IAEA Marine Environment Laboratories

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B. IMAP MONITORING GUIDELINES FOR CI17

B-1. Monitoring Guidelines for sampling and determination of contaminants in sediments

Table of Contents

1 Guidelines for sampling and sample preservation of sediment

1.1 Introduction

Determination of the concentrations of targeted heavy metals and organic contaminants in different marine matrices is a key component of the IMAP, since the analytical results will contribute to the assessment of the environmental status of the water body under consideration. Sediment is one of the proposed matrices for the analysis of heavy metals since the establishment of the UNEP/MAP – MED POL Monitoring programme in 1981 (MED POL Phase II), because heavy metals and persistent organic contaminants in seawater tend to become insoluble and precipitate with the particulate fraction on the seafloor. Therefore, since sediment is the ultimate sink of most heavy metals and persistent organic contaminants, which are introduced into the marine environment, their analysis will provide a clear view of the pollution state of the specific water body. Furthermore, in areas with undisturbed sediments, the yearly deposited sedimentary material integrates the pollution load during this specific time period, and the analysis of different sedimentary layers is providing a historical trend of pollution processes in the region.

The UNEP/MAP Integrated Monitoring and Assessment Programme (IMAP) (UNEP/MAP, 2019[1]; UNEP (2019a[2]), requires sediment sampling from the top layer of the seafloor, because this layer reflects the recently deposited material, therefore the actual status of pollution at the specific location. The depth of the "recently" deposited sediment varies from one location to another, influenced by the sedimentation rate but also by bioturbation, but in the coastal zone it is usually the top 1 to 5 cm from the seafloor surface. In open sea, the sedimentation rate is lower than in the coastal zone, therefore often the 1st cm of the sediment may be representing several deposition years. It is of paramount importance to collect the undisturbed top layer of the sediment for analysis. Therefore, the use of appropriate sampling equipment is very important, as well as the proper handling during sampling to collect a representative sediment sample.

Until now, UNEP/MAP – MED POL pollution monitoring programme was focussing on the marine coastal zone, which was affected by land-based pollution sources. Therefore, sediment sampling was mainly done in relatively shallow waters, although some Contracting Parties were also collecting sediment samples from deeper waters. In a view of extending monitoring to much deeper offshore areas in the framework of the IMAP, sediment collection protocols are also addressing sediment sampling procedures from such offshore environments. Box corers and multiple corers are mostly suitable for such offshore sediment sampling, while gravity corers can be mainly used for tracking historical pollution trends. It has to be underlined that sedimentation rates at offshore sediments are much lower than in the coastal zone, leading to a much lower yearly deposition of sediment material on the seafloor. Therefore, in order to decide on the appropriate sediment depth to be collected for recording recent contaminants' concentrations, as well as on the required sampling frequency in offshore sediments in view of detecting possible changes in contaminants accumulation, the determination of the sedimentation rate at the sampling stations is highly needed.

Once a representative sediment sample has been collected, it has to be transported to the laboratory for analysis. However, transportation has to be done in such a way as to avoid any alteration of the physical and chemical characteristics of the sample. Sediment characteristics and contaminants distribution in the sample may be altered if the sediment storage and transportation is not done under specific procedures, in order to avoid sample alteration and cross contamination from the material of the containers and the sampling and transportation environment.

The Protocols prepared in the framework of this Monitoring Guidelines for Sampling and Sample Preservation of Sediment for IMAP Common Indicator 17, as provided here-below, describe appropriate methodologies for sampling, processing and storage of marine sediment under controlled conditions to ensure the representativeness and the integrity of the samples. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

These Protocols aim at streamlining sediment sampling and sample preservation in order to assure comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes. They provide a step-by-step guidance on the methods to be applied in the Mediterranean area for sampling and sample preservation of sediments in a view of their subsequent analysis for heavy metals and organic contaminants. Quality Assurance requirements for sediment sampling and preservation in view of producing analytical data of the required quality are presented in Chapter E of these Guidelines.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent laboratories participating in IMAP implementation. They build upon

[1] UNEP/MAP (2019). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27;

[2] UNEP/MAP (2019a). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution;

the UNEP/MAP (2011[3]) Manual on sediment sampling and analysis (Annex IV), as well as similar Guidelines/Protocols for marine sediment sampling which were developed by other Regional Seas Organisations, such as ICES/OSPAR (2018[4]) CEMP Guidelines for Monitoring Contaminants in Sediments and HELCOM (2012[5]) Manual for marine monitoring in the COMBINE programme, as well as EC (2010[6]) Guidance on chemical monitoring of sediment and biota under the Water Framework Directive, given their suitability for application in the context of IMAP. Given the suitability of any of these Guidelines in the context of IMAP, they could be further used by interested IMAP competent Mediterranean laboratories for developing their laboratory specific sampling and sample processing methodologies.

1.2 Technical note for the sampling of sediment for the determination of heavy metals and organic contaminants

Sediment sampling for pollution monitoring aims at the collection of a representative sediment sample from the top layer of the seafloor, because this layer reflects the recently deposited material, therefore the actual status of pollution at the specific location. The depth of the "recently" deposited sediment varies from one location to another, influenced by the sedimentation rate but also by bioturbation. Usually it is recommended (EC, 2010) to sample the top layer of the sediment, from 1 to 5 cm depth, depending on the deposition rate. In open sea, the sedimentation rate is lower than in the coastal zone, while at coastal areas at the vicinity of large rivers the sediment sampling depth for recently deposited sediments could be more than 5 cm. During the initial phase of the IMAP (identification of key sampling sites/stations) sediment sampling should be done every two years, while during the advanced phase (when it is a fully completed MED POL Phase IV implementation with the ongoing reporting of IMAP data sets) sampling should be done every 3 to 6 years, depending on the characteristics of sedimentation areas and the chemical concerned known through previous MED POL assessments (UNEP, 2019).

To avoid erroneous sampling, it is of paramount importance to sample the undisturbed top layer (1-5 cm) of the sediment using the appropriate sampling equipment. Box corers are the most appropriate equipment to sample undisturbed top layers sediments in the coastal zone and the open sea, but they are relatively heavy and require adequate shipping facilities. In relatively shallow coastal areas, a grab sampler is a good solution, because it is portable and can be used from a coastal vessel, without special equipment for lowering and lifting the sampler from the seafloor. In very shallow sampling sites with a water depth less than 30 cm, surface sediment samples (5 cm) can be collected with a shovel, spatula or scoop, if no other sampling equipment is available.

Sediment monitoring generally addresses the top layer of the sediment because this layer indicates the actual deposited material and the actual status of pollution. Furthermore, the top layers of the sediment form the habitat of benthic organisms and therefore may affect their contaminants' uptake (EC, 2010, UNEP/MAP, 1999[7], UNEP/MAP, 2011). Surface sediments can be collected with grabs and box corers, while gravity corers can be used to collect cores to study historical pollution trends at a specific site. Also, corers could be used in order to collect deeper sediment layers in view of establishing the background concentration of contaminants at a specific area.

Under this technical Note, the Guideline for Sampling and Sample Preservation of Sediment for IMAP Common Indicator 17 provides the following IMAP Protocols:

- Protocol for the use of a grab for collecting sediments;
- Protocol for the for the use of a box corer for collecting sediments;
- Protocol for the use of a multi-corer for collecting sediments;
- Protocol for the use of a gravity corer for collecting sediments;
- Protocol for the hand collection of sediment with a shovel/scoop and a hand-held corer.

These Protocols are based on methods for sediment sampling and processing developed by UNEP/MAP (Annex IV: UNEP/MAP (2011), Manual on sediment sampling and analysis), EC (2010) Guidance Document No 25, HELCOM (2012) Technical note on the determination of heavy metals and persistent organic compounds in marine sediment, and ICES/OSPAR (2018) CEMP Guidelines for Monitoring Contaminants in Sediments. In each protocol the operation and the proper deployment and recovery of the sampling equipment is presented, and guidelines are provided for the appropriate taking of the sediment sample in order to preserve its integrity and to avoid contamination.

[3] UNEP/MAP (2011). UNEP(DEPI)MED WG.365/Inf.9. Manual on sediment sampling and analysis

[4] ICES/OSPAR (2018). CEMP Guidelines for Monitoring Contaminants in Sediments

[5] HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-13 Appendix 3.: Technical note on the determination of heavy metals and persistent organic compounds in marine sediment

[6] EC (2010). Guidance Document No: 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive

[7] UNEP/MAP (1999). MED POL Phase III. Programme for the assessment and control of pollution in the Mediterranean Region.

1.2.1 Protocol for the use of a grab for collecting sediments

a. Grab operation

A tightly closing grab, which is handled with care, can collect relatively undisturbed surface sediment samples. Grabs are not the preferable sampling equipment for collecting undisturbed sediment samples because their penetration in the sediment may disturb the recently deposited sediment layers. However, grabs may provide a workable sampling solution in relatively shoal waters, which are out of the reach of an oceanographic vessel, or when an oceanographic vessel equipped with a box corer is not available. A light-weight hand-held grab is suitable for collecting approximately 250 ml of sediment, which is an appropriate volume for sediment analysis (Figure 1).

Figure 1. Van Veen Grab

To improve the sampling procedures the sampling vessel should be equipped with some sampling facilities, such as a winch, davit or other such lifting equipment. However, in very shallow coastal waters (for example less than 20 m depth) a small hand-held grab can be used with success from a small boat.

The grab is lowered locked-open and upon hitting the sediment's surface the lock is released and the grab's jaws are closing penetrating thus into the sediment to a depth depending on the size and the weight of the grab, as well as the hardness of the sediment.

Grabs can be used efficiently in sand or consolidated sediments collecting a good volume of undisturbed sample. On the other hand, in hard clays the grab may not be able to penetrate the hardened sediment, while in un-consolidated soft sediments the grab will sink through the top layer disturbing sediment stratigraphy.

b. Taking the sample

The water depth at the sampling station should be recorded before the deployment of the grab in order to ensure that appropriate wire/rope length is available.

During the descent of the grab through the water column it is important to control the speed of deployment, to allow the grab arriving at the sediment floor jaws-first. If the grab falls aside on the sediment, sampling will be unsuccessful and the grab has to be lifted, locked-open again and lowered once more. Controlling the speed of the grab's deployment will keep the wire stretched and the grab in a vertical position, as needed.

Another factor affecting the successful deployment of the grab is the existence of near-bottom currents that may deflect the grab from the vertical line, resulting in unsuccessful sampling. Additional weight on the grab sampler, as well as longer wire than the actual depth at the station, may be needed.

Once the grab is closed at the sediment floor it has to be lifted to the surface. At this stage it is important to avoid any leakage of fine-grained sediment from the grab. If the grab is well designed, no loss of collected sediment should occur. However, leakage can occur if the grab is not tightly closed because of ill-design or because of partial closure of the jaws, caused by obstruction from coarse material (for example coarse sand or shell).

When the grab is lifted on-board it has to be positioned on a clean surface and handled with care to ensure that no alteration of the sediment characteristics will occur because of contamination.

i) Pose the grab on a clean surface (plastic).

ii) Visually inspect the collected sample from the small trap doors on top of the grab to make sure that the sediment collected is undisturbed. If water is trapped on the top of the sediment remove it using a glass tube or allow to be slowly drained in order to avoid washing off the top fine-grained layers that may be present.

iii) Record the visual characteristics of the sediment, such as grain size (fine or coarse grained), colour, smell and the presence of organisms. Taking a photo of the collected sediment is also recommended, in order to keep a visual record of the collected sample. If required, you can measure additional parameters, such Eh and pH.

c. Avoiding contamination

Grabs are made of metal therefore the best solution for trace metal determinations is to use a stainless-steel grab and, as an additional precaution, use plastic tools to collect subsamples from the central part of the sample, avoiding the sediment which is in contact with the grab's walls. If possible, use grabs with Teflon coatings on all surfaces that come into contact with the sediment. The use of lowering cables coated with plastic (polyethylene) or of synthetic ropes will further minimize possible contamination.

After the water is drained, open the grab carefully on a clean and metal-free area (for example a plastic sheet) to collect the samples for heavy metal analysis. For the

analysis of organic contaminants, the grab should be open in a dust-free area avoiding contact with possible sources of contamination from organic pollutants (such as exhaust gases).

Remove with a plastic or stainless-steel spoon the top layer, which is representing recent sedimentation. The depth of this layer may vary from 1 to 5 cm depending on the sedimentation rate in the sampling site and has to be decided by the institution that is responsible for the sampling.

It is important to ensure that enough sediment material is collected to allow for analysis of heavy metals, organic contaminants, as well as additional sediment analyses (such as grain size). The EC Guidance on sediment sampling (EC, 2010) suggests to collect 50 ml of wet sediment for heavy metal analysis. Taking into consideration that a small hand-held grab can collect approximately 250 ml of sediment, it is a suitable equipment to collect sediment samples for contaminants' analysis at shallow waters. When a larger grab is used, the collected sediment provides enough material for further analysis.

Surface sediment samples are transferred into wide-mouth, pre-cleaned containers:

i) Zip-lock bags, plastic (polyethylene, polypropylene), or glass are suitable container's materials for sediments to be analysed for heavy metals;

ii) Glass or aluminium are suitable container's materials for sediments to be analysed for organic contaminants.

Containers and zip-lock should be filled to the top to reduce the likelihood of oxidation during transport.

Sediment samples have to be stored at 4°C in a cooler box and transported to the laboratory for further processing and analysis.

1.2.2 Protocol for the use of a box corer for collecting sediments

a. Box corer operation

A box corer is a sediment sampling equipment, which collects large diameter undisturbed cores, from which replicate sub-samples may be collected by a hand-operated corer (Figure 2). Box corers are relatively heavy and are operated from a ship with appropriate equipment (heavy winch) in water depths more than 3 m (EC, 2010). Usual models collect sediment samples with a penetration of 0.75 m with a surface of 0.25 m^2, although there are smaller box corers available on the market. The big advantage of box corers is that they collect a virtually intact sediment core. If properly handled box corers operate efficiently in all kinds of bottoms, hard, soft or unconsolidated, retrieving undisturbed sediment cores. Therefore, if available, they are the preferable equipment for sediment sampling.

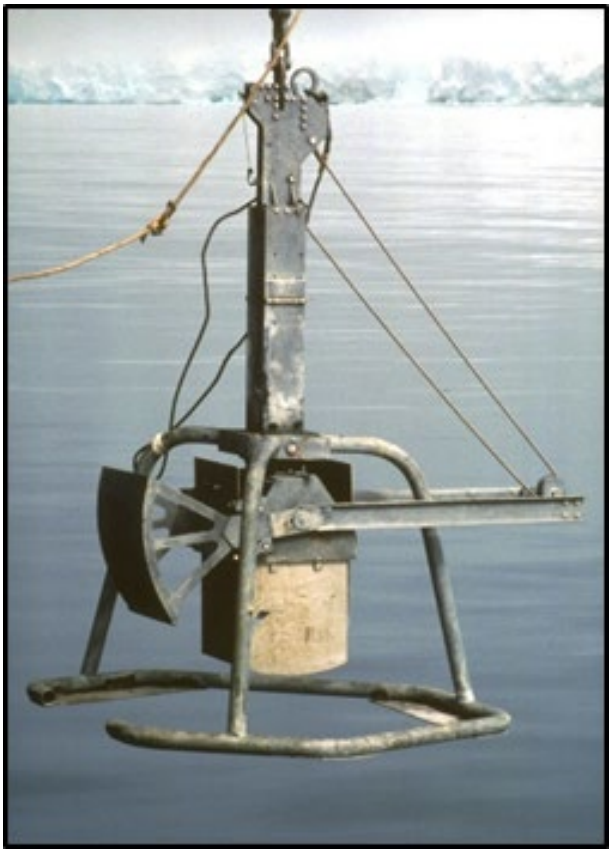

Figure 2. Box corer

b. Taking the sample

The water depth at the sampling station should be recorded before the deployment of the box corer in order to ensure that appropriate wire length is available.

The box corer is armed (locked-open) and is lowered from the ship with a controlled speed to allow the corer arriving upright at the sediment floor. Controlling the speed of the box corer's deployment will keep the wire stretched and the equipment in a vertical position, as needed. Another factor affecting the successful deployment of the box corer is the existence of near-bottom currents that may deflect it from the vertical line, resulting in unsuccessful sampling. Additional weight on the box corer, as well as longer wire than the actual depth at the station, may be needed.

Upon arriving at the sediment's surface, the box corer is penetrating the sediment depending on the hardness of the bottom.

Once the core box is filled with sediment, the winch operator slowly recovers the lifting wire and box corer ensuring the lowering of the cutting edge of the spade into the sediment to close the bottom of the box.

Once the box corer is lifted on board it has to be positioned on a clean area and secured.

i) Visually inspect the collected sample from the inspection door on top of the box corer to make

sure that the spades have are closed tightly and the sediment collected is undisturbed.

ii) Siphon the supernatant water off the sample with a plastic or glass tube and stored it in pre-cleaned bottles, if additional seawater analysis is planned.

iii) Record the visual characteristics of the sediment, such as grain size (fine or coarse grained), colour, smell and the presence of organisms. If required, you can measure additional parameters, such Eh and pH.

Record the depth of the core penetration in order to decide if the sampling can be considered successful (appropriate sediment penetration).

c. Avoiding contamination

Box corers are made of metal (usually stainless steel) therefore they have to be handled with care, to avoid contamination in the determination of heavy metals. Once the box corer is open on a clean area on the deck of the ship, subsamples can be taken by hand-held plastic coring tubes for metal analysis, and by metallic tubes for organic contaminants analysis. The diameter of these coring tubes depends on the surface of the sediment retrieved with the box corer, as well as the number of subsamples required. The depth of the sediment retrieved for analysis may vary from 1 to 5 cm depending on the sedimentation rate in the sampling site and has to be decided by the institution that is responsible for the sampling. In all cases it is important to ensure that enough sediment material is collected to allow for analysis of heavy metals, as well as additional sediment analyses (such as grain size). The EC Guidance on sediment sampling (EC, 2010) suggests to collect 50 ml of wet sediment for heavy metal analysis and 250 ml for the analysis of organic contaminants. All tools for handling sediment for metal analysis should be made by plastic tools, while metallic tools have to be used for handling sediment for organic contaminants' analysis.

Sediment sub-samples are transferred into wide-mouth, pre-cleaned containers:

i. Plastic (polyethylene, polypropylene) or glass are suitable container's materials for sediments to be analysed for heavy metals;
ii. Glass or aluminium are suitable container's materials for sediments to be analysed for organic contaminants.

Containers and zip-lock bags should be filled to the top to reduce the likelihood of oxidation during transport.

If the contaminants profile will be studied, the cores collected from the box-corer have to be sliced on board to preserve their integrity. If un-sliced cores are transported in horizontal position, the profile characteristics may be lost because of mixing of layers. On the other hand, if cores are transported in vertical position, they may be compacted because of vibration altering the thickness of core's depositional layers. The core sub-samples are transferred to pre-cleaned containers: plastic (polyethylene, polypropylene) or glass are suitable container's materials for sediments to be analysed for heavy metals, while glass or aluminium are suitable container's materials for sediments to be analysed for organic contaminants.

Samples have to be stored at 4°C in a cooler box and transported to the laboratory for further processing and analysis.

1.2.3 Protocol for the use of a multi-corer for collecting sediments

a. Multi-corer operation

A multi-corer is a sediment sampling equipment, with several corers joined together (usually 4 to 12 corers) (Figure 3). The multi-corer is lowered from a ship and when it touches the seafloor, its weight pushes the assembled cores into the sediment. When the multi-core is lifted, individual corers' tops and bottoms are closed in order to bring an undisturbed sediment on board. Multi-corers are relatively heavy and can be operated from a ship with appropriate equipment (heavy winch) in water depths more than 3 m, as well as in offshore waters (EC, 2010). Usual models collect sediment cores of 0.7 m length and a coring tube diameter 0.1 m, although there are smaller multi-corers available in the market. The big advantage of multi-corers is that they collect several virtually intact sediment cores, which can be used for the analysis of different parameters (heavy metals, organic contaminants, grain sizes, etc.). Multi-corers can also be used for dating sediment layers. If properly handled multi-corers operate efficiently in all kind of bottoms, hard, soft or unconsolidated, retrieving undisturbed sediment cores.

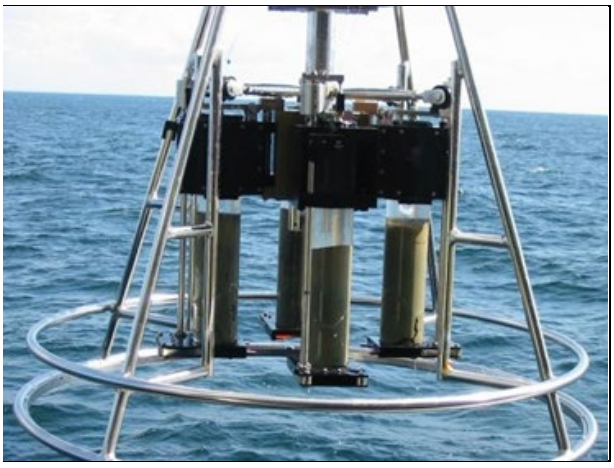

Figure 3. Multiple corer

b. Taking the sample

The water depth at the sampling station should be recorded before the deployment of the multi-corer in order to ensure that appropriate wire length is available.

The multi-corer is armed (locked-open) and is lowered from the ship with a controlled speed, in order to arrive at the bottom in an upright position.

During the descent of the multi-corer through the water column it is important to control the speed of deployment, to allow the corer arriving upright at the sediment floor. A speed of descent of 1 m/s is considered appropriate for the deployment of the device. Controlling the speed of the multi-corer's deployment will keep the wire stretched and the equipment in a vertical position, as needed.

Upon arriving at the sediment's surface, the individual corers are penetrating the sediment driven by the weights. Penetration depth depends on the hardness of the bottom.

Once the cores have penetrated the sediment, the winch operator slowly recovers the lifting wire and multi-corer, and upon detaching from the sediment, the core tubes are sealed, being capped both top and bottom, preserving the integrity of the samples, and the multi-corer is recovered to the surfaces.

Once the multi-corer is lifted on board it has to be positioned on a clean area and secured.

i) Visually inspect the collected cores to make sure that both ends of the coring tubes are properly closed and the sediment collected is undisturbed;
ii) Siphon the supernatant water off the samples with a plastic or glass tube and stored it in pre-cleaned bottles, if additional seawater analysis is planned;
iii) Record the visual characteristics of the sediment, such as grain size (fine or coarse grained), colour, smell and the presence of organisms. If required, you can measure additional parameters, such Eh and pH;
iv) Record the depth of the core penetration in order to decide if the sampling can be considered successful (appropriate sediment penetration).

c. Avoiding contamination

Multi-corers have coring tubs made of plastic (acrylic or polycarbonate) for heavy metal analysis or stainless steel for organic contaminants or granulometric analysis. Therefore, appropriate coring tubes should be used for specific measurements. The sizes of the individual coring tubes vary, however usual models collect sediment cores of 60-70 cm length with a coring tube diameter of 10 cm. The depth of the sediment which represents the surface, recently deposited material may vary from 1 to 5 cm depending on the sedimentation rate in the sampling site and has to be decided by the institution that is responsible for the sampling. However, in all cases it is important to ensure that enough sediment material is collected to allow for analysis of heavy metals, as well as additional sediment analyses (such as grain size). The EC Guidance on sediment sampling (EC, 2010) suggests to collect 50 ml of wet sediment for heavy metal analysis and 250 ml for the analysis of organic contaminants. As an example, if the top 5 cm are retrieved from a coring tube with an internal diameter of 10 cm, the sediment volume collected is 390 cm^3.

Multi-corers can also be used to collect deeper sediment layers cores for dating historic pollution trends. The length of the core is restricted to 70-100 cm, which may be enough for recent pollution studies. All sediment handling tools for metal analysis, including core slicer to retrieve specific sediment layers, should be made by plastic, while metallic tools have to be used for handling sediment samples for organic contaminants' analysis. Samples are transferred into pre-cleaned containers: plastic bags or containers for heavy metal analysis and glass or aluminium for organic analysis.

In order to preserve the integrity of cores, it is preferable to slice them on board and to store the samples of the different sediment layers. If un-sliced cores are transported in horizontal position, the profile characteristics may be lost because of mixing of layers. On the other hand, if cores are transported in vertical position, they may be compacted because of vibration altering the thickness of core's depositional layers

Sediment samples are stored at 4 ^oC on board in a cooler box and are transported to the laboratory for further processing and analysis.

1.2.4 Protocol for the use of a gravity corer

a. Gravity corer operation

A gravity corer consists of a metallic corer tube with a plastic internal liner and attached weights that enables penetration into the sediment (Figure 4). The gravity corer is used for taking relatively long cores to study sediment layers. It is a heavy equipment (could be hundreds of kilograms), which is usually operated from a ship equipped with a heavy winch for relatively deep waters. Smaller gravity corers may be available but, they also need a boat and a winch to be handled. Gravity corers are mostly used to study contaminants' variation between sediment layers, or to record pre-industrial background concentrations of contaminants, rather than studying recent pollution changes. They can be used in both coastal and offshore sediments, taking into consideration the respective sedimentation rates, in order to evaluate the analytical results.

