ISO 23040:2021-12 (E)

Marine environment impact assessment (MEIA) - Specification for marine sediments in seabed areas - Survey of interstitial biota

Contents

Page

Foreword ..... **viii**

Introduction ..... **ix**

1 Scope **1**

2 Normative references **1**

3 Terms and definitions **1**

4 General **2**

4.1 Technical design 2

4.2 Basic recommendations for the surveys 2

4.2.1 Survey object 2

4.2.2 Auxiliary parameters 3

4.2.3 Recommendations for the sampling equipment 3

4.2.4 Auxiliary equipment on board ship 3

4.2.5 Sampling method and scope of application 3

4.3 Sampling 3

4.3.1 Sediment sampling 3

4.3.2 Trawl sampling 3

4.3.3 Water sampling 4

4.3.4 Records 4

4.4 Sample analysis 4

4.4.1 Sample treatment 4

4.4.2 Sample measurement 4

4.4.3 Sample treatment and storage 4

4.4.4 Sample identification and enumeration 4

4.4.5 Sample data analysis 4

4.5 Basic recommendations of data organization 5

4.5.1 Organization of data 5

4.5.2 Data archiving, acceptance and achievements appraisal 5

4.6 Survey results 5

4.6.1 Original records 5

4.6.2 Maps or drawings 5

4.6.3 Investigation report 5

4.7 Data archiving 6

4.8 Program and quality control 6

5 Survey of the sediment **6**

5.1 Principle 6

5.2 General provisions 7

5.3 Collection and preservation of the samples 7

5.4 Measurement of environmental factors 7

5.5 Measurement of the age of the sediments 7

5.6 Measurement of the contents of heavy metals, organic pollutants and oils in the sediments 7

5.7 Measurement of the grain size of the sediments 7

5.8 Analysis of the mineral compositions in the sediments 8

5.9 Classification of the substrate type in offshore sediments 8

5.10 Classification of the deep-sea sediments 8

5.11 Survey of the biological components in the sediments 8

5.11.1 Summary of the methods 8

5.11.2 Technical recommendations 8
5.11.3 Collection and preservation of the samples 9
5.12 Organization of data 9

6 Survey of foraminifera 9
6.1 Principle 9
6.2 General provisions 9
6.3 Collection and preservation of the samples 9
6.3.1 Sampling and sample processing 9
6.3.2 Collection and preservation of the sedimentary tests 10
6.3.3 Collection and preservation of living foraminifera 10
6.4 Tools and reagents 10
6.5 Processing and analysis of the samples 10
6.5.1 Numbering and weighing the crystallizing dish or beaker 10
6.5.2 Drying and weighing the sediment sample 11
6.5.3 Sample soaking 11
6.5.4 Washing and drying 11
6.5.5 Suspension and concentration 11
6.5.6 Bottling and sealing 11
6.5.7 Specimen preparation and analysis 11

7 Survey of ostracoda 12
7.1 Principle 12
7.2 General provisions 12
7.3 Collection and preservation of the samples 12
7.3.1 Sampling and sample processing 12
7.3.2 Collection and preservation of dead ostracod tests 13
7.3.3 Collection and preservation of living ostracods 13
7.4 Tools and reagents 13
7.5 Processing and analysis of the samples 13
7.5.1 Numbering and weighing the crystallizing dish or beaker 13
7.5.2 Drying and weighing 13
7.5.3 Sample soaking 14
7.5.4 Washing and drying 14
7.5.5 Suspension and concentration 14
7.5.6 Bottling and sealing 14
7.5.7 Microscopic examination and enumeration 14

8 Survey of radiolaria 15
8.1 Principle 15
8.2 General provisions 15
8.3 Collection and preservation of the samples 15
8.3.1 Sample processing 15
8.3.2 Collection and conservation of deposited empty shells 16
8.4 Tools and reagents 16
8.5 Processing and analysis of the samples 16
8.5.1 Sample pretreatment 16
8.5.2 Preparation of microscope slide specimens 16

9 Survey of sedimentary diatom 17
9.1 Principle 17
9.2 General provisions 17
9.3 Collection and preservation of the samples 17
9.3.1 Sampling and treatment 17
9.3.2 Collection and preservation of sedimentary remains 18
9.4 Tools and reagents 18
9.5 Processing and analysis of the samples 18
9.5.1 Sample pretreatment 18
9.5.2 Preparation of microscope slide specimens 19
9.6 Organization of data 19

10 Survey of coccoliths 19
10.1 Principle 19
10.2 General provisions 19
10.3 Collection and preservation of the samples 20
10.4 Tools and reagents 20

10.5 Processing and analysis of the samples ... 20
10.5.1 Pre-preparation of the equipment and the samples ... 20
10.5.2 Method of preparing simple smear slides ... 21
10.5.3 Pre-concentration of coccolith samples ... 21
10.6 Organization of data ... 21

