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Monitoring Protocols for Common Indicators related to Pollution

Agenda item 6: Monitoring Protocols for IMAP Common Indicators Related to Pollution

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Note by the Secretariat

The 19th Meeting of the Contracting Parties (COP 19), held in February 2016, adopted the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria (Decision IG. 22/7), with a list of regionally agreed good environmental status descriptions, common indicators and targets, with principles and clear timeline for its implementation.

In line with IMAP, Guidance Factsheets were developed, reviewed and agreed by the Meeting of the Ecosystem Approach Correspondence Group on Pollution Monitoring (CorMon on Pollution Monitoring) held in Marseilles, France, 19-21 October 2016 and the Meeting of the MED POL Focal Points, held in Rome, Italy, 29-31 May 2017, for the Common Indicators to ensure coherent monitoring. The Guidance Factsheets provide concrete guidance to the Contracting Parties supporting implementation of their respective national monitoring programmes aligned with IMAP.

The comments received by the Contracting Parties were considered and approved by the 6th Meeting of the Ecosystem Approach Coordination Group, held in Athens, Greece, on 11th September 2017. It must be noted that the Guidance Factsheets were used during the elaboration of the Mediterranean Quality Status Report 2017 (Med QSR 2017).

Taking into account evolving needs to fill the gaps, in particular related to assessment component of the Guidance Factsheets, the UN Environment/MAP Programme of Work (PoW) adopted at COP 20, included under Output 2.4.1 "National pollution and litter monitoring programmes, provides for undertaking important monitoring activities supported by data quality assurance and control, including further development of the IMAP Guidance Factsheets."

In order to achieve this output, further development of the IMAP Guidance Factsheets requires a revision of the common scientific methods and monitoring protocols to ensure that the relevant practices applied in the monitoring strategies under IMAP are based on the scientific methodologies, as well as to allow a consensus among the Contracting Parties towards a harmonized and standardized monitoring protocols in the Mediterranean Sea.

The present document presents a summary of known practices employed in the marine monitoring networks for Ecological Objectives 5 (Eutrophication) and Ecological Objective 9 (Pollution) for consideration by present Meeting of CorMon on Pollution Monitoring, altogether with knowledge and practices obtained over 40 years of MED POL monitoring implementation, recent publications highlighting the current practices by the Contracting Parties' marine laboratories as well as in other Regional Seas Conventions. It does not pretend to be exhaustive at this stage, but it outlines future work to be considered to ensure monitoring protocols are regularly updated in line with the continues technological advances and a necessity to ensure frequent revision of the methodologies for measurements, including Quality Assurance.

List of Abbreviations / Acronyms

CI	Common Indicator
COP	Conference of the Parties
CORMON	Correspondence Group on Monitoring
EcAp	Ecosystem Approach
EEA	European Environmental Agency
EO	Ecological Objective
EU	European Union
FAO	Food and Agriculture Organization of the United Nation
GES	Good Environmental Status
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
MSFD	Marine Strategy Framework Directive
MRU	Marine Reporting Unit
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
RSP	Regional Seas Programme

1. Introduction

1. Monitoring protocols for the in-situ environmental monitoring process (namely, sample collection, sample processing, measurements and data reporting) should be understood as the ultimate performing cost-effective and science-based methodological package to allow the fit-for-purpose data and information gathering in the marine environment.

2. Within the monitoring process there are some methodological steps that would rarely change as they are basic procedures (e.g. collecting biota from the shoreline); although, these might need refinements over time, including surely considerations such as statistical compliance, spatial representativeness, number of control samples, sample field replicates, contamination blank control samples, sizes of the organisms, grain fractions to be analysed, reporting units, sample preservation and storage, to mention few. On the other side, however, within the process of monitoring, the sample measurement methods are highly dynamic and rapidly changing for some (e.g. analytical chemistry measurements); thus, continuous improved measurements methods and instrumentation are available. Nevertheless, in essence, break ground changes in the monitoring process will not occur as routine monitoring requires robust measurements with controlled uncertainty (ca. harmonised, stable and validated methods) to understand the status of the marine environment from local to regional scales.

3. For these reasons above, the in-situ monitoring protocols are live documents and the exhaustive revision of the past 40 years of monitoring of the marine environment would not help to construct the future IMAP monitoring common approach. Alternatively, the selection of the most recent methodologies, as well as the generally accepted ones, will better guide the users to perform changes and consider the wider spectrum of monitoring approaches and options; and ultimately, to switch to some of them (ca. improved). This selection of the monitoring steps is provided in this report, and the structure of the document aims to differentiate as well between monitoring steps which have a different degree of complexity or those that require larger scientific-technical knowledge (see the Table 1 below) for each of the IMAP Common Indicators within the EO5 (Eutrophication) and EO9 (Pollution). Therefore, in each section, the same approach is followed based on Table 1.

Table 1. Scheme of the monitoring steps selection table for EO5 and EO9 Common Indicators in this document.

Monitoring CI_n	**Purpose/ Rationale**	**Guidelines/References (including research publications)**
1.Sample collection		
2.Sample processing		
3.Measurements		
4.Reporting and QA		

4. Present monitoring strategy within the Mediterranean Sea is provided in IMAP Guidance Factsheets. However, as mentioned above, the continuous changes primarily in the measurement methods and techniques require an update on best monitoring tools, protocols and practices to be reviewed. To that effect, it should be noted that a collective contribution towards a common monitoring activity should be performed primarily in the framework of applied science (i.e. validated methods) rather than research science (i.e. experimental methods).

5. An updated and continuously revised compendium of the selected marine protocols used for monitoring in a single document has not yet been performed for the IMAP in the Mediterranean Sea comparable to the monitoring manual, for example, used as a guide to perform all the monitoring activities within the HELCOM Convention, namely the COMBINE Programme (HELCOM-COMBINE, 2017). Therefore, further work should take an advantage of a large number of guidelines and methodologies that have been published in the past decades towards the harmonization of the monitoring processes (e.g. UNEP/IOC/IAEA/FAO Guidelines).

6. Similarly, the monitoring and assessment under the activities of the OSPAR Convention is strategically managed by the OSPAR's Coordinated Environmental Monitoring Programme (CEMP, 2016) which aims to deliver comparable data from across the OSPAR Maritime Area for six thematic monitoring programmes, whilst the OSPAR's Joint Assessment and Monitoring Programme (JAMP, 2018) served to address specific questions raised by monitoring and assessments which could be considered collectively by the CEMP as well.

7. In the Mediterranean Sea, the basis for these monitoring documentation and revision efforts are the fulfilment of the commitments by the Contracting Parties with regard the monitoring of the marine environment, which is set by the Integrated Monitoring and Assessment Programme (IMAP) and Related Assessment Criteria, as well as necessarily taking into account the EU Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC) for those Contracting Parties members of the European Union.

8. As described in the most recent EU policy as well for the management of the marine environment, namely the EU Marine Strategy Framework Directive and their monitoring programmes (JRC, 2012; JRC, 2014), the UNEP/MAP IMAP (Decision IG.22/7 related to IMAP, COP19) takes into consideration in a similar way some overarching recommended principles for monitoring activities to be considered, as listed below:

- Adequacy (overarching principle 1);
- Coordination and coherence (overarching principle 2);
- Data architecture and interoperability overarching (principle 3);
- Adaptive monitoring programme (overarching principle 4);
- Risk-based approach to monitoring and assessment and where appropriate applying the precautionary principle (overarching principle 5);
- Precautionary principle (overarching principle 6).

9. These are key principles that monitoring programmes should follow. In any case, the implementation of the monitoring and assessment is to be performed under a 6-year cyclic programme with the primary objective to assess the progress towards the targets of the Good Environmental Status (GES) for each of the IMAP Common Indicators, as well as altogether from a holistic approach by integrating different Common Indicators and Ecological Objectives under IMAP.

2. General monitoring considerations under IMAP Pollution cluster

10. First of all, it should be noticed that the monitoring under IMAP Pollution Cluster is a transition to an enlarged monitoring activity from the MED POL four-decades experiences on eutrophication and contaminants monitoring (namely, IMAP EO5 and EO9); with the addition of the contemporary threat of marine litter which has been considered under EO10. Furthermore, each Ecological Objective within the IMAP Pollution cluster is composed of different Common Indicators which represent the basis for the monitoring and assessment within IMAP.

