

D3.2.1

Handbook for common approaches and methodologies

Field sampling and study measuring micro- and macro-plastic input in partner coastal regions

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1. Introduction

Marine litter is found and accumulates in shorelines, sea floor and water column of all the oceans of the world, from densely populated regions to remote places far away from any obvious sources (e.g. Haynes 1997; Convey et al., 2002). It has been estimated that 80% of marine litter is from land-based sources and the remaining 20% is from ocean based sources. There are four major groups of marine litter sources: tourism related litter at coast, sewage-related debris, fishing related debris and waste from ships and boats (Allsopp et al., 2006). To these may be added poor or inadequate waste management (Sheavly, 2005). Marine litter causes a wide spectrum of environmental, economic, safety, health and cultural impacts. The very slow rate of degradation of most marine litter items, mainly plastics, together with the continuously growing quantity of the litter and debris disposed, is leading to a gradual, but dramatic increase in the quantities of marine litter in our oceans and world shores. Despite international, national and local regulations (e.g. ANZECC 1996a, GESAMP 2001, Kiessling 2003), the level of manufactured litter lost or deliberately discarded into the world's seas and oceans is substantial and represents a growing threat to marine environments, industries and economy (e.g. ANZECC 1996a, b; Barnes 2002, Kiessling 2003). Marine litter is now present in every ocean (Cheshire et al., 2009) and is defined as *"any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment. Marine litter consists of items that have been made or used by people and deliberately discarded into the sea or rivers or on beaches; brought indirectly to the sea with rivers, sewage, storm water or winds; accidentally lost, including material lost at sea in bad weather (fishing gear, cargo); or deliberately left by people on beaches and shores."* (UNEP 2005: 3). The Mediterranean Sea is particularly vulnerable to this environmental threat: about 10% of the global coastal population lives on the Mediterranean coasts (CIESIN, Oct. 2012). It represents a population of nearly 150 million (UNEP/MAP, 2012) to who should be added 100 million of tourists visiting the coasts every year (UNEP/MAP/MED POL, 2005). The Mediterranean basin is by far the largest global tourism destination, attracting almost a third of the world's international tourists (UNEP/MAP, 2012). Coastal tourism is then fundamental for the economy of many Mediterranean countries and islands. However it represents a tremendous environmental pressure including contribution to the marine litter and plastic discharge (UNEP/MAP, 2012). These observations show that there is an urgent need to develop a new model for a more responsible and sustainable tourism in the Mediterranean Sea (Sabban, 2013).

In this context, one of the aims of the BLUEISLANDS project is to properly identify, address and mitigate the seasonal variation of waste generated on Mediterranean islands as an effect of tourism by assessing, amongst other, the seasonal dynamics of marine litter, with a special attention paid to both the micro- (<5mm) and macro-plastics (>5mm), in highly touristic coastal areas (i.e. beaches). The aim is to propose strategies to reduce the amount of litter related to touristic and recreational activities on beaches and to reduce its negative effects on the coastal environment. Not only the beaches will be monitored, but the surface waters off these beaches and the underlying sediment will be surveyed too. A total of 9 islands from the Mediterranean Sea (Mallorca, Sardinia, Sicily, Rab (Croatia), Malta, Crete, Mykonos, Rhodes and Cyprus) are involved. For each of these islands 3 specific beaches will be periodically monitored to assess the seasonal variation of litter as an effect of tourism.

In the following are detailed the specific criteria required to select the beaches to conduct the monitoring, the different types of monitoring which will be implemented and the methodologies related to each of them.

2. Selection of the study beaches

In order to assess the impact of tourism on the generation of marine litter on the beaches of the participating islands, a periodical monitoring of the marine litter, including the low and high touristic seasons, will be conducted on selected beaches. In order to encompass different case-scenarios, it has been decided to monitor 3 beaches for each island. The beaches will be selected to include 1 impact sites where tourists represent most of the visitors to the beach and 2 control sites including a beach where locals represent most of the visitors and a remote/preserved beach where the frequentation of both tourists and locals is low. Moreover, the selected beaches should comply with the following criteria: be composed of sand or gravel and exposed to the open sea; be accessible all year round; be accessible for ease

of marine litter removal; be a minimum length of 100m and if possible over 1km; be free of buildings all year round, and ideally not be subject to any other litter collection activities.

