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Agenda item 12: Harmonization and standardization of IMAP Pollution Cluster Monitoring

Monitoring Guidelines/Protocols for Sampling and Sample Preservation of Seawater for IMAP Common Indicator 17: Heavy and Trace Elements and Organic Contaminants

UNEP/MAP
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Note by the Secretariat

In line with the Programme of Work 2020-2021 adopted by COP21 the MED POL Programme has prepared the Monitoring Guidelines related to IMAP Common Indicators 13, 14, 17 and 20 for consideration of the Integrated Meeting of the Ecosystem Approach Correspondence Groups on Monitoring (December 2020), whilst the Monitoring Guidelines for Common Indicator 18, along with the Monitoring Guidelines related to data quality assurance and reporting are under finalization for consideration of the Meeting on CorMon on Pollution Monitoring planned to be held in April 2021.

These Monitoring Guidelines present coherent manuals to guide technical personnel of IMAP competent laboratories of the Contracting Parties for the implementation of the standardized and harmonized monitoring practices related to a specific IMAP Common Indicator (i.e. sampling, sample preservation and transportation, sample preparation and analysis, along with quality assurance and reporting of monitoring data). For the first time, these guidelines present a summary of the best available known practices employed in marine monitoring by bringing integrated comprehensive analytical practices that can be applied in order to ensure the representativeness and accuracy of the analytical results needed for generation of quality assured monitoring data.

The Monitoring Guidelines/Protocols build upon the knowledge and practices obtained over 40 years of MED POL monitoring implementation and recent publications, highlighting the current practices of the Contracting Parties' marine laboratories, as well as other Regional Seas Conventions and the EU. A thorough analysis of presently available practices of UNEP/MAP, UNEP and IAEA, as well the HELCOM, OSPAR and European Commission Joint Research Centre was undertaken to assist an innovative approach for preparation of the IMAP Monitoring Guidelines/Protocols.

The Monitoring Guidelines/Protocols also address the problems identified during realization of the Proficiency testing being organized by UNEP/MAP-MEDPOL and IAEA for two decades now, given that many unsatisfactory results within inter-laboratory testing may be connected to inadequate laboratory practices of the IMAP/MEDPOL competent laboratories.

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 the implementation of a monitoring programme for the determination of heavy metals and organic contaminants in seawater is a country-based decision. In order to support national efforts, this Monitoring Guidelines provides one Technical Note for the sampling and pretreatment of seawater for the analysis of heavy metals and organic contaminants, which includes the following six Protocols: i))Protocol for seawater sampling for heavy metals analysis; ii) Protocol for seawater filtration (heavy metals); iii) Protocol for the on-board storing of seawater samples for heavy metal analysis; Protocol for seawater sampling for organic contaminants analysis; iv) Protocol for seawater filtration (organic contaminants); and v) Protocol for the on-board storing of seawater samples for organic contaminants analysis.

The Monitoring Guidelines/Protocols, including this one related to sampling and sample preservation of seawater for the analysis of IMAP Common Indicator 17, establish a sound ground for further regular update of monitoring practice for the purpose of successful IMAP implementation.

In accordance with the Conclusions and Recommendations of the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (CORMONs) (Videoconference, 1-3 Dec. 2020), and in particular paragraph 22, this Meeting requested the Secretariat to amend this Monitoring Guideline by addressing agreed technical proposals that were described in the Report of the Meeting in line with its agreement to proceed with submission of this document to the Meeting of MEDPOL Focal Points. Requested amendments included technical written suggestions that were provided by several Contracting Parties up to 10 days after the Integrated Meeting of CORMONs. The amended document was shared by the Secretariat on 19 February 2021 for a period of 2 weeks for the non-objection by the Integrated Meetings of CORMONs on the introduced changes. Further to no objection from the Integrated Meeting of CORMONs, this Monitoring Guideline is submitted for consideration of present Meeting of MEDPOL Focal Points.

List of Abbreviations / Acronyms

CI	Common Indicator
COP	Conference of the Parties
CORMON	Correspondence Group on Monitoring
EcAp	Ecosystem Approach
EEA	European Environmental Agency
EC	European Commission
EU	European Union
FAO	Food and Agriculture Organization of the United Nation
GEOTRACES	An international study of the marine biogeochemical cycles of trace elements and isotopes
HELCOM	Baltic Marine Environment Protection Commission - Helsinki Commission
IAEA	International Atomic Energy Agency
IOC	International Oceanographic Commission
IMAP	Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria
MAP	Mediterranean Action Plan
MED POL	Programme for the Assessment and Control of Marine Pollution in the Mediterranean Sea
MED QSR	Mediterranean Quality Status Report
OSPAR	Convention for the Protection of the Marine Environment for the North-East Atlantic
PoW	Programme of Work
QA/QC	Quality Assurance/Quality Control
QSR	Quality Status Report

1 Introduction

1. 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 2019a[1],UNEP 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.

2. 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.

3. 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.

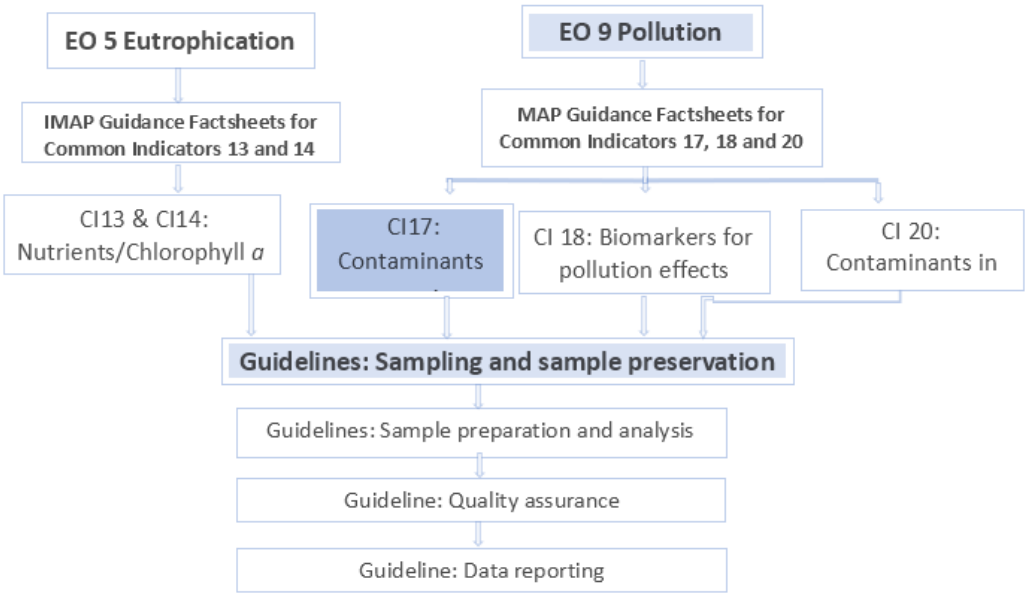
4. To assist countries which plan to include seawater monitoring in their respective national monitoring programmes for CI17, as a country-based decision, Protocols for seawater sampling and sample processing have been prepared. They are not intended to be analytical training manuals, but guidelines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

5. 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.

6. 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 I to III. 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.

7. The below flow diagram informs on the category of this Monitoring Guideline related to related to sampling and sample preservation of seawater for the analysis of IMAP Common Indicator 17 within the structure of all Monitoring Guidelines prepared for IMAP Common Indicators 13, 14, 17, 18 and 20.

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



Flow Diagram: Monitoring Guidelines for IMAP Ecological Objectives 5 and 9

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

8. 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.

9. 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 I) 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 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]).

10. 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

[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
[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.

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.

11. 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]).

12. Detailed Guidelines for seawater sampling and processing can be found in documents issued by ICES/OSPAR (2012) (I), HELCOM (2012a[7]) (Annex II), HELCOM (2012b[8]) (Annex III) 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 analysis;
- Protocol for seawater filtration (heavy metals);
- Protocol for the on-board storing of seawater samples for heavy metal analysis;
- Protocol for seawater sampling for organic contaminants analysis;
- Protocol for seawater filtration (organic contaminants);
- Protocol for the on-board storing of seawater samples for organic contaminants analysis.

2.1 Protocol for seawater sampling for heavy metal analysis

a) Sampling equipment for seawater collection

13. 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.

14. 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.

15. 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.

16. Use sampling equipment (such as GO-FLO or Niskin style bottle with a capacity 12-30 l) attached individually at hydrographic wire or placed in a metal-free rosette system (Figure 1).

[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.

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

17. 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.

18. 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).

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

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

20. 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)

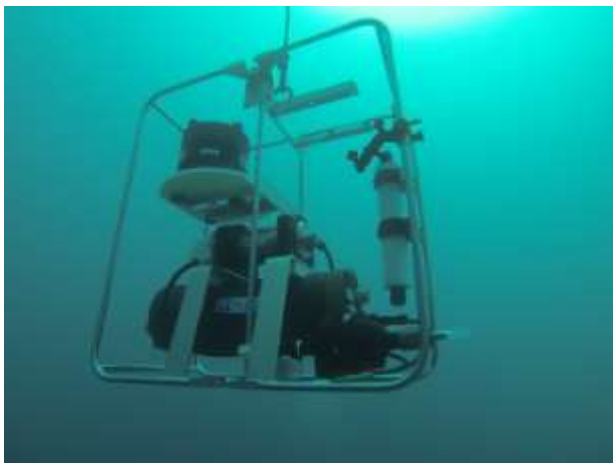


Figure 2. In-situ seawater pumping system (Marine Environmental Studies Laboratory, IAEA)

21. 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

22. 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

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

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 ^oC) 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).

24. 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 ^oC. 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.

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

2.2 Protocol for seawater filtration for heavy metals analysis

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

26. 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

27. 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).

28. 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.

[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.

b) Filters

29. 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]).

30. 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]).

31. 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.

32. 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

33. 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.

34. 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 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.

35. 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

[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.

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).

2.3 Protocol for the on-board storing of seawater samples for heavy metals analysis

36. 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.

37. Seawater samples (filtered or unfiltered, if total metals are to be analysed) 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 ºC 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 analysis, in addition of acidification oxidation agents should be added (such as $Cr_2O_7^{2-}$). (HELCOM, 2012a)

38. 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

39. 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 80ºC for 5 hours;

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

2.4 Protocol for seawater sampling for organic contaminants analysis

a) Sampling equipment for seawater collection

40. 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 I). 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.

41. For seawater sampling or the analysis 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.

42. Glass bottles are an appropriate sampling equipment for the analysis 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)

43. 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.

44. 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

45. 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).

46. 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.

2.5 Protocol for seawater filtration for organic contaminants analysis

a) Filtration/extraction procedure

47. 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

48. 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

49. 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.

50. 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

51. 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

52. 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.

53. 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.

54. 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.