11. The IMAP monitoring developments should consider the spatial and temporal scales for the scalability of the results to the extent possible, and to correlate pressures, status and impacts (ca. DPSIR framework). Furthermore, it should consider the existing national monitoring programmes to be adapted and combined (if considered adequate) for the monitoring of IMAP Common Indicators and Ecological Objectives. Table 2 below tentatively includes common national programmes which should allow their alignment to integrate IMAP Pollution cluster monitoring requirements (including marine litter), whilst the simplified summary of the monitoring programme structure for Common Indicator 14 and Common Indicator 17 is presented in Annex I.

12. The rationale behind the optimization of the monitoring practices for different Common Indicators, with the objective to be undertaken during the same periods of time and resources, is clearly a cost-effective purpose. However, the cost-effective optimization is not straight forward and

should be planned in detail before any attempt to integrate monitoring. In the next sections, the essential considerations for the cost-effective monitoring of different Common Indicators under EO5 and EO9 and their steps are explained and accompanied by the literature source citations.

13. The scope of the differences between traditional ongoing MED POL monitoring programme and IMAP implementations are provided below, with regard the EO5 and EO9 and their Common Indicators within the Pollution Cluster:

i) Eutrophication and contaminants, equivalent to IMAP EO5 and EO9, respectively, the MED POL IV monitoring programmes have generally focused on narrow coastal areas (ca. shorelines mainly for EO9);

ii) Monitoring is extended under IMAP to offshore areas (including, water column, biota and sediments); and therefore, requires changes in terms of monitoring protocols;

iii) Collection of biota (e.g. fisheries), sediment and water samples in offshore areas are challenging operations that requires research/adequate vessels, heavy sampling equipment, detailed planning and additional financial resources;

iv) Reference, coastal, hotspot stations remain within IMAP and the number of units to be monitored are enlarged to coastal and offshore areas in accordance with marine pressures putting a serious threat in monitoring planning that needs to be resolved by updating monitoring plans;

v) Spatial and temporal coverage of the monitoring programmes for EO5 and EO9 under IMAP should be integrated with the other relevant EOs to put in practice the integrated assessment of the marine environment as a whole (ca. Ecosystem Approach), through the achievement of the Good Environmental Status (GES). Compared to MED POL which was solely focused on tackling human pressures to ecosystem (namely, land-based sources of pollution), the IMAP has been built towards the achievement of GES and should be based on robust and accurate monitoring data and their trend analysis.

14. In line with the IMAP requirements, the Secretariat is making an effort to compile the exiting knowledge within MED POL monitoring programme and other elements of marine environment monitoring established within MAP and provide an upgrade, as appropriate. The most relevant actual developments under different policies in EU (e.g. EU MSFD, OSPAR and HELCOM), as well as relevant experiences in the realization of marine monitoring programmes worldwide (e.g. United States, Japan, New Zeland, South Africa) are also considered.

Table 2. Potential IMAP Common Indicator that could be tentatively monitored within existing national programmes.

Type of Programme	**Objectives and implementation**	**Ecological Objectives (EOs) and Common Indicators (CIs) potentially covered**
MED POL Programme (Eutrophication and Chemical pollution)	Monitoring, control and assessment of land-based sources of pollution (e.g. hotspots, coastal sites and reference areas scattered through national coastlines)	**EO5-CI13**.Concentration of key nutrients in water column; **EO5-CI14**.Chlorophyll-a concentration in water column; **EO9-CI17**. Concentration of key harmful contaminants measured in the relevant matrix (biota, sediment, seawater); **EO9-CI18**. Level of pollution effects of key contaminants where a cause and effect relationship has been established;
MED POL Programme (Bathing	Monitoring and control of microbial pathogens in recreational areas (e.g. selected beaches	**EO9-CI21**.Percentage of intestinal enterococci concentration measurements within established standards; ***EO10-CI22**.Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its

Type of Programme	Objectives and implementation	Ecological Objectives (EOs) and Common Indicators (CIs) potentially covered
Waters Quality)	during the touristic season)	composition, spatial distribution and, where possible, source);
Fisheries and aquaculture management programmes (driven by FAO)	Monitoring, control, statistics and surveillance of commercial fisheries and aquaculture activities (e.g. sampling in commercial ports/fish markets, ship observers, catch quota)	***EO9-CI17**. Concentration of key harmful contaminants measured in the relevant matrix (biota, sediment, seawater); ***EO9-CI18**. Level of pollution effects of key contaminants where a cause and effect relationship has been established; ***EO9-CI20**. Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels in commonly consumed seafood; ***EO10-CI23**.Trends in the amount of litter in the water column including microplastics and on the seafloor; ***EO10-Candidate CI24**: Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds and marine turtles;
Marine Protected Areas (MPAs) Programmes	Surveillance and environmental control (e.g. protected species, marine ecosystems, etc.)	***EO5-CI13**.Concentration of key nutrients in water column; ***EO5-CI14**.Chlorophyll-a concentration in water column; ***EO9-CI17**. Concentration of key harmful contaminants measured in the relevant matrix (biota, sediment, seawater); ***EO9-CI18**. Level of pollution effects of key contaminants where a cause and effect relationship has been established; ***EO10-CI22**.Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its composition, spatial distribution and, where possible, source); ***EO10-CI23**.Trends in the amount of litter in the water column including microplastics and on the seafloor; ***EO10-Candidate CI24**: Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds and marine turtles;
National Programmes to combat Marine and Coastal Pollution	Surveillance, Oil spill response	***EO9-CI19**. Occurrence, origin (where possible), and extent of acute pollution events (e.g. slicks from oil, oil products and hazardous substances) and their impact on biota affected by this pollution;

*Tentative CIs to be monitored along existing national programs (see text).

3. Monitoring Protocols for Eutrophication (EO5)

15. The tabular forms in the following section provide detailed both scientific and technical considerations related to the current practices for monitoring the marine environment, in accordance with IMAP Guidance Factsheets, but presenting specificities for each of the parameters within Common Indicators for Eutrophication which are necessary for an appropriate monitoring, including outstanding research publications in the field.

3.1. Key nutrients in the water column (CI13)

Monitoring CI13 Concentration of Key nutrients in water column	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	A variety of sampling bottles can be used for the collection of nutrient samples. These are commonly deployed on either a CTD -rosette or are clamped to a hydrographic wire and lowered to the prescribed depth. It is important to use suitable bottles to collect and store samples, i.e. glass bottles may leach silicate and phosphate into samples. Polyethylene or polypropylene bottles may be used. The sampling bottles and storage containers should always be rinsed with sample water before filling.	EN ISO 5667-3
Sample processing	Nutrient determinations should be carried out as soon as possible after sampling. Ammonia should be determined immediately after sampling, while nitrate, phosphate, and silicate should be determined within a few hours after sampling, with samples protected from light and stored in a refrigerator. "If analysis is not possible within a few hours then samples must be preserved. Commonly used preservation methods are freezing (for silicate preferable at temperatures between –18 °C and –20 °C) or adding a preservative, e.g. $HgCl_2$ (EN ISO 5667-3). Since no preservation method for nutrients can presently be recommended for general use, each laboratory must validate and document its storage methods for each nutrient, taking account of the likely differences in properties of estuarine, coastal, and offshore waters.	UNEP/MAP/MED POL, 2005
Measurements	The determination of nutrients is mostly based on colorimetric methods (e.g. Grasshoff et al., 1999). There are also fluorometric methods available, e.g. for the analysis of ammonia in seawater (Holmes et al., 1999; Aminot et al., 2001), and UV spectrophotometric methods for the direct determination of nitrate (Johnson and Coletti, 2002). The detailed procedure is fully described in MAP Technical Reports Series No. 163 (UNEP/MAP/MED POL, 2005). Most methods commonly used are manual methods adapted to automated analytical equipment (continuous flow analysis or flow injection analysis; Kirkwood, 1996). In addition to the validation of the chemical method itself, the validation of the handling procedures and maintenance of the automated equipment is important. Aditional publications and manuals are available that provide detailed guidance for working at sea with continuous flow analysis of nutrients (Aminot and Kerouel, 2007; Hydes et al., 2010).	UNEP/MAP/MED POL, 2005 Strickland and Parsons, 1972 http://www.ioccp.org/index.php/nutrients
Reporting and Quality Assurance	*Ammonium:* Symbol: $c(NH_4^+)$ *Nitrite:* Symbol: $c(NO_2^-)$ *Nitrate:* Symbol: $c(NO_3^-)$ *Total Nitrogen:* Symbol: c(TN) *Orthophosphate:* Symbol: $c(PO_4^{3-})$ *Total Phosphorous:* Symbol: c(TP) *Orthosilicate:* Symbol: $c(SiO_4^{3-})$	EN ISO/IEC 17025

