MOOPAM

Manual of Oceanographic Observations and Pollutant Analyses Methods



Regional Organization for the Protection of the Marine Environment (ROPME)

MANUAL OF OCEANOGRAPHIC OBSERVATIONS

AND

POLLUTANT ANALYSES METHODS

(MOOPAM)

Fourth Edition

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In the Name of God, the Compassionate, the Merciful

P R E F A C E

I am pleased that the Fourth Edition of the Manual of Oceanographic Observations and Pollutant Analyses Methods (MOOPAM) is now ready. MOOPAM is a comprehensive Manual intended to develop the fundamental capacity in the Region for the analyses of oceanographic and pollutant parameters through recommended and standard methods. The earlier editions have found a desired place in the scientific institutions of the Region and there has been much anticipation amongst the scientific community in and outside the Region to receive this updated version of MOOPAM. As is customary to the series, this volume is updated and upgraded in comparison to the earlier ones and has been validated by Regional and International scientists, institutions and ROPME-NFPs.

The basic premise on which the MOOPAM is prepared is that good policy decisions can only come out of dependence on accurate and reliable scientific data and information. It is with this objective that the methods suggested in MOOPAM have gone through due diligence and meticulous peer reviews. The Experts who have worked on this revised edition of MOOPAM have been careful in revising and selecting methods that have been internationally approved, accepted and routinely used. Further, while preparing this Manual, the Experts have taken every care that only those procedures which are suitable to our Region are incorporated. The contributions of Member States, ROPME Experts, Qatar University and Dr. Jae Oh of MEL-IAEA to this edition of MOOPAM are acknowledged and appreciated.

Besides the new design and colourful illustrations, some added features of this edition of MOOPAM are:

- A method for normalization of contaminants' concentration in sediments
- A new method to assess phytoplankton population using pigment markers
- Additional procedures for nutrients' analyses in seawater
- A new chapter for radionuclides' monitoring in marine sediments
- Additional procedures for the analyses of plankton and benthos

As always, I sincerely hope that MOOPAM will play a strong contributing role in enhancing the capacity of the Region to generate reliable data and information, which can be judiciously used in implementing Regional programmes and policies that help in collectively protecting the ROPME Sea Area.

Kuwait,
December 2010

Dr. Abdul Rahman Al-Awadi
Executive Secretary of ROPME

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I INTRODUCTION



I INTRODUCTION

I.1 INTRODUCTION

The basic objective of the Monitoring Programme is to provide baseline data on oceanographic characteristics, petroleum hydrocarbons, selected chlorinated hydrocarbons and heavy metals in the ROPME Region.

The Manual is designed primarily as a guide to the field surveys, sampling and analytical methods to be used by the participating institutions for implementation of the Monitoring and Research Programme for oceanographic characteristics and oil and non-oil pollutants in the Kuwait Action Plan (KAP) Region. The first edition of MOOPAM was prepared in co-operation with KISR-Kuwait and published in 1983. It was designed to meet the requirements for sampling and analysis set by the Second Task Team Meeting on Oceanography and Baseline Studies on Oil and Non-Oil Pollutants (January 1982). In this revised edition of MOOPAM several considerations were taken into account, i.e. the development of analytical capabilities in the Region, notes and suggestions submitted by scientists in the Region, results of the intercalibration exercises, advances in analytical methods on the international level and the revision of sampling and analysis program by the Seventh Task Team Meeting (January 1989).

I.1.1 Matrices and Parameters to be Monitored

The oceanographic parameters to be determined in seawater during the monitoring programme are summarized in Figure (I.1) which also includes the general analytical approaches to be used. Figure (I.2) presents the same for sediment.

The parameters to be determined during the baseline studies of oil and non-oil pollutants are illustrated in Figures (I.3), (I.4) and (I.5). Figure (I.3) presents the parameters, together with the general analytical approaches for water and Figure (I.4) and Figure (I.5) show the same for the sediment and biota, respectively.

I.1.2 Sampling Locations and Frequencies

The Task Teams also agreed on the sampling locations and the sampling frequencies for examining the various matrices and parameters. The oceanographic characteristics and the oil and non-oil pollutants in seawater and sediment will be monitored at the selected Reference and Quarterly Stations.

The exact locations of these stations together with the sampling locations for biota, for the ecological surveys and for the tar ball collections on beaches are given in the individual agreements between the National Focal Points of the Member States and ROPME.

I.1.3 Organization of the Manual

The manual is organized into four major sections covering all the tasks required for the regional studies. The sections are as follows :

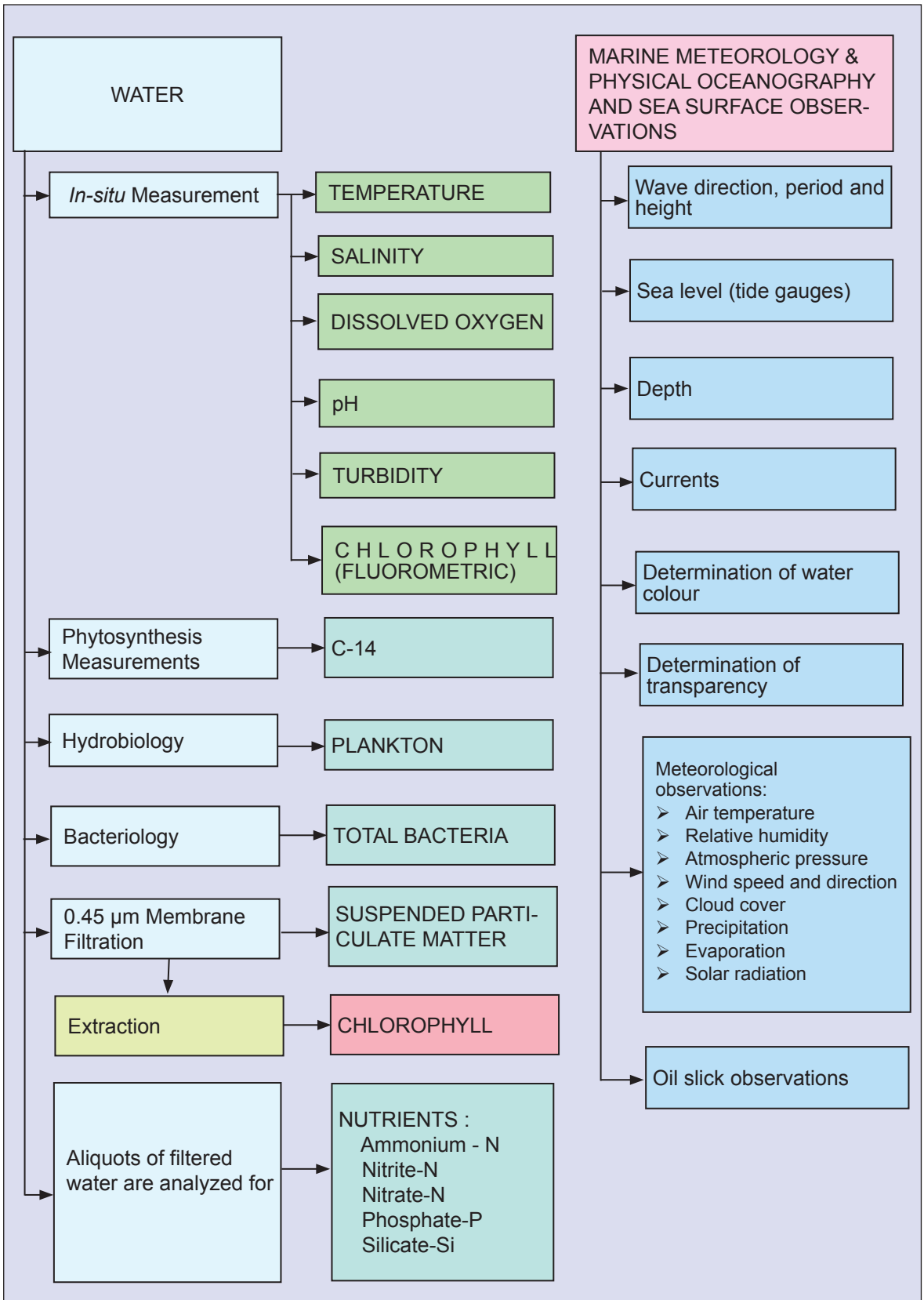


Figure (I.1) : Flow chart of *in-situ* measurements, analytical steps and parameters for water analysis for baseline oceanographic studies

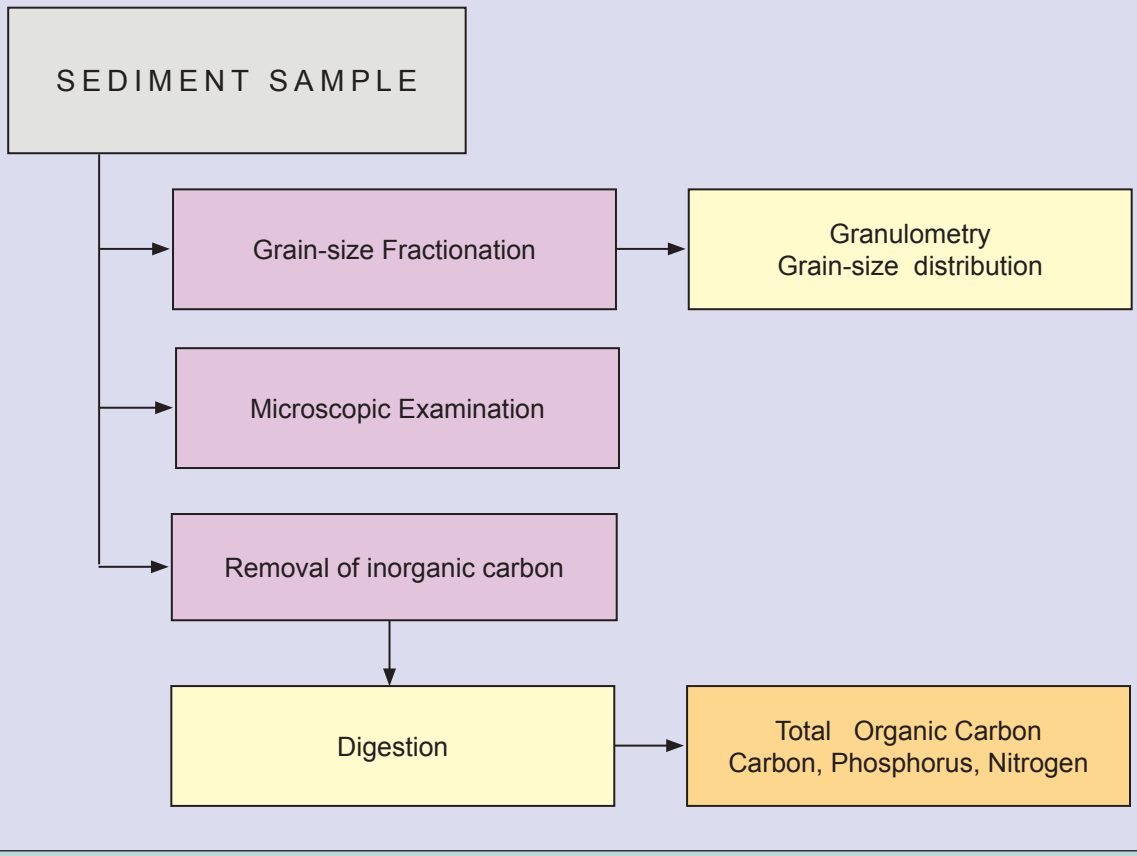


Figure (I.2) : Flow chart of analytical steps and parameters to be analyzed in sediment samples for oceanography

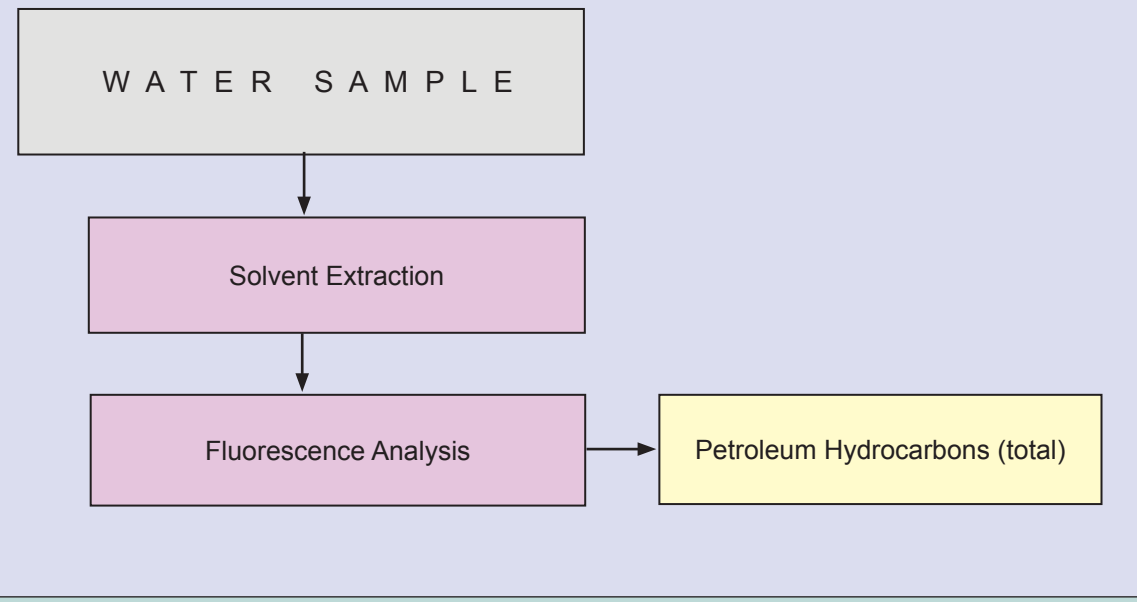


Figure (I.3) : Flow chart of analytical steps to be analyzed in water samples for oil pollutants

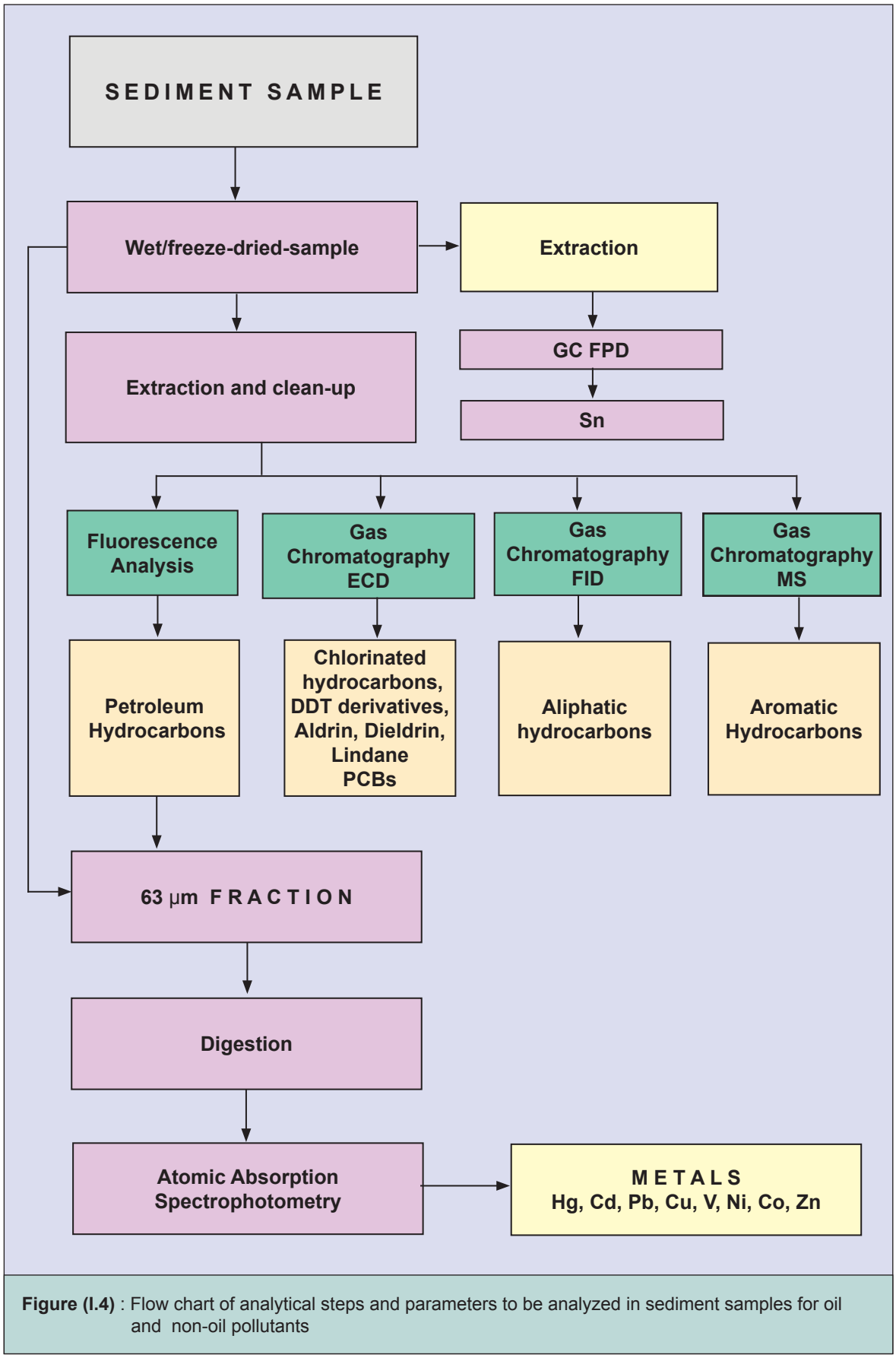


Figure (I.4) : Flow chart of analytical steps and parameters to be analyzed in sediment samples for oil and non-oil pollutants

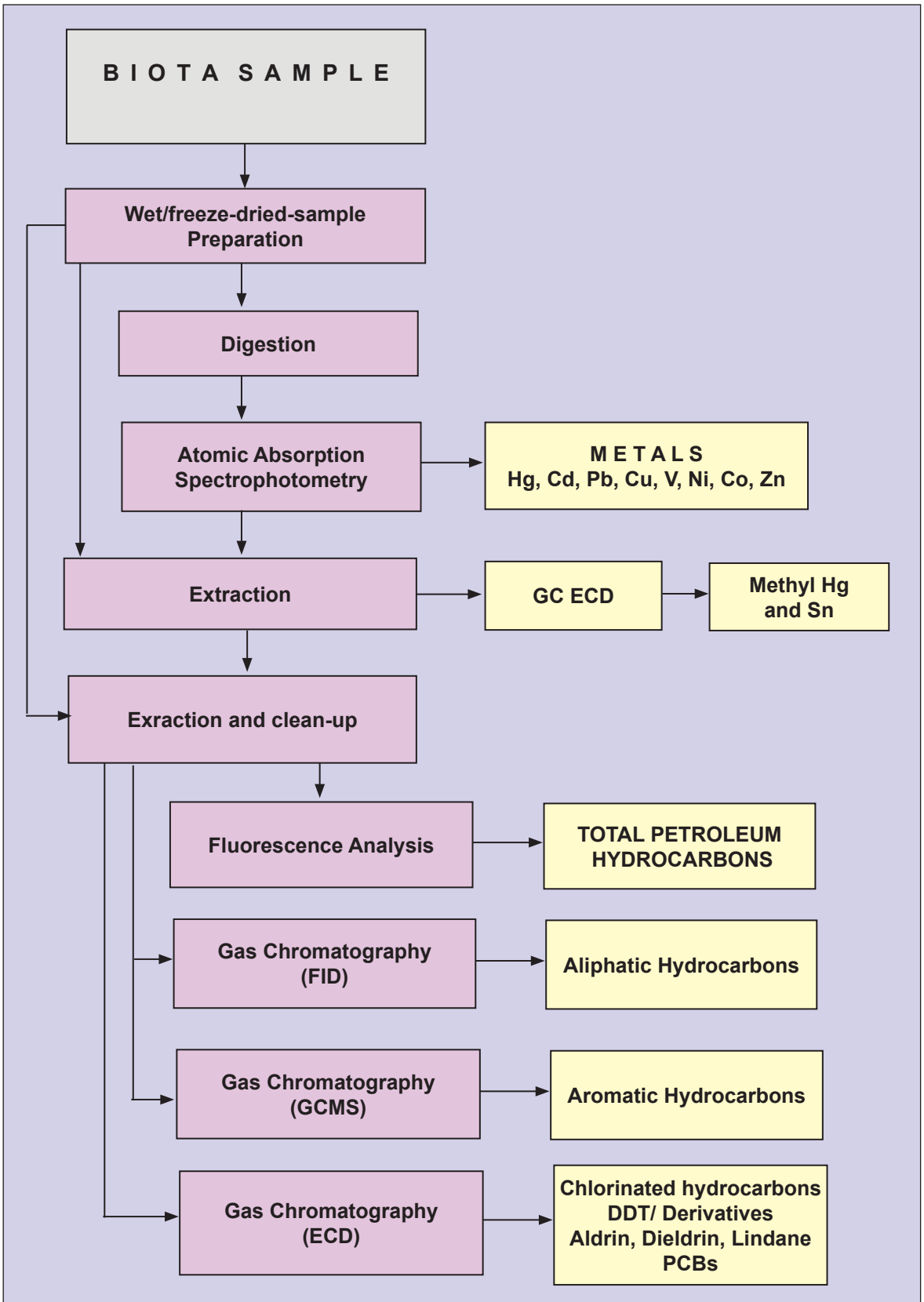


Figure (I.5) : Flow chart of analytical steps and parameters to be analyzed in biota samples for oil and non-oil pollutant

- Marine meteorology, physical oceanography and sea surface observations
- Sampling and *in-situ* measurements
- Laboratory analysis for water quality, oil and non-oil pollutants, sediment and dust fallout associated pollutants
- Ecological survey

Although the majority of the included methods were adopted from U.N. specialized agencies and other universally accepted sources, special considerations and precautions have been included in the form of nota bene (N.B.) where appropriate. Methods for environment contaminant analysis being developed by IAEA (MESL) for UNEP was relied upon in revising the relevant sections. Each section also includes a brief introduction as well as detailed instructions for the measurement of the specific parameters.

I.2. QUALITY ASSURANCE AND GOOD LABORATORY PRACTICE INRELATION TO MARINE POLLUTION MONITORING PROGRAMMES

Many laboratories are actively participating in marine pollution monitoring programmes under the auspices of the Intergovernmental Oceanographic Commission (IOC) and the United Nations Environment Programme (UNEP). Both organizations have expressed concern about the quality of data arising from such programmes, particularly the accuracy and comparability of data produced by individual laboratories. Until quite recently, they have dealt with this problem by encouraging laboratories to participate in intercomparison exercises, by arranging for less experienced laboratory staff members to attend analytical workshops, and by arranging for reference analytical methods to be produced and distributed to participants in the various regional seas programmes.

It should be stressed, however, that the acquisition of reliable and relevant data of suitable quality for contaminants in marine samples is not solely dependent on the production of accurate analytical measurements. The overall quality of data is also dependent on three other factors, which are:

- a meaningful sampling programme
- a suitable storage and pre-treatment procedure for samples following collection and prior to analysis
- a data assessment procedure

Unless all four factors are given adequate consideration before and during the monitoring programme, the aims of the programme may not be achieved and valuable time, staff, facilities and other resources may be wasted. The term used to describe the approach to this work is “quality assurance.”

The purpose of this document is to provide general guidance on quality assurance and to outline the approach that could be taken by laboratories to achieve the specific aim(s) for each marine pollution monitoring programme (see also Chapter V.1)

Since quality assurance work starts from the time when a decision is taken to get involved with marine pollution monitoring, it is appropriate to begin by discussing the different aims, which laboratories might wish to pursue under this work.

I.2.1 Aims of Monitoring Programmes

Designing a monitoring programme using marine organisms

There are several items to be considered in the detailed planning of an environmental monitoring programme.

i) Which contaminants are to be measured?
ii) Which species are to be selected?
iii) Where are the samples to be collected?
iv) When is sampling to be done and how frequently?
v) How many specimens (of what size) are to be taken for each sample?
vi) Which tissue(s) are to be taken for analysis?

The head investigator, field biologist, analytical chemist, and statistician must consider each question during the detailed designing and planning of the monitoring programme (e.g., prepare sampling instructions, estimate workload, prepare the necessary instruction sheets for the field staff, develop safeguards to ensure sample integrity, develop analytical quality control needs and data handling methods). All participants in the study must be fully trained in their roles within the programme and recognize how their efforts unite with those of the other participants. The investigator must:

1. Design a sampling programme: select sufficient numbers of specimens with the required biological attribute (usually size, but others, e.g., sex may be used); define sampling sites; specify sampling frequency and period; and design sample handling methods. This is based on the results of the pilot study and any other relevant information. A statistically sound base is required for sample design. Once the sampling programme design is complete, instruction sheets (sampling instructions) must be issued to field staff. Training is probably required at this point to ensure consistent sample collection and handling

2. See that samples are collected, stored, and transported in a way that ensures sample integrity (i.e., sample continuity [see point 4], no loss and gain of contaminants before analysis). Advice on this can be found in the UNEP Reference Methods series No. 6, 65 and 7 (Rev.2). Again it will be necessary to prepare instruction sheets for field and laboratory staff

3. Arrange for sample processing and storage (Uthe and Chou [1988] have described problems encountered in sample handling) and arrange for sample analysis by methods with the required accuracy, precision, and lower limits of detection. Collaboration between the head investigator and all participants is essential for success. The investigator and the analyst should consult UNEP Reference Method No 57, which gives directions on Quality Assurance, if they are in doubt how to achieve and maintain analytical data quality

4. Ensure that there is a proper documentation system for tracing (tracking) samples from sampling to final data recording. The investigator must make sure that all team members are aware of, and comply with, the documentation system

Selection of contaminants

The contaminants to be studied are determined by: I. The aims of the monitoring programme; 2. The findings of the pilot study; and, 3. The analyst's ability to measure these contaminants with the required accuracy and precision. In practice the last factor dictates which contaminants can be included.

It is necessary that the statistician specify the accuracy, precision, and limits of detection for all measurements. This defines the necessary standards of analysis needed to detect changes in contaminant concentrations. It is pointless to consider monitoring contaminant concentrations using an analytical method with an inadequate level of precision, accuracy, or lower limit of detection.

If the analytical methods do not meet the required standards, the analyst must either set up methods that meet these standards or abandon the programme to prevent waste of resources. However, an analytical method that has the theoretical performance to meet the aims does not guarantee success. It must be proven within the analyst's laboratory. Also, other factors have to be considered in obtaining the required analytical data quality. These are discussed in "Quality Assurance and Good Laboratory Practice in Relation to Marine Pollution Monitoring Programmes," UNEP Reference Method No 57. Again, investigators should obtain a copy for analysts at the start of the work.

It is obvious that the principal investigator also must ensure that all other techniques associated with analysis are of the required quality (e.g., species selection and other biological measurements, sample handling, including dissection and storage) to yield high quality. They must be proven along with the analytical methods discussed above.

It may be appropriate to measure other contaminants that meet regional and international needs. This should be considered only if additional data are useful to the laboratory or Regional Studies, and does not impede the laboratory's main aims.

The final selection of core contaminants should be related to knowledge of their sources and inputs to the study area and their transport and persistence. Two examples: 1. An extensive monitoring programme for pesticides along a deserted coastline would be unwarranted; and 2. Many organophosphorus pesticides persist in sediments but are rapidly metabolized by organisms. It would be pointless to measure these pesticides in biota but important to instead measure them in sediments, where they will be a representative indicator of pollution.

Species selection (see also Annex I)

Sampling species with the following attributes yields the most reliable data on contaminant trends in organisms (Butler *et al.*, 1971, Haug *et al.*, 1974)

1. A simple relationship exists between contaminant concentrations in the species and average concentrations in the surrounding environment

2. The species accumulates the contaminant without being affected by the concentrations encountered in its environment (other than in polluted areas)

3. The species is sedentary and thus represents the collection area

4. The species is widespread and abundant in the study region, to allow comparisons among different areas

5. The species lives long enough so that more than one year-class can be sampled, if desired

6. The species is large enough to yield sufficient tissue for analysis

7. The species is easy to collect and hardy enough to survive unfavorable conditions (e.g., harbors) or within the laboratory (e.g., for deputation before analysis, for studies of contaminant dynamics)

8. The species exhibits high bio-accumulation factors, to allow analysis without pre-concentration

9. The species tolerates brackish water, to allow comparisons between estuarine and offshore sites. However, offshore animals may show surprisingly high contaminant concentrations and burdens (total amount of contaminant in the animal or tissue)

10. The species must be easy to identify with certainty (Phillips *et al.*, 1980). This was a problem in some early programmes where *Mytilus trossulus* was an unrecognized species in sample collections (McDonald and Koehn 1988)

These attributes tend to limit the useful species to a group of moderately large, abundant, widespread, well-studied, inter-tidal species, mainly mollusca. Filter-feeding mollusca tend to reflect contaminants in the water column: deposit feeders are also influenced by sedimentary contamination. Sediment working both by organisms and water currents will average short-term variations in contaminant loading that some scientists suggest for use in monitoring various metallic and organochlorine contaminants in rocky and muddy intertidal areas in United Kingdom waters.

In practice, availability, scientific knowledge, and an understanding of the ability of a species to reflect environmental contaminant concentrations, determines which species are useful as monitors. If the latter information is unknown, it must be obtained either from the scientific literature (e.g., Phillips 1980) or by a pilot study. Final selection should be made in consultation with a knowledgeable biologist. Common mussels (*Mytilus edulis, M. trossulus, M. californianus* and *M. galloprovincialis*) are generally suitable for spatial and trend monitoring programmes in temperate coastal waters. Oysters have been suggested in tropical waters, but their speciation may be a problem (Phillips *et al.*, 1980).

Other species can be used for environmental monitoring purposes, provided they accumulate contaminants in relation to environmental contaminant concentrations. Several studies have employed fin fish (e.g., Misra *et al.*, 1993; ICES, 1989 & 1991). Aquatic plants have also been used.

Location of sampling sites

i) Spatial environmental monitoring

Hot spots usually exist in estuarine and coastal areas where anthropogenic wastes are discharged. The offshore areas where hot spots are most likely to occur are waste dumping sites and oil platforms. However, Uthe and Chou (1987) found higher cadmium concentrations and burdens in scallops (*Placopecten magellanicus*) collected offshore compared with concentrations in equivalently-sized nearshore scallops, including ones from a cadmium-contaminated area. They suggested nutrition and growth factors accounted for this. Therefore, caution is needed when comparing inshore and offshore populations.

A decision to monitor contaminant concentrations in hot spots should be taken only after consideration of the discharges to them. If the pilot study leads to action to reduce inputs, it is appropriate to monitor to see if the new controls result in lower contaminant concentrations in organisms. If no regulation of inputs is considered, monitoring is justified only to update the results of the pilot study, or where there is knowledge that discharges might increase in the future. Other sampling sites may be included in the programme to include clean and moderately contaminated areas. All sampling should be done by scientific personnel using research or chartered boats instead of fishermen, to ensure sample continuity and integrity.

Precise sampling locations must be recorded, because small spatial changes (resulting in large biological or environmental change) may strongly influence the final data (i.e., "mussels from the harbor wall" must specify the exact point on the harbor wall). It is essential to record details identifying the sampling site, including a map and a photograph (with identifying marks and scale).

ii) Trend monitoring

In hot spots, once contaminant inputs are reduced, contaminant concentrations in monitoring organisms should decrease over a fairly short interval. One can employ a simple technique (e.g., the use of pooled samples), if a large decrease is expected. In situations (e.g., more distant areas), where smaller changes are expected, more complex methods are required to detect them with statistical confidence (i.e., the variance must be reduced). Methods are available for reducing variance by using measured biological factors (e.g., length, weight, sex, fat content) and contaminant concentrations in individual specimens (Misra *et al.*, 1993; Uthe *et al.*, 1991a&b). These techniques are much more expensive to employ; therefore, monitoring for small magnitude changes must be carefully justified before using them. Usually, it is better to use a single pool, remembering that only very large changes will be detected.

Period and frequency of sampling

For spatial monitoring, all samples should be collected during a short period (within 1-4 weeks) to yield an overview of contaminant concentrations in the study area. This also helps to ensure that all specimens are in a similar physiological state. If one expects large annual changes in the quantity and composition of inputs, annual or biennial samplings are appropriate. Experience has shown that changes in contaminant inputs often affects only the immediate discharge area (hot spot). It is these areas where more frequent monitoring should be conducted.

For trend monitoring, the frequency of sampling must reflect : 1. The time scales over which the changes must be detected; 2. The degree of confidence required in the measurement of these changes; and, 3. The available laboratory resources. A monitoring programme in which the proposed work is less than that needed to detect the desired changes in contaminant concentrations is frustrating and wastes resources. Also, if the original programme aims cannot be met with the available resources, they must be replaced with ones that can be.

It is sensible to sample an area once every five years or so if no change in inputs is expected. More frequent sampling can be justified if more data is required for other purposes (e.g., to reassure the public that contaminant concentrations are not changing).

Seasonal variations in food supply, reproduction, behavior, and other physiological functions cause changes in tissue weights, fat content and composition, and water content. These influence tissue contaminant concentrations. Sampling should be during the pre-spawning period to minimize these variations. The timing of the spawning period varies with species and location. A knowledgeable biologist should be consulted.

Size of sample

If either the analytical resource or the amount of sample material is limited, all specimens in a sample are often pooled for analysis. If the specimens were not sorted (e.g., selecting a small size range), no information will be obtained on the effect of animal size on contaminant concentrations. Site-to-site differences can still be assessed with some level of confidence by pooling enough specimens to reduce the pool-to-pool variance and analyzing each pool several times. This minimal technique (Single Pool Technique) pools sufficient specimens (e.g., 20-25) to justify an assumption that the analytical variance is now the major variance. If contaminant concentration is significantly related to animal size (i.e., size explains much of the animal-to-animal variation in contaminant concentrations), select a small size range to reduce number of animals required.

For monitoring, to estimate the pool-to-pool variance, it is better (Van der Meer 1989 & 1990) to use a few equivalent pools at each site, instead of one large pool. The number of specimens per pool and the number of pools per sample site will be determined by the size of the change considered significant (*Multiple Pool Technique*).

Analysis of individual animals (*Individual Specimen Technique*) is costly and recommended only for studies in which it is essential to detect small changes (e.g. of the order of.10-25%) in contamination. Sampling a small size range may reduce the contaminant variance; however the effects of the other biological and physiological factors cannot be easily controlled or considered. Techniques and measurements that enable the statistician to reduce the effects of these factors on the variance can be employed (Misra *et al.*, 1993; Uthe *et al.*, 1991a&b), if it is essential to reduce the variance further.

Selection of one of these three techniques for an environmental monitoring programme must be made with great care, in consultation with a statistician who can weigh the costs and benefits of each with respect to the specific situation being studied. The basic rule is, “The smaller the change in mean contaminant concentration that one wishes to detect with a fixed level of statistical confidence, the greater the costs in both time and resources.” It is our opinion that the Single Pool Technique is sufficient for environmental monitoring in most areas, with the others needed only in specific, critical areas.

Selection of tissue

i) Spatial and trend monitorin

For mollusca, usually the whole soft tissue is analyzed; in large crustaceans, the digestive gland (hepatopancreas) is often used because it concentrates both metallic and organic contaminants. Uthe and Chou (1987) noted the importance of removing this gland immediately (within 1 minute) after death to prevent autolysis and cadmium loss from the tissue. It is essential that investigators determine the effect of their post-mortem procedures on tissue contaminant concentrations. Procedures may have to be modified to prevent such changes (e.g., freeze drying tissue can only be used if the laboratory has shown that freeze drying does not cause loss of volatile contaminants, such as organochlorine pesticides).

For fish, muscle is usually the tissue of choice (e.g., ICES 1989), probably because of its public health concern. However, the laboratory must use storage and handling procedures that do not alter contaminant concentrations (e.g., the problem of “drip”, i.e. the loss of fluid from thawing tissue, which can be as great as 40% of the tissue mass, from frozen fish muscle tissue [Uthe and Chou, 1988]). Sampling muscle tissue must be carefully defined (e.g., take: 1. The entire musculature comprising the red and white muscle from both sides of the fish; 2. Only the white muscle; or, 3. A portion of the white muscle from a defined site within the musculature). Liver and kidney tissues have also been used (ICES 1991) because most toxic trace metals accumulate in them. Fatty tissues accumulate the organochlorines and hydrocarbons of concern.

ii) Normalization procedures

Tissue contaminant data can be reported on a dry weight basis (i.e. µg/g dry wt.) or a wet (un-dried) weight base (e.g. µg/g wet wt., generally used for public health data). Because drying is a common part of many analytical protocols (see RM. No. 7 “Sampling of Selected Marine Organisms and Sample Preparation for Trace Metal Analysis” and RM. No. 12 “Sampling of Selected Marine Organisms and Sample Preparation for the Analysis of Chlorinated Hydrocarbons”), the reader should record wet weight:dry weight ratios.

For lipophilic organic contaminants (e.g., organochlorine pesticides) concentrations are often expressed in µg/g fat wt. (where the fat content is measured or estimated, e.g., as Hexane-Extractable Organic Matter). This results in some normalization for seasonal or spatial differences in the fat content of the organisms. Both dry and fat weight measurements also must be subject to quality control.

In a recent review of marine pollution monitoring work, conducted through the distribution of a questionnaire to a number of IOC and UNEP laboratories, four main aims were identified for the programmes involving the collection and analysis of marine organisms for the three main groups of contaminants (metals, organochlorine compounds and petroleum hydrocarbons), these are:

1) The measurement of contaminant levels in edible marine organisms in relation to public health
2) The identification of heavily contaminated areas of the sea (“hot spots”) where levels of contaminants are at least an order of magnitude higher than levels in ‘clean’ or uncontaminated areas
3) The establishment of present levels of contaminants in marine organisms (i.e. a ‘baseline’)
4) The assessment of changes in concentrations of contaminants in organisms over a period of time (trends)

Each of these programmes places a different demand on the laboratories regarding the method and care to be taken in the collection, storage and analysis of samples. These aspects are considered below in detail in the context of the main aims of each programme.

It is very important that the investigator should prepare a clear and unambiguous statement on the aims and objectives of the work before embarking on any monitoring programme. Only by doing this will it be clear what type of information will be required, and consequently what criteria should be laid down for the collection, storage and analysis of samples. Time spent on defining aims and objectives and the planning of the various parts of the field and laboratory work will always be rewarded by a more efficient and effective programme.

Good planning can avoid unnecessary sampling and analyses i.e. it is sensible to initially aim for a programme, which satisfies essential rather than desirable objectives. It is easy to expand such a programme if the necessary resources are available. Also, if plans are made in advance as to how the data to be collected is to be used, this will assist in the design of field and laboratory work. Finally, there is a need to regularly review the programme of work to assess how well the aims are being met. Such reviews might lead to a reduction of sampling and analyses, and the time gained can be usefully employed elsewhere. Equally it might identify the need to put in more effort. Early warning of such extra work will allow time to plan the necessary manpower and other resources.

I.2.2 Sampling Strategy

If the sampling strategy is not designed with great care, the data from the measurements may not meet the needs of the programme. On the basis of previous studies considerable effort can be devoted to long-term monitoring, so it is important to ensure that this is done in the most efficient and effective way.

Good planning needs considerable time and thought; never be persuaded to rush this aspect of the work and ensure that all disciplines involved are consulted before plans are finalized.

In designing a meaningful sampling programme involving marine organisms there are a number of questions, which have to be considered.

a) Where should the samples be collected?
b) Which organism(s) should be selected for study?
c) When should the sampling be done and what is the appropriate frequency of sample collection?
d) How many individual organisms should be collected on each sampling occasion and which size(s) should be included in each sample?
e) Which tissue(s) of the organism(s) should be taken for analysis?

Each of these points will be addressed below for each of the main aims referred to above i.e. public health, hot spots, baseline and trend studies.

I.2.3 Public Health

a) Since the aim of the programme is to assess the level of contaminants in edible species than samples should ideally be taken at the point of sale to the general public i.e. at the fish market. Alternatively the scientist(s) can collect the fish/shellfish from the main fishing areas.

b) The selection of organisms will be dictated by the eating patterns of the population. These can be identified by a survey of the species sold at the market, by obtaining information from colleagues in government departments who deal with such matters or in the absence of such information, by distributing a questionnaire to a representative section of the general public.

c) Unless there is a seasonal fishing pattern for some species, then samples may be taken at any time of the year. Ideally all species should be sampled at the same time so that a synoptic picture can be obtained for the contaminant levels. A typical monitoring programme might be a survey every 5 years. However, available resources may allow more frequent surveys to be conducted; assuming that the results indicate that this is required (i.e. if the concentrations of contaminants in foodstuffs are found to exceed permissible limits). This increased sampling should be confined to species in which permissible limits of contaminants are exceeded.

d) The size(s) of organisms to be sampled should be based on information collected at the fish market. If small and large sizes are sold to the general public, this should be reflected in the sample. The number of individual organisms in each sample may partly be influenced by the importance of the species as a foodstuff, by the availability of scientific manpower and by the need to sample sufficient of each species and of each size category to cover the range of values encountered in a typical population or catch. Generally, a sample of 5-10 individuals could be collected for each size range of fish and large shellfish (crabs, lobsters) and ca 50 individuals for smaller shellfish (e.g. mussels, shrimps).

e) Only edible tissue need to be analyzed for contaminants - usually this means muscle tissue for fish and large crustaceans and whole soft tissue (less viscera) for other shellfish.

Every opportunity should be taken to collect data on the size (or length) and age of the species. This may assist decision making at a later date if the programme is to focused on a particular area and/or species.

I.2.4 Hot Spots

a) Hot spots are usually found close to estuarine and coastal areas where anthropogenic wastes are discharged. The only offshore areas where hot spots are likely to occur are those, which are used for the dumping of wastes from ships (and sites where net deposition of fine material takes place).

b) Although one theoretically can select any type of organism to monitor hot spots, in practice the most useful organisms for this purpose are the invertebrates. These species, by virtue of their feeding behavior, can reflect levels of contamination in the soluble phase, in the suspended particulate phase and in the sediment phase (see Annex I for the list of characteristics for organisms to be used in monitoring studies). [It should be noted however, that levels of lipophilic organic compounds are most likely determined by water: lipid distribution equilibria. This factor may be more important than feeding habits for such compounds].

It should be stated that no single organism can be used to monitor levels in the above three phases. It may be necessary to use a seaweed for the soluble phase, a filter feeder (e.g. mussel) for the particulate phase and a detritus feeder for the sediment phase. The investigator should therefore consult biologists to determine which species are best for the type of waste(s) and phase(s) under examination.

If a single preferred organism has to be monitored, the common mussel (*Mytilus edulis* or the equivalent local species) would be recommended.

c) Seasonal variations in food supply, and the spawning cycle, are known to cause changes in total body weight as well as lipid concentration and composition and consequently contaminant levels in the tissues of invertebrates. In order to minimize these variations it is suggested that sampling be undertaken at the pre-spawning period. Provided that sampling covers the area under investigation, one survey is normally sufficient for the identification of hot spots.

d) Ideally the number of individuals collected should cover the size range of organisms encountered at the sampling site in order to establish the variations of contaminant levels with size. Although this approach needs not be taken at each of the sampling sites it should be carried out at least at one site in order to allow comparisons to be made with other sites where sampling is restricted to a small size range. Depending on analytical resources the organisms from each site can either be analyzed individually or bulked. In the latter case no information will be derived on the variation with size but in terms of the aim of the programme the results can be used to compare data from site to site with some level of confidence (this assumes that a number of replicate analyses are performed on the bulked sample to allow differences to be detected above and beyond those produced by sample variation).

e) Whole soft tissue (less viscere) should be taken for analysis.

Note : For the analysis of lipophilic contaminants it is important to measure the lipid content of each sample (e.g. n-hexane extract) in order to ensure that comparisons of data on a lipid basis can be made for different regions and times of sampling (i.e. in relation to the seasonal changes of lipid concentrations in organisms).

I.2.5 Baseline and Trends

a) Sampling sites should include estuarine, coastal and offshore areas to ensure that both clean and contaminated areas are covered in this programme. Sampling at sea should be done by trained personnel operating from research or chartered vessels, rather than by fishermen, to ensure that contamination of the samples during and after collection is kept to an acceptable level.

b) Since the aim of a baseline study is to establish the current state of contamination of marine organisms generally, there are no restrictions on the species that can be included in such a study; other than that imposed by the resources available to the investigator. If the laboratory wishes to conduct trend monitoring or compare levels of contaminants at different sites then it is necessary to include those organisms, which will provide the data with which to achieve such aims, e.g. invertebrates.

c) Collections should be made over a short interval of time (i.e. weeks rather than months) to ensure as synoptic a picture as possible for the baseline survey and to enable comparisons of concentrations of contaminants at different sites. This also ensures that organisms are in the same physiological state (i.e. lipid metabolism, spawning, etc.).

It is appropriate to repeat a baseline study every 5 years unless major changes in discharges occur, or are anticipated, in the intervening period, which are considered to influence levels of contaminants in organisms over the entire study area. In most cases the effects of increasing or decreasing inputs of contaminants are usually confined to the area in the immediate vicinity of the discharge. More frequent monitoring at these locations would fall under the category of trend studies.

For trend studies the minimum frequency of monitoring for fish and shellfish would consist of an annual sampling whereas the maximum sampling for invertebrates might be as much as 4-12 times per year. Each laboratory will clearly establish the frequency of sampling, which meets the aims of its programme. This frequency will reflect both the changes it wants to measure over a particular period of time, and the resources it has at its disposal.

d) Baseline studies should attempt to cover all size ranges of the species under examination in order to produce a comprehensive picture for the state of the environment. A sample of 5-10 individuals for each size range would be an appropriate number for each fish and major shellfish species. A sample of 25-50 individuals is usually required for smaller shellfish.

Having established the relationship of contaminant levels with size of organisms it is usual to select a particular size or size range for trend studies to reduce variability. The numbers of individuals required for each sample will be determined by the differences in contaminant levels one wishes to be able to differentiate, i.e. the smaller the differences the greater the number of individuals required for each sample. In a recent study of trace metal variability in two populations of *Mytilus californianus*, through the random sampling of organisms at two sites, the investigators (see below) found coefficients of variation of 18-40%. They concluded that a sample of 20-100 individuals from each site was required to detect a concentration difference of 20% between sites. Differences of 40% could be detected by analyzing ca 1/3 as many samples (Gordon, M; Knauer, G.A. and Martin, J.H., 1980. "*Mytilus californianus* as a bio-indicator of trace metal pollution: variability and statistical considerations." Marine Pollution Bulletin 11, 195-198).

e) For fish, muscle is the most useful tissue for all purposes. However, liver and kidney tissues have been used for both baseline and trend studies. Both hepatopancreas and muscle tissue have been used in studies of large crustaceans. In general, whole soft tissue is taken for smaller shellfish.

I.2.6 Storage and Pre-Treatment of Samples

Guidelines and recommended procedures dealing with the storage and pre-treatment of samples following their collection have been produced by UNEP in its reference methods series (Nos. 7 and 12, which deal with metals and organohalogens respectively).

The guidelines cover the following :

- The types of storage containers to be used to avoid contamination of samples in transit and the recommended temperature for storage prior to dissection and analysis
- The precautions to be taken during the removal of soft tissue from the organism and during the preparation of the sub sample for analysis to avoid contamination from dissection tools, reagents, laboratory environment and the person carrying out these procedures

Analysts should ensure that adequate time and effort are allowed for this work since improper storage of samples and poor sample handling prior to analysis will lead to the production of unacceptable data, irrespective of how well the subsequent analytical measurements are carried out.

Good planning is essential if this work is to be done efficiently and effectively. The following guidance is given in this respect :

Sampling
(see Chapter III.1)

- Staff involved in sampling work should be given clear written instructions concerning the methods of collection, particularly the precautions that have to be taken to avoid contamination of samples during the transfer from the site to the storage container
- Experienced staff should accompany new staff on their first site visit and occasionally on later visits to monitor their work
- A check-list of sampling and storage equipment should be compiled for each type of sampling programme and this should be used before each site visit
- Adequate time should be allocated for each site visit to ensure that unexpected delays do not cause field staff to give less attention to sampling and storage requirements
- Log books should be provided to record essential details of samples and of any site characteristics that are considered necessary. These logs should be checked on completion of the site visit
- On arrival at the laboratory, samples should be catalogued and securely stored in order to avoid loss of samples

Dissection of samples

- Ideally dissection of samples should be done on fresh material (cooled but not deep frozen). If these samples of tissue cannot be analyzed within one or two days they should be deep frozen until they can be analyzed

  However, if the above procedure cannot be followed the samples should be deep frozen following collection and only removed for thawing and dissection when the chemical analyses of soft tissue can be done. Repeated freezing and thawing of samples can lead to loss of body fluids and water content. This cannot only effect the form and concentration of contaminants in soft tissue but can make the determination of wet to dry weight conversion very inaccurate.

The dissection of organisms by inexperienced staff, under poorly controlled laboratory conditions, can result in the production of unrepresentative and contaminated samples of soft tissue for analysis. Staff responsible for dissection must be given proper training in dissection and sub-sampling. They must be issued with the appropriate dissecting tools and must work in an area of the laboratory specially kept for this work. It is good practice to assign two people to this work - one to dissect and to transfer tissue to the sub-sample container, the other to catalogue the sample and the weight of tissue taken for each sub-sample.

Note : Adequate time must be allowed for this work to avoid staff making errors

I.2.7 Analytical Quality Assurance

The earlier section of this document dealt with the collection of representative and meaningful samples of marine organisms and the pre-treatment and storage procedures, which ensure that losses and additions of contaminants prior to chemical analysis are kept to a minimum. This section deals with the aspect of quality assurance concerned with the measurement of contaminants in biological tissue, namely, analytical quality control and assessment.

It is assumed that the analyst has chosen a suitable analytical procedure to provide the appropriate performance characteristics of precision, accuracy and limit of detection (see Annex II).

Before an analyst employs an analytical procedure on a regular basis for the measurement of contaminants in a sample of biological tissue it is essential to check its performance characteristics to ensure that the method will produce data of the required accuracy and precision. This applies to any methods developed by the laboratory or other published methods used by the laboratory.

This check on accuracy and precision is done by analyzing Certified Reference Materials (CRMs) of known matrix and composition. (A list of relevant CRMs is given in Annex III). In the absence of such materials the analyst should check the accuracy and precision by the method of “standard addition” i.e. the analysis of samples of the tissue to which known quantities of the contaminants of interest have been added (i.e. “spikes”). Total recovery of “spikes” does not however guarantee that the method will produce accurate data for samples since the chemical form of the analyte in the sample and in the spike may not be the same or the concentration/ extraction procedure may be inadequate to release the contaminant from its site in the sample. It is advisable therefore, whenever possible, to employ CRMs for the evaluation of analytical methods since this is the only true test for accuracy of a method for the sample and analyte(s) under examination. Analysts should choose the CRM which is not only of a similar matrix to the sample but which also has a similar concentration of the analyte(s) under examination. In this respect it is often necessary to analyze two CRMs to cover the upper and lower ranges of concentrations likely to be encountered. This ensures that the method will provide data of the required accuracy and precision over the expected range of concentrations.

It must be emphasized however, that the use of a validated method, suitable instrumentation, experienced personnel, etc. does not guarantee the regular production of reliable data. All analytical work should be done under a system of quality control (the steps taken to minimize errors) and a system of quality assessment (the procedure adopted to verify that the errors are within the acceptable limits).

I.2.7.1 Quality Control

The elements of good quality control are as follows:

- Consistent use of reliable qualified personnel, and well-maintained instrumentation
- Appropriate calibrations and standards
- Close supervision of all operations by senior personnel
- In addition to using CRMs for evaluating methodology the analyst should employ reference materials on a regular basis throughout the monitoring programme to check that analytical performance is maintained
- Whenever possible analysts, encouraged by senior management, should participate in interlaboratory checks of analytical performance - this is the only independent method available to check on a laboratory's analytical capability (see Annex IV)

I.2.7.2 Quality Assessment

- The establishment of a system of control charts is the main element of this check system
- Control charts are plots of the results of analyses of the same sample over a period of time (see Chapter V.1). They allow analysts to check whether their results are falling within the acceptable limits of accuracy and precision
- Data for control charts may consist of the analyses of CRMs and other reference materials. The latter may include homogeneous materials based on real samples, which have been prepared by the analyst (known as internal reference materials)
- Regular analyses for control chart purposes are essential to avoid waste of valuable time and effort spent on analytical work. Ideally, daily analyses of check samples should be performed so that only one batch of analytical data is lost if the analysis gets out of control

(See also data assessment under section I.2.8 entitled "Documentation, assessment and reporting of data")

I.2.8 Documentation, Assessment and Reporting of Data

The adoption of the following guidelines by a laboratory should provide adequate documentation to support any data report or decisions on the results of its monitoring programmes, and to allow it to trace samples from the collection stage to the completion of its analyses by providing a record of the appropriate data in logbooks or in computer files.

I.2.8.1 Documentation

1. Descriptions of the sampling strategy, methods of collection, procedures of storage, pre-treatment and analytical procedures
2. Sample documentation [description of organisms, numbers of individuals collected for each sample, weights of tissue taken for analysis (individual tissue or homogenate)]
3. Evidence of successful evaluation and testing of analytical procedures, including details of accuracy, precision and limit of detection
4. The approach used for quality control and quality assessment and evidence that these procedures have been used and have provided acceptable data
5. Calibration details, a description of working standards used on each occasion and calculations of results
6. A secure system for the long-term storage of data either in logbooks or in computer files is essential. It is therefore advisable to have a duplicate set of records in case one is lost, mislaid or accidentally destroyed

   Advice should be sought on the correct method of storing computer hard disks, CD, DVD discs to ensure the long-term stability of the data files.

I.2.8.2 Preliminary Assessment, Tabulation and Storage of Data

It has been shown that even the most experienced personnel can make simple arithmetic errors in calculating data. Thus a check should be made for such errors before compiling tables of results. Once this check has been carried out it is appropriate to carry out a preliminary assessment of the quality of the data, prior to its evaluation and publication, to ensure that no erroneous results are included in the data set. This assessment can include a comparison of the results with existing data (i.e. data previously collected by the laboratory or data published in the literature for the study area). Before consigning data to long-term storage a final check should be made to ensure that no transcriptional errors have been made in transferring the data (i.e. the re-typing of data sets by typists or data processors can sometimes lead to such errors).

I.2.8.3 Publication of Data

Data held in logbooks or in computer files which has not been published is effectively lost to the scientific community. Every attempt should be made to evaluate and publish these results, even in the simple form of a data report. In addition to the results, the contents of a data report would normally include a statement on the aims of the work, a description of the methods used to collect and analyze the samples together with a statement on findings and conclusions.

In general it is inadvisable to supply a third party with a copy of your raw data, unless:

a) It is accompanied by information on its accuracy, precision and limits of determination and details of the sampling, or
b) It is accompanied by a report from the analyst or the principal investigator in which the results have been evaluated.





II PHYSICAL OCEANOGRAPHY

II PHYSICAL OCEANOGRAPHY

II.1 MARINE METEOROLOGY

This section provides guidelines for making meteorological measurements at sea, which are useful to assess the fluxes of pollutants in regional seas programmes. The fluxes of pollutants between sea and land are brought about by horizontal and vertical transport, where both ordered and turbulent flows are effective. Hence the monitoring of several meteorological parameters is important if the contributing mechanisms are to be evaluated.

The meteorological observations are primarily designed to obtain quantitative information about various meteorological aspects of the Region. Typically, such measurements include ocean surface and air temperatures, atmospheric pressure, wind speed and direction and relative humidity.

The methods described in the present documents follow WMO established routines and use the instrumentation maintained by marine meteorological services of national weather offices as much as possible.

II.1.1 Air Temperature

Air temperature is normally measured at sea at the height of the bridge. A thermometer is the usual instrument for monitoring variations in air temperature. The most common type of thermometer is a liquid-in-glass tube attached to a graduated scale, Figure (II.1). The liquid is either mercury (which freezes at -39°C, or -38° F) or alcohol (which freezes at -117°C or -179° F). As the air warms, the liquid expands and rises in the glass tube; as the air cools, the liquid contracts and drops in the tube.

It is recommended to prepare a thermometer which is engraved directly upon the thermometer stem, the back being whitened to allow easy reading and enclosed in an outer glass tube.

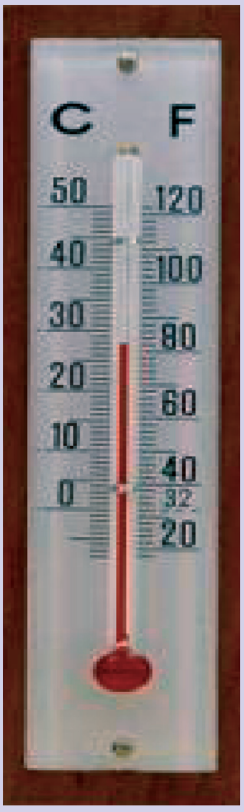


Figure (II.1) : A liquid-in-glass thermometer graduated in both the Celsius (°C) and Fahrenheit (°F) scales. In this case, the liquid is alcohol.

Reading the thermometer :

a. The thermometer should be read with care and recorded to the nearest tenth of a degree Celsius (°C)
b. When reading a thermometer, care should be taken to keep the eye at the same level as the end of the column, otherwise there will be an error due to parallax
c. The mercury column of a thermometer occasionally separates in one or more places. The thermometer should therefore be examined before each observation to see if the column is continuous. If there is break in the column, take the instrument down, swing it briskly at arm's length with the bulb end from you till the column is again continuous, and replace it. After this, give the thermometers another ten minutes to equilibrate before taking the reading.

II.1.2 Relative Humidity

Relative humidity is the most familiar way of describing the water vapor content of air. It compares the actual concentration of water vapor in the air with the concentration of water vapor in that same air at saturation and it is usually expressed as a percentage.

Today an instrument commonly used to measure atmospheric humidity is the psychrometer. It usually consists of two identical mercury-in-glass thermometers mounted side by side. The bulb of one thermometer is wrapped in a muslin wick. Readings are taken by first soaking the wick in distilled water, and then whirling both thermometers (or aerating them by a fan) until the wet-bulb thermometer reading steadies. The dry-bulb thermometer measures the ambient air temperature. Water vaporizes from the muslin wick into air streaming past the wet-bulb thermometer, and evaporation cooling lowers the wet-bulb temperature. The drier the air, the greater the evaporation, and the lower the reading will be on the wet-bulb thermometer compared with the dry-bulb reading. The temperature difference between the two thermometers, called the wet-bulb depression, is calibrated in terms of percent relative humidity on a psychorometric table, Table (II.1).

Two versions types of the psychrometer are in use: The aspirated psychrometer after Assmann, and the sling psychrometer. With the Assmann type psychrometer, the necessary ventilation is achieved by a mechanical spring driven aspirator. The spring assembly is sensitive to rough handling and can withstand 100 to 200 uses only if it is completely wound up very carefully. Though the Assmann psychrometer is a fine instrument, it is recommended for use only if sufficient spare parts (aspirator heads) are available.

The sling psychrometer is recommended for use in this study, Figure (II.2). The psychrometer can be suspended in a covered outside passage way and fastened at eye level.

Units for dry and wet bulb temperatures are °C, the thermometer scale is usually divided into 1/5 of °C and should be read to 1/10°C.

When converting from psychrometer-measurements to other measures of humidity, data should be presented to a similar number of significant figures in order to avoid misleading representation of their accuracy: 0.1 hPa for water vapor pressure, 0.1 g/kg for specific humidity, 0.1°C for dew point temperature.

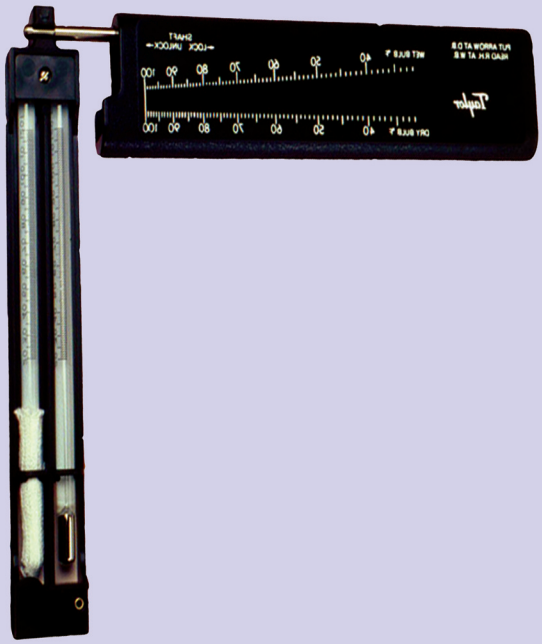


Figure (II.2) : Sling psychrometer consisting of dry (left) and wet bulb thermometer (right) in a common housing

Another instrument that is less accurate than a psychrometer, but measures atmospheric humidity more directly and conveniently, is the hair hygrometer. As the name implies, this device uses hair as the humidity sensor. The hair lengths slightly as the relative humidity increases and shrinks slightly as the relative humidity drops. Usually, a sheaf of blond human hairs is linked mechanically to a pointer on a dial that is calibrated to read in percent relative humidity. Hair typically changes length by about 2.5 percent over the full range of relative humidity from 0 to 100 percent. Use of hair hygrometers is not recommended.

Table (II.1) : Relative humidity (Percent)

Dry-bulb Temp (°C)	Wet-bulb depression (°C)														
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	7.5	10.0	12.5	15.0	17.5
- 10.0	85	69	54	39	24	10	-	-	-	-	-	-	-	-	-
- 7.5	87	73	60	48	35	22	10	-	-	-	-	-	-	-	-
- 5.0	88	77	66	54	43	32	21	11	0	-	-	-	-	-	-
- 2.5	90	80	70	60	50	41	31	22	12	3	-	-	-	-	-
0.0	91	82	73	65	56	47	39	31	23	15	-	-	-	-	-
2.5	92	84	76	68	61	53	46	38	31	24	-	-	-	-	-
5.0	93	86	78	71	65	58	51	45	38	32	1	-	-	-	-
7.5	93	87	80	74	68	62	56	50	44	38	11	-	-	-	-
10.0	94	88	82	76	71	65	60	54	49	44	19	-	-	-	-
12.5	94	89	84	78	73	68	63	58	53	48	25	4	-	-	-
15.0	95	90	85	80	75	70	66	61	57	52	31	12	-	-	-
17.5	95	90	86	81	77	72	68	64	60	55	36	18	2	-	-
20.0	95	91	87	82	78	74	70	66	62	58	40	24	8	-	-
22.5	96	92	87	83	80	76	71	68	64	61	44	28	14	1	-
25.0	96	92	88	84	81	77	73	70	66	63	47	32	19	7	-
27.5	96	92	89	85	82	78	74	71	68	65	50	36	23	12	1
30.0	96	93	89	86	82	79	76	73	70	67	52	39	27	16	6
32.5	97	93	90	86	83	80	77	74	71	68	54	42	30	20	11
35.0	97	93	90	87	84	81	78	75	72	69	56	44	33	23	14
37.5	97	94	91	87	85	82	79	76	73	70	58	46	36	26	18
40.0	97	94	91	88	85	82	79	77	74	72	59	48	38	29	21

II.1.3 Atmospheric Pressure

A barometer is the instrument used to monitor changes in air pressure. There are two basic types of barometer : mercury and aneroid.

The more accurate, though cumbersome, of the two is the mercury barometer. This instrument consists of a glass tube a little less than 1 m (39 in.) long, sealed at one end, open at the other end, and filled with mercury, a very dense liquid. The open end of the tube is inverted into a small open container of mercury, as shown in Figure (II.3). Mercury settles down the tube (and into the container) until the weight of the mercury column exactly balances the weight of the atmosphere acting on the surface of the mercury in the container.

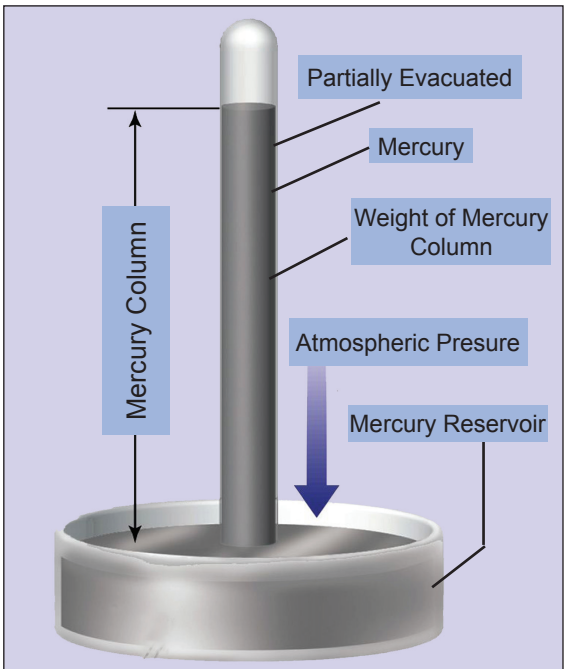


Figure (II.3) : Schematic drawing of a mercury barometer

The average atmospheric pressure at sea level will support the mercury column in the tube to a height of 760 mm. When air pressure changes, however, the height of the mercury column changes. Falling air pressure allows the mercury column to drop, and increasing air pressure forces the mercury column to rise. This, then, is the origin of the practice of expressing air pressure in units of length.

Precision aneroid barometers have now been adopted as the standard atmospheric pressure reading instrument for ships. They have proven to be of similar accuracy to the marine mercury barometer, yet are more compact, easier to read, handle and calibrate. Therefore, an aneroid barometer is recommended.

The aneroid (non-liquid) barometer, pictured in Figure (II.4) consists of a coil of tubing from which much of the air has been evacuated. A spring keeps the chamber from collapsing. As air pressure changes, the coil flexes, bending in when pressure rises and bowing out when pressure drops. A series of gears and levers transmits these movements to a pointer on a dial, which is calibrated to read in equivalent millimeters (or inches) of mercury, or to read directly in units of air pressure.



Figure (II.4) : An aneroid barometer, a portable instrument used to measure variations in air pressure

Note : *Units* : Physicists use the Pascal as the metric unit of pressure, while meteorologists designate atmospheric pressure in millibar units (1 mb = 100 Pa)

Correction

Good quality aneroid barometers are factory compensated, for expected working temperatures, either by leaving a calculated small amount of air in the vacuum chamber, or by use of a bimetallic lever. Therefore, such barometers do not require temperature corrections. No latitude correction is required, as the principle on which they are based is the balancing of atmospheric pressure by the elasticity of metal, so that the force of gravity does not affect the readings. The only corrections which should be applied to an aneroid reading are those for altitude and where necessary for index error.

It may be necessary, in some cases, to readjust the pointer to coincide with a known standard. To adjust the pointer, insert the key into the adjusting hole and turn very slowly in either direction to move the pointer to the desired reading. Tap the case lightly and readjust again if necessary. When adjustment is necessary, it is recommended that it be made against a known reading, from a meteorological station.

II.1.4 Wind Measurement

Most wind-monitoring instruments are designed to measure only the horizontal component of the wind since it is usually considerably stronger than the vertical component. For some specialized research purposes, very sensitive instruments are available that measure vertical wind speeds or a combination of vertical and horizontal wind components.

Beaufort scale

Wind speed can be estimated by observing the wind's effect on lake or ocean surfaces or on flexible objects such as trees. Such observations are the basis of the Beaufort scale, which is a graduated sequence of wind strength ranging from 0 for calm conditions to 12 for hurricane-strength winds, Table (II.2).

Wind speed

A cup anemometer, such as the one shown in Figure (II.5) is usually used to provide an accurate measurement of horizontal wind speed. The wind spins the cups (usually 3 or 4), thus generating a weak electric current, which is calibrated on a dial in meters per second, kilometers per hour, miles per hour, or knots. Several other types of anemometer are available, including the very sensitive hotwire anemometer. In this instrument, the wind blows past a heated wire, or wires, and the heat lost to the air is then calibrated in terms of wind speed. A cup anemometer is recommended to use in this study.



Figure (II.5) : Wind speed is measured by a cup anemometer. The faster the wind speed, the faster the cup spins

Note : One knot = 1 nautical mile (1.85 km) per hour; 1 knot = 0.51 meters per second; 1 mile per hour = 0.44 meters per second

Table (II.2) : Beaufort Scale of Wind Force

Beaufort number	General description	Land and sea observations for estimating wind speeds	Wind speed 10 m above ground (km/hr)
0	Calm	Smoke rises vertically. Sea like mirror	Less than 1
1	Light air	Smoke, but not wind vane, shows direction of wind. Slight ripples at sea	1-5
2	Light breez	Wind felt on face, leaves rustle, wind vanes move. Small, short wavelets	6-11
3	Gentle breeze	Leaves and small twigs moving constantly, small flags extended. Large wavelets, scattered whitecaps	12-19
4	Moderate breeze	Dust and loose paper raised, small branches moved. Small waves, frequent whitecaps	20-28
5	Fresh breeze	Small leafy trees swayed. Moderate waves	29-38
6	Strong breeze	Large branches in motion, whistling heard in utility wires. Large waves, some spray	39-49
7	Near gale	Whole trees in motion. White foam from breaking waves	50-61
8	Gale	Twigs break off trees. Moderately high waves of great length	62-74
9	Strong gale	Slight structural damage occurs. Crests of waves begin to roll over. Spray may impede visibility	75-78
10	Storm	Trees uprooted, considerable structural damage. Sea white with foam, heavy tumbling of sea	89-102
11	Violent storm	Very rare; widespread damage. Unusually high waves	103-118
12	Hurricane	Very rare; much foam and spray greatly reduce visibility	119 and over

Wind direction

Wind direction is always designated as the direction from which the wind blows. For example, a wind blowing from the east toward the west is described as an “east wind,” and a wind blowing from the northwest towards the southeast is a “northwest” wind. Measured clockwise from true north, an east wind is specified as 90 degrees, a south wind as 180 degrees, a west wind as 270 degrees, and a north wind as 360 degrees. The wind is recorded as 0 degrees only under calm conditions.

Wind direction should be measured by the anemometer when the vessel is stationary. If it is measured when the vessel is moving, corrections have to be made according to the relative speed of the vessel and its relative direction with respect to the wind.

II.1.5 Cloud Cover

Observation of cloud cover is carried out by non-instrumental methods. Therefore, it is dependent upon the personal judgement of the observer. This judgement is the product of training and experience at sea, together with practice in making the observations. To acquire the required experience and comparability of data, the following procedure is recommended:

In the past, the amount of cloud cover was estimated as the number of tenths of sky covered. At a conference of the International Meteorological Organization (Washington, 1947) it was recommended that the amount of cloud cover be reported in eighths (oktas) instead of tenths. This change of procedure was brought into force with the introduction of the revised International Code (Washington) in 1949.

In making the observations it is necessary to stand in a position affording an uninterrupted view of the whole sky. It is recommended that one of the members of each sampling team be trained by a meteorologist. It is convenient to imagine the sky divided into quadrants by two arcs drawn at right angles through the zenith. Each quadrant represents two-eighths of the total sky. If we choose the most appropriate of the figures:

0 = clear or almost clear of cloud
1 = about half covered
2 = completely or almost completely covered with cloud

for each separate quadrant, then the total amount of cloud for the whole sky is obtained simply by adding the amounts in the separate quadrants.

II.1.6 Precipitation

Precipitation is a variable not routinely measured on board ships yet because of its importance for the determination of wet deposition, efforts have to be made to obtain reasonable marine values. It could be collected and measured using the rain gauge.

The standard National Weather Service rain gauge, Figure (II.6) is equipped with a cone-shaped funnel at the top that directs rainwater into a long, narrow cylinder, which is seated inside a larger, outer cylinder. The narrow cylinder magnifies the scale of the rainwater, so the observer can resolve rainfall in increments of 0.2 mm.

Total rainfall of less than 0.1 mm is recorded as a “trace”. Rainwater that accumulates in the inner cylinder is measured by a stick, which is graduated in mm. Rainfall is measured at some fixed time once every 24 hours, and the gauge is then emptied.

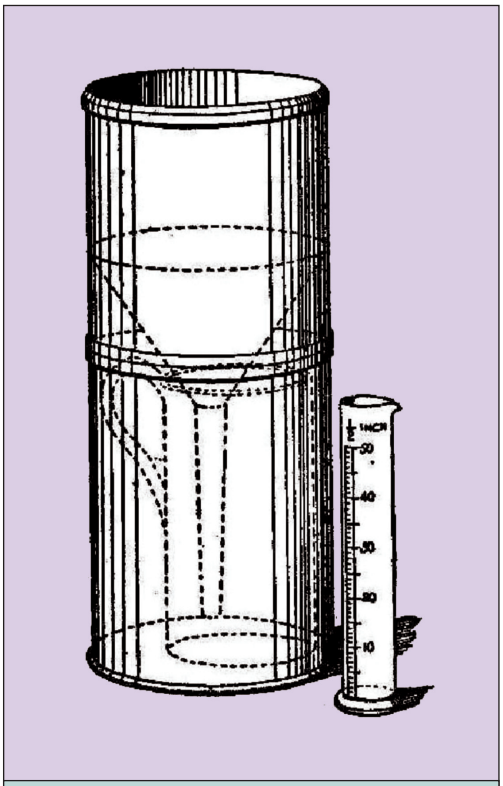


Figure (II.6) : A standard national weather service rain gauge

Some rain gauges are designed to provide a cumulative record of rainfall with time. A weighing-bucket rain gauge consists of a continuously recording scale that calibrates the weight of accumulating rainwater as water depth. Cumulative rainfall is recorded by either a device that punches a paper tape or a pen that marks a chart on a clock-driven drum.

A tipping-bucket rain gauge is somewhat more accurate than a weighing-bucket rain gauge. The instrument features two small, free-swinging containers, each of which can collect the equivalent of 0.2 mm of rainfall. Alternating with one another, each container fills with water, tips and spills its contents, and thereby trips an electric switch that either marks a clock-driven chart or sends an electric pulse for recording by a computer or magnetic tape.

The sitting of precipitation gauges is particularly important in order to ensure accurate and representative readings. The site should be as high as possible above the main superstructure of the ship and be sheltered from strong winds, as shown in Figure (II.7).

Note : *Units* : Precipitation is given in height of rain (mm) per observing period

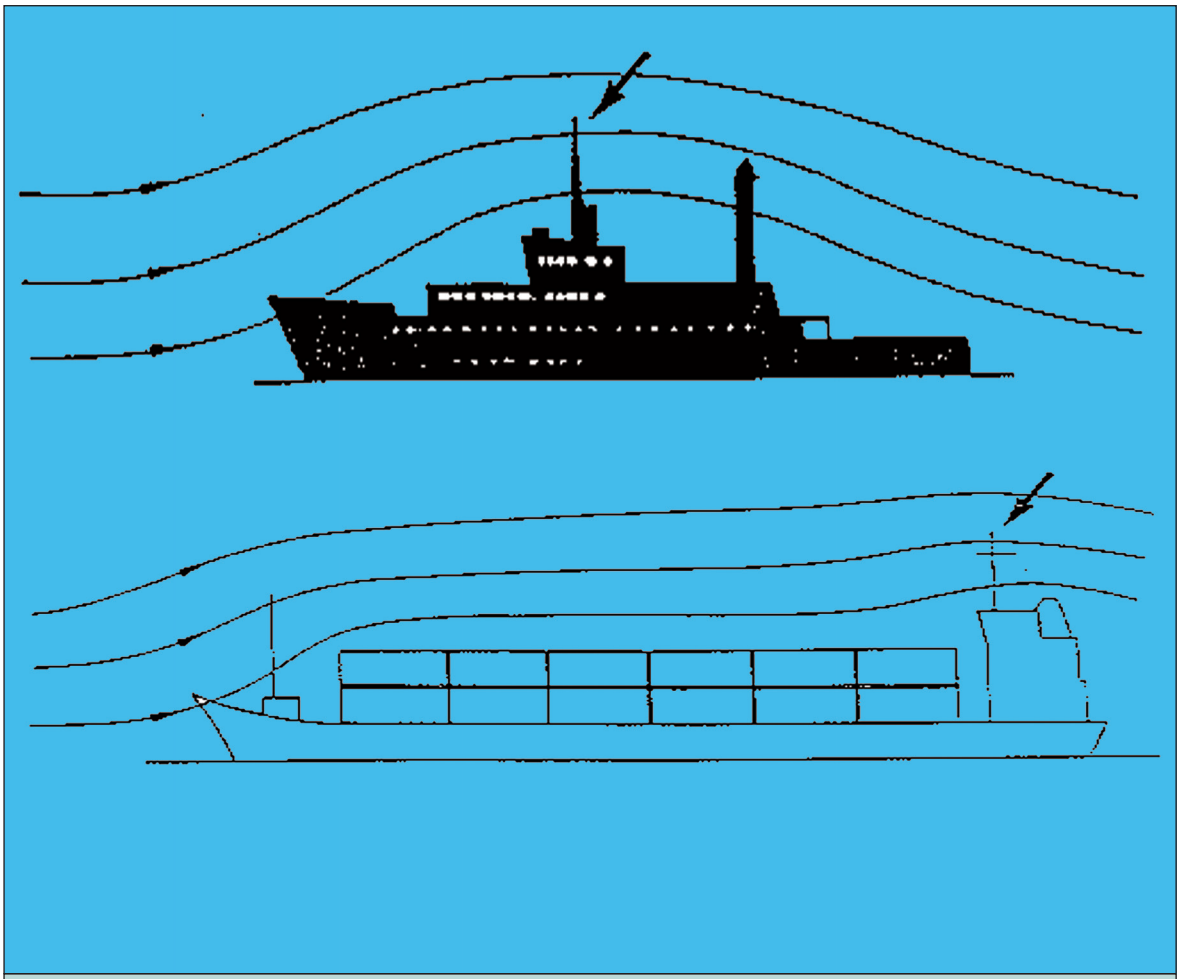


Figure (II.7) : Sketch showing schematically the air flow around typical ship superstructures. The best position for a rain gauge is a site with level air flow well above the main superstructure as indicated by thick arrows

II.1.7 Evaporation

There is a continual exchange of water molecules at the sea surface. They move backwards and forwards between the water and the atmosphere. Evaporation takes place when more molecules leave the water than return to it. This increases net water vapor in the atmosphere and evaporation is the net loss of water by this molecular motion. On the other hand, if more molecules are moving from air to water, then condensation occurs.

You measure evaporation with a ruler. The amount of water that has disappeared from a standard, vandal-proof, container is the evaporation; provided you have made allowance for any rain that fell in the interim. The most common standard container is a cylindrical pan of unpainted galvanized iron 122 cm in diameter and 25.4 cm deep, Figure (II.8). This is known as a class a evaporation pan and usual practice is to fill it to a depth of 20 cm every day, after noting the daily evaporation rate. Many people have investigated whether this measured evaporation rate represents open water conditions. The consensus is that it does not, and that the pan measurement must be multiplied by a coefficient, known as the pan factor, to truly represent open water conditions. The value of this coefficient varies with location and time of year, but is generally about 0.7 in temperate climates. It has been estimated that the oceans lose the equivalent of a 1.25 m layer of water per year through evaporation.



Figure (II.8) : An Evaporation pan

The heat energy lost by evaporation is the largest of the three principal oceanic sources of heat loss, being about 97 W/m^2 (watts per square metre) on average. The other two sources are long-wave radiation - mainly in the infra-red (and hence used in remote estimation of sea surface temperature) - which averages 63 W/m^2 and sensible heat loss (a term used to cover conduction and convection) which averages 10 W/m^2.

Evaporative heat transfer pumps heat energy into the lowest portion of the atmospheric boundary layer. The boundary layer is turbulent and so the energy flux must depend on turbulent fluctuations in specific humidity, q', and in vertical velocity w'. If these two quantities are correlated then the evaporative heat loss can he expressed as :

$$E = \rho_a q'w'$$

where q' w' is the mean value of the cross product of the fluctuations, and ρ_a is the air density, Figure (II.9). This way of considering evaporative energy loss is the turbulent boundary layer approach. Though theoretically attractive, the complexity and cost of instrumentation and analysis prevents this approach being routinely used. Numerous empirical formulas have been derived which express evaporation as a function of atmospheric elements and which parameterise the turbulent

transport in some way. Many of these are based on Dalton's law of partial pressure and the form most commonly used in oceanography gives the evaporation loss as:

$$E_{\alpha}\ (mm/day) = 0.143\ (e_w - e_{\alpha})\ W / 100$$

where e_{α} and e_w are the vapor pressure (in Pascal) in the air at ship height (10 m) and at the water surface respectively; and W is the wind speed in m/s. The last equation which represent the mass transfer approach, applies only to moderate and strong wind conditions (W> 5 m/s), for if the wind drops to zero, evaporation continues to take place, albeit more slowly. The extension to this situation will be dealt with subsequently.

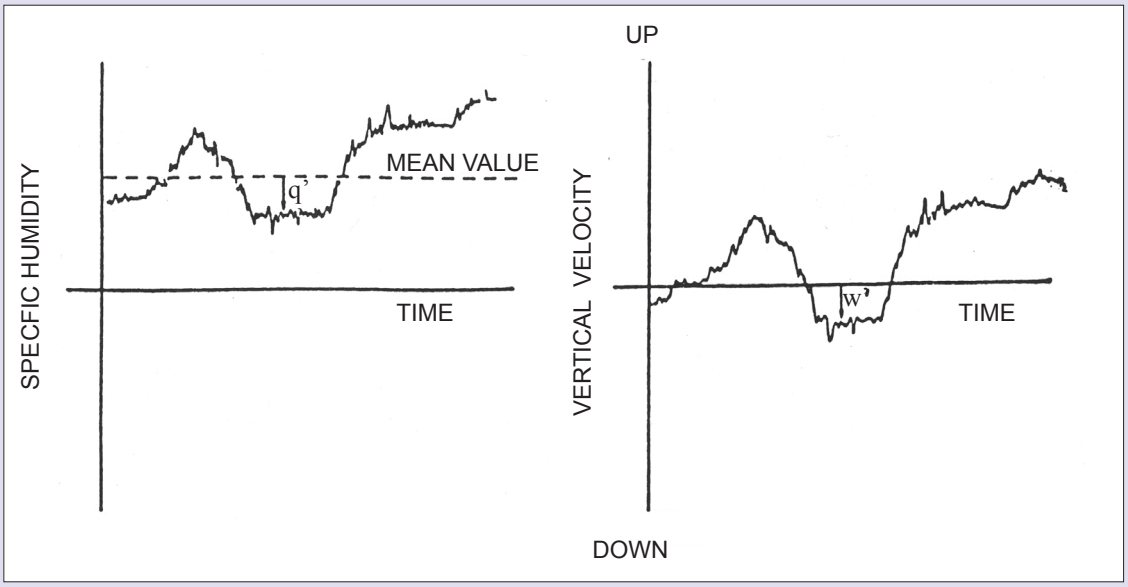


Figure (II.9) : The turbulent boundary layer approach treats evaporation as a consequence of the fact that humidity fluctuation (q') about their mean value are correlated with vertical wind velocity fluctuations (w') so that wet patches move up whilst dry patches move down

If water is warmer than the air above it, the water will heat the air and cause turbulence. Evaporation will also take place and the greatest evaporation occurs when cold air flows over warm water. If the air is very much colder than the water (a situation that can only occur near the coast) then the air becomes saturated and steam fog forms.

When the sea surface is colder than the air above it, both turbulence and evaporation are suppressed. Warm moist air passing over a cold sea surface induces condensation, which brings heat energy into the water surface. This situation can also lead to a type of fog, known as advection fog, when contact with the sea lowers the air temperature to dew point.

Maximum evaporation outside of the tropics occurs in winter. This seems contrary to experience that evaporation is greatest from heated water. This is, however, not quite correct. Evaporation is greatest when a water surface is warmer than the air above it. In winter, the sea surface outside of the tropics is much warmer than the air and hence evaporation is then a maximum.

II.1.8 Energy Budgets

A third way of studying evaporation is to consider the energy budget required to maintain it. As 2.46 X 10^6 joules of heat energy are lost with each kilogram of evaporated water, one should be able to calculate evaporation from a knowledge of the heat input and its subsequent transformations.

Energy is imparted to the sea by sunlight, with most of the insolation energy being in the visible spectrum. Let us call this Q_S. A certain proportion of this will be reflected - as if the sea surface were a grimy distorted mirror. The total reflected energy we shall represent by AQ_s where A, the proportion reflected, is called the albedo. Finally, lesser amounts of heat energy can reach a particular location through advection, which is transport by streams, currents, rain, snow, and so on. The income of energy to the water, Q_{in} is then

$$Q_{in} = Q_s (1 - A) + Q_A$$ which is illustrated in Figure (II.10).

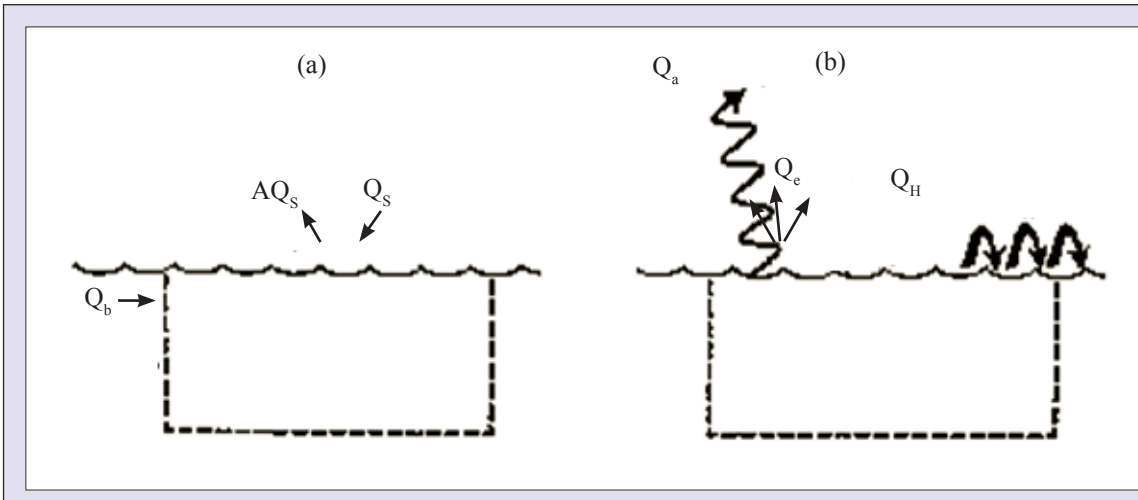


Figure (II.10) : The energy budget approach to evaporation treats a balance between incoming energy sources Q_s (solar insolation) and Q_a (advected heat) and outgoing energy losses. AQ_s due to reflection. Q_e (evaporation), Q_h (sensible heat loss) and Q_b (radiation)

Energy is removed from the sea by conduction, convection, radiation and evaporation. For a known water temperature the radiative loss, Q_b is proportional to the fourth power of the absolute temperature. The expenditure of energy is then:

$$Q_{ex} = Q_b + Q_e + Q_h$$

produced an analogue record on a smoked glass slide. This was done by a stylus scratching a trace into precalibrated slides. The BTG was lowered and raised from either a stationary or moving vessel. This general idea was extended by the XBT which consists of a specially designed sensor unit, whose rate of fall is known. A thin wire is attached to the unit, which relays temperature information to a chart recorder, whose speed of paper advance is geared such that it records depth linearly. After all the thin wire has played out of its spool it unplugs itself and is discarded.

There has been increasing concern in recent years over the accuracy of near-surface current meters when they are moored to record subsurface currents, Figure (II.11).

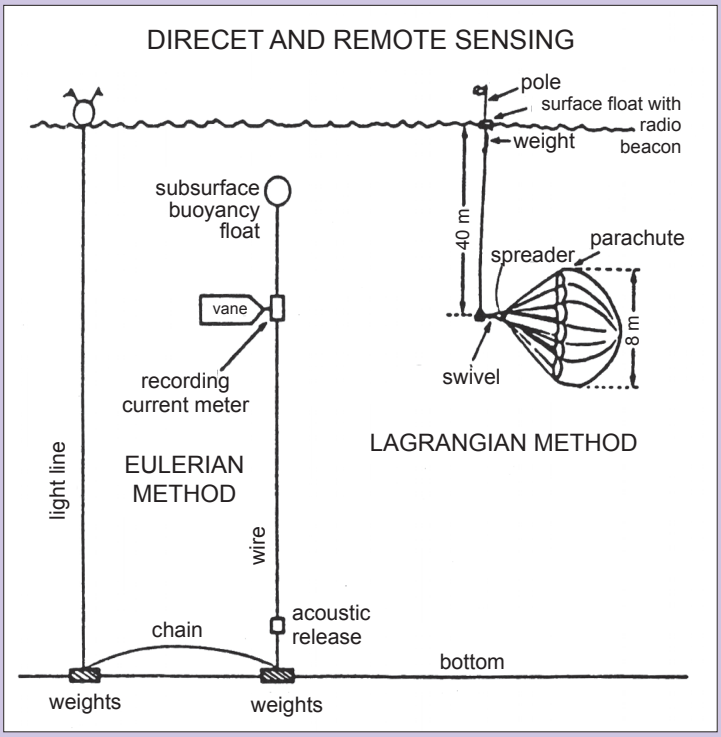


Figure (II.11) : Subsurface currents may be measured by Eulerian methods which moor a recording current meter at the appropriate depth (40 m in this case), or Lagrangian methods such as the parachute dragged buoy. The chain in the picture is floating to emphasize that grappling hooks can be used to locate it if the surface marker is lost

Their readings may be contaminated by surface waves and, even when this is not the case, their records need a great deal of smoothing before being useful. They are popular because a recording current meter gives a computer compatible output. However, these devices record only what happened at a single point (known as a Eulerian representation), not where the water mass went. If the vectors from such a meter are added up, then the progressive vector may seem to show that the water ended up several miles inland, Figure (II.12). Meter measure ment, therefore, should be supplemented by drogue tracking - or even discarded in favour of drogue tracking, which produces what is known as a Lagrangian representation of the currents. Figure (II.12) was an example of results obtained by Lagrangian methods.

A parachute some 8 m in diameter is a convenient form of drogue in deep water, whereas a bucket with a hole in the bottom can be used in shallower water. Usually these drogues are followed for many hours or several days - at the end of which they may either be many kilometers away or have returned to the starting point. When calculating the currents, the aerodynamic effects on the mariner pole need to be subtracted.

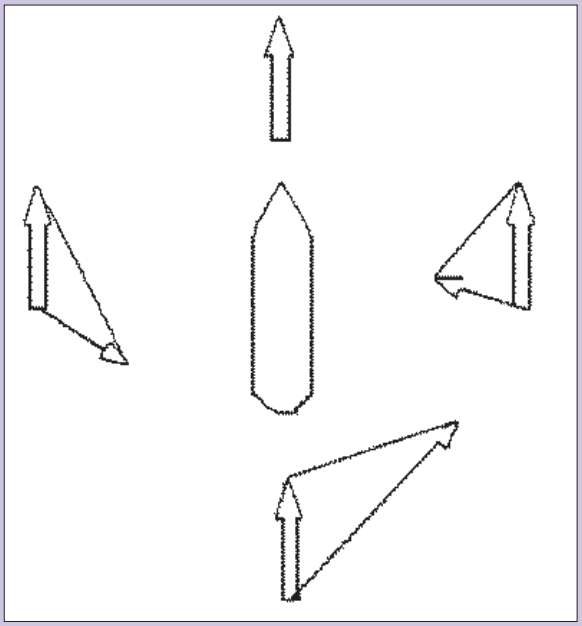


Figure (II.12) : Vector addition of true wind (full thick arrow) and ship velocity (open arrow) to yield the apparent wind (thin arrow)

II.1.9 Solar Radiation Measurement

The pyranometer is the standard instrument for measuring the intensity of solar radiation striking a horizontal surface. The instrument, as described by Moran and Morgan (1991), consists of a sensor enclosed in a transparent hemisphere Figure (II.13) that transmits total (direct plus diffuse) short-wave (less than 3.5

μm wavelength) insolation. The sensor is a disk consisting of alternating black and white wedge-shaped segments that form a starlike pattern. The black wedges are highly absorptive, and the white wedges are highly reflective of solar radiation. Differences in absorptivity and albedo mean that the temperatures of the black and white portions of the sensor respond differently to the same intensity of solar radiation. The temperature contrast between the black and white segments is calibrated in terms of radiation flux.

A pyranometer may be linked electronically to a pen recorder that traces a continuous record of insolation or the instrument's output signal may be recorded on a magnetic tape cassette for processing and storage.

Special care must be taken in mounting and maintaining a pyranometer. The instrument should be situated where it will not be affected by shadows by any highly reflective surfaces nearby, or by other sources of radiation. The glass bulb must also be kept clean and dry.



Figure (II.13) : The pyranometer is the standard instrument used to measure solar radiation

II.1.10 Automatic Weather Station

Recently, the automatic weather stations are widely used for measuring the above mentioned meteorological parameters. It is a rugged self-contained station suited for use in remote places without electricity supply. Data can be recorded on site or be transmitted in real-time, Figure (II.14).

The standard four meters station is fitted with sensors for :

- wind direction
- air temperature
- relative humidity
- air pressure
- sunshine duration
- net radiation
- rainfall



Figure (II.14) : Automatic weather station

II.1.11 Procurement and Calibration of Instruments; Log Books

In many industrialized countries several independent suppliers of marine meteorological equipment are available. It is not possible to give an unbiased selection of suppliers. If in doubt one should seek the help of the national marine meteorological service for the procurement of reliable instrumentation. Close cooperation with the official weather service is strongly advised with regard to calibration. Regularly repeated calibration is necessary with most types of meteorological instrumentation. Especially where ships from several nations are participating in a field study it is important to secure consistent calibrations. This can be achieved through calibration against a national secondary standard that itself is referenced to a common standard e.g., of the World Meteorological Organization.

The type of instrumentation, the instrument numbers, corrections applied or not, should be recorded in the instrumentation log book. It is good practice to also note any circumstances, which might be relevant to the quality of data obtained, e.g. reasons for replacing one instrument by the next; change of exposure of instruments; cleaning or other type of maintenance. Meteorological log books for recording measurements and observations are commonly booklets of entry forms corresponding to the code for transmission of observations. The layout of meteorological log books is usually a national responsibility.

II.1.12 Additional Sources of Data

Several national and international services provide regular dissemination of analyzed fields (and/or raw data), that can be obtained on board ship by telecommunications, particularly telefacsimile (schedules are usually printed and updated in the captains/radio officers handbooks of telecommunications). These fields - which generally consist of weather forecast charts or satellite picture transmissions - can advantageously be used for decision making on the conditional sampling of contaminants. Despite this service, the regular schedule of taking meteorological data should not be interrupted. The time and space resolution of the transmitted charts are fairly coarse, hence for interpretation of local observations of contaminant fluxes the knowledge obtainable from local meteorological observations is a necessary prerequisite.

II.2 *IN-SITU* MEASUREMENTS

This section presents the procedures for investigating those parameters which can only be measured or observed *in-situ* and those which should be analyzed immediately after collection, whenever possible. Also included are general descriptions of portable instruments for *in-situ* measurement of salinity, temperature, dissolved oxygen and depth. Although such instruments are in common use in many monitoring programmes, they all require periodic calibration against the chemical methods described in this Manual (see Chapter III). Therefore, such instruments may be used in lieu of the standard chemical analyses only if they are carefully handled and routinely maintained and calibrated. If such instruments are used, they should be calibrated by the same method used for chemical determinations.

II.2.1 Depth

Hourly depth measurements may be recorded simply by using either a lead-weight sounding line marked in 1 meter intervals and a wire angle indicator to correct the true depth or by employing any commercial type of acoustic echo-sounder, depth recorder or fathometer. Depth should always be reported in meters.

II.2.2 Seawater Temperature

Two methods for determining seawater temperature are presented: reversing thermometers and *in-situ* temperature sensors. Seawater temperature can also be measured using a multi-parameters system (see Section II.2.5).

A) Surface water temperature

The surface water temperature is measured either as a 'bucket' or 'intake' temperature. By the bucket method, the observer takes up a sample of water and determines its temperature. While originally the temperature of a freshly drawn bucket of water was determined on the ship's deck using a conventional thermometer, this method introduces known biases and is no longer recommended. Instead, the bucket temperature is measured with a water thermometer situated in the bucket and provided with an insulated container around it, Figure (II.15). Care should be taken that container and thermometer adjust to the temperature of the surface water, either by lowering it to the sea for a sufficient period of time or by taking up a few samples, reading the thermometer and emptying the bucket before lowering again. Repeated readings ensure that the bucket has adjusted to the surface temperature. Faster adjustment is achieved if the bucket thermometer is kept between measurements at about water temperature outside the direct sun.

Alternatively, the water temperature is read from a thermometer inserted into the intake of cooling water for the engine. Make sure that the water is not heated up before it reaches the thermometer. Also check the real depth of the intake, which may be somewhat below sea level.

Note : Units for sea surface temperature are degree Centigrade, the thermometer should be read to 0.1°C

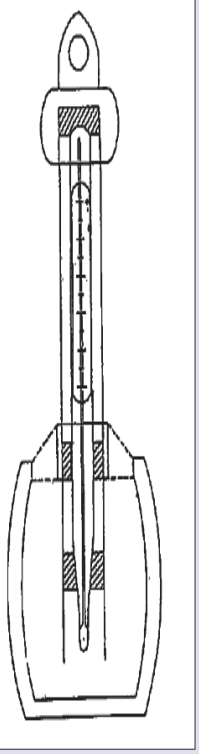


Figure (II.15) : Marine bucket thermometer. The rubber coated insulation helps to protect the thermometer against shocks when lowering into the water and reduces heating or cooling of the water sample by radiation and heat exchange. Because of the thermal inertia of insulation it is necessary to make sure that the assembly has adjusted to water temperature so that reading of the thermometer gives unbiased sea surface temperature

B) Reversing thermometers

Reversing thermometers are the mercury thermometers, which are especially designed for research in oceanography, marine biology, limnology, fisheries and many other fields concerning measurement of water temperature. There are two types of reversing thermometers, the protected and the unprotected. Reversing thermometers are sent down to the required depth in the set position as in Figure (II.16). A reversing

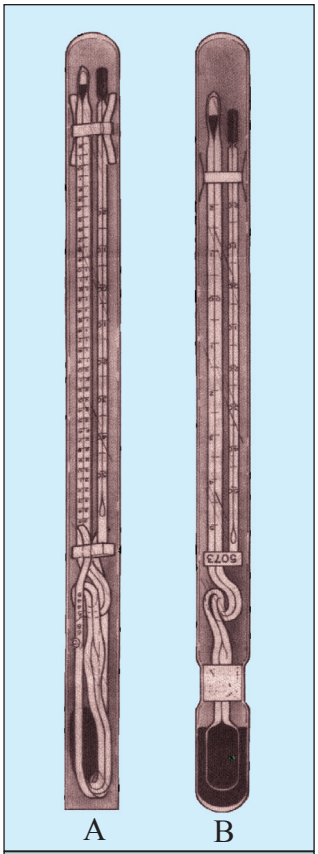


Figure (II.16) : Protected (A) and unprotected (B) reversing thermometers in set position, that is, before reversal

thermometer consists of a large reservoir of mercury connected by means of a fine capillary to a small bulb at the upper end. Just above the reservoir the capillary is constricted and branched with an appendix dead arm and above this the thermometer tube is bent in a loop (pigtail), from which it continues straight and terminates in the smaller bulb. The reversing thermometer is so constructed that in the set position mercury fills the reservoir, the capillary and part of the bulb. The amount of mercury above the constriction depends upon the temperature. When the reversing thermometer is then reversed by turning through 180 degrees, the mercury column breaks at the point of constriction if the temperature is raised after the reversing thermometer has been reversed.

Protected thermometer

Protected thermometers are not subject to pressure effects and record the true temperature (+ calibration correction) of the surrounding water at the moment of reversal. They may be safely used down to 6000 meters.

Unprotected thermometers

Unprotected thermometers are open to pressure effects and are used purely as a measure of the pressure at the moment of reversal. Roughly speaking, the difference between the protected thermometer temperature and the unprotected in centigrade, multiplied by 100 is the depth in meters.

Auxiliary thermometer

Auxiliary thermometers measure the air temperature at the time of reading the main reversing thermometer. The difference between this temperature and that shown by the main thermometer is a measure of the amount the mercury in the small bulb of the latter will have expanded since reversal and is used to compute the expansion correction. For this reason, it is important that time be allowed for both main and auxiliary thermometers to equilibrate with the normally higher air temperature after being taken out of the water.

Care of thermometers

The main point to bear in mind is that much unseen damage can be done to a reversing thermometer by careless handling without actually breaking it. Excessive shock can cause small air bubbles to form in the mercury and even distort the core. Thermometers should never be left in any other but the vertical position and when not reversed for reading, should face bulb down. They should never be transported unaccompanied although there is no objection to sending it by air if enclosed in a case and kept upright at all times by the person accompanying the consignment.

Breaks in the mercury column or the formation of gas bubbles may occur through mishandling. The continuity can, usually, be restored by gentle warming and reversal of the thermometer, but in this

case the thermometer should be 'rested' bulb down until the mercury and air have settled down, preferably for three months.

It is very important that reversing thermometers should be recalibrated before use if the last calibration is more than a year old and in any case at three years intervals. Calibration in itself does nothing to improve the performance of a thermometer and once issued to a ship it should not be returned for calibration unless three years have elapsed since the data of the last certificate or there is reason to suppose that a permanent change has occurred in an individual instrument's characteristics.

Anomalous readings

When a pair of protected thermometers disagree in the final corrected temperature by more than 0.04°C on two or more successive occasions of use, they should be paired with other thermometers next time they are used. If one of them is again involved in an excessive temperature difference it should be taken out of use and rested for at least a month.

Examination

On receipt, or after being out of use for some time, thermometers should be carefully examined for breaks and/or bubbles in the mercury. They should be smartly reversed and a check made that the break occurs at the "appendix" as shown in Figure (II.16).

Selection of thermometer

Reversing thermometers are commercially available and broadly fall into the following scale categories :

a. -2° to +20°C in 1/20
b. -2° to +35°C in 1/20

The two thermometers are recommended as they cover the required ranges.

Note : Any existing thermometer can be used provided the range is appropriate.

Unprotected range

c. +20° to +50°C in 1/10
d. -2° to +60°C in 1/5

Note : Unprotected thermometers are used to check the sampling depth

Considering that it is desirable to use the largest scale thermometer which will include the expected temperature range at the point of reversal, the above types of thermometer are recommended for the Region.

Selecting thermometer sets

It is strongly recommended that only one person be responsible for thermometers. He or she should keep a separate written record of how the sets are made up. Each reversing holder should carry two protected thermometers (normally left and right in the holder). The number of unprotected thermometers will depend on the number available and the nature of the work. In general, attach one unprotected thermometer on the lowest bottle only.

It is recommended that the thermometer holder on each water sampling bottle should not be altered except to test doubtful thermometers. Once the thermometers have been made up into sets, the serial numbers should be marked with a marker pen both on water sampling bottle and reversing thermometer holder.

Waiting time and messenger time

Five minutes should be allowed for thermometers to equilibrate to ambient temperature after they have reached the down position. It should be assumed that messengers travel at 200 meters a minute, so allow this time plus a 20% safety margin. In practice, messengers will probably travel faster than this speed with a vertical wire but it should be borne in mind that, due to current shear there may be a considerable wire angle below the surface. When wire angles exceed 35°, messenger time should be estimated as 100 meters a minute.

Reading the thermometer

Since there is liable to be a considerable difference between the thermometer reading on emerging from the water and ambient air temperature, and that the expansion time for the mercury in the main thermometer and the auxiliary may be different, it is now general practice to allow the thermometers to equilibrate for at least 15 minutes after being taken off the wire before being read.

During this period it is essential that the thermometers should be in an even temperature and not exposed to wind and sun. The errors likely to arise from failure to observe these precautions are admittedly small but every possible source of error must be eliminated if valid results are to be achieved.

After the equilibration period the thermometers can be read using the special tubular lens supplied and with light provided by a torch placed against the thermometer above or below the lens. This process requires the utmost care and concentration; it must on no account be hurried.

It is essential that the water sampling bottle with holder is at a convenient height, that a separate person records data and that all figures including the serial number of the thermometer be called out and repeated back. The most common sources of error are:

a) Wrong thermometer number written on the form
b) Parallax error, particularly with old-fashioned thermometers with a round scale. This should be eliminated by using the special lens but as a further check the observer should make sure that the degree of graduation mark nearest the top of the mercury appears as an absolutely straight line with no hint of a curve
c) Failure to notice that the graduation of a particular thermometer is in fifths rather than 10ths of a degree
d) Generally hurried and careless reading

It is recommended that the reader calls out in order the bottle serial number, the left thermometer number, the main thermometer reading and finally the auxiliary thermometer reading - followed by the equivalent figures for the right hand thermometer and lastly for the center unprotected thermometer if fitted.

Note : If this task takes less than 90 seconds per bottle the reader is certainly trying to go too fast

When all thermometers have been read, a second observer should re-read the main thermometer temperatures with the first observer checking against the figures listed on the form. If these agree within 0.02°C there is no need to re-read the auxiliaries and the process need not take long.

Difficulty is often experienced in reading unprotected thermometers owing to a mist forming on the inside of the outer tube. This may be eliminated by plugging the bottom end (when in the reading or reversed position) of the thermometer jacket with a cork so that water is retained in it. Care must, of course, be exercised that the opposite end is clear to admit pressure when the instrument is in use.

Manual temperature correction

Although there are several different, recognized methods of applying error corrections to thermometer scale calibration and correction for expansion of mercury after reversal, the method described below should be regarded as the standard formula.

a) Correction of protected thermometers :

The corrected value of the protected reversing thermometer is calculated by the following formula:

$$Tw = T^1 + C + I$$

where,

Tw = the corrected true water temperature to be determined.
T^1 = the reading of main thermometer.
I = the index correction for scale errors of main thermometer that is given on the calibration sheet of the thermometer.
C = the correction for thermal expansion as follows:

$$C = \frac{(T^1 + Vo)(T^1 - t)}{K - 100}$$

where :

Vo = the volume of mercury below the zero centigrade mark determined at zero centigrade in the reversed thermometer expressed in degrees Celsius, which is given on the calibration sheet
t = the temperature reading of the auxiliary thermometer corrected for index errors according to the calibration sheet
K = $1/\alpha$ = the reciprocal thermal expansion co-efficient for the thermometer system, given on the calibration sheet. For most reversing thermometers K = 6100
α = the apparent thermal expansion co-efficient of mercury

For example with a protected thermometer

I = 0.07, Vo = 67, K = 6100, these factors will be given in the certification sheet of each protected thermometer, main and auxiliary temperature readings were 3.40°C and 19.5°C respectively

The corrected true water temperature is as follows:

$$C = \frac{(T^1 + Vo)(T^1 - t)}{K - 100} = \frac{(3.40 + 67)(3.40 - 19.5)}{6100 - 100}$$

$$= \frac{(70.40)(-16.10)}{6000} = -0.19$$

$$Tw = T^1 + C + I = 3.40 + (-0.19) + (-0.07)$$

$$= 3.16 \text{ (the true temperature at reversal)}$$

b) Correction of unprotected thermometers :

The corrected reading of the unprotected reversing thermometer which depends on both temperature and pressure is given by the following formula :

$$T\mu = T\mu^1 + C + I$$

where

$T\mu$ = the corrected reading of the unprotected reversing thermometer
$T\mu^1$ = the uncorrected reading of the main thermometer
I = the index correction for scale errors of main thermometer given on calibration sheet
C = the correction of thermal expansion of the thermometer system given by the following formula

$$C = \frac{(T\mu^1 + Vo)(Tw - T\mu)}{K}$$

where :

Vo = the volume of mercury below the 0°C mark determined at 0°C in the reversed main thermometer given on the calibration sheet
Tw = the corrected value of the protected thermometer
$T\mu$ = the temperature reading of the unprotected auxiliary thermometer corrected for index errors given on the calibration sheet
K = the reciprocal thermal expansion co-efficient of the thermometer system. This is also given on the calibration sheet

For example with an unprotected thermometer :

I = -0.15, Vo = 159, K = 6300, these factors will be given in the calibration sheet of each unprotected thermometer and Tw = 3.16 from the corrected value of the protected thermometer reading. The main and auxiliary temperature readings of the unprotected thermometer were 13.50°C and 19.7°C

The corrected reading of the thermometer was as follows :

$$C = \frac{(T\mu^1 + Vo)(Tw - T\mu)}{K} = \frac{(13.50 + 159)(3.16 - 19.7)}{6300}$$

$$T\mu = T\mu^1 + C + 1$$

$$= 13.50 + (-0.45) + (-0.15)$$

$$= 12.90 \text{ (true temperature at reversal plus pressure effect)}$$

Corrections of protected and unprotected thermometers may also be made using graph developed by Theisen, 1947, which saves considerable time. It should be noted that not only the graph but also its plotting paper must be prepared for each individual thermometer as described by Theisen (1947). To simplify the calculations of reversing thermometers, a special oceanographic slide rule can also be used, such as the type extensively used by the U.S. Naval Hydrographic Office. The correction formula can easily be programmed for a computer and even a small programmable desk calculator. It is strongly recommended that a computer be used to calculate the corrections.

c) Thermometric depth

Depth determination by the thermometers is based on the principle of using the unprotected thermometer as a kind of pressure gauge in that, being open to the sea pressure, the mercury in the thermometer is forced up the tube by this means as well as by changes in temperature. This pressure effect changes the main thermometer readings by approximately 1°C for 100 meters depth so that the difference between the protected and unprotected thermometer may provide a direct measurement of the depth at time of reversal. The reversal depth can be determined by the following formula:

$$Z = \frac{(T\mu - Tw)}{\rho m \times Q}$$

where :

Z	=	the reversal depth in meters
$T\mu$	=	the corrected reading of the unprotected reversing thermometer
Tw	=	the corrected reading of the protected reversing thermometer
ρm	=	the mean density of the water column above the level of reversal. This value may be obtained from standard hydrographic tables
Q	=	the pressure co-efficient of the unprotected thermometer expressed in Degrees Celsius increase in the reading per 0.1 kg/cm^2 increase in pressure.

