



ADVANCING TOWARDS LITTER - FREE
ATLANTIC COASTAL COMMUNITIES
BY PREVENTING AND REDUCING
MACRO AND MICRO LITTER

GUIDELINES FOR MICROPLASTICS QUANTIFICATION AND ANALYSIS

WORK PACKAGE 2: ASSESSING AND MONITORING MICRO AND MACRO
LITTER CONTAMINATION. ACTIVITY 2: IMPROVED MICROPLASTICS
MONITORING, TASK 4: MICROPLASTIC QUANTIFICATION AND ANALYSIS



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1. BACKGROUND

Primary and secondary microplastics are increasingly becoming a focus of concern due to their pervasive presence in the environment (Kamel et al., 2024). Despite the numerous studies already published on microplastic monitoring, there is still an urgent need to establish harmonised guidelines for the collection, quantification and reporting of plastic particles. Such guidelines are essential to enable comparisons among different locations/studies and to support the implementation of effective mitigation actions (Bessa et al., 2019; Bhardwaj et al., 2024; Borja & Elliott, 2021).

Microplastic monitoring involves several steps that must be fully optimised and validated to ensure reliable reporting on the occurrence and characterisation of microplastics. This is particularly relevant for baseline assessments, for instance within the framework of the Marine Strategy Framework Directive (MSFD) (MSFD Technical Group on Marine Litter, 2023). However, monitoring microplastics remains a major challenge. Owing to its small size, microplastics is hard to detect, highly pervasive and associated with numerous potential impacts that are still poorly understood (Ramos et al., 2024). In recent years, growing efforts have focused on improving the detection and monitoring of microplastics pollution, including attempts to standardise sampling, sample processing and analytical methods (Bessa et al., 2019; MSFD Technical Group on Marine Litter, 2023; OSPAR, 2024). Nevertheless, further work is still required to strengthen monitoring capabilities through the development and implementation of harmonised protocols.

In this context, Work Package 2 (WP2), Activity 2 of the Free LitterAT project aims to propose harmonised guidelines for microplastic monitoring in aquatic environments across multiple matrices, including water, sediment and biota. Activity 2 focuses on proposing optimised protocols for sampling, sample processing and preparation for microplastic analysis, as well as on testing, improving and refining both current and innovative methods used for microplastic quantification and characterisation (WP2 A2 D2.2.3; WP2 A2 D2.2.4). These developments will contribute to the generation of validated, comparable and reproducible data on microplastic contamination in aquatic environments, supporting the establishment of robust baselines in line with the MSFD.

This Deliverable addresses the different methodologies available for the quantification and characterisation of microplastic, including visual assessment and microplastic polymers analysis, highlighting the analytical challenges associated with microplastic identification across diverse environmental matrices. It follows WP2



Deliverable 2.2.1. *Harmonised Sampling Guidelines to Monitor Microplastics in Different Matrices*, that addresses the complexities and methodological challenges of microplastics sampling and Deliverable 2.2.3 *Harmonised Protocols for Sample Preparation: Microplastics Extraction and Isolation* that proposes guidelines for sample preparation for microplastics extraction and isolation. All three deliverables are available online on the Free LitterAT project website (<https://freelitterat.eu/assessing-and-monitoring-marine-litter/>) and in Zenodo:

- D2.2.1. - <https://doi.org/10.5281/zenodo.21035158>;
- D2.2.3 - <https://doi.org/10.5281/zenodo.22771751>;
- D2.2.4. - <https://doi.org/10.5281/zenodo.22771805>.

This document provides a comprehensive overview of the main analytical techniques used for polymer characterisation, including spectroscopic and chromatographic methods, outlining their principles, required materials and instrumentation, and applicability to different types of microplastics. The constraints, limitations and suitability of each technique are also discussed in relation to the study objectives and sample characteristics. In addition, this Deliverable includes a dedicated section focused on quality control and assurance, to minimise contaminations and ensure consistent and robust characterisation results across studies.

The content of this Deliverable is built upon the European Union's Guidance on Monitoring of Marine Litter in Europe (MSFD Technical Group on Marine Litter, 2023) which addresses harmonised approaches for microplastic monitoring, including recommendations on visual classification, polymer identification methods, selection of particles for analysis and quality assurance procedures. These are complemented by other official guidelines, such as GESAMP (GESAMP, 2019) and OSPAR Microplastic Expert Group (OSPAR, 2024). In addition, it is supported by a comprehensive literature review (Araujo et al., 2018; K  ppler et al., 2016; L  der et al., 2015; Ourgaud et al., 2022; Primpke et al., 2018; Renner et al., 2019) and inputs from the practical expertise gained by the project team (Almeida et al., 2023; Espincho et al., 2024; Moura et al., 2025; Rodrigues et al., 2025; Rodrigues et al., 2019; Silva et al., 2022) and its partners.

