Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water

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1.0 Scope and Application

1.1 This method is for the determination of microplastics (State Water Resources Control Board, 2020) greater than 50 µm in size in treated drinking water using visual microscopy for particle counts, and Infrared (IR) spectroscopy for chemical identification of counted particles. IR spectroscopy can include, but is not limited to, Fourier Transform IR (FTIR), Laser Direct Infrared (LDIR) Imaging, Optical-Photothermal IR (O-PTIR), and other techniques capable of measuring microplastic particles as small as 50 µm. This method is for use in the California EPA's data gathering and monitoring programs and Section 116376 of the California Health and Safety Code. The method is based on peer-reviewed literature and the results and recommendations from an international microplastic method evaluation study carried out by the Southern California Coastal Water Research Project Authority (SCCWRP).

1.2 Sample collection protocols are not within the scope of this method. Example procedures for sampling drinking water are available (ASTM, 2020), e.g., volumes that can range up to 1500 L collected by inline sieving in which water is passed through sieves directly from the site sampled. This protocol presumes that samples are in a form amenable to sieving and filtration in the laboratory (i.e., relatively low volumes of up to 20 L, including those in samples collected from inline sieving into collection containers that can then be processed by this method).

1.3 The lowest particle size reliably detected by this method is 50 µm, with a maximum size of 5000 µm (i.e., the State definition of microplastics, State Water Resources Control Board, 2020) based on the sieves specified in the method. While the extraction procedures in this method have been applied to particles <50 µm (De Frond et al., 2021), this method has not been validated for this size fraction.

1.4 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The lowest concentration MRL (LCMRL) is the lowest true concentration for which a future analyte recovery is predicted with at least 99 percent confidence to fall between 50 and 150% (Martin et al., 2007). Single laboratory LCMRLs for microplastics in this method, based on analysis of 0.450 L interlaboratory comparison single-concentration spike (Foreman et al., 2021) blind-samples (De Frond et al., 2021) are 6.95 particles (>500 µm), 3.70 particles (212-500 µm), and 5.80 (50-212 µm) particles. These values are independent of the extracted water volume. Each laboratory must establish its own LCMRL, which may differ from the values noted here from the SCCWRP interlaboratory evaluation study (De Frond et al., 2021).

1.5 Microplastics are present in indoor air, and it is impossible to eliminate background contamination from airborne particles within the laboratory. This method includes suggestions for improvements in facilities and analytical techniques to maximize the ability of the laboratory to report reliable microplastic particle counts and minimize particle contamination throughout sample

processing and analysis (Section 4.0).

1.6 This method shall be used only by analysts who are experienced in the use of microscopic and spectroscopic techniques and who are thoroughly trained in the sample handling and instrumental techniques described in this method. Each analyst who uses this method must demonstrate the ability, using this procedure as detailed in Section 11.0, to generate acceptable results as noted in the quality assurance in Section 9.0.

2.0 Summary of Method

This method extracts microplastic particles from drinking water samples, and other water samples with low levels of suspended particulate matter and organic material, using sieving and vacuum filtration. Each sample is split into size fractions with separation at 500 µm, 212 µm and 20 µm (to maintain consistency between Raman and IR methods), and particles are collected onto filters or into glass containers prior to microscopic and spectroscopic analysis. Processed samples are viewed using stereomicroscopy and microplastic particles are identified. For the identification of material type, a representative subsample of particles is selected and prepared for IR spectroscopy by presentation either on a filter surface or on a glass slide. Each subsampled particle is measured and photographed to make a permanent record of the sample, then chemically identified individually using IR spectroscopy. The instrument is calibrated and run through performance checks prior to use, and spectra are matched using relevant spectral reference libraries. The proportion of particles confirmed to be microplastics via IR spectroscopy is applied to total counts from microscopy to provide an estimate of microplastic particles per liter. This method can reliably detect microplastic particles down to 50 µm in size.

3.0 Definitions

Analysis batch – A set of samples, excluding QC samples, extracted together by the same person(s) during a workday (e.g., 8 hours) using the same lot of solvents, reagents, and consumables. The specific number of samples in an analysis batch is dependent on the volumes of samples collected. For small volume samples, such as those from the SCCWRP intercalibration study (ca. 0.45 L), an analysis batch can consist of 20 field samples at maximum.

Dry Sorting – The process of identifying, counting and visually characterizing suspected plastic particles directly on the filter paper surface following sample extraction, using visual microscopy. The base of the petri dish that holds the filter can have a grid sticker attached to the outer base, allowing systematic counting of particles within each grid until the sample has been fully analyzed and all suspected particles on the filter have been identified and visually characterized using microscopy.

Field reagent blank (FRB) – An aliquot of MAG water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if

method analytes or other interferences are introduced into the samples during shipment and collection. At least one FRB must be collected and analyzed for each set of field samples from a sample collection period. The volume of the FRB must be similar to that of actual samples collected and processed by this method. FRBs differ from trip blanks in that the former evaluate contamination during both shipment and collection, while the latter only account for contamination during shipment.

HEPA filter – high-efficiency particle absorbing filter, capable of removing 99.97% of atmospheric particles of 0.3 mm diameter.

High-purity water - Reverse osmosis water, 18 MΩ-cm nanopore/MilliQ water, or deionized water.

Laboratory Fortified Blank (LFB) – Sample of MAG water of the same volume as test samples, to which known quantities of microplastic particles have been added. These particles may be derived from the laboratory using this procedure. The LFB is analyzed in the same manner as a sample, including the preservation procedures in Section 8. The LFB is used during the Initial Demonstration of Capability to verify method performance for precision and accuracy. Procedures for generating LFBs are available for particles between 100-300 mm (ASTM, 2021) and 30-200 mm (Seghers et al., 2021).

Laboratory Fortified Matrix (LFM) – Sample of MAG water of the same volume as test samples, to which known quantities of microplastics particles have been added. Unlike an LFB, these particles must come from approved sources and may not arise from the laboratory using this procedure. The LFM is analyzed in the same manner as a sample, including the preservation procedures in Section 8. The LFM is used during the Initial Demonstration of Capability of a laboratory in the accreditation process to verify method performance for precision and accuracy. Procedures for generating LFMs are available for particles between 100-300 mm (ASTM, 2021) and 30-200 mm (Seghers et al., 2021).

Laboratory Reagent Blank (LRB) - Sample of MAG water of the same volume as test samples and run through the same laboratory procedures as test samples. The laboratory reagent blank is used to monitor particles introduced via procedural contamination.

Lowest Concentration Minimum Reporting Level (LCMRL) – is the lowest true concentration for which a future analyte recovery is predicted with at least 99 percent confidence to fall between 50 and 150% (Martin et al., 2007, Winslow et al., 2006).

