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Podgorica, Montenegro, 4-5 April 2019

**Agenda item 3: State of Play of IMAP Implementation Related to Marine Litter (EO10) and its further
Development**

Methodological Elements for Monitoring Floating Microplastics

The meeting has been organized in collaboration with the European Union funded Project ENI SEIS II South Implementation of the Shared Environmental Information System (SEIS) principles and practices in the ENP South region – SEIS Support Mechanism

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1. Introduction

1. The basic elements, describing sampling methodology and laboratory techniques and analysis, are reported below aiming to provide technical guidance and to facilitate the Contracting Parties to evaluate the abundance and composition of microplastic, found afloat 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, less than 5 mm (i.e. 0.1 µm - 5 mm) in length, or in diameter, found dispersed in the marine and coastal environment as a consequence of plastic pollution.

3. Microplastics are present in a variety of products, from cosmetics to synthetic clothing to plastic bags and bottles. Microplastics are divided into two types: 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., nylon). Primary microplastics enter the environment directly through any of various channels—for example, product use (e.g., 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). Secondary microplastics form from the breakdown of larger plastics; this typically happens when larger plastics undergo weathering, through exposure to, for example, wave action, wind abrasion, and ultraviolet radiation from sunlight.

4. Due to their small size, lightweight properties and diversity in density, microplastics may be found afloat on the sea surface, or even deeper in the water column along the basal region of the thermocline, as a result of intense sea water mixing (i.e. mixing of surface layers with deeper layer of the sea) caused by the waving.

2. Sampling of Microplastics at Sea

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

6. The Manta Net or Manta Trawl is the most commonly used sampling tool. This tool is specifically designed to take 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 material of interest (i.e. microplastics coupled with organic matter).

7. 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

¹ EC/JRC 2013: Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

EC/JRC 2014. Technical guidance on monitoring for the Marine Strategy Framework. JRC Scientific and Policy Report. Scientific and Technical Research series, Report EUR 26499.

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).

dimensions of the mouth of the metal device are not pre-determined, it is however advisable to always maintain a ratio equal to $\frac{1}{2}$ between the height and the width of the mouth of the metal device. However, the most commonly used internal dimensions of the mouth of the Manta Net are 60 cm in width and 25 cm in height. 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. The dimensions of the wings depend on the weight of the metal floating device. In most cases, the wings have the same dimension in length as it is the length of the metal mouth.

8. 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.

9. Use of the Manta Net: The Manta Net is lowered slowly from the boat or the vessel to the sea and is left afloat, being secured to the boat by a rope up to a distance of 50-70 m from it. 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 resulted abundance figures (Figure 1). Wherever possible, it is therefore advisable to lower the net sideways, passing the towing end through a suitable pole installed on one side of the boat. The pull of the manta net from the side of the vessel or the zodiac may be another option.



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

10. A proper design of the monitoring surveys should include at least 3 sampling stations placed in different distances from the coastline (i.e. 0.5, 1.5, 6 Naut. Miles). Once the boat/vessel is positioned at the sampling point, the manta net is lowered and trawled for approximately 30 minutes along a rectilinear transects, with a speed approximately to 2-3

knots. Under no circumstances the speed should exceed the 3 knots, in order to allow the manta net to properly filter the water and thus having its whole mouth submerged into the sea. The 30-minute trawl must be conducted in the opposite direction to the surface current or in any case opposite to the wind direction.

11. 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.

12. The position of the transects along which the sampling will be carried out, must be determined according to the characteristics of the survey area (i.e. the following characteristics should be taken into account: 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). The number and position of the survey transects 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.

13. 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 of surveyed water (S) is calculated using the following formula:

$$S = L \times l$$

Where:

L: is the length of the sampled rectilinear transect

l: is the width of the mount of the Manta Net

14. Sample Collection and Storage: Once brought back to the boat or the vessel, the net must be rinsed with sea water from the outside to the inside, from its part close to the mouth towards the collection sock, in order to collect all the material towards the collection sock.

