HELCOM Guidelines on monitoring of microlitter in seabed sediments in the Baltic Sea

Baltic Marine Environment
Protection Commission

Co-funded by the
European Union

BLUES

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For bibliographic purposes this document should be cited as:
"HELCOM Guidelines on monitoring of microlitter in seabed sediments in the Baltic Sea. HELCOM (2022)"

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Content

Pre-Notes

In the context of the HELCOM BLUES project (DG Environment, MSFD, https://blues.helcom.fi/) a survey on existing and planned method approaches for the monitoring of microlitter in the Baltic Sea was conducted and compiled. This draft document on guidelines for sampling, sample treatment and analysis of microlitter within HELCOM is based on the outcomes of the discussions during three workshops with national experts on microlitter held on June 30, 2021, February 8, 2022 and September 6, 2022.

1.   Introduction

Marine Litter and Microlitter are defined according to Commission Decision 2017/848 (2017) and UNEP (2022): "Marine litter is any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2022).

Marine microlitter is marine litter with a length of its maximum dimension below 5 mm.

The scope of microlitter monitoring within HELCOM is in accordance with MSFD Com Dec: D10C2: "micro-litter shall be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored on the coastline. Micro-litter shall be monitored in a manner that can be related to point-sources for inputs (such as harbours, marinas, waste-water treatment plants, storm-water effluents), where feasible" (Commission Decision (EU) 2017/848, 2017).

2.   Sampling of seabed sediments for microlitter monitoring

2.1   Sampling conditions

2.1.1   Number and location of monitoring stations

The number of monitoring stations surveyed by each country depends on the heterogeneity across stations and areas as well as on how many sub-basins each respective country encompasses. For shared sub-basins, there is a shared monitoring responsibility. The distribution of monitoring stations should represent variation within HELCOM sub-basins and should, where possible, integrate stations for target and measure monitoring[1] (i.e. near coast locations that are related to potential point-sources or locations of potential accumulation areas) as well as state monitoring[2] (i.e. open water or offshore-locations) according to the technical guidance on monitoring for the Marine Strategy Framework Directive (Zampoukas et al. 2014). It is further suggested to include preferably stations with known sediment deposition rates.

Where feasible, stations for monitoring of microlitter should correspond to existing monitoring stations from other monitoring programmes such as contaminants in sediments.

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[1] "Target and measure monitoring (relating to Art. 10 and 13 MSFD) which compares to WFD operational monitoring: This requires additional monitoring (in terms of indicators/parameters, sampling frequency and stations) in those areas and for those ecosystem components for which GES has been failed and for those pressures, which are responsible for failing GES and for which environmental targets have been set. Monitoring should enable to assess progress towards GES and achieving targets and the efficiency of measures." (Zampoukas et al. 2014: 15).

[2] "State monitoring (relating to Art. 8, 9 MSFD) which compares to WFD surveillance monitoring: It aims at long-term monitoring and at surveillance monitoring for an overview of the state of the environment and is the backbone of MSFD monitoring. It is sufficient where GES is achieved for the individual ecosystem component. State monitoring includes the features, activities and pressures relevant for GES. It includes monitoring of additional parameters under Annex III MSFD to assess the extent and intensity of human activities and resulting pressures and their changes as well as changes in natural conditions." (Zampoukas et al. 2014: 15).
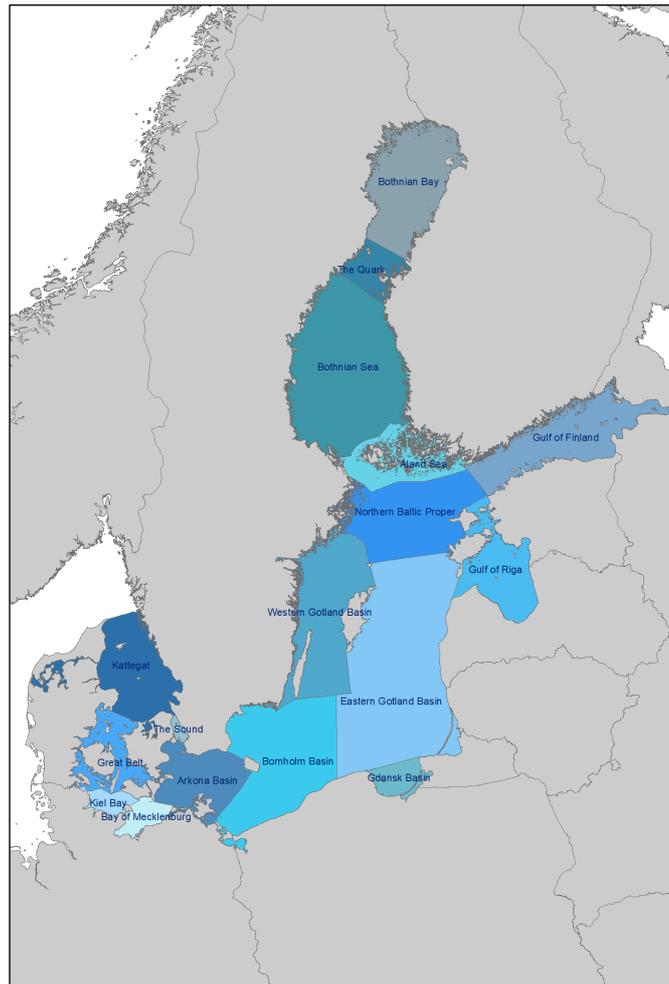
Figure 1.   Map of the Baltic Sea presenting the HELCOM sub-division into 17 open sea sub-basins (HELCOM 2022).