Microplastics - Solid[1] polymeric materials[2] to which chemical additives or other substances may have been added, which are particles[2] which have at least three dimensions that are greater than 1 nm and less than 5,000 µm. Polymers that are derived in nature that have not been chemically modified (other than by hydrolysis) are excluded. (State Water Resources Control Board, 2020).

Microplastics-analysis-grade (MAG) water – high-purity water filtered through a filter with pore-size 1 µm or smaller (of any appropriate material; glass fiber filters are suitable) and used as reagent water and to rinse apparatus in this procedure.

Minimum Reporting Level (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value in a sample following analysis. Please see LCMRL for this method, except as noted (Section 9.2.4).

Trip Blank – A sample of MAG water of the same volume as test samples, taken from the laboratory to the sampling site and returned without having been exposed to sampling procedures. The trip blank is to assess contamination introduced during shipping and storage only and must be present for each set of field samples from a sample collection period.

Wet Sorting – The process of identifying, counting and visually characterizing suspected plastic particles from a sample that has been extracted into size fractions and transferred from a sieve into a glass jar following sample extraction. Using a metal teaspoon, the contents of the glass jar (i.e., the extracted size fraction of the sample) is transferred into a clean glass petri dish in small amounts, (e.g., one spoonful at a time). By placing a grid sticker on the outer base of the petri dish used for particle sorting, suspected plastic particles within each grid and around the inner edge of the petri dish are counted and visually characterized. The petri dish is rinsed after all suspected plastic particles are counted, before moving on to the next spoonful. The process is repeated until the jar is empty.

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[1] 'Solid' means a substance or mixture which does not meet the definitions of liquid or gas. 'Liquid' means a substance or mixture which (i) at 50 degrees Celsius (˚C) has a vapor pressure less than or equal to 300 kPa; (ii) is not completely gaseous at 20 ˚C and at a standard pressure of 101.3 kPa; and (iii) which has a melting point or initial melting point of 20 ˚C or less at a standard pressure of 101.3 kPa.
'Gas' means a substance which (i) at 50 ˚C has a vapor pressure greater than 300 kPa (absolute); or (ii) is completely gaseous at 20 ˚C at a standard pressure of 101.3 kPa.

[2] '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 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; (b) less than a simple weight majority of molecules of the same molecular weight. 'Monomer unit' means the reacted form of a monomer substance in a polymer. 'Monomer' means a substance which is capable of forming covalent bonds with a sequence of additional like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process.

4.0 Interferences

4.1 Physical interferences

4.1.1 Preventing water samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in quantifying microplastics within drinking water samples. It is not possible to confidently eliminate all contamination from samples during laboratory processing. It is imperative that extreme care be taken to minimize contamination when collecting and analyzing water samples for microplastics. Controlling particle contamination during sample processing requires strict adherence to protocols for contamination control as outlined below in section 4.2.

4.1.2 Major sources of particle contamination within the laboratory include, but are not limited to: fibers from clothing and textiles (including lab coats, apparel worn by lab personnel, carpets, and furniture), particles deposited from the air within the laboratory environment, particles settled on equipment prior to or during use, reverse osmosis water, water used to clean equipment prior to use, sponges or brushes used to clean equipment prior to use, synthetic polymer gloves, and plastic sample container lids from abrasion during use.

4.2 Contamination Control

4.2.1 Laboratories must use as much plastic-free equipment as possible, except where allowed as noted in sections 4.2.1.3 to 4.2.1.7.

- 4.2.1.1 Laboratory personnel must use equipment throughout the process composed of glass (e.g., beakers, petri dishes) or metal (e.g., foil, forceps), except as noted in sections 4.2.1.3 to 4.2.1.7.
- 4.2.1.2 All materials used for cleaning of equipment prior to use must be made of natural/non-plastic materials (e.g., natural-based material sponge).
- 4.2.1.3 If plastic materials are used, inspect their integrity. LRBs exist to help account for any procedural contamination from plastics used during processing. Examples of plastics commonly used in microplastics analysis that are acceptable as they do not shed polymer particles are listed in Sections 4.2.1.4 and 4.2.1.5.
- 4.2.1.4 Use of hard plastic tubing (e.g., Tygon® or clear PVC tubing) to dispense high-purity water is acceptable.
- 4.2.1.5 Typical laboratory-grade solvent squeeze bottle (e.g., Teflon or polyethylene) are also suitable to dispense high-purity water for the rinsing of sieves, filters, and equipment as long as they are used similarly for QA/QC samples. Minimal contamination has been attributed to these sources.
- 4.2.1.6 Purple nitrile gloves (e.g., Kimtech®) have minimal contamination potential.
- 4.2.1.7 All plastic apparatus shall be evaluated periodically on a monthly basis for potential to shed microplastics by the procedures noted in Section 9.

4.2.2 Keep a clean environment.

4.2.2.1 Wipe surfaces down before and after use with water and a towel made from natural low-shedding natural fibers that do not meet the definition of 'microplastics' (e.g., cotton and paper towels).

4.2.2.2 Clean laboratory floors regularly (e.g., daily when microplastics analysis is being done), and maintain a record of cleaning frequency. Cleaning can be done by mopping with clean water and mops made of natural-fiber materials. Ideally, a High-Efficiency Particulate Air (HEPA) filter vacuum cleaner can also be used to clean floors at the end of each working day to minimize interference from the possible resuspension of particles into the air.

4.2.2.3 Clean all labware thoroughly with soap and water, and triple-rinse with MAG water before use. Pre-washing glassware (except volumetric flasks) and metal items at ≥ 450 °C for at least 1 hour is acceptable.

4.2.2.4 Installing a HEPA filtration system in your laboratory is recommended to minimize airborne particulates. Be sure to change the HEPA filter regularly based on manufacturer recommendations.

4.2.3 Minimize use of synthetic textiles in the laboratory

4.2.3.1 Do not wear synthetic clothing when processing samples. Wear cotton lab coats, ideally of a noticeable color not commonly found in environmental samples (e.g., pink) to allow clear identification within samples as contamination.

4.2.3.2 Remove furniture (e.g., chairs, stools, carpets) with padding or fabric. If removal is not possible, then synthetic surfaces may be covered with natural materials, or a material that does not shed plastic particles.

4.2.4 Clean all equipment thoroughly before use.

4.2.4.1 Before using any glassware or tools, and between processing individual samples, wash with soap and hot water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with MAG water.

4.2.4.2 Heavy-duty aluminum foil can be used to cover apparatus to protect from airborne particulate contamination. Foil must be pre-washed at ≥ 450 °C for at least 1 hour before use to destroy all organic material, then stored in a covered non-plastic container. Ash heavy-duty foil only, as the lightweight foil will disintegrate at high temperatures. Discard foil after use.