15. The collected material is then detached from the net and the sample is poured preferably into a 1000 ml, 500 ml or 250 ml glass jars for subsequent qualitative and quantitative analysis (Figure 3). If for any particular reason glass jars are not available, then rigid plastic containers can be used instead. In the latter case, special attention should be put while transferring the collected content/material to avoid contamination with microplastic fibers or particles generating from the plastic jars. The samples can then be stored in refrigerators (but not in freezers), protected from light and heat. It is advisable 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.

3. Laboratory Analyses of Samples Collected at Sea:

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

17. All laboratory equipment should be ensured to be made of glass or metal 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;
- 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.



Figure 3: Microplastic and organic matter Collected in a metal sieve just after the sampling
(Photo: © Christos Ioakeimidis).

18. The following equipment will be required during the laboratory analysis:
- 5 mm metal sieve;
 - 1 mm metal sieve;
 - 300 µm metal sieve;
 - Drying oven;
 - Filtration device;
 - Petri dishes (glass);
 - Jars/Beakers (glass);
 - Tweezers;
 - Distilled water;
 - Micrometre;
 - Stereoscope.
19. The following five steps should be followed during the analysis of the samples:
20. Step 1: Wet Sieving:
- Pour the sample through a stacked arrangement of 5mm, 1mm and 0.3 mm stainless steel mesh sieves
 - Pinse the container several times with distilled water, in order to recover all the microplastics.
21. Step 2: Transfer Sieved Solid Material:
- The fraction consisting of plant or animal residues of more than 5 mm (retained by the sieve with the larger meshes) must be thoroughly rinsed.
 - Weigh a clean and dry glass jar to the nearest 0.1 mg;
 - Transfer solids collected in the 1mm and 0.3 sieves into separate glass jars using a spatula and minimal rinsing with a squirt bottle containing distilled water;
 - Ensure all solids are transferred into the glass jars;
 - Place glass jars in 90°C drying oven for 24 hours or longer to sample dryness.
22. Step 3: Determine the Mass of Total Solids:
- Weigh the dry mass of the total solids collected on the sieves. This is the mass of all microplastic particles, including the weight of the organic matter;
23. Step 4: Sorting:
- Rinse the dry samples with distilled water, pour it through the sieves and place them into grass petri dishes;
 - Separate and sort the mesoplastics (> 5mm) and the microplastics (1mm < > 5mm) through visual observation. The use of a micrometer may be helpful at this stage;
 - Sort the microplastic of up to 300µm with the help of a stereoscope
24. Step 5: Wet Peroxide Oxidation and filtering (0.3 mm sample):
- Approximately 40 ml of 30% hydrogen peroxide per 3 gr of dry sample should be added in case of presence of significant organic matter. Boil at a hot plate (approx.65°C) until the digestion is complete (no natural organic material is visible) will be required.

25. Subsequently, the identified microplastic particles should be counted. The microplastic particles which are identified in the glass petri dish should be divided and counted based on the different shape (i.e. granule, pellet, foam, filament, fragment, sheet) and colour. The following is a brief description of the shape categories:

- Fragment: broken hard plastic piece; it can have a sub circular, angular, sub-angular contour;
- Sheet: broken soft plastic piece often of angular or sub-angular shape;
- Filament: filiform element, flexible, elongated, thin;
- Foam: spheroidal shape, soft consistency (polystyrene);
- Granule: irregular spherical shape or even smooth with a hard consistency;
- Pellets: cylindrical, ovoid, discoidal, spheroid, flat.

26. The colour of each microplastic particle should be recorded based on the following approach: white, black, red, blue, green, and other colour. Yellow must be counted and inserted in the white category and brown must be counted and inserted in the black category. The "other colour" category includes all the remaining colours which cannot be specified. Furthermore, always in the "other colour" category, a fragment should be inserted that has different colours on the two sides. Finally, for each colour counted, the transparency must be specified, with the next column showing if the pieces opaque or transparent.

27. The microplastic concentration in the sample, in terms of shape and colour, is expressed as the number of objects per m² of sampled seawater.

28. 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 (°C);
- Salinity (psu);
- Oxygen (dissolved oxygen – percentage of saturation);
- pH; and
- Transparency (m).