



Marine
Environment
Laboratories



Mediterranean Action Plan
Barcelona Convention



United Nations
Environment Programme

Integrated Monitoring and Assessment Programme (IMAP) Monitoring Guidelines

Introduction

The Monitoring Guidelines related to IMAP Common Indicators 13, 14, 17, 18 and 20 were prepared in line with the Programme of Work 2020-2021 adopted by COP21. The Monitoring Guidelines were prepared by the MED POL Programme in cooperation with IAEA/MESL, were reviewed by the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (CORMONs) and were approved by the MED POL Focal Points Meeting (UNEP/MAP 2021¹).

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 as HELCOM, OSPAR and European Commission Joint Research Centre was undertaken to assist an innovative approach for preparation of the IMAP Monitoring Guidelines/Protocols.

The MED POL Focal Points Meeting approved the here presented monitoring methodologies and practices elaborated for sampling, sample preservation and transportation, sample preparation and analysis, along with quality assurance and reporting of monitoring data, in their meeting on 6-7 October 2021 (UNEP/MAP 2021) for their use by the IMAP competent laboratories in order to ensure the representativeness and accuracy of the analytical results related to IMAP Pollution Cluster, including for the preparation of the 2023 MED QSR.

In line with the Recommendations of the MED POL Focal Points Meeting, the Secretariat in collaboration with IAEA/MESL, edited the approved Monitoring Guidelines in order to provide an optimal design for their practical use by the technical personnel of national IMAP competent laboratories.

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 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.

The Monitoring Guidelines/Protocols are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.

The IMAP Monitoring Guidelines are presented in Chapters as follows:

- A-1: Guidelines/Protocols for sampling and determination of hydrographic physical and chemical parameters;
- A-2: Guidelines/Protocols for sampling and determination of key nutrients and chlorophyll a in seawater
- B-1: Guidelines/Protocols for sampling and determination of contaminants in sediments
- B-2: Guidelines/Protocols for sampling and determination of contaminants in marine biota
- B-3: Guidelines/Protocols for sampling and determination of contaminants in seawater
- C-1: Guidelines/Protocols for sampling and determination of biomarkers in marine molluscs and fish
- D-1: Guidelines/Protocols for sampling and determination of contaminants in seafood
- E-1: Guidelines/Protocols for Analytical Quality Assurance
- F-1: Guidelines/Protocols for Reporting IMAP Monitoring Data

All relevant documents, which are cited in the IMAP Monitoring Guidelines are presented as Annexes in the Appendix to the Guidelines.

¹ (UNEP/MAP 2021) UNEP/MED WG.509/43/L.3. Conclusions and Recommendations of the Second Session of the Meeting of the MED

POL Focal Points, 6-7 October 2021, as reviewed and agreed by the Meeting

Table of contents	iii
Appendix – List of Annexes	vi
A. IMAP Monitoring Guidelines for CI13 and CI14	1
A-1. Monitoring Guidelines/Protocols for Sampling and Determination of Hydrographic Physical and Chemical Parameters	2
1 Guidelines for hydrographic physical parameters: sampling and measurement	3
1.1 Introduction	3
1.2 Technical note for measurement of temperature and salinity of seawater	3
1.3 Technical note for measuring Secchi depth	9
2 Guidelines for hydrographic chemical parameters: sampling and determination	11
2.1 Introduction	11
2.2 Technical note for measuring dissolved oxygen	11
2.3 Technical note for measuring pH	16
3 References	18
A-2. Monitoring Guidelines/Protocols for sampling and determination of key nutrients and chlorophyll a in seawater	22
1 Guidelines for seawater sampling and sample preservation	22
1.1 Introduction	22
1.2 Technical note for sampling of seawater for the determination of key nutrients and chlorophyll a	22
1.3 Technical note for the sample preservation of seawater for the determination of key nutrients and chlorophyll a	24
2 Guidelines for the determination of key nutrients in Seawater	28
2.1 Introduction	28
2.2 Technical note for determination of concentration of nitrite (NO ₂ -)	29
2.3 Technical note for determination of concentration of nitrate (NO ₃ -)	32
2.4 Technical note for determination of concentration of ammonium (NH ₄ ⁺)	37
2.5 Technical note for the determination of concentration of orthophosphate (PO ₄ ³⁻)	40
2.6 Technical note for the determination of concentration of orthosilicate (SiO ₄ ⁴⁻)	44
2.7 Technical note for the combined determination of concentration of total nitrogen and total phosphorous	48
3 Guidelines for the determination of Chlorophyll a in Seawater	51
3.1 Introduction	51
3.2 Technical note for determination of concentration of chlorophyll a	52
B. IMAP Monitoring Guidelines for CI17	62
B-1. Monitoring Guidelines/Protocols for sampling and analysis of contaminants in sediment	63
1 Guidelines for sampling and sample preservation of sediment	64
1.1 Introduction	64
1.2 Technical note for the sampling of sediment for the determination of heavy metals and organic contaminants	65

