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BSI Standards Publication

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

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National foreword

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

Évaluation de l'impact environnemental marin — Spécifications relatives aux sédiments marins dans les zones de fonds marins — Étude du biote interstitiel

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee 8, *Ships and marine technology*, Subcommittee SC 13, *Marine technology*.

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Introduction

Interstitial biota in marine sediments refers to the benthic life forms inhabited or deposited in the interstitial spaces between sediment particles, including marine microorganisms, benthic virus, microbenthos and meiobenthos. They cover the six "kingdoms" of life in the three-domain taxonomic system: Archaea, Bacteria, Fungi, Protista, Plantae and Animalia. Interstitial biota in marine sediments are so small that cannot be obtained and analysed by conventional methods for marine biological survey; they are numerous and complex; they have diverse functions, remarkable ecological significances and rich gene resources; they are ubiquitous and make up the basic components of the life system in marine sediments. Sediment interstitial biotas are the most abundant and complex life groups in the estuaries, intertidal zones, shelf shallow seas and deep sea. They play key roles in the regulation of material and energy flows in benthic ecosystems.

In seabed areas, a number of large international research programs have been carried out, such as the ocean drilling program (ODP) and the international ocean discovery program (IODP). Interstitial biota in marine sediments surveys have been key to solve scientific problems in relevant fields, such as marine biodiversity, oil and gas resource exploration, marine carbon cycle, global change, monsoon rainfall, ice melting, ocean acidification and deep-sea biological resources. But so far the lack of an International Standard leads different countries to use different regulations and technologies on the investigations, resulting in barriers to comparing research results in international cooperation.

This document provides relevant technical approaches for the investigation of sediment interstitial biota in seabed areas. Its purpose is to reflect the recent developments of modern marine science and technology to facilitate international cooperation. It is applicable to investigations and evaluations of marine sediment biodiversity in seabed areas, favouring the development and utilization of marine biological resources, the comprehensive environmental exploration, ecological environment assessment, protection and management, etc. The specifications in this document incorporate technical advances and technological key points reflecting current state-of-the-art and international practice.

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

1 Scope

This document provides requirements and recommendations for conducting marine surveys of interstitial biota in marine sediments. It includes the specification of technical methods for the investigation of marine sediments, foraminifera, ostracoda, radiolaria, diatoms, coccoliths, sedimentary sporopollen, benthic viruses, benthic microbes (including bacteria, archaea and fungi), benthic microalgae, benthic protozoa and metazoan meiobenthos.

This document is applicable to marine surveys in diverse benthic habitats at any seabed, such as benthic sediments of coastal zones, shallow seas, or deep-sea waters.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
marine sediment
substances under the action of crustal surface geology, where the original products such as weathered rocks, metamorphic rocks and pre-existing *sedimentary rocks* (3.2) of the parent rocks (i.e. magmatic rocks, metamorphic rocks and sedimentary rocks) are transported, settled or precipitated by biogenic, volcanic and cosmic phenomena as loose unconsolidated deposits on the sea floor

3.2
sedimentary rock
one of the three major types of rocks that make up the lithosphere (the other two are magmatic rocks and metamorphic rocks), which are formed from the weathering products of a parent rock (or any pre-formed rock), biogenic materials, volcanic material, cosmic material and other original material, and sedimentation after the formation of rock diagenesis

3.3
interstitial biota
benthic life forms that inhabit or are deposited in the interstices between sediment particles

Note 1 to entry: It includes *marine microorganisms* (3.6), benthic viruses, *microbenthos* (3.4), and meiobenthic organisms. In terms of individual sizes, interstitial biota in *marine sediments* (3.1) cover femto-level with a size of less than 0,2 µm, pico-level (0,2 µm to 2 µm), nano-level (2 µm to 20 µm) and micro- and meio-level benthic organisms of more than 20 µm.

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3.4
microbenthos
unicellular prokaryotic and eukaryotic microbes living on the surface, and within the interstices, of sediments, which can be trapped by 0,2 µm membrane filtration

Note 1 to entry: Mainly benthic bacteria, benthic microalgae and *benthic protozoa* (3.5). See Figure A.1 for examples of major groups. In terms of sizes of individuals, the microbenthos covers the pico-level of less than 2 µm, the nano-level (2 µm to 20 µm) and the micro-level of more than 20 µm.

3.5
benthic protozoa
unicellular eukaryotes whose life history is entirely or mostly associated with sedimentary environments

Note 1 to entry: It includes heterotrophic flagellates, ciliates, amoebae, etc.

3.6
marine microorganism
microeukaryotes and metazoans included in sedimentary investigations and marine geological surveys, including extant and fossil species of various groups

Note 1 to entry: It includes foraminifera, ostracoda, radiolaria, diatoms, calcareous fossils, sporopollen, pteropoda, ichthyoliths, etc.

3.7
benthic microbe
unicellular and small acellular organism with simple structure and a variety of physiological types that inhabits sedimentary environments

Note 1 to entry: It includes bacteria, archaea and fungi.

3.8
metazoan meiobenthos
metazoan meiofauna
small metazoa and larvae of large metazoans living in sedimentary environments that can pass through a 500 µm aperture mesh but are retained on a 42 µm to 31 µm aperture mesh

Note 1 to entry: The main groups include nematodes, copepods, tardigrades, ostracods, gastrotrichs, priapulid worms, bivalves, arthropods, acarina, polychaetes, kinorhyncha, rotifers, etc. Several major groups are shown in Figure A.2.

4 General

4.1 Technical design

Surveys of interstitial biota in sediments should be designed in terms of survey-related items, including survey section, station, object, detail, method, date, frequency, device, personnel quality, ship, equipment, expected results and survey plan. The establishment of the investigation plan shall refer to the requirements of the related survey plan.

4.2 Basic recommendations for the surveys

4.2.1 Survey object

The survey object can include marine sediments, foraminifera, ostracoda, radiolaria, sedimentary diatoms, coccoliths, sporopollen, benthic viruses, benthic microbes, benthic microalgae, benthic protozoa, and metazoan meiobenthos. Specific objects may be adjusted or designed according to the survey plan.

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4.2.2 Auxiliary parameters

Auxiliary observation objects can be added, if necessary, to the survey. These can be designed on the basis of the survey plan.

4.2.3 Recommendations for the sampling equipment

The sampling equipment should follow the recommendations of related clauses in this document.

The common equipment for sediment sampling includes box-corers, multicorers, grab samplers, gravity samplers or drills. Deep-sea sampling equipment can also include remotely-operated vehicles (ROV) and manned submersibles. The main disadvantage of grab sampling is that it is not possible to preserve the seafloor surface sediments, and it rarely preserves depositional sequences.

4.2.4 Auxiliary equipment on board ship

The auxiliary equipment on board ship includes the following:

a) winch systems: a winch system is required for the operation of general multicorers or box-corers; a winch system for armoured cable is required for the operation of television (TV) multicorers and TV box-corers;

b) large A frames: the lifting height should be 4,5 m or more.

