] Research articles on the application of NR in microplastic investigation have been constantly increasing during the last few years.

Some capability of FluoMic to differentiate between (some of) types of plastic via differences in fluorescence by use of NR has been reported.[108,115] The dye adsorbed onto the surface of different polymers was shown to have slightly different colour depending on the polarity of the polymer, which is associated to exhibiting some differences in emission spectra. However, this approach is not well investigated and thus its reliability is uncertain. Due to the findings that NR may also stain natural polymers in environmental samples (*e.g.* particulates made of wood, cotton, shells), it can be assumed that FluoMic can lead to an overestimation of the presence of microplastics, if other organic matter is present.[108,116,117] On the other hand, some polymers may not be detected by FluoMic due to the staining being too weak or failed completely.[117,118] Such flaws could potentially be avoided by carefully choosing the staining and measurement conditions as well as an appropriate sample treatment approach with the aim to remove/reduce the interfering organic matter. However, the authors of this report are not aware of any comprehensive investigation of such aspects or validation of corresponding procedure(s).

In general, after sample treatment, FluoMic can be used as screening technique for microplastic investigation, and it is often applied for preliminary investigation of the entire sample, while only a portion undergoes to further evaluation such as polymer recognition by vibrational microscopy techniques (see also paragraph 8.5).

7.2 Spectro-microscopic techniques using IR and Raman

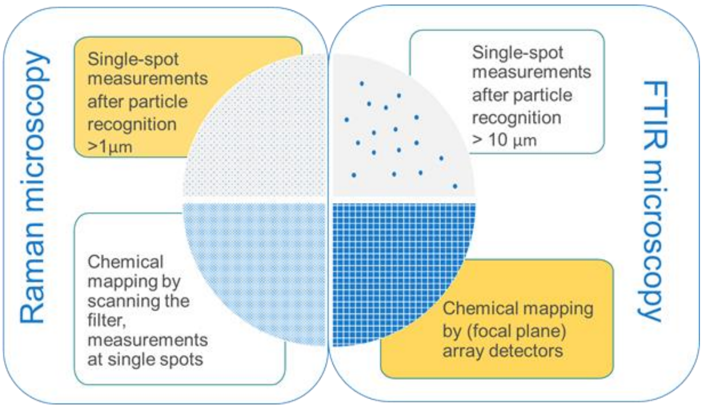
Spectro-microscopic techniques allow not only the counting, sizing and morphological characterisation of particles collected on a filter but, in contrast to FluoMic, can also identify the chemical composition of individual particles – within limitations that are specific to the technique and the instrument used. The two most applied techniques are Raman and Fourier-transform infrared (FTIR) spectro-microscopy with various commercial instruments available on the market. The expected limit of detection (LOD) of these techniques regarding detectable particle number is lower (*i.e.* better) than that for mass quantification techniques such as pyr-GC/MS. While spectro-microscopic techniques are supposed to be able to detect even a single 20 µm microplastic particle on a filter, the mass of a particle like this is below the expected LOD of pyr-GC/MS.[119] On the other hand, other (non-plastic) particles present in the water matrix might interfere stronger with the quantification by spectro-microscopic techniques than by thermo-analytical techniques. Additionally, data collection - and in some cases also data analysis – is quite time consuming. The throughput (in terms of analysed samples per day) strongly depends on instrumentation and the applied strategy. Sample handling is fully manual (the authors have no information about technology that would allow automated sample positioning); thus all measurements require sample positioning by a trained and skilled operator. In most cases, also data analysis needs input and decisions by an experienced operator, potentially leading to individual bias affecting the results.

While calibration is not needed for quantification (*i.e.* counting of particles), it is necessary for the spectra-based identification. The latter require only traditional calibrants for sizing and the wavelength accuracy in spectroscopy, which are commercially available. At the moment, there is no existing standardised method for microplastic analysis using FTIR or Raman microscopy. Ongoing efforts (for instance, ISO working group) are focused on providing guidelines on good practices rather than developing a single standardised method (see paragraph 4.1), because of the diverse instrumental solutions, data collection and analysis strategies already applied or being under development by the researchers of the field.

7.2.1 Data acquisition and analysis strategies

The strategy selected for particle recognition and polymer identification by spectro-microscopy users depends strongly on the specific combination of hardware, analysis software and spectral library(ies). However, the choice can be also affected by the targeted particle size classes, matrix properties or polymer types to be analysed. In general, two main measurement strategies can be adopted with the spectro-microscopic techniques (Figure 12): i) single spot analysis of preselected individual particles and ii) chemical mapping by scanning over extended areas with post-measurement data analysis to locate and identify particles.

Figure 12. Summary of typical data acquisition strategies in spectro-microscopic methods. Yellow boxes indicate the preferred options.



Source: own production

In the first case, particles are recognised in the optical image of the filter surface typically based on optical contrast between the particles and the filter. Then, all particles or a selected set of particles are chosen for data acquisition, which is done at multiple individual positions (coordinates that correspond to the single particles). Morphological analysis and sizing is based on the optical image. This image can be collected in transmission or reflection mode, depending on the instrument. If reflection mode is used, dark field illumination might be beneficial in order to improve the optical contrast between particles and the filter.[78] Composition of the particles is typically evaluated by comparing the acquired spectrum from a particle to the spectra in a spectral library or applying a decision algorithm developed for a certain sample type.[120]

In the second case, the full or a representative partial filter area is covered by the instrument acquiring a spectrum for each of an array of spots on the filter surface investigated. The resulting hyperspectral image is a kind of 'chemical map' composed of spectra corresponding to the individual points of the covered surface. Chemical mapping is supported by FTIR instruments using focal plane or linear array detectors that allow faster data collection. It is possible to apply this strategy also in Raman microscopy using very short integration times or for FTIR instruments equipped with single element detector if a limited filter area is mapped. Particles size and morphology can be analysed based on optical image, based on their chemical image, (*i.e.* the group of pixels touching each other and corresponding to the same polymer or polymer group) or based on their combination with the help of an operator. Composition of the particles can be evaluated by comparing the spectra of each pixel to a spectral library or using more complex approaches like principal component analysis or machine learning based approaches.[121–124]

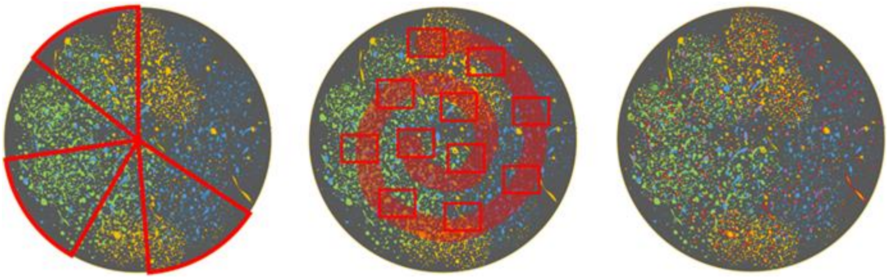
Both optical and chemical images are two dimensional and carry no information about the thickness of the particles. Still, some authors suggest that volume of particles can be estimated supposing a certain theoretical ratio between their 2D projection and thickness. The calculated volume multiplied with the density of the identified polymer type gives the estimated mass of the particle.[125]

7.2.2 Filter materials and filter analysis

In spectro-microscopical methods, particles are usually directly analysed on the surface of a filter. Thus, the selection of the filter material is crucial, as in transmission mode or working with small particles very close to the support surface, the filter material itself might contribute to the signals of the acquired spectra. Because of this, the use of polymer based filters is not recommended. Raman users often apply aluminium-oxide, silicon, metal coated, or cellulose based filters.[32] Working with µ-FTIR in transmission mode requires a filter material that is transparent for IR light. Such materials are silicon that is fully transparent in the spectral range of interest or aluminium-oxide that is transparent above 1250 cm^{-1}. The loss of the part of the spectrum below that wavenumber is balanced by the advantage that the thin aluminium oxide filters are also partially transparent for visible light. This helps better focusing and condenser adjustments and allows to collect good contrast optical image of the sample in transmission mode. Based on these partial transmission spectra, polymeric materials can still be well identified.

