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MARLESS - MARine Litter cross-border awarenESS and innovation actions

Priority Axis: Environment and cultural heritage
Specific objective: Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

MICROPLASTICS PROTOCOL

PROJECT MARLESS

11.10.2021

Work Package:	**3. Monitoring optimization**
Activity:	**3.1 Harmonization of the monitoring plan and methodology**
Phase Leader:	**ARFVG – Autonomous Region of Friuli-Venezia Giulia**
Deliverable:	**3.1.3 Agreement on a method to use in the following activity**

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1. INTRODUCTION

Microlitter is a term used to describe solid material that is less than 5 mm in length, differently dispersed in the environment. The sampling activities and laboratory analyses listed below are aimed at assessing the abundance and, if possible, the composition of the microlitter present in seawater, especially that of microplastics.

2. SAMPLING

Due to their exceedingly small size, lightweight properties and relative density, microplastics tend to accumulate mostly on the sea surface and then in the basal zone of the thermocline. For this reason it is necessary to detect the chemical-physical variables along the water column by lowering the multiparametric probe at the starting point of microplastic sampling. It is also important to consider the mixing effects caused by wave movement on the distribution of microplastics; therefore, it is preferable to take samples when the sea is calm.

For sampling, a "manta trawl net" is deployed. This type of net is specifically designed to be towed on the surface layer of the water column and hence to collect samples within the layer affected by the wave motion. In general, the manta net allows sampling of large volumes of water and retains the material of interest. The manta trawl net (figure 1) consists of a rectangular metal aperture, or mouth, with a cone-shaped net attached to it and a collecting cup or container at the cod end. There are two hollow metal wings on the external sides of the mouth to keep it floating on the surface.

Mouth size and length. The dimensions of the mouth are not predetermined, given that they depend on the tonnage of the towing boat. It is, however, advisable to always maintain a ratio equal to ½ between height and width of the mouth. The most commonly used mouth size is 25cm high by 50cm wide with a net length of about 2.5 m. The dimensions refer to the internal dimensions of the mouth; that is, the part to which the net is connected. The external part is wider assuming an overall truncated pyramid shape.

Mesh of the net. The mesh size should be approximately 330 µm.
In order to avoid problems of regurgitation following clogging, especially in eutrophic waters, the effectiveness of sampling must be constantly checked.

Dimensions of the wings. The dimensions of the wings depend on the weight of the metal floating device since the purpose of the wings is to keep the tool afloat. A length of 40–70 cm is suggested.