4.2.5 Sampling method and scope of application

4.2.5.1 Sediment sampling

Sediment sampling can be used to survey marine sediments for foraminifera, ostracoda, radiolaria, sedimentary diatoms, coccoliths, sporopollen, benthic viruses, benthic microbes, benthic microalgae, benthic protozoa, and metazoan meiobenthos. Sampling should follow the recommendations of the related clauses in this document.

4.2.5.2 Trawl sampling

Trawling can be used for auxiliary sampling of planktonic foraminifera, ostracoda, radiolaria, protozoa, etc.

4.2.5.3 Water sampling

Water sampling can be used to survey planktonic foraminifera, living radiolaria, etc. Sampling shall satisfy the requirements of related clauses in this document.

4.3 Sampling

4.3.1 Sediment sampling

Sediments should be collected by the specified sampler. Staff should strictly abide by the operating procedures and pay attention to the condition of the sampler. Surface samples or core samples shall be collected, treated and stratified according to the requirements of the survey. Sediment sampling procedures in offshore and coastal areas should follow the recommendations in ISO 5667-19:2004, 5.1. Stratigraphic equipment for processing surface samples is shown in Figures B.1 and B.2. In abnormal situations, replicate samples should be collected.

4.3.2 Trawl sampling

Sampling nets can be used in trawl sampling. The speed of net deployment and net withdrawal should be strictly controlled, and the time of net arrival at the target should be determined accurately. The

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condition of the net should be noted, and effective measures should be adopted if the situation is abnormal. The net should be washed, and the samples collected carefully, especially biological samples that can stick to the net and pipe.

4.3.3 Water sampling

Water can be collected using a water sampler. Before deployment, check that the cover is open and the faucet is closed. Deploy the water sampler to the target area and maintain its position for a precise time. Water sampling and sample treatments shall satisfy the requirements of related clauses in this document.

4.3.4 Records

All survey objects should be recorded according to the related clauses in this document. Examples of sample record tables are shown in Annex C (Tables C.1, C.2, C.3).

Take additional photos and videos in the case of abnormal phenomena or new discoveries.

4.4 Sample analysis

4.4.1 Sample treatment

Treat the samples according to the related clauses in this document.

4.4.2 Sample measurement

Measurement items should be determined according to the related clauses in this document.

4.4.3 Sample treatment and storage

Samples can be stratified, fixed and dyed on-site according to the related clauses in this document.

Preserve samples after analysis, measurement and identification, either complete or partial, according to the application and academic value. Samples storage should comply with the survey plan.

4.4.4 Sample identification and enumeration

Identify the organisms to the species level wherever possible, and enumerate according to the related requirements in this document. Observe and analyse the samples by microscopy, and record in a table similar to Tables D.1, D.2, D.3, D.4.

4.4.5 Sample data analysis

Data to be analysed are mainly related to community structure, abundance, relative abundance, or dominant groups of interstitial biota in marine sediments. Data analysis can refer to the related clauses in this document.

Analyse data with statistical software. Annex E provides methods of calculation of the community parameters. Table E.1 shows biomass conversion factors.

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4.5 Basic recommendations of data organization

4.5.1 Organization of data

4.5.1.1 Quantification and statistics

Quantify and analyse the results of identification and enumeration using the formulas and formatting given in this document.

4.5.1.2 Fill in forms

Fill in the forms according to related provisions of this document.

4.5.1.3 Draw charts

Draw charts to show data according to the requirements of this document.

4.5.1.4 Voyage report

A voyage report, including related requests according to the survey plan, should be completed by the scientist responsible to the object after the survey.

4.5.2 Data archiving, acceptance and achievements appraisal

Data archiving, acceptance and appraisal of achievements should be carried out with related requests in this document.

4.6 Survey results

4.6.1 Original records

Original records made during the survey, including those of sediment, sedimentary rock, organisms, and water samples, field descriptions, location records, etc. shall be retained. This is first-hand information and includes the primary results of the survey.

4.6.2 Maps or drawings

Draw maps or figures on a prescribed scale based on data analysis and calculation.

4.6.3 Investigation report

The investigation report should include the following.

a) Preface, including the survey plan, survey area, content and workload of the objects, work time (indoor and outdoor).

b) Survey and data consolidation, including working methods, station design, quality of original data, methods of data consolidation, accuracy of results.

c) Results, including sediment types, suspension concentration, structure, abundance and distribution of main biota.

d) Data analysis, including method and its basis, distribution characteristics and comprehensive analysis of every element.

e) Conclusions, including suggestions for further work.

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4.7 Data archiving

The following data should be archived:

a) survey contract or survey plan;

b) reports, technical design, program report and statements of approval;

c) executable plan and sampling stations;

d) original record of the survey, experiments undertaken and analysis;

e) report and explanation of the results;

f) tables, figures (including base map), photographs with explanatory legends;

g) voyage report and objects summary report;

h) investigation report and acceptance of the results;

i) tables of objects members and reconciliation of budget.

Related requests concerning data archiving, file quality and acceptance of the results can refer to the related clauses in this document.

4.8 Program and quality control

The institution executing the objects can provide the quality prospectus, including quotations of specifications and articles, summary of survey plan, quality target, organization and responsibilities of the execution institution and assurance measures of quality prospectus. Quality control can refer to the related clauses in this document.

Measures for ensuring quality control include the following.

a) Establish a quality control system: In addition to accepting supervision from administrations and technical supervision agency, a process of self-checking or quality control can be adopted. Formulate the quality control systems. Define the duty of quality control and programs of quality supervision and examination. Execute provisions of quality control strictly.

b) Execute quality control: There shall be clear quality requirements in the survey plan. Analyse specific quality of articles and data. Instruments, equipment, tools and materials can conform to the quality standards. Take specific field records for samples and data obtained at sea. Check original samples and data after the survey. Analysis and identification of samples, data consolidation and counts can be based on facts. Archiving of documents, data and results shall fulfil these requirements.

c) Full participation in quality control: Staff participating in the survey can have relevant professional skills. It is the duty of all staff to maintain quality control requirements.

5 Survey of the sediment

5.1 Principle

Analyses of sediment characteristics, including sediment classification, physicochemical characterization and granulometry, to obtain information on the substrate environment for surveying the interstitial biota.

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5.2 General provisions

The general provisions include the following:

a) qualitative and/or quantitative analyses should be selected based on the survey plan;

b) the samples shall not be mixed or contaminated;

c) the characteristics surveyed should primarily be the grain size, the type of sediment and its biological components.

5.3 Collection and preservation of the samples

Seafloor surface sediments, columnar cores, or undisturbed drill cores can be collected. A site description of the samples should be made, and certain physicochemical factors of the samples should be measured on site. The samples can be then sealed for refrigeration- or cryo-preservation, depending on the survey plan.