2.6 Protocol for on-board storage of seawater samples for organic contaminants analysis

55. 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).

56. Suspended Particulate Matter (SPM) samples after filtration should be refrigerated (-20 ^{o}C) and kept stored frozen until further analysis.

Annex I

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, (3.1.1)

1.5.5.4 Special request, Advice May 2012

ECOREGION General advice
SUBJECT Development of a JAMP guideline on monitoring of contaminants in seawater

Advice summary

ICES has developed a guideline document on monitoring of contaminants in seawater under the Joint Assessment and Monitoring Programme (JAMP) (Annex 1). The document also includes a technical annex on specifics of suitable sampling equipment. ICES advises that the document is included in the JAMP guidelines.

Request

Development of a JAMP guideline on monitoring of contaminants in seawater (OSPAR 2011/1)

To develop the general text for a JAMP guideline on monitoring contaminants in seawater, which could act as the overarching chapeau to technical annexes concerning specific substances. The technical annex on analysis of PFC compounds in seawater developed by ICES in 2009 is the first such document. The development of the overarching text should take into account the need to address the following issues: purposes; quantitative objectives; sampling strategy; sampling equipment; storage and pre-treatment of samples; analytical procedures; analytical quality assurance; reporting requirements.

ICES advice

ICES has developed guidelines for monitoring of contaminants in seawater (Annex 1), complementing the corresponding JAMP Guideline for Monitoring of Contaminants in Sediment and JAMP Guideline for Monitoring of Contaminants in Biota. The guideline document in Annex 1 covers monitoring for organic contaminants and trace metals and is structured along the sections outlined in the request (purposes, quantitative objectives, sampling strategy, sampling equipment, storage and pre-treatment of samples, analytical procedures, analytical quality assurance, and reporting requirements). In addition, an annex to the guideline has been developed on technical specifics of the sampling equipment suitable for subsequent analysis of organic contaminants and trace metals. The document includes references to the EU Water Framework Directive (WFD) and EU Marine Strategy Framework Directive (MSFD) where applicable.

ICES advises that this document is included in the JAMP guidelines.

Source

ICES. 2012. Report of the Marine Chemistry Working Group (MCWG), 20–24 February 2012, Southampton, UK. ICES CM 2012/SGHIE:05.

Annex 1: Guidelines for Monitoring of Contaminants in Seawater

1. Introduction

These guidelines provide advice on the sampling and analysis of seawater, for determination of trace metals and organic contaminants, including oceanic, coastal, and estuarine waters. Monitoring contaminants in seawater is a complex task which requires carefully designed and conducted sampling campaigns, appropriate sampling equipment and its correct handling, as well as suitable pre-treatment and storage methods for the analytes in question. There are numerous steps that will affect data quality prior to the chemical analysis itself.

Contaminants in seawater can originate from direct point sources, riverine discharges, and atmospheric dry and wet deposition. Their distribution in seawater depends on the physical-chemical characteristics of the compound or element, interactions with the water matrix, sediment and biota as well as hydrographical conditions, such as mixing of water masses. Organic contaminants and metals can occur freely dissolved in water, bound to colloids, or suspended particulate matter. Trace metals can form complexes with organic or inorganic material. This partitioning is the result of environmental conditions and the partitioning may change during sampling and storage, and has implications for analysis and interpretation.

These guidelines are general recommendations on contaminant monitoring in seawater. The techniques described are useful for routine monitoring and ship/campaign-based work. However, this guideline is not intended as a complete laboratory manual. Requirements for specific contaminants or contaminant groups should be further specified by expert groups, for example in associated technical annexes, in order to meet the objectives of the monitoring programme and to ensure consistent and comparable data sets.

2. Purposes

Monitoring of contaminants in seawater of the Northeast Atlantic Ocean is performed within the framework of OSPAR as the regional convention for the protection of the marine environment of this area. OSPAR monitoring also can assist member states of the European Union to fulfil their obligations under the relevant EU directives, such as the Marine Strategy Framework Directive (MSFD) (EU, 2008) and the Water Framework Directive (WFD) (EU, 2000) with its related directives such as the daughter directive on Environmental Quality Standards in the field of water policy (2008/105/EC).

One of the aims of OSPAR's Hazardous Substances Strategy is that concentrations of naturally occurring chemicals should approach background concentrations, and concentrations of man-made chemicals should be zero. Progress on the implementation of this strategy is monitored through the Joint Monitoring and Assessment Programme (JAMP) of chemicals for priority action and hazardous substances in general. The main objectives of the JAMP for the period 2010–2014, which seek to support the implementation of the OSPAR strategies and the EU MSFD are:

1. the continued implementation and development of existing OSPAR monitoring programmes and, where necessary, the development of additional coordinated monitoring programmes to take account of criteria, methodological standards and indicators for good environmental status, and the pressures and impacts of human activities;
2. development of tools for the delivery of integrated environmental assessments of the OSPAR maritime area or its regions, linking human activities, their pressures, the state of the marine environment, and management responses. Where relevant, these tools should support the exploration of new and emerging problems in the marine environment;
3. the preparation of integrated environmental assessments of the implementation of the OSPAR strategies, including in particular the assessment of the effects of relevant measures on the improvement of the quality of the marine environment. Such assessments will provide additional information and assessments in respect of the MSFD, enhance the OSPAR quality status reports (QSRs), take into account the Directive's obligations for regional cooperation, and help inform the debate on the development of further measures.

Aqueous inputs (direct or riverine) of contaminants, together with atmospheric deposition, are important sources of contaminants to OSPAR marine waters. Dynamic equilibria exist between the dissolved fractions of the total burden of contaminants, such that contaminants are partitioned between the dissolved state and particulate and colloidal phases in the water column, as well as becoming associated with bottom sediments and biota. The rates of exchange of contaminants between the water and the sediment or biota mean that changes in inputs are likely to be reflected more rapidly in the water than in, for example, bottom sediments. However, this sensitivity to change, and the partitioning between components of the aqueous phase, are also reflected in relatively high spatial and temporal variances in the observed concentrations. The selection of water as a monitoring matrix can therefore be appropriate for a number of reasons. These include the ability to observe short-term variations in contaminant pressure on organisms. Focusing on contaminants that partition strongly into the water rather than the sediment or biota can lead to water being the preferred

matrix for monitoring. OSPAR background documents on chemicals for priory action may provide valuable information with regard to the preferred monitoring matrix. In the context of the JAMP, coordinated monitoring of contaminants in seawater may be carried out in relation to the temporal changes in the degree of pollution, its spatial variation, or as an element of integrated monitoring and assessment of contaminants and biological effects.

Temporal trend monitoring can assess the effectiveness of measures taken to reduce contamination of the marine environment. The statistical assessment of a trend over a longer period also supplies a more reliable assessment for the environmental status within a certain period. The fitted value of the last year measured has been used in OSPAR CEMP assessments as the optimum value for comparing against assessment criteria and hence for assessment of the actual environmental status. In such a way, the within- and between-year variability is taken into account.

Spatial distribution monitoring can describe the existing level of marine contamination widely through the convention area. The measured levels can be compared to background or close to background concentrations, as well as to levels describing thresholds below which no chronic effects are expected to occur in marine species, i.e. environmental assessment criteria (OSPAR, 2009).

Contaminant analysis of seawater can be an element of integrated monitoring and assessment, where chemical and biological effects measurements are combined, in order to assess potential harm to living resources and marine life (OSPAR, 2012). The role of chemical measurements in integrated chemical and biological effects monitoring programmes is to support biological effects programmes by providing information to help identify the chemical causes of observed biological effects. In general, chemical measurements in seawater should contribute to improve and extend OSPAR's monitoring framework and better link it with the understanding of biological effects and ecological impacts of individual substances and the cumulative impacts of mixtures of substances.

Furthermore, beyond the objectives of the JAMP, monitoring of contaminants in water can provide information on the fate of contaminants in the environment, e.g. transformation, partitioning, and transport processes.

3. Quantitative objectives

Seawater monitoring should provide concentrations of target analytes in water, which are representative of the location and time of sampling. General considerations regarding the specification of quantitative objectives for monitoring are given in the JAMP (OSPAR, 2010). More specifically, the following issues should be considered prior to water monitoring: contaminant speciation, detection limits, detectability of temporal and spatial trends, and costs.

3.1. Contaminant speciation

Trace metals and organic contaminants can exist as freely dissolved species in water or bound to colloids and suspended particulate matter (SPM). Trace metals can also exist as inorganic and organic complexes. The targeted contaminant fraction determines which sampling and/or pre-treatment method to use:

- Analysis of unfiltered water samples yields the sum of the concentrations of contaminants that are freely dissolved, complexed, and bound to colloids and SPM. These samples are also referred to as total water or whole water samples.
- Filtered water samples can yield the concentrations in SPM (by analysis of the residue on the filter) and the concentrations of contaminants that are freely dissolved, complexed, and bound to colloids (filtrate). However, many organic contaminants are known to exchange freely between dissolved and other phases in the water. The removal of components of the particulate matter is very likely to alter the position of these equilibria, while the introduction of filter material, container walls, etc. provides additional phases taking part in the equilibration processes. The complete separation of dissolved, colloidal, particulate matter is therefore a difficult task.
- Passive sampling yields the concentrations of freely dissolved contaminants (organics) or freely dissolved and complexed contaminants (trace metals).

The choice of the targeted contaminant fraction may be pre-defined by legal obligations. For example, monitoring under the Water Framework Directive requires the monitoring of metal concentrations in filtered water, and of organic contaminants in total (i.e. unfiltered) water.

3.2. Detection limits

The sample size has to be sufficient to support the desired detection limits for the contaminants of interest, for example to enable descriptions of spatial and temporal trends. For example, one litre discrete water samples may be sufficient for time trend monitoring of PAHs in contaminated harbours, but may be insufficient for monitoring programmes in open waters. For consistency with Commission Directive 2009/90/EC, a limit of quantification (LOQ) should be equal to or below a value of 30% of the relevant assessment criterion, e.g. the Environmental Quality Standard.

3.3. Statistical significance and power

In the context of temporal trend monitoring, it is important to know the statistical power of a time-series to detect changes, i.e. the probability of detecting true trends in concentration in the presence of variance associated with sampling, analysis, and field variability. The necessary or possible power of a monitoring programme will vary with the contaminant and area being investigated. One approach would be to estimate the power of the time series based on the "random" between-year variation. Alternatively, the lowest detectable trend could be estimated at a fixed power. A quantifiable objective could be to detect an annual change (dC/dt) of 5% within a time period of 6 years with a power of 90% at a significance level (α) of 5%. In the case of an expected decrease, the null hypothesis would be chosen as $dC/dt=0$ and the alternative hypothesis as $dC/dt< 0$.