The local partners will provide a list of pre-selected beaches, including when possible pictures of the sites, complying with the above mentioned criteria. After discussion with the WP coordinator (UAB), the final 3 beaches will be selected to conduct the periodical monitoring of marine litter on each of them and for each island. For each of the selected beach, the local partners will have to identify a fixed 100m portion of the beach where all the surveys will be conducted.

3. Monitoring

The surveys conducted on the different beaches will include 1) a monitoring of the marine litter found on a fixed 100m portion of each beach (Figure 1), 2) a sampling of the sand within the fixed 100m portion of each beach to assess the concentration and composition of micro-plastics (Figure 1), 3) a sampling of the surface waters off the selected beaches to assess the concentration and composition of micro-plastics (Figure 2) and 4) a sampling of the underlying sediment off the selected beaches to assess the concentration and characteristics of micro-plastics (Figure 2).

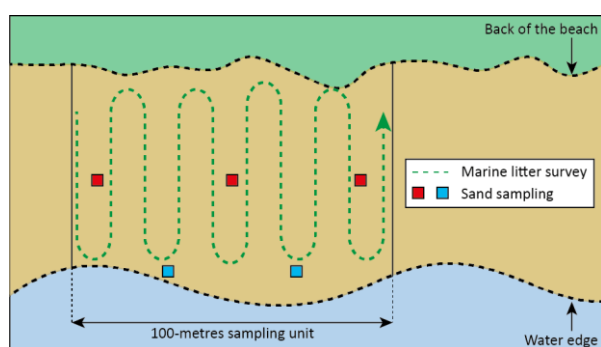


Figure 1: drawing showing the disposition of the survey for marine litter and the collecting sites for the micro-plastics.

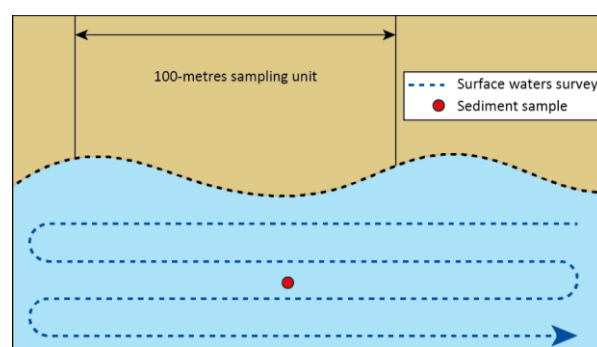


Figure 2: Drawing showing the disposition of the survey for both surface waters and sediment sampling. Important: the drawing is not to scale; water and sediment sampling will be conducted in allowed zones.

3.1. Marine litter survey

3.1.1 Protocol

The protocol for the marine litter surveys has been adapted for the BLUEISLANDS project from the OSPAR¹ *Guideline for monitoring marine litter on beaches*. The WP coordinator will share with the local partners 3 documents which will be used to monitor the marine litter on the fixed 100m portion of beach:

- The Questionnaire form which has to be filled only once for each beach during the very first survey. It will compile all the relevant information regarding the beach such as the name of the beach, its location, its general characteristics (total length, width, slope...), the usage made of this beach (tourists, locals, swimming, fishing...), the distance to the nearest town, its size and seasonal population, the cleaning activities, etc.
- The survey form which will be used to report all the items found during the survey of the marine litter on the fixed 100m portion of beach. It has to be done for every survey. It will compile some information related to the survey such as the presence of stranded animals or the occurrence of particular events affecting the marine litter (party, sporting event, etc.).
- The photo-guide which provides a picture of the most common items found in the marine litter. The items are divided into 12 different categories such as plastic, rubber, paper, metal, etc. The photo-guide is here to help the participants to the monitoring to fill the survey form.