	Unit: µmol/L (micromole per litre) Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats, together with QA information on methods used, detection limits, reference values, and any other comments or information relevant to an assessment of the data. It is recommended that laboratories carrying out analyses of nutrients have to establish a quality management system according to EN ISO/IEC 17025. An accreditation by a recognized accreditation authority is also recommended. The quality assurance procedures must cover all steps of the nutrient determinations, including sampling, storage of samples, analytical procedures, maintenance and handling of the equipment as training of the personnel. The laboratory should also take part in interlaboratory comparisons and proficiency testing, e.g. QUASIMEME, to provide external verification of laboratory performance. Currently certified reference materials (CRMs) for nutrients in seawater are commercially available from: • KANSO Technos in Japan - currently for nitrate plus nitrite, nitrite, phosphate, and silicate. http://www.kanso.co.jp/eng/production/index.html • National Research Council of Canada - for nitrate plus nitrite, nitrite, phosphate, and silicate. https://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/moos_3.html • Eurofins, Denmark - for ammonia, total nitrogen, total phosphorous, nitrate plus nitrite, nitrite, phosphate, and silicate. https://www.eurofins.dk/miljoe/vores-ydelser/certificerede-vki-referencematerialer/information-in-english/certificates-in-english/	

Literature:

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Aminot, A., and Kérouel, R. 2007. Dosage automatique des nutriments dans les eaux marines. Editions Quae, Versailles, France, 188 pp. ISBN 978-2-7592-0023-8.

EN ISO 5667-3: Water quality - Sampling - Part 3: Preservation and handling of water samples.

EN ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories.

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Kirkwood, D. S. 1996. Nutrients: Practical notes on their determination in sea water. ICES Techniques in Marine Environmental Sciences, No. 17.

Moore, T. S., Mullaugh, K. M. Holyoke, R. R. Madison, A. S., Yucel, M., and Luther, G. W. 2009. III. Marine Chemical Technology and Sensors for Marine Waters: Potentials and Limits. Annual Review of Marine Science, 1: 91–115.

UNEP/MAP/MED POL 2005 Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.

Additional Literature (Provided manual for other Conventions or Countries):

- HELCOM, 2017. Manual for Marine Monitoring in the COMBINE Programme of HELCOM;
- OSPAR, 2013 Revised JAMP Eutrophication Monitoring Guideline: Oxygen;
- Socal, G., Buttino, I., Cabrini, M., Mangoni, O., Penna, A., Totti, C., 2010. Metodologie di studio del planctonmarino. Manuali e LineeGuida 56/2010, ISPRA, 658 pp;
- GO-SHIP, 2019 Repeat Hydrography Nutrient Manual: The precise and accurate determination of dissolved inorganic nutrients in seawater; Continuous Flow Analysis methods and laboratory practices.

3.2. Chlorophyll-a in the water column (CI14) and related parameters

Monitoring CI14 Concentration of Chlorophyll *a*	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	A variety of sampling bottles can be used for the collection of nutrient samples. These are commonly deployed on either a CTD -rosette or are clamped to a hydrographic wire and lowered to the prescribed depth. A non-transparent sampling device is recommended and because chlorophyll is photolabile (is broken down to colourless compounds in the light).	EN ISO 5667-3
Sample processing	It is recommended that the sample drawn from the water sampler should be filtered immediately on board. However, samples may be stored for short periods in the dark and at ~4°C (for longer storage see note below). The volume of sample required depends on the amount of phytoplankton present; with ocean water, about four to five litres should be used but with coastal and bay waters, sometimes one tenth of this amount is sufficient. Chlorophyll samples should be filtered immediately after sampling and filtering should be carried out under green or low light conditions. Filters should be extracted immediately, and the extract should be kept deep-frozen. If it is not possible to follow this procedure the filters should be kept frozen at < -20 °C for no longer than 21 days. If stored longer a temperature of < -80 °C should be maintained to avoid degradation of chlorophyll.	UNEP/MAP/MED POL, 2005
Measurements	Extraction procedures and measurements should be carried out in low light. Standard procedures for the determination of chlorophyll *a* are given in Strickland and Parsons (1968), UNESCO (1994), ISO 10260 (1992) and resumed in UNEP/MAP/MED POL, 2005. It is important to report the method used. It should be ensured, that the same method of measuring chlorophyll concentrations is used and the same procedure (sample collection, filtration, extraction and storage) is followed during the surveys. Changes must be well documented. If HPLC is used for chlorophyll analysis the method by Wright et al (1991) should be used. This HPLC method has been commonly used, and it is accepted that it does	UNEP/MAP/MED POL, 2005 Strickland and Parsons, 1968 UNESCO, 1994 ISO 10260 (1992)

	not distinguish between chlorophyll a and divinyl chlorophyll a derivates. If that level of differentiation is required an interlaboratory comparison of different HPLC methods should be consulted (e.g. Claustre et al 2004). If in-situ chlorophyll fluorometers are used, they should be calibrated with local natural water samples with a range of chlorophyll concentrations. All measuring instruments should be calibrated with filtered water samples and standard chlorophyll *a*.	
Reporting and Quality Assurance	Symbol: *c*(Chl*a)* Unit: µg/L (microgram per litre) Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats, together with QA information on methods used, detection limits, reference values, and any other comments or information relevant to an assessment of the data. It is recommended that laboratories carrying out analyses of Chlorophyll *a* have to establish a quality management system according to EN ISO/IEC 17025. An accreditation by a recognized accreditation authority is also recommended. The recommendations of should be kept in mind. The quality assurance procedures must cover all steps of the concentration of chlorophyll *a* determination, including sampling, storage of samples, analytical procedures, maintenance and handling of the equipment as training of the personnel. The laboratory should also take part in interlaboratory comparisons and proficiency testing, e.g. QUASIMEME, to provide external verification of laboratory performance. Because a Certified Reference Material (CRM) for chlorophyll is not available the laboratories should take part in interlaboratory comparisons on a regular basis. Internal methods should be properly validated. As a routine procedure for controlling systematic errors, the use of control charts is recommended. It is common practice in analytical laboratories to run duplicate analyses at frequent intervals as a means of monitoring the precision of analyses and detecting out-of-control situations in R-charts so called Range (control) charts or Precision charts. This is often done for determinants for which there are no suitable control samples or reference materials available. For chlorophyll *a* analyses it is recommended to run at least one duplicate sample within every batch of samples.	EN ISO/IEC 17025 EN 14996 (2006)

Literature:

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UNEP/MAP/MED POL, 2005 Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.

UNESCO, 1994. Protocols for the Joint Global Ocean Flux Study (J GOFS) Core Measurements. Manual and Guide No 29. 179.

Wright, S.W.; Jeffrey, S.W.; Mantoura, R. F. C.; Llewellyn, C.A.; Bjornland, T.; Repeta, D.; Welschmeyer, N., 1991 Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton, Mar. Ecol. Prog. Ser., 77, 183–196.

Additional Literature (Provided manual for other convention or Countries):

- HELCOM, 2017. Manual for Marine Monitoring in the COMBINE Programme of HELCOM;
- OSPAR, 2012 JAMP Eutrophication Monitoring Guidelines: Chlorophyll *a* in Water;
- Socal, G., Buttino, I., Cabrini, M., Mangoni, O., Penna, A., Totti, C., 2010. Metodologie di studio del planctonmarino. Manuali e LineeGuida 56/2010, ISPRA, 658 pp.