When measuring the physicochemical characteristics and grain sizes, surface sediments and columnar or drill core samples can be collected from various strata at different depths. Each collected sample can be sealed in a polyethylene bag and related information including sampling site, sampling time and location coordinates should be marked on the bag. Parameters including pH, Eh, temperature and Fe^{3+}-to-Fe^{2+} ratios should be measured at the sampling site. For the measurement of other physicochemical characteristics, the samples should be stored in a refrigerator at a temperature of -20 °C and transported to the laboratory for analysis.

5.4 Measurement of environmental factors

The environmental factors measured include suspended particulate matters, moisture content (%), total organic carbon content (%), chlorophyll-a (Chl a) and pheophytin-a (Ph a) content, pH, Eh, temperature and the ratio of Fe^{3+} to Fe^{2+}. The items for measurement can increase or decrease according to the survey plan.

5.5 Measurement of the age of the sediments

As per the requirements of the survey, the sediments of columnar or drilling cores shall be dated by the methods appropriate to each sample.

For sediments from columnar cores and the top of drilling cores, ^{210}Pb or ^{137}Cs dating shall be conducted in accordance with the requirements of the survey plan; for drilling cores, ^{14}C dating, optically stimulated luminescence (OSL) dating, and palaeomagnetic chronology can be selectively conducted depending on the survey plan.

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

The survey for heavy metals (Hg, Cu, Pb, Cd, Cr, Zn and As), organic pollutants (arsenite, cyanide, organic-chlorine pesticide and volatile phenol) and oils shall be carried out with related provisions in this document. The specific analyses conducted for each sample can be selected on the basis of the specific conditions of the sea bed.

5.7 Measurement of the grain size of the sediments

The grain size of the sediments should be measured.

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5.8 Analysis of the mineral compositions in the sediments

In accordance with the recommendations of the survey, the contents of detrital and clay minerals in the sediments can be selectively measured.

5.9 Classification of the substrate type in offshore sediments

According to the requirements of the survey, the sediments should be classified and named using the ternary diagram classification methods proposed by References [15], [22] and [52].

a) On the basis of the Shepard's ternary diagram, the sediments are classified into nine types: clay, sandy clay, silty clay, clay sand, clay silt, sand, silty sand, sandy silt, and silt. The classification is based on the analysis of the grain size of sediments.

b) Using Folk's detrital-sediment classification method, the sediments are classified into gravel-bearing and gravel-free types. Specifically, through a ternary diagram classification, the gravel-bearing sediments are divided into 14 types: gravel, sandy gravel, argillaceous sandy gravel, argillaceous gravel, gravelly sand, gravelly argillaceous sand, gravelly silty mud, gravel-bearing sand, gravel-bearing argillaceous sand, gravel-bearing mud, sand, argillaceous sand, sandy mud, and mud.

c) Using the ternary diagram method, gravel-free sediments are divided into the following ten types: sand, silty sand, argillaceous sand, clay sand, sandy silt, sandy mud, sandy clay, silt, mud, and clay.

d) In addition to the above classifications, the percentages of gravel, sand, silt and clay components in the sediments are determined based on the results of grain-size analysis.

5.10 Classification of the deep-sea sediments

Deep-sea sediments should be classified and named using the ternary diagram classification method for deep-sea sediments (see Reference [15]).

5.11 Survey of the biological components in the sediments

5.11.1 Summary of the methods

This study can include the identification and analysis of foraminifera, ostracoda, radiolaria, sporopollen, and calcareous nanofossils (such as coccoliths). The identification of these fossils can be carried out regarding the identification of palaeontology in sediments.

The preparation, identification and analysis of the other sedimentary biodetritus, such as ichthyoliths, fish otoliths, stoneworts, corals, bryozoans and pteropods, can be performed regarding the identification of sedimentary palaeontology.

5.11.2 Technical recommendations

The technical recommendations include the following:

a) qualitative and/or quantitative analyses should be selected based on the survey plan;

b) the samples shall not be mixed or contaminated;

c) the abundance of a single species should be denoted as the number per gram of dry sample, or the number per 50 g of dry sample or the number per 10 cm^2;

d) the primary factors to be surveyed are the compositions, abundances and dominant species of major biological components.

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5.11.3 Collection and preservation of the samples

According to the requirements for the different objects, seafloor sediments, columnar or undisturbed drilling cores can be selectively collected, sealed on site, and refrigerated or frozen for preservation.

5.12 Organization of data

The organization of data from the surveyed sediments should follow the specific recommendations of this document.

6 Survey of foraminifera

6.1 Principle

Based on the collection, preparation, preservation, identification and analysis of living specimens and tests of the foraminifera, the distribution and preservation of foraminifera in the sediments are investigated to reflect the hydrological and environmental changes in the area.

6.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 20 g to 50 g samples for continental shelf and shallow water depths, and 2 g to 10 g samples for the slope and deeper water depths, because the abundances of foraminifera differ according to depth.

c) Ensure that the samples are not mixed or contaminated. Record the station and sample information

d) Observe the foraminifera specimens (>0,150 mm) under a microscope. Generally, identify the planktonic foraminifera and the dominant species of benthic foraminifera to species level, and identify the others can be to genus level or as ecological categories. Record the abrasion, breakage and dissolution of foraminifera specimens.

e) Count all the specimens if the number of specimens is less than 300. Divide the samples by the riffle or diagonal sample method if the sample volume is too great and identify at least 300 foraminifera for each subsample. About 300 planktonic foraminifera specimens and 150 benthic foraminifera specimens are recommended for microscopic examination.

f) Record the relative abundance of each species, showing its percentage (%), or record its absolute abundance as individuals in per gram dried sample (individuals/g), or record its abundance as individuals in per square centimetre dried sample (individuals/cm^2) for surface sediment sample.

6.3 Collection and preservation of the samples

6.3.1 Sampling and sample processing

Based on different marine sediment types, divide the sampling and processing methods into two types, as follows.

a) Offshore and shoal water type: these sediments are composed mainly of terrigenous clast that have fast deposition rates and low foraminifera content. For obtaining foraminifera specimens, the sediment is placed in a beaker and soaked in clean water. Sodium hexametaphosphate is added for dispersing the sediment particles. After heating and disaggregation, the organic matter is dissolved by adding a moderate amount of hydrogen peroxide. The sample is then washed using

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a 0,063 mm sieve. Clean foraminifera specimens are separated from coarse particles by flotation in tetrachloromethane. Foraminifera tests are suspended by stirring and collected by passing through a 0,063 mm filter paper.

b) Slope and deeper sea type: these sediments have less terrigenous clast, obvious fine sediment particles, relative slow deposition rates, and a higher foraminifera content. The sediment is placed in a beaker and soaked in distilled water or tap water for dispersing the sediment particles. After this process, the remnant is washed through a 0,063 mm sieve and oven-dried for specimen preparation.

6.3.2 Collection and preservation of the sedimentary tests

Collect the foraminifera tests using a sediment sampler (box-corer, multicorer, gravity sampler, etc.) on the survey ship. The samples can be preserved by cold storage or treated in the field (dyed with Rose Bengal to distinguish living specimens from empty tests) then seal and preserve at 4 °C or according to the survey plan.