Q has a magnitude of roughly 0.01°C cm^2. This value is given on the calibration sheet as the pressure factor K which is equivalent to 10Q

For example, from the computations given above, values for protected and unprotected thermometers are :

$T\mu = 12.90$
$Tw = 3.16$

Thermometric depth is given by a.

$$Z = \frac{(T\mu - Tw)}{\rho m \times Q} = \frac{(12.90 - 3.16)}{1.0294 \times 0.00954} = 992 \text{ meters}$$

where:

$Q = 0.00954$ from calibration sheet
$\rho m = 1.0294$ obtained from salinity data

II.2.3 Salinity

Until about 1960, nearly all salinity measurements were made by chemical titration. The ratio of chemical elements in a water parcel varies little from surface to depth and from one ocean basin to another. Thus, titrating for chlorinity, the major negative ion in seawater and multiplying by a constant (see method Section III.2.4.A).

$$\text{Salinity} = 1.80655 \times \text{chlorinity}$$

Another method to determine salinity is to collect water samples from appropriate depths using standard water samplers. Salinity is determined on board the vessel or in the laboratory using an electric salinometer and utilizing standard seawater ampoules (see Section III.2.4.B). If the instrument is handled and standardized with care, the results are accurate and precise. Inductive salinometers with built-in-temperature compensation for a limited temperature range, are commonly used. Inductive salinometers are manufactured by several companies and differ only slightly in features. The basic procedure for the determination of the salinity, described in the manufacturer's manual, is the same for all types of inductive salinometers.

Almost all salinity observations are made today by measuring the electrical conductivity of seawater. This technique is now the international standard. Present instruments that measure temperature and electrical conductivity *in-situ* (CTD) can provide an accuracy of ±0.002 psu (see Section II.2.5).

Note : *Units* : Much of the present oceanographic literature has substituted the term practical salinity units (psu) rather than following the traditional parts per thousand (‰)

II.2.4 Dissolved Oxygen (Membrane Electrode Method)

Dissolved oxygen (DO) concentration of water samples can be determined with a multiparameter *in-situ* (see Section II.2.5) system. If it is to be determined separately then the determination from the water sample should be done as soon as possible after collection. However, due to rough seas, lack of facilities or insufficient manpower, it is often not possible to analyze samples aboard the vessel (i.e., by the Winkler method). Under such circumstances, *in-situ* measurements should be made using a carefully pre-calibrated oxygen sensor.

The majority of *in-situ* oxygen sensors for determination of dissolved oxygen in water employ the membrane electrode technique for analysis. The most common instrumental probes are dependent upon electrochemical reactions. Under steady-state conditions, the current or potential can be correlated with DO concentrations. Interfacial dynamics at the probe-sample interface are a factor in probe response and a significant degree of interfacial turbulence is necessary. For precision performance, turbulence should be constant.

The application of membrane electrodes can be suggested :

- for those samples containing material which interfere with the Winkler procedure (such as sulphite, thiosulphate, polythionate, mercaptans, free chlorine or hypochlorite, organic substances readily hydrolyzed in alkaline solutions, free iodine, intense colour or turbidity and biological floes)
- as a substitute for the Winkler procedure (see Chapter III.2.1.1) where it is desired to obtain a continuous record of the dissolved oxygen content of the water under observation

Because it may be used under any circumstances as a substitute for the Winkler procedure it is important that the probe itself is standardized against the Winkler method on samples free of interfering materials.

Determination of dissolved oxygen with membrane electrode

Sample handling, calibration and calculation procedures are given in the manufacturer's instructions, however, the membrane electrodes **should always be calibrated with the Winkler method**. The electronic readout meter for the output from dissolved oxygen probes is normally calibrated in convenient scale (0 to 10, 0 to 15, 0 to 20 mg/l for example) with a sensitivity of approximately 0.05 mg/l. The possible interferences are given below :

Interferences

a) Dissolved inorganic salts are a factor in the performance of dissolved oxygen probe

Probes with membrane respond to partial pressure of oxygen which, in turn is a function of dissolved inorganic salts. Conversion factors for seawater and brackish waters may be calculated from dissolved oxygen saturation versus salinity data. Conversion factors for specific inorganic salts may be developed experimentally. Board variations in the kinds and concentrations of salt in samples can make the use of a membrane probe difficult.

b) Reactive compounds can interfere with the output or the performance of dissolved oxygen probes

Reactive gases, which pass through the membrane probes may interfere. For example, chlorine will depolarize the cathode and cause a high probe-output. Long-term exposures to chlorine will coat the anode with the chloride of the anode metal and eventually desensitize the probe. Alkaline samples in which free chlorine does not exist will not interfere. Hydrogen sulphide will interfere with membrane probe if the applied potential is greater than the half-wave potential of the sulphide ion. If the applied potential is less than the half-wave potential, an interfering reaction will not occur, but coating of the anode with the sulphide of the anode metal can take place.

c) Dissolved oxygen probes are temperature sensitive, and temperature compensation is normally provided by the manufacturer. Membrane probes have a temperature coefficient of 4 to 6 percent/°C dependent upon the membrane employed.

II.2.5 Multiparameter System (CTD)

Systems for measuring water temperature, conductivity, salinity, pressure and other parameters like DO and pH are available, and can measure these parameters to a high degree of accuracy. The system can be self-contained whereby it records results internally, or displays results on deck or records them in a computer compatible format. In that case, a special winch with electric cable is to be used. The system can also be provided with a special water sampler [called rosette water sampler, Figure (II.17)], which can be activated from the ship.

These systems should be calibrated before and after each cruise and sometimes during long cruises by direct measurement of temperature using reversing thermometers and other parameters using samples collected by water sampling bottles.



Figure (II.17) : A conductivity-temperature-depth recorder (CTD) attached to a rosette sampler equipped here with Niskin bottles. The CTD records conductivity, temperature and depth continuously. The rosette sampler is activated electronically from the surface and collects discrete water samples at different depths

II.2.6 pH

The pH is usually determined on board the collecting vessel.

Note : For further information on the determination of pH and scope and field of application see section III.2.2

II.2.7 Determination of Water Colour

The Forel scale is the basic means used in determination of water colour from blue to green. Normally the Forel scale is used in connection with Secchi disc readings. The Forel scale consists of a series of 11 sealed glass vials filled with separate fixed colour solutions, numerically designated and mounted in a sturdy two piece hinged aluminum housing with a handle.

The Secchi disc, with its white surface upwards, is suspended about one meter below the water surface. The number of the Forel scale vial which most closely matches with the water colour against the white Secchi disc is recorded. It is important that these operations be conducted from the shady side of the ship and that the vials are shaded from open sunlight when determinations are made.

II.2.8 Determination of Transparency

The Secchi disc is designed to measure transparency and is dependent upon the available illumination, which varies with the time of day, cloud formation, and amount of cloud cover. The Secchi disc is a circular white plate, with a diameter of 30 cm, and a ring attached at the center of the upper surface to allow a marked line to be secured. Opposite the ring, on the under surface, a lead weight is attached so that the disc will sink rapidly and vertically.

This weight should be at least 2 kg and may be increased when conditions of drift and current warrant. The line attached to the Secchi disc should be marked off in one meter intervals to at least 50 meters. It is recommended that heavy cotton sash cord or heaving line be used.

The Secchi disc should be lowered into the water from the shaded side of the ship until the disc is just perceptible, and the depth in meters noted. The lowering is then continued until the disc is no longer visible. The disc is then slowly raised until it is again barely visible. The depth recording of this point is then averaged with the reading obtained on lowering and is recorded on the data sheet. A Secchi disc reading must be taken at the same time the associated meteorological data is taken.

The extinction coefficient of water can be determined by the formula:

$$\text{Extinction coefficient} = \frac{1.7}{D}$$

where D is the depth in meters, where the secchi disc disappears

A more quantitative instrument for determining the beam attenuation coefficient is called a transparency meter or a turbidity meter (see Section II.2.9). The instrument as described by Pickard and Emery (1990) consists of a sensing head, which is lowered into the water. It has a lamp and photoelectric cell in separate watertight housings mounted 0.5 to 2 m apart and opposite to each other. The principle of the instrument is that the current from the photocell is a measure of the amount of light falling upon it. The lamp has a lens and colimator to ensure a parallel beam of light, which passes through the water and falls upon the photocell. The light output is kept constant by regulating the current through the lamp. The light beam is usually arranged horizontally because the absorption characteristics in the sea are often horizontally stratified. Also the photocell must not face upward or it would pick up direct sunlight which would give too high an apparent transmission by the water.

The photocell is connected by an electrical cable to a microammeter on deck. The current from the photocell is recorded when the sensing head is on deck with the light passing through air whose attenuation is taken to be zero. In the water the photocurrent decreases due to the attenuation of light by the water. The decrease from the air path value is a measure of the attenuation by the water. If the initial light intensity is Io and that in water after a path length x is Ix, the beam attenuation coefficient C may be obtained from the $I_x = I_o \exp(-Cx)$ or in logarithms to base 10 as $C = 2.3 (\log I_o - \log I_x)/x$. The length x used in these formulae is the length of the water path between the lamp and photocell in meters (usually from 0.1 to 2 m). The instrument is lowered in stages and the attenuation coefficient measured at a series of depths to give a vertical profile for this water property. The attenuation is often associated with particulate material present in the water independently of its temperature and salinity and therefore may be useful as an independent tracer of water masses. The attenuation coefficient C, or the "turbidity" as it is sometimes called, has been used to distinguish water masses both in the open ocean and in coastal fjords.

II.2.9 Turbidity

The turbidity of seawater cannot be measured by chemical methods due to the fact that the turbidity of seawater is caused by substances, which are not truly dissolved but rather present as colloidally dispersed or coarsely dispersed. Most of the instruments commercially available are true nephelometers, working on the principle that light, passing through a non-homogeneous medium, is scattered. Turbidity is not related directly to the mass or weight of the particles, but rather to their number, size distribution and surface structure. Usually turbidity sensors based on nephelometry can be incorporated in a CTD or STD system as an option.

For calibration of the instrument one has to use arbitrary units with some kind of standard. Such a standard is formazine, a substance that can be prepared synthetically and reproduced repeatedly to within one percent. The standardization and the analytical results are expressed as FTU (Formazine Turbidity Units), which are equivalent to JTU (Jackson Turbidity Units). Another unit, which is used and is based on a Formazine standard is NTU (Nephelometric Turbidity Units).

Calibration

A Formazine stock solution of 4,000 NTUs can be prepared as follows and calibration is carried out following the manual provided by the manufacturer:

a) Dissolve 5 g of reagent grade hydrazine sulphate in about 40 ml of distilled water
b) Dissolve 50 g of pure hexamethylenetetramine in about 400 ml of distilled water
c) Pour the two solutions into a one liter volumetric flask and dilute to the mark with distilled water
d) Allow the solution to stand for 3-4 hours at 20 to 22°C during which time the suspension will develop

The stock suspension can then be diluted with demineralized water to achieve a solution of the desired NTU value. It is important to thoroughly mix the stock suspension prior to removing a portion for dilution.

II.2.10 Light Intensity Measurements

The penetration of radiation into the sea is of interest to biologists in connection with the photosynthetic activity of phytoplankton and algae and with the behavior of zooplankton and fish.

The measurement of the light intensity as was described by Pickard and Emery (1990) could be done by mounting a photocell behind a filter in a watertight housing to face upward. A microammeter, connected to the cell, is read first with the cell on the deck facing upward to give a measure of the sun and sky light falling on the sea surface in the spectral range passed by the filter. The cell is then lowered to the depth of interest and the photocurrent again measured. The ratio of the photocurrent with the cell in the water to that on deck is then a measure of the ratio of light intensity at depth to that at the surface. If the photocell indications have been calibrated against a standard light intensity meter then the actual light intensity at depth will be known. It is usual to measure the light intensity at a series of depths below the surface in order to obtain a profile of light intensity as a function of depth It is usual to mount an opal glass or hemisphere in front of the photocell so that it will collect light from all directions above its plane.

The Weston type of photovoltaic cell is recommended to use for measuring the light intensity for the following reasons:

1. Its response can be made substantially linear with light intensity at a particular wavelength
2. It responds over the full range of visible light, and
3. It does not require a separate source of power

II.3 WATER CIRCULATION

II.3.1 Sea Level

Changes in sea level occur on different time scales: the time scale of hours in the case of tides and of months in the case of seasonal variations and in terms of years in the case of long term sea variations. Tide gauges deployed in suitable locations can provide information on all these scales. The location of tide gauges in the ROPME Sea Area should be:

1. On an open stretch of coast
2. Far from known amphidromic points
3. Easily connected with land-survey datums

The Aanderaa Water Level Recorder (WLR), designed to measure ocean water levels, Figure (II.18), is recommended for ROPME Sea Area. Placed on the seabed, the instrument records pressure, temperature and salinity at regular intervals. On the basis of these data, precise variations in water level can be calculated.

The instrument consists of a high precision quartz pressure transducer, an electronic board, a Data Storage Unit, wiring and hardware, all fastened to the top end plate and housed in a cylindrical pressure case.



Figure (II.18): A commercially available water level recorder (WLR)

A measurement cycle, triggered by a high precision clock, starts with a forty second integration time of the pressure measurements. This eliminates pressure fluctuations due to the waves. When the integration is completed, the data words are recorded. The first data word is a fixed reference reading followed by the temperature of the ambient water. The pressure is recorded as two ten-bit words and finally a ten-bit word for the water conductivity (optional sensor).

The data is stored in the Data Storage Unit (DSU), which also records the time of the first measurement and subsequently the time of every first measurement after midnight. The data is simultaneously transmitted acoustically into the sea by keying on and off a 16.384 KHz carrier. These acoustic signals can be monitored at the surface by the use of Hydrophone Receiver.

Information on procurement, installation, calibration and analysis of tidal records are available from International Organizations (IOC, etc.)

II.3.2 Wave Direction, Period and Height

The study of waves has recently been put on a more scientific basis by the utilization of an automatic method of recording data and the subsequent analysis of the records into component simple waves. The measurements require the use of sophisticated electronic wave recorders manufactured commercially under the names of “wave rider”, “wavecrest”, etc. The quality of the observations is dependent upon the personal skill and judgement of the observer. There are several difficulties inherent in visual observations. For example, should all the waves be counted or only the big waves. Since wave characteristics vary so much, what average values shall be taken? It is obvious that if comparable results are to be obtained the observer must follow a definite procedure.

The flat and badly formed waves between the wave groups cannot be observed accurately by eye and different observers would undoubtedly get different results if an attempt was made to include them in the record. Therefore, the recommended method is to observe only the well formed waves in the center of wave groups. The observation of waves entails the estimation of wave direction, wave period and wave height.

Reliable average values of period and height can only be obtained by observing at least twenty waves. These cannot be consecutive; a few must be selected from each succeeding group until the required number has been obtained. Only measurements or reliable estimates are valuable. Rough guesses have little value and should not be recorded. While observing waves from a moving ship, care must be taken to ensure that the observations, especially those of period, are not influenced by the waves generated by the motion of the ship.

II.3.2.1 Wave Direction

Wave direction means the direction from which the waves come. This is easily obtained either by sighting directly across the wave front or by sighting along the crests of the waves and remembering that the required direction differs from this by 90 degrees. Direction is always recorded true, not magnetic.

II.3.2.2 Wave Period

For measurements of period, a stop-watch is required. The observer selects a distinctive patch of foam or a small object floating at some distance from the ship and notes the time at which it is on the crest of each successive wave. This procedure is repeated for the larger waves of each successive group until at least twenty observations are made. The period is then taken as the average time for a complete oscillation from crest to crest. With practice, suitable waves can easily be picked out and the timing from crest to crest becomes quite simple.

II.3.2.3 Wave Height

The measurement of wave height is strictly based on estimations. The procedure to be adopted depends on the length of the waves relative to the length of the ship. If the length of the wave is short in comparison with the ship's length, the height should be estimated from the appearance of the waves.

II.3.3 Currents

The Aanderaa-type and tethered-type current meters are recommended: the operating principles of the equipment are similar to each other. The velocity of the current is measured by correlating it with the speed of rotation of a vane configured wheel or propeller which is actuated by the impinging current, and the revolutions are recorded for a given length of time along with the direction by compass-reading, Figure (II.19).

The Aanderaa-type current meter is designed to be anchored in the sea below the wave zone. It has proved to be a rugged and reliable means of current measurement. Its natural response to currents, however, rectifies any water movement to a rotary action of the impeller. Because of this characteristics, this meter is unable to provide a measure of the vector averaged currents, but instead gives a scaler average of current magnitude over the recording period.

The tethered-type current meter is designed specifically to operate in

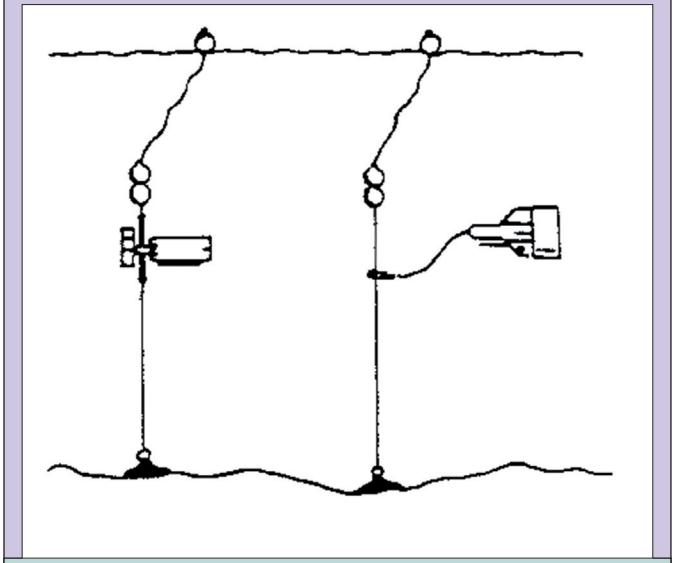


Figure (II.19) : Recording current meters (A) Aanderaa-type (B) Tethered-type

wave zones. The configurations of this meter use a tether as an integral part of the mooring system. This tether gives freedom to the meter's movement within the limits of the tether's length.

The tether mooring system allows lateral and vertical movement of the meter in response to short term oscillations such as passing waves without a change in polar orientation. It is, therefore, recommended that the choice of a particular type depends on the depth of the area to be monitored. Usually temperature and salinity sensors are incorporated in the current meters, and simultaneous recording of temperature and salinity on these current meters can be used as reference data.

Each *in-situ* array will consist of two meters: one at five meters below the lowest tide mark and one at one meter above the sea bed if the water depth is not more than 20 meters. In deeper waters, more current meters can be deployed at depths according to the need.

Additional back up meters should be prepared along with spare parts kit. The station should be carefully selected considering various factors: security of the mooring, shipping traffic, local fishing activities, bottom materials, etc.

Low cost anchors can be made locally by filling discarded automobile tires with concrete and a steel loop that can be joined to the mooring wire by a shackle.

Detailed instruction on handling, operation procedure and mooring are in the manufacturer's manual. The instrument should be calibrated prior to deployment. Usually new meters are calibrated in the factory and should not need further calibration unless they are damaged.

When selecting the meters, it is very important to know the measuring ranges of each parameter. The following ranges are required for the area:

a.	Current speed	:	2.5-250 cm/sec
b.	Direction	:	0-360°
c.	Conductivity	:	0-80 mmho/cm
d.	Temperature	:	10-37°C
e.	Range 1	:	down to 100 meters for shallow waters
f.	Range 2	:	down to 1000 meters for deep waters
g.	Clock	:	various sampling intervals

Ten minutes sampling interval is recommended for one month mooring duration. However, for short mooring durations shorter sampling intervals can be chosen.

Data recorded on a magnetic tape should be processed for the following parameters:

- Current speed
- Current direction
- Salinity (optional)
- Temperature (optional)
- Depth (optional)

Translation and processing facilities of current meter magnetic tapes are available in some ROPME Member States. The final data presentation should include:

- Current speed scatter plots
- Current direction scatter plots
- Current speed frequency plots (histograms)
- North-South and East-West current component plots
- Temperature time plots
- Salinity time plots
- Depth time plots

RCM 9 Doppler Current Meter is another recommended device. The measurement by this device is made by emitting a set of high frequency acoustic pulses into the sea. When these pulses hit some of the almost always present minute objects in the water, some of this acoustic energy is reflected back to the instrument and sensed by the ultra sensitive acoustic sensors. By measuring the frequency shift (the Doppler shift) of the reflected acoustic energy, the current of the water volume is calculated.

For the objects to reflect acoustic energy, their acoustic impedance must be different from that of water. In acoustic terms, water is neither soft nor hard. This means that soft objects, such as minute gas or air bubbles, as well as hard objects, such as minute sand or even plankton particles, will reflect energy.

However, the requirement for the presence of particles in the water column is one of the limitations when using this kind of measuring principle.

Aanderaa instruments has, over the years, improved the acoustic system of the RCM 9 by increasing the acoustic power level about 4 times, and lowering the sensitivity of the receiver to below that of the natural acoustic background noise.

Flexible design allows for several mooring techniques

The flexible design of the RCM 9 allows it to be used in sea bed, pelagic and surface installations. One reason for its flexibility is the built-in inclinometer that enables it to operate even when tilted up to 15 degrees from vertical position without degrading its accuracy, and to 35 degrees with only a slight degradation in accuracy.

Pelagic monitoring

When the RCM 9 is moored for pelagic measurements, there are several mooring options. The one shown below is often used and provides a flexible and easily installed and retrieved setup. The absence of a vane system, and the inclusion of a depth sensor, allows it to be positioned at the desired depth by adjusting the length of the retrieving rope.

A typical problem with instruments installed in this way is the disturbance of the current speed imposed by the instrument when the water passes the instrument's container. As a general rule of thumb, a downstream water volume of about 30 times the instrument's volume is disturbed by the presence of the instrument itself. The RCM 9 avoids this problem by including smart software algorithms that concentrate the measurements upstream, in the undisturbed, water volume.

Surface installation

Another interesting application for the RCM 9 is surface installation. The narrow acoustic beam allows it to be placed close to the surface without disturbing the current measurements. The surface, and near surface water columns are also effected by daylight and have increased organic activity. The optional turbidity sensor measures degradation of visibility by means of back-scattered light, making the sensor helpful for monitoring this water layer.

The compact and rugged design of the RCM 9 makes it equally suited for inland water measurements. The ability to obtain measurements in shallow waters is also crucial when making measurements in rivers and small lakes.

Real-time monitoring

One of the major advantages, compared to older designs, is RCM 9's capability of providing real-time monitoring of the sea current. The older design employed a van system that aligned the instrument according to the sea current direction. The RCM 9 does not need such a vane and obtains correct measurements even when rotating freely in the mooring system.

This new capability allows for direct read-out of data by means of a cable. The cable can further be connected to a VHF or UHF radio transmission system, or you may use a modem that can be called up at regular intervals. Both systems are available from Aanderaa Instruments.

Mooring configuration

The instrument can either be mounted on a purpose built bed frame, for near bottom measurements or on risers for mid water measurements. Generally, a L or U shape mooring is used consisting of a surface marker buoy (with a fishing beacon and radar reflector attached), a buoy line attached to a ballast weight (typically 300 – 400 kg) a ground line and a bed frame or weights and risers with sub surface buoys. Mooring configurations will vary depending on Client specifications and local conditions. Examples of moorings are shown in Figure (II.20). All mooring risers and ground lines shall be purpose constructed from new materials.

II.4 SEA SURFACE OBSERVATIONS

Valuable information concerning oil pollution distribution patterns and seasonal trends may be obtained through a programme of regular reporting of oil slicks and other floating pollutants. The procedures outlined below, if followed consistently and accurately, should identify areas of chronic oil pollution, which will require more intensive investigation. To ensure the consistency of data submitted by the various regional institutions, the responsible institution or ministry department should follow these guidelines as closely as possible.

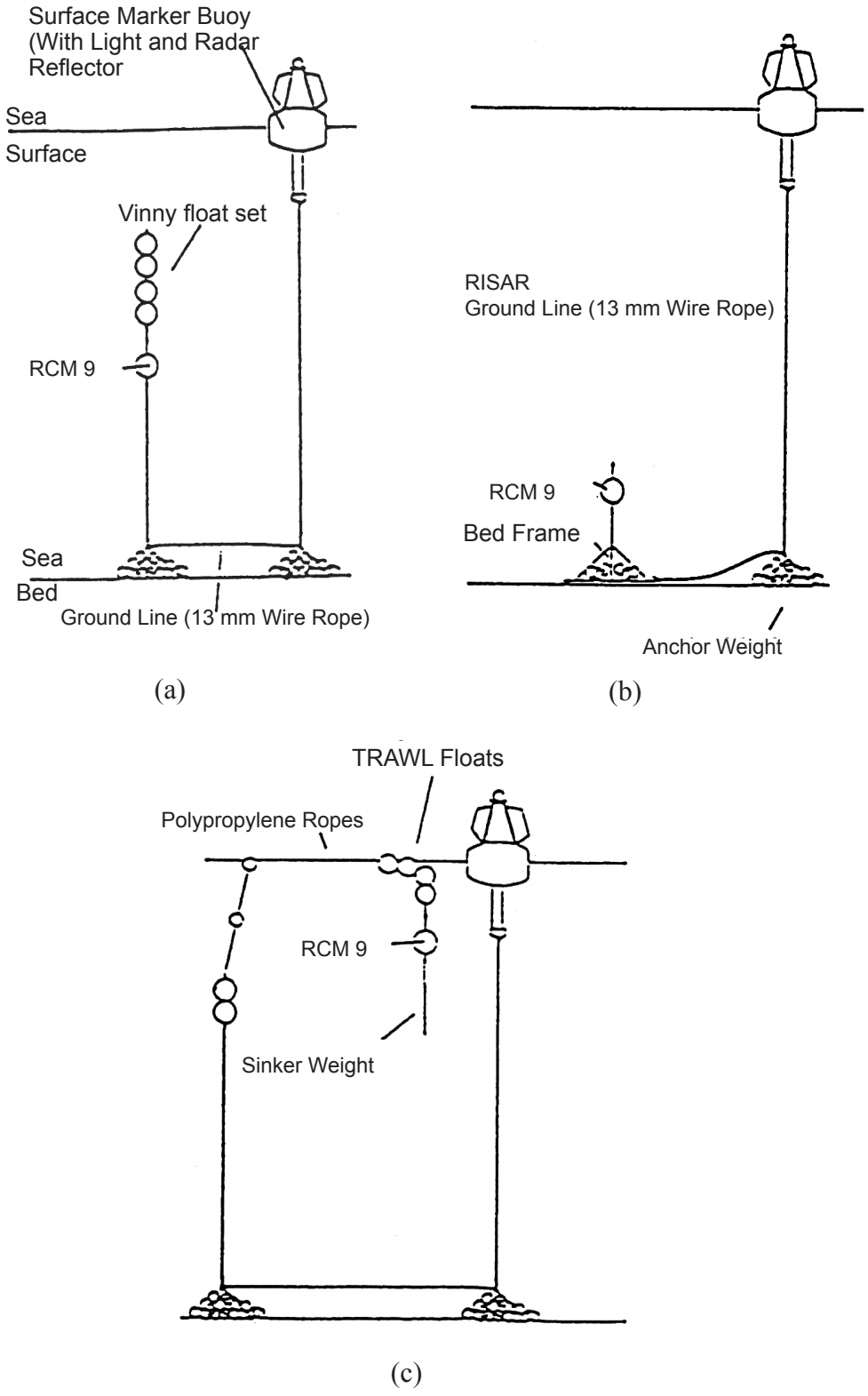


Figure (II.20) : Example of current meter moorings. U-shaped mooring for a) Mid-depth current meter; b) L-shaped for bed frame mounting; and c) U-shaped mooring for surface current meter

II.4.1 Observation Method

The observation method involves simple visual inspection during any regular cruise, flight or visit to offshore structures. Local personnel, not involved in the RAP Projects who are regularly at sea or fly over coastal areas (especially helicopter crews) should be contacted and enlisted in this task. The more information, the better. The method involves filling out the attached sample data form using the observation codes provided below.

Whenever floating oil, petroleum residues or slicks, and other floating pollutants (plastics, rubbish, etc.) are sighted, they should be reported on the data log form. It is equally important to record the observation periods during which no oil is sighted. Observations made from offshore structures such as oil rigs, pumping platforms, etc. should be reported once in 24 hours and aerial observations should include a description of the flight path. The observation method does not require any special instruments or equipment although a good pair of polarizing sunglasses may help to distinguish petroleum derived slicks from natural films. In addition, a lightweight pair of binoculars is also useful for observing slicks at distance.

a. Status of observations

0	=	sea surface observed but no slicks or pollutants sighted
1	=	sea surface not observed due to high seas, poor visibility or other reasons
2	=	oil slick and/or pollutants sighted and recorded

Note : A negative report (0 status) is as valuable as a positive sighting

b. Pollutant categories

1	=	thin oil film (may include minor patches or lumps of thick oil)
2	=	thick oil layer (may be surrounded by oil film which should be included under this same code)
3	=	plastic material
4	=	other (specify in remarks column)

c. Configuration

1	=	continuous cover
2	=	patches
3	=	in a line or lines
4	=	patches and lines

d. Extent of cover

The portion of the sea surface covered by a slick should be reported in eighths of the sea surface.

0 = Sea surface completely clean
1 = 1/8 (slight presence of oil or other pollutants)
2 = 2/8
3 = 3/8
4 = 4/8 (half the surface is covered)
5 = 5/8
6 = 6/8
7 = 7/8
8 = 8/8 (complete cover)

e. Ancillary data

Wind direction and speed :

a. true wind direction; enter the true direction, in tenths of degrees from which the wind is blowing.
b. wind speed in m/sec.

Wave period and height :

a. enter the average period of wind waves (the time in seconds between one wave crest or trough and the next).
b. wave height to the nearest half meter should be recorded using the following WMO code :

00	=	calm
01	=	<1 meter
02	=	1 meter
03	=	1½ meter
04	=	2 meters
05-99	=	increasing heights at id meter intervals

Air and water temperature :

Enter the air temperature and the sea surface temperature to the nearest tenth of a degree Celsius (°C).

II.4.2 Characteristics of Oil Slicks

A large crude or refined oil spill is conspicuously visible. If it has not weathered to tar like residues, there will be central zones, which are brown or black in colour which represent thick oil layers. These will be surrounded by thinner films sometimes showing an iridescence or sheen (rainbow coloured, metallic looking reflections on the water surface). At the outer edges of a slick thinner films may be present without obvious colour but which are noticeable since they dampen adjacent waves.

Subsequent weathering of crude and fuel oils will lead to tar residues (tar balls) within the oil slick, usually at the downwind end of the slick.

Characteristics of other types of slicks

It is often difficult to distinguish between natural sea slicks and those found by accidental or deliberate oil spills. This is especially true if the oil spilled is a refined petroleum product such as fuel oil, diesel oil or lubricating fluid. Since an oil film of this type dampens surface ripples as do natural sea slicks, the following guidelines should assist the observer to distinguish between petroleum oils and natural films.

a. When wind speeds are greater than 8 knots (4.1 m/sec)

Natural slicks are easily dispersed; under these conditions natural slicks will be rare and visible films should be oil pollution. However, a long narrow, isolated slick, sometimes containing seaweed and refuse should not be considered an oil slick.

b. Under relative calm wind conditions

A large area of the visible sea surface may contain natural surface slicks, as noted by areas of water without ripples. Pollutant slicks may easily be confused with natural films under these conditions. Use the following criteria to decide:

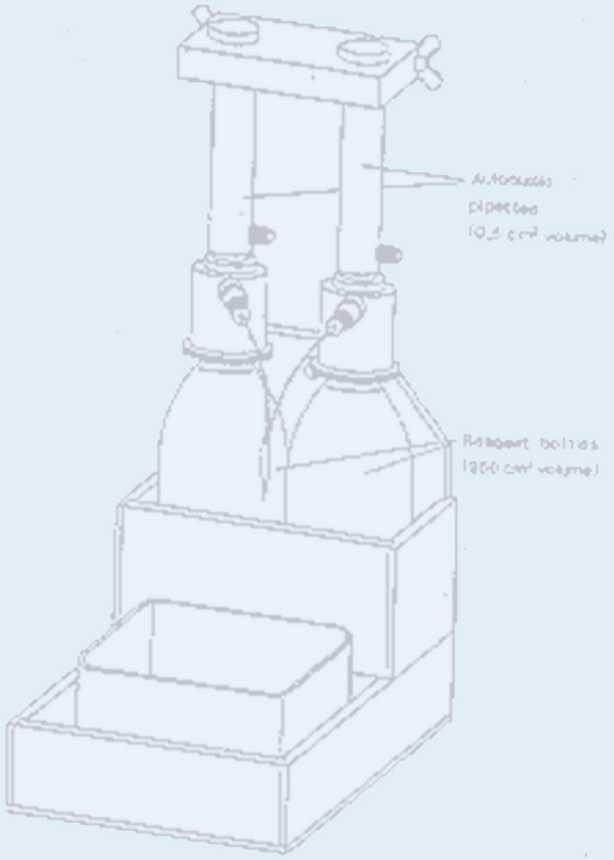
i) if the slick shows signs of dark layers and/or tar residue or if an oily odor is evident, the slick should be considered of petroleum origin

ii) if the sea is calm and the slick not obviously petroleum, it should be considered natural and recorded as “0” in the column under Status of Observation. When it is not possible to distinguish between natural and petroleum films it should be recorded as natural

c. A natural slick is usually caused by a film of recent biologically produced organic matter

Too thin to be seen but noted by its ability to dampen and resist the formation of wind generated ripples. This property produces a pattern of light reflection, which renders the slick visibly different from surrounding waters. Natural slicks are usually lighter in colour than rippled water or may appear darker when viewed towards the sun. During calm periods without ripples, the entire sea surface may appear slicked, however, there will be no colour to the film, no oily odor, nor thick patches unless oil is present.

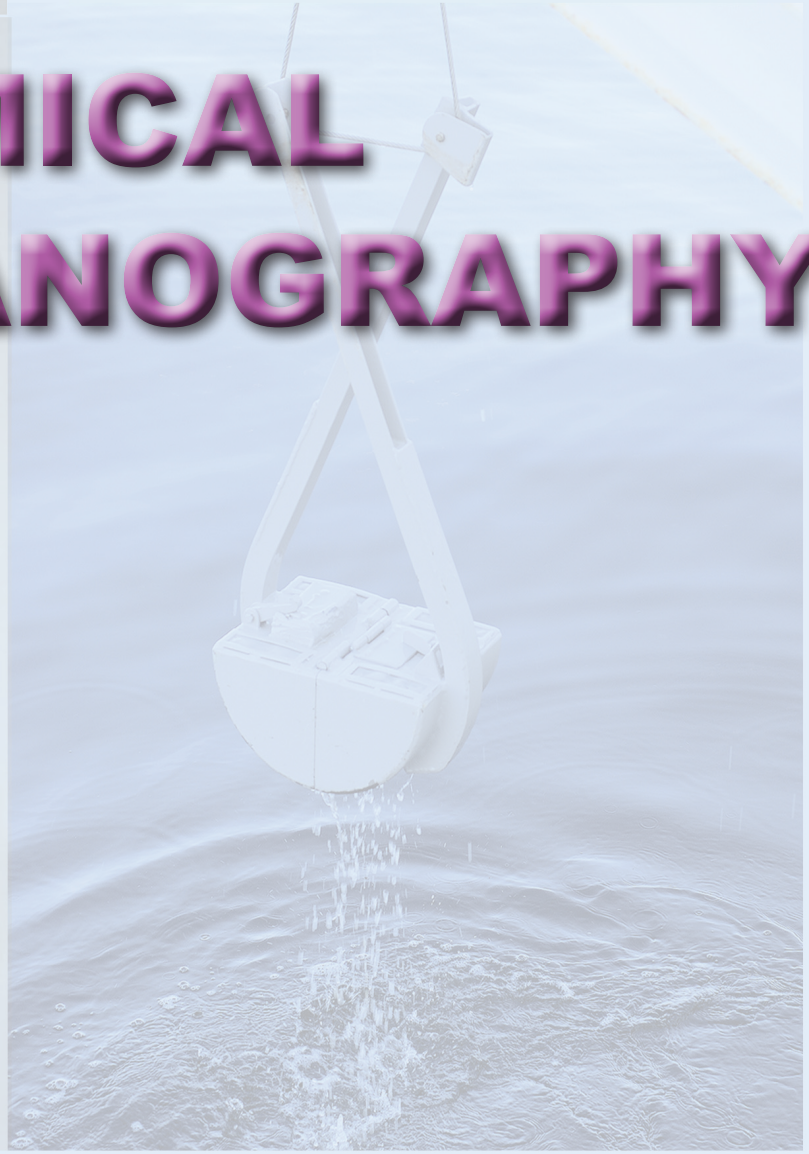




III

CHEMICAL OCEANOGRAPHY





III CHEMICAL OCEANOGRAPHY

III.1 SAMPLING OF WATER, SEDIMENT, BIOTA AND ATMOSPHERIC COMPONENTS

This section describes the techniques recommended for the collection of water, sediment and biota samples. The list of essential collecting equipment, laboratory equipment and suppliers are depicted in Annex V. This section is organized so that samples for several parameters can be collected simultaneously. For example, as illustrated in Figures (I.1) and (I.3), water samples are required for nutrient analyses, dissolved and dispersed petroleum hydrocarbons, suspended sediments, chlorophyll, etc.

III.1.1 Water

The following general considerations should be reviewed.

III.1.1.1 Sampling Apparatus

Water samples from specified depths are collected and analyzed for a variety of physical, chemical and biological parameters as well as for pollutant analyses. Water samplers of various designs have been used and there are numerous designs, which are commercially available.

Generally, they can be divided into two groups: reversing type and non-reversing type. Considering the depths in the Region and the sampling depths specified for the various parameters, it is recommended that non-reversing water samplers be used.

The non-reversing type sampler - such as Niskin or van Dorn type-usually consists of a PVC cylinder and top and bottom caps as in Figure (III.1.A). The top and bottom caps are held open by a clamp against the tension of a spring or a rubber connecting them through the bottle.

The action of a messenger releases the clamp, and the caps are pulled into position, closing off the top and bottom of the bottle. When the bottles are used in a series, the closure of one bottle releases a messenger below it, which travels down the wire and trips the next bottle. The samplers, which are lowered to depths with both caps open are adequately flushed during the lowering.

Another type of non-reversing water sampler recommended for the regional programme is the “Go-Flo” type water sampler as in Figure (III.1.B). This type of sampler was designed for use in pollution studies to collect uncontaminated water samples from specified depths. The sampler enters the water closed, to avoid surface film contamination, is opened by a pressure actuated valve and is re-closed by a messenger at the proper depth. Since this type of sampler has no internal closing springs, which contact the sample, they are highly recommended for both the general water samples and pollutant samples.

Most of the samplers commercially available are made from PVC. It should be noticed that for metal sampling, for C-14 productivity and any other procedure that must be metal free, only the Teflon coated samplers are suitable. Non-metallic sampling wire should also be used. PVC Niskin or Go-Flo bottles should not be used to sample organic materials, e.g., chlorinated hydrocarbons, etc. All-



Figure (III.1) : Non-reversing water samplers (A) Niskin (B) Go-Flo

metal or glass bottles should be used, such as the Bodman or Bodega-Bodman, and these should preferably go through the surface closed, as is the case with the dissolved/dispersed petroleum hydrocarbon sampler, Figure (III.3). The sampler should have an appropriate capacity to provide adequate sample for various analyses. It is recommended that each participating group responsible for conducting the project should have a sufficient number of samplers with spare parts to account for mechanical breakdown and loss during operations. Two samplers are needed as specified by ROPME but spare units should be available as a back up. The recommended number of samplers and accessories are as follows :

Water sampler (Niskin & “Go-Flo” type)	2-3 of each
Reversing thermometer holder	6 sets
Messengers	10
Spare Kit	6 sets
Rack for water sampling bottles	1 unit
Winch and hydrowire	1 unit
Weight - depressor weight for wire	2
Wire angle indicator	1
Sample bottles for aliquots	100

Water sampling bottles with reversing thermometer holders should be kept in a rack which enables the holder to be reversed to keep the thermometer bulb down when not being used, and which has enough space for all the bottles to be held on the ship. Below the bottles, there should be a compartment sub-sample bottle rack with one compartment for sub-sample bottles under each water sampler. The rack should be designed to accommodate the sampling bottles. If an inside wet laboratory is not available for the rack to be installed, canvas screens must be rigged to protect it from sunlight and wind.

The above equipment is available from several manufacturers.

III.1.1.2 Sampling Depths

Sampling should be done at 3 depths (1 meter below surface, mid-depth and 1 meter above sea bed) and at 10 meters when the depth allows. In practice, the nominal depth of a sampling bottle as indicated on a meter wheel as “wire out”, seldom coincides with the true depth of the sampler. True depth can be determined using a set of protected and unprotected thermometers as discussed under seawater temperature measurements (Section II.2.2) or estimated from the amount of wire out and wire angle at the time of collection using the formula :

True depth = w x (Cos°)

Where

w = is the length of wire (in meters) out below the sea surface, and
Cos° = is the cosine value of the angle of wire with the vertical at the time bottles are tripped (messenger time), measured with a wire angle indicator.

III.1.1.3 Formation of Sampling Team

A team should be organized for the sampling programme. It is desirable that there should be as few changes as possible in the team and that a rigid routine should be insisted upon as to who does what. Besides overall supervision, the following duties are crucial and must be carried out or closely supervised by a cruise leader :

- recording meter wheel readings, bottle numbers and thermometers serial numbers on working log sheets
- final checking of samplers and thermometers after clamping on wire
- operation of a winch and final checking of wire angle
- drawing water samples, and
- reading and recording thermometer values

Normally a team of one cruise leader, one or two specialists (to take the special samples such as for bacteria, etc., and to carry out *in-situ* measurements), one experienced seaman rated to operate a winch, one to act as bottle hanger plus one seaman as bottle carrier should be adequate. It is recommended that the cruise leader and/or the specialists themselves perform the crucial work of reading thermometers, drawing water samples, etc. It is highly important that the task of sampling should be approached in a calm and deliberate manner and that those concerned should have a proper amount of rest between stations.

III.1.1.4 Winch and Hydro-wire

Operating the winch requires considerable care and concentration and should not be entrusted to men other than those of proven reliability and common sense. The hydro-wire, like all running rigging, must be carefully watched and the outer turns, which receive most use and exposure, may have to be cut off after a few months use. Excessive greasing of the wire should be avoided for fear of contaminating water samples. Stainless steel or synthetic fiber ropes are best. It is very important that the wire should be wound on evenly as the wedging effect of the turns creates great pressure and any loose turns will be crimped with subsequent loss of strength.

When winding on a new wire an artificial tension must be created by improvised break on the drum on which the new wire was supplied. Before use, the new wire should be lowered with a sinker in deep water and rewound on with the natural tension due to its weight.

Although superficially the wire may appear to be in good condition, it will deteriorate in use due to corrosion and fatigue. It is important that a portion of all wire held both in use and as spare should be tested for strength annually. Should the breaking strain have deteriorated below 80% of its nominal value it should be replaced or returned to the store as defective as the case may be. To avoid wear occurring in the same place on the wire every time it is used, slight variations should be made in the amount of wire veered before attaching the first sampler. Standard oceanographic metal wire rope is normally 4 mm in diameter with a weight of 60 kg per 1000 meters and a breaking strain of 1000 kg.

Excessive speed of lowering of the hydro-wire is liable to cause a tangle of wire. A safe, conservative speed is 100 meters a minute. With experience it may be found possible to increase this speed; in bad weather it may have to be reduced considerably. A sinker of 30 to 45 kg weight on the outboard end of the wire will assist in lowering the wire at an even speed. The speed of hoisting will depend on the weather and the load on the wire. Use common sense.

III.1.1.5 Water Sampling Routine

The following is a summary of the sequence of events before, during and after a typical station.

Preparation

a) Arrange water sampling in order of bottom and mid-depth, 10 m and surface together with thermometer holders (Section III.1.1.2). Check reversing mechanism of the reversing thermometer holder. Leave holders in “thermometer bulb down position” with hanging mechanism ready to go on wire. Check that mercury columns in main thermometers are without breaks

b) Lay out sub-sample bottles under water samplers

c) Record wire depths, sampler numbers, thermometer numbers, sample bottle numbers, etc. on the log form

d) Record meter wheel readings and final lowering depths for each sampling

e) Prepare all deck personnel responsible for sampling, etc

On station

a) When captain gives permission, lower weight below surface
b) Set meter wheel to zero. Sampler carrier provides the first sampler for bottom
c) Sampler-hanger then clamps sampler to wire
d) Sampler-hanger checks that sampler cannot slip, mercury column in thermometers is unbroken, cock is closed. He gives a signal to winch man
e) Winch man lowers slowly for first 10 meters and then continues at normal speed until next meter wheel reading on his board is reached (if bottom sample taken).
f) Repeat c, d and e for each bottle
g) Lower the lowest sampler to the depth nearest bottom
h) Measure the wire angle. This should be done by cruise leader who notes this down on working log sheet; he works out time of messenger, angle correction and of heaving in
i) Sight and surface respectively are called by sampler-hanger as first bottle comes up
j) Slow winch progressively at first sight and at surface; winch man stops at convenient working height of bottle
k) Sampler-hanger unclamps sampler and gives winch man signal to continue heaving in
l) Keeping sampler upright, sampler-carrier returns it to its numbered position in the rack
m) Repeat j, k and 1 for remainder of bottles in cast
n) Read thermometers from the surface, 10 m and bottom samplers. Draw water samples into respective sub-sample bottles

Post sampling

a) Cruise leader should check over temperature to make sure they look reasonable before signaling completion to Captain
b) Open caps and reverse thermometer holder in each sampler in the rack so that thermometers are bulb downwards. If the sampler rack is in a secure sheltered place, bottles and thermometers will be safest left in-*situ* until next station; if not, thermometer holders should be removed from the bottles complete and stowed on a rack in a safe place
c) If equipment is not to be used for some time, rinse all of it in freshwater and dry thoroughly

III.1.1.6 Collection of Samples

Although extremely simple, the process of rinsing and filling sub-sample bottles must follow a strict routine and should always be supervised by a cruise leader. The general procedures are as follows :

a) Empty previous contents of bottle, if any
b) Fill bottle one third full taking care to keep fingers clear of the water
c) Replace cap loosely and shake vigorously
d) Pour out
e) Repeat b, c and d - repeat twice, if bottles are used for the first time during the cruise
f) Fill bottle to just above the shoulders of the bottle
g) Wipe the screw thread on the outside of the top of the bottle and the inside of the cap with a new tissue paper and then discard the paper
h) Screw on the cap gently but firmly

The above procedures outline the general considerations of water sampling and should be treated as guidelines for the following detailed collection methods.

The following sections describe the detailed procedures required to collect water samples for those measurements and analyses which will be carried out on shore or which are best performed aboard a research vessel as soon as possible after collection when facilities and sea conditions allow. The parameters are listed in the order they should be drawn from the samples.

III.1.1.6.1 Dissolved Oxygen

Sampling and oxygen fixation procedures

The oxygen sample should be the first sample drawn from the water sampler, and the sub-sampling should be carried out as soon as possible after the sampler has been recovered. (In oxygen deficient areas, oxygen sub-sampling should be carried out starting with the bottom sampler). Special 50 ml glass bottles should be used (see Section III.2.1). For this purpose the 50 ml glass bottles are unstoppered, and the nozzle tube of the sampler is inserted to the bottom of the sample bottle. (The nozzle should be preferably of transparent tubing, sufficiently narrow to ensure that the sample stream carries away any air bubbles in the tube, but wide enough to fill the bottle at a moderate speed). The bottle is flushed with at least twice the volume of the bottle (ca. 100 ml). At the final stages of filling the tube is carefully removed from the bottle. The bottle must be brim full. If air bubbles have nevertheless been trapped on the walls of the bottle, they should be driven out by gently tapping at the bottle.

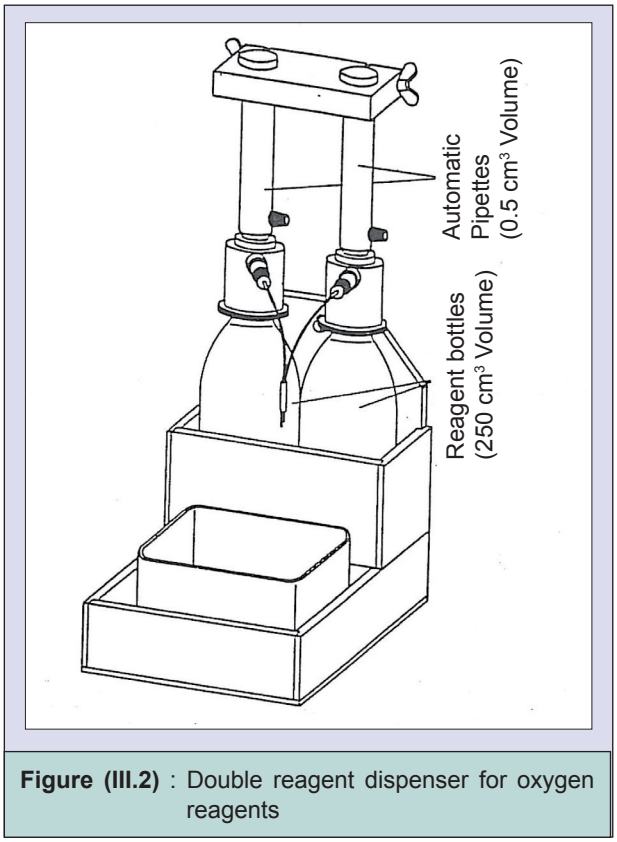


Figure (III.2) : Double reagent dispenser for oxygen reagents

The reagents are added simultaneously (0.5 ml each of manganous chloride and alkaline iodide solution) with the special dispenser, Figure (III.2), or with micropipette. The syringe pipette tips (or micropipette tips) should extend deep into the bottle. The tips are slowly withdrawn while the reagents are added. Then the stopper is inserted avoiding the trapping of air bubbles. The bottles are shaken vigorously for at least 1 min to achieve the chemical fixation of oxygen. The precipitate is then allowed to settle (ca. 10-20 min) before analysis is continued.

Oxygen samples may be stored up to 10-20 h after addition of the reagents. The bottles, however, should be kept in the dark and at constant temperature. If titration cannot be achieved within 12 hours, or, if a large temperature difference exists between sub-samples and the (e.g. air-conditioned) laboratory, the bottles should be kept underwater (in order to prevent contamination from atmospheric oxygen by expansion and contraction of the samples).

Reagents

Manganese (II) chloride solution

Dissolve 40 g $MnSO_4.4\ H_2O$ in distilled water and dilute to 100 ml.

Alkaline iodide solution

Dissolve 60 g KI and 30 g KOH **separately** in a minimum amount of distilled water and combine the 2 solutions. The solution is diluted to 100 ml with distilled water. (If the saturated solution contains some residual salts add some more distilled water for dissolution).

III.1.1.6.2 pH/Alkalinity

The samples for determination of pH/Alkalinity should be drawn from the water sampler with the same amount of care as for the oxygen samples. Glass bottles with glass stoppers with a volume of 150 to 200 ml should be used. At least one full volume of the pH bottle should be allowed to run over before stoppering. The outlet tube of the water sampler should be inserted to the bottom of the pH flask and then slowly withdrawn during the final stages of filling. Contamination with air must be rigorously avoided. No air bubble should be trapped under the stopper.

The pH/Alkalinity samples should be drawn from the water samples immediately after the oxygen sampling routine (if no sub-sampling for other dissolved gases is required).

III.1.1.6.3 Salinity

Water samples to be analyzed for salinity concentration should be drawn into salinity sample bottles. The type of glass bottle used by many institutes has a glass or porcelain stopper with a rubber gasket, which is attached to the bottle neck with a wire clamp-on device.

The bottles are numbered on top of the stopper or on the side of the bottle. They are shipped and stored in compartmented wooden cases. Follow general instructions for cleaning bottles. Draw the water samples directly from drain cock, rinse the bottle twice with greater than 25 ml each and fill the bottle to within 2.5 cm of the top. A 2.5 cm air space must be left to prevent the bottle from breaking due to warming of the sample. The stopper is then placed in the mouth and clamp-on device pulled securely down.

After all salinity samples are drawn and logged, they are placed in their shipping cases. If they are to be analyzed aboard ship they must be placed in the laboratory for a minimum of six hours before analysis. Eight to twelve hours is preferable in order to allow them to come to the same temperature as the room, apparatus and chemical solutions to be used in the analysis.

III.1.1.6.4 Chlorophyll

It is recommended that sample should be drawn into a polyethylene or other plastic container and should be filtered as soon as possible after collection (see Section III.2.5).

III.1.1.6.5 Suspended Particulate Matter

Water samples for suspended particulate matter determination should be collected from the defined depth in the sampling programme, using any of the samplers identified in section III.1.1.1. The sample will be analyzed for total suspended particulate matter (SPM) weight, which in turn is used to correlate with turbidity measurements.

Equipment

1. Filtering apparatus includes the following items :
   - Vacuum Pump (0-30 psi)
   - Manifold (3 or 6 places)
   - Funnel assembly (3 or 6)
   - Plastic filter forceps
   - Vacuum hose
2. Polyethylene measuring cylinder (1000 ml)
3. Polyethylene bottle (1000 ml)
4. Membrane filters (0.45 µm in pore size)
5. Petri dishes
6. Precision balance
7. Oven

Sampling and filtration of the sample

It is recommended that water samples drawn from the water sampler should be filtered immediately on board.

Set up the filtering system on board. Place a pre-weighed filter on the base of the filter holder and install funnel and clamp together.

The required volume of a sample (1 litre) is measured in a polyethylene measuring cylinder and transferred to a polyethylene bottle. The bottle should be shaken before filtration which should then be started at not more than two thirds of full vacuum.

After completing the filtration, rinse the membrane filter with distilled water and drain it thoroughly under suction before removing it from the filtration equipment.

Remove the membrane filter carefully from the filtration unit and transfer it to a clean petri-dish and keep in a freezer. However, if the analysis can be done within few hours, a refrigerator is sufficient.

Dry the membrane filter in an oven at (103-105°C) for 2 hours to constant weight.

Transfer to a desiccator and after cooling to room temperature, measure the weight again.

Calculations

$$\text{Total SPM (mg/l)} = \frac{(A - B) \times 1000}{V}$$

where A = The weight of the filter with residue in (mg)
B = The weight of the membrane filter before filtration in (mg)
V = Volume of the sample filtered in (ml).

III.1.1.6.6 Nutrients

Since water samples collected for nutrient analyses must often be filtered prior to analysis, it is recommended that aliquot of the water sample which is filtered for suspended particulate matter determination be used for nutrient analyses in order to minimize difference between regional sampling programmes (see Section III.1.1.6.5).

Filtration

If a sample has a visible turbidity, it must be centrifuged or filtered immediately after collection. Centrifugation is difficult to perform on board. Filters, however, can affect samples by absorption or leaching of components, or the filtration characteristics of the filter will change after the deposition of the first layer of particulate matter. Unfortunately, only very few systematic studies have been performed on this subject so far. We have obtained good results with 0.4 µm Nuclepore filters (cleaned in cold 6 mol/l e.g. HCl for 3 days and rinsed with distilled water) or with glass fibre filters from Sartorius (Type S 13400, cleaned for a maximum of 4 hours in 0.1 mol/l HCl). Filtration can be carried out by suction or with a pressure filtration system [an all-plastic filtration apparatus, for example, is available from Sartorius (Type SM 16510)]. In any case, however, the applied filtration procedures should be examined carefully and independently for each nutrient component of interest. Furthermore, analytical results from filtered samples should only be reported when “filtration blanks” are quoted.

In this section only sample collection, preservation and storage will be discussed while detailed analytical methodology is presented in Section III.2.8.

A) Phosphate - P

Water samples for phosphate analysis should be collected in stoppered glass or “aged” polyethylene bottles of 50 to 100 ml volume directly from the outlet tube of the in line filter used to collect suspended particulates. The samples are stored in a cool dark place until the analysis can be performed. For phosphate, the analysis should be commenced as soon as possible, preferably within half an hour, certainly before 2 hours and only glass bottles should be used for intermediate storage of the samples. The samples should not be stored in new polyethylene or polyvinylchloride containers since phosphate has been shown to disappear rapidly in these containers. Therefore, aged high-density polyethylene bottles or other plastic e.g. polycarbonate may be satisfactory but all sample containers should be thoroughly tested before use. Once collected, samples should be stored out of the light in a refrigerator until required for the analysis.

The addition of acid to unfiltered samples cannot be recommended since this cause hydrolysis of any polyphosphates and release of phosphate from plankton and bacteria. The addition of all

of the reagents of the analytical procedure to the sample and postponement of the photometric measurement is also not possible, since arsenic and silicate will also react and cause erroneous phosphate readings.

Summarizing, it can be stated that storage of samples for the analysis of dissolved phosphate for more than one hour should be avoided.

B) Ammonium - N

Samples for ammonium analysis should only be taken and stored in tightly sealed seawater-aged glass or high-density polyethylene bottles, which should only be used for the analysis of ammonia. Filtration of samples should also be avoided, if possible, because it is nearly impossible to obtain filters free of ammonium. Waters with high turbidity frequently contain high concentrations of ammonia and may therefore be diluted before the analysis (the residual turbidity may then be compensated by subtraction of the absorbance of the appropriately diluted sample without addition of reagents).

Ammonium is a nutrient compound, which rapidly undergoes biological conversion, i.e., oxidation into nitrite and nitrate and fixation as amino-bound nitrogen in organisms. The analysis of ammonia should be commenced without delay after sampling. Chemical methods for preservation have been proved unsatisfactory because of the fact that organisms rapidly release ammonia. It is therefore strongly recommended that the ammonia reagents be added within one hour after sampling.

C) Nitrite - N

Nitrite is an intermediate compound, which occurs if ammonia is oxidized or nitrate is reduced. The presence of higher amounts of nitrite (>1.5 µmol/l of NO_2) signifies the presence of high bacterial activity in the seawater sample. Storage of samples for nitrite analysis can therefore not be recommended. Chemical preservation (e.g. addition of chloroform) also seems to be unsatisfactory. In turbid waters a filtration step is necessary. Therefore, collect the sub-sample for nitrite determination directly from the outlet of the in-line filter described above in a 100 - 150 ml glass container. The nitrite reagents should, if possible, be added to the sample within one hour. Intermediate storage of the sample in glass bottles in a refrigerator for up to 3 hours causes, in most cases, no significant changes in the nitrite content, if the original ammonia level is low (<0.07 µmol/l). Samples should be stored in tightly sealed glass or polyethylene bottles only. Sulphide ions have been reported to interfere with the determination of nitrite and, thus, when hydrogen sulphide is suspected to be present in a sample, the gas should be expelled with nitrogen after the addition of the acid sulphanilamide reagent (Grasshoff *et al*., 1983).

D) Nitrate - N

Nitrate is the final oxidation product of nitrogen compounds. Changes of the original nitrate content of a seawater sample can, therefore, only result from oxidation of ammonia and of nitrite or from adsorption of nitrate to the material of the sample container. Adsorption of nitrate into particles seems to be insignificant since the analytical procedure liberates any nitrate, which may be adsorbed. For reasons yet unknown, the nitrate content of a sample decreases rapidly if stored in polyethylene bottles, and at a level of 1.4 µmol/l NO_3 about half of the nitrate disappears within seven days after storage at room temperature. This indicates that only glass or “aged” high-density polyethylene bottles with tight screw caps (preferably with Teflon liners) should be used.

If larger plastic bottles are used for sub-sampling for all nutrient analysis, the amount needed for nitrate should be transferred into a glass or "aged" high-density polyethylene bottle within one hour after the sampling. The analysis should not be delayed for more than 5 hours. In this case the samples should be stored in a refrigerator. If longer storage is unavoidable, the sample should be quickly frozen to -20°C after the addition of the ammonium chloride buffer solution (Grasshoff *et al*., 1983).

E) Silicate - Si

It is obvious that glass bottles should not be used for storage and analysis of seawater samples for reactive silicate. The sub-sampling for silicate analysis should be performed with plastic bottles (made of polyethylene or polypropylene). A few days storage of the sample in the dark in a refrigerator does not lead to significant changes in the silicate content. However, during seasons of high productivity, do not store them for longer than a day. Polymerization of orthosilicate during storage of frozen samples has been reported from fresh water samples but does not occur in seawater. If kept frozen, it is recommended to thaw the sample gradually at room temperature for at least 24 hours. However, as with all nutrients immediate analysis of sample is the preferred option.

The best procedure for storage and preservation of fresh-water samples seems to be the acidification of the seawater sample with sulfuric acid to a pH of 2.5 and storage in tightly sealed, seawater-aged, high density polyethylene bottles in the dark at about 4°C. However, as with all nutrients immediate analysis of the sample is the preferred option.

III.1.1.6.7 Dissolved and Dispersed Petroleum Hydrocarbons

Water samples for dissolved and dispersed petroleum hydrocarbon determination must be collected separately from general water samples.

A device such as that illustrated in Figure (III.3) is recommended for collecting the water samples. It consists of a weighted bottle holder with a clean amber glass bottle of 3-4 liters (an amber 1 gallon solvent bottle is excellent) held in place by a wire frame. The bottle holder is attached to a float by a line of 1 m length. (**Remarks**: Sample taken from 1 m below surface can be considered as surface sample for petroleum hydrocarbons). A similar device can be used for sampling below the surface. A second retrieving line of suitable length is attached to the float and is used to recover the assembly after the sample has been taken. The bottle should be stoppered with a Teflon stopper or a bottle cap (prepared as in d(i) below) which can be removed when the device reaches the approximate depth by means of a monofilament fishing line.

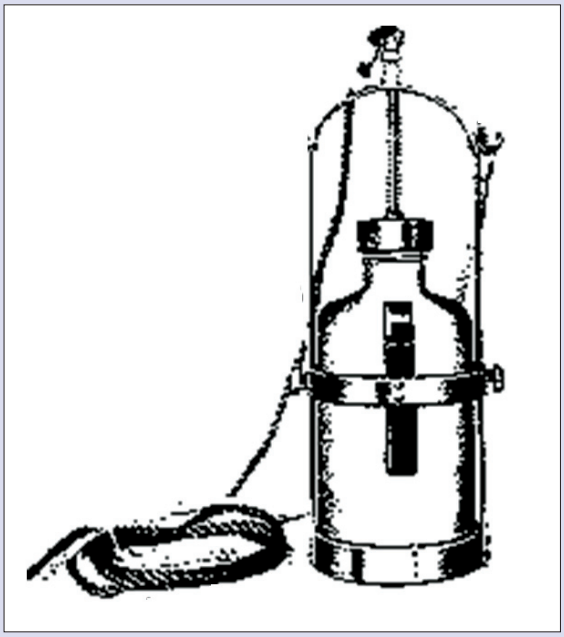


Figure (III.3) : Surface and subsurface water sampler for dispersed and dissolved petroleum hydrocarbons

Prior to use, the sample bottles should be thoroughly cleaned with dichromate-sulphuric acid or detergent and rinsed according to the following procedure :

a) Remove the dichromate-sulphuric acid, or detergent, rinse thoroughly with tap water (wear goggles). A continuous liquid film on the wet bottle should be an indication of the absence of grease
b) Rinse the bottle with acetone, and leave the bottle to dry
c) Rinse the bottle with solvent to be used in extraction
d) i) For shallow waters the bottle cap liner should be replaced with a piece of clean aluminum foil between the cap and the top with the dull side of the aluminum foil down. Do not touch with fingers
   ii) For deeper water use an underwater close-open-close device of the recommended materials only

Samples should be taken in triplicate. When the ship is at a complete stop, the assembly is thrown overboard from the bow as far as possible away from the ship to avoid water that has been disturbed or contaminated by the ship. Make sure the lead weight hits the water surface first. If the assembly hits sideways, the bottle may be forced through the frame and thus be lost. The bottle will sink and when it reaches the appropriate depth pull out the bottle cap with the fishing line. Sufficient time should be allowed for the bottle to fill completely. Upon retrieval, some water is spilled (sufficient to allow for possible thermal expansion, approximately 50 ml) out of the bottle. If the extraction is not started within 3-4 hours, add 10 ml of dichloromethane and hexane mixture (6/4;v/v) into the bottle and shake for a minute. Before capping, a piece of aluminum foil should be placed over the bottle top with dull side down and the cap screwed down over it; this will prevent contamination from the cap. The bottle containing the water (and the solvent mixture) is then allowed to settle and the bottle stored away.

Note : Since the concentration of dissolved/dispersed petroleum residues in the open sea are generally in the range of a few micrograms per liter, or less, throughout the procedure **great care must be taken to avoid contamination**

For preservation of the water sample, the samples should be kept in the dark. When solvent mixture is added, freezing is not necessary since this solvent is an effective bacteriostat. Samples should be analyzed as soon as possible.

III.1.1.6.8 Microbiological Sampling

A) Surface water

Label sample bottle at collection site indicating the sampling station, time of sampling and other factors relevant to the interpretation of the results. Attach clean sterilized sample bottle to the clean sampling rod. Immediately before submerging the sample bottle, remove the ground glass stopper from the bottle without touching the inner surface of the bottle and stopper or screw cap. Immerse the bottle from the bow of the boat or from the windward side while the boat is moving forward slowly. Push the bottle with the sampling rod 25 cm under the water surface film, then turn the sample bottle upward and take the sample Figure (III.4).

Retrieve the bottle and discard some water, if necessary, so that some air space remains in the closed bottle. This space is needed for homogenizing the water sample at the receiving laboratory. Replace the glass stopper or screw cap and store the samples in the clean, thermo-insulated sample bottle container with pre-frozen packs of chemical gel at about 4°C, avoiding exposure to more than + 10°C. Keep samples in the dark. Separate bottles from each other with clean wrapping paper to avoid breakage.

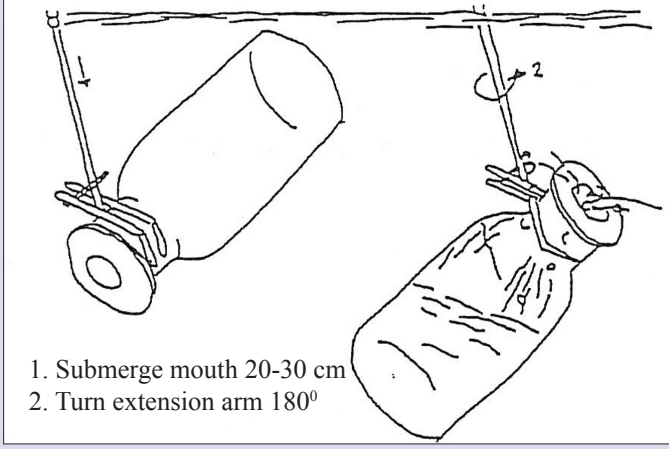


Figure (III.4) : Surface water sampler

B) Subsurface water

Subsurface (1 m or more) water samples should be collected using a simple closed bottle device such as shown in Figure (III.5). This sampler is lowered to 1 or 10 meters and a weighted messenger is used to break a small glass tube connecting the water inlet and air vent. The glass tube should be clamped to the wire or rope in a position where it will be struck forcibly by the messenger to ensure breakage. Label sample bottle at collection site indicating the sampling station, time of sampling and other factors relevant to the interpretation of the results. Lower the sterilized subsurface sampler after attaching it to a clean plastic rope, without letting the weight disturb the bottom sediments. Release the messenger and after one minute retrieve the sampler and store it in a thermo-insulated sample collection container. Proceed as for sampling of surface water.

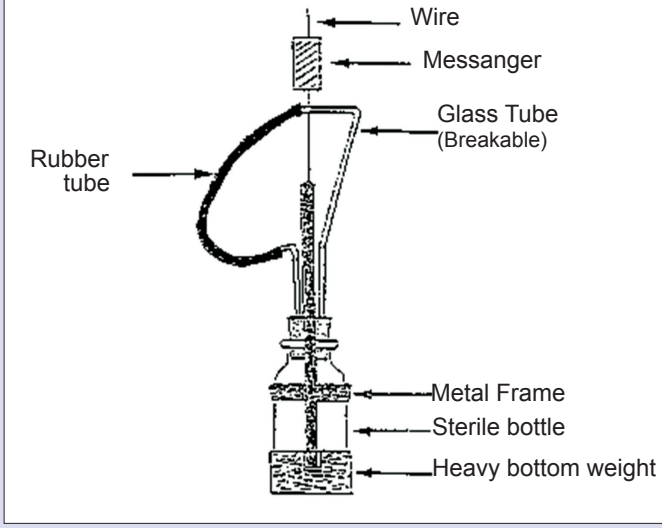


Figure (III.5) : Subsurface water sampler

Note : It is known that the die-away rate of faecal coliforms at ambient temperature in the presence of light is very high. Therefore, all efforts should be made so as not to collect more samples than can be analyzed and incubated the same day. If this is not possible, the samples should be stored at 4°C and analyzed not later than 24 hours after sampling

Before a new sample is taken, the glass tube is replaced with a new tube and the entire inside of the sampler including glass tubes and plastic hose are rinsed with 90% methanol or ethanol.

After collection of both surface and subsurface water samples, approximately 20-30 ml of sample should be discharged from the sample bottles to ensure thorough mixing of sample when the bottles are shaken prior to laboratory examination.

Keep all samples in a refrigerator or ice chest at 4°C for storage and transport to the laboratory. The sampling crew should be careful to do everything possible to maintain low temperature inside the cooling containers by keeping them in the shade, with the lids tightly secured.

The samples should be transported from the sampling vessel to the laboratory for processing within 6 hours of collection, provided they have been kept under refrigeration during this period. Samples should be processed as soon as possible after reaching the laboratory.

III.1.2 Sediments

III.1.2.1 Estuarine Sediment Classification

Estuarine and coastal sediments may be regarded as a mixture of inorganic and organic material that has arrived at the site of deposition as solid particulate matter (detritus) or has been incorporated into the sediments from solution (non-detrital) in a variety of ways. They may be classified according to their grain size and relative proportion of the sediment particles, their colour, the relative proportion of their organic and inorganic components, and their predominant chemical (siliceous, calcareous), and mineralogical composition. In most northern latitude sediments, detrital material dominates the non-detrital material and determines the broad chemical composition of the sediments. The organic component of such sediments is normally less than 10% by weight.

III.1.2.2 Sample Collection and Field Observations

The purpose is to collect a representative undisturbed sample of the sediment to be investigated. Samples of marine sediments and suspended particulate matter may be collected from the sea floor and from the water column using specific types of sampling apparatus. This is done to ensure that the sample is fully representative of the substrate or water column at a specific geographical location and that it is uncontaminated by any substance that is being analyzed for or that will interfere with the subsequent treatment of the sample.

III.1.2.3 Sampling Apparatus

Two types of sampling techniques are used for collecting sediments from the sea floor: grab sampling which collects surface and near surface sediments and coring which collects a column of the subsurface sediment. In all grab and core operations, a slow approach to the sea floor should be ensured to avoid the creation of “bow wave” that disturbs the sediment-water interface prior to sampling.

a. Grab sampling of surface sediments

Undisturbed surface sediment samples can provide an immediate assessment of the present levels of contamination in the area in relation to the textural and geochemical characteristics of the sediment. The sampler, Figure (III.6) used must consistently collect relatively undisturbed samples to a required depth below the sediment surface and of sufficient volume to permit subsequent analyses.

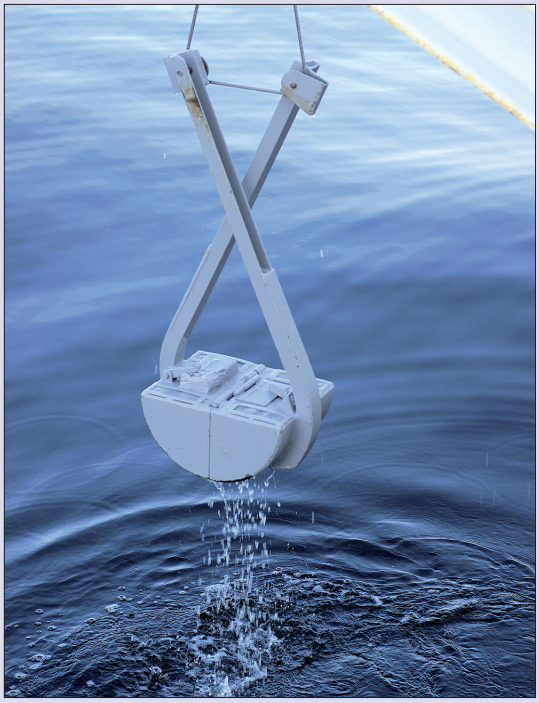


Figure (III.6) : Van Veen grab

Tightly closing grab samplers, of which there are many designs (Bouma, 1969; Murdoch and MacKnight, 1994) are usually adequate for studies of the most recently deposited layer. The 0.1 m2 Van Veen grab, Figure (III.6) is recommended simply as an inexpensive, reliable and effective type of sampler for sediments. Other samplers such as the Shipek and Ekman grabs are very efficient too to obtain relatively undisturbed samples of sediment (Mudroch and MacKnight, 1994). If the sampler is well designed, no loss of the entrapped sediments and water should occur from the grab after recovery from the sea floor. It is especially important to avoid leakage of fine-grained sediments between the sea-surface and the deck, because this will result in erroneous grain size and compositional determinations.

This procedures outlined below assume that these samples will be collected from a vessel equipped with the basic collection facilities such as a winch, davit or other such lifting equipment and adequate refrigerated storage space.

Regardless of the equipment chosen for the sampling, it is useful to know the water depth at each station before starting the sampling (see Section II.2.1). The purpose is to ensure adequate cable length for operation of the correct equipment and to control the speed of entry of the sampler into the sediment. The speed of deployment of the sampler can be critical to good operation and sample recovery. It is also useful to have some understanding of the currents at the sampling site. Strong near-bottom currents can lead to poor equipment deployment, deflect a grab sampler, or require a long cable/wire to be deployed. Care should be taken to ensure that the weight of the sampler is adequate for working at the particular current conditions.

On-board, the sediments contained in the grab sampler require attention to ensure that essential components are neither lost nor augmented by contamination through improper handling. The most critical sampling and storage techniques relate to the avoidance of chemical contamination and change in the physico-chemical characteristics of the sediments. Special steps should be taken to minimize contamination of the samples. For trace metal determinations, the use of a stainless steel grab sampler with Teflon coatings on all surfaces that come into contact with sediments, and polyethylene coated lowering cables are highly recommended. All samples should be collected into

cleaned plastic or glass vials (see Section V.2). For trace organic analyses (see Section VI.2).
The actual collection procedure is quite simple :

a) Prepare all sample containers for sedimentological and chemical samples as mentioned in Section V.2 and VI.2

b) Clean the sediment grab thoroughly with hot soapy water, rinse with tap water. Avoid placing the grab sampler on the open deck, keep in a large plastic or aluminum tub while not in use

c) Clean a large sized plastic tub

d) Cock the grab sampler

e) Haul sampler on-board

f) Initially, a visual inspection should be made of the sample by means of the small trap doors on top of the grab to ensure that the sample has been collected in an undisturbed state and to determine if there is water on top of the sample. If water is present, it can be siphoned off with a glass tube or slowly drained so as not to wash the sample unduly

Note : Plastic bags or wide-mouth jars (polypropylene or borosilicate glass) should be used for temporary storage of sediments for trace metal analysis. Prior to their use, containers and glass or plastic parts associated with the sampling equipment should be cleaned with detergent and acid then rinsed with metal-free water (see Section V.2). For trace organic analysis samples should be stored in cleaned wide-mouth borosilicate glass or aluminum containers (see Section VI.2). The samples should be stored frozen, or at a sufficiently low temperature (~ 4°C) to limit biological and chemical activity. It is recommended that a minimum sub-sample size be 50 grams.

g. Once the top of the sediment is exposed, visual estimates of grain-size (coarse, medium, fine grained), colour (according to the Munsell colour chart code), and the relative proportions of the components should be made and recorded. *In situ* measurements such as Eh or pH can be made by inserting the appropriate electrodes into the sample

h. Most fine grained sediments usually have a thin, dark yellowish brown surface layer resulting from the oxidization of iron compounds at the sediment-water interface. Since in most cases this layer represents the material being deposited at the present time, it should be sampled carefully with a non-contaminating utensil such as a plastic spatula for trace metals determination. About 1-3 g should be placed in a numbered polyethylene vial, sealed and frozen for transport to the laboratory. After the surface layer has been sampled, the grab can be opened and an additional sample, representative of the subsurface, can be obtained. Only cleaned plastic utensils should be used to collect sediment samples for trace metals determination, and cleaned stainless steel utensils for organics analyses (see Sections V.2 and VI.2)

i) After the surface layer has been sampled, the grab can be opened and an additional sample, representative of the subsurface, can be obtained. Observations of this material should include colour and textural characteristics. To ensure a representative sample, about 100 grams or more should be collected and placed in a numbered vial. The sample should be frozen quickly for return to the laboratory. Larger samples of about 1 kg are required for admixtures of gravel sand and mud

j) Store all sediment samples under refrigeration (4°C) until they are transported to the laboratory (see Section IV.1)

b. Barrel core subsurface sediment sampling

Sediment subsurface samples are often taken using barrel or box corers to determine the change in lithology and chemical composition with depth in order to assess environmental changes in metal fluxes with time. Cores are usually collected in areas of fine-grained sediments but specialized corers are available for coarse-grained sediments.

The main types of corers having cylindrical barrels are : a) the gravity corer which free-falls from the ship and penetrates the sea floor by gravity, and b) the piston corer, Figure (III.7a) which is released a set distance above the sea floor, penetrates the sediment by free fall, and sucks the sediment into the core barrel by an upward moving piston as the core is retrieved.

For Trace Metal analysis, plastic core liners are placed inside the core barrels to contain the sediment core sample and to avoid the problems of extrusion and contamination that occur in unlined barrels. In general, the greater the diameter of the liner, the less will be the amount of distortion of the subsurface sediment by the corer penetrating the sediments. Core liners with internal diameters >50mm are usually satisfactory for obtaining samples for geochemical purposes.

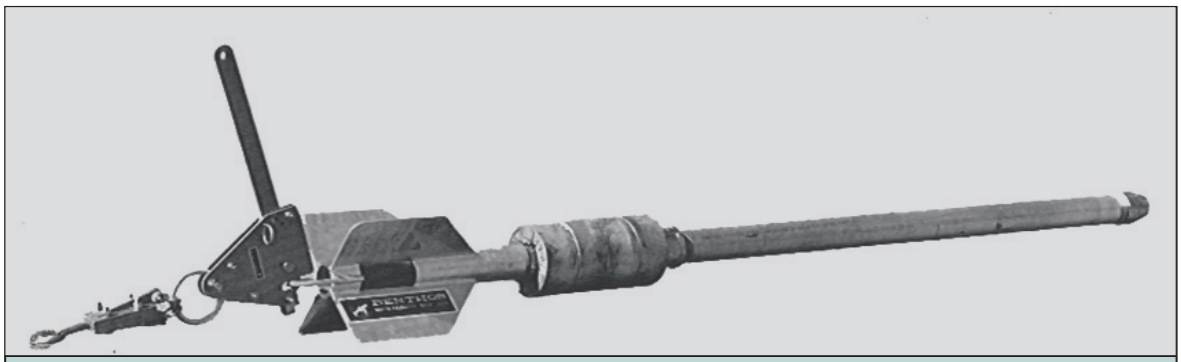


Figure (III.7a) : Piston corer sampler

After the corer is retrieved, the liners are capped at the bottom; the liner is removed from the barrel; the top is capped, and the core stored in a vertical position until all the water inside the liner has risen to the top. The liner is cut off at the sediment - water interface, capped and placed in a deep freezer or a cold room (4°C) for transport to the laboratory. Visual observations and measurements of sediment core samples should include information on the site number and location, depth, time, core length, lithology, stratigraphy, and any distortions in sediment layers.

In the laboratory, core sampling is best carried out by extruding the core upwards and slicing off layers (~ 1 cm) using a non-contaminating cutter (e.g. stainless steel, plexiglass) (Mudroch and MacKnight, 1994) or splitting the plastic core liners lengthwise, avoiding the smeared zone around the inside of the core liners and sampling the interior section of the core. The samples are placed in containers for transport to the laboratory.

c. Box core sediment sampling

Rectangular sampling devices which obtain cores about 15-25 cm square and 15-60 cm deep are known as box corers Figure (III.7b) and can be recommended for detailed sampling at or below the sediment-water interface. The advantage of the various types of box or square corers (Bouma, 1969 and Mudroch and MacKnight, 1994) is that they can recover the surface sediment and fauna virtually

intact. They can be subsampled by inserting several 5 cm diameter tubes into them. When sub-sampling, however, the core material should be taken from the mid-part of the core to avoid any "edge effects". Such samples are treated in the same way as the core samples described above.

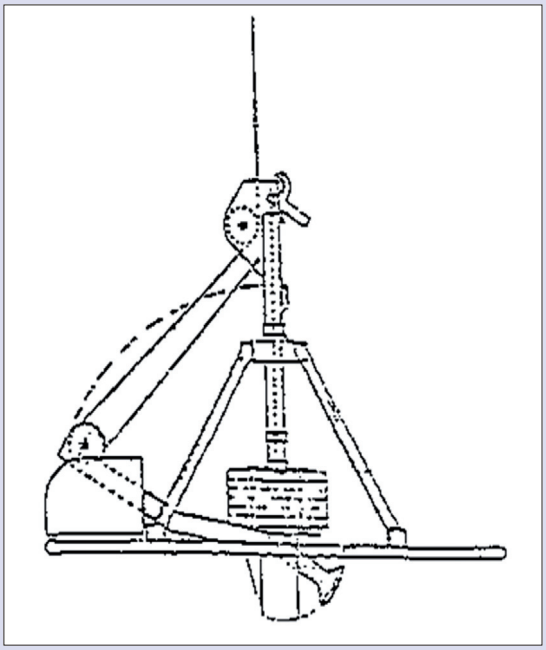


Figure (III.7b) : Reineck box corer. The square coring tube is closed by a knife edge actuated by pulling up on the lever to the left of the diagram. An attachment can be fitted to show the inclination and compass orientation of the core

III.1.2.4 Sediment Sampling for Meiofauna Monitoring Programme

Before sampling, the beach profile should be measured using Emery (1961) method or sight level. Meteorological parameters including air temperature, humidity, wind speed, wind direction and cloud cover should be determined.

Three stations of $2m^2$ should be established in each location along the transect line extending from the mean high water (MHW) to the mean low water (MLW). From each station triplicate sediment samples should be collected down to 15-cm depth using Perspex core of 3.5 cm internal diameter.

One sediment sample for biological analyses should be used for grain size analysis and another for the determination of total organic carbon (TOC), total organic nitrogen (TON), total organic matter (TOM) and petroleum hydrocarbons (PHCs). The water content of sediment samples should be determined using a separate quantity. The water temperature and the black layer depths should be recorded in data sampling sheet. Interstitial water from trench should be collected for the determination of pH and salinity.

The samples for biological analysis should be placed separately in plastic containers, preserved and stained by mixed solution of 5% buffered formaline (50 ml of formaldehide in one liter filter seawater), and Rose Bengal dye (1 g/l) for staining the organisms.

III.1.3 Biota

III.1.3.1 Plankton (Phyto and Zooplankton)

A) Sampling

The Bongo net (Posgay *et al*., 1968) proved satisfactory for collection of fish eggs and larvae as well as zooplankton for the surveys. It is recommended to adopt the Bongo net for the present survey in the ROPME Region. The towing frame of the net is made of anodized aluminum and consists of two circular frames (0.61 m in diameter), connected by a central yoke to which the towing wire is attached by a wire fastener. Therefore, there are no bridles in front of the mouth of the net.

It is well known that the errors, which can arise during collections, vary according to the mesh size of the nets. It is recommended to have two nets with different mesh size;* 0.333 mm and 0.064 mm. Sample spillage back into the net is less likely to occur with “soft” cod ends than with a “hard” cod end. The cod ends should be made of the same type of netting and mesh size as the main part of the net. It is strongly recommended that the nets and cod ends should be colour coded for easy recognition and match up of mesh size.

B) Preparation for sampling

It is essential that samples be properly labeled. Information contained on the labels should be sufficient to identify the sample with certainty. The label should be carefully fixed on the sample jar

This label should contain the following information: name of ship, cruise number, date, time, station designation, depth of tow, gear used, mesh size, type of haul, duration of haul, collector's name and number of jars in the sample.

C) Sampling operation

After oceanographic observations are recorded the sampler is assembled and the label for the plankton is prepared as outlined above. Towing may then commence. Tows off the stern are not recommended because of turbulence from the ship's propeller. Oblique tows made off either side of the ship are recommended as follows :

i) After oceanographic sampling, bottom depth is requested again from the bridge and recorded on the data sheet. The bottom depth will determine the depth to which the nets will be lowered and thus the length of wire to be let out

ii) The flowmeter is read and checked against the recorded initial meter reading. If there has been a previous tow, this should be the final meter reading. If the reading has changed between tows, the last recording is crossed out and the new recording is inserted

iii) The 22 kg weight is lowered below the surface of the water. If the ship is still slightly underway the wire (0.48 cm in diameter) is pulled to and fastened close towards the side platform

iv) Clamp the net wire to the tow line

v) The wire angle indicator is attached to the wire above the Bongo net. Enough slack is left on the line to the wire angle indicator so that when the proper angle is achieved during the tow, it will not ride up on the cable and hit the towing block

vi) Zero the winch meter or meter wheel. Signal to the bridge to start the tow. Towing speeds between 1-3 knots are recommended, depending on sea conditions, to maintain 45° wire angle. The blocks or pins, which keep the blades of the flowmeters from revolving between tows are removed. Some flowmeters have automatic blocks that release the impeller blades when water flows through them

vii) The nets are allowed to stream out briefly before lowering. As soon as it is obvious that they are not tangled, the wire is immediately let out to the predetermined amount at a constant rate of 50 $m.min^{-1}$

viii) The stop watch is started as soon as the flowmeters are seen to sink below the surface of water. The stop watch is used to record sinking time and towing time in seconds; enter these values on the data sheet. The duration of the two is used to calculate the mean velocity of towing

ix) The time the net enters the water is recorded in nautical time (2400 hours) to the nearest 5 minutes. The time of the day is used for analysis and correlation of day-night differences in fishing efficiency of the net

x) When the desired amount of wire has been let out, the stop watch is stopped, the sinking time is recorded in seconds and the stop watch is zeroed and restarted immediately. Since the net is sampling on the way down, sinking time is as important as that of retrieval

xi) When the stop watch is restarted, the nets are left at the desired depth for 30 seconds (assuming that falling nets will straighten out at depth in the 30 seconds interval)

xii) At the end of the 30 seconds, the stop watch is allowed to run, the wire angle is recorded for that depth, and retrieval is begun. Retrieval duration of 10 minutes is recommended in the Region, but if there are heavy phytoplankton blooms, shorten the time to five minutes to avoid clogging of the nets. Wire retrieval rate is calculated and held constant at each station to insure that the retrieval duration is 10 minutes and to ensure that all depths were sampled equally. During sinking, at depth, and during retrieval, ship speed is maintained to keep the wire angle at 45°. It is essential to maintain the 45°(+3°) wire angle to ensure that the proper amount of water is being filtered from each depth for the purposes of data quantification

xiii) The nets are brought directly out of the water at a steady rate. It is important not to allow the nets to sample too long at the surface because of the bias that results from over-sampling surface waters. When the flow-meters break the water surface, the stop watch is stopped and its reading in seconds is recorded as the towing time. If the flow-meters are the kind that do not have automatic blocks on the impeller blades, blocks or pins should be placed in them to stop the impeller blades from rotating as soon as possible after the flow-meters break the water surface

xiv) The nets are washed down from the outside to g*et al*l the plankton into the cod ends, keeping the net rings at rail height while the cod end is dangling. A seawater hose is used for the washing. Water pressure should be great enough to loosen the plankton adhering to the net mesh but not so high as to damage the plankton organisms. This operation usually takes only a few minutes per net

xv) After all the plankton has been washed into the cod ends, the nets are brought aboard. The colour-coded ends are removed, keeping the plankton from spilling back into the nets

xvi) The plankton sample should be preserved immediately in 5% formalin-seawater solution. This is especially critical in tropical waters

xvii) After the samples have been preserved, labeled, and stored, the cod ends are washed and replaced on the nets with matching colour codes in preparation for the next tow

xviii) Before leaving the station, the flow-meters are read and final values recorded. The initial readings, which were made before the tow began, are subtracted from the final readings to get the difference, which is the number of revolutions for the tow for each net

xix) Total towing time is recorded as the sum of sinking time and towing time. The most usual variation will be in the sinking time, caused by a slightly faster or slower rate in laying out the wire than the recommended 50 m per minute. In certain conditions, such as poor control of the ship, counter currents below the sea surface adversely controlling the net as it falls, etc., the winch operator may have to depart from the sinking time procedures to slow the falling net in order to keep it from becoming tangled. Such departures from normal procedures must be recorded in the data sheet

xx) Total towing time is added in minutes. This is actually the time the net comes out of the water

xxi) The station position is recorded in the data sheet. This may be done when the station is occupied, but is usually done at the end of the cruise when the captain has compiled a complete list of positions of all stations

It is very important that the proper amount of water has been filtered for quality of tow and data quantification. For each flow-meter, the proposed central number of revolutions should be calculated before the cruise to serve as a guide for the observer to tell if the tow has been correctly conducted. If the meter readings are not normal, the net tow may have to be repeated. A very high reading may have been caused by too great a ship's speed; check for many high wire angles. A low reading may have been due to too slow a ship's speed; check for many low wire angles. Another reason for low meter readings may be clogging of the nets. This may be cumulative if a net is not rinsed properly or it may occur at a single station. If a meter shows a trend toward lower and lower readings, and it is not malfunctioning, the net should be washed. The net tow need not be repeated if it is obvious that heavy clogging is the reason for low readings or if the ship's speed has caused low or high angles. If wire angles are normal, the net is clean, and the towing time is routine but the meter reading is low, the cause could be that a bit of detritus, a fish, or even a large jelly fish became entangled in the meter blades for a portion of the tow. Under these conditions, the tow should be repeated. If the reading is again very low and it is obvious that the flow-meter is not functioning properly, replace the meter and repeat the tow. Do not oil or grease any meter or make any repairs that might alter the rotation of the blades. Repairs of this type would seriously affect the calibration of the meter. If clogging is apparent, the amount is recorded in the appropriate box on plankton tow data sheet.

D) Checking the nets

Checks are made on the net to see if replacement is needed. If the net cannot be replaced then repair should be considered. Mending the net should only be considered when the holes are small and the damage is close to the ring. Any change should be recorded in the data sheet.

For washing the net, one of the following three methods may be used:

- The net is averted, still on its ring and brushed down with an ordinary sweeping broom and running seawater
- The rings are stood on edge, the net is tightened along its length by tying down the and hosed down with a high pressure fire hose. This is very effective provided that plankton has not dried in the meshes
- The net is detached from the ring and put in a washing machine using a 30 minute cycle

E) Preserving the sample

Glass jars holding 1000 ml are recommended as sample storage containers. Normally they are closed by a screw on plastic lid with an inside coating to prevent leakage and evaporation. The plankton sample is carefully poured from the cod end into the jar in which it will be stored. The whole net including the cod end is rinsed by spraying water from the outside to gather the remaining plankton into the jar in which it will be stored. Repeat the process, if necessary.

The jar containing the plankton is then filled three-fourths full with seawater before adding full strength formalin and sodium borate. To obtain the recommended 5% solution of formalin in a one-liter jar, 50 ml of concentrated commercial formalin is added. To assure proper buffering, 20 ml of a saturated solution of sodium carbonate in seawater is added for each liter of preserved sample. The sample jar is then filled almost to the top with seawater, capped and shaken lightly including inversion to obtain immediate, uniform preservation of the plankton organisms throughout the sample. It should always be kept in mind that formaldehyde is a poison that can irritate skin and cause serious cases of dermatitis.

F) Storage on the vessel

Sample jars should be completely filled. This is to prevent agitation of plankton in the jars during rough weather, as will happen if they are only partially filled. Even when filled, some movement of the organisms within a jar is inevitable due to rolling, pitching, etc. Hence to minimize damage to the delicate organisms during storage on the vessel, samples should be stored in the most stable part of the ship.

Temperature of storage may be important, particularly in tropical regions. This factor has seldom been given proper attention. Collections from tropical expeditions often are of poor quality, even when care has been taken in their preservation and handling. It is recommended to store the sample in air-conditioned storage rooms, if available, or in ice chests or refrigerators. See Section VIII.2 for Plankton analysis

III.1.3.2 Bivalves

The term bivalves is generally used to describe those mollusc species which typically have two shells (valves) of which mussels, clams, oysters, and scallops are common examples. Three types of bivalves have been selected for the Regional Baseline Survey of Oil and Non-Oil pollutants: pearl oysters, rock oysters, and clams. Although all three types are useful for monitoring pollutants in ambient waters, the selection of the most appropriate species for a particular area must be given careful consideration. In general, the species selected should:

- be present at a site inshore from the monthly and quarterly oceanographic collection stations
- be found in sufficient abundance to allow periodic collection of at least 25 individuals of one size range during each collection period without eliminating the species from the site
- be reasonably accessible from shore locations to allow rapid collection and transport to the laboratory
- collect enough mature organisms to provide approximately 250 grams (wet weight) of tissue
- not to be collected from metal pipes or other structures which may be sources of metal or petroleum and chlorinated hydrocarbon contamination

Note : In order to have comparable results for contaminant levels in bio-indicators on a regional basis, attempts should be made to analyze 2 kinds of bivalves by the Member States whenever possible.

The recommended sampling methods for each of the above species type are presented below :

A) Pearl oysters

Two common species of pearl oyster of the genus *Pinctada* are found in the KAP Region, *P. margaritifera* and *P. radiata* (= *P. fucata*). The precise distribution of the species in the Region are uncertain but existing information indicates that one or both species are locally abundant in coastal waters from the Kuwait-Saudi Arabia border to Oman. Both species are found subtidally on rocky substrates, commonly in relatively shallow water (5-15 meters) although deeper populations have been reported.

The simplest and most economical method of collection is by SCUBA diving. Therefore, this bivalve should be selected only in areas where they are very abundant (perhaps in Bahrain and Oman) and where qualified SCUBA divers or experienced pearl divers are available.

Care should be taken to collect specimens intact by severing the byssal threads, which anchor this species to the substrate. Once collected, rinse the outside of the shells free of any adhering sand or debris with clean ambient seawater and place in a clean plastic bag. Do not keep the samples in seawater during transport since they will open and may be contaminated by on board wastes. The best method of transporting the samples is to place them together in one large plastic bag inside a second bag in which a small amount of water is placed to maintain a moist environment. These bags should then be labeled and kept in an insulated box containing dry ice or frozen chemical ice packs. Oysters should remain closed and alive for several hours under these conditions.

B) Rock oysters

The most common rock oysters found in the Region are members of the genus *Crassostrea*. Although there is some confusion as to their exact species identification, two of these species have tentatively been identified as *C. margaritacea* and *C. glomerata*. However, if this bivalve type is selected for monitoring, exact identification should be made either by local experts or by sending representative specimens to an “international organization”.

Rock oysters are commonly found on rocky substrates in the lower intertidal zones throughout coastal areas of the Region; hence sample collection should present no problems. This type of bivalve forms dense aggregations on rocks and other permanent structures. Their colonial nature leads to the formation of clumps of individuals overgrowing each other, hence, it is difficult to collect individual organisms without crushing adjacent specimens. Therefore, the best collection method is to use a chisel and hammer to break off intact sections of these clumps taking care to avoid crushing the upper specimens. Once detached from the rocks, rinse the remaining clumps in clear seawater and place intact clumps into clean, heavy plastic bags and label. Return the specimens to the laboratory in an insulated box with dry ice or frozen chemical ice packs. At the laboratory or a suitable shore facility repack as described below.

C) Clams

There are numerous species of clams, which occur in the Region, several of which should be suitable for monitoring oil and non-oil pollutants. However, a species which is found in sandy sediments is preferred, particularly those which are filter feeders rather than sediment feeders, in order to

reduce the possibility of including ingested sediment in later analyses. Suitable clam species such as *Cercinita* spp. are typically found in sandy to muddy sediments in the mid and lower intertidal zone. Almost all the suitable clam species are found buried under the sediment. The filter feeding species are usually buried just below the surface under or alongside of boulders.

The best method of locating likely clam habitats is to look for an abundance of empty shells of the selected species, which will at least indicate its presence in the area. Collections should be made during low tide periods. Once the empty shells are located, begin turning over the medium to small rocks in the area and begin scraping the surface sediment with a three or four-fingered garden fork. These are usually available at hardware and agricultural supply stores. The type made of stainless steel or chromed steel are the strongest. If live specimens are located with little effort, sampling should be randomized along the beach face while remaining at approximately the same relative height in the intertidal zone; do not mix samples from the high and low intertidal area. Once collected, the clams should be rinsed thoroughly with clean seawater from the area and stored in moistened plastic bags in an insulated box with either dry ice or frozen chemical ice packs. Repack as described below as soon as possible.

Note : The following precautions are emphasized :

1. Do not allow samples to contact the decks of vessels or vehicles used during collection since they are significant sources of the pollutants under investigation
2. Do not include bivalves, which remain open when collected, or broken shells, which expose the underlying flesh in the sample
3. Do try to collect individuals of approximately the same size at all sampling locations

D) Mussels

Remove mussels from their attachments with the clean scraper or the rust-free knife.

Transfer a suitable number of undamaged mussels into clean thermo-insulated boxes with grid on the bottom. Collect, from the sampling site, a clean seawater sample in a suitable container to keep the mussels moist if a long transport (more than 2 hours in hot climates) is envisaged. Keep the mussels moist with the clean seawater without submerging them.

If the mussels have to be transported and stored before sample preparation for more than 24 hours place a suitable number of mussels in plastic bag. Squeeze out the air and close the bag airtight with a knot, thermo-seal, or similar. Place the bag into another bag together with a sample identification note, close airtight the second bag and deep-freeze.

This represents the “specimen sample”.

Note : The transport of mussels collected near the laboratory will not present special transport and storage problems. Mussels should be kept exposed to air and moistured with clean seawater during the transport to the laboratory. When gathered from the intertidal zone, they will survive aerial exposure for 24 hours. Mussels submerged in seawater during transport will open their valves, start pumping water and excreting waste products, while during aerial exposure their valves will remain closed and their metabolic rate is greatly reduced; therefore their submersion in seawater during transport should be avoided.

III.1.3.3 Shrimps and Small to Medium-size Fish

Place in a clean plastic bag a suitable number of the undamaged specimens collected from a fishing vessel, fish market, etc., taking care that the legs, spines, etc. will not puncture the plastic. Squeeze out the air and close the bag airtight with a knot, thermo-seal, or similar. Place the bag into another bag together with a sample identification note, and close the second bag airtight also. Deep-freeze the bag whenever possible. Use a refrigerator or a cooled thermo-insulated box only if the storage period is not too long (48 hours in hot climates).

This represents the “specimen sample”.

III.1.3.4 Large-size Fish

Determine and note the fork-length, the body weight and sex of the collected specimen.

Remove skin. Separate with a clean rust-free metal knife a portion of at least 100 g of muscle tissue. This portion must be at least 5 cm thick so that during sample preparation contaminated and dirty tissue can be sliced off. Place each portion into a separate clean bag, squeeze out the air and close the bag airtight. Place it together with the sample identification note into a second bag and close it airtight also. Deep-freeze the bag whenever possible, otherwise use a refrigerator or a cooled thermo-insulated box if the storage period is not too long (48 hours in hot climate).

This represents the “specimen sample”.

III.1.3.5 Sample Preparation

Note : All utensils and containers used should be cleaned and of appropriate material : plastic or glass for trace metal determination, stainless steel, glass or aluminum for organic analysis (see Sections V.2 and VI.2).

a. Preparatory activities

If necessary, partially thaw deep-frozen samples by placing them overnight in a refrigerator at -2°C to 4°C (partially frozen samples are easier to cut than completely thawed or even fresh samples).

Clean the knives, the dishes, the tweezers, the length-measuring board and weighing containers with detergent, rinse with distilled water or clean seawater. Cover the working area with pre-cleaned plastic sheets/aluminum foil. Clean hands carefully with detergent and rinse them with distilled water or clean seawater.

Note : If hands are cleaned and precautions are taken not to touch the dissected part with hands, bare hands are preferred to hands covered with gloves, since the operator has a much better control of instruments, etc. If possible a clean room should be used for preparatory activities.

b. Preparation of mussels, clams, oysters

Scrape off all foreign materials attached to the outer surface of the shell with a clean plastic/stainless steel knife, to be used only for this purpose or with a strong plastic/metal brush. Handle the mussels as little as possible.

Rinse each mussel with distilled water or alternatively with clean seawater and let the water drain off.

Pull out the byssus which extrudes from between the closed shells on the concave side of the shells. Weigh the whole mussel and note the weight.

Insert a second clean plastic/stainless steel knife into the opening from which the byssus extrudes and cut the adductor muscles by turning the knife as indicated in Figure (III.8) and open the mussel. Do not try to break the mussel open with the knife; if the muscles are cut, the mussel will open easily. Check if the byssus has been eliminated completely; if not, remove the remainder with clean tweezers.

Rinse the soft part of the mussel in its shells with distilled water or clean seawater.

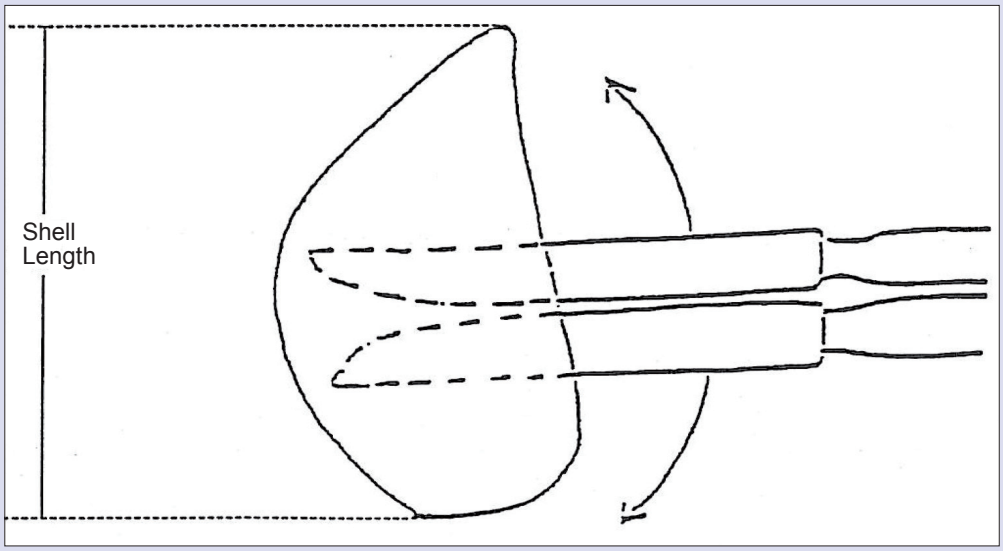


Figure (III.8) : Cutting the adductor muscle

Loosen all tissue with the second clean knife, remove the soft tissue from the shell with a pair of clean plastic/stainless steel tweezers without touching the outer part of the shells, and l*et a*ll the water drain off.

i) Single specimen sample: Weigh a clean empty container on the balance and note the weight. Then put the soft part of the mussel in it and reweigh. Note the flesh weight of the soft part. Close the container airtight, label it with the sample preparation code. Determine the length of the mussel's shell, Figure (III.8) by placing it with the inner part facing the cm scale. Note the length of the shell and the weight of the soft part of the mussel

ii) Composite sample: Fill a container of known weight with at least 10 soft parts of mussels prepared as described above. Reweigh the plastic container and note the composite fresh weight of the mussels. Homogenize the specimens in a cleaned blender, and return the homogenate in the plastic container. Note the total weight again and recalculate the fresh weight of the homogenate. Label the plastic container with the sample code

Note : i) When preparing composite samples, use mussels of similar size. The length and weight of each specimen should be determined separately before the soft parts are pooled

ii) The use of a blender with stainless steel blades should be avoided for TM analyses (in particular Ni, Cr). Instead homogenize after drying with agate mortar

Place several containers in a clean plastic bag, include an identification note with the containers sample codes, seal the bag airtight and deep-freeze. This represents the “tissue sample”.

c. Preparation of shrimps

i) Single specimen sample: Determine the length of the shrimp from rostrum to uropod [see Figure (III.9)] using the appropriate length-measuring device. Weigh the shrimp after placing a clean weighing container (plastic or aluminum foil) on the balance and note its length and fresh weight.

Separate the abdomen from the cephalothorax and the “tail” (telson and uropod) with a first knife taking care that no viscera remain in the abdomen, Figure (III.9). Cut off all legs. Turn the abdomen with the ventral side up and cut with a knife along the edges of the sterinites (ventral exoskeleton); lift the sterinites off with a pair of tweezers and discard.

Loosen with a second clean knife the abdomen muscle and lift it from the exoskeleton with a clean pair of tweezers. Determine and note the sex by examining the gonads.

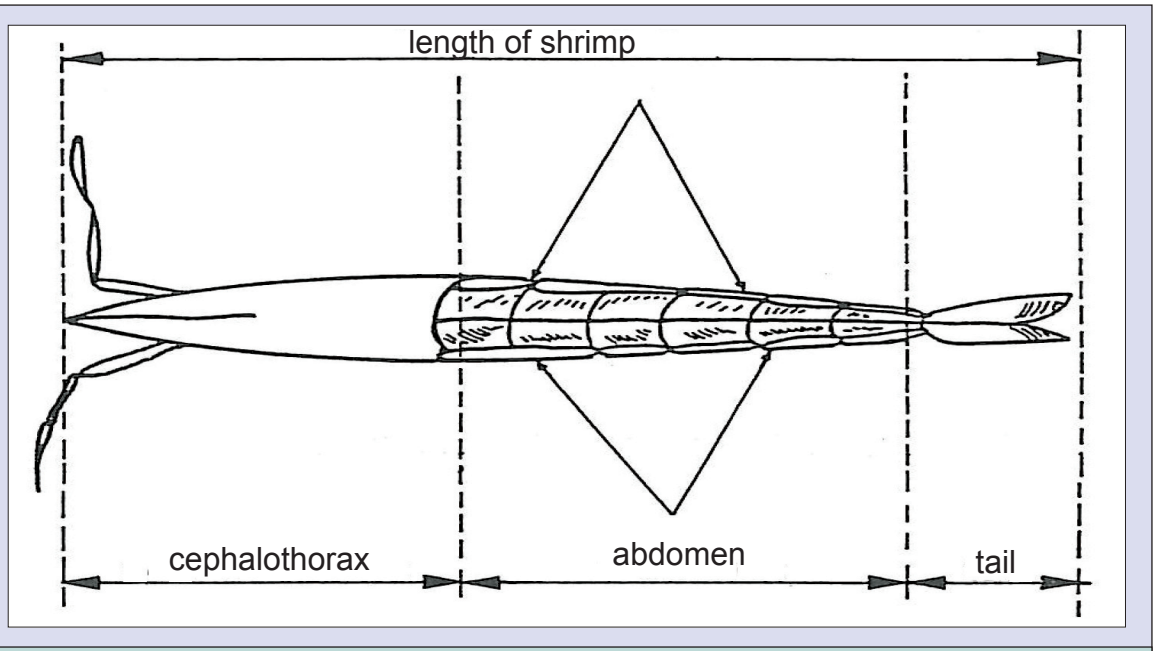


Figure (III.9) : Schematic diagram of a shrimp

Transfer the muscle with a clean pair of tweezers into a pre-weighed container, determine and note the fresh weight of the muscle. Close the container airtight, label it with the sample code, place a suitable number of containers in a plastic bag, add a sample identification note to the containers, and close the bag airtight and deep freeze the samples.

ii) Composite sample: Start sample preparation as described for single specimen sample above taking care to record length, fresh weight, tail muscle weight and the sex of each specimen separately. Reduce the tail muscle(s) of the large specimens to the weight of the smallest tail muscle. A composite sample should not contain less than 6 tail muscles from 6 different specimens of the same sex and size. Homogenize the tail muscles in a blender. Transfer the homogenate into a suitable clean container, which has been weighed empty. Close the container airtight, label it and weigh the container with the homogenate. Note the weight of the homogenate together with the other data in a protocol. Place a suitable number of containers in a plastic bag, add a sample identification note, close the bag airtight and deep-freeze the containers. This represents the “tissue sample”.