Figure 4. Gravity corer

The gravity corer is lowered from a ship and when it touches the seafloor, its weight pushes the corer tube into the sediment. Penetration depth depends on the hardness of the bottom and the weight added on top of the corer's tube. It has to be noted that because of the gravity-driven penetration of the corer into the sediment and the relatively small diameter of the coring tube, the retrieved sediment layers may be compressed and/or stretched, which may result in misleading geochronology results.

b. Taking the sample

The water depth at the sampling station should be recorded before the deployment of the gravity corer in order to ensure that appropriate wire length is available.

During the descent of the corer through the water column it is important to control the speed of deployment, to allow the corer arriving upright at the sediment floor. Controlling the speed of the gravity corer's deployment will keep the wire stretched and the equipment in a vertical position, as needed.

Once the corer has penetrated the sediment, the winch operator slowly recovers the lifting wire and when the corer is lifted from the seafloor the "orange peel" closing system prevents the loss of the collected sediment, preserving the integrity of the sediment layers.

Once the gravity corer is lifted on board it has to be positioned on a clean area and secured.

i) Remove the inner liner of the corer and record the visual characteristics of the sediment, such as grain size (fine or coarse grained), colour, smell and the presence of organisms. If required, you can measure additional parameters, such Eh and pH.

ii) Record the depth of the core penetration in order to decide if the sampling can be considered successful (appropriate sediment penetration).

iii) Slice the core using a core slicer, according to predefined sections. Surface sediment, which represents recent deposition, may correspond to the upper 1 - 5 cm, according to the sedimentation rate in the area, while the core intervals, which correspond to past deposition times will be defined by the leading scientist.

c. Avoiding contamination

Gravity corers collect only one core at a time, therefore if a plastic liner is used the collected sediment can be used for heavy metal analysis only. Stainless steel or Teflon liners can be used for collecting sediments for organic contaminants analysis. The depth of the sediment which represents the surface, recently deposited material may vary from 1 to 5 cm depending on the sedimentation rate in the sampling site and has to be decided by the institution that is responsible for the sampling. It is important to ensure that enough sediment material is collected to allow for analysis of heavy metals, as well as additional sediment analyses (such as grain size). The EC Guidance on sediment sampling (EC, 2010) suggests to collect 50 ml of wet sediment for heavy metal analysis and 250 ml for the analysis of organic contaminants.

Gravity corers are mainly used to collect deeper sediment layers for dating historic pollution trends. All sediment handling tools for metal analysis, including core slicer to retrieve specific sediment layers, should be made by plastic, while metallic tools have to be used for handling sediment samples for organic contaminants' analysis. Samples are transferred into pre-cleaned containers: plastic bags or containers for heavy metal analysis and glass or aluminium or other non-contaminating material for organic analysis.

In order to preserve the integrity of cores, it is preferable to slice them on board and to store the samples of the different sediment layers. If un-sliced cores are transported in horizontal position, the profile characteristics may be lost because of mixing of layers. On the other hand, if cores are transported in vertical position, they may be compacted because of vibration altering the thickness of core's depositional layers

Sediment samples are stored at 4 ^{o}C on board in a cooler box and are transported to the laboratory for further processing and analysis.

1.2.5 Protocol for hand collection of sediment with a shovel/scoop and a hand-held corer

a. Hand shovel/spatula operation

In mud flats or in very shallow water zones with a water depth less than 30 cm, surface sediment samples (5 cm) can be collected with a shovel, spatula or scoop, if no other sampling equipment is available (Figure 5). This method can be used to collect both unconsolidated and consolidated sediment; however, it is more accurate when used in relatively calm waters. The person who will take the sample has to walk with care into the water, avoiding disturbing the site to be sampled and using a shovel/spatula/scoop he/she collects the desired thickness of the sediment. The depth of the sediment which represents the surface, recently deposited material may vary from 1 to 5 cm depending on the sedimentation rate in the sampling site, and it has to be decided by the institution that is responsible for the sampling. It is important to ensure that enough sediment material is collected to allow for analysis of heavy metals, as well as additional sediment analyses (such as grain size). The EC Guidance on sediment sampling (EC, 2010) suggests collecting 50 ml of wet sediment for heavy metal analysis and 250 ml for the analysis of organic contaminants.

The sample collected is transferred to a pre-cleaned container. The excess water should be removed before closing the container with the sediment sample.

Figure 5. Hand-held scoop sediment sampler

b. Avoiding contamination

To avoid contamination during sampling the sampling utensil (shovel/spatula/scoop) has to be made of plastic for heavy metal analysis and of stainless steel for organic contaminants analysis. The containers used to store the sediment samples should be pre-cleaned and made of plastic for heavy metal analysis or of metal/glass for organic contaminants analysis.

Sediment samples are stored at 4 ºC on board in a cooler box and are transported to the laboratory for further processing and analysis.

1.3 Technical note for the preservation of sediment sample to be analysed for heavy metals and organic contaminants

After collection wet sediment samples have to be treated in order to be preserved unaltered until transfer to the analytical laboratory for heavy metals analysis. Sediment sample preservation include: i) Storage of wet samples on board; ii) Wet sieving to collect the grain size fraction < 2 mm, which will be further analysed for organic contaminants; iii) Freeze drying to prepare the sample for the analysis and iv) Homogenization and storage of dried sediments. Wet sieving may also include an additional step to define the percentage (weight) of the silt and clay fraction of the sediment (< 63 μm), which is a useful parameter in assessing pollution in sediments. For the processes, the Protocol includes all necessary precautions to avoid cross-contamination of the sediment samples from tools, equipment and the laboratory environment.

The IMAP Protocol 3.1. addresses the preservation of sediment samples prior to analysis for heavy metals and organic contaminants.

1.3.1 Protocol for the preservation of sediment sample

a. Storage of wet samples on board

Upon collection wet samples have to be stored on board in such a way as to preserve them from deterioration that will affect the subsequent analysis of contaminants. Keeping the samples in low temperature (at 4 ºC) and away from light and air (as much as possible) will slow down oxidation and bacterial activity, helping in maintaining sediment's initial characteristics. The first few hours after sampling are the most critical for changes to occur in the sample, therefore preservation steps should be taken, where possible, immediately upon sample collection (HELCOM 2012).

b. Wet sieving

Sediment texture may differ among locations, from very fine clay in the open sea to coarse sandy sediments close to the shoreline. Finer sediments indicate net depositional areas, which are preferable sampling stations for studying pollution impact, while coarse sand, pebbles or rocky substrates are not favourable sampling locations. For pollution studies, the most informative fraction of the sediment is the silt and clay fraction (< 63 μm) because contaminants are mainly associated with finer particles (EC 2010, ICES/OSPAR 2018) and coarser sediments (sand fraction) have much lower concentrations of heavy metals and organic contaminants. Therefore, the distribution of contaminant's concentrations in sediments will generally follow the distribution of fine-grained sediments. However, sieving over 63 μm mesh adds another step in the processing of the sample and, consequently, an additional source of potential contamination. Also, sieving over 63 μm mesh may be influenced by the unsuccessful disaggregation of particle conglomerates, which may affect the efficient quantitative segregation of silt + clay from the sand fraction.

The IMAP Common Indicator Guidance Fact Sheets (UNEP, 2019) requires the separation of the sediment fraction less than 2 mm, as the appropriate sediment fraction for the determination of heavy metals and organic contaminants. Also, an additional sieving over a 63 μm mesh is requested, in order to record the percentage of the silt and clay fraction in the sediment. This data will be used for normalizing contaminants concentrations in the whole sediment (< 2 mm) for the grain-size effect, evaluating pollution levels and comparing between areas with different sediment texture.

Upon arrival on board, sediment samples should be wet sieved using a 2 mm mesh-size sieve as soon as possible in order to remove large detritus and benthic organisms, which may affect the sediment characteristic during subsequent sample handling and processing (storage, freezing or ultrasonic treatment) (EC, 2010).

It is preferable to use seawater from the sampling site for wet sieving in order to avoid any possible alteration of the sediment equilibrium (such as adsorption or desorption of metals). If this is not possible, wet sieving could take place in the laboratory using seawater with approximately the same salinity with the sampling location. Sieving over 63 μm mesh, if not implemented on board, it can be done in the laboratory.

For heavy metal analysis, sieving for both 2 mm and 63 μm mesh sizes may be carried out using sieves made of polymer (PVC or acrylic rim, with nylon or polyester mesh).

For organic contaminants analysis sieving may be carried out using sieves made of stainless steel (rim and mesh).

The sediment material is placed on the mesh, water is poured, and the sieve is moved manually. For the processing of larger numbers of samples, sieves may be placed on vibrator tables. Clays often tend to form larger lumps if dried, therefore wet sieving should be done when the sediment is still wet. In case the sediment is becoming dry, it has to be pre-soaked in seawater for at least 2 hours to disaggregate the lumps (EC, 2010). However, this procedure may result in the release of contaminants, which are adsorbed on particles' surface and should be avoided, if possible. In case that pre-soaking is needed, use seawater from the sampling area and sieve disaggregated particles as soon as possible.

For heavy metal analysis, the sieved sediment is collected with a plastic spatula and stored in a plastic container for further processing (drying). For organic contaminants analysis the sieved sediment is collected with a stainless-steel spatula and stored in a glass or aluminium container.

c. Drying

Prior to the instrumental detection, sediment samples must be dried. For metal (except volatile mercury) analysis, sediments should be freeze-dried, which is the preferable procedure. Alternatively, the sediments may be dried at any temperature below 105°C until constant weight. For mercury analysis, to minimise losses due to evaporation, a sediment sub sample could be air dried at temperature <50°C (EC, 2010).

For organic compounds analysis drying procedures depends on the compounds to be analysed. For chlorinated hydrocarbons sediments can be freeze-dried taking care to avoid determinant loss through evaporation by keeping the temperature in the evaporation chamber below 0°C (OSPAR, 2018). For PAH determination, freeze-drying sediment samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore, drying the samples may result in losses of the lower molecular weight, more volatile PAHs through evaporation. To protect sediments samples during freeze drying from cross-contamination from particles and vapours, the sample containers could be covered with a lid or filter paper perforated with a small hole (HELCOM, 2012).

d. Homogenization and storage of dried sediments

After drying, the samples are homogenized using a ball mill and are stored in a cool and dark place, for further analysis. Temperature is the most important factor affecting the samples, from the time of sample collection through handling to the final analyses. Also, contamination from the laboratory's air should be avoided.

Freeze-dried sediment samples can be stored in pre-cleaned wide-mouth bottles with a screw cap. Samples intended for the analysis of metals can be stored in plastic or glass containers. For mercury analysis, samples must be stored in acid-washed borosilicate glass or quartz containers, as mercury can move through the walls of plastic containers. Samples intended for the analysis of organic contaminants must be stored in amber glass, stainless steel or aluminium containers (EC, 2010).

Containers with sediment samples should be archived and kept in storage after the completion of the analysis, in order to be used as a replicate sample in case crosschecking of the results are required or additional determinations are needed in the future. Freeze-dried sediments remaining after analyses could be are stored in the original sample bottle, closed with an airtight lid to protect against moisture. When stored in a cool, dark place, samples may be archived and stored for 10-15 years (EC, 2010).

2 Guidelines for the determination of contaminants in sediment

2.1 Introduction

Determination of the concentrations of targeted heavy metals and organic contaminants in different marine matrices is a key component of the IMAP, since the analytical results will contribute to the assessment of the environmental status of the water body under consideration. Sediment is one of the proposed matrices for the analysis of heavy metals and organic contaminants since the establishment of the UNEP/MAP – MED POL Monitoring programme in 1981 (MED POL Phase II), because many heavy metals and persistent organic contaminants in seawater tend to become insoluble and precipitate with the particulate fraction on the seafloor. Therefore, since sediment is the ultimate sink of most heavy metals and persistent organic contaminants, which are introduced into the marine environment, their analysis will provide a clear view of the pollution state of the specific water body. Furthermore, in areas with undisturbed sediments, the yearly deposited sedimentary material integrates the pollution load during this specific time period, and the analysis of different sedimentary layers is providing a historical trend of pollution processes in the region.

Contaminants may enter the marine environment form land- and sea-based sources as well as through atmospheric deposition. Land-based sources are mainly affecting coastal sediments, where the higher metal and organic contaminants concentrations are usually found at the vicinity of pollution "hot spots" (coastal cities and industrial areas, river mouths draining highly populated and/or industrialized basins). Offshore sediments are mainly influenced by atmospheric deposition, which play globally a very important role, especially for some metals (such as Hg) and organic contaminants (such as PAHs).

Heavy metal sources are both natural and anthropogenic. Therefore, it is important to be able to differentiate between metal enrichments caused by natural causes (such as sediment's mineralogy and granulometry) and those originating from human activities (urban, industrial). To that end, normalization of the heavy metal data is often used, in view of detecting the human imprint on the heavy metal distribution in sediment. On the other hand, persistent organic contaminants sources are solely anthropogenic, therefore the total contaminant's load is of anthropogenic origin.

In line with IMAP requirements (UNEP/MAP, 2019a, UNEP/MAP, 2019b), mandatory contaminants to be analysed in the marine sediment include: heavy metals (Cadmium (Cd), Lead (Pb) and total Mercury (THg)), organochlorinated compounds (PCBs, hexachlorobenzene, lindane and ΣDDTs) and Polycyclic Aromatic Hydrocarbons (US EPA 16 Reference PAHs compounds). Also, additional parameters to be analysed in sediment are: Aluminium (Al), Total Organic Carbon (TOC), grain size (<2 mm and <63 μm).

The UNEP/MAP assessment criteria (Background Assessment Criteria -BAC and Environmental Assessment Criteria - EAC) for targeted heavy metals and organic contaminants in sediments are presented in the Annex V.

The Protocols prepared in the framework of this Monitoring Guidelines/Protocols for Sample Preparation and Analysis of Sediment for IMAP Common Indicator 17, as provided here-below, describe appropriate methodologies for the analysis of marine sediments for the determination of heavy metals and organic contaminants, in order to ensure quality assured data. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

These Protocols aim at streamlining marine sediment sample preparation and analysis for heavy metals and organic contaminants in a view of assuring comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes. They provide a step-by-step guidance on the methods to be applied in the Mediterranean area for sampling and sample preservation of sediments.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent laboratories participating in IMAP implementation. They build upon the UNEP/MAP - IAEA Recommended Methods for the determination of heavy metals and organic contaminants, such as: IAEA (2011a) Recommended method on microwave digestion of marine samples for the determination of trace element content (Annex VI); IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry (Annex VIII); IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace (Annex IX); IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry (Annex XI); IAEA (2012b) Recommended method for the determination of mercury in samples of marine origin by cold vapour atomic absorption spectroscopy (Annex XII); UNEP/IAEA (2011d) Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment: Reference Methods for Marine Pollution Studies No 71 (Annex XIV); , which were prepared in the framework of the MED POL monitoring programme. They are also streamlined with similar Guidelines/Protocols for marine sediment sample preparation and analysis which were developed by other Regional Organisations, such as OSPAR (Annex VII, XIII, XV, and XVII) and HELCOM (Annexes XVI and XVIII), therefore any of these Guidelines are equally suitable to be applied in the context of IMAP, as well as and US EPA (Annex X). Given the suitability of any of these Guidelines in the context of IMAP, they could be further used by interested IMAP competent Mediterranean laboratories for developing their laboratory specific sampling and sample processing

methodologies. The Contracting Parties' laboratories should accommodate and always test and modify each step of the procedures to validate their results.

2.2 Technical note for the determination of heavy metals in sediment

Analysis of marine sediment samples for the determination of heavy metals[8] include: i) digestion of sediments and ii) analysis of the digested sample for heavy metals. Cd, Pb and THg are the mandatory metals to be determined in marine sediment samples (UNEP/MAP, 2019a). However, the Contracting Parties to the Barcelona Convention may decide to include in their national monitoring programmes the determination of additional heavy metals according to their national priorities.

National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of the Contracting Parties, the IMAP Protocols in order to be used as guidelines for the determination of heavy metals and trace elements in marine sediment samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the Protocols in order to validate their final results. Quality Assurance requirements for heavy metals analysis in sediments are presented in Chapter E of these Guidelines. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

Regardless of the analytical method used, heavy metal determination follows some procedures common to all analytical methodologies, such as the calibration of the analytical equipment and the cleaning and handling procedures to avoid the contamination of the samples from the laboratory's environment and the tools and containers used in the analysis.

a) Calibration

Calibration standards prepared from single standard stock solutions or multielement standards, by dilution of the stock solution using dilute acid, as required. All standard solutions have to be stored in polyethylene, borosilicate or quartz volumetric flasks. Standard solutions with lower concentrations, if prepared correctly and controlled in a QA system (checking of old versus new standards, and checking with standards from a different source), can be kept for a period no longer than one month

The calibration procedure has to meet some basic criteria in order to provide the best estimation of the true element concentration of the sample analysed (HELCOM, 2012a[9]):

i) the concentrations of standards for the preparation of the calibration curve should cover the range of anticipated concentrations;

ii) the required analytical precision should be known and achievable throughout the entire range of concentrations;

iii) the measured value at the lower end of the range has to be significantly different from the procedural analytical blank;

iv) the chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation.

b) Avoiding contamination

To avoid metal contamination in the laboratory all glassware and plastic vessels used should be carefully cleaned. The general cleaning guidelines include:

i) Allow the vessels to soak overnight in a plastic container in a soap solution (solution 2% in tap water);

ii) Rinse thoroughly first with tap water then with ultrapure deionised water;

iii) Leave the vessels to stand in 10% (v/v) concentrated HNO_3 solution at room temperature for at least 6 days;

iv) Rinse thoroughly with Milli-Q water (at least 4 times);

v) Allow the vessels to dry under a laminar flow hood;

vi) Store the vessels in zip-lock plastic polyethylene bags to prevent the risk of contamination prior to use.

This procedure should be used for all plastic ware use in the laboratory as tips, cup for autosampler, plastic containers.

Under this Technical Note, the Guideline for Sample Preparation and Analysis provides the following IMAP Protocols:

- Protocol for sediment digestion using nitric acid and hydrofluoric acid (microwave assisted digestion in closed systems and digestion on hot plate);
- Protocol for the determination of heavy metals with Flame Atomic Absorption Spectroscopy (F-AAS;

[8] In the Guideline text the term "heavy metals" is used to designate both heavy metals and trace elements

[9] HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-13 Appendix 3: Technical note on the determination of heavy metals and persistent organic compounds in marine sediment.

- Protocol for the determination of heavy metals with Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS);
- Protocol for the determination of heavy metals with Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS);
- Protocol for the determination of Total Mercury with solid Hg analyser;
- Protocol for the determination of Total Mercury in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry;
- Protocol for the normalization of heavy metal concentrations using Al.

These Protocols are based on Analytical Methods developed by IAEA (Annex VI: Recommended method on microwave digestion of marine samples for the determination of trace element content ; Annex VIII: Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry; Annex IX: Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace; Annex XI: Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry and Annex XII: Recommended method for the determination of mercury in samples of marine origin by cold vapour atomic absorption spectroscopy), OSPAR (Annex VII: CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods, Annex XIII: CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalisation of contaminant concentrations in sediments), and US EPA (Annex X: US-EPA Method 6020B. ICP-MS method for the determination of elements in water samples and in waste extracts or digests).

2.2.1 Protocol for sediment digestion using nitric acid and hydrofluoric acid

Sediment samples have to be digested (wet ashing) prior to analysis. The rate of digestion and the efficiency of acid decomposition increase substantially with elevated temperatures and pressure, therefore microwave digestion in closed vessels is the preferred method. However, in case no such equipment is available, sample digestion in open vessels over a hot plate is an alternative method.

IMAP requires the complete disintegration of the silicate matter of sediments using Hydrofluoric acid (HF) in order to measure the total metal load in sediments, including Al, which is needed for normalization purposes. Furthermore, Certified Reference Materials (CRMs) of sediments provide certified values for total metal concentrations, therefore their use to strengthen data quality assurance requires the measurement of the total metal content in sediment samples

a) Microwave acid digestion in closed systems for heavy metals for AAS, GFAAS and ICP-MS analysis

Sediment digestion can be performed in Teflon, or equal quality vessels, which are metal free and resistant to strong acids including HF (Loring and Rantala, 1991[10]). Dried sediment samples (0.1 to 0.5 g) are weighted in the microwave vessel and placed in a laminar hood compatible with acid fume. Approximately 5 ml of analytical grade nitric acid and 2 ml of analytical grade hydrofluoric acid are added and each vessel and let to react for at least 1hour (or more if possible). After the room temperature pre-digestion, 2ml of hydrogen peroxide (analytical grade) are added carefully, the vessels are closed and placed in the microwave apparatus and digestion steps are performed, following the IAEA's Recommended method on microwave digestion of marine samples for the determination of trace element content (Annex VI. IAEA 2011a[11]). Oxygen peroxide and organic matter can promote an explosive reaction, so this acid must be treated with great caution when added to the sediment. Also because closed vessels retain the HF, boric acid is added after the HF digestion to complex the remaining HF and make the resulting solution less hazardous, as well as preventing aluminium fluoride precipitation. After digestion the vessels are removed from the microwave apparatus and placed in a ventilated fume hood to cool. When the pressure is adequate, the vessels are opened, and their content is transferred into a 50 ml polypropylene graduated tubes. At least one Certified Reference Material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

Microwave assisted acid digestion of sediments are also proposed by OSPAR (2018a[12]) (Annex VII), HELCOM (2012a) and US EPA (1996[13]) (Method 3052).

b) Acid digestion over a hot plate

In case no microwave digestion system is available, it is possible to perform digestion over a programmable heating plate placed inside a metal free and acid resistant fume hood, allowing HF and other acids treatment. Sediment samples are treated in closed Teflon vessels with hydrofluoric acid (HF) in combination with aqua regia in order to decompose the samples. The use of HF

[10] Loring, DH and Rantala RTT (1991). Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Review, 32: 235:283. Elsevier Science Publishers B.V

[11] IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)

[12] OSPAR (2018a). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods

[13] US EPA (1996). Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices.

is essential because it is the only acid that completely dissolves the silicate lattices and releases all the metals. However, it should be noted that digestion in open systems may lead to loss of Hg (Delft and Vos, 1988[14]), while great care should be made to avoid loss of material because of violent boiling reactions. Therefore, digestion over a hot plate is not a recommended method and should be avoided if possible.

Several acid mixtures (together with HF) have been used for sediment digestion over a hot plate, such as aqua regia, nitric acid or perchloric acid (Loring and Rantala, 1991; Cook et al, 1997[15]). In case perchloric acid is used solutions are left to stand for a period of 1 hour – overnight to avoid problems with violent reactions, which may be prompted by the presence of organic matter in the sediment. Then the vessels are closed and placed on a hot plate at 120 °C or in boiling water for 1-2hdepending on the method followed. Then the samples are allowed to cool to room temperature, the tubes are opened and boric acid is added to complex the remaining HF (OSPAR 2018a).

2.2.2 Protocol for the determination of heavy metals with Flame AAS

In most marine sediments Al, Cu, Cr, Fe, Ni, Zn, as well as other metals, can be determined by Flame Atomic Absorption Spectroscopy, which has adequate sensitivity for these determinations.

In Atomic Absorption Spectrometry the sample solution is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the element in the flame. Each metal has its own characteristic wavelength so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

Metal standard solutions for the calibration curve are prepared from stock standard solution (1000 mg l^{-1} or an intermediate stock standard). Depending on the element, it may be necessary to make an intermediate stock standard solution. This intermediate stock is prepared as described in the Technical Note in a 2% HNO_3 matrix. The calibration curve is determined according to the expected concentrations of the samples, and the linearity of the AAS response for the element is considered (absorbance versus concentration curve given in the analytical methods book). If ionization or interferences are likely, the right option according to the analytical method book has to be chosen, e.g. use of correction for non-atomic absorption by using deuterium lamp background corrector; use of oxidizing air-acetylene flame; use of nitrous oxide-acetylene flame; addition of a releasing agent or ionization suppressant.

A detailed analytical protocol for the determination of heavy metals in sediments with flame AAS prepared by IAEA (2011b[16]) is presented in the Annex VIII.

2.2.3 Protocol for the determination of heavy metals with GF-AAS

In marine sediments Cd, Pb, Cu as well as other metals, can be determined by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), which has adequate sensitivity for these determinations. For GF-AAS analysis, after the digestion of the sediment sample, an aliquot of sample solution (10-50 µl) is introduced into a graphite tube of the GF-AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

The reagents used include: argon, standard solution of the element of interest 1000 mg l^{-1} , deionized water. All reagents should be of analytical grade.

A detailed analytical protocol for the determination of heavy metals in sediments by GF AAS prepared by IAEA (2011c[17]) is presented in the Annex IX.

The AAS software generally gives typical electrothermal programs for each element for 10 µl of sample in diluted HNO_3 (0.1%) and indications concerning maximum ashing and atomization temperatures. More specific information may also be found in the literature, such as recommendations regarding matrix modifiers and the use of partition tubes or tubes with platform. When a program is optimized for the determination of an element in a specific matrix, all information should be reported in the logbook of methods of the laboratory.

For some elements and some matrices, the results obtained are still not satisfactory (e.g. maximum ashing temperature is not sufficient to eliminate the background), this procedure should be redone with the addition of a matrix modifier. Different matrix modifiers could be tried before finding the best solution.