Pore size, diameter, roughness and flatness of the filter are also very important parameters determining lower particle-size cut-off, affecting clogging, optical imaging, particle recognition, data collection and analysis times. Scanning only partial filter areas is a common strategy to decrease analysis times. However, as particles are usually not evenly distributed on the filter surface but tend to accumulate close to the borders or certain areas, the non-homogeneity of their spatial distribution may affect the quantification. The work of Schymanski et al.[78] highlights the most reliable strategies for subdividing the total area of a filter (Figure 13). In case of the first model, radial sectors are selected for measurement.[78] The second model selects multiple small rectangles following a spiral track, while the third model uses random single particle selection.

Figure 13. Strategies for subdividing the filter area for spectro-microscopic analysis



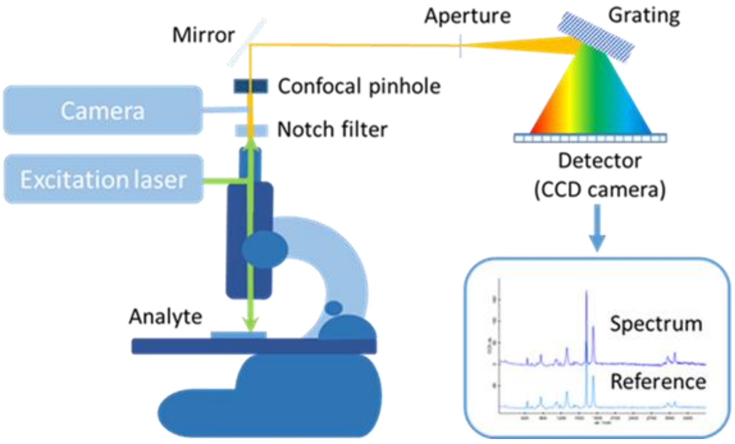
Source: Schymanski et al.[78]

The second model might be the best solution in cases when selection of partial areas is not the free choice of the operator, but the software allows only to map rectangular area(s) of interest or the analysis software is not able to deal with more complex data than a simple spectral hypercube.

7.2.3 Raman spectro-microscopy

Raman spectro-microscopy (µ-Raman) combines the capabilities of an optical microscope to visualise objects (down to 1 µm) with the analytical capabilities of Raman spectroscopy (Figure 14). The filter, on which the particles are collected, is placed on the (motorised) stage of the microscope and after imaging the filter, Raman spectra are acquired at single particle positions or the selected area of interest is mapped (see paragraph 7.2.2).

Figure 14. Schematic drawing of Raman spectro-microscopy.



Source: JRC, Technical report JRC125383[32]

During spectrum acquisition, monochromatic laser light is focused on the material, using the same objective as for imaging, or using an objective with larger magnification. In general, long distance objectives are recommended for signal collection, and the applied objective and possible confocal features will determine together the “spot size”, the volume fraction of the sample from which the reflected laser light is collected.[78] Raman microscopes are reported to detect single microplastic particles even with diameters below 1 µm, but proper sizing of objects in this size range is not feasible due to the limitations of optical microscopy.[126] The reflected laser light might pass a confocal pinhole if data collection from very small particles is required, or if confocal mode helps to decrease background fluorescence. Finally, the reflected light passes a monocromator and arrives to the detector or passes a grating that sends the light to different pixels of a charge-coupled device (CCD) camera (Figure 14). The resulting Raman spectrum is unique for a specific polymer (or other material with Raman active vibrations) and serves as a fingerprint in chemical identification by comparison with spectra of a spectral library.

The main advantage of µ-Raman compared to µ-FTIR is its higher spatial resolution but also that it is less sensitive to the presence of water. On the other hand, in the analysis of dry filters, this advantage is less relevant; at the same time, the weakness of Raman spectroscopy, being disturbed by fluorescence emission, becomes more significant. Fluorescence in microplastic particles might originate from additives (such as dyes and filler materials) of the plastic, but also from matrix components even in case of such simple matrices as clean water. Algae and other microbes that contain chlorophyll or other plant pigments, might be present in water samples and interact with the exciting laser light. Proper selection of the applied laser wavelength can help to decrease interference by fluorescence, but such a choice might not always be possible due to the specification of the instrument used. Photo-bleaching is another known strategy to decrease the effect of fluorescence, but in case of automatic or semi-automatic analysis of large number of particles the tailored treatment of individual particles is not realistic.

Both data collection at selected particle positions and chemical mapping (Figure 12) are applied in the analysis of microplastic particles by µ-Raman as confirmed also by the inter-laboratory comparison study run by JRC in 2021.[32] However, because of practical reasons, more Raman users combine optical image analysis for sizing with identification from single spot measurements.[126]

µ-Raman can be equipped with one or more laser sources. Instruments constructed for general use with non-specific application are most often equipped with a 532 nm or 785 nm laser; less often a 633 nm light source is applied. Raman spectra of polymeric materials do not depend strongly on the selected excitation wavelength. The position of the spectral peaks does not change, but variations in intensity ratios might happen – with potential impact on the chemical identification by comparison to the spectra in the applied database. This means that spectral databases from literature or provided by instrument manufacturers can be still used also when spectra are accumulated applying another laser. However, proper calibration of the instrument before measurements, or post-accumulation correction of the data is indispensable to get spectral features at their proper position.

Various light sources might work at very different laser power (milliwatt, mW) and transmit different amount of energy to the analysed particles per accumulation time. Too high laser power settings might result in thermal decomposition of dry heat-sensitive materials in air. Small polymeric particles with a high surface area are vulnerable from this point of view.

The recorded spectral range depends on the laser wavelength as well as the type and position of the optical grating. When applying a 532 nm laser, the full wavenumber range containing the fingerprint region and also C-H stretching vibrations at around 3000 cm^{-1} is typically covered at a single, well selected grating position.

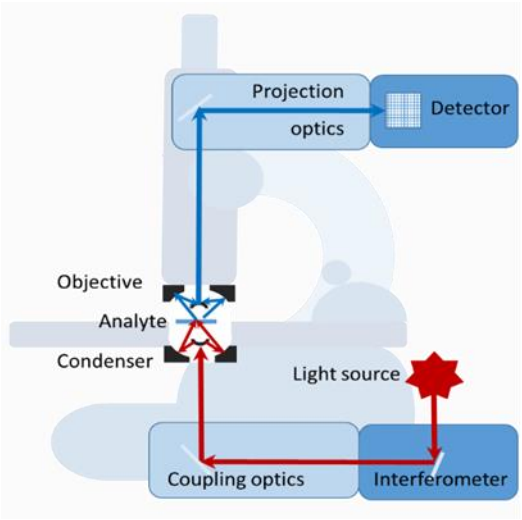
7.2.4 FTIR spectro-microscopy

In Fourier Transform Infrared spectro-microscopy (µ-FTIR), the polychromatic IR light beam source and interferometer is usually coupled with a standard microscope set-up equipped with an automated stage for scanning large areas (Figure 15). When IR light interacts with a sample, a certain portion of the IR radiation is absorbed due to the interaction with the vibrational modes of the irradiated molecules. The non-absorbed light is transmitted by or reflected from the sample. Depending on the instrument set-up, transmitted or reflected light is captured by the detector. The resulting interferogram is transformed by an algorithm, called Fourier-transformation, to a spectrum. The features of the FTIR spectrum serve as a fingerprint in chemical identification of materials by comparison with spectra of a spectral library.