6.3.3 Collection and preservation of living foraminifera

For surveying living foraminifera, collect the surface sediment sample (usually 0 cm to 2 cm of sediment from seafloor, but can be 0 cm to 15 cm according to the survey plan) and treat with ethanol in the field. For distinguishing living foraminifera from empty tests, stain the sample with Rose Bengal solution within 48 h then process and analyse.

For molecular identification of living foraminifera, the samples shall be refrigerated or cryopreserved and stored for further processing and analysis in the laboratory.

6.4 Tools and reagents

The tools and reagents include the following:

a) major equipment: quantitative layering sampler, thermostatic drier box, stereomicroscope, reefer, refrigerator;

b) other tools: mesh sieve (0,063 mm, 0,150 mm), crystallizing dish (50 ml, 100 ml), straw, funnel, glass rod, filter paper, beaker, bottle, brush, writing brush, riffle sampler, glass plate, blade, sampling needle, specimen slice, specimen box;

c) reagents: sodium hexametaphosphate, ethanol, hydrogen peroxide, tetrachloromethane, Rose Bengal, sodium salt ($C_{20}H_2Cl_4I_4Na_2O_5$), distilled water.

6.5 Processing and analysis of the samples

6.5.1 Numbering and weighing the crystallizing dish or beaker

The procedure for numbering and weighing the crystallizing dish includes the following steps:

a) clean the crystallizing dish;

b) dry the crystallizing dish at 60 °C in an oven;

c) number and weigh the crystallizing dish (accurate to 0,01 g);

d) record the mass of the crystallizing dish and the corresponding sample number on the record sheet (Table F.1).

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6.5.2 Drying and weighing the sediment sample

The procedure for drying and weighing the sediment sample includes:

a) put the original sediment sample into the pre-weighed crystallizing dish and number it;

b) record the wet mass of the sample;

c) dry the sample at 60 °C in an oven, then weigh and determine the dry mass.

6.5.3 Sample soaking

The procedure for sample soaking includes:

a) add distilled water or tap water to the beaker containing the sediment sample and soak for 1 or 2 days in order to disperse the sediment particles and foraminifera tests;

b) for sediment with high organic content, soak the sample with 3 % hydrogen peroxide for 2 h then wash with clean water (for offshore sediments, add a small amount of sodium hexametaphosphate to make the sample more loose. This step is not suitable for deep-sea sediments or for living foraminifera).

6.5.4 Washing and drying

The procedure for washing and drying includes:

a) transfer the sample into a 0,063 mm sieve and wash repeatedly with running water;

b) transfer the washed sample to a numbered crystallizing dish;

c) dry the crystallizing dish in an oven at 60 °C;

d) record the mass of the coarse fraction of the dried sample.

6.5.5 Suspension and concentration

The procedure for suspension and concentration includes:

a) number the beaker according to the sample number;

b) suspend with tetrachloromethane;

c) pour the sediment residue into a crystallizing dish dry and number the dish;

d) bag and label the sediment residue after microscopic examination.

6.5.6 Bottling and sealing

The procedure for bottling and sealing includes:

a) sieve the weighed and dried samples through 0,150 mm and 0,063 mm meshes respectively;

b) bottle and label the two samples and mark the sample layer and size fraction.

6.5.7 Specimen preparation and analysis

Apply the following procedure:

a) examine the specimens (>0,150 mm) by light microscopy;

b) select 150 to 300 tests at random for specimen preparation;

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c) carry out the classification and biocoenosis statistics (species number, abundance, diversity and group ratio, etc.) according to the survey plan;

d) for offshore sediment samples, carry out the classification and statistics of 0,063 mm specimens selectively according to the survey plan;

e) fill in the tables for the identification and statistics of foraminifera (tables are designed according to the survey plan).

7 Survey of ostracoda

7.1 Principle

Based on the collection, preparation, preservation, identification and analysis of living specimens and tests, the distribution and preservation of ostracods in sediments are investigated to reflect the hydrological and environmental changes in the area.

7.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 20 g to 50 g samples for continental shelf and shallow water depths, and 2 g to 10 g samples for the slope and deeper water depths, because the abundances of ostracods differ according to depth.

c) Ensure that the samples are not mixed or contaminated. Record the station and sample information.

d) Identify ostracods with a stereomicroscope, where possible to species level but otherwise to genus level. Record any abrasion, breakage or dissolution of ostracod specimens.

e) For statistical analyses of biocoenosis, identify all the specimens if the number present is less than 100. If the volume is large, divide the samples by the riffle or diagonal sample method and identify at least 100 specimens for each subsample.

f) Record the relative abundance of each species, showing its percentage (%), or record its absolute abundance as individuals per gram dried sample (individuals/g), or record its abundance as individuals per square centimetre dried sample (individuals/cm^2) for surface sediment sample.

7.3 Collection and preservation of the samples

7.3.1 Sampling and sample processing

Based on different marine sediment types, divide the sampling and processing methods into two types, as follows.

a) Offshore and shoal water type: these sediments are composed mainly of terrigenous clast that have fast deposition rates and low ostracod content. For obtaining ostracod specimens, the sediment is placed in a beaker and soaked in clean water. Sodium hexametaphosphate is added for dispersing the ostracods and the sediment particles. After heating and disaggregation, the organic matter is dissolved by adding a moderate amount of hydrogen peroxide. The sample is then washed using a 0,063 mm sieve. Clean ostracod specimens are separated from coarse particles by flotation in tetrachloromethane. Ostracods are suspended by stirring and collected by passing through a 0,063 mm filter paper.

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b) Slope and deeper sea type: these sediments have less terrigenous clast, obvious fine sediment particles, relative slow deposition rates, and a higher ostracod content. The sediment is placed in a beaker and soaked in distilled water or tap water for dispersing the ostracods and sediment particles. After this process, the remnant is washed through a 0,063 mm sieve and oven-dried for specimen preparation.

7.3.2 Collection and preservation of dead ostracod tests

Collect dead ostracod tests by sediment sampler (box-corer, multicorer, gravity sampler, etc.) on the survey ship.

The samples can be preserved by cold storage or treated in the field (dyed with Rose Bengal to distinguish living specimens from empty tests) then sealed and preserved (4 °C) according to the survey plan.

7.3.3 Collection and preservation of living ostracods

For surveying living ostracods, collect the surface sediment samples (usually 0 cm to 2 cm of sediment from seafloor, but can be 0 cm to 10 cm according to the survey plan) and treat with ethanol in the field. For distinguishing living ostracods from empty tests, stain the samples with Rose Bengal solution within 48 h then process and analyse.