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[14] Delft W. van; Vos. G. (1988) Comparison of digestion procedures for the determination of mercury in soils by cold-vapor atomic absorption Spectrometry; Analytica Chimica Acta, 209, 147-156.

[15] Cook JM, Robinson JJ, Chenery SR and Miles DL (1997). "Determining cadmium in marine sediments by inductively coupled plasma mass spectrometry: attacking the problems or the problems with the attack?" Analyst, 122, 1207-1210

[16] IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

[17] IAEA (2011c). Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

2.2.4 Protocol for the determination of heavy metals with ICP-MS

Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS) is currently state-of-the-art instrumentation for metal analysis, with the possibility to determine at sub-µg L^{-1} concentrations of a large number of elements in water and acid digested sediment samples.

Typical limits of detection for the determination of trace metals with ICP-MS (in mg kg^{-1} d.w.) based on typical sample intakes (0.5 –1 g), are as follows (OSPAR, 2018):

Al	Li	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
40	0.1	1	0.01	0.2	0.1	0.05	0.2	0.2	2

Inductively coupled plasma attached to a mass spectrometer (ICP-MS) allows a rapid analysis of a wide range of heavy metals. Most routine instruments utilize a quadrupole mass spectrometer, so mass resolution is not high enough to avoid overlap of double charged elements or multi-element ions (mainly hydrides, oxides and hydroxides) formed in the plasma. The main concern is for the Ar interferences as the plasma is usually an argon plasma. Some elements are prone to memory effects (particularly Hg) and needs extra precautions to avoid carry over effects. Modern ICP-MS instruments software includes all the tuning and correction formulas needed and described above to perform the analysis (HELCOM 2012a).

A multi-elemental determination of heavy metals by ICP-MS in water and solid samples after acid digestion, is described in the US EPA Method 6020B (2014 revision). Metal species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix. The US EPA Method 6020B is presented in Annex X (US EPA 2014[18]).

2.2.5 Protocol for the determination of Total Mercury with solid Hg analyser

Total mercury in the sediment can be analysed by solid Hg analyser, which has adequate sensitivity for this determination. The sample is dried and then chemically decomposed under oxygen in the decomposition furnace. The decomposition products are carried out to the catalytic section of the furnace, where oxidation is completed (halogens and nitrogen/sulfur oxides are trapped). The mercury present in the remaining decomposition products is selectively trapped on an amalgamator. After flushing the system with oxygen, the mercury vapour is released by rapid heating of the amalgamator, and carried through the absorbance cell in the light path of a single wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of mercury quantity (ng). The typical working range is 0.1–500 ng. The mercury vapour is carried through a long (first) and a short path length absorbance cell. The same quantity of mercury is measured twice with different sensitivity resulting in a dynamic range that spans four orders of magnitude. The typical detection limit is 0.01 ng of mercury.

Calibration standards should be prepared from single standard stock solutions or multielement standards by dilution of the stock solution using dilute acid, as required. All standard solutions have to be stored in Teflon, borosilicate or quartz volumetric flasks in 0.5-1 % HNO_3 and 0.1% (v/v) potassium dichromate. An alternative calibration curve can be performed using a solid certified reference material.

A detailed method describing the protocol for the determination of total mercury (inorganic and organic) in sediment prepared by IAEA (2012a[19]) Annex XI): Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry. With this method, Total Hg is determined without any chemical pre-treatment of the sample, minimising possible contamination and/or additional errors due to sample handling. The method is based on the US EPA 7473 method (US EPA, 2007a[20]).

2.2.6 Protocol for the determination of Total Hg in sediments by CV-AAS

In the Cold Vapour Atomic Absorption Spectrometry (CV-AAS) method, the inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapour is then passed through the quartz absorption cell of an AAS instrument where its concentration is measured. The light beam of Hg hallow cathode lamp is directed through the quartz cell, into a

[18] USEPA (2014 revision) Method 6020B, ICP-MS. Environmental protection Agency, Washington, DC.

[19] IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry

[20] US EPA (2007a). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

monochromator and onto a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

The method is simple, rapid and applicable to a large number of environmental samples with a typical working range 0.25–100 ng mL^{-1} for direct injection of cold vapour, using "batch" system. CV-AAS analysis can be performed manually using batch CV-AAS or automatically using flow injection (FIAS) techniques. FIAS is a very efficient approach for introducing and processing liquid samples in atomic absorption spectrometry, reduces sample and reagent consumption, and has a higher tolerance of interferences, lower determination limits and improved precision compared with conventional cold vapour techniques (HELCOM, 2012b[21]).

A detailed Recommended Method prepared by IAEA (2012b[22]) describing the protocol for the determination of total mercury in sediment using CV-AAS is presented in Annex XII. Methods for the determination of Total Hg in marine biota using CV-AAS are also prepared by HELCOM (2012b) and US EPA (2007b[23]).

2.2.7 Protocol for the normalization of heavy metal concentrations using Al

Normalization is defined here as a procedure to adjust heavy metal concentrations for the influence of the natural variability in sediment composition, grain size and mineralogy. In non-polluted sediments heavy metal concentrations usually increase with decreasing grain size of the sediment and therefore any differences in metal concentrations caused by pollution sources will be obscured by grain size differences. Normalization is therefore applied to differentiate between natural variability and anthropogenic input of contaminants.

A normalization approach is to consider that in sandy sediments heavy metals concentrations are considered as negligible, therefore metal concentration determined in the <2 mm fraction could be subsequently normalized to a sample consisting of 100% of the <63 µm fraction. However, this approach cannot always successfully compensate for metal variability, because natural trace metal concentrations and their variability in sediments are determined not only by grain size distribution, but also by the composition of minerals and secondary compounds.

To overcome this drawback, a geochemical normalization approach is often used. This technique consists in establishing the mathematical relationships between metal concentrations and the concentrations of a conservative element, which represents a certain mineral fraction of the sediment. Elements of natural origin which are structurally combined to one or more of the major fine-grained trace metal carriers are considered conservative and have been used for normalization purposes. Aluminium (Al) has been the most widely used element for normalization, because it is a major constituent of fine-grained aluminosilicates with which the bulk of trace metals are associated. However, this assumption may not be valid in all cases, since there are components in sediment which may also serve as hosts for contaminants with an even higher sorptive capacity but contain neither Si nor Al, such as organic matter, Fe/Mn oxides, or sulfide minerals (Kestern and Smedes, 2002[24]). Furthermore, when the sediment is derived from glacial erosion of igneous rocks, with significant amounts of aluminium present in feldspar minerals contributing to the coarse fraction, it is preferable to use lithium as a conservative element for normalization (Loring 1991[25]).

The main assumption for the application of a geochemical normalization to a conservative element is the existence of a linear relationship between the normalizer and other metals. Such a relationship suggests that, in the natural sediments of an area, the concentration of the metal will change proportionally to the concentration of the normalizer. Also, a linear relationship must exist between the normalizer's concentration and the percentage of fine-grained (silt and clay) content of the samples. Such a relationship would allow the use of the normalizer concentrations as a proxy for granulometric variability of the sediments, in order to distinguish the pollution-related metal enrichment from the natural enrichment caused by grain size variability (Loring and Rantala 1991).

Normalization procedure

Heavy metals (Me) concentrations are divided by the concentration of the normalizer (or co-factor) in each sample. The Me/Al ratio in the references stations represent the natural relationship between the two metals in the sediments of the area, while higher Me/Al ratios indicate metal enrichment, which cannot be explained by the natural textural variability, and should be attributed to anthropogenic inputs. A more detailed approach will calculate the regression line (and the slope) between the metal and the normalizer (Al) concentrations in the sediments of an area. In order to ensure that the changes in the normalizer's concentration reflect the differences in finer material content, it is necessary to also establish a statistically significant regression between the normalizer (Al) and

[21] HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy.

[22] IAEA (2012b). Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry

[23] US EPA (2007b). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

[24] Kestern, M. and Smedes, F. (2002). Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. J. Environ. Monit. 4, 109-115.

[25] Loring, DH (1991). Normalization of heavy metal data from estuarine and coastal sediments. ICES J. Mar. Sci. 48, 101-115

the finer fraction of the sediments (i.e. clay < 2 µm or even silt+clay < 63 µm), in order to check that the normalizer is suitable to be used as a proxy of the finer sediment (Loring 1991).

Kestern and Smedes (2002) propose to analyse also the sand fraction (>63 µm) in order to calculate the Al (or Li) concentrations in the coarse sediments. They propose a model as presented in Figure 1. "C_X and N_X represent the co-factor and the contaminant contents possibly present in the coarse material (*e.g.*, Al in feldspar) and can be estimated from samples without fine material. The regression line between the contaminant and co-factor will originate from that point. Regressions of co-genetic data sets but with a different contamination levels will have this point in common but tend to develop different slopes from this "turning point". In principle, therefore, only one additional sample is required to estimate the slope for a co-genetic sample set if this turning point is known. The slope for this sample with a contaminant content C_S and a co-factor content N_S can be expressed as follows":

$$P_L = \frac{dC}{dN} = \frac{C_S - C_X}{N_S - N_X}$$

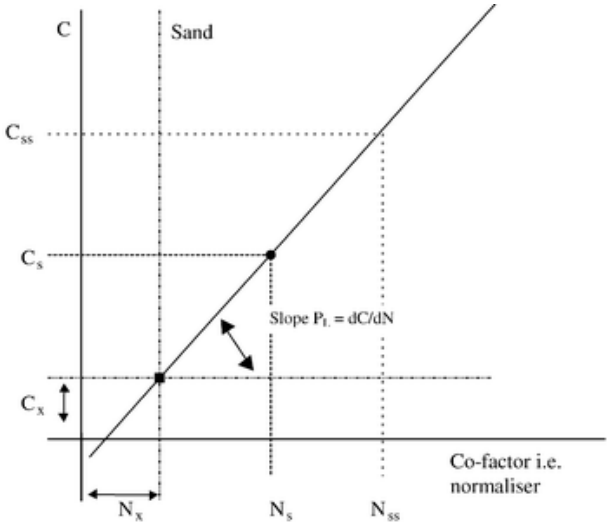

Figure 1. Contaminant content C_S and a co-factor content N_S (Kestern and Smedes, 2002, in OSPAR, 2018b[26])

The slope of the regression represents the natural relationship between the metal and the co-factor (normalizer). Therefore, "regression lines drawn for samples from different areas may thus be used to compare their degree of contamination. The steeper the gradient, the more contaminated an area is considered to be Positive residuals that plot above this line indicate that the concentrations are greater than would be predicted from the contaminant/co-factor relationship, and may represent hot-spot samples" (Kestern and Smedes, 2002, in OSPAR 2018b).

Kestern and Smedes (2002) also underline that "the precision of the result strongly depends on the natural (or analytical) variability of N_X. For coarse-grained samples, a significant standard deviation in both the C_X coefficient and the slope may arise from propagation of the errors of the analytical variation due to the overall low concentrations. The C_X coefficient of the regression may differ significantly from site to site, in particular, when using coarser grain size fractions. For some areas, Al contents in the coarse fractions are found at the same level as in the fines, and therefore the intercept N_X becomes very high. This implies that the denominator is the result of subtracting two relatively large numbers, N_S and N_X. Consequently, due to their individual uncertainties, the result has an extreme error." (OSPAR, 2018b). In MED POL IMAP, it has been decided to analyse the < 2 mm fraction of the sediment, therefore it is possible that *Ns* and *Nx* will be relatively high.

A similar approach to calculate the regression between metal and the normalizer is presented by Loring and Rantala (1992). Using data form the non-polluted (or reference) stations scatter plots of the regression lines between the metals and the normalizer (Al or Li) with 95% confidence bands are drawn. The regression line represents the natural variability of the metal concentrations in relation to the normalizer (Al or Li) content and the stations located within the confidence bands can be considered as non-polluted. On the other hand, stations located above the upper limit of the 95% confidence band may be considered as polluted.

A detailed discussion on normalization procedures can be found in OSPAR's Technical Annex 5: Normalisation of contaminant concentrations in sediments (OSPAR, 2018b) (Annex XIII).

The purpose of normalization is to reduce the variability between samples arising from differences in bulk sediment properties in order to draw conclusions on the level of metal contamination in a specific area, and/or to compare pollution levels between different areas. However, in some areas that the correlations between contaminant and cofactor concentrations may be weak or even absent. Therefore, normalization should be used taking into consideration its limitations and having a good knowledge of the characteristics of the sediments in the area under investigation.

2.3 Technical note for the determination of organic contaminants in marine sediments

The mandatory organic contaminants to be determined in sediments in the framework of IMAP are: Organochlorinated compounds (PCBs [28, 31, 52, 101, 105, 118, 138, 153, 156, 180], Hexachlorobenzene, Lindane and ΣDDTs) and polycyclic aromatic hydrocarbons (US EPA 16 individual PAHs congeners – Acenaphene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene,

[26] OSPAR (2018b). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalisation of contaminant concentrations in sediments.

Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(ghi)perylene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthene, Indeno(1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Pyrene) (UNEP/MAP, 2019a; UNEP/MAP 2019b). However, Parties may decide to include in their national monitoring programmes the determination of additional heavy organic compounds according to their national priorities.

Analytical protocols for the determination of organic contaminants in sediment samples include: i) extraction; ii) concentration; iii) clean-up; iv) fractionation; and v) quantification of contaminants.

National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of Mediterranean Parties, a list of Protocols has been drafted to be used as guidelines for the determination of organic compounds in marine sediment samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the Protocols in order to validate their final results. Quality Assurance requirements for organic contaminants determination in sediments are presented in Chapter E of these Guidelines. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

The laboratory area used for organic trace determination must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc. The emergency cut-off switch should be accessible from both inside and outside the laboratory. Firefighting equipment should be mounted in obvious places and laboratory personnel trained in their use.

Calibration of equipment for the determination of organic contaminants follows the same procedures as in the determination of heavy metals. All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances and their concentrations vary from batch to batch and with supplier. Powdered or crystalline reagents, such as anhydrous sodium sulphate (Na_2SO_4), potassium hydroxide (KOH), glass wool, must be extracted with hexane in a Soxhlet apparatus. Adsorbents, such as silica gel, alumina and Florisil have also to be solvent extracted. All glassware should be vigorously scrubbed with brushes in hot water and detergent and rinse five times with tap water and twice with distilled water. Then, glassware should be rinsed with acetone or methanol followed by hexane or petroleum ether and baked overnight in an oven at 300 °C. All glassware should be stored in dust free cabinets and tightly sealed with pre-cleaned aluminium foil when not in use. Ideally glassware should be cleaned just before use. More information on cleaning reagents and glassware is provided in UNEP/IAEA (2011[27]) (Annex XIV.).

In the framework of this Technical note, this Guideline provides the following IMAP Protocols for the analysis of organic compounds in marine sediment samples:

- Protocol for the determination of organochlorine pesticides and PCBs in sediment using Gas Chromatography-Electron Capture Detector (GC-ECD);
- Protocol for the determination of organochlorine pesticides and PCBs in sediment using Gas Chromatography – Mass Spectrometry (GC-MS);
- Protocol for the determination of PAHs in sediment using High Performance Liquid Chromatography - Fluorescence (HPLC-UVF);
- Protocol for the determination of PAHs in sediment using GC-MS;
- Protocol for the normalization or organic contaminants concentrations in sediment using Total Organic Carbon (TOC).

These protocols are based on Analytical Methods developed by UNEP/IAEA (Annex XIV: Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71; HELCOM (Annex XVI: Manual for marine monitoring COMBINE programme, Annex B-12, Appendix 2. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in marine sediment; Annex XVIII: Manual for marine monitoring in the COMBINE programme, Annex B-12, Appendix 1. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in sediment) and OSPAR (Annex XV: CEMP Guidelines for monitoring contaminants in sediments, Annex 2 Analysis of PCBs in sediments; Annex XVII: CEMP Guidelines for monitoring contaminants in sediments, Annex 3: Determination of parent and alkylated PAHs in sediments).

[27] UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71.

2.3.1 Protocol for the determination of organochlorine pesticides and PCBs in marine sediments using GC-ECD

The determination of PCBs and organochlorine pesticides (OCPs) in sediment samples involves the extraction from the matrix with organic solvents, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection. The samples can be extracted dry or wet. The extracts are then concentrated in a rotary evaporator to about 15 ml, and cleaned for the removal of lipids (whenever present at a significant amount) and the removal of elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation. An adsorption chromatography step (using Florisil columns) could be used to remove interfering lipids and to fractionate the extract into classes of compounds.

To minimize systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment, it is essential that the sources of systematic errors are identified and eliminated as far as possible (HELCOM, 2012c[28]). All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances; their concentrations vary from batch to batch and with supplier. Reagent quality should be checked by injection of 2 µl of a 100 ml batch of solvent, after concentration to 50 µl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 – 250 °C) should be larger than that for 1pg of lindane. Otherwise, the solvent must be distilled.

Quantitative analysis with Electron Capture Detector (ECD) is performed by comparing the detector signal produced by the sample with that of defined standards. Due to incomplete separation, several co-eluting compounds can be present under a single detector signal, therefore, the shape and size of the signal have to be critically examined. The relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases, or by the use of multi-dimensional GC techniques. The GC should be calibrated before each batch of measurements. Since the ECD has a non-linear response curve, a multilevel calibration is strongly advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a PCB which is not present in the sample and which does not interfere with other PCBs. All 2,4,6-substituted PCB congeners are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or the homologues of dichloroalkylbenzylether can be used (HELCOM, 2012c).

For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from sediments using hexane and dichloromethane. Following initial clean-up treatments (removal of sulphur from sediment extracts), extracts are fractionated using column chromatography with an Electron Capture Detector (ECD). It is suggested, when using GC-ECD (and to a certain extent GC-MS), two columns with stationary phases of different polarity should be used, as column-specific co-elution of the target CBs with other CBs or organochlorine compounds occurs.

A protocol for the determination of organochlorine pesticides and polychlorinated biphenyls in sediment samples using CG-ECD prepared by UNEP/IAEA (2011) is presented in Annex XIV. Similar analytical protocols using GC-ECD are also proposed by OSPAR (2018c[29]) (Annex XV) and by HELCOM (2012c) (Annex XVI.).

2.3.2 Protocol for the determination of organochlorine pesticides and PCBs in sediments using GC-MS

The determination of PCBs and organochlorine pesticides (OCPs) in sediment samples involves a similar extraction from the matrix with polar and non-polar organic solvents, followed by clean-up and gas chromatographic separation with mass spectrometric (GC-MS) detection.

Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards, using a mass spectrometer (MS). Often, due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MS detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. The GC should be calibrated before each batch of measurements. Since the MS has a non-linear response curve, a multilevel calibration is advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a PCB which is not present in the sample and which does not interfere with other PCBs. All 2,4,6-substituted PCB congeners are, in principle, suitable. Alternatively,

[28] HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-13 Technical note on the determination of heavy metals and persistent organic compounds in marine sediments. Appendix 2. Technical note on the determination of chlorinated biphenyls in marine sediment

[29] OSPAR (2018c) CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 2: technical annex on the analysis of PCBs in sediments.

1,2,3,4-tetrachloronaphthalene or the homologues of dichloroalkylbenzylether can be used. For GC/MS, 13C-labelled PCBs should preferably be used as internal standards (HELCOM, 2012c).

A method for extraction, concentration, cleanup and fractionation for the determination of organochlorine pesticides and polychlorinated biphenyls in sediment samples is prepared by UNEP/IAEA (2011) (Annex XIV.), including the list of reagents, the solvents, standards and examples for the preparation of the stock, intermediate and working solutions. All reagents, including the distilled water should be of analytical quality. Also, the analysis of PCBs and organochlorinated pesticides can be done by GC-ECD followed by confirmation using GC-MS.

Guidelines for the determination of organochlorine pesticides and polychlorinated biphenyls in sediment samples using CG-MS are also proposed by OSPAR (2018c) (Annex XV) and by HELCOM (2012c) (Annex XVI.).

2.3.3 Protocol for the determination of PAHs in sediments using HPLC

PAHs in the marine environment may derive from combustion processes and from oil and oil products releases. Combustion PAHs are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present.

For the determination of PAHs in sediments, samples are defrosted and prepared for solvent extraction, which can be performed on wet or dried sediment. Wet sediment are Soxhlet extracted in two steps: first, using a polar solvent, such as acetone, to extract the water from the sediment, then the extraction continued with a less polar solvent or solvent mixture (e.g., acetone/hexane). Dry sediments can be Soxhlet extracted using medium-polar solvents such as dichloromethane or toluene, or mixtures of polar and non-polar solvents (OSPAR 2018d[30] Annex XVII, HELCOM 2012d[31] Annex XVIII).

Following extraction, the extract is concentrated and any polar solvents used in the extraction step are removed using a rotary evaporator to a volume of about 15 ml (the temperature of the water bath does not exceed 30 °C). The extract is dried with anhydrous sodium sulfate and transferred in a graduated tube and concentrated down to 4 to 5 ml using a flow of clean nitrogen.Then a clean-up is undertaken with purposes to remove of lipids, whenever present in significant amount; remove elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation. To remove polar interferences from the extract in view of using HPLC-UVF for subsequent analysis, a chromatographic procedures using disactivated aluminum oxide (10 % water) eluted with hexane as well as silica or modified silica columns can be used (HELCOM, 2012d)

Detailed methods for the extraction, clean-up and determination of parent PAHs using High Performance Liquid Chromatography – Fluorescence developed by OSPAR, 2018d) and HELCOM (2012d) are presented in Annex XVII and Annex XVIII, respectively.

2.3.4 Protocol for the determination of PAHs in sediments using GC-MS

For the determination of PAHs in sediments using GC-MS, the extraction, concentration and clean-up procedures are similar to the procedures described for the analysis with HPLC. Solvent extraction can be performed on wet or dried sediment. Wet sediment is Soxhlet extracted in two steps: first, using a polar solvent, such as acetone, to extract the water from the sediment, then the extraction continued with a less polar solvent or solvent mixture (e.g., acetone/hexane). Dry sediments can be Soxhlet extracted using medium-polar solvents such as dichloromethane or toluene, or mixtures of polar and non-polar solvents (OSPAR 2018d, HELCOM 2012d).

When the extraction is completed, the extract is evaporated with a rotary evaporator to a volume of about 15 ml (the temperature of the water bath does not exceed 30 °C). The extract is dried with anhydrous sodium sulfate and transferred in a graduated tube and concentrated down to 4 to 5 ml using a flow of clean nitrogen. For GC-MS analysis sulphur should be removed from the extracts in order to protect the detector. This can be achieved by the addition of copper powder, wire or gauze during or after organic solvent extraction. Ultrasonic treatment might improve the removal of sulphur (OSPAR 2018d, HELCOM, 2012d).

Detailed methods for the extraction, clean-up and determination of PAHs using GC - MS developed by

[30] OSPAR (2018d). CEMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments

[31] HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 1. Technical note on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in sediment

(OSPAR, 2018d) and HELCOM (2012d) are presented in Annex XVII and Annex XVIII respectively.

2.3.5 Protocol for the normalization of organic contaminants using Total Organic Carbon (TOC)

Normalisation is defined as a procedure to adjust contaminant concentrations for the influence of the natural variability in sediment composition, grain size, organic matter and mineralogy. Most natural and anthropogenic substances, metals and organic contaminants, show a much higher affinity to fine particulate matter compared to the coarse fraction Grain size and organic matter are important factors controlling the distribution of natural and anthropogenic components in sediments. Therefore, normalizing contaminant's data for the effects of grain size or organic carbon is used to allow meaningful comparisons of the occurrence of substances in sediments of variable bulk properties (OSPAR, 2018b).

In the European Commission's Guidance Document No: 25 on chemical monitoring of sediment and biota under the Water Framework Directive (EC, 2010), is mentioned that organic contaminants in sediment can be normalized using the total organic carbon (TOC) concentration, because organic matter coatings of fine particles is more effective in bounding lipophilic substances such as chlorinated compounds and PAHs. It is suggested that usually coarser (sand) sediments are less important carriers of lipophilic substances because of their smaller relative surface area. Therefore, it is possible to use the ratio of [concentration of the organic compound]/[TOC] as a normalised value.

In many cases the mobility and partitioning of organic contaminants in the environment can be predicted based on their partitioning into the bulk organic carbon in the sediment, which may be presented using different normalizers (Total Organic Carbon - TOC; Elemental Organic Carbon - EOC; particulate organic carbon - POC; loss-on-ignition - LOI). However, Kestern and Smedes (2002) underline that because organic contaminants may enter the marine environment via different pathways, "the key issue for normalization is thus proper characterization of the Organic Matter by as many parameters as possible. The types of information that can be obtained by the utilization of at least the few key parameters are often complementary and extremely useful, considering the complexity and diversity of Organic Matter encountered in the sediment environment." They also note that "Due to its variability, Organic Matter will occur in both the fine and the coarse sediment fraction. Unlike Al in the case of metals, some Organic Matter in the coarse fraction may contribute to the affinity for organic contaminants as a co-factor as well, albeit of limited environmental significance."