A spatial monitoring programme should enable Contracting Parties to describe the distribution of contaminant concentrations in the survey area, for example to draw maps. These data can provide information to assist in the identification of representative stations for temporal trend studies, or for refinement of spatial surveys, and to implement measures where considered necessary. Statistical procedures can be used to estimate the number of samples and sampling sites needed to meet the required confidence level (i.e. to avoid Type I errors) and statistical power (to avoid Type II errors).

3.4. Costs

The concentrations of contaminants in water, as determined by discrete sampling, are commonly found to be quite variable, both in space and time, and meeting ambitious quantitative objectives may require extensive replication. Seawater sampling for contaminant analysis often requires equipment that is expensive to buy and maintain in good condition to keep the process blanks at low levels. The need for, and cost, of replicate water samples should be carefully considered in determining achievable quantitative objectives for a water-based monitoring programme. Therefore, it is often necessary to balance the scope and performance of monitoring programmes with available budgets.

4. Sampling strategy

The sampling strategy should reflect the purpose of the monitoring programme according to the JAMP (OSPAR, 2010) in relation to the OSPAR Hazardous Substances Strategy. Where applicable, the sampling strategy should consider requirements of the EU WFD (EU, 2000) and MSFD (EU, 2008); in all cases the quantitative objectives of the monitoring programme should be met (see Section 3). In accordance with the JAMP Guideline on Integrated Monitoring of Contaminants and Their Effects, seawater sampling should be carried out at the same time and locations as the sampling of other matrices (sediment, biota) and biological effects measurements (OSPAR, 2012).

A coherent approach to the detailed definition of a sampling strategy should take into account knowledge of the physical and biological oceanography of the area and requires consideration of temporal sources of field variance, such as seasonal factors, and spatial factors, such as the changes in location and water depth within the survey area. The analyte in question (its physical-chemical characteristics and expected concentration), as well as environmental conditions and practicalities, will further determine how samples are taken, e.g. what equipment is used and what volumes are required. However, sampling strategies also include compromises between scientifically advisable approaches and the economical and logistical frames of the sampling effort (see Section 3). It is therefore important that the objectives of monitoring programmes are expressed in quantitative terms and that they are achievable.

4.1. Temporal trend monitoring

The ability of a programme to identify temporal trends strongly depends on the extent to which unwanted sources of variability can be controlled. The short-term (< 1 year) temporal variability of contaminant concentrations in water is potentially very large. Concentrations may be subject to day-night variations in input and removal processes (Jaward *et al.*, 2004). In addition, concentrations at a fixed geographical position may vary over the tidal cycle (e.g. in estuaries). Further temporal variability may arise from variation in local inputs, such as discharges from ships, seasonality in the riverine discharge, changes in atmospheric deposition during rainfall events, and seasonal differences in seawater stratification. Some measures can be taken to reduce short-term temporal variability. These include sampling at pre-defined times of the year and at the same phase of the tidal cycle (e.g. always at high tide), although for ship-based discrete sampling it should be recognized that logistic constraints do not always allow such measures to be taken.

4.2. Spatial distribution monitoring

Analyte concentrations in seawater will vary between locations and with water depth, due to various physical and biogeochemical processes and the distribution of inputs. The expected spatial variability is an important factor in the development of an adequate geographical sampling scheme, i.e. the outline of the station grid and its vertical resolution (Brügman and Kremling, 1999). It should be recognized that the identification of spatial patterns may be obscured by

temporal variability (see Section 3.1), and that the same measures to reduce this source of variability also apply here. If the aim of the programme is to identify local sources of contaminants, then the sampling grid should be denser in the vicinity of suspected sources. Often, the variability of salinity or SPM content of the water can give an indication of the variability of pollutants and may even act as "normalization" factors.

4.3. Sampling method considerations

The proportion of the total concentration of a contaminant which is freely dissolved in the water phase increases with polarity of the pollutants (see Section 3). On the other hand, non-polar pollutants sorb to SPM and sediments and are thereby removed from the water column by sedimentation. For these contaminants, additional factors that should be taken into account are the SPM content and the volume of water that is sampled (see Section 3). These factors are important in filtration-extraction methods because the particle-bound and colloidally bound contaminant fractions that escape phase separation depend on the extent of filter clogging (Hermans *et al*., 1992). The measurement of SPM concentrations is even more important for monitoring contaminants in total water. The required water volume should be estimated before the sampling campaign, taking into account the method detection limits (see Section 3).

4.4. Supporting data

It is important that as much information as possible is collected concerning the waterbody being sampled. This includes co-factors such as salinity, SPM concentrations, and temperature. Whenever possible, sampling should be done as part of an integrated monitoring programme that includes the measurement of biological effects. These data should be obtained at the same time and locations as sampling for contaminant analysis.

4.5. Statistical considerations

Prior to starting a full-scale monitoring study, the available information on temporal variability should be carefully evaluated, possibly amended by a small-scale pilot programme. This evaluation should include a statistical assessment certifying that the objectives of the monitoring study can be met (see Section 3).

If no previous information exists, the sampling strategy can be based on a combination of general statistical principles and expert knowledge about sources and fate of the studied substances in the investigated sea basin. The statistical approach could include the principles of stratified sampling: First, the sampling area under consideration is partitioned into smaller more homogeneous areas, so-called strata. This can be based on simple information, such as depth, distance to land, or measured or modelled salinity. A successful stratification is characterized by a small variation of the measured concentrations within each stratum and a substantial variation between strata. For optimal allocation of the samples, the size (volume or area) of each stratum should be determined. Assuming that there are m strata with volumes $V_1, \ldots, V_m$ and that the standard deviation of the target variable is about the same in all strata, the number of samples n_j in stratum j shall be taken approximately proportional to the volume V_j, i.e.

$$n_j \approx n\frac{V_j}{V}$$

where V is the total volume of the investigated sea basin and n is the total number of samples.

If the standard deviation of the target variable varies from stratum to stratum, more samples should be taken in strata with high standard deviation. More specifically, the sample numbers chosen should aim at making n_j proportional to S_jV_j, where S_j is the standard deviation in the jth stratum, i.e. letting

$$n_j \approx n\frac{S_jV_j}{\sum_{j=1}^{m} S_jV_j}$$

Finally, the average concentration in the study area is estimated to be

$$\sum_{j=1}^{m} V_j\overline{X}_j / V$$

where $\overline{X}_j$ is the average observed concentration in the jth stratum.

4.6. Discrete sampling versus time-integrated sampling

Concentrations of contaminants in water respond quickly to changes in inputs and other environmental conditions, unlike concentrations in sediments and biota. This low level of time integration can be of advantage in detecting peak events but, on the other hand, concentrations in water are likely to show relatively high variability, which can have drawbacks in long-term monitoring and may require high sampling frequencies, causing high costs.

The influence of temporal variability may be reduced by time-integrated sampling. However, continuous water intake over a prolonged time period, followed by filtration and extraction, may often prove to be impractical and costly, particularly for ship-based sampling programmes. Unattended integrative devices, such as passive samplers (PSDs) also yield a time-integrated concentration if the necessary calibration parameters are available for the target analytes. Considerations for evaluating whether the necessary PSD calibration parameters are available for non-polar organic analytes are given by Lohmann *et al.* (2012). PSDs for polar contaminants (pharmaceuticals, detergents, and personal care products) are insufficiently mature for quantitative spatial and temporal trend monitoring at present, but may be useful in initial surveys. Diffusive gradients in thin films (DGT) is a mature PSD technique for trace metals, but its application in the marine environment has been quite limited so far (Mills *et al.*, 2011). All PSDs require suitable deployment sites, such as jetties, buoys, bottom landers, long-term moorings, etc, which always have to be visited twice and some losses due to other marine activities may be expected. If the monitoring programme requires sampling of total water, this will limit the applicability of PSDs.

5. Sampling equipment

The choice of sampling equipment depends on the physical-chemical properties and expected concentrations of the analytes, on the depth and location of the sampling site, and on the available infrastructure. All materials used for the sampling equipment (sample containers, tubing, connectors, valves, pumps, filters) should neither absorb nor release the target analytes, or any non-target substance that interferes with the chemical analysis. Contaminants are held in a range of dissolved, colloid, and particulate phases. These have a potential to interact differently with sampling equipment, and also for contaminants to exchange between phases during sample processing. Sampling equipment and processing therefore needs to be rigorously tested before adoption in large-scale monitoring programmes.

Since concentrations of organic contaminants and metals in seawater are usually very low, large volumes of water must be sampled. Contamination of the sample by compounds that leach out of the sampling equipment as well as analyte loss due to wall sorption are serious issues which may affect the integrity of seawater samples.

Sample contamination from the atmosphere should be avoided (e.g. paint and rust particles, engine exhausts, atmospheric background). To minimize contamination from the atmosphere, the surfaces of the sampling equipment in contact with the sample should be isolated from the atmosphere before and after the sampling, including storage of the equipment. These surfaces should be cleaned using appropriate solvents prior to sampling. Equipment blanks and recovery samples yield important quality control information that can be used to assess sample contamination and analyte losses, bearing in mind the potentially site-specific nature of airborne contamination.

Concentrations of target analytes in the water may be elevated because of leaching from the sampling platform itself (e.g. polyaromatic hydrocarbons (PAHs), organotin, polychlorinated biphenyls (PCBs), iron, and chlorofluoroalkanes can be released from the ship during ship-based sampling). The ship's keel should be at an angle of 20 to 40 degrees to any current coming from the bow at the sampling side (typically starboard side), to minimize any influence from the ship's hull.

Since the sampling equipment passes through the air-water interface, contamination from the sea surface microlayer is a significant risk. Concentrations of dissolved and particulate matter are elevated in this microlayer, and the associated analytes may therefore contaminate samples that are taken at larger depth. Sample contamination from the microlayer can be avoided by closing the sampling equipment during passage through the sea surface and only allowing sample intake at the intended depth.

5.1. Trace metals (including MeHg)

Contamination from the ship has to be avoided at all times. For analyses of trace metals, all contact between the seawater sample and metal must be avoided. On approaching a station, the sampling for trace metals has to be performed immediately. Hydrographical information about water depth and the stratification of the water column should be available.

Discrete samplers that are specially designed for trace metal analysis should be used, e.g. GO-FLO (from General Oceanic), available in sizes from 1.7 to 100 litres, or MERCOS samplers (from Hydrobios; or modified version, size 0.5 litre). They are typically operated on a Teflon, polymer, or Kevlar jacketed stainless steel hydrographic wire, tensioned

by a coated bottom weight. The messengers should also be free of metals; any essential metal parts should be of seawater resistant stainless steel (V4A).

Samples should be taken so as to avoid contamination by leachate from the hull of the ship. Sampling bottles should be made of plastic with low metal content, e.g. special low-density polyethylene (LDPE) bottles. For mercury, glass should be preferred if the samples are stored for a longer period. Teflon bottles may also be used, but they are relatively expensive and, depending on the manufacturing process, may have a relatively rough inner surface.