By addressing visual and polymer-based characterisation in environmental matrices, recognising current analytical challenges and methodological limitations, and indicating harmonised quality control and assurance recommendations, this Deliverable provides a comprehensive framework to strengthen the reliability, consistency and comparability of microplastic characterisation across studies.



2. MICROPLASTIC VISUAL CHARACTERISATION

2.1. Optical microscopy

Optical microscopy is, generally, the first step of microplastic quantification and visual characterisation, performed using a stereomicroscope. Through this method it is possible to quantify the microplastics present in a sample, characterising them by morphology, size, colour and transparency. This technique is non-destructive and can be carried out prior to polymer identification.

Microplastics are visually categorised, when applicable, according to the following characteristics:

1. Size
2. Morphology
3. Colour
4. Transparency (optional)

2.1.1. Size classification

Microplastic particles are commonly classified according to their largest dimension. In accordance with MSFD recommendations (MSFD Technical Group on Marine Litter, 2023), microplastic sizes are grouped into classes based on particle dimensions (Table 1). These size classes are prioritised for routine monitoring and reporting. Nevertheless, further subdivision may be applied depending on the specific objectives of the study. For instance, the 1000-4999 μm size class may be subdivided into 1000-1999 μm and 2000-4999 μm . The smaller size classes listed as optional in Table 1 may require more advanced analytical capabilities than those of simple optical microscopy.

Size classification should preferably be based on direct measurements of individual particles, when technically feasible. The size assigned to each particle should correspond to its largest measured dimension, such as the maximum particle length or maximum Feret diameter, unless a different approach is explicitly justified and consistently applied.

Direct image-based measurement is generally the preferred approach for size classification of microplastics and may be performed using optical microscopy, stereomicroscopy, $\mu\text{-FTIR}$, $\mu\text{-Raman}$, LDIR, or image-analysis software. Sieving may be



used as practical pre-fractionation step, especially for high-volume samples. However, sieve mesh size should not automatically be considered equivalent to the actual size of individual particles. This is particularly important for elongated, flexible, or irregular particles, such as fibres, which may pass through a mesh smaller than their real length due to their small diameter or flexible shape. Gridded filters may support visual inspection and approximate size estimation by comparing particles with the known grid spacing of the filter. This approach can be useful for assigning particles to broad size classes, but it should be reported as an approximate estimation rather than a precise dimensional measurement.

TABLE 1: SIZE CLASSES USED FOR MICROPLASTIC CLASSIFICATION

Size range classes (µm)	Classification
1000 – 4999	Recommended
300 – 999	Recommended
100 – 299	Recommended
50 – 99	Optional
20 - 49	Optional
< 20	Optional

Source: Adapted from MSFD Technical Group on Marine Litter, 2023

2.1.2. Morphology

Microplastic morphology can be a key parameter for understanding sources, transport pathways and potential ecological impacts, with microplastics shape influencing buoyancy, degradation behaviour and interactions with biota.

Table 2 presents the main microplastic morphological classes, defined in accordance with the framework of the MSFD and complemented by additional morphologies commonly encountered based on the team's experience. These categories reflect the most reported and observed shapes and forms of microplastics in environmental samples.



TABLE 2: MORPHOLOGICAL CLASSES USED FOR MICROPLASTIC CLASSIFICATION

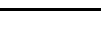
Class	Description	Possible sources
Filament	Threads	Fragmentation of ropes, strings, fishing gear, nets, textiles and other fibrous plastic materials associated with marine, agricultural, industrial and domestic activities
Fibre	Thin, elongated	Wearing, use and wash of textiles
Fibre bundle	Bundle of fibres Cannot be easily disentangled	Wearing, use and wash of textiles
Fragment	Rigid and thick Irregular shape With sharp crooked edges	Breakdown of larger plastic materials
Film	Thin, typically bends without breaking Thinner and more flexible than a fragment	Plastic sheets or film packaging
Foam	Compressible Generally, maintains shape when pressured Various shapes	Breakdown from larger foam products (e.g. expanded polystyrene)
Pellet	Spherical to cylindrical Diameter > 1 mm	Primary industrial pellets from plastic manufacturing and bio-beads or plastic media from wastewater treatment plants
Sphere	Spherical Diameter ranging from a few μm to a few mm	Personal care products, abrasives, etc

2.1.3. Colour

The colour of microplastic particles should be recorded whenever possible, as it can provide relevant information regarding the origin and weathering state. In accordance with MSFD recommendations (MSFD Technical Group on Marine Litter, 2023), microplastics can be classified into 13 standardised colour categories (Table 3). However, colour classification may not always be possible. For example, when Nile Red staining is used for microplastic quantification, the original colour of the particles may be masked or altered by the fluorescent dye. Therefore, colour should be recorded only when particles are analysed without staining.