11 Survey of sporopollen ... 22
11.1 Principle ... 22
11.2 General provisions ... 22
11.3 Collection and preservation of the samples ... 23
11.4 Tools and reagents ... 23
11.5 Processing and analysis of the samples ... 23
11.5.1 Sample disaggregation ... 23
11.5.2 Alkali-soluble digestion ... 24
11.5.3 Sieving ... 24
11.5.4 Carbonate digestion ... 24
11.5.5 Silicate digestion ... 24
11.5.6 Ultrasonic cleaning and sieving ... 24
11.5.7 Acetolysis ... 25
11.5.8 Storage of processed sample ... 25
11.5.9 Mounting sporopollen specimens ... 25
11.5.10 ... Glycerine jelly preparation ... 25
11.5.11 ... Precautions ... 25
11.6 Organization of data ... 26

12 Survey of benthic viruses ... 26
12.1 Principle ... 26
12.2 General provisions ... 26
12.3 Collection and preservation of the samples ... 26
12.3.1 Samples for epifluorescence microscopy ... 26
12.3.2 Samples for flow cytometry ... 27
12.3.3 Samples for environmental measurements and molecular diversity of viruses ... 27
12.4 Tools and reagents ... 27
12.4.1 Equipment and reagents for epifluorescence microscopy ... 27
12.4.2 Equipment and reagents for flow cytometry ... 28
12.4.3 Equipment and tools for molecular diversity of viruses ... 28
12.5 Processing and analysis of samples ... 28
12.5.1 Enumeration of viruses by epifluorescence microscopy ... 28
12.5.2 Procedure for flow cytometry ... 30
12.5.3 Estimation of the molecular diversity of viruses ... 30

13 Survey of benthic microbes ... 32
13.1 Principle ... 32
13.2 General provisions ... 32
13.3 Collection and preservation of the samples ... 33
13.3.1 Sampling stations and sample collection ... 33
13.3.2 On-site sample process ... 33
13.3.3 Sample storage ... 33
13.4 Tools and reagents ... 34
13.4.1 Equipment ... 34
13.4.2 Reagents ... 34
13.5 Processing and analysis of the samples ... 36
13.5.1 Assessment of molecular diversities of benthic microbes ... 36
13.5.2 Benthic microbial abundance ... 41

14 Survey of benthic microalgae ... 44
14.1 Principle ... 44

14.2 General provisions 44
14.3 Collection and preservation of the samples 45
14.3.1 Sampling design 45
14.3.2 Sampling methods 45
14.3.3 Sample preservation and fixation 46
14.4 Tools and reagents 46
14.5 Processing and analysis of the samples 46
14.5.1 Sample processing 46
14.5.2 Sample analysis 48
14.5.3 Abundance and biomass calculation 48
14.5.4 Cyst culture and identification 50
14.5.5 Fluorescence determination of demagnesium chlorophyll and chlorophyll a 50
14.5.6 Algal toxins determination 51

15 Survey of benthic protozoa 51
15.1 Principle 51
15.2 General provisions 51
15.3 Collection and preservation of the samples 52
15.3.1 Sampling equipment 52
15.3.2 Sediment core collection 52
15.3.3 Sediment layers 52
15.3.4 Sample fixation and preservation 52
15.3.5 Collection and treatment of living protozoa 53
15.4 Tools and reagents 54
15.4.1 Equipment and reagents for silica sol centrifugation 54
15.4.2 Equipment and reagents of quantitative protargol staining 54
15.4.3 Equipment and reagents for molecular analysis 54
15.5 Processing and analysis of the samples 55
15.5.1 Silica sol density centrifugation 55
15.5.2 Quantitative protargol stain (QPS) 56
15.5.3 Estimation of molecular diversity 59

16 Survey of metazoan meiobenthos 64
16.1 Principle 64
16.2 General provisions 65
16.3 Collection and preservation of the samples 65
16.3.1 Sampling in intertidal zones and shallow waters 65
16.3.2 Deep-sea sampling 65
16.3.3 Sample slicing 66
16.3.4 Sample fixation and preservation 67
16.3.5 Labelling 67
16.3.6 Record 67
16.4 Tools and reagents 67
16.5 Processing and analysis of the samples 67
16.5.1 Quantification and taxa analysis 67
16.5.2 Molecular analysis of biodiversity 68
16.6 Organization of data 69
16.6.1 Precision 69
16.6.2 Abundance 69
16.6.3 Biomass 70
16.6.4 Complete the report 71
16.6.5 Plotting data 71

Annex A (informative) **Photographs of several groups of communities of interstitial biota 72**

Annex B (informative) **Several stratified sampling devices for the survey of interstitial biota 74**

Annex C (informative) **Tables for sample labelling and sampling record — Examples 76**

Annex D (informative) **Tables of microscopy records — Examples 78**

Annex E (informative) **Calculation of community parameters 83**

Annex F (informative) **Record sheet for sample treatment — Examples** **86**
Annex G (informative) **Characteristics for the classification of coccoliths** **87**
Annex H (informative) **Flow diagram for the enumeration of viruses by epifluorescence microscopy** **88**
Annex I (informative) **Filter unit used in microbenthos survey** **89**
Annex J (informative) **Technical flowcharts for the investigation of microbenthos** **90**
Annex K (informative) **Solutions and regents** **93**
Bibliography **99**