Pumping using metal-free devices may be an alternative to discrete sampling, e.g. for separating SPM by subsequent centrifugation, but is not preferable when sampling from a ship at distinct sampling depths or in the open sea where concentrations are very low. More details on sampler types are described in the Technical Annex.

After sampling, the sampler should be placed immediately in a plastic bag or box or an aluminium container (if aluminium is not determined), followed by transport to a clean-room or laboratory with a clean-air bench. These measures are particularly critical for open sea samples where the expected concentrations of trace metals are very low.

5.2. Organic contaminants

Concentrations of organic contaminants in seawater are usually very low. In order to reach the projected LOQs in the low pg l^{-1} range, large water volumes (10 to 100 l or more) have to be collected and extracted. With modern analytical equipment, these LOQs are often not limited by the signal intensity in the instrumental analysis, but by blank levels and interferences from the matrix background.

Hydrophobic compounds occur in a continuum of dissolved, colloidal, and particulate-bound forms. Unless a total concentration is to be determined, the compound partitioning must not be altered during sampling and subsequent treatment. This is very challenging, as the separation process must be contamination-free and should not change the concentration distribution. It should be applied during or immediately after sampling. For details, see Section 6.2.

Sometimes blank problems can only be overcome by increasing the sample size. However, the maximum sample size may be limited by operational constraints, such as container size for discrete samplers, pumping time, and the ability to process large water volumes. Blank levels can be reduced by minimizing the size of the sampling equipment (e.g. short inlet tubes) and by using sampler designs and handling procedures that minimize exposure to the atmosphere (short assembly/disassembly times). The use of *in situ* filtration/extraction equipment that is both compact and easy to operate combines the advantages of small size and short exposure to the atmosphere. This holds even stronger for passive samplers (see Section 4.6), provided that the sampling phase is sufficiently clean and that times of exposure to the atmosphere during deployment and retrieval are sufficiently short.

The materials used for the sampling equipment depend on the target contaminants. Sampling equipment for organic contaminants in seawater is preferably made of glass or stainless steel. Teflon parts are often used for legacy persistent organic pollutants (POPs), while they cannot be used for sampling of fluorinated compounds. Before use, the equipment has to be cleaned, e.g. rinsed with appropriate organic solvents. Examples of sampling equipment suitable for organic contaminants are presented in the Technical Annex.

6. Storage and pre-treatment of samples

The storage and pre-treatment of samples should be carried out in full awareness of the risks of contamination or analyte loss if samples are handled incorrectly. Appropriate measures should be taken to avoid contamination, such as wearing clean gloves, pre-cleaning equipment, etc. All storage and pre-treatment steps should be fully documented for each sample. Field control samples (for assessing sample contamination) and surrogate spikes (for assessing analyte losses) should be processed regularly as part of the quality assurance and control procedures (see Section 8). All storage and pre-treatment steps should be fully validated prior to the start of a monitoring programme.

6.1. Storage

It is advisable to process samples as soon as possible rather than store them for a longer period of time. Storage of samples increases the risk of changing concentrations, by microbial degradation or sorption processes. However, appropriate laboratory facilities for handling of samples for trace analyses need to be available. If this is not the case, samples may have to be conserved. Water samples for metal analysis are typically acidified for conservation purposes. Sub-sampling of seawater, if required, should preferably be performed immediately after sampling.

Water samples for organic pollutants generally are impractical to store because of their large volumes. Instead, they are extracted onboard by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) and the extracts or adsorbent cartridges are stored under cool (< 4°C) and dark conditions. If water samples must be stored, this should also be in the

dark and in a refrigerator (4°C). Preferably, internal standards (e.g. isotopically labelled analogues) should be added before extraction or/and storage. Storage times should be kept as short as possible and the stability of all compounds during storage must be checked.

Only appropriate (pre-cleaned) containers should be used for short- or long-term storage. The analytes of interest determine the appropriate container material (plastic, glass, metal), the need for acidification, and the optimal storage temperature. All storage conditions should be fully validated by the laboratory that carries out the monitoring, since sample contamination and loss of analyte may be affected by subtle changes in the materials and procedures for sample storage. SPM samples should always be stored frozen until further analysis.

6.2. Sample pre-treatment

The need for filtration of samples is mainly determined by the monitoring programme which typically will specify the analysis of either filtered or unfiltered water (total water, whole water). No pre-treatment is required for the analysis of whole water, although acidification may be necessary as part of the extraction procedure, depending on the analyte and on the extraction method used.

Filtration is the preferred technique to separate the dissolved phase from the SPM for small volume samples (e.g. for metal analysis). Polycarbonate or cellulose acetate filters with a pore size of 0.45 µm are frequently used for trace metal determinations, whereas glass fibre filters (0.7 µm or 1.2 µm pore size) are commonly used in the analysis of non-polar and polar organic contaminants. The efficiency of the separation between dissolved and particulate contaminants depends on the pore size of the filters, and may also depend on SPM content of the water and on the sample intake (see Section 4). Adsorption of dissolved analytes to the filter may be an issue for some compounds, and should be addressed during method validation.

A flow-through centrifuge is suitable for obtaining SPM from large volume samples, but less suitable for obtaining particle free water as the separation is incomplete. In general, the efficiency of the separation depends on the geometry and operating conditions of the centrifugation equipment (residence time, effective gravity force), as well as on the density and size of the SPM. Filtration is more effective in this respect, but also more susceptible to artefacts and more time consuming. Ideally, filtration should occur online while sampling or immediately after sampling.

7. Analytical procedures

Analytical methods should be specific to the target analytes and sufficiently sensitive to allow analyses of seawater samples which generally have low concentrations of contaminants. They should meet minimum performance criteria consistent with Commission Directive 2009/90/EC, including an uncertainty on measurements < 50%, estimated at the level of the relevant Environmental Quality Standard, and an LOQ ≤ 30% of the Environmental Quality Standard. If no method meets the minimal performance criteria, the best available analytical method, not entailing excessive costs, should be used. All analytical methods should be capable of being brought under statistical control to ensure adequate quality assurance and quality control. It should be noted that analyses at such low concentrations require extensive experience.

7.1. Trace metals

Analysis of trace metals in seawater generally includes pre-treatment and pre-concentration steps, followed by detection using element-specific spectrometric instrumental procedures, e.g. graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma mass spectrometry (ICP–MS), anodic stripping voltammetry (ASV), and total reflection x-ray fluorescence (TXRF). For mercury, further methods and instruments are used, such as cold vapour atomic absorption spectrometry (CVAAS) and cold vapour atomic fluorescence spectrometry (CVAFS). These techniques are usually combined with a pre-concentration by amalgamation. ICP–MS is also used for mercury analysis.

7.2. Organic contaminants

Organic contaminants are usually found in the water phase at low concentrations, entailing the need for an extraction and enrichment step (e.g. SPE, LLE, solid-phase micro extraction (SPME)) and a selective chromatographic/detection step (e.g. GC–$MS^{(n)}$, GC–ECD, LC–$MS^{(n)}$, LC–Fl.) within every analytical procedure. Depending on the analytes chosen, the water body studied and expected pollutant concentration, clean-up may be necessary. Although GC–MS/MS and HPLC–MS/MS are very selective techniques, it is good practice to use a second MS transition as a qualifier.

8. Quality assurance (QA)

The quality assurance programme should ensure that the data conform to the quantitative objectives of the programme (see Section 3). The laboratory must establish a quality assurance / quality control system, if necessary consistent with

requirements in Commission Directive 2009/90/EC. All field and laboratory procedures should be fully validated, and the laboratory should also participate in intercalibration exercises and proficiency testing to provide external verification of results. The quality assurance procedures should cover sampling design, sampling, sample storage, analytical procedures (including field controls, analytical blanks, and recoveries), equipment maintenance and handling, training of personnel, data management, and an audit trail.

The use of a second (and different) sampling method, carried out simultaneously to the routine procedure, can be included in the validation process. All QA and QC data should be fully documented.

Because of the extremely low concentrations of pollutants in seawater, blank problems are generally more relevant and more difficult to control than in other matrices. Even ultra-pure chemicals and solvents used sometimes have to be purified before use. Concentrations are often close to the LOQs, which means difficult calibration and integration, and reduced analytical precision.

In addition, the following problems are encountered specifically in seawater analyses of organic contaminants:

- Because of the large sample volumes, it is not possible to analyze replicate samples on a routine basis or to take samples for back-up analysis. However, it is often possible to make a plausibility check by comparing the results with those of samples taken from adjacent stations in a homogeneous water body. Homogeneity can be assessed from oceanographic parameters, like salinity.
- No certified reference materials are available for organic contaminants in seawater. Therefore, laboratory reference materials have to be used, which should preferably be a natural or spiked extract from a typical monitoring station. Extraction efficiencies should be checked by standard addition tests.
- Laboratory performance studies (e.g. by QUASIMEME) are difficult to perform and to evaluate because sample volumes in these studies (max. 1 l) differ from those used in real analysis (>10 l). Thus, concentration ranges in the tests are often higher than in real-life samples.

For temporal trend monitoring in particular, it is extremely important to perform reliable and reproducible high-quality analyses over decades. Therefore, such analyses require well-documented procedures and experienced analysts (see Section 7).

9. Reporting requirements

Secure data storage and appropriate access to the data should be ensured by submission of data to national databases and to the ICES database. Reporting requirements will depend on the database. For entry of OSPAR data into the ICES database, data of trace metals and organic contaminants should be reported in accordance with the latest ICES reporting formats.

The calculation of results and the reporting of data can be major sources of error. Control procedures should be established in order to ensure that data are correct and to avoid transcription errors. This could include comparisons with independently obtained results for the same area or with typical concentration intervals. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases.

Concentrations of trace metals and organic contaminants in seawater should be given in weight per volume (e.g. ng l^{-1}). To ensure correct interpretation, reporting should include information on the sampling method, filtration (filter type and pore size), storage/conservation, and analytical method. Minimum performance criteria such as LOQ and uncertainty measurement along with relevant QA/QC data such as reference material analyses should be included in the report.

The purpose of the monitoring, geographical coordinates, and the name of the sampling stations should be reported in the data as well as being defined in the OSPAR Station Dictionary (http://www.ices.dk/datacentre/accessions/). Sample depth, suspended particulate matter concentration, and physicochemical parameters at the time of sampling, such as air and water temperatures, salinity, pH, and weather conditions, should also be reported.

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Technical Annex: Sampling equipment for analysis of trace metals and organic contaminants in seawater

1. Trace metals

1.1 Discrete sampling

An example of a discrete sampler is the GO-FLO sampler by General Oceanics (Figure 1). This sampler consists of a cylinder with an inner Teflon-coating which can be closed and lowered into the water column and opens automatically at a certain depth (ca. 10 m) by hydrostatic pressure. This avoids contact of the sample with the water surface where some contaminants can accumulate. At the desired depth, a messenger is sent on the hydrographic wire (made of Teflon coated stainless steel, polymer, or preferably Kevlar) to release the closing valves in both ends of the sampler. Each bottle can be equipped with a second messenger that is released when the valves close. Water samples can be collected from a range of depths by mounting a series of bottles along the cable.