7.4 Tools and reagents

The tools and reagents include the following:

a) major equipment: quantitative layering sampler, thermostatic drier box, stereomicroscope, reefer, refrigerator;

b) other tools: mesh sieve (0,063 mm, 0,150 mm), crystallizing dish (50 ml, 100 ml), straw, funnel, glass rod, filter paper, beaker, bottle, brush, writing brush, riffle sampler, glass plate, blade, sampling needle, specimen slice, specimen box;

c) reagents: sodium hexametaphosphate, ethanol, hydrogen peroxide, tetrachloromethane, Rose Bengal sodium salt ($C_{20}H_2Cl_4I_4Na_2O_5$), distilled water.

7.5 Processing and analysis of the samples

7.5.1 Numbering and weighing the crystallizing dish or beaker

Apply the following procedure:

a) clean the crystallizing dish;

b) dry the crystallizing dish in an oven at 60 °C;

c) number and weigh the crystallizing dish (accurate to 0,01 g);

d) record the mass of the crystallizing dish and the corresponding sample number on the record sheet (Table F.1).

7.5.2 Drying and weighing

Apply the following procedure:

a) place the original sediment sample into the weighed crystallizing dish and number it;

b) record the wet mass of the sample;

c) dry the sample at 60 °C in an oven, then weigh and calculate the dry mass.

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7.5.3 Sample soaking

Apply the following procedure:

a) add distilled water or tap water to the beaker containing the sediment sample and soak for 1 or 2 days in order to disperse the sediment particles and ostracods;

b) soak the sample with 3 % hydrogen peroxide for 2 h then wash with clean water if samples contented high organic matter;

c) for offshore sediments, add a small amount of sodium hexametaphosphate to make the samples more loose. This step is not suitable for deep-sea sediments or for living ostracods.

7.5.4 Washing and drying

Apply the following procedure:

a) transfer the sample into a 0,063 mm sieve and wash repeatedly with running water;

b) transfer the washed sample to a crystallizing dish and number it;

c) dry the crystallizing dishes at 60 °C in an oven;

d) record the mass of the coarse fraction of the dried samples.

7.5.5 Suspension and concentration

Apply the following procedure:

a) number the beaker according to the sample number;

b) suspend with tetrachloromethane;

c) pour the sediment residue into a crystallizing dish and number it;

d) bag the sediment residue and record the sampling information during microscopic examination.

7.5.6 Bottling and sealing

Apply the following procedure:

a) pour the weighed and dried sample through 0,150 mm and 0,063 mm meshes respectively;

b) bottle and label the two subsamples with information of sample layer and size fraction.

7.5.7 Microscopic examination and enumeration

Apply the following procedure:

a) examine the specimens by microscopy (>0,150 mm);

b) select 100 to 200 ostracods for specimen preparation, or select all if there are <100;

c) carry out the identification, classification and biocoenosis statistics (species number, abundance, diversity, etc.) according to the survey plan;

d) for offshore sediments, carry out the identification, classification and statistics of the 0,063 mm fraction selectively according to the survey plan;

e) fill in the tables (designed according to the survey plan) for the identification and statistics of ostracods.

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8 Survey of radiolaria

8.1 Principle

Survey of the preservation status, abundance and community structure of planktonic radiolarians deposited in the sediment of related sea area.

8.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 5 g to 10 g samples for continental shelf and shallow water depths, and 1 g to 2 g or 5 g samples for the slope and deeper water depths.

c) Identification of radiolarian specimens is carried out using diascoptic lighting under a biomicroscope. In general, identify to species level for the 60 dominant or common species, other specimens can be enumerated only for statistical analysis. But if the survey plan requires species diversity data, identify all specimens to species level.

d) For biocoenosis statistics, all samples should be counted if the total number of shells is less than 300. If the number of shells is large, sample can be divided by a sampler or a diagonal sampler.

e) Calculate the relative abundance of each species and record its percentage (%) or record the absolute abundance as individuals in per gram dried sample (individuals/g).

8.3 Collection and preservation of the samples

8.3.1 Sample processing

Based on the sediment type, samples should be processed in one of the two following ways.

a) Shelf and shoal water type: these sediments are composed mainly of terrigenous clast and organisms with calcareous shells; they have a fast deposition rate and the radiolarian content is low. For obtaining radiolarian specimens, sediment is soaked in a beaker with clean water. Tetrasodium pyrophosphate is added for dispersing the particles. After heating and disaggregation, organic matter and calcareous fractions are eliminated and dissolved by adding moderate amounts of hydrogen peroxide and hydrochloric acid. The sample is then washed over a 0,063 mm sieve and oven-dried. In order to separate the clean radiolarian specimens from coarse particles, flotation in tetrachloromethane while stirring is carried out. Radiolarian shells in suspension are poured onto a boulting cloth of 0,063 mm mesh or a filter paper.

b) Slope and deeper sea type: these sediments have less terrigenous clast, fine sediment particles, relatively slow deposition rates, and a higher radiolarian content. The sediment is soaked in a beaker with clean water. Tetrasodium pyrophosphate is added for dispersing the particles. Based on the content of calcareous particles, superadd moderate amounts of hydrogen peroxide and hydrochloric acid in order to dissolve organic matter and calcareous fractions. Continue until the reactions stop. The remnant is washed through a 0,063 mm sieve and oven-dried. The dried material may be directly used for making radiolarian specimen slides for species identification and enumeration.

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8.3.2 Collection and conservation of deposited empty shells

Empty radiolarian shells deposited on the surface of the sediment are collected from the top 0 cm to 2 cm layer using a box-corer, multicorer or gravity sampler. The sample is stored in a plastic bag, plastic box or glass bottle either at 4 °C or at ambient temperature.

8.4 Tools and reagents

The tools and reagents include the following.

a) Equipment: thermostatic drier box, ultrasonic oscillator, heating plate, beaker, straw, mesh sieve, filter paper, tweezers, glass slide, coverslip and glass rod.

b) Reagents: tetrasodium pyrophosphate, hydrogen peroxide, hydrochloric acid, tetrachloromethane, ethyl alcohol, neutral balsam, xylene (used for dilution or clearing remnant balsam).

8.5 Processing and analysis of the samples

8.5.1 Sample pretreatment

Apply the following procedure.

a) Weigh the dried sample (1 g to 2 g or 5 g), place into a 300 ml beaker, add 200 ml of clean water and 0,5 g to 1 g of tetrasodium pyrophosphate, soak about 10 h.

b) After dispersion of the sediment in water, add a moderate amount of H_2O_2 (a volume fraction of 30 %, analytical pure), place in a water bath at 80 °C, soak for several minutes until the reaction finishes. Monitor and adjust the temperature to avoid loss of radiolarian specimens by foam overflow.

c) Add a moderate amount of analytical pure a volume fraction of 36 % HCl according to the content of calcareous materials and leave for about 10 min or until the reaction stops.

d) Add clean water and allow to stand for about 5 min. Pour off the supernatant. Repeat this process 2 to 3 times in order to eliminate redundant HCl and H_2O_2 and reduce the acidity of the water.

e) Place the beaker in an ultrasonic oscillator for 1 min to 2 min in order to remove clay from the surface of the radiolarian shells.

f) Wash specimens over a 0,063 mm mesh copper sieve. Adjust water flow to avoid loss of specimens by overflowing.

g) Transfer specimens into a small beaker and allow to dry.