Monitoring CI14 Transparency by Secchi disk	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection and processing	(does not apply)	(does not apply)

Measurements	The methodology is based on the forthcoming ISO/WD 7027-2 standard.
	• *Testing disk (Secchi disk).* A white disk with a diameter of 30 cm. The disk should weigh at least 1.7 kg so as to descend quickly and not be affected by horizontal water movements.
	• *Measuring tape/rope* of non-elastic material. Depth recognition:
	  • colour-coded marks at 10 cm intervals. The upper side of the disk equals 0 cm. Half and full meters should be marked so as to be easily distinguishable.
	  • depth indicator of a winch
	• *Optional devices* for suppression of reflections, e.g., polarized glasses for the observer. Note: Secchi depth measurement is dependent on the observer's eyesight, and any aids for vision tend to increase Secchi depth, which should be considered, e.g., in the context of long-term data series.
	The observer should try to ensure that the measuring rope stays in an as upright position as possible. Measure the Secchi depth on the shaded side of the ship to avoid direct sunlight reflections from the water surface.
	Allow sufficient time (preferably 2 min) when looking at the disc near its extinction point for the eyes to completely adapt to the prevailing luminance level. Lower the disc further until it is no longer visible. The achieved depth is to be read and written down. After that, the disc is lowered by another 0.5 m. Then, during a slow elevation, the disc becomes visible as a greenish-bluish spot. The achieved depth is to be read and written down. It is recommended to repeat the test two times as a minimum. The Secchi depth is the arithmetic average of all readings.
		ISO/WD 7027-2
		UNEP/MAP/MED POL, 2005

	In the waters of high turbidity, the precision can approach 0.1 m under calm seas. In clearer waters, the precision ranges 0.2 to 0.5 m, depending on actual conditions.	
Reporting and Quality Assurance	Symbol: z_{SD} Unit: m (meter) Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats.	…..
Literature: Cialdi, M. and Secchi, P. A., 1865. Sur la transparence de la mer. Comptes Rendu de l'Acadamie des Sciences 61: 100–104. ISO/WD 7027-2: Water quality – Determination of turbidity – part 2: Semi-quantitative methods. Draft version. UNEP/MAP/MED POL, 2005 Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.		

Monitoring CI14 Temperature and Salinity	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	Samples are only collected when Salinity are measured with Bench salinometer. A variety of sampling bottles can be used for the collection of salinity samples. These are commonly deployed on either a CTD-rosette or are clamped to a hydrographic wire and lowered to the prescribed depth.	EN ISO 5667-3
Sample processing	For general requirements for sampling, preservation, handling, transport and storage of water samples, see EN ISO 5667-3. Samples for determination of salinity are subsampled into glass bottles with tight fitting caps. Bottles with a plastic screw cap and a disposable plastic insert are preferred. A large sample volume (>200 ml) decreases the risk of contamination during subsampling and handling and provides enough sample for thorough rinsing of the measuring cell (UNEP/MAP/MED POL, 2005).	EN ISO 5667-3 UNEP/MAP/MED POL, 2005
Measurements	*Temperature* Mercury reversing thermometer Electronic reversing thermometer For both thermometers follow standard oceanographic procedures *Salinity* Bench salinometer Follow the procedure from Muller, 1999 and summarized in UNEP/MAP/MED POL, 2005. *CTD (Conductivity, Temperature, Depth)* CTDs are equipped with conductivity and temperature sensors for *in situ* measurements. There are many protocols for CTD measurements (WOCE 1991, UNESCO 1994, UNESCO, 1988,). Decide for one and follow consistently in line with the manufacturer's recommendation.	WOCE, 1991 UNESCO, 1994, UNEP/MAP/MED POL, 2005.

	The Practical Salinity Scale of 1978 (PSS-78) has to be used. Practical Salinity (*S*) is calculated from the ratio of conductivity between sample and reference solution. Since the scale is based on a ratio, no unit is assigned to it. The equations used in calculation of Practical Salinity from conductivity are valid for practical salinity ranging from 2 to 42.	
Reporting and Quality Assurance	*Temperature:* Symbol: *t* Unit: °C (degree Centigrade) *Salinity:* Symbol: *S* Unit: - (dimensionless) Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats. It is recommended that laboratories carrying out analyses of salinity have to establish a quality management system according to EN ISO/IEC 17025. Laboratory calibration of CTD sensors need to be performed with regular intervals. Manufacturers normally provide calibration services for pressure, temperature and salinity sensors. In between calibrations, performance of the CTD conductivity and temperature sensors is monitored by comparison to data from reference samples/reference instruments.	EN ISO/IEC 17025

Literature:

Müller T J. Determination of salinity. Chapter 3 p 41-73 in Grasshoff K, Kremling K and Erhardt M. Methods of Seawater Analysis 3rd ed. Wiley-VCH 1999. ISBN 3-527-29589-5.

EN ISO 5667-3*: Water quality – Sampling – Part 3: Preservation and handling of water samples.

IOC, SCOR, and IAPSO. The international thermodynamic equation of seawater – 2010: Calculation and use of thermodynamic properties. Intergovernmental Oceanographic Commission, UNESCO 2010.

UNEP/MAP/MED POL 2005 Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.

UNESCO 1991. Processing of oceanographic station data. JPOTS editorial panel.

UNESCO 1994. Protocols for Joint Global Flux Study (JGOFS) Core Measurements. Manual and Guides 29.

UNESCO 1988. The acquisition, calibration, and analysis of CTD data. A report of SCOR Working Group 51. UNESCO Technical Papers in Marine Science, 54, 94pp.

WOCE 1991. WOCE Operational Manual, Vol. 3. WOCE Report 68/91, July 1991.

Additional Literature (Provided manual for other convention or Countries):

- HELCOM, 2017. Manual for Marine Monitoring in the COMBINE Programme of HELCOM
- Socal, G., Buttino, I., Cabrini, M., Mangoni, O., Penna, A., Totti, C., 2010. Metodologie di studio del planctonmarino. Manuali e LineeGuida 56/2010, ISPRA, 658 pp.

Monitoring CI14 pH measurement	Purpose/Rationale	Guidelines/References (including research publications)
Sample collection	A variety of sampling bottles can be used for the collection of nutrient samples. These are commonly deployed on either a CTD -rosette or are clamped to a hydrographic wire and lowered to the prescribed depth.	EN ISO 5667-3
Sample processing	Subsamples for pH should be drawn from sampler bottles as early as possible (after samples for oxygen but before samples for nutrients and salinity) to avoid gas exchange between water and air. Samples should be collected in gas-tight bottles. Bottles should be rinsed thoroughly with sample water before filling. Bottles are filled with a laminar flow of sample water, allowing 2-3 bottle volumes to overflow before capping. Bottles should be completely filled, leaving no headspace. Avoid trapping bubbles of air when capping bottles. Samples should preferably be analysed as soon as possible directly after sampling.	HALCOM, 2017
Measurements	pH is measured using a glass/combined electrode. Determination of pH using a glass electrode is described in ISO 10523. The NBS pH scale should be used, although not ideal, the NBS scale has to this day been considered to be the best option for the wide range of salinity. Temperature is measured and recorded both during pH measurement and at sampling depth. A correction for in situ pH (Gieskes 1969) is sometimes applied. A better option is to report measured pH, temperature from pH measurement and *in situ* temperature.	ISO 10523 HALCOM, 2017 Wedborg et al, 2007
Reporting and Quality Assurance	Symbol: pH Unit: - (dimensionless) Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats. It is recommended that laboratories carrying out measurement of pH have to establish a quality management system according to EN ISO/IEC 17025.An internal reference material (IRM) should be analysed daily.	EN ISO/IEC 17025

Literature:

Gieskes J M 1969. Effects of temperature on the pH of seawater. Limnology and Oceanography Vol 14 Issue 5, p 679-685.

HELCOM, 2017. Manual for Marine Monitoring in the COMBINE Programme of HELCOM.

Wedborg, M. , Turner, D. R., Anderson, L. G. and Dyrssen, D., 2007. Determination of pH. In Methods of Seawater Analysis (eds K. Grasshoff, K. Kremling and M. Ehrhardt).doi:10.1002/9783527613984.ch7

ISO 10523: Water quality – Determination of pH.

EN ISO/IEC 17025*: General requirements for the competence of testing and calibration laboratories.

Additional Literature (Provided manual for other convention or Countries):

- Socal, G., Buttino, I., Cabrini, M., Mangoni, O., Penna, A., Totti, C., 2010. Metodologie di studio del planctonmarino. Manuali e LineeGuida 56/2010, ISPRA, 658 pp.