As both Raman and FTIR spectroscopy provide information on vibrational energies in the material, Raman and FTIR spectra of molecules contain features at the same spectral positions, but the intensity ratios of the peaks in the Raman and FTIR spectra of the same molecule are different. FTIR spectroscopy is sensitive to hetero-nuclear functional group vibrations and polar bonds, among them the O-H stretching in water. Because of this, FTIR cannot be used for the analysis of wet samples.

Similarly to µ-Raman, FTIR spectra can be collected at single particle positions or the selected area of interest is mapped (see Figure 12). Special array detectors (focal plane array – FPA, and line array detectors) capable of simultaneously acquiring spectra at several portions (pixels) of the surface, can be added to the system to speed-up analysis time. This makes µ-FTIR more suitable for chemical mapping. The spatial resolution of µ-FTIR is lower than that of µ-Raman, which means that with µ-Raman smaller particles can be investigated than with µ-FTIR. The spatial resolution depends strongly on instrument configuration and measurement settings, but achievable values reported in literature for µ-FTIR in routine measurements are in the range of 10-20 µm.[71,126] The schematic drawing of a FTIR spectro-microscope (in transmission mode) is presented in Figure 15.

Figure 15. Schematic drawing of FTIR spectro-microscope (in transmission mode)



Source: own production

Depending on the instrument set-up, FTIR spectro-microscopes can be used in transmission, reflection and attenuated-total-reflectance (ATR) mode. All of them have their inherent advantages and disadvantages. The main advantage of measuring in transmission mode is that most of the available spectral databases contain reference spectra acquired in this mode. Disadvantage is that above a certain size, plastic particles are no longer transparent for the IR light. Very low transmission values appear as truncated peaks in the absorbance spectrum, distorting the ratios between the peaks of the spectral fingerprint. This leads to lower scores at comparison to reference spectra and possible misinterpretation or false negatives. If the full optical cross-section of the particle is scanned – like in chemical mapping – and each particle is covered by more chemical image pixels, there is better chance to collect some good quality spectra at the edges of the particle where the total thickness of the material is lower. Searching for more transparent regions in large particles can be also done by manual measurements with a single element detector.

Because of the limited IR transmission of thick samples, measuring in reflection mode is frequently chosen as strategy of automatic analysers. In this case, reflected IR light is collected by the same objective that is used to focus the beam on the sample. In case of a flat, well reflecting surface, the resulting spectra are reflectance spectra that can be transformed into a classical absorbance spectrum using the Kramers-Kronig transformation. Unfortunately, microplastic particles are usually far from being flat and well reflecting. The light reaching the particle is often partially transmitted and reflected from various curved surfaces before leaving the particle – and interacting with the reflected light. The resulting spectrum is a so-called transflectance spectrum. As it is strongly affected by the geometrical features of the particle, no commercial databases exist to compare in-house results with reference transflectance data. Thus, FTIR instrument users applying reflectance measurement mode are advised to build their own database – containing more spectra of the same polymer or other material.

ATR measurements are possible with a special objective, containing an IR transparent crystal with a very fine tip. This means that preliminary imaging of the filter should be done with a normal objective, and the ATR crystal is pressed on the sample at the selected measurement positions and acts as a kind of solid immersion lens. The very fine tip allows to get high quality spectra and good spacial resolution. However, scanning large areas or large number of particles by ATR is much more time consuming because of the crystal has to be lifted and landed again at the various positions. Moreover, it might also be contaminated by particles that are not strongly attached to the surface. Additionally, ATR provides reflectance spectra. Therefore, polymer identification usually requires further transformations or the use of a home built database. All in all, ATR is not the technique of choice for high number of loosely attached microparticles on a filter, but might become useful for the individual analysis of larger objects that are not transparent enough in transmission measurements.

Basic µ-FTIR and automatic instruments are usually equipped with a single element detector. These detectors allow the collection of spectra at selected measurement positions. For more efficient mapping, instruments can be equipped with line array or focal plane array (FPA) detectors. They create high-resolution images of a defined pixel format and the collected pixel groups are later stitched to larger spectral images. Spatial resolution of an FPA detector depends on the size of the individual detector pixels while the covered area depends on the number of pixels. Averaging the spectra of adjacent pixels, the so called binning (2x2, 4x4, etc...) can improve spectral quality but it is accompanied by the decrease of spatial resolution. Mapping by FPA detectors is much faster than by single element detectors and allows to scan full filters or larger representative filter areas in reasonable time. Moreover, microplastic particles might be identified based on more pixels, more spectra belonging to the same visible object.

The recorded wavelength range should cover the spectral fingerprint region(s) used for identification. Usually, the 400-4000 cm^{-1} range is applied also by most of available spectral databases. The recorded spectral range might be also wider: for example, in case of reflectance spectra the wider spectral range (up to 8000 cm^{-1}) helps to improve the quality of approximations during Kramers-Kronig transform. The final spectral range considered at spectral identification might be also different: for example, the always changing peaks of carbon dioxide (CO_2) gas that appear in a spectral region 2200-2500 cm^{-1} - and do not carry any information on the sample - or regions where the sample or reference spectrum values are not available can be excluded from the calculation.

7.2.5 Quantum cascade laser spectroscopy

Quantum cascade laser infrared (QCL-IR) spectroscopy[127] uses a tunable laser as the IR light source. It operates at lower power than lasers used in Raman spectroscopy, thus there is less probability for damaging the sample. The only commercially available instrument to date, applies a detector that is cooled electrically, thus no liquid nitrogen is needed. For microplastic analysis, the instrument offers an automated workflow including an initial scan of a large surface area in reflection mode using IR light at a fixed wavelength to determine the location, size, and shape of particles. Spectra are then collected from selected locations and compared to the spectral library. Like all single-spot-measurement strategies, it has the advantage of faster spectrum acquisition, analysis speed and lower data object size. The lower size detection limit for microplastics is reported to be 10 µm and is thus similar to µ-FTIR. However, the covered spectral range that is used for determining the chemical identity is limited (about 950-1800 cm^{-1}) which makes the identification of certain polymers (for example polyamides) less trustable. The instrument acquires transflection spectra. As reported in the previous paragraph, this makes the use of commercial spectral databases difficult. Moreover, to this date, there is no information about specific open source QCL-IR spectral databases on polymers.