8.5.2 Preparation of microscope slide specimens

The preparation of microscope slide specimens includes the following steps.

a) Preparation of dry samples: take all (or a proportion of) specimens of every sample and place in the centre of a glass microscope slide. Add several drops of alcohol to disperse specimens and use a needle to spread the samples evenly over an area similar to that of a cover glass (24 mm × 50 mm). Allow the alcohol to volatilize, or place onto a hot plate to accelerate drying. When completely dry, add 3 to 5 drops of neutral balsam and apply a cover glass with light pressure causing the balsam to spread slowly over the whole area. Number the slide.

b) Preparation of specimens in water: Using a small pipette, mix the sample, place a drop onto a glass microscope slide, and spread evenly over an area similar to that of a cover glass. Place onto a hot plate for drying. Continue as above.

c) The temperature of the hot plate or thermostatic drier box should be maintained at about 60 °C to 80 °C.

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d) Allow the slide to dry naturally, or place in a thermostatic drier box (60 °C to 80 °C, being careful to avoid bubbling). Remove surplus balsam from the slide by using xylene after balsam consolidation and hardening.

e) Label the slide.

9 Survey of sedimentary diatom

9.1 Principle

Based on the collection, identification and analysis of community composition of diatom shells in the sediment, the distribution and preservation of diatoms are investigated to reflect the hydrological, climatic and environmental information in the area.

9.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) For wet samples, take about 5 g to 10 g, for dry samples take about 1 g to 5 g.

c) Diatom identification is carried out using transmitted light microscope. Each sample is observed by random row number. It is suggested to identify the species or variant, otherwise identify to genus level. For incomplete diatom frustules, if more than half of the central diatom is complete, or if the longitudinal furrow side of feathery diatoms is complete, the specimen should be identified. Dominant or common species can be selected for identification with other specimens only being enumerated, however, if the survey plan requires details of species diversity, all species in each sample should be identified.

d) For statistical analyses, at least 300 specimens per gram of dry sample or per square centimetre should be identified for every sample. If there are fewer than 300 diatom specimens in the sample, all should be identified. The relative abundance of each species should be recorded.

9.3 Collection and preservation of the samples

9.3.1 Sampling and treatment

According to differences in diatom content in different types of seabed sediments, sample collection and processing are divided into two types, as follows.

a) Continental shelf and shallow water type: these sediments are composed mainly of terrigenous clast and organisms with calcareous shells; they have a fast deposition rates and the diatom content is relatively low. Dispersion of diatom specimens is by soaking the samples in distilled water. Appropriate amounts of hydrogen peroxide and hydrochloric acid are added in order to remove calcareous particles and organic matter. Allow to stand until the reaction stops. Add zinc bromide or other heavy liquids with a specific gravity of about 2,4 in order to separate the diatoms from other particles by flotation. Stir to keep the diatoms in suspension. These can be used directly for making specimen sheets.

b) Slope and deep-sea type: these sediments have less terrigenous detritus, fine sediment particles, relatively slow deposition rates, and a higher diatom content. Diatoms are dispersed by soaking in distilled water. Based on the calcium content, appropriate amounts of hydrogen peroxide and hydrochloric acid are added in order to remove organic matter and calcium particles. Allow to stand until the reaction stops. This material can be directly applied to the preparation of specimen slices.

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9.3.2 Collection and preservation of sedimentary remains

Surface sediments: scrape sediments from the top 0 cm to 2 cm of newly collected samples using a plastic knife or spoon. Place about 5 g of fresh sediment into a polyethylene bag and seal, label with site numbers and layer positions and store in a sample box in the shade until for further processing.

Core sediments: collect about 5 g fresh sample, or 1 g dry sample, from each layer. Place the sample into a polyethylene bag, seal, label with site number and layer position and store in a sample box in the shade until further processing.

9.4 Tools and reagents

The tools and reagents include the following.

a) Equipment: long-handled spoon, balance, glass beaker, centrifuge, electric heating plate, oven, slides, glass, glass rod, tube, cylinder, tweezers, ventilation cabinet.

b) Reagents: hydrogen peroxide, hydrochloric acid, alcohol, distilled water, neutral gum, sodium pyrophosphate, and zinc bromide.

9.5 Processing and analysis of the samples

9.5.1 Sample pretreatment

9.5.1.1 Centrifuge method

The centrifuge method includes the following steps.

a) Place about 2 g of wet sediment ample into a 5 ml tube with a long-handled spoon. Add about three times the volume of 10 % HCl to that of the sample in order to remove calcium. Allow to stand until the reaction stops, i.e. until no bubbled form. Note that the duration of this reaction depends on the calcium content in the sediment.

b) Centrifuge at 2 500 r/min for 5 min. Remove residual HCl and wash 3 times with distilled water.

c) To remove organic matter, add about 3 ml to 4 ml volume fraction of 30 % H_2O_2, and place in a constant temperature water bath at 60 °C for 1 h to 2 h, i.e. until no bubbles form.

d) Centrifuge at 2 500 r/min for 5 min. Remove residual H_2O_2 and wash 3 times with distilled water.

NOTE If time allows, the sediment can be separated from the supernatant by static precipitation (see 9.5.1.2).

9.5.1.2 Static precipitation method

The static precipitation method includes the following steps.

a) Place about 1 g to 2 g of sample into a beaker. Add a volume fraction of 10 % to 15 % hydrochloric acid. When the reaction stops, distribute the mixture evenly and place for 12 h to 24 h.

b) Remove the residual hydrochloric acid. Add distilled water and place for at least 24 h and pour off the supernatant. Repeat 3 times.

c) Add a volume fraction of 30 % H_2O_2 and place in a constant temperature water bath at 60 °C for 1 h to 2 h.

d) Wash 3 times with distilled water.

If the sample is dry, it is advisable to take about 1 g to 5 g dry sample, put it into 300 ml beaker, add 200 ml of distilled water and 0,5 g to 1 g of sodium pyrophosphate and allow to stand for about 10 h. After the sediment has dispersed, continue as above.

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9.5.2 Preparation of microscope slide specimens

The procedure for the preparation of microscope slide specimens includes the following.

a) Immerse coverslips in a volume fraction of 10 % to 20 % HCl solution for at least 24 h, and then immerse in alcohol to remove HCl, before use.

b) Spread diatom suspension evenly on coverslip with a glass rod and allow to dry. Add 1 or 2 drops of neutral resin onto the glass slide. Place the coated coverslip gently onto the resin drop, avoiding air bubbles.

c) Slide can be dried naturally or in an oven (45 °C to 55 °C, temperature should not be too high so as to avoid bubbling) for 48 h.

d) Label the slide and store it in a sample box.

e) Prepare replicate slides for each sample for identification and analysis.