A variety of the GO-FLO sampler is the reversing water sampler. The messenger releases the sampler from the upper attachment, it rotates, and closes the two valves. If a special thermometer type is attached to the sampler, it fixes the actual temperature at the sampling depth, which can be determined later on board. This accessory can be used when no CTD-sensor is used to record the temperature profile.

Generally, all samplers must be cleaned before the first use by rinsing the inner surfaces with diluted hydrochloric acid. In the open sea, this may not be necessary between sampling where rinsing with deionised water is sufficient in most cases. In the open sea, seawater is sufficiently clean to rinse the outer surface. Samplers with rubber parts which cannot be acid-cleaned or cannot be closed during deployment should be avoided.

Figure 1 Picture of a GO-FLO sampler (General Oceanics; photo courtesy of IFREMER, France).

The MERCOS sampler (Hydrobios Kiel) is designed for two 500 ml thick-walled cylindrical or ball-shaped Teflon bottles, which are closed by two silicone tubes of different diameters in the water. As the bottles are filled with air, the operating depth is restricted to about 50 m for the cylindrical and about 200 m for the globular type. However, this sampler is no longer offered by the manufacturer (http://www.hydrobios.de, 2012).

A modified version for four bottles was developed by the Bundesamt für Seeschifffahrt und Hydrographie (BSH, Germany), maintaining the triggering device, but using LDPE bottles of low metal content material (NALGENE) that are protected against the water pressure by a polyacrylate mantle. The LDPE bottles are cheaper and easier to clean due to the smooth inner surface compared to the relatively rough texture of the thick-walled Teflon bottles. Therefore, the LDPE usually show much lower blank values.

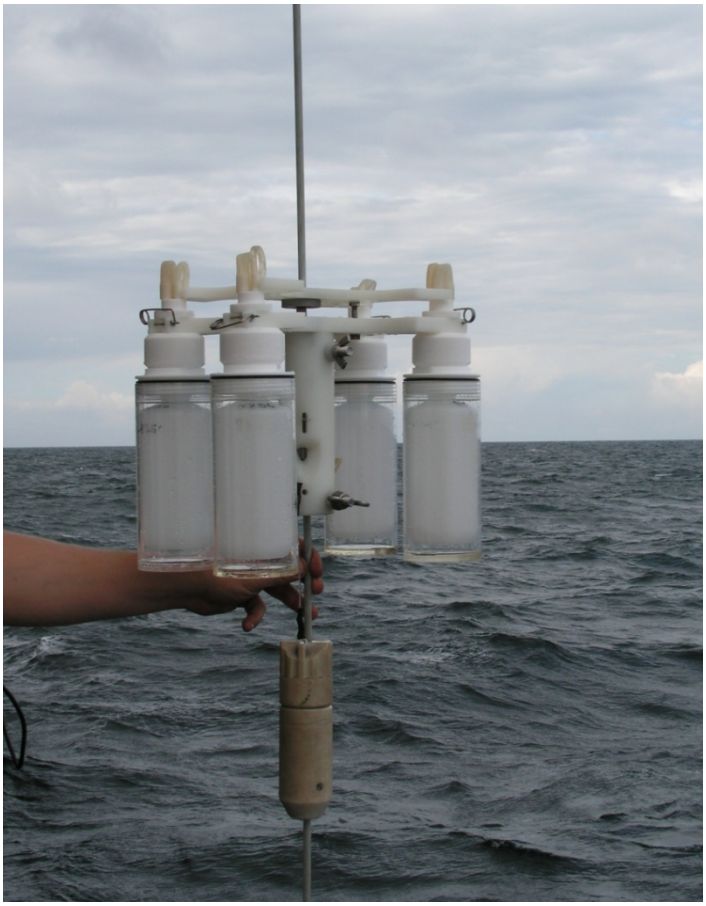


Figure 2 **Modified MERCOS water sampler of the second generation for four bottles, manufactured by BSH, Germany (photo courtesy of S. Schmolke, BSH, Germany).**

1.2 Sampling by pumping

For depths down to 100 m, perhaps even 200 m, it can be practicable to pump seawater up through silicone or Teflon tubing, optionally including in-line filtration. The tubing should be cleaned by pumping acid (e.g. 10% hydrochloric acid) prior to sampling. The first litres of seawater sampled should be subsequently discarded. A peristaltic pump or Teflon piston pumps are suitable. The peristaltic pump can be placed between the sampling tube and the filter. The outflow from the in-line filter can then be collected in polyethylene bottles, Teflon bottles, or in glass or quartz bottles for mercury analyses.

2. Organic contaminants

Large volumes of seawater samples are usually needed for the analysis of organic contaminants. Sampling devices depend on the amount of sample to be processed and the method of extraction (liquid–liquid extraction (LLE) or solid-phase extraction (SPE)).

LLE and SPE do not yield exactly the same concentrations as they use different extraction principles. While SPE effectively extracts only freely dissolved compounds, LLE extracts freely dissolved compounds and also compounds complexed with humic acids and, in part, compounds bound to particles (Sturm *et al.*, 1998). Non-polar compounds can be extracted by either LLE or SPE, whereas the extraction of polar compounds generally requires SPE.

Volumes of 1 to 100 l can be sampled by discrete sampling and/or pumping and are usually extracted either by LLE or SPE. Sample volumes >100 l are generally sampled by pumping and extracted by SPE.

2.1 Discrete sampling

Several different sampling devices have been designed for discrete sampling depending on the volumes needed and the extraction techniques to be applied.

All-glass bottle samplers for volumes of 10 L and 100 L are shown in Figure 3. They are mounted in a stainless steel cage and lowered on a hydrographic wire down to the desired sampling depth and opened under water. After filling, the sampler is brought on deck of the ship and the sample can be extracted by LLE directly in the sampler (using a non-polar solvent) or by SPE. For example, non-polar pollutants like organohalogen pesticides (e.g. DDx, HCH, HCB, dieldrin, endrin) can be extracted and enriched from seawater by means of LLE using hexane or pentane.

Gaul and Ziebarth (1983) described a 10 l glass sampler allowing extraction in the sampling flask itself, thereby minimizing uncertainties arising from sample handling, blanks, adsorption, etc. Later, the same principle was expanded to a 100 l flask, thus increasing the sample volume and lowering the limit of quantification (LOQ) by a factor of 10 (Theobald *et al.*, 1990). Figure 3 shows pictures of 10 l and 100 l sampling bowls. Extraction is done by agitating the samplers with 0.2 and 1 liter of pentane, respectively, using a stirrer. The glass sampler can be used to a depth of 2000 m (10 l) and 100 m (100 l).

Collecting samples at greater depth can be done with stainless steel bottles (Figure 4) holding about 30 litres. This type of sampler was developed based on experience with Niskin and Go-Flo type bottles, and has been used in analyzing dissolved herbicides in water samples collected down to 3000 m depth.

Figure 3 **Left: BSH all-glass bottle water sampler (10 l). Right: 100 l glass flask sampler for sampling seawater for the analysis of organic contaminants.**

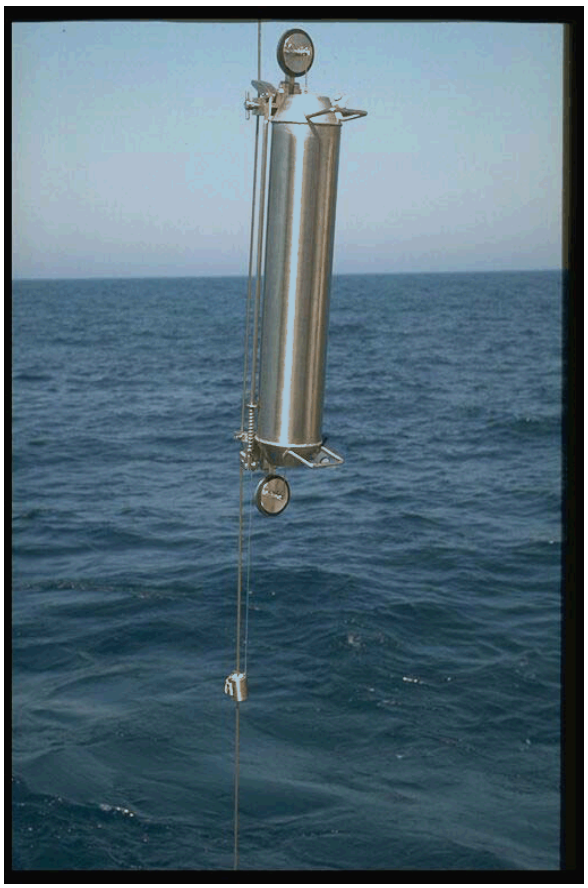


Figure 4 **A stainless steel sampling bottle, for subsequent analysis of organic contaminants in seawater.**

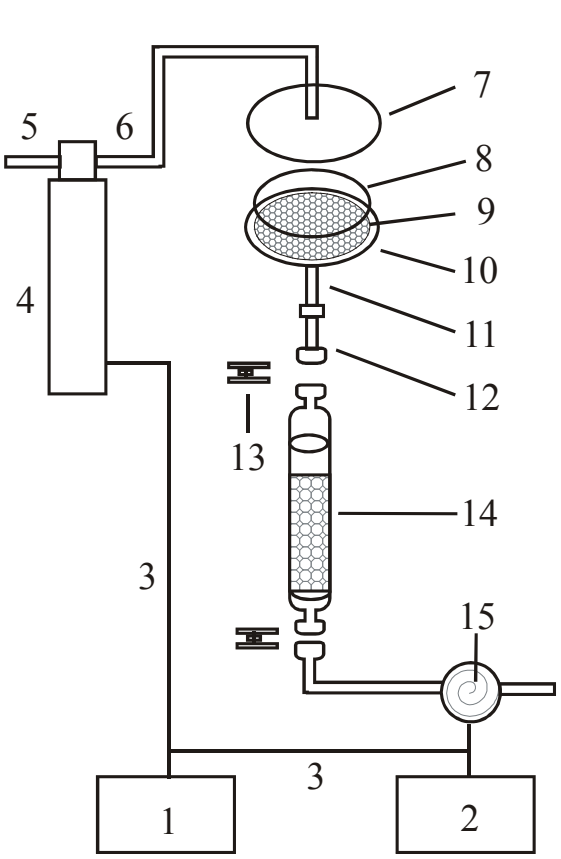
2.2 Sampling by pumping – *In situ* filtration and extraction

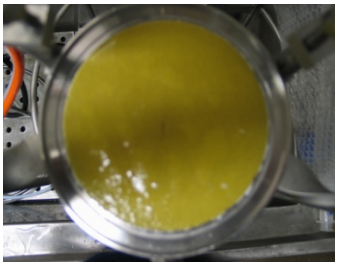
For larger volumes of 200 to 1000 l, Schulz-Bull *et al.* (1995) described an SPE procedure using large extraction cartridges filled with XAD resins. With this adsorbent, they obtained good extraction recoveries for PCBs, DDT, and PAHs, but not for HCH.