<table>
<tr><th>Monitoring CI14 Concentration of Dissolved Oxygen and Saturation</th><th>Purpose/Rationale</th><th>Guidelines/References (including research publications)</th></tr>
<tr><td>Sample collection</td><td>Water samples from specified depths are normally collected with water samplers. Various designs are commercially available. The non-reversing type sampler such as Niskin is the most widely used.</td><td></td></tr>
<tr><td>Sample processing</td><td>Oxygen in water samples for Winkler analysis must be fixed immediately after collection to eliminate the removal or production of oxygen in the sample. DO samples should be the first to be drawn from the sampling bottles. After fixation, samples should be stored in a dark place at a constant temperature - if possible the same as the in-situ temperature - for at least one hour. The fixed sample should be titrated within 24 hours of collection. In some cases, longer storage of the fixed sample is unavoidable, but storage conditions and handling procedures must be validated and clearly documented. Zhang et al. (2002) noted that storage under seawater is advisable in such circumstances. For general requirements for sampling, preservation, handling, transport and storage of water samples see EN ISO 5667-3.</td><td>EN ISO 5667-3.</td></tr>
<tr><td>Measurements</td><td>Determination of Oxygen by Winkler Titration Method

The reference method for the determination of DO is the Winkler titration to the iodine endpoint. The procedure according to ISO 5813:1983is fully described in MAP Technical Reports Series No. 163 (UNEP/MAP/MED POL, 2005). Modifications of this method, which have been verified in intercalibration exercises, are described elsewhere (e.g. Carpenter, 1965; Grasshoff et al., 1999; Strickland and Parsons, 1968). Modifications mainly concern composition of the reagents, titration devices (manual titration, automatic systems), and the method used for detecting the end point of the titration (e.g. visible colour change of indicator dyes, conductivity measurement, photometric detection). As verified by intercalibration exercises, reliable results can be obtained with many methods, providing proper procedures are followed.

In situ Determination of Oxygen

Oxygen is normally determined using electrochemical or optical sensors. A standard procedure for the determination of oxygen in water with electrochemical sensors is given in EN ISO 5814. Dissolved oxygen sensors can be deployed from vessels and may be used attached to a CTD systems.</td><td>ISO 5813:1983
UNEP/MAP/MED POL, 2005
Strickland and Parsons, 1968
EN ISO 5814</td></tr>
<tr><td>Reporting and Quality Assurance</td><td>Concentration of Dissolved Oxygen
Symbol: $c(O_2)$
Unit: µmol/L (micromole per litre)

Transformations:
<table><tr><th>Unit A</th><th>Unit B</th><th>Transformation factor</th></tr><tr><td>mg/L</td><td>mL/L</td><td>0.7</td></tr><tr><td>mL/L</td><td>mg/L</td><td>1.429</td></tr><tr><td>µmol/L</td><td>mL/L</td><td>11.196</td></tr><tr><td>mL/L</td><td>µmol/L</td><td>0.0893</td></tr><tr><td>mg/L</td><td>µmol/L</td><td>0.06251</td></tr><tr><td>µmol/L</td><td>mg/L</td><td>15.997</td></tr></table></td><td>EN ISO/IEC 17025
UNESCO, 1973
WOCE, 1994</td></tr>
</table>

	Saturation of Dissolved Oxygen Symbol: $\varphi(O_2/O_2')$ Unit: % (percent) Saturation is calculated from tables of oxygen saturation values in Volume II of the International Oceanographic Tables (UNESCO, 1973). With Dissolved Oxygen Temperature and Salinity has to be reported. Data reporting to the IMAP database should be in accordance with the requirements of the latest reporting formats, together with QA information on methods used, detection limits, reference values, and any other comments or information relevant to an assessment of the data. It is recommended that laboratories carrying out analyses of oxygen have to establish a quality management system according to EN ISO/IEC 17025. An accreditation by a recognized accreditation authority is also recommended. There is no Certified Reference Material for oxygen in water. The reference method is the properly performed Winkler method (Grasshoff *et al*, 1999). Several publications contain descriptions of how the calibration should be performed and quality assurance can be achieved (WOCE, 1994) The calibration of sensors is dependent on the Winkler method and therefore it is recommended to use internal laboratory procedures according to Grasshoff *et al.* (1999) for quality assurance of the chemical analysis.	

Literature:

Carpenter, J. H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnology and Oceanography, 10: 141–143.

EN ISO 5667-3: Water quality - Sampling - Part 3: Preservation and handling of water samples.

EN ISO 5814: Water quality - Determination of dissolved oxygen - Electrochemical probe method.

EN ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories.

ISO 5813:1983 Water quality – Determination of dissolved oxygen – Iodometric method.

Grasshoff, K., Kremling, K., and Ehrhardt, M. (Eds.) 1999. Methods of Seawater Analysis. 3rd ed. Wiley–VCH.

Strickland, J. D. H., and Parsons, T. R. 1968., A Practical Handbook of Seawater Analysis. Ottawa: Fisheries Research Board of Canada, Bulletin 167. Pp. 23-28.

UNEP/MAP/MED POL 2005 Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.

UNESCO, 1973. International Oceanographic Tables, Vol. 2 Oxygen Solubility In Seawater.

WOCE, 1994. Operational Manual. Volume 3: The Observational Programme.

Zhang, J., Berberian, G., and Wanninkhof, R., 2002. Long-term storage of natural water samples for dissolved oxygen determination. Water Research, 36: 4165–4168.

Additional Literature (Provided manual for other convention or Countries):

- Langdon, C., 2010 Determination of Dissolved Oxygen in Seawater by Winkler Titration Using the Amperometric Technique. The GO-SHIP Repeat Hydrography Manual: A collection of Expert Reports and Guidelines, IOCCP Report No. 14, ICPO Publication Series No. 134, Version 1, 2010.
- HELCOM, 2017. Manual for Marine Monitoring in the COMBINE Programme of HELCOM
- OSPAR, 2013 Revised JAMP Eutrophication Monitoring Guideline: Oxygen.
- Socal, G., Buttino, I., Cabrini, M., Mangoni, O., Penna, A., Totti, C., 2010. Metodologie di studiodelplanctonmarino. Manuali e LineeGuida 56/2010, ISPRA, 658 pp.

4. Monitoring Protocols for Contaminants (EO9)

16. The tabular forms in the following section provide detailed both scientific and technical considerations related to the current practices for monitoring the marine environment, in accordance with IMAP Guidance Factsheets, but presenting specificities for each of the parameters within Common Indicators for Contaminants are necessary for an appropriate monitoring, including outstanding research publications in the field.

4.1. Heavy metals, trace elements and organic chemicals (CI17)

Monitoring CI17	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	To collect marine organisms, where the whole soft tissues or dissected parts are processed for to perform analytical measurements of chemical contaminants (primarily, in bivalve species and/or fish) In the Mediterranean the most common sample species are bivalves, (e.g. *Mytilus galloprovincialis*, *Donaxtrunculus)* and fish (e.g.*Mullus barbatus).* Further, sediment samples should be collected in coastal and marine areas, the continental platform and offshore by mechanical means (grab or corer) according the sampling strategy.	No 6 Rev. 1 UNEP/FAO/IOC/IAEA: Guidelines for monitoring chemical contaminants in marine organisms. (25 p) No 12 Rev. 2. UNEP/FAO/IAEA: Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. (23 p) HELCOM-COMBINE, 2017. Manual for Marine Monitoring in the Programme of HELCOM (last update July 2017 JAMP, 2018 (OSPAR). Joint Assessment and Monitoring Programme (JAMP) 2014 – 2021 JRC, 2014. Technical guidance on monitoring for the Marine Strategy Framework Directive. JRC Scientific and Policy Report, EUR 26499 EN.
Sample processing	Some additional parameters need to be recorded in biota are the biometrics (e.g. size/length, age), biological parameters such as condition index (e.g. mussels) and condition factor according established protocols and scientific literature and knowledge. For sediments the standard sieving fraction processed at the laboratory and analysed should be < 2 mm particle size fraction after freeze-drying (e.g. in-house mesh validated methods and/or geological sieving methods). The < 63µm sediment fraction is also recommended to be complementary for metals.	No 71UNEP/IAEA/IOC/FAO: Sample work-up for the analysis of chlorinated hydrocarbons in the marine environment. (52 p) León V.M., García I., Martínez-Gómez C., Campillo J.A., Benedicto J., 2014. Galgani, F.; Chiffoleau, J.F.; Barrah, Mahmoud; Drebika, Usama; Tomasino, C., Andral, B., 2014. Benedicto, J., Andral, B., Martínez-Gómez, C., Guitart, C., Deudero, S., Cento, A., Scarpato, A., Caixach, J., Benbrahim, S.,