Therefore, the use of TOC as a normalizing factor should be used cautiously and only if field data support the usefulness of normalizing organic contaminants concentrations, as the means to enhance environmental information for pollution assessment. Normalization to TOC is not a mandatory information to be reported to UNEP/MAP and is up to country to decide if it will be done. However, Parties are encouraged to analyse TOC in sediments as an additional information, which could be used in better understanding of the pollution processes in the areas under investigation in the framework of IMAP.

a) TOC analysis with Carbon Analyser

Total Carbon (inorganic and organic) in sediments can be determined with a Carbon Analyser. The sample is injected into a heated reaction chamber packed with an oxidative catalyst (Pt/Al_2O_3) the water is vaporized and both organic and inorganic carbon are oxidized to CO_2, which is measured by means of an Infrared (IR) analyser. Then the inorganic carbon is measured separately, by acidifying the sediment sample with HCl acid at pH <3 and all carbonates are transformed to CO_2 measured in the IR analyser. TOC can be calculated as the difference Total Carbon – Inorganic Carbon. Alternatively, inorganic carbonates are converted to CO_2 with acid, which is removed by purging before the sample injection. The remaining sample contains only the organic carbon fraction of total carbon, which is measured in the IR analyser.

b) TOC analysis with wet oxidation

The wet oxidation technique is the complete oxidation of organic carbon using $K_2Cr_2O_7$ and concentrated H_2SO_4 and the titration of excess dichromate with 0.5N ferrous ammonium sulphate solution to a sharp one drop end point (Schumacher, 2002[32]). The method is based

[32] Schumacher, B.A (2002). Methods for the determination of Total Organic Carbon (TOC) in soils and sediments. EPA/600/R-02/069

on the Walkley and Black (1934[33]) protocol as modified and described by Nelson and Sommers (1996[34]).

0.5 g of dried sediment is placed in a 500 ml Erlenmeyer flask and 10 ml of 1 N $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 are added and mixed for 20 min. The mixture is diluted to 200 ml volume with distilled water and 10 ml of 85% H_3PO_4, 0.2 g NaF and 15 drops of diphenylamine indicator. The solution is back titrated with 0.5 N ferrous solution.

[33] Walkley, A. and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-38

[34] Nelson, D.W and Sommers, L.E. (1996). Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America Book Series no.5, pp. 961-1010.

3 References

- Cook JM, Robinson JJ, Chenery SR and Miles DL (1997). "Determining cadmium in marine sediments by inductively coupled plasma mass spectrometry: attacking the problems or the problems with the attack?" Analyst, 122, 1207-1210
- Delft W. van; Vos. G. (1988) Comparison of digestion procedures for the determination of mercury in soils by cold-vapor atomic absorption Spectrometry; Analytica Chimica Acta, 209, 147-156
- EC (2010). Guidance Document No: 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive
- IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)
- IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry
- IAEA (2011c). Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace
- IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry
- IAEA (2012b). Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry
- ICES/OSPAR (2018). CEMP Guidelines for Monitoring Contaminants in Sediments
- HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-13 Appendix 3.: Technical note on the determination of heavy metals and persistent organic compounds in marine sediment
- HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-13 Appendix 3: Technical note on the determination of heavy metals and persistent organic compounds in marine sediment
- HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy
- HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-13 Technical note on the determination of heavy metals and persistent organic compounds in marine sediments. Appendix 2. Technical note on the determination of chlorinated biphenyls in marine sediment
- HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 1. Technical note on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in sediment
- Kestern, M. and Smedes, F. (2002). Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. J. Environ. Monit. 4, 109-115
- Loring, DH (1991). Normalization of heavy metal data from estuarine and coastal sediments. ICES J. Mar. Sci. 48, 101-115
- Loring, DH and Rantala RTT (1991). Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Review, 32: 235:283. Elsevier Science Publishers B.V
- Nelson, D.W and Sommers, L.E. (1996). Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America Book Series no.5, pp. 961-1010
- OSPAR (2018a). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods
- OSPAR (2018b). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalisation of contaminant concentrations in sediments
- OSPAR (2018c) CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 2: technical annex on the analysis of PCBs in sediments
- OSPAR (2018d). CEMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments
- Schumacher, B.A (2002). Methods for the determination of Total Organic Carbon (TOC) in soils and sediments. EPA/600/R-02/069
- UNEP/IAEA (2011d). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71

- UNEP/MAP (1999). MED POL Phase III. Programme for the assessment and control of pollution in the Mediterranean Region
- UNEP/MAP (2011). UNEP(DEPI)MED WG.365/Inf.9. Manual on sediment sampling and analysis
- UNEP/MAP (2019). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27
- UNEP/MAP (2019a). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution
- US EPA (1996). Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices
- US EPA (2007a). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf
- US EPA (2007b). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf
- USEPA (2014 revision) Method 6020B, ICP-MS. Environmental protection Agency, Washington, DC.
- Walkley, A. and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-38

B-2. Monitoring Guidelines/Protocols for sampling and determination of contaminants in marine biota

Table of Contents

1 Guidelines for sampling and sample preservation of marine biota

1.1 Introduction

Heavy metals and organic contaminants are entering the Mediterranean marine environment discharged from land-based and sea-based pollution sources, as well as from atmospheric deposition. The UNEP/MAP Integrated Monitoring and Assessment Programme (IMAP) (UNEP/MAP, 2019a[1]; UNEP/MAP (2019b[2]) includes the analysis of specific sedentary marine sentinel organisms (bivalves and benthic feeding fish) in order to assess pollution impact on the marine organisms. The suggested species for monitoring contaminants are a benthic feeding fish (e.g. *Mullus barbatus*) and bivalves (e.g. *Mytilus galloprovincialis*, *Donax trunculus*). However, in case different species of fish and bivalves are used by the Contracting Parties to the Barcelona Convention for assessing marine pollution, explanation has to be provided to UNEP/MAP Secretariat on the reason behind the selection of a different sentinel species for CI17 monitoring.

Standardize protocols for sampling and processing of marine biota samples is important in view of assuring comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes. Also, sampling protocols provide guidance on the suitability of selected sampling sites, the number of required samples, the biometric indices to be recorded, the appropriate handling to avoid cross-contamination, and the storage conditions in view of maintaining the sample's integrity during the transfer from the sampling site to the analytical laboratory. Furthermore, protocols are providing guidance on the procedures to dissect the organisms (fish and bivalves) in order to collect the appropriate tissue for analysis (muscle and liver of fish and whole body of bivalves), taking care to avoid cross-contamination by metals or organic contaminants, depending on the foreseen analysis.

The Protocols on of this Guidelines, as provided here-below aim at streamlining sampling and processing of marine biota samples in view of assuring comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes. They also provide the guidance on the suitability of selected sampling sites, the number of required samples, the biometric indices to be recorded, the appropriate handling to avoid cross-contamination, and the storage conditions in view of maintaining the sample's integrity during the transfer from the sampling site to the analytical laboratory to ensure the representativeness and the integrity of the samples. Furthermore, they guide on the procedures to dissect the organisms (fish and bivalves) in order to collect the appropriate tissue for analysis (muscle and liver of fish and whole body of bivalves), taking care on a need to avoid cross-contamination by metals or organic contaminants, depending on the foreseen analysis. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent laboratories participating in IMAP implementation. Namely, the six here-below elaborated IMAP Protocols build on previous UNEP/MAP - IAEA Recommended Methods, such as Reference Methods No 6 on sampling of selected marine organisms and sample preparation for trace metal analysis (UNEP/FAO/IOC/IAEA, 1987, Annex XIX) and Reference Methods No 7 (Rev. 2) on sampling and dissecting marine organisms (UNEP/FAO/IOC/IAEA, 1988, Annex XX), which were prepared in the framework of the MED POL monitoring programme. IMAP Protocols are also streamlined with similar Guidelines/Protocols for marine biota sampling, sample processing and preservation, which were developed by other Regional Seas Organisations, such as HELCOM (2012[3]) (Annex XXI) and ICES/OSPAR (2018[4]) (Annex XXII) as well as the European Commission's guidance documents (EC 2010[5] and 2014[6]). Given the suitability of any of these Guidelines in the context of IMAP, they could be further used by interested IMAP competent Mediterranean laboratories for developing their laboratory specific sampling and sample processing methodologies.

1.2 Technical note for the sampling of marine biota for the determination of heavy metals and organic contaminants

Sampling is a very important step in the analysis of marine biota, since it affects the representatives of the sample, which is the basis of every Quality Assurance scheme. The fish and bivalves collected should reflect the condition of other organisms of the same species in the marine area under consideration. The sampling location and conditions (including seafloor nature,

[1] UNEP/MAP (2019a). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27

[2] UNEP/MAP (2019b). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution

[3] HELCOM (2012). Annex B-12, Appendix 1. Technical note on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements

[4] ICES/OSPAR (2018). CEMP Guidelines for Monitoring Contaminants in Biota

[5] EC (2010). Guidance Document No: 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive

[6] EC (2014). Guidance Document No: 32 Guidance on biota monitoring under the Water Framework Directive

sampling depth, location of pollution sources) have to be chosen carefully, taking into consideration other oceanographic data (such as temperature, turbidity, trophic level) in the sampling area. The handling of biota after collection is also of primary importance, in order to follow appropriate procedures to avoid cross contamination of the samples from the ship's environment and the storage of samples. Also, the appropriate preservation of samples during transportation from the sampling site to the laboratory for further analysis is crucial, in order to avoid the deterioration of the biota tissues that may result in loss of determinant or contamination from the packaging materials. Finally, once the biota samples arrive at the laboratory, additional processing is required to dry and homogenize the samples and to store the dried samples in appropriate conditions in order to avoid any alteration of the contaminants' concentrations in the samples.

Under this Technical Note, this Guidelines for Sampling and Sample Preservation of Marine Biota for IMAP Common Indicator 17 provides the following Protocols:

- Protocol for the collection of fish for heavy metal and organic contaminants determination;
- Protocol for the collection of bivalves for heavy metal and organic contaminants determination;
- Protocol for the dissection of fish to collect muscle and liver;
- Protocol for the dissection of bivalves.

1.2.1 Protocol for the collection of fish for heavy metal and organic contaminants determination

The most common fish species used for marine pollution monitoring in the Mediterranean region is the mullet (*Mullus barbatus*) (UNEP, 2019b). However, in different areas, according to local conditions, other benthic fish may be used for monitoring contaminants. A list of available reference species (Code list) for Data Dictionaries and Data Standards of the IMAP (Pilot) Info System for E09 (CI17 and CI20) is presented in the document UNEP/MED WG.467/8 (UNEP/MAP, 2019c[7]).

For fish sampling, in line with the IMAP Monitoring Protocols for CI17 (UNEP, 2019b), 3-5 parallel composite samples (5-6 specimen for each fish sample) are collected from the same size class at each site. During the initial phase of the IMAP (identification of key sampling sites/stations) fish sampling should be done every 4 years and bivalves sampling yearly, while during the advanced phase (when it is a fully completed MED POL Phase IV implementation with the ongoing reporting of data sets) biota sampling should be done every 1 to 3 years, according the trends and levels assessed at the different stations/sites (UNEP, 2019a). EU requests Member States to determine the frequency of monitoring in sediment and/or biota so as to provide sufficient data for a reliable long-term trend analysis (2008/105/EC[8]). As a guideline, the Directive suggests a monitoring frequency of three years for sediment and biota, unless technical knowledge and expert judgment justify another interval.

Fish having a length of 12-16 cm should be included if possible in the selected size classes, to be in line with the Protocol for fish collection for the CI18. Fish can be collected by gill net fishing or trawling using a square-meshed net of 40 mm or, if justified, by a diamond meshed net of 50 mm as required by the EU legislation (EC 1967/2006[9]). Guidelines for collection of fish are presented in UNEP/FAO/IOC/IAEA (1987) (Annex XIX) and UNEP/FAO/IOC/IAEA (1988) (Annex XX). Fish could be sampled from a research vessel or from a small fishing boat. Guidelines on sampling and processing of fish samples are also provided by HELCOM (2012) (Annex XXI) and ICES/OSPAR (2018) (Annex XXII).

It has to be underlined that concentrations of chemical pollutants in marine biota tissues can be influenced by many environmental factors (such as seasonal fluctuations of temperature, organic matter, nutrients) and biological factors (such as the phase of reproductive cycle, weight fluctuations, changes in relative tissue composition, the massive development of gonadic tissues during gametogenesis and the loss of weight during spawning). In order to avoid such variations, it is recommended that sampling take place in the off-spawning period (EC, 2010). Also, in order to evaluate the influence of common biological and environmental factors it is suggested to record the date, seawater temperature, salinity, phytoplankton development, at sampling time.

Fish samples should be protected from contamination, which may occur during sampling, sample handling, storage and transfer to the laboratory for further analysis. In case fish are dissected on board, the work must be carried out by personnel capable of identifying and removing the desired organs according to the requirements of the investigation. Fish samples have to be handled with care to avoid any contact with metals (for heavy metal analysis) or possible sources of organic contaminants (for chlorinated hydrocarbons and PAHs analysis). Detailed procedures for fish dissection and the measures to be taken in order to avoid sample contamination during handling, are presented in Protocol for the fish dissection to collect muscle and

[7] UNEP/MAP (2019c) UNEP/MED WG.467/8. Data Standards and Data Dictionaries for Common Indicators related to pollution and marine litter.

[8] EC Directive 2008/105/EC (2008) on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council

[9] EC Council Regulation No 1967/2006 concerning management measures for the sustainable exploitation of fisheries resources in the Mediterranean Sea

liver. Upon fish collection additional information on length, wet weight and sex should be recorded. In case of pooling, number of specimens and length range should also be recorded.

In case fish samples have to be transported to the laboratory for dissection, they have to be handled and stored in such a way, as to avoid sample deterioration or contamination. A ship has several potential metal contamination sources (metallic hull and superstructures, paint). To prevent metal contamination fish samples intended for heavy metal analysis should be handled in metal-free areas (working surfaces with plastic coatings or cover) and stored in plastic bags for transport to the laboratory. Regarding PAHs and chlorinated hydrocarbons, possible contamination sources in a ship include fuel and lubrication, as well as exhaust from the ship's engines. Fish samples intended for organic contaminants analysis have to be stored in metal containers for their transport to a stainless steel of aluminium clean working surface in the ship's laboratory. Before starting the handling of fish samples, it is important to identify possible contamination sources in the ship and the samples handling area, in order to take appropriate measures to avoid contamination.

In case fish transport to the laboratory is done in less than 24 hours, samples can be stored on ice. However, for longer periods, fish samples have to be frozen (-20 ^{o}C) and transported frozen to the laboratory for further processing. Each sample should be labelled with the sample's identification number, the type of tissue, and the date and location of sampling.

1.2.2 Protocol for the collection of bivalves for heavy metal and organic contaminants determination

Mytilus galloprovincialis and *Donnax trunculus* are the bivalve species suggested to be analysed for heavy metals and organic contaminants in the framework of CI17 (UNEP/MAP, 2019a). If the Contracting Party decides to analyse other bivalve species, it has to provide UNEP/MAP the rationale behind its decision. To facilitate reporting a list of available reference species (Code list) is provided in the document UNEP/MED WG.467/8 (UNEP/MAP, 2019c).

In line with the IMAP Monitoring Protocols for CI17 (UNEP, 2019b), 3-5 parallel composite samples of bivalves (10 specimens for each bivalves sample) are collected yearly from the same size class at each trend monitoring. Minimum bivalves sampling is once per year, although twice per year may be applied if possible to be in line with CI18 sampling frequency. The most adequate sampling period is during the post winter months, but before the spawning period. Usually, in most Mediterranean coastal areas, April-June is an appropriate sampling period, but local climatic characteristics have to be taken into consideration for the fixing of the sampling period.

The bivalves' size to be collected should be 4-5 cm, to be in line with the sampling protocol for CI18. However, a length-stratified sampling could be applied, which is generating data that can also be used in monitoring programmes for temporal trends of contaminants in biota (HELCOM, 2012). The HELCOM methodology requires that at least 20 mussels in the largest length interval can easily be found and the length stratification should be determined in such a way that it can be maintained over many years for the purposes of temporal trend monitoring. It is also requiring that the length interval shall be at least 5 mm in size. The length range should be split into at least three length intervals (small, medium, and large) which are of equal size after log transformation and the number of specimens selected for analysis depends on their length, e.g. 80-100 individuals are necessary to suffice material within the length range 4-5 cm (HELCOM, 2012).

Bivalves sampling sites should host an abundant population of the targeted species in order to take appropriate size of sample and to be reasonably accessible in order to easily and rapidly transport biota samples to the laboratory. Bivalves growing on metal structures (i.e. underwater pipes) or substrates, which may be enriched in metals or organic contaminants, should be exempted from collection. Divers will collect manually the mussels living at a 4-5 m under the water surface. Mussel byssus threads should be cut from the substrate, since pulling the animals from the rocks (threading) can result in damage to internal tissues. Using mussels living at the water/air interface, the physical contamination by lipophilic contaminants present on the water surface may alter the evaluation of the chemical's content in mussel soft tissues.

Detailed guidelines for bivalves' collection and samples processing for the determination of contaminants, are presented in the recommended methods developed by UNEP/FAO/IOC/IAEA (1987) (Annex XIX) and UNEP/FAO/IOC/IAEA (1988) (Annex XX). Also, similar guidelines are published by HELCOM (2012) (Annex XXI) and OSPAR (2018) (Annex XXII).

In places were no wild bivalves populations are found, caged bivalves can be used as an alternative option for monitoring (UNEP, 2019b). Adult mussels (4-5 cm) are collected from a mussel farm, transported to the marine area under investigation and re-immersed for 10 days to permit them to re-cluster and reduce mortality risk during transplantation at the sampling site. Then cages with mussels are transported to the sampling site, where cages are suspended at 6m to 8m from the sea surface, anchored at the bottom with a 30 kg ballast, and exposed for 12 weeks. During recovery of cages, the biometric parameters shell height and wet weight (w.w.) of soft tissues are measured at least in 15 mussels per each cage. Details on the protocol for using caged bivalves in monitoring heavy metals and organic contaminants in

the marine environment are presented in Galgani et al. (2011)[10] and Galgani et al (2014)[11].

The undamaged bivalves are transported to the laboratory moist and alive in appropriate closed containers to avoid contamination (i.e. plastic containers for organisms to be analysed for heavy metals and metals containers for organisms to be analysed for chlorinated hydrocarbons and PAHs), at temperatures between 5 °C and 15 °C (24 hours is the maximum transport time in these conditions). Bivalves should be kept moist using clean seawater from the sampling site without submerging them. For a transportation time of more than 24 hours, bivalves should be placed in appropriate container and frozen. Frozen, samples can be stored in a deep freezer at temperatures of -20ºC. Each sample should be labelled with the sample's identification number, the type of tissue, and the date and location of sampling.

1.2.3 Protocol for the dissection of fish to collect muscle

a) Dissection

Muscle tissues of fish has to be dissected while the organism is in good condition, otherwise the decay of the tissues will affect the concentration of contaminants. Therefore, it is preferable to dissect collected fish on board, by experienced personnel able to perform the dissection and remove the fish tissues to be analysed (muscle and liver). The on-board dissection should be done in a clean area free from possible contamination of the sample by metals or organic contaminants respectively. If no on-board dissection capability is available (because of lack of experienced personnel and/or lack of adequate clean dissection area), collected fish should be transferred to the laboratory taking care to prevent tissue decay. If the laboratory is reachable within 24 hours, fish could be preserved on ice during the transfer. For longer periods, fish should be frozen immediately and transferred frozen to the laboratory, where they will be thawed before dissection.

Detailed guidelines for the dissection of fish and collection of samples for the determination of contaminants are presented in the UNEP/FAO/IOC/IAEA Reference Method No 6 (1987) (Annex XIX) and UNEP/FAO/IOC/IAEA Reference Method No 7 (1988) (Annex XX).

HELCOM (2012) and OSPAR (2018) propose a similar procedure for fish dissection and removal of muscle for further analysis. The method requires the removal of the epidermis and the collection of a sample from the right side dorso-lateral muscle in order to ensure uniformity of samples (Figure 1). It is also suggested to take the entire right dorsal lateral filet as a uniform sample, from which subsamples can be taken after homogenizing for replicate dry weight and contaminant determinations. If the amount of material obtained by this procedure is too large to be easily handled, a specific portion of the dorsal musculature should be chosen for the sample. It is recommended that the portion of the muscle lying directly under the first dorsal fin should be utilized in this case. It is important to obtain the same portion of the muscle tissue for each sample, because both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish.

In case fish samples are frozen for their transfer from the field to the laboratory, they have to rest until thawed. It is often suggested that the dissection of fish is easiest when the material, at least the surface layers of the muscle tissue, is half frozen. Extreme care has to be demonstrated during dissection because any loss of liquid or fat due to improper cutting or handling of the tissue makes the determinations of dry weight and fat content less accurate, which is also affecting the accuracy of the reported contaminants' concentrations.

In all cases fish dissections should be undertaken by trained personnel.

[10] Galgani, F., Martínez-Gómez, C., Giovanardi, F., Romanelli, G., Caixach, J., Cento, A., Scarpato, A., BenBrahim, S., Messaoudi, S., Deudero, S., Boulahdid, M., Benedicto, J., Andral, B. (2011). Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (Mytilus galloprovincialis) from the western basin of the Mediterranean Sea. Environ. Monit. Assess. 172 (1–4), 301–317. https://doi.org/10.1007/s10661-010-1335-5.

[11] Galgani, F., Chiffoleau, J.F., Barrah, M., Drebika, U., Tomasino, C., Andral, B. (2014). Assessment of heavy metal and organic contaminants levels along the Libyan coast using transplanted mussels (Mytilus galloprovincialis). Environ. Sci. Pollut. Res. 21, 11331–11339. https://doi.org/10.1007/s11356-014-3079-1.

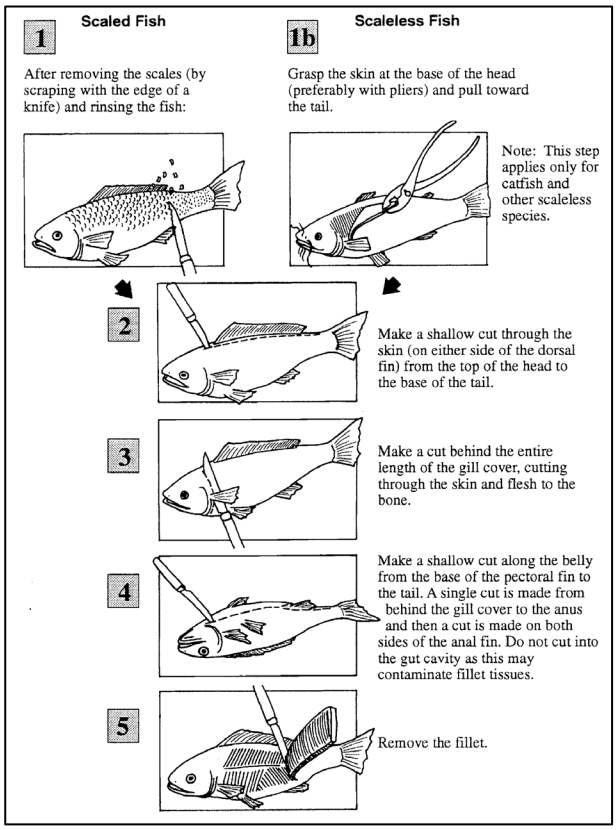

Figure 1. Fish filleting procedure (from US EPA, 2000[12])

In case liver tissue is sampled for analysis (not a mandatory tissue in the framework of IMAP), HELCOM guidelines underline that "the liver must be identified in the presence of other organs such as the digestive system or gonads. After opening the body cavity with a scalpel, the connective tissue around the liver should be cut away and as much as possible of the liver is cut out in a single piece together with the gall bladder. The bile duct is then carefully clamped and the gall bladder dissected away from the liver."

b) Avoiding contamination

For metal determination, handling of fish should be made on a metal-free bench, using plastic knives and tweezers for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

For organic contaminants determination, handling of fish should be made on a metallic (stainless steel or aluminium) bench, using stainless steel knives and tweezers for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

After the removal of a tissue sample from a fish, the tools have to be cleaned before being used to remove another organ (i.e. liver) of the same individual or being used on a different individual.

HELCOM (2012) recommends the following procedures for cleaning tools used for preparing samples:

For analysis of heavy metals, tools should be:

i) Washed in acetone or alcohol and high purity water.

ii) Washed in HNO_3 diluted (1+1) with high purity water. Tweezers and haemostates should be washed in diluted (1+6) acid.

iii) Rinsed with high purity water.

For analysis of organochlorine pesticides

i) Washed in acetone or alcohol and rinse in high purity water.

The glass plate used during dissection should be cleaned in the same manner. The tools must be stored in a dust-free area when not in use. Also, the dissection room should be kept clean and the air should be free from particles. If clean benches are not available on board the ship, the dissection of fish should be carried out in the land-based laboratory under conditions of maximum protection against contamination.

1.2.4 Protocol for the dissection of bivalves

a) Depuration

Collected bivalves should be left to void the gut contents and any associated contaminants before freezing or sample preparation, because gut contents may contain significant quantities of contaminants associated with food and sediment particles which are not truly assimilated into the tissues of the mussels (HELCOM, 2012). Bivalve's depuration over a period of 24 hours is usually sufficient and should be undertaken under controlled conditions and in filtered sea water in the laboratory. The aquarium should be aerated, and the temperature and salinity of the water should be similar to that from which the animals were removed.

b) Bivalve dissection

According to the UNEP (2019b) UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution, the whole soft tissue of bivalves has to be collected for analysis. Detailed guidelines for the dissection of fish and collection of samples for further analysis is presented in the UNEP/FAO/IOC/IAEA Reference Method No 6 (1987) (Annex XIX) and UNEP/FAO/IOC/IAEA Reference Method No 7 (1988) (Annex XX). Guidelines for sampling and processing of bivalves is also prepared by

[12]US EPA (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories Volume 1 Fish Sampling and Analysis. Third Edition.

