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Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring (CORMON Marine Litter)

Meeting held by videoconference, 30 March 2021

Agenda item 7: Monitoring Guidelines/Protocols for Floating Microplastics

Monitoring Guidelines/Protocols for Floating Microplastics

Note by the Secretariat

The 19th Meeting of the Contracting Parties (COP 19), held in February 2016, adopted the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria (Decision IG. 22/7), with a list of regionally agreed good environmental status descriptions, common indicators and targets, with principles and clear timeline for its implementation.

In line with IMAP, Guidance Factsheets for Marine Litter were developed, reviewed and agreed by the Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring (CorMon on Marine Litter Monitoring) (Madrid, Spain, 28 February – 2 March 2017), the Meeting of the MED POL Focal Points (Rome, Italy, 29-31 May 2017) and by the 6th Meeting of the Ecosystem Approach Coordination Group (Athens, Greece, 11 September 2017). The Guidance Factsheets for Marine Litter provide concrete guidance to the Contracting Parties supporting implementation of their respective national monitoring programmes in line with IMAP requirements and were used during the elaboration of the 2017 Mediterranean Quality Status Report (2017 MED QSR).

Moreover, the Data Standards (DS) and Data Dictionaries (DD) for Common Indicators related to Marine Litter (IMAP EO10) were reviewed and agreed during the Meeting of MED POL Focal Points (Istanbul, Turkey, 29-31 April 2019) and the 7th Meeting of the Ecosystem Approach Coordination Group (Athens, Greece, 9 September 2019)

Considering the needs to fill the methodological gaps on all different aspects of marine litter monitoring, UNEP/MAP introduced in 2019 the Information Document "*Methodological Elements for Monitoring Floating Microplastics*" (UNEP/MED WG.464/Inf.4) during the Joint Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring and ENI SEIS II Assessment of Horizon 2020/National Action Plans of Waste Indicators (Podgorica, Montenegro, 4-5 April 2019). An updated version of the document was presented to the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (CORMONs) (Videoconference, 1-3 December 2020) and further to its Conclusions and Recommendations the informal Online Working Group on Marine Litter (OWG-ML) was established with the scope, amongst other issues, to further improve and elaborate the revised version of the present document.

The OWG-ML consisting of the following countries (in alphabetical order): Croatia, France, Israel, Italy, Spain, Tunisia, and Turkey, had 2 online meetings. To that effect the guidelines for monitoring floating microplastics were reviewed and updated during these meetings of the OWG-ML, as well as in intersessional period, under the leadership of Italy, with outmost scope to address the recommendations of Integrated CORMONs` session related to Marine Litter.

The document at this stage extracts from the most commonly applied methodologies for monitoring floating microplastics and presents the basic methodological elements for monitoring floating microplastics in the Mediterranean in line with IMAP requirements. The present guideline aims to guide the technical personnel of the IMAP competent laboratories for the implementation of the standardized and harmonized monitoring practices on IMAP EO10 (Marine Litter) Common Indicator 23 (Floating Microplastics).

The revised version of the present working document (i.e. WG.490/6) since has received major revision, including elements extended beyond the initial Conclusions and Recommendations of the Integrated CORMON Meeting (1-3 Dec. 2020), but of added value for the proposed guidelines, is thus submitted to the Participants of the present CORMON Marine Litters Meeting for review and approval in order to be subsequently submitted to the Meeting of MED POL Focal Points foreseen in May 2021.

The Secretariat wishes to warmly thank and acknowledge all the marine litter experts that volunteered to participate to the informal OWG-ML, for providing substantive input, scientific knowledge, and

most importantly for revising the present document within a very short timeframe which is expected to largely support monitoring of floating microplastics in the Mediterranean.

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1. Introduction

1. The basic elements, describing sampling methodology for floating microplastics as well as laboratory techniques and analysis for identification, characterization and quantification, are reported below aiming to provide technical guidance and to facilitate the Contracting Parties to evaluate the abundance and composition of microplastic, found in sea surface waters around the Mediterranean. The present chapter has been based on a number of guidance documents for monitoring floating microplastics.[1]

2. Definition of Microplastics: Microplastics includes all sorts of small particles of plastic with a diameter smaller than 5 mm and that pass through a 5 mm mesh screen but are retained by a lower mesh size, according to the chosen size class[2] (i.e. 330 μm - 5 mm). Microplastics can be found dispersed in the marine and coastal environment as a consequence of plastic pollution.