Note : The concentration of trace metals/organics in a composite sample should represent the mean value of trace metals/organics concentrations of single specimens. In order to avoid overrepresentation of large specimens, only shrimp of similar size (age) should be used for the preparations of composite samples. In addition, the weight of the tail muscles of all specimens to be included in the composite sample should be reduced to that of the tail muscle of the smallest specimen. As there might be considerable differences in the trace metal content of male and female specimens, use them in separate composite samples.

d. Preparation of small and medium size fish

i) Single specimen sample: Determine the fork-length (from tip of snout when the mouth is closed to the apex or the fork of the tail) of fish, Figure (III.10) to the nearest mm on the length-measuring board. Weigh the fish on a clean weighing container (plastic or aluminum foil) with an accuracy of 0.1% of its total weight and note both the fork-length and the fresh weight of the specimen.

Rinse the fish with distilled water or clean seawater and place it on a clean working surface. Remove the pectoral fin and cut the skin of the fish with a first knife near the dorsal fins, starting from the head to the tail, Figure (III.10).

Cut near the gills across the body, along the ventral edge from the gills to the tail and, finally, across the body near the tail. These four cuts should be carried out first on one side only taking care not to cut too deep in order to avoid cutting into the viscera and thus contaminating the fillet. It is advisable that a second person hold the fish by the head and tail during this operation.

Pull the skin from the fillet with a pair of tweezers, taking care that the outer skin does not contaminate the fillet.

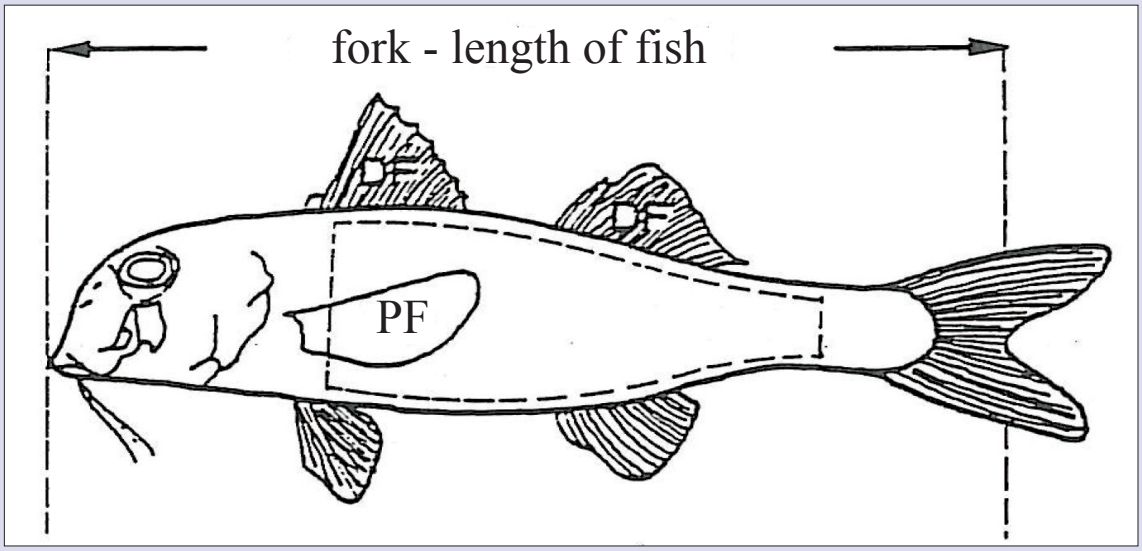


Figure (III.10) : Schematic diagram of a fish
(PF- Pectoral fin, DF = dorsal fins, dashed line shows where the cuts should be made)

With a second clean knife, cut the fillet from the vertebral column (backbone) starting from the cut near the gills. Lift the fillet with a second clean pair of tweezers, so that the fillet will not touch the working surface (e.g. the Pyrex dash) or other parts of the fish. Weigh the fillet in a clean container and note its fresh weight.

If one fillet does not yield enough material for analysis, put the fish, skin side upwards, on a clean portion of the working surface or on a new working surface and remove the second fillet from the other side of the same fish as described above, add it to the first sample and record their total weight. Close the container airtight. Identify the container with a code number and/or label, record all data in the protocol and deep-freeze.

This represents the “tissue sample”.

Determine and note the sex of fish by examining the gonads.

Note : Comparing the weight of the container holding the fillet sample(s) determined at this point with the weight of the container before the digestion step will show if the tissues have lost moisture during prolonged storage.

ii) Composite sample: Start sample preparation as described for single specimen sample above taking care to record the length, the fresh weight and fillet (sample) weight of each fish separately. Determine and note by examining the gonads the sex of each specimen separately.

Reduce the fillet(s) of the large specimens to the weight of the smallest fillet. A composite sample should not contain less than 6 fillets from 6 different specimens of the same sex and size. Homogenize the fillets in a blender. Transfer the homogenate into a suitable clean container, which has been weighed empty. Close the container airtight, label it and weight the container with the homogenate. Note the weight of the homogenate together with the other data in a protocol [see below, Table (III.1)] and deep-freeze the container. This represents the “tissue sample”.

Note : The concentration of trace metals/organics in a composite sample should represent the mean value of metals/organics concentrations of single specimens. In order to avoid overrepresentation of large specimens, only fish of similar size (age) should be used for the preparation of composite samples. In addition, the weight of the fillets of all specimens to be included in the composite sample should be reduced to that of the fillet of the smallest fish. As there might be considerable differences in the trace metal content of male and female specimens, use them in separate composite samples.

e. Preparation of large-size fish

If necessary, thaw partially, e.g. overnight in a refrigerator (-2 to 4°C), the sub-sample taken in the field during sampling.

Rinse the sub-sample with distilled water or clean seawater and place it on a clean working surface. Remove any skin and bone that may be present. Cut off thin slices from all surfaces with clean plastic knife and discard them. Repeat the operation with a second clean knife in order to obtain a clean uncontaminated block of homogeneous tissue.

Note : It has been recognized that differences in trace metal concentrations may exist between different muscles in large fish, therefore as much information as possible on the actual sample should be recorded.

Transfer the tissue into an airtight container, close and label it, weigh it, note all data together with data of the sub-sample in the protocol, and deep-freeze.

This represents the “tissue sample”.

III.1.3.6 Sampling and Sample Preparation Protocol

Fill in the sampling and sample preparation protocol, Table (III.1) giving full details in every column. This protocol should be attached to the test report on the determination of trace metals/organics in the analyzed sample.

The following guidelines should be kept in mind when completing the protocol (the numbers refer to those used in Table (III.1) :

1.1 Use the scientific name for the species sampled. If necessary indicate subspecies or variety

1.2 Indicate the name under which the species is known locally

1.3 Use any code adopted by your institution. Never use the same sample code for more than one sample

3.2 For samples obtained on fish market, indicate the town (village) where the market is. For samples taken at standard sampling stations or areas, indicate the name (code) of the station or area

3.3 If the sampling point does not coincide with a standard sampling station or area, it may be advisable to code (name) it, in particular when the sampling point is used more frequently (e.g. particular fish market). Never use the same sampling point code for more than one sampling point

3.4 Always indicate the longitude and latitude of the sampling point to the nearest minute. For samples obtained from fish market, enquire about their provenance and try to reflect it also as geographic coordinates. Circle either E or W and N or S as appropriate

3.5 Give any additional information, which may be relevant for the interpretation of the results (e.g. sampling point in vicinity of outfalls or similar)

4.l Indicate the difference between data given under 2 and 5

4.2 Mark the storage conditions used. If none of them applicable, give additional explanations in 4.3

4.2 Identify sex of the specimen whenever possible. As for specimen length, determine shell length for mussels, fork length for fish and total length for shrimp as indicated in Figures (III.8), (III.9) and (III.10). Specimen weight always refers to the fresh weight of the whole mussel, of the whole shrimp and of the whole fish. Note that sample weight, in the case of mussels, refers to the total weight of soft tissues. In the case of shrimp, the sample weight refers only to the fresh weight of the muscle, and in the case of fish, to the fresh weight of the fillet or of the combined weight of fillets removed from the same fish

4.3 Whenever possible use six or more specimens of the same sex and size (age) in preparing composite samples. Mean length and weight refers to the arithmetical mean of the weight and length of individual specimens, as explained above. Always calculate the standard deviations

Table (III.1) : Sampling and Sample Preparation Protocol

1. Sample (specimen):
1.1 Scientific name: _____
1.2 Common name: _____
1.3 Sample code: _____

2. Date of sampling: day : _____; month : _____; year : _____

3. Sampling point:
3.1 Country: _____
3.2 Type of sampling point: _____ fish market;
_____ sampling area/station
3.3 Sampling point code: _____
3.4 Longitude: _____° _____' E or W
3.5 Latitude: _____ ° _____' N or S
3.6 Conditions at sampling point which may be relevant for the interpretation of results:
_____
_____
_____

4. Sample storage:

4.1 Duration of storage: _____ hours; _____ days

4.2 Storage: deep-freezing _____; cooling _____

4.3 Factors relevant to sample storage which may be important for the interpretation of results:

_____

_____

_____

5. Date of sample preparation: day _____; month _____; year _____

6. Sample preparation:

6.1 Tissue type (kind): _____

6.2 Single specimen sample: sex _____; specimen length _____ cm; specimen weight _____ g; sample weight _____ g

6.3 Composite sample:

- number of specimens _____; sex _____
- mean length of specimens _____ cm; stand. dev. _____
- mean weight of specimens _____ g; stand. dev. _____
- total weight of composite sample _____ g
- total net weight of homogenate _____ g

6.4 Factors relevant to sample preparation which may be important for the interpretation of results :

_____

_____

_____

7. Full address of the institution carrying out the sampling and sample preparation:

_____

_____

_____

8. Name(s) and signature(s) of the person(s) who carried out the sample preparation:

_____

_____

_____

Date: _____

III.1.4 Atmospheric Component

Sampling of precipitation may be achieved manually and automatically. Manual collection equipment consists essentially of a funnel and collection bottle. An automatic precipitation collector includes a cover, which makes a tight seal around the orifice of the sample container to prevent contamination and evaporation during dry periods. The usual sampling period is one week. For special applications, the sampling period is selected according to meteorological conditions (event sampling).

Suspended particulate matter is monitored with high-volume samplers (Hi-Vols.). A High Volume sampler consists of three basic units: housing, filter adapter and motor blower. The determination of the mass of the aerosol sample collected on the filter is done by weighing it under defined conditions before and after exposure.

III.1.4.1 Sampling of Precipitation

The determination of the chemical constituents of precipitation, either dissolved or suspended, consists of two distinct phases: the collection of samples and the analysis of constituents. It is essential that the collection procedure results in samples of wet deposition only and not in bulk samples, which consist of wet deposition plus materials, which have entered the collector during dry periods. For the measurement of chemical constituents in wet deposition, it is necessary to have the collector exposed only during periods of precipitation and to have it tightly closed at other times. This may be achieved manually or automatically.

Matter is transferred from the atmosphere to the surface of the earth by a number of processes during both wet and dry periods. During wet periods, the transfer occurs mainly by scavenging (in-cloud “rain-out” and below-cloud “wash-out”) and gravitational sedimentation of the precipitation elements. During dry periods, matter in both gaseous and particulate phases is transferred by several processes, including gravitational sedimentation, turbulent diffusion and molecular diffusion. The final removal processes are adhesion (impaction, absorption) and chemical reaction. Collectively, these latter mechanisms are referred to as dry deposition processes.

An important process for removal of trace constituents is the mechanism of precipitation. On the most general sense, wet deposition is that which is removed from the atmosphere and deposited on the earth’s surface in precipitation. Dry deposition is gaseous and particulate matter, which is transferred to the earth’s surface by means other than precipitation. To remain representative on a regional basis, precipitation monitoring must exclude dry deposition. If dry deposition is avoided, rain-out (removal process in the cloud), and wash-out (removal process below the cloud) are the mechanisms, which control the composition of the sample. Both aerosols and some reactive gases are collected by precipitation. Proper sampling and analysis of precipitation provides a measure of the burden of trace substances in the lower troposphere and of the ability of the atmosphere to cleanse itself.

a. Manual sampling equipment

The advantages of manual operation are :

i) it is economical if manpower is already on site
ii) no power source or batteries needed
iii) no maintenance or repair is required
iv) there is a limited splash effect in very heavy rain
v) it is more suitable for “event” sampling
vi) it has a good collection efficiency

Manual collection equipment consists essentially of a funnel and collection bottle. The funnel may be made of stainless steel or resistant glass-like Pyrex or Jena glass. Any glass from which sodium or any other element may be extracted should be avoided. The collection bottle may be of polythene. It has been found that a funnel diameter of 15 cm, coupled with a collection bottle of two liters, is satisfactory in temperate climates, with 10 mm of precipitation giving about 200 ml of water sample. However, the size of the collector should be chosen to best meet local rainfall conditions, with care being taken to ensure that normal extremes in participation can be accommodated.

The size of the funnel opening is not considered significant with respect to sampling composition of precipitation. For comparative purposes, however, it is necessary that precipitation measurements be made with a standard national gauge at the same site. Such an arrangement will allow for the normalization of the deposition amount.

Airborne particles bigger than about 10 μm radius that remain un-dissolved in the sample are not to be regarded as representative. To avoid interaction with the liquid sample during the exposure period, and during mailing and storage prior to analysis, a suitable filter should be inserted between the funnel and the collection bottle. A 20 μm-mesh, chemically inert screen will remove insects and debris. Small-mesh screens may be necessary for the removal of finer particles. If this is not practicable because of high intensities of rainfall and low filtration velocity, fiber filters or other coarser filters may be used. The porosity should not exceed 30 - 50 μm, otherwise the pollen and spores that may exert an effect on the liquid samples are not eliminated.

Where a funnel and a polyethylene bottle are used, it is recommended that these be mounted in a cabinet. Besides offering protection, the cabinets also make it possible to supply heat to the unit for the collection of occasional snow. Well-insulated cold boxes, such as those for picnics, have been found suitable for this purpose.

In warm climates, the darkness within the box helps to suppress the growth of algae in the water sample. In some cases, however, it will be necessary to add a chemical suppressant, such as toluene, chloroform or hydrogen peroxide.

Since the cabinet exerts an aerodynamic influence on the funnel, it is preferable to mount the funnel inside a cylindrical case, much in the same way as certain recording rain gauges are mounted. This cylinder may have a conical-shaped top part with the funnel protruding slightly above it. The lower end of the cylinder opens into the cabinet, so that warm air during winter has access to the outer walls of the funnel, melting any snow, which has been deposited. This prevents formation of “snow bridges” in the funnel, which may occur in winter when the upper part of it is exposed to outside air.

The outside of the funnel should be painted black to prevent light from entering the cabinet. The funnel is connected by a glass tube to a 2-litre polyethylene collection bottle, through a rubber stopper with a small air vent.

The cabinet is conveniently and safely heated with 20-30 watt heater supplied by a 24-volt AC line. If the cabinet is well insulated and not unduly large, the required power should not exceed 25 watts, even during severe cold spells. The thermostat should have an accuracy of 2°C.

The collection efficiency of an arrangement, as described above, is somewhat impaired if the site is exposed to strong winds. This problem, however, is not different from that of ordinary rain gauges and wind screens can be used to reduce this effect.

b. Automatic sampling equipment

The advantages of automatic operation are:

i) it can be sited with more flexibility, i.e. not necessary to place at a 24-hour manned station
ii) human error is eliminated
iii) the possibility of human contamination is reduced
iv) samples should be more uniform (chemically)

It is recognized that automatic collectors have not yet reached a degree of reliability necessary for unconditional recommendation of their use: also, there may be financial constraints, lack of technical service and expertise, or lack or a power source that makes manual collection more desirable.

The following assessment of the current state of automatic precipitation collectors is based upon experience gained and results obtained from collector intercomparison studies carried out by Cornell University in the United States, the Atmospheric Environment Service in Canada, and the Finnish Meteorological Service. Testing in all cases was carried out in similar northern temperature climates and thus the findings may not be directly applicable to other climates.

In general, desirable features of an automatic precipitation collector include the following :

i) rugged construction, reliable mechanical and electrical operation to withstand long periods of operation in the field with minimum loss of samples from malfunctions
ii) high collection efficiency, proper aerodynamic design and a reliable sensing unit, which opens the collector at the onset of precipitation and closes it at the end
iii) a cover which makes a tight seal around the orifice of the sample container to prevent contamination and evaporation during dry periods
iv) construction of materials which do not cause sample contamination
v) design that minimizes splash

Collectors must be of sufficiently rugged construction and mechanical strength to withstand conditions such as large temperature differences, wind buffeting, heavy snow loads and freezing precipitation. Materials and mountings must be corrosion resistant. The cover assembly, in particular, must be strong enough to withstand snow-load and freezing precipitation and must position itself accurately and positively.

The cover must make a tight seal around the sample container orifice in order to prevent the entry of dust, insects, etc., and also to prevent sample evaporation and the exchange of pollutant gases with the atmosphere during dry periods. A cover which can be moved down vertically to sit firmly on the orifice appears to be superior to one which slides across the opening.

The cover must be designed to prevent any splashing from occuring between it and the sample container during rainfall. The complete removal of the cover from the vicinity of the orifice is

the best solution. A less desirable technique is to put screening on top of the cover to reduce splashing. Again, it should be stressed that the present automatic collectors have not been tested in tropical climates and torrential rain may cause problems.

Some of the collectors tested were supplied with two containers, one for wet deposition and the other for dust-fall. Such a gauge, in particular with regard to dry deposition, is influenced by wind direction.

The sample collecting orifice must retain the same geometry in order to allow calculation of the area used in determination of the deposition rate. In cold climates, the collector must be deep enough to prevent “capping” during snowfall although a funnel is excellent for rain. A heater in the sample housing, which melts snow causes evaporation and is not desirable.

The motor, which moves the cover, must be sufficiently strong to move it during heavy snowfall or freezing rain, and to close it firmly on the orifice. Switches, which activate the motor for covering positioning, must be precise and reliable. To prevent damage to the motor, they should automatically disengage if the lid fails to open.

The sensing unit, consisting of the sensor, heating unit and thermostat, must retain its temperature setting to ensure reproducible opening and closing. Desirable features include a sensor with an AC mode of operation to prevent electrolysis and corrosion. Sensor-grid mountings require protection against short-circuiting from excess water.

c. Collector material

Sample container materials must be selected on the basis of the species being analyzed and the type of material should be standardized throughout a network. Plastics (polyethylene) are normally recommended for collection of inorganic ions, glass (pyres, pretreated, or aged) for organics, and stainless steel for organic and some inorganic species. Absorption of some metals occurs in plastic; there is breakage problem with glass and a cleaning problem with stainless steel. It is claimed that acidification of collections in plastic will fix the metals for subsequent analyses. Obviously this will prevent accuracy in other determinations.

The outer surfaces of collectors should be resistant to chipping and should not be able to contaminate the sample.

d. Sampling procedure

The usual sampling period is one week. For special applications, the sampling period is selected according to meteorological conditions (event sampling). To avoid deterioration and evaporation of the precipitation sample during the exposure period, precipitation samples at the end of each main precipitation event can be transferred to special storing bottles kept in a cool and dark place until the end of the total exposure period.

On the first day of a new sampling period, a well-mixed portion of the precipitation caught is transferred to a clean one-liter or half-liter polyethylene mailing bottle (as appropriate), and sent to the laboratory carrying out the analysis. The laboratory should normally provide the mailing bottles and they should be carefully labelled. The information should contain the station name,

code or number, the sampling period, the total amounts of precipitation collected in the collector and the national gauge. A lead pencil can be used for paper labels; however, the safest technique may be to write directly on the bottle with a felt pen containing black, quick-drying ink.

During the handling of sampling equipment, all possible contamination of samples must be avoided. Precipitation may have a very low concentration of dissolved substances, and any additional contamination by careless handling can easily give erroneous results.

The importance of proper handling can be seen when considering perspiration. It contains about one per cent sodium chloride, whereas precipitation contains about 1000 times less. One drop of perspiration added to a 900 ml sample could double the concentration of sodium chloride. If perspiration evaporates, the salts accumulate and may even form tiny particles coating the hands and fingers. The station operator should, therefore, always rinse his hands with water and dry them on a towel before working with the sampling cabinets. In addition, the following general precautions should be taken in order to avoid undue contamination of samples :

i) never touch the inside of the funnel with hands
ii) never touch the inside of the neck of the samples bottles with fingers
iii) when pouring liquid precipitation from the collector into the mailing bottle, do not use a funnel - pour it directly. If some water spills, no harm is done as the collection bottle normally contains ample excess
iv) never touch the glass tube from the tunnel below the rubber stopper
v) before inserting a new collecting bottle, clean the funnel with distilled water

It is advisable to inspect the sampling unit once a week to see that everything is in order.

The technician should also have a suitable form to record any unusual events, which may influence the results; for example, very high winds (storms), severe snow drifts, large objects found in funnels, failure of heating, etc. This can be done on a form supplied by the laboratory containing a few pertinent headings. A suitable form for this is illustrated in Table (III.2).

e. Collection efficiency

While recognizing that standard meteorological rain and snow gauges have collection efficiencies of less than 100%, it is necessary to strive for high efficiency in precipitation collectors. The following factors contribute to low collection efficiency in collectors, which accumulate samples over the period of a month:

i) poor aerodynamic design (irregular shape)
ii) evaporation of sample
iii) lack of sensitivity of sensing unit

The first point is a general problem for standard meteorological gauges, particularly in snow conditions. Wet deposition collectors, because of their lid mechanisms, cannot be as efficient at collection; however, lid design should interfere as little as possible with the air flow. Sample evaporation may be minimized by a tight cover seal. The collection of the initial precipitation during an event is necessary because the highest concentration of constituents generally

occurs at this point. Thus, a very sensitive sensor setting is required. However, during periods of intermittent light drizzles, a sensitive setting will result in frequent opening and closing of the cover. Thus, adjustable lurid sensitivity is essential and must be regulated for different times of the year and for various rainfall regimes.

Table (III.2) : Forms for station attendants

Record below unusual events by inserting dates in the proper places :

Duration dates : Day : ----------

Month : ----------

Year : ----------

Event : ----------

High winds (storms) : ----------

Heavy snow drift : ----------

Objects found in funnels (indicate what kind) : ----------

Power failure : ----------

Precipitation events missed (estimated amount) : ----------

Any other unexpected event (please specify) : ----------

It is noted here that a sample volume reduced by evaporation will result in an erroneous (high) concentration value, whereas, a sample volume which is too low, as a result of poor - collection - efficiency, will result in an erroneously low deposition value. To aid interpretation, it is essential that total sample volume be reported, and that a standard meteorological gauge be used and read at the site, and reported in order to establish a correlation between the gauge find the contents in the collector.

f. Sample storage

Sample swindling in the field must be kept to an absolute minimum. Procedures such as taking aliquots or transferring to shipping containers, greatly increases the risk of contamination. It is preferable to use the same container type consistently throughout the year.

Sample volume or mass should be determined in the field without removing the sample from the container. Although a field measurement of pH and/or conductivity would be valuable in checking the integrity of samples, this requires more handling, trained personnel and added expenses. It is not recommended as a routine procedure.

All samples should be labelled in the field with the following information :

i) day, month, year and time of sample collection period (beginning and end)
ii) site name, collector type, orifice diameter
iii) sample amount
iv) catch of standard gauge offer collection period
v) any visible contamination or unusual events
vi) handler's name

Samples received at the laboratory may still contain particles bigger than 10 µm radius or particles that have been formed during exposure, storage and mailing, especially algea and fungi. Before sub-dividing the sample, to start the analysis, these particles should be removed by filtration.

If analysis is to be done within a month, the samples can be stored in a dark cupboard or dark-room. If analysis will take place much later, it is advisable to keep the bottles in a storage compartment kept at about +5°C.

The composition of a sample changes in the collector over the collection period of a month. NH_4, NO_3 and pH are particularly susceptible to changes. Sample stability varies with location and should be checked from time to time.

III.1.4.2 Sampling of Wet Deposition

The continuous deployment of open collectors will result in the sampling of both wet and dry deposition (generally called “bulk” sampling). This procedure has the advantage of simplicity, but, by integrating sampling over long periods of time, increases the potential for local contamination. Furthermore, this approach loses a lot of useful information, which can be derived from sampling wet and dry deposition separately. In general wet-only collection procedures, in which sampling is only carried out during periods of precipitation, are to be preferred, though it is recognized that in some situations deployment of open collectors is the only available practical method. The procedures for deployment, collection and preservation of bulk samples is the same as that for manual sampling of wet-only precipitation described below, except for the longer sampling intervals. Wet-only deposition can be sampled by either manual or automatic procedures.

Manual sampling involves manually opening collectors as soon as precipitation starts. This is usually done by deploying the collector encased in a plastic bag and simply removing the bag at the start of precipitation. This approach does not require the purchase and maintenance of automated collectors

or the availability of power on site but does require manning of the site by trained operators. In practice it is usually impossible to define exactly when the precipitation starts and finishes, and periods of several hours can elapse between the cessation of precipitation and closure of the collectors. Manual sampling is thus labor intensive and often does not generate as distinct definition of wet and dry deposition as automatic procedures. If the site is not to be manned continuously it may be possible to sample on pre-selected precipitation events. However, this may be difficult if sophisticated meteorological facilities are not available nearby for predicting events. Despite these problems manual operation is in some circumstances a useful and cost effective procedure for sampling wet deposition.

The dimensions of the sampling system can be varied to suit local conditions. Unless the design is matched to that of a standard rain gauge, the collection efficiency for precipitation will be uncertain. However, it is common practice to use a collector of this type to sample the precipitation and then use information from a standard rain gauge to calculate wet-deposition rates.

The sampling process can be made more sophisticated by the introduction of switching gear to allow sampling only when the wind is from certain directions and atmospheric condensation nuclei counts are low. Such devices can be very useful, but such sophistication should only be contemplated in a large and well-developed programme, due to the inevitable complications, which arise with more complex equipment. It is important to carry out regular “sampling blank” determination. For this procedure filters are deployed by the normal procedures and for the normal lengths of time but the pumps are switched off. The sampling blank is determined from any increase in trace metal concentrations on the filter compared to filters stored in the laboratory. The sampling blank should be small compared to sample concentrations and if they are not the source of contamination should be investigated and eliminated.

In the laboratory under clean room conditions, filters are removed from filter assemblies, folded over with the exposed face inward and stored in acid cleaned plastic bags or vials.

Prior to GF-AAS determination, the atmospheric particles on the filters must be dissolved and this can be done by several procedures.

a) Most contaminant elements are relatively soluble and soaking the filter in 20 ml of l% nitric acid will dissolve most of the metal, and this solution can then be analyzed directly. This approach offers the advantages of simplicity and safety and can be useful in a semi-quantitative regional survey, but such an approach will present uncertainties in absolute concentrations and may be inappropriate for intercomparisons between various groups.

b) The alternative, therefore, involves total digestion of the filter and the particles. This can be done by placing the filter in a PTFE beaker, adding 5 ml of concentrated high purity nitric acid and slowly boiling the mixture to dryness. A 1:1 (v/v) mixture of high purity nitric and hydrofluoric acid is then added (2 ml) and the solution gently boiled to dryness The residue is then re-dissolved in 1% hydrochloric or nitric acid This approach should be adequate to completely dissolve a membrane filter and about a quarter of a glass fiber filter and associated particles, but since this does depend on the temperatures used this should be checked. Several safety features must be noted. First PTFE beakers will only tolerate gentle heating high temperatures melt them. Secondly concentrated hydrofluoric and nitric acid are dangerous, particularly hydrofluoric acid and great care must be exercised. All

handling must be done wearing highly acid resistant plastic gloves and in a very efficient fume cupboard. Persons unfamiliar with the use of these acids must consult appropriate texts on laboratory safety before using this method.

It should be noted that hydrofluoric acid cannot be redistilled in a glass apparatus since it destroys glass. The best available quality hydrofluoric acid should be purchased and stored safely.

c) In many cases concentrated nitric acid alone is an adequate digestion method, though this will not be the case for aluminum and silicon.

d) Some alternative techniques for dissolving solid samples collected on fibrous, porous and glass fiber filters are described by Rovinsky and Wiersma (1987).

Blanks of cleaned but unused filters should regularly be carried through the digestion and analytical method.

III.1.4.3 Sampling of Aerosol

Particulate trace metal aerosols are measured by drawing known volumes of air through a filter- In general two types If samplers are available - high volume samplers (Hi-Vols) with flows of 1-2m^3 air/min and low volume samplers which displace 1 m^3 air/hr or less.

High volume samplers are described in reference method 24, and these systems have been adapted for trace metal sampling by replacing the stainless steel intakes and filter supports with cleaned plastic materials and by separating the filter assembly from the motor via a plastic pipe in order to avoid contamination from the motor. The filters used in these samplers are 500cm^2 glass fiber or cellulose filters and these should be pre-cleaned by acid washing. In areas of high aerosol concentration, filters may sometimes not need washing. Cellulose filters such as Whatman 41 have lower blanks for trace metals and are therefore usually preferred. Earlier concerns about the efficiency of collection of this type of filter appear to have been overcome. The full acid cleaning system described earlier will destroy the filter but if the filters are supported on polyethylene screens they can be soaked in more dilute acid solutions. Soaking for 24 hours in 10% (v/v) reagent grade concentrated nitric acid, rinsing with de-ionized water, soaking for 24 hours in 10% (v/v) reagent grade concentrated hydrochloric acid, soaking for 24 hours in high purity-de-ionized water and final rinsing in high purity-de-ionized water followed by air drying, with all procedures carried out under clean room conditions, should yield filters with very low blanks. High volume air samplers can sample relatively large volumes of air in relatively short periods of time and thus have considerable advantages. In addition it is often possible to sub-sample the filter and analyze for several different purposes though this will depend on the computability of sampler design and cleaning procedures with other analyses (e.g., plastic samplers are incompatible with trace organic analyses and the sample cleaning procedures say compromise the filter for use in sampling for acid aerosols).

A simpler relatively inexpensive alternative to high volume sampling is a low volume sampling system. A standard 47 mm polycarbonate filter holder with 0.45 µm membrane filters designed for conventional water filtration can be used. The filter head should be sheltered from precipitation and a shelter can either be made from polypropylene sheet or from 2 liter wide mouth polyethylene bottles. A plastic tube then leads from the filter assembly to a sir pump with a gas meter mounted on the outlet to monitor the air flow. This should be located far enough away to avoid contamination of the sample. Flow rates

of the order of 1m^3/hr are used and in most environments this yields a suitable sample within 24 hours, though it will be necessary to experiment with sampling times at any location to determine to appropriate sampling interval. Flow rates should be regularly monitored. In order to minimize the risks of contamination filters should be loaded in filter holders (both low and high volume systems) under clean room conditions then transported to the field in a plastic bag. The used filter assembly is then removed and placed in a plastic bag and the new one installed. Disposable plastic gloves should be worn throughout the filter changing procedure.

Manual collection systems consist of a collection funnel attached to a sample bottle. If the collection programme is for wet-only precipitation, the use of mesh coverings to prevent large pieces of debris entering, or devices to minimize evaporation are not necessary. For bulk sampling programmes such additions may be useful, though it should be recognized that the simplest apparatus is the easiest to keep clean, and that a mesh covering can be a source of contamination and of reduced sampling efficiency.

One successful design for manual collection is the global-bulk collector designed by Keene and Galloway. This consists of 2 liters polyethylene bottle attached directly to a 27.9cm diameter polyethylene funnel via a screw thread machined into the plastic base of the funnel. A drip shield over the join prevents water running down the outside of the funnel and into the bottle. For samples for mercury analysis, glass funnels and PTFE bottles are recommended with a glass shroud to act as a drip shield.

Automatic wet-only collectors are available. These incorporate a precipitation sensor, which opens the lid of the collector as precipitation starts and then closes it at the end of the precipitation event. Such equipment is available commercially or can be manufactured. Such collectors offer a standardized process of collection and only require visits from trained personnel at appropriate sampling intervals. Several of the available machines are reliable though interruptions of sampling programmes due to instrumental failure are inevitable. The equipment requires an electricity supply. The features of these instruments are reviewed in Reference Method 24 of this series.

Commercial wet-only collectors require some modification prior to use for trace metal work and in some cases such options are available at the time of purchase. Such modifications include the use of a plastic covered lid, covering of all other exposed metal with plastic or PT9E and the displacement of the metal precipitation sensor grid to a side arm away from the sample collector. The buckets provided for sample collection are a source of contamination but this can usually be eliminated by lining the inside of the bucket with an acid cleaned heavy-duty clear plastic bag and collecting the sample in this bag. Some experience and care is necessary when installing bags to ensure that they do not blow out of the bucket under windy conditions. The lid should move away from the collecting bucket sufficiently far to avoid drops from the lid falling into the bucket. For this reason the collecting bucket should be upwind of the rest of the collector under normal prevailing wind conditions. The underside of the lid should be regularly wiped clean with de-ionized water and paper tissue, and checked to see that it forms a good fit over the bucket.

Both manual and automatic collectors can suffer problems with heavy snow falls. Many of these can be overcome by minor modifications to the collectors (see Topol, 1986), which are now standard on many models, though in some cases a heater system nay need to be installed.

Collectors of all types should be deployed on non metal, plastic/PTFE coated or painted metal supports with the collectors well above the support. If collectors are to be deployed for extended periods,

biological growths can occur in the samples. This effect can be reduced by darkening the collectors via black outer bags. The addition of biological inhibitors is probably counter-productive because of their potential for contamination. Shorter deployment times are the best solution.

In the case of rainwater samples collected for mercury analysis the possible loss of mercury by volatilization is usually countered by putting high purity nitric acid in the collection bottle prior to deployment - The volume of acid required depends on the rainfall, which is of course unknown. However, 2 ml of concentrated acid should be sufficient to maintain a very acid solution under all but the heaviest rainfall conditions. In the case of prolonged deployments when losses of mercury nay be more of a problem, further additions may be necessary, and both gold chloride and potassium permanganate have been used. In the case of long deployments the aim will be to achieve conditions that are similar to those used for storage. For mercury recommended conditions are pH 1.0 and 30 ppm Au/l.

For all other trace metals, for which volatility is not a problem, pre-acidification of the samplers before deployment is not recommended for three reasons. Firstly concentrated acids will damage any plastic sampler other than PTFE. Secondly the first rain collected will be subject to very acid conditions, which may affect the particulates associated with the rain. The third reason is that volatilization of the acids can damage the collector and contaminate other samplers on the site, which may be being used for the collection of rainwater to determine the acids in the rain. For these reasons post-depositional acidification is recommended. Samplers are collected from the field, covered with plastic bags and returned to the laboratory. The samplers are weighed to estimate the volume of water collected and then acidified, under clean bench conditions, with high purity concentrated nitric or hydrochloric acid to give a final solution that is 0.4% (v/v) in acid. If the sample is to be stored in a different container, then the sample should be left soaking in the sampler for 24 hours in order to desorb any adsorbing trace metals. The sample is then decanted into a storage bottle and under these acid conditions the sample is stable indefinitely.

Under the above acid storage conditions most particulate matter is dissolved, though clay lattices will remain intact. However, agitation of samples prior to injection into the GFAAS system usually ensures that representative samples of such fine particulate matter are injected into the furnace and hence analyzed.

Blank procedures to check for contamination during sampling should be carried out at regular intervals. This can be done by deploying a collector, opening it briefly and pouring in 200 ml of high purity water and then leaving the collector closed (and electricity disconnected for automatic collectors) for the normal period of sampling. After this time the sample is retrieved and treated as a sample. The sampling blank" is assessed by comparing the concentration of trace metals in the acidified high purity water after storage in the sampler, with acidified high purity water that is stored in the laboratory. The sampling blank should be small compared to the concentrations found in samples and if this is not the same then the source of the contamination should be traced.

Samples of storage acid should be retained for analysis so that any blank from this source can be quantified. Occasionally samples will be recovered which contain obvious signs of contamination such as bird droppings, insects, visible soil dust or plant debris. In general, such visibly contaminated samples will be contaminated for trace metals, and in many laboratories it is considered pointless to analyze such samples. They are simply noted as having been collected, judged to have been contaminated and discarded. Regardless of the protocol, the samples should be inspected and any visible debris noted.

Precipitation Samples: Field Reporting Form

1	Sampling station	_____
2	Sample number	_____
3	Type of sampler	_____
4.	Date and time sampler deployed	_____
5.	Date and time sampler opened (if known)	_____
6.	Date and time sampler closed (if known)	_____
7.	Date and time sampler retrieved	_____
8.	Weight of sample collector	_____
9.	Weight of sample collector + sample	_____
10.	Weight of sample	_____
11.	Amount, type and batch of acid added	_____
12.	Date and time of acid addition	_____
13.	Date and time of transfer of sample from sampler to storage bottle	_____
14.	Rainfall measured by rain gauge	_____
15.	Estimated wind speed and direction at deployment	_____
16.	Estimated wind speed and direction at retrieval	_____
17.	General observations on sample (e.g., debris, collector stuck, etc.)	_____
18.	General observations on weather	_____

Aerosol Samples : Field Reporting Form

1.	Sampling station	_____
2.	Sample number	_____
3.	Type of sampler	_____
4.	Date and time sampler deployed	_____
5.	Date and time sampler switched on	_____
6.	Date and time sampler switched off	_____
7.	Date and time sampler retrieved	_____
8.	Total sampling time	_____
9.	Initial air flow	_____
10.	Final air flow	_____
11.	Note any intermediate measurements of air flow	_____
12.	Volume of air sampled	_____
13.	Estimated wind speed and direction at deployment	_____
14.	Estimated wind speed and direction at retrieval	_____
15.	General observations on sample (e.g. colour of filter)	_____
16.	General observations on weather	_____

III.2 ANALYSES OF WATER

III.2.1 Dissolved Oxygen

Scope and field of application

The chemical determination of oxygen concentrations is based on the well-known method first proposed by Winkler in 1888, and which has been modified for the determination of oxygen in seawater by several authors. The chemical procedure outlined here mainly follows the description by Grasshoff (1983). For the application of electrochemical methods, which are of special interest for a continuous record of oxygen consumption of oxygen profiles, it is referred to the literature (e.g. Whitfield and Jagner, 1981).

Principle

The Winkler method is based on the following principle: Manganese (II) ions are precipitated in an alkaline solution as manganese (II) hydroxide, which is oxidized by the dissolved oxygen (in a known amount of seawater) to manganese (III) hydroxide (first step of the procedure). After complete fixation of oxygen, the hydroxide is dissolved with acid. The liberated manganese (III) ions then react with previously added iodide ions. These are oxidized to iodine, which in turn forms a complex with the surplus iodide, thus protected from partial evaporation (second step of the procedure). In the third step of the method, the iodine (or I_3 produced) is then determined by titration with thiosulphate.

The stoichiometric equations for the reactions described above are :

First step : $Mn^{2}+ + 2OH^{-} \longrightarrow Mn(OH)_2$

$2Mn(OH)_2 + ½O_2 + H_2O \longrightarrow 2Mn(OH)_3$

Second step : $2Mn(OH)_3 + 2I^{-} + 6H^{+} \longrightarrow 2Mn^{2+} + I_2 + 6H_2O$

$I_2 + I^{-} \longrightarrow I_3^{-}$

Third step : $I_3^{-} + 2S_2O_3^{2-} \longrightarrow 3I^{-} + S_4O_6^{2-}$

The equation shows that one mole (two atoms) of oxygen is equivalent to four moles of thiosulphate.

Reagents

Sulphuric acid (1:1)

Add 50 ml concentrated sulphuric (d = 1.84 g. ml) acid slowly to 50 ml distilled water with constant mixing and cooling.

Sodium thiosulphate stock solution (ca. 0.2 mol/l=49.5x10^{3}mg/l)

Dissolve 49.5 g $Na_2S_2O_3.5H_2O$ in distilled water and make up to 1000 ml. This stock solution is diluted with distilled water 1:10 for use; i.e. 100 ml of the stock solution are diluted with distilled water to a total volume of 1000 ml to give around 0.02 mol/l ($4.95x10^3$mg/l).

Iodate standard

Dissolve 325 mg exactly ($0.0833/10^2$ mol) $KH(IO_3)_2$ or 356.7 mg ($0.1667/10^2$ mol) KIO_3, after drying at 115°C to constant weight, in distilled water and dilute to 1000 ml. The solution has an "oxidation concentration" of 0.0100 mol/l of electrons.

Starch solution

Dissolve 1.0 g soluble starch in 100 ml distilled water and heat quickly until the solution becomes clear (The solution may be stabilized with 1 ml phenol). Instead of this solution, a commercially available zinc starch solution (readily dissolvable in water) may be used.

Apparatus

a) Glass bottles (ca. 50 ml)

A special type of bottle should be used for the sub-sampling of oxygen samples [see Figure (III.11)]. Only glass stoppers should be used, and each bottle must have its own stopper. The volume of each stoppered bottle must be determined by weighing the bottle empty and filled with distilled water to ± 0.05 g. Measure the water temperature immediately after weighing in order to apply the appropriate water density for calculating the bottle volume.

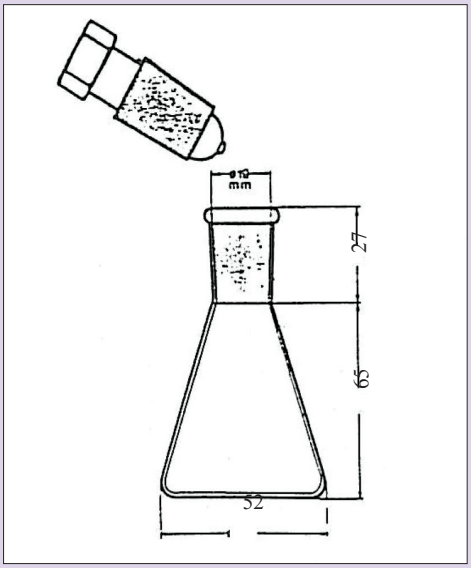


Figure (III.11) : Recommended sub-sampling bottle for the determination of oxygen (Ca, 50cm³; Duran glass)

b) Reagent dispenser

Automatic or semi-automatic syringe pipettes (with long plastic or stainless steel tips) should be used for reagent additions. They do not require filling via the dispenser nozzle and allow swift additions [see Figure (III.2)]. Micropipettes can also be used.

c) Precision burette (10 ml)

A calibrated piston burette (hand-operated or motor-driven) with reading to 0.01 ml should be used for titration. The piston burette offers the advantage of better precision, higher speed of work and is less fragile in comparison to ordinary burettes.

The titration procedure can be optimized by positioning the whole equipment in a box, as illustrated in the diagram of Figure (III.12). The equipment can also easily be transported without risk of damage.

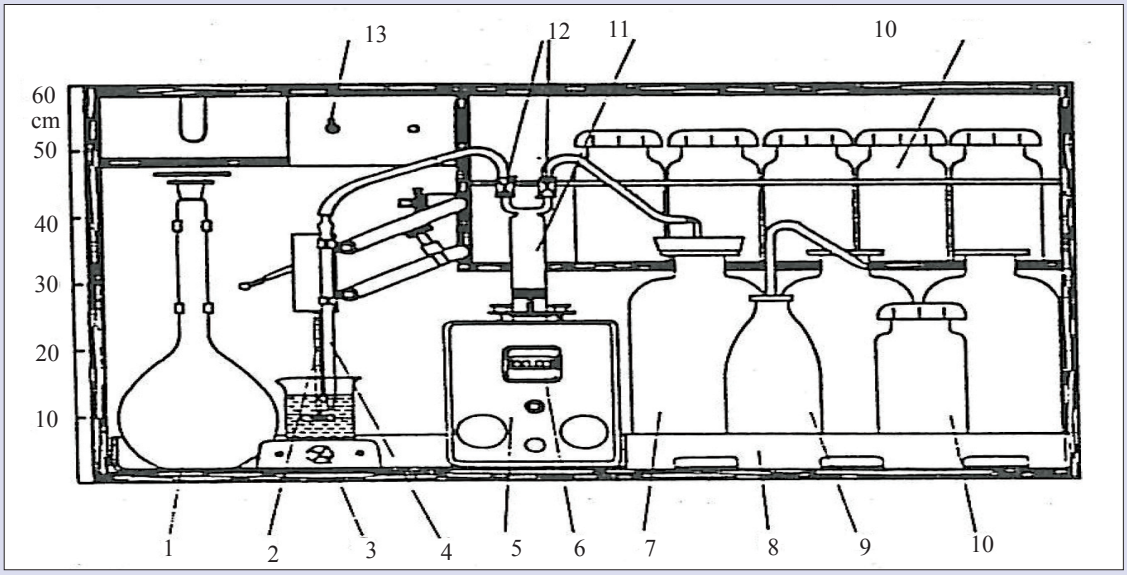


Figure (III.12) : Diagram of complete set-up for oxygen determination

1. 1L volumetric flask
2. Stirrer
3. Illumination from below
4. Burette tip
5. Burette
6. Digital reading
7. $Na_2S_2O_3$ bottle
8. Plastic lining
9. Splashing bottle
10. Reagent bottles for H_2SO_4, KOH+KI, $MnCl_2$
11. Burette cylinder
12 Valves
13. Main switch

Sampling and oxygen fixation procedures (See details in Section III.1.1.6.1)

The oxygen sample should be the first sample drawn from the water sampler, and the sub-sampling should be carried out as soon as possible after the sampler has been recovered. For this purpose the 50 ml glass bottles are unstoppered, and the nozzle tube of the sampler is inserted to the bottom of the sample bottle. The bottle flushed with at least twice the volume of the bottle (ca. 100 ml). At the final stages of filling the tube is carefully removed from the bottle. The bottle must be brim full. If air bubbles have nevertheless been trapped on the walls of the bottle, they should be driven out by gently tapping at the bottle.

The reagents are added simultaneously (0.5 ml each of manganese (II)-chloride and alkaline iodide solution) with the special dispenser [see Figure (III.2)]. The syringe pipette tips should extend deep into the bottle. The tips are slowly withdrawn while the reagents are added. Then the stopper is inserted avoiding the trapping of air bubbles. The bottles are shaken vigorously for at least 1 min to achieve the chemical fixation of oxygen. The precipitate is then allowed to settle (ca. 10-20 min) before analysis is continued.

Oxygen samples may be stored up to 10-20 h after addition of the reagents. The bottles, however, should be kept in the dark and at constant temperature. If titration cannot be achieved within 12 hours, or, if a large temperature difference exists between sub-samples and the (e.g. air-conditioned) laboratory, the bottles should be kept underwater (in order to prevent contamination from atmospheric oxygen by expansion and contraction of the samples).

Method

a) Standardization of the thiosulphate solution

The exact titre of the sodium thiosulphate is determined by titration against the iodate standard. This standardization is based on a quantitative co-proportionation reaction (only in acidic solutions) of iodide with iodate resulting in the formation of iodine. The next steps - the fixation of the liberated iodine and subsequent titration with thiosulphate - are identical with the second and third step described in paragraph III.2.1 (Principle). The stoichiometric equations involved in the standardization are :

$$IO^-_3 + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$3I_2 + 3I^- \longrightarrow 3I^-_3$$

$$3I^-_3 + 6S_2O_3^{2-} \longrightarrow 9I^- + 3S_4O_6^{2-}$$

For the standardization procedure [with the equipment shown in Figure (III.12)] place about 80 ml distilled water in 150 ml titration beaker and add separately 1 ml sulphuric acid (1:1), 0.5 ml alkaline iodide solution and 0.5 ml manganese (II) chloride solution respectively. Mix thoroughly after each addition. (Do not add manganese (II) chloride solution before alkaline iodide solution). Then add 10 ml (with the calibrated pipette) of the iodate standard solution, and titrate the liberated iodine with the thiosulphate solution as described below for oxygen analysis. Note the volume of thiosulphate solution consumed.

b) Analysis of the sample

Dissolve the precipitated hydroxides with sulphuric acid. For this, pipette 1 ml sulphuric acid (1:1) into the sample bottle immediately after unstoppering. Insert the tip of the pipette almost to the level of the precipitate and then slowly withdraw without disturbing the precipitate. Stopper the bottle again and dissolve the precipitate by vigorous shaking. Then remove the stopper and rinse the film of the sample on it (containing liberated iodine) carefully into the titration beaker. Transfer the content of the bottle quantitatively into the 150 ml beaker by rinsing twice with a few ml of distilled water.

Titrate [see Figure (III.12)] the liberated iodine with the thiosulphate solution to a light yellow colour. Then add 1 ml starch indicator solution and continue (at an even speed) until the blue colour disappears. (Diffuse illumination from below and moderate room illumination facilitate detection of the end point). Note the volume of thiosulphate solution consumed.

Note : 1. The liberated iodine should be titrated quickly and without delay in order to prevent losses by evaporation. It is also important, for the same reason that the entire volume of the sample is titrated and representative aliquots are not taken

2. Microtitration within the oxygen bottle and using strong thiosulphate solution but no starch and photometric detection can now be used

c) Determination of the reagent blank

Determine the overall reagent blank by the following procedure: Fill three oxygen bottles with distilled water or any seawater sample. Use the same water for all bottles, and take extreme care that the oxygen content in the three bottles is identical. Then add to the first bottle one set of fixation reagents; to the second two sets; to the third three sets. Proceed with titration as described above. Note the volumes of thiosulphate solution consumed.

Calculation of results

a) Titre of thiosulphate solution

The exact concentration of the thiosulphate solution, C, is determined from the titration with the iodate standard as :

$$C = \frac{10 \text{ ml} \times 0.01 \text{ mol/l}}{V \text{ ml}} = \frac{0.1000 \text{ mol/l}}{V}$$

where V is the volume of thiosulphate solution consumed. Since C should be around 0.02 mol/l, the factor f, with which the consumed volume (V) must be multiplied to get the corresponding amount of 0.02 mol/l solution is then :

$$0.02 = \frac{0.1000}{f \cdot V}$$

or

$$f = \frac{5}{V}$$

b) Concentration of dissolved oxygen

One mole of thiosulphate corresponds to ¼ mole oxygen (or 8 g). Therefore, 1 ml of 0.02 mol/l thiosulphate solution corresponds to 0.160 mg oxygen or 0.122 ml oxygen (at 0°C and normal pressure). If a ml is the amount of thiosulphate solution used to titrate a sample and b ml is the volume of the oxygen bottle [actually (b-l) will be employed in the formula shown below in order to allow for the added volume of added reagents], then the oxygen in the water sample (enclosed in the oxygen bottle) is :

$a \cdot f \cdot 0.160 = \text{mg } O_2$ or

$a \cdot f \cdot 0.112 = \text{ml } O_2$

Referred to a sample volume of 1 L, the concentration is :

$$\frac{a \cdot f \cdot 0.112 \cdot O^3}{b - 1} = \text{ml/l } O_2$$

(For convenience a "bottle factor" $F = 0.112 \cdot 10^3/(b-1)$ may be calculated and listed together with the bottle number. The final calculation is then :

$a \cdot f \cdot F = \text{ml/l } O_2$)

Note : In compliance with SI units it is recommended to report the oxygen concentrations in mmol/m^3 in which case these values should be multiplied by 44.66

Sometimes it is necessary to express the results as percent saturation at normal atmospheric pressure (760 mm Hg). For this calculation, use the solubility data in Table (III.3).

Table (III.3) : Oxygen solubility in ml/l

Salinity ‰ Temp °C	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
0	10.22	10.08	9.94	9.81	9.67	9.54	9.41	9.29	9.16	9.04	8.91	8.79	8.67	8.56	8.44	8.32	8.21	8.10	7.99	7.88	7.77
1	9.94	9.8	9.67	9.54	9.41	9.28	9.16	9.04	8.91	8.79	8.68	8.56	8.44	8.33	8.22	8.11	8.00	7.89	7.78	7.68	7.58
2	9.67	9.54	9.41	9.28	9.16	9.04	8.92	8.80	8.68	8.56	8.45	8.34	8.22	8.11	8.01	7.90	7.79	7.69	7.59	7.48	7.38
3	9.41	9.28	9.16	9.04	8.92	8.80	8.68	8.57	8.45	8.34	8.23	8.12	8.01	7.91	7.80	7.70	7.60	7.50	7.40	7.30	7.20
4	9.16	9.04	8.92	8.81	8.69	8.57	8.46	8.35	8.24	8.13	8.02	7.92	7.81	7.71	7.61	7.51	7.41	7.31	7.22	7.12	7.03
5	8.93	8.81	8.70	8.58	8.47	8.36	8.25	8.14	8.03	7.93	7.83	7.72	7.62	7.52	7.42	7.33	7.23	7.14	7.04	6.95	6.86
6	8.70	8.59	8.48	8.37	8.26	8.15	8.05	7.94	7.84	7.74	7.64	7.54	7.44	7.34	7.25	7.15	7.06	6.97	6.88	6.79	6.70
7	8.49	8.38	8.27	8.16	8.06	7.95	7.85	7.75	7.65	7.55	7.45	7.36	7.26	7.17	7.08	6.98	6.89	6.81	6.72	6.63	6.55
8	8.28	8.17	8.07	7.97	7.86	7.76	7.66	7.57	7.47	7.37	7.28	7.19	7.09	7.00	6.91	6.82	6.74	6.65	6.57	6.48	6.10
9	8.08	7.98	7.88	7.78	7.68	7.58	7.48	7.39	7.30	7.20	7.11	7.02	6.93	6.84	6.76	6.67	6.59	6.50	6.42	6.34	6.26
10	7.89	7.79	7.69	7.60	7.50	7.41	7.31	7.22	7.13	7.04	6.95	6.86	6.78	6.69	6.61	6.52	6.44	6.36	6.28	6.20	6.12
11	7.71	7.61	7.52	7.42	7.33	7.24	7.15	7.06	6.97	6.88	6.80	6.71	6.63	6.54	6.46	6.38	6.30	6.22	6.14	6.07	5.99
12	7.53	7.44	7.35	7.26	7.17	7.08	6.99	6.90	6.82	6.73	6.65	6.56	6.48	6.40	6.32	6.24	6.17	6.09	6.01	5.94	5.87
13	7.37	7.27	7.18	7.10	7.01	6.92	6.84	6.75	6.67	6.59	6.50	6.42	6.34	6.27	6.19	6.11	6.04	5.96	5.89	5.82	5.74
14	7.20	7.12	7.03	6.94	6.86	6.77	6.69	6.61	6.53	6.45	6.37	6.29	6.21	6.14	6.06	5.99	5.91	5.84	5.77	5.70	5.63
15	7.05	6.96	6.88	6.79	6.71	6.63	6.55	6.47	6.39	6.31	6.24	6.16	6.08	6.01	5.94	5.87	5.79	5.72	5.65	5.8	5.52
16	6.90	6.81	6.73	6.65	6.57	6.49	6.41	6.34	6.26	6.18	6.11	6.03	5.96	5.89	5.82	5.75	5.68	5.61	5.54	5.48	5.41
17	6.75	6.67	6.59	6.51	6.44	6.36	6.28	6.21	6.13	6.06	5.99	5.91	5.84	5.77	5.70	5.64	5.57	5.50	5.43	5.37	5.31
18	6.61	6.54	6.46	6.38	6.31	6.23	6.16	6.08	6.01	5.94	5.87	5.80	5.73	5.66	5.59	5.53	5.46	5.40	5.33	5.27	5.21
19	6.48	6.40	6.33	6.25	6.18	6.11	6.03	5.96	5.89	5.82	5.75	5.69	5.62	5.55	5.49	5.42	5.36	5.29	5.23	5.17	5.11
20	6.35	6.28	6.20	6.13	6.06	5.99	5.92	5.85	5.78	5.71	5.64	5.58	5.51	5.45	5.38	5.32	5.26	5.20	5.14	5.07	5.02
21	6.23	6.15	6.08	6.01	5.94	5.87	5.80	5.74	5.67	5.60	5.54	5.47	5.41	5.35	5.28	5.22	5.16	5.10	5.04	4.98	4.93
22	6.11	6.04	5.97	5.90	5.83	5.76	5.69	5.63	5.56	5.50	5.44	5.37	5.31	5.25	5.19	5.13	5.07	5.01	4.95	4.89	4.84
23	5.99	5.92	5.85	5.79	5.72	5.65	5.59	5.52	5.46	5.40	5.34	5.28	5.21	5.15	5.10	5.04	4.98	4.92	4.87	4.81	4.75
24	5.88	5.81	5.74	5.68	5.61	5.55	5.49	5.42	5.36	5.30	5.24	5.18	5.12	5.06	5.01	4.95	4.89	4.84	4.78	4.73	4.67
25	5.77	5.70	5.64	5.58	5.51	5.45	5.39	5.33	5.27	5.21	5.15	5.09	5.03	4.98	4.92	4.86	4.81	4.75	4.70	4.65	4.30
26	5.66	5.60	5.54	5.48	5.41	5.35	5.29	5.23	5.17	5.12	5.06	5.00	4.95	4.89	4.83	4.78	4.73	4.67	4.62	4.57	4.52
27	5.56	5.50	5.44	5.38	5.32	5.26	5.20	5.14	5.08	5.03	4.97	4.92	4.86	4.81	4.75	4.70	4.65	4.60	4.54	4.49	4.44
28	5.46	5.40	5.34	5.28	5.23	5.17	5.11	5.05	5.00	4.94	4.89	4.83	4.78	4.73	4.67	4.62	4.57	4.52	4.47	4.42	4.37
29	5.37	5.31	5.25	5.19	5.14	5.08	5.02	4.97	4.91	4.86	4.81	4.75	4.70	4.65	4.60	4.55	4.50	4.45	4.40	4.35	4.30
30	5.28	5.22	5.16	5.10	5.05	4.99	4.94	4.89	4.83	4.78	4.73	4.68	4.62	4.57	4.52	4.47	4.43	4.38	4.33	4.28	4.24
31	5.19	5.13	5.07	5.02	4.96	4.91	4.86	4.80	4.75	4.70	4.65	4.60	4.55	4.50	4.45	4.40	4.36	4.31	4.26	4.22	4.17
32	5.10	5.04	4.99	4.94	4.88	4.83	4.78	4.73	4.68	4.63	4.58	4.53	4.48	4.43	4.38	4.33	4.29	4.24	4.20	4.15	4.11
33	5.01	4.96	4.91	4.86	4.80	4.75	4.70	4.65	4.60	4.55	4.50	4.46	4.41	4.36	4.31	4.27	4.22	4.18	4.13	4.09	4.04
34	4.93	4.88	4.83	4.78	4.73	4.68	4.63	4.58	4.53	4.48	4.43	4.39	4.34	4.29	4.25	4.20	4.16	4.11	4.07	4.03	3.98
35	4.85	4.80	4.75	4.70	4.65	4.60	4.55	4.51	4.46	4.41	4.36	4.32	4.27	4.23	4.18	4.14	4.10	4.05	4.01	3.97	3.93

Equations for correcting the solubilities to barometric pressures other than mean sea level are given below :

Calculate the solubility of oxygen in distilled water at any barometric pressure, P (mm Hg), temperature, t, and saturated vapor pressure, μ (mm Hg), for the given t, between the temperature of 0 and 30°C, by :

$$\text{mg/l DO} = \frac{(P - \mu) \times 0.678}{35 + t}$$

and between 30 and 50°C by:

$$\text{mg/l DO} = \frac{(P - \mu) \times 0.827}{49 + t}$$

c) Reagent blank value

The apparent amount of oxygen in each bottle (from the three blank titrations) is calculated as described above, but a correction is applied for the additional volume of the double and triple sets of reagents. The results are plotted on a graph (apparent concentration against number of sets). The slope (if any) of the resulting straight line gives the reagent blank which must be subtracted from the estimated oxygen concentration of the sample. (It is convenient to calculate the blank correction for a sample volume of 1 L, assuming an average value of the oxygen bottles). If the reagent blank has a higher concentration than 0.1 ml/l O_2, the reagents should be discarded and new ones prepared.

d) Estimation of precision and accuracy

The accuracy in oxygen analysis is very difficult to ascertain since the major sources for systematic errors originate from the sampling and sub-sampling procedures. The effect of possible out-gassing of oxygen during retrieval of the sampler, for example, can hardly be estimated and can only be minimized by proper equipment and by a careful sampling routine.

If considerable care is taken to avoid handling errors, the precision (standard deviation) for the remaining procedure can be as good as ± 0.02 ml/l O_2 for concentrations of less than 2 ml/l and ± 0.04 ml/l for higher oxygen concentrations.

III.2.2 pH

Scope and field of application

pH can be determined by the multi-parameter system (see Section II.2.5). If it is to be determined separately, the following procedure, which requires the use of a good quality pH meter, is recommended.

The determination of pH described here is performed electrometrically by means of ion specific electrodes (glass electrodes) with a silver-silver chloride or calomel electrode as reference. The potentiometric procedure represents normal practice in oceanography given the ready availability

of glass electrodes and reliable voltmeters with an impedance of more than 10^{12} "ohms". Other techniques (e.g., spectrophotometric measurement with suitable indicators) are, therefore, not considered here.

Principle

The pH is defined by the equation:

$pH = -\log a_{H+}$

where a_{H+} is the activity of the hydrogen ions. The relationship of a_{H+} with the hydrogen ion concentration (C_{H+}) is given by $a_{H+} = C_{H+}. r_{H+}$ where r_{H+} is the thermodynamic activity coefficient of hydrogen ion solution. This activity coefficient is a function of the solvent (e.g. water), the ionic strength, and the specific ions dissolved in the solvent.

The fundamental principle of the glass electrode is based on the fact that special conductive glasses are able to build up a hydrated boundary layer at the glass surface when in contact with aqueous solutions. Because of ion exchange processes between the boundary layer and the solution, a membrane potential exists which is sensitive to a_{H+} in the solution. The weak current in the glass is conducted by the alkali ions.

The potential of the glass electrode (indicator electrode) is measured against a pH-independent reference electrode (usual types are the silver-silver chloride and calomel electrodes). However, since the electrode chain potential of this glass-reference electrode couple is not defined in absolute terms, the pH electrode couple must be calibrated with solutions of known hydrogen ion activities. The unknown pH_x value is then found from the equation :

$$pH_x = \frac{pH_s + \in_x - \in_s}{N_F}$$

where

pH_s = pH of the standard solution,

$\in_x$ and $\in_s$ = electrode chain potential in the test and standard, respectively,

N_F = Nearest factor (slope of the pH couple at the working temperature in mV/pH).

Usually, the pH meter automatically converts the measured potential into units of pH.

The presently described technique should generate data easily comparable with that of other authors for the purpose of providing supporting environmental information to contamination monitoring programmes. It should be noted, however, that there is a considerable debate in the scientific literature concerning the poor reproducibility of the liquid junction when calibrating the electrodes with low ionic strength National Bureau of Standards (NBS) buffers and subsequently employing them for seawater, which has a completely different major ion composition. An alternative seawater based buffer scale is available but is not as yet widely employed. pH values obtained with the two techniques are somewhat different and extreme caution should be employed when making thermo-dynamic interpretations of pH (NBS). For further information, the review by Dickson (1984) should be consulted.

Reagents

The NBS has compiled a list of standard buffer solutions for pH calibration. Some suitable ones are listed in Table (III.4) together with their temperature dependencies.

Table (III.4): pH of NBS standard solutions

	0.05 mol/kg KH-phthalate	0.025 mol/kg KH_2PO_4 ($3.402x10^3$ mg/kg) 0.025 mol/kg Na_2HPO_4 ($3.550x10^3$ mg/kg)	0.00869 mol/kg KH_2PO_4 ($1.183x10^3$ mg/kg) 0.03043 mol/kg Na_2HPO_4 ($4.321x10^3$ mg/kg)
5°C	3.999	6.951	7.500
10°C	3.998	6.923	7.472
15°C	3.999	6.900	7.448
20°C	4.002	6.881	7.429
25°C	4.008	6.865	7.413
30°C	4.015	6.853	7.400

a) Phosphate standard (1:1)

Dry 3.402 g potassium dihydrogen phosphate (KH_2PO_4) and 3.550 g disodium hydrogen phosphate (Na_2HPO_4) at 80°C, dissolve in CO_2-free distilled water and dilute to a total weight of 1 kg. The solution contains 0.025 mol/kg of each salt.

b) Phosphate standard (1:3:5)

Dry 1.183 g potassium dihydrogen phosphate (KH_2PO_4) and 4.321 g disodium hydrogen phosphate (Na_2HPO_4) at 80°C, dissolve in CO_2-free distilled water and dilute to 1 kg. The solution contains 0.008695 and 0.03043 mol/kg of each salt, respectively (1.183×10^3 mg/kg KH_2PO_4 and 4.321×10^3 mg/kg Na_2HPO_4).

c) Potassium hydrogen phthalate

Dissolve 10.211 g potassium hydrogen phthalate in distilled water and dilute to 1 kg. The solution contains 0.05 mol/kg.

Note : All standard solutions should be prepared in CO_2-free distilled water (preferably prepared by boiling for a while and passing nitrogen gas through during the cooling) and should be stored in tightly stoppered glass bottles. They are stable for several weeks but should be discarded if they eventually become turbid.

d) Commercially available buffer solutions

These are readily available from a number of suppliers. They should be of very high quality (accurate to ± 0.001 pH units) and careful attention must be paid to the maker's instructions on storage and the calibration temperature.

Apparatus

a) Electrodes

Combined electrodes, where the glass electrode and the reference electrode are combined in one shaft, should be used. The reference electrode should be of the same type as the "pick-up electrode" in the glass electrode (symmetrical chain). The glass bulb should never dry out, i.e. the electrode should be immersed in seawater during pauses (or in distilled water during longer storage). Normally, the manufactures (e.g., W. Ingold or Metrohm) provide guidance on how their electrodes should be treated in order to produce reliable readings.

b) pH meter

Numerous pH meters of good quality are commercially available. An instrument with digital display to at least two decimal places is preferred for work at sea. It should have controls for temperature, slope, and zero adjustment, and should be insensitive to slight changes of the line voltage.

c) Water bath and measuring cell

Since pH is dependent on temperature, measurements must be performed with thermostated samples. For high precision experiments, a measuring cell with connected heat exchanger is recommended, Figure (III.13). They are made of glass and require a water bath with a pump for external circulation of the thermostated water. Connecting tubes should be as short as possible in order to reach thermal equilibrium (+ 2°C) quickly and to keep the sample volume small.

It is also general practice today to equilibrate the samples in a simple water bath. It offers the advantage of faster measurement (due to simultaneous thermal equilibrium of a greater amount of samples) but may cause problems during severe movements of the ship implying lower previsions in pH measurement.

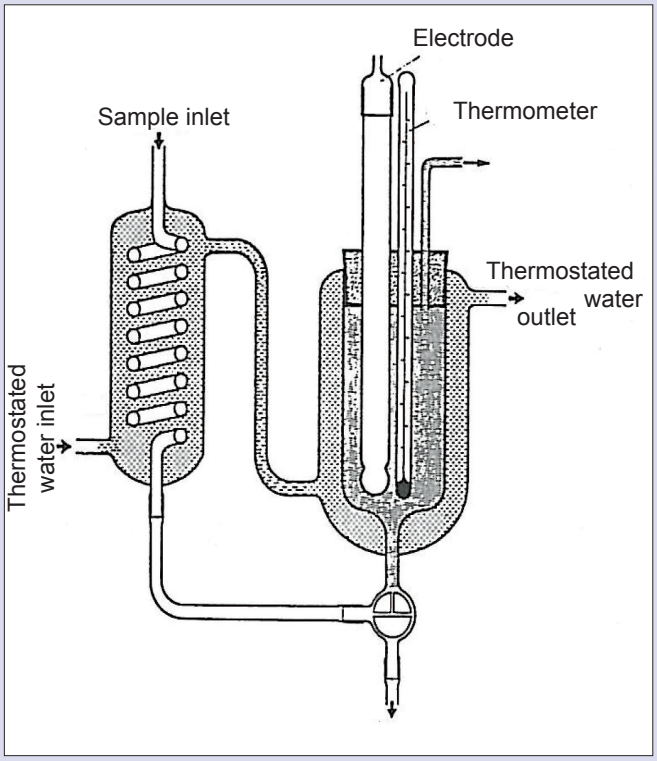


Figure (III.13) : Set-up for thermostated pH cell

Method

a) Calibration of pH meter

Set the temperature control of the thermostat to 20 or 25°C (selection depends on the water temperature and cooling facilities on board). Rinse the measuring cell carefully with distilled water and the standard solution (phosphate standard 1:1) before filling with the latter. Set the

slope control of the pH meter (if any) to 98% of the theoretical slope and adjust the pH meter to the assigned value of the standard, Table (III.4) by the pH control. Drain the cell and fill it again with the phosphate standard (1:1) solution. The reading should be within ± 0.002 pH units (or ± 0.01) if a reading of three (or two) decimal places is available at the pH meter.

Drain the cell again and rinse at least twice with distilled water and then twice with phthalate standard solution before finally filling it with this buffer. Set the pH meter to the theoretical value, Table (III.4) by means of slope control (if any) or otherwise with the temperature control (the latter is only allowed if calibration and measurement are performed at the same temperature). Drain and rinse the cell and fill it again with the 1:1 phosphate standard. If the pH reading deviates more than + 0.003 units from the theoretical value then adjust by means of the pH control and repeat the calibration procedure also with phthalate standard solution.

After completion of the calibration procedure rinse the cell thoroughly with distilled water and keep it filled with seawater of about the salinity to be expected in the samples.

Note : 1. Instead of the phthalate standard solution, the phosphate standard 1:3:5 can be used for calibration. This offers the advantage that the second calibration point is closer to the pH range of seawater, but has the disadvantage of being quite close to the first calibration point, set by the 1:1 phosphate standard. The use of the alkaline N.B.S. borax standard solution (about pH 9.2 at 25°C) is not recommended because of its instability as a result of carbon dioxide uptake.

2. Samples from very cold waters can de-gas when warmed. If this appears to be a problem (for example in arctic, or deep-sea samples) samples should then be kept cool and measurement made at a low, constant (e.g., 5°C) temperature

b) Measurement of pH in seawater samples

Drain the cell and rinse at least twice with the sample. Fill the measuring cell completely (slowly through the heat exchanger; [see Figure (III.13)] and let the sample reach constant temperature. The pH reading should be constant after about one minute if the electrode is working correctly. Proceed with the adjacent sample in uninterrupted order from surface to bottom or vice-versa.

When simply a water bath is used for thermal equilibrium, the electrode is directly immersed into the sampling bottles (remove the stopper just before pH measurement). After pH reading is constant proceed with the adjacent sample without further rinsing of the electrode. The remaining seawater film on the electrode from the previous measurement has been proved to be of negligible influence when the difference from one sample to another is only a few tenths of a pH unit. This method can be considered almost as good as the procedure described in Figure (III.13).

Calculation of results

The measured pH value must be corrected for in-situ conditions. The correction for the temperature difference between measurement conditions and those at the sample depth in the water can be calculated from :

$$pH\ in\text{-}situ = pH\ measured + 0.0114\ (t_2 - t_1),$$

where :

t_1 = *in-situ* temperature
t_2 = measurement temperature

Note : The temperature coefficient is uncertain by about ± 0.0010, i.e., the accuracy of the correction will be ± 0.01 pH units, if t_1 and t_2 differ by 10°C.

For samples taken from 500 m or deeper a further correction for the difference in hydrostatic pressure should be applied. However, the effects of pressure on pH changes (i.e., pressure effects on the dissociation, ion pairing formation or activity coefficients of the different components) are far from understood. Because of these uncertainties and because of the fact that monitoring studies are performed, in general, in shallower water bodies, a correction factor will not be given here.

Estimation of precision and accuracy

It is clear that precision and accuracy of the pH measurement are dependent not only on the handling of samples and the quality of the standard solutions, but also on the pH meter, the electrodes as well as its cable connections. It is, therefore, essential that these are of good quality and maintained according to instructions given by the manufacturers. The precision (standard deviation) of the measurement can be, under optimal conditions, such that ± 0.002 pH units may be obtained with proper equipment.

III.2.3 Alkalinity

An unaltered sample is titrated with standard acid solution to an electrometrically determined end point of pH 4.5.

Equipment

a) pH meter or electrically operated titrator that uses a glass electrode and can be read to 0.05 pH units. Standardize and calibrate according to manufacturer's instructions. If automatic temperature compensation is not provided, make titration at 25 ± 2°C

b) An appropriate sized vessel to keep the air space above the solution at a minimum. Use a rubber stopper fitted with holes for the glass electrode, and burette

c) Magnetic stirrer, pipettes, flasks and other standard laboratory equipment

Reagents

a) Sodium carbonate solution, approximately 0.05 N: Place 2.5 + 0.2 g (weighted to nearest mg) Na_2CO_3 (dried at 250°C for 4 hours and cooled in desiccator) into a 1 liter volumetric flask and dilute to the mark

b) Standard acid (sulphuric or hydrochloric), 0.1 N: Dilute 3.0 ml concentrated H_2SO_4 or 8.3 ml concentrated HCl to 1 liter with distilled water by titrating potentiometrically to pH of about 5. Lift electrode and rinse into beaker. Boil solution gently for 3-5 minutes under a watch glass cover. Cool to room temperature. Rinse cover glass into beaker. Continue titration to the pH inflection point. Calculate molarity using :

$$M = \frac{A \times B}{53.00\ XC}$$

where :

A = g Na_2CO_3 weighted into 1 liter
B = ml Na_2CO_3 solution
C = ml acid used to inflection point

c) Standard acid (sulphuric or hydrochloric), 0.02 N: Dilute 200.0 ml of 0.1000 N standard acid to 1 liter with distilled water. Standardize by potentiometric titration of 15.0 ml 0.05 N Na_2CO_3 solution as above

Analytical procedure

Place 100 ml sample in flask by pipetting with pipette tip near bottom of flask and measure pH of sample with a calibrated pH meter (see Section II.2.1.2), determination of pH value).

Add standard acid being careful to stir thoroughly but gently to allow needle to obtain equilibrium. Titrate the sample to pH 4.5 with 0.02 N acid solution for less than 1000 mg/l $CaCO_3$ and with 0.1 N acid solution for more than 1000 mg/l $CaCO_3$ alkalinity.

A preliminary titration is helpful. For alkalinity of less than 20 mg/l $CaCO_3$, titrate 200 ml sample using a 10 ml micro-burette and 0.02 N acid solution. Stop titration at pH in the range of 4.3-4.7, record volume and exact pH. Very carefully add titrant to lower pH exactly 0.3 pH units and record volume.

Calculation

Calculate the alkalinity of the sample as follows :

- Potentiometric titration to pH 4.5

$$\text{Alkalinity, mg/l } CaCO_3\ M = \frac{A \times N \times 50{,}000}{\text{ml of sample}}$$

where :

A = ml standard acid
N = normality standard acid

- Potentiometric titration of low alkalinity :

$$\text{Total Alkalinity, mg/l } CaCO_3 = \frac{(2B\text{-}C) \times N \times 50{,}000}{\text{ml of sample}}$$

where :

B = ml titrant to first recorded pH
C = total ml titrant to reach pH 0.3 units lower
N = normality of acid

III.2.4 Salinity

A) Titrimetric method

The titrimetric method for determination of chlorinity and calculation of salinity of seawater is still capable of giving very accurate and precise results. It is reported that a significant difference between the chlorinity as defined by Knudsen (1901) and chlorinity derived from the conductometrically determined salinity may occur in in-shore waters or epicontinental seas. This is one of the reason why many marine chemists still prefer the old fashioned chlorinity determination.

Equipment

When using the Knudsen titration method, there is no marine chemist who does not have an endless struggle against dirty and thus poorly draining burettes, intrusion of stopcock grease into the burettes, loose stopcocks and spillage of the silver nitrate. Therefore, an automatic piston burette system is recommended. Such a system includes a Metrohm Dosimat (20 ml), a small stirrer, a 2 L reservoir bottle for the silver nitrate and a clamp for the 10 ml Knudsen pipette which are all mounted in a box in such a way that the working procedure during the titration is optimized by the location of the equipment, Figure (III.14). The arrangement is constructed to give the best working conditions within a relatively small container. The equipment is easy to transport without risk of damage and is ready to work immediately after installation and connection to an electrical power supply.

Reagents

a) Silver nitrate: Dissolve 50 g of $AgNO_3$ in distilled water which is completely free of traces of chloride and made up to one 1. The solution is stored in an amber glass bottle
b) Potassium chromate solution: Dissolve 8 g of K_2CrO_4 in 100 ml of distilled water. This solution is stable indefinitely if kept in a ground glass stoppered bottle

Note : Connections between the silver nitrate reservoir bottle and the burette should be made of glass as far as possible. The connections and the piston burette should be either amber glass or printed black to avoid any photochemical reaction of the silver ions

Standardization of silver nitrate solution

a) 10 ml of standard seawater are transferred with the 10 ml Knudsen pipette into a 150 ml titration beaker. The pipette must be cleaned thoroughly prior to use
b) About 25 ml of chloride free distilled water and 6 drops of potassium chromate indicator solution are added
c) The stirrer is started. The speed of the stirrer must be adjusted to provide thorough mixing without any spattering
d) The silver nitrate is added from the piston burette, until the first sign of a constant colour change occurs. The tip of the burette is formed of a polyethylene capillary tube and should just touch the sample surface
e) The piston speed is slowed down. The silver nitrate is now added in small increments. The titration is continued until the orange colour persists
f) The sample pipette is removed from the stand, the tip is placed on the surface of the titrated

sample and the last drop, which has collected in the tip is expelled from the pipette by warming the bulb with one hand

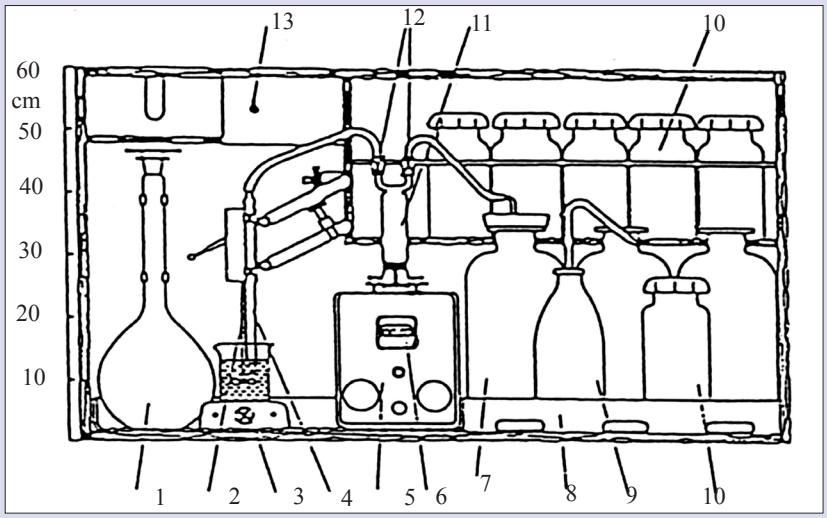
g) After this addition any droplets from the wall of the beaker are washed down with distilled water and the titration is finished by adding the last increments of the silver nitrate solution. The final end point is reached when the colour has changed from yellow to a shade of rather dirty orange

h) The digital reading of the burette is recorded (C_1)

i) The titration of the standard is repeated at least twice (C_2 and C_3)

j) After completion of the titration, drain and rinse the beaker and the stirrer

k) Calculation of the calibration factor (f)



1. Measuring flask
2. Stirrer and burette tip
3. Light
4. Burette
5. Digital readout
6. Silver nitrate
7. Plastic linear
8. Wash bottle
9. 10 ml Knudsen pipette
10. Bichromate solution
11. Spare pipettes
12. 20 ml burette cylinder
13. Valves

Figure (III.14) : Diagram of portable salinity titration equipment

The mean of three titrations is determined by :

$$\frac{C_1 + C_2 + C_3}{3} = C_m$$

The mean C_m must be corrected for a positive volume error (C_e) of the burette, which is obtained from a graph, if the deviation between nominal volume and real volume exceeds 0.0005 ml.

When $C = (C_m + C_e)$

$$\text{then } f = \frac{N}{C}$$

where N is the indicated chlorinity of the standard seawater.

Titration of the sample

After thorough rinsing of the pipette with the sample, 10 ml of the sample are transferred into the beaker with the same Knudsen pipette as used for calibration and titration is performed in the same way and with the same precautions as described under standardization. It is very important that the temperature of the sample, of the standard seawater and of the room should not deviate by more than $\pm$ 1.5°C.

Calculation of chlorinity and salinity

The numerical reading of the burette (v_m) is corrected (v_c) for the burette error (v_e).

$v_c = (v_m - v_e)$ and

multiplied by the calibration factor (f) to give

$V = f \times v_c$

The normalized volume of the $AgNO_3$ used for the titration (V) must be corrected to give the chlorinity/kg of seawater. The correction value (k) is taken from Table (III.5). (The correction factor (k) is identical with the (k) in the Knudsen Hydrographical Tables for $\alpha = 0$).

$$Cl = V + k$$

$$Cl = \frac{N}{C}(V_m + V_e + k)$$

The salinity of the sample is calculated as follows:

$$S = 1.80655\ Cl$$

Table (III.5) : The correction k for the calculation of the chlorinity

v	k	v	k	v	k
0.00		10.29		19.57	
	0		+ 0.12		- 0.01
0.18		12.39		19.54	
	+ 0.01		+ 0.11		- 0.02
0.58		13.32		20.31	
	+ 0.02		+ 0.10		- 0.03
0.99		14.40		20.67	
	+ 0.03		+ 0.09		- 0.04
1.42		15.13		21.01	
	+ 0.04		+ 0.08		- 0.05
1.88		15.78		21.35	
	+ 0.05		+ 0.07		- 0.06
2.36		16.37		21.66	
	+ 0.06		+ 0.06		- 0.07
2.88		16.91		21.98	
	+ 0.07		+ 0.05		- 0.08
3.45		17.21		22.29	
	+ 0.08		+ 0.04		- 0.09
4.08		17.89		22.59	
	+ 0.09		+ 0.03		- 0.10
4.79		18.34		22.89	
	+ 0.10		+ 0.02		-0.11
5.64		18.77		23.18	
	+ 0.11		+ 0.01		
6.74		19.17			
	+ 0.12		0		
8.82		19.57			
	+ 0.13				

B) Salinometer method

The majority of present day salinity determinations are carried out with salinometers, Figure (III.15). If the instru ment is handled and standardized with care, the results are accurate and precise. The manufacturer's manual should be consulted in addition to the method explained below.

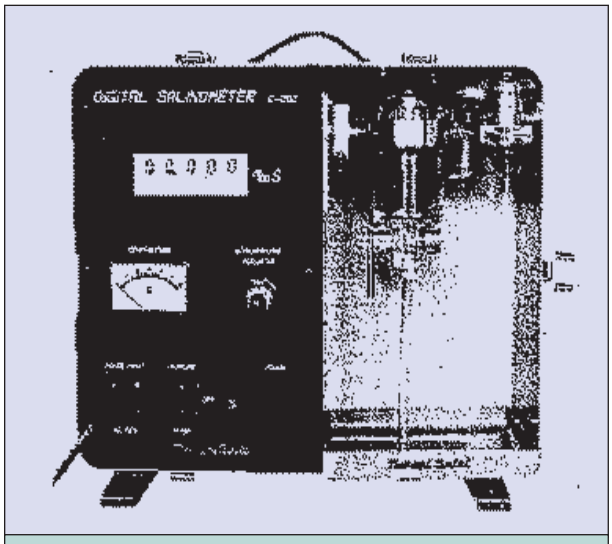


Figure (III.15) : Digital Salinometer

Preparation

a) Allow all standards, samples and the salinometer to equilibrate to room temperature for at least one day prior to measurement.

b) At least two hours before measuring, the sample bottles should be shaken vigorously and the stopper removed quickly in order to release any pressure, which reduces the risk of out-gassing of the sample in the salinometer. The stopper is then replaced.

Standardization

a) Drain the distilled water in the cell of the salinometer

b) Carefully rinse the cell at least twice with used Standard Seawater and then rinse again twice with the contents of a new ampoule of Standard Seawater. Fill the cell with new Standard Seawater. Make sure that the cell is entirely filled so that water can be seen at the overflow. If air bubbles are trapped in the cell, the readings will not be stable

c) Switch on the stirrer and measure the temperature of the Standard Seawater. Set the two dials of the temperature compensation control to a value corresponding to the recorded temperature as indicated in the instrument's operation manual

d) Set the conductivity ratio dials to the ratio corresponding to the labelled salinity of the standard which is usually 35.00‰ corresponding to a dial setting of 1.00000

e) Set the indicator switch to conductivity ratio and adjust standardization dials so that the instrument reads zero. If the reading is unstable this is usually an indication of air bubbles trapped in the cell. In this case the filling procedure must be repeated after the stirrer has been switched off. After the first standardization the procedure should be repeated and the standardization dials should not be turned until the standardization process has to be repeated. In the record sheet for the instrument, the standardization reading is recorded together with the measurement temperature. The instrument is now standardized and ready to measure samples

Note : After standardization, the temperature compensation dial and the standardization dial of the salinometer must not be changed during the measuring period. Samples are measured as above. A portion of sample is stirred and drained to rinse the cell and a second portion is measured. The temperature of the sample should be within the range in which the built-in compensating circuit is able to balance the change in conductivity. The range for each working temperature is given in a table provided by the manufacturer

f) The conductivity ratio and the temperature for each sample is recorded on the data sheet. That portion of the sample, which has been measured in the cell should be drained back into the sample bottle. This ensures enough water is saved for checking salinities, which seem to be dubious

g) After about half an hour or at the end of a series of measurements, the instrument drift is controlled by measuring Standard Seawater. In order to conserve the somewhat expensive Standard Seawater, subsequent and intermediate standardization and instrument drift control can be checked with a sub-standard. The sub-standard is Seawater usually taken from the area where the samples are taken. Preparation of a substandard should be as follows:

The primary standard for this method must be a sample of Standard Seawater with a stated chlorinity value. To conserve this primary standard, it is best to prepare a large volume (10-20 liters) of a secondary standard consisting of filtered seawater with a chlorinity exceeding 1.8‰. This sample is stabilized by adding a few crystals of thymol and is then quickly put into sample bottles. Every tenth bottle is opened and analyzed in duplicate by the titrimetic method using a silver nitrate solution that has been standardized by Standard Seawater. The mean of ten or more such duplicates is taken as the chlorosy of the secondary standard

h) It is important that the salinities of subsequent samples do not vary greatly to minimize a memory effect within the cell

i) After the measurement, the cell should be thoroughly cleaned with distilled water

Calculation of the salinity

By international agreement, the conductivity ratio is converted to a salinity value by the use of international oceanographic tables [The international oceanographic Tables (Table Ia), Vol.3, 1981] definition of practical salinity,

$$S = 0.0080 - 0.1692\,K_{15}^{1/2} + 25.3851\,K_{15} + 14.0941\,K_{15}^{3/2} - 7.0261\,K_{15}^{2} + 2.7081\,K15^{1/2,}$$

where K_{15} is a conductivity of seawater equivalent to that of a KCl solution having exactly a concentration of 32.435 KCl/kg of solution in vacuum at 15°C.

The above equation is used to compute an uncorrected salinity from the ratio Rt* measured by a salinometer at any given temperature between -2° and +35°C. The uncorrected salinity obtained from the above equation is then corrected by addition of the following equation to give the practical salinity.

$$\Delta S = \frac{t - 15}{1+0.0162(t-15)(0.0005-0.0056\,Rt^{1/2}-0.0066\,Rt-ý0.0375Rt^{3/2}+0.0636Rt^{2}-0.0144\,Rt^{5/2}}$$

Note : *Rt = Ratio of electrical conductivity of a given seawater at t°C to that of 35 psu seawater at the same temperature

Note : Units: Much of the present oceanographic literature has substituted the term practical salinity units (psu) rather than following the traditional parts per thousand (‰)

III.2.5 Chlorophyll

III.2.5.1 Determination of Chlorophyll using Spectrophotometer

The procedure agreed to by a SCOR/UNESCO working group and published by UNESCO in Monographs on Oceanographic Methodology; No.I, is adopted for the determination of chlorophyll pigments. It is also possible to determine chlorophyll by using *in-situ* fluorometer.

Equipment

a) Filtering apparatus consisting of the following items :

- vacuum pump
- Manifold (3 or 6 place)
- funnel assembly (3 or 6)
- filter forceps
- vacuum hose

b) Polyethylene measuring cylinder (1 L)

c) Polyethylene bottle (2 L)

d) Nylon net (0.3 mm mesh size)

e) Filters (0.45 µm in pore size: acetone-soluble; e.g. plain cellulose)

f) Spectrophotometer with cell having a path length of 4 - 10 cm

g) Swing out type centrifuge

h) Stoppered graduated centrifuge tubes of 15 ml capacity having both glass and polyethylene stoppers

Reagents

a) Silica gel

b) Magnesium carbonate suspension:
Add approximately 1 g of fine magnesium carbonate (A.R., light weight or “Levis” grade) to 100 ml of distilled water in a stoppered Erlenmeyer flask. Shake vigorously to suspend the powder immediately before use.

c) Acetone 90% solution :

Distill reagent grade acetone over about 1% of its weight of both anhydrous sodium carbonate and anhydrous sodium sulphate. Collect the fraction boiling at a constant temperature near 56.6°C. Put 100 ml of distilled water into a liter volumetric flask and add acetone to make the volume exactly 1000 ml. The redistilled acetone should be stored in a tightly stoppered dark glass bottle and the 90% reagent prepared in moderately small amounts for use. If good quality acetone is available, it should be shaken with a little granular anhydrous sodium carbonate and decanted directly for use

Filtration of the sample

It is recommended that water samples drawn from the water sampler should be filtered immediately on board. However, samples may be stored for short periods. The amount of sample depends on the amount of phytoplankton in the sample; with ocean water, about four to five liters should be used but with coastal and bay waters, sometimes one tenth of this amount is sufficient.

Set up the filtering system on board or in the laboratory. Place a filter on the base of the filter holder, install funnel and clamp together. Add about 10 mg $MgCO_3/cm^2$ of filter surface either as a powder or as a suspension in filtered seawater.

The required volume of a sample should be measured in a polyethylene measuring cylinder, transferred to a polyethylene bottle and shaken vigorously, before filtration.

Invert the sample bottle into the funnel through a small piece of clean 0.3 mm mesh nylon netting to remove the larger zooplankton and then commence filtration with no more than two thirds of full vacuum.

Drain the filter thoroughly under suction before removing it from the filtration equipment. The filter is ready for chlorophyll measurement.

Note : It is preferable to extract the damp filter immediately and make photometric measurements without delay, however, the filter can be stored in the dark at freezer 18°C or less for up to two months

Procedure

Fold the filter (sample inside) and place it in a small (5-15 ml) glass, pestle type homogenizer.

Add 2-3 ml 90% acetone. Grind it for one minute. Transfer to a stoppered centrifuge tube and wash the pestle and homogenizer two or three times with 90% acetone so that the total volume is 5-10 ml. Seep it for ten minutes in the dark at room temperature. During the extraction period pigments are very photosensitive and neither extracts nor the un-extracted sample should be exposed to strong sunlight or else chlorophyll values will be reduced to a small fraction of their initial level.

Centrifuge for ten minutes at 4000-5000 rpm, but efficiency should be tested with each instrument used. It is recommended to adopt a swing-out type centrifuge, because it gives better separation than an angle centrifuge. If a stoppered, graduated centrifuge tube is used, the extract can be made up to volume and the supernatant, carefully poured or pipetted into the spectrophotometer cell.

If turbid, try to clear by adding a little 100% acetone or by centrifuging again. If necessary dilute to a convenient volume. This depends on the spectrophotometer cell used. Dilute with 90% acetone if the extinction is greater than 0.8.

Prior to measurements, the wavelength setting of the spectrophotometer used should be checked against a standard hydrogen or neon light source. Use a spectrophotometer with a band width of 3nm or less, and cells with a light path of 4-10 cm. Read the extinction at 750, 663, 645 and 630 nm against a 90% acetone blank. Correct the extinctions at each wavelength against blank value. If the 750 nm is greater than 0.005/cm light path, reduce the turbidity as described above.

Calculation

Subtract the extinction at 750 nm from the extinction at 663, 645 and 630 nm. Divide the answers by the light path of the cells in centimeters. If these corrected extinctions are E_{663}, E_{645} and E_{630} the concentrations of chlorophyll in the 90% acetone extract as µg/ml are given by the following equations:

Chlorophyll a = $11.64E_{663} - 2\ 16E_{645} + 0\ 10E_{630}$
Chlorophyll b = $20.97E_{645} - 3.94E_{633} - 3.66E_{630}$
Chlorophyll c = $54.22E_{630} - 14\ 8E_{645} - 5\ 53E_{663}$

If the values are multiplied by the volume of the extract in milliliters and divided by the volume of the seawater sample in liters, the concentration of the chlorophyll in seawater is obtained as µ g/l (= mg/m^3).

III.2.5.2 Determination of Chlorophyll and their Degradation Products using HPLC

In marine ecology, an accurate knowledge of the concentration of chlorophyll-a rather than of the full suite of chlorophylls, carotenoids and degradation products is often all that is required. Some instances are: when monitoring phytoplankton biomass in seasonal studies or in cultures, doing large scale oceanographic mapping or profiling of chlorophyll, and calibrating bio-optical instruments (such as *in situ* fluorometers and transmissometes).

If the determination of chlorophyll-a is to be reliable, it must be separated from any interfering chlorophyll degradation products. One of the objectives of SCOR Working Group 78 was to develop a simple, isocratic HPLC system capable of rapidly separating chlorophyll-a from interfering pigments. Annex VI-Appendix-1 gives a critical summary of isocratic HPLC methods from the literature, and describes two new 'simple' but efficient HPLC systems developed during the SCOR Workshops. The first is an isocratic HPLC for rapidly separating chlorophyll-a from other pigments, and the second is a step-isocratic HPLC for separating and selectively detecting three principal chlorophylls a, b and c_{1+2} and ten additional chlorophyllides and pheopigments.

Note: For further details see Annex VI - Appendix-1.

III.2.5.2.3 Analysis of Phytoplankton Populations Using Pigment Markers

HPLC is used to separate, identify and quantify the various chlorophylls and carotenoids in phytoplankton. Many of these are markers for particular taxa and can be used to estimate their contributions to the phytoplankton community. HPLC is an excellent technique for mapping populations, since it is feasible to analyse more than 1000 samples per cruise. However it does not identify taxa directly and must be combined with microscopy to determine the key species present. Special softwares are available to calculate the relative contributions of different groups of phytoplankton from the pigment content of field samples.

Note: For details of the method see Annex VI - Appendix-2.

III.2.6 C-14 Primary Production

The C-14 method of determining primary production is considered the best available method to date. However, it is only a relative measurement and results should be evaluated carefully in conjunction with chlorophyll and plankton data. The method involves incubation time of a light day (from true noon to sunset).

Filtering

Water samples should be filtered immediately after the incubation period has been stopped under conditions, which are as dark as possible. The vacuum pressure should not exceed 0.3 kg/cm^2. The pore size of the filters should not exceed 0.45 µm. The whole procedure should not exceed one hour for the whole series of bottles. If the samples cannot be filtered within two hours after the incubation has been stopped, the samples should be preserved with concentrated formalin (40%), 0.5 ml per 100 ml sample to avoid loss of ^{14}C due to respiration. Preserved samples can be stored in darkness and at low temperature for 1-2 days before filtering. Other preservation agents should not be used.

Counting

As mentioned earlier, liquid scintillation counting is recommended. During drying, a certain loss of radioactivity has been observed. Therefore, after filtration, filters should be exposed immediately to fuming HCl for about five minutes and then be placed in the scintillation counting vials. It is necessary to use a solubilizer before placing the filters in the fluor. This is done to minimize the problems connected with quenching. If wet filters are not used directly for liquid scintillation counting it is necessary to place the filters in the vapor of 40% formaldehyde in order to minimize the loss of counts during drying.

Total CO_2 concentration

The total CO_2, temperature, pH and salinity of the water sample must be measured and recorded during sampling. (Data should be available from the above analyses).

Reporting

a) General information : data, location and scientist responsible
b) Weather conditions : wind, sky, wave
   Depth : sample depths in meters and Secchi disc reading
c) **Time** : sample collected, ^{14}C added. Incubation period. Start and end of filtration
d) Type of experiment : *in-situ*
e) Temperature : seawater
f) Solution batch used
g) pH and salinity
h) If subsamples are filtered : volume of sample and volume filtered
i) Filter number and pore size

Calculation of primary production

The total carbon uptake dp/dt is calculated from the following equation :

$$dp/dt = \frac{cpm(a) \bullet total_{12}\ CO_2(c) \bullet 12(d) \bullet 1.05(e) \bullet 1.06(f)}{cpm(b)} \bullet k_l \bullet k_2 \bullet k_3$$

where :

(a) = filter cpm - background cpm = net cpm/filter
(b) = the activity of the added solution, cpm
(c) = total concentration of $^{12}CO_2$ in the water sample in mM/l
(d) = 12 = the atomic weight of carbon
(e) = a correction for the effect of ^{14}C discrimination. The uptake of the ^{14}C-isotope is 5% slower than that of the ^{12}C- isotope
(f) = a correction for respiration of organic matter produced during the experiment. This has empirically been found to represent 6% at optimal photosynthesis. The production rate is thus corrected to represent the rate of gross production
k_l = a correction for subsampling factor. Filter 30 ml from a bottle of 128 ml, and add 1 ml of $NaH_{l4}CO_3$, K_1 = 129/30 = 4.3
k_2 = a time factor, e.g. an experiment has an exposure time of half a light day, $k_2 = 2$ if the production should be expressed per light day
k_3 = a dimension factor, e.g. to cover mg C/1 to ma C/m^3, $k_3 = 10^3$

No correction should be made for different total values of the experimental bottles if the entire content of the bottles is filtered. If 1 ml of $NaH_{14}CO_3$ is added to each bottle, any difference in the bottle size only means a more or less dilution of the $NaHCO_3$. The absolute amount of $NaH_{14}CO_3$ will thus be the same in all the bottles. It is usual to express the production as mg C per volume and time unit

III.2.7 Suspended Particulate Matter (SPM)

See Section III.1.1.6.5.

III.2.8 Nutrients

The following procedures assume the samples have been filtered using 0.45 µm membrane filter (see Section III.1.1.6.6).

Calibration techniques applied in the following procedures are based on the traditional way of calibration using a series of different concentrations of calibration standards chosen so that they brack*et al*l sample concentrations. Absorbance readings are blank corrected, plotted against concentration and a best fit calibration line determined.

Calculations based on molar absorbtivities can also be used though these are regarded as less satisfactory by most analysts.

In the case of any nutrient the calibration standards are prepared by diluting the standard stock solution. A blank and at least four calibration standards in graduated amounts in the appropriate range should be prepared. Modern procedures use low nutrient seawater (LNSW) for the final dilution stages. If this is not available then to avoid the complexity of the sample matrix the best way is to use the method of standard addition in which aliquots of standard are added to a series of sub-samples. The use of distilled water for making up the final dilutions of standards can in some circumstances leas to salt errors.

Generally, in the method of standard addition, equal volumes of sample are added to the de-ionized distilled water blank and at least to three standards containing different known amounts of the determinant. The volume of the blank and the standards must be the same. The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled at the same as on the right side, but in the opposite direction from the ordinate. The slope of the plot can be considered as the slope of the calibration curve.

A) Phosphate - P

Principle

The present methods in the analysis of inorganic phosphate in seawater follow essentially the colourimetric method by Murphy and Riley (1962) which is based on the formation of a highly coloured blue phosphomolybdate complex. The modified procedure described here mainly follows the method outlined by Koroleff (1983).

The method shows no measurable effects from salinity but interference may be observed with relatively high concentrations of silicate, arsenate or hydrogen sulfide. The absorbances are proportional to the phosphate concentrations up to about 28 µmol/l when measured in a 1 cm cell.

The phosphate ions in the sample react in acidic solution with ammonium molybdate to yield a phosphomolybdate complex. This heteropoly acid is reduced by ascorbic acid with trivalent antimony ions as catalyst to a blue-coloured complex (with molar absorbtivity of about 22,700), the absorbance of which is then measured in a spectro or filter photometer at 882 nm. In order to obtain a rapid colour development and to depress the interference to silicate, it is important that the final reaction pH is less than 1, and that the ratio between sulfuric acid (in mol/l) and molybdate is kept between 2 and 3 percent.

Reagents

a) Sulfuric acid (4.5 mol/l)

Add 250 ml concentrated acid (d=1.84 g/ml) slowly, under cooling and mixing, to about 700 ml distilled water. Finally, adjust the volume to 1000 ml with distilled water.

b) Ammonium heptamolybdate solution

Dissolve 9.0 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in about 90 ml distilled water and dilute to 100 ml.

The solution should be stored in a polyethylene bottle and should be renewed if any precipitation occurs.

c) Potassium antimonyl tartrate solution

Dissolve 3.25 g $K(SbO)C_4H_4O_6$ in distilled water and dilute to 100 ml.
The solution should be renewed if any precipitation occurs.

d) Mixed reagent

Mix 200 ml sulfuric acid (as above) under continuous stirring with 45 ml molybdate solution. Then add 5 ml tartrate solution.

If the reagent is stored in cool conditions, it is stable for several months.

e) Ascorbic acid solution

Dissolve 7.0 g $C_6H_8O_6$ in distilled water and dilute to 100 ml. Note that solid ascorbic acid is not stable indefinitely and should be rejected if it gives rise to coloured solutions.

The solution is stable for at least a week (as long as it remains colourless) if stored in a dark bottle and in a refrigerator.

f) Phosphate stock solution (10 mmol/l PO_4) (A)

Potassium dihydrogen-phosphate (KH_2PO_4, a.g.) is dried in an oven at 110°C. Then, exactly 1.3609 g of KH_2PO_4 are dissolved in distilled water, 2 ml of 4.5 mol/l H_2SO_4 is added and the mixture diluted to 1000 ml with distilled water in a volumetric flask.

The solution is stable for at least several months.

g) Phosphate working solution (10 µmol/l PO_4) (B)

Dilute 10 ml of the stock solution with distilled water to 1000 ml in a volumetric flask.
This solution should be prepared daily.

Apparatus

a) Glass stoppered bottles (ca. 50 ml) or other suitable containers (e.g. polypropylene bottles, Nalgene type No. 2105-002) for sub-sampling.

b) Graduated cylinder (glass or plastic made) for the quick sub-sampling of 50 ml nutrient sample portions (with a hole as sample overflow at 50 ml, see Figure (III.16) for the quick sub-sampling of 50 ml portions in nutrient analysis (with the hole as sample overflow). These devices are less accurate than say a 50 ml pipette but better than multiple uses of a smaller pipette and safer to use on a small boat than large glass pipettes

c) Automatic syringe pipettes of 1 ml or 2 ml volume for reagent additions

d) Spectro- or filter photometer with filter at or close to 882 nm and cells of 10 cm length (Shorter cells e.g. 5 cm may be used but will give proportionally more important detection limits)

Note : All glass and plastic ware to be used must be cleaned and should be reserved solely for phosphate analysis. The procedure used for cleaning must be tested for phosphate contamination (Common detergents also usually contain phosphates, so that care must be taken when choosing cleaning chemicals). This is also necessary for the distilled or de-ionized water used in the cleaning and analytical procedure described here. It is also recommended to store the sample bottles, when not in use, in 0.1% v/v HCl solution (after the previous major cleaning) and to rinse them, before sub-sampling, three times with the sample solution.

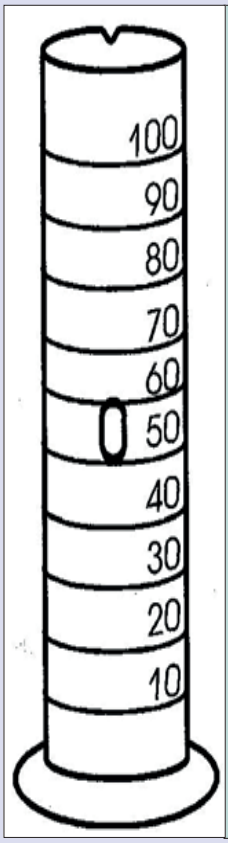


Figure (III.16) : Special type of graduated cylinder (glass or plastic made) for the quick sub-sampling of 50 ml portions in nutrient analysis (with the hole as sample overflow). These devices are less accurate than say a 50 ml pipette but better than multiple uses of a smaller pipette and safer to use on a small boat than large glass pipettes

Method

a) Calibration

Prepare a series of working standards from the phosphate working solution (B). To 100 ml volumetric flasks add (by means of pipette or burette) the following volumes and fill up with low nutrient seawater (distilled water may also be used, but salt effects should be considered) to the 100 ml mark. Then the resulting standard concentrations are:

0.5 ml of working solution = 0.5µmol/l PO_4^{3-}
1.0 ml of working solution = 1.0 µmol/l PO_4^{3-}
2.0 ml of working solution = 2.0 µmol/l PO_4^{3-}
3.0 ml of working solution = 3.0 µmol/l PO_4^{3-}
4.0 ml of working solution = 4.0 µmol/l PO_4^{3-}
5.0 ml of working solution = 5.0 µmol/l PO_4^{3-}

To 50 ml portions of these working standards add the reagents and follow the same procedure as described below for analysis of the sample. Prepare a reagent blank from the same volume of distilled water and reagents. Plot the standard concentrations as abscissa versus the absorbances (corrected for the reagent blank). The calibration curve should be linear over the range of concentration.

b) Analysis of samples

Transfer two 50 ml portions of the sample to two reaction flasks by means of the graduated cylinder. One of the portions is regarded as the sample, the other one as the turbidity blank. To each of the portions add 1.5 ml of acid-molybdate solution (mixed reagent) and afterwards 1.5 ml of the ascorbic acid solution is added only to the sample. Mix well after each addition to both portions. After 10 minutes (but within half an hour) measure the absorbance of the sample and the turbidity blank at 882 nm against distilled water as reference (the turbidity blank may be negligible especially if filtered samples are employed, and can therefore be omitted at high phosphate concentrations and insignificant turbidity).

c) Interferences

There are some other ions occurring in seawater, which potentially may interfere with the formation of the blue phosphomolybdic complex. The major interferences are briefly considered in the following paragraph.

It is well know that silicate and arsenate ions gradually form similar blue heteropoly acid complexes with molybdate ions. As a general rule, however, it may be stated that if the colour is measured after 10 minutes, there are practically no interferences caused by silicate (up to 200 µmol/l) or arsenate (at “normal” total arsenic seawater concentration of around 25 µmol/l). If measurements are performed after 30 minutes for example, 200 nmol/l silicate gives - according to Koroleff (1983) - a net but almost negligible increase in absorbance of 0.003 in a 10 cm cell.

The analysis of phosphate may also be influenced by high concentrations of hydrogen sulfide. It has been found that sulfide concentrations up to about 60 µmole/l do not interfere with the phosphate determination. At higher concentrations antimony sulfide is formed (with greenish colour) when

the acid molybdate reagent is added to these waters. Since high sulfide concentrations are mostly associated with elevated phosphate concentrations, the effect of sulfide can be simply eliminated by diluting the sample with distilled water. If this step is not possible, the sulfide ions should be oxidized by adding bromine water (0.9 ml of bromine in 100 ml of water) drop by drop to an acidified sample (add 0.2 ml of 4.5 mol/l acid to 100 ml sample). The excess bromine is then removed by passing a stream of air or nitrogen through the sample (for about 15 minutes) before commencing the phosphate determination.

Calculation of results

Note that some modern spectrophotometers calculate the concentrations automatically from the absorbances of the standards, however, much software cannot deal with two separate blanks (e.g. a reagent and a turbidity blank). In such cases, alternate methods of calculation are preferred.

The concentration of phosphate is determined from the standard curve as described in the calibration section. Calculate the slope b of the calibration curve (for the individual cell length used), which follows the equation:

$$\text{Absorbance} = b \times \text{concentration}$$

Then the phosphate concentration of the sample is obtained from:

$$C\ (\mu mol/l) = (A_s - Ab_l - A_t) / b$$

Where A_s, A_{bl} and A_t are the absorbances of the sample, the reagent blank and the turbidity blank, respectively (Note: A_t is normally very close to 0 for filtered samples).

Estimation of precision and accuracy

Systematic errors in the phosphate analysis mainly originate from an improper cleaning of the glassware, from difficulties during the sub-sampling and from prolonged storage of the untreated samples. The precision of the method can be considered as being between ± 2% at a relatively high concentration level (about 3 µmol/l) and ± 15% at the low level of around 0.22 µmol/l.

Recommendation : Dissolved inorganic phosphate concentrations for some surface waters in the Eastern Mediterranean may be lower than 0.1 µmol/l and in order to apply sensitive and reproducible techniques for these type of waters, once can refer to MAGIC (magnesium-induced co-precipitation) method (Karl and Tien, 1992).

B) Ammonium - N

Principle

The method is specific for ammonium and is based on the formation of the blue coloured indophenol complex, by phenol and hypochlorite in the presence of the $NH_4^+$and NH_3 species. The reaction requires an elevated temperature or a catalyst. The colour is measured at 630 nm and is stable for at least 30 hours.

The procedure outlined here, mainly follows the methods described by Koroleff (1983) as described by Hansen and Koroleff (1999). The detection limit of the method is about 0.05 µmol/l (in a 10 cm cell), and the Lambert-Beers's Law is followed up to an ammonia concentration of about 40 µmol/l.