HELCOM (2012) (Annex XXI) and OSPAR (2018) (Annex XXII).

In general, foreign materials attached to the outer surface of the shell have to be removed using a clean plastic/stainless steel knife and a strong plastic/metal brush. Handle the mussels as little as possible. Rinse each mussel with clean seawater and let the water drain off. Then pull out the byssus which extrudes from between the closed shells on the concave side of the shells; weigh the whole mussel and note the weight.

For removing the soft tissue for further analysis, bivalves should be shucked live and opened with minimal tissue damage. Insert a clean plastic/stainless steel knife into the opening from which the byssus extrudes and cut the adductor muscles. Avoid forcing the mussel to open, if the abductor muscle is cut, the bivalve will open easily (Figure 2). Rinse the soft part of the mussel in its shells with clean seawater. The soft tissues should be removed and homogenized as soon as possible, frozen and kept in plastic containers (for metal analysis) or in metal containers at -20°C until analysis. Homogenization can be done using stainless steel blades (for organic contaminants analysis) or using an agate mortar, following the drying of the sample.

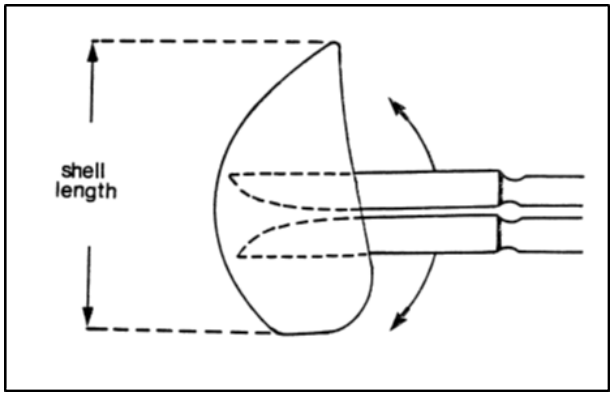

Figure 2. Cutting the abductor muscle

For metal determination, the handling of bivalves should be made on a metal-free bench, using plastic knives and tweezers for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned with a tissue and rinsed with clean water.

For organic contaminants determination, the handling of bivalves should be made on a metallic (stainless steel or aluminium) bench, using stainless steel knives and tweezers for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned with tissue and rinsed with solvent

In all cases bivalve dissection should be undertaken by trained personnel.

1.3 Technical note for the sample preservation of marine biota for the determination of heavy metals and organic contaminants

Once the biota samples arrive at the laboratory, additional processing is required to dry and homogenize the samples and to store the dried samples in appropriate conditions. During the processing of the samples it is important to avoid any cross contamination (metal or organic contaminants) from the equipment and the containers used to store the dried samples. Analysis may be performed at a later stage, it is therefore important to avoid any alteration of the contaminants' concentrations in the samples during storage.

Under the Technical Note, this Guidelines for Sampling and Sample Preservation of Marine Biota for IMAP Common Indicator 17 provides the following Protocols:

- Protocol for the treatment of biota samples prior to determination of heavy metals;
- Protocol for the treatment of biota samples prior to determination of organic contaminants.

1.3.1 Protocol for the treatment of biota samples prior to determination of heavy metals

a. Storage of wet samples on board

Upon collection wet samples have to be stored on board in such a way as to preserve them from deterioration that will affect the subsequent analysis of contaminants. When the fish transport to the laboratory is done in less than 24 hours, samples can be stored on ice. However, for longer periods, fish samples have to be frozen (-20 °C) and transported frozen to the laboratory for further processing. Each sample should be labelled with the sample's identification number, the type of tissue (if already dissected), and the date and location of sampling.

b) Drying of biota tissues

Drying biota tissues is a procedure to establish the dry/wet weight (dw/ww) ratio of the tissues, in order to express metal concentrations accordingly enabling comparisons between different data sets. Dried biota tissues can then be digested for heavy metal analysis. For metal (except volatile mercury) analysis, biota freeze-drying is the preferable procedure. Alternatively, the biota tissues may be dried at any temperature below 105°C until constant weight. For mercury analysis, to minimise losses due to evaporation, a biota tissue sub sample could be air dried at temperature <50°C (EC, 2010).

Frozen biota samples are placed in clean wide-mouth glass or plastic containers suitable for freeze-drying and are freeze-dried for 24 hours taking care to protect them from cross-contamination from particles and vapours. A possible way to protect samples from contamination is

to cover the sample containers with a filter paper perforated with a small hole (HELCOM, 2012). Then the containers with the samples are weighted and freeze-dried again for another 24 hours and weighted. If the difference between the 2 weighing is less than 0.5%, drying is completed and the dw/ww ratio can be calculated. Otherwise the drying cycle can be repeated (24 hours) until the difference between successive weighing is less than 0.5%.

Freeze dried biota tissues are then grinded and homogenized using a metal-free ball mill.

Guidelines for processing biota samples for metal determination are provided by OSPAR (2018) and HELCOM (2012).

c) Storage of dried biota tissues

Freeze-dried tissue samples can be stored in pre-cleaned wide-mouth bottles with a screw cap. Samples intended for the analysis of metals can be stored in plastic or glass containers. For mercury analysis, samples must be stored in acid-washed borosilicate glass or quartz containers, as mercury can move through the walls of plastic containers (EC, 2010).

Containers with biota tissue samples should be archived and kept in storage after the completion of the analysis, in order to be used as a replicate sample in case crosschecking of the results are required or additional determinations are needed in the future. Freeze-dried biota tissues remaining after analyses could be stored in the original sample bottle, closed with an airtight lid to protect against moisture and stored in a cool, dark place. Under these conditions, samples may be archived and stored for 10-15 years. (EC, 2010).

1.3.2 Protocol for the treatment of biota samples prior to determination of organic contaminants

a) Storage of wet samples on board

Upon collection wet samples have to be stored on board in such a way as to preserve them from deterioration that will affect the subsequent analysis of contaminants. When the fish transport to the laboratory is done in less than 24 hours, samples can be stored on ice. However, for longer periods, fish samples have to be frozen (-20 ^{o}C) and transported frozen to the laboratory for further processing. Each sample should be labelled with the sample's identification number, the type of tissue, and the date and location of sampling.

b) Drying of biota tissues

For organic contaminants analysis drying procedures depends on the compounds to be analysed. For chlorinated hydrocarbons biota can be freeze-dried taking care to avoid determinant loss through evaporation by keeping the temperature in the evaporation chamber below 0°C. (OSPAR, 2018). For PAH determination, freeze-drying of biota tissues may be a source of contamination due to the back streaming of oil vapours from the rotary vacuum pumps. Furthermore, drying may result in losses of the lower molecular weight, more volatile PAHs through evaporation. A possible way to protect samples from contamination is to cover the sample containers with a filter paper perforated with a small hole (HELCOM, 2012). Frozen biota samples are placed in clean wide-mouth glass containers suitable for freeze-drying and are freeze-dried for 24 hours taking care to protect them from cross-contamination from particles and vapors. Then the containers with the samples are weighted and freeze-dried again for another 24 hours and weighted. If the difference between the 2 weighing is less than 0.5%, drying is completed and the dw/ww ratio can be calculated. Otherwise the drying cycle can be repeated (24 hours) until the difference between successive weighing is less than 0.5%.

Freeze dried biota tissues are then grinded and homogenized using a plastic-free ball mill.

c) Storage of dried biota tissues

Freeze-dried tissue samples can be stored in pre-cleaned wide-mouth bottles with a screw cap. Samples intended for the analysis of organic contaminants should be stored in glass containers.

Containers with biota tissue samples should be archived and kept in storage after the completion of the analysis, in order to be used as a replicate sample in case crosschecking of the results are required or additional determinations are needed in the future. Freeze-dried biota tissues remaining after analyses could be stored in the original sample bottle, closed with an airtight lid to protect against moisture and stored in a cool, dark place. Under these conditions, samples may be archived and store stored for 10-15 years. (EC, 2010).

2 Guidelines for the determination of contaminants in marine biota

2.1 Introduction

Marine biota uptake contaminants from the marine environment through food and the water medium (breathing, skin exchange). Depending on their physicochemical properties and the organism's metabolism, contaminants may be bioaccumulated in the organism's body and, in some cases (such as Hg and persistent organic pollutants), they may be biomagnified in the top levels of the marine food chain. Since the establishment of the UNEP/MAP – MED POL Monitoring programme in 1981 (MED POL Phase II), the benthic fish *Mullus barbatus* and the bivalve *Mytilus galloprovincialis* have been used as sentinel species to assess the accumulation of contaminants in marine organisms of the Mediterranean Sea. In the framework of the Integrated Monitoring and Assessment Programme (IMAP) Common Indicator 17 (CI17), the same organisms are recommended for analysis, namely the benthic fish *Mullus barbatus* (muscle tissue) and the

bivalves *Mytilus galloprovincialis* or *Donnax trunculus* (whole body). Parties may decide to include in their national monitoring programmes the collection and analysis of additional species of national interest. In all cases, contaminants data, along with relevant metadata, have to be reported to the UNEP/MAP Secretariat using the appropriate format.

Both organisms are encountered in the coastal marine environment and are absent in the offshore marine areas. Therefore, they cannot be used as sentinel organisms to assess the impact of contaminants in the offshore marine environment. For such offshore areas, appropriate sentinel species to be used for pollution assessment, will be designated by Parties at a later stage.

In line with IMAP requirements (UNEP 2019a, UNEP 2019b), mandatory contaminants to be determined in the muscle of fish and the whole body of bivalves include: heavy metals (Cadmium (Cd), Lead (Pb) and Mercury (Hg)), organochlorinated compounds (PCBs, hexachlorobenzene, lindane and ΣDDTs) and Polycyclic Aromatic Hydrocarbons (US EPA 16 Reference PAHs compounds). Also, additional parameters should be measured, such as: length, sex, and total wet weight of organism, as well as lipid content of the tissue to be analysed.

Heavy metals and organic contaminants are encountered in marine biota at trace levels (ng/kg - mg/kg); therefore it is of paramount importance to avoid cross contamination from the laboratory environment (dust particles and the analyst), from sample containers or packing materials, from instruments used during sample pre-treatment and sample preparation, and from the chemical reagents used for analysis. Accordingly, sample handling and analysis should be made in a clean laboratory, to eliminate cross contamination and to control the total analytical blank. To that purpose, if the laboratory is not specifically designed as “clean lab” (class 100 US Federal Standard 209), it has to be equipped with appropriate laminar flow rooms, clean benches, and fume hoods, specifically designed for trace metal analysis.

The UNEP/MAP Proposed assessment criteria (Background Assessment Criteria - BAC and Environmental Assessment Criteria - EAC) for targeted heavy metals and organic contaminants in fish and bivalves are presented in the Annex V.

The Protocols on of this Guidelines, as provided here-below, aim at streamlining marine biota sample preparation and analysis for heavy metals and organic contaminants, including step-by-step guidance on the methods to be applied in the Mediterranean area for sample preparation and analysis of marine biota tissues for the determination of heavy metals and organic contaminants, in a view of assuring comparable quality assurance of the data, as well as comparability between sampling areas in different national monitoring programmes. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties’ competent laboratories participating in IMAP implementation. Namely, here-below elaborated IMAP Protocols build on previous UNEP/MAP - IAEA Recommended Methods, for the analysis of heavy metals and organic contaminants, such as: IAEA (2011a) IAEA (2011) Recommended method on microwave digestion of marine samples for the determination of trace element content (Annex VI); IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry (Annex VIII); IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace (Annex IX); IAEA (2012a) Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry (Annex XI); IAEA (2012b) Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry (Annex XII); UNEP/IAEA (2011d) Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment: Reference Methods for Marine Pollution Studies No 71 (Annex XIV);, which were prepared in the framework of the MED POL monitoring programme. They are also streamlined with similar Guidelines/Protocols for marine biota sample preparation and analysis, which were developed by other Regional Seas Organisations, such as OSPAR (Annexes XXVII and XXIX) and HECLOM (Annexes XXIII, XXV, XXVI, XXVIII). The analytical method developed by US EPA is also considered (Annex XXIV). Given the suitability of any of these Guidelines in the context of IMAP, they can be further used by competent Mediterranean laboratories for developing their lab-specific sampling and sampling processing methodologies. The Parties’ laboratories should accommodate and always test and modify each step of the procedures to validate their results.

2.2 Technical note for the determination of heavy metals in marine biota

Analysis of marine biota samples for the determination of heavy metals include: i) digestion of biota tissues and ii) analysis of the digested sample for heavy metals.

National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of Mediterranean Parties, a non-exclusive list of Protocols has been drafted to be used as guidelines for the analysis of heavy metals in marine biota samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

Regardless of the analytical method used, heavy metal analysis follows some procedures common to all analytical methodologies, such as the calibration of the analytical equipment and the cleaning and handling procedures to avoid the contamination of the samples from the laboratory's environment and the tools and containers used in the analysis.

a) Calibration

Calibration standards prepared from single standard stock solutions or multielement standards, by dilution of the stock solution using dilute acid, as required. All standard solutions have to be stored in polyethylene, borosilicate or quartz volumetric flasks, depending on the best suitability for the respective analytes. Standard solutions with lower concentrations, if prepared correctly and controlled in a QA system (checking of old versus new, and checking with standards from a different source), can be kept for a period no longer than one month.

The calibration procedure has to meet some basic criteria in order to give the best estimate of the true element concentration of the sample analysed (HELCOM, 2012a[13]):

i) The concentrations of standards for the preparation of the calibration curve should cover the range of anticipated concentrations;

ii) The required analytical precision should be known and achievable throughout the entire range of concentrations;

iii) The measured value at the lower end of the range has to be significantly different from the procedural analytical blank;

iv) The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation;

v) The analytical instruments should be recalibrated regularly (every 10-20 samples) to correct for instrumental drift and analytical efficiency.

b) Avoiding contamination

To avoid metal contamination in the laboratory all glassware and plastic vessels used should be carefully cleaned. The general cleaning guidelines include:

i) The vessels are allowed to soak overnight in a plastic container in an alkaline surfactant solution (e.g. Micro solution 2% in tap or even better distilled water).

ii) Vessels are rinsed thoroughly first with tap or even better distilled water then with ultrapure deionised water (18 MΩ cm,).

iii) Vessels are left to stand in 10% (v/v) concentrated HNO_3 solution (analytical grade) at room temperature for at least 6 days.

iv) Vessels are thoroughly rinsed with ultrapure deionised water (at least 4 times).

v) Vessels are allowed to dry under a laminar flow hood.

vi) Vessels are stored in closed plastic polyethylene bags (e.g. zip-lock variety) to prevent the risk of contamination prior to use.

This procedure should be used for all plastic ware use in the laboratory as tips, cup for auto-sampler, plastic containers.

Under this Technical Note, this Guidelines for sample preparation and analysis of marine biota for IMAP Common Indicator 17 provides the following IMAP Protocols for the determination of heavy metals in marine biota samples:

- Protocol for biota tissues digestion using nitric acid (microwave assisted digestion in closed systems and digestion on hot plate);
- Protocol for the determination of heavy metals with Flame Atomic Absorption Spectroscopy (F-AAS);
- Protocol for the determination of heavy metals with Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS);
- Protocol for the determination of heavy metals with Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS);
- Protocol for the determination of Total Hg by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry;

[13] HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota.

- Protocol for the determination of Total Hg with Cold Vapour Atomic Absorption Spectrometry (CV-AAS).

These Protocols are based on Analytical Methods developed by IAEA (Annex VI: IAEA (2011a) Recommended method on microwave digestion of marine samples for the determination of trace element content; Annex VIII: IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry; Annex IX: IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace; Annex XI: IAEA (2012a) Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry (AAS); Annex XII: Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry), HELCOM (Annex XXIII: Manual for marine monitoring in the COMBINE programme: Technical note on the determination of trace metallic elements in biota; Annex XXV: COMBINE Programme: Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy) and US EPA (Annex XXIV: US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry).

2.2.1 Protocol for biota tissues digestion using nitric acid

Biota tissues samples have to be digested (wet ashing) prior to analysis. The rate of digestion and the efficiency of acid decomposition increase substantially with elevated temperatures and pressure, therefore microwave digestion in closed vessels is the preferred method. However, in case no such equipment is available, sample digestion in open vessels over a hot plate is an alternative method. Biota samples can be digested in wet or dried condition, however regardless of the method applied, it is of paramount importance to secure the complete destruction of all organic material of the sample, as well as to avoid metals losses and the contamination of the sample (HELCOM, 2012a).

The existence of residual dissolved organic carbon compounds in the digested sample would change the viscosity of the solution and therefore may lead to erroneous results when calibration of the AAS instrument is made using aquatic calibration standard solutions. Also, in the GF-AAS, residual organic carbon may undergo secondary reactions with the analyte prior to or during the atomization process causing matrix interferences (Harms, 1985[14]).

a) Microwave acid digestion in closed systems (for heavy metals analysis with AAS, GFAAS and ICP-MS analysis)

Biota tissue digestion can be performed in Teflon, or equal quality vessels of pure material, which are metal free and resistant to strong acids, therefore loss of elements through volatilisation and contamination by desorption of impurities from the vessel surface are significantly reduced. Also, since only small quantities of high-purity nitric acid are used, extremely low analytical blanks can be obtained. Microwave systems enable a very fast energy transfer to the sample and a very rapid build-up of high internal vessel temperature and pressure, with the advantage of an enormous reduction in digestion time occurs (HELCOM, 2012a)

Digestion reagents for the analysis of Cd, Pb and other heavy metals analysis

The following reagents are required:

i) HNO_3 (65%, e.g., Suprapur);

ii) H_2O_2 (analytical grade) to be kept in the fridge after opening;

iii) Ultrapure deionised water (> 18MΩ cm, e.g. Millipore).

Dried biota tissue samples (approximately 0.2. g) are weighted in the microwave vessel and placed in a laminar hood compatible with acid fume. Approximately 5 ml of nitric acid (HNO_3) are added and each vessel and left to react for at least 1hour (or more if possible). After the room temperature pre-digestion, 2ml of hydrogen peroxide (H_2O_2) are added carefully, the vessels are closed and placed in the microwave apparatus and digestion steps are followed. Detailed methods for biota tissues microwave digestion with strong acids are presented in Annex VI (IAEA 2011a[15]) and Annex XXIII (HELCOM 2012a).

All chemicals used in the analysis should be kept extremely clean once opened. Double bagged and only to be opened in a clean bench or clean room. It is also strongly advised not to use any pipettes or other devices to take out chemicals from the main container, but to subsample the chemicals into pre-cleaned containers for daily use. This is paramount to avoid contamination of the very expensive ultra clean chemicals needed for this analysis.

Digestion reagents for Mercury analysis

[14] Harms, U. 1985. Possibilities of improving the determination of extremely low lead concentrations in marine fish by graphite furnace atomic absorption spectrometry. Fresenius Journal of Analytical Chemistry, 322: 53-56.

[15] IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)

For Mercury analysis the following reagents are required:

i) HNO_3 (65%, analytical grade, certified low in mercury);

ii) Ultrapure deionised water (> 18MΩ cm,);

iii) 10% $K_2Cr_2O_7$ (w/v) solution (e.g. 10 g $K_2Cr_2O_7$ analytical grade diluted into 100 ml with ultrapure deionised water).

iv) V_2O_5 analytical grade

Dried biota tissue samples (approximately 0.2. to 1.5 g depending of the expected concentration) are weighted in the microwave vessel and placed in a laminar hood compatible with acid fume. If processing high weight of bivalve (> 1g), add 40 mg of V_2O_5 to each tube (including blanks). Five ml of concentrated nitric acid (HNO_3) are added and let to react for at least 1hour. If large amount of sample is used more acid should be added until the mixture becomes liquid. To control the performance of the digestion procedure, at least 2 blanks should be prepared in a similar manner as the samples for each batch of analysis. Also at least one Certified Reference Material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used. After digestion, the vessels are removed from the microwave apparatus and placed in a ventilated fume hood to cool. When the pressure is adequate, the vessels are opened 1 ml of $K_2Cr_2O_7$ solution is added (final concentration should be 2% v/v) and their content is transferred to a volumetric flask, preferably of Teflon, but glass is also good, and made to a known volume.

b) Acid digestion in open systems

In case no microwave digestion system is available, it is possible to perform a digestion over a programmable heating plate placed inside a specially designed fume hood, allowing acid treatment. However, for the complete destruction of the organic matter, large quantities of reagents and voluminous apparatus with large surfaces are usually needed and the method is subject to contamination problems (too high blank values) if insufficiently purified acids are used. Also, the rate of reaction and efficiency of acid decomposition in open vessels is lower than in closed vessels under pressure. Therefore, digestion over a hot plate is not a recommended method and should be avoided if possible.

Dried biota tissue samples (approximately 0.2. g) are weighted in the microwave vessel and placed in a laminar flow hood compatible with acid fume. Approximately 5 ml of concentrated nitric acid (HNO_3) are added to each vessel and let to react at room temperature for at least 1 hour. The tubes are closed and placed in an aluminium block on a hot plate at 90°C for 3hrs. The samples are allowed to cool to room temperature, and the tubes are opened carefully, and the samples are transferred to the labelled 50 ml polypropylene graduated tubes or volumetric flasks.

A method for biota tissues digestion in open systems, using aqua regia, HNO_3 / $HClO_4$ can be found in Black et al, (2013[16]).

2.2.2 Protocol for the determination of heavy metals with Flame AAS

Flame Atomic Absorption Spectroscopy (F-AAS) has adequate sensitivity for the determination of a wide range of metals in marine biota tissues. The sample solution is aspirated into a flame and atomized. In case of flame-AAS, a light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the element in the flame. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

A detailed analytical protocol for the analysis of heavy metals in biota tissue samples prepared by IAEA (2011b[17]) is presented in the Annex VIII.

2.2.3 Protocol for the determination of heavy metals with GF-AAS

In marine biota tissues Cd, Pb, as well as other heavy metals, can be determined by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), which has adequate sensitivity for these determinations. For GF-AAS analysis, after the digestion of the biota sample, an aliquot of sample solution (10-50 µl) is introduced into a graphite tube of the GF-AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

The AAS software generally gives typical electrothermal programs for each element for 10 µl of sample in diluted HNO_3 (0.1%) and indications concerning maximum ashing and atomization temperatures. More specific information may also be found in the literature, such as recommendations

[16] Black, K., Kalantzi, I., Karakassis, I., Papageorgiou, N., Pergantis, S., Shimmield, T. (2013). Heavy metals, trace elements and sediment geochemistry at four mediterranean fish farms, Science of the Total Environment. Elervier, 444, 128–137.

[17] IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

regarding matrix modifiers and the use of partition tubes or tubes with platform. When a program is optimized for the determination of an element in a specific matrix, all information should be reported in the logbook of methods of the laboratory.

For some elements and some matrices, the results obtained are still not satisfactory (e.g. maximum ashing temperature is not sufficient to eliminate the background), this procedure should be redone with the addition of a matrix modifier. Different matrix modifiers could be tried before finding the best solution.

A detailed analytical protocol for the analysis of heavy metals in sediments by GF AAS prepared by IAEA (2011c[18]) is presented in the Annex IX.

2.2.4 Protocol for the analysis of heavy metals with ICP-MS

Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS) is currently state-of-the-art instrumentation for metal analysis, with the possibility to determine at sub-µg L^{-1} concentrations of a large number of elements in acid digested biota tissue samples. ICP-MS allows a rapid analysis of a wide range of heavy metals. Most routine instruments utilize a quadrupole mass spectrometer, so mass resolution is not high enough to avoid overlap of double charged elements or multi-element ions (mainly hydrides, oxides and hydroxides) formed in the plasma. The main concern is for the Ar interferences as the plasma is usually an argon plasma, overlapping with As. Some elements are prone to memory effects (particularly Hg) and needs extra precautions to avoid carry over effects. Modern ICP-MS instruments software includes all the tuning and correction formulas needed and described above to perform the analysis (HELCOM 2012).

A multi-elemental determination of heavy metals by ICP-MS in water and solid samples after acid digestion, is described in the US EPA Method 200.8 (1994[19]). The method was initially intended for inorganic solid samples (soils and sediments) but can also be directly applied to organic samples. According to Enamorado-Baez et al. (2015[20]), for biota tissues the digestion step could use only nitric acid (similar to the US-EPA 3051 method established for sediments, sludge, soils, and oils) but increasing the sample mass to acid volume ratio.

Metal species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed, and valid corrections applied. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix. The US EPA Method 200.8 is presented in Annex XXIV.

2.2.5 Protocol for the determination of Total Mercury with by thermal decomposition, amalgamation and AAS

Total mercury in biological tissues can be determined by solid Hg analyser, which has adequate sensitivity for this determination. A detailed method describing the protocol for the determination of total mercury (inorganic and organic) in biota prepared by IAEA (2012a[21]) ("Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry" Annex XI). With this method, Total Hg is determined without any chemical pre-treatment of the sample, minimising possible contamination and/or additional errors due to sample handling. The method is based on the US EPA 7473 method (US EPA, 2007[22]).