4.2.4.3 Cover all equipment when not in use with glass or clean aluminum foil, or store upside down.

4.2.4.4 Pressurized air can be used to remove possible contamination on the surface of equipment prior to use. If compressed gas is used to blow-dry equipment or samples for microplastics, ensure that the air is clean (e.g., put a 1 µm metal filter between the source and the outlet).

4.2.5 Recommend working in a covered environment.

4.2.5.1 Process samples in a biosafety cabinet, laminar flow hood, a clean cabinet, or other fully enclosed space. A covered environment, even without active air convection, helps to reduce airborne particulate contamination. While chemical fume hoods can reduce airborne particulate contamination by up to 50%, a laminar flow hood, clean

cabinet, or enclosed spaces can reduce contamination by 95% (Brander et al., 2020). Caution and characterization of blank levels (Section 9) is needed with the use of only a chemical fume hood unless its air source is filtered, as it will continuously move air, and any suspended particulates present, up and across all surfaces in the hood.

5.0 Safety

5.1 No analytes or reagents of concern are used within this method.

5.2 The following Personal Protective Equipment (PPE) are mandatory for method sections 11.1 and 11.2:

a. Cotton lab coat
b. Nitrile gloves
c. Safety glasses or goggles

5.3 IR instrumentation suitable for this method may need small quantities of liquid nitrogen to cool its detectors. Appropriate PPE (safety goggle, cryogenic gloves, cotton lab coat), cryogenic flasks and transfer equipment, and ventilation are required when the instrument's liquid nitrogen reservoir is refilled. Cryogenic safety equipment is not required during instrument operations, so long as manufacturer instructions regarding the use of cryogenic fluids are followed.

6.0 Equipment and Supplies

References to specific brands or catalogue numbers are included as examples only and do not imply endorsement of the product. Such reference does not preclude the use of other vendors or suppliers.

6.1 Cleaning

Item	Suggested Materials
Low foam dish soap	-
Sponge made of natural materials	Loofah, cellulose, natural sponge.
Cotton cloths and paper towels	-
Mop with natural-fiber head or HEPA-filter vacuum cleaner (consumer-grade is ok)	-

6.2 Sieving

Item	Suggested Materials or equivalent
Heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog No. 15901A
Squirt bottle (Teflon, polypropylene or LDPE)	-
Metal sieve (8" diameter)	VWR Catalog no. 57334-568 (500 µm mesh size) VWR Catalog no. 57334-578 (212 µm mesh size) VWR Catalog no. 57334-604 (20 µm mesh size)
Metal sieve pan	Same diameter as sieve
Glass beakers or jars	>500 mL size One for each size fraction that will be wet sorted. Non-plastic lids (e.g., metal) preferred (use washed heavy-duty aluminum foil to cover containers that do not have lids, such as beakers)

6.3 Vacuum filtration

Item	Suggested Materials or equivalent
1 µm pore-size filters	47 mm diameter *Material not specified as the 1 µm filter is only used for filtering high-purity water for the rinsing of apparatus. GF/F filters are suitable as they resist clogging, are not made of plastic polymer, and can be readily cleaned by washing at 450 °C for at least an hour.*
20 µm pore-size filters	47 mm diameter Polycarbonate recommended
Vacuum filtration system (without plastic parts exposed to sample water; Teflon O-rings are acceptable). The following describes typical systems for sample sizes of 1-2 L. Different sizes of funnels and flasks may be used for other sample sizes as appropriate. Systems typically consist of: 1 ´ Vacuum pump 2 ´ Plastic tubing 2 ´ 1000 mL Glass filtering flasks with rubber stopper 1 ´ filtering funnel 1 ´ filter holder with glass support 1 ´ metal clamp	GAST model DOA-P704-AA or an equivalent vacuum system Tygon® S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858 *(For use with a 47 mm diameter filter)*

Item	Suggested Materials or equivalent
1´ venting valve or T-adapter with shut-off valve that connects tubing between the filtering flask and vacuum pump (or laboratory bench-vacuum valve).	
Glass Petri dish(es) (55 mm bottom diameter)	VWR Catalog no. 25354-025 *(For use with a 47 mm diameter filter)*

6.4 Visual Microscopy

Item	Suggested Materials or equivalent
Glass Petri dishes (95 mm bottom diameter)	VWR Catalog no. 25354-069
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)” *The suggested item for systematic counting*
Heavy-Duty aluminum foil	-
Glass microscope slides	VWR Catalog no. 48300-026
2% dextrose solution	Sigma-Aldrich Catalog no. G8270-100G
Square glass petri dishes (100 mm diameter)	VWR Catalog no. CA25378-115
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon, Set of 6”
Stereoscope	Interchangeable black and white base preferable for picking with bright light source. A magnification of ca. 45´ is useful.

6.5 Images and Measurements

Description	Example
Microscope digital camera attachment	e.g. ToupTek® touptek.com/product/product.php?lang=en&class2=56
Computer software for digital imaging and measurements	e.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

6.6 QC materials

Item	Suggested Materials or equivalent
NIST Material Standards for Microplastic Research	Polymer Kit 1.0 https://www.hpu.edu/cncs/cmdr/cmdr-new/cmdr-new-images/polymer_kit_brochure.pdf
Chromosphere-T Certified Size Standards (polymer microspheres)	https://www.thermofisher.com/order/catalog/product/BK050T#/BK050T
NIST-traceable monosized and monodispersed polymer beads	https://assets.thermofisher.com/TFS-Assets/CDD/Specification-Sheets/PS-10021649-MTL-SIZE-STANDARDS-EN.pdf

7.0 Reagents and Standards

7.1 MAG water is required throughout the sieving and filtration process to rinse sieves and filter apparatus, and to ensure that all particles from the sample have been collected. The clean water is be collected in a clean vessel (see Section 4.2.4.1) and covered (Section 4.2.4.2) until use.

7.2 The LFB is prepared by procedures outlined in ASTM (2021) and Seghers et al. (2021). In brief, generation of stock suspension involves cryo-milling of particles (e.g., the NIST Material Standards for Microplastic Research), adding water and surfactant to sieve particles into size fractions, washing to remove excess surfactant, and resuspension of particles into water with surfactant to generate the stock suspension. The Chromosphere-T and NIST-traceable beads are microspheres of known size that can also be used for this purpose, if spiked into an LFB at a concentration near the laboratory's MRL.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection is by procedures noted in ASTM Method 8332-20 (ASTM, 2020), following protocols for low-suspended-matter waters. Samples collected from inline sieving following ASTM Method 8332-20 consist of particulates suspended in water, and go into glass sample containers, as noted below, prior to processing steps in the laboratory (Section 11).