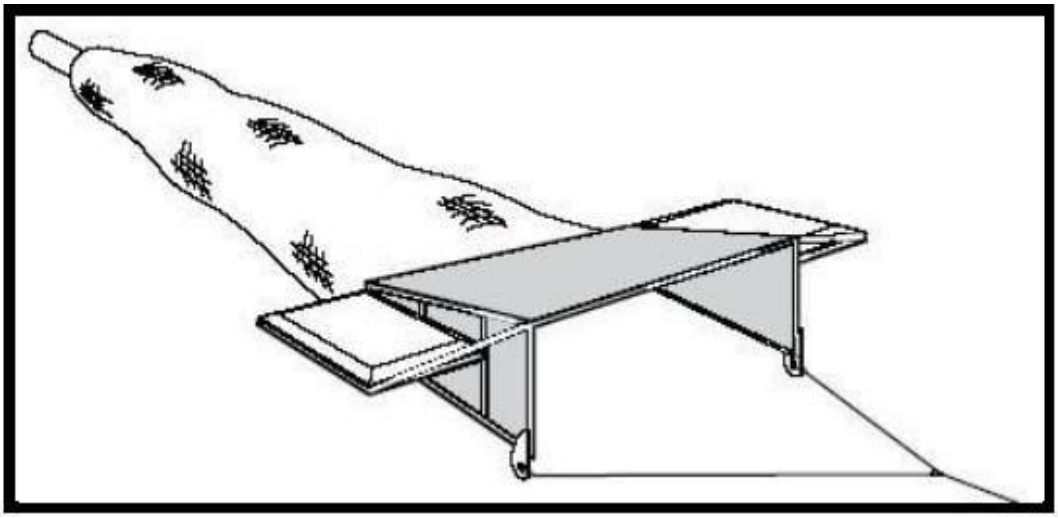

Figure 1. *Manta Trawl Net (swfsc.noaa.gov)*

Use of the manta trawl net: The net is slowly lowered from the boat or vessel to the sea and is left afloat, being secured to the vessel by a rope up to a distance of 50–70 m from it. The manta net should, however, be left out of the boat's wake since the induced turbulence will significantly influence the real abundance figures of the microplastics (Fig. 2–3). Where possible, it is therefore advisable to lower the tool sideways, passing the towing end through a suitable pole installed on one side of the boat.

Figure 2. *Manta Trawl Net (Photo A. Camedda Cnr-IAMC Oristano)*

Figure 3. *Manta Trawl Net (Photo A. Camedda Cnr-IAMC Oristano)*

Flowmeter: a flowmeter with back run stop to quantify water filtered by the manta net used has to be installed. The flowmeter must have a measuring range from 0,3 to 10 m/s with an accuracy of 5%.

Sampling mode. The monitoring surveys should include at least 2 sampling stations located at different distances from the coastline (i.e. from 0.5 to 1 and from 3 to 5 Naut. Miles) along transects (or sampling units) that are orthogonal to the coastline.
Once in position at the sampling point, the net is lowered and hauled for 20 minutes along a linear path at a speed of between 1 and 2 knots, but never more than 3 knots, so as to allow the net to filter the water without regurgitation (*avoidance*). The 20-minute drop must be made in the opposite direction to the surface current or, in any case, to the direction of the wind.
For each haul, the GPS coordinates (degrees and thousandths; DD°, DDDDD) at the beginning and at the end of sampling must be recorded correctly in the WGS 84 UTM 32 geographic and projected coordinate systems. In the presence of high quantities of mucilage or other organic substances in the sea during sampling, it is suggested to divide the sampling time per transect into two 10-minute hauls.

Position of the survey transects (or sampling units). The position of the transects for monitoring the coastal strip must be determined according to the characteristics of the survey area (i.e. the following must be taken into consideration: *upwelling* and *downwelling* areas, accumulation areas

for local hydrodynamic conditions, distance from direct input sources, such as river mouths, distance from port structures 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 maximum and minimum anthropogenic impact. The criteria for choosing the position of the sampling units must be recorded on dedicated sampling sheets.

Calculation of the amount of microparticles/m^2
– The surface of filtered water (S) is calculated using the following formula:

$S = L \times l$

where:

L is the length of the linear transect, and
l is the width of the manta trawl net mouth

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

$V = N \times A \times c$

where:

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;
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.

3. SAMPLE COLLECTION AND STORAGE

Once the net has been brought back to the surface, it must be rinsed with seawater from the outside to the inside in order to convey all the collected material towards the collection cup. The cup is then detached from the net and the sample is poured into a 1000 ml, 500 ml or 250 ml glass jar for subsequent qualitative-quantitative analyses (Fig. 4). Should it not be possible to use glass containers on board the vessel for safety reasons then containers made of rigid plastic material can be used instead. In the latter case, special attention should be paid when transferring the collected contents to avoid microparticles sticking to the container. The sample can be stored in the refrigerator (but not in freezers), however, always away from sources of light and heat. It is advisable to add a fixative (i.e. 70% ethyl alcohol), for the sole purpose of preventing the decomposition of any organic matter (such as zooplankton, phytoplankton, etc.) which would release unpleasant odours when analysing the sample.

4. LABORATORY ANALYSIS OF SAMPLES

The analysis is aimed at identifying and quantifying the microplastics (as such non-degradable) present in the sample.

Figure 4. *Collected material (Photo S. Coppa Cnr-IAMC Oristano)*

4.1 EQUIPMENT

- 5 mm mesh sieve (metal);
- 300 µm mesh sieve (metal);
- filtering device;
- Petri dishes (glass);
- beakers (glass);
- tweezers;
- stereoscope.