3. Microplastics are present in a variety of products, ranging from cosmetics to synthetic clothing to fragmentation of larger products such as plastic bags and bottles into smaller items. Consequently, microplastics are divided into two types according to their origin: primary and secondary. Examples of primary microplastics include microbeads found in personal care products, plastic pellets used in industrial manufacturing, and plastic fibres used in synthetic textiles (e.g. polyester, acrylic, nylon). Primary microplastics enter the environment directly through any of various channels, for example personal care products being washed into wastewater systems from households, unintentional loss from spills during manufacturing or transport, or abrasion during washing (e.g., laundering of clothing made with synthetic textiles). On the other hand, secondary microplastics originate form from the breakdown of larger plastics; this typically happens when larger plastics in the marine environment undergo weathering, through exposure to wave action, wind abrasion, and ultraviolet radiation from sunlight, amongst others.

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[1] Galgani F., G. Hanke, S. Werner, et al. (2013) Guidance on Monitoring of Marine Litter in European Seas. EU/JRC editor, EUR 26113, 123 pages, doi:10.2788/99475 (pdf).

Zampoukas N., Palialexis A., Duffek A., et al. (2014) Technical guidance on monitoring for the Marine Strategy Framework Directive. EUR – Scientific and Technical Research series – ISSN 1831-9424, 166 pages, doi: 10.2788/70344.

Ryan, PG, 2013. A simple technique for counting the sea steep gradients between the Straits of Malacca and the Bay of Bengal. Marine Pollution Bulletin 69 (1), 128-136.

UNEP, 2015. Marine Litter Assessment in the Mediterranean. UNEP / MAP Athens, 45 pp.

UNEP / MAP MEDPOL, 2011. Results of the Assessment of the Status of Marine Litter in the Mediterranean Sea, (UNEP / MAP (DEPI) / MED WG.357 / Inf.4).

[2] Galgani F., Giorgetti A., Vinci, M., Le Moigne M., Moncoiffe, G,. Brosich, A., Molina, E., Lipizer, M., Holdsworth, N., Schlitzer, R. Hanke, G., Schaap, D., 2019. Proposal for gathering and managing data sets on marine micro-litter on a European scale, 07/06/2019, 34 pp., DOI: 10.6092/8ce4e8b7-f42c-4683-9ece-c32559606dbd.

GESAMP, 2016. “Sources, fate, and effects of microplastics in the marine environment: part two of a global assessment” (Kershaw, P. J., and Rochman, C. M., eds). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep.Stud. GESAMP No. 93, 220 p.

4. Due to their small size, lightweight properties and diversity in density, microplastics may be found on the sea surface, or even deeper the entire. Microplastics can also sink to seafloor bottom due to their specific density, fouling by organisms or weathering. Monitoring of microplastics in sediment are not considered in this document.

2. Sampling of Microplastics at Sea

5. When focusing on sampling floating microplastic, it is advisable to conduct the sampling in calm sea conditions, preferably when the wind intensity is less than three (3) Beaufort (approximately 13 - 19 km/h).

2.1 Manta Net Properties

6. The Manta Net or Manta Trawl is the most commonly used sampling equipment. This tool is specifically designed to collect samples from the surface layer of the sea. The use of Manta Net allows the sampling of large volumes of water, retaining at the same time the target material (i.e. microplastics). High Speed Manta Net is also used but given that its use is not so common it is not taken into consideration in this document.

7. Mouth size and length: The Manta Net (Figure 1) consists of a rectangular metal floating device from which a net cone is attached, having a final collection sock (or any other relevant collection equipment) at its very end where the microplastics and the organic matter are collected. The dimensions of the mouth of the metal device are not pre-determined, it is however advisable to always maintain a ratio equal to ½ between the height and the width of the mouth of the metal device. The most common dimensions of the mouth of the Manta Net are 50 cm in width and 25 cm in height, however other dimension are possible. This dimensions refer to the inside size of the mouth, the part to which the 2.5 m net in length is connected. The outer part is wider assuming an overall truncated pyramid shape.

8. Mesh of the net and cup/sock: The net cone, which is attached to the floating metal device, should be made out of a net with a mesh size of approximately 330 μm. In order to avoid problems of regurgitation following clogging, especially in eutrophic waters, it is necessary to constantly check the effectiveness of sampling. Optional, for areas with high gelatinous organisms and zooplankton, a metal net (mesh 1-2 cm) could be added in front of the mouth of the manta net.