In moderately alkaline solution ammonia reacts with hypochlorite to form monochloramine which, in the presence of phenol, catalytic amount of nitroprusside ions and excess hypochlorite, gives indophenol blue (with a molar absorbtivity of about 20,000). The reaction is quantitative in the pH range between 10.8 and 11.4. The reaction mechanism, however, is complicated and not yet fully understood.

The mentioned salt effect depends on the fact that the final reaction pH is a function of the sample salinity (i.e. increasing salinity with increasing buffer capacity decrease the final reaction pH). Therefore the pH of the samples from estuaries should be measured after the addition of reagents. The precipitation of magnesium and calcium hydroxides in the sample solution (occurring at a pH higher than 9.6) is avoided by the addition of a complexing reagent (citrate), which keeps the Mg and Ca ions in solution.

Reagents

a) "Ammonia-free" water

There is no standard procedure for the preparation of water with very low ammonia content. De-ionized water may sometimes be used without subsequent distillation, but it must be noticed that ion exchange resins potentially bleed out organic substances and ammonia. In case the ammonia blank concentrations are higher than 0.3 µmol/l., the water should be subjected to subsequent distillation. In this second step, 0.3 g NaOH and 1 g $K_2S_2O_8$ are added to 1000 ml of water (in a 2 l flask). The solution should be boiled for 10 minutes to remove ammonia (without the condenser) and then distilled until a residue of about 150 ml. The distilled water should be stored in a tightly sealed container, preferably made of glass. The method of preparation of ammonia-free water should be regularly checked and appropriate blanks must be analyzed with every batch of samples. As an alternative, "open sea surface water" can be used as "ammonia-free" water.

b) Buffer solution

Dissolve 240 g tri-sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$), 20 g of disodium EDTA and 0.4 g NaOH in about 600 ml distilled water. The solution is boiled (to remove ammonia) until the volume is below 500 ml. It is then cooled and diluted to 500 ml with "ammonia-free" water.

The solution is stable and should be stored in a well-stoppered polyethylene bottle.

c) Phenol reagent

Dissolve 80 g colourless phenol (C_6H_5OH) in 300 ml of ethanol, add 600 ml of distilled water and 600 mg sodium nitroprusside dihydrate [$Na_2Fe(CN)_5NO.2H_2O$] in "ammonia-free" water and dilute to 1000 ml. When stored in a tightly closed dark bottle and in a refrigerator, the solution should be stable for several months.

Warning : Phenol is a particularly toxic compound and safety glasses and gloves should be worn and all handling conducted in a fume cupboard

d) Hypochlorite reagent

Dissolve 1 g "Trione", the commercial name for the sodium salt of dichloroisocyanuric acid [dichloro-s-triazine-2, 3, 6 (1H, 3H, 5H)-trione] and 8 g NaOH in "ammonia-free" water and dilute to 500 ml. The reagent "Trione" is employed as a hypochlorite donor (in comparison to generally used commercial hypochlorite solutions) has the advantage of being a stable solid, and that it is easy to prepare.

The solution should be stored in a dark bottle in a refrigerator and is stable for at least a week.

e) Ammonia stock solution (A) (10 mmol/l NH_3)

Ammonium chloride (NH_4Cl) is dried at 100 ºC to constant weight. Then dissolve 0.0535 g NH_4Cl in "ammonia-free" water and dilute to 100 ml in a volumetric flask.

When kept in a glass bottle (protected from sunlight) and in a refrigerator, the solution should be stable for at least several weeks.

f) Ammonia working solution (B) (100 µmol/l NH_3)

Exactly 10.0 ml of the stock solution is diluted with "ammonia-free" water to a final volume of 1000 ml in a volumetric flask made of glass.

This solution must be prepared daily.

Apparatus

a) Stoppered 50 ml flasks (of glass, reserved solely for this determination and stored closed and filled with "ammonia-free" water between analysis)
b) Automatic syringe pipettes of 2 ml volume for reagent additions
c) Spectro- or filter photometer with a filter having maximum transmission at 630 nm, and cells of 1 cm, 5 cm and 10 cm length as required

Note : All flasks and tubes to be used should be cleaned with hot HCl, rinsed well with "ammonia-free" water and kept closed between analyses. The analysis should be performed in a well-ventilated room with no ammoniacal solutions stored (Note this should include any cleaning agents containing ammonia and used by laboratory cleaning staff during or outside normal working hours). This includes the NH_4Cl reagent used for nitrate analysis. **Smoking should be forbidden**.

Alternative : Before use, all flasks should be treated by performing the reaction in them with the addition of chemical reagents to the "ammonia-free water" or "open sea surface water". The reaction should proceed at least for 6 hours and the flasks should be shaken time to time during the reaction period. Later, the flask should be rinsed with ammonia-free water and kept stoppered when not in use. The flasks should not be washed between the analysis of different sets of samples/standards, but just rinsed with ammonia-free water and kept closed.

Method

a) Calibration

Prepare a series of working standards from the ammonia working solution (B). To 100 ml volumetric flasks add (by means of pipette or burette) the following volumes and fill up with "ammonia-free" water or "open sea surface water" to the 100 ml mark. In this instance, it is probably best not to use low nutrient seawater unless it is known to have a suitably low ammonia concentration. Then the resulting standard concentrations are :

0.5 ml of working solution = 0.50 µmol/l NH_3
1.0 ml of working solution = 1.00 µmol/l NH_3
2.0 ml of working solution = 2.00 µmol/l NH_3
3.0 ml of working solution = 3.00 µmol/l NH_3
5.0 ml of working solution = 5.00 µmol/l NH_3
7.0 ml of working solution = 7.00 µmol/l NH_3
10.0 ml of working solution = 10.00 µmol/l NH_3

To 50 ml portions of these working standards add the reagents and follow the procedure outlined below for analysis of the sample. In addition, prepare a "blank sample" from the same volumes of the distilled water or "open sea surface water" used and the reagents. Use a cuvette of similar length (preferably at least 5 cm) filled with distilled water as reference. Plot the measured absorbances (corrected for the blank) versus the standard concentrations.

b) Analysis of samples

Measure 50 ml of the sample with a graduated cylinder and transfer it into the reaction flasks. Add 2 ml phenol reagent, 1 ml buffer solution and 2 ml hypochlorite reagent. Mix well by swirling between the additions. Close the reaction bottles properly and keep them in a dark place during the reaction time which is at least 6 hours at room temperature but which is reduced to 30 minutes if samples are incubated in a water bath at 37 ºC ± 1 ºC. Note that standards and samples of the same series must be treated simultaneously, and in the same way.

Measure the absorbance after 6 hours in a cell of suitable length at 630 nm, and use a cuvette of similar length filled with distilled water as reference. (In ocean waters the reaction requires about 5 hours at room temperature; since the colour of the indophenol blue is stable for at least 30 hours, it is convenient, in routine analysis, to let the sample react overnight).

With detectable traces of ammonia in the re-distilled water or "open sea surface water", the blank from the reagents added is determined in the following way: First, measure the absorbance (A_b) by carrying out the above procedure using 50 ml of distilled water. Repeat the determination by adding, however, 3 ml phenol reagent, 1.5 ml buffer solution and 3 ml hypochlorite reagent to only 47.5 ml of the same distilled water or "open sea surface water". Measure the absorbance ($A_{1.5b}$). Then the blank caused by the reagent only is calculated from:

$$A_{rb} = 2\,(A_{1.5b} - A_b)$$

Determine the reagent blank at regular intervals (and for each new batch of solution), because the reagents may absorb ammonia from the air.

c) Interferences

Interferences from amino acids and urea (at seawater levels) can be neglected but may be significant in estuarine or brackish waters, especially where these are contaminated with urban waste. Hydrogen sulfide can be tolerated up to about 60 µmol/l. Samples with higher H_2S concentrations should be diluted. The blue colour of the indophenol, however, is influenced by salinity, which has to be compensated by the application of a salt factor (see below).

Calculation of results

Calculate the slope b of the calibration curve described above (for the length used) which follows the equation :

Absorbance = b x Concentration

As already mentioned, for any given concentration of ammonium the blue colour produced in seawater is less intensive than in distilled water. Thus, for each sample a correction has to be made with respect to its salinity and the resulting pH. In many circumstances a simple correction may be used where the correction is given by:

$NH_{3(corr)} = [1 + 0.0073S_s]NH_{3(uncorr)}$, where S_s is the salinity of the sample.

Estimation of precision and accuracy

Good accuracy and precision in the analysis of ammonia is difficult to obtain, and highly dependent on how successfully the contamination control is achieved during cleaning procedures, sample handling and analytical steps. The main pitfalls are caused by contamination from airborne ammonia (especially tobacco smoke) as well as from the reagents and glassware.

The precision of the method (under ideal circumstances) can be considered as being between 2 and 5% at a concentration level of about 5 µmol/l.

C) Nitrite - N

Principle

The method is specific for nitrite ions (NO_2) and is based on the formation of a highly coloured azo dye which is measured colourimetrically at 540 nm. The procedure outlined here is applicable to all types of marine waters and follows the methods by Bendscheider and Robinson (1952) and Grasshoff (1983). It shows a detection limit of 0.02 µmol/l and a linearity between the amount of the azo dye formed and the initial concentration of nitrite over a wide range of concentration (0 - 10 µmol/l).

The reaction is widely free from interferences of compounds normally present in ocean or in inshore waters. Salinity does not affect the absorbance significantly. If hydrogen sulfide is suspected to be present in a sample (nitrite and sulfide cannot co-exist for long periods in natural seawater), the gas must be expelled with nitrogen after addition of the acid sulfanilamide reagent.

The determination of nitrite is based on the reaction of nitrite with an aromatic amine (sulfanilamide) which leads (at pH 1.5 - 2.0) to the formation of a diazonium compound. This diazo compound then couples with a second aromatic amine N-(1-naphthyl)-ethylenediamine to form the azo dye with a molar absorbtivity of about 46,000.

Reagents

a) Sulfanilamide reagent

Dissolve 10 g crystalline sulfanilamide ($NH_2C_6H_4SO_2NH_2$) in a mixture of 100 ml concentrated hydrochloric acid (HCl, a.g.) and about 500 ml distilled water. (Moderate heating accelerates the dissolution). After cooling, the solution is diluted to 1000 ml with distilled water.

The reagent is stable for several months.

b) N-(1-naphthyl)-ethylenediamine solution

Dissolve 0.5 g of N-(1-naphthyl)-ethylenediamine dihydrochloride [$C_{10}H_7NH(CH_2)2NH_2.2HCl$] in distilled water and dilute to 500 ml.

The solution should be stored in a dark glass bottle in a refrigerator, and must be renewed as soon as it develops a brown colour (usually stable for 1 month).

c) Nitrite stock solution (A) (10 mmol/l)

Anhydrous sodium nitrite ($NaNO_2$, a.g.) is dried at 100°C (for about 1 hour) to constant weight. Then 0.690 g of the dry salt is dissolved in distilled water and diluted to 1000 ml in a volumetric flask.

The solution should be stored in a glass bottle and is stable for at least several months.

Note : Aged solid reagents, even of analytical grade, may contain less than 100% $NaNO_2$ and should, therefore, not be used for the preparation of the nitrite standard solution. For calibration purposes (in distilled water) silver nitrite may be preferred instead because of its higher stability

d) Nitrite working solution (B) (100 µmol/l)

Transfer exactly 10.0 ml of the nitrite solution to a volumetric flask and dilute to 1000 ml with distilled water.

This solution must be renewed daily.

Apparatus

a) Stoppered glass bottles with a capacity of about 100 ml
b) Reagent dispensers (automatic syringe pipette or piston pipettes)
c) Spectro- or filter photometer, with filter at or close to 540 nm, and cuvettes of 10 cm

Method

a) Calibration

Prepare a series of working standards from the nitrate working solution (B). To 100 ml volumetric flasks add (by means of micropipettes) the following volumes and fill up with low nutrient seawater (or distilled water) to the 100 ml mark. Then the resulting standard concentrations are :

0.10 ml of working solution = 0.1 µmol/l NO_2
0.20 ml of working solution = 0.2 µmol/l NO_2
0.40 ml of working solution = 0.4 µmol/l NO_2
0.60 ml of working solution = 0.6 µmol/l NO_2
0.80 ml of working solution = 0.8 µmol/l NO_2
1.20 ml of working solution = 1.2 µmol/l NO_2

To 50 ml of these working standards add the reagents and follow the procedure outlined below for analysis of the sample. In addition, prepare a “blank sample” from the same volumes of distilled water and the reagents. Measure the absorbances in (at least) a 5 cm cuvette. Plot the measured absorbances (corrected for the blank) versus the standard concentrations.

b) Analysis of the sample

Transfer 50 ml of the sample with a graduated cylinder into the reaction bottle and add 1 ml of the sulfanilamide reagent. Then mix well. After reaction time of about 1 minute, add 1 ml of the diamine solution. Shake the flask and allow the azo dye to develop for at least 20-30 minutes. Measure the absorbance in a cell of suitable length at 540 nm against distilled water as reference. The colour intensity is constant for about two hours. (The dye should not be exposed to bright daylight).

For precise measurements of low nitrite concentrations, any turbidity in the sample must be compensated by a “turbidity blank”. For this reason add 1 ml of the sulfanilamide reagent to 50 ml of the sample and measure the absorbance against distilled water as reference. (The addition of acid to a sample usually changes its turbidity; Therefore, it is important that the acidic sulfanilamide reagent is added not only to the sample, but also to the “turbidity blank” sample).

c) Calculation of results

Calculate the slope b of the calibration curve described above (for the individual cell length used) from the equation:

$$\text{Absorbance} = b \times \text{Concentration}$$

The concentration of nitrite is then calculated according to:

$$C\ (\mu mol/l) = (A_s - A_b - A_t) / b$$

Where A_s, A_b and A_t are the absorbance values from the sample, the “blank sample” and the “turbidity blank” sample respectively.

d) Estimation of precision

The precision of the method is ± 0.02 µmol/l though an increase of precision to ± 3 nmol/l is possible with a well setup continuous flow system.

D) Nitrate - N

Note : It is self evident that glassware must not come into contact with nitric acid in this procedure, but analysts should be aware that cigarette tobacco may contain potassium nitrate and that great care must be taken to avoid problems with this during both sample collection and analysis

The method is generally applied for the determination of nitrate (NO_3) is based on its reduction to nitrite, which is then determined colourimetrically via the formation of an azo dye. The method outlined here is based on a heterogeneous reaction with copper-coated cadmium granules and follows mainly the procedure by Grasshoff (1983). It has proved to be reliable and useful for work at sea and is widely free from interferences in nearshore and oceanic waters.

The method determines the sum of nitrite and nitrate, therefore, a separate determination of nitrite must be conducted, and concentration subtracted from that obtained with this method. At concentration levels higher than about 20 µmol/l, calibration curves for a low and high range must be established.

Nitrate is reduced to nitrite in a reduction column filled with copper-coated cadmium granules. The yield of the reduction depends on the pH of the solution and on the activity of the metal surface. The conditions of the reduction described in the method are adjusted to a pH of about 8.5, so that nitrate is converted to nitrite almost quantitatively (90-95%) and not reduced further. Ammonium chloride buffer is used to control the pH and to complex the liberated cadmium ions.

The nitrite formed is then determined colourimetrically (at 540 nm).

Reagents

a) Ammonium chloride buffer

Dissolve 10 g ammonium chloride (NH_4Cl, a.g.) in distilled water and dilute to 1000 ml. The pH is adjusted to 8.5 by adding about 1.6 ml of concentrated ammonium hydroxide (NH_4OH).

Note : The preparation ideally should be done in another laboratory to avoid contamination of the samples by the atmosphere

b) Sulfanilamide reagent (same reagent as for nitrite determination)

Dissolve 10 g crystalline sulfanilamide ($NH_2C_6SO_2NH_2$) in a mixture of 100 ml concentrated hydrochloric acid (HCl, a.g.) and about 500 ml distilled water and make up to 1000 ml with distilled water.

The reagent is stable for several months.

c) N-(1-naphthyl)-ethylenediamine : (same reagent as for nitrite determination)

Dissolve 0.5 g of N-(naphthyl)-ethylenediamine dihydrochloride [$C_{10}H_7NH(CH_2)_2NH_2.2HCl$] in distilled water and dilute to 500 ml.

The solution should be stored cool in a dark glass bottle and should be renewed as soon as it develops a brown colour (usually stable for 1 month).

d) Filling the reduction column

Commercially available granulated cadmium (e.g. coarse powder for reductors grade-BDH) is sieved and the fraction between 40 and 60 mesh (i.e. around 0.25 and 0.42 mm) is retained and used.

> **Warning** : Cadmium is poisonous. It should, therefore, be handled with great care. Never inhale the dust. Perform all operations on the dry metal in a fume hood

e) Copper sulfate solution

Dissolve 10 g copper sulfate pentahydrate ($CuSO_4.5H_2O$) in about 1000 ml distilled water.

f) Nitrate stock solution (A) (10 mmol/l)

Dissolve 1.011 g dry potassium nitrate (KNO_3, a.g.) in distilled water and dilute to 1000 ml in a volumetric flask.

The solution is stable for at least several months.

g) Nitrate stock solution (B) (1 mmol/l)

Transfer 10 ml of the nitrate stock solution (A) to a volumetric flask and dilute to 100 ml with distilled water.

This solution must be renewed daily.

h) Nitrate working solution (C) (10 µmol/l)

Transfer 10 ml of nitrate stock solution (B) to a volumetric flask and dilute to 1000 ml with low nutrient seawater (or distilled water).

This solution must be renewed daily.

Apparatus

a) Reduction to column [see Figure (III.17)]

The major part of the reduction column consists of a U-shaped glass tube with a total length of about 10-25 cm and an inner diameter of 3 mm. Connections to the 100 ml sample bottle and the 25 ml (marked) Erlenmeyer flask are made from flexible capillary tubing (tygon). The sample is drawn through the column by an aspirator or by a small peristaltic pump (with control of the flow rate by means of a three-way stopcock with a bypass). For practical purpose, the whole set-up can be mounted in a box. Suitable flow rates should be determined by experimentation.

b) Stoppered glass bottles, reagent dispensers, and a spectro- or filter photometer (with at least 5 cm cuvettes) as described for the analysis of nitrite are required.

Method

a) Preparation of the reduction column

Free the sieved cadmium granules from oxides by washing them in 2 mol/l hydrochloric acid. Then shake the granules in a 200 ml beaker vigorously (for about 3 minutes) with 100 ml of the copper sulfate solution. Afterwards rinse the copperized cadmium granules under gentle shaking, decant the water and continue washing until the water is free from finely dispersed copper.

Then pour the copperized granules into the reduction column (with the aid of distilled water and a funnel). Encourage effective packing by gently tapping the column with a pencil. When one arm is filled, connect the funnel to the other arm and repeat the procedure. Leave some space in both side arms in order to pack in some glass wool.

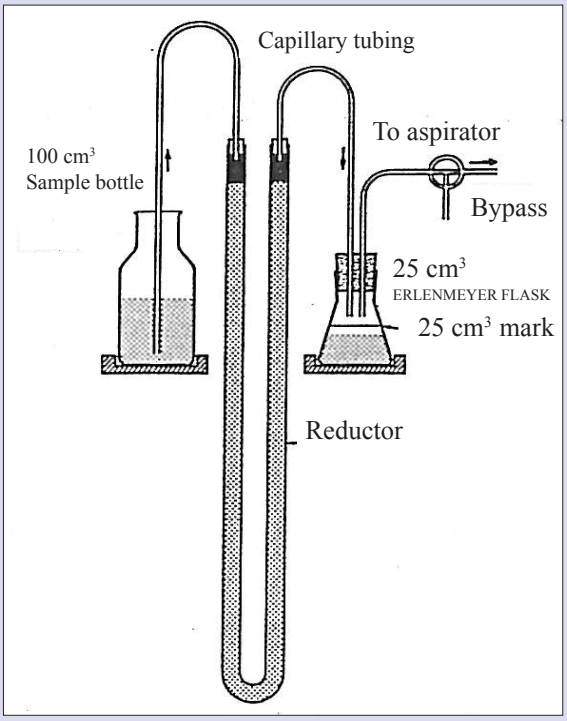


Figure (III.17): Reduction column for the analysis of nitrate

Activate the metal by passing through about 250 ml buffer solution (ammonium chloride) containing about 100 µmol/l nitrate. Then rinse thoroughly with buffer solution before the reducer is used for analysis.

Check the reduction efficiency of the reduction column by analyzing a nitrate standard solution of suitable concentration (e.g. equimolar). Compare the determined absorbance with that of a nitrite solution of the same concentration (e.g. if A_{NO3} = 0.200, A_{NO2} = 0.210, the reduction efficiency would be (0.200 x 100) / 0.210 = 95.2%.

Note : Repeat the activation procedure if the reduction column has not been used for several days, or, if the column has accidentally been filled up with air bubbles. When not in use, keep the column brimful with the ammonium chloride buffer solution. If the efficiency cannot be brought back to above 90% of the theoretical value, it is preferable to refill the column. If frequently used, however, it should last for several months.

b) Calibration (low and high concentrations)

Prepare, a series of working standards from the nitrate stock solution (B) and from the working solution (C) (section: Reagents g) and h) page 28). To 100 ml volumetric flasks add the following volumes of the nitrate standard solutions respectively and fill up with low nutrient seawater (or distilled water) to the 100 ml mark. Then the resulting standard concentrations are :

Stock solution	C µmol/l	B µmol/l
0.1 ml of working solution =	0.01	1
0.25 ml of working solution =	0.025	2.5
0.5 ml of working solution =	0.05	5
1.0 ml of working solution =	0.1	10
2.5 ml of working solution =	0.25	25
5.0 ml of working solution =	0.5	50

To 25 ml of the working standard add 25 ml of the buffer solution. In addition, prepare a "blank sample" from 25 ml distilled water and the same volume of buffer solution. Analyze the standard and blank solutions in the same way as described below for the analysis of the sample. Plot the measured absorbance (corrected for the blank value) versus the standard concentrations. Both curves should be linear over the entire range of concentrations.

c) Analysis of the sample

Transfer 25 ml of the sample into the 100 ml reaction flask, add 25 ml of the buffer solution (ammonium chloride buffer) and mix well. If nitrate concentrations of more than about 15 µmol/l are expected, 25 ml of the sample must be diluted with 75 ml of the buffer solution.

Pass about 20 ml of the mixture through the reduction column in order to rinse the system and to adjust the time of passing (3-5 minutes). Discard this fraction. Then pass (at unchanged speed) another fraction through the column until the level in the Erlemeyer flask has reached the 25 ml mark.

Stop the collection of the reduced sample and add 0.5 ml of the sulfanilamide reagent and 0.5 ml of the diamine solution in the same way as described for the analysis of nitrite. Determine the azo dye colour within about 1 hour (as 540 nm) in 1 cm or 5 cm cells against distilled water as reference.

Calculation of results

Calculate the slope b of the calibration curves described above (separately for the low and high concentration levels and for the individual cell lengths used) from the equation:

$$\text{Absorbance} = b \times \text{Concentration}$$

The concentration of nitrate is then calculated according to:

$$C\ (\mu mol/l) = ((A_s - A_b) / b) \quad - \quad C_{NO2}^-$$

Where A_s and A_b are the absorbances of the sample and of the "blank sample", and C_{NO2}^- is the nitrite concentration (in µmol/l) observed in the same sample.

Estimation of precision and accuracy

In routine analysis the precision (standard deviation) of the method with one and the same reduction column is about ± 0.05 µmol/l for nitrate concentration of 5 µmol/l, ± 0.2 µmol/l in the range

5 - 10 µmol/l and about ± 0.5 µmol/l in the higher concentration range. If different reduction columns are used, the deviation of results depends, of course, strongly on the reduction efficiency of the columns.

The accuracy depends on the reliability of the standard used for the calibration procedure, on the quality of sampling, and/or, if necessary, on the procedure of storage.

E) Silicate - Si

Principle

The determination of dissolved silicon compounds is based on the formulation of a heteropoly acid when the sample is treated with a molybdate solution. This silicomolybdic acid (occurring in two isomeric forms) is then reduced to an intensely blue-coloured complex by adding ascorbic acid as a reductant. The colour is formed within 30 minutes determined at 810 nm, and is stable for several hours.

The method outlined here mainly follows a procedure described by Koroleff (1983). It has a concentration range up to 30 µmol/l (samples with higher concentrations are diluted with distilled water), and a detection limit of about 0.04 µmol/l (in a 5 cm cell). However, only silicic acid and its dimer react with molybdate; therefore, the method gives only the amount of “reactive” silicate.

Similar to phosphate ions, “reactive” silicate forms, in acid solution, a heteropoly acid when treated with molybdate ions. This silicomolybdic complex exists in two isomeric forms (α- and β- isomer) depending on the pH at formation. The isomers have different stabilities, are both yellow-coloured, showing however, only low molar absorptivities in the range of about 1200 and 3300 absorbance units/mole for the (α- and β- isomer), respectively. Major analytical efforts were therefore dedicated to the development of methods in which the heteropoly acids are reduced to intensely blue-coloured complexes. The method outlined here involves the addition of oxalic acid (to avoid the reduction of any excess molybdate reagent and to eliminate the influence of any phosphate present), and the use of ascorbic acid as the reductant. The blue complex shows a molar absorptivity in ocean waters of about 19,000 absorbance units/mole, with stability of the colour for at least several hours.

Reagents

All solutions should be prepared from reagents of known analytical grade only, using high purity de-ionized water. The silicate content of this water should be checked (as a precaution) at frequent intervals according to the procedure described below, and the solutions must be stored in plastic bottles.

a) Sulfuric acid (3.6 mol/l)

Add slowly 200 ml concentrated acid (d = 1.84 g/ml). Under cooling and mixing, to about 700 ml distilled water. Finally the volume is adjusted to 1000 ml with distilled water. The solution should be stored in a polyethylene bottle.

b) Ammonium heptamolybdate solution

Dissolve 20 g $(NH_4)_6MO_7O_{24}.4H_2O$ in about 80 ml distilled water (in a plastic beaker) by moderate heating and dilute to 100 ml.

c) Mixed reagent

A measured volume of the molybdate solution is added to an equal amount of the sulfuric acid under mixing. DO NOT add acid to the molybdate solution.

The solution should be stored in a polyethylene bottle protected from sunlight and should be stable for several months.

d) Oxalic acid solution

Dissolve 10 g $(COOH)_2.2H_2O$ in 100 ml distilled water.

The saturated solution is stored in a plastic bottle and is stable indefinitely.

e) Ascorbic acid solution

Dissolve 1.75 g $C_6H_8O_6$ in 100 ml distilled water. The solution is stored in an amber glass bottle in a refrigerator.

The reagent is effective as long as it remains colourless.

f) Silicate stock solution (A) (10 mmol/l)

Disodium hexafluoro silicate, Na_2SiF_6, is dried at 105 ºC to constant weight. Then 1.8806 g of the salt is dissolved in distilled water (in a plastic beaker) and diluted to 1000 ml in a volumetric flask. The solution is immediately transferred to a polyethylene bottle.

Note that the silica content of this material may vary slightly and that the manufacturer's assay should be consulted and the weight of standard weighed out adjusted as necessary.

g) Silicate working solution (B) (500 µmol/l)

Dilute 4 ml of the stock solution with distilled water to a final volume of 100 ml in a volumetric flask, preferably made of plastic material.

This solution must be renewed daily.

Apparatus

a) Stoppered bottles made of plastic (e.g. polyethylene bottles of ca. 100 ml).
b) Automatic syringe pipettes of 2 ml volume for reagent additions.
c) Spectro- or filter photometer with filter at or close to 810 nm and cells of 1 cm, 5 cm and 10 cm length as required.

Method

a) Calibration

Prepare a series of working standards for the silicate working solution (B). To 100 ml plastic volumetric flasks add (by means of pipette) the following volumes and fill up with low nutrient seawater (or distilled water) to the 100 ml mark. Then the resulting standard concentrations are :

0.25 ml of working solution = 1.25 µmol/l Si
0.5 ml of working solution = 2.5 µmol/l Si
1.0 ml of working solution = 5 µmol/l Si
2.0 ml of working solution = 10 µmol/l Si
5.0 ml of working solution = 25 µmol/l Si
10 ml of working solution = 50 µmol/l Si

To 50 ml portions of these working standards add the reagents and follows the same procedures outlined below for analysis of the sample. In addition, prepare a blank sample from the same volumes of distilled water and reagents. The blank sample compensates for the silicate content in the reagents as well as in the distilled water used for the preparation of the standard solutions. Plot the measured absorbances versus the standard concentrations (corrected for the reagent blank). The calibration curve should be linear over the range of concentration.

b) Analysis of samples

Measure 50 ml of the sample with a graduated cylinder, and transfer it into the plastic reaction bottle. Add 1.5 ml of the mixed reagent and mix well. After 10-20 minutes add 1 ml oxalic acid immediately followed by 1 ml ascorbic acid. Mix well between the additions. Measure the absorbance after 30-40 minutes in a cell of suitable length at 810 nm against distilled water as reference.

As distilled water usually contains detectable amounts of silicate (of the order of 2 µmol/l Si), the reagent blank for analysis of the sample must be determined in a different way than described for the calibration procedure. For this reason the blank is best prepared by carrying out the above procedure using 50 ml of distilled water. The absorbance is denoted $A_{1.5}$. Repeat the determination, but add only 1.0 ml of the mixed reagent. Measure the absorbance ($A_{1.0}$). The absorbance caused by the reagent (A_{rb})only is calculated from :

$$A_{rb} = 3 (A_{1.5} – A_{1.0})$$

Determine the reagent blank for each new batch of mixed reagent.

c) Interferences

Interferences are observed from salinity which reduces the final colour intensity to some extent. Hydrogen sulfide can be tolerated up to about 150 µmol/l without problems.

For precise estimates of low silicate concentrations (using a 10 cm cell), measure a reference absorbance for every sample to compensate for its natural turbidity. For this reason add 3 ml sulfuric acid (0.25 mol/l) to 50 ml of the sample and measure the absorbance against distilled water as reference. (If the sample has a visible turbidity centrifuge or filter it, before analysis, through a well-rinsed 0.45 µm Nucleopore filter).

Samples containing more than 150 µmol/l sulfide must be treated with bromine water in the same way as already described (before) for phosphate.

Calculation of results

Calculate the slope b of the calibration curve described above (for the individual cell length used) which follows the equation:

Absorbance = b x Concentration

Then the "reactive" silicate concentration of the sample is obtained from:

$$C\ (\mu mol/l) = (A_s - A_{rb} - A_t) \times SF / b$$

Where A_s, A_{rb} and A_t are the absorbances of the sample, the reagent blank, and the turbidity blank, respectively.

Estimation of precision and accuracy

Several national and international intercalibration studies in the past have proven the blue silicamolybdic acid method as a very accurate procedure with a rather low tendency to systematic errors. The precision of the method can be considered as being between ± 2.5 and ± 4% at concentration levels of about 50 µmol/l and 5 µmol/l respectively.

F) Total phosphorus

Methods given in Grasshoff *et al*. (1983, 1999) and APHA-AWWA-WPWF (1992) can be used.

Reagents

An oxidizing reagent is required in addition to the reagents listed for phosphate:
Potassium persulphate solution. Dilute 5 ml sulphuric acid (4.5 mol/l) with water to 100 cm^3. Add and dissolve 5 g potassium peroxodisulphate, $K_2S_2O_8$. Store this saturated solution at room temperature in a polyethylene bottle protected from direct sunlight. The reagent is stable for about a week.

Potassium persulphate. $K_2S_2O_8$, solid

Sodium Hydroxide (1.0 mol/l): Dissolve 40.0 grams of NaOH in 1.0 liter of pure water.

Sampling and storage

The analysis should be commended as soon as possible, preferably within 2 h of sampling. When immediate analysis is not possible, the samples are acidified with 0.4 cm_3 of 4.5 mol/l sulphuric acid per 100 ml and stored cold in polyethylene bottles in the dark. Because of the observed uptake of phosphorus by glass, glass flasks should not be used for long storage. Deep-freezing in polyethylene bottles can also be recommended.

Sample and blank measurements

To 50 ml of the sample in the oxidation bottle is added 4 ml peroxodisulphate solution. Alternatively add 0.2 ml sulphuric acid (4.5 mol/l) and 220 ± 20 mg solid persulphate. If the sample is preserved with acid, no further acid is added. Dissolve the reagent by swirling. Close the bottles and place them in the autoclave, which has been filled with about 200 ml water. Autoclave the samples for 30 min. After cooling to room temperature, check that the stoppers are tight, then open the bottles and transfer their contents quantitatively to the phosphate flasks. The volume of the samples should remain unchanged. Neutralize the digested samples with NaOH. Then add 1 ml ascorbic acid, mix, wait for about half a minute and then add 1 ml of the mixed reagent prepared for the phosphate determination. Measure the absorbance at 880 nm after 10-30 minutes using 10 cm cell (or the longest available cell).

Free chlorine formed is reduced by first adding ascorbic acid in the subsequent phosphate analysis. The flow-analysis manifold for phosphate may be modified accordingly, i.e., ascorbic acid solution is introduced as the first reagent, or 0.5 ml of ascorbic acid is added to the sample and mixed before the flow-analysis. Then transfer the required volumes into the reaction flasks for phosphate determination or into sample containers for the flow-analyser.

Take at least three 50 ml potions of distilled de-ionized water (free of phosphorus) and pour into the oxidation bottles. Add 4 cm^3 peroxodisulphate solution. Alternatively add 0.2 ml sulphuric acid (4.5 mol/l) and 220 ± 20 mg solid persulphate. Dissolve the reagent by swirling. The blanks are then treated exactly as described for the samples. In near-shore waters the determination of turbidity blank may be necessary and is then made on a second portion of the sample, by omitting the reagents after the oxidation step. The phosphorus concentrations calculated after blank corrections represent all forms of phosphorus in unfiltered samples. When the analysis is performed using a filtered sample, the result includes all the dissolved forms of the element.

Alternatively, samples are digested on a preheated hot plate for 30 to 40 min or until a final volume of 10 ml is reached. Then, cool the sample and blank, dilute to 30 ml with P-free water, add 0.05 ml (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink colour with NaOH. Make up to 50 ml with distilled water and measure phosphorus concentration by adding ascorbic acid and mixed reagent. In some samples a precipitate may from at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colourimetric reactive phosphorus measurement.

The flow-analysis manifold for phosphate may be modified accordingly, i.e., ascorbic acid solution is introduced as the first reagent, or 0.5 ml of ascorbic acid (Section 10.2.5.4; reagent 4) is added to the sample and mixed before the flow-analysis. Transfer the required volumes into the reaction flasks for phosphate determination or into sample containers for the flow-analyser.

G) Total nitrogen and total phosphorus

For the determination of total nitrogen (TN) and total phosphorus (TP), the methods in Grasshoff *et al*. (1999) can be used.

Reagents

Oxidation mixture: Dissolve 50 g potassium peroxysulphate and 30 g of boric acid in 1 liter of 0.375 mol/l NaOH and store this saturated solution in a well-closed polyethylene bottle wrapped with aluminium foil at room temperature. This mixture is stable for several weeks. Since TN is determined all reagents must be very low in nitrogen.

In the simultaneous oxidation of samples, the reaction starts at about pH 9.7 and ends at 4-5. These conditions are established using a boric acid-NaOH system.

Sampling and storage

Samples can be stored for a long time in the oxidation bottle without adding any preservative if a sufficient number of bottles are available.

Preservation with acid should be avoided. Deep-freezing of the samples in polyethylene bottles is also recommended.

Analysis

To 50 ml of sample, 5 ml of the oxidizing agent is added. Close the flask and autoclave for at least half an hour. Then, open the autoclave and allow the flasks to cool to about room temperature. With a bulb pipette take from the flask 5 ml of the digested sample, on which TN as nitrate can be determined separately. Transfer the remaining 50 ml of sample to a phosphate reaction flask and add 1 ml of ascorbic acid and mixed reagent solutions. Mix swirling between additions. Measure the absorbance at 880 nm after 10-30 minutes using 10 cm cell (or the longest available cell).

H. Analysis of nutrients using continuous flow or other technologies

a) Continuous flow methods

The principle used by the continuous segmented-flow auto-analyzers is recognized as the most reliable and accurate method for determination of nutrients. Different systems are available and can be configured to meet the standard methods such as ISO, EPA, ASTM, etc… Wherever possible it is strongly recommended that such analyzers are used because of the considerable increase in precision and sample throughput that they offer. Ideally such analyzers can be used in laboratories on board a research vessel allowing problems of sample deterioration during storage to be circumvented.

Many different analytical techniques have been developed for these analysers and can be found in the scientific literature.

Note : 1. System reagents and configurations should be available with the operation manual of the instrument
2. Analytical quality control should be considered with each batch of samples to check the performance of the instrument

Important operating notes for automated measurements of nutrients :

1. *Ammonia*

Sodium salicylate has replaced phenol in the latest automated method of BANN+LUBBE.

i) For seawater analysis, use a sampler wash solution containing sodium hydrogen carbonate, magnesium chloride, magnesium sulphate and calcium chloride or "ammonia-free open sea surface water". If the salinity of seawater samples is markedly lower than in the artificial seawater formulation, the dilution of the wash water is required because of the different refractive index. If the concentration of calcium and magnesium ions is markedly lower than in the artificial seawater formulation, artificial seawater containing only sodium chloride (35 g/l) and sodium hydrogen carbonate (0.2 g/l) can be used

ii) For rain, river and waste water analysis, use only distilled-de-ionized water wash solution

iii) The diluent for the standards must have the same matrix as the sample and the sampler wash solution. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from ammonia content in the inorganic salts used for artificial seawater, using a zero calibration standard of low-nutrient seawater of known low concentration is recommended

iv) Final pH of (sample + reagent)

For optimum results, the pH of the final reaction must lie within certain limits. Therefore, collect the solution from the flowcell waste line to check the pH.

Final pH (with salicylate reagents): pH 12.8-13.1
Final pH (with phenate reagents): pH 11.5-11.9
If the final pH is too low, increase the sodium hydroxide concentration.

v) Warning for seawater analysis! Without salicylate and phenol, the hydroxides of magnesium and calcium precipitate after the addition of dichloro isocyanuric acid reagent. Therefore add salicylate and phenol first or remove it last when starting up or shutting down the system

During the day, it may occur that a slight precipitation occurs in the 5-turn coil after DIC addition. This does not affect performance. If it occurs, during the shutdown procedure clean the system with 1 N HCl.

vi) Ammonia is a common contaminant in the atmosphere and general environment. Take extra precautions to avoid contamination of the reagents. Do not touch any surfaces which will be in contact with reagents or samples. Check the reagent absorbance each time fresh reagents are made. Rinse sample cups (pre-washed with 1 N HCl) with samples before filling them. Fill and place the sample cups just before analysis in order to minimize contamination risk from atmosphere. Check sample cup blanks occasionally

2. *Phosphate*

i) For seawater analysis, use sampler wash solution containing sodium chloride and sodium hydrogen carbonate or “phosphate-low seawater”. For water and waste water analysis use only DDW as sampler wash solution

ii) The standard diluent must have the same matrix as the samples and the sampler wash solution. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from phosphate content in the inorganic salt used for artificial seawater, analysis of a zero calibration standard of low-nutrient seawater of known low concentration is recommended

3. *Silicate*

i) Prepare all reagents in polyethylene containers. Use plastic flasks to prepare working standards. If mixed standards (including nitrate, phosphate and ammonia) are prepared, use the same flasks

ii) For seawater analysis, use sampler wash solution containing sodium chloride and sodium hydrogen carbonate. For water and wastewater analysis, use only DI water

iii) For the most accurate results, the standard diluent should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis

4. *Nitrate and Nitrite*

i) For seawater analysis, use a sampler wash solution containing sodium chloride and sodium hydrogen carbonate. For water and wastewater analysis, use only DI water

ii) For the most accurate results, the standard dilutes should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis

iii) The nitrite value of samples can be determined by eliminating the reduction column and standardizing with an appropriate nitrite solution. In order to determine the nitrate values, the nitrite alone must be subtracted from the total (nitrite + nitrate). Check the efficiency of the reduction (cadmium) column daily by analyzing equi-molar nitrate and nitrite standards. If the reduction efficiency of the column is less than 90%, activate the column or replace it by a new one

b) Other technologies

A number of *in situ* multi-channel nutrient analyzers are now available on the commercial market and are being developed for deployment for extended periods at depths down to a few hundred meters. Flow injection systems are also an alternative to the larger scale continuous flow analyzers. Some high sensitivity techniques have been developed, for example using chemiluminescence technology to analyse nanomolar concentrations of nitrate and nitrite. More recently ammonium has been analysed using a combination of Teflon-membrane gas diffusion coupled with fluorescence detection, this again allows nanomolecular detection limits. Most recently there are developments

using the classical colourimetric analytical chemistries similar to those described above but with long path-length flow cells up to 2 metres in lengths called liquid waveguide capillary cells. These systems are now regularly used in some laboratories in their oceanic monitoring and research programmes.

I. Recommendations for water sampling, sample processing and chemical analysis

Contamination sources : Contamination from the sampling equipments, ship and on-board activities should be avoided while sampling is undertaken. Sampling bottles should be cleaned with dilute hydrochloric acid and washed with pure water and always be kept closed when not in use.

Sub-sampling protocol : Firstly, take DO and pH samples using tygon tubing; then in the order of nitrite, other nutrients, chlorophyll and phytoplankton samples. Water samples for dissolved inorganic and total nutrients are taken in high density polyethylene bottles. The bottle quality should be checked before their use by filling both Low Nutrient Sea Water (LNSW) and nutrient-spiked SW.

Sample Handling on board : It is recommended to analyze the samples as quickly as possible after collection and filtration (if visibly turbid). If immediate analysis is not possible, freeze the samples in “aged” and “tested” polyethylene bottles. Water samples for simultaneous analysis of total nitrogen and phosphorus can be stored for a long time in the oxidation bottle. Preservation with acid should be avoided. Samples taken for only total-P is acidified and kept cool until analysis.

Chemical Measurement : All spectrophotometric measurements should be referenced against distilled water. To avoid salinity effect, use Low Nutrient Sea Water (LNSW) for standard preparations and as wash-water in the automated method. It is also recommended to obtain full “procedural blanks” which includes sample bottle blank filled with Low Nutrient Sea Water (LNSW) and treated as samples. Pay attention to provide nutrient-free chemicals. Nitrate analysis (using NH_4Cl) should not be conducted in the same laboratory as ammonia analysis. Check Nitrate reduction column efficiency (>90%) before analysis. The colourimetric reactions of the nutrient measurements are quantitative only within certain pH ranges. Therefore, check the final pH for samples during analysis.

Quality Control : Replicate samples of 5 can be obtained both from low and high nutrient waters (e.g. reference and polluted sites) to ensure the repeatability of sampling and analysis. Laboratories are also encouraged to participate in regional or international Quality Control schemes such as those operated by IAEA/MEL or QUASIMEME.

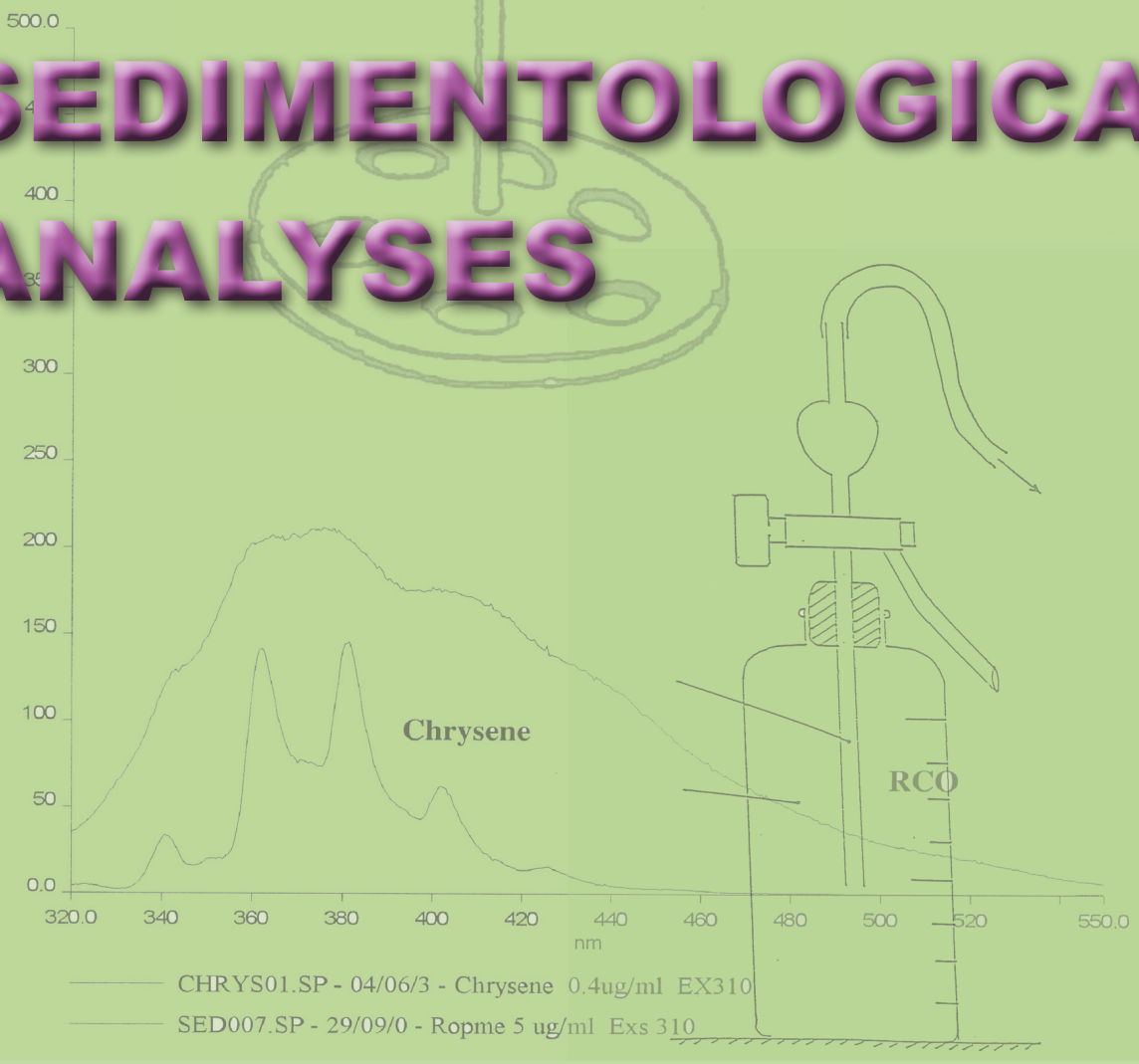
III.2.9 Petroleum Hydrocarbons

See Chapter VI (Analysis of Petroleum Hydrocarbons).

III.2.10 Microbiological Measurements

(See Chapter VIII)

IV
SEDIMENTOLOGICAL ANALYSES



IV. SEDIMENTOLOGICAL ANALYSES

Sedimentological analyses involve procedures for determining the granulometric and mineralogical composition of the sediments since bottom sediments are considered the ultimate depository of trace metal and other pollutant inputs. Numerous studies have shown a positive correlation between fine-grained sediments and elevated levels of these pollutants. Therefore, an accurate determination of the granulometry and mineralogy of sediments collected for pollutant analysis is essential in comparative studies.

The main purpose of the size analysis of sediments is to determine and understand their granulometric characteristics and textural properties. This information is essential for the assessment of depositional environment and the determination of some petrophysical and geo-technical characteristics.

In addition, regional comparisons of elemental concentrations can only be made by using textural equivalent sediments and/or size fractions. It is, therefore, often necessary to normalize for the 'grain size effect' and sometimes to determine the elemental concentrations in different size fractions of the sediments.

IV.1 SAMPLE PREPARATION

IV.1.1 Initial Preparation

The selection of sub-samples for sedimentological and chemical analyses involves procedures for sub-sampling, drying, water content determination, and removal of the sea salts.

Note : Extreme care must be taken during sample preparation for chemical analyses as always in order to avoid contamination :

- by metallic objects, un-decontaminated plastic and glassware, and uncovered hands for trace metal analyses (see Section V.2)
- by un-decontaminated glassware and/or plastic for trace organic contaminant analyses (see Section VI.2)

This implies that samples for the 2 types of chemical analyses are treated separately or that only clean glass or Teflon materials should be used for all preparation steps.

Initially, samples designated for chemical analyses of the total sediment are unfrozen and homogenized still wet. The samples are dried, crushed lightly, homogenized and sub-samples removed for archiving, and for grain size separation (see below). The coarse material >2 mm (-1.0 Ø) such as pebbles, coarse organic fibers and shells are removed by hand (with the help of tweezers) or by using a clean 2 mm sieve. (If analyzing trace elements, the sieve should be plastic or nylon.) The remaining material is again homogenized being split into sub-samples for the various chemical analyses by splitting and quartering (see Section IV.1.7). Representative samples are then obtained using plastic (for trace metal analyses) or stainless steel (for trace organics analyses) utensils Figure (IV.1).

Note : Extreme care must be taken during sample preparation as always to avoid metallic contamination by metallic objects, un-decontaminated plastic and glassware and uncovered hands

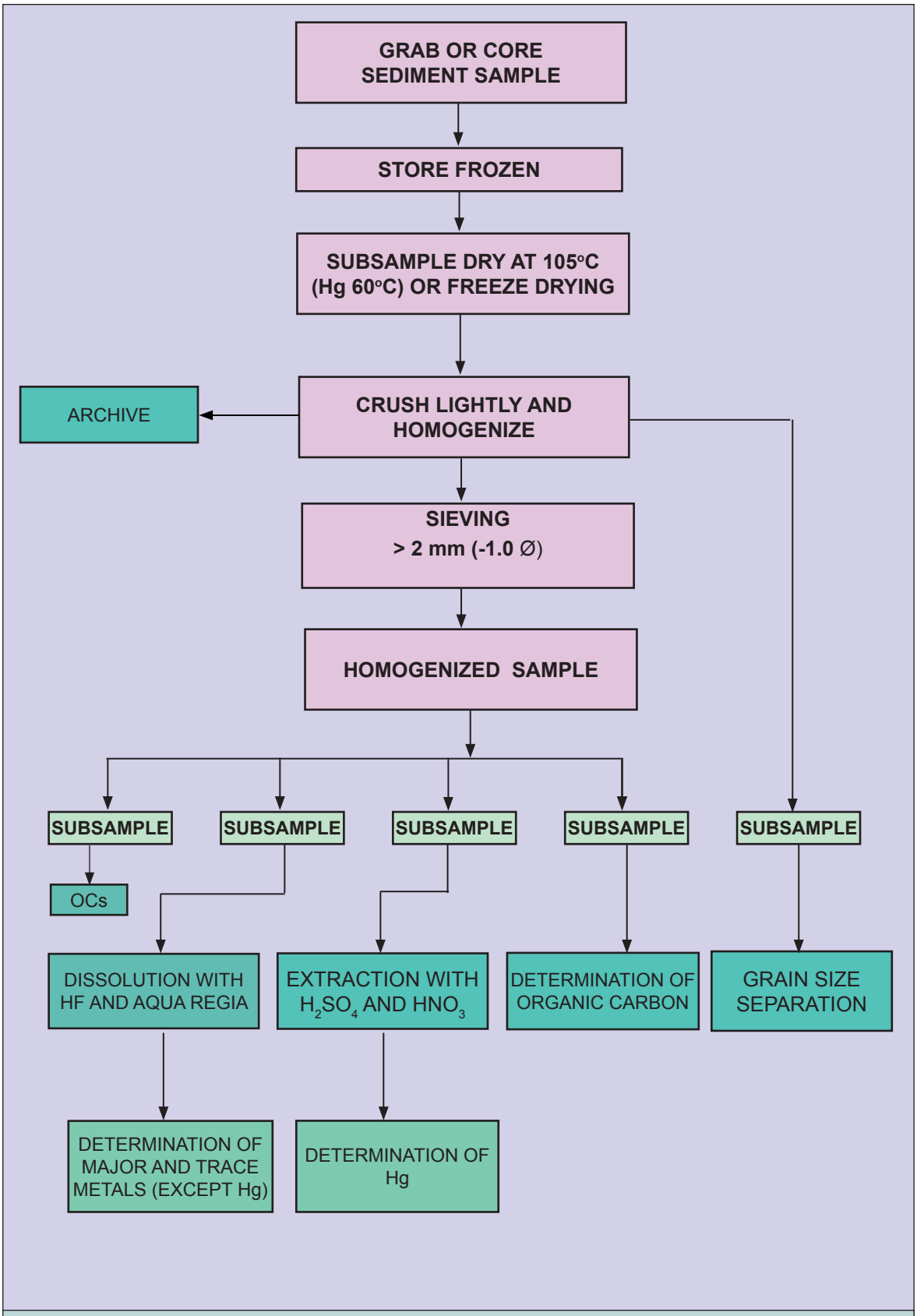


Figure (IV.1) : Sample preparation and analytical sequence for determinations of physical and chemical parameters in marine sediments

IV.1.2 Drying

Sediment samples must be dried before grain size and chemical analyses are conducted because analytical measurements are based on a dry weight basis. Sediments should be dried immediately after removal from frozen storage to prevent bacterial growth; dried sediments can be stored indefinitely at room temperature, preferably in a dark space.

The preferred method for drying is freeze drying (lyophilization), as it is compatible with all analyses. Freeze drying is one of the most useful methods for drying sediments because of the lower risk of losing volatile elemental components and providing a powdery material instead of hard clay aggregates. If freeze-drying is not an option, samples may be oven-dried; however, this should be done in an oven designated specifically for this purpose. If the samples will be analyzed for Hg, they should be dried at 60°C to avoid the loss of any volatile Hg. If this is the case, either the entire sample can be dried at 60°C (advisable for clay minerals), or the sample can be split before drying, with one fraction to be processed at 60°C for Hg and other volatile components and another fraction processed at 100-110°C for all other analyses.

It is important to note that whatever method of drying is employed, a representative sub-sample, which will **not** be analyzed, will need to be taken for oven-drying to a constant weight at the time of sample processing. This is done for the purpose of determining 'moisture' content (i.e., residual moisture in a previously dried sample, generally not more than a few percent) rather than 'water' content (i.e., quantity of water in a wet sample, generally 10's of percent). Freeze drying will not always remove all water to a constant weight, and even samples that have been completely dried will take on a small amount of moisture between the time they are dried and the time that they are processed for analysis. The value obtained for the moisture in the sub-sample will be used to correct the measured analyte concentrations so that they are reported on a constant dry-weight basis.

Note : Studies (Bartlett and James, 1980, Thompson *et al*., 1980, and Kersten and Forstner, 1987) indicate that air, oven, and freeze drying all introduce physical and chemical changes in the composition of the original sedimentary material after it has been removed from its in situ environment. Most of the changes relate to oxidation/reduction changes, and affect the proportion of metals weakly held in the sediments. For example, Kersten and Forstner (1987) found that oven drying and freeze drying, carried out separately under vacuum, change the original associations of the metals amongst the various sedimentary components. Since trace metal concentrations are relatively high in most sediments, such changes are most likely insignificant in relation to other errors in total trace metal determinations. In any event, such effects can be minimized by careful sampling, storage, and use of the material

IV.1.3 Water Content

Fresh sediment samples contain 30 to 95% by weight of water. In addition to the requirement for constant weight dry samples, the water content should be measured because it allows a salt content correction factor to be calculated in sediments of high water content, and allows one to go from measurements of sedimentation rates (mm/yr) to mass accumulation rates when radiochemical analyses are performed.

IV. 1.3.1 Determination of Water Content in Wet Samples (Oven drying)

a) Weigh out accurately 1 to 10 grams of the wet sample (x grams) into a glass, aluminum or Teflon weighing container that has been dried at 105°C and preweighed
b) Place in drying oven (pre-heated to 105°C) for 24 hours
c) After drying, place the samples in a desiccator until cool
d) When the samples reach room temperature, reweigh them (y grams)
e) Calculate the water content (expressed in percentages) from the equation:

water content % = weight loss [(x - y)/ x] • 100

IV.1.3.2 Determination of Water Content in Wet Samples (Oven drying)

All the results should be expressed on an oven dry weight basis so that the results are independent of variations in air humidity. This procedure should be also followed for oven-dried samples that have been stored at room temperature outside a desiccator.

a) Follow the procedure outlined for wet samples (Section IV.1.3.1)
b) Instead of determining the % moisture, instead determine the % solid, and divide the final result by the decimal percent solid
c) Correction to obtain oven dry weight:
Decimal % solid = weight after oven-drying / weight before oven-drying
Correction of results to oven-dry mass: Original result / Decimal % solid

IV.1.4 Salt Content Correction

Corrections for salt content are required for samples containing large amounts (>70%) of interstitial salt water because high salt contents introduce significant errors in metal determinations. In many cases, an average salinity (35 ‰) and a density of 1.025 g/ml may be assumed for pore water but for sediments with a high water content (>90%), the pore water should be squeezed out and its salinity determined.

The corrections for salt content are made from the data obtained from the determination of the water content of wet samples (Section IV.1.3.1).

Calculate the salt correction from the following equation :

Weight (wt.) of sample wet = X grams
Wt. of sample dry = Y grams
(X-Y) = wt of water = (wt. of sea-water - wt. of salt)
Wt. of water + salt (g) = (X-Y)/1 x 1.025 (density of pore water)
X - 1.025(X-Y) = wt. of dry mud (salt corrected)

Removal of soluble sea salts

In addition to correcting for the salt content of sediments containing high water contents, it is sometimes necessary to remove the soluble sea salts from the sediments before analyses. Removal of soluble sea salts is required for samples, which are used for the determination of the major cations Na, K, which are also present in seawater. This is done to ensure that there is no contamination of sediments by the seawater and that there are no chloride-induced interference in the chemical analyses.

a) Transfer 10 to 20 grams of dry sample to a 1 liter polypropylene centrifuge bottle
b) Disperse it by shaking for 30 minutes with 1 liter of water (distilled and de-ionized water)
c) Centrifuge the suspension at 2000 rpm until clear
d) Siphon the water off, repeating until the supernatant is chloride free (tested by adding $AgNO_3$ to a small water sample which precipitates the chloride as $AgCl_2$)
e) Transfer the sediment to an evaporating dish and dry in the oven at 105°C
f) Crush the dried sample in an agate mortar, mix well to avoid selective crushing, and store in air-tight vials until required (only used for pollution analysis)

IV.1.5 Storage of Samples

After drying, the various sub-samples are placed in a desiccator until cool, and stored in air tight plastic or glass vials until required for future use.

IV.1.6 Disaggregation

Most sediments can be placed in one of the following three categories: (A) sediment containing clay and cementing agents, (B) sediment containing clay, but not requiring freeing of cementing agents or pigments, (C) sediments containing no clay, cementing agents nor soluble salts. Marine sediments belong to types A or B since they will contain appreciable amounts of soluble salts, organic detritus, etc.

Whilst it is appreciated that for accurate grain size analysis the sample should be maintained in its “natural” state, in practice it might be necessary to treat the sample as described below, especially when sediments are oven-dried and not freeze-dried. It should, however, be stressed that “breaking up” of particles will change the particle size distribution, so this task should be performed with care.

IV.1.6.1 Unconsolidated Sediments

The dried sample should be spread out on a large sheet of wax paper (40 x 40 cm) and clumps gently broken with gloved hands / fingers. Examine the sample with a hand lens or binocular microscope to see that all aggregates are crushed.

If aggregates still occur, they should be separated from individual grains using a 2 mm (-1.0 Ø) sieve. Mash the aggregates with gloved hands / fingers, and repeat examining and sieving until the sample is completely disaggregated.

IV.1.6.2 Weakly Consolidated Sediments

Place the dried sample in a mortar and place a large sheet of paper under the mortar. Break the clumps with a rubber pestle. Examine with hand lens to check that aggregates are completely destroyed. If aggregates still occur, follow the procedure for unconsolidated sediments described above.

Note : Always use an up-and-down motion; never use a grinding motion or the individual grains may be broken

IV.1.7 Sample Splitting

Mix the dried, disaggregated sediment thoroughly and split the sample to obtain the desired weight.

IV.1.7.1 John Splitter Method

Use John's Splitter, Figure (IV.2) to divide the sample in half. Replace one of the pans containing one half of the sample with a clean pan, and pour this half through the splitter again. The clean pan will then receive one-fourth of the original sample. Repeat as needed to reach the desired weight of sample.

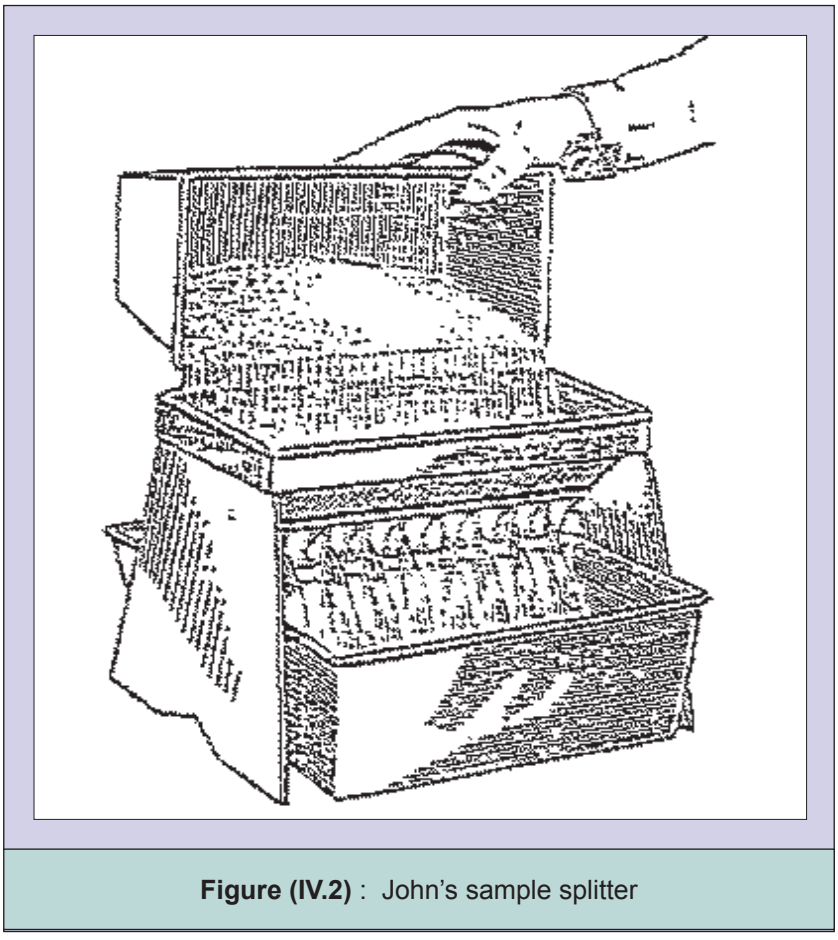


Figure (IV.2) : John's sample splitter

IV.1.7.2 Coning and Quartering Method

This involves the following steps [see Figure (IV.3)].

- pour the sample onto a flat surface to form a cone (1)
- use a clean straight edge to divide cone in half (2)
- divide halves into quarters (3)
- remove alternate quarters (4), reform cone and repeat steps until approximate desired weight is obtained

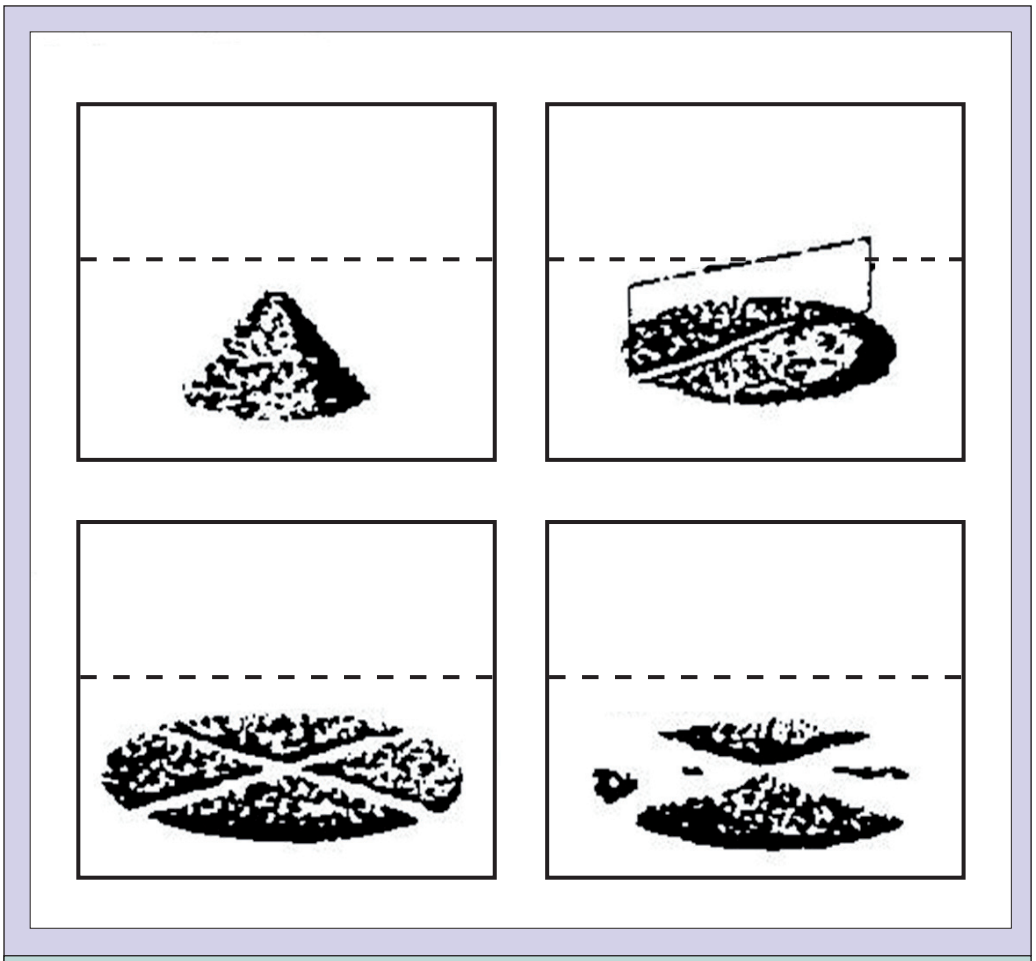


Figure (IV.3) : Diagram of coning and quartering techniques for obtaining sediment sub-samples

After taking the sample split needed for grain size analysis, keep the rest of the sample in a polyethylene bag, labeled with its number and location, and store it.

Note : Make sure that a considerable portion of the sample is stored as reference bond for other analyses

Decantation

This is a method used for removing soluble salts from the sediment sample. It is not recommended to use this method if the sediment contains more than 20-30% mud.

Place the sediment suspension in a high-form 500 ml beaker. Stir well and let it settle for the time required for 4 f (63 microns) particles to settle to the bottom of the beaker Table (IV.1).

Table (IV.1) : Time for quartz particles to settle 10 cm of water[a] (after Carver, 1971)

Diameter		Temperature			
Phi	mm	15°C	20°C	25°C	30°C
4	0.063	0 min 32 sec	0 min 29 sec	0 min 25 sec	0 min 23 sec
4.5	0.044	1 min 5 sec	0 min 57 sec	0 min 51 sec	0 min 45 sec
4	0.031	2 min 10 sec	1 min 55 sec	1 min 42 sec	1 min 31 sec

[a]For a distance of settling (s) other than 10 cm, multiply above times by s/10

When the appropriate time has elapsed, insert the drawing end of the siphon at a distance of about one inch above the layer of sediment that has settled to the bottom of the beaker.

Note : Do not draw any sediment off the bottom of the beaker

IV.1.8 Removal of Carbonate Cement (Anusher Method)

The removal of content of organic matter is done from mineralogical investigation. Place the dried sample split in a mortar and crush it to pea-size chunks.

Place the dried, crushed sample in a 500 ml beaker. Add 25 ml distilled water and stir.

Add 10% HCl slowly and stir until effervescence stops. The amount of acid required is proportional to the abundance of carbonate material. **BE VERY CAREFUL, VIGOROUS EFFERVESCENCE MAY RESULT**. In case of vigorous effervescence add distilled water from a squirt bottle to prevent frothing over. When the beaker is nearly full, concentrated acid may be added very slowly or the excess liquid may be decanted or siphoned off.

Place the beaker on a hot plate and heat it to 80-90°C until effervescence stops and a pH of 3.5 to 4 is reached. To check completion of the reaction, use an appropriate ranged pH paper as an indicator

After completion of the reaction decant or siphon off the solution. Then add distilled water, stir and decant. Repeat this process at least three times until the residual sand is properly washed.

Pour the residual sand onto a filter paper that has been previously weighed. Use distilled water from a squirt bottle to ensure complete transfer of the sand from the beaker to the filter paper.

Place the filter paper in a watch glass and dry it at a temperature of 110°C in an oven.

IV.1.9 Removal of Organic Matter

In case a little organic matter is present, place the sample in a 500 ml beaker and add 100 ml of 6% H_2O_2 slowly and with constant stirring. Cover the beaker with a watch glass and heat it on a hot plate at 40°C for one hour. Decant excess H_2O_2 and wash the sample with distilled water.

If much organic matter is present, use the same procedure but use 30% H_2O_2 instead of 6%. Do not let sample froth over. Avoid contact with the skin, as 30% H_2O_2 can cause burns.

IV.2 GRANULOMETRY (GRAIN SIZE ANALYSIS)

Granulometry is the classification of sediments into various grade sizes. The procedure commonly involves two steps (1) wet sieving of raw sediments on a graded series of metal sieves, to separate the coarser fractions above 4 Ø (0.062 mm), and (2) a further separation of the fine grained fraction (finer than 4 Ø) by repeated collection of particles settling in an aqueous solution (pipette or hydrometer). It should be noted that when determining grain size, as with moisture content, that the measurement is done on an aliquot of the sample that will not be analyzed.

The first step in measuring grain size of collected sediments is to select a scale. Natural systems can usually best be described in ratio scales rather than arithmetic scales; the grain size scale devised by Udden is based on a constant ratio of 2 between successive classes. Wentworth and Krumbein devised the phi (Ø) scale as a logarithmic transformation of the Wentworth Scale. Almost all contemporary studies use the phi (Ø) scale since they simplify mathematical computation. Table (IV.2) presents the various equivalent scales.

All data for the granulometric analysis of regional samples should be reported using the phi (Ø) scale.

There are several techniques for the size analysis of sediments: the most widely used are sieve analysis and sedimentation analysis. The first is used for sands and gravels and the second is mainly used for the mud fraction. The analytical procedure is dependent on the nature and composition of the sediment Figure (IV.4).

In the case of recent muddy sediments (e.g., tidal flat mud, marine muddy sediments, desert playa mud), sediment samples should be air-dried. Using a sample splitter, obtain about 50 g of sample having a mud content of 20-30%. If the sediment sample has more than 50% mud fraction then obtain about 20 g. Place the dried sample split in a 500 ml beaker and disperse. After dispersion separate the sand from mud by wet sieving.

In the case of sub-recent muddy sediments (e.g., terrigeneous muddy sand or sandy mud; usually these sediments are friable), disaggregate the sample and obtain a sample split of about 50 g using John's splitter. Place the dried sample split in a 500 ml beaker and disperse. Separate the sand from the mud by wet sieving.

Table (IV.2) : Selected Wentworth (mm) and phi (Ø) scale equivalent with common nomenclature for each size fraction. Scale covers range of most marine sediments. Also included are the mesh numbers for both U.S. Standard and Tyler Sieve Mesh

US Standard Sieve Mesh #	Millimeters	Microns	Phi (Ø)	Wentworth Size Class	
			-20		
	4096		-12		GRAVEL
	1024		-10		
	256 ----------	----------	-8	Boulder (-8 to -12 Ø)	
	64 ----------	----------	-6	Cobble (-6 to -8 Ø)	
	16 ----------	----------	-4	Pebble (-2 to -6 Ø)	
5	4		-2		
6	3.36		-1.75		
7	2.83		-1.5	Granule	
8	2.38		-1.25		
10 ----------	2.00		-1.0		SAND
12	1.68		-0.75		
14	1.41		-0.5	Very coarse sand	
16	1.19		-0.25		
18	1.00		0.0		
20	0.84		0.25		
25	0.71		0.5	Coarse sand	
30	0.59		0.75		
35 -------- 1/2 ---	0.50	500	1.0		
40	0.42	420	1.25		
45	0.35	350	1.5	Medium sand	
50	0.30	300	1.75		
60 -------- 1/4 --	0.25	250	2.0		
70	0.210	210	2.25		
80	0.177	177	2.5	Fine sand	
100	0.149	149	2.75		
120 -------- 1/8 --	0.125	125	3.0		
140	0.105	105	3.25		
170	0.088	88	3.5	Very fine sand	
200	0.074	74	3.75		
230 -------- 1/16 --	0.0625	62.5	4.0		
270	0.053	53	4.25		MUD
325	0.044	44	4.5	Coarse silt	
	0.037	37	4.75		
------ 1/32 ---	0.031	31	5.0		
Anlayzed 1/64 --	0.0156	15.6	6.0	Medium silt	
------ 1/128 --	0.0078	7.8	7.0	Fine silt	
by 1/256	0.0039	3.9	8.0	Very fine silt	
	0.0020	2.0	9.0		
Pipette	0.00098	0.98	10	Clay (Some use 2 Ø or	
	0.00049	0.49	11	9 Ø as the clay	
or	0.00024	0.24	12	boundary)	
	0.00012	0.12	13		
Hydrometer	0.00006	0.06	14		

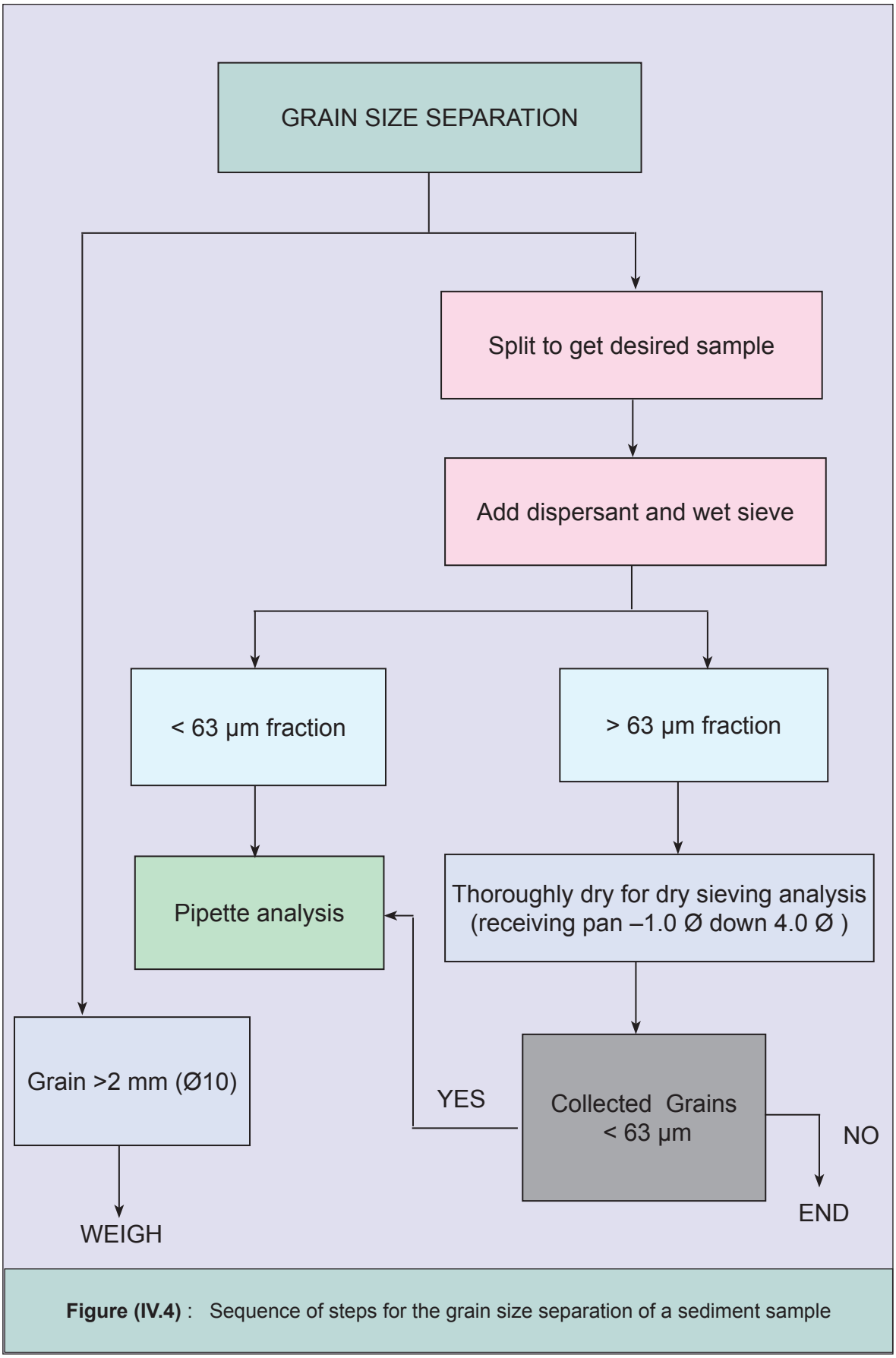


Figure (IV.4) : Sequence of steps for the grain size separation of a sediment sample

In the case of calcareous muddy sediments (e.g., marl), disaggregate the dried sample and mix the disaggregated sample well to ensure proper homogenization. Obtain a sample split of 100 g using John's splitter. Remove the carbonate material. Disperse the insoluble residue and then separate the sand from the mud by wet sieving.

IV.2.1 Equipment and Supplies

1. U.S. Standard; Tyler or equivalent sieve set containing -1.0, -0.5, 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 Ø sieves with lid, collecting pan and sieve brushes
2. 1000 or 500 ml cylinders, glass or plastic
3. Laboratory balance accurate to 0.001 g
4. Top loading balance accurate to 0.01 g (optional)
5. Mechanical shaking device (Ro-Tap or equivalent)
6. Mechanical or electrical stirrer (milk shake machine, drink stirrer, etc.)
7. Funnel (large size plastic or metal)
8. Drying oven
9. Washing bottles
10. Dispersing agent, e.g. sodium hexametaphosphate (Calgon ®); NH_4OH
11. Distilled water
12. Large sheets of clean paper 50 x 50 cm
13. 50 ml evaporating dishes or beakers
14. Stirring rod or equivalent [see Figure (IV.5)]
15. 20 ml volumetric pipettes with rubber hose
16. Thermometer (accuracy 0.5°C)
17. Stop watch
18. Log normal graph paper
19. Marking pens and diamond pencil (for marking evaporating dishes)
20. Centrifuge: A large capacity centrifuge is highly desirable, although not absolutely necessary, for washing samples free of sea salts and for separating clay size fractions. The same centrifuge with a smaller head, or a centrifuge of smaller capacity, is also needed for separating the acetic acid soluble fraction.
    Polypropylene or glass centrifuge bottles.
21. Brushes
    - soft brass wire sieve brush
    - nylon bristle sieve brush
22. Glass or plastic vials 30 and 60 ml
23. Aluminum foil
24. Hot plate
25. pH paper
26. Filter paper
27. Watch glass

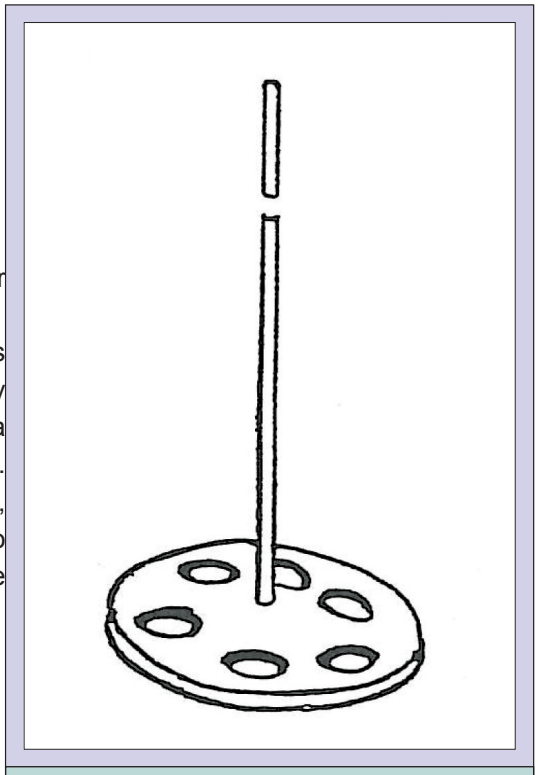


Figure (IV.5) : Stirring rod for pipette analysis. Rod should be constructed to fit the particular 1000 ml graduated cylinder used. Base can be of plastic, metal or rubber; diameter should be 5 mm less than inside diameter of cylinder

28. Rubber gloves, disposable
29. Wide form beaker 500 ml
30. John's splitter or equivalent
31. Andreason sedimentation apparatus or 1 L cylinder, Figure (IV.6)
32. 30 ml beakers
33. 50 ml beakers
34. 2" (5 cm) watch glass
35. Water bath

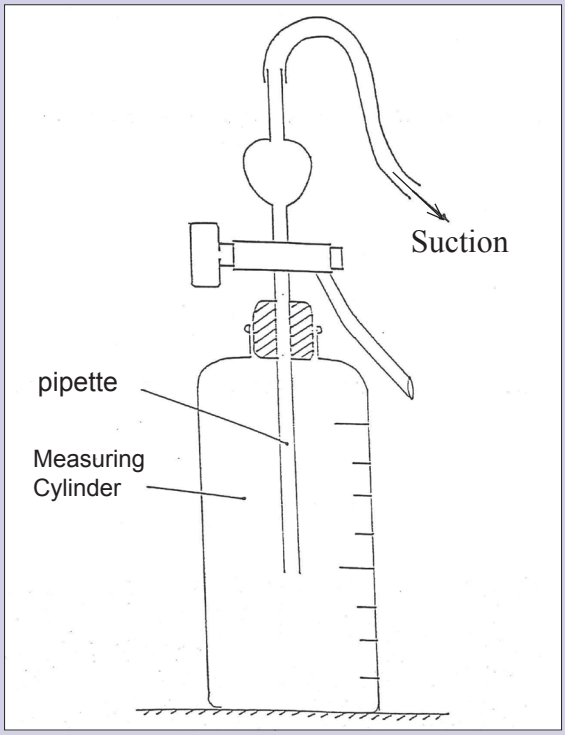


Figure (IV.6) : Andreason sedimentation apparatus (500 ml)

IV.2.2 Dispersion of Muds and Clays

To ensure complete separation of grains prior to sieve analysis, the samples should be dispersed by the following steps:

- Place the pre-weighed dried sample in a 500 ml beaker, add 200 ml of the dispersant solution (0.5% of sodium hexametaphosphate) and let it soak overnight. Muddle the sample with a rubber gloved finger until all the lumps, even the tiniest ones, have been crushed

Note : Be careful not to lose any of the material that clings to the glove

- Pour the suspension into a 500 ml bottle and cap it. Shake vigorously for 2-4 minutes. Pour the suspension from the bottle into the beaker again and stir with an electrical stirrer for 10-60 minutes, making sure that none is lost. The suspension now is ready for separation of the sand fraction, and further dispersion of the silt and clay fraction

IV.2.3 Determination of Sand and Mud Fractions by Wet Sieving

Determination of the amount of sand and mud size material by weight is essential for the basic classification and identification of the sediment texture.

Separation of sand from mud by wet sieving is considered to be the most accurate method. It requires the equipment shown in Figure (IV.7), which consists of a 230 mesh sieve (4.0 Ø or 63 µm), a glass funnel with a diameter larger than that of the sieve, a holder for the funnel, a receiving beaker and a washing bottle for the dispersant solution.

After proper dispersion of the pre-weighed dry sediment sample (a), pour the suspension on the sieve (4.0 Ø). Move the sediment in the sieve by hand in a round motion while squirting a fine jet

of dispersant solution from the squirt bottle to wash the mud through the screen. Wash down the underside of the screen periodically with the dispersant jet. Continue washing until sediment is free of fine particulates. The sand fraction collected on the sieve will be taken for size analysis.

Note : Make sure that only dispersant solution is used, the volume of the mud suspension does not exceed 400 ml; and none is lost

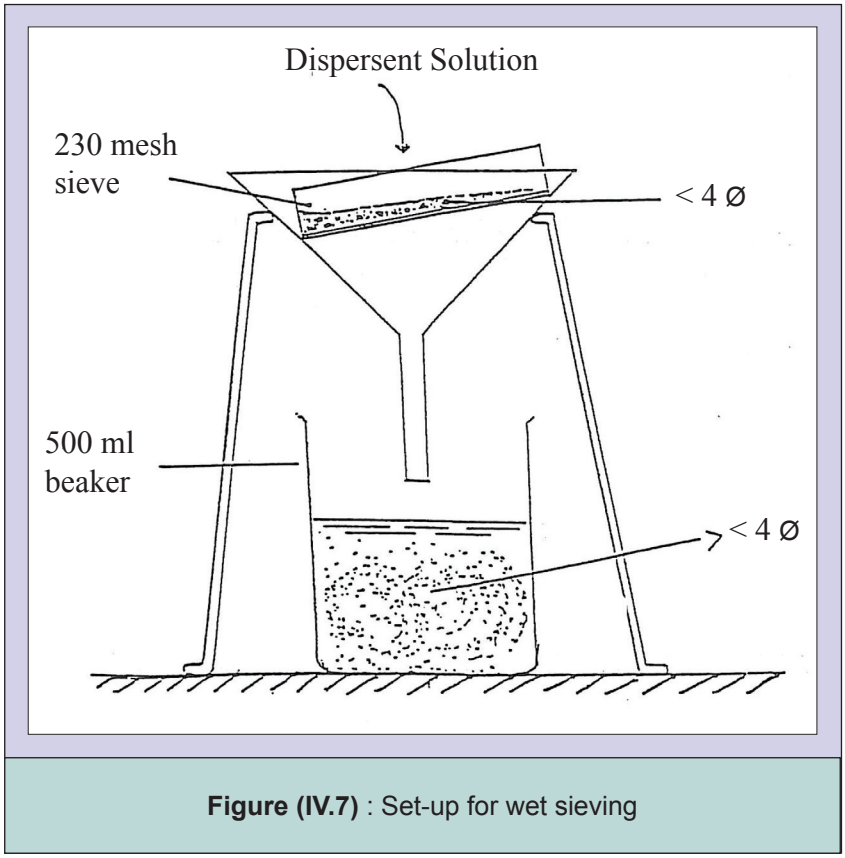


Figure (IV.7) : Set-up for wet sieving

The muddy water collected in the beaker will be ready for processing for pipette analysis.

Transfer the sand retained on the sieve to a large, pre-weighed evaporating dish. This is a very delicate process; a water jet may be used to assist the transfer of the sand from the sieve to the evaporating dish.

Note : Make sure that none is lost

Place the evaporating dish in oven at 105°C to dry the sand fraction (b). Cool the evaporating dish in a desiccator, and reweigh.

Calculate the solids content (a - b).

Prior to sieving, ensure that the sand has already been dried and disaggregated and that mud, if present in considerable amounts, has been removed. Now the sand fraction is ready for detailed size analysis (sieve analysis).

Note : If the absolute chemical compositions of separated sub-fractions are required, the dispersant must be non-contaminating, such as a weak solution of metal-free ammonium hydroxide (NH_4OH). All laboratory-ware should be pre-cleaned (see Section V.2 and VI.2)

Alternatively, for samples designated for the analyses of trace metals in separate size fractions, the bulk sample can be unfrozen and the wet sample sieved though a 63 µm nylon or stainless steel sieve. A separate determination of water content is required to calculate the amounts of the sand and mud size material in the sample (see also Section V.3.4)

IV.2.4 Sieve Analysis of Sand

Using a sample splitter, obtain about 50 g of sample. Weigh the split sample to the nearest 0.01 g.

Select a clean set of sieves 8" (20 cm) in diameter and preferably half height with ½ Ø interval. Nest the sieves in order, the coarsest one (-1 Ø, mesh 10.2 mm) at the top, pan on the bottom.

Note : Make sure that the sieves are arranged in the following order from top to bottom: 10, 14, 18, 25, 35, 45, 60, 80, 120, 170, 230 (US Standard sieve mesh #)

Pour the sample into the top sieve and shake gently by hand. Remove all the screens that are too coarse to catch any grains. Place cover on the stack.

Place the screens in the Ro-Tap or any equivalent mechanical shaker, Figure (IV.8). **Fasten very tightly**, and sieve for 15 minutes.

Empty each sieve onto a large sheet of paper (40 x 40 cm). The removal of sand is helped by striking the rim of the sieve with the palm of the hand or a wooden rod along the general direction of the diagonals of the wire mesh and brushing the bottom of the sieve with a sieve brush. Use a soft

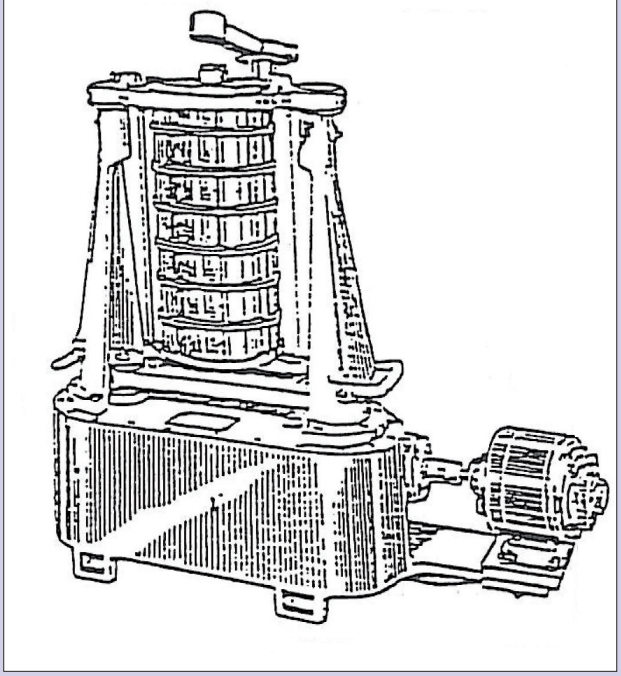


Figure (IV.8) : Ro-Tap mechanical shaker for the sieve analysis of sand.

brass wire sieve brush on sieves coarser than 100 mesh. For sieves finer than 100 mesh, use only a nylon bristle sieve brush.

Note : Be careful not to brush the sieve wires apart

Weigh each size fraction to the nearest 0.01 g. and enter data on log sheet.

Calculate the dry weight percentage for each size fraction from the equations (see Section IV.1.3).

Add the fine material caught in the bottom pan to the sediment in the beaker during wet sieving (see Section IV.2.3).

Store the sample in corked vials. Label each fraction as both coarser and finer limits and sample number.

IV. 2.5 Sieve Analysis of Gravelly Sediments

To obtain a representative sample of the gravel fraction, it is necessary to have a large sample, between 0.5 and 3 kg (depending on the size and amount of gravel).

Separate the sand from the gravel fraction using a (-1.0 Ø mesh sieve and weigh the sand fraction.

Sieve the gravel fraction using coarser sieves and a set of wire squares of the following diameters: 4, 16, 64, and 256 mm. Weigh each gravel fractions.

Split the total amount of sand to 30 to 70 g and sieve normally.

Multiply each sieved sand fraction by a “splitting factor” which is the total weight of sand in the sample divided by the weight of the sand split that was actually sieved.

Note : Care of the sieves is extremely important for accurate reproducible data. After each use, all sieves should be carefully cleaned and stored. Occasionally more thorough cleaning is required. In this case, wash sieves in warm, soapy water using the special nylon and brass sieve brushes. If this fails to remove most of the lodged particles, dip sieves in a boiling 5% solution of acetic acid and then use sieve brushes. Wash sieves thoroughly with distilled water to remove acid

IV.2.6 Pipette Analysis of Silt and Clay

This method is used for sediments containing more than 20% mud.

Sedimentation or pipette analysis is the most common method used for determining the grain size distribution of sediments less than 4 Ø (63 µm). This method relies on the relationship between the settling velocities and spherical diameters of particles in an aqueous solution. The first sample taken during pipette analysis determines the total amount of silt and clay in the cylinder since it is taken at

such a time and depth that no particular size fraction has completely settled past the sampling point. Subsequent samples are taken at specific times and depths so as to calculate the percentage of various size fractions present in the sample.

Pipette analyses take time, however, several samples may be processed concurrently by setting up an “assembly line” system. The sediment in the beaker which collected the less than 4 Ø fraction during wet sieving and to which has been added the particles collected in the bottom pan of the sieve stack during sieve analysis, can be used for this procedure.

Analytical method

Take the fraction passing the 230 mesh, which is already a dispersed suspension of silt and clay. Pour this suspension in the Andreason sedimentation cylinder [see Figure (IV.6)]. Do not add more dispersant solution, and try not to let the suspension exceed 400 ml in volume. In the case of a 1 L cylinder, the suspension should not exceed 800 ml in volume.

Stir vigorously and let the cylinder stand for a day. If it starts to flocculate (presence of a soapy-looking layer near bottom of cylinder with very clear water overlying it), dispersion will have to be repeated or the sample discarded. If flocculation occurs, the sample must be collected by filtration onto a suitable sized filter paper, dried in a 40°C oven overnight, and re-dispersed. Flocculation sometimes occurs if too much sediment is present in the cylinder. Approximately 15 g of dry sediment is best for these analyses. If it shows no sign of flocculation, it is ready for analysis.

Add dispersant solution to bring it up exactly to the upper marker of the Andreason cylinder (1000 ml). Stir the cylinder vigorously. Cover the cylinder and turn it upside-down several times until all the material is distributed uniformly throughout the column. As soon as the cylinder is turned to the right position for the last time, start the stop watch or use the stirring rod, Figure (IV.5). This methodology can also be substituted by a stirring glass rod.

Begin by vigorously stirring the sample for 2-3 minutes starting from the bottom and working up to the top. The last minute of stirring should consist of long smooth strokes the full length of the column, from the very bottom until the stirrer just breaks the surface. **THIS IS VERY IMPORTANT**. As soon as the stirrer is withdrawn from the surface, begin timing.

At the end of 20 seconds, insert the pipette to a depth of 20 cm and withdraw exactly 10 ml. This first withdrawal is the most important step, as it represents the total fraction finer than 4 Ø , i.e., total mud, so make it as close to 10 ml as you possibly can.

Remove the pipette, expel the suspension into a weighing beaker of 30 ml. Draw 10 ml of distilled water to rinse out the pipette, and expel the rinsing water into the same beaker.

Continue withdrawals at the specified time intervals [see Table (IV.4)]. It is unnecessary to stir the suspension after the first stirring; however, if needed, stirring can recommence. Timing should be started from the new stirring (Table IV.3).

Table (IV.3) : Settling time for quartz and clay minerals at 24°C

Depth (cm)	Size		Time of Withdrawal
	Ø	mm	
20	4.0	0.06250	20 seconds
20	4.5	0.04400	1 minute 35 seconds
R E S T I R			
10	5.0	0.03100	1 minute 45 seconds
10	5.5	0.02210	3 minutes 28 seconds
10	6.0	0.01560	6 minutes 58 seconds
10	7.0	0.00780	28 minutes
10	8.0	0.00390	1 hour 51 minutes
10	9.0	0.00200	7 hours 24 minutes
5	10.0	0.00098	14 hours 50 minutes

Place the beakers in an oven with cover glass on top, and evaporate them to dryness (24 hours at 100-130°C). Remove them from the oven and let them cool to room temperature. Weigh them to 0.001 g on a precision balance. Weigh all beakers at nearly the same time.

Record data on data form. A form for recording sieve analysis data is shown in Table (IV.4).

IV.2.7 Calculation

a) Use the data sheet in Tables (IV.3) and (IV.5) for data recording.
b) Determine the weight of the solid fraction (S) retained on 4 Ø sieve during wet sieving (IV.2.3).
c) Determine the weight of the whole mud fraction (F) by multiplying the weight of the first pipette sample (after subtraction of the amount of Calgon - 0.005 g) by a factor, which is the volume of the whole suspension in the cylinder divided by the volume of the pipette sample (this factor is 55 for the Andreason cylinder).
d) F = Multiply the weight of each later pipette sample by 55 (P)
e) Obtain the cumulative percentages of the total sample directly from pipetting data by the following equation :

$$\text{Cumulative Percent Coarser} = \frac{100\,(S + F - P)}{(S + F - P)}$$

A form for recording the percentages of all size classes, as indicated by sieve analysts and pipette analysis, is shown in Table (IV.6).

Table (IV.4) : Form for recording sieve analysis data

SIEVE ANALYSIS RESULTS

Sample location : **Depth :** **Sample No. :**

Sample Description : **Date of Sampling :**

Weight of Sampling

Sieve Opening			Size Class	Weight retained	Weight %	Cumulative weight %
Phi	mm	Mesh				
-2.0		5	Pebble			
-1.0		10	Granule			
-0.5		14	Very Coarse Sand			
0.0		18				
0.5		25	Coarse Sand			
1.0	0.5	35	Sand			
1.5	0.35	45	Coarse			
2.0	0.25	60	Sand			
2.5	0.177	80	Medium			
3.0	0.125	120	Sand			
3.5	0.088	170	Very fine			
4.0	0.052	230	Sand			
>4.0	<0.062	>230	Mud			

Date of Analysis : _____ Analyst : _____

Table (IV.5) : Form for recording pipette analysis data

RESULTS OF PIPETTE ANALYSIS

Sample location : Depth :

Date of Sampling : Sample No. :

Sample Description : Date of Sampling :

Weight of sand fraction (S) :

Withdrawal depth (cm)	Time at 24°C	Size Ø	Beaker Wt.	Sample Wt.	Sample Wt. x 50	Cumulative Wt. %
		< 4				(1)
20	20 sec	4			(F)	
20	1 min 45 sec	4.5			(P)	(2)
Restir / 10	1 min 45 sec	5			(P)	
10	3 min 28 sec	5.5			(P)	
10	6 min 58 sec	6			(P)	
10	28 min	7			(P)	
10	1 hr 51 min	8			(P)	
10	7 hr 24 min	9			(P)	
5	14 hr 50 min	10			(P)	

$$(1)\quad \text{Cumulative \% of } <4\ Ø = \frac{100 \times \text{sand fraction (S)}}{\text{Sand fraction(S)} + F}$$

$$(2)\quad \text{Cumulative \% of of coarser} = \frac{100 \times (S + F - P)}{S + F}$$

Date of Analysis : _____ Analyst : _____

Table (IV.6) : Form for recording percentages of main size classes [see Table (IV.2) also]

Date : Location :

Project : Data Sheet No. :

Sample No.	Depth (m)	Gravel %	Sand %	Mud % >4.0 Ø		
				Silt %	Clay %	Total

IV. 2.8 Data Presentation

Mean size, sorting, skewness and kurtosis are the main grain size parameters used in sedimentological studies. The most commonly used method for the calculation of statistical size parameters is the graphical method. In this method, certain size percentiles are measured from the cumulative size distribution curve. Many formulas have been designed to calculate the size parameters. A summary of these formulas is presented in Table (IV.7).

A form for recording statistical size parameter is shown in, Table (IV.8).

Table (IV.7) : Formulas for computing "Graphical" parameters (after Carver, 1971)

Parameter	Formula	Efficiency %
1. Mean		
Trask (1930)	Median, Ø50	64
Otto (1939), Inman (1962)	MF = (Ø16 + 84)/2	74
*Folk and Ward (1957)	Mz = (Ø16 + Ø50 + Ø84)/3	88
Folk (1974)		
McCammon (1962)	(Ø10 + Ø30 + Ø50 + Ø70 + Ø90) / 5	93
2. Sorting		37
Krumbein (1934)	QDØ = (Ø75 - Ø25) / 1.35	54
Otto (1939), Inman (1962)	(Ø84 - Ø16) / 2	79
*Folk and Ward (1957)	(Ø84 - Ø16) / 4 + (Ø95 - Ø5) / 6.6	
Folk (1974)		
McCammon (1962)	(Ø70 + Ø80 + Ø90 + Ø97 - Ø3 - Ø10 - Ø20 - Ø30) / 9.1	87
3. Skewness		
Krumbein and Pettijohn (1938)	SkqØ = [Ø25 +Ø95 - 2(Ø50)] / 2	87
Inman (1962)	$\alpha 1Ø = \frac{Ø16 + Ø84 - 2(Ø50)}{Ø84 - Ø16}$	
	$\alpha = \frac{Ø5 + Ø95 - 2(Ø50)}{84 - Ø16}$	
*Folk and Ward (1957) Folk (1974)	$SK_I = \frac{Ø16 + Ø84 - 2(Ø50)}{2(Ø84 - Ø16)} + \frac{Ø5 + Ø95 - 2(Ø50)}{2(Ø95 - Ø5)}$	
4. Kurtosis or Peakedness		
*Folk and Ward (1957) Folk (1974)	$KG = \frac{Ø95 - Ø5}{2.44\ (Ø75 - Ø25)}$	

Table (IV.8) : Form for recording statistical size parameters

Date : STATISTICS SIZE PARAMETERS (moment statistics) **Location :**

Project : **Data sheet No :**

Sample No.	Depth (m)	Mean M_z	Sorting σ_I	Skewness SK_I	Kurtosis K_G

IV.3 MINERALOGY

IV.3.1 Heavy Minerals

"**Heavy mineral suite**" of sediments is a term to define all classic mineral grains that have a specific gravity greater than 2.9. The heavy mineral components of terrigeneous sediments are commonly used for the identification of potential source rocks. There are various methods used for the separation of heavy mineral grains. In this manual, the method of separation by heavy liquids is described.

Equipment and materials

a) Glass funnels
b) Dark glass bottles
c) Filter paper
d) Sensitive balance (+0.1 mg)
e) Bromoform or any equivalent
f) Alcohol/Acetone
g) 500 ml separation funnel

Sample preparation

a) The sediment sample should be dried, disaggregated and sieved in the normal way for the separation of the sand fraction
b) After the sample has been disaggregated and sieved, take the fine sand fraction (2Ø - 3Ø) for heavy mineral separation

Method

Set up the heavy mineral separation apparatus, Figure (IV.9). A funnel that will hold the heavy liquid and sample is supported above a filtering funnel and receiving flask. A short piece of rubber tubing is fitted over the end of the holding funnel and a pinch cork placed on the rubber tubing.

Fill the holding funnel with bromoform and pour a known weight of the sediment sample in the bromoform.

Note : Make sure that the rubber tubing is tightly fitted with a strong clamp

Using a glass rod, stir the sample periodically until heavy minerals are no longer seen settling into the stem of the holding funnel. Due to stirring of the sample, some of it will adhere to the wetted area above the liquid on the funnel side. This procedure may be repeated to ensure complete separation of the heavy minerals from the entire sample.

Note :Wash the funnel walls with heavy liquid, remix the washed sediment and allow heavy minerals to settle

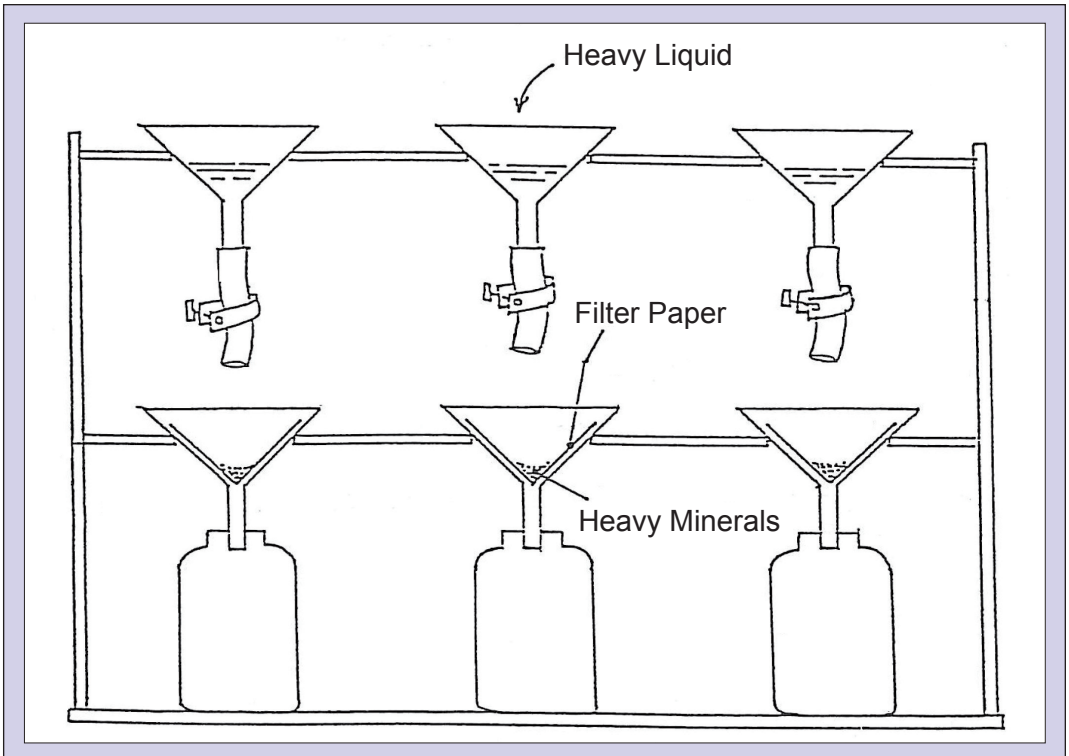


Figure (IV.9) : Heavy mineral separation battery

A funnel (of the same size as the holding funnel) with filter paper is placed under the holding funnel, and a dark colored bottle reserved for pure heavy liquid is placed under the lower funnels.

Open briefly the clamp on the tubing of the upper funnel to drop the accumulated heavy minerals in the lower receiving funnel.

Note : Be very careful when opening the clamp to avoid splashing the heavy minerals and heavy liquid out of the filter paper

Heavy liquid is allowed to filter into the clearly marked flask for undiluted heavy liquid. Close the clamp before the floating light mineral fraction reaches the vessel of the funnel.

Transfer the filter paper containing the heavy minerals to another funnel rack, which has a flask below it reserved for washing. Wash the filter paper with alcohol. Place the filter paper in a watch glass and let it dry, then weigh for obtaining the heavy mineral fraction.

Place another filter paper in the lower funnel and open the clamp on the tubing of the upper funnel to receive the rest of the bromoform and the light mineral fraction. Receive the bromoform in the dark bottle reserved for undiluted bromoform. Wash the upper funnel with bromoform. Allow the bromoform to filter into the dark bottle.

Transfer the filter paper, which now contains the light mineral fraction, to the other funnel rack for washing with alcohol, and receive the washed bromoform in a flask reserved for washing. Place the filter paper in a watch glass and let it dry.

Place both the heavy and light mineral fractions in small, labeled glass vials.

The diluted bromoform, which resulted from washing with alcohol, can be recovered by placing it in a 500 ml separating funnel, adding distilled water and shaking well. Leave it for about 15 minutes to allow the bromoform to settle at the bottom of the funnel. Separate the clean bromoform from the alcohol-water mixture. You may repeat this process several times to ensure that the bromoform is well cleaned.

Calculation

Calculate the heavy mineral index of the sample as follows:

$$\text{Heavy mineral index} = \frac{\text{Weight of heavy mineral}}{\text{Total weight of sample}}$$

Semi-quantitative analysis of heavy minerals

The identification of the various heavy minerals and estimation of the relative frequency percentage are very useful for the identification of sediment provenance as well as for correlation purposes.

Materials

a) Polarizing microscope
b) Glass slides and glass covers
c) Canada balsam
d) Counter
e) Hot plate

Methods

Take a representative sample of the heavy mineral fraction and mount it with Canada balsam and a glass slide. Make sure that the heavy mineral grains are uniformly spread on the glass slide. Try to identify the main heavy minerals in the studied sample.

Note : Do not start counting prior to clear identification of the main heavy mineral grains

Heavy minerals are commonly counted in ribbon traverses randomly selected on a mechanical stage where each grain that falls within the field of view is identified and tabulated. Numbers of grains will be converted to mineral percentages.

Note : You should count at least 200 grains

The non-opaque suite of minerals is most useful for genetic interpretations and should be recalculated to total 100% by omitting micas and opaque grains. In this case you have to count 200 non-opaque grains.

A form for recording heavy mineral counts is shown in Table (IV.9).

Table (IV.9) : Form for recording heavy mineral counts

Date : _____ Location : _____

Project : _____ Data Sheet No : _____

Sample No	Depth (m)	opaques	Pyroxenes					Amphiholes					Epidotes	Zircon	Grantes	Rutile	Sphene	Sillimantite	Dolomite	Tourmaline	Others	Total No of grains
			Ortho px		Clino Px			Ortho Px		Clinio Px												
			Enstatitle	Hypersthene	Diopside	Augite	Total	Anthophylite	Florblende	Basalta	Others	Total										

Insoluble residues

Insoluble residues are materials remaining after the digestion of a sediment sample in acid. Generally, these are the materials left after dissolving carbonate rocks by hydrochloric acid, but siliceous rocks treated with hydrofluoric acid can also produce insoluble residues. Insoluble residues can be used for stratigraphic correlation. This section deals only with insoluble residues from calcareous rocks.