7.2.6 Data analysis and spectral databases

As mentioned above, Raman databases collected using different laser sources are applicable in general, independently from instrumentation, at least in the 532-785 nm laser wavelength range. For Raman and transmission-FTIR spectra, instrument providers usually offer also commercial databases including spectra of pure polymer and other materials alike. Thus, the most convenient choice is to use an already existing, quality controlled database that is compatible with the search features of the instrument's software applied by the user. However, experts of the field suggest - and also the findings of the JRC ILC study confirmed - that microplastics quantification results are better, if the database contains more than one spectrum for a certain polymer as well as spectra of weathered plastic, particles with various sizes and possible matrix components.[32,78,120] In case of commercial databases, software features usually allow to run a search using several databases, thus other materials, like inorganic crystalline materials or dye databases can also be used in addition to the polymer spectra database. In case of in-house databases, the laboratory should consider not only collecting the spectra of other materials that might appear in the sample, but possibly also to record more spectra of the same type of material obtained from different sources and/or at different weathering stages. Other materials that can be found in DW samples include carbonates (calcium and magnesium salts), oxides (sand, iron oxide) and all possible materials that might appear as contaminants from sample treatment process (*e.g.* cellulose from paper, plastic contaminants).[120]

For some FTIR data formats, free-to-use software solutions are available together with microplastic specific databases and documentation in scientific literature.[128] Other open or free databases might be also available online or from literature.[129,130] In combination with certain FTIR instrument types, a commercial software solution was applied which is based on a machine learning approach developed specifically for microplastic quantification by chemical image analysis.[122]

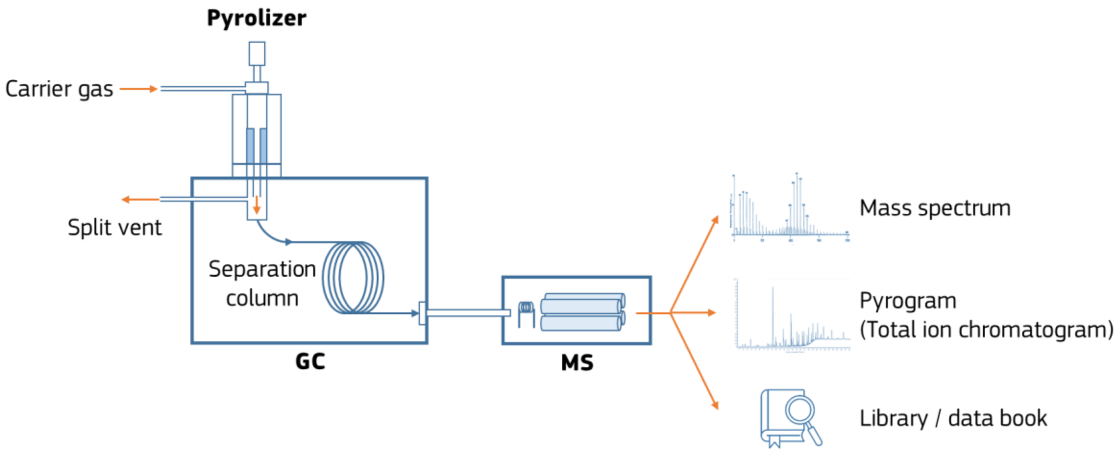
7.3 GC/MS-based thermo-analytical techniques

Thermo-analytical techniques such as thermal extraction desorption GC/MS (TED-GC/MS) and pyrolysis GC/MS (pyr-GC/MS, Figure 16) were introduced relatively recently for the mass-based quantification of microplastics.[95,131–134] In these techniques, high temperatures and oxygen-free conditions lead to the thermal cracking of polymers (pyrolysis) and the production of a range of volatile decomposition products characteristic of each polymer. Their subsequent gas chromatographic (GC) separation and mass spectrometric (MS) detection enable the identification and quantification of individual polymer types.

Both methods share a common analytical principle (GC/MS) for the identification and quantification of the pyrolysis products, but they vary in how the thermal products are generated and introduced into the GC/MS. The traditional pyr-GC/MS method involves a direct coupling of the pyrolyser unit to the GC/MS. This unit heats the sample in an inert atmosphere, producing volatile pyrolytic products that are immediately fed into the GC/MS system for analysis.

The TED-GC/MS system, developed more recently, decouples the pyrolysis and GC/MS steps. First, sample pyrolysis is carried out in a thermogravimetric analyser, with volatile decomposition products purged from the furnace and transferred to a solid sorbent material for capture. In the second and separate step, the sorbent material is transferred to the thermal desorption (TD) unit of a GC/MS analysis system. Polymer breakdown markers are thermally desorbed in the TD unit and transferred to the GC/MS system for determination. By decoupling the two steps the TED-GC/MS can simplify sample handling and can be applied to larger, more complex samples.

Figure 16. Configuration of a typical pyr-GC/MS system



Source: own production

7.3.1 Sample treatment for pyr-GC/MS

Following filtration to collect the microplastic particles from the water sample, the entire filter or part(s) thereof is/are transferred to the sample holder which is subjected to pyrolysis. The selection of appropriate filter materials is important because filters pyrolyze along with the sample and could produce interferences with the decomposition products of the polymeric materials. Some of the most common filter types are aluminium oxide membranes, borosilicate glass fibre filters or stainless steel filters.[32] Pyrolysis requires solid samples. Therefore, wet samples must be dried before analysis.

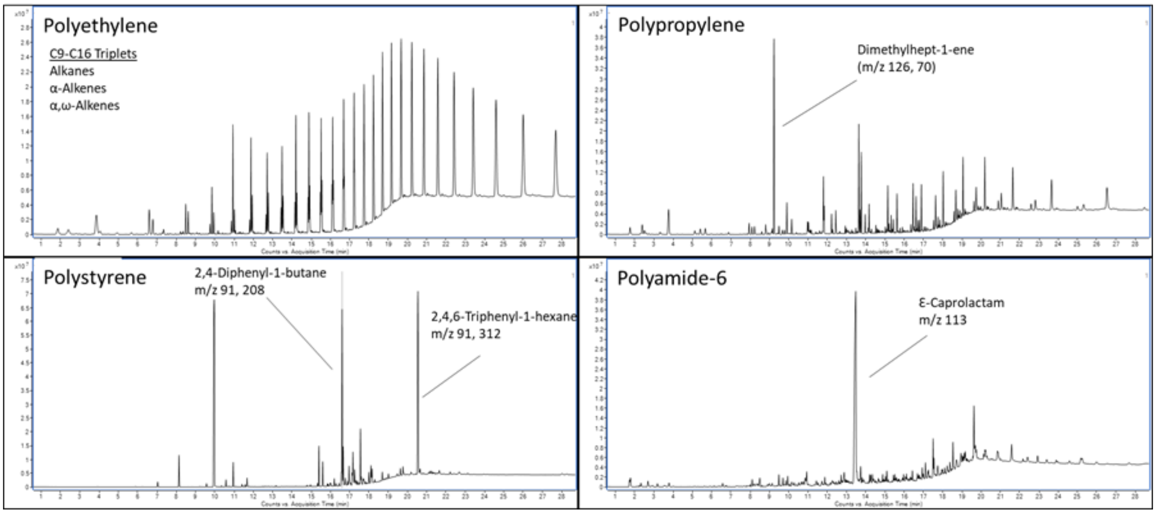
Besides this, if the DW to be analysed contains very low levels of natural organic solids, no additional sample treatment (*e.g.* matrix removal) is required.