The sample is dried and then chemically decomposed under oxygen in the decomposition furnace. The decomposition products are carried out to the catalytic section of the furnace, where oxidation is completed (halogens and nitrogen/sulfur oxides are trapped). The mercury present in the remaining decomposition products is selectively trapped on an amalgamator. After flushing the system with oxygen, the mercury vapour is released by rapid heating of the amalgamator, and carried through the absorbance cell in the light path of a single wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of mercury quantity (ng). The typical working range is 0.1–500 ng. The mercury vapour is carried through a long (first) and a short path length absorbance cell. The same quantity of mercury is measured twice with different sensitivity resulting in a

[18] IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

[19] US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry.

[20] Enamorado-Báez, S.M., Abril, JM and Gómez-Guzmán, JM (2013) Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples. Hindawi Publishing Corporation, ISRN Analytical Chemistry, Volume 2013, Article ID 851713, 14 pages. http://dx.doi.org/10.1155/2013/851713

[21] IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry

[22] US EPA (2007). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

dynamic range that spans four orders of magnitude. The typical detection limit is 0.01 ng of mercury.

2.2.6 Protocol for the determination of Total Hg in samples of marine origin by CV-AAS

The Cold Vapour Atomic Absorption Spectrometry (CV-AAS) method is widely used for the determination of total mercury in biological tissues and it is simple, rapid and applicable to a large number of environmental samples. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapour is then passed through the quartz absorption cell of an AAS instrument where its concentration is measured. The light beam of Hg hallow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

The typical working range is 0.25–100 ng mL^{-1} for direct injection of cold vapour, using "batch" system (IAEA, 2012b[23]). CV-AAS analysis can be performed manually using batch CV-AAS or automatically using flow injection (FIAS) techniques. FIAS is a very efficient approach for introducing and processing liquid samples in atomic absorption spectrometry, reduces sample and reagent consumption, and has a higher tolerance of interferences, lower determination limits and improved precision compared with conventional cold vapour techniques (HELCOM, 2012b[24]).

A recommended method describing the protocol for the determination of total mercury in biota prepared by IAEA (2012b) is presented in Annex XII. (Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry). A method for the determination of Total Hg in marine biota using CV-AAS is also proposed by HELCOM (2012b) (Annex XXV)

2.3 Technical note for the determination of organic contaminants in marine biota

In line with IMAP requirements (UNEP/MAP, 2019; UNEP/MAP 2019a), the mandatory organic contaminants to be monitored in marine biota in the framework of the IMAP are: Organochlorinated compounds (PCBs [28, 31, 52, 101, 105, 118, 138, 153, 156, 180], Hexachlorobenzene, Lindane and ΣDDTs) and polycyclic aromatic hydrocarbons (US EPA 16 individual PAHs congeners – Acenaphene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(ghi)perylene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthene, Indeno(1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Pyrene). However, Contracting Parties to the Barcelona Convention may decide to include in their national monitoring programmes the analysis of additional heavy organic compounds according to their national priorities.

Analysis of marine biota samples for the determination of organic contaminants include: i) extraction; ii) concentration; iii) clean-up; iv) fractionation; and v) quantification of contaminants.

National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of the Contracting Parties, the IMAP Protocols have been drafted to be used as guidelines for the analysis of organic compounds in marine biota samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the IMAP Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

Under this Technical note, this Guideline sfor sample preparation and analysis of marine biota for IMAP Common Indicator 17 provides the following five IMAP Protocols:

- Protocol for the determination of organochlorine pesticides and PCBs in biota using Gas Chromatography-Electron Capture Detector (GC-ECD);
- Protocol for the determination of organochlorine pesticides and PCBs in biota using Gas Chromatography – Mass Spectrometry (GC-MS);
- Protocol for the determination of PAHs in biota using High Performance Liquid Chromatography– Fluorescence (HPLC –UVF);
- Protocol for the determination of PAHs in biota using Gas Chromatography – Mass Spectrometry GC-MS;
- Protocol for the normalization or organic contaminants concentrations using the lipid content.

These protocols are based on Analytical Methods developed by UNEP/IAEA (Annex XIV: Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71;), HELCOM (Annex XXVI: Manual for marine monitoring COMBINE programme. Annex B-12, Appendix 3. Technical note

[23] IAEA (2012b). Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry.

[24] HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy.

on the determination of chlorinated biphenyls and organochlorine pesticides in biota; Annex XXVIII: Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota) and ICES/OSPAR (Annex XXVII: CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 8: Determination of chlorobiphenyls in biota; Annex XXIX: CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 3: Determination of parent and alkylated PAHs in biological materials).

2.3.1 Protocol for the determination of organochlorine pesticides and PCBs in marine biota using GC-ECD

The determination of PCBs and organochlorine pesticides (OCPs) in marine biota samples (fish muscle and bivalve whole body) involves extraction from the matrix with organic solvents, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection. To minimize systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment, it is essential that the sources of systematic errors are identified and eliminated as far as possible (HELCOM, 2012c[25]).

For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from biota using hexane or petroleum ether. Following initial clean-up treatments (treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography.

All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances; their concentrations vary from batch to batch and with supplier. Therefore, reagent quality should be checked by injection of 2 µl of a 100 ml batch of solvent, after concentration to 50 µl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 - 250 °C) should be larger than that for 1pg of lindane. Otherwise, the solvent must be distilled.

The laboratory used for organic trace determination must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc. The emergency cut-off switch should be accessible from both inside and outside the laboratory. Firefighting equipment should be mounted in obvious places and laboratory personnel trained in their use.

Quantitative analysis with Electron Capture Detector (ECD) is performed by comparing the detector signal produced by the sample with that of defined standards. Due to incomplete separation, several co-eluting compounds can be present under a single detector signal, therefore, the shape and size of the signal have to be critically examined. The relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases, or by the use of multi-dimensional GC techniques. The GC should be calibrated before each batch of measurements. Since the ECD has a non-linear response curve, a multilevel calibration is strongly advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a PCB which is not present in the sample and which does not interfere with other PCBs (HELCOM, 2012c).

A step-by-step method for the determination of organochlorine pesticides and polychlorinated biphenyls in biological samples is prepared by UNEP/IAEA (2011d[26]) (Annex XIV), including the list of reagents, the solvents, standards and examples for the preparation of the stock, intermediate and working solutions. A method for the analysis of organochlorine pesticides and PCBs in biota tissues is also proposed by HELCOM (2012c) (Annex XXVI) and OSPAR (2018a[27]) (Annex XXVII).

[25] HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota.

[26] IAEA (2011d). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71

[27] ICES/OSPAR (2018a). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 8. Determination of chlorobiphenyls in biota

2.3.2 Protocol for the determination of organochlorine pesticides and PCBs in marine biota using GC-MS

The determination of PCBs and organochlorine pesticides (OCPs) in marine biota samples (fish muscle and bivalve whole body) involves extraction from the matrix with organic solvents, followed by clean-up and gas chromatographic separation with mass spectrometric (GC-MS) detection. For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from biota using hexane or petroleum ether. Following initial clean-up treatments (treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography (UNEP/IAEA. 2011d).

Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards, using a mass spectrometer (MS). Often, due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MS detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. The GC should be calibrated before each batch of measurements. Since the MS has a non-linear response curve, a multilevel calibration is advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure.

A method for extraction, concentration, clean up and fractionation for the determination of organochlorine pesticides and polychlorinated biphenyls in biological samples is prepared by UNEP/IAEA (2011d) (Annex XIV), including the list of reagents, the solvents, standards and examples for the preparation of the stock, intermediate and working solutions. The analysis of PCBs and organochlorinated pesticides can be done by GC-ECD followed by confirmation using GC-MS. A method for the analysis of organochlorine pesticides and PCBs in biota tissues using GC-MS is also proposed by HELCOM (2012c) (Annex XXVI) and ICES/OSPAR (2018a) (Annex XXVII).

2.3.3 Protocol for the determination of PAHs in marine biota using HPLC-Fluorescence

PAHs emitted from combustion processes are predominantly parent (un-substituted) compounds, while PAHs from petroleum and its by-products contain a range of alkylated compounds in addition to the parent PAHs. HPLC has the capacity to determine parent PAHs but has not the required selectivity to be used for alkylated PAHs' determination. However, this is not a handicap for the analysis of the EPA 16 PAHs, which are parent compounds.

PAHs are lipophilic and so are concentrated in the lipids of an organism, therefore they have to be extracted with Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. For Soxhlet extraction, wet tissues should be dried by mixing with a chemical agent (e.g., anhydrous sodium sulphate). Non-polar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective. Tissue extracts will always contain many compounds other than PAHs, and a clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. In order to reduce the sample volume to 2 cm^3 solvents are evaporated using a rotary-film evaporator at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. Evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If such compounds are found, then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods (HELCOM, 2012d[28]).

If Soxhlet extraction was used residual lipids have to be removed before the analytical determination, with an additional clean-up stage, using column chromatography with silica and alumina.

Detailed guidelines for the determination of Petroleum Hydrocarbons in biological samples using HPLC are prepared by HELCOM (2012d) (Annex XXVIII) and ICES/OSPAR (2018b[29]) (Annex XXIX).

2.3.4 Protocol for the determination of PAHs in marine biota using GC-MS

GC-MS analytical method has the sufficient selectivity to determine the full range of PAHs including parent (unsubstituted) PAH compounds (combustion derived) and alkylated PAH compounds (petroleum spill derived). (ICES/OSPAR, 2018).

Samples are Soxhlet extracted using methanol. Tissue extracts will always contain many compounds other than PAHs, and a clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. In order to reduce the sample volume to 2 ml solvents are evaporated using a rotary-film evaporator at

[28] HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annxe B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota.

[29] ICES/OSPAR (2018b). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 1. Determination of parent and alkylated PAHs in biological materials.

low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. Evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If such compound are found, then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods.

If Soxhlet extraction was used, residual lipids have to be removed before the analytical determination, with an additional clean-up stage, using column chromatography with silica and alumina.

Quantification is done by GC-MS. The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. (HELCOM, 2012d).

Detailed methods for the determination of PAHs in biological samples using GC-MS are proposed by HELCOM (2012d) (Annex XXVIII) and ICES/OSPAR (2018b) (Annex XXIX).

2.3.5 Protocol for the normalization of organic contaminants concentrations using the lipid content

Normalisation to the total lipid content of marine biota is a means to reduce the variability of pollution level. For organic contaminants that accumulate through hydrophobic partitioning into the lipids of organisms, measured concentrations of contaminants in biota can be normalised to fish with a lipid content of 5% (European Commission 2014[30]). This default lipid content of 5% has been incorporated in the OECD (1996[31]) 305 Guideline for bioconcentration to ensure comparability between results of bioconcentration tests. The rationale behind this lipid normalisation is that the whole body biota concentration is linearly correlated with the lipid content of the species (EC 2014). Other taxonomic groups, such as bivalves, have different lipid contents than fish. For marine bivalves a lipid content of approximately 1% is proposed (European Food Safety Authority, 2009[32]).

There is evidence that using lipid contents for normalization purpose may not always be appropriate, because it requires a linear correlation between the concentration of contaminant and the lipid content, which may not be the case for PAHs (León et al., 2013[33]). Normalization can be useful in specific areas with similar oceanographic conditions and/or for contaminants with a predominant diffuse input in the marine environment (such as PCBs), but not to compare areas subjected to different exposition to pollutants, food availability. Therefore, normalization to lipid content is not a mandatory parameter to be reported in the framework of IMAP, but is to the Contracting Parties to decide if such an exercise is useful in facilitating pollution detection in specific areas. However it is useful to include normalization procedures in the Guidelines, making clear that the method should be tested before being applied, using sufficient data from the area under investigation.

In case it is decided to apply normalise measured data to lipid content, a detailed procedure is described in the EC Guidance No 32 (EC 2014) (Annex XXX). In such cases, European Commission's suggests that contaminant concentrations are normalised to lipid contents of 5% in fish and 1% in bivalves, or to dry weight contents of 26% in fish and 8.3% in bivalves, on the basis of the measured lipid content or dry weight, or on the basis of generic values for lipid content or dry weight for the relevant species obtained from FishBase Global Information System on Fish[34]

To calculate the normalised concentrations *concnorm, lipid* or *concnorm, dry weight* from measured concentrations *concmeas* for a fish species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$concnorm,\ lipid = concmeas \cdot 0.05/lipid\ content_x$$

or

$$concnorm,\ dry\ weight = concmeas \cdot 0.26/dry\ weight_x$$

[30] European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083.

[31] OECD (1996). OECD Guidelines for Testing Chemicals: Proposal for Updating Guideline 305. Bioconcentration: Flow-Through Fish Test Paris 1996.

[32] EFSA. 2009 Guidance Document on Risk Assessment for Birds and Mammals. Parma, Italy: European Food Safety Authority. Authority EFS.358 pp.

[33] León V.M., Martínez-Gómez, C., García, I., Campillo, J.A., Benedicto J. (2013). Spatial distribution and temporal trends of polycyclic aromatic hydrocarbons in Mytilus galloprovincialis from the Iberian Mediterranean coast. Environmental Monitoring and Assessment, 185, 2, 1055-1070.

[34] FishBase: A global information system on fishes (www.fishbase.in)

Similarly, to calculate the normalised concentrations *concnorm, lipid* or *concnorm, dry weight* from measured concentrations *concmeas* for a bivalve species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$concnorm,\ lipid = concmeas \cdot 0.01/lipid\ content_x$$

or

$$concnorm,\ dry\ weight = concmeas \cdot 0.083/dry\ weight_x$$

It is also noted that using the exact lipid or dry weight content of the biota samples is always preferred over generic values for the species (such as those available from FishBase).

The total lipid content of fish or bivalves can be determined using the method of Bligh and Dyer (1959[35]) using chloroform/methanol extraction techniques (OECD, 1996) or, alternatively the method proposed by Smedes (1999[36]), which has a comparable efficiency of extraction and high accuracy, but is using less toxic organic solvents (propan-2-ol–cyclohexane–water (8 + 10 + 11 v/v/v) mixture to avoid the use of chloroform).

3 References

- Black, K., Kalantzi, I., Karakassis, I., Papageorgiou, N., Pergantis, S., Shimmield, T. (2013). Heavy metals, trace elements and sediment geochemistry at four mediterranean fish farms, Science of the Total Environment. Elervier, 444, 128–137.
- Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.
- EC (2010). Guidance Document No: 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive
- EC (2014). Guidance Document No: 32 Guidance on biota monitoring under the Water Framework Directive
- EC Directive 2008/105/EC (2008) on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council
- EC Council Regulation No 1967/2006 concerning management measures for the sustainable exploitation of fisheries resources in the Mediterranean Sea
- European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083.
- EFSA. 2009 Guidance Document on Risk Assessment for Birds and Mammals. Parma, Italy: European Food Safety Authority. Authority EFS.358 pp.
- Enamorado-Báez, S.M., Abril, JM and Gómez-Guzmán, JM (2013) Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples. Hindawi Publishing Corporation, ISRN Analytical Chemistry, Volume 2013, Article ID 851713, 14 pages. http://dx.doi.org/10.1155/2013/851713
- FishBase: A global information system on fishes (www.fishbase.in)
- Galgani, F., Martínez-Gómez, C., Giovanardi, F., Romanelli, G., Caixach, J., Cento, A., Scarpato, A., BenBrahim, S., Messaoudi, S., Deudero, S., Boulahdid, M., Benedicto, J., Andral, B. (2011). Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (Mytilus galloprovincialis) from the western basin of the Mediterranean Sea. Environ. Monit. Assess. 172 (1–4), 301–317. https://doi.org/10.1007/s10661-010-1335-5.
- Galgani, F., Chiffoleau, J.F., Barrah, M., Drebika, U., Tomasino, C., Andral, B. (2014). Assessment of heavy metal and organic contaminants levels along the Libyan coast using transplanted mussels (Mytilus galloprovincialis). Environ. Sci. Pollut. Res. 21, 11331–11339. https://doi.org/10.1007/s11356-014-3079-1.
- Harms, U. 1985. Possibilities of improving the determination of extremely low lead concentrations in marine fish by graphite furnace atomic absorption spectrometry. Fresenius Journal of Analytical Chemistry, 322: 53-56.
- HELCOM (2012). Annex B-12, Appendix 1. Technical note on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements
- HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota.

[35] Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.

[36] Smedes F (1999). Determination of total lipid using non-chlorinated solvents. Analyst, 124:1711-1718.

- HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy.
- HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota.
- HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annxe B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota.
- IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)
- IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry
- IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace
- IAEA (2011d). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71
- IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry
- IAEA (2012b). Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry.
- ICES/OSPAR (2018). CEMP Guidelines for Monitoring Contaminants in Biota
- ICES/OSPAR (2018a). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 8. Determination of chlorobiphenyls in biota
- ICES/OSPAR (2018b). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 3. Determination of parent and alkylated PAHs in biological materials.
- León V.M., Martínez-Gómez, C., García, I., Campillo, J.A, Benedicto J. (2013). Spatial distribution and temporal trends of polycyclic aromatic hydrocarbons in Mytilus galloprovincialis from the Iberian Mediterranean coast. Environmental Monitoring and Assessment, 185, 2, 1055-1070.
- OECD (1996). OECD Guidelines for Testing Chemicals: Proposal for Updating Guideline 305. Bioconcentration: Flow-Through Fish Test Paris 1996.
- Smedes F (1999). Determination of total lipid using non-chlorinated solvents. Analyst, 124:1711-1718.
- UNEP/MAP (2019a). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27
- UNEP/MAP (2019b). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution
- UNEP/MAP (2019c) UNEP/MED WG.467/8. Data Standards and Data Dictionaries for Common Indicators related to pollution and marine litter.
- US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry.
- US EPA (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories Volume 1 Fish Sampling and Analysis. Third Edition.
- US EPA (2007). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

B-3. Monitoring Guidelines/Protocols for sampling and determination of contaminants in seawater

Table of Contents

1 Guidelines for sampling and sample preservation of seawater

1.1 Introduction

Seawater is not included in the mandatory matrices to be analysed in the framework of the UNEP/MAP's Integrated Monitoring and Assessment Programme (UNEP/MAP 2019a[1], UNEP/MAP 2019b[2]), therefore the implementation of a monitoring programme for the determination of heavy metal and organic contaminants in seawater is a country-based decision. It has to be emphasized that heavy metals and organic contaminants' concentrations in seawater are very low, especially in offshore waters, so improper sample collection and handling could easily result in loss of determinant and/or contamination of the sample before analysis. Therefore, if a country decides to implement a seawater monitoring programme, it has to develop and test a very strict sampling and preservation protocol, using appropriate equipment and shipping infrastructure. Also, laboratory facilities should be adapted accordingly for the quality assured analysis of ultra-low contaminant's concentrations in seawater samples.

Seawater sampling can be equally implemented in coastal and offshore marine areas, since sampling equipment and sample preservation methodologies to avoid determinant's loss and/or cross-contamination are similar for both areas. Therefore, the suggested sampling and preservation protocols are equally applicable in coastal as well as in offshore sampling stations, taking into consideration that concentrations of heavy metals and organic contaminants in offshore waters are expected to be lower than in coastal seawater samples. If transects are sampled, the sampling should be done from the open ocean to the coast and not the other way around to avoid contamination of samples from sampling equipment.

It is important to collect representative seawater samples from the sampling area, but it is equally important to avoid any alteration of the physical and chemical characteristics of the samples during transportation form the field to the laboratory. Therefore, seawater storage and transportation have to be done under specific procedures, in order to avoid sample alteration and cross contamination from the material of the containers and the transportation environment.

To assist countries which plan to include seawater monitoring in their respective national monitoring programmes for CI17, as a country-based decision, Protocols for seawater sampling and sample processing have been prepared. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

The Protocols aim at streamlining sampling and processing of seawater samples in a view of assuring comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes. They provide a step-by-step guidance on the methods to be applied in the Mediterranean area for sampling, sample handling to avoid cross-contamination, as well as the storage conditions in a view of maintaining the sample's integrity during the transfer from the sampling site to the analytical laboratory to ensure the representativeness and the integrity of the samples for analysis.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent laboratories participating in IMAP implementation. Namely, the six here-below elaborated IMAP Protocols build upon the relevant Guidelines developed by GEOTRACES, ICES/OSPAR and HELCOM on seawater sampling and analysis, as provided in Annexes XXXI, XXXII and XXXIII. Given the suitability of any of these Guidelines in the context of IMAP, they can be further used by competent Mediterranean laboratories for developing their lab-specific sampling and sampling processing methodologies.

1.2 Technical note for the sampling and pretreatment of seawater for the determination of heavy metals[3] and organic contaminants

Seawater sampling should be carried out at the same time and locations as the sampling of other matrices (sediment, biota) and biological effects measurements (ICES/OSPAR, 2012[4]). Sampling, pretreatment and analysis is a complex endeavour requiring careful design and implementation. Due to the very low concentrations of heavy metals in seawater (especially in open sea stations), improper sample handling could easily result in loss of determinant and/or contamination of the sample before analysis. Appropriate sampling and pretreatment protocols are therefore a crucial step in any seawater monitoring programme.

The size of the seawater sample has to be sufficient to support the desired detection limits for the contaminants of interest. ICES/OSPAR (2012) guidelines for seawater analysis (Annex XXXI) suggests to collect appropriate seawater volume for analysis in relation to the contaminant's concentration in the specific station (polluted or non-polluted) in such a way that the limit of

[1] UNEP/MAP (2019a). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27.

[2] UNEP (2019b). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution.

[3] The term "heavy metals" is used indicating both heavy metals and trace elements

[4] ICES/OSPAR (2012). JAMP guideline on monitoring of contaminants in seawater: Annex 1: Guidelines for Monitoring of Contaminants in Seawater. ICES Advice 2012, Book 1

quantification (LOQ) to be equal to or below a value of 30% of the relevant assessment criterion (i.e. the Environmental Quality Standard, Commission Directive 2009/90/EC[5]).

There are two ways to approach seawater analysis: a) unfiltered seawater and b) filtered seawater. The analysis of unfiltered water samples gives results on the total concentration of contaminants in seawater, regardless of the chemical forms or particle size (i.e. dissolved, complexed and bound to colloids and to suspended particulate matter (SPM)), therefore important information on the distribution and availability of contaminants is lost. On the other hand, filtration over 0.45 µm mesh separates the filtered seawater (i.e. freely dissolved, complexed and bound to), from the particulate phase of contaminants, which is retained in the filter. However, due to exchanges of contaminants between the chemical forms in the dissolved and particulate phases, as well as to potential influence of the sampling and filtration equipment (filters, containers walls, etc.) the equilibria between dissolved and particulate phases may be altered during the process. Therefore, filtration should be performed in such a way as to minimize the alteration of the seawater sample and the distribution of contaminants between dissolved and particulate phases. Also, in the case of organic contaminants, their distribution between the dissolved and the particulate phases is influenced by their polarity, which can be expressed by their octanol/water coefficient (log Kow; Kow = Concentration in octanol phase / Concentration in aqueous phase). The more hydrophilic compounds with log Kow values of 3 to 4 (such as 2- and 3-ring aromatics and HCH isomers) are mainly found in water, while pollutants with log Kow values >5 (4- to 6-ring aromatics, DDT group, PCBs) manly found in suspended particulate matter (SPM). Non-polar hydrophobic compounds are associated with SPM, which are separated by filtration, but they are also present in the filtrate adsorbed on colloids. As a consequence, the validation of the phase separation procedures is very difficult.

Filtration could be done in-line (from the sampling bottle or the seawater pumping system) or off-line in the laboratory. In-line filtering systems have the advantage of reducing the risk of loss of determinant and/or contamination of the sample from storage bottles or the air. In all cases filtration should be done in an area free of particles as much as possible. Working in a laminar flow hood is the preferable solution. Recommended conditions for a 'clean bench' or a 'cleanlab' are ISO Class 5 (GEOTRACES, 2017[6]).

Detailed Guidelines for seawater sampling and processing can be found in documents issued by ICES/OSPAR (2012) (Annex XXXI), HELCOM (2012a[7]) (Annex XXXII), HELCOM (2012b[8]) (Annex XXXIII) and GEOTRACES (2017). Further building on these documents, under this Technical Note, the Guidelines for Sampling and Sample Preservation of Seawater for IMAP Common Indicator 17 provide the following IMAP Protocols for seawater sampling that:

- Protocol for seawater sampling for heavy metals determination;
- Protocol for seawater filtration (heavy metals);
- Protocol for the on-board storing of seawater samples for heavy metal determination;
- Protocol for seawater sampling for organic contaminants determination;
- Protocol for seawater filtration (organic contaminants);
- Protocol for the on-board storing of seawater samples for organic contaminants determination.

1.2.1 Protocol for seawater sampling for heavy metals determination

a) Sampling equipment for seawater collection

Usually for metal analysis seawater samples from different depths are collected using GO-FLO bottles (General Oceanics). The sampler consists of a cylinder with an inner Teflon-coating which can be lowered closed into the water column and opens automatically at a certain depth by hydrostatic pressure. This avoids contact of the sample with the water surface film which is enriched in contaminants. Other types of sampling bottles can also be used (such as Niskin bottles) properly modified for avoiding metal contamination.