Sampling by pumping can be performed with compressed air Teflon pumps (not suitable for subsequent analysis of perfluorinated compounds). In order to equilibrate the system with the sampling water, the water is pumped for about ten minutes before the actual sampling begins. Then the sampling bottles are thoroughly rinsed with the sample, before beginning the sampling itself. The hose is kept away from the ship's hull while the system is being rinsed, and during the collection of the sub-surface samples.

In situ filtration and solid-phase extraction sampling devices may minimize the risk of sample contamination during sampling. A typical *in situ* pump system, the Kiel In-Situ Pump (KISP), has been widely applied to the extraction of organic contaminants in seawater (Petrick *et al.*, 1996). A modified KISP has been described for seawater sampling on-board research vessels (Ebinghaus and Xie, 2006). Briefly, as shown in Figure 5, KISP includes a filter holder, a polymeric resin column, a pump, and a flowmeter. A glass fibre filter (pore size 0.7 µm) is used to recover the particulate phase and a glass column packed with polymeric resin for the dissolved phase. The KISP can be easily operated on board by connecting it to the ship's seawater intake system for sampling seawater at certain depths. The pump system assembly with batteries can be deployed at different depths on a hydrographic wire, and the pumping can be started and ended by remote control.

The original KISP contains some plastic parts and connections, which may present a contamination risk for some organic contaminants, such as brominated flame retardants, alkylphenols, and plasticizers. Low blanks and detection limits have been obtained from KISP samples for legacy persistent organic pollutants (POPs), such as PCBs, DDTs, and HCHs (Lakaschus *et al.*, 2002; Sobek and Gustafsson, 2004). However, it is recommended that these parts are replaced by stainless steel or glass if KISP is to be applied for sampling seawater for the determination of other organic contaminants. Surrogate standards can be added to the resin column before sampling to control the extraction recoveries and storage. It should be noted that the validation of the *in situ* pump sampling method is difficult, and extraction efficiency may depend on dissolved organic matter and humic substances.





GF filter after sampling of 300 l seawater

Figure 5 **Schematic presentation of the Kiel In-Situ Pump (KISP). 1: flowmeter controller; 2: flowmeter; 3: cable connections; 4: pump; 5: pump inlet; 6: pump outlet; 7: stainless steel deck of filter holder; 8: GF 52 filter; 9: glass plate; 10: filter holder; 11: stainless steel tubing; 12 glass connect; 13 adjustable clip; 14: resins column; 15: counter of flow meter.**

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Annex II:

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 (3.1.2)

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-11, APPENDIX 1. TECHNICAL NOTE ON THE DETERMINATION OF TRACEMETALS (CD, PB, CU, CO, ZN, NI, FE), INCLUDING MERCURY, IN SEAWATER

Introduction

General techniques which address the questions of water sampling, storage, filtration procedures and determination of trace metals in natural sea water are described by Sturgeon and Berman (1987) and Gill and Fitzgerald (1985, 1987).

For the determination of mercury in sea water, the chemical species of this element are of importance. Therefore, a differentiation between the several Hg species, including ionic, volatile, dissolved (organic) complexes or particulate adsorbed Hg, has to be considered during sample preparation.

Several definitions of mercury compounds are common (Cossa et al., 1996, 1997), for example:

- Reactive mercury (HgR): A methodologically defined fraction consisting mostly of inorganic Hg(II).
- Total mercury (HgT): Mercury content of an unfiltered sample, after digestion with an oxidizing compound (e.g., K MnO4).
- Total dissolved mercury: Mercury content of a filtered sample, after digestion with an oxidizing compound (e.g., K MnO4).
- Dissolved gaseous mercury (DGM): This includes elemental mercury (Hg), monomethylmercury (MM-Hg) and dimethylmercury (DM-Hg).

1. CLEAN LABORATORY; CLEAN BENCHES

Particles are everywhere, including dust in the air or on clothes, hair or skin. Owing to the clothes, the person who is working with the samples for trace metal analysis is the main source of contamination because this person is a particle producer. One of the most important things during sample pretreatment for trace metal analysis is to eliminate particles that can contaminate the samples or the sample containers from the laboratory environment.
The best way to eliminate most of this contamination is to work under a laminar flow box with a laminar horizontal flow (sample protection). Recommended conditions for a 'clean bench' or a 'clean lab' are class 100 (US Norm) which means that there are still about one hundred particles present per cubic foot or class 3 (DIN-Norm), which equals 3000 particles per m3 (corresponding to class 100 US Norm).

2. PREPARATIONS

Chemicals

High purity water (e.g., 'Milli-Q water', 18 M cm-1) freshly prepared, is termed 'water' in the following text.

A sub-boiling quartz still is recommended for the distillation of highly purified acids and solvents. A teflon still is recommended for the distillation of HF.

Amalgamation (filtration of oversaturated solutions with goldnet) and volatilization (bubbling with ultrapure argon) are effective methods to purify (clean) chemicals and solutions for mercury analysis.

In order to avoid contamination problems, all plastic ware, bottles and containers must be treated with acids (HCl or HNO3) for several weeks and then rinsed with water and covered in plastic bags until use.

The following procedures (Patterson and Settle, 1976) are suggested:

Laboratory ware

Store in 2M HCl (high purity) for one week, rinse with water, store in water for one week and dry under dust-free conditions (clean bench).

Samplers and bottles

Sampling devices: Fill with 1% HNO3 (high purity), store at room temperature for three weeks, and rinse with water .

Teflon/quartz bottles: Store in warm (40 C ±5 C) 1:1 diluted HCl for one week. Then rinse with water and store with 1M HNO3 (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). Bottles are filled with a solution of 0.1 % KMnO4, 0.1% K2S2O8 and 2.5 % HNO3 and heated for 2 hours at 80 C. The bottles are then rinsed with water and stored with 2 % HNO3 containing 0.01 % K2Cr2O7 or KMnO4 until ready for use.

Filters

Polycarbonate filters (e.g., Nuclepore) (0.4 m, 47 mm diameter) are recommended for trace metals except mercury. Store the filters in 2M HCl (high purity) for a minimum of three weeks. After rinsing with water, store for one more week in water.

For the determination of mercury, glass microfibre filters (GF/F grade, Millipore type) and teflon filters are recommended for the filtration of natural water samples. Cleaning of these filters is comparable to the procedure used for polycarbonate filters. For GF/F filters, an additional drying step has to be considered (450 C for 12-24 hr) to volatilize gaseous mercury. This procedure is described in detail by Queremais & Cossa (1997).

If trace metals in suspended particulate matter (SPM) are to be determined, filters have to be placed in precleaned plastic dishes, dried in a clean bench for two days, and stored in a desiccator until they are weighed using an electronic microbalance with antistatic properties. Each filter has to be weighed daily for several days until the weight is constant. The same procedure for drying and weighing should be applied to the filters loaded with SPM (Pohl, 1997).

3. SAMPLING AND SAMPLE HANDLING

The basis for the reliable measurement of extremely low concentrations of trace metals in sea water is a well-performed sampling to avoid contamination risk from the ship. Careful handling is recommended because copper and tin are still the main substances used in antifouling paints on ships and there is also a risk of contamination by zinc (anodes of the ship), iron or lead.
In coastal and continental shelf waters, samples are collected using 30 l teflon-coated GO-FLO (General Oceanics, close-open-close system) bottles with teflon O-rings deployed on Kevlar or on a Hostalen coated wire. Niskin bottles deployed on rosettes using standard stainless steel hydrowire are also acceptable. For surface waters, an all-teflon MERCOS-Sampler (Hydrobios) could be chosen.

PVC gloves should be worn during subsampling into the precleaned quartz or teflon bottles (teflon has an extra low content of trace metals). Subsampling should be carried out in a clean lab or a clean-lab container, if available.

Pumping of samples using peristaltic or teflon piston pumps must be carried out using precleaned silicon- or teflon-lined tubes.

In the absence of clean-lab conditions, sampling and sample handling must be carried out in a closed system, or contamination cannot be avoided.
For mercury analysis, it should be noted that the integrity during sampling and storage may be jeopardized by the addition of mercury to the sample as well as by unexpected losses owing to volatilization.

4. FILTRATION PROCEDURE

In the environmental and geochemical scientific community concerned with water analysis, it has generally been accepted that the term 'dissolved' refers to that fraction of water and its constituents which have passed through a 0.45 m membrane filter. This is an operationally defined fraction. Coastal and shelf water samples have to be filtered to eliminate particles from the water. A number of metal species pass through this filter pore size, including metals bound to colloids or clays or to humic, fulvic, amino, and fatty acids.

To prevent desorption of metal ions from particle surfaces or from biological degradation of SPM, separation between the dissolved phase and the particulate phase has to be done immediately after sampling by filtering the water through a 0.45 m polycarbonate filter. This procedure should be carried out under clean conditions (clean benches are recommended on board the ship). If metals in both the dissolved and particulate phases are to be analysed, pressure filtration with nitrogen is recommended. After filtration the filter should be rinsed with high purity isotonic solution to remove sea salt residues. Only a few millilitres are necessary because a change of pH could cause desorption of metal ions from the particles. In pumping systems, on-line filtration is possible.

5. STORAGE OF SAMPLES

To avoid wall adsorption of metal ions, 1.5 ml HNO3 or HCl (high purity) should be added per litre of seawater sample immediately after filtration for acidification to pH 1.0-1.6. The sample containers should be stored in plastic bags under controlled environmental conditions. The filters should be stored in plastic dishes at -18 C or below. Under these conditions, both water samples and SPM on filters can be stored for at least one year.

Special consideration must be given to samples destined for Hg determinations. It is necessary to add either oxidants (Cr2O72-) in addition to acidification or complexing agents (cysteine) to neutral or alkaline samples to prevent Hg losses during storage.

6. SAMPLE PRETREATMENT

Water samples

Depending on the expected concentration range (10-7-10-9 gkg-1) of trace metals (dissolved) in Baltic Sea water and because of the salt matrix interfering during the measurement process, preconcentration techniques and/or the elimination of sea salt has to be carried out prior to the analytical measurement. Detailed method information is available in the open literature (e.g., Danielsson et al., 1978; Kremling et al., 1983; and Pohl, 1994).

Filters

Different methods to analyse the material on the filter are described by Hovind and Skei (1992) and Loring and Rantala (1991). Pressure decomposition with an acid mixture (HCl, HNO3, HF) is recommended. If the silica content is high due to diatoms, the HF concentration should be increased accordingly. If the organic content increases, it is advisable to work with perchloric acid.