After selecting the beaches where the marine litter will be monitored, it is first necessary to fill the Questionnaire form and to define the fixed 100m portion of beach to be examined. The coordinates of the starting and ending

¹ <http://www.ospar.org/documents?v=7260>

points of the portion of the beach are reported. It is important to note that all the subsequent surveys will be done on the exact same portion of beach. Before starting any survey, it is important to walk along the portion of the beach to roughly evaluate the amount of marine litter and to pay attention to the small items such as the micro-plastics (<5mm) or the cigarette butts, which could represent most of the items. Once this first inspection is done, the participants will start to collect the marine litter: each item found will be put in a collection bag, with the exception of the visible micro-plastic (<5mm) that will be collected in a separate bag. Each item found will be reported in the Survey form. It is recommended that surveyors work in pairs, one collecting the items and the other one completing the Survey form. The surveys ends when all the items present on the 100m portion of beach were collected and reported in the Survey form.

After each survey, the Survey form has to be sent to the WP coordinator as well as all the collected micro-plastics.

In the following are some recommendations:

- In the case of an item does not appear in the Survey form and the Photo-Guide, it is asked to the participants to take a picture of it, if possible with scale such as a pen or a coin, and to report it in the appropriate "Other item box" of the Survey form with reference to the picture.
- If during the first inspection before starting the survey, the visible micro-plastics particles and/or the cigarette butts are numerous (>5-10 per m²), then at least one surveyor should only collect these items in 2 separates collection bags along ± 1.5 m of width transects.
- All the collected items, with the exception of the micro-plastics, should be disposed of properly.
- In any case the safety of the participants has to come first. It is important to check the forecast before the survey. During the summer, the surveys should not be done during the warmest hours of the day. Dangerous or suspicious looking items, such as ammunition, chemicals and medicine should not be removed: instead the participants should inform the police or authorities responsible.

3.1.2 Schedule

In order to assess the seasonal variation of litter as an effect of tourism, it is necessary to conduct periodically the surveys during both the low and high touristic seasons. Two intensive periods of monitoring are scheduled for 2017 and 2019 and a relaxed period of sampling is scheduled for 2018:

- 2017: February, May, June, July, August, September and November
- 2018: February and August
- 2019: February, May, June, July, August, September and November

3.2. Microplastics sampling on the beaches

3.2.1. Protocol

The survey for the micro-plastics on the beach will consist of the collection of 5 samples of sand on the fixed 100m portion of each beach. The sampling will be done during the marine litter surveys. Each sample will consist of ± 1 liter of sand. Among the 5 samples, 3 will be collected in the middle of the beach (Figure 3) and 2 will be collected in the high water mark (Figures 3 and 4). The samples will be stored in clean glass jars with specific labelling (including the island, the name of the beach, the reference of the sample (Figure 3) and the coordinates of the sampling site).

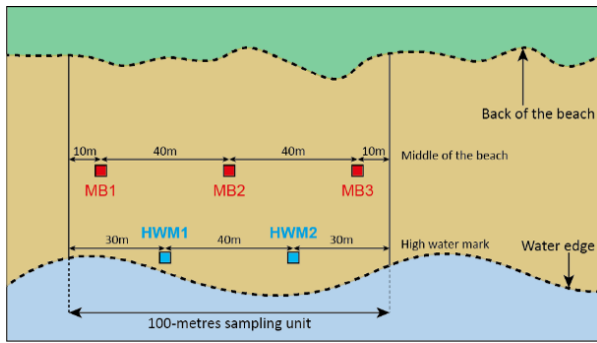


Figure 3: Distribution of the sand samples on the 100m portion of beach. MB (Middle of the Beach) and HWM (High Water Mark) are used as a reference for the samples.

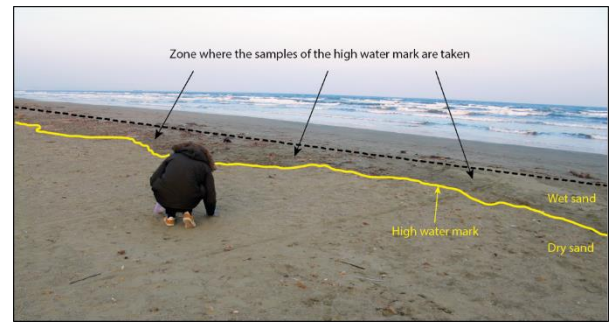


Figure 4: Picture showing the zone for the sampling in the HWM

Briefly, for each sample, a quadrat of 20x20cm (Metal or wood) will be put on the ground and the sand will be removed from the 2 – 3 upper cm using a metal trowel (or table spoon) and stored in a clean glass jar. It is strongly recommended to wear cotton clothing to reduce risk of contamination from micro-plastic fibers from surveyor's clothing. In case participant are not sure of the material of their clothing, it is possible to "sample" them for comparison with what will be found in the sand samples: for each of the clothing articles, the participants should collect a sample of fibers by putting a piece of tape on it. These "samples" will be stored in a zip lock bag (with the name of the beach and the day of the sampling) and ship with the sand samples to the WP coordinator.