TABLE 3: COLOUR CLASSES USED FOR VISUAL CLASSIFICATION OF MICROPLASTICS. COLOUR SWATCHES ARE ILLUSTRATIVE REFERENCES ONLY.

Colour class	Colour swatch
Black	
Grey	
White (including cream)	
Red	
Orange	
Yellow	
Green	
Blue (including cyan)	
Purple (including violet)	
Pink (including magenta)	
Brown (including tan)	
Multicolour (mix of two or more)	
Colourless (without added dyes, pigments or additives)	



NOTE: Colour should be assessed independently from transparency - a particle may be colourless but not transparent or it may have a visible colour while translucent.

2.1.4. Transparency

Transparency is included as one of the visual descriptors used for the preliminary characterisation of microplastic particles, supporting standardised characterisation of microlitter. It may be influenced by factors such as polymer type, particle thickness, surface weathering, degradation state and the presence of pigments or additives. The classes are presented in Table 4.

TABLE 4: TRANSPARENCY CLASSES USED FOR MICROPLASTIC CLASSIFICATION

Classification	Definition
Opaque	Particles that do not allow the light to travel through them
Transparent/translucid	Particles that allow light to travel through them either almost unaltered (transparent) or with some diffraction (translucid or semi-transparent)

Source: Adapted from MSFD Technical Group on Marine Litter, 2023

NOTE: Transparency should not be confused with being colourless, as a particle may have a visible colour while still being transparent or translucent.

2.2. Fluorescence staining and detection

Fluorescence staining is a distinct analytical approach that can be used either as a complementary or as an alternative approach to conventional visual detection. This approach uses fluorochromes to generate a fluorescent signal associated with stained particles, facilitating their detection and quantification. When used as a complementary approach, fluorescence staining can enhance the detection of microplastics that may be difficult to identify under the stereomicroscope, and the detected particles can subsequently be characterised according to the morphological criteria described above. Alternatively, fluorescence-based detection may be used as the primary screening approach for microplastics quantification, depending on the objectives of the study.



The use of fluorochromes provides a rapid and cost-effective approach for the quantification of microplastics in environmental matrices. These dyes exhibit an affinity for polymeric materials and emit a fluorescent signal when excited at an appropriate wavelength. Among the available fluorochromes, Nile Red is one of the most widely used due to its high staining efficiency and extensive application in microplastic analysis protocols (Maes et al., 2017; G rigny et al., 2024).

Glass fibre filters containing the environmental samples are treated with a fluorochrome solution prior to examination under a fluorescence stereomicroscope. The fluorescence filter cube (prism), including the excitation and emission filters, must be selected according to the spectral properties of the fluorochrome used to maximise the contrast between stained particles and the sample background.

This approach enables the rapid quantification of microplastics and, where applicable, their classification according to morphological categories. However, fluorescence staining does not provide chemical confirmation of the observed particles. Despite this limitation, its simplicity, low analytical cost, and high sample throughput make it a valuable complementary or alternative approach for environmental monitoring programmes, particularly where a cost-effective assessment of microplastic contamination is required.



3. MICROPLASTIC POLYMER ANALYSIS

Polymer analysis is performed to determine the chemical composition of suspected microplastic particles and to distinguish synthetic materials from natural or non-plastic materials. This step is essential to improve the reliability of the results, as visual identification alone may lead to misclassification, particularly for small and weathered particles.

Depending on the number of particles identified, the available analytical capacity and the objectives of the study, polymer analysis may be performed on all particles or on a representative subset. When only a subset is analysed, particle selection should reflect the diversity of the visually identified suspected microplastics in each sample, considering the time and resource constraints associated with the analysis.

Polymer identification is typically carried out after visual characterisation using spectroscopic techniques, such as Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, Laser Direct Infrared spectroscopy (LDIR). These techniques provide information on the polymer composition of individual particles and are generally minimally destructive or non-destructive. With these methods, polymer identification can be performed on a group of particles, for instance, directly on particles retained in a filter when using μ -FTIR or μ -Raman.