A crucial step in this process is the transfer of the sample to the sample holder (cup/vial/crucible/beaker), which is then transferred to the pyrolysis device. This can be done in a number of ways. One possibility is to pyrolyze all or parts of the filter containing the sample. For example, alumina-based filters can be crushed/homogenized, followed by pyrolysis of known aliquots of this homogenised material. Thin, small (diameter ≤15 mm) glass fibre filters can be folded and pyrolyzed as a whole. However, this depends on the size/capacity of the pyrolysis sample holder. A second way to transfer the sample material is to dissolve the particles caught on the filter surface with a suitable solvent. The resulting solution (or an aliquot thereof) is transferred to the sample holder, the solvent is evaporated and the residue is pyrolyzed. The difficulty here is choosing the right solvent, as each polymer requires a different solvent. With an unknown sample, this latter approach is rather impracticable, unless only a specific (limited set of) polymer(s) shall be detected and quantified.

Measurement procedure, calibration and data analysis

The sample holder containing the loaded filter or the polymer solution, as appropriate, is transferred to the pyrolysis unit, which can be of different designs, comprising a furnace or a filament. The sample is then pyrolyzed at temperatures typically around 600°C. This temperature is maintained until the sample has undergone complete pyrolysis. The volatile compounds formed during the cracking process are transferred to a gas chromatographic column, where they are separated by boiling points and affinity for the stationary phase, and finally detected with a mass-selective detector (mass spectrometry).

Figure 17. Characteristic fingerprint chromatograms and specific indicator ions of exemplary polymers: polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide-6.



Source: own production

Each polymer produces specific decomposition products (Figure 17) that can be used for identification. Polymer identity is confirmed by the specific marker fragment ions of the characteristic decomposition product, the respective qualifier ions and the retention time. The intensity of the specific maker fragment ions is proportional to the amount of pyrolyzed product and is used for quantification against external calibration curves generated with pure polymers of known composition.

Each polymer to be studied necessitates its own individual calibration curve. There are various methods for creating calibration curves, one of which involves weighing known quantities of pure polymer into sample holders with the help of an ultra-microbalance, which can provide accurate measurements to within about 5 µg. For smaller masses, finely ground powders made up of small particles can be mixed with inert materials like silica or calcium carbonate to achieve the desired measurements. A microplastic calibration standard (Frontier Lab)[135] has recently become commercially available. This standard includes 12 commonly used polymers that are uniformly dispersed with a solid diluent for easy weighing on microbalances. Another method for creating calibration curves involves dissolving precise amounts of polymers in solvents. Aliquots of these solutions are then transferred to sample holders, and after the solvent has evaporated, the remaining polymer residues are pyrolyzed. It is important to note that each polymer requires a different solvent, and dissolution is often only achievable under harsh conditions.

7.3.2 Overview of capabilities and limitations of pyr-GC/MS and TED-GC/MS

Pyr-GC/MS has been applied for the analysis of polymers for a long time.[136] However, its use to identify and quantify microplastics is relatively new (~ 5 years) and is currently still in the development stage. It is relevant to note that that many existing GC/MS instruments can be upgraded with a pyrolyzer as an instrument accessory. The use of the alternative TED-GC/MS to analyse microplastics is also a recent evolution but in this case the higher cost and the lower availability of suitable instruments has limited the more widespread use in the field. The information that can be obtained from the GC/MS based thermo-analytical techniques includes absolute mass and identification of polymers, while it does not offer information on particle shape, size and number. Compared to the spectroscopic techniques, thermo-analytical ones provide information about the properties of the entire particle and are less sensitive to interferences such as pigments or surface oxidation phenomena. In case of interference by (organic) matrix compounds, it may be necessary to reduce their presence by sample treatment procedures such as digestion, density separation and/or filtration (see paragraph 6.2).

The limit of quantification of microplastics using thermo-analytical techniques is typically around 0.1 µg (≈ 1 spherical particle of 60 µm in diameter) – theoretically higher than the equivalent mass of single particles detectable by spectro-microscopical techniques (1-5 µm by µ-Raman). However, as the limit of quantification is also polymer dependent (0.02 µg – 4 µg) high sampling volumes may be required to compensate for the reduced sensitivity to some common polymers such as PE and PET. If robustness is proven, these techniques might allow higher throughput (lower analysis time) compared to most of the other techniques. However, the techniques are still in the developmental stage and no routine methods, standards or standard operating procedures are available for microplastics analysis.

In addition, the technique requires reference materials for the calibration that allows quantification. No specific size or shape of the particles is required, but the most convenient calibrants are homogeneous polymer powders that are not easy to dose, particularly for pyr-GC/MS, which has more stringent limits on the maximum acceptable size of sample. The calibration procedure can be challenging, especially if more than one polymer is present in the sample. Interference between common polymer types (PVC and PET) during pyrolysis is also known from literature.[137]

In the case of TED-GC/MS, decoupling the pyrolysis and GC/MS steps can simplify sample handling and permit the introduction of larger, more representative, and more complex samples in the pyrolysis stage. In particular, the additional flexibility in the thermal processing of the sample can be exploited to help reduce the presence of interfering organic matter. Non-volatile residues never enter the analysis system. The main disadvantages of TED-GC/MS, compared to pyr-GC/MS are the much higher equipment cost, the more limited availability in existing laboratories and the longer analysis time (2h for a complete analysis cycle vs 45 mins for pyr-GC/MS).

Finally, it should be noted that the thermo-analytical techniques are destructive: samples cannot be re-analysed or transferred for further analysis with an orthogonal technique.

8 Analysis strategies

In practice, the most complex, time consuming and costly step of an analytical methodology is the instrumental technique applied for detection and quantification of the microplastic particles. As discussed previously (section 7), there are a limited number of techniques available and each has different capabilities in terms of specificity, measurement metric, detectable size range, sensitivity, throughput and cost. Unfortunately, no single technique has clear advantages in all of these aspects.

The consequence of this is that it will be necessary to either accept, as component of the analysis process, a single technique (paragraphs 7.1, 7.3, 7.2.3 or 7.2.4) with certain limitations or to combine two (or even three) different techniques to obtain more complete information. The following options describe possible strategies which could be considered as a means to either compensate for the technical limitations of individual analytical techniques or to minimise the financial and manpower burden. This latter is specifically significant for routine monitoring, where it is relevant also to consider cost and time.

8.1 Screening technique

An option to reduce cost and time would be to pre-screen samples using the previously described fluorescence microscopy to produce number size distributions. A discrimination between plastic and non-plastic particles is often possible due to the ability of the fluorescent dye to bind to hydrophobic surfaces, like most synthetic polymers. Size and shape of the particles can be determined as well. The technique, if supported by microscope automation and image analysis, could allow the analysis of entire filters in time periods of less than 1 hour per sample (plus the timing for sample treatment). Since the detection limit is theoretically one particle and size detection limit of a few microns is achievable, the mass detection limit could be in the sub-pg range. Additional advantages are that the cost of materials and instrumentations are relatively low compared to other methods, in particular spectroscopic techniques, as previously described in this report.

However, the disadvantage of using this technique alone is that it cannot determine polymer identity and potentially will produce an undefined number of false positive or/and false negative results. Little or no reliable information on the performance of such method in validation or inter-laboratory comparison studies is available in the open literature. Consequently, it would be necessary to investigate systematically the reliability of the technique's discrimination power. Such a study would be valuable but complex, as it would require the consideration of real-life samples which would have to be thoroughly analysed using techniques that can identify the particles' composition, such as µ-FTIR or µ-Raman, for comparison with the results gained from fluorescence microscopy.

Other potential screening techniques which may be applicable in principle (*e.g.* thermogravimetry, Differential Scanning Calorimetry, determination of Total Organic Carbon) are even less well investigated for the analysis of microplastics.