9. Dimensions of the wings: Two metal wings are attached right and left from the metal device to ensure that the Manta Net is always kept floating on the sea surface (Figure 1). The dimensions of the wings depend on the weight of the mouth, since they are used to ensure the buoyancy of the instrument. Therefore, it depends on the weight of the metal floating device. In most cases, each wing has the same dimension in length as the metal mouth. A size of 40–70 cm in length is generally expected, In any case, they should be sufficiently large to keep the Manta Net afloat.

2.1 Use of Manta Net

10. The Manta Net is lowered slowly from the boat or the vessel to the sea and is left afloat. According to the dimension of the boat it is possible to tow the net from stern or from the side. If the net is lowered to stern, the distance between the boat and the Manta Net should be at least 50-70 m. If the net is lowered on the side, of the boat the net should be kept at the distance of around 3 m. When possible, it is suggested to use non plastic material rope in order to avoid contamination. The pull of the manta net from the side of the vessel or the zodiac may be another option (Figures 2 and 3). It is extremely important for the manta net to be left outside of the bow wave caused by the spinning of the

propeller because this turbulence will significantly influence the amount of collected microplastics as well as the contamination due to paint chips from the vessel (Figure 1).

Figure 1: Manta net being operated in calm sea, outside of the bow wave caused by the spinning of the propeller (Photo: © Christos Ioakeimidis, UNEP/MAP).

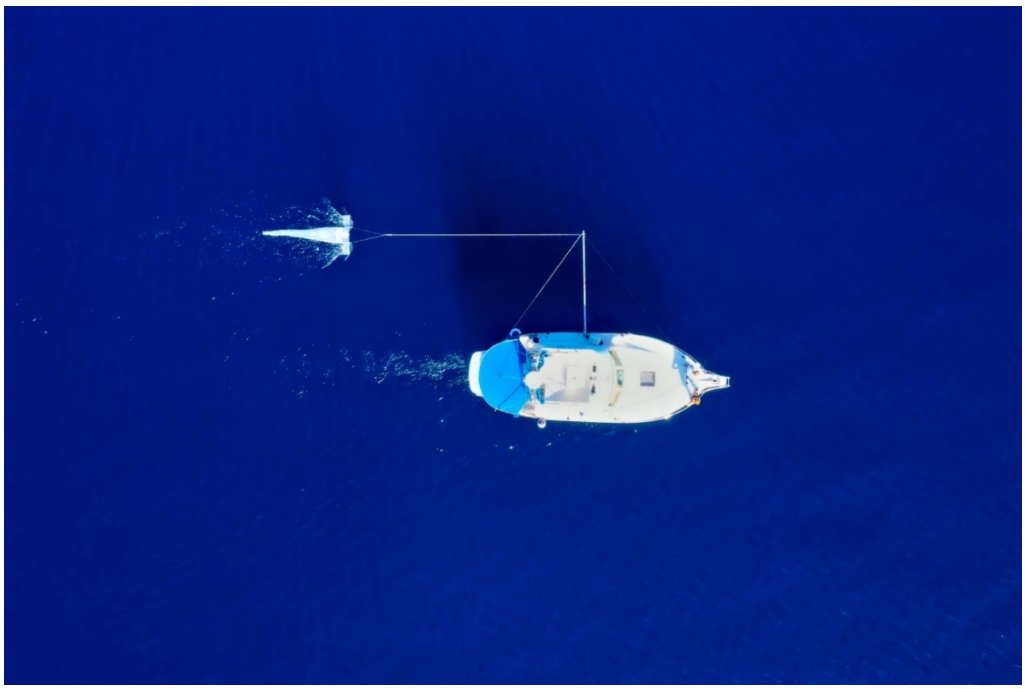

Figure 2: A manta net being pulled from the side of the vessel (Credit © Stipe Muslim, Croatia)

Figure 3: A manta net being pulled from the side of the vessel (Credit © Cecilia Silvestri & Marco Matiddi, Italy)

2.2 Designing a Monitoring Campaign

11. Method for sampling: A proper design of the monitoring surveys should include at least coastal and offshore sampling. For coastal surveys, sampling should be carried out at 3 stations located at different distances from the coastline (e.g. 0.5, 1.5, 3 Naut. Miles) set along an orthogonal line to the coast. For offshore surveys, the samples should be carried out at 3 stations located at 6, 12 and 24 Naut. Miles following the trajectories of the coastal ones. Once the boat/vessel is positioned at the sampling point, the manta net is lowered and trawled for approximately 20 minutes or more (according to the Manta Net clogging) along a straight transects, with a speed approximately of 1-2 knots. In order to allow the Manta Net to properly filter the water and thus have its whole mouth submerged into the sea, under no circumstances the speed should exceed 3 knots. The 20-minute trawl must be conducted in the opposite direction to the surface current or in any case opposite to the wind direction.