2.1.2   Frequencies and time of sampling

The frequency of monitoring for microlitter in seabed sediments is still under discussion. It is suggested that monitoring frequency should be determined on the basis of further analysis e.g. on sampling methods, variance in microlitter concentrations and local conditions. It is further proposed to consider different frequencies in the case of parallel investigations at target and measure monitoring versus state monitoring stations.

The sampling time depends on feasibility and whether the sampling is carried out in accordance with other monitoring programmes that require a specific season for sampling.

2.2   Sampling techniques

2.2.1   Sampling device, sample volume, replicates

Sampling of seabed sediments can be done via grabs or corer-based approaches (e.g. Van Veen grab, box corer, Gemax corer, Kajak corer).

Samples are taken within the upper 2 to 5 cm layer of the sediment by means of stainless-steel equipment (spoons, trowels) and are transferred to pre-cleaned labelled glass or metal/aluminium jars. For monitoring stations with known sedimentation rate and absence of bioturbation processes

the sample depth may be adjusted in order to take into account the sedimentation of specific periods or assessment cycles.

The total sample volume relates to the sampling device. It is recommended to retrieve a minimum volume that allows for repetitions of the analyses and the determination of additional sediment-related parameters.

It is discussed to sample in duplicates or replicates (2-3 hauls) and/or to combine the resulting samples to one composite laboratory sample for further analyses. It is also discussed if replicates are only taken at state monitoring stations.

2.2.2   Recording of basic parameters, sampling protocol
Basic parameters during sampling shall be recorded and include:

a)   Mandatory: date, station name and internal identification code (ID), coordinates, water depth, depth of sampled sediment surface layer.

b)   Optional: weather and sea conditions, substrate, station classification.

Sample codes and parameters are documented in the sample documentation form. The respective sample containers are labelled with (at least): date, station code, station name and the internal code for laboratory processing (ID).

2.2.3   Sample transportation, preservation and storage
Samples are stored in glass, aluminium or metal containers providing light-absence and low temperatures. The use of plastic bags or containers is to be avoided. The storing conditions depend on the storage time and conditions during the sampling campaign and/or on the schedule of the laboratory conditions.

2.3   Sampling QA/QC

To minimise background contamination, the following measures should be considered within the sampling campaigns, also when they are carried out in parallel to other monitoring campaigns:

c)   Use of glass or aluminium/metal materials only, avoid the use of synthetic materials.

d)   Pre-cleaning of sample containers and instruments with filtered water and/or ethanol or isopropanol. Glassware can also be subject to baking within a muffle oven at 500 °C.

e)   Operators to avoid wearing synthetic clothes . Operators to position facing the wind while retrieving the sample. Operators to take care that potential contamination sources during sampling and sample processing are avoided.

f)   Integration of blank samples: a representative number of blank samples are integrated to account for contamination during sampling. The number of blank samples should be at least 3. The total number of blank samples should be representative for varying sampling conditions during the respective sampling campaign and thus, should reflect the specific contamination potential e.g. through varying weather conditions, varying operators wearing varying clothes. It is recommended to retrieve material from any device of synthetic polymer origin implemented during sampling. These comparative materials should be investigated for their polymer composition to enable exclusion of clearly identified contamination from sampling devices.