All samplers have to be cleaned before the first use by rinsing the inner surfaces with diluted hydrochloric acid. In the open sea, the bottles should be rinsed with seawater between samplings, while in polluted stations they could be rinsed with deionized water.

The metallic hull of the ship is a potential source for metal contamination (iron and lead), as is the use of antifouling paints (copper and tin) and the ship's anodic protection (zinc). To avoid metal contamination the ship should be positioned in such a way in relation to the wind and sea current directions, as to minimize any influence from the ship's hull on the seawater samples.

Use sampling equipment (such as GO-FLO or Niskin style bottle with a capacity 12-30 l) attached

[5] EC (2009). Commission Directive 2009/90/EC laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status.

[6] GEOTRACES (2017). Sampling and Sample-handling Protocols for GEOTRACES Cruises (Version 3), edited by the 2017 GEOTRACES Standards and Intercalibration Committee.

[7] HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-11, Appendix 1. Technical Note on the determination of trace metals (Cd, Pb, Cu, Co, Zn, Ni, Fe) including mercury in seawater.

[8] HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-11, Appendix 2. Technical note on the determination of persistent organic pollutants in seawater.

individually at hydrographic wire or placed in a metal-free rosette system (Figure 1).

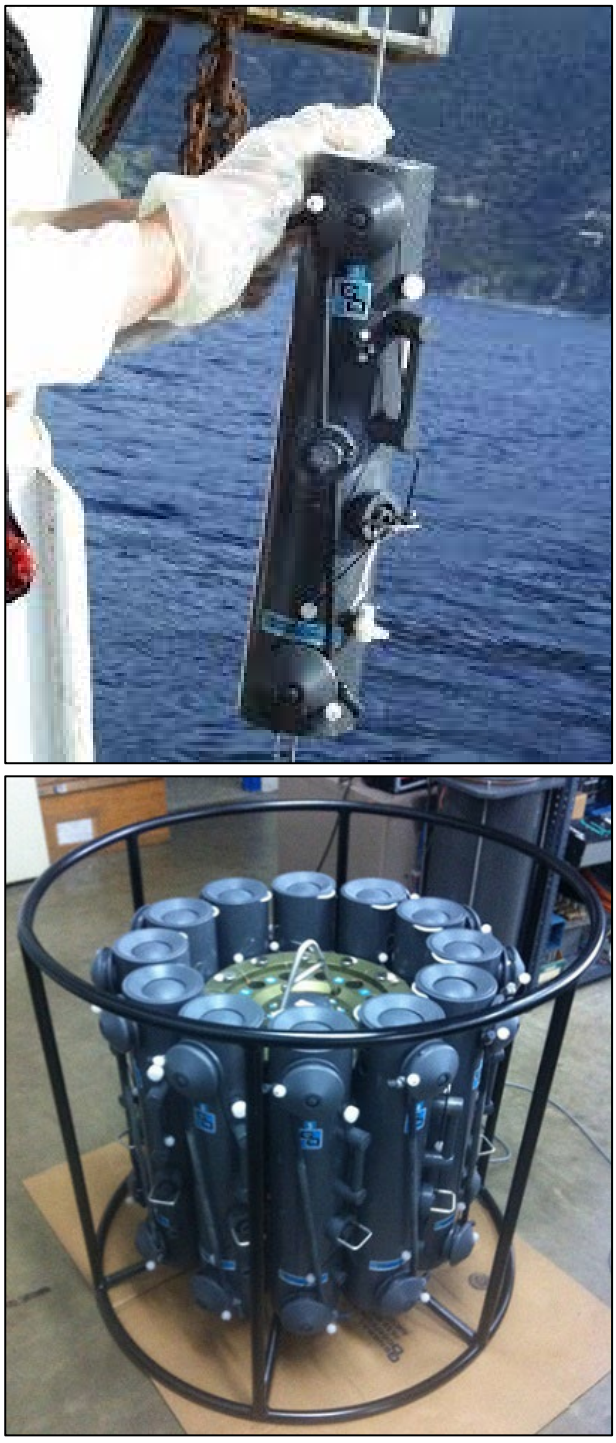

Figure 1. Individual GO FLO seawater sampler and rosette system with multiple samplers

The hydrographic wire should be made of Teflon coated stainless steel, polymer, or Kevlar to avoid metal contamination. All weights used as ballast for lowering the bottles/rosette should be non-metallic or coated with epoxy resins to avoid metal contamination

i) The sampling bottles are lowered to the designated depths. A depth recorder is fitted to the individual bottles or the rosette system to monitor the sampling depth;

ii) A non-metallic messenger (or coated with epoxy resins) is used to release the closing valves in both ends of the sampler for individual bottles or use a triggering system to close bottles in a rosette system at the ascending path;

iii) Once seawater samples have been collected form all sampling depths, the bottles/rosette system is lifted on board;

iv) Once the sampling equipment is lifted on board, it should be placed in a pre-cleaned plastic bag or other container and then transported to an ISO Class-5 area (or a hood with metal-free filtered air) for further handling;

v) Seawater samples are transferred from the GO-FLO (or similar sampling equipment) to a pre-cleaned (with dilute HCl or HNO_3) Teflon (or polyethylene) bottles for total metals analysis;

vi) In case SPM will be anaysed separately from the dissolved metal fraction, seawater sample is transferred to the filtration unit, using a pre-cleaned Teflon tubing.

Sample contamination from the atmosphere (such as paint and rust particles, engine exhausts and atmospheric background) could be very important and measures have to be taken to avoid it. Therefore, entire seawater handling has to be performed in a dust-free and metal-free environment, under controlled conditions (ISO Class-5 area).

Use unpowered latex or nitrile gloves for handling seawater samples to avoid contamination.

b) <u>In-situ seawater pumping (profiles)</u>

In-situ seawater pumping from designated depths is an alternative method for seawater collection, which minimises sample's handling, which may result to loss of determinant or/and sample contamination from the air. The pumping system can optionally include in-line filtration, to separate SPM from the seawater filtrate. The method can be used for relatively shallow depths (up to 100 m) using a peristaltic pump or Teflon piston, or diaphragm pumps and tubes made of silicone, polyethylene or Teflon, in order to avoid metal contamination. Prior to use, the tubing should be cleaned by pumping diluted acid (such as HCl or HNO_3). During sampling, the first litres of seawater should be discarded in order to rinse the whole pumping system before the collection of seawater samples. The rinse volume depends on the length of tubing used and one should rinse with at least 3 times the volume of the tubing before taking the actual sample. Before its use in the field, the pump's operation and performance have to be thoroughly checked and optimized. (Figure 2)

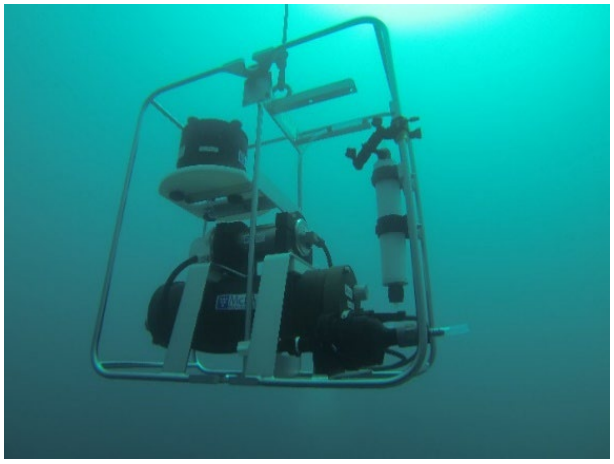

Figure 2. In-situ seawater pumping system (Marine Environmental Studies Laboratory, IAEA)

The outflow from the pumping system is collected in metal-free bottles (polyethylene, Teflon, glass). For Mercury analysis water should be collected in glass or quartz bottles. If an in-line system is attached to the pumping device, the filtrate should be stored in metal-free bottles (as above), while the filters with the SPM samples should also be placed in metal free containers.

c) In-situ surface seawater sampling

For surface sampling of seawater GEOTRACES (2017) recommends surface pump sipper/tow fish system which consists of:

i) PTFE Teflon diaphragm pump with silicone pump tubing;

ii) PFA Teflon sample tubing;

iii) PVC depressor vane 1 m above a 20 kg weight enclosed in a PVC fish (alternatively a 50 kg stainless steel fish) which does not require a separate depressor;

iv) Polyester braided line connecting the fish to the depressor (if required) and then to the ship; the Teflon sampling tubing is run along this line;

v) PFA Teflon tubing is used on the other side of the pump to deliver seawater directly into a clean area for sampling;

vi) For underway surface sampling at speeds from 1 to 12 knots, the sipper system is deployed off the side of the ship using the ship's crane to suspend the fish outside of the bow wake with the intake at approximately 2-m deep. Faster speeds are possible with this sipper design if there is little or no swell and the sipper remains outside of any breaking bow waves. The sipper design also allows near-stationary sampling (moving forward into clean water at 0.5 to 1 knots) in order to collect large volumes of trace metal–clean seawater at depths up to 25 m.

Figure 3. Surface pump sipper/tow fish system (GEOTRACES, 2017)

d) Cleaning of equipment and lab ware prior to sampling

A protocol of lab ware cleaning for seawater sampling equipment for metal analysis is proposed by HELCOM (2012a) (Annex XXXII)

i) Lab ware is stored in 2M HCl (high purity) for one week, rinsed with water, stored in water for one week and dried under dust-free conditions (clean bench).

ii) Sampling devices are filled with 1% HNO_3 (high purity), stored at room temperature for three weeks, and rinsed with water.

iii) Teflon/quartz bottles are stored in warm (40 C ±5 °C) 1:1 diluted HCl for one week. Then rinsed with water and stored with 1M HNO_3 (high purity) until the final use (a minimum of three weeks).

Modified cleaning procedures are required for mercury. Glass containers (borosilicate, quartz) used for the collection and storage of samples for the determination of mercury are usually cleaned using an oxidizing procedure described by Sturgeon and Berman (1987[9]). Bottles are filled with a solution of 0.1 % $KMnO_4$, 0.1% $K_2S_2O_8$ and 2.5 % HNO_3 and heated for 2 hours at 80 °C. The bottles are then rinsed with water and stored with 2 % HNO^3 containing 0.01 % $K_2Cr_2O_7$ or $KmnO_4$ until ready for use.

Detailed protocols for cleaning of sampling equipment and storage bottle are also proposed by GEOTRACES (2017) and ICES/OSPAR (2012) (Annex XXXI).

[9] Sturgeon, R., and Berman, S. 1987. Sampling and storage of natural water for trace metals. In Critical reviews in Analytical Chemistry. 18(3): 209-244. CRC Press.

1.2.2 Protocol for seawater filtration for heavy metals determination

a) Filtration procedure: Seawater should be filtered as soon as possible after the samples were taken as otherwise ratio between dissolved and particulate contaminant concentration may change.

In-line filtration

Seawater can be directly filtered from pressurized GO-FLO bottles using a low overpressure (<50 kPA, or <7 psi, maximum) of filtered high-quality nitrogen gas or compressed air to obtain a sufficient flow across the filters (GEOTRACES, 2017). Before starting filtration, it is recommended to gentle mixing the GO-FLO bottles because particle settling can occur continuously during the period between GO-FLO closing at depth and initiation of filtration. A pre-cleaned capsule filter or membrane filter holder is connected to the GO-FLO's Teflon plug valve with Teflon PFA tubing (or clean equivalent) and the sample bottles are filled with the effluent from this filter (capsule filters should be rinsed with ca. 0.5 L of sample water prior to collection of the filtrate).

Off-line filtration

After collection from the GO-FLO sampling bottles, seawater is transferred to a secondary bottle, from which it is sent to the filtration equipment. Off-line filtration yields similar results than in-line filtration if strict trace metal clean working procedures are followed. Therefore it can be used if required by sample handling limitations on board of the sampling vessel. Before starting filtration, it is recommended to gentle mixing the GO-FLO bottles because particle settling can occur continuously during the period between GO-FLO closing at depth and initiation of filtration. Then drain the seawater into a pre-cleaned transfer bottle, which is cupped and transferred to the filtration area. Volume to filter is suggested to be 5-10 L, which is sufficient to load filters with enough material to exceed filter blanks for nearly all samples and all analytes (GEOTRACES, 2017).

Once filtration is completed, the residual seawater can be forced to through the filter using a polypropylene syringe filled with air. This will avoid spillage and loss of particulate material from face of filter when filter holder is opened. The filter holders can then be disassembled and filters carefully removed using Teflon forceps and stored in Petri-slide or similar suitable container and frozen at -20° C.

b) Filters

Polycarbonate filters (0.45 µm) are often used for seawater filtration for the analysis of heavy metals (except mercury). The main purpose for choosing a filter is low metal blanks, mechanical strength and ease of handling, relatively high particle load capacity, low tendency to clog completely, and good filtration flow rate. The filters have to be cleaned with 2M HCl (high purity) for a minimum of three weeks, rinsed with deionized water, and stored for one more week in water (HELCOM, 2012a). Then the filters have to be dried in a clean bench and stored in a desiccator until constant weight. The same procedure for drying and weighing should be applied to the filters loaded with SPM (Pohl, 1997[10]).

For the determination of mercury, glass fibre filters (GF/F grade, Millipore type) and Teflon filters are recommended. Cleaning of these filters is comparable to the procedure used for polycarbonate filters (Queremais and Cossa 1997[11]).

Filter diameters depend on the quantity of SPM in the sampling stations. While a filter diameter of 25 mm is sufficient to filter 10 L of seawater without clogging at open sea stations, 47 mm is preferred for shelf-slope stations where particle concentrations are higher. Effort is made to minimize the filter's diameter in order to maximize the particle loading per filter area, and thus lower the filter's blank in relation to the metal concentrations in the SPM.

Filter holders made of polypropylene are often used because they compatible with trace metal clean procedures. It important to have perfect sealing capabilities under pressure.

c) Cleaning Filters and filter holders

GEOTRACES (2017) proposes the following protocol for cleaning filters and filter holders for trace metal analysis in seawater samples:

i) A 1000 mL Low Density Polyethylene (LDPE) pre-cleaned bottle is further pre-cleaned by filling with 10% (v/v, or 1.2M) of TM Grade HCl, double bagging in heavy duty (e.g. 4mm) Ziploc polyethylene bags, and placing in oven at 60°C for 4 hrs to overnight.
ii) The bottle is removed to fume hood and placed inverted so that lid is acid-leached while acid cools. Acid is poured-out and the bottle is rinsed thoroughly at least 3 times with TM-clean deionized water (e.g., Milli-Q).
iii) The clean bottle is filled 90% full with TM-clean deionized water.

Filters should be removed from the original box using metal-free forceps, grasping filters only on the edge so that the sample region is not damaged, and are carefully dropped into the bottle. Make sure any separator papers from the original packaging are not included. When 100

[10] Pohl, C. 1997. Trace Metals (Cu, Pb, Zn, Cd, Al, Li, Fe, Mn, Ni, Co) in Marine Suspended Particulate Matter: An International ICES Intercomparison Exercise. Accreditation and Quality Assurance, 2: 2-10

[11] Quémerais, B., and Cossa, D. 1997. Procedures for sampling and analysis of mercury in natural waters. Environment Canada-Quebec region, Environmental Conservation, St. Lawrence Centre. Scientific and Technical Report ST-31E, 34 pp.

filters have been immersed in the water, the last 10% of bottle volume are filled with concentrated TM Grade HCl, caped tightly, mixed gently so that the filters do not crease, and the double bagged bottle is placed in a 60°C oven overnight, as for bottle cleaning.

When bottle of filters is cool, acid is slowly poured off to waste, retaining filters with the cap held against the bottle mouth. Filters are kept in suspension by gentle hand-agitation while pouring off acid, to minimize folding and creasing while all the solution is removed. The bottle is slowly filled with DI water running gently down the inside wall, while swirling gently, and the water is poured out, retaining filters with the cap. The procedure is repeated 5 times. Leave the last rinse in the bottle and allow to sit at room temperature overnight so that any residual acid diffuses from the pore spaces of the filters. Three more rinses are repeated the next day. Always the pH has to be checked to ensure no acid remains as filters can take many rinses to remove all traces of acid. Filters can be left in the DI water suspension until used on ship, or can be loaded in advance into individual Petri-slides for easy access and storage in the same Petri-slide. Caution has to be used to avoid getting doubled filters, as the filters tend to stick to each other (GEOTRACES, 2017).

1.2.3 Protocol for the on-board storing of seawater samples for heavy metals determination

Seawater samples should be stored in such conditions as to avoid metal loss or contamination during the transfer from the ship to the laboratory for further pre-treatment and analysis. The usual process for conserving seawater samples for the analysis of trace elements is acidification and freezing. However, any sub-sampling of the seawater samples has to be done on board immediately after sampling. If filtration is required to separate the SPM from the dissolved phase of the sample, it should also be done immediately after sampling and before any acid addition for preservation causes.

Seawater samples (filtered or unfiltered, if total metals are to be determined) are acidified by adding 1.5 ml HNO_3 or HCl (high purity) per litre of seawater sample immediately after filtration, for acidification to pH 1.0-1.6. The bottles are stored at 4 °C in the dark. Filters with SPM should be stored in plastic dishes at -20 C. Under these conditions, both water samples and SPM on filters can be stored for at least one year. For Hg determination, in addition of acidification oxidation agents should be added (such as $Cr_2O_7^{2-}$). (HELCOM, 2012a)

The bottles used for seawater storage should be made of Low-Density Polyethylene (LDPE) or High-Density Polyethylene (HPDE). Bottle caps are usually made of polypropylene, which is suitable material for seawater storage. For Hg, polyethylene bottles are not recommended and instead, glass or Teflon bottles can be used (GEOTRACES, 2017).

a) Sample Bottle Cleaning

The cleaning of the bottles used for storage of seawater samples for trace element analysis, should be very thorough to avoid sample alteration from the container. GEOTRACES (2017) proposes a very rigorous protocol for bottle cleaning, which is used by research groups with a long history of successful trace metal clean sampling. The GEOTRACES protocols are as follows:

GEOTRACES protocol for LDPE and HDPE bottles (dissolved and dissolvable trace elements):

i) The bottles may need to be rinsed with methanol or acetone to release oils from manufacturing.

ii) Soak bottles for one week in an alkaline detergent (e.g. Micro, Decon). This process can be sped up by soaking at 60°C for one day

iii) Rinse 4x with Reverse Osmosis/Deionized Water.

iv) Rinse 3x with Ultra High Pure Water (UHPW) under clean air.

v) Fill bottles with 6M HCl (reagent grade) and submerge in a 2M HCl (reagent grade) bath for one month. Again, this can be sped up by heating for one week.

vi) Rinse 4x with UHPW under clean air.

vii) Fill bottles with 1 M HCl (trace metal grade) for at least one month. Should be stored doubled bagged. Bottles should be emptied of all acid before transporting to the ship.

viii) Rinse with UHPW and ship the bottles empty and double bagged.

GEOTRACES protocol for PFA Teflon bottles:

i) Soak bottles for one day in an alkaline detergent;

ii) Rinse 7x with Deionized Water (DIW) thoroughly until there is no trace of detergent;

iii) Rinse 3x with UHPW;

iv) Soak in 6 M reagent grade HCl bath for 1 day;

v) Rinse 5x with UHPW;

vi) Fill bottles with 1M nitric acid (analytical grade) and keep them at 100°C for 5 hours in a fume hood'

vii) Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood;

viii) Fill bottles with UHPW water and keep them at 80°C for 5 hours;

ix) Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood. Should be stored doubled bagged.

1.2.4 Protocol for seawater sampling for organic contaminants determination

a) Sampling equipment for seawater collection

Concentrations of organic contaminants in seawater are usually very low, therefore in order to reach the Limit of Quantification (LOQs) required for such contaminants (in pg l^{-1}) large water volumes should be collected (sometimes more than 100 litres) to be extracted to avoid

interferences from the matrix background (ICES/OSPAR, 2012) (Annex XXXI). However, large seawater volumes cannot be easily handled and transported, therefore on-board seawater extraction solves a lot of logistics' problems as well as avoids alteration of seawater samples characteristics. The in-situ filtration/extraction equipment has in addition the advantage of short exposure of the seawater sample to the atmosphere.

For seawater sampling for the determination of organic contaminants, equipment is preferably made of glass or stainless steel. Teflon-coated equipment can also be used for Persistent Organic Compounds and PAHs.

Glass bottles are an appropriate sampling equipment for the determination of organic contaminants. The bottles are mounted in a stainless-steel cage and are lowered on a hydrographic wire down to the desired sampling depth, opened under water and then lifted to the deck of the ship. The glass sampler can be used to a depth of 2000 m (10 l) and 100 m (100 l) (ICES, 2012) (Figure 4). For greater depth stainless steel bottles, based on the Niskin and GO-FLO design can be used. A depth recording system is fitted on the steel case, to allow seawater collection from the desired depth.

Figure 4. Glass bottle for seawater sampling for organic contaminants analysis (ICES/OSPAR 2012)

All samplers have to be cleaned before the first use, with appropriate organic solvents. In the open sea, the bottles should be rinsed with seawater between samplings, while in polluted stations they could be rinsed with deionized water.

Once the sampling equipment is lifted on-board, it should be placed immediately in an aluminium or stainless-steel container and transported to a clean-room (or a hood with dust-free filtered air) in the ship's laboratory, for further handling. Sample contamination from the atmosphere (such as PAHs from the engine exhausts) or the ship (i.e. PCBs in lubricating oil) can lead to sample contamination, therefore measures have to be taken to avoid it, including the positioning of the ship in relation to the wind and sea current directions in order to minimize any influence from the ship. All seawater handling has to be performed in a dust-free environment, under controlled conditions.

b) Sampling by pumping – *In situ* filtration and extraction

In-situ seawater pumping from designated depths is an alternative method for seawater collection, which minimises sample's handling that may result to loss of determinant or/and sample contamination from the air. The pumping system can optionally include in-line filtration, to separate SPM from the seawater filtrate. The in-situ filtration followed by a solid-phase extraction minimizes the risk of sample contamination during sampling. The pumping system includes a glass fibre filter (pore size 0.7 μm) to collect the particulate phase and a glass column packed with polymeric resin for the dissolved phase. The pumping system is operated in a similar manner as for heavy metal analysis (paragraph 17). Volumes of 1 to 100 l can be sampled by discrete sampling and/or pumping and are usually extracted either by liquid-liquid extraction (LLE) or solid phase extraction (SPE), while larger volumes are generally sampled by pumping and extracted by solid phase extraction (ICES/OSPAR, 2012).

Details on the calibration of the situ pumping system are provided by the pump's manufacturer. Before its use on the field, the pump's operation and performance has to be thoroughly checked and optimized.

1.2.5 Protocol for seawater filtration for organic contaminants determination

a) Filtration/extraction procedure

The concentrations of organic contaminants in seawater are very low (LOQ are at the pg l^{-1} range). Therefore, large water volumes (10 to 100 l or more) need to be filtered and extracted to overcome blank problems. Because hydrophobic compounds occur in dissolved, colloidal, and particulate-bound forms, filtration should be done in such a way as to avoid the alteration of the organic compounds partitioning between dissolved and particulate phases because of handling artefacts. It is therefore preferable that filtration is done immediately after sampling.

In-situ filtration/extraction

In order to minimize alteration of organic contaminants partitioning between phases, as well as contamination from the air, in-situ filtration/extraction can be done with a submersible water pump. The in-situ filtration/extraction is compact and combines the advantages of small size and short exposure to the atmosphere (HELCOM, 2012b). The pump, which includes a filter holder, a polymeric resin column, a pump, and a flow-meter, is deployed at a designed depth on a hydrographic wire and the pumping is started and ended by remote control. A glass fibre filter (pore size 0.7 μm) recover the particulate phase and a glass column packed with polymeric resin the dissolved phase. Since the submersible pumps have usually some plastic parts and connections, before use the pump should be checked for targeted organic contaminants blanks, in order to

make necessary replacements of parts with stainless steel or glass (if possible) to reduce contamination. Surrogate standards can be added to the resin column before sampling to control the extraction recoveries and storage. The in-situ pump sampling method has to be validated before its use (ICES/OSPAR, 2012).

Off-line filtration

Storage of seawater samples for the determination of organic contaminants is impractical because of the large seawater volumes required for the quantification of the determinants. Furthermore, the storage period of seawater samples before extraction should limited (less than 2 hours, HELCOM, 2012b) and it is recommended to extract the water sample as soon as possible after sampling. Also, it is preferable to avoid transfer of seawater to another container, as well as unnecessary manipulation that may lead to the alteration of the sample's characteristics. Sampling bottles have to be carefully moved to the clean area of the on-board laboratory (IMAP Protocol 2.4. on seawater sampling for organic contaminants analysis) to proceed to filtration and extraction.

The sampling bottles are connected to a glass fibre filter (pore size 0.7 µm) for recovering the particulate phase and the dissolved phase in extracted on board by liquid–liquid extraction (LLE) or solid-phase extraction (SPE). The extracts or adsorbent cartridges are stored under cool (< 4°C) and dark conditions.

b) Filters

Filtration is done using Glass Fibre filters (GF/F) (0.7 µm pore size). Flat-bed filters have a very limited capacity, therefore coiled glass fibre filters are often used for volumes larger than 10 l and water samples with high amounts of suspended matter. A pump is necessary to force the water through the filter (HELCOM, 2012b).

c) Cleaning Filters and filter holders

In many cases, the procedural detection limit is determined by the blank value. In order to keep the blank value as low as possible, the compounds to be analysed or other interfering compounds should be removed from the filters and all glassware and tubing used in filtration.