8.2 During storage, water samples must be kept liquid at low temperature (e.g., <6 °C), to prevent bacterial growth. Samples must also be kept away from direct sunlight or bright light.

8.3. Glass containers with non-plastic lid liners (PTFE is acceptable), pre-cleaned as with other apparatus in this method and of a size appropriate to the volume needed, must be used to collect and store samples to minimize microplastic contamination from the container when feasible. Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts if possible; if

not, then ensure that containers are sealed prior to shipment. Shipping samples on ice (<6 °C) is preferred, but samples may be shipped at room temperature.

8.4 Water samples should be processed as soon as possible after collection and extraction to minimize the opportunity for algal growth. A maximum 28-day holding time, from sample collection to analysis, for treated drinking water is allowed. Freezing of samples is not permitted.

8.5 Trip Blanks shall accompany empty bottles to the sampling site and back to the laboratory. Do not open Trip Blanks in the field; Trip Blanks must remain sealed until analysis. Trip Blanks may be used to identify potential sources of contamination occurring from shipping the sample container to the site and back, and do not need to be analyzed unless evidence of contamination during shipment arises from analysis of LFBs.

8.6 Field Reagent Blanks must accompany each sample taken from the laboratory to the sampling site. During the duration of the sampling event, keep the FRB open at the site while collecting the sample. At least one FRB must be collected and analyzed for each test series.

9.0 Quality Control

QC requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements. This section describes each QC parameter, its required frequency and the performance criteria that must be met in order to satisfy the method's quality objectives. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs. Compliance with the requirements of the IDC must be demonstrated for each size fraction that the laboratory intends to report.

9.1 Quality control measures for this method include collection and analysis of laboratory reagent blank samples (LRBs), use of laboratory fortified blanks (LFBs), the use of color and morphology keys to standardize particle characterization (see section 17), and the documentation in variability of analyst count, color, and morphology characterization.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – The IDC must be successfully performed prior to analyzing any field samples. Prior to conducting the IDC, the analyst shall be familiar with the calibration requirements outlined in Section 10. The IDC must be completed for each size fraction. Prior to conducting the analysis, the laboratory must analyze at least 7 LFBs, spiked with particles >50 mm. Average recovery efficiency by visual microscopy of particles (> 212 µm) must be 50%, with a precision of 40% RSD. An LRB must also be analyzed and results must be < MRL.

9.2.1 DEMONSTRATION OF LOW SYSTEM BACKGROUND – Analyze at least 7 LRBs. Confirm that the blank is free of contamination as defined in Section 9.3.1.

9.2.2 DEMONSTRATION OF PRECISION – Prepare and analyze at least 7 replicate LFBs. Fortify these samples near the midrange of the initial calibration curve. The percent relative standard deviation (%RSD) of the concentrations of the replicate analyses must be ≤ 40% for all size fractions greater than 50 µm.

$$\%RSD = \frac{Standard\ Deviation\ of\ Measured\ Concentrations}{Average\ Concentration}\ x\ 100$$

9.2.3 DEMONSTRATION OF ACCURACY – Calculate the average percent recovery using the same set of replicate data generated for Section 9.2.2. The average recovery of the replicate analyses for particles > 212 µm must be at least 50% of the true value and must not exceed 150%.

$$\%Recovery = \frac{Average\ Measured\ Concentration}{Fortified\ Concentration}\ x\ 100$$

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL (i.e., the LCMRL) based on the intended use of the data. Establishing the LCMRL concentration too low may cause repeated failure of ongoing QC requirements. Method analytes that are consistently present in the background should be reported as detected in field samples only after careful evaluation of the background levels. In such cases, a LCMRL must be established by determining mean and standard deviation values from spiking LFB measurements with at least 7 replicates. This guidance is intended to minimize the occurrence of reporting false positive results.

9.2.4.1 Collect the particle count data for each size fraction. Calculate spiking recovery. Particle count data with recovery less than 50% and larger than 150% is outlier that should be deleted.

9.2.4.2 Calculate “reducing factor” (= spiked particle number ÷ expected detection limit (e.g., 2 particles, the minimum possible value)) for each size fraction. Calculate the reduced particle count value from the measured particle count that is divided by the “reducing factor”. Calculate the mean and standard deviation for the reduced particle count data.

9.2.4.3 Calculate half range prediction interval of results (HRPIR) as:

$$s \times \sqrt{1 + \frac{1}{n}} \times t_{df,1-\left(\frac{1}{2}\right)\alpha}$$

where: *s* is the standard deviation of n replicate samples; *n* is the number of replicates; *t* is the Student's *t* value with *df* degrees of freedom and confidence level (1-α) (i.e., α = 99%).

9.2.4.4 Calculate the LCMRL value.

9.2.4.4.1 Determine the "regression line". The slope *k* is the ratio of the data point of the mean value (*y*) and the spiking level (*x*) (i.e., *k* = *y*/*x*). This regression line is a straight line through the origin.

9.2.4.4.2 Determine the linear equations for the upper and lower prediction interval boundary lines. Both these lines have the same slope as the "regression line" in the previous step. The y-intercept is +HRPIR for the upper boundary line, and -HRPIR for the lower boundary line.

9.2.4.4.3 Determine linear equations for the recovery lines for 50% (i.e., $y = 0.5x$) and 150% (i.e., $y = 1.5x$).

9.2.4.4.4 Calculate the *x*-value for the intersection point of the recovery line and the "regression line" (i.e., $0.5x = kx$ - HRPIR (lower bound), $1.5x = kx$ + HRPIR (upper bound) as per Figure 2.

9.2.4.4.5 The larger value of the two *x*-values is the LCMRL (Figure 2).

9.3 ONGOING QC REQUIREMENTS – This section describes the ongoing QC procedures that must be followed when processing and analyzing field samples.

9.3.1 LABORATORY REAGENT BLANK (LRB) – Analyze a LRB with each Analysis Batch. Laboratory reagent blanks must be quantified for particle count, size, color (see section 17 for guide), and morphology (see section 17 for guide). The content of the blank samples must be recorded, and this data shall be associated with the samples processed in the same analysis batch. Blank correction is not permitted, given the many factors that can go into blanks (e.g., it is possible to have many blue fibers, for example, in the blank, but few such fibers in actual samples). One LRB is analyzed for every batch, and the microplastics level must be less than the MRL. Take corrective action with regards to reducing contamination (Section 4) and repeat until this criterium is met.

9.3.2. LABORATORY FORTIFIED BLANK (LFB) – LFBs consist of MAG water samples spiked with microplastic particles of known and representative count, size range, color, morphology, and material composition, with known spectra. Appropriate particles for creating LFBs are available from NIST (see Section 6.6).