3.2.2. Schedule

In order to assess the micro-plastics concentration and composition in sand samples as an effect of tourism, it is necessary to collect periodically the samples during both the low and high touristic seasons. Two intensive periods of monitoring are scheduled for 2017 and 2019 and a relaxed period of sampling is scheduled for 2018:

- 2017: February, June, August and November
- 2018: February and August
- 2019: February, June, August and November

3.3. Microplastics sampling in the surface waters

3.3.1. Protocol

Before starting this activity, a boat (~3-5 m) has to be identified in each area to support the deployment of a net to collect all the floating particles. The surface waters off the selected beach will be sampled using a manta net (or any other type of net suitable for this purpose) with a mesh size of less than 250µm (0.25mm) dragged by the boat. The net will be deployed along one or more transects parallel to the selected beach. The transect should cover the total length of the beach and not only the fixed 100m portion. The surface waters will be sampled during 30 minutes at a speed of 2-3 knots representing a distance of ~1.8 to ~2.9 km. The number of transects will depend of the total length of the selected beach: if the length of the beach is more than 2km, then only one transect is needed. If the total length of the beach is less than 2 km at least 2 transects should be considered. In any case for each transect, the initial and final coordinates as well as the deploying time are reported on a table sheet. To ensure a good sampling it is necessary to comply with the following steps:

- Report the coordinates of the starting point and the time
- Deploy the net from the side of the vessel to avoid collecting water affected by turbulence
- Move in one straight direction at 2-3 kt for 30 min
- Report the coordinates of the ending point and the time
- Rinse the net thoroughly from the outside and from top to bottom to concentrate the particles with sea water

- Safely remove the cod end of the net
- Rinse thoroughly the cod end from the outside
- Sieve the water collected with the same mesh size as the net
- Pour the rest of the sample through the sieve until no particles remain in the cod end
- With a funnel, rinse the sieve into a glass jar with 70% ethanol
- Close and label the glass jar.

A video detailing step by step the whole procedure can be found here (from Kovač Viršek et al., 2016):
<https://www.jove.com/video/55161/protocol-for-microplastics-sampling-on-sea-surface-sample>

The samples will be shipped to the UAB for analysis.

3.3.2. Schedule

In order to assess the concentration and composition of floating particles in the surface waters off the selected beaches, it is necessary to collect periodically the samples during both the low and high touristic seasons. Two intensive periods of monitoring are scheduled for 2017 and 2019 and a relaxed period of sampling is scheduled for 2018:

- 2017: February, June, August and November
- 2018: February and August
- 2019: February, June, August and November

3.4. Microplastic sampling in the underlying sediment

3.4.1. Protocol

Before starting this activity, a boat (~3-5 m) has to be identified in each. It is recommended to do the sampling of the sediment during the surface water survey. For each selected beach, one surface sediment samples will be collected. The samples will be retrieved using a sediment grabber such as Eckman grab or Van Veen grab. In the case this type of device cannot be used or if a boat is not available, it is possible to take samples by hand few meters behind the breaking waves. The samples will be stored in labelled and clean 1L glass jars. Depending of the volume collected more than one jar may be needed.

The samples will be shipped to the UAB for analysis.