A cleaning procedure for all equipment and materials used in handling and processing seawater samples for organic contaminants analysis is proposed by HELCOM (2012b):

i) Glassware should be thoroughly washed with detergents and rinsed with an organic solvent prior to use. Further cleaning of the glassware, other than calibrated instruments, can be carried out by heating at temperatures > 250 °C.
ii) All solvents should be checked for impurities by concentrating the amount normally used to 10 % of the normal end volume. This concentrate is then analysed in the same way as a sample by HPLC or GC and should not contain significant amounts of the compounds to be analysed or other interfering compounds.
iii) All chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glass fibre thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 coarse efficiency glass filter at the bottom, can be used. The storage of these super-cleaned materials for a long period is not recommended, as laboratory air can contain PAHs that will be adsorbed by these materials. Blank values occurring despite all the above-mentioned precautions may be due to contamination from the air.

As the concentrations of the PAHs and chlorinated hydrocarbons in seawater are very low, it is very difficult to control blank and contamination problems. Therefore, it is recommended to rewash all equipment (vials, pipettes, glass bottles) with solvent just before use. If possible, critical steps should be done in a clean bench.

1.2.6 Protocol for on-board storage of seawater samples for organic contaminants determination

Seawater can be stored in glass bottles to avoid contamination and minimize the adsorption of the organic contaminants on the surface of the bottle. However, because very lipophilic compounds such as 4- to 6-ring PAHs, DDT, PCBs, tend to adsorb on every surface, samples should be extracted as soon as possible after sampling. The best procedure is to extract the samples by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) and to store the extracts or adsorbent cartridges under cool (< 4°C) and dark conditions. The extracts in organic solvents are less susceptible to adsorption onto surfaces (HELCOM, 2012b). If, however, seawater samples must be stored, this should also be in the dark and in a refrigerator (4°C) (ICES/OSPAR, 2012).

Suspended Particulate Matter (SPM) samples after filtration should be refrigerated (-20 ºC) and kept stored frozen until further analysis.

2 Guidelines for the determination of contaminants in seawater

2.1 Introduction

According to IMAP requirements (UNEP/MAP, 2019a and UNEP/MAP, 2019b) seawater is not included in the mandatory matrices to be analysed in the framework of the UNEP/MAP's Integrated Monitoring and Assessment Programme (IMAP), therefore no list of contaminants has been designated as mandatory for analysis. However, seawater pollution is an issue of concern that might be introduced at latter stage of the IMAP implementation. Therefore, at this stage of IMAP implementation, it is recommended that seawater monitoring is carried out on a country decision basis, including contaminants that countries consider more appropriate and technically feasible to be monitored.

Seawater analysis is a complex endeavour including sampling, sample processing and analysis, requiring

careful design and implementation. The major analytical challenge of heavy metals and organic contaminants analysis in seawater, is their extremely low concentrations (especially in offshore areas), which requires an ultra-clean laboratory's environment to avoid cross-contamination of the samples, appropriate analytical equipment to accurately measure ultra-low concentration and appropriate staff expertise for this kind of analysis.

The Protocols prepared in the framework of this Monitoring Guidelines for Sample Preparation and Analysis of Seawater for IMAP Common Indicator 17, as provided here-below, describe appropriate methodologies for the analysis of seawater for the determination of heavy metals and organic contaminants, in order to ensure quality assured data. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results. These Protocols aim at streamlining marine seawater sample preparation and analysis for heavy metals and organic contaminants in a view of assuring comparable quality assurance of the data, as well as comparability between sampling areas and different national monitoring programmes, by providing a step-by-step guidance on the methods to be applied in the Mediterranean.

In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent laboratories participating in IMAP implementation. Regarding the analysis of heavy metals, here-below elaborated IMAP Protocols build on the Guidelines/Protocols developed by GEOTRACES, HELCOM (Annexes XXXII and XXXIII), ICES/OSPAR (Annex XXXI) and US EPA (Annex XXXIV), as well as on analytical methods which have been developed by IAEA for sediment (Annexes VI and IX and XIV). Given the suitability of any of these Guidelines in the context of IMAP, they could be further used by interested IMAP competent Mediterranean laboratories for developing their laboratory specific sampling and sample processing methodologies. The Contracting Parties' laboratories should accommodate and always test and modify each step of the procedures to validate their results.

2.2 Technical note for the determination of heavy metals in seawater

Given no list of heavy metals has been agreed as mandatory for analysis in seawater, at this stage of IMAP implementation, Contracting Parties to the Barcelona Convention may decide to include in their seawater monitoring programmes the analysis of metals according to their national priorities. However, since Cadmium (Cd), Lead (Pb) and Total Mercury (THg) are the mandatory metals to be determined in marine sediment and biota samples in the framework of IMAP (UNEP/MAP, 2019a), it makes sense to include these contaminants in any voluntary seawater monitoring programme.

National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of the Contracting Parties, the IMAP Protocols were developed in order to be used as guidelines for the analysis of heavy metals in seawater samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

a) Determination of heavy metals

Seawater analysis could be performed using unfiltered or filtered (0.45 μm) seawater samples. If the analysis is performed on unfiltered seawater, the sample is analysed following directly the appropriate protocol. In this case a seawater sub-sample has to be filtered to record the suspended particulate matter (SPM) content. If both the filtered seawater and the relative SPM are analysed, the later has to be digested following the protocols for sediment digestion, as presented in Protocol for SPM digestion using nitric acid and hydrofluoric acid of the present Guideline.

Because of the expected dissolved metal concentration range (10^{-4}-10^{-6} mg kg^{-1}) and the salt matrix interference during the measurement process, preconcentration techniques and/or the elimination of sea salt has to be carried out prior to the analysis of the dissolved phase. For the analysis of the SPM retained in the filter, a first step of digestion is required, using an acid mixture (HCl, HNO_3 and HF). The determination of metals in seawater and digested SPM samples could be done with analytical techniques, such as GF-AAS, ICP-MS, ICP-AES (ICP-atomic emission spectrometry), electrochemical methods, or total-reflection X-ray fluorescence (TXRF).

Regardless of the analytical method used, heavy metal analysis follow some procedures common to all analytical methodologies, such as the calibration of the analytical equipment and the cleaning and handling procedures to avoid the contamination of the samples from the laboratory's environment and the tools and containers used in the analysis.

b) Calibration

Calibration standards should be prepared from single standard stock solutions or multielement standards by dilution of the stock solution using dilute acid, as required. All standard solutions have to be stored in polyethylene, borosilicate or quartz volumetric flasks. Standard solutions with lower concentrations, if prepared correctly and controlled in a QA system (checking of old versus new standards, and checking with standards from a different source), can be kept for a period no longer than one month.

The calibration procedure has to meet some basic criteria in order to give the best estimate of the true element concentration of the sample analysed (HELCOM, 2012a) (Annex XXXII):

i) The concentrations of standards for the preparation of the calibration curve (function) should cover the range of concentrations as related to practical conditions; the mean of the range should be roughly equal to the expected analyte concentration in the sample;

ii) The required analytical precision should be known and achievable throughout the entire range of concentrations;

iii) The measured value (instrument signal) at the lower end of the range has to be significantly different from the procedural analytical blank;

iv) The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation, i.e. the difference in density between the standard and environmental sample should be minimized (this is of particular importance in flame atomic absorption determinations);

The concentrations of standards for the preparation of the calibration curve should cover the range of concentrations as related to practical conditions; the mean of the range should be roughly equal to the expected analyte concentration in the sample.

c) Avoiding sample contamination

To avoid metal contamination in the laboratory all glassware and plastic vessels used should be carefully cleaned. The general cleaning guidelines include:

i) Allow the vessels to soak overnight in a plastic container in an alkaline surfactant solution (e.g., Micro solution 2% in tap or distilled water).

ii) Rinse thoroughly first with tap water then with ultrapure deionised water.

iii) Leave the vessels to stand in 10% (v/v) concentrated analytical grade HNO_3 solution at room temperature for at least 6 days.

iv) Rinse thoroughly with ultrapure deionised water (at least 4 times).

v) Allow the vessels to dry under a laminar flow hood.

vi) Store the vessels in closed plastic polyethylene zip-lock bags to prevent the risk of contamination prior to use.

This procedure should be used for all plastic ware use in the laboratory as tips, cup for autosampler, plastic containers.

Under this Technical Note, this Guidelines for sample preparation and analysis of sea water samples for heavy metals provides the following IMAP Protocols:

- Protocol for SPM digestion using nitric acid and hydrofluoric acid;
- Protocol for the determination of heavy metals in seawater with Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS);
- Protocol for the determination of heavy metals in seawater with Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS);
- Protocol for the determination of THg in seawater with Cold Vapour- Atomic Fluorescence Spectroscopy (CV-AFS).

These Protocols are based on Guidelines developed by GEOTRACES, HELCOM (2012a) (Annex XXXII), ICES/OSPAR (2012) (Annex XXXI) and US EPA (1995[12]) (Annex XXXIV). Analytical methods are also based on similar methods, which have been developed for other media (sediment) (IAEA, 2011a[13] (Annex VI) and 2011b[14] (Annex IX).

2.2.1 Protocol for SPM digestion using nitric acid and hydrofluoric acid

Suspended Particulate Matter (SPM) samples have to be digested prior to analysis. The rate of digestion and the efficiency of acid decomposition increase substantially with elevated temperatures and pressure, therefore microwave digestion in closed vessels is the preferred method. However, in case no such equipment is available, sample digestion over a hot plate is an alternative method. The digestion method dissolves completely the filter material, therefore it is of paramount importance to use a filter material with very low metal content, to avoid misinterpretation of the results (polycabonate or cellulose acetate).

The use of hydrofluoric acid (HF) is required for a complete disintegration of the silicate matter of SPM and the determination of the total metal load. Furthermore, Certified Reference Materials (CRMs) of sediments, which can be used also for SPM analysis, provide certified values for total metal concentrations, therefore their use to strengthen data quality assurance requires the measurement of the total metal content in SPM samples.

a) Microwave acid digestion in closed systems (for heavy metals for GFAAS and ICP-MS analysis)

SPM digestion can be performed in Teflon closed vials, under heat and pressure, following the methodology

[12] US EPA (1995). Method 1640: Determination of trace elements in ambient waters by on-line chelation preconcentration and Inductively Coupled Plasma Mass Spectrometry.

[13] IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content

[14] IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

proposed for sediments (Loring and Rantala, 1991[15]). Filters with SPM, with already known weight of SPM, are transferred to a Teflon vial inside a laminar hood compatible with acid fume. Then the protocol for sediment digestion is followed (IAEA, 2011a, Annex VI). Approximately 5 ml of nitric acid and 2 ml of hydrofluoric acid are added and each vessel and let to react for at least 1hour (or more if possible). After the room temperature pre-digestion, 2ml of hydrogen peroxide are added carefully, the vessels are closed and placed in the microwave apparatus and digestion steps are followed, following the IAEA's "Recommended method on microwave digestion of marine samples for the determination of trace element content" (IAEA 2011a). Because closed vessels retain the HF, boric acid is added after the HF digestion to complex the remaining HF and make the resulting solution less hazardous, as well as preventing aluminium fluoride precipitation. After digestion the vessels are removed from the microwave apparatus and placed in a ventilated fume hood to cool. When the pressure is adequate, the vessels are opened and their content is transferred to a volumetric flask and made to a known volume. All reagents used are analytical grade.

b) Acid digestion on a hot plate

A method for the digestion of filters and SPM using HF and HNO_3 in Teflon containers on a hot plate are proposed by GEOTRACES (2017).The use of HF is essential because it is the only acid that completely dissolves the silicate lattices and releases all the metals.

Digestion procedure with complete destruction of the filter material

i) Ideally, one filter is to be digested per digestion vial.
ii) 10% HF/50% HNO_3 (v/v) digest solution is recommended in order to achieve complete dissolution of all particle types, and in particular to bring all lithogenic material in solution.
iii) MF-Millipore filters are placed in the bottom of the vial because a complete digestion of the cellulose filter is achieved in under these conditions.
iv) 47 mm filters are cleanly cut in half using a ceramic blade scalpel, or rotary cutter and the halves placed on opposite sides of the vial for refluxing.
v) Typically, for a 25 mm diameter filter, add 1 mL of 50% HNO_3/10% HF solution to each vial. Roll acid around inside vial to ensure full contact with filter.
vi) Close the caps tightly and place vials on a Teflon or silicone surface hot plate at 130° C for 4 hours.
vii) After a cool down period, collect all the droplets from the cap and inside of the vials down to the bottom of the vial by either tapping the sealed vials or rolling the solution around.
viii) Dry down the solution on the hot plate at 130° C. Watch it until near dryness, reducing heat as necessary. Remove when droplet is reduced to <5 µL volume.
ix) This step reduces the HF in the sample and allows the matrix to be switched to dilute nitric acid for analysis. Heat lamps cleanly mounted above the hot plate may help prevent condensation on vial walls.
x) If desired, add 100 µL concentrated HNO_3, directly onto residual droplet, and dry down again to same size droplet. This ensures sufficient HF removal so that glass and quartz components of the introduction system of the analytical instrument are not etched or degraded.

2.2.2 Protocol for the determination of heavy metals in seawater with GF-AAS

In seawater Al, Cd, Pb, Cu, Cr, Ni, as well as other metals, can be determined by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), which has adequate sensitivity for these determinations. Direct analysis of seawater is limited by very low metal concentrations and spectral and non-spectral interferences caused by the sea water matrix, therefore a preconcentration step for matrix removal is often used before analysis.

Prior to analysis, dissolved metals can be pre-concentrated on Chelex-100 resin (Kingston, et al, 1978[16]). The pH of the seawater samples is adjusted to 5 – 5.5 and the sample is passed through a Chelex-100 resin. Alkali and alkaline earth metals are then eluted from the resin with ammonium acetate (CH_3COONH_4) and the trace elements are eluted with two 5 ml aliquots of 2.5 M HNO_3. The whole processing of seawater samples, including metal pre-concentration has to be done under clean conditions (ISO Class 5 clean room) taking precautions to avoid any metal contamination of the samples (appropriate clothing including gloves). All reagents are analytical grade. The pre-concentration system consists of a column of a chelating resin, a sample loop constructed for a narrow-bore, high pressure inert tubing (such as ethylene tetra-fluoroethylene - ETFE), an eluent pumping system to deliver one or two eluents, argon gas supply and solution reservoirs (US EPA Method 1640, 1995; Annex XXXIV).

[15] Loring DH and Rantala RTT (1991). Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Review, 32: 235:283. Elsevier Science Publishers B.V

[16] Kingston, H.M., Barnes, I.L., Brady, T.J., Rains, T.C., and Champ, M.A. (1978). Separation of eight transition elements from alkali and alkaline earth elements in estuarine and seawater with chelating resin and their determination by graphite furnace atomic absorption spectrometry. Analytical Chemistry, 50 (14): 2064-2070.

Automatic pre-concentration of metals in seawater can be achieved using the SeaFAST system, which improves elemental detection limits in undiluted seawater by both preconcentrating analyte and eliminating matrix components. The system can be operated off-line using a chelation column to pre-concentrate metals prior to analysis.

The pre-concentrated seawater sample is then analysed for heavy metals by GF-AAS, following the analytical protocol prepared by IAEA (2011b) presented in the Annex IX, Analysis of trace metals in biological and sediment samples

2.2.3 Protocol for the determination of heavy metals in seawater with ICP-MS

Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS) is currently state-of-the-art instrumentation for metal analysis, with the possibility to determine at sub-µg/L concentrations of a large number of elements in water. However, direct analysis of seawater is limited by spectral and non-spectral interferences caused by the sea water matrix, therefore a preconcentration step for matrix removal is often used before analysis.

ICP-MS allows a rapid analysis of a wide range of heavy metals. Most routine instruments utilize a quadrupole mass spectrometer, so mass resolution is not high enough to avoid overlap of double charged elements or multi-element ions (mainly hydrides, oxides and hydroxides) formed in the plasma. The main concern is for the Argon (Ar) interferences as the plasma is usually an argon plasma, overlapping with As. Some elements are prone to memory effects (particularly Hg) and needs extra precautions to avoid carry over effects (HELCOM 2012a).

A multi-elemental determination of heavy metals by ICP-MS in water samples is described in the US EPA Method 1640 (1994). The method includes a first preconcentration step with a chelating resin (i.e. Chelex 100) using a system consisting of a column with the chelating resin, a sample loop constructed for a narrow-bore, high pressure inert tubing, an eluent pumping system to deliver one or two eluents, argon gas supply and solution reservoirs. The preconcentration system is linked with the ICP-MS for metal determination The US EPA Method 1640 is presented in Annex XXXIV. The automate SeaFAST metal preconcentration system can be operated in-line, linked to the ICP-MS.

2.2.4 Protocol for the determination of Total Mercury in seawater with CV-AFS

Total mercury in seawater can be determined efficiently using Cold Vapour Atomic Fluorescence Spectroscopic (CV- AFS) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (with isotope dilution). Cold Vapour Atomic Absorption Spectrometry (CV-AAS) is not a preferable method for Mercury determination because according to GEOTRACES's (2017) intercalibration exercises, the method does not exhibit adequate sensitivity to detect total Hg. CV-AFS has the advantage to allow rapid determination of total Hg and DGM (Hg^o + $(CH_3)_2Hg$) at sea, while ICP-MS has the potential for a lower absolute detection limit. A recommended Hg workflow for the determination of total Hg in seawater with CV-AFS is presented in the Sampling and Sample-handling Protocols for GEOTRACES Cruises (GEOTRACES, 2017).

2.3 Technical note for the determination of organic contaminants in seawater

As already elaborated above for metals, the Contracting Parties to the Barcelona Convention may decide to include in their seawater monitoring programmes the determination of organic contaminants according to their national priorities, given no list of organic contaminants has been agreed as mandatory for analysis in seawater, at this stage of IMAP implementation. However, since chlorinated hydrocarbons and PAHs are mandatory contaminants to be determined in marine sediments and biota in the framework of IMAP (UNEP/MAP, 2019a; UNEP/MAP 2019b), it makes sense to include these contaminants in any voluntary seawater monitoring programme.

Same analytical methods can be used for the determination of lipophilic pollutants in extracts of water samples as are used for extracts of sediments. However, the distribution of contaminants in seawater are influenced by their polarity. Therefore, more hydrophilic organic compounds (such as 2- and 3-ring PAHs and HCH isomers) are distributed in the dissolved phase, while more lipophilic compounds (such as 4- to 6-ring PSHs, DDT group and PCBs) are mainly found in SPM.

In monitoring programmes, total seawater (unfiltered) is usually analysed for organic contaminants. The analytical procedure includes the simultaneous extraction of organic contaminants from seawater, clean-up and analytical determination. The extraction of the organic contaminants is also concentrating the compounds enabling their enrichment in the solution to be analysed. This is an important step, since the concentrations of the organic contaminants is total seawater are extremely low (from 10 pg L^{-1} to 10 ng L^{-1}, HELCOM, 2012b). Extraction can be done by liquid-liquid extraction (LLE) (using a non-polar solvent such as hexane) or by solid-phase extraction (SPE). It has to be emphasized that all steps of the procedure are susceptible to insufficient recovery and/or contamination. Therefore, regular quality control procedures must be applied to check the performance of the whole method.

A description of the procedures for the extraction seawater by liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are presented in the "Technical Note on the determination of persistent organic pollutants in seawater" of HELCOM (2012b) (Annex XXXIII). It is noted that the SPE has the advantage of being able to extract very large water volumes (up to 1000 l) and to incorporate a phase separation to obtain separate samples for SPM and the solute phase. However, the method requires longer sampling time, more complex instrumentation, and problems with validation and control of the extraction efficiency. On

the other hand, the LLE has the advantage that it can be easily validated and controlled, as internal standards can be added before extraction. The limitation in sample volume is only relative, since sampling volume of 100 l is sufficient for nearly all monitoring tasks. HELCOME (2012b) concludes that "Because of the robustness of the method, there is a preference LLE for routine monitoring purposes for all lipophilic organic contaminants".

Although there are less interferences from matrix compounds in seawater samples than in sediments or biota, extracts require a clean-up before the chromatographic separation and determination. A clean-up procedure using short silica gel chromatography columns that can be applied with GC-ECD and GC-MS methods, is proposed by HELCOM (2012b), using silica dried at 200° C and subsequently washed with CH_2Cl_2 and hexane. The hexane sample extract is applied on top of the column and eluted with CH_2Cl_2/hexane and then with acetone. Fraction 1 contains all lipophilic compounds of interest (PAHs and all chlorinated hydrocarbons (from HCB to HCH)); this fraction can be used for GC-MS determination after concentration to 50–300 µl. All reagents are of analytical grade.

Following the simultaneous extraction and clean-up, the determination of organochlorine pesticides - PCBs and PAHs will be done following the respective analytical procedures. National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). The here-below proposed IMAP analytical Protocols are based on the HELCOM (2012b) (Annex XXXIII) guidelines for organic contaminants (chlorinated hydrocarbons and PAHs) analysis in seawater and the analytical method developed by UNEP/IAEA (2011[17]) (Annex XIV), for the analysis of chlorinated hydrocarbons in sediment. Analytical laboratories should accommodate, test and modify each step of the procedures presented in here-below provided IMAP Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

Under this Technical Note, this Guidelines for sample preparation and analysis of sea water samples for organic compounds provides the following IMAP Protocols:

- Protocol for the determination of organochlorine pesticides and PCBs in seawater using Gas Chromatography - Electron Capture Detector (GC-ECD) or Gas Chromatography - Mass Spectroscopy (GC-MS);
- Protocol for the determination of PAHs in seawater using Gas Chromatography - Mass Spectroscopy (GC-MS).

These protocols are based on Analytical Methods developed by UNEP/IAEA (2011b): Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71, HELCOM (2012b): Manual for marine monitoring in the COMBINE programme. Annex B-11, Appendix 2. Technical note on the determination of persistent organic pollutants in seawater and ICES/OSPAR (2012): JAMP Guidelines for monitoring contaminants in seawater.

2.3.1 Protocol for the determination of organochlorine pesticides and PCBs in seawater using GC-ECD or GC-MS

Following extraction and clean-up, as described in the Technical Note for the determination of organic contaminants in seawater, organochlorine pesticides and PCBs can be determined by GC-ECD or GC-MS following the guidelines for the analysis of sediment and biota matrices proposed by UNEP/IAEA (2011c) (Annex XIV), HELCOME (2012b) (Annex XXXIII) and ICES/OSPAR (Annex XXXI).

2.3.2 Protocol for the determination of PAHs in seawater using GC-MS

Following extraction and clean-up, as described in the Technical Note for the determination of organic contaminants in seawater, PAHs can be analysed by GC-MS following the guidelines for the analysis of sediment and biota matrices proposed by HELCOME (2012b) (Annex XXXIII) or ICES/OSPAR (Annex XXXI).

3 References

- EC (2009). Commission Directive 2009/90/EC laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status.
- GEOTRACES (2017). Sampling and Sample-handling Protocols for GEOTRACES Cruises (Version 3), edited by the 2017 GEOTRACES Standards and Intercalibration Committee.
- HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-11, Appendix 1. Technical Note on the determination of trace metals (Cd, Pb, Cu, Co, Zn, Ni, Fe) including mercury in seawater.
- HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-11, Appendix 2. Technical note on the determination of persistent organic pollutants in seawater.

[17] UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71

- IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content
- IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace
- ICES/OSPAR (2012). JAMP guideline on monitoring of contaminants in seawater: Annex 1: Guidelines for Monitoring of Contaminants in Seawater. ICES Advice 2012, Book 1
- Kingston, H.M., Barnes, I.L., Brady, T.J., Rains, T.C., and Champ, M.A. (1978). Separation of eight transition elements from alkali and alkaline earth elements in estuarine and seawater with chelating resin and their determination by graphite furnace atomic absorption spectrometry. Analytical Chemistry, 50 (14): 2064-2070.
- Loring DH and Rantala RTT (1991). Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Review, 32: 235:283. Elsevier Science Publishers B.V
- Pohl, C. 1997. Trace Metals (Cu, Pb, Zn, Cd, Al, Li, Fe, Mn, Ni, Co) in Marine Suspended Particulate Matter: An International ICES Intercomparison Exercise. Accreditation and Quality Assurance, 2: 2-10
- Quémerais, B., and Cossa, D. 1997. Procedures for sampling and analysis of mercury in natural waters. Environment Canada-Quebec region, Environmental Conservation, St. Lawrence Centre. Scientific and Technical Report ST-31E, 34 pp.
- Sturgeon, R., and Berman, S. 1987. Sampling and storage of natural water for trace metals. In Critical reviews in Analytical Chemistry. 18(3): 209-244. CRC Press.
- UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71
- UNEP/MAP (2019a). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27.
- UNEP/MAP (2019b). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution.
- US EPA (1995). Method 1640: Determination of trace elements in ambient waters by on-line chelation preconcentration and Inductively Coupled Plasma Mass Spectrometry.