	The liophilization ratio (dry/wet sediment ratio) should be considered for datasets reporting and data should be reported in dry weight.	Chouba, L., Boulahdidi, M., Galgani F., 2011.
Measurements	Trace/Heavy Metals (TM) and Aluminium: Spectrometry, Mass Spectrometry (MS) Organic compounds: Gas or Liquid Chromatography (GC/LC) coupled to a variety of detectors, such as Flame Ionization Detector (FID) Electron Capture Detector (ECD) or Mass Spectrometry (MS)	Guidance Document No. 33 ON ANALYTICAL METHODS FOR BIOTA MONITORING UNDER THE WATER FRAMEWORK DIRECTIVE, Technical Report - 2014 – 084, ISBN 978-92-79-44679-5 León V.M., García I., Martínez-Gómez C., Campillo J.A., Benedicto J., 2014. Galgani, F.; Chiffoleau, J.F.; Barrah, Mahmoud; Drebika, Usama; Tomasino, C., Andral, B., 2014. Ahmeda I., Mostefa B., Bernard A., Olivier R., 2018. Note: a number of UNEP Regional Sea edited Guidelines could serve as well as a guide to measurement performance, although for some the instrumental techniques have been improved (e.g. from cold-vapour AAS to Solid-AAS sample analysis for total Hg).
Reporting and QA	• TM: ug/Kg (e.g. Cadmium), mg/Kg (e.g. Zinc), g/Kg (e.g. Aluminium) • OC: ug/Kg (ppb) or mg/Kg (ppm) • TOC: Elemental Analyser (as %) • Particle fractions (as %) Selected analytical methods and measurements are subject to internal Quality Assurance through National Laboratories QA/QC Protocols and Laboratory accreditations, as well as external Quality Assurance by performing regional interlaboratory QA/QC exercises organized by theUNEP/MAP MED POL/IAEA MESL.	No 7 Rev. 2 1988 UNEP/FAO/IOC/IAEA: Sampling of selected marine organisms and sample preparation for trace metal analysis. (21 p). No 57 UNEP/IOC/IAEA: Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice. (30 p).
Literature: No 6 Rev. 1 UNEP/FAO/IOC/IAEA: Guidelines for monitoring chemical contaminants in marine organisms. (25 p)		

No 12 Rev. 2.UNEP/FAO/IAEA: Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. (23 p)

No 71UNEP/IAEA/IOC/FAO: Sample work-up for the analysis of chlorinated hydrocarbons in the marine environment. (52 p)

Guidance Document No. 33 ON ANALYTICAL METHODS FOR BIOTA MONITORING UNDER THE WATER FRAMEWORK DIRECTIVE, Technical Report - 2014 – 084, ISBN 978-92-79-44679-5

HELCOM-COMBINE, 2017. Manual for Marine Monitoring in the Programme of HELCOM (last update July 2017, http://www.helcom.fi/action-areas/monitoring-and-assessment/manuals-and-guidelines/combine-manual)

JAMP, 2018 (OSPAR). Joint Assessment and Monitoring Programme (JAMP) 2014 – 2021. Update 2018 (Agreement 2014-02)
https://www.ospar.org/work-areas/cross-cutting-issues/jamp

CEMP, 2016 (OSPAR). Coordinated Environmental Monitoring Programme (Agreement 2016-01).
https://www.ospar.org/work-areas/cross-cutting-issues/cemp

JRC, 2012. Monitoring for the Marine Strategy Framework Directive: Requirements and Options. EUR 25187 EN
http://mcc.jrc.ec.europa.eu/document.py?code=201409261130

JRC, 2014. Technical guidance on monitoring for the Marine Strategy Framework Directive. JRC Scientific and Policy Report, EUR 26499 EN.
http://mcc.jrc.ec.europa.eu/document.py?code=201406241353

León V.M., García I., Martínez-Gómez C., Campillo J.A., Benedicto J., 2014. Heterogeneous distribution of polycyclic aromatic hydrocarbons in surface sediments and red mullet along the Spanish Mediterranean coast. Marine Pollution Bulletin 87, 352–363.

Galgani, F.;Chiffoleau, J.F.; Barrah, Mahmoud; Drebika, Usama; Tomasino, C., Andral, B., 2014. Assessment of heavy metal and organic contaminants levels along the Libyan coast using transplanted mussels (Mytilus galloprovincialis). Environmental Science and Pollution Research, 21, Issue 19, 11331–11339.

Ahmeda I., Mostefa B., Bernard A., Olivier R., 2018. Levels and ecological risk assessment of heavy metals in surface sediments of fishing grounds along Algerian coast. Marine Pollution Bulletin 136, 322–333

Benedicto, J., Andral, B., Martínez-Gómez, C., Guitart, C., Deudero, S., Cento, A., Scarpato, A., Caixach, J., Benbrahim, S., Chouba, L., Boulahdidi, M., Galgani F., 2011. A large-scale survey of trace metal levels in coastal waters of the Western Mediterranean basin using caged mussels (Mytilus galloprovincialis). Journal of Environmental Monitoring. DOI: 10.1039/c0em00725k

4.2. Biomarkers and toxicology related methods (CI18)

Monitoring CI18	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	The marine organisms collected to perform biomarker and toxicology evaluations should be collected exactly as for CI17. In this way, the integrated chemical-biological assessments of the contaminant effects in the marine environment might better support the achievement of GES. As for chemical monitoring, sample collection should focus on selected	UNEP (1997), The MED POL Biomonitoring Programme Concerning the Effects of Pollutants on Marine Organisms Along the Mediterranean Coasts. UNEP(OCA)/MED WG.132/3, Athens. UNEP/RAMOGE (1999). Manual on the Biomarkers Recommended for the UNEP/MAP MED POL

	locations such as hotspots and reference stations.	Biomonitoring Programme. UNEP, Athens. ICES Cooperative Research Report. No.315. Integrated marine environmental monitoring of chemicals and their effects. I.M. Davies and D. Vethaak Eds., November 2012.
Sample processing	Preservation, storage and transportation to the laboratory from remote locations are key factors to undertake toxicological measurements in live organisms (e.g. Lysosomal Membrane Stability-neutral Red Retention method). Further, dissections of the parts from marine organisms according the standard methodologies for biochemical parameters and organism parts will be also undertaken (e.g. gills in *Mytilus galloprovincialis*. Additional parameters to be recorded in this step (in the field or at the laboratory) are: biometrics (size/length, age), biological parameters such as condition index (mussels), condition factor, gonadosomatic index, hepatosomatic index (fish) and data on temperature, salinity and oxygen dissolved.	ICES Cooperative Research Report. No.315. Integrated marine environmental monitoring of chemicals and their effects. I.M. Davies and D. Vethaak Eds., November 2012. Cenov et al., 2018.
Measurements	In marine bivalves (such as *Mytilusgalloprovincialis*) and/or fish (such as *Mullus barbatus*): - Lysosomal Membrane Stability (LMS): Biological techniques (neutral red retention), including microscopy - Acetylcholinesterase (AChE) assay: Biochemical techniques, including spectrophotometry - Micronucleus assay: Biochemical techniques, including microscopy <u>Sub-indicators:</u> as indicated in the Guidance Factsheets for CI18, complementary biomarkers, bioassays and histology techniques and methods are also recommended to be carried out on a country basis (such as, hepatic pathologies assessment, reduction	European Commission, 2014. Technical report on aquatic effect-based monitoring tools. Technical Report - 2014 – 077. Moore, M.N. (1985); Moore, M.N. (1990) Tsangaris C., Kormas K., Strogyloudi E., Hatzianestis I., Neofitou C., Andral B., Galgani F., 2010. Ben Ameur, 2015.

	of survival in air by Stress on Stress (SoS), larval embryotoxicity assay, Comet assay, etc.). Metallothionnein in mussels and Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarkers of chemical exposures	
Reporting and QA	The main units for the agreed toxicological test under IMAP CI18 are: (retention) minutes - Lysosomal Membrane Stability (LMS); nmol/min mg protein in gills (bivalves) for Acetylcholinesterase (AChE) assay; and, Number of cases, ‰ in haemocytes for the Micronucleus assay	ICES Cooperative Research Report. No.315. Integrated marine environmental monitoring of chemicals and their effects. I.M. Davies and D. Vethaak Eds., November 2012. Martínez-Gómez, C., 2017. Regoli, F and Giuliani, M.E., 2014.

Literature:

UNEP (1997), The MED POL Biomonitoring Programme Concerning the Effects of Pollutants on Marine Organisms Along the Mediterranean Coasts. UNEP(OCA)/MED WG.132/3, Athens.
UNEP/RAMOGE (1999). Manual on the Biomarkers Recommended for the UNEP/MAP MED POL Biomonitoring Programme. UNEP, Athens.

ICES Cooperative Research Report. No.315. Integrated marine environmental monitoring of chemicals and their effects. I.M. Davies and D. Vethaak Eds., November, 2012.

European Commission, 2014. Technical report on aquatic effect-based monitoring tools. Technical Report - 2014 – 077.