Procedure

- Weigh the sample to be dissolved
- Place the sample in a glass beaker and add properly diluted HCl. Pour the acid carefully to avoid frothing over the top of the beaker
- After complete digestion, wash the residue well and decant several times
- Dry at a temperature below 100°C, and weigh to calculate the percentage of soluble residue
- Study the insoluble residue under the binocular microscope to identify its type

Staining

Staining is a very useful technique in the identification of the various mineral components in sedimentary rocks, particularly in carbonates.

a) Types of stain

i) Alizarine Red S: Dissolve 0.1 g of alizarine red S in 100 ml of 0.2 % cold hydrochloric acid (add 2 ml of commercial grade concentrated HC1 to 998 ml of distilled water)

ii) Feigl's Solution: Prepare a solution of $MnSO_4.7H_2O$ by dissolving 1.0 g of this solid material in 100 ml of distilled water. Add to this solution 1.0 g of solid (commercial grade) Ag_2SO_4. Boil the solution and let it cool, then filter the suspension. Add one or two drops of diluted sodium hydroxide solution. The precipitate is filtered off after one or two hours

Note : Do not use tap water, use only distilled water

iii) Titan Yellow: Add 0.5 g of titan yellow or Clayton yellow dye, 8 g of NaOH and 2 g of EDTA in 500 ml of distilled water

iv) Potassium Ferricyanide: Dissolve 5 g of Potassium Ferricyanide in distilled water containing 2 ml of concentrated HCl

v) Alizarine Red S and Potassium Ferricyanide: Dissolve 1 g of alizarine red S with 5 g of potassium ferricyanide in distilled water containing 2 ml of concentrated HCl and bring the solution to 1 liter with distilled water

vi) Stains Specific to Gypsum: Dissolve 0.1 g to 0.2 g of alizarine red S in 25 ml of methanol. Add 50 ml of 5X NaOH (5 g of NaOH in 95 ml of distilled water)

vii) Cobaltinitrite Solution: Dissolve 1 g of sodium cobaltinitrite in 4 ml of distilled water

viii) Amaranth : Dissolve 1 ounce of 92 % pure coal tar dye in 2.1 of distilled water

b) Staining of carbonates

Hand specimens, cores, drill cuttings, sand grains, and thin sections should be etched with diluted HCl and washed with running distilled water before staining. Never use tap water.

Place the specimens, grains, or the thin sections in a beaker filled with staining solution for the suggested time (varies with the type of staining solution). Then wash with running distilled water and watch the color.

Use flow chart, Figure (IV.10) for staining procedure.

c) Staining of silicates

Hand specimens, cores, drill cuttings, sand grains, thin sections should be etched with hydrofluoric acid.

Note : Hydrofluoric acid reacts rapidly with tissue and causes painful burns, and the effects may not be noticed for several hours. The use of nitrile gloves and goggles is a must, and hood ventilation is imperative. Have neutralizing eyewash and a water-soluble suspension of 2.5% calcium gluconate available. In case of accidental contact seek immediate medical attention

For thin sections, etch the uncovered thin section for 10 seconds over hydrofluoric acid at room temperature.

For sand grains, etch the grains in hydrofluoric acid vapor for 1 minute at 90°C.

After etching wash the specimen with distilled water. Then dip it in the staining solution. Cobaltinitrite will stain alkali feldspar grains yellow. Amaranth will stain plagioclase purple-red.

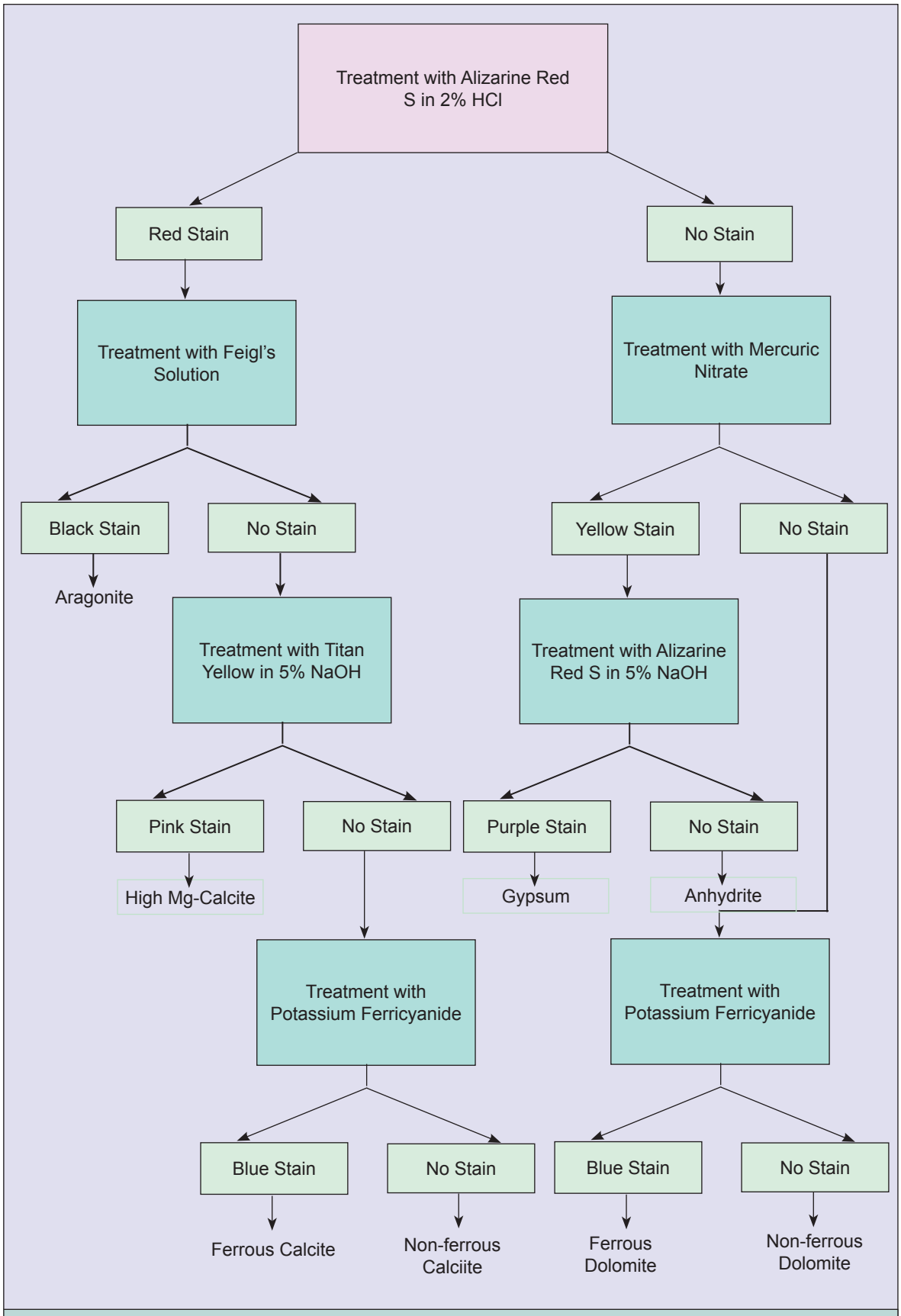


Figure (IV.10) : Staining tests for carbonates and evaporites

Figure (IV.11) depicts a flow chart showing the staining procedure for the differentiation between quartz, K-feldspar and plagioclase

IV.3.2 X-Ray Diffraction

X-ray diffraction is a very useful technique for mineral identification, especially when minerals occur as fine particles and optical microscopy is inadequate for their identification (e.g., clay minerals). Also, X-ray diffraction can be used for semi-qualitative analysis.

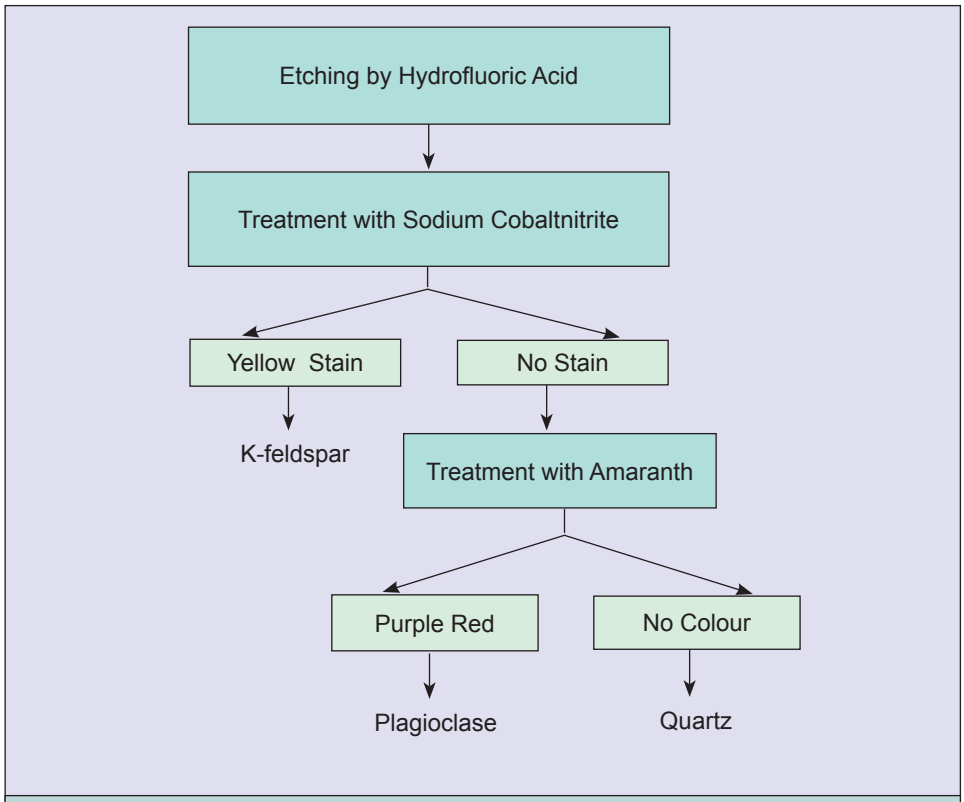


Figure (IV.11) : Staining tests for silicates (mixtures of quartz, K-feldspar and plagioclase)

The identification of minerals by X-ray diffraction is based on the determination of the interpolar space or lattice spacing “d” which is the distance between the various atomic planes in the atomic structure of minerals. These lattice spacings are identical throughout the atomic pattern of the mineral. The lattice spacing “d” can be calculated from Bragg's Law as follows:

$$n \lambda = 2 D \sin \Theta$$

where λ = wave length of the incident radiation
d = lattice spacing
Θ = the angle between an incident monochromatic X-ray beam and the chosen atomic plane.
n = an integer.

Equipment

The equipment used for this analysis is the X-ray diffractometer or any new equipment. This is composed mainly of three parts, namely,

a) X-ray tube
b) Sample holder, and
c) Radiation counter

A filtered monochromatic beam (Kα) from the X-ray tube passes through a slit and the collimating system on to the specimen. The size of the incident beam is controlled by the divergence slit. The specimen is a powdered sample and the sample holder is inclined at an angle Θ to the incident beam. The angle is adjustable by mechanical and hand gearing.

The diffracted beams are received at a receiving slit and pass through a plate collimator and finally enter the counter through a scatter slit. The receiving slit, collimator and scatter slit are all adjustable to allow variation in the diffracted radiation intensity entering the counter. The counter is electronically set to give maximum response to the Kα radiation in use, and the equipment is allowed to scan through the 2Θ range of interest. The pulses from the counter are fed into a chart recorder, which records 2Θ angles against the amount of radiation (count rate) falling on to the window of the counter.

The advantages of diffractometers are that they allow a quick manual scan or a reliable chart record. Should an area need to be examined more closely, the scan speed can be lowered and the chart recorder sensitivity increased allowing a very accurate determination of the 25 angle and incident intensity.

Sample preparation

Whole sediment

a) Dry the sample
b) Obtain a representative sample split of about 5 g
c) Grind the representative sample using an automatic agate mortar
d) Mix the powdered sample thoroughly and load it into an aluminum holder from the back, with the surface to be exposed to the X-ray beam placed face downward on the mounting plate

Clay Minerals

a) Separation of the <2 µm fraction

The technique used for the separation of the <2µm fraction is dependent on the nature of the studied rocks. In the case of carbonate rocks, obtain a representative sample of about 200 g, wash and scrub it with distilled water, then dry it. Crush the sample, then obtain a sample split of about 100 g (the weight of the sample split depends on the clay content). Treat the sample with 10% HCl to remove all carbonates. Wash the insoluble residue and separate the <2µm fraction using the sedimentation method.

In the case of terrigeneous sediments, obtain a representative sample, disperse it and then separate the <2µm fraction using the sedimentation method.

b) Preparation of oriented specimens

The method applied by Shaw (1971) for the preparation of oriented clay mineral specimens for X-ray diffraction analysis is recommended. This method involves filtering a clay suspension under suction through a suitable ceramic medium. The apparatus used consists of a cylindrical stainless steel filter funnel, attached to a conical flask joined to a vacuum pump. A ceramic disc of about 1.5" in diameter and ¼" thick is used as a filter bed. A battery of three filter funnels can be used, Figure (IV.12).

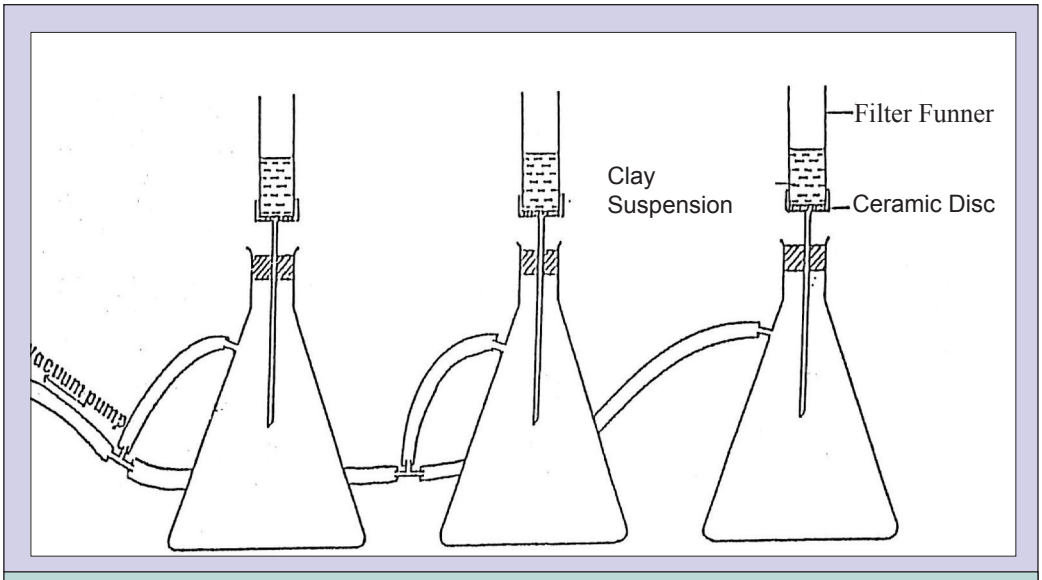


Figure (IV.12): Suction onto ceramic method of sample preparation

Analytical methods

The recommended experimental conditions to be used are :

a) Cobalt radiation (CoKα) with an iron filter placed before the receiving slit to eliminate possible β radiation
b) As supply, a 38 kV, 24 mA and 1 kW generator is normally used to give high intensity and a good peak to background ratio
c) Scanning speed 1° 2Θ per minute, divergence and scatter slit both in the case of oriented specimens, and ½° in the case of non-oriented specimens, receiving slit 0.1 mm

Generally, identification of the clay minerals by means of X-ray diffraction is facilitated by pre-treatment of the oriented mount in various ways before x-ray analysis (Brown, 1961).

The following experimental procedure should be carried out :

a) Air-dried specimen mounted on a ceramic disc, scanned from 2 to 34° 2Θ
b) Glycerate the specimen by spreading a thin film of liquid glycerol over the clay specimen and let it dry, scan from 2 to 20° 2Θ
c) Heat the specimen in muffled furnace at 550° for one hour, scan from 4 to 15° 2Θ

Clay mineral identification

The characteristic X-ray properties of clay minerals have been discussed in detail in the literatures such as Molloy and Kerr (1961) and Mackenzie and Mitchell (1966). The following is a brief description of the general X-ray properties of the most abundant clay minerals :

Illite

The term *illite* is used as a collective name denoting all 10 Å non-expanding clay minerals (Gaudette *et al*., 1966). In oriented mount it shows a relatively sharp basal reflection, which becomes slightly larger after heat treatment. When the mineral is associated with illite-montmorillonite mixed layer minerals, the first order basal reflections of both minerals partially overlap, forming a combined peak characterized by asymmetrical tailing towards the lower diffraction angle. Illite has a marked second order reflection of about 5 Å, though usually weaker than that of muscovite; in the trioctahedral 10 Å member of the mica minerals, such as biotite, this reflection is almost absent.

Mixed layer clay minerals

Two varieties of mixed layer clay mineral are most common. These are randomly interstratified illite-montmorillonite and regularly interstratified illite-montmorillonite. Randomly interstratified illite-montmorillonite is identified on the basis of its broad peak, which occupies the area between 13 Å and 10 Å in the case of an untreated sample. The peak shifts to a lower diffraction angle on glyceration and contracts to 10 Å during heat treatment. This mineral gives a non-integral series of basal reflections. The d spacing of its basal reflection (001) depends on the percentage of the swelling montmorillonite layers within the structure. The percentage of expandable (montmorillonite) layers in randomly interstratified illite-montmorillonite can be determined by using a curve based on Weaver's data (1956), Figure (IV.13).

Regularly interstratified illite-montmorillonite is characterized by a regular progression of its basal reflection.

Kaolinite

Kaolinite can usually be identified directly from its strong diffraction maxima at 7.1 Å, 3.5 Å and 2.38 Å. On heating at 550°C the kaolinite structure will break down and all the reflections due to kaolinite will disappear. Chlorite will give peaks at or near the position of the strongest basal reflection of kaolinite.

It is possible to determine the state of crystallinity of the kaolinite from the X-ray diffractograms. Disorder in the kaolinite lattice produces blurred and weak reflections. It also shows an asymmetric (002) band with a sharp head at 4.45 Å, fading away towards higher angles. On the other hand, well-ordered kaolinite shows little or no band in this region, and the reflections at 4.18 and 4.13 Å are resolved (Brindley, in Brown, 1961).

Dickite is better crystallized than kaolinite, and shows sharper reflections, Figure (IV.14). Unlike kaolinite, dickite is not affected by heating to 550°C. For dickite, the peak temperature is about 100°C higher than for kaolinite (Richardson, in Brown, 1961).

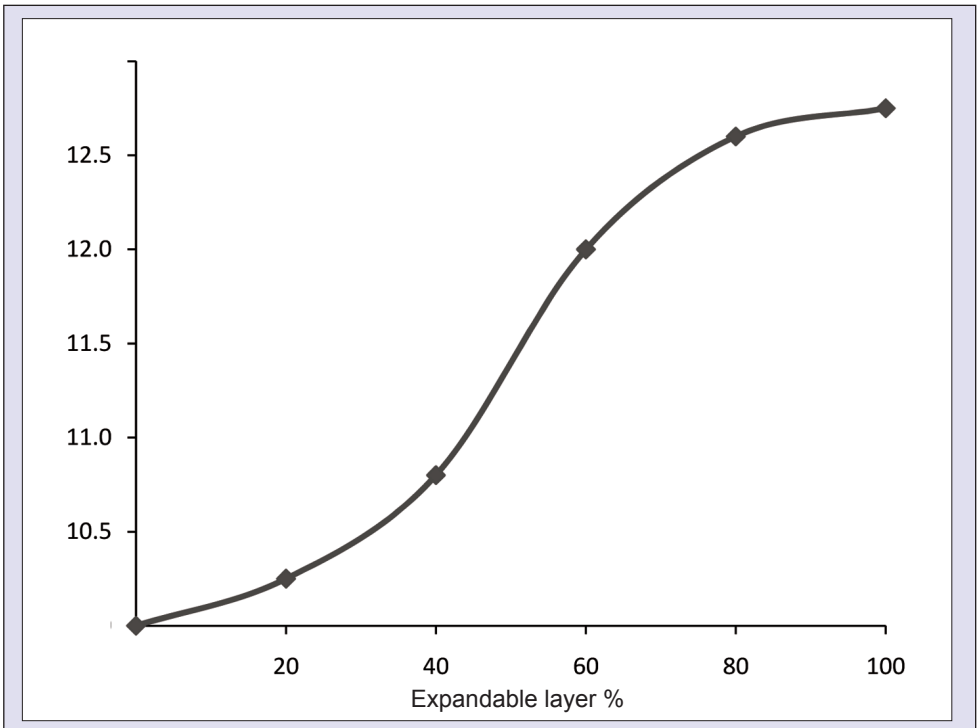


Figure (IV.13) : Curve showing migration of 001/001 peaks of randomly interstratified 10 Å and 12.4 Å layers (data after Weaver, 1956)

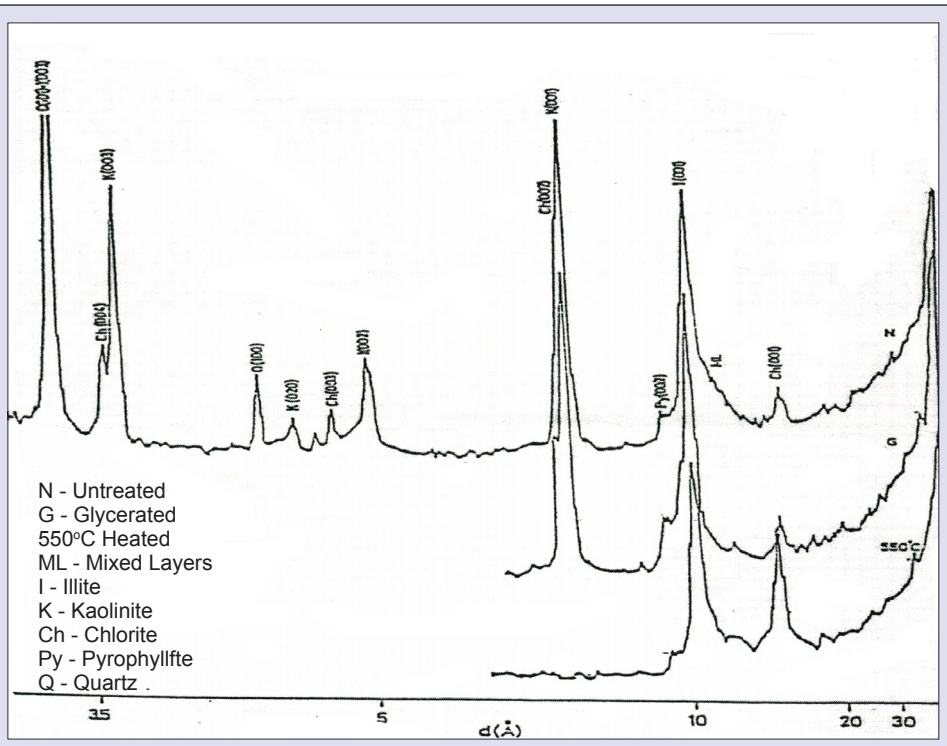


Figure (IV.14) : X-ray diffractograms of the clay fraction show the main diffraction peaks of dickite, well crystalline kaolinite and illite

Chlorite

Chlorite exhibits an integral series of basal reflections with a first order reflection of 14 Å. The spacings are not generally affected by glyceration. Carefully executed heating can yield valuable data for identifying chlorite. Generally, upon heating the intensity of the (001) chlorite reflection increases, combined with a slight peak shift to higher angles, while the (002), (003) and (004) reflections decrease as the brucite layer decomposes.

Pyrophyllfte

This mineral is mainly identified on the basis of its (002) reflection at 9.16 Å. It is not affected by either glyceration or heating.

Semi-quantitative analysis of clay minerals in oriented mount.

Quantitative X-ray diffraction analysts of mineralogical mixtures involves comparison of the intensities of strong reflections characteristic of each mineral present in the mixture. Many methods of quantitative X-ray diffraction analysis of clay minerals have been published, e.g. Gibbs (1967), Schultz (1964) and Shaw (1971).

Quantitative analysis of the clay minerals can be done by an adaptation of the methods described by Schultz (1964) and Shaw (1971). The following procedure is used:

1. Measure the peak areas of the 10 Å, 7 Å and 3.58 Å in the untreated air-dried oriented sample. The 10 Å peak area will include the area of the low angle shoulder. The peak area of the 10 Å reflection will be equivalent to the intensity of the (001) illite reflection plus the (001) reflection of the illite-montmorillonite mixed layer mineral. The 7 Å peak area will be equivalent to the integrated intensity of the (00l) kaolinite and (002) chlorite reflections. The 3.58 Å peak area will be equivalent to the intensity of the (002) kaolinite reflection
2. Measure the peak area of the 10 Å reflection after glyceration of the sample. This will be equivalent to the integrated intensity of the (001) illite reflection only
3. The (002) kaolinite reflection is adjusted to (001) equivalent by multiplying by 1.25. Also, the peak area of 10 Å in the untreated sample is increased threefold to allow direct comparison with the peak areas of chlorite and kaolinite

The following equations are used in the calculations :

% Illite + illite-montmorillonite mixed layer

$$= \frac{\text{I 10 Å untreated x 3}}{\text{I 10 Å untreated x 3 + I 7 Å}}$$

$$\% \text{ Illite} = \frac{\text{I 10 Å glycerated x 3}}{\text{I 10 Å untreated x 3 + I 7 Å}}$$

% Illite-montmordllonite = % (Illite + illite-montmorillonite) - % Illite

% Kaolinite + chlorite = 100 - % (Illite + illite-montmorillonite)

$$\% \text{ Kalonite} = \frac{\text{I } 3.58 \text{ Å x } 1.25}{\text{I } 7 \text{ Å}} \text{ x \% Kaolinite + chlorite}$$

% Chlorite = % (kaolinite + chlorite) - % kaolinite

Because of the semi-quantitative nature of the analysis, the relative clay mineral contents of the studied samples are only quoted to the nearest 5%.

Peak areas are measured using a polar planimeter. In measuring the peak areas the background level beneath the peaks must be projected by joining up the background levels on either side.

Quantitative analysis of the whole rock material

A semi-quantitative estimation of the relative amount of the non-clay minerals (quartz, calcite, dolomite, feldspars, etc.) is made based on the method outlined by Schultz (1964). Briefly, this involves comparing the intensities of particular diffraction peaks of various minerals when present as 100% of the specimen with the intensities of the same peaks in the samples being analyzed.

Measure the areas of the characteristic peaks of the minerals present. This will be equivalent to their intensities (peak heights may also be used). They are then multiplied by their correction factors before direct comparison. Table (IV.10) gives the characteristic peaks of each mineral and its correction factor after Schultz (1964). The forms used to record the data and report the results are shown in Tables (IV.11), (IV.12), (IV.13) and (IV.14).

Table (IV.10) : Factors for X-ray semi-quantitative estimation of minerals (after Schultz, 1964)

Mineral	Hk1	d(hkl) Å	Intensity Factor (Cps per 100%)	Correction Factor
Quartz	101	3.34	2000	x 1
Feldspars	040	3.18	1000	x 2
Calcite	104	3.04	1000	x 3
Dolomite	104	2.88	1000	x 4
Siderite	104	2.74	1000	x 5

Table (IV.11) : Form used to record peak height measurements of X-ray diffractograms

PEAK HEIGHT MEASUREMENTS OF
X-RAY DIFFRACTOGRAMS

Project : **Data sheet No :**

Sample No.	Depth (m)	Quartz	Feldspar	Calcite	High Mg Calcite	Arago-nite	Dolomite	Remarks

Table (IV.12) : Form used to report the mineral composition of sediments

WHOLE SEDIMENT MINERALOGY
(X-Ray Diffraction)

Project : **Data sheet No :**

Sample No.	Depth (m)	Quartz	Feldspar	Calcite	High Mg Calcite	Arago-nite	Dolomite	Remarks

Table (IV.13): Form used to record peak area measurement of X-ray diffractograms table

PEAK AREA MEASUREMENTS OF
X-RAY DIFFRACTOGRAMS
(Clay Minerals)

Project : **Data sheet No :**

Sample No.	Depth (m)	Untreated			Glycerated 10Å	Remarks
		10Å	7 Å	3.50Å		

Table (IV.14) : Form to be used to report the clay mineral composition of sediments table

PERCENTAGES OF CLAY MINERALS

Project : **Data sheet No :**

Sample No.	Depth (m)	Percentage					Remarks
		Illite	Montmorillire + Illite-Mont	Kaolinite	Chlorite	Others	

It is difficult to measure the peak area of the (001) Muscovite reflection due to the fact that it is superimposed on the broad diffuse (001) illite and illite-montmorillonite mixed layer mineral reflection. Therefore, it is calculated with the clay minerals.

For the estimation of the "clay minerals" present in the "whole rock" samples, the variation made by Shaw (1971) to the Schultz method was used. This involves measuring the intensities of the illite and muscovite (021) reflections, the kaolinite (001) and chlorite (002) reflections, correcting them for difference in diffracting ability relative to each other, and then comparing the total corrected intensity directly with that of the quartz (101) reflections.

IV.4 TOTAL ORGANIC CARBON

Organic carbon matter is determined to assess the role played by the organic fraction of sediments in the transport, deposition and retention of trace metals.

The readily oxidizable organic carbon content is determined by the Walkey-Black Method (1947), adopted and modified from Jackson (1958). This method differentiates humus matter from extraneous sources of organic carbon such as graphite and coal. Gaudette *et al*. (1974) found that this method provided excellent agreement with the LECO combustion method of organic carbon analysis.

The Walkey-Black method utilizes exothermic heating and oxidation with potassium dichromate and concentrated H_2SO_4 of the sample, followed by the titration of excess dichromate with 0.5N ferrous ammonium sulfate solution to a sharp, 1-drop end point. Oxidation of $C1^-$ can be prevented by the use of Ag_2SO_4 in the digestion mixture.

Apparatus

a) 2-50 ml burette with 0.1 ml graduations
b) Magnetic stirrer
c) 500 ml Erlenmeyer flasks

Reagents

a) 85% H_3PO_4
b) Solid NaF
c) Concentrated H_2SO_4 with Ag_2SO_4 (dissolve 2.5 g Ag_2SO_4 in 1 liter of H_2SO_4)
d) Standard 1N $K_2Cr_2O_7$ solution (dissolve 49.04 g of $K_2Cr_2O_7$ in water; dilute to 1 liter)
e) 0.5N Ferrous solution (dissolve 196.1 g of $Fe(NH_4)_2SO_4$ $6H_2O$ in 800 ml of water containing 20 ml of concentrated H_2SO_4; dilute to 1 liter)
f) Diphenylamine Indicator (dissolve approximately 0.5 g of reagent grade diphenylamine in 20 ml of water and 100 ml of concentrated H_2SO_4)

Determination of organic carbon

a) Place 0.5 grams of dried and sieved (200 µm sieve) sediment sample in a 500 ml Erlenmeyer flask
b) Add exactly 10 ml of 1 N $K_2Cr_2O_7$ solution by burette and 20 ml of concentrated H_2SO_4 with Ag_2SO_4 and mix by gently rotating the flask for about 1 minute

Note : This should be done carefully to ensure complete mixing of the reagents with the sediment, while avoiding splashing the sediments onto the sides of the flask, out of contact with the reagents

c) Allow the mixture to stand for 30 minutes
d) A standardization blank without sediment should be run with each new batch of samples
e) After 30 minutes, add 200 ml distilled water; 10 ml of 85% H_3PO_4, and 0.2 g NaF
f) Add 15 drops of the diphenylamine indicator to the sample flask
g) Back-titrate the solution with the 0.5 N ferrous ammonium sulfate solution to a one-drop end point (brilliant green)

Note: The color of solution will progress from an opaque green-brown, to green upon the addition of approximately 10 ml of the ferrous solution. The color will continue to shift upon titration to a bluish-black-gray; at this point the addition of 10-20 drops of ferrous solution will shift the color to a brilliant green giving a one-drop end point

Calculation of results

% organic matter (readily oxidizable) = 10 (1-T/S) x F

S = standardization blank titration, ml of ferrous solution
T = sample titration, ml of ferrous solution
F = factor derived as follows :

(1.0 N) x 12/4000 x 1.72 x 100/sample weight = 1.03 when sample weight is exactly 0.5 grams

Where 12/4000 = meq wt. carbon and 1.72= factor for organic matter from carbon.

Note: In the back-titration, the ferrous solution reduces the dichromate that has not been used up in the oxidation processes. Therefore, if it takes less than 4 ml of the ferrous solution to reach the end point, then more than 8 ml of the available 10 ml of dichromate has been consumed in the oxidation. If this is the case, then it is necessary to repeat the determination using less sediment

Standardization of organic carbon determinations

Dextrose ($C_6H_{12}O_{l6}$) is used as the standard. It should contain 39.99% carbon.

a) Weigh out exactly 0.01 g of dextrose and treat in the same manner as the sediment sample
b) The carbon in dextrose is calculated as follows: %C= l0(1-T/S) x F
F= (1.0N) x 12/4000 x 100/sample weight = 30 for 0.01g of dextrose

The theoretical value is 39 99% C in one gram of dextrose.

Following standardization with dextrose it is recommended that a well-homogenized sample be analyzed and be used for standardization instead of dextrose.

Precision of determinations

Replicate analyses (10) of a sample with an average of 0.36% organic matter gave a standard deviation of 0.04% (coefficient of variation of 11%). At a level of 3.17% organic matter, the standard deviation was 0 05% (coefficient of variation of 1.6%).

IV.5 DETERMINATION OF CALCIUM CARBONATE

Carbonate is often an important component of marine sediments and has been found to be an important indicator of provenance and dispersal of terrigenous material in the Gulf of St. Lawrence (Loring and Nota, 1973).

For a simple determination of calcium carbonate ($CaCO_3$), the sediment is placed in a pre-weighed stoppered flask and treated with HCl. By adding excess HCl to $CaCO_3$, a certain volume of CO_2 is evolved, while an equal volume of air is expelled. The loss of weight due to the escape of air expelled by the evolved CO_2 is determined. As both CO_2 and air follow the Boyle-Gay Lussac Law, and weights are determined instead of volumes, the temperature and pressure have no influence on the analysis, assuming that these quantities do not change between weighings.

Apparatus

a) Conical flask, 250 ml
b) Rubber stopper fitted with a glass tube filled with $CaCl_2$ to remove water vapor and plugged at both ends with cotton. Another similar tube is attached to the first tube by means of plastic tubing. This tube is used to ensure that outside moisture does not enter the main tube.
c) Glass vials: 7 ml
d) Desiccator
e) Analytical balance

Reagents

4N HCl : dilute 330 ml of concentrated HCl to I liter with water.
$CaCO_3$: reagent grade, oven dried and stored in the desiccator.

Determination of carbonate

a) Transfer 1 g of oven dry ground sediment into a 250 ml conical flask [see A in Figure (IV.15)]
b) Place vial D filled with 5 ml of 4N HCl into the flask
c) Close the flask with a rubber stopper containing tube B filled with $CaCl_2$
d) Determine the total weight
e) Mount tube C also filled with $CaCl_2$ on top of tube B with the aid of a piece of plastic tubing
f) Tilt the flask so that the HCl in vial D is poured out onto the sediment sample
g) During the next 2 hours, swirl the contents of the flask occasionally so that the acid is in contact with the sediment
h) After 2 hours, remove tube C and weigh
i) Calculate loss of weight in grams (P)
j) Repeat the procedure using 100 mg of $CaCO_3$ instead of the sediment and calculate the loss of weight in grams (Q)

Calculation of calcium carbonate content

% $CaCO_3$ = P/Q x 0.100/ weight of dry sediment x 100

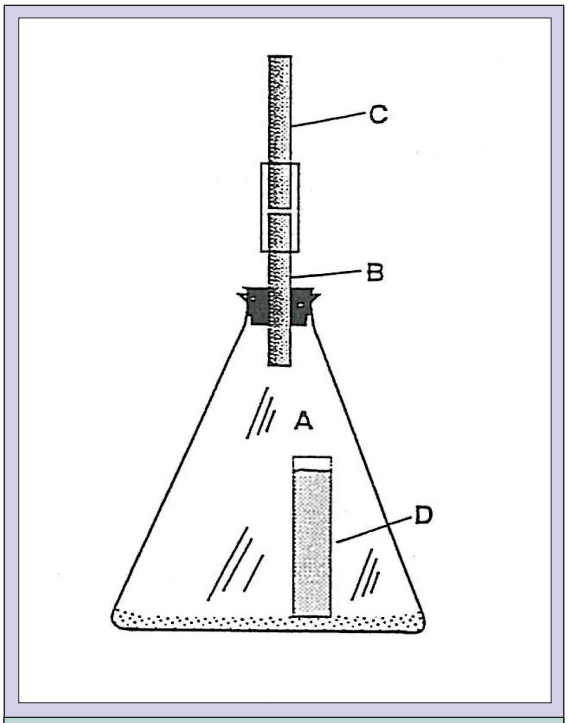
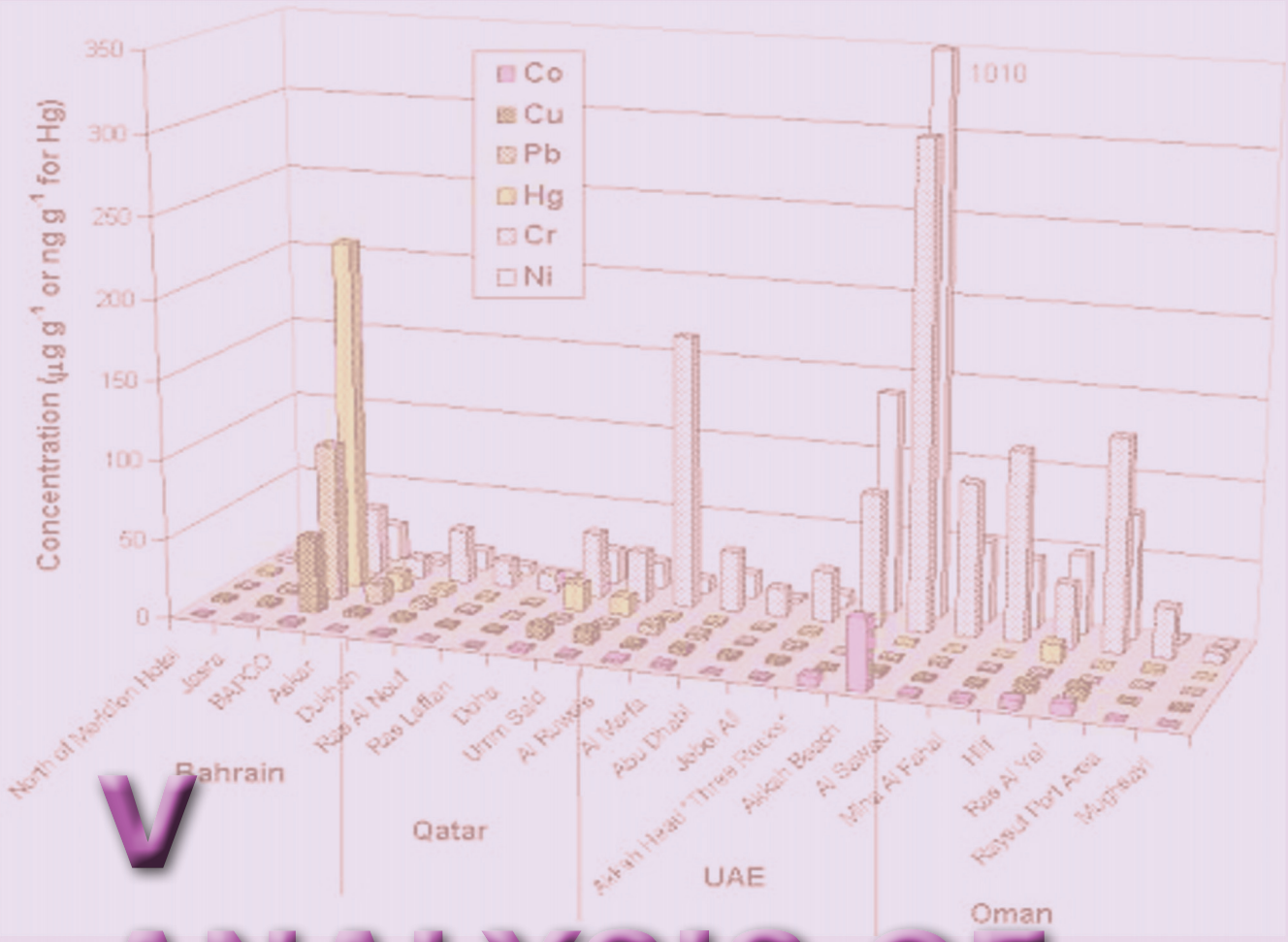


Figure (IV.15) : Apparatus used for the determination of $CaCO_3$. A = Flask; B = Rubber stopper fitted with glass tube filled with $CaCl_2$; C = Second glass tube filled with $CaCl_2$ attached to the tube B by means of plastic tubing; D = Vial filled with 4N HCl

Note : Tubes filled with $CaCl_2$ should be stored in a desiccator and the CaCl2 replaced if it is observed to be hard

Precision of carbonate determination

Replicate analyses (6) of a sample with an average of 6.84% $CaCO_3$ gave a standard deviation of + 0.18% (coefficient of variation of 2.6%).



V ANALYSIS OF TRACE METALS



V. ANALYSIS OF TRACE METALS

V.1 QUALITY ASSURANCE BASIS FOR OBTAINING QUALITY DATA

Guidelines for data acquisition and data quality evaluation in environmental chemistry were published by UNEP (Reference Method 57 and 63) and by the American Chemical Society (ASC, 1980). The following remarks are based on these guidelines (see also Section I.2).

V.1.1 Planning

It is extremely difficult to achieve accurate results in environmental analysis. It is of utmost importance that every step of the analytical programme is well planned. Such a plan or model is implicit or explicit in every measurement process. Data are generated in order to answer specific questions and to draw usable conclusions. If the data generated are not responsive to the questions posed, then the conclusions will be faulty even if the data are accurate. Thus the model or plan must be based on a collaborative effort by the analyst, who is an expert in the measurement techniques, the scientist who will use the data and the statistician who will help to evaluate the data. No measurement programme should be undertaken until such a plan is developed which includes the statement of the problem, the sampling and analytical protocols, and the statistical methods that will be used in data evaluation.

V.1.2 Quality Assurance

A quality assurance programme must be established by every analytical laboratory; it should be designed to assess accuracy and precision and to identify and correct problems as they arise. General elements necessary to quality assurance programmes can be listed as follows :

1. Maintenance of skilled personnel, written and validated analytical methods, and properly constructed and equipped laboratories
2. Acquisition of representative samples and controls
3. Acquisition of high quality analytical standards for instrument calibrations
4. Use of high quality and/or dedicated glassware, solvents and other reagents and equipment
5. Routine calibration and adjustments of instruments and maintenance of continuous records of these calibrations
6. Routine performance tests including complete procedural blank analyses and standard recovery experiments
7. Use of reference materials with certified values
8. Regular and critical review of analytical results
9. Use of replicate samples
10. Comparison of results with other laboratories (intercomparison)
11. Response to user complaints

These basic elements of quality assurance programmes define the framework, which supports written protocols, and all laboratory procedures.

Reference materials (RMs) are available from United Nations and national sources (see Annex III). These consist, among other items, of homogenized marine sediments or fish tissues which can

be distributed to interested laboratories on a regular basis. These materials should be used by the laboratories to establish their precision and accuracy for the determination of trace elements in marine samples by performing the initial analysis of three aliquots of the reference material (see Annex II). Levels should be tabulated on a dry weight basis. Means and standard deviations should be computed for each compound quantified. This data will establish the precision of replicate analysis for each result tabulated. Intercomparison with the results of other reputable laboratories will aid in the assessment of accuracy (see Annex IV).

V.1.3 Definitions of Some Relevant Statistical Terms (see Annex II)

The **specificity** of an analytical chemical method is the degree to which the mean value of the measurements is due to the substance to be determined and not to other substances that may be present in the sample being analyzed.

The **sensitivity** of an analytical chemical method is the smallest change in the quantity to be measured which produces a detectable change in the output. In this case it is synonymous with the term minimum detectability.

The **precision** of an analytical chemical method represents the reproducibility of the analytical results.

The **accuracy** of an analytical chemical method is the degree to which the mean value of the measurements obtained by the method approaches the true value for the measured substance (the effects of other substances interfering being eliminated physically or mathematically).

V.1.4 Use of Reference Materials (RMs)

The accuracy of a method can only be checked with a CRM for which the mean values and standard deviations are well documented. Depending on data quality objectives, the project might require that a certified RM be used (e.g., supplied by NIST, BCR, etc.). A variety of strict criteria must be met before a RM can attain the distinction of CRM, and not all RMs meet these rigorous requirements. For most applications, the use of well-characterized RM is sufficient for assuring data accuracy. Analysts who choose to use their own specially prepared RM (i.e., an Internal RM, IRM) for quality control purposes should note that they are primarily checking the precision of measurements and not their accuracy. These IRMs are very convenient, especially when the cost of CRMs for construction of QC charts would be prohibitive; but as they generally have not been characterized by many different laboratories using different analytical techniques (as commercially available RM's must be), IRM's cannot be used to verify method accuracy.

V.1.5 Analytical Quality Control Charts (AQCCs)

A) Purpose of AQCCs

It has been recommended that a reference material should be analysed periodically to provide a check on the quality of analytical data. The simplest way to assess the results of these analyses is

to examine them at the end of the analytical period and decide whether or not they are satisfactory, and thus whether or not the results for samples are acceptable. This approach is very subjective and a much better approach is to plot the results of the analysis of RMS on a simple chart, which contains guidelines that allow an objective decision to be made on the quality of the data. This chart is known as an analytical quality control chart (AQCC). AQCCs have the additional benefit of graphically depicting the historical performance of a method, so that analytical quality control over a designated period of time can quickly and easily be verified.

B) Construction of an AQCC

Analysts are reminded that before a method is used routinely for samples it must have been rigorously assessed to ensure that it will provide data of the required quality. Assuming that such a method is used the analyst should carry out the following procedure to construct an AQCC, along the lines of that given below in Figure (V.1).

i) Select the RM to be analyzed with samples on a regular basis

ii) Analyze the RM at least 10 times for the analyzes under examination. These analyses should not be done on the same day but spread out over a period of time in an attempt to ensure that the full range of random errors within and between batch analyses are covered

iii) Calculate the mean value (X), and the standard deviation (s) and then plot the following values on a blank control chart :

X, X+2s (UWL), X+3s (UCL), X-2s (LWL) and X-3s (LCL)

C) Using an AQCC

Assuming that the analytical measurements for RM(s) follow a normal distribution, 95% of them (19 in every 20) should fall within the area between UWL (upper warning limit) and LWL (lower warning limit). Similarly 99.7% of the results should fall within the area between UCL (upper control limit) and LCL (lower control limit).

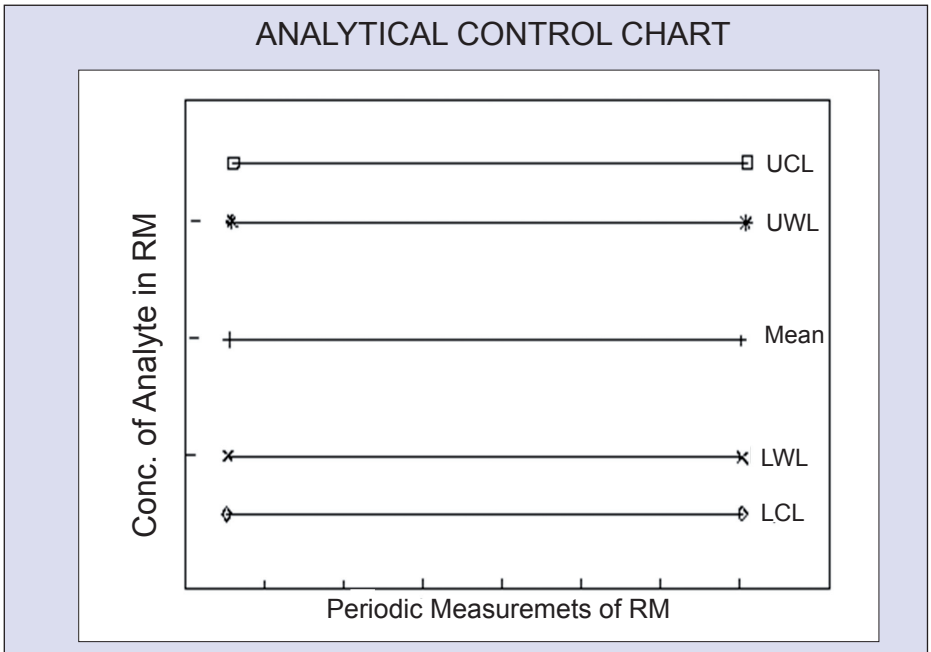


Figure (V.1) : Blank control chart

The analyst should plot the results of the analysis of RM(s) after each batch of analyses to check where the data lies in relation to these limits. An example of such a plot is given in Figure (V.2).

The following guidelines can be used to assess whether the data for the RM(s) and consequently the data for the samples are of acceptable quality, i.e., are the analyses under control ?

i) The mere fact that one result falls outside the warning limits need not require the analyst to doubt the result or take any action provided that the next result falls within the warning limits
ii) If the results fall outside the warning limits too frequently, particularly if the same warning limit has been crossed more than once on consecutive results, then the analyst needs to assess the source of this systematic error
iii) If the results on more than 10 successive occasions fall on the same side of the X line (either between X and UWL or X and LWL) then the analyst needs to check the analytical procedure to determine the cause of this error
iv) If the result falls outside the UCL or LCL lines then the analyst should check the analytical procedure to determine the cause of this source of error

If any of the above cases occur the analyst should reject the results of the analysis of the particular batch of samples and should not carry out any further analysis of samples until the sources) of the errors have been identified and he/she is satisfied that future analyses will be under control.



Figure (V.2): An example of a quality control chart

ANNOTATIONS

(A) A "consensus value" is established by repeated analyses of an IRM. Upper and lower warning limits are determined statistically from the standard deviations (s) of the n measurements made

(B) The routine measurements of the IRM are well within the warning limits. Measurements are under control

(C) Something appear to be contaminating the samples here. Reagents were investigated and a new batch of solvents was found to be at blame and was replaced immediately

(D) The process is back under control

(E) Here, a serious problem was discovered. The previous ten data were rejected and all analyses were discontinued until the fault (dirty glassware this time) was detected and corrected

V.1.6 Documentation and Reporting of Data

The adoption of the following guidelines by a laboratory should provide adequate documentation: to support any data report or decisions on the results of its monitoring programmes, and to allow it to trace samples from the collection stage to the completion of its analyses by providing a record of the appropriate data in logbooks or in computer files.

A) Documentation

The following documentation is necessary for a laboratory participating in pollutant monitoring activities:

i) Descriptions of the sampling strategy, methods of collection, procedures of storage, pre-treatment and analytical procedures

ii) Sample documentation (description of organisms, numbers or individuals collected for each sample, weights of tissue taken for analysis (individual tissue or homogenate)

iii) Evidence of successful evaluation and testing of analytical procedures, including details of accuracy, precision and limit of detection

iv) The approach used for quality control and quality assessment and evidence that these procedures have been used and have provided acceptable data

v) Calibration details, a description of working standards used on each occasion and calculations of results

vi) A secure system for the long term storage of data either in logbooks or in computer files is essential. It is therefore advisable to have a duplicate set of records in case one is lost, mislaid or accidentally destroyed

At the time this revision is beeing prepared, the preferred method of long-term computer file data backup is burning a CD-ROM. CD-R burners are relatively inexpensive, the medium can be read on the vast majority of modern computers, and the medium is known to be stable for many years. Advice should be sought on the correct method of storing computer tapes and/or discs to ensure the long-term stability of the data files.

B) Preliminary assessment, tabulation and storage of data

It has been shown that even the most experienced personnel can make simple arithmetic errors in calculating data. Thus a check should be made for such errors before compiling tables of results.

Once this check has been carried out it is appropriate to carry out a preliminary assessment of the quality of the data, prior to its evaluation and publication, to ensure that no erroneous results are included in the data set. This assessment can include a comparison of the results with existing data (i.e., data previously collected by the laboratory or data published in the literature for the study area). Before consigning data to long term storage a final check should be made to ensure that no transcriptional errors have been made in transferring the data (i.e., the re-typing of data sets by typists or data processors can sometimes lead to such errors).

C) Publication of data

Data confined exclusively to logbooks or on a computer is effectively lost to the scientific community. Every effort should be made to evaluate and publish these results, even in the simple form of a report. In addition to the results, the contents of a data report would normally include a statement on the aims of the work, a description of the methods used to collect and analyze samples together with a statement on findings and conclusions.

In general it is inadvisable to supply a third party with a copy of your raw data, unless:

i) it is accompanied by information on its accuracy, precision and limits of determination and details of the sampling record, or
ii) it is accompanied by a report from the analyst or principal investigator in which the results have been evaluated

V.1.7 Normalization of Contaminant Concentrations in Sediments

Normalisation is defined as a procedure to adjust contaminant concentrations for the influence of the natural variability in sediment composition, grain size, organic matter and mineralogy. Most natural and anthropogenic substances, metals and organic contaminants, show a much higher affinity to fine particulate matter compared to the coarse fraction. Constituents such as organic matter and clay minerals contribute to the affinity to contaminants in this fine material.

Fine material, both inorganic and organic, and associated contaminants are preferentially deposited in areas of low hydrodynamic energy, while in areas of higher energy, fine particulate matter is mixed with coarser sediment particles which are generally have weaker binding capacity for contaminants. This dilution effect will cause lower and variable contaminant concentrations in the resulting sediment. Obviously, grain size and organic matter are important factors controlling the distribution of natural and anthropogenic components in sediments. It is, therefore, essential to normalise for the effects of grain size or organic carbon in order to provide a basis for reliable assessments of temporal trends and for meaningful comparisons of the occurrence of substances in sediments of variable bulk properties with background (assessment) criteria and environmental assessment criteria derived for a defined sediment composition.

In sediment of varying bulk properties, contaminant concentrations will be closely related to the distribution of fine grained material, and any effects of other sources of contaminants, for example anthropogenic sources, will be at least partly obscured by grain size differences. Also in temporal trend monitoring, differences in sediment bulk properties can obscure trends. If samples have a considerable and constant percentage of fine material, the influence of grain size distribution is of minor importance and may probably be neglected.

Annex-X includes the updated OSPAR-JAMP guidelines and method for the normalization of contaminants in marine sediments.

V.2 CLEANING OF LABORATORY GLASSWARE FOR TRACE METAL ANALYSES

V.2.1 Laboratory Materials

Trace metal analyses in environmental matrices (especially in seawater) demand extreme precautions to avoid secondary sample contamination. All manipulations must strictly avoid contact of the sample with any metal surfaces, metal vapors, and materials which, under the conditions of sampling, sample storages, sample work-up, and analysis, release metals in quantities sufficient to cause significant blank levels, either in ionic form or uncharged. Contact with soft- or soda-lime glass should also be avoided. Because of high levels of trace metal impurities, and because its surface acts as an ion exchanger, equipment made of soda-lime glass cannot be used for trace metal analyses. The same applies to borosilicate glass with one exception: laboratory-ware made of borosilicate glass may be used for mercury analyses.

A variety of plastics are used in the construction of laboratory-ware, and careful selection is necessary to minimise interferences in trace metal analyses. A review of the chemical and physical characteristics of materials used in the manufacture of laboratory products has been published in: "Inorganic Trace Analysis: Philosophy and Practice" (Howard and Statham 1993). This is most valuable in selecting laboratory apparatus for a given analysis. In general, it is best to avoid all colored plastics as many of the coloring materials are major sources of metals. Blank tests must be made in order to check for possible contamination.

For trace metals other than mercury, low density polyethylene (LDPE) is the recommended material, as it should not be a major source of contaminating metals. Laboratory-ware made of Teflon (PTFE, PFA, FEP), and vitreous silica or fused quartz can also be used. High density polyethylene (HDPE), and polypropylene are less suitable, because the manufacture of these plastics include catalytic processes involving some trace elements. The risk or contamination may therefore be high.

For mercury, Teflon (PTFE, PFA, FEP) is the material of choice. Quartz or borosilicate glass can also be used. Plastics other than Teflon are not recommended because of adsorption of mercury onto the walls of the containers or because of diffusive losses through the walls.

For additional information on the suitability of laboratory-ware for trace metal analyses see: Inorganic Trace Analysis: Philosophy and Practice (Howard & Statham 1993); and Methods of Seawater Analysis (Grasshoff *et al*. 1983).

V.2.2 Cleaning Procedure

For trace metal analyses. new or conventionally cleaned apparatus is unsuitable. Almost without exception, a vigorous cleaning procedure is required when a new container is first put into use. The extent and nature of subsequent cleaning depends very much on the application for which the container is to be used. Blank tests must be run to check the efficiency of the chosen cleaning procedure.

The following procedure for cleaning is appropriate for laboratory-ware made of any of the materials described in Section V.2.1. It involves a degreasing step followed by sequential leaching of the containers with hydrochloric and nitric acids, which have been shown to provide complementary cleaning actions (Howard & Statham 1993).

Degreasing can normally be achieved using commercial laboratory detergents. Compatibility of the cleaning materials with both the final analysis and the chemical nature of the container to be cleaned must be considered at this early stage. The laboratory-ware is immersed for several hours (overnight) or for several minutes when using ultrasonication in a dilute aqueous laboratory detergent solution. Heating the bath to about 60°C enhances the cleaning process. The laboratory-ware is then rinsed with copious quantities of tap water (ten rinses) followed by three rinses with purified distilled or de-ionized water. A blank test must be run to ensure that the tap water is sufficiently clean. If it is not, purified water has to be used exclusively

The next step is a treatment with hydrochloric acid (1:1, v/v, analytical reagent grade, diluted with purified water). The laboratory-ware is immersed in the acid solution for one week at room temperature or 3 days at 50°C. It is then rinsed thoroughly (at least three times) with purified water.

The final treatment is immersion in nitric acid (1:1, v/v, analytical reagent grade diluted with purified water). The laboratory-ware is soaked in the acid for one week at room temperature or 3 days at 50°C. It is then, once again, rinsed thoroughly (at least three times) with purified water.

For certain applications not requiring the stringent cleaning protocols described above, a treatment with 1:1, (v/v) nitric acid solution followed by a 1:9, (v/v) nitric acid solution can be sufficient for the cleaning of plastic-ware used for the analysis of trace metals (with the exception of methylmercury, for which HCl cleaning is necessary). Adequacy of the protocol must, however. be checked using procedural blanks.

The laboratory-ware is then air dried in a laminar flow hood and sealed in polyethylene bags if not for immediate use. Sample containers, i.e. bottles and flasks, should be stored filled with 0.1% ultrapure hydrochloric acid (HCl) or nitric acid (HNO_3). Teflon bottles for Hg analysis should be tightly closed using pliers.

V.3 SAMPLE PREPARATION PROCEDURES

V.3.1 Laboratory Equipment

- Agate mortar and pestle
- Mechanical shaker
- Drying oven (drying ovens having teflon coated surfaces and a filtered nitrogen atmosphere are sometimes used to ensure against sample contamination)
- Nylon or stainless steel sieves (2000, 500, 63, 37 µm diameter)
- Freeze - dryer
- Stainless steel, glass or Teflon tissue homogenizer
- Analytical balance sensitive to 0.1 to 0.5% of the sample weight
- Centrifuge: A large capacity centrifuge is highly desirable, although not absolutely necessary, for washing samples free of sea salts and for separating clay size fractions. The same centrifuge with a smaller head, or a centrifuge of smaller capacity, is also needed for separating the acetic acid soluble fraction
- lypropylene or glass centrifuge bottles
- Eight or more Teflon digestion vessels of at least 25 ml capacity, single or in a “digestion block” e.g. Teflon bombs or tubes
- Teflon bombs (60 ml) or Teflon tubes (FEP, 60 ml. with caps)

Note : If smaller digestion vessels are used, the amounts digested must be accordingly reduced to avoid explosions

- Oven or hot plate with aluminum block for the digestion vessels, working temperature 120 - 150°C complete with double independent temperature control

Note : Oven or hot plate must have two independent temperature controls so that an overheating and consequent explosion can be avoided

- Laminar flow hood and/or "clean room"
- Analytical balance (100-200 g) with a precision of 0.001 g for weighing reagents; preferably a 'top-load' balance
- Microwave oven for laboratory use
- Plastic tweezers
- Plastic spatulas
- Desiccator
- Evaporating dishes
- Micropipettes for 0-200 µl; 200 -1000 µl, 1,2,10 ml
- Polypropylene tubes with caps (50 ml)
- 1 polyethylene bottle (500 ml) for Milli-Q water
- Graduated polypropylene cups for automatic sampler
- Teflon bottles 125 ml
- Polypropylene spatulas
- Glass bottle, 1 litre (preferably with screw)
- A range of volumetric flasks (borosilicate), graduated measuring cylinders
- 30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids
- 5 ml. capacity tapered vials with PTFE lined screw-capped lids (e.g. "Reactivials")
- A wrist action shaker capable of holding the centrifuge tubes described above

Note : i) All glassware to be used in the analyses (including new glassware) should be soap and acid cleaned (see V.2.2). The apparatus should then be thoroughly rinsed with water. Reagent blanks must be processed to ensure that analyses are free from contamination.

ii) Teflon bomb heating equipment (for example)

The teflon bomb in which the sediment sample is decomposed is normally heated by way of a microwave digestion system, which quickly and effectively produces the necessary heat and resulting pressure for the total decomposition of the sample. Alternatively, if a microwave digestion system is not available, the bomb can be heated in boiling water.

a) **For microwave heating** :

The microwave oven should be calibrated to detect any decrease in the power output in the future and to facilitate the calculation of the optimum heating time for samples. Once calibrated, recalibration should be necessary on a periodic basis, and is usually done as part of the regular maintenance by a qualified engineer. If necessary, calibration can be done by the analyst by measuring the temperature rise of 1 L of distileld water in a beaker with the oven set at full power for 2 min. Specifics for interpreting this data should be found in the user manual.

The heating time depends on the power output of the microwave oven and the load. The optimum heating time can be estimated (Kingston and Jassie, 1986) and verified experimentally by analyzing certified reference materials and comparing the results to those obtained by a 1 hour decomposition in boiling water. Teflon bombs without pressure relief should not be used for microwave heating due to the potential danger of over-pressurization and explosion.

b) **For heating the bomb by submersion in boiling water** :

Hot plate

Covered ceramic dishes (25cm x 25cm x 10cm deep). Each dish can accommodate 8 bombs at a time

Sufficient water to cover the teflon bombs is placed into the ceramic dishes and heated to boiling on the hot plate

- Atomic absorption spectrophotometer (AAS) with hollow cathode lamps for Cd, Cu, Pb and Zn and with background correction for use with acethylene/air flame or alternatively complete with programmable graphite furnace atomizer

Note : Lower detection limits can be reached using an electrodeless discharge lamp

- Signal indicator, recorder or similar
- Air compressor or high-pressure cylinder with compressed air (for flame atomization only)

Note : A source of clean, compressed air may be required for some spectrophotometers in the furnace mode to purge ozone which is generated by the deuterium lamp used for background correction from the optics

- High-pressure cylinder with acetylene of analytical grade purity (for flame atomization only)

Note : If acetylene intended for welding is utilized it should be checked against a pure supply. Nitrogen of adequate purity or any other gas or gas mixture specified by the M S manufacturer may be used

- Gas Chromatograph. Capillary column instrument with temperature programming and flame photometric detection, fitted with a 25 m x 0.32 mm ID fused silica capillary column and coated with a cross-linked 5% phenyl methyl silicone fluid with a film thickness of 0.52 µm. A retention gap consisting of one metre of deactivated, uncoated fused silica (0.53 mm ID) is attached to the injection end of the analytical column in order to allow on column injection and better chromatographic resolution. The use of an autosampler and electronic data capture system is beneficial.

V.3.2 Chemicals

All reagents, including the distilled water, should be of recognized analytical quality, with as low as possible trace elements concentration. All reagents must be checked for contamination with the elements of interest by analyzing blanks.

- Demineralized distilled water or water of equivalent quality, with trace metal content below detection limits of this method, e.g. Milli-Q deionised water (>18 DΩ cm, Millipore)
- Nitric acid (d_{20}°c 1-4 g^{-1})
- Hydrochloric acid (d_{20}°c = 1.19 g^{-1})
- Standard solution of the elements of interest 1000 mg/l
- HNO_3 (65% analytical grade, low in Hg)
- HNO_3 (65%, Suprapur)
- HF (48%, analytical grade ISO)
- HCl (30%. Suprapur)
- H_2SO_4 (95-97%, analytical grade, low in mercury)
- Hydrochloric acid, 35.4%, e.g. 1.18
- $K_2Cr_2O_7$ (analytical grade, low in Hg)
- KBr (Suprapur)
- $KBrO_3$ (Suprapur)
- $SnCl_2$ (analytical grade, normal or low in Hg)
- Boric acid crystals, H_3BO_3 (analytical grade ISO)
- Sodium borohydride powder. Purity 96% or better

- V_2O_5 (extra pure)
- Sodium hydroxide and methanol reagent. Prepared by dissolving 1 g sodium hydroxide (98% pure or better) in 1 l of methanol (99.8% pure or better) to give a 1% solution
- Authentic Standards
- Butyltin trichloride
- Dibutyltin dioxide
- Bis (tributyltin) oxide
- Tripropyltin chloride (Internal standard)
- Sodium Hydroxide 99%
- Hexane (glass distilled grade)
- Copper sulphate ($CuSO_4.5H_2O$)
- Sodium sulphate - anhydrous ($NaSO_4$). This reagent should be heated at 450°C for 4 hours to remove any contamination, and then stored in a sealed container
- L(+) - cysteine hydrochloride ($HSCH_2CH (NH_2) CO_2H.HCl.H_2O$)
- Sodium citrate ($NaO_2CCH_2C (OH) (CO_2Na) CH_2CO_2Na.2H_2O$)
- Sodium thiosulphate ($Na_2S_2O_3.5H_2O$)
- Ethanol (C_2H_5OH)
- Toluene ($C_6H_5CH_3$) (Distilled in glass)
- Methyl mercury chloride (CH_3HgCl)
- Ethyl mercury chloride(CH_3CH_2HgCl)
- Mercuric chloride ($HgCl_2$)
- Silver sulphate ($AgSO_4$)
- Acetone (CH_3COCH_3)

V.3.3 Reagents

V.3.3.1 Trace Metal Analysis (except Hg)

A) Standard solutions

1. Standard solutions

Individual standard solutions can be prepared either from concentrated spectrometric solutions purchased commercially, from dissolving an appropriate quantity of the metal salt (of adequate purity) into the desired volume of dilute acid (generally ~5% ultrapure acid in de-ionized water), or by dissolving an appropriate quantity of pure metal in concentarted acid, then diluting to the desired volume with de-ionized water. From these concentrated solutions, working solutions can then be prepared, either as individual metal or multi-element solutions.

Note : The concentration of metal in the standard solution should depend on the target metal levels anticipated in the samples to be analyzed.

2. Metal standard solutions for the calibration curve

1) Put about 10 ml of Milli-Q water into clean polypropylene tubes (50 ml)
2) Add reagents in order to obtain a similar matrix as in the sample (e.g., if sample is in 8% nitric acid, add 4 ml of nitric acid)
3) Add the appropriate quantity of stock standard solution with a micropipette.

4) Dilute to the mark (50 ml) with Milli-Q water
5) Shake well

There solutions can be kept for a few days if stored in a refrigerator (+4°C).

Depending on the element, it may be necessary to make an intermediate stock standard solution.

3. Multi-element standard solutions

The primary issues to be aware of when preparing multi-element standards are :

a) the concentration of the analyte should be in an appropriate concentration range to bracket the anticipated concentration in the standard solution. This will mean that a multi-element standard will not contain the same concentartion of all elements. For example, elements such as Cd, Pb, Sb, Sn, and Tl are generally found at lower concentrations in environmental samples than Cu, Cr, Ni and V. For sediments, Al, Fe and the minerals will be at far higher concentrations than the other elements. These factors should be taken into account; a working multi-element standard solution may, for example, contain 10 µg/l Cd and other low-level elements, 500 µg/l Cu and other similar concentration elements (e.g., most first-row transition elements), and 10 or 100 mg/l Al, Fe and minerals.

b) elements should be compatible with the matrix and with one another such that all remain stable in solution. The clearest example of this is ensuring that Ag is in a chlorine-free matrix, else it will precipitate as AgCl. Ensuring that no HCl is added is not sufficient; Ag will precipitate with very small quantities of chloride, e.g., from the matrix of another element standard that is being added.

In general, elements in like-acid primary solutions should be able to be prepared together, in a similar acid as they were originally received in (though possibly more dilute; ~5% is generally sufficient for a working solution). Also, ensure that if the standard was prepared from a salt, that the salt anion will not precipitate any of the other elements that will be added to the multi-element standard.

B) Special reagents

Cadmium

If a graphite furnace technique is used, to each 100 ml of calibration standard and sample alike add 2.0 ml of ammonium phosphate solution which is prepared as follows:

Dissolve 40 grams of ammonium phosphate, $(NH_4)_2HPO_4$ (analytical reagent grade) in W and dilute to 100 ml.

Lead

If a graphite furnace technique is used, to each 100 ml of calibration standards and sample alike add 10 ml of lanthanum nitrate solution which is prepared as follows :

Dissolve 58.64 g of lanthanum trioxide, La_2O_3 (analytical reagent grade) in 100 ml conc. HNO_3 and dilute to 1 litre with DDW.

Vanadium

To each 100 ml of calibration standard and sample alike, add 2 ml of aluminum nitrate solution which is prepared as follows:

Dissolve 139 g aluminum nitrate, Al $(NO_3)_3.9H_2O$ in 150 ml DDW; heat to effect solution. Allow to cool and make up to 200 ml.

V.3.3.2 Analysis of Total Hg

A) Standard solutions

Solution stock 1 : 1 mg/ml Hg in 10% nitric acid

1) Weigh exactly 1.354 g of $HgCl_2$ into a 1 litre glass volumetric flask
2) Add about 500 ml of Milli-Q water
3) Add l0 ml of concentrated nitric acid (low in Hg)
4) Complete to the mark with Milli-Q water
5) Shake well until complete dissolution is achieved
6) Transfer into a 1 litre Teflon bottle

Closed tightly with a torque wrench and keep in the refrigerator (+4°C).

Solution stock : 1 µg/ml Hg in 4% nitric acid

1) Weigh 95 g of Milli-Q water into a 125 ml Teflon bottle
2) Add 4 ml of concentrated nitric acid (low in Hg)
3) Add 1 ml of BrCl solution (or 2 ml. of $K_2Cr_2O_7$ solution)
4) Add l00 µl of solution stock 1 (1 mg Hg/ml)
5) Shake well

Closed tightly with a wrench and keep in the refrigerator (+4° C).

Calibration curve (at least 3 standards and zero calibration)

1) Put about l0 ml of Milli-Q water into a clean 50 ml glass volumetric
2) Add reagents as in the digested samples (HNO_3/H_2SO_4 2: l; or HNO_3)
3) Add the appropriate quantity of stock standard solution (stock I or stock 9 depending on the samples concentrations) with a micropipette
4) Add 1 ml of BrCl solution (or 2 ml of $K_2Cr_2O_7$ solution)
5) Dilute to the mark (50 ml) with Milli-Q water
6) Shake well

These solutions should be done fresh every day of analysis.

B) Special reagents

20% w/v $SnCl_2$ in 20% v/v HCl (200 ml)

1) Weigh accurately 40 g of $SnCl_2$ into a clean glass beaker using a plastic spatula (beaker and spatula are used only for $SnCl_2$)
2) Add 40 ml of concentrated HCl directly to the $SnCl_2$ and transfer to a 200 ml volumetric flask. Mix and wait for complete dissolution of $SnCl_2$
3) Add Milli-Q water to the mark (200 ml)
4) With older stock of $SnCl_2$ it may be necessary to warm up the solution on a hot plate to obtain complete dissolution of $SnCl_2$ (do not allow to boil)
5) In case of low concentration samples if $SnCl_2$ used is not "low in Hg", it should be purged with nitrogen for two hours before use
6) This solution should be made fresh for each day of analysis

Note : All glassware used for preparation of $SnCl_2$ solution should be kept separately from remaining laboratory ware in order to avoid cross contamination of ware for trace element determination

Nitric acid 10% v/v (500 ml)

1) Put about 400 ml of Milli-Q water into a 500 ml. volumetric flask
2) Add carefully 50 ml of concentrated nitric acid
3) Make up to the mark with Milli-Q water
4) Shake well
5) This solution can be stored if kept in a tightly closed flask

Two alternative choices for oxidizing solution

a) $K_2Cr_2O_7$ l0% (w/v) in Milli-Q water

1) Weigh 50g of $K_2Cr_2O_7$ into a clean 500 ml glass volumetric flask
2) Add about 250 ml of Milli-Q water and shake until $K_2Cr_2O_7$ is dissolved
3) Shake up to the mark with Milli-Q water

b) BrCl oxidizing solution

1) Weigh accurately 11 g of $KBrO_3$ and l5 g of KBr into a clean 1 litre glass bottle
2) Add 200 ml of Milli-Q water
3) Add carefully 800 ml. of concentrated HCl. The dilution has to be carried out in a well ventilated fume hood to prevent exposure to toxic fumes released during dissolution of $KBrO_3$
4) Keep the bottle wrapped with aluminum foil

Note : These 2 solutions can be kept for an unlimited time if stored in the dark at room temperature in a tightly closed Teflon or glass bottle, and in a Hg free area

V.3.3.3 Analysis of MeHg

A) Standard solutions

Methylmercury and ethylmercury standard stock solutions in toluene (1 mg Hg/ml)

Weigh 125.2 mg of CH_3HgCl or 132.1 mg of C_2H_5HgCl into 100 ml volumetric flasks and bring to volume with toluene. These solutions are stable for years.

Stock solutions for chromatography (10 µg Hg/ml)

Using a graduated pipette and suction bulb, transfer l.00 ml of the standard stock solutions to 100ml volumetric flasks and bring to volume with toluene

Standard solutions for chromatography

Prepare standard solutions of 20, 40, 60, 80 and 100 ng Hg/µl by adding 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of the stock solutions prepared to 100 ml volumetric flasks and bring to volume with toluene

Methylmercury and ethylmercury standard aqueous solutions

Weigh the same amounts of methylmercury and ethylmercury into 100 ml volumetric flasks and dissolved in 1-2 ml acetone. Make up to volume with 0.1 M hydrochloric acid. Working solutions (1 µg Hg/ml) are prepared by appropriate dilutions with 0.1M hydrochloric acid

Note : The stock and working standard solutions must be kept in darkness to prevent decomposition of MeHg by ultraviolet light

B) Special reagents and other preparations

Conditioning solution for chromatography

Add sufficient mercuric chloride to 10 ml toluene so that some solid compound remains undissolved.

Sulphuric acid solution saturated with copper sulphate

Prepare approximately 2M sulphuric acid by slowly adding 11 ml of concentrated acid to 90 ml of water. Saturate this solution with copper sulphate by adding sufficient crystals so that some remain in the bottom of the flask without dissolving.

Potassium bromide solution

Weigh 48 g of potassium bromide into a 100 ml volumetric flask and bring to volume with water. This constitutes a 4M solution.

1%, L (+)- cysteine hydrochloride in 20% sodium citrate clean-up solution

Weigh 1 g of L (+)- cysteine hydrochloride and 20 g of sodium citrate into a 100 ml flask and bring up to volume with water.

Note : Prior to use, this solution should be pre-extracted with toluene. THIS SOLUTION SHOULD BE PREPARED DAILY

Sodium thiosulphate clean-up solution

Into a small beaker or vial weigh 0.12 g of sodium thiosulphate. Using a graduated pipette add 4 ml of water and allow to dissolve. Using a graduated pipette transfer 1.0 ml of this solution to a 100 ml volumetric flask, add 50 ml of ethanol and bring to volume with water.

Note : This solution must be no older than 3 days

Silver sulphate solution

Dissolve 0.5 g $AgSO_4$ in 100 ml water.

6 M hydrochloric acid solution

Slowly add 500 ml of concentrated HCl to 500 ml of water.

0.1 M hydrochloric acid solution

Slowly add 7.3 ml conc. HCl to 1000 ml of water.

4 M sulphuric acid

Slowly add, with cooling, 22 ml of conc. H_2SO_4 to 60 ml water.

Filter papers

A 7 cm diameter circular filter papers of "medium" porosity are required in the preparation of "Cysteine paper". A narrow strip (2 mm in width) is cut from the circumference of the paper which is then impregnated with cysteine solution (0.2 ml). The paper is then dried at room temperature in an **ACID-FREE ATMOSPHERE**. (Papers for each analysis should be prepared just prior to their use).

Note : 1. Pore size is not a critical parameter
2. This method provides a length and shape of paper which is easily added so the extract. (A surface area of approximately 4 cm^2 is necessary to treat the 5 to 10 ml toluene extracts)

V.3.3.4 Preparation of Standards for TBT Analysis

- Individual stock solutions of standards are prepared by dissolving 0.1 g of each compound in 100 ml of methanol to produce a solution containing 1 mg/ml of each compound
- Individual working solutions are then prepared by dissolving 0.5 ml of stock solution in 50 ml of methanol to provide a 10 µg/ml solution

A composite working standard may be prepared by adding 50 µl of each working standard to a centrifuge tube containing 2 ml of distilled water, 8 ml of sodium hydroxide/methanol reagent, 100 mg of sodium borohydride and 2 ml hexane. The tube is then shaken for 15 min. and centrifuged at 2500 rpm. This composite may be used to determine relative response factors for each compound detected by the flame photometric detector.

V.3.4 Initial Preparation

Note : Extreme care must be taken during sample preparation, as always, to avoid metallic contamination by metallic objects, undecontaminated plastic and glassware and uncovered hands

V.3.4.1 Determination of Dry Weight

A) Biological material

Results expressed on the basis of the dry weight (DW) of the analyzed tissues are more reliable than results expressed on the basis of its fresh weight (FW) because the continuous loss of water from the fresh biological samples does not allow an accurate determination of FW. However, results expressed on the basis of FW are easier to interpret as most transfer models of pollutants are based on FW (see Section IV.1.2 and IV.1.3).

Note : Biological materials containing large amounts of lipids cannot be oven-dried to constant weight and must, therefore, be freeze-dried

Place a 1 – 2 g exactly weighed subsample of the specimen sample in the clean sample container suitable for freeze-drying and freeze-dry for 24 hours. Weigh the subsample exactly and freeze-dry for another 24 hours. Determine again the weight of the subsample. If the difference between the 2 weighing is less than 0.5% determine the DW and DW/FW ratio. Otherwise repeat the drying cycle until the difference between successive weighing is less than 0.5%.

B) Sediments

Sediment samples must be dried before chemical analyses are conducted because analytical measurements are based on a constant dry weight. All the results should be expressed on an oven dry weight basis so that the results are independent of variations in air humidity (see Section IV.1).

IV.3.4.2 Grain-size Separations for Trace Metal Determination in Sediments

The chemical composition of marine sediments changes with the grain size and mineralogical composition. Usually, the trace metal concentrations increase with decreasing grain size of the material. This is because the host minerals of the elements and the surface area of the particles making up the sediment change with grain size. In addition, regional comparisons of elemental concentrations can only be made by using texturally equivalent sediments and/or size fractions. It is, therefore, often necessary to normalize for the "grain size effect" and sometimes to determine the elemental concentrations in different size fractions of the sediments.

Geochemical analyses of sediments involves procedures for their decomposition and chemical analyses such as are outlined in Figure (IV.1). The number of steps that are followed will depend on the nature and extent of the investigation. It is recommended that, at least, the amount of material <63 µm and the total trace concentrations be determined for each sample.

A) Determination of sand and mud size material

Determination of the amount of sand and mud size material by weight is essential for the basic classification and identification of the sediment texture. The general scheme for the separation of the different grain size fractions is shown in Figure (V.3) and described in detail in Section IV.2.3.

B) Detailed grain size separations for trace metal determinations

For detailed grain size separations, the procedures described in Sections IV.2.4 and IV.2.6 can be followed. Alternatively, a simplified procedure as outlined in Figure (V.3) can be used.

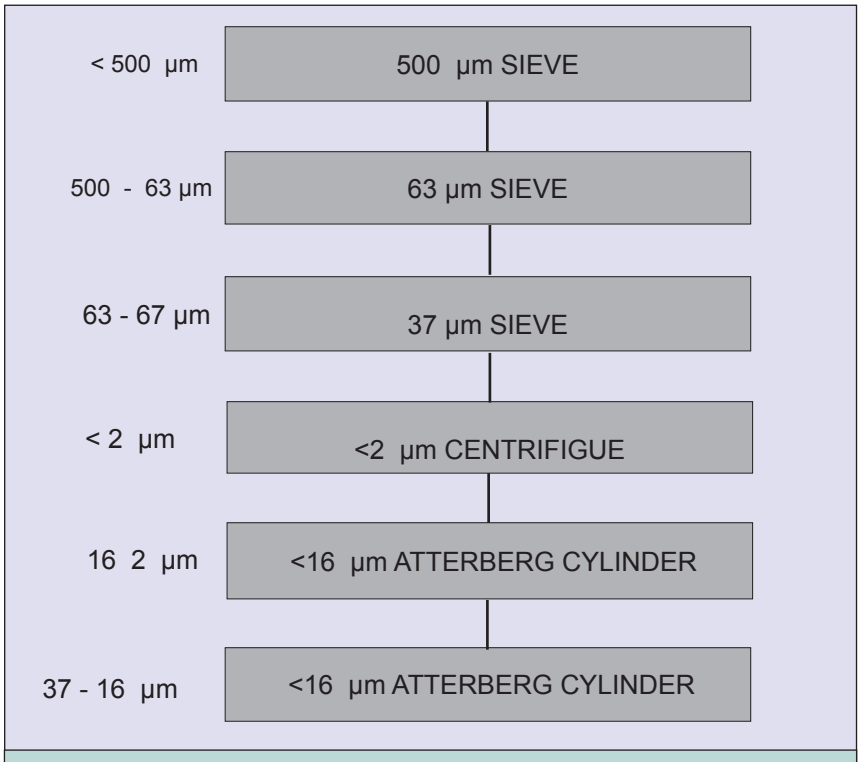


Figure (V.3) : General scheme for grain size separation

Simplified wet sieving procedure for separation of sand fractions :

a) Remove water soluble sea salts from 10-20 g of sample but do not dry (This step is not required for trace metal determinations, but is only necessary if the major cations Na, K, Mg, and Ca are to be determined) (see Section IV.1.4)
b) Wet sieve salt free sample through a set of 500 µm, 63 µm, and 37 µm stainless steel or nylon sieves, brushing carefully to prevent particles from aggregating or sticking to them
c) Collect the fractions from each sieve
d) Carefully remove the fraction retained on each sieve, and transfer it to a pre-weighed Teflon evaporating dish
e) Dry each fraction at 105°C, cool in a desiccator, and weigh (except the one from the >500 µm sieve)
f) Resieve the dry >500 µm fraction through a 2000 µm sieve to remove rock fragments >2 mm and weigh
g) Calculate the dry weight percentages for each size fraction from the equation : Size fraction = wt. of fraction / wt of sample X 100
h) Retain the liquid passing through the 37 µm sieve
i) Pour into a centrifuge bottle, and make up to l litre with 0.16N NH_4OH as the dispersive agent
j) Shake mechanically for 30 minutes (an ultrasonic probe could be used instead of NH_4OH to disperse the sample)
k) Separate the < 2 µm fraction by centrifuging (the centrifuging time is calculated from Stoke's law, Tanner and Jackson, 1947). If a centrifuge is not available, the < 2 µm fraction can be separated in a beaker according to the sampling times shown in Table (V.I)
l) Siphon the <2 µm material off and store in centrifuge bottles
m) Add 0.16N NH_4OH again, shake the centrifuge bottle mechanically, and centrifuge
n) Repeat the whole process until the supernatant siphoned off is clear. Usually four times is sufficient, although this depends on the amount of clay present
o) Centrifuge the stored < 2 mm fraction again to remove excess water
p) Transfer it to a pre-weighed teflon dish, dry at 105°C (60°C for Hg), cool in a desiccator, and weigh
q) Transfer the 37-2 µm fraction from the bottle into an Atterberg cylinder, marked 30 cm from the bottom of the siphon tube
r) Make up to the mark with 0.16N NH_4OH, shake, and let stand for the specified time intervals shown in Table (V.1)
s) Unclamp the siphon tube, and collect the 2-16 µm fraction in the centrifuge bottle;
t) Repeat until clear
u) Centrifuge off the excess water from the 2-16 µm fraction
v) Transfer the fraction to a pre-weighed evaporation dish, dry at 105°C, cool in a desiccator and weigh
w) Transfer the 16-37 µm fraction remaining in the Atterberg cylinder to a pre-weighed evaporation dish, dry at 105°C, store in a desiccator until cool, and weigh

Table (V.1): Sampling times for size fractions in Beaker or Atterberg cylinder

Size Temp °C	<2 µm 5 cm*	<4 µm 5 cm	<8 µm 10 cm	<8 µm 30 cm	<16 µm 30 cm
10	5 h 12'	1 h 18'	39' 00"	l h 57'	29' 15"
11	5 3	1 16	37 55	1 54	28 27
12	4 55	1 14	36 53	1 51	27 41
13	4 47	1 12	35 54	1 48	26 56
14	4 40	1 10	34 58	1 45	26 13
15	4 32.5	1 8	34 4	1 42	25 32
16	4 25.5	1 6.5	33 12	1 39	24 53
17	4 19	1 5	32 21	1 37	24 15
18	4 12	1 3	31 32	1 34	23 39
19	4 6	1 1.5	30 45	1 32	23 4
20	4 0	1 0	30 0	1 30	22 30
21	3 54	58.5	29 16	1 28	21 57
22	3 49	57	28 31	1 25	21 24
23	3 44	55.5	27 46		20 51
24	3 39	54	27 01		20 18

h - Hours
' - Minutes
" - Seconds
* - Column from which particles larger than indicated have settled down in a specified time and temperature

V.3.5 Digestion of Sediment for Determination of Trace Metals by AAS

V.3.5.1 Sediment Decomposition Methods

In order to determine the major and trace metal concentrations of marine sediments by wet chemical methods, it is necessary to dissolve all or part of the sample. Sample digestion methods commonly used are: (a) Total decomposition; (b) Strong acid digestion, or (c) Moderate or weak acid extractions.

A) Total decomposition

Total decomposition methods use hydrofluoric acid (HF) in combinations with concentrated oxidizing acids such as aqua regia. Alternatively, alkaline fusion followed by acid dissolution of the flux can be used.

Hydrofluoric acid decomposition has the following advantages :

- HF is the only acid that completely dissolves the silicate lattices and release the associated metals such as Al, Fe, and Li used for the grain size normalization of the data

- Accuracy can be assessed by analyzing reference materials certified for the metal content
- Intercomparable data, free from operationally defined bias, can be obtained

Some laboratories have been reluctant to use HF due to its corrosive nature 30 years use, it is generally accepted that HF poses no greater danger than other strong 2 when normal laboratory safety rules for handling acids are observed.

Warning : Hydrofluoric acid can cause serious injury – extreme care should be exercised whenever working with HF. As with any acid, eye protection should always be worn during handling, whenever pouring the acid or handling beakers or containers that contain HF. In addition, normal vinyl gloves will not provide adequate protection; nitrile gloves should be worn whenever handling HF.

B) Strong acid (non-HF) digestion

Strong acid digestions using nitric acid (HNO_3) or aqua regia (HNO_3 + HCl) commonly used to decompose marine sediments. They are NOT recommended for following reasons:

- Strong acid digestions without HF result in incomplete digestions because silicates and other refractory oxides are not dissolved
- The proportion of metals dissolved is variable and depends on the sample type, matrix, and element
- Accuracy of the results can not be determined since no reference materials certified for strong acid digestions
- Metal data obtained from strong acid digestions are not intercomparable with total metal data and are subject to operationally defined bias

C) Moderate or weak acid extractions

Moderate or weak acid extractions such as hydrochloric acid (HCl) and acetic acid (HOAc) are often used for chemical partition studies (Loring, 1978, 1981). They are strictly operationally defined procedures. Accordingly, they cannot be standardized using reference materials.

V.3.5.2 Total Decomposition Methods

Principle

Sediment samples are treated in closed Teflon vessels with hydrofluoric acid (HF) in combination with aqua regia in order to decompose the samples. The use of HF is essential because it is the only acid that completely dissolves the silicate lattices and releases all the metals.

Sample size

The size of sample required depends mainly on the expected metal concentrations. In general, a 0.1 g sample is used for the major elements and a 1 g sample is used for trace metal determinations if only flame AAS is available. A 200 mg sample containing normal amounts of major and trace metals,

however, has been found to be sufficient for most metal concentrations if the sample is homogeneous and graphite furnace AAS is available. It would be preferable to make only one decomposition for all elements using 0.1 – 0.3 g sample size and adjusting the dilutions accordingly. Certain trace metals occurring in very low concentrations may require larger sample sizes.

Maximum sample size used in the microwave decomposition is 200 mg. Larger samples may be feasible although small samples are best suited for microwave heating.

Procedure

1) Shake the vials containing the samples for about 2 min. for homogenization
2) Wait a few minutes before opening the vials
3) Weigh accurately from 0.1 to 0.3 g of dry sample in Teflon bombs (a) or 0.1 to 0.5g in Teflon tubes (b)
4a) Add slowly 5 ml of nitric acid (HNO_3) and 2 ml of concentrated hydrofluoric acid (HF). Leave samples at room temperature for at least 1 hour
4b) Or add slowly 1 ml of aqua regia (HNO_3 : HCl, 1:3 v/v) and 6 ml. of concentrated hydrofluoric acid (HF). Leave samples at room temperature for at least 1 hour
5a) Close the Teflon bombs tightly (with the aid of a torque wrench) and place the bombs in microwave digestion system and apply appropriate digestion routine. (See example routine, Table (V.2), below)
5b) Or close the tubes and place them in an aluminum block on a hot plate at 120°C for 2h and 30 min
6a) Weight 0.8 g of boric acid into 50 ml polypropylene graduated tubes then add about 20 ml of Milli-Q water and shake
6b) Weigh 3.70 g of boric acid into 50 ml polypropylene graduated tubes then add about 20 ml of Milli-Q water and shake
7a) Allow the samples to cool to room temperature, before releasing the pressure carefully, then open the bombs
7b) Allow samples to cool to room temperature then open the tubes
8) Transfer samples into the polypropylene graduated tubes (containing boric acid). Rinse the bombs or the Teflon tubes with Milli-Q water at least 3 times adding the wash to the polypropylene tubes.
9) Shake the tubes to complete the dissolution of H_3BO_3 (black carbon residue may remain but does not contain significant amounts of metals and does not interfere with subsequent metal determinations)
10) Dilute to the mark (50 ml) with Milli-Q water
11) Place in ultrasonic bath for 30 minutes to facilitate dissolution / resuspension of boric acid and precipitates
12) Allow particles to settle (usually overnight) before analysis
13) Sample solutions can be stored for several days before analysis, preferably in the cold (+4°C)

Table (V.2) : Example of sediment digestion routine

Step	Power (W)	Power %	Ramp time (min)	PSI	°C	Hold Time (min)
1	1200	100	5	600	50	5
2	1200	100	5	600	100	5
3	1200	100	10	600	200	12

Reagent blanks

At least 3 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as samples, except that no sample is added to the digestion vials.

Reference materials

At least one certified reference material should be used and prepared in triplicate for each batch of analysis. These digestions are prepared in a similar manner as samples.

Reference material should be of similar composition and concentration range as samples.

V.3.6 Digestion of Sediment for Determination of Total Mercury by Cold Vapour AAS

Principle

This method is applicable to all sediment samples with total mercury concentrations higher than about 0.05 µg/g. The strong acid digestion is meant to decompose the samples and to oxidize and convert any organic forms of mercury into inorganic mercury.

Procedure

1) Shake the vials containing the samples for about 2 min. for homogenization
2) Wait a few minutes before opening the vials
3) Weigh accurately from 0.1 to 1 g of dry sample in Teflon tubes (60 ml) depending on expected concentration
4) Add 4 ml of concentrated nitric acid (HNO_3) and 2 ml of concentrated sulfuric acid (H_2SO_4) for sample size from 0.1 to about 0.5 g
5) If more sample is taken add more acid in the same ratio (2:1) until the mixture becomes liquid
6) Close the tubes and leave 1 hour at room temperature (or longer if the reaction is very strong)
7) Heat for 3 hours at 90°C on an aluminum block on the hot plate (or in water bath)

8) Allow the samples to cool to room temperature before opening the tubes. Leave the tubes cooling down in a fume hood to avoid toxic acid fumes
9) Add about 20 ml of Milli-Q water
10a) Add 1 ml of $K_2Cr_2O_7$ solution (final concentration = 2% v/v)
10b) Or 0.5 ml of BrCl solution (final concentration = 1% v/v)
11) Dilute to the mark with Milli-Q water or transfer to volumetric flasks or to graduated tubes and dilute to 50 ml
12) Shake and wait for sedimentation of material before analysis

Note : 1. The samples can be kept for a few days before analysis if stored in the refrigerator (+4°C). The maximum storage time has to be determined by experience for each kind of samples
2. Reagents, including the distilled water, should be of recognized analytical quality, with as low as possible Hg concentration. All reagents must be checked for Hg contamination by analysing blanks

Reagent blanks

At least 3 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as samples, except that no sample is added to the digestion tubes.

Reference materials

At least one certified reference material should be used and prepared in triplicate for each batch of analysis. These digestion are prepared in a similar manner as sample.

Certified reference material should be of similar composition and concentration of Hg as samples.

V.3.7 Digestion of Sediment for Determination of Methyl Mercury (MeHg)

See Annex VII.

V.3.8 Digestion of Biological Material for Determination of Trace Elements by Atomic Absorption Spectrophotometry (AAS)

Principle

Biological samples are treated with concentrated nitric acid in order to decompose the samples and solubilize all metals.

Procedure

1) Shake the vials containing the samples for about 2 min. for homogenization
2) Wait a few minutes before opening the vials
3) Weigh accurately from 0.1 to 0.3 of dry sample in Teflon bombs (a) or in polypropylene tubes (b)

4) Add 5 ml of concentrated nitric acid. Leave samples at room temperature for at least 1 hour

5a) Close the Teflon bombs tightly (with the aid of a torque wrench) and place the bombs in microwave digestion system and apply appropriate digestion routine. [See example routine, Table (V.3), below]

5b) Or close the polypropylene tubes and place them in an aluminum block kept at 90°C for 3 hours (or on a water bath)

6a) Allow the samples to cool to room temperature, before carefully releasing the pressure, then open the bombs

6b) Allow samples to cool to room temperature then open carefully the tubes

7) Transfer samples into 50 ml polypropylene graduated tubes. Rinse the bombs with Milli-Q water 3 times adding the wash to the tubes

8) Dilute to the mark (50 ml) with Milli-Q water and shake

9) Samples can be stored for several days before analysis, preferably in the cold (+4°C)

Reagent blanks

At least 3 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as samples, except that no sample is added to the digestion vials.

Table (V.3) : Example of biota digestion routine: MARSX digestion procedure for 'FISH2T'

Step	Power (W)	% Power	Ramp time (min)	PSI	°C	Hold Time (min)
1	1200	100	5	600	50	5
2	1200	100	5	600	100	5
3	1200	100	10	600	200	12

Reference materials

At least one certified reference material should be used and prepared in triplicate for each batch of analysis. These digestions are prepared in a similar manner as samples. Reference material should be of similar composition and concentration range as samples.

V.3.9 Digestion of Biological Material for Total Mercury Analysis by Cold Vapor AAS

Principle

This method is applicable to all biological samples with total mercury concentrations higher than about 0.05 µg/g. The strong acid digestion is meant to decompose the samples and oxidize and convert any organic forms of mercury into inorganic mercury.

Procedure

1) Shake the vials containing the samples for about 2 min. for homogenization
2) Wait a few minutes before opening the vials
3) Weigh accurately from 0.2 to 1g (depending on expected concentration) of dry sample into Teflon vials (60 ml)
4) Weigh 45 mg of V_2O_5 into these vials
5) Add 5 ml of concentrated HNO_3 or more if it is necessary (the mixture must be liquid)
6) Close the caps and leave the vials to stand for at least 1 hour at room temperature. If the reaction is very strong, it may be safer to leave samples at room temperature overnight before heating
7) Put the tubes into an aluminum block on a hot plate at 90°C and leave for 3 hours or water bath
8) Allow the samples to cool to room temperature before opening the tubes. Leave the tubes cooling down in a fume hood to avoid toxic acid fumes
9) Add about 20 ml of Milli-Q water

10a) Add 1 ml of $K_2Cr_2O_7$ solution (final concentration = 2% v/v)

10b) Or 0.5 ml of BrCl solution (final concentration = 1% v/v)

11) Dilute to the mark with Milli-Q water or transfer to volumetric flasks or to graduated tubes and dilute to 50 ml
12) Shake the vials and wait for sedimentation of material before analysis.

Note : 1. The samples can be kept for a few days before analysis if stored in the refrigerator (+4°C). The maximum storage time has to be determined by experience for each kind of samples
2. Reagents, including the distilled water, should be of recognized analytical quality, with as low as possible Hg concentration. All reagents must be checked for Hg contamination by analysing blanks

Reagent blanks

At least 3 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as samples, except that no sample is added to the digestion tubes.

Reference materials

At least one certified reference material should be used and prepared in triplicate for each batch of analysis. These digestions are prepared in a similar manner as sample.

Certified reference material should be of similar composition and concentration of Hg as samples.

V.3.10 Extraction of Methyl Mercury in Biota

Extraction and clean-up

To 0.5 to 2.0 g of thoroughly homogenized fresh sample (or equivalent weight of dry sample) add 2 ml of 2M H_2SO_4 saturated with $CuSO_4$, 2 ml of KBr solution and 3 ml of toluene. Shake for 10 minutes and centrifuge for 10 minutes at greater than 2000 rpm (or 5 minutes at 6000 rpm). Repeat the

extraction with 3 ml of toluene. Only clear toluene phases should be transferred quantitatively. Add cysteine paper to the isolated toluene phase and shake for 15 minutes. Decant the organic phase and wash the cysteine paper with three 5 ml portions of toluene and dry the paper in an acid free atmosphere. Add to the paper 0.2 ml of 6M HCl and 0.2 - 1.0 ml of toluene. Equilibrate for 5 minutes and centrifuge. Transfer the toluene phase to a small test tube.

Alternative clean-up procedures

The clean-up procedure described above is recommended for ease of application and efficiency. Certain other techniques have, however, been successfully applied and are therefore listed below as alternatives.

a) At the state of the extraction where cysteine paper is recommended for use, alternatively 1 ml cysteine solution can be added to the toluene phase. This mixture should be shaken for 5 minutes and then centrifuged for l0 minutes at 2000 rpm (or 5 minutes at 6000 rpm). Following transfer of the aqueous phase the toluene is re-extracted with cysteine solution. (Additional washings with cysteine solution are recommended if difficulties occur with phase separation). To the combined aqueous phase add 0.5 ml of 4M H_2SO_4, 0.5 ml of 4M KBr and 1.0 ml of toluene. Following agitation for 10 minutes, the mixture should be centrifuged (2000 rpm; 10 minutes) and the toluene phase transferred into a small test tube awaiting GC analysis. Keep extracts well closed to avoid evaporation.

b) In place of the cysteine solution, 2 ml of sodium thiosulphate clean-up solution can be substituted. The protocol apart from this single modification is identical.

Note : The extraction and clean-up should be carried our in one run in order to minimize loss of MeHg. If the procedure has to be interrupted briefly then it should be discontinued only after the first centrifugation, i.e. when the MeHg is bound to cysteine. Longer interruption (i.e. overnight) should only occur when the sample is in toluene solution. Usually the final toluene extract (i.e. the test solution) is stable and can be stored for several weeks if closed weld but an extract kept longer may lose MeHg

Blanks

Follow procedure replacing the tissue sample with 2 ml of distilled water. If the blank gives a peak at the retention time of MeHg check all reagents and solutions for impurities. Blanks should be processed in duplicate in each run.

Recovery of standard compound

The technique should be calibrated to allow for any incomplete recovery of the extraction and clean-up technique. This parameter can be tested by spiking aqueous MeHg at varying concentrations either into distilled water for treatment as described in extraction and clean-up or into biological material according to the normal protocol (the latter is termed “standard addition” and partly allows for the influence of biological tissue). Methyl mercury recovered is then calculated by comparison with the response of the chromatograph to methylmercury directly injected. Recovery should typically exceed 75% (±10%).

Standard addition analyses should be performed for each different sample matrix (e.g., fish, mussels, oysters, etc.), with “spike” levels selected to approximate multiples of the anticipated environmental concentrations. The results are processed by plotting ng Hg.g dry wt^{-1} actually measured using the protocol, against ng Hg added/g dry wt. A straight line with a slope of 1 should be obtained to indicate negligible matrix interference. The intercept on the axis indicates the amount of methylmercury actually present in the sample.

Reproducibility

To enhance reliability of results, replicate (at least duplicate and preferably triplicate) analyses should be processed simultaneously for each sample.

V.3.11 Determination of Triorganotin Concentrations in Shellfish Tissue

Principle

Tissues are dissolved in tetramethylammonium hydroxide in a heated ultrasonic bath. The samples are simultaneously derivatized and extracted using sodium tetraethylborate ($NaBEt_4$) and *n*-hexane, and the organic phase is recovered. The extract is dried and concentrated to ~0.5 ml. The samples are the cleaned up using SPE (Solid Phase Extraction) Florisil cartridges and eluted with *n*-hexane. The purified samples are then again concentrated to about 0.5 ml prior to cGC-FPD injection.

Method

The procedure is based on a paper published by Cassi *et. al* (2002). Biota samples (0.5 g freeze-dried) are dissolved in 10 ml of tetramethylammonium hydroxide. To optimize the dissolution, samples are stirred in an ultrasonic bath for 1.5 hr at 50 °C. After complete dissolution of the tissue, buffer and acetic acid are added to stabilize the pH between 4 and 5. The samples are simultaneously derivatized and extracted using 1 ml of 1% sodium tetraethylborate ($NaBEt_4$) in 2% KOH and 5 ml of *n*-hexane. The samples are mechanically shaken for 20 min., then centrifuged at 5000 rpm for 15 min. at a temperature of 0 °C. The organic phase is recovered. A second extraction with 5 ml of n-hexane is performed, followed by a second centrifugation. The organic phase is recovered and combined with the first one. The combined extract is then dried with activated sodium sulfate and concentrated to ~0.5 ml by evaporation under a gentle stream of pure nitrogen. The samples are then cleaned up using SPE (Solid Phase Extraction) Florisil cartridges and eluted with 10 ml of *n*-hexane. The purified samples are then again concentrated to about 0.5 ml under a gentle stream of pure nitrogen prior to cGC-FPD injection. Duplicate procedural blanks and a quality control tissue are analysed with each batch of samples.

The sample extracts are reasonably stable, but if not analysed immediately after preparation should be stored in the dark and cool (4°C) to prevent photolytic decay and evaporative loss, and analysed within 24 hours.

Summary of method

Triorganotins in Shellfish

0.5 g sample/AQC material/blank/spiked sample

↓

dissolve in 10 ml of tetramethylammonium hydroxide in heated ultrasonic bath

↓

add buffer and acetic acid to stabilize the pH between 4 and 5

↓

add 1 ml of 1% sodium tetraethylborate ($NaBEt_4$) in 2% KOH and 5 ml of *n*-hexane

↓

shake 20 min, then centrifuge

↓

decant organic phase and add 5 ml of *n*-hexane

↓

shake 10 min

↓

centrifuge

↓

dry with activated sodium sulfate

↓

evaporate to ~0.5 ml with pure nitrogen

↓

elute with 10 ml of *n*-hexane

↓

evaporate to ~0.5 ml with pure nitrogen

↓

analyze by cGC-FPD

Calculation of results

The concentration of organotin (as TBT equivalents) (C) in the sample is given by:

$$C = \frac{T \times v}{W \times Rf} \times 2.5\ \mu g/g\ TBT$$

where:

T	=	concentration of tin measured in the toluene extract in µg Sn ml^{-1}
v	=	volume of extract in ml
W	=	weight of sample in g
Rf	=	recovery factor for the determination of a known amount of stannous chloride spiked into a tin free sample

Internal standardization

A quality control system based on three internal standards is used. Each critical step of the procedure is assessed by the recovery of the corresponding internal standard. Firstly, tripropyltin is used to indicate the derivatization reaction efficiency. All the chromatographic peak areas are normalized to that of tripropyltin. Low recoveries for tripropyltin usually indicates that the sodium tetraethylborate solution has not been correctly prepared or that it had been kept too long. Secondly, tetraoctyltin is used to check the overall solvent extraction efficiency. Low tetraoctyltin recoveries are usually associated with incomplete sample dissolution manifested as opaque solutions. Thirdly, tetrabuthyltin is used as a GC-internal standard to quantify the recoveries of both internal standards.

Precision and accuracy

Certified Reference Materials were used to assess the accuracy and the precision of the method described. NIES-11 (fish tissue) and BCR-477 (mussel tidssue) were analyzed several times and the results obtained for organotin compounds were compared with the certified values. Butyltin values obtained for both BCR-477 and NIES-11 were consistently in excellent agreement with the certified values. The available value for triphenyltin in NIES-11 is not certified but it is given as a reference value and the results obtained seemed to be slightly underestimated. However, since triphenyltin degrades rapidly and considering the age of the material, the low levels were possibly due to degradation of the material rather than to an underestimation.

V.3.12 Digestion of Sediment and Biological Tissue for Determination of TBT by GC

Method

a) **Sediment** preparation: Sub samples of 4 g of sediment are oven dried to constant weight at 105°C to determine moisture content. Extraction of alkyltin is then carried out on up to 4.0 g of wet sediment sample, ensuring that there is at least 2 ml of water (either natural or added) in the sample.

b) **Animal tissues** (shellfish and fish) are homogenised and 4.0 g of the homogenate is used for each analysis.

An appropriate amount of internal standard (tripropyltin chloride) is added to the sediment or tissue in a centrifuge tube; e.g. 50 µl for samples containing 0.01 to 1 µg/g TBT. Double distilled water is added to the sample if necessary (see above). Sufficient 0.1% sodium hydroxide in methanol is then added to the sample to obtain a 4:1 MeOH:water (v/v) ratio. The minimum volume of methanol reagent is therefore 8 ml and the maximum, below 16 ml for sediment samples. For shellfish, 10 ml of sodium hydroxide/methanol reagent is added for wet samples. The tube is capped and shaken for 45 min. then 2 ml of hexane and approximately 100 mg of sodium borohydride are added. The sample is then shaken for a further 15 min. After centrifugation (4000 rpm for 20 min.), the hexane phase is analysed by cGC/FPD.

The method does not employ a “clean up” procedure, and is therefore rapid, extremely robust and suitable for a high throughput of monitoring samples. Residues of extracted organic material do

however tend to accumulate at the injection end of the analytical column/retention gap and degrade the performance of even the highest quality capillary columns. The retention gap should be frequently changed and 15 cm of fused silica periodically removed from the injection end of the analytical column.

Stored extracts will revert to alkyl tin chlorides within days of preparation. Extracts may be stored at –20°C for several weeks. The hydrides may be reformed by shaking extracts with 300 µl of 5% aqueous sodium borohydride.

Summary of method

Alkyl tin hydrides in tissues and sediments :

4g tissue/sediment/AQC material/blank/spiked sample
↓
add internal standard
↓
adjust water content if necessary
↓
add 4 volumes of sodium hydroxide/methanol reagent
↓
shake 45 min.
↓
add 2 ml hexane and 100 mg sodium borohydride
↓
shake 15 min.
↓
centrifuge
↓
analyse by cGC/FPD

V.4 INSTRUMENTATION

V.4.1 Preparations of Standards Solutions

V.4.1.1 Preparations of Standards for Trace Metals

See section V.3.3

V.4.1.2 Calibration Standards

Prepare dilutions of the stock solutions to be used as calibration standards at the time of analysis.

The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

V.4.2 Determination of Trace Metals in Sediments and Biota

Analyses of sediments

Precise and accurate atomic absorption analyses for the determination of metals involves the use of flame (F-AAS) and graphite furnace atomic absorption spectrophotometry (GF-AAS).

F-AAS is the method of choice for most metal determinations when the metals are present in sufficient concentrations to allow precise and accurate determinations.

GF-AAS is generally used for the determinations beyond the sensitivity of F-AAS. Due to improved accuracy and precisions. GF-AAS should be used for all Cd, Cr and Ni determinations. Pb should be determined by F-AAS when concentrations are high enough otherwise GF-AAS should be used.