IMPORTANT: all laboratory equipment must be made of glass or metal in order to prevent microplastic fragments from adhering to the walls. In addition, particular attention should be paid to cleaning the work area in order to avoid contamination of the sample. In this regard, some important precautions are required to be taken to contain the risk of contamination such as:

- Avoid wearing synthetic clothes with plastic fibres (such as fleece or stretch fabrics in Lycra-polyamide) during laboratory analyses and wear pure cotton gowns.
- Avoid exposing the sample and the sub-samples to be analyzed to the air. Always take care to cover them so as to avoid contamination.
- Avoid leaving any windows open when analyzing samples.

4.2 PROCEDURE

The analysis is performed on the *whole* sample.
Use distilled water when transferring and washing the sample.

- Pour the whole content of the sample through a set of two stacked sieves with a 5 mm and 300 µm mesh, rinsing the container several times with distilled water to recover all the microplastics.
- The fraction consisting of plant or animal residues of more than 5mm (retained by the sieve with the larger mesh) must be thoroughly rinsed.
- Pour the sample fraction containing the microplastics into a glass beaker, then transfer the floating plastic fragments to a Petri dish with a mesh bottom and analyze with a stereomicroscope, taking note of the magnification used.
- Sort the floating component by separating the plastic material from other organic residues (plants, wood, etc.) with the help of a pair of tweezers.
- After having done this, sort the precipitate to check for any plastics with a higher density, or that have got "stuck" in the plant or animal residues.

- Select the microplastics to be counted with the aid of a sheet of graph paper under the Petri dish, so that the microplastics measuring between 5 mm and 0.3 mm can be extracted. This procedure can also be performed with a micrometer inserted in the eyepiece or with an imaging program. Filaments with a length of > 5mm must still be counted.
- Divide and count the microplastics identified in the sample based on shape (granule, pellet, foam, filament, fragment, sheet) and colour. Below is a brief description of the shape categories.

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

It is important to distinguish fibres from filaments so that they are not included in the count. Figure 5 highlights how fibres generally have a smaller diameter, with frayed edges and often you can see a slight helical winding. Furthermore, if fibres are poked with a needle they bend and deform (Fig. 5, 1 red and 2 blue fibres). Filaments, on the other hand, have a generally well-defined shape, cylindrical with clean well-defined edges, and a more even colour. Furthermore, filaments are stiffer than fibres and less deformable (Fig. 5, 2 filaments in blue).

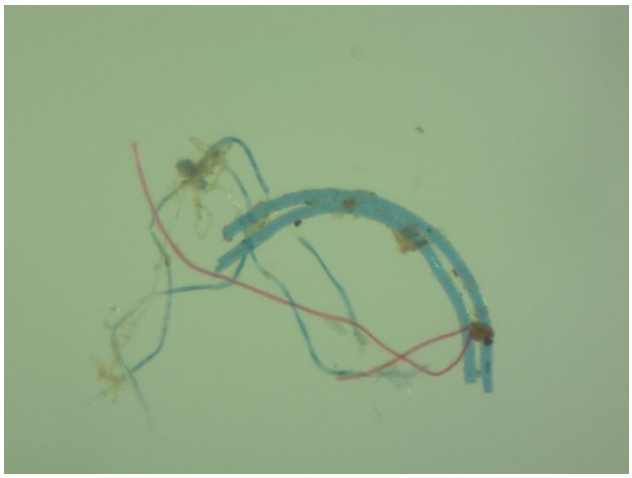

Figure 5. *Distinction between fibres and filaments*

The colour of each particle should be recorded according to the following colours: white, black, red, blue, green, brown and yellow. Furthermore, a fragment that has different colours on two sides must always be included in the "other colour" category. Finally, for each colour counted, the transparency must also be recorded by specifying whether the particle is opaque or transparent in the next column.

4.3 UNIT OF MEASURE

The microplastic concentration in the sample, in terms of shape and colour, is expressed as the number of objects per m^2 of sampled seawater