1.3	Technical note for the preservation of sediment sample to be analysed for heavy metals and organic contaminants	71
2	Guidelines for the determination of contaminants in sediment	73
2.1	Introduction	73
2.2	Technical note for the determination of heavy metals in sediment	74
2.3	Technical note for the determination of organic contaminants in marine sediments	80
3	References	85
B-2. Monitoring Guidelines/Protocols for sampling and determination of contaminants in marine biota		87
1	Guidelines for sampling and sample preservation of marine biota	88
1.1	Introduction	88
1.2	Technical note for the sampling of marine biota for the determination of heavy metals and organic contaminants	88
1.3	Technical note for the sample preservation of marine biota for the determination of heavy metals and organic contaminants	93
2	Guidelines for the determination of contaminants in marine biota	94
2.1	Introduction	94
2.2	Technical note for the determination of heavy metals in marine biota	95
2.3	Technical note for the determination of organic contaminants in marine biota	100
3	References	104
B-3. Monitoring Guidelines/Protocols for sampling and analysis of contaminants in seawater		106
1	Guidelines for sampling and sample preservation of seawater	107
1.1	Introduction	107
1.2	Technical note for the sampling and pretreatment of seawater for the determination of heavy metals and organic contaminants	107
2	Guidelines for the determination of contaminants in seawater	114
2.1	Introduction	114
2.2	Technical note for the determination of heavy metals in seawater	115
2.3	Technical note for the determination of organic contaminants in seawater	118
3	References	119
C. Monitoring Guidelines for CI18		121
C-1. Monitoring Guidelines/Protocols for sampling and analysis of biomarkers in marine molluscs (such as <i>Mytilus</i> sp.) and fish (such as <i>Mullus barbatus</i>)		122
1	Guidelines for sampling and sample preservation of marine molluscs and fish	124
1.1	Introduction	124
1.2	Technical note for the sampling and sample preservation of marine molluscs (such as <i>Mytilus</i> sp.) for biomarker determination	124
1.3	Technical note for the sampling and sample preservation of marine fish (<i>Mullus barbatus</i>) for biomarkers determination	129
2	Guidelines for the determination of lysosomal membrane stability (LMS) in marine molluscs and fish	132
2.1	Introduction	132
2.2	Technical note for the determination of Lysosomal membrane stability (LMS) a) on cryostat sections in mussel digestive gland and fish liver and b) in vivo evaluation in mollusc haemocytes	132

3	Guidelines for determination of micronuclei (MNi) frequency, Acetylcholinesterase (AChE) activity and Stress on Stress (SoS) in marine molluscs and fish	139
3.1	Introduction	139
3.2	Technical note for the determination of micronuclei (MNi) frequency in fish (<i>Mullus barbatus</i>) blood cells and in mussel (<i>Mytilus</i> sp.) gill cells and haemocytes	139
3.3	Technical note for the determination of Acetylcholinesterase (AChE) activity in mussel gills and fish muscle	145
3.4	Technical note for the determination of Stress on Stress (SoS) in mussels	149
4	References	151

D. Monitoring Guidelines for CI20 **155**

D-1. Monitoring Guidelines/Protocols for Sampling and determination of contaminants in seafood **156**

1	Guidelines for seafood sampling and sample preservation	157
1.1	Introduction	157
1.2	Technical note for the sampling of seafood for the determination of heavy metals and organic contaminants	158
1.3	Technical Note for the dissection of seafood for the determination of heavy metals and organic contaminants	160
1.4	Technical note for the sample preservation of seafood for the determination of heavy metals and organic contaminants	163
2	Guidelines for the determination of contaminants in Seafood	164
2.1	Introduction	164
2.2	Technical Note for the determination of heavy metals in seafood samples	165
2.3	Technical note for the determination of organic contaminants in seafood samples	170
3	References	175

E. Monitoring Guidelines for Quality Assurance **177**

E-1. Monitoring Guidelines/Protocols for Analytical Quality Assurance for IMAP Common Indicators 13, 14, 17, 18 and 20 **178**

1	Guidelines for Analytical Quality Assurance	179
1.1	Introduction	179
1.2	Technical note for a Quality Assurance scheme	179
2	References	187

F. Monitoring Guidelines for Reporting IMAP Monitoring Data **189**

F-1. Monitoring Guidelines/Protocols for Reporting Monitoring Data for IMAP Common Indicators 13, 14, 17, 18 and 20 **190**

1	Guidelines for Reporting IMAP Monitoring Data	191
1.1	Introduction	191
1.2	Technical Note for reporting Monitoring Data for IMAP Common Indicators 13, 14, 17, 18 and 20	193
2	References	196

IMAP Monitoring Guidelines – List of Annexes

Annex I: Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.), 2007. Ch: 4. Recommended standard operating procedure, SOP6a: Determination of the pH of sea water using a glass/reference electrode cell. In: Guide to best practices for ocean CO₂ measurements. PICES Special Publication 3, 7 pp.