Analyze at least one LFB spiked with particles > 50 µm, for every batch. Recovery efficiency by visual microscopy of particles in the LFB must be at least 50% and must not exceed 150% for particles > 212 µm. Document recovered microplastic count, size range, color, and morphology. These values can be used for IDC and ODC, as well as routine analysis batch-to-batch QC analysis.

9.3.3 FIELD REAGENT BLANK (FRB) – A Field Reagent Blank must be included with each set of samples collected at the same site and time, and analyzed to assess contamination during shipping and storage. Microplastics levels must be below the MRL; if not, the batch of samples associated with the FRB must be flagged accordingly.

9.3.4 TRIP BLANK – Trip blanks do not need to be analyzed unless the FRB shows evidence of contamination. In that event, the requisite trip blank must be analyzed to determine if the contamination was due to shipping.

9.3.5 LABORATORY FORTIFIED MATRIX (LFM) – A LFM needs to be analyzed every batch. Recovery efficiency by visual microscopy of particles in the LFM must be at least 50% for particles > 212 µm. Document recovered microplastic count, size range, color, and morphology.

9.3.6 COUNTING VARIABILITY – Analysts in a laboratory shall quantify the total number of each color and morphology (as per the keys in Section 17, which include appropriate collapsing of some colors and morphologies) on a sample of known content via visual microscopy once per month or every 100 samples, whichever is more frequent. Use samples from LFMs, which can be saved after analysis. If a single analyst is present, replicate counts must be done and be within 5% of the known sample composition. If there is more than one analyst in the laboratory, a comparison among analyst's count of particle enumeration must fall within 20% of each other. If not, then analysts shall perform additional counts, until this quantification falls within 20% between analysts for at least three consecutive LFM samples.

10.0 Calibration and Standardization

Calibration procedures for spectroscopy are vendor-specific, in which the instrument measures spectra of a built-in reference material for comparison to reference spectra and shall be recorded in the laboratory SOP. Calibration shall be performed daily according to the manufacturer's instructions to ensure an accurate collection of spectra. Any vendor changes to instrument calibration shall be documented to allow for traceability.

For particle photographs and measurements, the use of imaging software is recommended. For this purpose, the microscope should be connected to a camera and computer. Here, the scale bar must be calibrated before use. This can be done by placing a ruler or optical micrometer under the microscope, comparing ruler measurements to the scale bar on the screen and adjusting accordingly. Any appropriate ruler will suffice.

Analysts shall be familiar with the color and morphology keys in Section 17. Particles in LFMs and LFBs shall be characterized using these keys for reference (See Section 9.3.7).

11.0 Procedure

11.1 Filtering (See Figure 1 for flow diagram)

11.1.1 Rinse off the outside of the sample container with MAG water, to remove all particulates that may interfere, including those from packing materials. Discard this rinse. If the sample has not been size-fractionated, then proceed with step 11.1.2. If the sample has already been size-fractionated, skip step 11.1.2, and go to 11.1.3.

11.1.2 Place the 500 µm sieve on top of the 212 µm sieve, which in turn is on top of the 20 µm sieve, which in turn is placed on top of the sieve pan.

11.1.2.1 Pour the sample into the sieve.

11.1.2.2. Triple rinse the inside and rim of the sample container and lid into the sieve using MAG water. Rinse the sieve stack with water and tap the sieve gently to move everything through to its appropriate size fraction.

11.1.2.3 Rinse the contents of each sieve into a (cleaned and labelled) glass container using MAG water. This will collect a size fraction >500 µm, a size fraction 212-500 µm, and a size fraction 20-212 µm. Aim to rinse minimal amounts of water into each size fraction/jar. To do this, tilt the sieve towards you and rinse the contents of the sieve to pool all particles into one area. Then rinse the pooled particles into the jar, using as little water as possible.

11.1.2.4 Pour the contents of the sieve pan into a clean beaker and cover. This will collect a size fraction <20 µm, if this is desired. If collection of this size fraction is not desired, then the contents of the sieve pan may be discarded.

11.1.3 Decide on which size fractions will be wet sorted, and which will be dry sorted, based on the following guidance:

Particles >212 µm may be wet sorted. Size fractions >212 µm may be left in their glass container with the rinsed MAG water. Particles can be transferred from the wet container and sorted appropriately (i.e., go to 11.2). Alternatively, if it is found that wet picking particles in the size fraction 212-500 µm is challenging, this size fraction may be filtered (11.1.4) and dry sorted.

Particles <212 µm should be dry sorted, as small particles are difficult to manipulate manually. Following size fractionation and wet sorting, particles are filtered onto appropriately sized filter paper (11.1.4). Particles can be subsampled from the filter paper and sorted appropriately (Section 11.2).

11.1.4 Assemble the vacuum filtration system without the filtering funnel and clamp.

11.1.4.1 Turn on the vacuum pump. Pour MAG water onto the glass filter holder to pre-clean the system.

11.1.4.2 Turn the vacuum pump off. Empty the waste from the bottom flask and rinse the flask with MAG water, then reassemble.

11.1.4.3 Rinse the filtering funnel with MAG water.

11.1.4.4 Rinse a 20 µm polycarbonate filter with MAG water and place onto the glass filter holder and secure the filtering funnel on top using the metal clamp. If measurement of the < 20 mm size fraction is desired, then the 20 mm polycarbonate filter for this step is replaced for this size fraction with a 1 mm polycarbonate filter.

11.1.5 Turn the vacuum pump on and pour the appropriate sample (e.g., sieve pan contents that have been transferred to a beaker) through the filtration system. Triple rinse the beaker with MAG water into the filtration system once the final sample volume has been emptied. Ensure that the filtering flask does not overfill, as this may lead to sample loss.

11.1.5.1 Keeping the vacuum pump on, triple rinse the sides of the filtering funnel with MAG water in a circular motion. Turn off the vacuum pump, triple rinse the filtering funnel with MAG water in a circular motion. Turn on the vacuum pump again and triple rinse the filtering funnel with MAG water in a circular motion. Gently break the vacuum by turning on the venting valve or the shut-off valve of the T-adapter to balance the pressure on both sides of the filter prior to turning off the vacuum pump or laboratory bench vacuum valve.

11.1.5.2 Turn off the vacuum pump, then remove the metal clamp and carefully lift the filtering funnel away from the base. Forceps may be used to ensure the filter is not removed with the filtering funnel as you do this.

11.1.5.3 Turn on the vacuum pump and carefully rinse the base of the filtering funnel onto the filter, using MAG water. Aim to rinse small sections of the funnel base onto the center of the filter so that particle loss is minimized.
11.1.5.4 Turning off the vacuum pump, remove the filter paper from the filtration system with tweezers and place it into a clean, labeled petri dish and cover. If necessary, use two sets of tweezers to pull the filter paper away without losing particles.

