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CORMON Marine Litter)

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Agenda Item 5: Supporting the Initiation of an Intercalibration Exercise for the Collection and Analyses of Floating Microplastic Samples from Mediterranean Laboratories

Preliminary Elements for an Intercalibration Exercise for Collection and Analyses of Floating Microplastic Samples between Mediterranean Laboratories

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Note by the Secretariat

Floating microplastic is part of the Integrated Monitoring and Assessment Programme for the Mediterranean Sea and Coast (IMAP) and its Ecological Objective 10 (EO10) – Common Indicator 23 (CI23).

With the aim to fill the methodological gaps regarding monitoring floating marine litter, more specifically microplastics, the Secretariat and its MED POL Programme introduced in 2019 the "Methodological Elements for Monitoring Floating Microplastics" (UNEP/MED WG. 464/Inf.4), which was further transformed in 2021 (CORMON Marine Litter, 30 March 2021) into the region-wide approved "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34).

At the request of the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (Videoconference, 1-3 December 2020), the Online Working Group on Marine Litter (OWG-ML) was established to support the Secretariat in finalizing the said guidelines, and under the leadership of Spain initiated the process for preparing a draft outline for an intercalibration exercise for collection and analyses of floating microplastic samples between Mediterranean laboratories.

Since then and in the framework of preparation of the 2023 Mediterranean Quality Status Report (MED QSR) a number of important developments are in place for floating microplastics, including the establishment of updated Baseline Values (BV) and Threshold Values (TV), and subsequently the preparation of a well-elaborated chapter on floating microplastics with the contribution of raw data, being officially uploaded to IMAP InfoSystem from 14 Contracting Parties.

The present document supplements the guidelines for monitoring floating microplastics through a two-phase process including: (a) a dedicated in situ training session in two reference laboratories; and (b) an Intercalibration exercise between the different Mediterranean laboratories.

The present document is submitted to the Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring (CORMON Marine Litter) for review and most importantly for supporting the establishment of an intercalibration exercise for collection and analyses of floating microplastic samples between Mediterranean laboratories.

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1. Introduction

1. Floating microplastics are widely spread throughout the Mediterranean Sea and have ecological, socio-economic and aesthetic implications. Microplastics (MPs) (man-made artificial polymers) 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 and are distributed throughout all sea compartments from the sea surface to the seafloor.

2. The most prominent types of floating MPs found in the marine environment include fiber, filament, film/sheet, fragment, granule, pellet, and foam which can be classified as primary or secondary MPs. Primary MPs are intentionally produced at a microscopic scale through the process of extrusion or grinding, either as precursors to other products (e.g., plastic pellets) or for direct use as abrasives in cleaning products or rotomilling. On the other hand, secondary MPs result from the degradation of mesoplastics (5-<25 mm) or macroplastics (≥ 25mm) due to mechanical, photolytic and/or chemical degradation processes in the marine environment and biodegradation under environmental conditions (weathering, photo-oxidation) of larger items to smaller pieces.

3. In order to compare abundances of floating microplastics across the Mediterranean basin and to evaluate and locate exposed and accumulation areas of this type of pollution, it is essential to follow standardized sampling protocols enabling comparison of scientific data across different Mediterranean sub-regions. In line with the above, the "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34) provides advice and recommendations on how to sample floating microplastics at sea as well as the posterior laboratory analyses of collected samples.

4. This document should be complemented with an inter-calibration exercise amongst different laboratories including a Quality Assurance (QA) procedure and good laboratory practices for monitoring floating microplastics.

5. The scope of this document is to supplement the guidelines for monitoring floating microplastics through a two-phase process including:

- A dedicated *in situ* training session in two reference laboratories
- An Intercalibration exercise between the different Mediterranean laboratories

2. Methodology

6. In order to assure that the monitoring guidelines/protocol for floating microplastics are being applied in a harmonized way across the Mediterranean Basin, a two-fold approach is considered which includes: (a) a first phase with in-situ training sessions; and (b) a second phase with an intercalibration exercise.

2.1 Dedicated in-situ training sessions

7. For the dedicated in-situ training sessions, two reference laboratories should be selected around the Mediterranean, preferable one laboratory based on the northern coast and another one based on the southern coast of the Mediterranean Sea. The training sessions will be conducted in situ and will be held in English and French. It is recommended to conduct the two dedicated sessions within the same period of the year. Each in situ training session will have a duration of three days and will include both field- and laboratory-related work, following all the steps described within "The Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34), including sampling of MPs at sea, samples collection and storage and laboratory analyses of samples.

8. For the progress of the in-situ training session, two experts are needed to conduct and moderate the work and preferably, these two experts should be the same for both in-situ training sessions. It is also recommended that these two experts are involved in the field work in addition to a boat skipper.

Also, it is necessary to use a research vessel well equipped to trawl the Manta net, collect samples and host crew, experts and training participants.

9. For the in-situ training sessions, it is advisable that the reference laboratories are close or have a good access to the sea in order to facilitate the instruction of the sessions. Before the beginning of each training session, a minimum of two (optimum three) sampling sites at the same location should be selected, considering upwelling and downwelling areas, storage areas for local hydrodynamic conditions, distance from direct input sources (i.e., river mouths, distance from port facilities, wastewater treatment plants or relevant urban settlements) and distance from the coastline. Given that this is a training session, and to optimize time, sampling sites should be selected within 4 nautical miles from the coast.

10. Field and laboratory work during the training session will be recorded in order to produce detailed and illustrative material for posterior trainings as these reference laboratories will become *training laboratories of trainee laboratories* with the intercalibration exercise, enhancing capacity building across the Mediterranean basin.