Annex II: Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.), 2007. Ch: 4. Recommended standard operating procedure, SOP6b: Determination of the pH of sea water using the indicator dyem-cresol purple. In: Guide to best practices for ocean CO₂ measurements. PICES Special Publication 3, 7 pp.

Annex III: Automated methods for determination of concentration of key nutrients in seawater

Annex IV: UNEP/MAP (2011), UNEP(DEPI)MED WG.365/Inf.9. Manual on sediment sampling and analysis

Annex V: UNEP/MAP Proposed assessment criteria (Background Assessment Criteria -BAC and Environmental Assessment Criteria - EAC) for targeted heavy metals and organic contaminants in sediments

Annex VI: IAEA Recommended method on microwave digestion of marine samples for the determination of trace element content

Annex VII: OSPAR CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods

Annex VIII: IAEA Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

Annex IX: IAEA Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

Annex X: US-EPA Method 6020B. ICP-MS method for the determination of elements in water samples and in waste extracts or digests

Annex XI: IAEA Recommended method on the determination of Total Hg in marine samples by

Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry

Annex XII: IAEA Recommended method for the determination of mercury in samples of marine origin by cold vapour atomic absorption spectroscopy),

Annex XIII: OSPAR CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalization of contaminant concentrations in sediments

Annex XIV: UNEP/IAEA Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71;

Annex XV: OSPAR CEMP Guidelines for monitoring contaminants in sediments, Annex 2 Analysis of PCBs in sediments;

Annex XVI: HELCOM Manual for marine monitoring COMBINE programme, Annex B-12, Appendix 2. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in marine sediment;

Annex XVII: OSPAR CEMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments

Annex XVIII: HELCOM Manual for marine monitoring in the COMBINE programme, Annex B-12, Appendix 1. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in sediment

Annex XIX: UNEP/FAO/IOC/IAEA (1987). Reference methods No 6 (Rev. 1): Guidelines for monitoring chemical contaminants in marine organisms

Annex XX: UNEP/FAO/IOC/IAEA (1988). Reference methods No 7 (Rev. 2): Sampling of selected marine organisms and sample preparation for trace metal analysis

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

Annex XXII: ICES/OSPAR (2018). CEMP Guidelines for Monitoring Contaminants in Biota

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

Annex XXIV: US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry

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

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

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

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

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

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

Annex XXXI: 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

Annex XXXII: 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.

Annex XXXIII: 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.

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

Annex XXXV: JRC (2010). Indicative Tables of most consumed species of fish and seafood for different (sub) regions. JRC Technical Report 2010.

Annex XXXVI: EU Commission Regulation (EC) No 1881/2006, setting maximum levels for certain contaminants in seafood

Annex XXXVII: EU Commission Regulation (EC) No 835/2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs

Annex XXXVIII: EU Commission Regulation (EC) No 333/2007, laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

Annex XXXIX: EU Commission Regulation (EC) No 836/2011 amending (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

Annex XXXX: EU Commission Regulation (EC) No 644/2017, laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs

Annex XXXXI: UNEP/FAO/IOC/IAEA (1987). Reference methods No 6 (Rev. 1): Guidelines for monitoring chemical contaminants in marine organisms

Annex XXXXII: UNEP/FAO/IOC/IAEA (1988). Reference methods No 7 (Rev. 2): Sampling of selected marine organisms and sample preparation for trace metal analysis

Annex XXXXIII: US EPA (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories Volume 1 Fish Sampling and Analysis. Third Edition

Annex XXXXIV: EU Commission Regulation (EC) No 1259/2011, amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs

Annex XXXXV: US EPA (1994). Method 1613, Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Office of Water, US Environmental Protection Agency, Washington, DC

Annex XXXXVI: US EPA (2008) Method 1668, Revision B: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS, EPA-821-R-08-020. Office of Water, US Environmental Protection Agency, Washington, DC

Annex XXXXVII: ICES (2004a). ICES Techniques in Marine Environmental Sciences, No 35. Chemical measurements in the Baltic Sea: Guidelines on quality assurance.

Annex XXXXVIII: List of available Reference Materials (RMs) and Certified Reference Materials (CRMs) prepared by IAEA (Marine Environmental

Studies Laboratory) for heavy metals and organic contaminants in marine matrices (sediment and biota)

Annex XXXXIX: UNEP/MED WG.492/6. Monitoring Guidelines/Protocols for IMAP Common Indicator 18: Implementation of IMAP Common Indicator 18 on Biomonitoring

Annex XXXXX: Data Standards and Data Dictionaries for IMAP Common Indicators 13, 14, 17, 18 and 20

Annex XXXXXI: Elements proposed for preparation of Data Standards and Data Dictionaries for IMAP Common Indicator 18 as amended by the Meeting of CorMon on Pollution Monitoring

Annex XXXXXII: Elements proposed for preparation of Data Standards and Data Dictionaries for IMAP Common Indicator 20

Annex XXXXXIII: Proposal of Data Standards and Data Dictionaries for IMAP Common Indicators 18 and 20 as prepared after the Meeting of CorMon on Pollution Monitoring for consideration of the 8th Meeting of Coordination Group Meeting