A proportion of 10 % blank samples of the total number of sediment samples is recommended for homogeneous conditions during the sampling campaign.
For generating blank samples, an empty sampling container is positioned next to the sample and opened while retrieving the sample. The resulting blank sample is subject to laboratory analyses in the same manner as sediment samples.

3. Sample treatment / laboratory analyses

Sample treatment and laboratory analyses can be done applying different methods when specific quality criteria are ensured. Any sample treatment needs to ensure not to harm synthetic polymers by applying strong chemicals and high temperatures. The treatment process and methods applied need to be controlled via contamination control and recovery tests with reference samples.

3.1 Laboratory QA/QC

3.1.1 Contamination control

Appropriate measures to reduce air contamination, cross-contamination and contamination control must be taken during laboratory analysis. These include:

- Wearing of personal protection equipment made of natural materials (cotton laboratory coats, avoid plastic fibre face masks).
- Ensuring clean laboratory conditions (regular cleaning, regulated air circulation, minimized presence of staff, use of clean room and laminar flow chambers combined with fume hoods if possible).
- Avoidance of any plastic materials during analyses (preferred use of glass and stainless steel materials).
- Pre-filtration of water and chemical solutions with filter pore size significantly lower than minimal cut-off size of targeted particles in the samples.
- Pre-cleaning of beakers and instruments.
- Pre-cleaning of filters (rinsing, annealing according to filter material).
- Covering samples and working solutions throughout the sample processing.
- Reduction of processing steps as far as possible.
- Inclusion of a relevant number of blank samples analysed in parallel with each sample series.
- Inclusion of a relevant number of reference samples analysed in parallel with each sample series.

3.1.2 Blank samples and recovery tests (mandatory)

g) A relevant number of blank samples is to be analysed in parallel with each sample series (set of samples investigated in parallel in one laboratory processing cycle). Combining field blank and laboratory blank samples is not recommended since the number of samples processed within one sample series may differ from the number of samples being representative for the respective field blank sample.

h) Microlitter particles detected within both, field and laboratory blank samples, are used to calculate the limit of detection (LOD - mean + 3 x standard deviation of the particle concentration) according to McDougall et al. (1980). LOD thus reflects the efficiency of the precautionary methods during sampling and sample processing of the respective laboratory. LOD is reported within the data to EMODnet. Blank values are not subtracted from the results on sediment samples.

i) A relevant number of reference samples is to be analysed in parallel with each sample series. Reference samples reflect the efficiency of the respective laboratory protocol and are treated in the same manner and throughout all steps as the sediment samples.

j) Reference samples should encompass real sediment samples that are spiked with a relevant number of synthetic polymer particles that are representative for dominating size categories, morphologies and polymer composition of the particles to be detected within the sediment samples. The number of added reference particles is to be discussed. The number of reference particles will affect the resolution of the recovery

rates, thus, a number of at least 50 reference particles for both fragments and fibres could be recommended leading to a resolution of 2 %.

k) The recovery ratio (%) is calculated for re-detected added reference particles as the mean value accounting for different size categories, morphologies and polymer composition. It is recommended to include reference material containing three types of polymer with different densities, three morphologies and a similar size to the targeted lower cut-off size (i.e. 100 µm) of particles according to Cui et al. (2022). The mean recovery ratio is reported together with the data to EMODnet. Results on sediment samples are not corrected for recovery rates.

3.2 Sample volume and weight for laboratory analyses

In general, the sample volume for laboratory analyses is dependent on sample composition, sample storage conditions and further sample processing methods.

Field samples are homogenised by stirring with glass or metal spatulas or spoons. The respective volume for laboratory analyses is determined (e.g. by using a metal measuring spoon) and weighed to a pre-cleaned beaker with an analytical balance (accuracy minimum 0.01 mg).

In parallel, a second aliquot of the field sample is investigated for water content in order to determine the dry weight of sediment. Therefore, an aliquot of approximately 10 ml is transferred and weighed into an evaporating dish and dried at 105 °C. Following cooling to room temperature within a desiccator, the samples are reweighed, and dry weight is calculated. Equally, dry weight of sediment can be calculated from weight difference derived through freeze drying.