V.4.2.1 Determination of Trace Metals in Sediment and Biological Materials by Flame AAS

Principles

Samples are digested with strong acids (see procedure V.3.4 or V.3.8). Atomic absorption spectrometry resembles emission flame photometry in that the sample solution is aspirated into a flame and atomized. In case of flame AAS, a light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the element in the flame. Each metal has its own characteristic wavelength so a source hallow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

In case of flame emission, the amount of light emitted at the characteristic wavelength for the element analyzed is measured.

Before analysis

Analytical conditions may change for each element, so it is necessary to first carefully read the analytical methods book of the AAS before starting analyses.

Determine the calibration curve according to the expected concentrations of the samples and the linearity of the AAS response for the element considered (absorbance versus concentration curve given in the analytical methods book).

If interferences or ionization are reported choose, the right option according to the analytical method book e.g. use of correction for non atomic absorption by using deuterium lamp background corrector, use of oxidizing air-acetylene flame; use of nitrous oxide-acetylene flame; addition of a releasing agent or ionization suppressant.

A) Analysis by flame-AAS with normal calibration curve

Calibration curve

Prepare standards with at least three concentrations plus zero. The zero calibration solution is prepared as other standard solutions without adding analyte.

If samples are not within the calibration range, dilute them in the same matrix, or prepare a new calibration curve.

Sample measurements

Measure a blank and reslope every 4 or 5 samples depending on the stability of the instrument.

Measure one reference material at regular intervals during analysis

B) Analysis by flame-AAS using standard additions

If matrix effects are suspected and cannot be removed by other means (e.g. background correction; addition of releasing agent; nitrous oxide flame), it may be necessary to use standard additions calibration.

Calibration curve

The calibration curve should have 3 points (2 additions plus the zero addition) and one blank. The first addition must be half of the expected concentration of the sample solution. If possible this addition is done without dilution of sample (i.e. less than 250 µl of standard solution for 50 ml of sample). If a larger amount of standard solution is needed the sample has to be diluted (i.e. put 45 ml of sample, 2 ml of standard solution and dilute to 50 ml).

The second addition should be the double of the first. In case of dilution, the same volume of sample is used (i.e. put 45 ml of sample, 4 ml of standard and complete to 50 ml).

In case of dilution of the sample solution, this dilution factor should be applied to the calculate result.

> **Note :** The calibration curve obtained should be used for samples of the same matrix. If there are different matrices in the batch of analysis, several standard addition calibration curves should be prepared

Calculation

$$C\ (\mu g/g) = \frac{(Cd - Cb) \times V \times F}{W}$$

The units of concentrations are the same as for she standards entered in the AAS program.

C	=	Concentration of element in original sample (µg/g or dry weight)
Cd	=	Concentration of element in sample solution (µg/ml)
Cb	=	Mean concentration of element in reagent blanks (µg/ml)
V	=	Volume of dilution of digested solution (ml)
W	=	Dry weight of sample (g)
F	=	Dilution factor if needed (=1 in case of no further dilution than initial dilution during digestion procedure)

V.4.2.2 Determination of Trace Metals in Sediment and Biological Materials by Graphite Furnace or Electro-thermal AAS

Principle

In case of graphite furnace (GF) AAS an aliquot of sample digested solution (10/50 µl) is introduced into a graphite tube of the GF-AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength so a source hallow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

Before analysis

Analytical conditions may change for each element, so it is necessary to first carefully read the analytical method for GF book before to starting.

Determine the calibration curve according to the expected concentrations of the samples and the linearity of the AAS response for the element considered (absorbance versus concentration curve given in the analytical method book).

The AAS book also gives typical electrothermal programs for each element generally for 10 µl of sample in diluted HNO_3 (0. 1%) and indications concerning maximum ashing, and atomization temperatures. More specific informations may also be found in the literature, such as recommendations concerning the need of matrix modifier and the use of partition tube or tube with platform.

Calibration curve for GF-AAS

The automatic sampler is doing the calibration curve by mixing appropriate volume of standard and zero calibration solutions; so only one standard solution have to be prepared. It can be the highest standard solution of the calibration curve or a solution more concentrated in case of standard additions. The solution must be chosen so that the volumes pipetted by the automatic sampler to make the standards are not lower than 2 µl.

Optimization of electrothermal programmed

Temperature and time of drying, ashing and atomizing steps have to be optimized in a systematic manner. The absence of interferences has to be verified by the use of a reference material of similar

matrix as samples or by standard addition method if reference material of appropriate matrix are not available. If matrix effect are observed and can not be eliminated by optimization of electrothermal program, it may be necessary to use standard addition calibration.

When a program is optimized for the determination of an element in a specific matrix all informations are reported in the log book of methods of the laboratory such as quality control charts.

Calculation

If the same volume is always injected.

$$C\ (\mu g/g) = \frac{(Cd - Cb) \times V \times F}{W}$$

The units of concentrations are the same as for the standards entered in the AAS program.

C	=	Concentration of element in original sample (µg/g dry weight)
Cd	=	Concentration of element in sample solution (µg/ml)
Cb	=	Mean concentration of element in reagent blanks (µg/ml)
V	=	Volume of dilution of divested solution (ml)
W	=	Dry weight of sample (g)
F	=	Dilution factor if needed (=1 in case of no farther dilution than initial dilution during digestion procedure)

V.4.2.3 Determination of Total Mercury in Sediment and Biological Materials by Cold Vapour AAS

Principle and application

Sediment or biological samples are mineralized with strong acids. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapor is then passed through the quartz absorption cell of an AAS where its concentration is measured. The light beam of Hg hollow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapor in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

Sample digestion procedure

- Sediment: see procedure in Section V.3.6
- Biological samples: procedure in Section V.3.9

Analysis by CV-AAS

Calibration curve

Prepare standard solutions with at least three standard concentrations plus one zero. The zero calibration is prepared as standard solutions without adding the mercury standard.

If samples are not within the calibration curve, dilute them in the same matrix, or prepare a new calibration curve.

V.4.2.4 Measurement of Methyl Mercury in Biological Materials by GC/ECD

Chromatographic system

- Gas Chromatograph (GC). Equipped for a glass/packed column, fitted with an electron capture detector and with a suitable recorder/integrator.
- GC Column. Glass column 1.6 m length x 2 m mid-packed with 5% DEGS-PS on 100-120 mesh (or equivalent). Columns should be purchased either prepared or packed according to manufacturers instructions. On installation of the new column into the GC, the column should be conditioned by heating at 200°C; and a flow rate of carrier gas of 50 ml. min-1, for 12 hours WITHOUT attaching the end of the column to the detector.

Note : Whilst it is appreciated that capillary column GC should theoretically provide better resolution and sensitivity than packed column GC, to date no adequately robust and proven capillary column analysis is available for routine use. For this reason the present manual has retained packed column GC methodology

- GC Conditions. Carrier gas: nitrogen; flow rate 60 ml/min. Injector temperature: 190°C. Electron Capture Detector temperature: 280°C. Column temperature: 160°C isothermal (see note below).

Note : Analyses are performed isothermally generally between 145°C and 170°C. Column, injector and detector temperatures should be selected according to the packing used and should be adjusted to obtain a satisfactorily sensitive and stable response (for 50 pg methylmercury injected reproducibility for replicate injections should not exceed + 3%) and an appropriate peak shape with minimum tailing

Calculation of results

The concentration of MeHg in the sample is obtained from sequential chromatograms of the authentic standard compound followed by those of the sample extract as follows:

$$\text{Concentration of MeHg (ng . g}^{-1}\text{ as Hg)} = \frac{h_x \bullet a_s \bullet V \bullet 100}{h_s \bullet m_x \bullet V_x \bullet R}$$

where :

a_s	=	the number of ng of standard MeHg injected into the gas chromatograph (ng)
h_s	=	the height of the MeHg peak resulting from “as” ng of standard (mm)
m_x	=	the weight of sample processed (g)
V	=	the volume of the final sample extract (µl)
V_x	=	the volume of final sample extract injected into the gas chromatograph (µl)
h_x	=	the height of the peak at the retention time corresponding to MeHg resulting from injection of V_x µ1 of sample extract (mm)
f	=	a dilution factor in case that not all of the sample (m_x) is processed through the entire protocol; the final extract represents only a proportion of the original sample weight. As an example, if only half of the tissue extract is located using cysteine paper and then processed through to `GC analyses, f = 2. If, however, complete transfers at each stage are performed, f = 1
R	=	recovery of extraction and clean-up procedure

V.4.2.5 Speciation of Alkyl Tin Hydrides in Sediments and Animal Tissues by Gas Chromatography

Principle

Alkyltin compounds are extracted from animal tissues or sediments by sodium hydroxide and methanol, converted to hydrides and partitioned into hexane. The derivatives are then analysed by gas chromatography with flame photometric detection. The method was developed by Waldock *et.al*. (1989).

An appropriate amount of internal standard (tripropyltin chloride) is added to the sediment or tissue in a centrifuge tube; e.g. 50 µ1 for samples containing 0.01 to 1µg/g TBT. Double distilled water is added to the sample if necessary (see above). Sufficient 0.1% sodium hydroxide in methanol is then added to the sample to obtain a 4:1 MeOH:water (v/v) ratio. The minimum volume of methanol reagent is therefore 8 ml and the maximum, below 16 ml for sediment samples. For shellfish, 10 ml of sodium hydroxide/methanol reagent is added for wet samples.

The tube is capped and shaken for 45 min. then 2 ml of hexane and approximately 100 mg of sodium borohydride are added. The sample is then shaken for a further 15 min. After centrifugation (4000 rpm for 20 min.), the hexane phase is analysed by cGC/FPD.

The method does not employ a "clean up" procedure, and is therefore rapid, extremely robust and suitable for a high throughput of monitoring samples. Residues of extracted organic material do however tend to accumulate at the injection end of the analytical column/retention gap and degrade the performance of even the highest quality capillary columns. The retention gap should be frequently changed and 15 cm of fused silica periodically removed from the injection end of the analytical column.

Stored extracts will revert to alkyl tin chlorides within days of preparation. Extracts may be stored at -20°C for several weeks. The hydrides may be reformed by shaking extracts with 300 µ1 of 5% aqueous sodium borohydride.

Summary of method

Alkyl tin hydrides in tissues and sediments :

4.0 g tissue/sediment/AQC material/blank/spiked sample
↓
add internal standard
↓
adjust water content if necessary
↓
add 4 volumes of sodium hydroxide/methanol reagent
↓
shake 45 min.
↓
add 2 ml hexane and 100 mg sodium borohydride
↓
shake 15 min.
↓
centrifuge
↓
analyse by cGC/FPD

Calculation of results

The retention times of standards are used for identification purposes. The peak heights or areas of known quantities of standards are then used to calculate a response factor for each compound to the tripropyltin internal standard. The concentration of each alkyltin compound in the sample (Cy) is then given by :

$$Cy = \frac{Hy \times Ci \times R}{Hi \times W} \ \mu g/g \text{ (dry wt. sediment, wet wt. tissue)}$$

where:

Hy = the peak height or area given by the alkyltin compound
Ci = concentration of the internal standard (as the cation) in the tissue or sediment sample in µg/g
R = the relative response of the internal standard (Ri) to that of the alkyltin compound Rx

$$R = \frac{Ri}{Rx}$$

Hi = peak height or area given by the internal standard
W = weight of sample in grams (dry weight for sediments, wet weight for tissues)

Precision and accuracy

The recovery of tributyltin was determined by standard additions of tributyltin hydride to samples previously spiked with TBTO, and comparing the resultant GC peak areas over a range of spiked concentrations. The calculated recovery ranged from 90-97% (mean 93%) for sediment and 95-104% (mean 98%) for shellfish tissue. However the use of an internal standard (tripropyltin chloride) which behaves in a similar way to TBT allows for loss of sample without compromising the precision and accuracy of the determination. The relative recovery for TBT, DBT and MBT to the internal standard is close to 100%. The detection limit for the method is 0.005 µg/g for TBT, 0.010 µg/g for DBT and 0.02 µg/g for MBT. These limits may be reduced by concentration of the hexane extracts before GC analysis, however since a "clean up" procedure is not employed, concentrated samples more rapidly degrade the performance of the capillary column.

V.4.2.6 TBT Speciation of Organotin Hydrides by GC/MS

GC/MS forms another suitable detection system for alkyltin hydrides extracted by the method described above. The flame photometric detector is replaced with a mass spectrometer set up in the electron impact mode with unit resolution.

Figure (V.4a - V.4e) show a GC/MS separation and the mass spectra of di and triorganotin compounds. Characteristic tin isotopic clusters give readily identifiable fragmentation patterns. The prominent peaks around masses 119 and 177 provide a useful confirmatory technique in identification of the presence of tributyltin at single nanograms per litre in water samples.

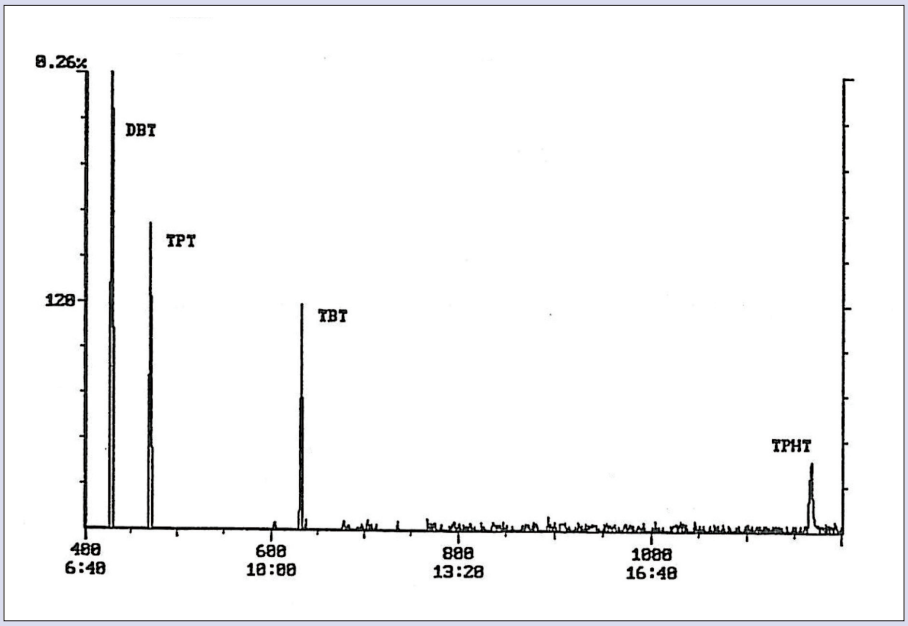


Figure (V.4a): Scanning GC/MS of di and triorganotin hydrides. Total ion current

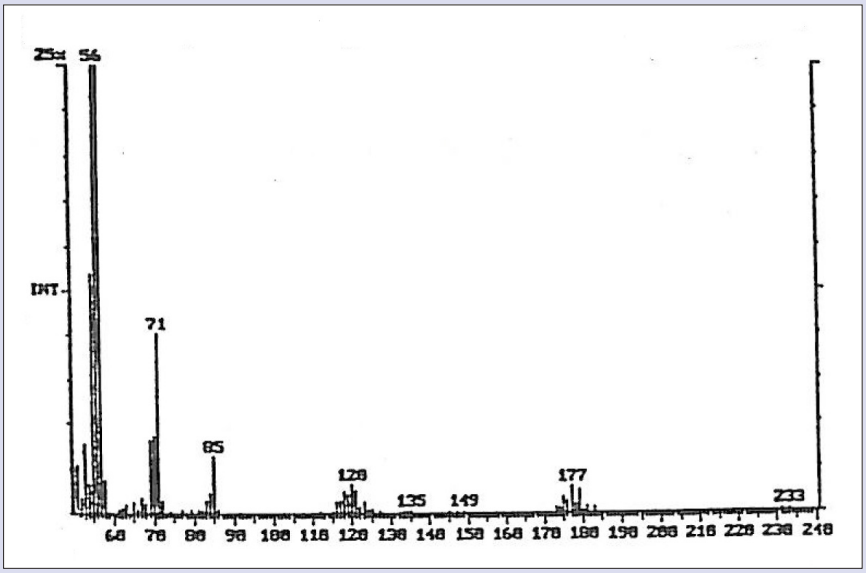


Figure (V.4b) : Scanning GC/MS of di and triorganotin hydrides. Mass spectrum for DBT

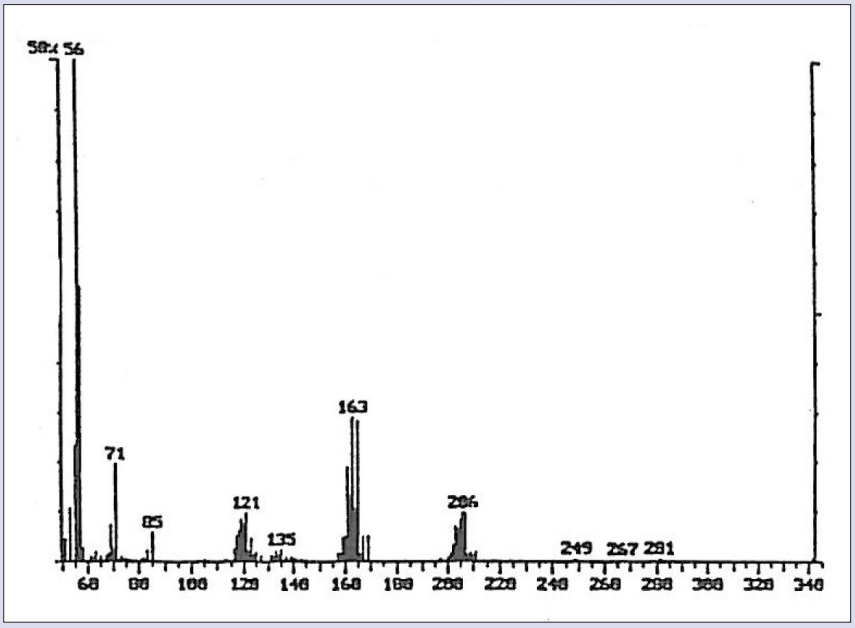


Figure (V.4c) : Scanning GC/MS of di and triorganotin hydrides. Mass spectrum for TPT

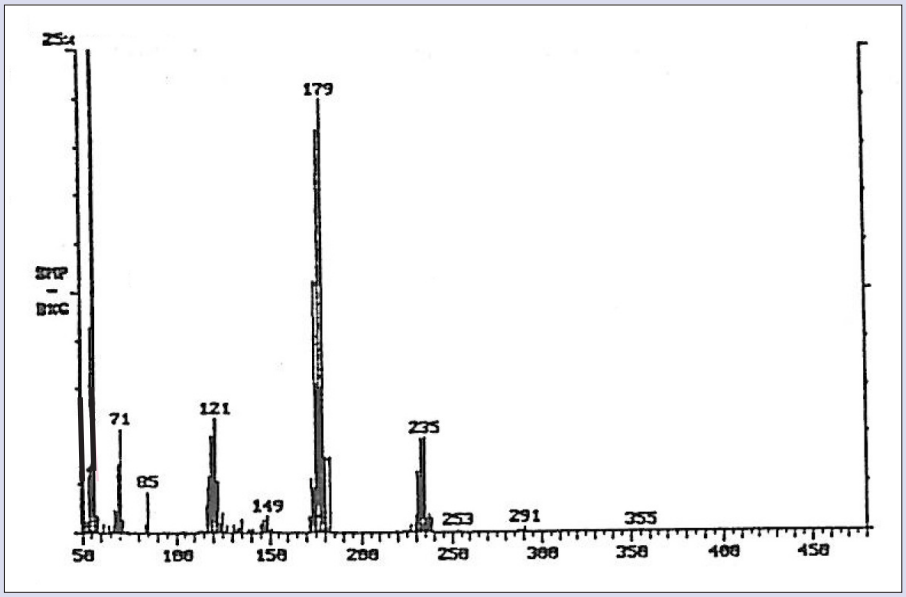


Figure (V.4d) : Scanning GC/MS of di and triorganotin hydrides. Mass spectrum for TBT

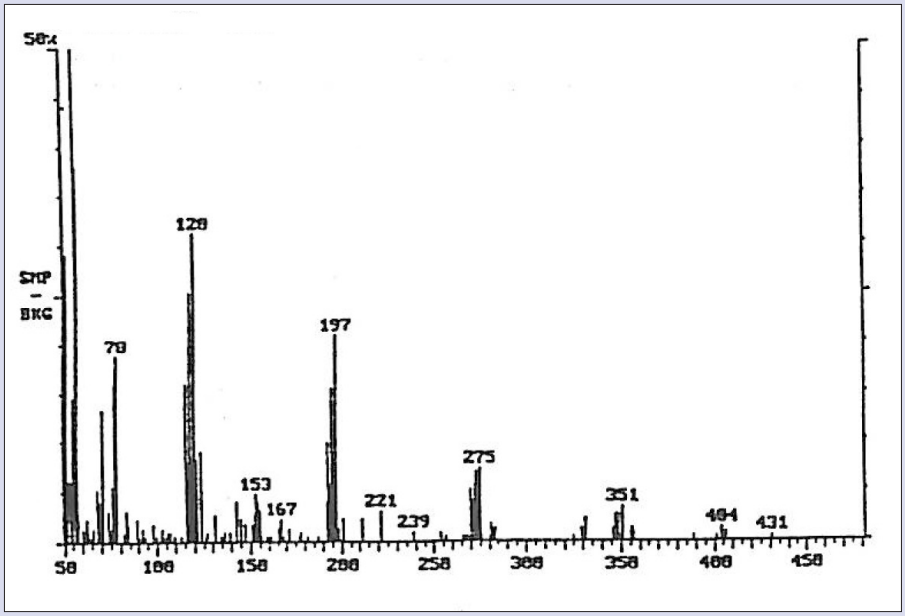
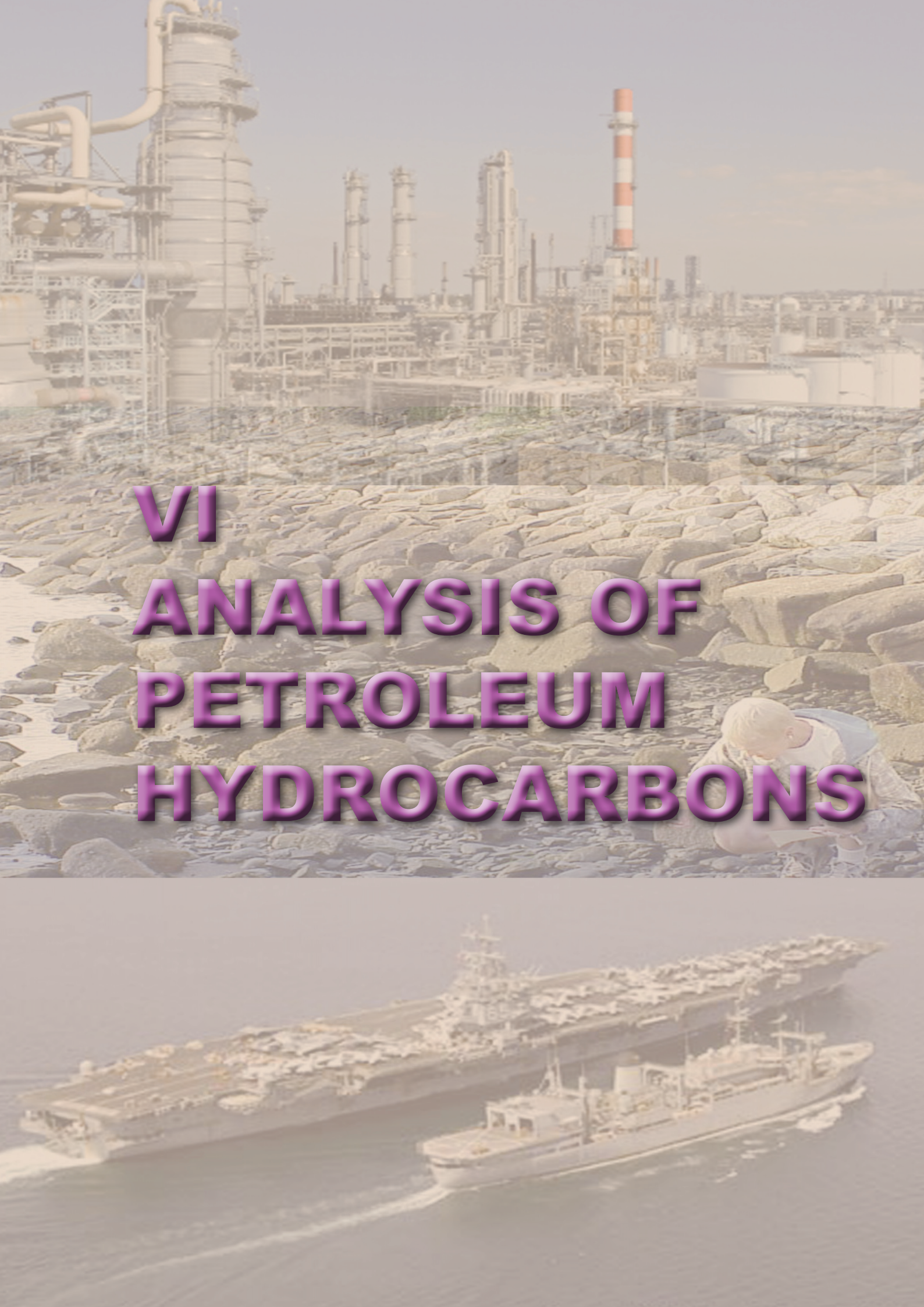


Figure (V.4e) : Scanning GC/MS of di and triorganotin hydrides. Mass spectrum for TPhT



VI ANALYSIS OF PETROLEUM HYDROCARBONS

VI ANALYSIS OF PETROLEUM HYDROCARBONS

VI.1 QUALITY ASSURANCE BASIS FOR OBTAINING QUALITY DATA

See Section V.1

VI.2 CLEANING OF LABORATORY GLASSWARE AND MATERIALS

VI.2.1 Cleaning of Laboratory Glassware

The material of choice for analytical equipment used in trace organic analyses is borosilicate glass (Pyrex, Duran, etc.). Soft glass (also called soda-lime glass) is suitable as well, but it breaks more easily under mechanical or heat stress. For special purposes, mainly photochemical investigations, flasks, etc. are used made of vitreous silica. It is cleaned using the same procedure as described for “glassware”.

Glassware, if visibly dirty, is first cleaned with a household cleaning powder such as Ajax® or similar with tap water and a bottle brush followed by copious rinsing with hot tap water. The glassware is then immersed for several hours (overnight) in a dilute aqueous laboratory detergent solution such as Alconox® (available through Aldrich Chemical Company or Cole Palmer Co), Extran MA® (Merck), etc. It should be noted that if organophosphorus compounds are to be analyzed, phosphate free detergents (such as Micro® which is available through Bioblock Scientific or Cole Palmer Co) should be used. Small pieces of glassware may be cleaned effectively by ultra-sonication for 10 minutes in detergent solution. Following detergent treatment, the glassware is rinsed with abundant hot tap water, organic free ion exchange water, and is rinsed with methanol and then with hexane. The glassware in then heated at 200°C in a drying oven overnight. When cold, all openings are closed with pre-cleaned aluminum foil in order to prevent the dust to enter the glassware.

Aluminum foil used for protection from dust and vapors can be a source of contamination because lubricants used in manufacture may adhere to its surface. Rinsing with solvents such as acetone and hexane and drying in the oven at 200°C usually removes extraneous material.

An oven for drying cleaned glassware must not be used for any other purposes. Otherwise it may too easily itself become a source of contamination.

Checks must be run at regular intervals to ensure that the chosen cleaning procedure is adequate for a given purpose. This is done by rinsing the cleaned glassware with the amount of solvent (or the mixture of solvents) used for a given analytical procedure followed by the same work-up and analysis as for the analyte.

VI.2.2 Cleaning of Reagents

Powdered or crystalline reagents, such as anhydrous sodium sulphate (Na_2SO_4)*, potassium hydroxide (KOH), glass wool* and carborundum boiling chips*, are thoroughly cleaned before use. They are extracted with hexane in a Soxhlet apparatus for 8 hours and then with methanol or dichloromethane for another 8 hours. For those indicated by an *, this will require pre-combustion in a muffle furnace at approximately 400°C.

VI.2.3 Cleaning of Adsorbents

Silica gel, Alumina and Florisil are treated chemically and are not clean when received from the supplier. Reagents are first refluxed with methanol or dichloromethane in a Soxhlet apparatus for 8 hours, then with n-hexane for the same period. The solvent is removed in a rotary evaporator at a low speed, until the sorbent starts falling down as fine particles. Reagents are then dried in a drying oven at 120°C for 4 hours. When dried, they are stored in amber glass bottle in a laboratory cupboard.

When needed, silica and alumina are activated at 200°C for 4 hours. They are allowed to cool in the oven. As active sorbents attract water and contaminants from the atmosphere, controlled deactivation is carried out by adding water to the fully active sorbent (5% by weight) and mixing by gentle shaking for a few minutes, the equilibration takes one day. They should be used just after equilibration (because of the humidity of the atmosphere they will be fully deactivated very quickly and could not be used at such).

The same procedure is applied for Florisil: when needed, Florisil is activated at 130°C for 12 hours, partially deactivated with 0.5% water, the water is mixed by gentle shaking for a few minutes and the Florisil is kept in the laboratory for the water to equilibrate for 24 hours. If not used the following day, the activation procedure has to be done again.

VI.3 SAMPLING

VI.3.1 Water

A) Dissolved and dispersed petroleum hydrocarbons

See Section III.1.1.6.7

B) Surface micro-layer

There are several methods available for sampling the sea-surface micro-layer. The importance of this interface layer and the methods used to sample it, have been reviewed by GESAMP (1995). One method (IOC Manual and Guides No 15) which has been widely used in oceanographic studies, is presented in Annex VIII.

C) Beach tar surveys

Beach tar surveys are recognized as an important aspect of coastal oil pollution monitoring programmes for assessing the level of oil pollution in offshore waters and the relative threat posed to coastal resources. They also provide information in support of coastal oil pollution regulatory measures and provide some indication as to where oil would accumulate in the event of an accidental spill in offshore waters.

In addition, beach tar surveys are relatively simple to conduct and allow those areas with only basic facilities to participate in regional monitoring programmes. The method described in this manual is based on the procedures recommended by UNESCO, (1977) with slight modifications.

Tar balls are formed when crude oil and other heavy petroleum hydrocarbon products introduced into coastal waters, whether deliberately or accidentally, eventually conglomerate into viscous masses or lumps after several stages of weathering. These pelagic or floating tar balls may be transported by wind and currents to adjacent shores where they accumulate. Periodic quantification of this accumulated tar at selected coastal locations may provide valuable information on offshore oil pollution. The method for conducting beach tar measurements requires periodic monitoring of at least one representative site in order to determine both seasonal variation and accumulation trends.

Sampling strategy

Selection of sampling sites should be carefully considered. All sites should be on sandy beaches with :

- a uniform shoreline (no breakwaters or cuts)
- a gentle slope, however, avoid beaches where the highest tides wash over the back beach into lagoons or low areas
- a minimum of human activity, such as recreational beaches or areas where vehicles cross the beaches, etc.
- no local land-based sources of petroleum pollution, such as adjacent to refinery effluents, wastewater drains, etc

If more than one sampling site is selected, sites should be spaced at regular intervals along relatively uniform coastlines with similar orientation such as on north or south facing beaches. At each station, at least three randomly selected 1 meter wide strips are marked running from the water's edge, at time of collection, to the highest tide line (identified by observing the most landward accumulation of detritus, shells and rubbish).

Visual observation of the selected beach should indicate whether three transects would provide representative samples. If the tar balls are more abundant only in one area of the beach, either another site should be selected or the number of transects increased to include several sections of the same beach. Each section should then be treated separately (i.e. three or more transects per section).

Sampling method

a) After randomly selecting the positions of the transect lines, use any convenient means of marking a 1 meter wide strip up the beach face from the edge of the water.

Note : Avoid walking within this strip while marking the transect to avoid pushing any tar balls below the sand surface

b) After marking the transect, proceed up the beach collecting all solid or semi-solid tar balls on the sand surface within the transect which can be picked up between thumb and forefinger and placed in a clean plastic bag or other suitable container.

Try to remove as much sand and shell fragments as possible from the tar. Avoid including extra weight.

Note : On most sandy beaches, tar will accumulate along tide wash lines, so, concentrate on these areas more than on the relatively clean areas between tide marks. On heavily polluted beaches, where collecting tar balls by hand is either impractical or where the tar is too fresh to pick up, other methods may have to be used. If the tar is solid, it may be brushed into pills with a long handled floor brush. These pills should then be sieved through a 2 mm mesh screen to remove sand and other debris. If the tar is very fresh and wet, use a wide beaded putty knife to slide the tar into the collecting bags. Remember, the object of the survey is to obtain a semi-quantitative estimate of the accumulated tar on a representative beach. Therefore. Use the best method, which fits the local conditions

c) Keep the tar collected within a given transect in a separate container for transport to the laboratory. Individual bags from each transect at a given station may be placed in a single, larger bag for convenience of transport. Each bag be carefully labelled as follows :

i) Station location:…….
ii) Date of collection:……
iii) Prevailing wind direction:……..
iv) Length of transect:……...

VI.3.2 Sediments

See Section III.1.2

VI.3.3 Biota

See Section III.1.3

VI.4 SAMPLE PREPARATION

VI.4.1 Laboratory Apparatus and Equipment

- A coring device with liners and plunger or a grab sampler. Thoroughly cleaned with detergents and solvents before use
- Glass jars and aluminium foil, stainless steel knives, scoops, forceps, labels, marking pens, log-book
- Insulated plastic boxes for transporting samples. Ice or dry ice
- Deep freezer (-18 to -20°C) for sample preservation (frost free type freezers heat to above zero during frost removal cycles and they cannot be used for long term storage)
- Rotary evaporator
- Soxhlet extraction apparatus and heaters or microwave oven designed for solvent extractions
- Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer flasks, separatory funnels, centrifuge tubes, weighing bottles, pipettes, tissue grinders

- Drying oven (temperature range up to at least 300°C) for determining sample dry weights, baking of contaminant residues from glassware and reagents
- Centrifuge and tubes
- Freeze-dryer and porcelain mortar and pestle
- Analytical balance with a precision of 0.1 mg and an electro-balance with a precision of at least 1 µg
- Stainless steel tweezers and spatulas
- Dessicator - completely cleaned and with no grease applied to sealing edges
- Supply of clean dry nitrogen
- Columns for the silica/alumina chromatography
- Mechanical blender (food mixer)
- Vacuum pump (water-jet air pump)
- Ultrasonic bath
- Gas Chromatograph equipped with suitable detector

VI.4.2 Chemicals and Reagents

- Distilled water produced by distillation in a quartz distillation system. The water should, then be extracted with hexane (1 litre of water extracted twice with 80 ml of hexane). Milli-Q type water should be avoided, as it will contain traces of plasticizers such as phthalates
- Detergent
- Sulfochromic cleaning solution made from concentrated sulfuric acid and potassium dichromate
- HCl, 30%
- Hexane, “distilled in glass” quality
- Dichloromethane, “distilled in glass” quality
- Methanol, “distilled in glass” quality
- Acetone, “distilled in glass” quality
- Tetrahydrofuran (THF)
- Hexamethyldisilazane (HMDS)
- Bio-Beads SX-3 (200-400)
- Sephadex LX-20
- Anhydrous sodium sulfate
- Carborundum
- Copper powder (Aldrich, 200 mesh, 99% purity)
- Glass wool
- Silica gel Merck Kieselgel 60 (0.04-0.063 mm, 230-400 mesh)
- Aluminium oxide neutral Merck 90 Aktiv (0.063-0.200 mm, 80-200 mesh) KOH
- Hg
- Florisil PR (60-100)
- n-C_{19} d_{40}, n-C_{32} d_{66}
- Hexamethylbenzene, Cadalene : 1,6-dimethyl-4-(1-methylethyl) naphthalene, Naphthalene d_8
- Standard solutions of aliphatic and aromatic hydrocarbons
- PCB congener 29 and 198
- ε -HCH
- Endosulfan I d_4

- DDT reference solutions - Stock solution of the DDT series (pp' DDT, op DDT, pp' DDD, op DDD, pp' DDE, op DDE) are prepared by dissolving 50 mg of each compound in 100 ml of hexane. Stock solutions are stored in sealed glass ampoules

Other reference solutions - are prepared the same way, if other residues are to be quantified in these procedures.

Note : Working solutions from the stock reference solutions are prepared on a regular basis and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that standards have not changed their concentrations through solvent evaporation. In order to achieve a good accuracy of the standard solutions, at least 50 mg of pure individual compound should be weighted and dissolved into 100 ml of hexane. This will give stock solutions of 500 ng/µl

VI.5 DETERMINATION OF DRY WEIGHT

See Section V.3.4.1

VI.6 ANALYSIS OF WATER

VI.6.1 Dissolved and Dispersed Petroleum Hydrocarbons and Micro-surface Layer

Sample extraction should be performed as soon after collection as possible. Ideally, this is done at sea if conditions and space allows, otherwise it is done as soon as the vessel reaches shore. Samples should never be stored more than 3-4 hours (if it is impossible, add solvent mixture into the sample as preservative, see under sampling) and should be kept in refrigerator or ice-box during the storage. The extraction is performed with hexane. One hundred ml of the solvent is used in two successive 50 ml extractions and the extracts are combined. There are two convenient methods for extracting water.

a) Separatory funnel method

This technique requires two clean and solvent rinsed 2 litres separatory funnels with Teflon stopcocks and stoppers. Assuming a maximum of 4 litres sample (or 1 gallon), the following procedure is applied. Half the sample is transferred to separatory funnel No 1 and 50 ml of solvent is added. The funnel is then shaken vigorously for a few seconds and the cap loosened to allow solvent vapours to escape. This process is repeated for about five times after which the funnel is racked to allow the solvent phase to separate. The water phase is then withdrawn and added to funnel No 2. 50 ml of fresh solvent is then added to funnel No 2 and the shaking process repeated. After allowing the solvent phase to separate in funnel No 2, the water phase is drained and discarded. The second half of the sample is then added to the funnel No 1 (which contains the first 50 ml of solvent), shaken, allowed to separate, and the water phase transferred to funnel No 2 where it is shaken again, allowed to separate and the water phase drained off and discarded. The 50 ml of extract in funnel No 1 is then drained into the funnel

No 2, and a few grams of anhydrous Na_2SO_4 is added to break any emulsion and to remove excess water. Once the combined extract is dry, it is poured out of the top of the funnel into a clean, solvent rinsed 100 ml volumetric flask, the funnel rinsed with fresh solvent and the rinsing used to make the volume up to the 100 ml mark. Then the flask should be labelled, and stored for later analysis.

b) Pipette method

Approximately 75 ml of the sample is poured into a clean hexane rinsed beaker and set aside. Fifty ml of solvent is then measured (a graduated cylinder is accurate enough) and poured into the screw-capped sampling bottle. The cap, carefully line with solvent rinsed aluminium foil (to prevent contamination of solvent from the cap material) is replaced and the entire sampling bottle shaken vigorously. After a few seconds, the bottle is placed upright and the cap loosened slightly and slowly, to allow solvent vapors to escape. This process is repeated over and over again for about 5 minutes. The bottle is then placed upright and the solvent phase allowed to separate. The solvent phase should be up in the narrow neck of the bottle, if not some of the water poured out previously is added until it is. Then, a clean, solvent rinsed pipette is used to withdraw the solvent phase and to transfer it to a clean, solvent rinsed, glass stoppered storage bottle. It is convenient to carefully measure the length of the pipette needed to reach to solvent water interface and then placed a pinch clamp or cloth pin on the pipette to mark that length. The process is repeated with the second 50 ml aliquot of solvent. The glass stoppered storage bottle is then labelled, visible quantities of water removed with a clean pipette if possible, a few grams of anhydrous Na_2SO_4 added to remove excess water, and carefully stored for future analysis.

It is important that blanks also be collected and taken through the analytical procedure. One blank of 100 ml of the solvent should first be used to rinse the already clean and empty sampling bottle and then transferred to the graduated cylinder and then in a second clean, solvent rinsed, glass stoppered bottle in the same place the extraction is performed, whether this is on ship or on shore. A second blank should be “collected” in the laboratory when the analysis is performed.

VI.6.2 Beach Tar

The sample analysis consists of weighting the tar collected within each transect to the nearest 0.1 gram. If the samples contain a high percentage of sand and other debris, it is suggested that samples are dissolved in CCl_4, filtered, and, after evaporation of CCl_4, the weight of tar is recorded.

Sample reporting

The mean weight and the deviation should be calculated from the triplicate or more samples collected at a given station and recorded as tar weight (g/m) of shoreline. Reporting the mean tar weight as g/m of shoreline, rather than g/m^2 of beach surface as recommended by UNESCO (1977), has been shown to provide more accurate and inter-comparable means of assessing the relative extent of tar pollution. Data in this form (g tar/meter of shoreline) may also be used to calculate the “standing stock” of tar along a given coastline as a kg/km of coast value, which is more representative of a particular period.

The guidelines for completing data reporting forms used for sampling and reporting tar on beaches are summarized in Table (VI.1).

Table (VI.1) : Guidelines for completing data reporting forms for beach tar surveys

Data reporting forms should be used to report the results of the following general tasks :

- Beach Tar Survey
- Meteorological and Oceanographic Observations
- 24 hours Oceanographic Observations (hourly, three & six hourly, phytoplankton)
- Oil and Non-Oil Pollutant Analyses
- Sedimentological and Mineralogical Analyses

Standard data forms for Ecological Surveys are not provided since each country will investigate a unique habitat. Data forms for C-14 and plankton analyses are also not included and should be developed by participating institutions.

The following general explanations relate to all of the supplied forms :

1. **COUNTRY** : Name of country reporting, e.g. Bahrain, U.A.E., etc
2. **INSTITUTION** : Name of institution conducting observations, analyses
3. **SAMPLE LOCATION** : Latitude and Longitude will be given to each sampling station and these should be used on all data forms where required
4. **DATE OF SAMPLING (TIME)** : Date and, where required, time should be recorded as follows :
   - Day, month, year
   - the 24 hours notation for time should be used (00:01 – 24:00)
5. REMARKS: Any additional information or data that will assist in later identification of samples or locations as well as unexpected or unusual events should be recorded as footnote on the reporting forms

Most of the items in the data reporting forms are self explanatory, however, detailed instruction for filling in the forms where necessary are given in the following example:

BEACH TAR SURVEY

1. SAMPLING LOCATION - record the latitude and longitude of a point at the centre of a surveyed beach

2. BEACH NAME - record names of beaches surveyed as they appear on hydrographic charts or identify according to closest name of point or settlement given on the chart

3. BEACH LENGTH - measure the length of the beach being sampled on a hydrographic chart of suitable scale, report as kilometres

4. SLOPE OF BEACH - refer to slope of beach sampled, code as follows :
(1) steep, greater than 45°
(2) medium, between 30°– 45°
(3) shallow, les than 30°

5. TEXTURE OF SEASHORE - refer to texture of seashore surface, code as follows :

(1) fine sand
(2) coarse sand
(3) pebbles
(4) rock slabs

6. STATE OF TIDE - record the state of the tide at time of collection, following codes :

- low tide
- mid tide
- high tide
- from low to mid tide
- from mid to low tide
- from mid to high tide
- from high to mid tide

7. CONDITION OF TAR - refer to condition of majority of tar, code as follows :

(1) very soft and fresh, unable to pick up by hand, use spatula or putty knife
(2) soft and fresh, unable to collect by hand
(3) soft but with hard exterior
(4) hard without sand coating
(5) very hard with sand coating

8. WEIGHT OF THE TAR - (Grams tar/meter of shoreline)

Transect 1 (g/m)
Transect 2 (g/m)
Transect 3 (g/m)
Mean (g/m)

9. OTHERS - rubbish, cans, plastic spheres or containers and any information about beach or conditions

METEOROLOGICAL AND OCEANOGRAPHIC OBSERVATIONS :

- WIND DIRECTION – direction from which wind is coming
- CLOUD – cloud cover reported as 1/8ths of cover

OCEANOGRAPHIC OBSERVATIONS (THREE & SIX HOURLY) :

- TURBIDITY – record instrument reading in units – FTU (Formaline Turbidity Units) or NTU (Nephelometric Turbidity Units) – indicated on particular instrument

OIL AND NON-OIL POLLUTANT ANALYSES :

- WATER – SEDIMENT – BIOTA: - delete matrices if not applicable
- RESULTS – report results in the appropriate units listed in the manual

VI.7 ANALYSIS OF SEDIMENTS

VI.7.1 Analysis of PHCs in Sediments

10 to 20 grams of freeze-dried sediment sample, ground and sieved at 63 µm, are extracted in a Soxhlet extractor with a mixture of hexane and dichloromethane (50:50). Internal standards are added to the sample for recovery: 50 µl of a mixture containing about 20 ng/µl each of n-C_{19} d_{40}, and n-C_{32} d_{66} for the first fraction and about 20 ng/µl each of Hexamethylbenzene, Cadalene and Naphthalene d_8 for the second fraction.

Extraction is realised in the Soxhlet with 250 ml of the mixture hexane/ dichloromethane, the siphon cycle is about 10 minutes during 8 hours.

When the extraction is finished, the extract is evaporated with a rotary evaporator to a volume of about 15 ml (the temperature of the water bath does not exceed 30°C).

Note : In fact the parameters to take into account for the concentration with the rotary evaporator are: the temperature of the water bath and the vacuum obtained with the vacuum system used. These parameters have to be considered as a couple, if one is not powerful enough, then the other one could be increased. As an example, in case the vacuum is very poor, then, the temperature of the water bath could be increased to 35°C or even to 40°C. The observation plays an important role, the distillation of the solvent should be slow, but solvent condensation should be seen in the flask. The parameters (temperature, vacuum) are adjusted in such a way that it takes about 20 minutes to concentrate an extract from 250 ml down to 15 ml

Clean-up and separation

Purposes of the clean-up: removal of lipids, whenever present in significant amount; removal of elementary sulfur and sulfur compounds. Both these compound classes can interfere with the gas-chromatographic separation.

Sulphur and sulphur compounds removal :

Preparation of copper :

Transfer about 20 grams of the copper powder in an Erlenmeyer. Add enough concentrated HCl to cover the copper powder, agitate. Sonicate for 10 min., agitate, put again in ultrasonic bath and sonicate for 10 min. Throw the used HCl, add some fresh HCl, transfer in ultrasonic bath and sonicate for 20 min. Repeat that procedure four times in total. Wash with distilled water, agitate, discard, add water again, transfer in ultrasonic bath and sonicate for 15 min., discard the used water, do it again, up to pH neutral. Wash with acetone, agitate, transfer in ultrasonic bath and sonicate for 15 min. Repeat that procedure four times in total. Then use the same procedure with hexane as a solvent.

Keep in hexane (use it immediately, avoid Cu to be in contact with air).

Transfer 3 to 4 Pasteur pipettes per sample in the flasks containing the hexane extracts. Let the copper react all night. The presence of sulphur compounds in the sample will be detected by the tarnishing of the copper powder.

The extract is dried with anhydrous sodium sulfate and transferred in a graduated tube and concentrated down to 1 to 2 ml using a flow of clean nitrogen.

Extractable organic matter (EOM)

The EOM is determined in the following manner. On the weighting pan of an electro-balance, a known volume of the sediment extract is evaporated (up to 100 µl) and the residue is weighted with a precision of about ±1 µg. If the residue is less than 2 µg, pre-concentration of the original extract is required. The quantity of EOM is :

$$\text{EOM (µg/g)} = \frac{\text{Weight of residue (µg) x volume of the extract (ml) x 1000}}{\text{volume evaporated (µl) x quantity of sample extracted (g)}}$$

Separation of compounds

The clean-up and separation are achieved by a simple column chromatographic partition as follows :

Preparation of silica and alumina : silica gel and alumina are pre-cleaned by Soxhlet extraction, first for 8 hours with methanol and then for 8 hours with hexane. They are dried at 60°C to remove the solvent, then at 200°C for 8 hours and then stored in amber bottle.

Before use, they are activated at 200°C for 4 hours and partially deactivated with 5% water.

A chromatography column is prepared using 50 ml burette in which a piece of glass wool is added near the stopcock to maintain the packing material. Then, 5 grams (or 10 ml) of silica are transferred into the column, then 10 grams (or 10 ml) of alumina and on top 1 gram of sodium sulfate is added in order to avoid the disturbance of the first layer when solvents are poured into the column.

The sample (maximum 100 mg lipids) is applied on top of the column. A first fraction is obtained by eluting the sample with 20 ml of hexane (F1), this fraction will contain the saturated aliphatics. The second fraction (F2) is obtained by eluting with 30 ml of a mixture of hexane and dichloromethane (90:10), this fraction will contain the unsatured and aromatic hydrocarbons.

Sonic or Shaker extraction

As an alternative method to Soxhlet extraction, laboratories equipped with a sonicator can follow the US-EPA SW846 Method 3550 or NSTP procedures described by Krahn *et al*., (1988). In this method the sediment is mixed with Na_2SO_4 as above and spooned into a 250 ml glass or Teflon jar. Add surrogate standards to each jar. Add 100 ml of 1:1 v/v dichloromethane/acetone. Sonicate for about 3 minutes at settings appropriate to yield efficient extraction. Alternatively, the extracts can be shaken several hours on a mechanical shaker. After extraction, decant the solvent into a clean flask. Repeat the extraction two more times combining all extracts into the flask. Filter and dry the extract through glass wool and Na_2SO_4. Reduce the extract volume as described previously. Use the same quantity of Na_2SO_4 for the blank than the quantity used for mixing the sample.

Alternate method : Microwave extraction

Another alternative method for the extraction of PH's in sediment samples is the use of the Microwave oven instead of the Soxhlet extractor. The main advantage of the microwave oven is the fact that, for one sample, only 40 ml of solvent mixture are used instead of 250 ml for clean-up of extraction thimbles and 250 ml for the extraction itself.

10 to 20 grams of freeze-dried sediment sample, ground and sieved at 250 µm, are put in the glass tube of the reactor. Internal standards are added to the sample for recovery: 50 µl of a mixture containing about 20 ng/µl each of n-C_{19} d_{40}, n-$C_3$2 d_{66} for the first fraction and about 20 ng/µl each of Hexamethylbenzene, Cadalene and Naphthalene d_8 for the second fraction and samples are extracted with 40 ml of a mixture of hexane / dichloromethane (50:50).

Extraction is realised within the following cycle :

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour

The carrousel containing 14 reactors, 12 samples could be extracted together with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The sediment is poured in a glass funnel containing a plug made of glass wool. The extracted sediment is washed with 10 - 20 ml of hexane. The extract is then cleaned with copper.

Note : In this context "surrogate standard" is meant to be a compound added to the sample, which will behave in an identical manner to the compounds to be quantified and which is used to correct the results for the recovery through all steps of the analytical procedure. Since the hydrocarbons are to be separated into fractions, it is necessary to add at least two standards, but as the volatility range is rather important it would be better to have 2 standards in each fraction. The two standards considered for the aliphatic fraction are: n-C_{19} d_{40} and n-C_{32} d_{66}. The three standards for the aromatic fraction are: Naphthalene d_8, Hexamethylbenzene and Cadalene. If samples are to be analysed by GC/MS there is a variety of deuterated aromatic hydrocarbon standards that could be added just before the injection in the GC/MS, such as Anthracene d_{10}, Pyrene d_{10}, Perylene d_{12} and Chrysene d_{12}.

Since the basis of all quantitative work is the analytical balance, control must be exercised over the care of the balances. They must be routinely checked for accuracy against a set of high quality calibration weights. Also, since the standards for hydrocarbon analysis are made up in volatile solvents, extreme care must be taken to ensure that concentrations are not altered by evaporation. Solutions should be made up by weight into tight sealed vials and the total weight recorded on each vial. Working solutions are then weighed before every use. The weight of every stock vial should be recorded for future evaluation of the usefulness of the solutions. Iso-octane is recommended as the dilution solvent for stock solutions while hexane dilutions of these should be added to samples as surrogates.

High purity nitrogen gas should be used to evaporate samples. A molecular sieve trap should be placed in-line. Either a single line could be used or a manifold could be constructed. Flows must be adjustable and all materials in-line must be non-contaminating (glass, Teflon, stainless steel). If necessary, high purity air could be substituted to the N_2. Prevent cross contamination of samples by solvent rinsing the evaporation tips between each sample.

VI.7.2 Analysis of Chlorinated Hydrocarbons

Sample extracts obtained for the analysis of petroleum hydrocarbons can also be used for analysis of chlorinated hydrocarbons. In this case, internal standards should be added directly to the extract. Alternatively, the following protocols could also be followed.

A) Extraction

i) Extraction of freeze-dried samples

A 50 to 100 grams sub-sample of the sediment is selected, weighted and freeze-dried. When dried, it is weighted again and the dry to wet ratio is calculated. Then the sample is pulverised using the mortar and pestle and sieved using a 250 µm stainless steel sieve. About 20 grams of ground sample is weighted accurately and placed in the pre-cleaned extraction thimble. 1 ml of a solution containing about 20 pg/µl each of CB 29, CB 198, Endosulfan I d_4 and ε-HCH as internal standards is added to the sample. The sample is extracted for 8 hours in a Soxhlet apparatus with 250 ml of a mixture hexane / dichloromethane (50:50). An empty thimble is extracted in the same way as the samples for purposes of generating a procedural blank.

ii) Extraction of wet samples

The sediment is thawed, sieved at 250 µm and homogenized manually with a stainless steel spatula or clean glass rod. A sub-sample of 1-2 grams is weighted into a flask and placed in a drying oven at 105°C for 24 hours, then allowed to cool to room temperature and re-weighted. Calculate the dry to wet ratio and discard the dry sediment.

Place a 30 to 40 grams sub-sample of thawed, homogenized sediment in a blender. Slowly add 100 grams of dried anhydrous sodium sulphate (desiccant) and blend the mixture at maximum speed for 10 minutes. Transfer the dried sample quantitatively to the pre-cleaned extraction thimble in the Soxhlet apparatus, add the internal standard solution (see above) and apply the same extraction procedure as above. Extract the same amount of sodium sulphate as a procedural blank making sure to add an appropriate amount of internal standard solution.

iii) Alternate method: Microwave extraction

An alternative method for the extraction for chlorinated pesticides and PCBs in sediment samples is the use of the Microwave oven instead of the Soxhlet extractor. The same advantage (see VI.7.1, PH in sediment sample) of the microwave oven is the fact that, for one sample, only 40 ml of solvent mixture are used.

A 20 to 100 grams sub-sample of the sediment is selected, weighted and freeze-dried. When dried, it is weighted again and the dry to wet ratio is calculated. Then the sample is pulverised using the mortar and pestle and sieved using a 250 µm stainless steel sieve. About 5 - 15 grams of ground sample is weighted accurately and placed in the pre-cleaned extraction glass tube of the reactor. 1 ml of a solution containing about 20 pg/µl of CB 29, CB 198, Endosulfan I d_4 and ε-HCH as internal standards is added to the sample.

Extraction is realised with 40 ml of a mixture hexane / dichloromethane (50:50) within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour

The carrousel containing 14 reactors, 12 samples could be extracted with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The sediment is poured in a glass funnel containing a plug made of glass wool. The extracted sediment is washed with 10 - 20 ml of hexane. The extract is then cleaned with copper (or mercury in case only OC's are of interest) and concentrated with rotary evaporator.

B) Concentration of the extract

After extraction, the extracts are concentrated on a rotary evaporator to about 15 ml. The temperature of the water bath does not exceed 30°C (see Note at VI.7.1). The extract is dried with anhydrous sodium sulphate. The dried extract is collected in the graduated tube and adjusted to 1 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen. The sample extract is analysed gravimetrically for extractable organic matter (EOM) content at the 1 ml volume as a starting point. If measurements of the EOM are outside of the calibration range of the balance, the total volume of the extract is adjusted accordingly using either dilution with hexane or evaporating under a stream of nitrogen gas.

C) Extractable Organic Matter (EOM)

The EOM is determined in the following manner. On the weighting pan of an electrobalance, a known volume of the sediment is evaporated (up to 100 µl) and the residue is weighted with a precision of about ±1 µg. If the residue is less than 2 µg, preconcentration of the original extract is required. The quantity of EOM is :

$$\text{EOM (µg/g)} = \frac{\text{Weight of residue (µg) x volume of the extract (ml) x 1000}}{\text{volume evaporated (µl) x quantity of sample extracted (g)}}$$

D) Clean-up procedure and fractionation

Purposes of the clean-up: removal of lipids, whenever present in significant amount; removal of elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation.

Sulphur and sulphur compounds removal

a) Elementary sulphur and sulphur compounds such as mercaptans are removed using mercury.

Note : Mercury method should be used only if chlorinated pesticides and PCBs are analysed. If the combined method is used for petroleum and chlorinated hydrocarbons, then the copper method should be used instead of mercury that will destroy some of the PAHs

One drop (a few ml) of mercury is added with a drop counter to the sediment extract and vigorously shaken for one minute. The extract is carefully recovered in another tube. If the mercury is tarnished, the treatment is repeated with another drop of mercury. This treatment is repeated until the mercury stays brilliant in the extract. Then, the resulting solution is concentrated to 1 ml with a gentle stream of nitrogen.

b) Removal of sulphur and sulphur compounds using copper :

Preparation of the copper :

Transfer about 20 grams of the copper powder in an Erlenmeyer. Add enough concentrated HCl to cover the copper powder, agitate. Sonicate for 10 min., agitate, put again in ultrasonic bath and sonicate for 10 min. Throw the used HCl, add some fresh HCl, transfer in ultrasonic bath and sonicate for 20 min. Repeat that procedure four times in total. Wash with distilled water, agitate, discard, add water again, transfer in ultrasonic bath and sonicate for 15 min., discard the used water, repeat that procedure again, up to neutral neutral pH. Wash with acetone, agitate, transfer in ultrasonic bath and sonicate for 15 min. Repeat that procedure four times in total. Then use the same procedure with hexane as a solvent.

Keep in hexane (use it immediately, avoid Cu to be in contact with air).

Transfer 3 to 4 Pasteur pipettes per sample in the flasks containing the hexane extracts. Let the copper react all night. The presence of sulphur compounds in the sample will be detected by the tarnishing of the copper powder.

Fractionation

i) Florisil

Preparation of the columns: 50 ml glass burettes (1 cm diameter) with Teflon stopcocks make convenient adsorption columns. The column is plugged with pre-extracted glass wool. Separate columns are prepared for each sample and blank determination. A slurry method to fill the columns is recommended. The column is partially filled with hexane. The appropriate amount of sorbent is mixed with hexane in a small beaker. A glass funnel and a glass rod are used to pour the adsorbent into the column. Several rinses with hexane are necessary to fill the column to the desired height. Each column is prepared freshly immediately before use.

A Florisil column is used for this separation, which is prepared in the following way. The Florisil is pre-extracted in the Soxhlet apparatus to remove any contaminants using methanol or dichloromethane for 8 hours, then hexane for another 8 hours. It is then dried in an oven. Activation is achieved by heating the dried Florisil at 130°C for 8 hours. It is then partially deactivated with 0.5% water by weight and stored in a tightly sealed glass jar with ground glass stopper. The water is well mixed into the Florisil and the mixture is allowed to equilibrate for

one day before use. The activation/deactivation procedure is made one day before use. A 50 ml glass burette with Teflon stopcock is plugged with pre-cleaned glass wool. 18 grams of Florisil are weighted out in a beaker and covered with hexane. A slurry is made by agitation and poured into the glass column. The Florisil is allowed to settle into an even bed and any Florisil adhering to the column is rinsed down with hexane. The solvent is drained to just above the Florisil bed. It is rinsed by a further 5 ml of hexane, one gram of anhydrous sodium sulphate is added to the top of the column in order to protect the surface of the Florisil. Individual columns are prepared immediately before use, and a new column of Florisil used for each sample.

The extract, reduced to 1 ml, is applied to the Florisil column. It is carefully eluted with 65 ml of hexane and the first fraction collected. Then the column is eluted with 45 ml of a mixture containing 70% of hexane and 30% of dichloromethane and the second fraction collected. The third fraction will be eluted with 55 ml of pure dichloromethane.

Fraction one contains the PCBs, pp' and op DDE and some other pesticides such as HCB, aldrin, heptachlor, DDMU.

Fraction two contains the DDTs, DDDs, most of the toxaphene, and some pesticides such as the HCH isomers and chlordane components.

Fraction three contains mainly dieldrin, endrin and endosulfan components.

ii) Gel permeation chromatography

Low pressure GPC can be used as an alternative clean-up technique to remove high molecular weight co-extractable lipidic material from polycyclic aromatic compounds and halogenated aromatics. Concurrently, elemental sulphur could be also removed from the whole organic extract.

The main feature of the semi-preparative-GPC as a clean-up technique relies on the comparability of this analytical procedure with labile components of the extract (i.e. DDTs, chlorinated cyclohexadiene derivatives) which are not stable in other types of extract clean-up procedures. Further, GPC as a clean-up technique has already been automated enabling a high sample throughput, taking into account the short analysis time involved.

The GPC retention mechanism may involve adsorption, partition and size exclusion mechanisms. The predominance of one mechanism over the others is largely determined by the choice of the mobile phase and the pore size of the packing. In the case of GPC packings with large pore size (1000-2000 daltons), size exclusion and adsorption mechanism prevail (Bio-Beads SX-3 using cyclohexane, dichloromethane-hexane, dichloromethane-cyclohexane, toluene-ethylacetate and ethylacetate-cyclohexane) (Erickson *et al*., 1986). On the other hand, when smaller pore sizes (400 daltons) are used in combination with highly polar solvents, (THF, DMF), size exclusion predominates (Lee *et al*., 1981). While using the first approach, a chemical class fractionation could be obtained, however, if smaller pore sizes are used, it should be combined with another fractionation technique (i.e. adsorption chromatography) to achieve this selectivity. It has yet to be demonstrated that using GPC as a single clean-up step produces a completely clean extract for GC-ECD determination. Nevertheless, taking into account the increasing availability of high-resolution low molecular weight exclusion packings, they could definitely integrate fractionation and clean-up in a single step.

Low resolution packings (Sephadex LH and Bio-Beads SX 200, 100 mesh size) are the most widely used because they are inexpensive and afford relatively high sample loading (500 mg in 10 mm i.d. columns). The implementation of low resolution GPC requires a solvent delivery system and a UV detector and may be useful. For method development, it is advisable to inject a broad range of standard compounds covering the whole range of molecular weights of the analytes to be determined in order to determine the cut-off points to fractionate real samples. Reported recoveries of PCBs and PAHs range from 60 to 80% for the concentration level (ng) injected. (Fernandez and Bayona, 1992).

iii) Alumina and HPLC (silica column)

The first step in this clean-up procedure is an adsorption step using an alumina column to remove most of the lipid material. Prepare an alumina column (4 x 0.5 cm i.d., made from a Pasteur pipette). Apply the concentrated extract to the top of the column and elute with 10 ml of hexane. Concentrate the eluate to about 200 µl. It is followed by a second step to more completely remove interfering compounds and at the same time to separate compounds of interest into different fractions, containing aliphatics, PCBs, PAHs, pesticides and toxaphene. Between 20 and 200 µl of the extract (after alumina clean-up) are eluted on a stainless steel column (200 x 4 mm i.d.), packed with Nucleosil 100-5 with n-pentane, 20% dichloromethane in n-pentane and finally dichloromethane. The eluate is collected in fractions containing 1) n-hydrocarbons, 2) PCBs, 3) PAHs and toxaphene, 4) pesticides and toxaphene and 5) acids, etc. (polar compounds). The size of the fractions has to be determined with standard solutions containing the compounds of interest, collecting the eluate in 0.5 ml fractions. Each fraction is then analysed by GC-ECD. Full details have been given in the literature (Petrick *et al*., 1988 and IOC, 1993).

iv) High pressure liquid chromatography

High pressure liquid chromatography (HPLC) columns packed with micro-particles are available and have the advantages of high reproducibility, low consumption of solvents, high efficiency and high sample loading capacity.

This method can be used to separate fractions containing aliphatic hydrocarbons, PCBs and aromatic hydrocarbons from interfering compounds. These fractions can then be analysed separately for their constituents by GC-FID and/or GC-ECD.

HPLC methods have been developed using synthetic solutions of n-alkanes, PAHs, pesticides, PCBs and toxaphene and have been applied to samples in which interfering substances were present in such high concentrations as to render the analysis of HC and PCB extremely difficult without this clean-up procedure (e.g. sediments and biological tissues with OCs in the ng/g range). The samples are eluted with *n*-hexane, subjected to clean-up with alumina, concentrated down to 20-200 µl and treated by HPLC. With the use of *n*-hexane, n-pentane and 10%, 20% and 50% dichloromethane in *n*-hexane, respectively, the following five fractions are obtained: 1) n-hydrocarbons and alkenes, 2) PCBs and alkylbenzenes, 3) PAHs and toxaphene, 4) pesticides, 5) acids, etc. (polar compounds) (Petrick, *et al*., 1988).

VI.7.3 Analysis of Sterols

A) Extraction

To the freeze-dried sample, before the start of the extraction of sediment in the Soxhlet, together with the internal standards used for petroleum hydrocarbons and chlorinated pesticides, 10 to 20 µg of 5α-androstan-3β-ol should be added as internal standard. Samples are Soxhlet extracted for 8 hours with 250 ml of a mixture (1:1) of hexane / dichloromethane.

B) Concentration of the extracts

The extract is concentrated in a rotary evaporator to about 15 ml. The temperature of the water bath must not exceed 30°C (see Note at VI.7.1). Concentration down to 5 or 6 ml are carried out under a flow of clean nitrogen.

C) Clean-up procedure and fractionation

i) Removal of sulphur

Sulphur is removed by copper powder (see above for petroleum hydrocarbons). The extract is then separated into aliquots: 1/3 for hydrocarbons and sterols and 2/3 for chlorinated pesticides and PCBs.

ii) Fractionation

The extract is passed through a silica / alumina column (silica and alumina are activated first at 200°C for 4 hours and partially deactivated with 5% water). The chromatography column is prepared by slurry packing 5 grams (or 10 ml) of silica, then 10 grams (or 10 ml) of alumina and finally 1 gram of sodium sulphate is added to the surface to avoid disturbance of the top layer when pouring the solvents. Elution is performed using 20 ml of hexane to yield the first fraction (which contains the aliphatic hydrocarbons), then, 30 ml of hexane/ dichloromethane (90:10). Sterols are then eluted with 40 ml of a dichloromethane/methanol (90:10) mixture.

iii) Injection

Extract should be dried under nitrogen and 100 µl of BSTFA [N, O-bis (trimethylsilyl) trifluoroacetamide] added for 1 hour at 50-60°C (Readman *et al*., 1986a).

Before injection, the extract is dried and iso-octane is added (between 100 µl and 3 ml).

VI.8 ANALYSIS OF BIOTA

VI.8.1 Analysis of Petroleum Hydrocarbons

Following collection of biological samples using appropriate techniques, samples are stored in non-contaminating jars at -20°C until analysis. For analysis, the samples are defrosted and prepared for solvent extraction. To achieve a satisfactory recovery of the petroleum hydrocarbons, samples are freeze-dried. Lipids are then Soxhlet extracted from samples using methanol. Following initial clean-up treatments (partial removal of lipids by saponification), extracts are fractionated using column chromatography with silica and alumina. Quantification is done by GC-FID.

A) Extraction

5 to 10 grams of freeze-dried sample are extracted with a Soxhlet extractor with 200 ml of methanol. Internal standards are added to the sample in the extraction thimble before extraction starts (same I.S. mixture than sediment sample).

After the extraction is completed, 20 ml of 2 mole/l KOH are added to the flask and the extraction is continued for 2 more hours to saponify the lipids.

The content of the extraction flask is transferred into a separatory funnel with 30 ml of water (distilled and extracted with hexane) and extracted with 90 ml of hexane and re-extracted again twice with 50 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulphate.

(The aqueous phase is not discarded as it is used for total lipid weight determination).

B) Concentration of the extracts

The hexane fraction is concentrated with a rotary evaporator down to about 15 ml (maximum temperature: 30°C). Then transferred in a graduated tube and concentrated with nitrogen down to a volume corresponding to1 ml/gram of freeze-dried sample extracted (this will avoid the precipitation of the lipids in the tube).

The aqueous phase is acidified with 1 mole/l sulphuric acid and extracted 3 times in a separatory funnel with 30 ml of hexane. Hexane is then concentrated and the lipids are weighed with the electro-balance. The total lipid content is the sum of the lipid found in the first hexane fraction and this one. Then this fraction is discarded.

C) Extractable Organic Matter (EOM)

See Section VI.7.2, Part (C).

D) Clean-up procedure and fractionation

Especially in the case of biota samples, it is necessary to clean-up the extract before proceeding with the analysis. The clean-up should remove non-petroleum hydrocarbons material that fluoresces under certain conditions. Furthermore, materials that may cause quenching will be removed simultaneously.

Fractionation

The clean-up and separation are achieved by a simple column chromatographic partition as follows :

Preparation of silica and alumina: silica gel and alumina are pre-cleaned by Soxhlet extraction, first for 8 hours with methanol and then for 8 hours with hexane. They are dried at 60°C to remove the solvent, then at 200°C for 8 hours and then stored in amber bottle.

Before use, they are activated at 200°C for 4 hours and partially deactivated with 5% water.

A chromatography column is prepared using a 50 ml burette in which a piece of glass wool is added near the stopcock to maintain the packing material. Then, 5 grams (or 10 ml) of silica are transferred into the column, then 10 grams (or 10 ml) of alumina and, on top, 1 gram of sodium sulfate is added in order to avoid the disturbance of the first layer when solvents are poured into the column.

Separation of compounds

The sample (maximum 100 mg lipids) is applied on top of the column, then a first fraction is obtained by eluting the sample with 20 ml of hexane (F1), this fraction will contain the saturated aliphatics, the second fraction (F2) is obtained by eluting with 30 ml of a mixture of hexane and dichloromethane (90:10), this fraction will contain the unsatured and aromatic hydrocarbons.

VI.8.2 Chlorinated Pesticides and PCBs

Following collection of biota samples using appropriate techniques, samples are stored in non-contaminating jars at -20°C until analysis. For analysis, the samples are defrosted and prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are freeze-dried. Lipids are then Soxhlet extracted from biota using hexane. Following initial clean-up treatment (treatment of extracts with concentrated sulphuric acid to destroy interfering lipids), extracts are fractionated using column chromatography.

A) Extraction

A 50 to 100 grams fresh weight sub-sample is selected from the sample. This sub-sample is weighted and freeze-dried. When the sub-sample appears to be dry, it is weighted again and the dry to wet ratio is calculated. The freeze-dried sub-sample is carefully pulverised in a cleaned pestle and mortar. About 5 to 10 grams of this pulverised material is accurately weighted, the weight to be extracted is noted, and it is placed into a pre-cleaned extraction thimble in a Soxhlet apparatus. The size of the sub-sample is adjusted so that about 100 mg of extractable organic matter (“lipid”) will be obtained. Smaller sub-samples are used if residue concentrations are expected to be high. A known amount of internal standard is added to the sub-sample in the thimble before Soxhlet extraction. Candidate internal standards are the same than for sediment samples (see VI.7.2, Part A). About 250 ml of hexane are added to the extraction flask with a few carborundum boiling chips, and the sample is extracted for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. An empty thimble is extracted as a procedural blank.

Alternate method : Microwave extraction

A 50 to 100 grams fresh weight sub-sample is selected from the sample. This sub-sample is weighted and freeze-dried. When the sub-sample appears to be dry, it is weighted again and the dry to wet ratio is calculated. The freeze-dried sub-sample is carefully pulverised in a cleaned pestle and mortar. About 3 to 8 grams of this pulverised material is accurately weighted, the weight to be extracted is noted, and it is placed into the pre-cleaned glass tube of the reactor. The size of the sub-sample is adjusted so that about 100 mg of extractable organic matter ("lipid") will be obtained. Smaller sub-samples are used if residue concentrations are expected to be high. A known amount of internal standard is added to the sub-sample in the tube before extraction. Candidate internal standards are the same than for sediment samples.

Extraction is realized with 30 ml of a mixture hexane / acetone (90:10) within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour

The carrousel containing 14 reactors, 12 samples could be extracted with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The powder of biota is poured in a glass funnel containing a plug made of glass wool. The extracted biota is washed with 10 - 20 ml of hexane. The extract is then concentrated with rotary evaporator.

B) Concentration of extracts

After extraction, the extracts are concentrated on a rotary evaporator to about 15 ml. The temperature of the water bath does not exceed 30°C. The extract is dried with anhydrous sodium sulphate. The dried extract is collected in a graduated tube and the extract is concentrated to exactly 1 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen. The sample extract is analysed gravimetrically for extractable organic matter (EOM) content at the 1 ml volume as a starting point. If measurements of the EOM are outside of the calibration range of the balance, the total volume of the extract is adjusted accordingly using either dilution with hexane or evaporating under a stream of nitrogen gas.

C) Extractable Organic Matter (EOM)

See Section VI.7.2, Part (C)

D) Clean-up procedure and fractionation

Purposes of the clean up: removal of lipids, whenever present in significant amount, these compound classes can interfere with the gas-chromatographic separation.

Removal of lipids by concentrated sulphuric acid

In case the lipid content of the extracts is higher than 100-150 mg, a preliminary step for the removal of the lipids is necessary before further sample purification. This removal is carried out by using concentrated sulphuric acid. The treatment with sulphuric acid is used when chlorinated hydrocarbons are determined. However, sulphuric acid will destroy dieldrin and endrin so that an aliquot of the untreated extract is used for the determination of these compounds.

An aliquot of the concentrated extract, containing about 200 mg of “lipids” is taken, transferred into a separatory funnel and enough hexane (40 to 50 ml) is added to this extract in order to dilute the sample, this permits to recover the hexane after acid treatment. 5-10 ml concentrated sulphuric acid is added to the extract and the separatory funnel is vigorously shaken. The separatory funnel is placed in a rack and the phases are allowed to separate. The hexane phase is recovered into a glass beaker. Dried with sodium sulphate and the hexane is transferred into a graduated tube. The volume of the extract is reduced by evaporating the solvent with a gentle stream of pure nitrogen to about 1 ml.

Fractionation

Preparation of the columns: 50 ml glass burettes (1 cm diameter) with Teflon stopcocks make convenient adsorption columns. The column is plugged with pre-extracted glass wool. Separate columns are prepared for each sample and blank determination. A slurry method to fill the columns is used. The column is partially filled with hexane. The appropriate amount of sorbent is mixed with hexane in a small beaker. A glass funnel and a glass rod are used to pour the adsorbent into the column. Several rinses with hexane are necessary to fill the column to the desired height. Each column is prepared freshly immediately before use.

A Florisil column is used for this separation, which is prepared in the following way. The Florisil is pre-extracted in the Soxhlet apparatus to remove any contaminants using methanol for 8 hours, then hexane for another 8 hours. It is then dried in an oven. Activation is achieved by heating the dried Florisil at 130^{o}C for 8 hours. It is then partially deactivated with 0.5% water by weight and stored in a tightly sealed glass jar with ground glass stopper. The water is well mixed into the Florisil and the mixture is allowed to equilibrate for one day before use. The activation/deactivation procedure is made one day before use. A 50 ml glass burette with Teflon stopcock is plugged with pre-cleaned glass wool. 18 grams of Florisil are weighted out in a beaker and covered with hexane. A slurry is made by agitation and poured into the glass column. The Florisil is allowed to settle into an even bed and any Florisil adhering to the column is rinsed down with hexane. The solvent is drained to just above the Florisil bed. It is rinsed by a further 5 ml of hexane, one gram of anhydrous sodium sulphate is added to the top of the column in order to protect the surface of the Florisil. Individual columns are prepared immediately before use, and a new column of Florisil used for each sample.

The extract, reduced to 1 ml, is applied to the Florisil column. It is carefully eluted with 65 ml of hexane and the first fraction collected. Then the column is eluted with 45 ml of a mixture containing 70% of hexane and 30% of dichloromethane and the second fraction collected. The third fraction will be eluted with 55 ml of pure dichloromethane.

Fraction one contains the PCBs, pp’ and op DDE and some other pesticides such as HCB, aldrin, heptachlor, DDMU.

Fraction two contains the DDTs, DDDs, most of the toxaphene, and some pesticides such as the HCH isomers and chlordane components.

Fraction three contains mainly dieldrin, endrin and endosulfan components.

For the acid-treated aliquot, only Fractions one and two are recovered, as the compounds in Fraction three are destroyed by the concentrated sulphuric acid, Table (VI.2).

VI.9 MEASUREMENTS

VI.9.1 Determination of PHCs by UVF

The fluorescence intensity of the sample analysed is compared with the fluorescence of a reference solution of almost the same concentration as the unknown extract or to a series of reference solutions.

After measurement of fluorescence, the extracts are concentrated to an appropriate volume (50-100 µl) using a gentle flow of clean nitrogen and injected into the gas chromatograph. Quantification of the internal standards should be made first in order to assess the % recovery of the extraction.

a) Calibration

The fluorescence intensity of the sample analysed is compared with the fluorescence of a reference solution of almost the same concentration as the unknown extractor to a series of reference solutions. When the measurement of fluorescence of the sample takes more than one day, the fluorescence of the reference solution must be measured at least once a day under identical instrumental conditions. To achieve comparability of results in the Region, each laboratory must use chrysene and the same crude oil standard. The standard crude oil will be provided to the participating laboratories by ROPME [ROPME Crude Oil, (RCO)]. From the stock solution prepared, a range of standard solutions of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 µg/ml are prepared. At least 100 ml of each solution should be prepared to allow adequate supply. This will require the use of a micro-pipette. The intensity of the fluorescence of the standards is then measured on a spectrofluorometer in a stoppered 1cm silica cell using a wavelength of 310 nm and an emission wavelength of 360 nm. The results are plotted as intensity of emission versus concentration. The plot should be linear to at least 5 µg/ml.

Chrysene standard solutions are prepared in the same way as for the crude oil. These standards are then run on the spectrofluorometer at the same sensitivity at which the crude oil standards were run, and a calibration plot prepared. An intercomparison ratio (R) can be calculated from the crude oil and chrysene standardisation curves according to the following formula :

$$R = \frac{\text{Fluorescence intensity of crude oil standard}}{\text{Fluorescence intensity of chrysene standard}} \times \frac{\text{Concentration in µg/ml of chrysene standard}}{\text{Concentration in µg/ml of oil standard}}$$

Typical runs of ROPME Crude Oil and Chrysene in a scanning spectrofluorometer are shown in Figure (VI.1).

Table (VI.2): Stability of different compounds to different treatment

Compounds	Treatment H_2SO_4	Treatment KOH/ MeOH	Transformation product
HCB	+	+	
Lindane	+	_	
2,4,5 trichlorobiphenyl (PCB 29)	+	+	
Alpha-HCH	+	-	
Aldrin	+	-	
pp' DDE	+	+	
Heptachlor epoxide	-	_	
Dieldrin	-	+	
op' DDE	+	+	
op' DDT	+	-	op' DDE
pp' DDD	+	-	pp' DDMU
op' DDD	+	-	
pp' DDT	+	-	pp' DDE
PCBs	+	-	
Toxaphene	+	+	
Phthalate esters	-	-	

+: Stable against treatment

-: Not stable against treatment

b) Fluorescence analysis of sample extracts and blanks

The fluorescence of sample extracts and blanks is measured in the same way as for the standard, i.e., at an excitation wavelength of 310 nm and an emission wavelength of 360 nm with the instrument set at the same sensitivity at which the chrysene and crude oil standards were run. This provides a detection limit of approximately 0.4 µg ROPME crude oil equivalent per liter (corresponding to 0.05 µg chrysene equivalent per liter).

It is recommended that a simple standard addition technique is also used to check the quantification of the results.

More information on differences in extract composition can be obtained from fluorescence emission spectra. Synchronous excitation and emission scanning (25 nm offset) is especially useful to provide information on relative aromatic ring number composition, Wakeham (1977).

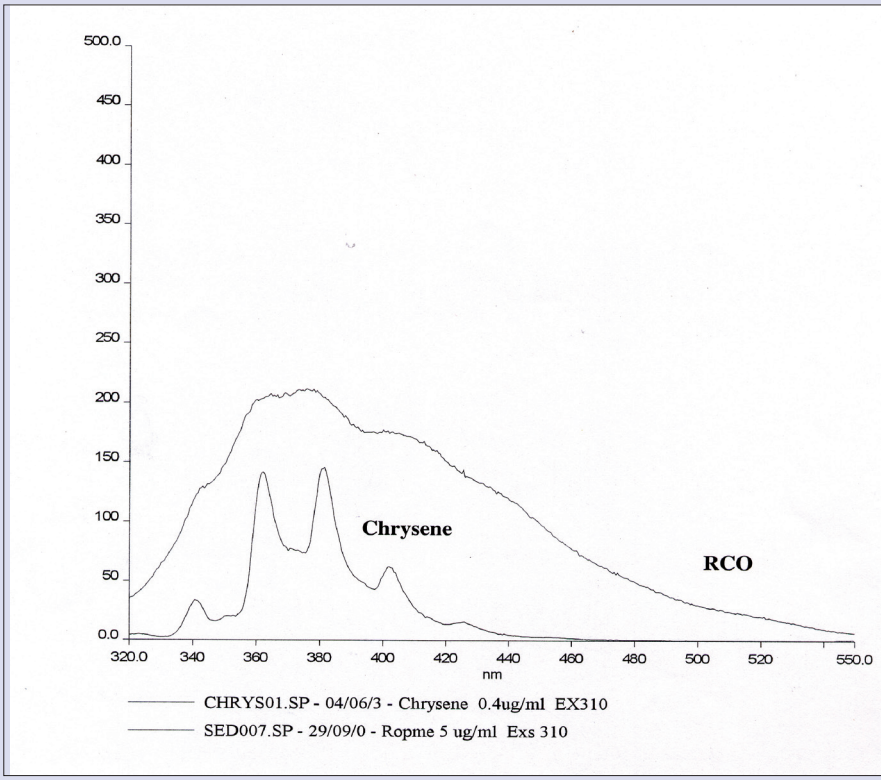


Figure (VI.1) : Typical fluorescence for ROPME Crude Oil (RCO) and Chrysene

c) Calculation of original sample concentration

The concentration of hydrocarbons in the sample extract, in crude oil equivalents, is read from the calibration curve and corrected for blank fluorescence. The original sample concentration in crude oil equivalents is then calculated according to the following :

$$A = \frac{B \times C}{D}$$

A = Concentration of hydrocarbons in original samples (µg/l water, µg/g sediment and biota)
B = Concentration of hydrocarbons in samples extract (µg/ml)
C = Volume of extract (ml)
D = Volume of original water sample (liters) or weight of original sediment or biota sample (grams)

The result is reported in crude oil or in chrysene equivalent concentrations (µg/l water, or µg/g of dry sediment or biota).

d) Testing for fluorescence quenching

Instances may occur where high concentrations of compounds, other than hydrocarbons, may be present in the sample that could quench or inhibit the fluorescence of the hydrocarbons in the sample. An experiment to test whether this is the case can be performed as follows:

a) the fluorescence of the sample extract is measured
b) the fluorescence of the crude oil standard is measured
c) equal volume of the sample extract and crude oil standard are combined and the fluorescence of the mixture is measured

The fluorescence of the mixture should be equal to :

$$\text{Fluorescence of the mixture} = \frac{\text{Fluorescence of crude oil standard}}{2} + \frac{\text{Fluorescence of crude oil standard}}{2}$$

If the fluorescence of the mixture is over 20% less than calculated from the above formula, significant quenching is occurring and sample clean-up should be attempted.

VI.9.2 Determination of Chlorinated Hydrocarbons by Gas Chromatography

Electron capture detector

High-energy beta particles (β^-), emitted by a radioactive source within the detector (e.g. a ^{63}Ni foil), are subjected to repeated collisions with carrier gas molecules, producing secondary electrons. These electrons (with the low energy of the thermal e^-), can be captured by sample molecules, eluting from a GC column. The resulting reduction in cell current is the operating principle of the electron capture detector. The detector current produced is actually a non-linear function of the concentration of electron-capturing material. However, the useful linear range of an ECD may be greatly improved if the instrument is operated at a constant current but in a pulsed mode, i.e. with short voltage pulses being applied to the cell electrodes. The current in the cell is kept constant by varying the frequency of the pulses.

The optimum flow for the “make-up” gas in an ECD is about 30-50 ml per minute (depending on the GC brand). Contamination of the detector (and thus lower sensitivity) may result from high boiling organic compounds eluting from the column. Periodic heating to 400°C may overcome this problem. The ^{63}Ni ECD can be used at 300°C under normal operational conditions, in order to limit such contamination.

Note : It is easy to check the response factor of one compound every day, when standards are injected, and by following it on a graph in the log book of the GC, to judge when it is necessary to heat the detector to clean it before it gets too dirty

i) Preparation of Standard Solutions

In order to achieve acceptable accuracy for the standard solutions, at least 50 mg of pure individual compound should be weighed and dissolved into 100 ml of hexane. This will give a stock solution of 500 ng/µl.

Example of preparation of stock solution :

Preparation of a stock solution of pp' DDE at approximately 500 ng/µl

The pp' DDE stock solution is prepared by dissolving approximately (but weighed accurately) 50 mg of pp' DDE in hexane in a 100 ml volumetric flask and bringing the volume to exactly 100 ml with hexane. If the actual weight of pp' DDE is 52 mg, then

$$\frac{52 \text{ mg DDE}}{100 \text{ ml solvent}} \times \frac{1000 \text{ µg}}{\text{mg}} \times \frac{\text{ml}}{1000 \text{ µl}} = \frac{52 \text{ µg DDE}}{100 \text{ µl solution}}$$

52 µg/100 µl => 0.52 µg/µl => 520 ng/µl

The concentration of the stock solution will be : 520 ng/µl

Preparation of the intermediate solution

Use the stock solution to prepare the intermediate solution. The concentration of pp' DDE in the intermediate solution should be approximately 5 ng/µl. To prepare the 5 ng/µl intermediate solution, transfer 1 ml of the stock solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1 \text{ ml DDE stock solution}}{100 \text{ ml final volume}} \times \frac{520 \text{ ng DDE}}{\text{µl}} = \frac{5.2 \text{ ng}}{\text{µl intermediate solution}}$$

The concentration of the intermediate solution will be : 5.2 ng/µl

Preparation of the working solution

Use the intermediate solution to prepare the working solution. The concentration of pp' DDE in the working solution could be approximately of 50 pg/µl (but depending on the sensitivity of the electron capture detector, it would be necessary to prepare less concentrated working solution such as 10 pg/µl or even less).

To prepare the 50 pg/µl working solution, transfer 1 ml from the pp' DDE intermediate solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1 \text{ ml DDE interm. solution}}{100 \text{ ml final volume}} \times \frac{5.2 \text{ ng}}{\text{µl}} \times \frac{1000 \text{ pg}}{\text{ng}} = \frac{52 \text{ pg}}{\text{µl working solution}}$$

The concentration of the working solution will be: 52 pg/µl

Note : Working solutions from the stock solutions are prepared on a regular basis and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that standards have not changed their concentrations through solvent evaporation. As all volumetric flask containing the standard solutions are kept in a fridge and are sealed with Teflon tape, the evaporation occur when the solution is taken out of the fridge and is opened. The more concerned one will be the working solution being used in the everyday work, it will be more subjected to evaporation and should then be changed every 6 months. Then as it is prepared from a more concentrated solution. This more concentrated solution will be opened only every 6 months, so it will be evaporated after a few years (4-5). Then this solution will have to be prepared again from the stock solution. The stock solution could be used many years before being discarded and re-prepared

ii) Quantification

PCBs represent a complex mixture of 209 compounds that cannot be resolved on capillary column. Also, there is no simple standard available for their quantification. Each peak in a sample chromatogram might correspond to a mixture of more than one individual compound. These difficulties have led to the recommendation of various quantification procedures. The usual method to quantify PCBs is to compare the chromatograms of commercially available industrial formulations such as Aroclor 1254 and Aroclor 1260, then within the Aroclor 1254, peaks that belong only to that mixture (at the beginning of the chromatogram) could be chosen to calculate the contribution of Aroclor 1254 to the sample. Then the peaks at the end of the chromatogram of Aroclor 1260 (that belong only to Aroclor 1260 mixture) could be used to calculate the contribution of the Aroclor 1260 to the sample.

Of course, the best way would consist to quantify PCBs as individual congeners when possible. In this case the quantification will follow the same method used for any individual compounds, using the peak area (or the peak height) of the standard and comparing them with the peak area (or the peak height) of the same compound in the sample. The formula to be used is the following:

$$|\text{Concentration}| = \frac{h \times C \times V \times 1000}{h' \times V(inj) \times M \times R} \text{ ng/g (or pg/g)}$$

Where:

V = total volume extract in ml
M = weight of sample extracted in gram
h = peak area or peak height of the compound in the sample
h' = peak area or peak height of the compound in the standard
C = quantity of standard injected in ng or pg
V(inj) = volume of sample injected in µl
R = recovery obtained for the sample

iii) Gas chromatographic conditions

- Gas chromatograph with a split/splitless injection system, separate regulation system for inlet and column pressures and temperatures, multi-ramp temperature programming facilities (preferably microprocessor controlled), electron capture detector interfaced with the column with electronic control unit and pulsed mode facilities. An integrator with a short response time (0.25s) is essential

- Narrow bore (0.22 mm internal diameter), 30 m long, fused silica open tubular column coated with 0.17 µm film thickness, chemically bonded 5% phenyl - methyl silicone phase (such as SE-54 or DB-5 or OV-5 or equivalent) with sufficient resolution to separate the relevant peaks in the standards provided for the PCB analysis

- Carrier gas should be high purity H_2. If this is not available or if the GC is not equipped with a special security system for hydrogen leak, He may be used. Gas purification traps should be used with molecular sieves to remove oxygen, moisture and other interfering substances

- High purity nitrogen gas (99.995%) as ECD make-up gas should be used (Argon/methane high purity gas is another option, but the ECD response will be lower than with nitrogen)

Conditions

- H_2 or He carrier gas at inlet pressure of 0.5 to 1 kg/cm^2 to achieve a flow rate of 1 to 2 ml/min. (in case H_2 is used as a carrier gas, the flow rate could be much higher than 1 or 2 ml/min. but, anyhow, the exhaust from the electron capture detector should be connected by a tube to the exterior of the laboratory to avoid any build-up of hydrogen in the room)

- Make-up gas N_2 or Ar/CH_4 (second choice) at the flow rate recommended by the manufacturer (between 30 and 60 ml/min.)

- ECD temperature of 300°C

- Injector temperature: 250°C (lower temperature could be used depending on the quality of the septum used)

- Septum purge: 1 ml/min

- Injector purge: 20 ml/min. (valve to be opened 50-60 seconds after injection)

- Temperature programme: 70°C for 2 min., then, increase at 3°C/min. to 260°C, then maintained at isothermal conditions until the end of the analysis (15 to 20 min)

Column preparation

Fused silica columns are the columns of choice for their inertness and durability (they are extremely flexible). They are made of material that is stable up to 360°C. The 5% phenyl-methyl silicone gum (SE-54, DB-5 or OV-5) liquid phase is present as a thin (0.17 µm), uniform film which tolerate temperatures up to 300°C. SE-54 is relatively resistant to the detrimental effect of solvents, oxygen and water, at least at low temperatures. These columns are even more resistant and durable if the liquid phase is chemically bonded to the support by the manufacturer.

For GC/MS work it is advised to restrict the film thickness to 0.17 µm because with thicker film some of the phase could be released, resulting in an increase of the noise signal in the GC/MS. Special columns for GC/MS could be found on the market, they are indicated by a "MS" after the name of the phase (ex: HP-5 MS).

The flexible fused silica columns can be conveniently connected to the inlet and outlet system without the transfer lines used in conventional glass capillary chromatography which often lead to increased dead volume. Low bleed graphite or Vespel ferrules provide a good seal.

The presence of extraneous peaks and elevated baseline drift will result in poor detector response performance. This can be caused by components, which elute from the column, such as residual solvents and low molecular weight liquid phase fractions on new columns, and build-up of later eluting compounds on old columns. Conditioning is a necessary step to remove these contaminants. New columns are connected to the inlet (while left unconnected to the detector). Columns are flushed with carrier gas at low temperature for 15 min. to remove the oxygen, then heated at 70-100°C for 30 min. and finally at 170°C overnight. The column can be then connected to the detector. Old columns can be heated directly to elevated temperatures overnight. The final temperature is selected as a compromise between the time required to develop a stable baseline and exceed column life. Thus, it may be necessary for older columns to be heated to the maximum temperature of the liquid phase resulting in shorter column life. The temperature of the ECD, when connected to the column, should always be at least 50°C higher than the column, in order to avoid condensation of the material onto the detector foil. It is essential that carrier gas flows through the column at all time when at elevated temperatures. Even short exposure of the column to higher temperature without sufficient flow will ruin the column.

Caution : If H_2 is used as a carrier gas, position the column outside of the oven to avoid explosion risk

Column test

A column performance test should be carried out at regular intervals.

The column remains in optimal condition as long as the liquid phase exist as a thin, uniform film. The quality of the film at the inlet side may be degraded as a result of repeated splitless injections. Decreased column quality may be remedied by the removal of the end of the column (10 to 20 cm) at the inlet side. Chemically bonded liquid phase require less maintenance. These columns can be washed with hexane (10-20 ml of hexane pushed through the capillary column with the help of nitrogen pressure) to remove impurities. It is very important that the hexane is injected on the

detector side so the impurities, being at the inlet side, will travel quickly out of the column.

Quality assurance/Quality control

Guidelines on the QA/QC requirements for analysis of sediments and marine organisms are detailed in Reference Method No 57, “Contaminant monitoring programme using marine organisms: Quality assurance and good laboratory practice”.

Precision

The precision of the method is established by the replicate analysis of samples of the appropriate matrix. The precision of the entire analytical procedure is estimated by extracting 5 sub-samples from the same sample after homogenisation. The principal advantage of using a RM is that the material permits the simultaneous evaluation of accuracy while offering a well homogenised sample. Precision is evaluated as a matter of course during the initial implementation procedure just before initiation of sample analysis.

Accuracy

The accuracy of the method is confirmed by analysis of a suitable RM (i.e. appropriate matrix, analytes) prior to initiation of sample analysis. Agreement between measured and certified concentrations for any individual analyte should be within 35% and on average, within 25%. Further description of the preparation of control charts and criteria for data acceptance are elaborated in Reference Method No 57.

Blanks

Blanks represent an opportunity to evaluate and monitor the potential introduction of contaminants in the reagents, those arising from passive contact between the sample and the environment (e.g. the atmosphere) and those introduced during sample handling by hands, implements or glassware. It is essential to establish a consistently low (i.e. with respect to analytes) blank prior to initiating analysis or even the determination of the method detection limit. In addition, it is necessary to perform blank determinations on a regular basis (e.g. with every batch of samples).

Recovery

Recovery reflects the ability of the analyst to fully recover surrogate compounds introduced to the sample matrix or blank at the beginning of the procedure. The primary criteria for selection of compounds to be used for testing recovery are that they: 1) have physical (i.e. chromatographic/ partitioning) properties similar to and if necessary spanning those of the analytes of interest, 2) not suffer from interferences during gas chromatographic analysis, 3) be baseline resolved from the analytes of interest. Recovery is tested on all samples and blanks as a routine matter of course.

VI.9.3 Determination of Aliphatic and Aromatic Hydrocarbons by GC/FID and GC/MS

Gas Chromatography Conditions

Gas Chromatograph	:	
Detector	:	FID
Injection Technique	:	On column
Injector temperature	:	30°C
Injection Volume	:	1 µl
Carrier gas	:	Helium or Hydrogen
Flow rate	:	1-2 ml/min. for Helium, 10 ml/min. for Hydrogen

Column used :

Type of column	:	Capillary
Length	:	25 m (but analysts are encouraged to use longer ones)
Diameter	:	0.32 mm (for on-column injector)
Phase	:	SE - 54 (or equivalent: DB-5, OV-5, etc.)
Film thickness	:	0.17 µm

Temperature program :

Initial temperature	:	60°C
Rate	:	3°C/min
Final temperature	:	300°C
Isothermal	:	20 min.
Detector temperature	:	310°C
Air flow	:	300 ml/min
Hydrogen flow	:	30 ml/min

Confirmation

GC/MS	:	
Detector	:	MS - SIM
Injection Technique	:	Splitless
Injector temperature	:	270°C
Injection Volume	:	1 µl
Splitter closing time	:	1.5 min.
Carrier gas	:	Helium
Flow rate	:	1-2 ml/min

Column used :

Type of column	:	Capillary
Length	:	30 m (or longer)
Diameter	:	0.25 mm
Phase	:	SE - 54 (or equivalent, better if specified “MS” quality)
Film thickness	:	0.17 - 0.25 µm

Temperature program :

Initial temperature	:	60°C
Isothermal	:	1 min
First rate	:	10°C/min
To	:	100°C
Second rate	:	4°C/min
To	:	285°C
Isothermal	:	10 min
Interface temperature	:	285°C

Gas chromatography using fused silica capillary columns

Better resolution is achieved by using an instrument manufactured to accommodate fused silica capillary column. In order to achieve stable response factors, all of the gases must be of ultra pure grade. Each must have accurate flow control based on multiple staged constant pressures. Extreme caution to eliminate oxygen and water in the lines is necessary. Columns useful for hydrocarbon analysis are 5% phenyl methyl silicone (SE-54 or equivalent) chemically bonded onto fused silica. Optimum performance is achieved with a “Grob” type on-column injector, which requires 0.32 mm i.d. columns. Alternatively, a split/splitless injector using 0.25 mm i.d. columns can be used. Carrier gas can be hydrogen, helium or nitrogen, depending on the availability of high quality grades. Safety cut offs must be included if hydrogen is used. The carrier gas should be plumbed independently of the detector gases. As flow rates are low, make-up gas is essential. The flow rate of the carrier gas should be optimised. The following procedure is useful for 25 m columns and H_2 carrier gas. Set oven temperature to 150°C. If using splitless injection, time the valve opening for 60 seconds after injection. Set carrier flow rate to approximately 10 psi or 1 to 2 ml/min. Inject 1 µl of a solution containing 20 ng/µl n-tetradecane (C_{14}). Time the emerging of the C_{14} peak from the leading edge of the solvent peak. Adjust the carrier flow rate to make this difference approximately 2.7 min. Then adjust the flow of H_2, air and N_2 through the detector to give optimum response. With these optimum flows, the system is now ready for calibration. Under the conditions given in Table (VI.3), it will take approximately 30 min. to elute C_{17}. C_{17}/pristane and C_{18}/phytane should show baseline resolution and the entire run will take approximately 70 min.

Successful analyses using gas chromatography is a skill acquired by years of practice. The novice is referred to Freeman (1981) for further details.

Table (VI.3) : Conditions for a capillary gas chromatograph

Carrier gas	:	Helium at approximately 8 psi or 60 kPa (1 to 2 ml/min.)
Make-up gas	:	N_2 at 40 ml/min. (not for all GCs)
Column	:	Fused silica SE-54 (or equivalent: DB-5, OV-5, etc.)
Injector temperature	:	On-column: 30°C
		Splitless: 250°C
Detector temperature	:	310°C
Initial oven temperature	:	60°C
Programme rate	:	3°C/min
Final Temperature	:	300°C
Final Time	:	20 min
Injection volume	:	0.3 - 0.5 µl for On-column
		1 µl for splitless

Calculations for resolved components

First the chromatograph is calibrated by injecting an appropriate standard mixture which includes all the surrogate standards used in the analysis. The Response factors (RFs) for the aliphatic fraction are calculated in area/ng (or area/pg):

$$RFc_{10} = \frac{\text{Area of } c_{10} \text{ by integrator}}{\text{Quantity of } c_{10} \text{ injected}}$$

Then the same calculation is done for RFc_{12}, RFc_{14}, etc. All RF are expressed in unit area given by the integrator/ng. Then the average RF is calculated for the aliphatic fraction :

$$\text{Average RF}_{\text{aliphatics}} = \frac{\text{Sum of all RF}}{\text{Number of compounds used}}$$

Calculation of the recovery

When the extraction started, 2 internal standards were added for the first fraction: n-C_{19} d_{40} and n-C_{32} d_{66}. The calculation of the recovery for the first fraction is done by calculating the quantities of n-C_{19} d_{40} and of n-C_{32} d_{66} recovered through the complete procedure.

$$\text{Quantity } C_{19}d_{40} = \frac{\text{Area n-}C_{19}\ d_{40} \text{ in sample}}{\text{Average RF}} \times \frac{\text{Volume of extract}}{\text{Volume injected}}$$

$$\text{Quantity } C_{32}d_{66} = \frac{\text{Area n-}C_{19}\ d_{40}\text{ in sample}}{\text{Average RF}} \times \frac{\text{Volume of extract}}{\text{Volume injected}}$$

$$\text{The recovery for n-}C_{19}\ d_{40}\text{ will be : } \frac{\text{Quantity of n-}C_{19}\ d_{40}\text{ recovered}}{\text{Quantity added when extraction began}} \times 100$$

$$\text{The recovery for n-}C_{32}\ d_{66}\text{ will be : } \frac{\text{Quantity of n-}C_{32}\ d_{66}\text{ recovered}}{\text{Quantity added when extraction began}} \times 100$$

The recovery for the aliphatic fraction will be the average of the two recoveries :

$$\text{Recovery Aliphatics} = \frac{\text{Recovery for n-}C_{19}\ d_{40} + \text{recovery for n-}C_{32}\ d_{66}}{2}$$

Doing this, you take into account the great variability of volatility for the aliphatic fraction.

Calculation of individual aliphatic compounds

For all identified compounds within the first fraction, the quantity of these compounds could be calculated as :

$$\text{Quantity}_{\text{compound}} = \frac{\text{Area of compound in sample} \times \text{Final volume of extract}}{\text{Average RF} \times \text{Volume injected} \times \text{Recovery for aliphatic fraction}}$$

Quantity of compound in the sample = Quantity $_{\text{compound}}$ in sample - Quantity $_{\text{compound}}$ in Blank

The concentration can be calculated by :

$$|\text{C compound}| = \frac{\text{Quantity of compound in the sample}}{\text{Quantity extracted}}$$

This calculation has to be done for all aliphatic compounds from n-C_{10} to n-C_{34}.

Calculation of the total resolved aliphatics (TRA)

The area of the total resolved aliphatics is given on the integrator by the total area to which should be removed what has been added, such as the solvent and of course, the areas of the internal standards added at the beginning of the extraction. The best way to do it for the solvent peak is to start the integration after the solvent peak has eluted (at a retention time of 5-10 min.)

$$\text{TRA} = \frac{[\text{Total Area - Area of I.S. - Area solvent peak}] \times \text{Final volume of extract}}{\text{Average RF} \times \text{Volume injected} \times \text{Recovery of the aliphatic fraction}}$$

$$\text{Concentration of TRA} = \frac{\text{TRA in sample} - \text{TRA in Blank}}{\text{Quantity extracted}}$$

Calculations for unresolved components

The calculation of the UCM is the same than the calculation of all peak, the Response Factor should be calculated first:

$$RF_{UCM} = \frac{\text{Peak Area of the Standards}}{\text{Quantity of Standards injected}}$$

The peak area should be expressed in mm^2

Recalculate, for all aliphatics, the Response Factors, using the peak height and peak width at mid-height, Figure (VI.2).

Peak Area = (Peak height) x (peak width at mid-height)

Then, calculate the average Response Factor in mm^2/ng.

Cut, on a piece of integrator paper, a square portion of paper of 10 cm x 10 cm, this will represent: 100 cm^2, or 10^4 mm^2.

Weight this piece of paper to the mg.

You obtain a factor in mm^2 /mg by dividing 104 by the weight obtained.

With another piece of the integrator paper, draw the UCM and then, cut it and weight it to the mg.

The area of the UCM will be : Weight of UCM x Factor in mm^2 / mg

The Quantity of UCM will be :

$$UCM = \frac{\text{Area UCM in } mm^2 \times \text{Final Volume of Extract}}{\text{Average RF in } mm^2/ng \times \text{Volume injected} \times \text{Recovery of the aliphatic fraction}}$$

The real quantity of UCM in the sample will be :

Real Quantity $_{UCM}$ = (Quantity $_{UCM}$ in the sample) - (Quantity $_{UCM}$ in the Blank)

The concentration of the Unresolved Complex Mixture in the sample will be :

$$|C_{UCM}| = \frac{\text{Real Quantity}_{UCM}}{\text{Quantity extracted}}$$

Note : Some computer programmes are now available for acquisition of GC data and calculation of the results. For petroleum hydrocarbon analysis, the software must be capable of calculating the UCM independently of the peaks (e.g.: Agilent Technologies Inc. and their Chemstation software)

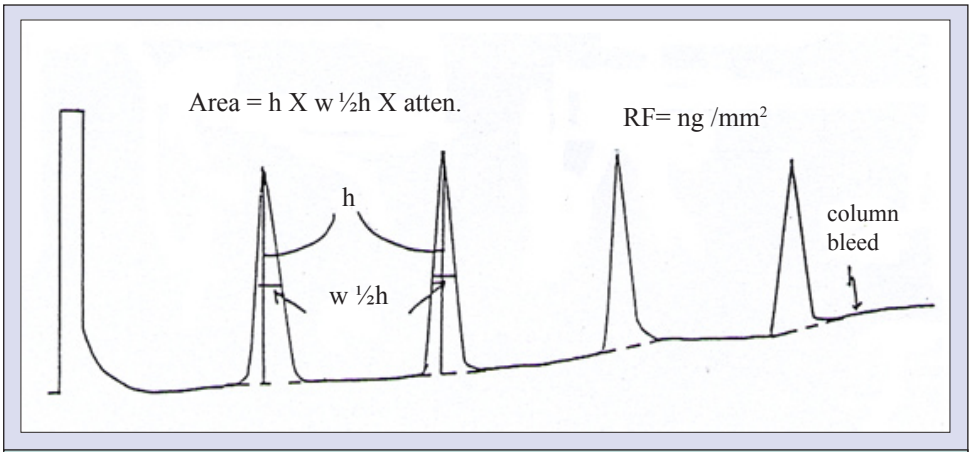


Figure (VI.2) : Measuring peaks areas and calculating response factors manually

Computerized gas chromatography/Mass spectrometry (GC/MS)

A computerized GC/MS system capable of selected ion monitoring (SIM) greatly extend the scope for determining the presence and concentration of specific marker compounds that may not be visible in a complex mixture of hydrocarbons seen by GC with FID. This technique requires extensive investment in analytical equipment and scientific expertise. The information below is intended as a starting point for applying this expertise to analysis of samples for petroleum pollution. Conditions useful for hydrocarbon analysis are given in Table (VI.4).

Table (VI.4) : Conditions for the GC attached to the MS

Carrier gas	1 ml/min. flow rate at 200°C oven temperature
Column	HP - 5 MS bonded fused silica (or equivalent)
Injector temperature	280°C for split/splitless; 30°C for On-column
Initial oven temperature	50°C
Initial time	1 Min
Program rate	4 to 6 °C/min
Final temperature	280°C
Final time	20 Min
Injection volume:	0.3 to 1 µl

The mass spectrometer is operated in the electron impact ionisation mode with an electron energy of 70 eV. It is tuned to optimum sensitivity according to the manufacturer specifications. The column is extended all the way through to the ion source on most modern GC/MS instruments and on the mass selective detectors (MSDs). Mass to charge ratios (m/z) for the most abundant fragments or "target" ions of aromatic hydrocarbons found in the light to medium weight oils are listed in Table (VI.5). These are the major compounds of usual interest in the determination of petroleum hydrocarbons in marine sediments.

The range of hydrocarbons to be quantified will need to be established in each sample set. Benzenes are relatively soluble and volatile and may not be expected to persist in oil contaminated marine sediments unless the residues are isolated from oxidation, solution and volatilisation processes. Thus, it will be important to determine the concentrations of the low molecular weight aromatics only in sediments contaminated with relatively fresh crude oil or light fuel oils. With weathering, these fractions are expected to evaporate or dissolve and the analytical strategy then emphasizes the higher molecular weight compounds.

Prior to use a quantitative instrument for low level analysis of PAH, the linear range of response must be determined by injecting a series of standard mixtures of PAH and by constructing calibration curves. Quantitative analysis can only be conducted when the concentrations of analytes are within the linear calibrated range.

Similarly to GC calibration data, continuous records of the response factors generated must be used to evaluate daily performance. Daily verification checks should generate RFs, which deviate from initial average calibration by no more than 40% for each analyte.

Scan mode GC/MS

In samples of relatively fresh oil, the aromatics are likely to be present in concentrations suitable for analysis by scanning mode GC/MS. This is especially true if saturated and aromatic fractions have been separated using the preparative chemistry procedures.

Quantification by GC/MS can be achieved in a similar manner to GC/FID when the mass spectrometer system is calibrated with standard solutions of known concentrations. However, response factors are determined from the ion current generated and will depend on the molecular structure of the compounds and their fragmentation patterns. Response factors are generated for each compound of interest from the integrated areas of specific parent ions acquired in scan mode.

The calibration solution containing the parent hydrocarbons and as many alkyl substituted compounds as available is injected into the GC/MS operated in the scan or total ion current (TIC) mode. This establishes the elution time of the PAH. Table (VI.6) is an example of RT data for a standard PAH mixture. The positions of the alkyl substituted peaks for which pure standards are unavailable are determined by programming the computer to generate reconstructed ion chromatograms using m/z ratios specific to each class of compounds (128 for naphthalenes, 178 for phenanthrenes, etc.).