11.1.5.5 Pour and triple rinse the contents of the filtering flask into a clean beaker and cover.

11.1.5.6 Repeat all steps in 11.1.3, 11.1.4, and 11.1.5 as appropriate for the remaining size fractions.

11.2 Visual microscopy

11.2.1 Prepare materials for sorting and visual identification of particles; attach the grid sticker to the outer base of the 95mm diameter petri dish and remove the excess, prepare glass slides (Section 17.3).

11.2.2 Bring all size fractions of the sample and materials for visual identification over to the microscope to perform wet or dry sorting.

11.2.3. Using appropriate magnification, background and illumination settings, start with the largest size fraction and identify, count, number and visually characterize (by color and morphology) all suspected microplastic particles that are observed within the sample by either wet sorting (following 11.2.3.1) or dry sorting (following 11.2.3.2) depending on the size fraction and decisions made in 11.1.3. Use the color and morphology keys in Section 17 for guidance on visual characterization and categorization of particles and refer to Lusher et al. (2020) for guidance on differentiating between plastic and natural particles. Adjust illumination until the grid lines of the grid sticker are clearly visible through the filter.

11.2.3.1 Wet sorting: For size fractions that have been left in their glass containers, extracted particles may be transferred one spoonful at a time directly from the glass jar into a glass Petri dish using a small metal teaspoon. Rinse the contents of the metal teaspoon into the Petri dish. Work through each grid square, from the top left to the bottom right identifying and characterizing particles. Then thoroughly check for particles around the inner edge of the petri dish (i.e., outside of the grid area). Once the spoonful has been thoroughly checked for microplastic particles, and all particles are counted and visually characterized, and/or picked for subsampling (11.2.4), empty and rinse the Petri dish with MAG water and continue with another spoonful. This process is repeated until the jar is empty. At this point, rinse the inside of the empty jar with MAG water three times to ensure any particles that may be stuck to the inside of the glass jar have been transferred to the petri dish for visual identification, and sort these particles as well.

11.2.3.2 Dry sorting: For size fractions that have been filtered onto filter paper, particles may be directly counted and visually characterized from the filter surface. Be sure also to check visually for particles around the inner edges of the petri dish that might have moved from the filter surface during transition to the microscope.

11.2.4 Whilst visually identifying, counting and characterizing particles using microscopy, randomly select (subsample) a minimum of 30 particles per sample (irrespective of color and morphology) (De Frond et al. 2021, 2021a), ensuring particles are selected representatively from each size fraction. The subsampled particles should be placed on proper substrate to facilitate further instrumental measurement and be numbered. For particles smaller than 212 µm that may be dry counted, the particles can be marked and left on the filter surface for later images, measurements, and chemical identification (Sections 11.4, and 11.5).

11.2.5 Repeat 11.2.3 and 11.2.4 with remaining size fractions.

11.3 Images and Measurements

11.3.2 For each sub-sampled particle, record a clear image and measure the longest dimension, using computer software such as Image J, or software that is compatible with the camera attached to the microscope. For fibers, do not measure frayed projections and use segmented/curved lines to measure length where necessary. If a particle has broken apart, use your best judgment e.g., measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.

11.4 IR Spectroscopy

11.4.1 All subsampled particles from 11.2.4 must be chemically identified using IR spectroscopy.

11.4.2 Calibrate the instrument as per procedures in Section 10, relevant to each make and model prior to use.

11.4.3 Method specifics to be used, based upon findings from De Frond et al. (2021b), include:

11.4.3.1 Spectral Collection:

11.4.3.1.1 Use ATR or reflectance mode for particles >212 um, and reflectance or transmittance modes for particles <212 um, as applicable.
11.4.3.1.2 Spend a maximum of 10 mins on the spectral collection of each particle.

11.4.3.3 Spectral Matching:
11.4.3.3.1 Include reference libraries relevant to both microplastics (virgin and environmentally aged) and natural materials that may be misidentified as microplastics via microscopy. Examples of reference libraries for plastic particles include the polymer library from Primpke et al. 2018, and the SLoPP and SLoPPe libraries from De Frond et al. (2021b).
11.4.3.3.1 A hit quality of 70% or above should be used as the threshold for an accurate spectral match. In cases where a spectral match result is reported that is below this threshold, notes must be provided on why this match was reported.

11.5 Storage of samples

Samples shall be stored in containers that will minimize disturbance of picked particles or filters and avoid contamination (e.g., picked particles on glass slides shall be stored in new clean glass petri dishes with the cover on, and filters shall be stored in original clean, glass petri dish with the cover on). It is impossible to guarantee that particles on filters will never be jostled, moved, or dislodged, so all images and measurements must be taken prior to long-term storage of samples.

12.0 Data Analysis and Calculations

12.1 Data to be recorded for microscopy
For every particle: Particle ID, color, morphology, size fraction
Total suspected microplastic particle count (e.g., sum of all suspected microplastic particles within each sample) and total suspected microplastic particle count within each size fraction.

12.2 Images and Measurements
For all subsampled particles: length and width and one clear image of the particle with a scale bar.

12.3 Data recording for spectroscopy
For all subsampled particles: the chemical ID result for each particle. Save a file of the raw spectrum along with a file of the spectral match result.

12.4 Microplastic particle counts
Visual microscopy alone cannot provide information on particle material types. Therefore, analysis of particles using IR spectroscopy is required to confirm which particles are microplastic, and which may be false-positive counts, i.e., natural or anthropogenic particles mistaken for microplastic via visual identification. IR spectroscopy results are used to determine the proportion of microplastic particles within the subsample, The number of subsampled particles from each sample has been shown to be representative for environmentally relevant samples, if a minimum of 30 particles per sample are picked irrespective of color and morphology (De Frond et al., 2021a). The proportion of microplastic samples within the subsample is then used to calculate the estimated count of microplastic particles present in each sample.

Estimated number of plastic particles in sample = Proportion of particles chemically confirmed as plastic within the subsample $\times$ Total number of suspected plastic particles counted via microscopy.

13.0 Method Performance

This method was validated via a blind sample intercalibration study organized by SCCWRP in 2019-2020, with 26 participating laboratories (De Frond et al., 2021). The clean water matrix used was deionized water, to which microplastic particles of various sizes (3-2000 µm), colors, polymers, and morphologies (e.g., fragments, spheres, fibers) were added along with natural particles (e.g., sand, shell fragments, cotton fibers, animal fur) serving as false-positive materials. Most microplastic particles came in individual gelatin capsules containing sodium bicarbonate and malic acid to facilitate dissolution; others were added manually. Laboratories analyzed these samples up to 11 months after creation. Method performance data from these samples is applicable for reagent water, finished drinking water, and raw source water. This method has not been evaluated for water high in ionic strength or total dissolved solids (> 0.2 M), or water containing substantial levels of natural matrices (e.g., surface water, wastewater).