Moore, M.N. (1985), Cellular responses to pollutants. *Mar.Pollut.Bull.*, 16:134-139;
Moore, M.N. (1990), Lysosomal cytochemistry in marine environmental monitoring. *Histochem.J.*, 22:187-191

Scarpato, R., L. Migliore, G. Alfinito-Cognetti and R. Barale (1990), Induction of micronuclei in gill tissue of *Mytilusgalloprovincialis*exposed to polluted marine waters *Mar.Pollut.Bull.*, 21:74-80

UNEP (1997). The MED POL Biomonitoring Programme Concerning the Effects of Pollutants on Marine Organisms Along the Mediterranean Coasts. UNEP(OCA)/MED WG.132/3, Athens.

UNEP/RAMOGE (1999). Manual on the Biomarkers Recommended for the UNEP/MAP MED POL Biomonitoring Programme. UNEP, Athens.

Cenov et al., 2018. A baseline study of the metallothioneins content in digestive gland of theNorway lobster Nephrops norvegicus from Northern Adriatic Sea: Body size,season, gender and metal specific variability. Marine Pollution Bulletin 131 (2018) 95–105

Ben Ameur, 2015. Oxidative stress, genotoxicity and histopathology biomarker responsesin Mugil cephalus and Dicentrarchuslabrax gill exposed to persistentpollutants. A field study in the Bizerte Lagoon: Tunisia. Chemosphere 135 (2015) 67–74

Martínez-Gómez, C., 2017. Biomarkers of general stress in mussels as common indicators for marine biomonitoring programmes in Europe: The ICON experience. Marine Environmental Research, 124, 70-80

Regoli, F and Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Marine Environmental Research, 93,106-117.

4.3. Oil spills and hazardous substances (CI19)

Monitoring CI19	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection Sample processing Measurements (visual, aerial, satellite imagery)	The current monitoring and reporting are performed by national authorities in the event of an spill of oil or other hazardous chemical substances in the marine environment. There are some specific and internationally agreed protocols to address the steps to report on those pressures. Both visual and satellite imagery are current practices to perform estimations of the quantities of oil that enter the marine environment (e.g. tons/year)	ITOPF. "*Aerial Observation of Marine Oil Spills*", Technical Information Paper 1. ITOPF. "*Recognition of Oil on Shorelines*", Technical Information Paper 6. ITOPF. "*Fate of Marine Oil Spills*", Technical Information Paper 2. ITOPF. "*Response to Marine Chemical Incidents*", Technical Information Paper 17. Bonn Agreement. "*Bonn Agreement Oil Appearance Code*". IPIECA/IMO/IOGP/CEDRE. "*Aerial Observation of Oil Spills at Sea: Good practice guidelines for incident management and emergency response personnel*" (February 2015). CEDRE. "*Surveying Sites Polluted by Oil: An Operational Guide for Conducting an Assessment of Coastal Pollution*" (March 2006). REMPEC. "*Mediterranean Guidelines on Oiled Shoreline Assessment*" (September 2009). GESAMP. "*Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships"* (2014).
Reporting and QA	Reporting is required according the set standards and requirements established through REMPEC and the Contracting Parties.	GESAMP. Report n° 75: "Estimates of Oil Entering the Marine Environment from Sea-Based Activities", IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (2007). The Guidelines for Co-operation in Combating Marine Oil Pollution in the Mediterranean (UNEP/IG.74/5, UNEP/MAP, 1987) recommended Contracting Parties to the Barcelona Convention to report to REMPEC all spillages or discharges of oil in excess of 100 cubic metres. To align with the revised reporting formats for a mandatory reporting system under MARPOL ("one-line" entry format) adopted by IMO in 1996 (see MEPC/Circ.318), the Joint Session of MED POL and REMPEC Focal Points Meetings, which was held in Attard, Malta on 17 June 2015, discussed the appropriate threshold and concluded that spills of 50 cubic metres should be reported, whereas countries could also opt to report on spillages of lower amounts.

Literature:

GESAMP. Report n° 75: "Estimates of Oil Entering the Marine Environment from Sea-Based Activities", IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (2007).

ITOPF. "Aerial Observation of Marine Oil Spills", Technical Information Paper 1.

ITOPF. "Recognition of Oil on Shorelines", Technical Information Paper 6.

ITOPF. "Fate of Marine Oil Spills", Technical Information Paper 2.

ITOPF. "Response to Marine Chemical Incidents", Technical Information Paper 17.

Bonn Agreement. "Bonn Agreement Oil Appearance Code".

IPIECA/IMO/IOGP/CEDRE. "Aerial Observation of Oil Spills at Sea: Good practice guidelines for incident management and emergency response personnel" (February 2015).

CEDRE. "Surveying Sites Polluted by Oil: An Operational Guide for Conducting an Assessment of Coastal Pollution" (March 2006).

REMPEC. "Mediterranean Guidelines on Oiled Shoreline Assessment" (September 2009).
GESAMP. "Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships" (2014).

4.4. Seafood contaminants (CI20)

Monitoring CI20	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection	To collect marine organisms, mainly commercial species, and similarly to CI17. The sample collection for CI20 could be easily integrated with CI17 in terms of sample monitoring (e.g. from dedicated fish vessels or from artisanal fleets at port). To be noticed, that in any case, the origin (i.e. area) of the fish captures should be exactly known, including detailed field information (e.g. coordinates)	No 6 Rev. 1 UNEP/FAO/IOC/IAEA: Guidelines for monitoring chemical contaminants in marine organisms. (25 p)
Sample processing	Sample processing refers to the dissection of the selected parts (e.g. liver, flesh fillet tissue, etc.) or the whole organism (e.g. soft parts) to be performed previously to the analytical determination of contaminants. Samples can be pooled to obtain sufficient sample material; however, this approach should be consistent over time and therefore, specific sample processing protocols should be recorded. Additional general parameters required might include: sample	Spada, L. *et al.* 2014.

	identification, location, date and biometrics.	
Measurements	Trace/Heavy Metals (TM) and Aluminium: Spectrometry, Mass Spectrometry (MS) Organic compounds: Gas or Liquid Chromatography (GC/LC) coupled to a variety of detectors, such as Flame Ionization Detector (FID) Electron Capture Detector (ECD) or Mass Spectrometry (MS) <u>Sub-indicators:</u> other relevant chemicals and emerging pollutants are recommended to be carried out on a country decision basis as greed under the IMAP Guidance Factsheets	Maulvault, A.M. et al. 2015.. Perello, G. et al., 2015. Zaza, S. et al. 2015.
Reporting and QA	Percentages of occurrence of contaminants (e.g. number of detected regulated contaminants in commercial species, number of detected regulated contaminants exceeding regulatory limits (the European Regulation EU 1881/2006). With regard analytical QA and determinations, the same approach for CI17 should be followed. Note: the assessment of this indicator should take advantage of the knowledge by the GFCM/FAO in the Mediterranean Sea, as well as off the methodologies developed for the EU MSFD (Descriptor 9).	Maggi, C. et al., 2014. Vandermeersch, G. et al. 2015.

Literature:

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Perello, G. *et al.*, 2015. Human exposure to PCDD/Fs and PCBs through consumption of fish and seafood in Catalonia (Spain): Temporal trend. Food and Chemical Toxicology, 81, 28-33.

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Spada, L. *et al.* 2014. Mercury and methylmercury concentrations in Mediterranean seafood and surface sediments, intake evaluation and risk for consumers. International Journal of Hygiene and Environmental Health, 215, 418-42.