3.3 Sample digestion

In general, the order of digestion and density separation depends on the sample treatment protocol and particle analysis technique of the processing laboratory.

Optional digestion protocols cover oxidative, enzymatic, alkaline or mixed treatments. The implementation of acid digestion is not recommended since especially strong acids proofed to affect synthetic polymers. The duration of the sample digestion depends on the selected digestion protocol.

The application of low temperatures and stirring of the samples are optional add-ons within sample digestion. The application of temperatures >40°C (>50°C if enzymatic digestion is applied) is to be avoided since it may damage synthetic polymers.

After digestion, the digestion solution is rinsed-off over a sieve with the mesh size of the minimum size of targeted particles (100 µm mandatory, see chapter 3.5 and 4.3 for further options).

If particle dimensions are not determined by single particle a size separation step with a sieving cascade encompassing at least 100, 300 and 1000 µm can be applied at this stage (smaller mesh sizes are optional). If pre-sieving at the beginning of the laboratory processing has not been applied, an additional sieve with a mesh size of 5000 µm can be integrated at this stage.

3.4 Density separation

The choice of the density solution and the device used for density separation depends on the respective protocol applied. Density solutions cover zinc chloride ($ZnCl_2$), sodium iodide (NaI), and sodium polytungstate ($NaWO_4$) with a minimum density of 1.5 g/cm³. The application of solutions with densities >1.7 g/cm³ is recommended since this will distinctly improve the recovery rates of synthetic particles of higher material densities. The use of sodium chloride (NaCl) is not recommended since a relevant number of synthetic polymers will not be recovered due to low solution density.

In general, samples are introduced into the density separation solution, stirred for 10 minutes and left for settling for 24 h. The supernatant suspension is rinsed thoroughly with filtered water and

transferred onto filters applicable for the further particle identification. Filters are left to dry in pre-cleaned glass petri dishes. It is recommended to repeat the density separation process at least once.

3.5 Particle identification

The identification of synthetic particles depends on the device available and varies between optical microscopic identification, spectroscopic approaches like FTIR and Raman spectroscopy and staining approaches like Nile red staining in combination with fluorescence microscopy. Particles are identified according to numbers, size classes, morphology, colours (optional) and polymer composition (on at least a subset). The minimum cut-off size for data to be reported is 100 µm (see 4.3 for size classes and options).

3.6 Polymer identification

The determination of at least a subset of particles for their polymer composition via FTIR or Raman spectroscopy is mandatory. Device settings and minimum library match (%) attributed is to be recorded within the metadata to EMODnet. Spectra libraries utilised for polymer composition determination should integrate spectra from synthetic and organic components and weathered synthetic polymers. It is suggested to agree on one or several libraries that are used by all processing laboratories and/or to generate a combined FTIR and/or Raman spectra library for HELCOM microlitter monitoring.

It is recommended to analyse the polymer composition on a representative subset with a minimum of 10 % (preferably at least 20 particles per sample) of synthetic particles identified within the size categories from 100 to 1000 µm. The subset size of particles identified in any smaller size category is to be discussed. The particles integrated in the subset are to be selected representatively according to size categories and morphologies.

4. Parameter and data recording

Parameters are to be recorded according to EMODnet requirements (see section 5). Data can also be reported to ICES DOME when parameters and attributes and e.g. harvesting of data from EMODnet will be harmonised (and the consent of the country is given). The reporting to or harvesting of data through ICES DOME is under discussion.

Parameters to be recorded encompass the following:

4.1 Numbers

The recording of number of particles identified as synthetic polymers/microlitter is mandatory.

Data are calculated to number of particles and optionally weight in grams per kg dry weight of sediment. At this stage, no recommendation on re-calculating number of particles into mass is given. The development of conversion algorithms based on polymer composition and particle size/volume is to be evaluated.

4.2   Morphology

The morphology of all identified particles is to be recorded according to the following morphology classes:

Table 1 Morphology classes to be used to report all identified particles.