Depending on the digestion system used (high pressure autoclave, microwave digestion, wet ashing in an open system, or dry ashing), the completeness of the digestion is a function of temperature, time, digestion material and pressure, and has to be tested and validated in pilot studies with (certified) reference materials (see the detailed remarks in Annex B-7, Section 4.3). Digestion of samples for mercury analysis must always be carried out in a closed system to prevent losses by evaporation.

7. INSTRUMENTATION

For the analytical measurements, several analytical techniques can be used, such as GFAAS (graphite furnace atomic absorption spectrometry), electrochemical methods, ICP-MS (inductively coupled plasma-mass spectrometry), ICP-AES (inductively coupled plasma-atomic emission spectrometry), or total-reflection X-ray fluorescence (TXRF). Because of the very low mercury concentrations in sea water, the most widely used technique for mercury is the cold vapour technique (reduction of mercury with SnCl2 to elemental Hg) and preconcentration of mercury by amalgamation on a gold trap. This is followed by atomic absorption spectrometry or by atomic fluorescence spectrometry, with detection limits adequate for the purpose. In the case of anoxic (sulfur-containing waters), see Annex B-11.

8. QUALITY CONTROL

The internal quality control is described in Chapter B.5 of the Manual.

Blank

Particularly in the case of trace metal analysis, with high contamination risks at each step of the analytical work, a satisfactory blank control is necessary. Therefore, it is important to control the blank daily, for reproducibility and constancy over a longer time. The blank should include all analytical pretreatment procedures, including the addition of the same quantities of chemical substances as for the sample.

Calibration

For calibration purposes, single element standard stock solutions at a concentration of 1000 mg dm-3, purchased from a qualified manufacturer, should be available. Preparation date and concentration should be marked on the bottle. From this stock solution, a multi-element working standard solution can be prepared using dilute HCl or HNO3 as required (normally 1M acid is used).

Traceability can be ensured by the use of CRMs or participation in intercomparison exercises. The working standard should be prepared from the stock standard solution for every batch of samples and kept no longer than two weeks. Precleaned teflon containers are preferable for storage.

To evaluate effects from the matrix, the method of standard addition can be used, particularly in connection with the analytical method of voltammetric stripping. For other techniques, the method of standard addition should generally be used with care (Cardone, 1986a, 1986b).
Reference materials

Owing to problems in defining the blank, the use of a low-concentration CRM is important. Regular participation in intercomparison exercises should be considered mandatory.

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Annex III:

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. (3.1.3)

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-11 APPENDIX 2: TECHNICAL ANNEX ON THE DETERMINATION OF HEAVY METALS AND PERSISTENT ORGANIC COMPOUNDS IN SEAWATER

TECHNICAL NOTE ON THE DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS IN SEAWATER

1. INTRODUCTION

These guidelines concentrate on the sampling and extraction of lipophilic persistent organic pollutants from seawater and special aspects of the sampling matrix. This group of pollutants comprises the group of polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (e.g., HCH, HCB, DDT group, chlorinated biphenyls (PCBs)).

For general aspects and the analytical determination, reference is made to the following guidelines:

• "Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediments: Analytical Methods", ICES ACME Report 1997;

• "Guidelines for the determination of chlorobiphenyls in sediments: Analytical methods", ICES ACME Report 1996;

• "Determination of Polycyclic Aromatic Hydrocarbons (PAH)s in Biota", ICES ACME Report 1998; and

• Annex B-14 (these Guidelines).

As the same analytical methods can be used for the determination of lipophilic pollutants in extracts of water samples as are used for extracts of sediments, it is felt that it is a useful way to unify analytical procedures to refer to these publications only.

However, it should be taken into consideration (e.g., for calibration) that the relative concentrations of the individual pollutants are generally quite different in water and sediment samples. The concentration patterns of the pollutants are mainly influenced by their polarity which can be expressed by their octanol/water coefficient (log Kow; Kow = Concentration in octanol phase / Concentration in aqueous phase). Thus, in water samples the more hydrophilic compounds with log Kow values of 3 to 4 predominate (e.g., 2- and 3-ring aromatics and HCH isomers), while in sediments and biota the pollutants with log Kow values >5 are enriched (4- to 6-ring aromatics, DDT group, PCBs).

These guidelines provide advice on lipophilic persistent organic pollutant (POPs) analyses in total seawater with a log KOW > 3. The analysis of POPs generally includes:

• sampling and extraction of the water;
• clean-up; and
• analytical determination

The extraction of the POPs simultaneously enables an enrichment of the analytes. Because of the very low concentration range of 10 pg l−1 to 10 ng l−1, the enrichment of the contaminants is a very important step in the procedure. Extraction and enrichment can be done by solid phase extraction (SPE) or liquid-liquid extraction (LLE).

Determination depends on the chemical structure of the compounds. PAHs can be determined by high performance liquid chromatography (HPLC) with fluorescence detection or gas chromatographic (GC) separation with flame ionization (FID) or mass spectrometric (MS) detection (Fetzer and Vo-Dinh, 1989; Wise et al., 1995). Chlorinated hydrocarbons are generally analysed by gas chromatographic (GC) separation with electron capture detectors (ECD) or mass spectrometric (MS) detection.

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. These guidelines are intended to encourage and assist analytical chemists to critically reconsider their methods and to improve their procedures and/or the associated quality control measures, where necessary.

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. In addition, the use of a second (and different) method, carried out concurrently to the routine procedure, is recommended for validation. The participation in analytical proficiency tests is highly recommended.

2. SAMPLING AND STORAGE

Plastic materials must not be used for sampling and storage owing to possible adsorption on the container material or contamination. Especially the very lipophilic compounds (4- to 6-ring aromatic hydrocarbons, DDT, PCBs) tend to adsorb on every surface. Therefore, the seawater samples should not be stored longer than 2 h and should not be transferred into other containers before extraction. It is highly recommended to extract the water sample as soon as possible after sampling and to use as little manipulation as possible. It is recommended that sampling and extraction should be done in the same device. Extracts in organic solvents are less susceptible to adsorption onto surfaces.

3. BLANKS AND CONTAMINATION

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 all glassware, solvents, chemicals, adsorption materials, etc., that are used in the analysis. The following procedures should be used:

- 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.

- 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.

- 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. Glassfiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used. The storage of these supercleaned 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. The most volatile compounds will usually show the highest blanks (Gremm and Frimmel, 1990).

As the concentrations of the PAHs and chlorinated hydrocarbons in seawater are very low, possible blank and contamination problems might be even more difficult to control than with sediment samples. 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.

The more volatile compounds (especially naphthalene and phenanthrene) show the largest blank problems.

4. PRE-TREATMENT

For the extraction of whole water samples, no pre-treatment is necessary.
If the suspended particulate material (SPM) will be analysed separately from the solute phase, a phase separation has to be done. Because of the necessary additional manipulation step, this is a difficult operation which affords a number of additional quality control procedures (adsorption losses, contamination problems). There are two possible ways for phase separation: filtration and centrifugation.

Filtration is done by GF/F glass fibre filters. As flat-bed filters have a very limited capacity, the use of coiled glass fibre filters is recommended 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. Centrifugation needs a high volume centrifuge which must be operable onboard a ship. Such centrifuges with a throughput of 1 m^3 $h^{-1}$and more are commercially available and used for sampling SPM; however, they are expensive and generally not a standard equipment. For centrifugation, blanks and adsorption problems have to be controlled as well as the separation efficiency.

The sampled SPM is analysed like a sediment. The solute phase is analysed like the whole water sample.

Validation of the phase separation procedures is very difficult; thus, it might be wise to analyse the whole water sample for monitoring purposes and to determine separately only the amount of SPM in the water for reference or normalization purposes.

5. EXTRACTION

The volume of the water sample is the most important parameter which influences the limit of determination of the method. As POP concentrations down to 10 pg l^{-1} and less are observed in seawater, large water volumes of 10 l to 100 l have to be sampled and extracted. Large volumes are required not only to obtain a sufficiently high detector signal, but also to discriminate from blank problems.

Principally, there are two different extraction principles in current use: solid phase extraction (SPE) and liquid-liquid extraction (LLE). Unfortunately, the two procedures do not always yield comparable results, as the physical extraction principles are quite different (Sturm et al., 1998, Gomez-Belinchon et al., 1988).

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. The

drawbacks of the method are a longer sampling time demand, a more complex instrumentation, and problems with validation and control of the extraction efficiency.

LLE has the advantage that it can be easily validated and controlled, as internal standards can be added before extraction. Also, standard addition techniques can be used for accuracy testing. As LLE is a classical extraction technique, a great deal of experience is available and the robustness of the principle is proven. The limitation in sample volume is only relative, as techniques have been described for sampling 10 l and 100 l on a routine basis (Gaul and Ziebarth, 1993; Theobald et al., 1990). It has been shown that a sampling volume of 100 l is sufficient for nearly all monitoring tasks. Because of the robustness of the method, there is a preference LLE for routine monitoring purposes for all lipophilic organic contaminants.

5.1 Solid phase extraction

The extraction device consists of a filter holder, an adsorption column filled with an adsorbing material (e.g., XAD resin, C18 modified silica gel), a pump which forces the water sample through the column, a flow meter, an electronic control unit, and a power supply. Sampling can be done either by deploying the whole extraction device into the water (in situ pumping) or by pumping the water with a separate pump onboard a ship and then through the extraction device. A suitable in situ system is described in detail in Patrick et al. (1996). After sampling, the columns are stored at 4 °C and the filters at –20 °C.

The adsorption column is eluted with an organic solvent (acetone or acetonitril). Prior to the extraction, internal standards are added to the solvent. The extract obtained is pre-cleaned and analysed.

Analytical procedures for the use of XAD-2 adsorption resins are published by the IOC (1993), Ehrhardt (1987), and Bruhn and McLachlan (2001).

Although the SPE technique has many advantages, one has to be aware of some problems. Especially for large volume sampling, validation of the method is extremely difficult and has not yet been achieved. Some publications have shown that the extraction efficiency is dependent on, e.g., the amount and kind of humic substances which can complex lipophilic compounds (Johnson et al., 1991; Kulovaara, 1993; Sturm et al., 1998).

5.2 Liquid-liquid extraction

The decision to sample 10 l, 20 l, or 100 l of water depends on the anticipated concentrations of the compounds to be analysed in natural samples . For remote sea areas with expected concentration of 10 pg l^{-1} or less, a volume of 100 l is recommended. The technique and principle are identical for all volumes, only the sampling bottle and the equipment are different. Details of the sampling and extraction techniques are described in Gaul and Ziebarth (1993) for the 10 l sampler and in Theobald et al. (1990) for the 100 l sampler.