4.5. Bathing water quality (CI21)

Monitoring CI17	**Purpose/Rationale**	**Guidelines/References (including research publications)**
Sample collection Sample processing	The measurements are made in selected monitoring stations during the summer season focusing in the touristic beaches and other sites of concern. The full description of indications to prepare a monitoring strategy can be found in Directive 2006/7/EC of the European Parliament and of the council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC.	UNE/MAP MED POL, 2010. Assessment of the state of microbial pollution in the Mediterranean Sea. MAP Technical Reports Series No. 170 (Amended). Cabelli VJ, Dufour AP, Levin MA, McCabe LJ, Haberman PW. 1979. R Byappanahalli, MN. *et al.*, 2012.
Measurements	As in the case of analytical chemistry, the data confidence originates in the maintenance of internal QA/QC programmes by national laboratories, as well as regular proficiency testing exercises. However, the majority of laboratories performing microbiology in a routine basis should be nationally accredited. It should be mentioned that the level of uncertainty in measurements could be considered low, provided the above is fulfilled.	ISO 7899-1[Water quality – Detection and enumeration of intestinal enterococci: Part 1: Miniaturized method (Most Probable Number) for surface and wastewater] ISO 7899-2 [Water quality – Detection and enumeration of intestinal enterococci: Part 2: Membrane filtration method].
Reporting and QA	The 90^{th} and 95^{th} percentiles of the log10 normal probability density function of the CFU datasets measured at one single location according established monitoring and assessment protocols and standards. A methodology has been proposed by Directive 2006/7/EC, as well as by UNEP(DEPI)/MED IG 20/8. Decision IG.20/9with the following specification: 1) Take the log10 value of all bacterial enumerations in the data sequence to be evaluated. (If a zero value is obtained, take the log10 value of the minimum detection limit of the analytical method used instead) 2) Calculate the arithmetic mean of the log10 values (μ).	Kay D, et al. 1994. Prüss A. 1998. UNEP(DEPI)/MED IG 20/8. Decision IG.20/9. Criteria and Standards for bathing waters quality in the framework of the implementation of Article 7 of the LBS Protocol. COP17, Paris, 2012. WHO, 2003. Guidelines for safe recreational water environments. VOLUME 1: Coastal and fresh waters. WHO Library. ISBN 92 4 154580. World Health Organisation, 2003. Directive 2006/7/EC of the European Parliament and of the

	3) Calculate the standard deviation of the log10 values (σ). The upper 90- percentile point of the data probability density function is derived from the following equation: upper 90- percentile = antilog (μ + 1,282 σ). The upper 95- percentile point of the data probability density function is derived from the following equation: upper 95- percentile = antilog (μ + 1,65 σ). Next, assess the obtained 90^{th} and 95^{th} percentile values against the reference values to obtain the classification category of the Bathing Water Quality at the studied site.	council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC

Literature:

Cabelli VJ, Dufour AP, Levin MA, McCabe LJ, Haberman PW. 1979. Relationship of microbial indicators to health effects at marine bathing beaches. Am. J. Public Health, 69, 690–696

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WHO, 2003. Guidelines for safe recreational water environments. VOLUME 1: Coastal and fresh waters. WHO Library. ISBN 92 4 154580. World Health Organisation, 2003.

Directive 2006/7/EC of the European Parliament and of the council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC

5. Way forward

17. The selection and description of the main methodological steps accompanied by literature sources and known guidelines should improve the common understanding and monitoring practices for each IMAP Common Indicator with regard Eutrophication (EO5) and Pollution (EO9). The main needs to be further addressed include the following:

1) The continuous technological advances require the monitoring protocols to be updated regularly, especially with regard the measurement methodological steps, whilst the published

standardized methods and guidelines for sample collection and sample processing remain valid in general terms.

2) There is a need for the methodologies for measurements, including Quality Assurance (e.g. instrumental analysis) to be frequently revised, as well as for common methodologies to be agreed when necessary.

3) There is a strong need to develop an IMAP Practical Monitoring Manual to collate and agree on the selected methodologies in use in the Mediterranean Sea, including the technical details and recommendations of use.

Annex I.
Simplified summary of the monitoring programme structure for Common Indicator 14 and Common Indicator 17.

Annex IA Simplified example scheme of monitoring structure for CI14.

CI	Parameter	Sample collection	Depth (m)	Sample frequency	Analysis method/ QAQC*	Assessment method
CI 14 Chlorophyll a concentration	Chlorophyll a	Niskin sampler	0, 5,10, 20, bottom -2	Monthly	Spectrophotometric Internal Reference Material	IMAP reference and boundary thresholds
	Dissolved Oxygen				Winkler titration No CRM, laboratory practice	Not applicable
	Temperature				Seabird CTD, regular calibration	Not applicable
	Salinity					Not applicable
	Transparency	Secchi disk	-		Secchi depth	Not applicable

Annex IB. Detailed example scheme of a biota and sediment monitoring structure for CI17.

Matrix	Cont. Group	Analytes	Sample collection	Sample processing (frequency)		Analysis method/ QAQC*	Assessment method
BIOTA	Heavy metals	TCd TPb	Bivalve species/ Caged bivalves**/ Fish	3-5 x pools of organisms, whole soft tissue, dry weight, acid digestion	Yearly	GF-AAS, ICP-OES, ICP-MS	IMAP BACs and EACs and sub-regional observation
		THg				Solid Hg analyser	
	Organic contaminants	PCBs (28, 31, 52, 101, 105, 118, 138, 153, 156 and 180)	Bivalve species/ Caged bivalves**/ Fish	3-5 x pools of organisms, whole soft tissue, dry weight, organic solvents extraction	Yearly***	GC-ECD, GC-MS, GC-NCI-MS	IMAP BACs and EACs and sub-regional observation
		HCB					
		Lindane					
		ΣDDTs					
		PAHs (individual congeners, 16 EPA)	Bivalve species/ Caged bivalves**/ Fish/	3-5 x pools of organisms, whole soft tissue, dry weight organic, solvents extraction	Yearly	HPLC-UV-Flu, GC-MS	IMAP BACs and EACs and sub-regional observation

***CRMs for metals and organic contaminants NIST 2976 (mussel), NIST 1566b (oyster), IAEA, etc.**
****Caged-bivalves could be an operational monitoring alternative option.**

Matrix	Cont. Group	Analytes	Sample collection	Sample processing (frequency)		Analysis method/ QAQC*	Assessment method
***Baseline studies show no occurrence of this compounds recently, and therefore if initially confirmed, their measurement frequency could be expanded or targeted/suspicious locations monitored Sub-indicators: other relevant chemicals (such as tributyltin, TBT; low molecular weight PAHs; etc.) and non-regulated or emerging pollutants are recommended to be carried out on a country decision basis. Observations for biota: a) Recommended to include also As, Cu, Zn, Cr and Ni, V (oil related); b) Also report biometric parameters for individual species (averaged); c) It would be recommended Cadmium measurements in other species and matrices to elucidate the background levels of Cd in Libyan coastlines and coastal waters.							
SEDIMENT	Heavy metals	TCd TPb	Fine, silt and mud fraction <2mm Top cover grab sampler or Box-corer	3 sample replicates dry weight basis reported	Biannual (spatially alternate), Off-shore coast (50-80 m depth)	GF-AAS, ICP-OES, ICP-MS	IMAP BACs and EACs and sub-regional observation
		THg				Solid Hg analyzer	
	Organic contaminants	PCBs (28, 31, 52, 101, 105, 118, 138, 153, 156 and 180)	Fine, silt and mud fraction <2mm Top cover grab sampler or Box-corer	3 sample replicates dry weight basis reporting	Biannual** (spatially alternate), Off-shore coastal areas (50-80 m depth)	GC-ECD, GC-MS, GC-NCI-MS	IMAP BACs and EACs and sub-regional observation
		HCB					
		Lindane					
		ΣDDTs					
		PAHs (individual congeners, 16 EPA)	Fine, silt and mud fraction <2mm Top cover grab sampler or Box-corer	3 sample replicates dry weight basis reporting	Biannual (spatially alternate), Off-shore coast (50-80 m depth)	HPLC-UV-Flu, GC-MS, GC-FID	IMAP BACs and EACs and sub-regional observation
*CRMs for sediment: NIST 1941b (organic), IAEA 457 (inorganic), BCR 277R (inorganic), etc. **Baseline studies show little occurrence of this compounds recently, and therefore, if confirmed their measurement frequency could be expanded to five years or targeted/suspicious locations monitored Sub-indicators: other relevant chemicals (such as tributyltin, TBT; low molecular weight PAHs; etc.) and non-regulated or emerging pollutants are recommended to be carried out on a country decision basis. Observations for sediment: a) Recommended to include also As, Fe, Li, Mn, Al, Cu, Zn, Cr and Ni, V (oil related); b) Additionally, Total Organic Carbon (TOC); c) Dry/Wet ratio (lyophilisation ratio); d)It would be recommended Cadmium measurements in other and matrices (dust, particulate matter in the water column) to elucidate the background levels of Cd in Libyan coastlines and coastal waters.							
SEAWATER SAMPLES	Heavy metals	According IMAP Common Indicator Guidance Factsheets - UNEP(DEPI)/MED WG. WG.439/12 the systematic long-term monitoring is a country-based decision to the complexity and high cost-effectiveness of seawater monitoring for reliable assessments					
	Organic contaminants						

Annex II:
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