Method performance can be divided into two aspects: (1) optical microscopy alone, and (2) spectroscopic confirmation of particle composition. These parameters are applicable for particles 50-5000 µm in size.

For optical microscopy, the accuracy of reported suspected plastic particle counts (i.e., recovery) was 92 ± 57% for 22 laboratories for all particle sizes > 50 mm (De Frond et al., 2021). This protocol, however, requires the use of spectroscopy to confirm the composition of particles analyzed. For particles > 50 mm, FTIR spectroscopy can accurately identify the surface composition of a particle 93% of the time; if it is a plastic particle, correct identification is 95%. Precision data for IR (using FTIR in the SCCWRP study, De Frond et al., 2021) is not available due to the large variation in the number of particles identified by the various participants in the validation study. Fibers are a morphology more difficult to identify correctly by FTIR (76% accuracy overall for both plastic and natural fibers). Further details on accuracy and precision of this method, based on De Frond et al. (2021), are listed in Table 1 for the size fractions used in this method.

14.0 Pollution Prevention

14.1 All extracted microplastics may be disposed of as stated in Section 15.3 so as not to contribute to microplastic pollution of waterways.

15.0 Waste Management

This section describes the minimization and proper disposal of waste and samples.

The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrix of concern is finished drinking water. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations and that laboratories protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15.1 Clean drinking water remaining after particle extraction is unlikely to contain microplastics > 20 mm, and removal of those particles < 20 mm remaining is not economically feasible. This water can be disposed down the drain.
15.2 All waste including used filter papers, projector paper and tape can be disposed of in solid waste intended for landfill.
15.3 When appropriate (i.e., when all particles have been identified, results reported and the samples are no longer required), dispose of the extracted and identified particles in solid waste intended for landfill.

16.0 References

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17.0 Tables, Diagrams, Forms, Flowcharts, and Validation Data

This section contains all the method, tables, figures, diagrams, example forms for data recording, and flowcharts. This section will also contain validation data referenced in the body of the method.

Table 1: Summary of LCMRLs, recovery by visual microscopy with RSDs, overall accuracy of identifying particle as plastic vs. non-plastic, and accuracy of correctly identifying the type of polymer for a microplastic particle. Data is based on 0.45 L spiked blind-samples of clean water matrix from the SCCWRP measurement intercalibration exercise (De Frond et al., 2021). Spectroscopy data was normalized by pooling results from all laboratories (De Frond et al., 2021) to determine the proportion of correct chemical IDs for certain particle types (i.e., overall, plastic, natural), classified into size fractions by the measured largest dimension. Relative standard deviations of accuracy results are not available as all analyzed particles were pooled across laboratories and no standard deviation may be calculated. Method Performance Criteria were not developed in De Frond et al. (2021) for the <50 um fraction given the limited amount of data reported by participating laboratories. N/A = not applicable. n = total number of particles chemical identified among all laboratories.

Size fraction	LCMRL (particles)	Recovery (%)	Recovery (%RSD)	Accuracy (overall, n)	Accuracy (plastic, n)
>500 µm	5.32	125.82	95.74	97.99%, 547	99.42%, 522
212-500 µm	6.40	122.30	63.63	90.54%, 391	92.72%, 371
50-212 µm	10.2	79.91	74.95	86.57%, 201	89.14%, 175
< 50 µm	N/A	31.90	153.26	N/A	N/A

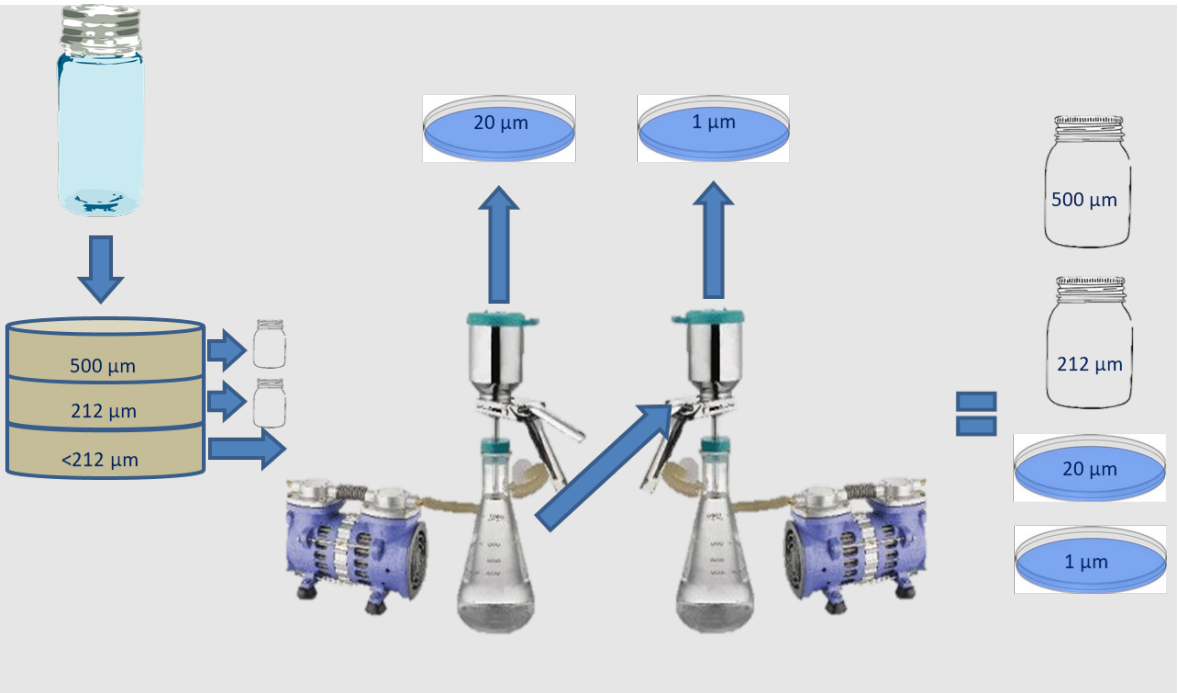


Figure 1: Flow diagram schematic of filtration procedure (Section 11.1).