EMODnet identifier "micro-litter morphology"	Name	Definition according to EMODnet	Definition according to GESAMP 2019 (Kershaw et al. 2019)
H0100004	Filaments	Slender thread-like micro-litter particles	"Line" (Fibre, filament, strand): long fibrous material that has a length substantially longer than its width
H0100005	Films	Micro-litter particles derived from plastic sheets or thin plastic films	"Film" (sheet): flat, flexible particle with smooth or angular edges
H0100006	Foams	Any kind of micro-litter particle made of plastic foam, including styrofoam	"Foam" (EPS, PUR): near spherical or granular particle, which deforms readily under pressure and can be partly elastic, depending on weathering state
H0100002	Fragments	Irregularly-shaped plastic micro-litter particles with broken off edges that may be rounded or angular	"Fragment" (granule, flake): irregular shaped hard particles having appearance of being broken down from a larger piece of litter
H0100003	Pellets	Micro-litter particles from industrial origin only. In comparison with granules, pellets are usually flat on one side, rough surface and irregular, round shapes	"Pellet" (resin bead, Mermaids tears): hard particle with spherical, smooth or granular shape
H0100009	Granules	Micro-litter particles with smooth spherical shape. In comparison with pellets, they have a rounder shape	

It is to be evaluated whether microbeads are to be reported as a single class or identified from the data set as morphology: granules and the (smaller) dimension in size compared to pre-production resin pellets.

It is to be evaluated whether "pellets" and "granules" should be separate classes. In addition, it has to be considered that "film" and "foam" might not be identified due to restrictions of devices or protocols especially within the smaller size fractions.

4.3   Particle dimensions

The dimensions of identified particles should be recorded according to the following size classes:
- 100 – 299 µm
- 300 – 999 µm

- 1000 – 4999 µm

The reporting of size classes below 100 µm is optional according to the following size classes:
- 50 – 99 µm
- 20 – 49 µm
- <20 µm

It is to be pointed out that results may be biased if particle dimensions are retrieved from mesh sizes from sieving and filtering or measuring of actual particle length and width dimensions.

The reporting of absolute dimensions on particle length and/or particle width is optional.

Sizes of particles are defined according to:
    l)   Length (maximum Ferret diameter in longitudinal orientation)
    m)   Width (maximum Ferret diameter perpendicular to the identified length transect)

Fibres with a length > 5000 µm are considered "mesolitter" and are therefore excluded from the data analysis.

4.4   Polymer composition

Polymer composition is to be reported according to polymer classes and is to be defined for at least a subset of identified synthetic particles.

It is suggested to align the polymer types according to the list modified from AMAP 2021 (see Table 2) but to set up a short list with prioritised synthetic polymers that are predominantly found in environmental samples and that at least have to be reported when occurring.

Table 2 Polymer types for data reporting (modified from AMAP 2021: 225)

Polymer type name	Examples of materials included (detailed level)	Modifications compared to AMAP (2021)
Acrylonitrile based	e.g. acrylonitrile butadiene styrene (ABS), polyacrylnitrile (PAN)	Modified to "Acrylonitrile based", PAN removed from polymer type and integrated here as an example
Cellulose based	e.g. cellulose acetate (CA), cellulose nitrate (CN)	Modified to "cellulose based", examples added
Polyamide based	e.g. all types of polyamide (PA) like various nylons	
Polycarbonate based	e.g. polycarbonate (PC)	Modified to "polycarbonate based"
Polychlorinated polymers	e.g. polyvinyl chloride (PVC), chlorinated PE, various chlorinated polymers	
Polyester based	e.g. polyethylene terephthalate (PET), all other types of polyesters	Modified to "polyester based"
Polyethylene based	e.g. high density polyethylene (HDPE), low density polyethylene (LDPE), and copolymers with a major PE fraction including ethylene-vinyl acetate copolymer (EVA)	EVA removed from polymer type and integrated into polyethylene based.
Polyfluorinated polymers	e.g. polytetrafluoroethylene (PTFE)	
Polymeth(ester)acrylate based	e.g. all types of polymeth(ester)acrylate (PM(ester)A)	
Polypropylene based	e.g. polypropylene (PP), and copolymers with a major PP fraction	
Polystyrene based	e.g. polystyrene (PS), and copolymers with a major PS fraction	