12. *Optional:* In case of large quantities of organic matter, mucilage and gelatinous zooplankton are present during the sampling, it is suggested to split the sampling time into two 10 minutes hauls. Both samples will be merged to have an equivalent to one 20-minutes trawl.

13. GPS Coordinates: For each trawl the GPS coordinates (grades and thousandths, GG°, GGGGG) at the beginning and end of sampling must be recorded in WGS 84 UTM 32. Additional GPS coordinates (e.g. every 10 minutes) are most welcomed as will allows us to confirm, or not, the rectilinear transect and even to determine a more accurate length of the trawl. In case of large quantities of organic matter and relevant organic gel are present during the sampling, it is suggested to decrease the sampling time into two 10-15 minutes hauls.

14. Wind direction and intensity should be recorded along with sea condition.

15. Position of the survey stations: The position of the stations for coastal monitoring must be determined according to the characteristics of the survey area (i.e. upwelling and downwelling areas, storage areas for local hydrodynamic conditions, distance from direct input sources, such as river mouths, distance from port facilities or relevant urban settlements etc.). The position of the stations for offshore sampling must be complementary to those of the coastal ones, along the trajectories of the coastal stations at 6;12 and 24 miles and/or fall within the accumulation areas envisaged by the predictive models. The number and position of the survey stations will be established in order to have a better representation of the entire region, considering areas of both high and minimum anthropogenic

activity/impact. The criteria for choosing the position of the transects must be recorded on dedicated sampling sheets.

16. Replicates: Because of the variability of floating microparticles distribution, it is necessary to increase the data representativity. Further, it is strongly recommended to carry out replicates from the same sampling point. Three replicates for each station are recommended. Each replicates must be conducted following the transect in the opposite direction to the surface current or in any case opposite to the wind direction, approximately parallel to the first one. Using twin manta nets in order to collect duplicate samples in one time is suggested (less time consuming).

2.3 Calculating the Surveyed Areas

17. The surface area of the surveyed water: The calculation of the amount of microplastics should be expressed in number of microplastic particles per square meter based on the following methodological approach:

The surface areas of surveyed water (S) is calculated using the following formula:

$$S = D \times W$$

Where: D: is the distance of the sampled rectilinear transect
W: is the width of the mount of the Manta Net

* It is possible to calculate D by using a flowmeter, or GPS coordinates, or vessel instruments

18. *Optional:* It is also possible to calculate the filtered volume (m^3) by: (i) multiplying the area of the mouth of the net by the distance covered during the tow; or (ii) applying the appropriate formula of the flowmeter as follow:

(i) $V = D \times A$

(ii) $V = N \times A \times c$

D: is the distance of the sampled transect (m)
N: is the number of turns of the propeller recorded by the flow meter during the transect;
A: is the area of the mouth of the used Manta net;(width x height)
c: is a constant value, typical of each flowmeter.

It should be considered that the filtered volume using a flowmeter is more accurate, but the flowmeter needs a continuous maintenance and it can stuck during sampling. For this reason, the square meter measure must be always calculated.

2.4 Sample Collection and Storage

19. Once brought back to the boat or the vessel, the net must be rinsed each time, with sea water from the outside to the inside, from its part close to the mouth towards the collection sock, in order to concentrate all the natural and man-made materials to the cod-end. The collection sock is removed, and the material is transferred into a 250 or 500 ml glass bottles for subsequent qualitative and quantitative analysis (Figure 3). The sock/cup should be washed, from the outside, using distilled water or sea water, and from inside using only distilled water, in order to collect all the material stacked among the mesh. Larger pieces of biological material, including e.g. leaves, bugs, larger algae or wood are picked out of the samples with metal tweezers and carefully rinsed on a metal sieve (< 330 µm). Macro-plastics are picked

out and rinsed in the same way, but instead of discarding them, they could be counted and stored for further analysis. It is important to separate macro-plastic from the sample in order to avoid fragmentation.