8.2 Single polymer-specific technique

Another strategy would be to apply either a method using a single type of spectro-microscopy for particle counting or a single thermo-analytical technique for determining polymer mass. Key discriminating factors in the final choice would be the relevance of mass or number based data, sensitivity, cost, throughput and particle size range. Subsequently, it would be necessary to accept the specific limitations which are intrinsic to each of the method categories.

8.2.1 Polymer-specific number-based techniques

For the analysis of microplastics on a particle number basis with identification of the particle's composition (polymer type), there are only two classes of techniques in routine use at the moment: infra-red spectro-microscopy (µ-FTIR and QCL-IR) or Raman spectro-microscopy (µ-Raman). They permit the identification of the polymer composition of individual particle and the counting of all particles of interest. Additionally, these techniques permit the determination of particle size and shape, both of which might be factors influencing biological responses.

As discussed in section 7.2, spectro-microscopy techniques have the advantage of high absolute sensitivity and can provide particle identification as well as number size distributions and this would normally make them a good choice for monitoring applications. However, solids recovered from DW by filtration usually contain only a few plastic particles mixed together with many other non-plastic particulates. Consequently, obtaining statistically significant data[138] may require that several thousand individual particles be analysed. This makes methods using spectro-microscopy techniques time consuming. For routine monitoring, rather than basis research, sample throughput is a factor which must be taken into consideration. An additional disadvantage of these techniques is that they have minimum size limits which allow them to cover only a part of the particle size range which may be present in DW. In routine operation, as particle size decreases below 10 µm, first µ-FTIR and then µ-Raman methods become increasingly ineffective until at around 1 µm even µ-Raman identification becomes difficult or impossible. It should be noted that these size values are indicative and in practice depend on factors such instrument type and specification, operator skill and the nature of sample.

Both classes of techniques have medium-high investment costs and have a low throughput (typically 1-2 sample/day) but deliver more reliable and more extensive information about the targeted particles than the screening methods.

8.2.2 Polymer specific mass-based techniques

The thermo-analytical techniques such as pyrolysis gas chromatography/mass spectrometry (pyr-GC/MS) or thermal-extraction-and-desorption gas chromatography/mass spectrometry (TED-GC/MS) have a higher per-sample throughput[139] and, in the specific case of pyr-GC/MS, lower investments costs than methods using micro-spectroscopic techniques. However, the calibration which is required to obtain quantitative data is time consuming and challenging as it needs to be done for each type of polymer, which has to be quantified. Furthermore, real-life samples may contain mixtures of polymers and unknown quantities of natural organic matter both of which can have unpredictable effects on the reliability of the measurement. The thermo-analytical techniques have poorer detection limits (mass) than the optical spectroscopic techniques, which may be a critical limitation in the case of the low microplastics levels expected in DW. Thermo-analytical techniques are as such not dependent on the particle size and thus can be used for the analysis of smallest particles, provided that their number are high enough to exceed the (mass-based) detection limit. On the other hand this means that, in contrast to the microscopy-based techniques, the particles' size (and shape) cannot be determined with these techniques.

8.3 Combination of techniques

Multiple orthogonal techniques could be combined to compensate their individual disadvantages. For example, a filter cascade with different pore sizes (*e.g.* 100 µm, 20 µm and 1 µm) could be used to separate particulates into size ranges most appropriate for the two categories of techniques applied. The fraction 1-20 µm could be analysed using pyr-GC/MS while the fractions above 20 µm would then be investigated by micro-spectroscopy techniques such as µ-FTIR, QCL-IR or µ-Raman. The advantage of this approach is that it would provide information on the presence of microplastics over a much wider size range and, in particular, the sub-micron range which may have higher probability of being taken up by the human body though the gastro-intestinal system. The disadvantages of this approach are the significant increase in cost from the need to invest in two different techniques and the technical difficulties of sampling using fine filters with large volumes of water. Additionally, the use of multiple filters of different nominal sizes does not guarantee correct size separation, as phenomena such as filter cake formation may result in the retention of particles which are smaller than the nominal size cut-off of a filter. At this time the relevance of size to risk and hazard assessment is not clearly known and thus the benefits of this more complex and costly approach remain uncertain.

8.4 Polymer specific indicator approach

The analysis strategy would target the detection and quantification of a limited number of polymer types which are most commonly found in DW ('indicator polymers'). This approach could be particularly beneficial for the thermo-analytical techniques as it would simplify the time-consuming calibration steps. In the case of the spectro-microscopy it may provide some more limited benefit by simplifying the data analysis and thus decrease the analysis time. The disadvantage is that polymers not among the pre-defined list of 'indicators' would be missed.

8.5 Tiered approach

On the basis that the primary confirmatory technique would be spectro-microscopy and that these techniques have high cost and low throughput, it could be relevant to consider a tiered approach to the analysis. In such an approach, a screening technique, *e.g.* fluorescence microscopy, would be carried out first (1st tier). If a pre-set threshold is not exceeded, no further analysis is done, while in case the threshold is exceeded, a more specific analysis (µ-FTIR or µ-Raman) is carried out subsequently (2nd tier). Under the assumption that the threshold will not be passed in many (most of the) cases, the overall cost is much lower than applying spectroscopic techniques for all samples. The 2nd tier analysis may be even outsourced to another (specialised) laboratory.

To apply such a tiered approach, thresholds would need to be established and the selection of relevant values will pose some challenges. Considerations for setting the thresholds should include the screening technique's capacity to distinguish plastic from non-plastic materials – *i.e.* the probability of false positive and false negative results – potentially linked to some kind of 'safety margin' to compensate for the impact of the sample matrix and other factors causing deviations from the probability estimates. Since, to date, reliable information regarding such factors is not available in the scientific literature, some research is needed to establish the knowledge for setting suitable thresholds. In addition, the omission of the second tier, re precise analysis, triggered by a false negative result in the first (screening) step, should not lead to a risk for human health. However, there is still very little information on the latter either. Consequently, it is likely that scientifically based threshold values will not be available in the next 1-2 years.

9 Summary and conclusions

Globally, microplastics were shown to be present in drinking water (DW) with sizes ranging from a few µm to several mm. Reported concentrations typically ranged from several hundred particles per litre to values which are often much less than the equivalent of 0.1 particle per litre. In Europe, the analysis of particle composition indicated that about 90% were distributed amongst 7 polymer types with no single polymer type constituting more than 25% of the particle number. When considering specifically studies of EU water supplies, the particle concentrations were all below 1 particle per litre and the majority were significantly below 0.1 particle per litre. These low levels mean that instrument sensitivity will be a key limiting factor in establishing a suitable methodology for monitoring the occurrence of microplastics in drinking water in the EU.

Studies using small (<10 litres) sample volumes, typically reported higher calculated values of particle concentration (above 1 particle per litre) compared to when larger sampling volumes (>50 litres) were adopted. This is likely attributable to higher relative influence of background contamination (from sample processing and handling) on the final concentration values when the sample volumes are low. To reduce this source of error, large sampling volumes are a likely necessity with a minimum of 500-1000 litres being advisable.

In all the reported studies, the particles to be analysed were collected by passing DW through one or more filter(s) and thus reported only those particulates with a size above the cut-off of the finest filter. The majority of studies used filters with cut-off values of 5-10 µm or above and consequently there was little information about the presence of smaller microplastic particulates as these were not retained for subsequent analysis. Although the majority of studies used particle counting methods based on microscopy, very little information about particles size distributions was available with studies reporting only total particle numbers or particle numbers in a few broad size bins. Similarly, there was no information of the size distribution in relation to actual polymer types.