Table (VI.5) : "Target" ions to be used for quantification and "Confirming" ions. The relative percentage of the confirming ions with range shown vary as a result of different isomer configurations within the alkyl group

Compounds	Target	Confirming	% Abundance
Benzene	78		
C_1 - benzene	92		
C_2 - benzene	106		
C_3 - benzene	120		
C_4 - benzene	134		
Naphthalene	128	127	10
C_1 - naphthalene	142	141	80
C_2 - naphthalene	156	141	47 - 95
C_3 - naphthalene	170	155	61 - 300
C_4 - naphthalene	184	169	189
Acenaphthylene	164	162	32
Acenaphthylene	152	151	20
Fluorene	154	153	86
C_1 - fluorene	166	165	80
C_2 - fluorene	180	165	95 - 144
C_3 - fluorene	194	179	25
C_4 - fluorene	208	193	
d_{10} - phenanthrene	188	187	98
Phenanthrene	178	179	16
Anthracene	178	176	20
C_1 - phenanthrene/anthracene	192	191	39 - 66
C_2 - phenanthrene/anthracene	206	191	16 - 150
C_3 - phenanthrene/anthracene	220	205	
C_4 - phenanthrene/anthracene	234	219, 191	73 - 297
Dibenzothiophene	184	185	14
C_1 - dibenzothiophene	198	197	53
C_2 - dibenzothiophene	212	211	
C_3 - dibenzothiophene	226	211	
C_4 - dibenzothiophene	240	211	
Fluoranthene	202	200	17
Pyrene	202	200	21
C_1 - fluoranthene/pyrene	216	215	36 - 64
Benz[a]anthracene	228	226	19
Chrysene	228	226	21
C_1 - benzanthracene/chrysene	242	243	20
C_2 - benzanthracene/chrysene	256	241	75 - 131
C_3 - benzanthracene/chrysene	270	255	
C_4 - benzanthracene/chrysene	284	269, 241	
d_{12} - perylene	264	260	21
Perylene	252	253	22
Benzo[b or k]fluoranthene	252	253	23
Benzo[a or e]pyrene	252	253	22
Indeno[1,2,3-c,d]pyrene	276	138	50
Dibenz[a,h]anthracene	278	279	24
Benzo[g,h,i]perylene	276	138	37

Table (VI.6) : Example of retention time reproducibility achieved with manual injection of 1 µl aliquots of standard PAH solution into an SIP mass selective detector system

Peak	Target ion	Retention Time (min.)
Naphthalene	128	17.944 ± 0.031
2-methyl-naphthalene	142	22.010 ± 0.041
1-methyl-naphthalene	142	22.632 ± 0.041
Acenaphthene	153	28.566 ± 0.034
Fluorene	166	31.690 ± 0.039
Phenanthrene	178	37.518 ± 0.034
Fluoranthrene	178	44.918 ± 0.034
Pyrene	202	46.222 ± 0.038
1,2-benzanthracene	228	53.782 ± 0.034
Chrysene	228	54.017 ± 0.036
Perylene	252	62.242 ± 0.040
1,2,5,6-dibenzanthracene	278	69.537 ± 0.064

Peak confirmation is achieved by evaluating the retention times of the peaks on the reconstructed ion chromatograms and evaluation of the associated spectrum. The spectra are also used to determine if any co-eluting substances are contributing to the areas of target ions to base used for quantification.

For the peaks that can be calibrated against pure standards in the calibration mixture, the response factors are calculated directly by dividing the area of the parent ion peaks by the amount injected. Very few alkyl PAH compounds are available as pure standards. Thus to compute the amounts of the alkyl PAH, the response factor of the parent compound must be used. This is not rigorously accurate for quantification of the alkyl PAH and methods for estimating the response factors have been proposed.

Once a table of RFs has been constructed, the concentrations of PAH and alkyl PAH in samples can be calculated by acquiring data in the scan mode. Then reconstructed ion chromatograms are generated using each of the target ions. The integrated peak areas are then divided by the appropriate RF of each peak similar to the method for quantification by GC/FID. This is the external standard method.

Co-injection of internal standards increases quantitative accuracy in a manner similar to GC/FID analysis. The internal standard must not be present in the sample, but it must be structurally similar to the compounds of interest. Deuterated analogues of PAH are useful internal standards. Structural similarity is important for the following reason: the justification for using substances other than the compound to be quantified rests on the assumption that the standard yields equal, or very similar, total ion current per amount injected. This assumption is valid only for related structures. For the internal standard method, the external response factors are redefined in relation to the series of internal standards and a new table of RF_{IS} is generated. The internal standard must be included in the calibration runs. The quantification of the naphthalene series is based on the internal deuterated biphenyl (ion 164). Deuterated phenanthrene (ion 188) is used for the quantification of the phenanthrene, dibenzothiophene, and fluorine series. Deuterated perylene (ion 212) is used for the

quantification of the fluoranthene and pyrene series. Deuterated perylene (ion 264) is used for the quantification of the chrysene, benzanthracene, perylene and benzopyrene series.

$$RF_{IS}\ \text{naphthalene} = \frac{\text{Area ion 128 / ng naphthalene}}{\text{Area ion 164 / ng } d_{10}\text{-biphenyl}}$$

Sample calculations are done as follows from area based on reconstructed ion chromatograms from sample runs.

$$[C_{\text{naphthalene}}] = \frac{\text{Area ion 128}}{RF_{IS}\ \text{naphthalene}} \times \frac{\text{ng } d_{10}\text{-biphenyl}}{\text{Area ion 164}}$$

The practical detection limit for GC/MS analysis in scan mode is generally in the range of 5 to 10 ng per compound injected.

Selected ion monitoring mode

Sensitivity and signal to noise ratio is greatly increased by acquiring the data in the selected ion monitoring (or SIM) mode. In this procedure, the time windows for ion monitoring are established with scan data for pure compounds and standard oils. Then an acquisition program is written in which specific ions are scanned over the appropriate time windows in SIM mode. A sample SIM acquisition program developed for oil spill studies is shown in Table (VI.7).

As described above, compound identification is based on the relative retention indices and the full mass spectra of the compounds. The full mass spectra are required to avoid mistakenly integrating fragment ions of non-PAH compounds co-eluting with the PAH as if they were actual PAH molecular ions. The mass spectra can only be obtained in a full scan run since SIM mode does not collect mass spectra. For routine SIM acquisition, Table (VI.5) lists some confirmatory ions and the approximate percent relative abundance. These percentages can be used as a first level of confirmation of peak identity for SIM data.

Quantification by SIM mode GC/MS is done by external or internal standard methods as described for scan mode. A calibration solution containing PAH standards and the deuterated internal standard is injected and the data acquired using the SIM acquisition program.

The integrated peak areas of the specific ions within appropriate time windows are stored. The response factor table is then used to calculate the amount of each compound in a manner identical to that described for scan mode.

The detection limit for PAH in SIM mode is generally in the range of 40 pg per compound injected. The linear calibration range is generally up to approximately 5 ng. Samples should be concentrated or diluted to yield area counts within the linear range of instrument calibration.

Table (VI.7) : Example of a selected ion monitoring program useful for quantitative analysis of aromatic hydrocarbons in oil spill studies

Compounds	Target	Confirming	% Abundance
Group 1 (15 - 25 min.)			
Naphthalene	128	127	15.5
C_1 - naphthalene	142	141	19.5 - 20.1
C_2 - naphthalene	156	141	23.3 - 25
d_{10} - diphenyl	164	162	47-95
Acenaphthylene	152	151	24.7
Group 2 (25 - 35 min.)			
Acenaphthene	154	153	25.7
C_3-benzothiophene	176	161	25.9 - 27.6
C_4-benzothiophene	190	175	27
C_3 - naphthalene	170	155	26.6 - 29.2
C_4 - naphthalene	184	165	29.7 - 33.5
Fluorene	166	179	28.9
C_1 - fluorene	180	193	32.5 - 32.9
Dibenzothiophene	188	185	33.9
d_{10} - phenanthrene	188	197	34.6
Phenanthrene/anthracene	178	179	34.7
Group 3 (35 - 40 min.)			
C_2 - fluorene	194	179	36
C_3 - fluorene	208	193	39.6
C_1 - dibenzothiophene	198	197	36.7 - 37.3
C_2 - dibenzothiophene	212	211	39.1 - 41.0
O-terphenyl	229	230	37.7
C_1-phenanthrene/anthracene	192	191	37.9 - 38.5
Group 4 (40 - 55 min.)			
C_3 - dibenzothiophene	226	211	41.6 - 44.2
C_4 - dibenzothiophene	240	211	44.9
C_2-phenanthrene/anthracene	206	191	41.3 - 42.2
C_3-phenanthrene/anthracene	220	205	44.0 - 44.3
C_4-phenanthrene/anthracene	234	219/191	
Fluoranthene/pyrene	222	200	41.9 - 43.2
C_1 - fluoranthene/pyrene	216	215	45.5 - 46.8
Benzanthracene/chrysene	228	226	50.7 - 50.9
C_1 - benzanthracene/chrysene	242	243	54.0
Group 5 (55 - 70 min.)			
C_2 - benzanthracene/chrysene	256	241	55.0 - 55.7
Benzofluoranthenes	252	253	56.9 - 57.1
Benzopyrenes	252	253	58.8
d_{12}-perylene	264	260	58.8
Perylene	252	253	58.9
Indenopyrene	276	138	64.3
Dibenzanthracene	278	279	64.5
Benzoperylene	276	138	65.7

Potential corrections for alkyl PAH response factors

As stated above, the assumption that the response factors for the alkyl homologues are equal to that of the corresponding parent PAH is not rigorously accurate. It will be shown below that the use of RFs calculated from parent compounds may underestimate the content of alkyl PAH by as much as 50%. Several procedures for interpolating the response factors have been suggested and will be mentioned here.

At the time of writing this manual there is no standard method for quantification of compounds by GC/MS for which pure standards are unavailable. It therefore remains the responsibility of the expert analyst to defend the calculation procedures used or to follow the guidelines suggested in individual monitoring programs.

Calibration using standard oils

This procedure was described in the original version of this manual and is reproduced here. It relies on an interplay between GC/FID for determining amounts of alkyl PAH in fractions obtained from a standard oil and the internal standard method on the GC/MS.

The response factors of the different alkyl PAH relative to the appropriate deuterated standards are determined by injecting known amounts of the aromatic components in a mixture of Ekofisk Crude oil (rich in naphthalenes and phenanthrenes) and Arabian Light Crude oil (rich in dibenzothiophenes). The standard oils (20 mg) are first separated into saturated and aromatic fractions by adsorption chromatography. The aromatic fractions are then reduced to near dryness and retained in 100 µl hexane.

These two aromatic mixtures are then analyzed by high resolution GC/FID using the same conditions that will be used for the GC/MS analysis. The composition of the aromatic fraction (in percent) is calculated from the areas on the gas chromatograph. After the peak areas of the unspiked mixtures are calculated, exact amounts of naphthalene, 2,6-dimethyl naphthalene and 2,3,6-trimethyl naphthalene are summed so that the response factors of the naphthalene series can be determined. Phenanthrene is added to determine RFs for the methyl-phenanthrenes and methyl dibenzothiophenes. Fluoranthene is added for the determination of RFs for dimethyl phenanthrenes, di- and trimethyl dibenzothiophenes. The new mixtures are then analyzed by GC/FID and the peak areas integrated. The calibration information is now sufficient to determine the concentrations of naphthalenes, phenanthrenes, and dibenzothiophenes in the aromatic mixtures by GC/MS.

To calculate the RFs for these compounds on the GC/MS, known amounts of the deuterated standards are added to the aromatic mixtures and the samples are then analyzed by GC/MS in the SIM acquisition mode. Since both the amounts of the aromatic hydrocarbons and the deutereted standards are now known, the RFs can be calculated from the peak areas of target ions integrated by the computer. For example, the RF for naphthalene in relation to d_{10}-biphenyl is the number used to divide the area of the 128 fragment to obtain the true concentration.

$$RF_{IS}\ \text{naphthalene} = \frac{\text{Area ion 128 / pg naphthalene}}{\text{Area ion 164 / pg } d_{10}\text{-biphenyl}}$$

The average response factors for each class of PAH are then used to determine the concentrations of each compound within the class in sample extracts.

Interpolation using relative ion abundances

Another method that has been proposed is to calculate response factors for alkyl-PAH from the relative abundance of specific ions using scan data. The mass spectrum of the substance to be quantified is examined and the area (A) under the reconstructed ion chromatogram of its most intense fragment (usually, but not always, the molecular ion of PAH) is integrated. Using the full mass spectrum of the substance, the abundance (RI) of this most intense fragment is calculated relative to the sum of abundances of the 10 most intense fragments including itself. This ratio is independent of the amount of the alkyl-PAH present in the sample. Very few alkyl substituted PAH are available to determine this ratio from pure standards. It can be computed from tabulated spectra from a library generated on a similar instrument. If the required spectra are not available in tabulated libraries and if the peaks of interest are well resolved from interfering compounds, the ratios can be computed from a scan data obtained from the aromatic fraction of a standard oil run under the same conditions. Subtracting background interference should result in ratios of ion abundances similar to those obtained from pure standards. However, spectra obtained from complex mixtures cannot be used to compute the ion abundance ratios if the spectrum of the alkyl-PAH is contaminated with a co-eluting substance that contributes any peaks with ion abundance greater than the least intense of the 10 most abundant ions in the mass spectrum of the pure alkyl-PAH. The ion abundance ratios for the alkyl-PAH are called R1 values and the ratios for the parent PAH are called R2 values, Table (VI.8). The system is calibrated on a routine basis for quantification in SIM mode by injecting a calibration solution of the parent hydrocarbons and the alkyl-PAH for which pure standards are available. Areas for the major ions to be used in quantification are tabulated as are those of a few “confirming” ions Table (VI.7). Response factors (RFs) for the parent PAH are calculated as areas of the major ions per pg. The response factor (RF alkyl) for the alkyl-PAH is then equal to the response factor (RF) of the PAH multiplied by R1/R2.

$$RF_{alkyl} = RF \times R1 / R2$$

A table of RF and RF alkyl is constructed to include all the “target” compounds, Table (VI.9). The corrected response factor RF alkyl is usually smaller than the response factor RF (in unit of area / pg), because the parent peak is in the spectrum of an alkyl-PAH, in many cases, contributes less to the total ion intensity than the parent peaks in the spectrum of the corresponding un-substituted PAH.

The amount of alkyl-PAH in pg is :

$$[\text{PAH alkyl}] = \frac{\text{Area}}{RF_{alkyl}}$$

Table (VI.8) : Ion abundance ratios R1 or R2 (base ion / sum 10 ions) calculated from library spectra compared to spectra generated from direct injection of pure standards into an H.P. mass selective detector system

Compounds	R1 or R2 NBS.L	R1 or R2 HCARB.L	R1 or R2 Stand. Inj.
Naphthalene	57.3		57.3
2 methyl-napthalene	37.1		38.5
1 methyl-naphthalene	38.3		36.7
2,3 di-methyl-naphthalene	37.3		33.9
Acenaphthylene	52.4		51.1
Acenaphthene	32.1		29.7
Fluorene		40	37.2
Dibenzothiophene	58.8		59.4
Anthracene		53.2	49.0
6-terphenyl	35.1	33.6	33.9
3,6 di-methyl phenanthrene	43.4	43.4	41.5
Fluoranthene	54.7	54.7	53.5
Pyrene	44.8	50.4	51.0
1 methyl-pyrene	43.6	40.9	42.0
Benz[a]anthracene	51.2	51.6	
Chrysene	51.1	51.1	50.0
1 methyl-chrysene		49.0	49.0
Benzo[k]fluoranthene		46.3	46.2
Benzo[a]pyrene	48.2	55.9	48.4
Perylene		47.1	51.8
Indenopyrene	44.3		51.8
Dibenzanthracene	216		57.6

The accuracy of this interpolation method can be checked by direct determination of response factors for the alkyl-PAH for which pure standards can be obtained. Table (VI.8) shows the ion abundance ratios for the 'target' compounds obtained from unpublished libraries and confirmed where possible by scan data from direct injection of pure alkyl-PAH standards. Examples of the interpolated response factors for the alkyl-PAH are listed in Table (VI.9). Also shown in Table (VI.9) are the RFs for a few alkyl-PAH calculated from the direct injection of the standards. Agreement between the interpolated RF alkyl and those calculated from direct injection of standards confirms that the method appears to work reasonably well.

This quantification procedure is further improved if internal standards are included in both the calibration solutions and in sample. In this case the internal standards used to correct for the fraction of the total sample injected (dilution factor, XF) as per GC analysis. Including three standards, which elute at different times allows the analyst to note and correct for any discrimination over the elution range which may be due to the injector or detector configurations.

Table (VI.9) : Table showing R1/R2 ion abundance corrections for the alkyl-PAH and sample calculations of response factors. Compounds for which no published spectra or pure standards could be located are given the RF of the nearest alkyl-substituted or the parent PAH

Compounds	Target	**R1/R2**	Standard **RF**	**RF** $_{alkyl}$
Benzene	78			
C_1 - benzene	92			
C_2 - benzene	106			
C_3 - benzene	120			
C_4 - benzene	134			
Naphthalene	128		106	
C_1 - naphthalene	142	0.67	75	71
C_2 - naphthalene	156	0.59	58	63
C_3 - naphthalene	170	0.71	71	75
C_4 - naphthalene	184	0.69		73
Acenaphthylene	164			119
Acenaphthylene	152			
Fluorene	154		75	
C_1 - fluorene	166	0.85		63
C_2 - fluorene	180			63
C_3 - fluorene	194			63
C_4 - fluorene	208			
d_{10} - phenanthrene	188		77	
Phenanthrene	178		114	
Anthracene	178		114	
C_1 - phenanthrene/anthracene	192	0.80		92
C_2 - phenanthrene/anthracene	206	0.81		92
C_3 - phenanthrene/anthracene	220	0.67		77
C_4 - phenanthrene/anthracene	234	0.61		70
Dibenzothiophene	184		122	
C_1 - dibenzothiophene	198	0.83		101
C_2 - dibenzothiophene	212			101
C_3 - dibenzothiophene	226			101
C_4 - dibenzothiophene	240			101
Fluoranthene	202		132	
Pyrene	202		135	
C_1 - fluoranthene/pyrene	216	0.78		105
Benz[a]anthracene	228		79	
Chrysene	228		102	
C_1 - benzanthracene/chrysene	242	0.89		70
C_2 - benzanthracene/chrysene	256	0.61		48
C_3 - benzanthracene/chrysene	270			48
C_4 - benzanthracene/chrysene	284			48
d_{12} - perylene	264		34	
Perylene	252			
Benzo[b or k]fluoranthene	252			
Benzo[a or e]pyrene	252			
Indeno[1,2,3-c,d]pyrene	276			
Dibenz[a,h]anthracene	278			
Benzo[g,h,i]perylene	276			

Interpolation using assumed increments within classes

It has been suggested that the response factors within a class of compounds can be assumed to vary in a predictable manner with alkyl substitution. This variation can be determined on individual GC/MS systems from a series of PAH ranging from parent through substituted compounds for which standards are available. For example, the series: naphthalene; 1-methyl- and 2-methyl-; 2,3, and 2,6-dimethyl-naphthalenes; 2,3,6-trimethyl-naphthalene. The increment of variation within the series is then used to estimate the change in response factor with alkyl substitution. Data included in Table (VI.9) indicates that this method is not very accurate.

Other marker compounds

There are numerous other qualitative and quantitative GC/MS analysis that are possible for specific monitoring programs. For example, it may be possible to identify specific steroid or triterpenoid hydrocarbons indicative of a particular oil input from an offshore platform effluent to use as a tracer for that input. Figure (VI.3) shows the triterpane pattern determined by selected monitoring of ion 191 of the saturated hydrocarbons fractions of three different source oils. Peak identifications are listed in Table (VI.10). Ratios of specific compounds (such as Tm / Ts) may be useful for source identification. The obvious differences in patterns between the Venezuelan / Mexican Ismus Crude (VMIC) oil and the Sunniland crude oil from Florida demonstrates the usefulness of the technique in differentiating source oils. The visual similarity between the VMIC and the Prudhoe Bay Crude oil demonstrates that in some cases sophisticated pattern recognition methods would be required to distinguish

Table (VI.10): Triterpane peaks

Peak	Abbreviated Notation	Empirical Formula	Molecular Weight	Compound
A	Tricyclic A		Unknown	Tricyclic triterpanes
B	Tricyclic B		Unknown	Tricyclic triterpanes
1	C27α(Ts)	C27H46	370	18α(H)-22,29,30-trisnorneohopane
2	C27α(Tm)	C27H46	370	17α(H)-22,29,30-trisnorhopane
3	C29α,β	C29H50	398	17α(H), 21β(H)-30-norhopane
4	C29 β,α	C29H50	398	17β(H), 21α(H)-30-normoretane
5	C30α,β	C30H52	412	17α(H), 21β(H)-hopane
6	C30β,α	C30H52	412	17β(H), 21α(H)-moretane
7	C31α,β	C31H54	426	17α(H), 21β(H)-30-homohopanes (22S + 22R)
8	C32α,β	C32H56	440	17α(H), 21β(H)-30,31-bishomohopanes (22S + 22R)
9	C33α,β	C33H58	454	17α(H), 21β(H)-30,31,32 trishomohopanes (22S + 22R)

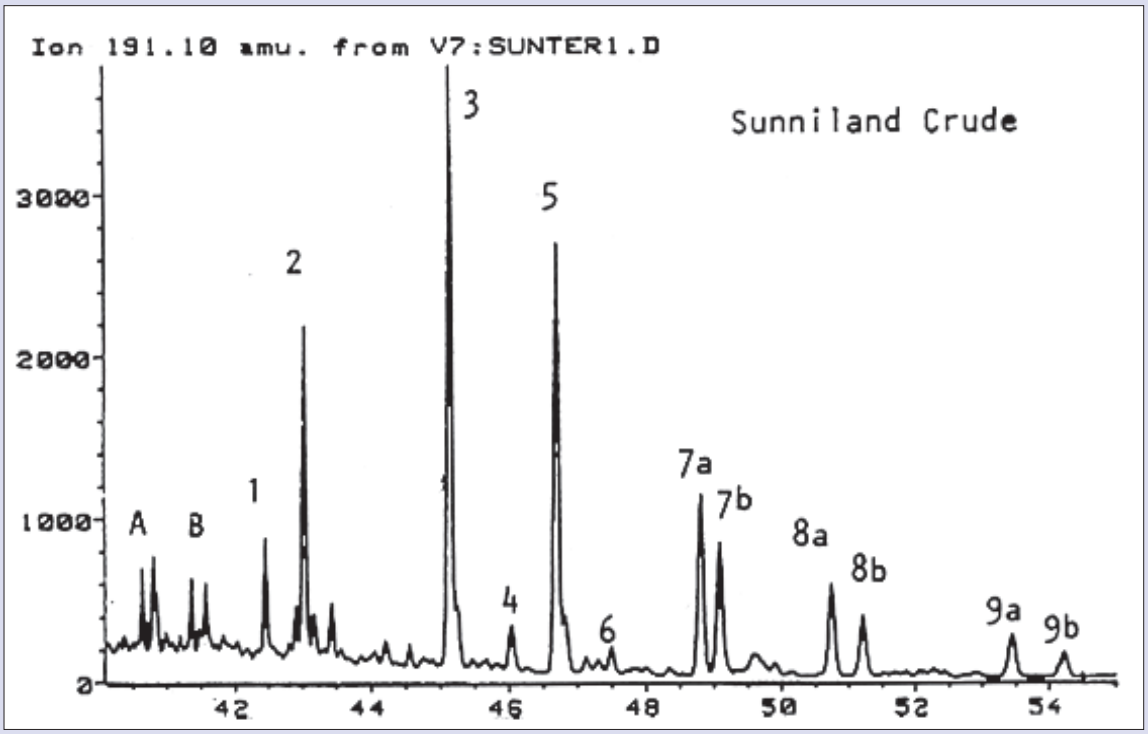
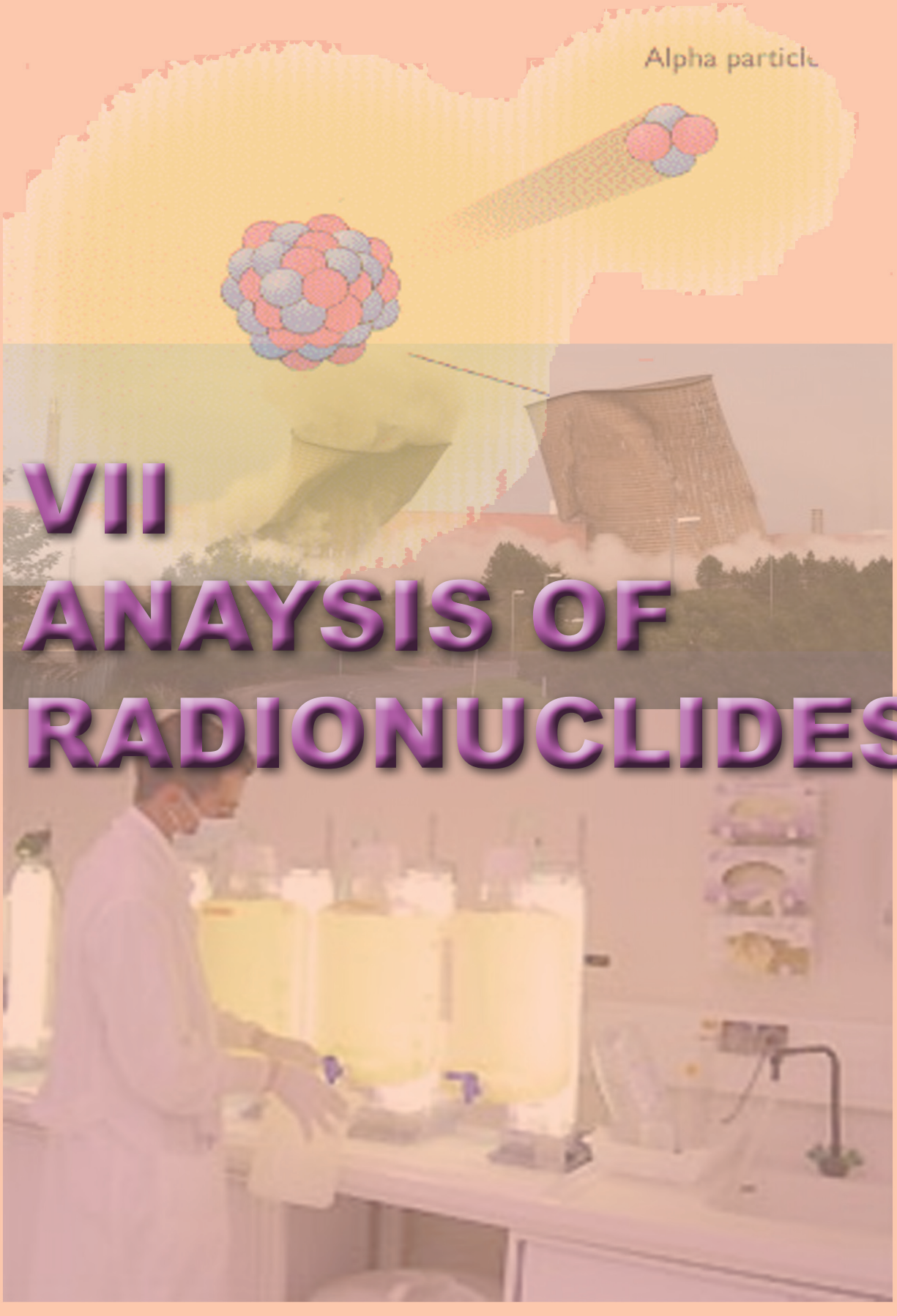


Figure (VI.3) : Reconstructed ion chromatograms of ion 191 from the saturated hydrocarbon fraction of sunniland crude

oils which may be closely related or which may have undergone similar diagenesis histories. Many references discuss the application of triterpane, steroid and isoprenoid hydrocarbon markers. Clearly these types of measurements require sophisticated instruments, elaborate calibration standards and analytical experts with the ability to interpret the data within the context of the organic geochemistry literature. These may be made available to regional monitoring programs via cooperative arrangements with advanced analytical facilities in a region or external to the region. Detailed description of these methods is beyond the scope of this manual.

VI.9.4 Determination of Sterols by GC/FID

Carrier gas : Helium
Flow rate : 1-2 ml/min
Column : DB - 5 (or equivalent)
Injector : Splitless mode
Injector temperature : 270°C
Initial oven temperature: 60°C
Initial time : 0.5 min
Temperature rate : 6°C/min
Final temperature : 290°C
Final time : 20 min



VII ANAYSIS OF RADIONUCLIDES

VII. ANALYSIS OF RADIONUCLIDES

VII.1 INTRODUCTION

VII.1.1 What is Radioactivity?

Radioactivity is the emission of particles, such as alpha, beta or neutrons, or the emission of ionizing electromagnetic radiation, such as gamma, from the nucleus. The emitting nucleus is known as a radionuclide.

VII.1.2 How is Radioactivity Measured?

Radioactivity is quantified using what is known as half-life, which is the period of time it takes for half of the given nuclei to decay. Half-life varies widely, from seconds (Astatine-218) to billions of years (Uranium-238). The amount of radioactivity is measured by the number of nuclei decaying each second; this number is called the activity of the radioactive source and has SI units of Becquerel (Bq). The historic unit, the Curie (Ci) = 3.7×10^{10} Bq. Radioactivity follows an exponential decay function known as Radioactive decay law given as follows:

$$N=N_o e^{-\lambda t}$$

This means that if we start by a number of nuclei, N_o, after a certain time, t, this number will be reduced and given by a smaller number N in the previous equation, where λ called the decay constant is inversely proportional to the half life ($T_{½}$); $\lambda = \ln 2/T_{½}$.

VII.1.3 Sources of Radioactivity

There are two types of Radionuclides; those found naturally in air, water and soil, and even in our bodies. Those are called Naturally Occurring Radioactive Materials (NORM). Those can exist in marine environment just like any other environment or they are sometimes released to the environment during mining of mineral ores, and nuclear fuel fabrication.

The second type is artificial radioactivity; in this case, radioactive nuclides can be found in marine environment artificial sources such as industrial or military. Release of low levels of artificial radionuclides can occur to the environment during normal operations of nuclear installations such as nuclear reactors, particle accelerators, production and application of radioisotopes in the fields of nuclear medicine, research, industry and agriculture (Merril & Gesell, 1997; UNSCEAR, 1982). Higher quantities of radioactive materials may be introduced into the environment due to atmospheric or underground nuclear weapon testing and nuclear accidents.

VII.1.4 Radioactive Series

Some naturally occurring nuclides like ^{232}Th follow a decay chain starting with ^{232}Th and ending with a stable isotope of lead. The chain looks something like: ^{232}Th --> ^{228}Ra --> ^{228}Ac --> ^{228}Th --> ^{224}Ra --> ^{220}Rn --> ^{216}Po --> ^{212}Pb --> ^{212}Bi --> ^{212}Po --> ^{208}Pb (stable).

There are 3 decay series in nature; ^{238}U, ^{232}Th, and ^{235}U. The important fact is that all members of a certain decay series are in a state of radioactive equilibrium. This means that they all have the same activity. This fact is useful since it makes it possible to calculate the concentration of one radioisotope by measuring the amount of the previous (parent) radioisotope or the next (daughter) radioisotope in the chain.

Potassium is essential to life and is thus found in all living, and formerly living, things.

The isotopic abundance of ^{40}K is small, only 0.012% of naturally occurring potassium which gives a specific activity of 855 pCi/g (31.6 Bq/g) of natural K (UNSCEAR, 1997; UNSCEAR, 2000).

VII.1.5 Natural Radioactivity in Soil

How much natural radioactivity is found in a volume of soil that is 1 square mile, by 1 foot deep? The following table is calculated for this volume (total volume is 7.894 x 105 m^3) and the listed activities. It should be noted that activity levels vary greatly depending on soil type, mineral make-up and density (~1.58 g/cm^3 used in this calculation). This table represents calculations using typical numbers.

Natural Radioactivity by the Square Mile, 1 Foot Deep			
Nuclide	Activity used in calculation	Mass of Nuclide	Activity found in the volume of soil
Uranium	0.7 pCi/g (25 Bq/kg)	2,200 kg	0.8 curies (31 GBq)
Thorium	1.1 pCi/g (40 Bq/kg)	12,000 kg	1.4 curies (52 GBq)
Potassium 40	11 pCi/g (400 Bq/kg)	2000 kg	13 curies (500 GBq)
Radium	1.3 pCi/g (48 Bq/kg)	1.7 g	1.7 curies (63 GBq)
Radon	0.17 pCi/g (10 kBq/m^3)	11 µg	1.7 curies (63 GBq)
		Total :	>17 curies (>653 GBq)

VII.1.6 Natural Radioactivity in the Ocean

How much natural radioactivity is found in the world's oceans?

All water on the Earth, including seawater, has some radionuclides in it. In the following table, the oceans' volumes were calculated from the 1990 World Almanac:

- Pacific = 6.549 x 10^{17} m^3
- Atlantic = 3.095 x 10^{17} m^3
- Total = 1.3 x 10^{18} m^3

The activities used in the table below are from 1971 Radioactivity in the Marine Environment, National Academy of Sciences (NAS, 1971):

Natural Radioactivity by the Ocean				
Nuclide	Activity used in calculation	Activity in Ocean		
		Pacific	Atlantic	All Oceans
Uranium	0.9 pCi/L (33 mBq/L)	6×10^8 Ci (22 EBq)	3×10^8 Ci (11 EBq)	1.1×10^9 Ci (41 EBq)
Thorium	300 pCi/L (11 Bq/L)	2×10^{11} Ci (7400 EBq)	9×10^{10} Ci (3300 EBq)	3.8×10^{11} Ci (14000 EBq)
Potassium 40	0.016 pCi/L (0.6 mBq/L)	1×10^7 Ci (370 PBq)	5×10^6 Ci (190 PBq)	2×10^7 Ci (740 PBq)
Radium	0.135 pCi/L (5 mBq/L)	8×10^7 Ci (3 EBq)	4×10^7 Ci (1.5 EBq)	1.8×10^8 Ci (6.7 EBq)
Radon	28 pCi/L (1.1 Bq/L)	1.9×10^{10} Ci (700 EBq)	9×10^9 Ci (330 EBq)	3.6×10^{10} Ci (1300 EBq)

Tables are adapted from the National Council on Radiation Protection (NCRP) report 94 (NCRP, 1987). References are listed at the end of this section. The tables were produced by calculations based on available data.

VII.1.7 International Limits of Activity Concentrations in Marine Samples

In order to assess the health effects from the radioactivity of the surface materials containing ^{226}Ra, ^{232}Th (in equilibrium with its daughter product ^{228}Ra) and 40K, Radium equivalent (Ra_{eq}) activity, air absorbed dose rates (D) and annual effective doses (H) are calculated. The annual effective dose for Ra_{eq} of 370 Bq/kg corresponds to the dose limit of 1.0 mSv for the general population (ICRP-60, 1990). The ground materials, for which radium equivalent activity concentration exceeds 370 Bq/kg may pose radiation hazards.

The range of annual effective doses due to activity concentration of natural radionuclides in sediments is 0.03 mSv to 0.04 mSv with mean value of 0.03 mSv. The mean value is less than the average external gamma dose of 1.0 mSv/y from natural radiation sources (NAS, 1971). As the values of absorbed dose and annual effective dose are calculated for air medium, these values will be even lower due to the radiation attenuation from overlying waters.

VII.2 SAMPLING

The types of samples considered include seawater, sediment and biota. Sampling methods are only briefly presented.

VII.2.1 Seawater

Large volumes of sub-surface seawater are taken with glass fiber reinforced plastic samplers of either two 270 liters or one 500 liters capacity. For surface water, a pump is used to collect 500 liters at the selected sites. These large volume samples are filtered under pressure through a 0.45 µm (293 mm diameter) membrane filter in a stainless steel holder to separate particulate matter. The particulate-loaded filters were reserved for possible analyses.

VII.2.2 Sediment

Sediment samples can be taken with a box corer or a multicorer. Plexiglas tubes are used to take sub-samples from a box corer. Sediment columns are meticulously sliced into sections with a special tools. Characteristics of each sediment slice (e.g. porosity, pore water content) is measured before any further treatment preceding radioanalytical work.

VII.2.3 Biota

Standard plankton nets of 1000, 500 and 250 µm mesh sizes can be used, and the total volume of seawater passing through the nets is estimated with a flowmeter attached to one of the nets during sampling. Fish are obtained by traditional fishing methods and dissected into appropriate parts. Both plankton and fish are frozen for transport to laboratory.

VII.3 PRE-CONCENTRATION FROM LARGE VOLUME SEAWATER SAMPLES

The determination of activity concentrations of Sr-90, Cs-137, Am-241 and Pu radionuclides in the large volume seawater samples needs the reduction of the sample size on the ship for economic transportation back to the home laboratory. This is carried out by three separation stages in which the desired radionuclides are efficiently co-precipitated with macroscopic amounts of insoluble precipitates.

VII.3.1 Pre-concentration of Actinides with Manganese Dioxide

In large tanks, 400 - 500 liters of filtered (0.45 µm) seawater are acidified with concentrated HCl to pH 2. A solution of mixed tracers (Sr-85: 1000 - 1500 Bq; Cs-134: 1 - 10 Bq; Pu-242; 0.02 Bq; Am-243: 0.01 Bq) and carriers (Sr: 1000 mg; Cs: 20 mg) is added to the acidified seawater and mixed by bubbling air for 15 minutes. Addition of 125 ml of $KMnO_4$ solution (60 g/liter) is followed by air bubbling for another 15 minutes.

In order to precipitate manganese dioxide, 250 ml of 0.5M $MnCl_2$ are added to the $KMnO_4$-treated seawater and followed soon by addition of 10M NaOH solution sufficient to make the mixture slightly basic. Hydrated manganese dioxide immediately comes out of solution from this medium as a brown, gelatinous precipitate which carries down the trace amounts of Pu and Am along with some other insoluble species. Limiting the excess of added NaOH prevents the precipitation of Ca and Mg hydroxides, thereby minimizing the bulk of the manganese dioxide. If inorganic phosphate is present, some basic Ca and/or Mg phosphate will be precipitated with the MnO_2. Hydrated silica and alkaline earth silicates will accompany the MnO_2 precipitate when present in the seawater. The MnO_2 suspension is stirred for perhaps 30 minutes then allowed to settle for 4-5 hours. After settling, the supernatant solution is pumped to another plastic tank, and the settled MnO_2 (Pu, Am) is removed from bottom of the tank and transferred as a suspension (usually ≤ 20 liters) into a plastic container (e.g. a flexible Cubitainer) for shipment to the home laboratory for final analysis.

VII.3.2 Pre-concentration of Caesium with Ammonium Molybdophosphate (AMP)

The supernatant solution from the manganese dioxide precipitation of the previous step is re-acidified to pH 1.5 - 2 with about 350 ml of concentrated HCl. Addition of a few ml of 30% H_2O_2 to dissolve a small amount of MnO_2 suspension carried over from the previous step is sometimes done. A slurry of 30 g of AMP in water is added and the suspension stirred for 30 minutes by bubbling to permit efficient sorption of caesium carrier and radioisotopes by the AMP. The AMP is permitted to settle out for 4 -5 hours in the tank, then the supernatant solution is pumped out into another tank (for subsequent precipitation of Ca(Sr) oxalate). The AMP(Cs) precipitate is removed from the bottom of the tank into a 20-liter container as a suspension for transportation to the home laboratory for final analytical work-up.

VII.3.3 Pre-concentration of Strontium with Calcium Oxalate

The supernatant solution (pH = 2) from the AMP(Cs) precipitation step is used for the pre-concentration of strontium. The strontium is co-precipitated with calcium as an insoluble oxalate from solution containing excess oxalic acid and adjusted to pH 5 - 6. The seawater should contain a reasonable concentration of calcium (ca. 0.1- 1 g of Ca per liter); if the Ca concentration is much less than 0.1 g/liter, a $CaCl_2$ solution is added to obtain the desired concentration of Ca which will produce a sufficient amount of calcium oxalate to carry the strontium oxalate efficiently. An excess of oxalic acid is needed to take into account the large Mg concentration in seawater and to ensure the complete precipitation of the Sr as oxalate. Magnesium ions can form complex ions with oxalate, thereby competing with the Ca and Sr for oxalate; furthermore, with an excess of oxalate and long settling times (e.g. overnight), some of the Mg can post-precipitate onto the mixed Ca-Sr oxalate crystals.

The mixed Ca-Sr oxalate precipitation is carried out by adding an appropriate quantity of oxalic acid dissolved in very hot de-ionized water (e.g. 1000 g dissolved in ca. 4 liters of hot water obtained from an electric hot water heating pot) to the AMP(Cs) supernatant solution, mixing well and adjusting to a final pH 5 -6 with the slow addition of enough 10M NaOH to neutralize all of the HCl and oxalic acid in the seawater solution (e.g. 500 liters of 0.01M HCl [pH =2] solution containing 1000 g of $H_2C_2O_4 \cdot 2H_2O$ would require ca. 2.1 liters of 10M NaOH) while continuing to bubble air for an extra 30 minutes. Sometimes a pink color appears in the suspension due to the presence of a small amount of manganese which has carried over from the MnO_2 precipitation step. The Ca(Sr) oxalate precipitate

is allowed to settle for 4 -5 hours in the reaction tank. Afterwards, the supernatant solution is pumped out and discarded, and the Ca-Sr mixed oxalate precipitate is recovered from the bottom of the tank and transferred as a suspension to a 20-liter container for shipment back to the home analytical laboratory.

VII.4 SEAWATER ANALYSES

VII.4.1 Strontium (Sr-90)

In the shipboard pre-concentration of Sr-90 from seawater, the Sr carrier and radionuclides (Sr-85 spike, Sr-90 analyte) are co-precipitated with calcium oxalate after the MnO_2(Pu, Am) and AMP(Cs) precipitations, as described earlier. In the laboratory, the calcium oxalate suspension is allowed to settle after transferring it to a large beaker. The supernatant solution is siphoned off, and the precipitate is washed several times with water to remove the seawater. It is then dried and ashed at 600°C to convert the oxalate to carbonate.

The ashed sample is weighed to determine the amount of 70% HNO_3 to be added. A volume of 70% HNO_3 in milliliters equal to seven times the ash weight in grams is slowly added to the calcium-strontium carbonate ash in an appropriate beaker. Much carbon dioxide gas and some red-brown gas (perhaps due to interaction of nitric acid and residual chloride or due to some ash component that reduces nitric acid to nitrogen dioxide) are generated in the dissolution. Strontium nitrate (1^{st}) precipitates from this medium while the calcium remains in solution. Barium, radium and lead are expected to accompany the strontium nitrate.

Natural Sr is present in most seawater; for example, Pacific Ocean seawater has approximately 8 mg Sr/liter (practically independent of depth). Therefore, 100 liters of seawater carries already ca. 800 mg of Sr, which is nearly equal to the amount of Sr carrier (1 g) added to the original sample before the pre-concentration steps are performed. This extra Sr carrier decreases the losses from chemical operations used to separate and purify the strontium fraction. If a gravimetric method is employed to determine the Sr chemical recovery, this additional source of Sr must be taken into account. In the present case, the use of Sr-85 tracer avoids problems associated with gravimetric methods.

The first strontium nitrate precipitate is separated from the mother solution containing the dissolved calcium-strontium ash by decanting the supernatant solution after settling. It has been found that heating the strontium nitrate suspension for some hours before allowing it to settle seems to improve the separation, perhaps by producing a $Sr(NO_3)_2$ precipitate of larger crystal size. This 1^{st} $Sr(NO_3)_2$ precipitate is transferred to a tared 150 ml beaker, then washed with three 30 – 50 ml portions of acetone to remove HNO_3 and more soluble calcium nitrate. The washed $Sr(NO_3)_2$ is dried under a heat lamp and weighed.

To the 1^{st} $Sr(NO_3)_2$ precipitate, a volume of water in ml equal to 1.5 times the weight of dried precipitate in grams is added. If the precipitate is truly $Sr(NO_3)_2$ with a small contamination of $Ca(NO_3)_2$, it will easily dissolve to give a clear solution. In some cases, there will be an insoluble residue. If there is a lot of undissolved residue, it may be due to Ca-Sr sulfate, which is very insoluble. Then the residue is separated from the soluble part by centrifugation, washing and filtration of finely suspended particles. The soluble fraction is evaporated to a salt residue of $Sr(NO_3)_2$. The insoluble residue is transferred to a small beaker and digested with an excess of 2.5M Na_2CO_3 (about 10 ml for every ml of residue)

to metathesize any Ca and Sr sulfate to carbonate. Sulfate passes into solution while Ca and Sr carbonates remain in the solid phase. The solution and insoluble residue are separated, and a second metathesis is carried out. If there is very much insoluble matter, a third metathesis is recommended. At the end of this treatment, the centrifuged residue is washed several times with water containing a drop of Na_2CO_3 solution to remove the carbonate-sulfate solution. Finally, concentrated HNO_3 is added to convert carbonates to nitrates until no further gas evolution is observed. At this point some nitrate precipitation may occur. If the residue dissolves easily when water is added, the conversion of sulfate to nitrate has been successful. If the metathesis was not complete, some insoluble residue remains. It is also possible that the original residue was not an alkaline earth sulfate, so digestion with Na_2CO_3 may not have altered it and it likewise remains undissolved. Experienced analysts will decide an appropriate course of action in such circumstances.

If a successful metathesis has been made, the recovered Ca-Sr nitrate is added to the salt residue from the evaporation of the soluble fraction, and the combined material is taken to dryness and weighed. Addition of water at this time invariably dissolves the salt completely. After the salt is dissolved, a second $Sr(NO_3)_2$ precipitate is obtained by adding a volume of 70% HNO_3 equal to ten times the volume of water used to dissolve the salt. This 2nd $Sr(NO_3)_2$ is separated by decantation of the supernatant liquid and washed again with small portions of acetone. After drying and weighing, the dissolution, concentrated nitric acid addition and acetone washings are repeated to give a 3rd $Sr(NO_3)_2$ precipitate.

Further purification of the Sr from barium, radium and lead contaminants is performed by a Ba chromate precipitation from an acetate-buffered solution at pH = 5. A final clean-up of the Sr from ingrown Y-90 and some other possible beta-emitting radionuclides (e.g. Bi-210) is made by an iron hydroxide scavenge using 10 – 50 mg of Fe(III) and concentrated ammonia to pH 9. The iron hydroxide precipitate is centrifuged off, and the Sr-acetate-chromate solution is acidified with concentrated HCl to pH = 1.

The purified Sr fraction is transferred to a plastic container and diluted with 0.1M HCl to the volume (typically 50 ml) required for the geometry of the gamma spectrometry system. Strontium-85 tracer is measured by means of its 514 keV photon emission in order to determine the chemical recovery of the Sr.

VII.4.1.1 Milking of Y-90 from Purified Sr Fraction

After an ingrowth period of 2 – 3 weeks, the purified, acidified Sr solution containing acetate and dichromate is transferred to a beaker. An accurately known amount of yttrium carrier (10 mg) is added, and the solution is partly evaporated down to 20 ml or less. During this evaporation, some or all of the dichromate is reduced by the acetate to green Cr(III). The solution is transferred to a centrifuge tube, 1 g of NH_4Cl is dissolved in it, and concentrated NH_4OH is added to pH = 9 to precipitate yttrium hydroxide accompanied by Cr(III) hydroxide. The mixed hydroxide precipitate is briefly digested in a hot water bath and centrifuged at 3000 rpm for 15 minutes. The supernatant solution is easily decanted away from the packed, gelatinous Y-Cr hydroxide precipitate. **The time at which the decantation occurs is noted as the Y-Sr separation time!**

The Y-Cr hydroxide is dissolved in several milliliters of concentrated HCl, diluted to 25 ml, and 1 g of NH_4Cl is dissolved in it. Ten mg of stable Sr holdback carrier are added, and a second mixed Y-Cr hydroxide is precipitated upon the addition of excess of NH_4OH. The flocculated precipitate

is centrifuged down, and the supernatant solution is decanted. (This supernatant solution can be acidified and combined with the first supernatant solution in case later Y-90 milkings are anticipated.) This 2nd Y-Cr hydroxide is dissolved in concentrated HCl (some ml of HCl in excess), diluted and filtered through a low-porosity filter paper or membrane filter to remove any insoluble material if necessary.

To the Y-Cr solution, 0.5 g of oxalic acid are added, the solution is heated (oxalic acid dissolves), and a white yttrium oxalate is precipitated by the addition of NH_4OH to pH = 1.5 – 2 (determined with short-range pH paper). The Cr(III) remains in solution as a purple oxalate complex. This preliminary yttrium oxalate is separated from the solution by filtration through a low-porosity paper or membrane filter under suction. Then it is dissolved by treatment with concentrated HCl and passed through the filter with rinses into a 100 ml beaker.

The final yttrium oxalate precipitate is obtained from 40 –50 ml of hot solution by dissolving 0.1 g of oxalic acid, adding 5 – 8 drops of 0.04% thymol blue (TB) indicator and adding concentrated ammonia until a salmon-pink color is obtained. The pH is between 1.5 and 2 (checked by short-range pH paper). The yttrium oxalate is digested for about 30 minutes before filtration to obtain a crystalline form. Then it is filtered onto a pre-washed, tared 0.2 µm polysulfone membrane filter (Gelman Sciences, Inc.) which has been punched to 22 mm diameter from 25 mm original diameter. (The filter is pre-washed with: (a) 50 ml of a solution prepared by dissolving 2 g of oxalic acid in 800 ml of 1M HCl, adding 5 ml of 0.04% TB indicator solution and bringing to pH = 1.5 – 2 with the addition of 30% NH_3 solution (ca. 50 ml), (b) 4 x 2.5 ml of 0.5% ammonium oxalate solution (pH = 8) and (c) 3 x 1 ml of 80% ethanol. These filters have been found to lose 0.5 to 1 mg of weight after this conditioning.) The yttrium oxalate is washed with four 2.5 ml portions of 0.5% ammonium oxalate solution to remove the mother liquor and to dissolve any TB which has collected on the filter (red color). Finally, three 1 ml portions of 80% ethanol are used to rinse the yttrium oxalate. A Millipore glass filtration assembly has been used with the following modification: a silicone rubber annulus with a 16 mm inside diameter, 30 mm outer diameter and 3.5 mm thickness is inserted between the glass chimney (also 16 mm inside diameter) and the fritted glass base. The assembly is held together by a spring-loaded clamp. The yttrium oxalate precipitate does not adhere to the silicone rubber, and the annulus also provides a leak-tight seal (gasket). Thus, the filtered yttrium oxalate is essentially quantitatively recovered and forms a sharply-defined, circular deposit on the membrane filter. (When the annulus is omitted, yttrium oxalate adheres strongly to the glass chimney and the corresponding deposit is very non-uniform and irregular.)

The final filtered yttrium oxalate is dried in a 70°C oven for ca. 30 minutes and weighed to determine the yttrium chemical recovery from the stable weighing form of $Y_2(C_2O_4)_3 \cdot 9H_2O$ (10 mg of Y gives 34 mg of yttrium oxalate).

VII.4.1.2 Beta Counting of Yttrium Oxalate Sources

The yttrium oxalate-loaded filter is mounted on a 23.5 mm x 4 mm thick plastic disk by covering with a ¼-mil Mylar plastic film and pressing a metal retaining ring around the perimeter to seal the source. These sources are placed in a Riso GM-25-5 Beta Multicounter System (Riso National Laboratory, Roskilde, Denmark) gas-flow proportional counter with anti-coincidence background reduction. This system operates at around 1100 volts with a gas mixture of 99% argon – 1% isobutane. The beta counting efficiency for Y-90 in yttrium oxalate sources which are in contact with the counter window has been measured experimentally to be about 48 ± 2%. Background count rates are typically

between 0.1 and 0.2 counts/minute. The yttrium oxalate sample sources are measured usually for 2 hour intervals in a continuous 60 hour period. The start of the counting is generally 6 to 8 hours after the actual Y-Sr separation time. Almost without exception, the measured beta activity decay curve is consistent with the known 64 hour half-life of Y-90 and which indicates the high radiochemical purity of the yttrium sources. The decay data are corrected for background, extrapolated to the Y-Sr separation time, corrected for the Y chemical recovery and corrected for percentage ingrowth of Y-90 to obtain the Sr-90 activity in the "cow" at the time of the milking. The total Sr-90 activity in the sample is then obtained by correcting for the Sr chemical recovery and for radioactive decay to a particular reference time.

Limit of detection : The detection limit for Sr-90 with a single Y-90 milking has been estimated based on the detection limit formula of Currie for paired observations {LD(counts) = 2.71 + 4.65 vμB, where μB is the total number of background counts in the measurement period} taking into account the following conditions:

proportional counter with a background of 0.11 counts/minute
Y-90 counting period of 60 hours starting from 8 hours after the Y-Sr chemical separation (μB = 396 counts)
50% Sr chemical recovery
90% Y chemical recovery
48% Y-90 beta counting efficiency

Here LD = 95.2 counts was taken to be the integrated number of counts in the 60 hour period. Assuming that the activity decayed with the 64 hour half-life of ^{90}Y, this gave an ^{90}Y source net count rate of 0.0392 counts/minute when it was extrapolated to the time of the Y-Sr chemical separation. The resulting detection limit is 3 mBq of Sr-90. For 100 liters of seawater, this gives a detection limit of about 30 μBq/liter of Sr-90.

VII.4.2 Caesium (Cs-137)

From the shipboard pre-concentration step with ammonium molybdophosphate (AMP), caesium carrier and radionuclides in several hundred liters of seawater have been efficiently collected on 30 g of AMP. A suspension of this AMP in 10 – 20 liters is received in the laboratory for final analysis. The AMP is allowed to settle, and the supernatant solution is decanted or siphoned away. The remaining amount of suspension is centrifuged, and the AMP solid can be washed a few times with small volumes of water acidified to pH = 2.

The separated AMP is then dissolved in a minimum amount of 10M NaOH. The yellow color disappears, leaving usually a small amount of insoluble matter which is often due to manganese dioxide. This fine-particle suspension of MnO_2 was carried over in the supernatant solution of the first pre-concentration step (MnO_2 precipitation for Pu and Am) and subsequently taken down by the AMP. The insoluble matter is separated by centrifugation and/or filtration. The Cs-AMP solution in NaOH is transferred to a beaker, heated and boiled to drive off ammonia. This will minimize precipitation of AMP when the solution is re-acidified.

The boiled Cs-AMP-NaOH solution is cooled and diluted to about 500 ml with water. Concentrated HCl is added to neutralize the NaOH and to acidify the solution to about pH = 2. The transition

from basic to acidic is signaled by the re-appearance of yellow color from the molybdophosphate; however, because the ammonia has largely been removed in the previous step, very little AMP will precipitate directly. To the acidic solution, 1 g of fresh AMP is added and stirred in to collect the Cs (2^{nd} AMP-Cs precipitation).

After settling and decantation, the 2^{nd} AMP-Cs is separated by centrifugation and washed. It is again dissolved in a minimum amount of 10M NaOH, and any insoluble can be centrifuged or filtered off. Precipitation of a third AMP-Cs is optional; normally, for gamma spectrometry, two AMP-Cs precipitations are sufficient. The dissolved 2^{nd} AMP-Cs is adjusted to a standard volume geometry for which an efficiency calibration has been performed with a particular detector. One or more gamma ray measurements are done to determine the Cs-137 content by way of its photon emission at 661 keV energy. The caesium chemical recovery is determined by measurement of the Cs-134 major gamma ray emissions at 605 and 796 keV. Because of serious coincidence summing effects in well counters, the Cs-134 efficiency calibration must be done with a known activity of Cs-134. Alternatively, a direct comparison with a standard containing the same amount of Cs-134 which was originally added to the sample before chemical processing can be done to assess the fraction of Cs-134 remaining in the purified Cs sample.

VII.4.3 Actinides (Pu, Am)

Manganese dioxide containing Pu and Am radionuclides from the shipboard pre-concentration step (see previous section) is separated from the suspension which is received in the laboratory (after checking the pH of the liquid) by a combination of settling, decantation/siphoning of supernatant liquid and centrifugation.

Dissolution of manganese dioxide : Two liters of 2M HNO_3 are added to the manganese dioxide in a 10-liter beaker and the suspension is heated. A solution of hydroxylammonium hydrochloride (NH_2OH•HCl, 0.1 g/ml) is added in small portions to the hot suspension with rapid stirring until all of the manganese dioxide has dissolved (typically 10 - 20 ml) by reduction from Mn(IV) dioxide to soluble Mn(II). Usually the resulting solution is quite clear and colorless; otherwise, light-colored turbidity at this point in the solution suggests the presence of silica.

Oxidation state adjustment of plutonium : Fifty milligrams of Fe(III) is added as 5 ml of a solution of 10 mg Fe(III)/ml as $FeCl_3$ in 0.1M HCl. Then the acid concentration is reduced to < 1M by the addition of 2M NaOH, typically 1.5 to 1.8 liters. A few milliliters of NH_2OH•HCl solution are added and the manganese-iron-Pu-Am solution is heated to reduce Fe(III) to Fe(II). This is often visibly evident by a change in solution color from yellow to colorless. The oxidation state of Fe in solution is qualitatively ascertained by means of so-called “spot tests” wherein 2-5 drops of the sample solution are mixed with the same number of drops of a test reagent solution. 2,2’-bipyridine (2,2’-BP) as a solution of 10 mg/ml of ethanol is used to test for the presence of ferrous iron; it gives a bright pink-red color with Fe(II) but no color with Fe(III). 5M NH_4SCN solution is used to test for the presence of ferric iron; it produces a red color with Fe(III) but no color with Fe(II). A well-reduced sample solution shows a bright pink-red spot test with 2,2’-BP and a faint color with the NH_4SCN spot test. The Fe(II), now the predominant iron species in solution, rapidly reduces all soluble Pu species to Pu(III). Without Fe(II), the reduction of Pu(V) and Pu(VI) to lower oxidation states by hydroxylammonium hydrochloride alone might be significantly slower, and it would be difficult to judge the extent of the Pu reduction.

After the reduction to Fe(II) and Pu(III), 2 g of $NaNO_2$ dissolved in 20 ml of water is added to the hot solution to oxidize the excess NH_2OH•HCl and to convert Fe(II) and Pu(III) to Fe(III) and Pu(IV), respectively. The yellow color of Fe(III) becomes easily visible again.

Iron hydroxide precipitation : Without delay 2M NH_4OH is then added to the hot solution still containing excess $NaNO_2$ to make the pH 8 - 9, causing precipitation of $Fe(OH)_3$ with associated co-precipitation of Pu(IV) and Am(III). Using 2M NH_4OH to precipitate the iron hydroxide assists by buffering the pH. The $Mn(OH)_2$ has a much higher solubility at this pH, and the excess $NaNO_2$ helps to limit the formation of MnO_2 by keeping the manganese in its reduced form. The freshly precipitated iron hydroxide (which should be dark red-brown if there is no $Mn(OH)_2$ or calcium phosphate precipitate) is flocculated by heating strongly and boiling for a few minutes. Immediately after boiling, the pH of the suspension is adjusted to 6 - 7 by addition of 1M HCl (typically 10 - 20 ml). If any dark color is visible in the solution, it may be due to some MnO_2 formation; addition of some drops of 25% $NaNO_2$ often lightens the color by reducing the Mn(IV) to soluble Mn(II). At pH 6 - 7, the large amount of manganese will stay in solution but the flocculated iron hydroxide and its co-precipitated Pu(IV) and Am(III) remain quite insoluble. Overnight standing permits the iron hydroxide (Pu, Am) to settle out, leaving a clear, usually colorless supernatant solution above it.

The $Fe(OH)_3$ (Pu, Am) is separated from 4 - 5 liters of supernatant solution by decantation or siphoning; filtration of this solution will usually recover a small amount of suspended iron hydroxide. The main $Fe(OH)_3$ is obtained by centrifugation of the 100 - 300 ml of suspension left in the precipitating vessel. Hot, concentrated HCl is used to dissolve the separated iron hydroxide, including that on a filter and on the wall of the precipitating beaker. If there is little or no insoluble matter, the HCl solution of Fe-Pu-Am is transferred to a 250 ml beaker and evaporated to dryness. Concentrated nitric acid additions and evaporations convert this to a nitrate salt residue which is finally dissolved in 1M HNO_3 and filtered, if needed, to remove any small amount of insoluble matter.

Occasionally, when the $Fe(OH)_3$ (Pu, Am) precipitate is treated with hot HCl, it produces a thick suspension due to the presence of considerable gelatinous hydrated silica. This presumably comes from silicates in the water due to biological activity. Such a thick suspension cannot be conveniently centrifuged or filtered, so it is transferred to a PTFE beaker. Concentrated HF is added to convert the silica to SiF_4, and the mixture is heated to evaporate the volatile silicon tetrafluoride. This operation is repeated one or two more times to remove the silica efficiently. The residue from the last HF evaporation, which is now essentially iron fluoride, is then digested with concentrated HCl + H_3BO_3 , evaporated, and converted to nitrate with concentrated HNO_3 digestions. The final nitrate-boric acid residue is dissolved in 1M HNO_3.

VII.4.3.1 Plutonium Separation and Purification

Preparation for Pu separation by anion exchange : To the hot 1M HNO_3 solution (usually 50 ml) of the Fe-Pu-Am, 1 or 2 ml of 25% hydrazinium hydrate (N_2H_4•H_2O) is added dropwise with rapid stirring. The solution is heated to promote the reduction of Fe(III) to Fe(II). As in the reduction step preceding the precipitation of $Fe(OH)_3$, spot tests with 2,2'-BP and 5M NH_4SCN can be done to assess the extent of the Fe reduction (see previous discussion under "Oxidation state adjustment of plutonium"). Successful conversion of at least a significant fraction of the iron to the ferrous state ensures that the dissolved Pu species are brought to their lower oxidation states of III and IV. Excess N_2H_4•H_2O is destroyed by adding 15 ml of 70% HNO_3 and heating the now approximately 4M HNO_3

solution strongly for ca. 20 minutes until the usual bright yellow color of ferric iron is restored and normal boiling without fine bubble formation takes place. Iron acts as a catalyst in the hydrazine destruction which would otherwise take place very slowly in pure 4M HNO_3. The Pu in this solution has most probably converted to Pu(IV) at this point. As further insurance, 2.5 g of $NaNO_2$ are added to the cooled solution; usually a blue to blue-green color is produced from the mixture of nitrous acid and Fe(III). Traces of $N_2H_4 \bullet H_2O$ are eliminated and any Pu(III) is oxidized to Pu(IV) by the nitrous acid. After 10 minutes, the solution is heated and boiled briefly to decompose the nitrous acid which would otherwise interfere in the subsequent column chromatography by causing formation within the column of gas bubbles that block the liquid flow. The sample solution, ca. 65 ml, is cooled to room temperature. Finally, 35 ml of ice-cold 70% HNO_3 are added to produce 100 ml of 7 - 8M HNO_3 solution containing Pu(IV), Am(III) and Fe(III) at about room temperature. This solution is now ready for loading into an anion exchange resin chromatography column for separation of Pu(IV).

Plutonium separation by column chromatography : The basis of the Pu separation and purification from Fe, Am and most of the common elements is the selective retention of its anionic nitrate complex, $Pu^{IV}(NO_3)_6^{2-}$, in 7 - 8M HNO_3 by strongly basic anion exchange resin. Only Th(IV) and Np(IV) are strongly retained under these conditions; Pa(V) is moderately retained while U(VI) is weakly retained in this medium. The anion exchange resin most commonly used by us is analytical grade AG 1-X8, 100 - 200 mesh bead size, supplied in the chloride form from either Bio-Rad or EiChrom. A water slurry of the resin is loaded into a 30 cm glass column (1 cm inside diameter) with glass fiber plugs at bottom and top. The resin is converted from chloride to nitrate form (“conditioning”) by passing 8M HNO_3 (50 ml) through the resin bed at a flow rate of ca. 1 ml/minute. The height of the resin bed in the column is left to the analyst’s choice, typically 7 - 14 cm corresponding to bed volumes of about 5.5 to 11 ml.

The sample solution in 7 - 8M HNO_3 from the previous step is filtered through a filter paper (fast or medium speed, sometimes both in succession) to remove any small amount of insoluble matter that might block the column flow. Gelatinous precipitates such as hydrated silica are particularly effective at stopping the liquid flow in the column since they will be filtered out at the top of the column. The filtered sample solution (100 to 200 ml) is loaded into the column reservoir (a plastic, detachable funnel) and the flow rate is adjusted to pass the solution at a moderately slow rate of 1 - 2 ml/minute. When the main sample solution has passed through the resin, three small rinses (ca. 2 -3 ml each) of the original sample solution container are put through the column. This is followed by 50 ml of 8M HNO_3 “wash” to rinse the feed solution thoroughly out of the column (removing non-retained species such as Am, Fe, Al, Ca, K) and to partly elute weakly retained U(VI). *The 7 - 8M HNO_3 feed, rinses and wash eluates are collected together and reserved for the Am separation.*

After the 8M HNO_3 column wash, 100 ml of 10M HCl are passed through the column to elute any thorium which might have been retained as $Th^{IV}(NO_3)_6^{2-}$ from the 8M HNO_3. Th(IV) does not form an anionic chloride complex, but Pu(IV) converts from anionic $Pu^{IV}(NO_3)_6^{2-}$ to anionic $Pu^{IV}Cl_6^{2-}$ which remains on the resin column in 10M HCl. Nitric acid is washed out of the column, and the resin is converted from nitrate to chloride form during this so-called Th strip elution. This conversion to chloride is important for the next step.

In order to remove Pu from the anion exchange column, 100 ml of freshly prepared 0.1M NH_4I-9M HCl is passed through the column as the so-called “Pu strip”. Even in strong HCl medium, iodide reduces Pu(IV) to Pu(III) in the column. Since Pu(III) does not form an anionic chloride complex, it promptly elutes from the column and is collected in a beaker. This solution is evaporated, the iodide

is removed as volatile iodine (I_2) vapor by repeated additions of concentrated HNO_3 with occasional small portions (ca. 5 - 10 drops) of 30% H_2O_2. (Some of the iodide is oxidized to iodate, IO_3^-, by concentrated HNO_3. H_2O_2 reduces iodate to iodine which is then visible as a violet vapor.) Eventually there remains a simple residue of NH_4NO_3 containing the trace of Pu.

Further purification of plutonium : The NH_4NO_3/Pu residue from the anion exchange separation of Pu may contain traces of U (U-234 and U-238), Th (Th-232, Th-230, and Th-228) and Po-210 activity which could interfere with the measurement of Pu-238, Pu-239+240 and Pu-242 (spike) by alpha spectrometry. This is especially likely if the original sample had activity concentrations of natural radionuclides much higher than that of artificial ones. Additional purification is therefore desirable to obtain a final Pu source which is free of these spectral interferences. In most cases, a second anion exchange column procedure is done under similar conditions to the first one discussed above. Adjustment of the Pu oxidation state before the column step is made, often by the hydrazine-reduction/nitrite-oxidation method described previously, or sometimes just by the addition of $NaNO_2$ to the 8M HNO_3 solution of the Pu. Alternatively, purification of Pu can be done using extraction chromatography with commercially available materials (e.g. from EiChrom, Inc.) where the Pu is sorbed and released depending on oxidation state and solvent medium conditions.

Source preparation for alpha spectrometry : To purified Pu fractions (often as NH_4NO_3 residues from anion exchange column 0.1M NH_4I-9M HCl strips after HNO_3-H_2O_2 evaporations), 2 ml of concentrated sulfuric acid and 1 ml of 0.3M Na_2SO_4 are added and the solution is evaporated to remove nitrate. This treatment also degrades organic residues from anion exchange resin or extraction chromatography materials. If the hot sulfuric acid solution becomes dark-colored due to charring of organic matter, a few drops of concentrated HNO_3 usually decolorizes the solution by oxidizing the degraded organic species. When the liquid sulfuric acid has been evaporated, the solid ammonium sulfate is heated to decompose it until only the Na_2SO_4 remains. Finally, 1 ml of 70% HNO_3, 1 ml of 60% $HClO_4$ and 2 ml of 96-98% H_2SO_4 are added to the Na_2SO_4 residue and heated to destroy any last traces of organic matter. This mixture is evaporated to dryness, leaving a white Na_2SO_4 residue containing Pu.

Electrodeposition of the Pu onto a polished stainless steel (SS) disk (18 mm diameter) is carried out according to a modification by L. Hallstadius to the method of Talvitie. The Na_2SO_4(Pu) residue from the HNO_3-$HClO_4$-H_2SO_4 treatment is dissolved in 0.3 ml of hot 96-98% H_2SO_4, diluted by adding 4 ml of water, and transferred to an electrodeposition cell made from an inverted 30 ml polyethylene screw-cap bottle (Nalge Co.) with an opening cut out of the bottom. The SS disk fits into the screw cap (which has a hole for an electrical connection to the SS disk), and the screw threads of the bottle are wrapped with PTFE tape to prevent leakage. The assembled system is leak-tested. The sample solution containing some drops of 0.04% thymol blue indicator is adjusted to pH 2.1 - 2.4 by the addition of concentrated NH_4OH until a salmon-pink color is obtained. The pH is checked with short-range (0 - 2.5) test paper.

A platinum anode wire with a small horizontal loop (0.5 - 0.8 cm diameter) is immersed in the sample solution and adjusted to a distance of ca. 0.3 - 0.5 cm from the SS disk. A regulated power supply with ca. 30 volt/1.5 ampere capacity (e.g. Hewlett-Packard model no. HP6289A) is set to deliver a constant current of 1 ampere, and the electrodeposition is carried out for 1 hour to deposit the Pu onto the SS disk as a hydrated oxide. (Other actinides can be electrodeposited under similar conditions.) At the end of the time period, 1 -2 ml of concentrated NH_4OH is added to make the sample solution basic, and the current is allowed to continue for another minute to mix the ammonia into the solution.

(Making the solution basic prevents possible re-dissolution of deposited Pu into the previously acidic sample solution when the power is turned off.) The electrodeposition is then stopped, the solution poured out (reserved) and the SS disk rinsed with 1% NH_4OH and acetone. The dried disk is then ready for alpha spectrometry measurements.

VII.5 SEDIMENT AND BIOTA ANALYSES

VII.5.1 Caesium (Cs-137)

If it is desired to measure Cs-137 in sediment and biota, it can often be done directly without chemical processing for small amounts of material. The sediment is dried and perhaps sieved for particle size ranges, or the entire sample is ground and homogenized. Biota are likewise sectioned, dried and ground to homogenize the material. Then all or part of the homogenized sample is taken, weighed and arranged in a plastic container in a fixed geometry. After measurements with a planar HPGe detector are carried out, the activity of Cs-137 can be calculated from the efficiency calibration and taking into account correction due to self-absorption in the sample itself.

For biota, if there is not sufficient sensitivity by this method, the sample can be ashed at 450°C. Sometimes the ash can then be directly taken for gamma spectrometry if a significant reduction in sample size is obtained. Otherwise, the ash must be dissolved or leached in the presence of Cs-134 and Cs carrier, and the Cs separated from the acidic solution by using AMP collection. Even in 200 ml of a 1M HNO_3 solution of 10 g of biota ash, the percentage of Cs that co-precipitates with AMP has been found to be surprisingly high.

VII.5.2 Actinides (Pu, Am)

After measurement of sediment characteristics such as pore water content, the sediment is dried. If desired, the dried sediment can be sieved into different particle size ranges and each of those groups analyzed separately; otherwise, the dried sediment is ground to homogenize it and to prepare it for subsequent dissolution. Some or all of the ground material is then ashed at 600°C in a programmable electric muffle furnace to burn off organic matter.

Biota wet weight is recorded. For large fish or other creatures, the animal may be dissected into component parts or specific organs. Shellfish can be separated into soft and hard parts. Freeze-drying is sometimes used if the tissue must be ground and divided into sub-samples. For Pu and Am analyses, a selected amount of dried material is carefully ashed in an electric muffle furnace programmed to slowly increase the temperature to 600°C. If the ignition is properly performed, only inorganic matter remains, including the non-volatile Pu and Am. Significant reductions in sample size from dry weight to ash weight are usually obtained.

Ash dissolution : Typically 10 g of the biota or sediment sample ash is taken for analysis of Pu and Am to obtain sufficient sensitivity. Dilute HNO_3, 10 to 15 ml, is added to create a slurry, and tracer spikes (usually Pu-242 and Am-243) are carefully added in known amounts (ca. 0.01 to 0.02 Bq generally for low activity samples). Concentrated acid is then added for digestion of the ash. There are two usual approaches to the sample ash decomposition: (1) leaching with concentrated acids excluding HF, and (2) aggressive digestion with concentrated acids including HF with the intent of total dissolution.

1) Leaching with concentrated acids. The sample ash (up to 10 g) is put into a borosilicate glass beaker, dilute HNO_3 is introduced to make a slurry, and spikes (tracers) are added. Then concentrated HNO_3 (or HCl) is added and the mixture is gently boiled for 1 – 2 hours. After settling, the supernatant liquid is decanted, and a second hot digestion with concentrated HNO_3 (or HCl) is carried out. The insoluble residue is washed several times with small portions of concentrated acid (separated by centrifugation), and all decantates and washes are combined. The liquid leach is filtered to remove small amounts of insoluble material. The filtered leach solution is evaporated, and the residue is dissolved in 8M HNO_3 in preparation for the anion exchange column separation of Pu. Sodium nitrite ($NaNO_2$) is added to adjust soluble Pu to the tetravalent oxidation state. After destruction of the excess nitrous acid formed in this solution, the sample solution is loaded onto the pre-conditioned anion exchange resin column for the separation of Pu(IV) (see previous section entitled "Plutonium separation by column chromatography" under **Seawater Analyses**). The plutonium separation, purification and final electrodeposition proceed in the same manner described under **Seawater Analyses**.

2) Dissolution with HF. When a more complete breakdown of the inorganic sample matrix is desired, concentrated HF is used. The chief benefits here are the dissolution of any refractory forms of Pu (e.g. PuO_2), which may be initially present in the sample or produced by ignition to a high temperature, and the decomposition of silica and silicates. Furthermore, there may be non-refractory Pu which is physically trapped (i.e., not chemically bound) inside a matrix which is difficult to solubilize in the absence of HF. Using concentrated HF necessarily requires a special reaction vessel, most commonly a fluoroplastic beaker such as PTFE or PFA. (Platinum vessels could be used but are usually too expensive for the sizes which are needed to handle more than 1 g.) Silica is conveniently removed as the volatile SiF_4 with an excess of concentrated HF present, and transition metals form strong fluoro-complexes that are generally soluble. On the other hand, Mg, Ca, Sr and Ba and rare earth elements (e.g. La, Ce) form insoluble fluoride compounds that precipitate even from moderately concentrated HNO_3 or HCl solutions.

The typical procedure used with a 10 g sediment ash sample is illustrated as follows. After making a sample ash slurry with dilute HNO_3 and adding tracer spikes (Pu-242, Am-243) in a 250 ml PTFE beaker, 70 ml of 40% HF are carefully introduced and mixed thoroughly. The beaker is covered and strongly heated for at least 2 hours to promote aggressive attack by the HF. During this time, the sample suspension often undergoes visible changes; for example, if the original ash is brick-red colored due to iron oxide, it becomes white due to the dissolution and complexation of the Fe(III) by fluoride. Fine-grained particles disappear and are replaced by a white or weakly purple-colored (from the iron-fluoride), gelatinous suspension due to precipitation of alkaline earth fluorides. If the samples are mainly silica, a large fraction of the original material will have actually dissolved. After the covered digestion, the PTFE cover is raised on three specially made PTFE "C-clips" which attach to the rim of the beaker. These C-clips enable the hot vapors to escape while the cover is present to catch any spray of the boiling solution/suspension. Condensation and refluxing of the condensed vapor continuously cleans the cover. Excess HF and some of the SiF_4 are vaporized from the sample suspension, which is reduced to a thick paste. A second portion of 40% HF (35 ml) is added, the mixture is stirred, and the suspension heated with raised cover to remove more SiF_4 and to promote the HF aggressive digestion. This is again evaporated to a thick, wet paste.

In the next step, 50 ml of 70% HNO_3 are added, and the covered mixture is digested with minimum evaporation for 1 – 2 hours. This begins the conversion of fluorides to nitrates and the oxidization of

carbonaceous matter that was freed from occlusion in the original ash matrix by action of the HF. The cover is again raised on the C-clips and the HF/HNO_3 mixture is evaporated to a wet paste residue. (Because the boiling points of concentrated HF and HNO_3 are not very different, the acids co-distill from the mixture.) Two more 50 ml portions of 70% HNO_3 are successively added and evaporated to oxidize and remove as much fluoride as possible. However, very insoluble compounds such as CaF_2 and rare earth fluorides are not converted to nitrates by this procedure. After the three HNO_3 treatments have finished, the resulting paste is usually bright yellow if much iron is present. This indicates the removal of a lot of the fluoride, since the iron-fluoride complex is nearly colorless.

Following the last HNO_3 evaporation, 30 – 50 ml of 37% HCl and 2 g of boric acid are added to the paste residue. The mixture is covered and heated for 1 –2 hours. In the presence of strong acid, the boric acid causes the dissolution of very insoluble fluorides such as CaF_2 and rare earth fluorides by formation of the fluoborate complex, BF_{4-}. It is important to remove free fluoride which would otherwise interfere with the plutonium chemistry. If the original sample is known to contain a very high concentration of calcium (e.g. coral), the amount of boric acid is increased to 4 g. The strong HCl converts nitrate salts to chloride salts and helps to dissolve transition metals like iron and titanium which form strong chloride complexes. The initial mixture of HNO_3 and HCl provides further oxidizing power to the solution. Often the sample solution at this point is quite clear but strongly colored by the iron-chloride concentration.

When the gas evolution subsides, the solution is partly evaporated (to perhaps 15 –20 ml). A 50 ml portion of 70% HNO_3 is introduced, the vessel closely covered to intercept spray from the gas evolution, and the mixture heated to convert from chloride back to nitrate. Again, after the vigorous gas evolution ceases, the solution is evaporated down to a wet paste. About 7 ml of 70% HNO_3 are added, the mixture is heated and then 0.1M HNO_3 is added to reach around 70 ml while heating. This solution is now 1 – 2M HNO_3 and usually contains some insoluble matter. It is transferred to two 50 ml centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant solution is decanted and saved. The insoluble matter is washed three times successively with 5- 10 ml of 1M HNO_3 with separation by centrifugation. The amount of the final insoluble residue is typically quite small compared to the original 10 g of sample ash. (If the residue is not small, another cycle of digestions with HF, HNO_3 and HCl may be done to see if the amount can be significantly reduced). Some elements such as Ti(IV) are more easily kept in solution in HCl medium; in low concentrations of HNO_3 medium, the Ti(IV) tends to precipitate as a form of hydrated titanium dioxide. However, if the HNO_3 concentration is at least 1M, soluble forms of Pu should not co-precipitate to any significant degree with this hydrated titanium dioxide.

The combined 1 – 2M HNO_3 sample solution (ca. 100 ml) is then subjected to the same reduction-oxidation treatment with hydrazine and $NaNO_2$ as previously described under “Preparation for Pu separation by anion exchange” in the section on **Seawater Analyses**. In the present case, the amounts of reagents are doubled. If little or no iron is present from the sample matrix, 10 – 20 mg of Fe(III) as nitrate in solution are added before the hydrazine hydrate reduction step. Usually it is observed that the reduction of Fe(III) to Fe(II) is incomplete; however, the presence of a significant concentration of Fe(II) (as detected qualitatively by the 2,2’-BP spot test) is considered sufficient to reduce higher oxidation states of Pu down to at least Pu(IV). The final 7 – 8M HNO_3 solution of Pu(IV) in ca. 200 ml is then passed through an anion exchange column to selectively separate the Pu, as described previously. The procedure then follows closely the scheme of the Pu separation and electrodeposition detailed in the **Seawater Analysis** section.

VII.5.3 Alpha Spectrometry

Instrumentation : The measurement of Pu-238 and combined Pu-239+Pu-240 and of Am-241 in purified plutonium and americium fractions, respectively, is carried out by means of solid-state alpha spectrometry.

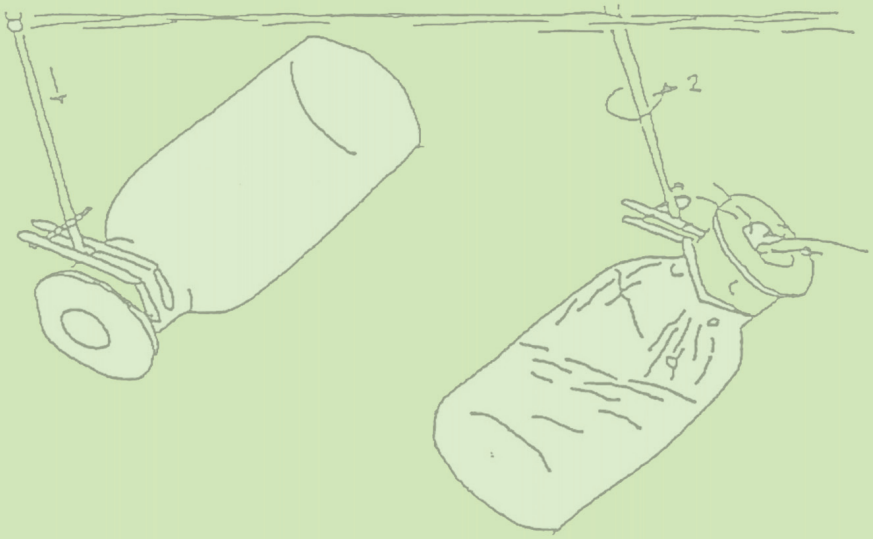
Alpha spectra characteristics : The plutonium and americium alpha sources are produced by electrodeposition of the chemically purified fractions onto stainless steel disks. Successfully prepared sources should display alpha spectra with excellent resolution for the signal peaks with almost no low-energy peak "tailing". Typically the range of alpha peak FWHM values for Pu and Am radionuclides is between 30 and 60 keV. These sources are measured at a distance of 3 – 5 mm from the detectors and had an alpha peak detection efficiency of 18 – 22%. Internal tracers of Pu-242 and Am-243, added in known amounts at the beginning of the chemical purification, are present in the final alpha spectra to indicate the chemical recoveries for Pu and Am, respectively. Since the efficiency for alpha detection is independent of energy in the 4 to 10 MeV range with these detectors, the alpha peaks of Pu-238, Pu-239+240 and Pu-242 all have the same detection efficiency. Therefore, the activities of Pu-238 and Pu-239+240 can be easily obtained by their observed peak area relative to the tracer Pu-242 peak area. The same is true for Am-241 and Am-243. Corrections for chemically processed blank sample and background contributions (obtained from independent measurements with new stainless steel disks) are subtracted from the sample data to give the net Pu radionuclide and Am-241 sample activities.

Re-processing of sources : In the rare case where a Pu or Am source is found to be significantly contaminated by foreign radionuclides (e.g. The series, U-234 and U-238, Po-210) or has an unacceptably poor spectral resolution, the activity is stripped from the disk by soaking in hot concentrated HNO_3 for 1 – 2 hours. Only a few milligrams of the stainless steel components (Fe, Ni, Cr) are dissolved by the HNO_3 while the Pu or Am is quantitatively leached. Then the Pu or Am is carefully re-purified by techniques known to remove the particular radionuclide contaminant(s) as well as the Fe, Ni and Cr. For example, Pu is purified of radioactive contaminants such as U-234 (alpha spectral interference with Pu-242) and Po-210, as well as Fe, Ni and Cr, by adjusting its oxidation state to Pu(IV) and by anion exchange column chromatography from 8M HNO_3 again. Finally, the re-purified Pu or Am sample is again electrodeposited.

Limit of detection : The detection limit for Pu-239+240 by alpha spectrometry has been estimated on the basis of Currie's formula for paired observations {LD(counts) = 2.71 + 4.65 $\sqrt{\mu B}$} under the following conditions :

- a background count rate of 10 counts in 10 days in the 5.15 MeV energy region of the alpha spectrometer (μB = 10 counts)
- a Pu chemical recovery of 50%
- an alpha detector efficiency of 20%

The resulting detection limit is 0.2 mBq of Pu-239+240. For 100 liters of seawater, this corresponds to a detection limit of 2 μBq/liter of Pu-239+240.



VIII BIOLOGICAL OCEANOGRAPHY



VIII BIOLOGICAL OCEANOGRAPHY

VIII.1 MARINE SAMPLING

General consideration

Sampling should be performed in a systematic manner to reduce variation between individual results. For this reason, it is necessary to maintain as many factors as possible constant. These include both the actual period of sampling (i.e. time of day) and the actual sampling method, as well as the location and depth of individual sampling points.

The actual sampling period (i.e. time of day) in the same station should not vary by more than 3 (preferably 2) hours. The actual optimum sampling time would depend on a number of factors. Two approaches are possible in determination of the optimum sampling time: (a) coincidence with peak use, and (b) correlation with the effects of intermittent-flow sources, such as sewage outlets. Eventual selection of the optimum sampling time will depend on the particular approach adopted in the light of the overall objectives of the monitoring programme, i.e. to ensure adequate health protection for bathers.

Examination of a large number of samples together with their analysis for a relatively small number of important microbiological parameters would be preferable to the reverse, i.e. a small number of samples analyzed for many parameters.

Every effort should be made to achieve the fullest possible degree of standardization between all sampling stations.

VIII.1.1 Sampling Point Locations

In large beaches, as well as in relatively extensive shellfish areas, multiple sampling (i.e. the taking of a number of samples from within the same overall location) will have to be performed. For recreational waters, normal locations of sampling points for multiple sampling are generally considered to be best spaced at intervals of 250 meters along the coastline, 10 meters seaward from the low tide mark. However, the exact location of sampling points in any monitoring programme, including the distance between them, would be expected to vary with each individual beach, the particular requirements of which would have to be determined by the local responsible authorities. In addition, the criterion for determination of the actual location relative to the shoreline should be water depth, rather than distance from shore. As the most critical part is that immediately near the shoreline, those areas where the water is 20-30 cm deep should invariably be monitored. One other good indication as to where to monitor is to look for those parts where bathers tend to congregate most. In sandy beaches, the two most important points to monitor from the point of view of distance from shore would be where the water is at approximately waist-high level (where most adults will be found) and in the shallows near the shoreline (where small children would normally be).

Rocky beaches present more variations, as there may be no regular bottom gradient, and the bottom could be rocky, sandy or mixed. Large shallow pools where children are found should be monitored. Where the water depth exceeds 5 meters, water samples should preferably be taken at surface, bottom and intermediate points. Where the bottom is composed of sand, silt or mud, a sediment sample should also be taken. Multiple sampling points should also include points remote from direct (outfalls) and indirect (rivers, creeks, etc.) wastewater discharges. Results from such points would serve as references for other observations in the area, and would also serve for comparison between areas. A seaward sampling point should also be established within one kilometer of point source.

The location of sampling points in areas used for recreational activities other than bathing, i.e. windsurfing, canoeing, scuba diving, etc., would have to be determined on an *ad hoc* basis, depending on the circumstances prevailing in each particular locality.

For shellfish areas, sampling point locations should be situated :

a) in the shellfish culture or harvesting area
b) in the culture or harvesting area surroundings
c) close to sources of pollution affecting the culture or harvesting area

Selection of the sampling locations will depend on the exact aims of the monitoring programme, on the resources made available for monitoring, and on the specific characteristics of the area itself. Where adequate resources for comprehensive monitoring are not available, or where the area itself does not permit the coverage described above, modifications should be made, keeping in mind that the primary aim of the whole programme is to determine the suitability of the area for recreational purposes or for the harvesting of shellfish for human consumption from the public health viewpoint, i.e. to ensure that no significant health risks are attached to bathing in, or consuming shellfish from, the area in question. .

Adjustments to the monitoring programme, such as alterations in the number or location of sampling points, may be realistically considered only after the completion of at least one season's monitoring.

VIII.1.2 Sampling Frequency

For coastal recreational waters, observations and measurements at the sampling points should be made at two-week intervals (as a minimum) as well as after very considerable rainfall in beaches located in the vicinity of an outfall. Under special circumstances, a higher frequency may have to be applied during specific periods. For example, measurements would have to be taken immediately after any accident or other circumstance (such as leaks in sewerage systems) likely to affect water quality and continued at a frequency dictated by the situation itself until it reverts back to normal.

For commercial shellfish areas observations/measurements should be carried out once a week during the peak consumption period when shellfish are harvested. When no harvesting occurs, monitoring may be carried out at longer intervals.
For natural shellfish areas, observations/measurements should be carried out once a week during the season in which the shellfish are collected, and at longer intervals for the rest of the year.

VIII.1.3 Sampling Equipment

For surface and subsurface sampling, the containers utilized should be bottles of dark-coloured borosilicate glass of 200 to 300 ml capacity, wide-mouthed and with ground glass stoppers. A common method of sampling directly beneath the surface is shown in Figure (VIII.1). The same type of bottle may be used for subsurface sampling with the addition of an extension arm and clamp, as in Figure (III.5). One type of equipment for sterile subsurface sampling is shown in Figure (III.6).

For sediments, two types of bottom samplers are shown in Figure (VIII.2) and Figure (VIII.3) respectively.

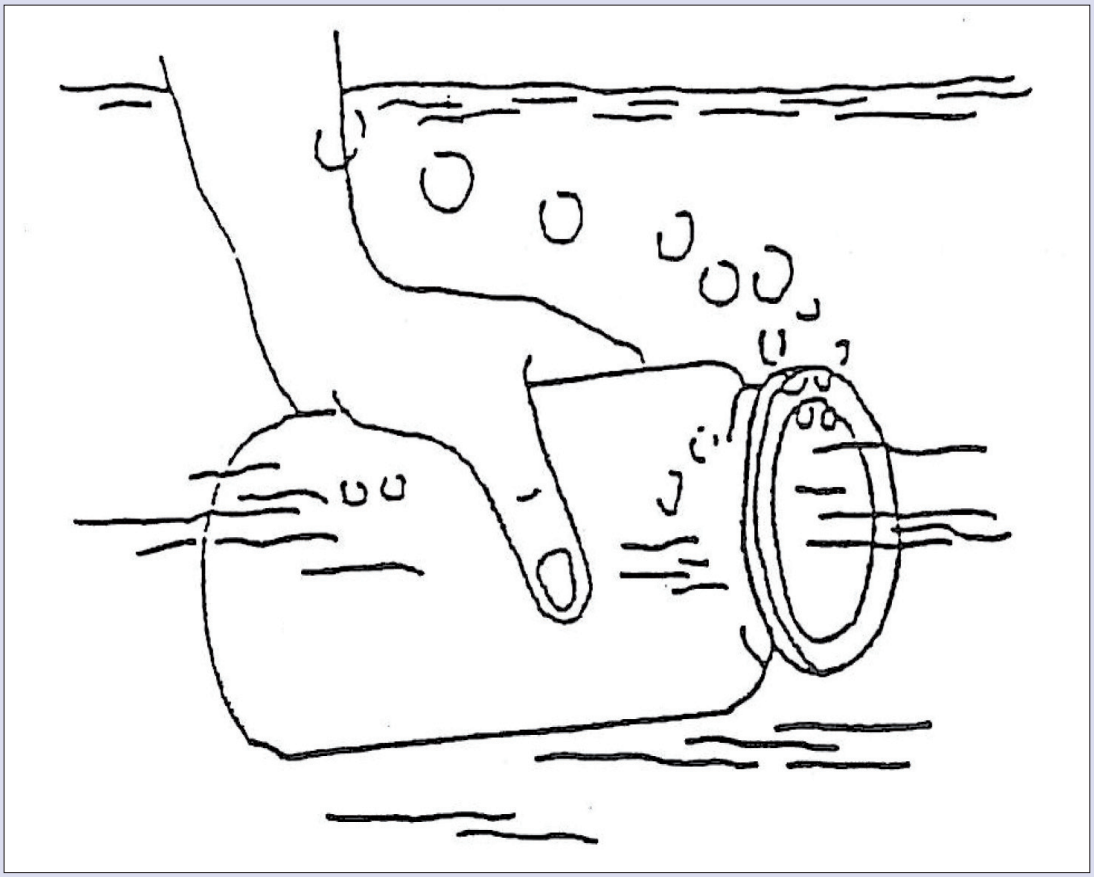


Figure (VIII.1) : Subsurface sampling by hand

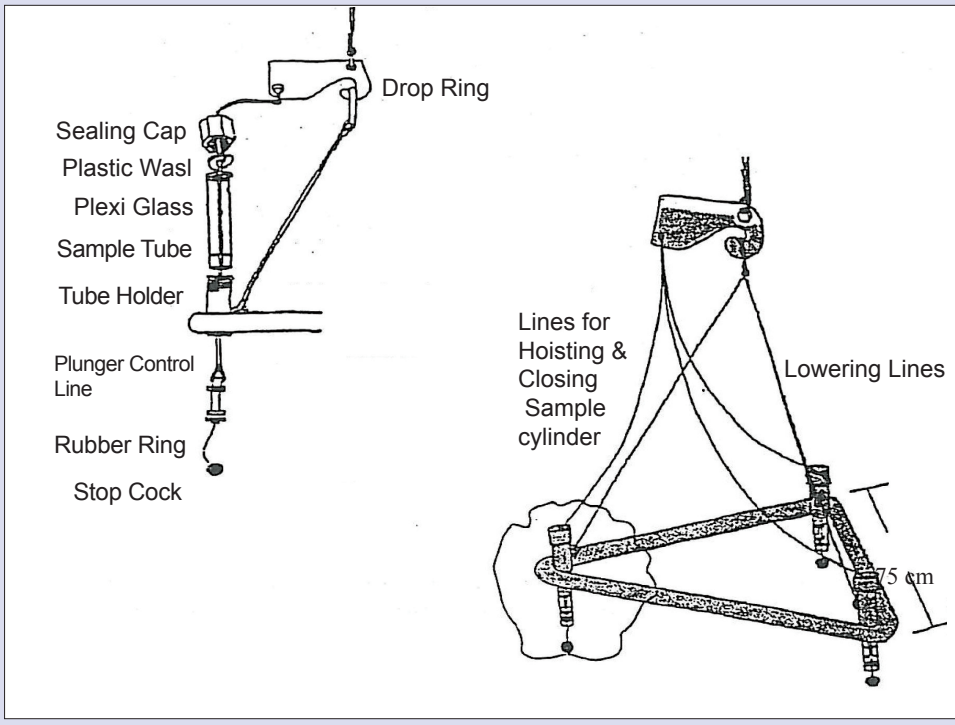


Figure (VIII.2) : Three-core Albrechtsen bottom sampler

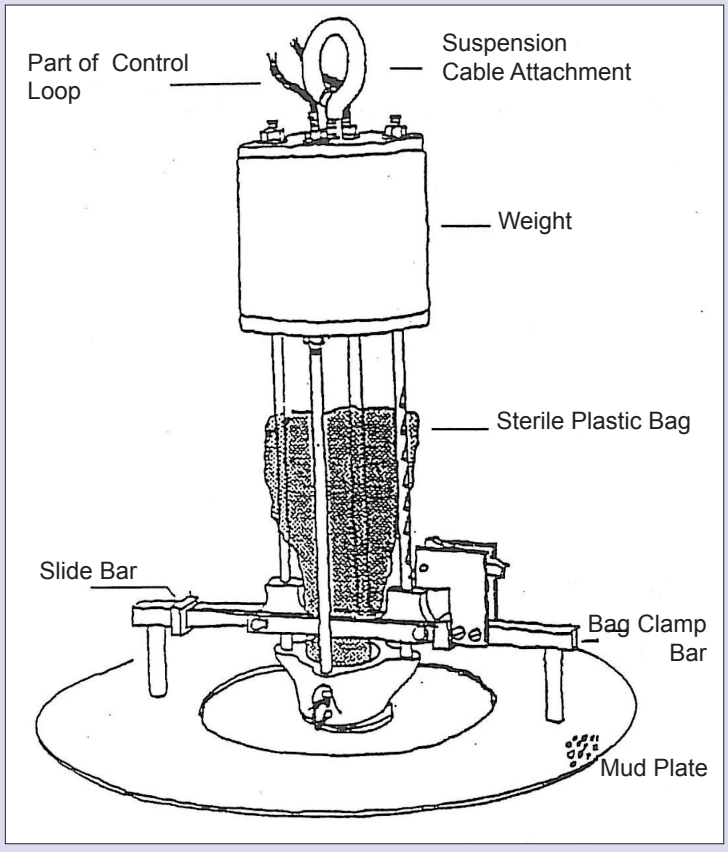


Figure (VIII.3) : The Van Donsel-Geldreich bottom sampler

Sterilization of glassware and equipment

Surface sample bottles. Clean all sample bottles as described above and dry. Autoclavable plastic sample bottles can only be sterilized in the autoclave (121°C for 15 minutes). Be sure screw-cap tops are loose fitting during sterilization to avoid bottle collapse due to steam pressure in autoclaving. Sterilize glass sample bottles preferably in a drying oven for two hours at 160°C. Before sterilization, place a small piece of filter paper in the neck of the bottle to prevent the ground glass stopper from sticking after cooling. The paper insert should be left in place until time of sample collection. At that time, discard the paper insert by flipping it out of bottle. Do not insert it into the sample or remove with fingers because of chance contamination. Put bottles into detergent-cleaned thermo-insulated boxes. Separate the bottles from each other with clean wrapping paper to avoid breakage.

Note : If residual chlorine is suspected in the water sample, add 0.1 ml of a 10 per cent sodium thiosulphate solution for each 100 ml of sample to the contents of the sample bottle before sterilization. This amount is sufficient to neutralize about 15 mg of residual chlorine per litre

VIII.1.4 Sampling of Water

A) Sampling of surface water

See Section III.1.1.6.8 (Part A).

B) Sampling of subsurface water

See Section III.1.1.6.8 (Part B).

VIII.1.5 Sediment Sampling

When monitoring marine sediments, the sealing characteristics of particles in sewage or in rivers may be of importance because the organisms adhere to or even constitute the settling particles. The discharge of a river into the coastal zone may significantly influence the marine environment, so that several different monitoring regions must be distinguished, as salinity, temperature, turbidity and other parameters vary greatly among them.

Sediment sampling can normally be carried out only where the bottom consists of relatively soft and homogeneous sands, silts, clays or mud. A rocky bottom should not be sampled by the equipment described below, and a quantitative interpretation of any such results would be most difficult.

Core sampling is the only technique advised for fine sediments (mud). The grab samplers commonly used for other biological sampling seem inappropriate as they immediately mix the sediment surface with deeper sediments. The microbiological precipitate normally concentrates in the sediment surface, which is consequently where sampling should take place.

The Albrechtsen sediment sampler, Figure (VIII.2) has been used widely in Scandinavian waters and it is simple to produce as well as to use. The van Donsel-Geldreich bottom sampler, Figure (VIII.3) also seems adequate at least for areas where the sea bosom is soft.

In spite of the compatibility of data that can be achieved by using a single sampling device, it should be noted that due to lack of homogeneity in the microbiological density in the sediment, the comparability of results from different samplings will always be questionable. For this reason, the use of devices of different types would jeopardize the comparability of results.

The Albrechtsen sediment sampler, where it can be employed, is consequently recommended for common use. 200-300 ml wide-mouth sterile plastic bottles for collecting samples.

Samples of dry sand from recreational beaches can be collected with a shovel and kept into sterile plastic bass or into 200-300 ml sterile plastic bottles. Approximately 100 g of sand should be collected for each sample. Finally, the sediment sampling could be performed in different locations as follows:

VIII.1.6 Beach Sampling

Dry or lightly humid sand at recreational beaches

The easiest way is to collect a sand sample of 100 g from the top 5-cm layer of the beach using a small clean shovel and to keep it into a plastic bag.

Very humid mud at shellfish growing areas

Obtain a core sample from the top 5-cm layer using a metal or glass sterile cylinders. The core sediment sample is kept into a sterile plastic bottle.

VIII.1.7 Sub-tidal Sampling

In this case sampling procedures are more delicate.

A sediment sample can be collected using a sediment samples (Albrechtsen or van Donsel-Geldreich) from a boat, or by skin diving

Shell fish (bivalves) sampling

Details of bivalves sampling are provided in Section III.1.3.1 taking into general considerations regarding sterilization for sampling equipment and handling.

VIII.2 BIOLOGICAL ANALYSIS

VIII.2.1 Phytoplankton

VIII.2.1.1 Analysis of Phytoplankton

Sampling

Collect surface waters (at approximately 0.5 m depth) using a Niskin bottle or, if this is not available, a bucket. If a subsurface chlorophyll a maximum is present, an additional sample has to be taken at this depth, always using a Niskin bottle.

Due to its substantial seasonal variability, phytoplankton should be sampled at least once a month, fortnightly in summer.

In presence of an oligotrophic monitoring area, nets are useful in filtering large volumes of water and could be employed to provide material for qualitative purposes. Transfer the sample in 250, 500 or 1000 ml dark glass bottles with screw cap. Do not fill up the bottle completely to allow a gentle mixing of the sample when in the lab. Keep the bottles in a dark, cool place.

Preservation and storage of samples

The most commonly used fixatives are formaldehyde and Lugol's solution, but it is good practice to observe an alive subsample first. This would allow a better characterization of cells morphology, colour and motility.

- *Lugol*

  Acidic Lugol's solution is recommended, since it is less toxic than formaldehyde. Lugol's solution is adequate for the preservation of dinoflagellates, diatoms and delicate species, such as small flagellates. It is less indicated for the preservation of coccolithophorids, because it can dissolve the $CaCO_3$ (if storage time is longer than one month).

  The use of Lugol's solution excludes the possibility to use epifluorescence microscopy and SEM.

  Add 0.5-1 ml of Lugol's solution to 250 ml of sample. If the cells become too dark it is possible to obtain a lighter colour by adding sodium thiosulfate.

  Store the fixed samples at room temperature, in the dark. Samples can be stored for up to 12 months, but it is recommended to check the colour of the solution, that tends to become lighter with time due to the oxidation of iodine, thus reducing fixation.

Acidic Lugol's solution

20 g potassium iodine (KI)
200 ml distilled water
10 g iodine (I_2)
20 ml acetic acid (CH_3COOH)

◊ *Formaldehyde*

Formaldehyde can be used as an alternative to Lugol's solution. The advantage is that it allows further investigation by epifluorescence microscopy or SEM.

Formaldehyde solution
Use 40% formaldehyde and add an equal volume of distilled water (20% final concentration).

100 g/L of hexamethylenetetramine to keep the final pH neutral or slightly basic.
Filter the solution before use. Store the solution at 5-6°C and add 20-40 ml of solution per litre of sample.

For a neutralized (weakly alkaline) solution add sodium borate or sodium carbonate.

Analysis

Perform a quali-quantitative analysis of the subsample using the inverted microscope method described by Utermöhl (1958) (Zingone *et al*., 1990).

On the basis of the observed chlorophyll a values, use 10, 25, 50 or 100 ml sedimentation chambers. Gently shake up and down the glass bottle for about 1 min (or about 100 times) before adding the subsample to the sedimentation chamber.

Counting must be performed when all the cells have settled to the bottom. A sedimentation time of 3 hours per centimetre chamber height commonly results in a complete sedimentation of all the organisms, but is suggested to use longer times especially for those samples containing many small species

Cell counts are to be performed on casual fields or on transects for abundant species, and on the whole bottom area for less abundant species.

- **casual fields** : the number of fields must be at least 1/100 of the surface area of the sedimentation chamber, according to the magnification used. If the total number of cells counted is less than 200, increase the number of fields. Use a magnification of about 400x
- **transects** : choose two perpendicular transects. If the total number of cells counted is less than 200, increase the number of transects. Use a magnification of about 400x

Use the following equations for estimating cell concentrations :

a) Cell counts performed on fields

$$C = (N \cdot 1000 \cdot A) / (n \cdot v \cdot a)$$

where :

C = cells concentration, expressed as cells/L
N = total number of cells counted on all fields
A = surface area of the bottom of the sedimentation chamber (in mm2)
n = number of fields used for cell counts
v = volume (in ml) of the sample
a = area of the visual field (in mm^2)

b) Cell counts performed on transects

$$C = (N \cdot \pi \cdot r \cdot 1000) / (2 \cdot h \cdot v \cdot n)$$

where :

C = cells concentration, expressed as cells/L
N = total number of cells counted on all transects
r = radius (in mm) of the sedimentation chamber
h = height (in mm) of the transect (diameter of the visual field)
v = volume (in ml) of the sample
n = number of transects used for cell counts

Recommendations

When studying eutrophicated environments it is very important to give an estimation of the phytoplankton biomass which is involved into the process. For this purpose it is recommended to transform cell abundance data into a more useful form, cell volume, by morphometric measurements and geometric formulae.

VIII.2.1.2 Direct Counting of Nanoplankton by Epiluorescence Microscopy

Sampling and preservation

1. Water sampling by Niskin bottles

2. Preservation

   Subsamples (100 ml) in plastic bottles are preserved with prefiltered (0.22 µm) glutaraldehyde (final concentration 1%) and stored in the dark at +4 °C until slides are prepared (within few days from sampling).

Direct counting

The analysis is performed using the Sherr method (Sherr *et al*.,1983), which involves the observation by epifluorescence microscopy of 20 ml of sample. This volume of water is prefiltered (low vacuum, 48 kPa) onto black policarbonate filters (2 µm pore size, 25 mm diameter). A Millipore filter (0.45 µm) was placed underneath the Isopore black filter to promote even dispersion of sample on the black filter. When 2 ml are left to filter, 2 µl of a DAPI solution (1 mg/ml) are added to the sample, which is finally left for 15 min at total darkness without vacuum. This allows the formation of a DNA-DAPI complex. The filter is then preserved at -20° (total darkness) until observation by epifluorescence microscopy. The filter is mounted on a glass slide and it is observed using a final magnification of 1000x (Figure VIII.4). Heterotrophs are counted using an UV-filter set (BP 340-380 exitation filter, RKP 400 dichroic mirror, LP 430 barrier filter). Autotrophs are counted using a Blue-filter set (BP 450-490 excitation filter, RKP 510 dichroic mirror, LP 510 barrier filter). The number of observed fields (20-30) depends on sample concentration (minimum cell number: 200).

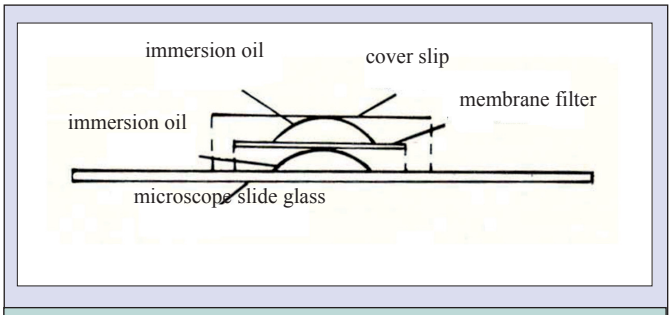


Figure (VIII.4) : Mounting of the filter on the microscope glass slide (in Maugeri T.L. *et al*., 1990)

Nanoplankton calculation :

$$cell/ml = N \bullet (A_f/A_c) \bullet 1 / V$$

where :

N = average number of cells counted in the field
A_f = filtration area
A_c = field area
V = volume of sample filtered (in mL)

VIII. 2.1.3 Direct Counting of Picoplankton by Epifluorescence Microscopy

Sampling and preservation

1. Water sampling by Niskin bottles
2. Preservation

Subsamples (100 ml) in plastic bottles are preserved with prefiltered (0.22 µm) formaldehyde (final concentration 2 %) and stored in the dark at +4 °C until slides are prepared (within few days from sampling).

Direct counting

The analysis is performed using the Porter & Feig method (Porter & Feig, 1980), which involves the observation by epifluorescence microscopy of 2-3 ml of sample. 2-3 µl of a DAPI solution (1mg/ml) are added to this volume of water, which is then stored in total darkness for 15 min. Subsequently the sample is filtered (low vacuum, 48 kPa) onto a black policarbonate filter (2 µm pore size, 25 mm diameter). A 0.45 µm Millipore filter was placed underneath it to promote even dispersion of sample on the black filter. The filter is then preserved at -20° (total darkness) until observation by epifluorescence microscopy. The filter is mounted on a glass slide and it is observed using a final magnification of 1000x. Heterotrophs are counted using an UV-filter set (BP 340-380 exitation filter, RKP 400 dichroic mirror, LP 430 barrier filter). Autotrophs are counted using a Blue-filter set (BP 450-490 excitation filter, RKP 510 dichroic mirror, LP 510 barrier filter). The number of observed fields (20-30) depends on sample concentration (minimum cell number: 200).

Picoplankton calculation :

$$cell/ml = N \bullet (A_f/A_c) \bullet 1 / V$$

where :

N = average number of cells counted in the field
A_f = filtration area
A_c = field area
V = volume of sample filtered (in mL)

VIII.2.2 Zooplankton

The zooplankton samples collected and preserved on board, are ready for volume determinations and identification.