Figure 3: Microplastic and organic matter collected in a metal sieve just after the sampling (Photo: © Christos Ioakeimidis, UNEP/MAP).

20. The samples can then be stored in refrigerators (but not in freezers), protected from light and heat. It is possible to add a fixative (i.e. 70% ethyl alcohol), solely in order to prevent the decomposition of the organic matter present (e.g. zooplankton, phytoplankton, etc.), which would release unpleasant odors during the analysis of the samples. This procedure it is not suggested because it can change the microplastic colour.

3. Laboratory Analyses of Samples Collected at Sea:

21. The analysis is aimed at identifying and quantifying the different microplastic particle (as non-degradable) found present in the sample/s.

3.1 Cross Contamination

22. All laboratory equipment should be ensured to be made of glass or metal as much as possible in order to prevent the contamination of the sample, with microplastic particles deriving from the potential plastic equipment, as well as to avoid microplastic fragments from sticking to the walls of the equipment. To avoid this carefully rinsing of the equipment with distilled water should be ensured. The use of distilled water during all the wash/rinsing steps should be ensured. during all laboratory steps. Furthermore, particular attention must be paid to the cleaning of the working area in order to avoid contamination of the sample with microplastic particles, mainly fibers, being present in the atmosphere or being generated from relevant plastic equipment. To this extent, important precautions should be taken to limit the risk of contamination such as:

- Avoid wearing synthetic clothes which could release plastic fibres (such as fleece or stretch fabrics in lycra - polyamide) during the laboratory analyses and wear pure cotton clothes. Always wear a 100% cotton lab coat.

- Avoid the exposure of the sample into the atmospheric air, and thus ensuring to cover the corresponding laboratory spaces to avoid contamination.
- Do not leave windows open while analysing the samples.
- Reduce personnel in the lab during operation.
- Use of laminar flow cabinet is recommended.
- Cover the petri dish during the first stereomicroscopic analysis with a glass.
- Place a damp filter paper in a petri dish in the working area for a blank control in every step representing the whole process of treatment.

3.2 Equipment at the Laboratory

23. The following equipment will be required during the laboratory analysis:

Requirement:

- 5 mm metal sieve;
- 300 µm metal sieve;
- Drying oven;
- Filtration device;
- Petri dishes (glass);
- Jars/Beakers (glass);
- Tweezers;
- Distilled water;
- Micrometre;
- Stereoscope.

Optional:

- *Micrometer;*
- *Additional sieves for size classes;*
- *Oxygen Peroxide or Potassium hydroxide;*
- *Drying oven or hot plat or hot bath;*
- *Laminar flow cabinet;*
- *Vacuum pump system and fiber glasses membrane;*
- *Hot needle, optical microscope, FT-IR or RAMAN spectroscopy.*

3.3 Five Steps at the Laboratory

24. The following **five steps** should be followed during the analysis of the samples:

25. <u>Step 1: Wet Sieving:</u>

- Pour the sample through a stacked arrangement of 5mm and 330 µm metal mesh sieves.
- *Optional: in order to subdivide the items in different size classes it is possible to stack additional sieves (e.g. 1 mm).*
- Pinse the container where the samples are stored several times with distilled water, in order to recover all the microplastics.
- The fraction consisting of plant or animal residues larger than 5 mm (retained by the sieve with the larger mesh) must be thoroughly rinsed with distilled water.
- *Optional: In the presence of large quantities of organic matter, incubate samples on hot plate, hot bath or oven (≤ 40°C) adding supplementary 15% H2O2 or KOH 10% to the sample while evaporate, until all organic matter is digested. Be careful not to exceed 40 °C degree.*
- For the digestion process, the jars with the collected samples should be kept at room temperature for 5 days or less according to the digestion rate. Jars should be covered with aluminum foil or glass dish during the digestion processes.
- *Optional: The digested matter can be filtered on GF/C fiber glasses membrane under vacuum pump, rinse the funnel several time and the membrane with distilled water to remove the organic matter.*

26. Step 2: Transfer Sieved Solid Material:

- Once the sample is filtered, transfer all solids collected in the 330 μm sieves into a Petri dish with the help of a spatula and minimum rinsing with a squirt bottle containing distilled water.
- Ensure all solids are transferred into the glass jars.