A detailed examination of the literature showed that there are four main analytical techniques currently used in microplastic analysis. Each of these have specific strengths and limitations in terms of the measurement metric (polymer weight or particle number), sensitivity, minimum particle size and ability to identify the polymer type (see Annex A and Annex B). Overall, it appeared that no single analytical technique was fully suited to characterise the nature and extent of microplastic contaminants in drinking water. Consequently, the adoption of a methodology will require some degree of compromise and pragmatic choices must be made regarding the parameters to monitor. Key factors in determining the most suitable method or combination of methods will be the measurement metric, size range, sensitivity and reliability (specificity, accuracy, precision), but additionally the throughput and cost per sample should also be considered.

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List of abbreviations and definitions

Abbreviations	Definitions
ABS	Acrylonitrile butadiene styrene
AC	Acrylic compounds
AFNOR	French Standardisation Association [Association Française de Normalisation]
ASTM	ASTM International, formerly known as American Society for Testing and Materials
ATR-FTIR	Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy
BAM	(German) Federal Institute for Materials Research and Testing [Bundesinstitut für Materialforschung und -prüfung]
CA	Cellulose Acetate
CCD	Charge-Coupled Device
co PE+PP	copolymer polyethylene-polypropylene
CEN	European Committee for Standardization [Comité Européen de Normalisation]
DIN	German Standardisation Organisation [Deutsches Institut für Normung]
DW	Drinking Water
DWD	Drinking Water Directive
EC	European Commission
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy-dispersive X-ray spectroscopy
EU	European Union
EVA	Ethylene-vinyl acetate
EtOH	Ethanol
FluoMic	Fluorescence Microscopy
FPA	Focal Plane Array

Abbreviations	Definitions
FTIR	Fourier-Transform Infrared (spectroscopy)
GC	Gas chromatography
GC/MS	Gas-Chromatography Mass-Spectrometry (analytical techniques combining gas-chromatography and mass spectrometry)
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HPLC	High-performance liquid chromatography
ILC	Inter-laboratory comparison
IR	Infra-Red
ISO	International Organisation for Standardization
JRC	Joint Research Centre
KOH	Potassium hydroxide
LOD	Limit of detection
LDIR	Laser Direct Infrared (imaging)
m	Minimum
MP	Microplastic (vertical axis in Figure 5, Figure 6, Figure 7, Figure 8)
MS	Mass spectrometry
MSFD	Marine Strategy Framework Directive
mW	milliwatt
n.a.	Not available
NMR	Nuclear Magnetic Resonance (spectroscopy)
NR	Nile Red

Abbreviations	Definitions
OECD	Organisation for Economic Co-operation and Development
OpMic	Optical microscopy
PA	Polyamide
PAM	Polyacrylamide
PAN	Polyacrylonitrile
PB	Polybutene
PBA	Poly(butyl acrylate)
PBT	Polybutylene terephthalate
PC	Polycarbonate
PE	Polyethylene
PEST	Polyester other than PET
PET	Polyethylene terephthalate
PI	Poly(isoprene)
PMMA	Poly(methyl methacrylate)
PMPS	Poly(methyl phenyl siloxane)
PMS	Poly alpha-methylstyrene
PP	Polypropylene
PPS	Polyphenylene sulfide
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PTT	Polytrimethylene terephthalate
PU	Polyurethane
PVC	Polyvinyl chloride

Abbreviations	Definitions
Pyr-GC/MS	Pyrolysis-Gas Chromatography Mass Spectrometry
QCL-IR	Quantum cascade laser-Infrared (IR) spectroscopy
SB	Styrene-Butadiene Copolymer
SD	Standard Deviation
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCWRP	Southern California Coastal Water Research Project
SE	Standard Error
SEBS	Styrene-ethylene-butyrene-styrene
SEM	Scanning Electron Microscopy
SEM-EDX	Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis
SPES	Single particle extinction and scattering
TED-GC/MS	Thermal extraction and desorption gas chromatography/mass spectrometry
TWA	Technical working area
TZW	Technologie Zentrum Wasser (German Centre for Water Technology)
UN	United Nations
VAMAS	Versailles Project on Advanced Materials and Standards
µ-FTIR	Fourier-Transform Infrared spectro-microscopy (FTIR spectroscopy coupled to a microscope)
µ-Raman	Raman spectro-microscopy (Raman spectroscopy coupled to a microscope)
µm	micrometre (micron)
WHO	World Health Organization

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Annexes

Annex A Comparison of sample treatment in the analysis of drinking water as described in literature

Sample type (T = tap water; B= bottled water)	Sample volume (L)	Size cut-off of filter (µm)	Filter type (n.a. = data not available)	Pre-Treatment	Recovery %*	Pre-treatment directly on filter	Transfer to analysis filter/unit	Analysis technique	Plastics found (abbreviations see page 66) (n.a. = data not available)	Reference
T	1200-2500	3	stainless steel	compressed air, HCl, EtOH, H_2O_2		+	+	µ-FTIR	PEST, PVC, PA, PE	Mintenig 2019[10]
T	1500	10	stainless steel	detergent, cellulase, tripsin, EtOH		-	+	µ-FTIR	PS, ABS	Johnson 2020[26]
T	1300	5,20,100	stainless steel	sonication	81	-	+	µ-Raman	PE, PET, PP, PA	Pittroff 2021[17]
T	500-1300	10	stainless steel	Hexane, concentrated HCl	55	+	+	Raman	PE,PP PET, PS	Weber 2021[18]
T	1000	1,10,100	stainless steel	detergent, protease, H_2O_2, EtOH	98.5	+	+	Pyr-GC/MS	PE, PA, PET, PP, PS	Gomiero 2021[22]
T	1000	5,50	stainless steel	ciric acid, EtOH	58	-	+	µ-FTIR	PVC, PEST, PS, PE	Weisser 2021[74]
T	200-1000	5	stainless steel	EtOH, detergent, density separation, sonication		-	+	µ-FTIR, Pyr-GC/MS	PEST, PA, PVC, PS, PE, PP, PU, AC	Kirstein 2021[23]
T	(1) 200	(5, 10) 20, 100, 500, 5000	stainless steel	H_2O_2 30%		-	+	µ-Raman	PP, PE PVC, PA, vinylchloride-vinylacetate-copolymer	Wu 2022[75]
T	100	50	paper, glass fibre	detergent, density separation, sonication, H_2O_2,		+	+	µ-Raman	PP, PE, PET, PS, PVC	Chanpiwat 2021[25]
T	100	20, 100, 300, 1000, 3500	stainless steel			-	+	µ-FTIR	PES, PP, PE, ABS, PA	Dalmau-Soler 2021[81]
T	50	10	stainless steel	30% acetic acid, EtOH	90	+	+	µ-FTIR	PE, PP, PET	Feld 2021[13]
T	10	0.22	PTFE	H_2O_2, HCl, EtOH		+	+	µ-FTIR, µ-Raman	PE, PP, PET	Shen 2021[16]
T	10	10, 45, 125, 300, 500	PC, stainless steel	KOH 10%, detergent		-	+	OpMic, µ-Raman	PET, PEST, PE, PAN, PP, PBT, PU, AC	Cherniak 2021[76]
T	9	10	PC		68	-	-	OpMic, µ-Raman	PET, PVC, PA	Yuan 2022[6]
B	4-8	1.5	glass fibre	Nile red		-	-	FluoMic, µ-FTIR	PP, PA, PS, PE, PEST	Mason 2018[140]
T	2.4-6	0.45	nitrocellulose	Nile red		-	-	FluoMic, µ-FTIR, µ-Raman	PET, PE, PP, PA	Kankanige 2020[77]
B	3	0.2	aluminum oxide	detergent, EtOH	86	-	-	µ-FTIR	PE, PS	Weisser 2021[74]
T	2.5	0.2	PTFE	H_2O_2 Fenton, 75 °C		-	-	OpMic, µ-FTIR	PES, PE, PP, cellophane, PA, polytrimolithicamide	Radityaningrum 2021[141]
B	0.7-1.5	3	Au coated PC			-	-	µ-Raman	PET, PE, PS, PP	Schymanski 2018[142]
T	1	1.7	glass fibre	H_2O_2 <1%, 24h		-	-	µ-FTIR	PEST, PS, PA	Chu 2022[7]