Polyurethane based	e.g. all types of polyurethane (PUR)	
Rubbers, automotive	e.g. styrene butadiene rubber (SBR), tire wear	SBR added as an example
Varnish/paint particles	If different from PM(ester)A	
Other plastics	e.g.polyether ether ketone (PEEK), polyoxymethylene (POM), polyvinyl acetate (PVA), polylactic acid (PLA), polyhydroxyalkanoate (PHA)	Examples added / moved from single polymer classes
Other rubbers	e.g. ethylene propylene diene monomer rubber (EPDM), nitrile rubbers, natural rubbers, silicone	Examples added / moved from single polymer classes / rubber types (refers to "rubbers sealing", "nitrile rubbers", "natural rubbers and derivates", "silicone rubbers and derivates"
Other microlitter materials	e.g. metal, glass	Examples added
Other semi-synthetic polymers	e.g. rayon	Polymer type added / introduced

4.5   Optional parameters

The recording of particle colours and/or transparency is optional. Colours and transparency are classified according to EMODnet:

Colour classes:

- black / grey
- blue / green
- brown / tan
- white / cream
- yellow
- orange / pink / red
- purple
- multicolour

It is suggested and discussed to include a class „colourless" in order to address particles derived from colourless and transparent foils or particles from e.g. (uncoloured) plastic bottles.

Transparency:

- yes
- no

4.6   Sediment parameters

Mandatory parameters: dry weight of sediment (g, weight after drying at 105°C, according to ISO 11465:1993 (2020).

Optional parameters: water content (%, weight difference between 40°C and 105 °C according to DIN ISO 11465), carbonate content (%), total organic carbon (%).

It is under discussion whether organic content (%, to be determined by loss on ignition at 550 °C) and grain size distribution according to sand (63-2000 µm, %) and clay + silt (2-63 µm, %) should be mandatory or optional parameters.

5. Data reporting to EMODnet

Data are to be reported to EMODnet according to current specifications provided by EMODnet (i.e. Vinci et al. 2021).

The reporting to or harvesting of data through ICES DOME is under discussion.

The following lists comprise parameters (mandatory and optional), EMODnet codes and descriptions where available and suggestions for modifications or the integration of further parameters following the discussions and suggestions provided within these draft guidelines and first evaluations through EMODnet.

Parameters and related attributes are under continuous development. Therefore, it is recommended to consult the latest tables and vocabularies online at the NERC Vocabulary Server (NVS).

Table 3 Current list of default (green), mandatory (orange) and optional (light orange) parameters to be reported (modified from Vinci et al., 2021, p7).

Label/column header	Concept id	Use	Comments
Cruise		metadata/mandatory (ODV Default)	
Station		metadata/mandatory (ODV Default)	
Type		metadata/mandatory (ODV Default)	The suggestion is to use type "B". From manual: 'B' for bottle profile data. For time series and trajectories set to 'B' for small (<250) row groups
YYYY-MM-DDThh:mm:ss.sss		metadata/mandatory (ODV Default)	Start date/time. Format must be adapted to the date value (for example YYYY-MMDDThh:mm is second are not available)
Longitude [degrees_east]		metadata/mandatory (ODV Default)	start point coordinates
Latitude [degrees_north]		metadata/mandatory (ODV Default)	start point coordinates
LOCAL_CDI_ID		metadata/mandatory (ODV Default)	
EDMO_code		metadata/mandatory (ODV Default)	EDMO_CODE of the data centre distributing the data (the one connected to the CDI service)
Bot. Depth [m]		metadata/mandatory (ODV Default)	Field empty if no data
MinimumObservation Depth [m]	MINWDIST	mandatory in ODV micro-litter	
MaximumObservation Depth [m]	MAXWDIST	mandatory in ODV micro-litter	
SampleID:INDEXED_TEXT	SAMPID01	mandatory in ODV micro-litter	
Microlitter_Type:IND EXED_TEXT	SDN:H01	mandatory in ODV micro-litter	Type of the item (H01 SDN vocabulary); MLITYPS
Microlitter_Size:INDE XED_TEXT	SDN:H03	mandatory in ODV micro-litter	Size classes (H03 SDN vocabulary), MLITSZS