27. Step 3: Visual Sorting of Samples:

- Place the Petri dish under the stereomicroscope and proceed with the identification of microplastics. For this, plastic items are counted through visual sorting of the sample and it is recommended to move the Petri dish top-down from the left to the right and vice versa, to facilitate the particle count, perform two rounds of visual sorting under the stereomicroscope
- Filaments with a length > 5mm must still be counted.
- In case of suspected micro-items, hot needle or optical microscope or spectroscopy equipment should be used to detect if it is plastic material.
- *Optional: For size categorization and in order to subdivide the collected items in different size classes put a sheet of graph paper under the Petri dish, this procedure can also be performed with a micrometre inserted in the eyepiece or with an image analysis software (i.e. Image J) which helps in the measurement of identified microplastics.*
- During the entire visual sorting of samples, a blank control will be done for this, an uncovered Petri dish with a filter inside it will be left beside the stereomicroscope and will be inspected for potential airborne contamination after each sample. Colour and shape of identified particles in the blanks will be recorded. If the blank is contaminated, micro-litter items with similar characteristics (e.g. shape, color, polymer type), the amount of this micro-items should be excluded from the results of the same bath.

28. Step 4: Categorization and Classification:

- The identified microplastic particles should be categorized and classified.
- The microplastic particles which are identified in the glass Petri dish should be divided and counted based on the shape (i.e., fiber, filament, film/sheet, fragment granule, pellet, foam) and colour (Figure 4).
- Types of shapes used in microplastics characterization:
    - Fiber: only from textile. They are very flexible with different thicknesses and colours. They can be made by natural or synthetic material.
    - Filament: filiform element elongated, threadlike, thin and less flexible than a fiber, made by artificial polymer (e.g. fishing line).
    - Film/sheet: broken soft plastic piece as foil, they are thinner and more flexible; than fragments (e.g. pieces of plastic bags).
    - Fragment: broken and hard plastic piece, thick, with an irregular shape.
    - Granule: spherical shape, with a regular round shape bead .
    - Pellet: only from industrial origin, irregular, round shapes, and normally bigger in size, than granule.
    - Foam: soft consistency irregular or spheroid shape (e.g. polystyrene, rubber silicone).

Figure 4: Common shapes of microplastics. (1: fibers, 2-3: filaments, 4-7: films, 8-11: fragments, 12-14: foams, 15: pellet, 16-17: granule) (Photo: © Ülgen Aytan, Turkey).

29. Attention should be given in distinguishing Fibres (from textile) and Filament (threadlike artificial polymer: i.e. fishing line), as the first one should pass through a 330 μm mesh and are more susceptible to originate from airborne contamination.

30. Figure 4 highlights the differences between Fibres and Filament, while fibers are generally thinner in diameter, with frayed edges and it is often ending in helical winding. In addition, the fibers, when approached with a needle bend and deform (Fig. 5, 1 red fiber and 2 blue fibers).

31. On the other side, filaments have generally a well-defined shape: cylindroid with clear margins, and the colour is more uniform. Furthermore, the filaments are stiffer than the fibers and less deformable (Fig. 5: 2 filaments in blue).