Sample type (T = tap water; B= bottled water)	Sample volume (L)	Size cut-off of filter (µm)	Filter type (n.a. = data not available)	Pre-Treatment	Recovery %*	Pre-treatment directly on filter	Transfer to analysis filter/unit	Analysis technique	Plastics found (abbreviations see page 66) (n.a. = data not available)	Reference
T	1	0.2	PTFE, aluminum oxide	1 M H_2SO_4		-	-	SEM, µ-Raman	CA, PET, PVC, PE, PP, EVA, PBA, PTT	Pivokonský 2020[79]
T	1	7	glass fibre	KOH 15%, density separation, Nile red		-	+	FluoMic	n.a.	Ferraz 2020[19]
T	1	2.7	glass fibre	Rose Bengal		+	-	OpMic	n.a.	Lam 2020[20]
T	1	0.22	nitrocellulose			-	+	FluoMic, SEM-EDX, µ-Raman	PTT, epoxy resin	Shruti 2020[24]
B	1	0.45, 53, 300, 500	nitrocellulose	Nile red		-	-	FluoMic, µ-FTIR	PC, PE, PP, PET	Kankanige 2020[9]
T	1	0.2	PC	Nile red HCl		+ -	+ +	FluoMic µ-Raman	PE, PP, PPS, PET, PB, PMMA, PA, PMS, PVC, PBT, PTFE (by µ-Raman analysis)	Tong 2020[8]
T	1	0.7, 1.2	glass fibre			-	-	Pyr-GC/MS	PVC, PE	Asmundsdottir 2020[21]
T	1	0.45	n.a.			-	-	OpMic, Pyr-GC/MS	PE	Panno 2019[84]
T	1	0.2, 5	PTFE	H_2O_2		-	+	µ-FTIR, µ-Raman	PAM, PET, PE, PP, PVC	Pivokonsky 2018[85]
T	0.5	26	stainless steel	drop of H_2O_2 30%, 60 °C		+	-	µ-FTIR	PS, PP, PES, PE, SEBS, PVC	Mukotaka 2021[15]
T	0.5	na	n.a.	no		-	-	FluoMic	n.a.	Pratesi 2021[14]
T	0.5	2.5	cellulose	Rose Bengal		+	-	OpMic	n.a.	Kosuth 2018[11]
T	0.25	2.5	cellulose	Rose Bengal		+	-	OpMic, FluoMic	n.a.	Paredes 2019[86]
B	0.25	0.4	Al coated PC	EDTA, detergent		-	-	µ-Raman	PET, PE, PP, SB	Oßmann 2018[83]
T	0.25	5	PTFE	H_2O_2		-	+	µ-Raman, SEM	PET, PE, PP, PAM, PS, PVC	Wang 2020[87]
B,T	0.2	0.2	aluminum oxide	no		-	-	µ-FTIR	PE, PS, PET, PP, PU, PA	Almaiman 2021[88]
T	0.05	8	silicon nitride	Nile red		+	-	FluoMic, EDX	n.a.	Madejski 2020[89]

Source: own production

* When recovery were reported with different values in relation to the plastic type, the minimum value was included in the table

Annex B Comparison of key aspects of main instrumental techniques

Aspects	Fluorescence Microscopy	Pyrolysis GC/MS	Raman Spectro-microscopy	Infrared Spectro-microscopy
Principle	Samples are treated with a dye which preferentially makes hydrophobic surface, such as plastic ones, fluorescent. When illuminated with light of a suitable wavelength and viewed through an optical microscope the stained particles (plastics) are highlighted with respect to the more hydrophilic material (such as mineral particles).	Thermal decomposition of polymers by heating in an oxygen-free atmosphere (pyrolysis) resulting in polymer-specific decomposition products, which are identified/quantified (mass) by gas chromatography-mass spectrometry (GC/MS).	Particles are illuminated with monochromatic light from a focused laser beam. Interactions with the molecular bonds in the material generates scattered light, which has higher or lower wavelength than the incident light. The scattered light is collected and analysed by a spectrometer resulting in a spectrum, which is characteristic for the material. Comparison of the spectrum with a library of known spectra permit the identification of the material.	The material is illuminated with infra-red light and the transmitted or reflected light is collected and analysed to determine the relative intensity at each frequency (or wavelength). The resulting IR spectrum is characteristic for the material analysed and comparison with a library of known spectra permit the identification of the unknown material.
Price (Estimate)	20-40,000 euro	Laboratories may be able to upgrade an existing GC/MS system by retrofitting a pyrolyser unit and optionally adding an autosampler. Pyrolysis unit: 30.000 euro. Autosampler for pyrolisis unit: 25.000 euro. GC/MS: 80.000 euro	200.000 euro	300.000 euro
Analysis time	<1 hour per sample	Approximately 0.5-1 hour per sample (depends on the GC settings). In addition, at least one calibration curve is required with at least 3 points that corresponds to 0.5-1 hour of analysis each. Total time: several hours, depending on number of identified polymers and of points on the calibration curve.	3-6 hours for the analysis of a sample with about 1000 particles.	For particles collected on a 13 mm filter: 8-24 hours for the analysis of one sample by chemical mapping by µ-FTIR. Times for QLC-IR method is significantly less but not known
Information that can be obtained	Number, size, size-distribution, shape of polymer particles	Identification of polymer(s), mass per polymer	Number, size, size-distribution, shape and identification of polymer(s)	Number, size, size-distribution, shape, identity of polymer(s)
Min. measurable size	1-5 µm	(None)	≈1 µm	≈5 -10 µm
Min. measurable size in routine operation	5-10 µm	(None)	5 -10 µm	15-20 µm
Min. mass that can be determined	Not applicable	Depends on polymer. Typically around 1 µg (correspond to 1 spherical particle with diameter 60 µm)	pg (estimate by calculation from size and polymer density)	ng (estimate form size and polymer density)
Destructive/Non-destructive	Non-destructive, but particles are modified (stained)	Destructive BUT resulting pyrograms can be re-evaluated retrospectively for further indicator ions of new polymers (semi-quantitative)	Non-destructive (at careful selection of laser power)	Non-destructive
Main interferences	The selectivity of the staining dye for polymers is not absolute and false positive/negative results may occur to an unknown extent.	Natural organic matter with decomposition products similar to those of plastics	Coloured particles, pigments, fluorescence, fatty acids, amides, proteins. Coloured particles and fluorescence can be seen as well by changing laser wavelength	Particles loaded with carbon black. Presence of water, difficulty identifying mineral particles, protein that can be confused with PA, fatty acids, amides.

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