The all-glass bottle sampler fixed in a stainless steel cage is lowered by a hydrographic wire down to the sampling depth and opened under water. After filling, the sampler is brought on deck of the ship and immediately extracted with a non-polar solvent such as pentane or hexane. Prior to extraction, a solution with appropriate internal standards (e.g., deuterated PAHs, e-HCH, PCB 185) is added to the water sample. After phase separation, the organic extract is dried with Na_2SO_4 and carefully concentrated to about 1 ml in a rotary evaporator. Further evaporation is done under a gentle stream of nitrogen.

Extreme care has to be taken to ovoid contamination during sampling, extraction, and work up. Blank samples must be taken in every sampling campaign; this can be done, e.g., by rinsing the cleaned sampling bottle with the extraction solvent and treating this extract like a normal sample. The sampling bottle must be cleaned with detergent, water, and organic solvents (acetone and hexane or pentane) before use. After using in open sea areas, it can be of advantage not to perform the whole cleaning/washing procedure but just to use the sampler directly after emptying the glass bottle from the extracted previous water sample.

Extracts should be stored in the refrigerator and in the dark.

6. CLEAN-UP

Interferences from matrix compounds in seawater samples are generally smaller than in sediment or biota samples. Nevertheless, the crude extracts require a clean-up before chromatographic separation and determination can be done. The clean-up is dependent on the compounds to be analysed, the sample, the determination method used, and the concentration range to be analysed. For all GC methods, it is essential to remove polar and non-volatile compounds in order to protect the GC column from rapid destruction. A detection system with low selectivity (eg., GC-FID) needs a far better clean-up than a detector with a high selectivity such GC-MS or even GC-MS/MS. HPLC with fluorescence detection (for PAH analyses) has a relative high selectivity but the method will fail if petrogenic aromatic compounds (from an oil spill) are present in the sample. GC-ECD (for chlorinated compounds) has a high selectivity but some interferences (e.g., phthalate esters) may disturb the detection; therefore, for GC-ECD a good clean-up is necessary as well.

A clean-up procedure for this is presented here that uses short silica gel chromatography columns that can be applied with any determination technique: HPLC, GC or GC-MS. The method is simple and is sufficient in most cases of PAH and chlorinated hydrocarbon determinations in seawater (ICES, 1996, 1997, 1999).

A 3 ml glass column with glass fibre frit (commercially available for SPE) is filled with 500 mg silica gel (dried for 2 h at 200° C) and subsequently washed with 30 ml CH2Cl2 and 30 ml hexane. The hexane sample extract (concentrated to 500 µl) is applied on top of the column and eluted with 5 ml CH2Cl2/hexane (15/85 v/v) and then with 5 ml of 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. If the water sample has been extremely rich in biological material (algae) or if detection limits far below 10 pg l−1 are requested, additional clean-up (HPLC, GPC) might become necessary.

7. CROMATOGRAPHIC DETERMINATION

Details for the chromatographic determinations are comprehensively described in the 1996 ACME report (ICES, 1996) for chlorobiphenyls in sediments (GC-ECD and GC-MS), the 1997 ACME report (ICES, 1997) for PAHs in sediments (HPLC-Fluorescence detection, GC-FID and GC-MS), and the 1998 ACME report (ICES, 1999) for PAHs in biota (HPLC and GC-MS).

As the cleaned extracts from the seawater samples can be analysed in the same way as the extracts from sediments and biota, the above guidelines can be used. When a GC-MS system can be used, all compounds can be determined in one single GC analysis; if not, the samples have to be analysed separately for PAHs (HPLC-F, GC-FID) and chlorinated hydrocarbons (GC-ECD).

7.1 Gas chromatography-mass spectrometry

As GC-MS has the advantage of being both very selective and quite universal, it is strongly recommended to use GC-MS as the determination method. It especially has the advantage that both PAHs and chlorinated hydrocarbons can be determined in one single analysis. This is not possible with any of the other techniques.

Because of the sensitivity required, the mass spectrometric detector must be operated in the selected ion mode (SIM). By this, absolute sensitivities in the range of 1 pg to 10 pg can be achieved for most compounds. Ion-trap instruments can be operated in full-scan mode and are in principle as sensitive as quadrupole detectors; however, with real samples and matrix underground they can lose considerably sensitivity.

With GC-MS, detection limits of 5–30 pg l^{-1} can be reached with water sample volumes of 10 l to 100 l. In most cases, it is not the absolute signal strength of the detector which limits the detection; therefore, the injection of a larger aliquot of the analysis solution would not improve it. For some compounds, blank values are the limiting parameter (especially naphthalene and phenanthrene and, to a lesser extent, other PAHs); for this, only a larger sample volume can improve the detection limits. Many other compounds do not exhibit blank problems, if appropriate care is applied; for these, matrix noise often limits the detection. For such situations, only a better clean-up (e.g., HPLC, GPC) or a more specific detection method (GC-NCI-MS or GC-MS/MS) will improve the detection limit. Negative chemical ionization (NCI) mass spectrometric detection can be used for highly chlorinated compounds (e.g., HCB, PCBs with five or more Cl atoms, HCH) and shows extremely high sensitivity and selectivity for these compounds. More universally applicable is tandem mass spectrometry (MS/MS), which yields a similar absolute sensitivity as normal MS but much higher selectivity. Some MS/MS transitions for the detection of selected chlorinated hydrocarbons are listed in Table 1 in Appendix 2 to Annex B-13: Technical note on the determination of polycyclic aromatic hydrocarbons in biota, from the full "Guidelines".

7.2 Quantification

A multilevel calibration with at least five concentration levels is recommended. The response of the FID detector is linear. For UV and fluorescence detection, the linear range is also large. The working range should be linear and must be covered by a calibration curve. Since the mass spectrometric detector often has no linear response curve, the use of stable deuterated isotopes is a prerequisite. Furthermore, the response of PAHs in standard solutions is often much lower than in sample extracts. Only a combination of different techniques, e.g., the use of internal standards and standard addition, might give reliable quantitative results.

The calibration curve can be checked by recalculating the standards as if they were samples and comparing these results with the nominal values. Deviations from the nominal values should not exceed 5%.

When chromatograms are processed using automated integrators, the baseline is not always set correctly, and always needs visual inspection. Because the separation of the peaks is often incomplete in HPLC analysis, the use of peak heights is recommended for quantification. In case of GC techniques, either peak heights or peak areas can be used.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract, the data from which should be ignored. In addition, standards used for multilevel calibration should be regularly distributed over the sample series so matrix- and non-matrix-containing injections alternate. A sample series should include:

• a procedural blank,
• a laboratory reference material,
• at least five standards,
• one standard that has been treated similarly to the samples (recovery determination).

The limit of determination should depend on the purpose of the investigation. A limit of 2 ng g^{-1} (dry weight) or better should be attained for single compounds. The method for calculating the limit of determination should reflect QUASIMEME advice (Topping et al., 1992). The limit of determination that can be achieved depends on the blank, the sample matrix, concentrations of interfering compounds, and the volume of water taken for analysis. The typical concentration ranges of PAHs and other POPs in seawater can be found in HELCOM assessments (HELCOM, 2003a, 2003b).

8. QUALITY ASSURANCE

A number of measures should be taken to ensure a sufficient quality of the analysis. Five main areas can be identified:

1. extraction efficiency and clean-up;
2. calibrant and calibration;
3. system performance;
4. long-term stability; and
5. internal standards.

8.1 Extraction efficiency and clean-up

A check on extraction efficiency and clean-up can be performed by analysing a reference material (Annex B-7). To determine the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution through the entire procedure. Additionally, at least one internal standard should be added to each sample before extraction, to check for recovery during the analytical procedures. If major losses have occurred, then the results should not be reported. CB29 is suggested as a recovery standard because, owing to its high volatility, losses due to evaporation are easily detected. CB29 elutes relatively late from alumina and silica columns. Small peaks that may be present in the gas chromatogram at the retention time of CB29 do not hinder the use of this CB because the recovery standard only indicates major errors in extraction or clean-up. In case of GC/MS, labelled CBs can be used as recovery standards. This allows correction for recovery, provided that each chlorination stage is represented.

8.2 Calibrant and calibration

PAH determinations should preferably be carried out using calibration solutions prepared from certified crystalline PAHs. However, the laboratory should have the appropriate equipment and expertise to handle these hazardous crystalline substances. Alternatively, certified PAH solutions, preferably from two different suppliers, can be used. Two independent stock solutions should always be prepared simultaneously to allow cross-checks to be made. Calibration solutions should be stored in ampoules in a cool, dark place. Weight loss during storage should be recorded for all standards.

CB determinations should always be carried out using calibration solutions prepared from crystalline CBs. Preferably, certified CBs should be used. Two independent stock solutions of different concentrations should always be prepared simultaneously to allow a cross-check to be made.

Calibration solutions should preferably be stored in a cool, dark place. For all containers with standards, the weight loss during storage should be recorded.
After clean-up and before GC analysis, both in PAH and CB analysis, an additional internal standard is added for volume correction. Internal standards should be added in a fixed volume or weighted to all standards and samples.

8.3 System performance

The performance of the HPLC or GC system can be monitored by regularly checking the resolution of two closely eluting PAHs or CBs. A decrease in resolution indicates deteriorating HPLC or GC conditions. The signal-to-noise ratio of a low concentration standard yields information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio. Additionally, the peak can be affected.

8.4 Long-term stability
One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected PAHs, e.g., fluoranthene (stable results), pyrene (sensitive to quenching), benzo[a]pyrene (sensitive to light), or, correspondingly, for selected CBs. If the warning limits are exceeded, the method should be checked for possible errors. When alarm limits are exceeded, the results obtained should not be reported. A certified reference material (CRM) should be analysed at least once a year, when available, and each time the procedure is changed. Each laboratory analysing PAHs and CBs in water should participate in interlaboratory analytical performance tests on a regular basis.

8.5 Internal standards

Internal standards should be added to all standards and samples either in a fixed volume or by weight. The PAH internal standards should preferably be non-natural PAHs which are not found in water and do not co-elute with the target PAHs; several predeuterated PAHs have proved to be suitable for GC/MS as well as for HPLC analysis. For example, for GC/MS it is recommended to add four internal standards representing different ring-sizes of PAHs.

The following compounds can be used (Wise et al., 1995):

• for HPLC analysis: phenanthrene-d10, fluoranthene-d10, perylene-d12, 6-methyl-chrysene;
• for GC/MS analysis: naphthalene-d8, phenanthrene-d10, chrysene-d12, perylene-d12;
• for GC/FID analysis: 1-butylpropylene, m-tetraphenyl.

Similarly the ideal internal standard for PCBs is a compound which is not found in the samples and does not co-elute with other CBs, e.g., CBs 29, 112, 155, 198 or all 2,4,6-substituted CB congeners. Alternatively, 1,2,3,4-tetrachloronaphthalene can be used.

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