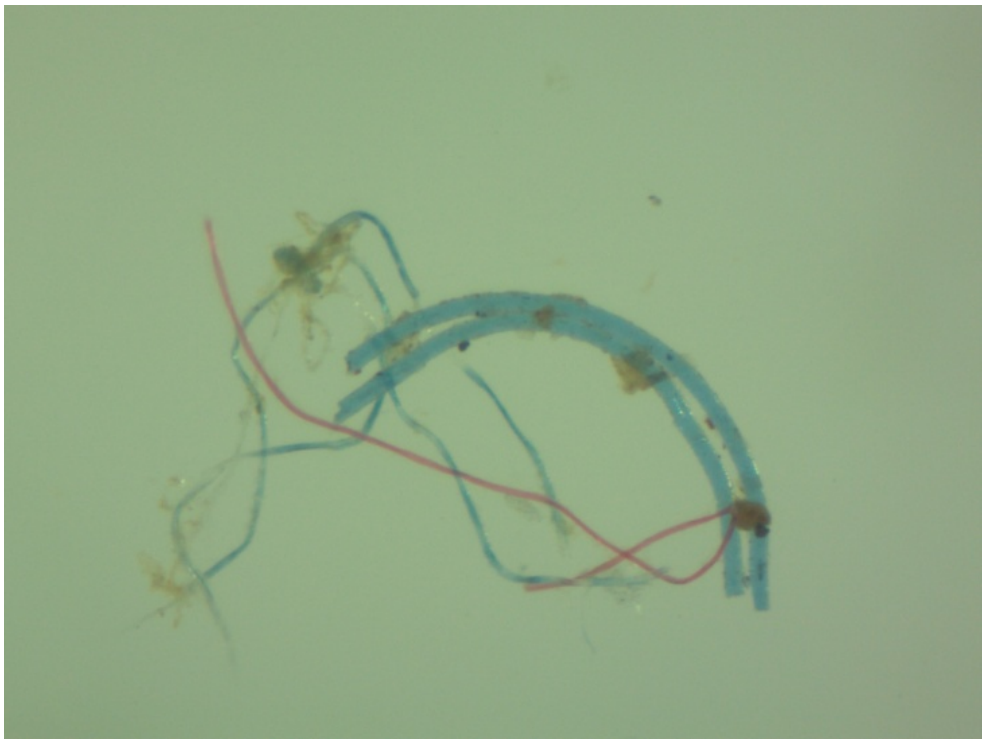

Figure 5: Differences between fiber and filament microplastics (Photo: © Marco Matiddi, Italy).

32. The colour of each microplastic particle should be recorded based on the following approach: white, black, red, blue, green, and other colour (Figures 6 and 7). In case of biofouling or degradation, yellow must be included in the white category and brown in the black category, whereas , orange and pink in the red category. The "*other colour*" category includes all the remaining colours which cannot be specified, or in case an item has different colour on two sides. Furthermore, when a fragment is made up of two different colours depending on the side this has to be always included in the "*other colour*" category. A more specific differentiation is possible when it has relevance for a specific purpose (e.g. project etc.)

33. Finally, for each colour identified, the transparency must be specified, with the proceeding column of the data file indicating if the pieces are opaque or transparent.

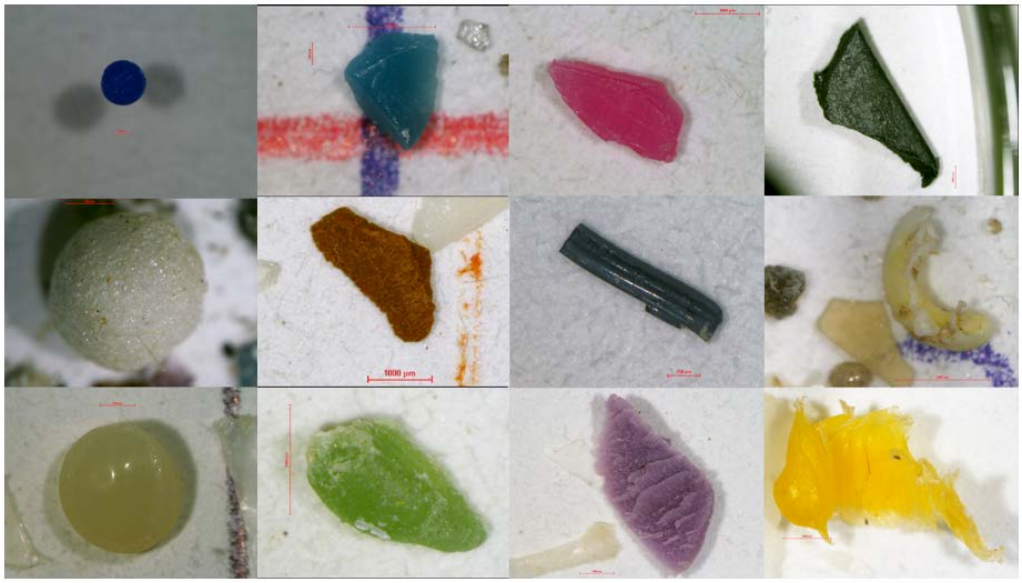

Figure 6: Different colors of microplastics (Photo: © Ofrat Rave, Israel)