Polymer identification can also be performed using other methods such as pyrolysis gas chromatography coupled with mass spectrometry (Py-GC/MS). Py-GC/MS is a quantitative and destructive method which can be used to identify and quantify mass of individual polymers in a sample (but not the number of particles). It can be used to analyse individual particles or groups of particles. To obtain the polymer composition of individual particles, each particle has to be analysed individually.

3.1. Analysed Sample

The polymer composition should be determined for at least a representative subset of visually identified suspected microplastic particles. The selected subset should reflect the diversity of particles observed in each sample and support the validation of visual identification.



It is recommended that polymer composition be analysed for:

- at least 10 % of the particles identified as potential microplastics across the full sample set;
- a minimum of 20 particles per sample, whenever possible;
- all particles in samples containing fewer than 20 potential microplastic particles.

When it is not feasible to analyse all suspected microplastic particles, the analysed subset should, whenever possible, be selected proportionally, i.e., considering the relative abundance of each morphological category observed in the sample and should, where applicable, include particles of:

- relevant size categories (should cover the 100 µm to 1000 µm size range);
- different morphologies, such as fibres, fragments, films, spheres or pellets;
- different colours;
- different transparency classes.

In addition, it should include:

- particles with uncertain visual identification;
- particles that may be confused with natural or non-plastic materials.

The number of particles analysed, the selection criteria applied, and the proportion of particles confirmed as synthetic polymers should always be reported. In accordance with Commission Regulation (EU) 2023/2055, non-chemically modified cellulose and other non-chemically modified natural polymers are excluded from the designation of “synthetic polymer microparticles”. Nevertheless, as they can have anthropogenic origin, they should always be reported. Where relevant, such particles should be recorded separately as natural or non-plastic particles, with indication of their material.

3.2. Techniques

Analytical techniques should be used for the polymer identification of suspected microplastic particles. The selected technique should be appropriate for the expected particle size range, sample matrix and monitoring objectives and should provide reliable information that can be compared with validated reference libraries.



FTIR, μ -FTIR, Raman spectroscopy, μ -Raman and LDIR are suitable spectroscopic techniques for routine polymer confirmation of individual particles (Table 5). Py-GC/MS may also be used for polymer identification, particularly for confirmatory analysis, complex matrices or mass-based determination. Other techniques may be used when appropriate and validated (as described in Deliverable 2.2.5 – *Report on the potential of Pyrolysis-GC/MS and Rock-Eval® techniques to monitor small microplastics*).

For spectroscopic methods, polymer assignment should be supported by an adequate spectral match with reference libraries. According to recommendations provided by the MSFD technical group on marine litter (Galgani et al., 2013), matches above 70% can be considered strong evidence of the polymer type, whereas matches between 60% and 70% require manual verification and matches below 60% should not be accepted as reliable polymer identification, although they can still be reported if the match percentage is clearly indicated. However, spectral match thresholds are technique-, sample type- and library-dependent and should therefore be established and verified on a case-by-case basis. Acceptance criteria should be validated using polymers of known identity and, ideally, reference materials that include partially degraded particles representative of environmental samples. Based on the research team's studies, a threshold of 75% is recommended for FTIR polymer analysis, exceeding the minimum guidance value (Almeida et al., 2023; Espincho et al., 2024; Rodrigues et al., 2019; Silva et al., 2022). For microplastics retrieved from biota samples, the match threshold may be lowered to 65%, considering the higher possibility of mismatch due to particle degradation (Moura et al., 2025; Rodrigues et al., 2025).

Spectral libraries used for polymer composition determination should include spectra from synthetic polymers, organic or natural materials, and, if possible, weathered synthetic polymers, to improve the reliability of polymer identification and reduce the risk of misclassification. For Py-GC/MS, polymer identification should be based on characteristic thermal degradation products, validated pyrograms, reference polymers and/or suitable mass spectral libraries. The technique used, instrument settings, spectral library, polymer match threshold and any deviations from the minimum acceptance criteria should be recorded and reported.