9.6 Organization of data

Identification of diatoms is made by observing under a microscope at 1 000× magnification. Enumeration can be carried out at 400× magnification. At least 300 diatom frustules (not including resting spores and auxospore) per sample should be identified. The relative abundance (%) of each species relative to the total number of diatom frustules (i.e. not including resting spores and auxospore) should be recorded for each sample.

Data processing should meet the relevant provisions of this document. For the calculation of biocoenosis analysis, refer to Annex E.

10 Survey of coccoliths

10.1 Principle

The objectives of investigating coccoliths in sediments are: to meet the need of chronostratigraphy and to date geological age of sediments by analysis and identification of coccoliths; to explore their oceanographical/palaeoceanographical significance through analysis of characteristics of coccolith assemblages; to infer sedimentary environments (including sediment sources) or diagenesis based on analysis and evaluation of coccolith preservation status.

10.2 General provisions

The general provisions include the following.

a) Sampling quality: the sample shall not be contaminated for example by cross-sampling, mixing with another sample, re-using disposable tools for sampling.

b) Sampling depth or depth interval: the topmost 0 cm to 2 cm layer of sediment should be collected for seafloor surface samples; depth intervals between 2 cm to 10 cm, or other intervals in accordance with the requirements of a survey plan can be selectively collected from box-corer, multicorer or gravity sampler, for subsurface sediment samples. For undisturbed sediments and well-preserved sediment sequences, a grab sampler can be used from which vertical tube subcores can be taken. Sampling can then continue in the same way as by multicorer.

c) Sampling amount: take about 5 g to 10 g of wet sediment for sampling shallow water regions of continental shelf; take about 1 g to 2 g of wet sediment for sampling continental slope and deep-water areas. The dry mass of samples should be calculated. It is suggested that the sampling volume be at least 3 cm^3, or the sampling mass more than 5 g, so that the amount of sediment is sufficient to meet the needs of the clean procedure (which includes removing the outer layer of the sample), and the needs for multiple slide-making or scanning electron microscopy.

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d) Requirements of identification and classification: identify dominant taxa at species or genus level.

e) Statistical requirements: all coccoliths within 5 fields of view (at a magnification of 1 000×) on a microscope slide shall be enumerated. At least 300 individuals should be identified and enumerated. At least 100 fields of view should be observed if there are fewer than 300 individuals on a slide.

f) Characteristic parameters of fossil community: the parameters include the number of species, dominant and common species, relative abundance and species diversity.

10.3 Collection and preservation of the samples

The common methods of sediment sampling are mainly carried out by box-corer, multicorer, grab samplers, gravity piston corer or drilling. After the sampling, the samples can be stored in sealed plastic bags, plastic boxes, or glass bottle; according to the conditions, the samples can be stored either at 4 °C or in a sample store room at room temperature.

10.4 Tools and reagents

The tools and reagents include the following.

a) Equipment for slide preparation: beaker (commonly 50 ml, 20 ml), wash bottle, electric heating plate, glass microscope slides, cover slips, straws (large and small), glass rods (large and small), ultrasonic cleaner, centrifuge, tweezers, toothpicks, labels, markers, kitchen paper or paper towels, microscope slide box.

b) Reagents and solvents: distilled water, ammonia (preparation of alkaline buffer of pH 9: add a few drops of ammonia into a bottle filled with 1 l distilled water, shake the bottle, test pH with pH test paper), a volume fraction of 10 % hydrochloric acid.

c) Slide mounting medium: the mounting medium should have a refractivity lower than or close to that of calcite, namely between 1,658 to 1,486; neutral balsam (or Canada balsam), or Norland optical adhesive (ultraviolet light is needed to dry it), or abienic balsam.

d) Observation instruments: a polarizing microscope (with magnifications of 200×, 400×, 1 000×). The use of contrast device on the microscope depends on the need. A scanning electron microscope or transmission electron microscope can be used depending on the need.

10.5 Processing and analysis of the samples

10.5.1 Pre-preparation of the equipment and the samples

As coccoliths are very small, the samples are prone to be contaminated. The following precautions should always be taken during sample preparation.

a) Clean the test bed: cover the preparation area of the test bed with disposable paper, replace with new paper after each usage, avoid solution spatters out during preparation.

b) Use fresh samples: scrape off the outer surface of the sample and subsample the interior sediments. Avoid touching the samples directly; wear disposable gloves or use disposable paper while manipulating the samples.

c) Clean the containers and equipment: use disposable tools (such as toothpicks, paper) where possible; glass containers and reusable equipment should be washed after each usage, soaked for 10 min in dilute hydrochloric acid (a volume fraction of 10 %), and rinsed.

10.5.2 Method of preparing simple smear slides

The procedure is as follows.

a) Mark a glass microscope slide.

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b) Scrape small fraction of the clean and fresh raw sediment (about the size of a mung bean) and place on a cover slip or glass slide. Add a few drops of distilled water to the sample and use a toothpick to mix into a slurry.

c) Use the toothpick to smear the sediment slurry and form a thin sample layer on the cover slip or glass slide. During the smearing process, large-sized or coarse particles should be moved to the edge of the glass slide and then discard. Put a cover slip or glass slide onto a heating plate (usually set at 70 °C to 90 °C) and allow to dry.

d) Place an appropriate amount of mounting medium onto the cover slip or glass slide. Place the cover slip onto a glass slide, or apply a cover slip onto the glass slide, and place onto the heating plate. Use a glass rod to gently compact the cover slip, squeezing any air bubbles out of the mounting medium. Remove from the heating plate and allow to cool. When Norland optical adhesive is used, place the slide under an ultraviolet light for at least 5 min to allow the adhesive to cure.

The above second step can be substituted as follows.

b) Put the sample into a beaker filled with distilled, buffered water, mix well and allow to soak in order to disperse the coccoliths. Place 1 or 2 drops of the sediment suspension onto a cover slip or glass slide.

10.5.3 Pre-concentration of coccolith samples

For sediments from shallow seas or deep-sea gravity flow environments, sorting and concentration methods are needed. The procedure is as follows.

a) Soak and disaggregate sediment sample: take about 1 g of sample, place it in a beaker, add an appropriate amount of buffer (pH 9), and soak. To disperse particles, either stir, or place the beaker in an ultrasonic bath for 10 s to 15 s.

b) Sort and separate coarse fractions: stir the sample solution, allow the solution to settle for 5 min to 10 min, so that coarse particles (sand and coarse silt) gradually precipitate onto the bottom of the beaker; pour the supernatant into another beaker and discard the settled coarse debris.

c) Sort and separate fine clay fractions: cover the beaker containing the supernatant and allow to settle for 15 h to 20 h. Pour off the supernatant liquid, which contains mainly clay minerals; stir the settled residue in the bottom of the beaker, and take a few drops to make a smear slide. If the sample contains abundant clay fraction, repeat this step 2 to 3 times. Centrifugation can also be used in this step, however, a pre-experiment should be conducted in order to determine suitable speed and time of centrifugation.