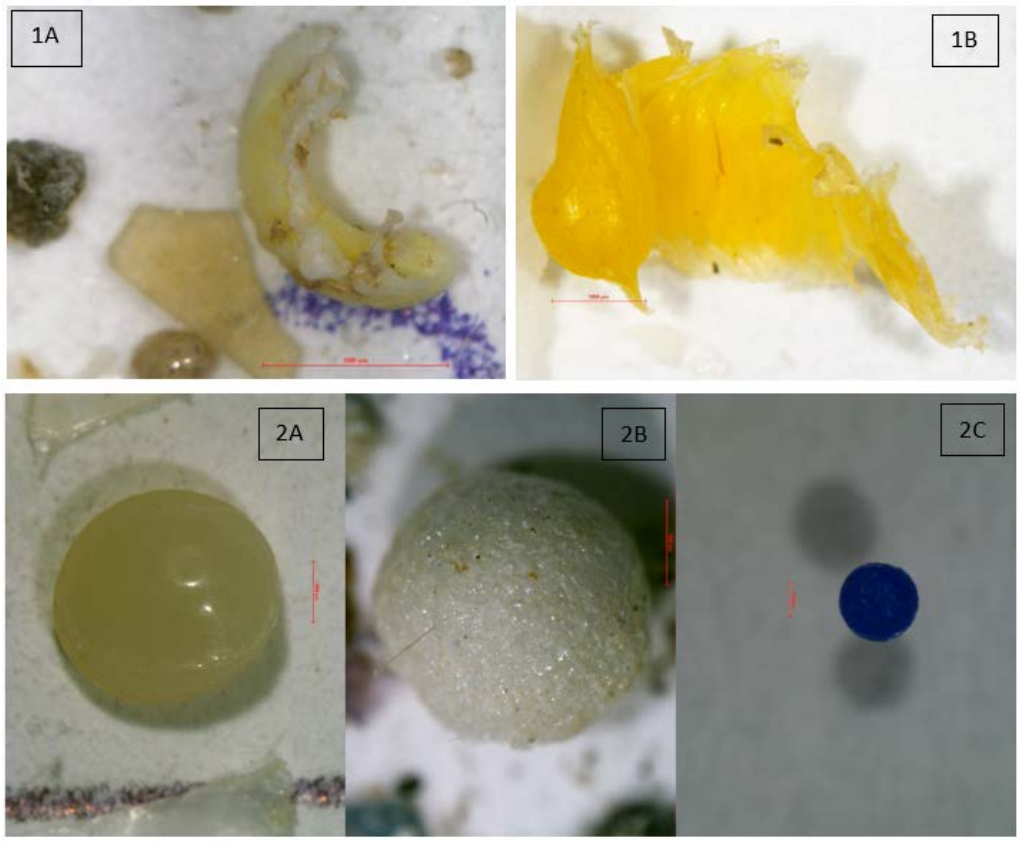

Figure 7: Examples of color categorization for microplastics: [1A] a colored microplastic degraded because of biofouling that should be regarded as "white"; [1B] a yellow-colored microplastic which should be considered as "other color";[2A] a pellet which should be considered as "white" (scale bar 1000µm); [2B] A white-colored foam (scale bar 1000µm); [2C] a colored blue granular (scale bar 250µm). (Photo: © Ofrat Rave and Yael Segal Israel)

Step 5.Reporting units

The reporting units for microplastics abundance from water samples are:

Option 1: Number of Microplastics per Surveyed Area
(No, Particles / km^2 | No, Particles/ m^2)
Option 2: Number of Microplastics per Volume
(No, Particles / m^3)

34. The first one is mandatory as required by the IMAP Common indicator 23 and the Criteria D10C2 of the MSFD. The second one is optional.

35. Information referring to shape and colour of microplastics identified, are useful for source identification.

4. Keynotes

36. Spectral optical procedures such as FT-IR or Raman spectroscopy are very important techniques to differentiated microplastics from non-plastic materials and further verifying plastic polymers which is also necessary for obtaining useful information regarding sources of sea surface plastics. These instruments can perform counting, shape measurement and material identification simultaneously but they are expensive so not all laboratories can afford them. For laboratories that have the possibility to use them, in the case that time and resources do not allow analysis of all samples, the recommendation is to proceed with a representative spectroscopic analysis for a subsample of 10% of the total, choosing the suspected microparticles to verify visual identification.

37. A list of additional physical and chemical parameters of the water column are recommended (non-mandatory) by means of a multiparametric, integrated sampling, which are hereunder listed:

- Depth (m);
- Temperature (^oC);
- Salinity (psu);
- Oxygen (dissolved oxygen – percentage of saturation);
- pH; and
- Transparency (m).