11. The proposed *in situ* training session with a duration of three days will have the following structure:

Day 1:

12. **Introduction to the training session**: During the first hour of the *in-situ* training session, the aims of the exercise and description of the monitoring guidelines/protocol for floating microplastics will be shared with the participants of the session. During this introduction, the importance of standardized and harmonized sampling of floating microplastics in order to obtain comparable scientific data around the Mediterranean Sea should be highlighted.

13. **Field work**: A total of two (up to three) previously selected sites will be sampled for floating microplastics. For sample collection, the "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34) document will be used as basis.

Day 2:

14. **Laboratory work**: Samples collected the previous day will be identified and quantified according to the "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34).

Day 3

15. **Laboratory work**: During the third day of the training session, laboratory work will continue. Microplastic particles will be identified and characterized according to the guidelines and all data will be introduced into a standardized datasheet in line with the agreed Data Standards and Data Dictionaries for floating microplastics (IMAP EO10 Common Indicator 23) under UNEP/MAP Barcelona Convention framework.

2.2 Designated Reference Laboratories

16. Two laboratories will be proposed as reference laboratories where the training session will take place:

a) Lab Name, Country (to be defined)
b) Lab Name, Country (to be defined)

2.3 Intercalibration Exercise

17. For the intercalibration exercise, the two designated reference laboratories will exchange water samples with initially four[1] different laboratories from countries along the Northern and Southern Mediterranean basin. In this regard, each reference laboratory will collect water samples following the "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34) and advice will be provided throughout the in-situ training session.

18. Two types of **reference samples** will be assessed:

a) Filtered sea water supplemented by known amounts of standard polymers of MPs with specific sizes and different shapes (Reference material for MPs analyses: CARAT GmbH, Harderhook 20, 46395 Bocholt, Germany). This will act as a **positive control**. A total of 6 samples will be prepared[2].
b) Filtered water from the sampling area but with no supplemented MPs. This will act as a **negative control**. A total of four samples will be prepared[3].

19. Prepared samples can be preserved for a very short period (i.e., facilitating transfer from sea to the laboratory) in 70% ethyl alcohol prior to being shipped to the corresponding laboratory. Prolonged preservation of plastics in alcoholic solutions may increase dryness, fragmentation, color or spectra alteration. Reference laboratories will send twelve water samples to each of the four laboratories involved in the intercalibration exercise of their corresponding region.

20. Each receiving laboratory should identify and quantify the different microplastics particles following the steps explained under section 3.3 (Laboratory Analyses of Samples Collected at Sea) in the "Monitoring Guidelines/Protocols for Floating Microplastics" (UNEP/MED WG.509/34). Given that not all steps described are mandatory, laboratories should carefully explain which steps they have followed, modified (and how) or not conducted. After excluding contaminating particles, results expressed in counts, shape and colour should be included in the common datasheet which will be shared by the two reference laboratories both in English and French.

21. Additional, optional parameters[4] (e.g., size etc.) may be considered by several laboratories under the understanding that this is beyond the UNEP/MAP protocol requirements.

22. The reference laboratories will receive the data for further analysis. Differences in microplastic count between the prepared samples and the shared samples will be calculated and a percentage error will be calculated for each sample for the total count and according to shape and colour. This percentage will help to evaluate overestimation or underestimation of microplastic analyses. It is

[1] Additional laboratories (>4) may be considered provided that the reference laboratories can support this work as well as that sufficient resources are made available.
[2] In this regard, once the water samples are collected from sea, these will be filtered through glass microfibers filters and microplastics simulating those found at sea will be added to 500 ml of this filtered seawater in order to prepare the sample to be used in the intercalibration exercise.
[3] In this regard, once the water samples are collected from sea, these will be filtered through glass microfibers filters following the same procedure as the positive control but with no addition of MPs
[4] Optional parameters are elaborated in UNEP/MED WG.509/34.

essential for continued communication between all laboratories involved in the intercalibration exercise.

2.4 Designated Competent Laboratories

23. For the intercalibration exercise, four competent laboratories will be proposed for each of the Northern and Southern region of the Mediterranean Sea making up a total of 8 laboratories:

Northern Mediterranean Sea	Northern Mediterranean Sea
a) Lab Name, Country (to be defined)	a) Lab Name, Country (to be defined)
b) Lab Name, Country (to be defined)	b) Lab Name, Country (to be defined)
c) Lab Name, Country (to be defined)	c) Lab Name, Country (to be defined)
d) Lab Name, Country (to be defined)	d) Lab Name, Country (to be defined)

2.5 Required Material

24. For the in-situ training sessions and the intercalibration exercise the following material will be needed:

- Field work material: this equipment only applies for the two reference laboratories involved in the *in situ* training sessions
- Semi-rigid boat with the capability to trawl the manta net and host crew, experts and training participants (approximately 6 persons).
- Manta Net
- Video Camera to document the sampling and take images of the area as well as possible accumulation areas of plastics.

25. Laboratory facilities with:

- Drying oven
- Filtration ramp
- Heating plate
- Precision balance
- Stereomicroscope

26. Laboratory material with:

- 5 mm metal sieve
- 1 mm metal sieve
- 300 µm metal sieve
- Petri dishes (glass)
- Jars/Beakers (glass)
- Tweezers
- Distilled water
- Micrometre
- Alcohol (70% ethyl alcohol)
- Graduated cylinder
- Aluminum Foil

- White coat 100% cotton
- Gloves
- H_2O_2
- Glass microfibers filters