Microlitter_Count [Dimensionless]	MLITCNTS	mandatory in ODV micro-litter	Number of items collected. It's the official mandate from MSFD to provide the count of collected microplastics.
EventEndDateTime [YYYY-MMDDThh:mm:ss.sss]	ENDX8601	additional/optional	End date/time
EventEndLongitude [degrees_east]	ENDXXLON	additional/optional	End point coordinates. Either End Lat/Lon or SamplingEffort are mandatory
EventEndLatitude [degrees_north]	ENDXXLAT	additional/optional	End point coordinates. Either End Lat/Lon or distance are mandatory.
Microlitter length	NEW	additional/optional	
Microlitter width	NEW	additional/optional	
Microlitter_Weight [g]	MLDWSD01	additional/optional	Weight of the collected items, not mandatory Information in grams
Microlitter_Color:IN DEXED_TEXT	MLITCOLS	additional/optional	Colour classes (H04 SDN vocabulary)
Microlitter_Transparency:INDEXED_TEXT	MLITROPS	additional/optional	Transparency classes (H06 SDN vocabulary)
Microlitter_Polymer_type:INDEXED_TEXT	MLITPOLS	additional/optional	Polymer type of the micro-litter (H05 SDN vocabulary)
WMO_Sea_State [Dimensionless]	WMOCSSXX	additional/optional	Sea conditions following the Douglas scale
Wind_direction [degT]	EWDAZZ01	additional/optional	Direction relative to true north from which the wind is blowing
Wind_speed [m/s]	WSBZZ01	additional/optional	Sustained speed of the wind (distance moved per unit time by a parcel of air) parallel to the ground at a given place and time.
Sampling_protocol	SAMPPROT	additional/optional	The name of, reference to, or description of the method or protocol used to produce the sample

6. References

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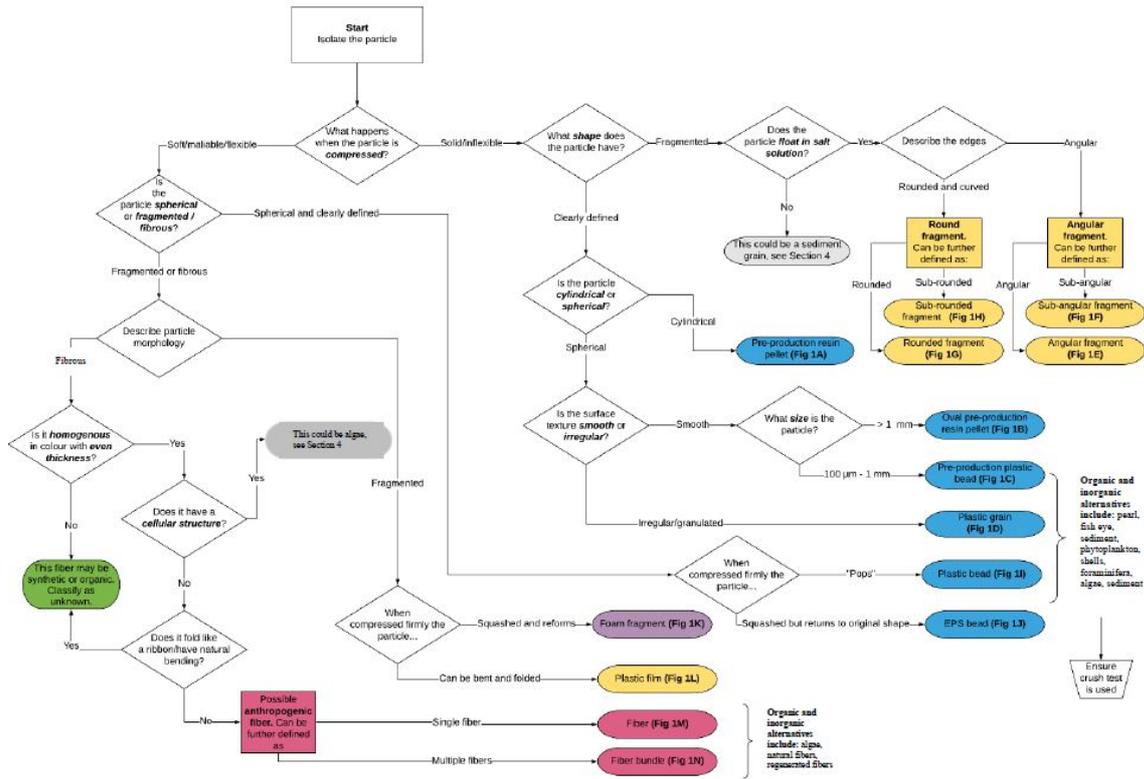
Annex

Figure A1   Proposed flow chart for the visual identification of microplastics. (AMAP 2021: 223, reproduced from Lusher et al., 2020).