TABLE 5: ANALYTICAL TECHNIQUES FOR MICROPLASTIC POLYMER IDENTIFICATION

Technique	Details
FTIR / μ -FTIR	Recommended method General acceptance for polymer match: >70%
Raman/ μ -Raman	Suitable particularly for smaller particles General acceptance for polymer match: >70%
LDIR (Laser Direct Infrared Spectroscopy)	Suitable for automated and high-throughput polymer identification
Py-GC/MS	Suitable for complex matrices and mass-based polymer determination
Other techniques	May be used when appropriate and properly validated

For FTIR analysis, suspected microplastic particles should be deposited or transferred onto a suitable infrared-compatible filter, window or substrate, depending on the analysis mode used, such as transmission, reflection or attenuated total reflectance. Sample preparation should minimise matrix residues, organic matter, particle overlap and substrate-related contamination, which may interfere with infrared spectral acquisition and polymer identification (Löder et al., 2015; Käßler et al., 2016; Primpke et al., 2018). For μ -FTIR analysis, suspected microplastic particles may be analysed directly on a suitable infrared-compatible filter or substrate, particularly in mapping or imaging modes. Where required, particles may also be transferred to an appropriate window or substrate depending on the analysis mode and instrument configuration. The FTIR mode, analysed or scanned area, size detection range, spectral match threshold, polymer library and quality control criteria should be documented.

In Raman and μ -Raman analysis, suspected microplastic particles should be deposited or retained on suitable low-background filters or substrates at a particle density that allows reliable detection and spectral acquisition. The substrate should not generate excessive fluorescence interference. Sample preparation should minimise fluorescence, matrix residues, organic matter and particle overlap, as these may compromise spectral quality and polymer assignment (Käßler et al., 2016; Araujo et al., 2018). μ -Raman is particularly useful for small particles, including those below the lower size range typically accessible by conventional Raman methods. Laser wavelength, laser power and acquisition time should be optimised to obtain spectra of sufficient quality while minimising thermal degradation or burning, especially for small, dark, weathered



or highly absorbing particles. Potential limitations, including fluorescence, thermal degradation and longer acquisition times, should be considered (Araujo et al., 2018; Renner et al., 2019). Laser wavelength, power, acquisition time, analysed area, size detection range, spectral match threshold, reference library and quality control criteria should be documented.

LDIR-based analysis requires filtration of the sample onto a suitable membrane filter or transfer to IR reflective low-e slides. Samples should be prepared to minimise matrix residues and organic matter and automated detection settings should be configured for the expected particle size range and sample matrix (Ourgaud et al., 2022). Since LDIR analyses particles directly on the filter or slide, it is also suitable for high throughput microplastic analysis. The filter or substrate type, scanned area, size detection range, spectral match threshold, polymer reference library, automated detection settings and quality control criteria, should be documented.

For Py-GC/MS analysis the filter material should be compatible with pyrolysis or the retained material should be transferred to a suitable pyrolysis cup or sample holder. This technique is particularly useful for determining polymer composition and mass-based concentrations. Pyrolysis conditions should be optimised to generate characteristic polymer degradation products while avoiding excessive degradation, carry-over or matrix-related interferences (recommendations provided in the Deliverable 2.2.5.). Appropriate polymer-specific marker compounds, calibration standards and reference materials should be used where quantitative analysis is required. Potential limitations include destruction of sample, limited particle-level information, matrix effects, overlapping pyrolysis products and the need for suitable calibration (Dümichen et al., 2017; Fischer & Scholz-Böttcher, 2017; Fries et al., 2013; GESAMP, 2019; Hermabessiere et al., 2018). The sample mass, pre-treatment steps, pyrolysis temperature and time, GC/MS conditions, target polymer markers, calibration, limit of detection and quantification, polymer reference library or marker database, blank correction and quality control criteria should be documented.



4. QUALITY ASSURANCE AND QUALITY CONTROL

4.1. Quality Assurance

To reduce contamination and ensure the reliability of the results obtained, some quality assurance measures should be applied during particle characterisation and polymer identification (Table 6).

TABLE 6: PARTICLE CHARACTERISATION QUALITY ASSURANCE

Category	Best Practices
Workspace	Clean the laboratory regularly Minimise air circulation Clean workspace with 70% ethanol filtered through the same type of filter used in analyses
Operators	Wear cotton lab coats (preferably bright or uncommon colours) Minimise the presence of operators in the work area
Material & Equipment	Use glass and metal tools Avoid plastics/synthetics Clean all equipment/tools with 70% ethanol and deionised water before use and between samples Clean the equipment used for polymer identification with ethanol 70% between each particle analysed (when applicable)

4.2. Quality Control

To ensure sample integrity and detect potential contamination during visual particle characterisation, a blank control should be placed near the sample during the analysis:

- place a Petri dish containing deionised water near the stereomicroscope;
- open the Petri dish only when the sample is uncovered;
- use one control per sample;
- report both control results and uncorrected sample results.

Additionally, the analysis of procedural blanks (at least one per batch of samples) allows the overall contamination introduced during the analytical process to be assessed. The levels measured in these blanks, as well as in the blanks mentioned above, should be reported but should not be subtracted from the values obtained for the samples.



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