3.4.2. Schedule

In order to assess the concentration and composition of micro-plastics in the surface sediments off the selected beaches, it is necessary to collect periodically the samples during both the low and high touristic seasons. For each year of implementation of the project, 2 samples will collected for each beach:

- 2017: February and August
- 2018: February and August
- 2019: February and August

3.5. Material required

3.5.1. For marine litter surveys and sand sampling

- | | |
|---------------------------------------|--|
| • The survey form and the photo guide | • Collection bags/zip lock bags (for the visible micro-plastics) |
| • Clip-boards | • Small ruler (for the visible micro-plastics) |
| • Pen/permanent film marking pen | |

- Camera/smartphone (to take pictures for identification)
- GPS/smartphone (free apps can be downloaded such as “My GPS coordinates” and “GPS Odometer” from GPS Tool for Android, as an example)
- Decameter
- 1 liter clean glass jars (5 per beach and per survey)
- 1 metal trowel/table spoon (with no coating)
- 1 quadrat of 20x20cm made of wood or metal
- Scotch tape
- Appropriate clothing and footwear (cotton if possible)
- Hat
- Sunscreen
- Wet weather gear
- Water and food
- First aid kit

3.5.2. For surface waters and surface sediment sampling

Apart from the boat and the specific devices to sample surface waters and surface sediments:

- Pen/permanent film marking pen
- Clipboards and paper
- GPS (if not available on the boat)
- 1 sieve (20cm diameter) with the same mesh size as the net
- 1 liter clean glass jars (number will depend of the amount of particles caught with the net and the amount of sediment retrieved)
- 70% ethanol
- Appropriate clothing and footwear

4. Laboratory protocols

Once the WP coordinators receives the samples from the marine litter surveys (visible micro-plastics), the sand samples, the surface water samples and the surface sediment samples, the collected material will be processed in order to extract the micro-plastics. Depending of the origin of the samples, different approaches will be undertaken.

4.1. Sand samples

For sand beach samples, micro-plastics will be extracted using the methodology developed by Thompson et al. (2004). For a given sample, three replicates of approximately 150 g of sand will be added to three different beakers with 250mL of concentrated NaCl solution (1.2g cm^{-3}). The mixture will be stirred for 20 min at 400 rpm, and left settling for two hours. Then, the supernatant will be collected and vacuum filtered (GF/F; 47mm $\varnothing$, 0.7 μm). This density separation process will be repeated three times for each replicate. The fiber glass filters will be stored in Petri dishes and dried at 40°C overnight. The filters will be analyzed under microscope to estimate the concentrations of the different types of micro-plastics (microbeads, fragments, fibers, foams, films, etc.).

4.2. Surface water samples

The surface water samples will poured in beakers with pure analytical grade water (MilliQ®) and vacuum filtered (GF/F; 47mm $\varnothing$, 0.7 μm) in the laboratory. The fiber glass filters will be stored in Petri dishes and dried at 40°C overnight. The filters will be analyzed under microscope to estimate the concentrations of the different types of micro-plastics (microbeads, fragments, fibers, foams, films, etc.).

4.3. Surface sediment samples

For surface sediment samples, the analysis will consist in the combination of density separation with wet peroxide oxidation (WPO): for each sample, three different replicates of approximately 10-20g of sediments will be added to three separated beakers with 200mL of concentrated NaCl solution (1.2g cm^{-3}) and 20 mL of 30% hydrogen peroxide (H_2O_2). The mix will be heated to 50°C and stirred for 20 min at 200 rpm. Then, it will be left settling for an hour at 50°C and one additional hour at laboratory temperature. Then, first the foam from the reaction and the supernatant will be transferred to a clean beaker, mixed with 50mL of concentrated NaCl solution and 20 mL of 30% H_2O_2 . Secondly, to the rest of the WPO solution and sediments will be added other additional 20 mL of 30% H_2O_2 , and both beakers followed

the same mentioned steps of heating, mixing and settling. In the case of WPO solution and sediments, the supernatant will be collected and transferred to a clean beaker following, once again, the oxidation, heating, mixing and settling for overnight steps. Finally, supernatants are collected and vacuum filtered (GF/F; 47mm ø, 0.7 µm). The fiber glass filters will be stored in Petri dishes and dried at 40°C overnight. The filters will be analyzed under microscope to estimate the concentrations of the different types of micro-plastics (microbeads, fragments, fibers, foams, films, etc.).

4.4. Micro-plastics composition

Finally, the composition of the visible micro-plastics as well as all the micro-plastics extracted from the sand samples, the surface water samples and the surface sediment samples will be assessed by infra-red spectroscopy.

5. Data sharing

All the data regarding the marine litter concentrations and composition along with the micro-plastics concentration and composition will be stored in a data base and shared with all the partners of the project.

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