10.6 Organization of data

Data analysis and record documentation should meet the relevant provisions of this document. The procedure includes the following.

a) Identification and classification of coccolith species: observe and identify the coccoliths, mainly using polarized light microscopy at a magnification of 1 000×. Generally, coccolith fossils should be identified to species or genus level, for a very small species, identification should be made using a scanning electron microscope at a magnification of at least 10 000×.

b) Evaluation of coccolith preservation status: provide a qualitative and quantitative estimate of the preservation status of coccoliths, e.g. if they have been damaged by chemical etching, physical breakage, or by secondary crystallization/recrystallization (Table G.1). Relative coccolith abundance in sediments is the proportion of coccoliths relative to the sediment clastic components and is expressed as a percentage (Table G.2). It is determined by referring to sediment particle abundance charts[50]. The evaluation should be made based on observations of at least 10 fields of view at a magnification of 1 000×. Observation of at least 100 fields of view is needed if sediments contain very few coccoliths.

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c) Enumeration of coccoliths. There are two minimum requirements; at least 300 coccoliths shall be observed and counted for each sample; and at least more than 5 fields of view (at a magnification of 1 000×) randomly located on the slide should be observed. Very commonly, the sediment samples analysed contain abundant coccoliths, and the number of coccoliths within one field of view is more than 100. In this case, a subdivision of one field of view into 4 parts can be made by using the cross-hairs in the eyepiece, and coccoliths within 1/4 field of view can be counted. In this case, at least 5 fields of view shall be observed. If a sediment sample contains very few coccoliths, at least 100 fields of view shall be observed.

d) Analysis of coccolith assemblage abundance: for basic marine geological survey objects, it is recommended to estimate the relative abundance of coccoliths.

e) There are several semi-quantitative estimation methods. For example, the method suggested by Reference [5], it is to obtain the number of coccoliths per area on a sample slide, with the purpose of analysing some selected coccolith species that have significance for ecology or geological age diagnosis. There are also several absolute quantitative methods of analysis to obtain the number of coccoliths per mass in the sample. For example, the random settling method that was suggested by Reference [7] and by Reference [55], the spray method suggested by Reference [8] and the microbeads method suggested by Reference [47]. These methods are commonly applied in palaeoceanographic studies, however, they need more time for sample preparation than simple smear slides. These methods can be applied if necessary in the marine survey objects.

11 Survey of sporopollen

11.1 Principle

The outer coat (exine) of pollen and spore is mainly composed of tough, resistant organic compounds, namely sporopollenin $(C_{10}H_{16}O_3)_x$ and chitin, which protect the sporopollen from desiccation and oxidation. The sporopollen assemblage in sediments reflects the community characteristics of the original vegetation and provides information on the temperature and humidity of terrestrial habitats around sedimentary basins. The methods employed for investigating sporopollen in sediments include sample collection, sample processing by both physical and chemical treatments in order to extract the sporopollen by removing organic materials, carbonate and siliceous minerals, the identification and enumeration of sporopollen and data analysis. Based on the sporopollen data, it is possible to reconstruct past climate change in the terrestrial habitats surrounding sedimentary basins.

11.2 General provisions

The general provisions include the following.

a) All samples should be collected and processed without contamination; only filtered or distilled water can be used; laboratory windows should be kept closed when the pollen filtration system is in operation; there should be no other sporopollen source in the laboratory. Detailed information of the field site and samples shall be recorded.

b) Samples should be collected from the upper 2 cm layer of the sea surface sediments, and at 2 cm intervals for the core sediments, or as required by the investigation program.

c) The investigation should include the identification of the sporopollen components and their quantity, and the total sporopollen concentration.

d) The nomenclature of sporopollen should follow modern classification systems for extant plants. All sporopollen should be identified to genus level (or family level if genus identification is not possible).

e) At least five slides should be observed to enumerate the sporopollen when the sporopollen is low in quantity (less than 100 grains). Otherwise, a minimum of 200 grains (spores not included) should be counted and a total of 100 grains for non-pine pollen is suggested.

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11.3 Collection and preservation of the samples

The procedure for collection and preservation of samples include the following steps.

a) Samples of surface or core sediments can be collected, packed and sealed in plastic bags with labels showing the site location, water depth, core number and depth, and sample number. All samples should be kept in a refrigerator or a cool room between 2 °C and 5 °C, or frozen. Samples can be taken in laboratory if the surface sediments and cores are whole packed and stored in refrigerator or frozen.

b) The volume of the sediments for sporopollen investigation is 10 ml or 10 g to 20 g, based on the lithology. A large sample volume is usually needed when the site location is far from the shore or the samples are made up of coarser components.

11.4 Tools and reagents

The tools and reagents include the following.

a) Equipment: balance, polytetrafluoroethylene (PTFE) or polypropylene beaker, glass beaker, funnel, ultrasonicator (40 kHz), standard sieve (7 µm and 180 µm), centrifuge, hot plate, water bath, microscope slides, cover slips (0,150 mm or thinner), watch glasses (Φ=20 cm), pipe, wooden spatulas, stainless steel or polytetrafluoroethylene (PTFE) spatulas and rods, glass rods, stereo microscope.

b) Reagents: *Lycopodium* tablets, glycerine, phenol, gelatine, Canada balsam or nail polish, sodium pyrophosphate ($Na_4P_2O_7$, 5 %), potassium hydroxide or sodium hydroxide (KOH or NaOH, 10 %), hydrochloric acid (HCl, 10 %), hydrofluoric acid (HF, 40 % or conc.), glacial acetic acid (CH_3COOH), mixture of acetolysis anhydride [$(CH_3CO)_2O$] and sulphuric acid (H_2SO_4, conc.) (9: 1 in volume).

11.5 Processing and analysis of the samples

11.5.1 Sample disaggregation

The procedure for sample disaggregation includes the following.

a) Record the site location, water depth, core number and depth, registered the sample number and water depth. Label the beaker.

b) Place the sediment (10 ml, or 10 g dry sample) and one tablet of *Lycopodium* into the beaker. Record the mass or volume of the sample.

c) Add a volume fraction of 5 % sodium pyrophosphate (two times the volume of the sediment sample). Place in a water bath at 100 °C for 30 min, stirring with a rod. The sediment particles are dispersed to maximize the reactive surface area. If the sediments are quite loose, this step can be skipped.

11.5.2 Alkali-soluble digestion

Add a volume fraction of 10 % potassium hydroxide (two times the volume of the sample). Place in a water bath at 100 °C for 10 min, stirring with a rod. This process dissolves humic materials.

11.5.3 Sieving

Add distilled water and suspend the sediments. Wash through a stainless-steel wire sieve (180 µm) into another labelled beaker. The sieving step removes large clasts and organic debris, which makes the reaction in subsequent treatments more efficient when digesting extraneous material. Transfer the suspension by passing through the sieve (180 µm) into a centrifuge tube and centrifuge for 5 min at 3 000 r/min. Decant the supernatant.

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