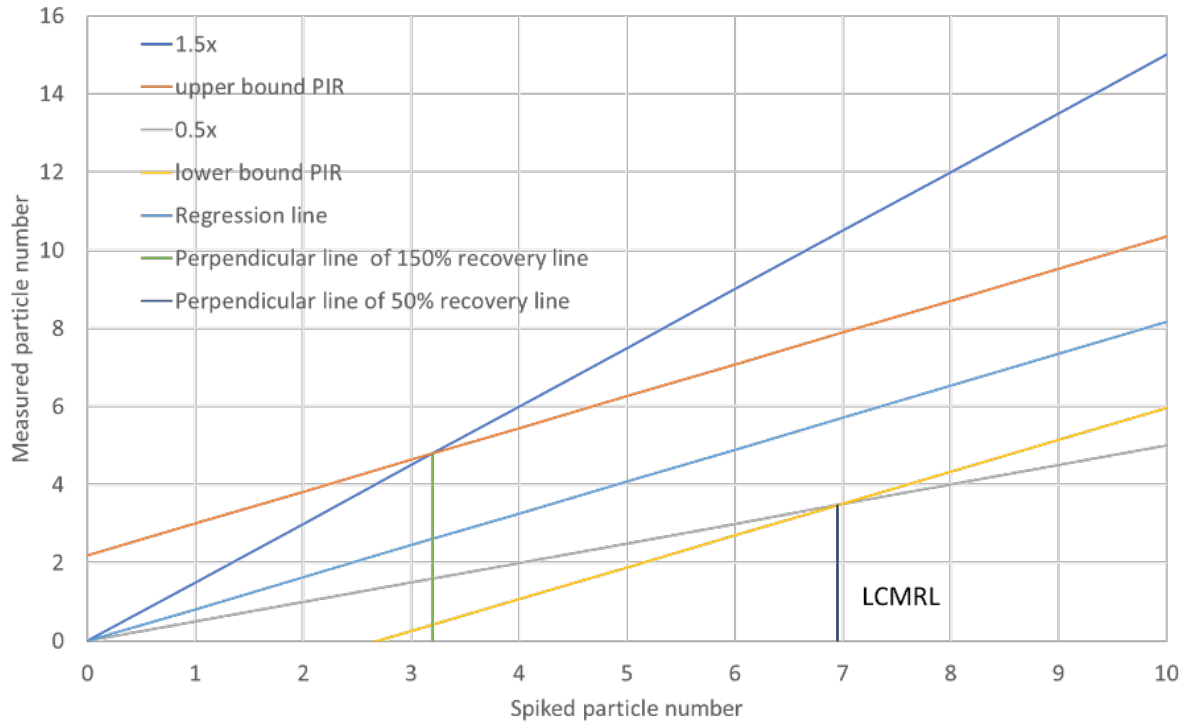


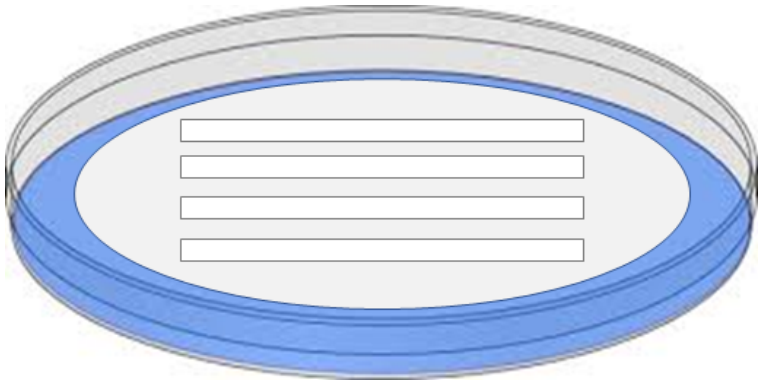
Figure 2: Example LCMRL plot.

Color Key	HEX Values	RGB
Black	#000000	rgba(0,0,0,255)
Blue	#add4ee #0ab2f0 #0b31d1	rgba(173,212,238,255) rgba(10,178,240,255) rgba(11,49,209,255)
Brown	#ad6800 #7f4800 #522e06	rgba(173,104,0,255) rgba(127,72,0,255) rgba(82,46,6,255)
Green	#00f727 #00a509 #005b01	rgba(0,247,39,255) rgba(0,165,9,255) rgba(0,91,1,255)
Multicolor (2+ colors)		
Pink	#fc9cf7 #e651d3 #c608b1	rgba(252,156,247,255) rgba(230,81,211,255) rgba(198,8,177,255)
PURPLE	#c887fe #b656e4 #7d0bc4	rgba(200,135,254,255) rgba(182,86,228,255) rgba(125,11,196,255)
Red	#fd3334 #e51c0f #bd0501	rgba(253,51,52,255) rgba(229,28,15,255) rgba(189,5,1,255)
Clear, Grey, Silver, White WHITE	#fcfcfc #c4c4c4 #787474	rgba(252,252,252,255) rgba(196,196,196,255) rgba(120,116,116,255)
Gold, Orange, Yellow ORANGE	#ffe501 #ffd600 #ffc001	rgba(255,229,1,255) rgba(255,214,0,255) rgba(255,192,1,255)

17.1 Color key. This color key is to be used to characterize colors of microplastic particles in samples. All particles described as clear, grey, silver or white are categorized as white, and all gold, orange or yellow particles are described as orange. For a more detailed breakdown of further developed color characterizations see Martí et al., 2020 (Figure 1).

17.2 Morphology Key. This morphology key is to be used to characterize microplastics particles in samples. All foams, films, fragments or pellets are categorized as fragments, and fibers and fiber bundles are categorized as fibers.

Specific Morphology	Morphology Name to use for Reporting
Foam Film Fragment Pellet	Fragment
Fiber Bundle Fiber	Fiber
Sphere	Sphere
Fragment with rubbery constituency, often black but not always	Rubbery fragment



17.3 Diagram of the method for particle preparation on double-sided tape, laid across projector paper within a petri dish.

17.4 Data table. Sample data reporting with suggested columns, as per De Frond et al. (2021). PHOTOID refers to the name of the file with an image of the particle. TIMEIMAGESMEASUREMENTS refers to the amount of time needed to analyze and image the particle.

sizefraction	particleid	morphology	color	photoid	instrumenT	chemid	length (mm)	width (mm)	timeimagesmeasurements (hours)	comments
>500 µm	CW_1_500_1	Sphere	Green	CW_1_500_1-1	Stereoscope	PE	0.123	0.60	0.05	comment
212-500 µm	CW_1_212-500_1	Fragment	White	CW_1_212-500-1-1	FTIR	PS	0.312	0.123	0.10	comment
212-500 µm	CW_1_212-500_2	Fiber	Brown	CW_1_212-500-2-2	Raman	PET	0.250	0.018	0.15	comment
20-212 µm	CW_1_20-500_1	Fiber	Red	CW_1_20-500-1-1	Raman	PP	0.120	0.010	0.15	comment