Volume determination

A measurement of wet plankton volume, determined by displacement, is made for each plankton sample soon after the samples are taken ashore. The volume measurement provides a rough measure of zooplanktonic biomass and it also has a practical value in the subsequent handling of the sample.

The preserving liquid is removed from the plankton by pouring the sample through a drainage cone, constructed of 0.33 mm NITEX (nylon screen). The plankton is retained in the cone until drainage of liquid from the cone diminishes to an occasional drop. The volume of the drained plankton is then determined by displacement in a graduated cylinder.

Even after drainage, the sample will retain a considerable amount of interstitial liquid, usually between 30 to 40% of the total volume according to Ahlstrsm and Trailkill (1963). Usually no allowance is made for interstitial liquid; rather the total displacement volume of the drained wet sample is reported. The sample is returned to the original preserving liquid. Divide the displacement volume by the volume of water filtered through the flowmeter and then the displacement volume is ml/m^3.

Examination and counting

Examination is probably the step that requires the most time. Before the identification is started, the preserving liquid should be drained off because the formaldehyde fumes, when breathed, can be irritating and even injurious to health. Precaution must be taken to prevent deterioration of the plankton material. If a sample has not been completely identified during the working hours, the unidentified plankton should be put back into 5% buffered formalin during the night

The examination and counting of zooplankton samples is done in several ways. First, the whole sample is examined for very large species (>10 mm), which are all sorted out and identified. It is advisable, although time consuming, to examine the whole sample also for rare species, which may not appear in a sub-sample. For this purpose successive portions of a sample are examined in a petri dish under a good dissecting microscope.

The bulk of the sample cannot be examined as a whole, at least not while doing serial work, and must, therefore, be sub-sampled. It is recommended to have sub-sample as follows :

1/128 - small sized animal with large number
1/64 - medium abundance
1/8 - large sized animal with small abundance

For this purpose the use of the Folsom plankton splitter is recommended. This device separates the whole sample into two equal portions. Half portion of the sample is diluted and returned to the splitter to obtain the 1/4 sample, and so on. Some sub-sample must also be examined in portions; suitable portions are pipetted into a counting tray with a Stempel pipette. Some original design of the counting tray by Bogorov (1927) was modified by Russell and Colman (1931), and has three grooves. Some tray can easily be made in a workshop out of perspex and a cleaned photographic plate glass. You can construct it with six grooves to accommodate more animals, Figure (VIII.5).

The counting of easily recognizable species and major taxonomic groups can be done straight away, but other groups or other taxa are transferred from the bulk sample into separate vials for later identification by experts. Skilled and patient technicians must assist planktonologists in this operation. Provide adequate time needed for taxonomic identifications, compilation of quantit at ive data and interpretation of results. Organisms should be identified to species wherever possible and the number per cubic meter is to be calculated from the amount of water filtered through the net and the volume of the sub-sample. (See section VIII.2.3 for flowmeter calculation)

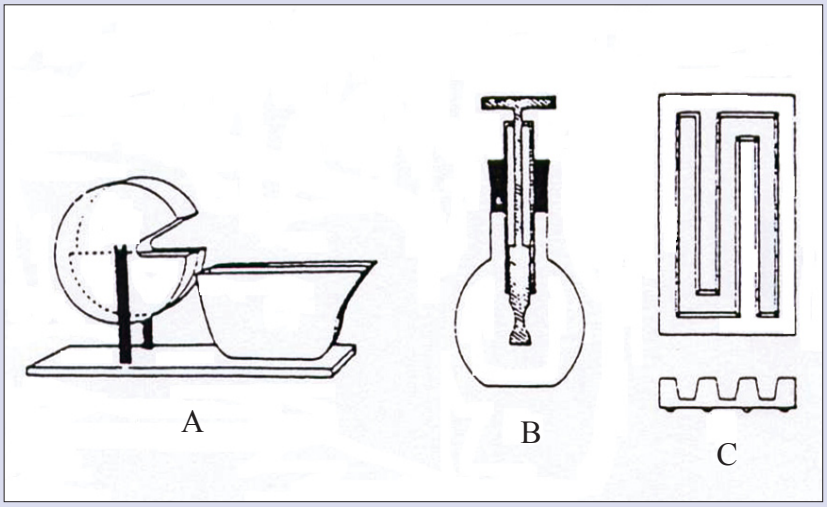


Figure (VIII.5) : Zooplankton sub-sampling and counting equipment
A) Folsom splitter
B) Stempel pipette
C) Bogorove counting tray

VIII. 2.3 Ichthyoplankton

In marine fisheries research the sampling of eggs, larvae and juvenile fish aims generally at the detection and appraisal of fishery resources and at studies on the population dynamics of fishes. Surveys of ichthyoplankton have been found suitable to explore for new resources, to locate spawning concentrations of important stocks, to describe relative abundances of commercially important stocks including comparison within and amongst regions, to monitor long-term changes in the composition and abundance of resources, and in spawning times and areas.

Eggs and larvae of most of the important marine fish species are entirely pelagic throughout a certain period of their life and can be found within the upper 200 or less meters of the water column. Most kinds of fish larvae are confined in their vertical distribution to the upper mixed layer. Hence they may be caught fairly easily with plankton sampling gear.

Many different gears have been used for quantitative collections of fish eggs and larvae on survey cruises since scientists started to study plankton. For standard hauls, nets are usually lowered at a ship's speed between 2 and 8 kn (depending on the type of gear) down to a depth at which fish larvae can be expected, then the net is retrieved. Oblique tows which are done in such a way, have the advantage that much more water is strained within each depth layer than in vertical tows.

Almost all of the extensive national and international fish eggs and larval surveys of the past ten to twenty years chose oblique tows for sampling fish fry. Bongo net is used for all kinds of fish eggs and larval surveys.

The Bongo is a paired net Figure (VIII.6). Two rings or cylinders of 60 cm diameter each are combined by an axis which also holds the towing wire. This assembly may be towed at low speeds as well as up to 6 knots.

The accuracy of ichthyoplankton survey results depends on knowledge of the sampling efficiency of the gear. In this respect not only filtration efficiency, avoidance and extrusion, but also handling of the gear has to be considered. Filtration performance in simple plankton nets without encasement is fairly well understood. If the ratio of effective straining (aperture) area to mouth area is not less than 5 to 1 the initial filtration efficiency approaches closely to 100 percent. This is the fact for all Bongo models.



Figure (VIII.6) : Bongo net

Clogging is of special importance as reduction in filtration efficiency during an oblique tow may affect the accuracy of sampling results in several ways :

i. It may lead to unequal amounts of water being filtered at different depths and hence organisms, not homogeneously distributed over the total depth range, will not be sampled representatively for that water column
ii. Selectivity of the net will change during a tow as larger fish larvae may avoid even high speed samplers with reduced filtration efficiency and the proportion of small organisms extruded through the meshes will be reduced when clogging occurs

Thus, it is necessary to measure the total amount of water filtered during a tow by means of a flowmeter mounted in the mouth of the net to determine the volume of water filtered during each tow. The filtering capacity of the net model must also be sufficient for the particular situation under study and the desired duration of tow. This may be checked by comparing initial and final readings of the flowmeter.

At high filtration efficiency avoidance by fish larvae is apparent only when sampling for the older more mobile larvae with low towing speeds (about 2 kn) whereas good evidence is given that sampling results will not be biased by avoidance when fishing at high speeds (about 5 kn). In the latter case, however, extrusion may affect accuracy of results by reducing the proportion of very small larvae in the samples.

In any case mesh size and towing speed have to be adjusted according to the size and mobility of organisms sampled for, and taken into account the specific filtration pressure of the gear used. With regard to sorting effort mesh size should, of course, not be smaller than necessary for retaining the desired organisms quantitatively. At low towing speed a mesh size of 0.5 mm will retain most fish larvae. At high speeds, on the other hand, several small forms of larvae will be pressed through meshes of this size as even organisms which are somewhat larger than mesh width may be extruded in this case. To allow for all possibilities it is recommended that the Bongo should be used with its nets having different mesh sizes (0.5 and 0.3 mm) and be towed at two different speeds (1.5 to 2 and 4 kn).

The samples should be preserved immediately with 5% neutralized formalin. In the laboratory, fish eggs and larvae should be sorted out from rest of the zooplankton. Numerical estimation of egg abundance of different species should be made individually using a microscope. If the eggs of a particular species are found in super abundance, sub-samples are to be used. All larvae samples should be counted irrespective of their abundance. The raw catch data of eggs and larvae at each collection should be standardized to number per m3 based on the flowmeter readings.

Flowmeter

The flowmeter is used to determine the volume of water passing through a plankton net Figure (VIII.7). A Hydro-Bios digital flowmeter can be used for this purpose. It has a three blade impeller which is coupled directly to a five digit counter. The pitch of the impeller is 0.3 meter per each revolution. Therefore, the number of revolutions multiplied by 0.3 gives the towing distance in meters.

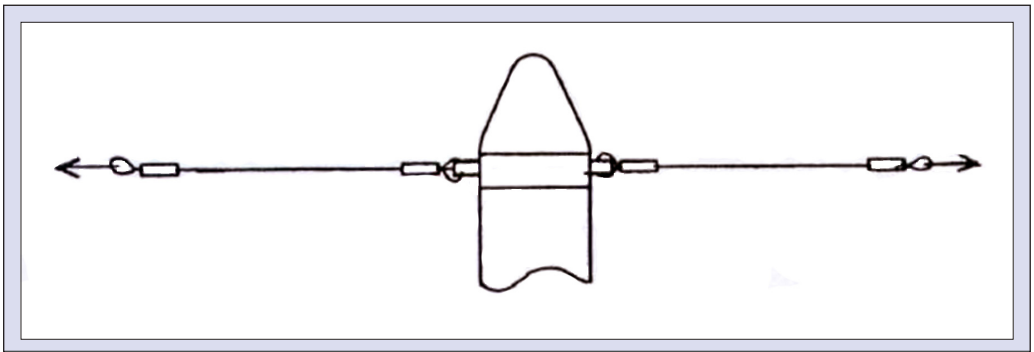


Figure (VIII.7) : Flowmeter

Example for the calculation :

a) Calculation of towing distance :

Initial reading of the flowmeter (I) = 00061
Final reading of the flowmeter (F) = 00181
No. of revolutions (F – I) = 00181 – 00061 = 120
Therefore towing distance = 0.3 x 120 = 36 meters

b) Calculation of water volume passing through plankton net :

Volume of water passed through net (vol. of water filtered) =
Flowmeter reading x area of the net x c.f (pitch of flowmeter 0.3)

$$\text{Standardizing for one } m^3 = \frac{1 \times \text{number of plankton or vol. of plankton (ml)}}{\text{Vol. of water filtered}}$$

Value will be represented as Number or volume / m^3

VIII.2.4 Benthic fauna

In the laboratory the samples are stirred gently by plastic rod and placed in ultrasonic water bath for 30 seconds to fix the stain of organisms (Theil *et al*., 1975). Sieve the samples by using 0.5 mm and 0.063 mm sieves (meiofauna size).

By using rinsing and floating method (Holme and McIntyre, 1984; Hulings Gray, 1971).

Among specimens of the fauna, which do not posses solid shells, the specific gravity is generally smaller than that of the coarser sediment particles. When stirred up by using dense solution of $CaCl_2$ or NaCl with specific gravity more than 1.17 g/ml prepared by dissolving a quantity of salt in filtered seawater. Its density can be determined using the hydrometer (1-1.2 ml).

When stirring up with the dense solution the animals will remain in suspension longer than do sand grains.

After seconds the sand grains settle on the bottom of the container and the dense solution which contains the fauna and other lighter particles in a suspension can be poured through a 63 µm sieve. Thus the rinsing procedure is followed by the sieving procedure.

Examination and counting

Organisms are sorted and counted under a stereoscopic microscope in petri-dishes, which can be marked with paralleled lines or squares etched on the bottom to aid scanning. For identification to species level under the high power microscope, the organisms must be picked out for mounting on slide using a fine pipette, fine tungsten needle, sharpened quill or similar instrument. Counting of higher tax (e.g. nematodes, copepods in site is far less time consuming and in this case the use of moveable stereomicroscope with an extended arm is recommended. Fix the petri-dish in its position and the organisms should not be disturbed during the scanning of the dish bottom and the surface film.

VIII. 3 MICROBIOLOGICAL ANALYSIS

There are two major methods for microbiological analysis, which are as follows:

a) Multiple Test Tube Method (MTM)
b) Membrane Filtration Method (MFM)

The Membrane Filtration (MF) Culture Method is preferred to the MPN Test depends on local conditions and personal preferences. In general the MF method is less labor intensive and due to the pre-concentration of the bacteria in the sample it is more suitable in situations where low numbers of coliforms are to be estimated. The MPN test should be given preference when the test sample contains high amounts of particulate matter, which will hinder the reading of the MF's after incubation.

VIII.3.1 The Multiple Test Tube Method (MTM)

Replicate tubes of the appropriate culture medium are inoculated with decimal dilutions of a water sample. The bacterial densities are then calculated from probability formulae that predict the most probable number of bacteria necessary to produce the combinations of positive and negative tubes actually observed when analyzing replicate decimal dilutions. The Most Probable Number (MPN) is obtained from the corresponding MPN tables.

VIII.3.1.1 Determination of Total Coliform in Seawater

The method described is suitable for the determination of total coliforms in coastal bathing waters of temperate and tropical seas and is designed to be used in sanitary surveillance of bathing beaches.

A) Definition

Total coliforms are aerobic and facultatively anaerobic, Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas at 35°C within 48 hours.

Since total coliforms die within hours when exposed to sunlight in seawater at temperatures above +4°C, their presence in seawater indicates only recent contamination by faecal material and by soil in storm-water runoff. Die-away rates (T-90).

B) Principles

Quantification of total coliforms in marine (seawater) samples is accomplished by testing multiple sample portions in a lactose-based medium for gas production at 35°C within 48 hours. Positive findings in the presumptive test phase are confirmed in a second medium for verification of coliform occurrence.

Five sample portions for each multiple tube dilution (10 ml, 1 ml, 0.1 ml, etc.) are inoculated into individual tubes of lactose broth. After 24 hours incubation, all lactose cultures are examined for gas production. Those that have visible evidence of gas production are confirmed by transferring a loop-full of culture from each positive tube to individual tubes of MacConkey broth or brilliant green broth and incubating at 35 ± 0.5°C. Presumptive tubes showing no gas production are re-incubated for an additional 24 hours, then inspected for gas production. Any new positive cultures are confirmed as previously described and incubated at 35 ± 0.5°C. All 48 hours negative presumptive tube results are recorded, then discarded. All confirmation cultures are inspected at 24 hours and 48 hours for gas production. These results are also recorded and the combination of confirmed positive reactions (gas development in brilliant green or gas + acid development in MacConkey broth at 35°C) in the different dilutions within 48 hours is used for the calculation of the most probable number (MPN) of total coliforms in the water sample.

C) Apparatus and glassware

i. Sample bottles of borosilicate glass or autoclavable plastic bottles with screw-cap tops, 200-250 ml capacity, wide-mouthed and with ground-glass stoppers are used to collect marine (seawater) samples
ii. Sample rod of non-corrosive material with a clamp to hold the sampling bottle
iii. Subsurface sampler of the type shown in Figure (III.6), or similar, complete with plastic rope and weight
iv. Thermo-insulated plastic container with pre-frozen packs of chemical gel (camping equipment) for storage of samples
v. Thermometer, 0 to 50°C, precision ±1°C, to be used for checking temperature in sample collection containers. Thermometer, 0° to 50°C, precision ±0.5°C, to be used for checking temperature during incubation
vi Air or water incubator adjusted to 35 ± 0.5° C with suitable test tube racks for incubating multiple tube cultures
vii. Autoclave, maximum 2 atm., electric or gas
viii. Drying oven for sterilization of glass sample bottles and pipettes at 160°C for 2 hours
ix. pH meter, precision ± 0.1 pH units

x. Balance for media preparation, precision + 1 mg
xi Refrigerator with temperature range of 2-8°C for storage of samples to be processed within 24 hours of collection
xii. Vibrator {shaker) for mixing sample aliquots in serial dilution (optional).
xiii Ehrlenmeyer flasks of borosilicate glass for media preparation, capacity 1 and 2 litters
xiv. Borosilicate glass bacteriological culture tubes with sufficient capacity to receive 20 ml of medium
xv. Small borosilicate glass tubes 6 x 50 mm ("Durham vials") to be inserted in culture tubes (5.15) for detection of gas formation in the multiple test tubes
xvi. Total volume (blow-out) borosilicate glass pipettes of 1, 9 and 10 ml capacity with stainless steel containers for sterilization

Note : 9 ml capacity pipettes are optional

xvii. Graduated borosilicate glass cylinders of 100, 500 and 1000 ml capacity with glass beakers, aluminum foil or wrapping paper for cover
xviii. Bacteriological transfer loops made from 22-24 Chromel gauge, nichrome or platinum-iridium. Diameter of the loop: 3 mm
xix. Test tube racks, rust resistant, with capacity for 15 or 25 test tubes (preferably)

D) Culture media, reagents and stock culture

Note : The composition of the media is based on one liter solutions or similar units. Before preparation, the actual needs have to be established and adequate amounts must be chosen accordingly

E) Preparation of materials, media, reagents

All glassware and apparatus should be washed with non-toxic detergent, followed by a thorough (complete) rinse with hot tap water and then rinsed at least three times with distilled water.

i) Lactose broth

	Strength	
	Single	**Double**
Beef extract	3.0 g	6.0 g
Peptone	5.0 g	10.0 g
Lactose	5.0 g	10.0 g
Distilled water	1.0 liter	1.0 liter

Preparation : Dissolve ingredients in distilled water. The pH of single strength broth should be between 6.8 and 7.0, but preferably 6.9 after sterilization. Double strength broth pH should be 6.7 ± 0.1.

Place 5 clean culture tubes in each of 3 rows of a test tube rack (more in case the expected MPN of total coliforms is high). Then add inverted vials to all culture tubes and dispense 10 ml of medium into the culture tubes or sufficient amount so that the inverted vials (Durham vials) are at least partially covered after then entrapped air in these vials has been driven out during autoclaving. Use double strength lactose broth in the first row of these culture tubes. Into the second and third rows (and if necessary into successive tows) transfer single strength broth. Close all prepared tubes with metal caps or cotton plugs. Autoclave the prepared media tubes at 121°C preferably for 12 minutes but not to exceed 15 minutes. Check tubes of sterile medium for a final pH of 6.9 ± 0.1 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures. If the non-lactose fermenting control culture produces gas in 24 hours at 35°C, medium has been overheated in autoclaving and should be discarded.

Note : Double strength broth must be used in the first row when 10 ml of sample is added to the culture tubes. If sample portions to be tested are greater than 10 ml, prepare medium concentration such that after sample addition, the medium will approximate 1 x (normal) strength

ii) MacConkey broth

Sodium taurocholate	5.0 g
Lactose	10.0 g
NaCl	5.0 g
Peptone	20.0 g
Distilled water	1.0 litre

Preparation : Dissolve ingredients by shaking and adjust pH to 7.1 ± 0.1 with diluted HCl or NaOH. Add 2 ml bromo-cresol purple solution to each liter of prepared MacConkey broth.

Place 5 clean culture tubes in each of 3 rows of a test tube rack. Then add inverted vials to all culture tubes and dispense sufficient medium into the culture tubes so that the inverted vials are at least partially covered after the entrapped air in these vials has been driven out during autoclaving. Close all prepared tubes with metal caps or cotton plugs. Autoclave the prepared medium in culture tubes at 121°C, preferably for 12 minutes, but not exceeding 15 minutes.

After sterilization, cool the broth as quickly as possible. Final pH of single strength medium should be 7.2 ± 0.2 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures. If the non-lactose fermenting control culture produces gas in 24 hours at 35°C, the medium has been overheated in autoclaving and should be discarded.

iii) Bromo-cresol purple solution

Preparation : Dissolve l g of bromo-cresol purple in 99 ml of 95% pure ethanol.

iv) Brilliant green bile broth

Oxgall, dehydrated	20.0 g
Lactose	10.0 g
Peptone	10.0 g
Brilliant green	0.0133 g
Distilled water	1.0 litre

Preparation : Dissolve the ingredients in one liter of distilled water.

Place 5 clean culture tubes in each of 3 rows of a test tube rack. Then add inverted vials to all culture tubes and dispense 10 ml of medium into the culture tubes, or sufficient amount, so that the inverted vials are at least partially covered after the entrapped air in these vials has been driven out during autoclaving. Close the tubes with metal caps or cotton plugs. Sterilize by autoclaving at 121°C, preferably for 12 minutes, but not exceeding 15 minutes. After sterilization, cool the broth as quickly as possible. Final pH should be 7.2 ± 0.2. Test a sample of the finished product for performance using control stock cultures. If the non-lactose fermenting control culture produces gas in 24 hours at 35°C, medium has been overheated in autoclaving and should be discarded.

v) Phosphate buffered water

Preparation : To prepare stock phosphate buffer solution, dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4), in 500 ml distilled water, adjust to pH 7.2 ± 0.5 with 1 N sodium hydroxide (NaOH), and dilute to 1 liter with distilled water.

Add 1.25 ml stock phosphate buffer solution and 5.0 ml magnesium chloride solution (38 g anhydrous $MgCl_2$/1 distilled water) to 1 liter distilled water. Dispense in amounts that will provide 99 ± 2.0 ml or 9 ± 0.2 ml after autoclaving for 15 minutes. Final pH should be 7.2 ± 0.2. If the anhydrous salt is not available use 81.8 $MgCl_2.6H_2O$ per 1 liter of distilled water.

Peptone water (alternative dilution water): Prepare a 10% stock solution of peptone in distilled water. Dilute a measured volume to provide a final 0.1% working solution. Dispense in amounts to provide 99 ± 2.0 ml or 9 ±0.2 ml after autoclaving for 15 minutes. Final pH should be 6.8.

Note : Do not suspend bacteria in any dilution water for more than 30 minutes at room temperature because death or multiplication may occur

vi) Distilled water

Use only water distilled in all-glass or all-quartz distillation apparatus or reverse osmosis (RO) water. De-ionized water is also acceptable if produced in apparatus that does not release toxic substances.

Note : Commercially available distilled water is often produced in copper and zinc apparatus and is highly toxic for coliforms

vii) Thiosulphate solution

Prepare a 10 per cent sodium thiosulphate ($Na_2S_2O_3$) stock solution. To a 120 ml sample bottle, add 0.1 ml of the 10% solution of $Na_2S_2O_3$ before sample bottle sterilization.

viii) Detergents for cleaning glassware and apparatus

Use only detergents recommended by the supplier for bacteriological use. If such a detergent is not available, check normal household detergents with a biotest using a stock culture of *E. coli*.

Note : Never use toxic chromic-sulphuric acid mixture for cleaning glassware

ix) 95 per cent pure ethanol (not denatured)

x) Stock cultures for quality control

Stock cultures of *E. coli* for positive control (lactose fermentation) and Proteus inirabillis or a Salmonella strain for negative control (glucose fermentation).

F) Test procedure

i) Selection of sample size and dilution series

Select a dilution series for each sample that will ensure positive tubes in the lowest dilution row and negative tubes in the higher dilution rows. If previous experience for planning the dilution series for clean seawater is not available, transfer 10 ml of the seawater to the first row containing 5 tubes of double strength lactose broth, 1 ml to the second row of 5 tubes and 0.1 ml to the third row of 5 tubes. All rows after the first row (if 10 ml sample portions are used) should contain only single strength lactose broth. In polluted seawater the dilution should be extended to 5 dilution rows (the first receiving 10 ml from the seawater sample into double strength lactose) or 5 dilution rows of single strength lactose broth into which the seawater sample is transferred after the appropriate serial (10 fold) dilutions, Figure (VIII.8).

ii) Incubation in lactose broth at 35 ± 0.5°C for 48 hours (Presumptive test)

Using sterile pipettes transfer 10 ml portions of the water sample into each of 5 sterilized culture tubes containing double strength lactose broth.

Next transfer with a sterile pipette 1 ml sample portions into 5 sterilized culture tubes containing single strength lactose broth.

For preparing further dilutions shake sample again then transfer with a sterile pipette 1 ml of water sample into a test tube containing 9 ml of phosphate buffer (dilution D-1). Mix vigorously by hand or optionally with vibrator. Transfer aseptically, using a sterile pipette, 1 ml of D-1 into each of the 5 culture tubes containing 10 ml single strength lactose broth.

If necessary, for further dilutions transfer 1 ml of the D-1 dilution into a test tube containing 9 ml of phosphate buffer to obtain dilution D-2, 1 ml of dilution D-2 into 9 ml of phosphate buffer to obtain dilution D-3, etc. Shake each succeeding dilute aliquot prior to transferring dilute sample portions to culture tubes containing single strength lactose broth.

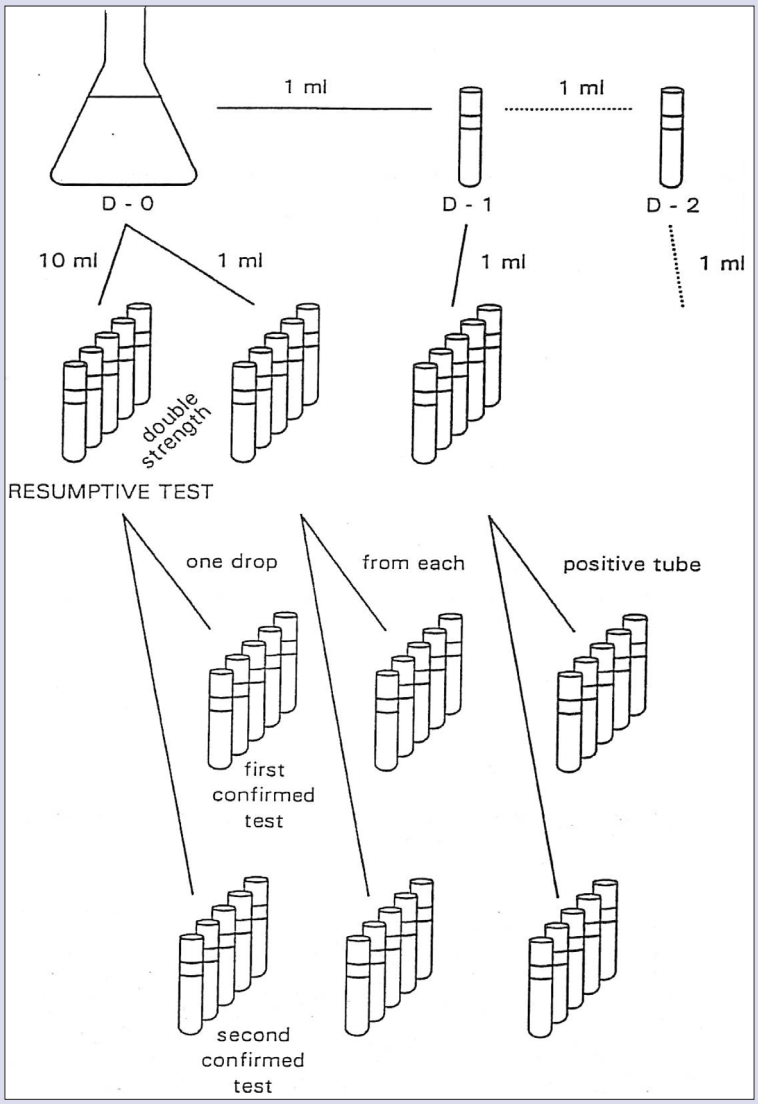


Figure (VIII.8): Scheme of preparation of dilution series

Incubate the series of lactose broth culture tubes in an incubator at 35 ± 0.5°C for 48 hours.

After 24 hours check for gas formation. The observation of gas formation in the Durham vials can be facilitated by slightly tapping on the walls of culture tubes. The appearance of an air bubble must not be confused with actual gas production. If the gas is formed as a result of fermentation, the broth will become cloudy. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial when the fermentation tube is shaken gently. Record the number of tubes showing positive reactions (gas formation after 24 hours of incubation) in Table (VIII.3), item 9, under (1 a).

After 48 hours check again for gas formation in the same tubes and record the results in Table (VIII.3), item 9, under (1b) (test report).

iii) Incubation in MacConkey or brilliant green bile broth at 35°C for 24 hours (Confirmed test)

Three row lactose broth presumptive test: after incubation for 24 hours in lactose broth at 35°C transfer with flame sterilized transfer loop one loop-full from each lactose culture tube that provided a positive reading into the test tubes situated in identical positions on the tube rack containing MacConkey or brilliant green bile broth.

Five row lactose broth presumptive test: after incubation for 24 hours in lactose broth at 35°C select the highest dilution in which the row of 5 tubes gives positive readings in all 5 tubes (no negative readings should exist in lower dilutions) and all positive tubes in succeeding higher dilutions, then transfer with flame sterilized transfer loop one loop-full from each lactose culture tube that provided a positive reading into test tubes situated in identical positions on the tube rack containing MacConkey or brilliant green bile broth.

Incubate all confirmation cultures (MacConkey or brilliant green bile broth) at 35 ± 0.5°C in an incubator for 24 hours.

Repeat this confirmation procedure with all other culture tubes containing lactose broth which have become positive in the 24 - 48 hours interval.

At the same time, record the number of tubes showing positive reactions in Table (VIII.3), item 9, under (2a) (test report). Coliforms will ferment lactose in either confirmatory medium, producing gas, which is trapped in the inverted vials and if MacConkey broth is used, acid produced in fermentation will turn the violet-like color of the medium to yellow.

After another 24 hours record the number of tubes showing positive reactions in Table (VIII.3), item 9, under (2b) (test report).

G) Expression of results

Calculation of total coliforms per 100 ml seawater sample

Based on the data in the test report (10) showing positive results in various serial dilutions, calculate the MPN (Most Probable Numbers) value using Table (VIII.2).

If sample portions of 10 ml, 1 ml and 0.1 ml per test tube have been used, take the number of recorded positive readings from the 2448 hour incubation in MacConkey or brilliant green bile broth at 35°C i.e. the highest readings from either 2a or 2b in Table (VIII.3), item 9, and find the corresponding most probable number (MPN) from Table (VIII.2). Enter this value in the test report, [Table (VIII.3), item 10].

When more than three dilutions are employed, the results of only three of these are used in computing the MPN. For MPN calculation purposes, select the highest dilution that has all tubes positive and the next two following dilutions that will have some negative tubes.

Occasionally, positive plates may be encountered beyond the three serial dilutions. In those cases, and for purposes of calculating the MPN, include such additional results in the third row. These skip combinations of positive plates should occur with a frequency lower than 1% of the MPN tests performed. Higher frequencies would suggest laboratory error in pipetting sample dilutions. Select from either 2a or 2b in Table (VIII.3), item 9, the dilution in which the row of five tubes gives positive readings in all 5 tubes (no negative readings should exist in lower dilutions) and the two next succeeding higher dilutions. Find the MPN, Table (VIII.2) corresponding to the number of positive tubes in these three dilutions and adjust the value by multiples of 10 depending on the starting dilution below 10 ml.

Table (VIII.1) illustrates an example of the numbers of positive test obtained with a series of 5 consecutive dilutions: 5-5-3-2-1. For MPN calculations the highest dilution selected would be that using 1 ml of sample. The final combination of positive plates reported would be 5-3-3, instead of 5-3-2. The MPN value corresponding to the 5-3-3 combination would be 170 total coliforms/100 ml, Table (VIII.2), and not the MPN value of 140 total coliforms/100 ml associated to the 5-3-2 combination. The MPN density thus obtained should be adjusted for dilution by multiplying for as many powers of 10 as dilutions were performed with the first plate series considered below 10 ml. For example, a MPN value of 5-3-3 would be expressed as 170 total coliforms/100 ml when the starting dilution is 10 ml, but it would be counted as 1700 total coliforms/100 ml when the starting dilution, is 1 ml, and 17000 total coliforms/100 ml when the largest sample volume used was 0.1 ml.

Table (VIII.1) : Example for computing the density of total coliforms in a water sample analyzed by the MPN method

Sample portion (ml)	Positive tubes
10	5
1	5 (X)
0.1	3 (X)
0.01	2 (X)
0.001	1

dilution factor = 10

MPN (5-3-3) = 170 x 10 = 1700 total coliforms/100 ml

95% confidence limits:

lower : 80 x 10 = 800 total coliforms/100 ml
higher: 410 x 10 = 4100 total coliforms/100 ml

Precision of results

Select the 95 per cent confidence limits for the MPN selected in from Table (VIII.2) and enter them in the test report, Table (VIII.3), item 10.

Table (VIII.2) : MPN index and 95% confidence limits for various combinations of positive and negative results when five 10-ml portions, five 1-ml portions and five 0.1-ml portions are used.

No. of Tubes giving Positive Reactions out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml each	5 of 10 ml each	5 of 10 ml each		Lower	Upper
0	0	0	< 2	-	-
0	0	1	2	1.0	10
0	1	0	2	1.0	10
0	2	0	4	1.0	13
1	0	0	2	1.0	11
1	0	1	4	1.0	15
1	1	0	4	1.0	15
1	1	1	6	2.0	18
1	2	0	6	2.0	18
2	0	0	4	1.0	17
2	0	1	7	2.0	20
2	1	0	7	2.0	21
2	1	1	9	3.0	24
2	2	0	9	3.0	25
2	3	0	12	5.0	29
3	0	0	8	3.0	24
3	0	1	11	4.0	29
3	1	0	11	4.0	29
3	1	1	14	6.0	35
3	2	0	14	6.0	35
3	2	1	17	7.0	40
3	3	0	17	5.0	46
4	0	0	13	5.0	38
4	0	1	17	7.0	45
4	1	0	17	7.0	46
4	1	1	21	9.0	55
4	1	2	26	12.0	63
4	2	0	22	9.0	55
4	2	1	26	12.0	65
4	3	0	27	12.0	67
4	3	1	33	15.0	77
4	4	0	34	16.0	80
5	0	0	23	9.0	86
5	0	1	30	10	110
5	0	2	40	20	140
5	1	0	30	10	120
5	1	1	50	20	150
5	1	2	60	30	180
5	2	0	50	20	170
5	2	1	70	30	210
5	2	2	90	40	250
5	3	0	80	30	250
5	3	1	110	40	300
5	3	2	140	60	360
5	3	3	120	80	410
5	4	0	130	50	390
5	4	1	170	70	480
5	4	2	220	100	580
5	4	3	280	120	690
5	4	4	350	160	820
5	5	0	240	100	940
5	5	1	300	100	1300
5	5	2	500	200	2000
5	5	3	900	300	2900
5	5	4	1600	600	5300
5	5	5	<1600	-	-

H) Test report

Table (VIII.3) : Total coliforms in seawater samples

1. Sampling area
   Country:_____
   Area: _____
2. Sampling point
   (Station)
   code number : _____
   longitude : _____
   latitude : _____

3. Time of sampling hour: __:__ day: _____ month: _____ year: _____
4. Sampling and environment conditions
   Sampling depth: _____ Container number: _____
   Temperature at sampling depth: _____ Duration of storage: _____
   Salinity at sampling depth: _____
   (other factors which may influence the results should be reported under 11)

5. Time of inoculation hour: _____ : _____ day: _____/ _____/ _____
6. Start of incubation hour: _____ : _____ day: _____/ _____/ _____
7. End of incubation hour: _____ : _____ day: _____/ _____/ _____
8. Confirmatory test MacConkey: _____ Brilliant green: _____

9. Number of positive reactions at 35.0°C

Volume in ml transferred	Lactose		MacConkey / BG	
	24 h (1a)	48 h (1b)	24 h (2a)	48 h (2b)
10	_____	_____	_____	_____
1	_____	_____	_____	_____
0.1	_____	_____	_____	_____
0.01	_____	_____	_____	_____
0.001	_____	_____	_____	_____
0.0001	_____	_____	_____	_____
0.00001	_____	_____	_____	_____

10. Test results:
10.1 MacConkey broth:
_____T.colif./100 ml
95% confidence limits:
____ : ____T.colif./100 ml

10.2 Brilliant green :
_____T.colif./100 ml
95% confidence limits :
____ : ____T.colif./100 ml

11. Anomalies observed in the test procedure:
_____
_____
_____

12. Full address of the institution which carried out the analysis:
_____
_____
_____

13. Name(s) and signature(s) of the person(s) who carried out the analysis:
_____
_____
Date: _____

VIII.3.1.2 Determination of Faecal Coliforms in Seawater

A) Definitions

Faecal coliforms are aerobic and facultatively anaerobic. Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas at 35°C and 44.5°C, in less than 24 hours. They produce indole in tryptone water containing tryptophane at 44.5°C.

Faecal coliforms exhibit a highly specific positive correlation with faecal contamination from warm-blooded animals and, therefore, are good indicators for the sanitary quality of coastal waters. Since faecal coliforms die within hours when exposed to sunlight in seawater at temperatures about +4°C, their presence in seawater indicates only recent contamination by faecal material. Die-away rates (T-90) depend on salinity, temperature, solar radiation, etc. and must be taken into consideration when interpreting the results.

B) Principles

Quantification of faecal coliforms in seawater samples is accomplished by testing multiple sample portions in a lactose-based medium for gas production at 44.5°C. There are two methods: (a) a direct test using A-1 broth with incubation at 44.5°C for 24 hours or (b) the supplemental confirmation of positive lactose tubes in the total coliform procedure.

Five sample portions for each multiple tube dilution (10 ml, 1 ml, 0.1 ml, etc.) are inoculated into individual tubes of A-1 broth (direct test) or tubes of lactose broth (total coliform procedure). The A-1 cultures are inoculated at 44.5 ± 0.2° C for 24 hours, the lactose cultures at 35.0 ± 0.5° C for 48 hours. The direct test (A-1 broth) is read for gas production after 24 hours incubation and faecal coliform density is determined from the combination of positive-negative tubes interpreted in the MPN Table, Table (VIII.7).

When the total coliform procedure is used to obtain a faecal coliform determination, confirm all positive lactose cultures in 24 and 48 hours by transferring a loop-full of culture from each positive tube to either MacConkey or EC broth. Brilliant green broth may be used, however, there may be some loss in detection of stressed faecal coliforms.

At the same time, one drop of all positive test tubes is transferred into a similar multiple test tube series containing tryptone water and incubated also at 44.5 ± 0.2°C (second confirmed test).

The frequency of dual confirmed positive reactions (lactose fermentation and positive indole) in the different dilutions is used for the calculation of the most probable number (MPN) of faecal coliforms in the analytical test sample.

C) Apparatus and glassware

See Section VIII.3.1.1, Part (C).

D) Preparation of materials, media, reagents

See Section VIII.3.1.1, Part (E), for other media and reagents needed for this determination.

i) A-1 broth (Direct test)

Lactose	5.0 g
Tryptone	20.0 g
Sodium chloride NaCl	5.0 g
Salicin	0.5 g
Polyethylene glycol p-	
iso-octylphenyl ether*	1.0 ml.
Distilled water	1.0 litre

*Triton X-100, Rohm and Haas Co., or equivalent.

Heat to dissolve ingredients, add polyethylene glycol p-iso-octylphenyl ether, and adjust to pH 6.9 ± 0.1. Before sterilization, dispense sufficient medium into fermentation tubes to cover the inverted vial (Durham vials) at least partially after sterilization. Sterilize by autoclaving at 121°C for 10 minutes. Store in dark at room temperature for not longer than 7 days. Ignore formation of precipitate.

Make A-1 broth of such strength that adding 10 ml sample portions to medium will not reduce ingredient concentrations below 1 x (normal) strength. For 10 ml samples prepare double-strength medium.

ii) EC medium

Tryptose or trypticase	20.0 g
Lactose	5.0 g
Bile salts mixture or bile salts No.3	1.5 g
Dipotassium hydrogen phosphate K_2HPO_4	1.4 g
Sodium chloride, NaCl	5.0 g
Distilled water	1.0 liter

Before sterilization, dispense in fermentation tubes with an inverted vial sufficient medium to cover the inverted vial at least partially after sterilization. pH should be 6.9 after sterilization. Test a sample of the sterilized medium for performance using control stock cultures (6.15). If the non-lactose fermenting control culture produces gas in 24 hours at 35°C, the medium has been overheated in autoclaving and should be discarded.

iii) Tryptophane broth

Tryptone	10.0 g
Distilled water	1.0 litre

Preparation : Dissolve the ingredients in distilled water (6.11).

Dispense 10 ml of tryptone water into each of 15 test tubes positioned in 3 rows of the dilution series. Autoclave (5.7) at 120°C for 15 minutes. The final pH should be between 7.2 and 7.4. If necessary, adjust the pH before sterilization.

iv) Kovac's indole reagent

Paradimethyl amino-benzaldehyde	5.0 g
Amyl alcohol	75.0 ml
Concentrated HCl	25.0 ml

Preparation : Dissolve the benzaldehyde in iso-amyl or normal amyl alcohol and add HCl. The reagent should be yellow. Some brands of paradimethyl aminobenzaldehyde are not satisfactory or become unsatisfactory (poor color reaction) or aging.

E) Test procedure

See Section VIII.3.1.1, Part (F).

Incubation in Tryptophane Broth at 44.5°C for 24 hours (Second confirmed test)

At the same time, when confirming in MacConkey broth, EC or brilliant green broth at 44.5°C from positive lactose tubes, also inoculate parallel tubes of tryptophane broth. Confirmation in tryptophane broth may also be applied to positive tubes of A-1 broth in the direct faecal coliform test.

Incubate the tryptophane cultures at 44.5° ± 0.2°C in a water incubator for 24 hours

After 24 hours add approximately 1 ml of Kovac's reagent into each of the test tubes and shake. Within 10 minutes positive reactions are indicated by a red colour of the iso-amyl alcohol surface layer in the test tubes. Record the results in Table (VIII.4), item 9, under (2c).

After 24 hours repeat this operation with all tubes which have become positive in lactose during the 24-48 hour interval and record the results in Table (VIII.4), item 9, under (2d).

F) Expression of results

See Section VIII.3.1.1, Part (G).

G) Test report

Table (VIII.4) : Faecal coliforms in seawater samples

1. Sampling area
   Country:_____
   Area: _____
2. Sampling point
   (Station)

code number : _____
longitude : _____
latitude : _____

3. Time of sampling hour: __:__ day: _____ month: _____ year: _____
4. Sampling and environment conditions
   Sampling depth: _____ Container number: _____
   Temperature at sampling depth: _____ Duration of storage: _____
   Salinity at sampling depth: _____
   (other factors which may influence the results should be reported under 11)

5. Time of inoculation hour: _____ : _____ day: _____/ _____/ _____
6. Start of incubation hour: _____ : _____ day: _____/ _____/ _____
7. End of incubation hour: _____ : _____ day: _____/ _____/ _____
8. Confirmatory test MacConkey : _____ Brilliant green: _____ Indole : _____

9. Number of positive reactions at 44.5°C

Volume in ml transferred	Lactose (A-1 broth) (Direct test)		MacConkey / BG or Br. Green or EC		Indole Test	
	24 h (1a)	48 h (ab)	24 h (2a)	48 h (2b)	24 h (2c)	48 h (2d)
10	_____	_____	_____	_____	_____	_____
1	_____	_____	_____	_____	_____	_____
0.1	_____	_____	_____	_____	_____	_____
0.01	_____	_____	_____	_____	_____	_____
0.001	_____	_____	_____	_____	_____	_____
0.0001	_____	_____	_____	_____	_____	_____
0.00001	_____	_____	_____	_____	_____	_____

10. Test results:

10.1 Direct test (A-1) plus indole test : _____F.col./100 ml

10.2 After 48h in MacConkey, or BG plus indole :. _____F.col./100 ml ml

95% confidence limits: ____ : ____ F.col./100 ml

11. Anomalies observed in the test procedure:
_____
_____
_____

12. Full address of the institution which carried out the analysis:
_____
_____
_____
_____

13. Name(s) and signature(s) of the person(s) who carried out the analysis:
_____
_____
_____
Date : _____

VIII.3.1.3 Determination of Faecal Streptococci in Seawater

The method described is suitable for the determination of faecal streptococci in coastal bathing waters of temperature and tropical seas and is designed to be used in sanitary surveillance of bathing beaches.

A) Definition

Faecal streptococci are Gram-positive oblong/oval occurring in pairs or in short chains. They will grow in acid dextrose at 35 ± 0.05°C, in brain-heart infusion broth within 2 days at 45°C; in 5 days at 10°C and confirm as catalase negative.

Faecal streptococci normally originate from the intestine of warm-blooded animals and when found in seawater indicate contamination with faecal material. Recent studies indicate that a few variant strains in the faecal streptococci group, may be found in certain plants or plant products. Therefore, wastes from food processing industries may be also a source of organisms yielding positive reaction when tested with this method. Die-away rates (T-90) depend on salinity, temperature, solar radiation, etc. and must be taken into consideration when interpreting the results.

Because of limited survival in the environment, it is not recommended that only faecal streptococci be used when determining water quality. In combination with faecal coliform data (UNEP/WHO 1983a/b), data on faecal streptococci may provide more specific information about pollution sources because certain faecal streptococci are host-specific.

B) Principles

Quantitation of faecal streptococci in marine (seawater) samples is accomplished by testing multiple sample portions in an azide based medium for growth at 35°C within 48 hours. Positive findings in the presumptive test phase are confirmed in a second medium (ethyl violet azide broth) for verification of faecal streptococci occurrence.

Five sample portions for each multiple test tube dilution (10 ml, 1 ml, 0.1 ml, etc.) are inoculated into individual tubes of azide dextrose broth. After 24 and 48 hours incubation, all culture tubes are examined for the presence of turbidity.

The frequency of positive reactions in the different dilutions of confirmed cultures is used for the calculation of the most probable number (MPN) of faecal streptococci in the water sample.

C) Apparatus and glassware

See Section VIII.3.1.1, Part (C).

D) Preparation of materials, media, reagents

See Section VIII.3.1.1, Part (E) and Section VIII.3.1.2, Part (D) for other media and reagents needed for this determination.

i) A-1 broth (Direct test)

	Strength	
	Single	Double
Beef extract	4.5 g	9.0 g
Tryptone or polypeptone	15.0 g	30.0 g
Glucose	7.5 g	15.0 g
Sodium chloride	7.5 g	15.0 g
Sodium azide NaN_3	0.2 g	0.4 g
Distilled water	1.0 liter	1.0 liter

Preparation: Dissolve ingredients in distilled water. The pH should be 7.2 ± 0.2 after sterilization.

Place 5 clean culture tubes in each of 3 rows of a test tube rack (more in case the expected MPN of faecal streptococci is high). Add 10 ml of double strength azide broth to the first row of these culture tubes. Into the second and successive rows transfer 10 ml of single strength azide dextrose broth. Close all prepared tubes with metal caps or cotton plugs. Autoclave the prepared media tubes at 121°C for 15 minutes. Check tubes of sterile medium for a final pH of 7.2 ± 0.2 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures. *S. faecalis* should produce turbidity within 24 hours incubation at 35°C. *E. Coli* growth (turbidity) should not occur.

Note : Double strength broth is only necessary in the first row where 10 ml of test tube solution is added to the culture tubes

ii) Ethyl violet azide broth

Tryptone or biosate	20.5 g
Glucose	5.0 g
Sodium chloride NaCl	5.0 g
K_2HPO_4	2.7 g
KH_2PO_4	2.7 g
NaN_3 (sodium azide)	0.4 g
Ethyl violet	0.00083 g
Distilled water	1.0 liter

Preparation: Dissolve ingredients in distilled water (6.6). The pH should be 7.0 ± 0.2 after sterilization.

Place 5 clean culture tubes in each of 3 rows of a test tube rack (more in case the expected MPN of faecal streptococci is high). Dispense 10 ml of single strength ethyl violet azide broth into the culture tubes. Into the second and successive rows transfer 10 ml of single strength azide dextrose broth. Close all prepared tubes with metal caps or cotton plugs. Autoclave the prepared media tubes at 121°C for 15 minutes. Check tubes of sterile medium for a final pH of 7.2 ± 0.2 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures. *S. faecalis* should produce turbidity within 24 hours incubation at 35°C. *E. Coli* growth turbidity) should not occur.

iii) Strains for quality control

Strains of *Streptococcus faecalis* or *Streptococcus faecium.*

E) Test procedure

See Section VIII.3.1.1, Part (F).

Incubation in azide dextrose broth at 35 ± 0.5°C for 48 hours (Presumptive test)

Each sample and all dilutions must be vigorously shaken to insure uniform dispersion of bacteria in each sample portion tested.

Using sterile pipettes transfer 10 ml portions of the water sample into each of 5 sterilized culture tubes containing double strength azide dextrose broth. Next transfer with a sterile pipette 1 ml sample portions into 5 sterilized culture tubes containing single strength azide dextrose broth.

For preparing further dilutions shake sample again then transfer with a sterile pipette. ml of water sample into a test tube containing 9 ml of phosphate buffer or peptone water (dilution D-1). Mix vigorously by hand or optionally with vibrator. Using a sterile pipette, transfer aseptically, 1 ml of D-1 into each of the 5 culture tubes containing 10 ml single strength azide dextrose broth.

If necessary, for further dilutions transfer 1 ml of the D-1 dilution into a test tube containing 9 ml of phosphate buffer or peptone water to obtain dilution D-2, 1 ml. of dilution D-2 into 9 ml of phosphate buffer to obtain dilution D-2, 1 ml of dilution D-2 into 9 ml of phosphate buffer to obtain dilution D-3, etc. Shake each succeeding dilute aliquot prior to transferring dilute sample portions to culture tubes containing single strength azide dextrose broth. Incubate the series of azide dextrose broth culture tubes in an incubator at 35 ± 0.5° C for 48 hours.

All azide dextrose broth tubes showing turbidity after 24 and 48 hours must be subjected to the confirmed test with ethyl violet azide broth. After 24 hours of incubation, enumerate the positive azide dextrose tubes (growth and resultant turbidity of the broth) in Table (VIII.5), Paragraph 8, Column 1a of the Analytical report. Between 24 and 48 hours, new tubes of azide dextrose broth may become positive. Therefore, note down the total number of positive dextrose azide tubes in Column 1b.

Incubation in ethyl violet azide broth at 35°C for 24-48 hours (Confirmed Test)

Three row azide dextrose broth presumptive test: after incubation for 24 hours in azide dextrose broth at 35°C, a second test tube series is transferred with flame sterilized transfer loop three loop-fulls from each azide dextrose broth culture tube that provided a positive reading into culture tubes situated in identical positions on the tube rack containing ethyl violet azide broth.

Five row azide dextrose broth presumptive test: after incubation for 24 hours in azide dextrose broth at 35°C select the highest dilution in which the row of 5 tubes gives positive readings in all 5 tubes (no negative readings should exist in lower dilutions) and all positive tubes in succeeding higher dilutions, then transfer with sterilized transfer loop three loop-fulls from each azide dextrose culture tube that provided a positive reading into test tubes situated in identical positions on the tube rack containing ethyl violet azide broth.

Incubate all confirmation cultures (ethyl violet azide broth) at 35 ± 0.5°C in an incubator for 24 hours. The presence of faecal streptococci is indicated by the formation of a purple bottom at the base of the tube, or occasionally by a dense turbidity. After 24 hours of incubation, record all positive tubes in Table (VIII.5), paragraph 8 under column 2a of the Analytical report (10) and discard them. At the same time inoculate all negative ethyl violet azide tubes again by transferring with flame sterilized transfer loop three loop-fulls from each original azide dextrose culture tube that provided a positive reading after incubation for 24 hours and any 48 hours positive culture (turbidity) in azide dextrose broth at 35°C. Under completion of a 48 hour incubation for all ethyl violet azide tubes, record positive and negative results on the sample test report, Table (VIII.5).

F) Expression of results

Calculation of faecal streptococci per 100 ml of seawater sample

See Section VIII.3.1.1, Part (G).

G) Test report

Table (VIII.5) : Faecal Streptococci in seawater samples

1. Sampling area
   Country:_____
   Area: _____
2. Sampling point
   (Station)

code number : _____
longitude : _____
latitude : _____

3. Time of sampling hour: __:__ day: _____ month: _____ year: _____
4. Sampling and environment conditions
   Sampling depth: _____ Container number: _____
   Temperature at sampling depth: _____ Duration of storage: _____
   Salinity at sampling depth: _____
   (other factors which may influence the results should be reported under 10)

5. Time of inoculation hour: _____ : _____ day: _____/ _____/ _____
6. Start of incubation hour: _____ : _____ day: _____/ _____/ _____
7. End of incubation hour: _____ : _____ day: _____/ _____/ _____

8. Number of positive reactions

Volume in ml transferred	Azide dextrose		Ethyl violet azide	
	24 h (1a)	48 h (ab)	24 h (2a)	48 h (2b)
10	_____	_____	_____	_____
1	_____	_____	_____	_____
0.1	_____	_____	_____	_____
0.01	_____	_____	_____	_____
0.001	_____	_____	_____	_____
0.0001	_____	_____	_____	_____

9. Test results:

Ethyl violet azide :
broth (24 + 48 h)
_____ F.strep./100 ml

95% confidence limits:
____ : ____ F. strep./100 ml

10. Anomalies observed in the test procedure:

_____
_____
_____

11. Full address of the institution which carried out the analysis:

_____
_____
_____
_____

12. Name(s) and signature(s) of the person(s) who carried out the analysis:

_____
_____
_____

Date : _____

VIII.3.1.4 Determination of Faecal Coliforms in Bivalves

The method described is suitable for the determination of faecal coliforms in bivalve (shellfish) specimens from temperate and tropical seas. It is designed to be used in sanitary surveillance of sea-food.

Faecal coliforms are specific indicators, exhibiting a high positive correlation with faecal contamination from warm-blooded animals. Filter-feeding shellfish concentrates coliforms from its marine environment. The concentration of faecal coliforms in edible shellfish tissue gives an indication of the potential health hazard to consumers of shellfish due to pathogens of faecal origin which may have been present in the marine environment surrounding the shellfish.

A) Definition

Faecal coliforms are aerobic and facultatively anaerobic, Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas, both at 36°C and at 44.5°C in less than 24 hours. At 44.5°C they produce indole in tryptone water containing tryptophan.

B) Principles

After the shellfish have been washed and brushed in the laboratory, their soft tissue, either with or without their inter-valvular fluid, is separated under sterile conditions from the shell and transferred to a sterilized blender where the shellfish sample is macerated and diluted by nine times its weight with phosphate buffer (or 0.1% peptone water). in this way a solution is obtained which contains 1 gram of mussel sample per 10 ml of homogenate. From this homogenate a first multiple test tube dilution series containing lactose broth is set up and incubated at 36 ± 1°C (presumptive test).

After 24 hours, one drop of all positive test tubes is transferred into a second multiple test tube dilution series containing MacConkey broth (or brilliant green broth) and incubated at 44.5 ± 0.2°C (first confirmed test). At the same time, one drop of all positive test tubes is transferred into a third multiple test tube series containing tryptone water and incubated also at 44.5 ± 0.2° C (second confirmed test). The frequency of positive reactions in the test tubes is used for the calculation of the most probable number (MPN) of faecal coliforms in the analytical test sample.

C) Apparatus and glassware

i) Thermoisolated plastic boxes (camping equipment) with cooling pads or similar cooling units for transport and keeping live mussel specimens
ii) Water incubator for 36 ± 1°C and for 44.5 ± 0.2°C
iii) Autoclave, max. 2 atm, electric or gas
iv) Drying oven for sterilization of glassware and equipment at 160°C
v) pH meter, precision ± 0.1 pH units
vi) Stainless steel forceps
vii) Balance for media preparation, precision ± 10 mg
viii) Refrigerator, 4 ± 2° C

ix) Vibrator for mixing liquids in culture tubes
x) Ehrlenmeyer flasks of borosilicate glass for media preparation, capacity 1 and 2 liters
xi) Borosilicate glass bacteriological culture tubes with autoclavable racks
xii) Small borosilicate glass tubes (“Durham vials”)
xiii) Total volume (blow-out) borosilicate glass pipettes of 1, 5, 9 and 10 ml capacity for transfer of culture media in test tubes, with stainless steel containers for sterilization

Note : 9 ml capacity pipettes are useful, but not essential

xiv) Graduated borosilicate glass cylinders of 100, 500 and 1,000 ml capacity with glass beakers for cover
xv) Stainless steel homogenizer or blender with several blender vessels, sterilizable in a drying oven or autoclave
xvi) Crush for Cleaning shellfish shells
xvii) Surgeon's scalpels or similar knives for opening mussels

D) Preparation of materials, media and reagents

See Section VIII.3.1.1, Part (E), Section VIII.3.1.2, Part (D) and Section VIII.3.1.3, Part (D) for other media and reagents needed for this determination.

i) Tryptone water

Tryptone	10.0 g
NaCl	5.0 g
Distilled water	1.0 liter

Preparation : Dissolve the ingredients in distilled water.

Dispense into each of the 5 test tubes in the 3 rows (more in case the expected PMN of faecal coliforms is high) of the dilution series 10 ml. of tryptone water. Autoclave at 120°C for 15 minutes. The final pH should be between 7.2 and 7.4. If necessary, adjust the pH before sterilization.

ii) Peptone water for dilutions

Preparation: Dissolve a sufficient amount of peptone to obtain a 0.1% solution of peptone in distilled water. Dispense 9 ml in test tubes used for dilution series and autoclave at 131° C for 15 minutes.

E) Preparation of test sample

Weigh the sterilized blender vessel. Select 10 bivalves at random from the specimen samples collected at each sampling station. (For example, a mytilus of 4 cm shell length contains a soft part of about 0.5 grams fresh weigh plus a variable volume of intervalvular fluid, hence 10 mussels of this size should yield about 5 g total weight). Before opening the shells, carefully clean them with a brush and alcohol. Then hold the closed bivalve with sterilized forceps for a short time over a flame in order to dry the outside of the shells.

Cut the valve open with a sterilized knife or scalpel by inserting the knife into the opening from which the byssus extrudes and cut the posterior adductor muscle by turning the knife as indicated in Figure (VIII.9). Then cut in the other direction and open the mussel with sterilized forceps.

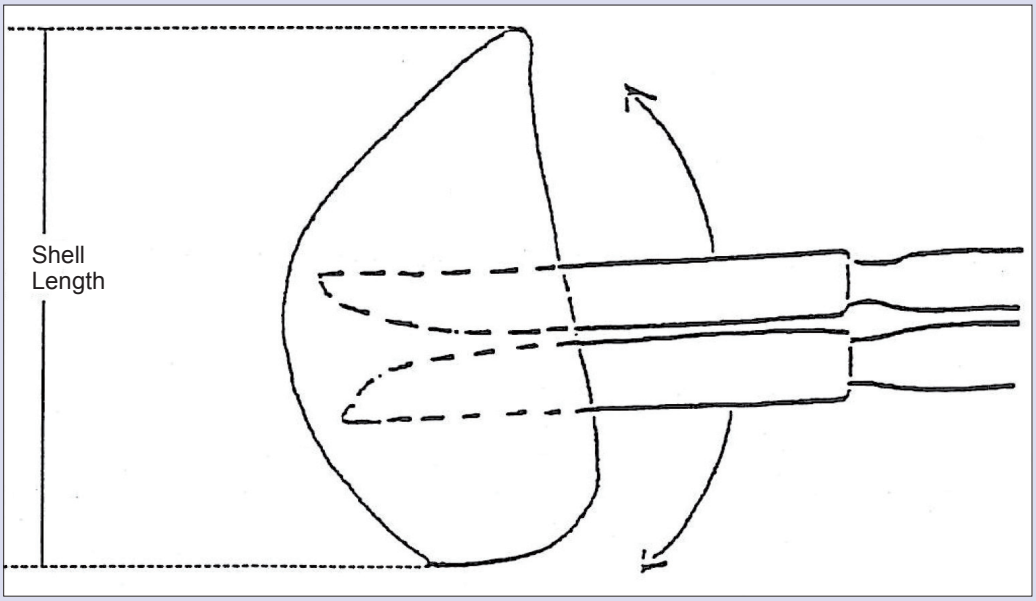


Figure (VIII.9) : Cutting the two adductor muscle

Do not try to break the mussel open with the knife. If the two muscles are cut the mussel will open easily.

The microbiological parameters can be determined on either a test sample of shellfish flesh and inter-valvular fluid or on a test sample of shellfish flesh only. The option adopted shall be indicated with the results of the analysis.

Test sample of shellfish flesh and inter-valvular fluid

Collect both the shellfish flesh (soft tissue) and their inter-valvular fluid into the sterilized blender flask with sterilized forceps. After transferring the soft parts and the inter-valvular fluid of all 10 specimens into the blender vessels, weigh the vessel and determine the weight of the test sample by subtracting the weight of the empty blender vessel from the weight of the vessel plus the soft parts and the inter-valvular fluid. Report the weight of the sort parts and the inter-valvular fluid obtained from the 10 mussels in Table (VIII.8), item 5. Then, (in order to arrive at a concentration of 1 g/10 ml) dilute the test sample with 9 times its weight using phosphate buffer or peptone water in our example, the test sample is now composed of 5 grams of mussel flesh and inter-valvular fluid plus 45 ml of dilution solution, resulting in a total of 50 ml of flesh solution homogenate. Homogenize the flesh and inter-valvular fluid for 2 minutes. The homogenate now contains 1 gram of test sample in 10 ml, or 0.1 g sample/ml. This homogenate represents the test sample (D-1 dilution).

Test sample of shellfish flesh only

Drain the inter-valvular fluid from the shells, discard it so that it will not be included in the test sample and transfer the flesh (soft tissue) into the sterilized blender flask with sterilized forceps. After transferring only the soft parts of all 10 specimens into the blender vessels, weigh the vessel and determine the fresh weight of the test sample by subtracting the predetermined weight of the empty blender vessel from the weight of the vessel plus the soft parts. Report the weight of the soft parts obtained from the 10 mussels in Table (VIII.8), item 5. Then, (in order to arrive at a concentration of 1 g/10 ml) dilute the test sample with 9 times its weight using phosphate buffer or peptone water in our example, the test sample is now composed of 5 grams of mussel flesh plus 45 ml of dilution solution, resulting in a total of 50 ml of flesh solution homogenate. Homogenize the flesh for 2 minutes. The homogenate now contains 1 gram of 43 test sample in 10 ml, or 0.1 g sample/ml. This homogenate represents the test sample (D-1 dilution).

F) Test procedure

i) Incubation in lactose broth at 36 ± 1°C for 48 hours (Presumptive test)

Before taking aliquots from the original sample or from the dilutions these must be vigorously shaken in order to guarantee that representative aliquots are taken. Using sterile pipettes transfer 10 ml of the test sample into five sterilized culture tubes containing double strength lactose broth. This test tube row contains now 1 g FW/tube, Figure (VIII.8). Next transfer with sterile pipettes 1 ml of the test sample into five sterilized culture tubes containing single strength lactose broth. This tube row contains now 0.1 g FW/tube.

For preparing further dilutions transfer with a sterile pipette 1 ml of test sample (dilution D-1) into a test tube containing 9 ml of phosphate buffer or peptone water (dilution D-2). Mix vigorously by hand or with vibrator. Transfer aseptically 1 ml of D-2 into each of the five culture tubes containing single strength lactose broth. This test tube row contains now 0.01 g FW/tube.

If necessary, for further dilutions transfer 1 ml of the D-2 dilution into a test tube containing 9 ml of phosphate buffer or peptone water to obtain dilution D-3, etc. Incubate the series of culture tubes in an air or water incubator at 36 ± 1°C for 48 hours.

After 24 hours, check for gas formation. The gas production is indicated by the broth turning turbid. The observation of gas formation in the Durham vials can be facilitated by slightly tapping on the walls of culture tubes. The appearance of an air bubble must not be confused with actual gas production. If the gas is formed as a result of fermentation, the broth must become cloudy. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial when the fermentation tube is shaken gently. Record the number of tubes showing positive reactions (gas formation after 24 hours of incubation) in Table (VIII.8), item 8 under (1a). After 48 hours, check again for gas formation in the same tubes and record the results in Table (VIII.8), item 8 under (1b).

ii) Incubation in MacConkey or brilliant green broth at 44.5°C for 48 hours (First confirmed test)

After incubation for 24 hours in lactose broth at 36°C a second test tube series is prepared by transferring with sterile pipettes one drop from each test tube that provided a positive reading into test tubes situated in identical positions on the tube rack containing MacConkey broth or brilliant green broth. Incubate this second test tube series at 44.5 ± 0.2°C in a water incubator for 24 hours.

After 24 hours record the number of tubes showing positive reactions in Table (VIII.8), item 8 under (2a). Coliforms will develop gas, which is trapped in the inverted vials (brilliant green broth) and acid, which turns the violet-like color of the original MacConkey broth into a yellowish color.

After incubation for 48 hours in lactose broth at 36°C with sterile pipettes one drop from each previously negative test tube, that after 48 hours provides a positive reading, is transferred into test tubes situated in identical positions on the tube rack containing MacConkey broth or brilliant green broth. After another 24 hours record the number of tubes showing positive reactions (acid and gas production) in Table (VIII.8), item 8 under (2b).

iii) Incubation in tryptone water at 44.5°C for 24 hours (Second confirmed test)

At the same time when the first confirmed test is prepared, transfer aseptically a drop from each test tube which provided a positive reaction after incubation for 24 hours in lactose broth at 36°C into the third series of test tubes situated in identical positions of a test tube rack containing tryptone water. Incubate this third test tube series at 44.5 ± 0.2°C in a water incubator for 24 hours.

After 24 hours add approximately 1 ml of Kovac's reagent into each of the test tubes and shake. Within 10 minutes positive reactions are indicated by a red color of the amyl alcohol surface layer in the test tubes. Record the results in Table (VIII.8), item 8 under (2c). Repeat this operation with the tubes from the presumptive test which have become positive during the 24-48 hours intervals and record the results in Table (VIII.8), item 8, under (2d).

G) Expression of results

Calculation of faecal coliforms per gram of shellfish sample

If the dilutions 1 g, 0.1 g and 0.01 g of shellfish sample per test tube have been used, take the highest number of recorded positive readings from the incubation in MacConkey broth or brilliant green at 44.5°C and in tryptone water, i.e. the highest readings from either 2a, 2b, 2c or 2d in Table (VIII.8), item 8 and find the corresponding most probable number (MPN) from Table (VIII.7).

When more than three dilutions are employed, the results of only three of these are used in computing the MPN. Select the dilution in which the row of five tubes gives positive readings in all five tubes (no negative readings should exist in lower dilutions) and the two next succeeding higher dilutions from either 2a, 2b, 2c or 2d in Table (VIII.8), item 8. Determine how many powers of 10 the highest dilution is smaller than 1 g, find the MPN corresponding to the number of positive tubes in these three dilutions and multiply the MPN found with number of times of 10. Enter this value in the test report, [Table (VIII.8, item 9].

Table (VIII.6), illustrates an example of the numbers of positive test obtained with a series of 5 consecutive dilutions: 5-5-3-2-1. For MPN calculations the highest dilution selected would be that using 0.1 g of sample. The final combination of positive plates reported would be 5-3-3, instead of 5-3-2. The MPN value corresponding to the 5-3-3 combination would be 180 faecal coliforms/g, Table (VIII.7), and not the MPN value of 140 faecal coliforms/g associated to the 5-3-2 combination.

Table (VIII.6) : Example for computing the density of faecal coliforms in a bivalve sample analyzed by the MPN method.

Sample portion (ml)	Positive tubes
10	5
1	5 (X)
0.1	3 (X)
0.01	2 (X)
0.001	1

Dilution factor : 1/0.1 = 10

MPN (5-3-3) = 18 x 10 = 180 faecal coliforms/g of shellfish sample

95% confidence limits:
lower: 4.4 x 10 = 44 faecal coliforms/g
higher: 50 x 10 = 500 faecal coliforms/g

Precision of results

Select the 95 per cent confidence limits from Table (VIII.7) and enter them in the test report, [Table (VIII.8), item 9].

Table (VIII.7) : MPN index and 95% confidence limits for various combinations of positive and negative results when five 10-ml. portions, five 1-ml and five 0.1-ml portions are used

No. of tubes giving positive reactions out of 5 tube containing			MPN index per 1 g	95 % Confidence limits		No. of tubes giving positive reactions out of 5 tubes containing			MPN index per 1 g	95 % Confidence limits	
1 g	0.1 g	0.01 g		Lower	Upper	1 g	0.1 g	0.01 g			
0	0	0	<0.2			4	2	1	2.6	0.9	7.8
0	0	1	0.2	<0.05	0.7	4	3	0	2.7	0.9	8
0	1	0	0.2	<0.05	0.7	4	3	1	3.3	1.1	9.3
0	2	0	0.4	<0.05	0.1	4	4	0	3.4	1.2	9.3
1	0	0	0.2	<0.05	0.7	5	0	0	2.3	0.7	7
1	0	1	0.4	<0.05	1.1	5	0	1	3.1	1.1	8.9
1	1	0	0.4	<0.05	1.1	5	0	2	4.3	1.5	11
1	1	1	0.6	<0.05	1.5	5	1	0	3.3	1.1	9.3
1	2	0	0.6	<0.05	1.5	5	1	1	4.6	1.6	12
2	0	0	0.5	<0.05	1.3	5	1	2	6.3	2.1	15
2	0	1	0.7	0.1	1.7	5	2	0	4.9	1.7	13
2	1	0	0.7	0.1	1.7	5	2	1	7	2.3	17
2	1	1	0.9	0.2	2.1	5	2	2	9.4	2.8	22
2	2	0	0.9	0.2	2.1	5	3	0	7.9	2.5	19
2	3	0	1.2	0.3	2.8	5	3	1	11	3.1	25
3	0	0	0.8	0.1	1.9	5	3	2	14	3.7	34
3	0	1	1.1	0.2	2.5	5	3	3	18	4.4	50
3	1	0	1.1	0.2	2.5	5	4	0	13	3.5	30
3	1	1	1.4	0.4	3.4	5	4	1	17	4.3	49
3	2	0	1.4	0.4	3.4	5	4	2	22	5.7	70
3	2	1	1.7	0.5	4.6	5	4	3	28	9	85
3	3	0	1.7	0.5	4.6	5	4	4	35	12	100
4	0	0	1.3	0.3	3.1	5	5	0	24	6.8	75
4	0	1	1.7	0.5	4.6	5	5	1	35	12	100
4	1	0	1.7	0.5	4.6	5	5	2	54	18	140
4	1	1	2.1	0.7	6.3	5	5	3	92	30	320
4	1	2	2.6	0.9	7.8	5	5	4	160	64	580
4	2	0	2.2	0.7	6.7	5	5	5	240		

H) Test report

Table (VIII.8) : Faecal coliforms in Bivalves

1. Sampling area
   Country:_____
   Area: _____
2. Sampling point
   longitude : _____
   description : _____
   code number : _____
   latitude : _____

3. Time of sampling hour: __:__ day: _____ month: _____ year: _____
4. Sampling and environment conditions
   Sampling depth: _____ Container number: _____
   Temperature at sampling depth: _____ Duration of storage: _____
   Salinity at sampling depth: _____
   (other factors which may influence the results should be reported under 10)
5. Test sample: number of mussels per sample: _____ Weight of sample: _____ g

6. Time of inoculation hour: _____ : _____ day: _____/ _____/ _____
7. Incubations:

	Start of incubation	End of incubation
Lactose broth at 36°C	Hour: __ : __ Day : __/ __/ __	Hour: __ : __ Day : __/ __/ __
MacConkey/BG at 44.5°C	Hour: __ : __ Day: __/ __/ __	Hour: __ : __ Day: __/ __/ __
Tryptone water at 44.5°C	Hour: __ : __ Day: __/ __/ __	Hour: __ : __ Day: __/ __/ __

8. Number of positive reactions :

Portions transferred in g	Lactose		MacConkey / or Brillant Green		Tryptone water	
	24 h (1a)	48 h (ab)	24 h (2a)	48 h (2b)	24 h (3a)	48 h (3b)
10	_____	_____	_____	_____	_____	_____
1	_____	_____	_____	_____	_____	_____
0.1	_____	_____	_____	_____	_____	_____
0.01	_____	_____	_____	_____	_____	_____
0.001	_____	_____	_____	_____	_____	_____
0.0001	_____	_____	_____	_____	_____	_____

9. Test results :

After 48h in MacConkey, BG or tryptone water:
_____F.col./g

95% confidence limits:
____ : ____ F.col./g

Option adopted:
Flesh only: _____

Flesh and fluid: ____

10. Anomalies observed in the test procedure:
    _____
    _____
    _____

11. Full address of the institution which carried out the analysis:
    _____
    _____
    _____
    _____

12. Name(s) and signature(s) of the person(s) who carried out the analysis:
    _____
    _____
    _____
    Date : _____

VIII.3.2 Membrane Filtration Method (MF)

The membrane filtration (MF) method for enumeration of total coliforms, faecal coliforms, and faecal streptococci in sediments to be included if more type of bacteria measurement are needed. These methods involve filtering a known volume of water sample through a MF of optimum pore size for full bacterial retention. As water passes through the pores, bacteria are entrapped on the surface of the MF. The MF is then placed in contact with either a paper pad saturated with liquid medium or directly over an agar medium to provide nutrients for bacterial growth. After incubation under prescribed conditions of time, temperature, and humidity, the MF cultures are examined for bacterial colonies of specific characteristics that are then counted and recorded as a bacterial concentration per 100 ml of water sample.

The presence of high concentrations of suspended matter in turbid waters, and that of high concentrations of non-coliform bacteria are two of the main limitations of the membrane filter methods. In those cases, the MPN is the recommended method for bacteriological analysis of water samples.

The MF method may be adjusted to promote recovery of attenuated coliform bacteria. The method frequently used consists in a two layer enrichment differential growth medium that allows for repair and subsequent reproduction of those faecal coliforms that have been stressed by exposure to chlorine, industrial wastes or marine waters. General procedures and considerations regarding recovery of stressed indicator organisms appear in Standard Methods (APHA, 1985).

The decision to use the double layer M-FC agar method or any other coliform MF method for the bacteriological assessment of coastal water quality must be based on comparative analyses that show at least an 80 percent agreement between parallel MF and MPN faecal coliform methods. Approximately 100 samples chosen from a variety of coastal sampling stations should be used in this MF-MPN comparative study.

VIII.3.2.1 Determination of Total Coliform in Seawater

The method described is suitable for the determination of total coliform bacteria, the coliform bacteria detectable by this method include *E. coli* type I which are of faecal origin and irregular type II and III which may not be of faecal origin.

From seawater samples taken under sterile conditions, a dilution series is set up according to the number of total coliforms expected in the water sample. Aliquots of this dilution series are filtered through 0.45 µ pore size membrane filters. The membrane filters are placed on the surface of M-endo-agar-MF contained in Petri dishes and incubated at 36 ± 1°C for 24 hours. The coliform colonies will appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage. Residual chlorine, if present, is neutralized by adding thiosulphate to the sampling bottle before sterilization. Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test using the MacConkey or brilliant greet broth.

Warning : All the preparations of the Media should be carried on under a laminar flow hood

A) Aparatus and glassware

See Section VIII.3.1.1, Part (C).

- Filtration apparatus for 4.7 cm diameter membrane filters (MF) consisting of at least three filter funnels for simultaneous filtration, made of borosilicate glass or other non-toxic sterilizable material, Complete with electric or water vacuum pump
- Stereomicroscope, magnification 10 - 50X; and/or dark field colony counter, magnification 2 - 3X
- Stainless steel forceps
- Analytical balance, precision + 1 mg
- Petri dishes of borosilicate glass, diameter 5 cm, complete with stainless steel containers for sterilization, or disposable pre-sterilized plastic Petri dishes
- Small borosilicate glass vials ("Durham vials") to be inserted in culture tubes
- Bacteriological loops made from 22-24 Chromel gauge, nichrome or platinum-iridium. Diameter of the loop: 3 mm
- Water bath 44.5 ± 0.2°C
- Heavy wrapping paper
- Aluminium foil (household quality)
- Membrane filters (MF), pore size 0.45 µm, diameter 4.7 cm, or similar, fitting filtration apparatus
- Metal boxes, which are water tight and suitable for incubating petri dishes in the water bath

Note :The 0.45 µm pore size Membrane filter (MF) should be certified by the manufacturer to be free from substances which may hinder the growth and development of bacteria. Maximum recoveries are obtained using membranes composed of mixed esters of cellulose

B) Preparation of media and reagents

See Section VIII.3.1.1, Part (E).

i) M-endo-agar-+F

Polypeptone	10.0 g
Thiopeptone	5.0 g
Casitone	5.0 g
Yeast extract	1.5 g
Lactose	12.5 g
NaCl	5.0 g
K_2HPO_4	4.375 g
KH_2PO_4	1.375 g
Sodium lauryl sulfate	0.05 g
Sodium desoxycholate	0.10 g
Sodium sulfite	2.10 g
Basic fuchsine	1.05 g
Distilled water	1.0 liter
Agar	15.0 g

Preparation : Dissolve the components of the medium in 1 liter of distilled water containing 20 ml ethanol. Heat to boiling point and promptly remove from heat. The final medium should have a pH of 7.2 ± 0.1. Cool to about 45°C and pour 4-5 ml into each Petri dish. After the agar has solidified in the Petri dishes invert the Petri dishes and store them in the refrigerator. The dishes with prepared medium can be kept in a refrigerator for up to 30 days if kept in the dark.

Note : 1) Do not autoclave the medium
2) The agar surface should not become too dry because if the agar is dry the MF will not adhere well to the agar surface and hinder the diffusion of nutrients to the colonies on the MF

ii) P-buffer for filtration

Preparation: Dissolve components and autoclave at 121°C for 15 minutes.

iii) P-buffer for dilutions

Preparation: Dissolve components and dispense 9 ml in test tubes used for dilutions in the dilution series and autoclave at 121°C for 15 minutes.

C) Test procedure

Selection of sample size and dilution series

The MFs ideally have from 20 to 80 colonies after incubation. If previous experience for planning the dilution series for clean seawater is not available, filter the following volumes of the original

sample: 100 ml, 10 ml, 1 ml and 0.1 ml, Figure (VIII.10). For contaminated waters the dilutions have to be greater.

Preparation of the dilution caries

Before taking aliquots from the original sample or from the dilutions these must be vigorously shaken in order to guarantee that representative aliquots are taken.

Prepare the dilution series by taking with a sterilized pipette, after vigorously shaking the sample, 1 ml from the original sample, Figure (VIII.10), dilution: D-0, and transfer this 1 ml into a culture tube containing 9 ml of p-buffer to make the first dilution (D-1). Agitate the tube on a mixer or shake it vigorously by hand. Continue the preparation of the dilution series by taking 1 ml from the first dilution (D-1) and mixing it in a new culture tube containing 9 ml of phosphate buffer in order to obtain the second dilution (D-2), etc.

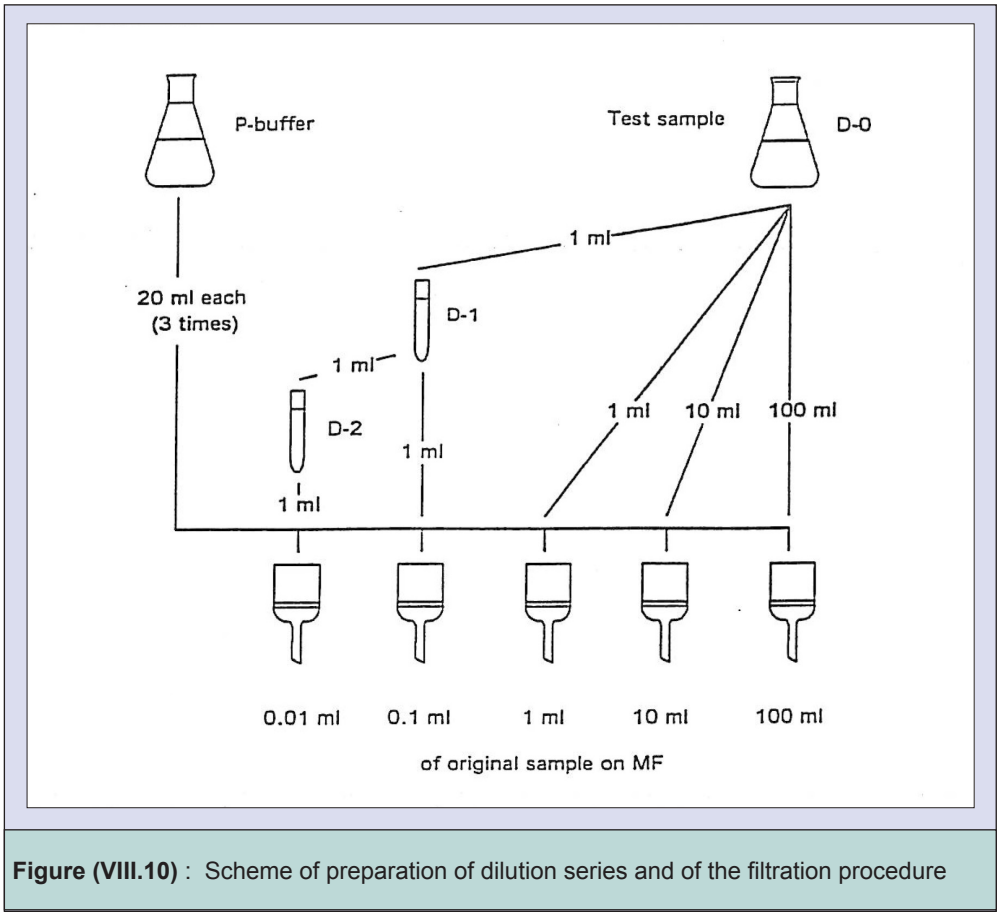


Figure (VIII.10) : Scheme of preparation of dilution series and of the filtration procedure

Filtration procedure

Begin filtration with the highest dilution (e.g. D-2) in order to avoid contamination from samples containing bacteria in higher concentrations. Use a sterilized filtration funnel for each dilution series. Place the sterilized MF with flamed sterilized forceps over the porous plate of the filtration apparatus. Carefully place the matching funnel unit over the receptacle and lock it in place.

Add into the funnel about 20 ml of buffer solution. With a sterilized pipette add 1 ml of the D-2 dilution into the buffer solution in the funnel. Filter with a partial vacuum. Wash the funnel walls with approximately 20 ml of buffer solution. Filter with a partial vacuum. Wash the funnel walls two more times with 20 ml of buffer solution and filter again. Unlock and remove the funnel, immediately remove the MF with flamed sterilized forceps and place the MF on the agar surface of the medium contained in the Petri dish with a rolling motion to avoid the entrapment of air. Before filtering the next dilution (D-1) in the same manner, pass 20 ml of buffer solution through the assembled filtration unit.

Incubation

The Petri dishes containing the MFs on agar are sealed and incubated immediately for 24 hours at 36 ± 0.1°C. As a sterility check, incubate also one blank (without MF), i.e. a Petri dish containing agar only.

Interpretation

Count with a stereomicroscope or similar only colonies, which appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage. If the number of dubious colonies is greater than 10 per cent of the total number of colonies, test dubious colonies either by MacConkey broth test or brilliant green bile broth test.

Estimation of precision

Check the precision of the technique at periodic intervals (at least once every season) by preparing three independent series of dilutions using the same sample, i.e. repeating the four last consecutive dilution steps described above, Figure (VIII.10). The water sample used should be collected during the routine monitoring programme at a coastal station typical of the area. The dilution series should be selected in such a way so that one dilution step yields three MF counts, which satisfy the 20 to 200 colonies.

Filter each individual dilution, incubate and report MF counts taking into consideration the interpretation method mentioned above. Results should be reported in the test report, Table (VIII.10), item 9. Calculate the total coliform concentrations of the original sample for each of the replicate results, and report the results in the test report, Table (VIII.10), item 10).

For each dilution step having the three MF counts between 20 and 200 total or faecal coliform colonies, calculate: the mean concentration, the concentration range, the standard deviation of the concentrations, and the coefficient of variation of the concentrations, and record them in the test report, Table (VIII.10), item 11. If the sample does not yield at least 20 colonies per membrane filter in one dilution, prepare a test solution from a stock culture and repeat the estimation of precision.

Note : Coefficient of variation (%) = $\frac{\text{standard deviation} \times 100}{\text{mean}}$

Confirmatory test

MacConkey broth test: With a flamed bacteriological loop transfer the suspected colony from the MF into a culture tube containing MacConkey broth and incubate at 36 ± 1°C for 48 hours. Coliforms will develop gas, which is trapped in the inverted Durham vials, and acid, which turns the violet-like color of the original broth into a yellowish color.

Brilliant green bile broth test: With a flamed bacteriological loop transfer the suspected colony from the MF into a culture tube containing brilliant green bile broth and incubate at 36 ± 1°C for 48 hours. Coliforms will develop gas, which is trapped in the inverted Durham vials.

Note : The MacConkey broth test is equivalent to the brilliant green bile broth test Either can be used for confirmation

D) Expression of results

Report the number of coliform colonies on individual MFs after the incubation has been completed and adjust this count after the confirmatory tests, if necessary, have been made. Use only MFs with a total number of colonies (i.e. coliforms plus non-coliforms) between 20 and 200. Retain only two significant digits of the counted number of coliform colonies per filter.

Indicate the results obtained from each filter separately in the test report, Table (VIII.9), item 9.

Express the results in terms of total coliforms per ml of sample, using the following equation:

$$\text{total coliforms (or faecal coliform) per 100 ml samples} = \frac{\text{adjusted number of coliform colonies}}{\text{ml of sample filtered}} \times 100$$

Indicate the results obtained for each dilution separately in the test report, Table (VIII.9), item 10. Report also the results obtained on MFs with less than 20 coliform Colonies per filter. If there are no coliform colonies on the filter report the results as “<1 coliform per 100 ml.”

Compute the number of total coliforms (or faecal coliform) per 100 ml sample and report it as the final test result, Table (VIII.9), item 11. If there are MFs containing between 20 and 200 characteristic colonies in two consecutive dilutions calculate the mean of these dilutions and report it as final test result.

Record in the test report, Table (VIII.9), item 12, anomalies observed in test procedure (confluent growth of colonies, deviation from temperature prescribed for sample storage and incubation, etc.).

E) Test report

Fill in the test report, Tables (VIII.9) and (VIII.10) giving full details in every column.

Table (VIII.9) : Test Report on Total Coliforms (or Faecal Coliform) in seawater samples

1. Sampling area :

1.1 Country: _____

1.2 Area: _____

2. Sampling point (station) :

2.1 Code number: _____

2.2 Longitude: _____

2.3 Latitude: _____

3. Time of sampling :

3.1 Hour : _____ 3.2 Day: _____

3.3 Month: _____ 3.4 Year: _____

4. Sampling and environmental conditions :

4.1 Depth of sampling: _____ (m)

4.2 Container number: _____

4.3 Temperature at sampling depth: _____ °C

4.4 Salinity at sampling depth: _____ S‰

4.5 Duration of storage: _____ hours

4.6 Other factors, which may influence the results should be reported under 12

5. Time of filtration :

5.1 Hour: _____ 5.2 Day: _____

6. Start of incubation :

6.1 Hour: _____ 6.2 Day: _____

7. End of incubation :

7.1 Hour: _____ 7.2 Day: _____

8. Confirmatory tests carried out (mark test used) :

8.1 MacConkey: _____ 8.2 Brilliant green: _____

9. Number of colonies per individual filter :

dilution	ml of original sample filtered	Total (or Faecal) coliform colonies
D-0	100	
D-0	10	
D-0	1	
D-1	0.1	
D-2	0.01	
D-3	0.001	
D-4	0.0001	

10. Number of total (or faecal) coliforms/100 ml sample :

dilution	n/100 ml

11. Test result : _____ total (or faecal) coliforms/100 ml samples

12. Anomalies observed in the test procedure :

_____

_____

_____

_____

_____

13. Full address of the institution, which carried out the test :

_____

_____

_____

_____

_____

14. Name(s) and signature(s) of the person(s) who carried out the test :

_____

_____

_____

_____

_____

Date: _____

Table (VIII.10) : Test Report on precision estimation

1. Sampling area :

1.1 Country: _____

1.2 Area: _____

2. Sampling point (station) :

2.1 Code number: _____

2.2 Longitude: _____

2.3 Latitude: _____

3. Time of sampling :

3.1 Hour: _____ 3.2 Day: _____

3.3 Month: _____ 3.4 Year: _____

4. Sampling and environmental conditions :

4.1 Depth of sampling: _____ (m)

4.2 Container number: _____

4.3 Temperature: _____ °C

4.4 Salinity: _____ S‰

4.5 Duration of storage: _____ hours

4.6 Other factors, which may influence the results should be reported under 12

5. Time of filtration :

5.1 Hour: _____ 5.2 Day: _____

6. Start of incubation :

6.1 Hour:_____ 6.2 Day: _____

7. End of incubation :

7.1 Hour: _____ 7.2 Day: _____

8. Confirmatory tests carried out (mark test used) :

8.1 MacConkey: _____ 8.2 Brilliant green: _____

8.3 Indole test: _____ (for faecal coliform)

9. Number of colonies per individual filter :

dilution	ml of original sample filtered	Number of total (or faecal) coliform colonies per filter in replicate dilution Replica		
		1st	2nd	3rd

10. Number of total (or faecal) coliforms per 100 ml :

dilutions	n/100 ml

11. Test results [total (or faecal) coliforms/100 ml sample] :

Mean	Range	Stand. Deviation	Coef. Var. (%)
_____	_____	_____	_____

12. Anomalies observed in the test procedure :

_____
_____
_____
_____
_____

13. Full address of the institution, which carried out the test :

_____
_____
_____
_____
_____

14. Name(s) and signature(s) of the person(s) who carried out the test :

_____
_____
_____
_____
_____

Date: _____

VIII.3.2.2 Determination of Faecal Coliforms in Seawater

From seawater samples taken under sterile conditions, a dilution series is set up according to the number of faecal coliforms expected in the water sample. Aliquots of this dilution series are filtered through 0.45 µ pore size membrane filters. The membrane filters are placed on the surface of M-FC agar contained in Petri dishes and incubated at 44.5 ± 0.2°C for 24 hours. Lactose fermentation will cause colonies of faecal coliforms to exhibit a characteristic blue color.

Residual chlorine, if present, is neutralized by adding thiosulphate to the sampling bottle before sterilization.

Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test using MacConkey broth or brilliant green bile broth. In areas where industries discharge polysaccharides (paper mills, sugar beet industries, etc.), further confirmation by the indole test may be necessary.

A) Apparatus and glassware

See Section VIII.3.1.1, Part (C) and Section VIII.3.2.1, Part (A).

B) Preparation of media and reagents

See Section VIII.3.1.1, Part (E).

i) M-FC agar

Medium

Tryptone	10.0 g
Proteose peptone No.3	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salt No.3	1.5 g
Aniline blue	0.1 g
Agar	15.0 g
Distilled water	1.0 liter

Preparation : Dissolve the ingredients of this agar in 1 liter of distilled water. Heat to boiling point until all ingredients are completely dissolved. If necessary, add 10 ml of 1% solution of rosalic acid, keep boiling for more than one minute and then cool. The final medium should have a pH of 7.4 ± 0.1. If necessary, adjust the pH with diluted analytical grade HCl. A volume of 200 ml of M-FC agar, with or without rosalic acid, is poured in

each sterile 250 ml flask. For immediate use, these flasks are kept in a 50°C water bath for a maximum of 3-4 hours. For later use, the flasks are kept in a refrigerator for a maximum of 1 week.

Note : 1) The addition of rosalic acid is facultative and should only be added, if necessary, to suppress excessive growth of non-faecal coliforms
2) Do not autoclave the medium
3) The agar surface should not become too dry because if the agar is dry the MF will not adhere well to the agar surface and hinder the diffusion of nutrients to the colonies on the MF

ii) Rosalic acid

Prepare a sufficient amount of 1% solution of rosalic acid in 0.2 N NaOH.

Note : Solution of rosalic acid should be prepared freshly each time. It should not be autoclaved

C) Test procedure

Interpretation

Count with a stereomicroscope or similar only colonies, which appear as blue colored. If the number of dubious colonies is greater than 10 per cent of the total number of colonies, test dubious colonies either by MacConkey broth test or brilliant green bile broth test.

In areas where industries discharge polysaccharides (paper mills, sugar beet industries, etc.), further confirmation by the indole test may be necessary.

Note : Colonies produced by faecal coliform bacteria are blue in color. The non-faecal coliform colonies are grey to cream-colored. Background colors on the membrane filter will vary from a yellowish cream to a faint blue, depending on the age of the rosalic acid salt reagent. Normally, only few non-faecal coliform colonies will be observed on M-FC agar because of the selective action of the elevated temperature and addition of the rosalic acid salt reagent

Indole test

With a flamed bacteriological loop transfer the suspected colony into culture tubes containing tryptone water and incubate at 44.5 ± 0.2°C in a water bath for 24 hours. Then add 0.2 - 0.3 ml of Kovac's indole reagent and shake. Let the tube stand for about 10 minutes and observe the results.

A dark red color in the amyl alcohol surface layer constitutes a positive indole test; the original color of the reagent, a negative test. An orange color probably indicates the presence of skatole and may be reported as a positive reaction.

D) Expression of results

See Section VIII.3.2.1, Part (D).

E) Test report

See Section VIII.3.2.1, Part (E).

General

About the dominant species of coliform, there are some recommended species which are:

a) Staphylococcus aureus (UNEP/WHO 1995, No. 28)
b) Mesophylic aeromonads (UNEP/WHO, 1995, No. 69)
c) Pseudomonas aeruginosa (UNEP/WHO, 1995, No. 29)
d) Camphylobacter (UNEP/WHO, 1995, No. 68)
e) Salmonella (UNEP/WHO, 1995, No. 30)

VIII.3.2.3 Determination of Faecal Coliform in Sediments

The method described is suitable for the determination of faecal coliforms in sediments of coastal bathing waters of temperate and tropical seas and is designed to be used in sanitary surveillance of bathing beaches and shellfish growing areas.

This method allows colonies determination, as the Membrane Filtration Culture Method, but the sample volume cannot be larger than 1 ml. because the culture medium must be able to absorb the volume of the material inoculated and solidify without difficulty.

From sediment samples taken in sterile conditions, a dilution series is set up made according to the quantity of faecal coliforms expected in the sediment sample. A 1-ml aliquot volume of this dilution series are transferred to empty Petri dishes (5.16). Then M-FC agar (6.1) is poured in each Petri dish. Once the agar has solidified, the Petri dishes are incubated at 44.5± 0.2°C for 24 hours. Lactose fermentation will turn faecal coliform colonies into their characteristic blue color.

Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test using the MacConkey broth or the Brilliant Green broth. In urban bathing areas where sewer outlets discharge rain water as well as wastewater, the confirmative test for indole production is necessary.

A) Apparatus and glassware

See Section VIII.3.1.1, Part (C) and Section VIII.3.2.1, Part (A).

B) Preparation of media and reagents

See Section VIII.3.1.1, Part (E).

C) Test procedure

Sampling bags and bottles

i) Plastic bags and sterile plastic bottles are disposable items

If these items are not available, similar size wide-mouthed glass bottles can be used. Then proceed as follows: clean sample bottles. Dry and sterilize them in a drying oven at 160°C for 3 hours. Before sterilization, place a small piece of filter paper in the neck of the bottle to prevent the glass stoppers from sticking after cooling. After cooling to ambient temperature in the drying oven, remove this filter paper with sterilized forceps and fit the ground glass stopper securely into the neck of the bottle. Place the bottles into detergent-cleaned thermo-insulated boxes. Separate the bottles from each other with clean wrapping paper to avoid breakage.

ii) Glass petri dishes and glass pipettes

Clean Petri dishes and pipettes, place a cotton plug in their mouth piece and put them into suitable stainless steel containers for sterilization in a drying oven at 160°C for 3 hours.

Note : The M-FC agar for determination of faecal coliforms should not be sterilized

Selection of sample size and dilution series

Inoculated Petri dishes should ideally present from 20 to 80 colonies after incubation. If previous experience for planning the dilution series for clean sediments is not available, inoculate dilutions D-1 to D-3. For more polluted sediments (as from estuaries and wastewater outfalls) inoculate dilutions D-2 to D4.

Preparation of the dilution series [see Figure (VIII.11)]

i) Preparation of dilution D-1 (stock solution)

Weigh 50 g of sediment and place them into a sterile bottle or flask with 447.5 ml of phosphate buffer and 2.5 ml of Tween 80. Homogenize the sediment suspension D-1 (stock solution containing 100 g sediment/liter). Use a sterilized laboratory blender. Homogenization must last for several minutes and must be repeated at least three times. Tween 80 is added to the phosphate buffer to promote the release of bacteria attached to sediment particles.

ii) Preparation of dilutions D-2, D-3 and D-4 [see Figure (VIII.10)]

These are 1/10 dilutions prepared using phosphate buffer, but without Tween 80. For each sediment sample, prepare 4-5 tubes each with 9 ml of phosphate buffer and autoclave them. Prepare the dilution series by taking with a sterilized pipette, after vigorously shaking the sample, 1 ml from the bottle or flask D-1 and transfer this 1 ml into a culture tube containing 9 ml of phosphate buffer to make the D-2 dilution. Agitate the tube on a

mixer for at least a minute or shake it vigorously by hand. Repeat agitation three times and then continue the preparation of the dilution series by taking 1 ml. from the previous dilution (D-2) and mixing it in a new culture tube containing 9 ml of phosphate buffer in order to obtain the third dilution D-3. Proceed until reaching the chosen dilution level.

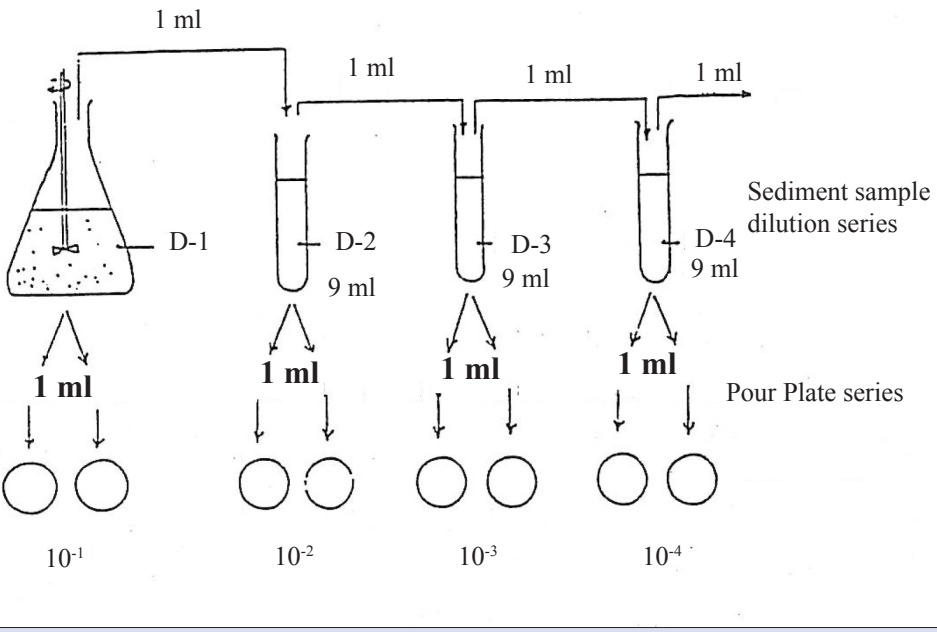


Figure (VIII.11) : Working diagram for the determination of faecal coliforms in sediments by the Pour Plate method

Inoculation procedure (Pour plate method)

During inoculation, or one hour before, liquify the M-FC agar previously prepared by immersing the flask in a water-bath at 55°C. A 250 ml flask should contain approximately 200 ml of agar. The M-FC agar should not be autoclaved. The agar can be kept liquified in a water bath at 48-50°C for three hours maximum.

Place 9 cm diameter Petri dishes and mark on their back the sample number and the dilution number, using a soft pencil or an alcohol felt pen. set up 2 Petri dishes for each dilution tube.

Begin inoculation with the highest dilution (for example D-4). With a sterilized pipette transfer 1 ml of that dilution to the middle of the corresponding empty Petri dish. (Repeat this operation with a second dish for the same dilution). Using the same pipette, for example of 2-ml capacity, transfer t ml of the next smallest dilution bottle, D-3 in this example, into each of the two corresponding Petri dishes. Proceed in the same manner until arriving at the D-1 dilution that should be vigorously shaken again.

Once the inoculation procedure is complete, each dilution tube should correspond to two Petri dishes, each containing 1 ml of sediment dilution.

Flame the mouth of the flask containing the liquified M-FC agar.

Pour 10 to 14 ml of liquified M-FC agar in each Petri dish while conferring it a circular motion from right to left and then from left to right to ensure a thorough mixing and an even distribution of the inoculum in all the agar volume. Close two thirds of the dish surface with the dish top and let the agar solidify.

Incubation

Petri dishes are inverted to avoid condensation water forming inside Petri dish lid from running.

Inverted Petri dishes are placed horizontally inside clean metal boxes. These metal boxes (wrapped in tight plastic bags) are then placed in a water bath (5.28) and incubated immediately at 44.5 ± 0.2 °C for 24 hours. As a sterility test, incubate also one blank (without diluted sediment sample), i.e. a Petri dish containing agar only (6.1).

Note : 1) The metal boxes must be suitably weighed to prevent them from floating
2) Water-bath incubation of Petri dishes is optional

Interpretation

Count with a stereoscopic microscope or similar equipment only colonies that appear as blue points and whose size vary from a pin head to a well formed colony. If the number of dubious colonies is higher than 10% of the total numbers of colonies on the Petri dish, test dubious colonies either with the MacConkey broth test or the Brilliant Green Bile broth test and confirm them with the indole test.

In urban bathing areas where sewer outlets discharge rain water as well as wastewater, the confirmative test for indole production is necessary.

Note : 1) Colonies produced by faecal coliform bacteria are blue. The non-faecal coliform colonies are grey to cream-colored. Background colors on the agar plate may vary from a yellowish cream to a faint blue, depending on the age of the rosalic acid. Normally, only few non-faecal coliform colonies will be observed on M-FC agar because of the selective action of elevated temperature, medium salts and the rosalic acid reagent
2) Possible precipitate accumulations on the agar should not be counted as colonies
3) If Petri dish reading (colony counting) must be suspended, place inverted Petri dishes in a refrigerator at 4°C for a maximum of 24 hours

Confirmatory tests

i) MacConkey broth test

With a flamed bacteriological loop, or a flamed sterile Pasteur pipette, transfer the suspected colony from the Petri dish into a culture tube containing MacConkey broth and incubate at 44.5 ± 0.2 °C for 24 hours. Coliforms will produce gas, that will be trapped as a big bubble in the inverted Durham vials, and acid, that will turn the violet-like color

of the original broth from into a yellowish and cloudy color (if the tube becomes yellow with a greenish taint at the top and a surface veil, there is a presumptive evidence of Pseudomonas).

ii) Brilliant green bile broth test

With a flamed bacteriological loop, or a flamed sterile Pasteur pipette, transfer the suspected colony from the Petri dish into a culture tube containing Brilliant Green Bile broth and incubate at 44.5 ± 0.2 °C for 24 hours. Coliforms will produce gas that will be trapped in the inverted Durham vials.

Note : The MacConkey broth test is equivalent to the Brilliant Green Bile broth test. Either can be used as a confirmatory test

D) Expression of results

See Section VIII.3.2.2, Part (D).

E) Test report

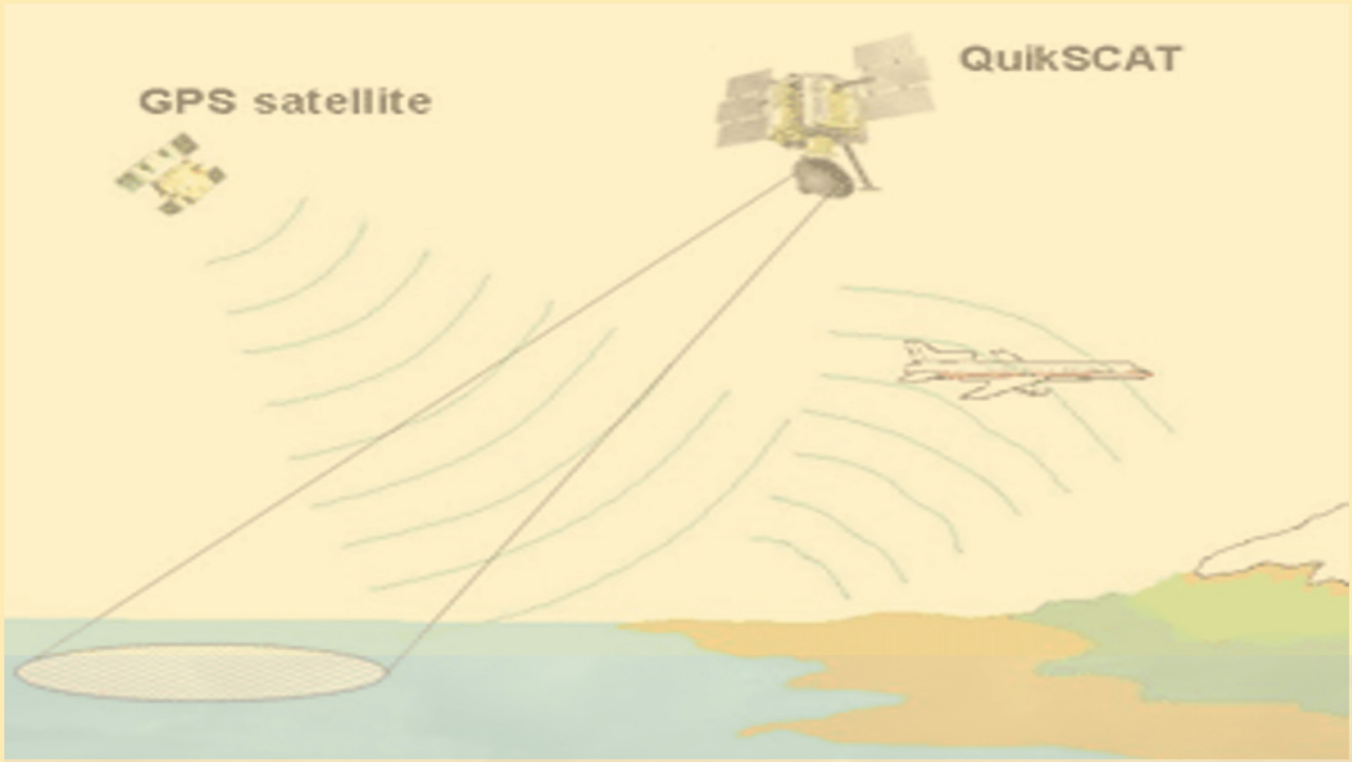
See Section VIII.3.2.2, Part (E).

VIII.4 QUALITY ASSURANCE PROGRAMME

The main objective of a quality assurance programme in a microbiological laboratory is to ensure the production of analytical results of high quality through the use of analytical procedures that are accurate, reliable, and adequate for the intended purpose.

The success of a quality assurance programme depends on the commitment and cooperation of the laboratory management and personnel, as well as on the adequacy of the objectives and foundation of the programme itself. Although the implementation of a quality assurance programme may initially represent a slight decline in productivity, and certainly requires human resources and time, the confidence and overall benefits derived from knowing that the results are reliable and comparable brings about higher personal satisfaction, improves laboratory performance and efficiency, and greatly contributes to the overall success of the monitoring programme, resulting thus in a worthwhile effort.

The development and implementation of a quality assurance programme requires basically: 1) a set of written guidelines and analytical procedures, 2) an organizational structure to carry out the quality assurance programme, and 3) a group of qualified and well trained laboratory personnel. For further information, see Annex IX.



IX REMOTE SENSING APPLICATIONS



IX REMOTE SENSING APPLICATIONS

Remote sensing is a method for obtaining information of the studied phenomenon based on measurements of : upward radiance, emerging out of the atmosphere in characteristic spectral intervals in optical and microwave spectral range (passive remote sensing systems) (ERTS/LANDSAT, 1972; NOAA, 1980; SPOT; SeaWiFs, 1992), or recorded echoes from active remotely sensed instruments (ERS1, 1991; JERS; ERS2).

IX.1 BASIC PRINCIPLES OF REMOTE SENSING

Consider that the remotely sensed sensor is outside the Earth's atmosphere and is looking down, the detected radiance will depend on the constituents and physical processes of the atmosphere and underlying surface. Depending on the spectral ranges and spatial resolution of the sensor there are different applications of satellite remote sensing. Some applications of remote sensing and the electromagnetic spectrum, ranging from gamma rays at 10 pm to radio waves at 1 km wavelength are shown on Figure (IX.1).

WAVE DESCRIPTION				REMOTE SENSING APPLICATION	
Wavelength λ (m)				Applications	Frequency(v) (Hz)
10^{-12}	-	1 pm	Cosmic Ray		10^{21}
	-		----------		
	-		Gamma Ray		

10^{-9}	-	1 nm	X Ray		10^{18}
	-		----------		
	-		Ultra Violet	Ultraviolet spectrometers for atmospheric sounding	
10^{-6}		1µm	Visible	Visible and near infrared systems for land and atmospheric sounding	
			Infrared	Infrared radiometes for surface and atmospheric sounding	
—	—	—	—	—	—
10^{-3}				Passive microwave radiometers for marine and atmospheric sounding	
	-			SAR, Scatterometer, Altimeter for land, marine and atmospheric sounding	
1	-	1 m	----------		
	-		----------	Telemetry	
	-		Radio wave	Radio and T.V. transmission	
10^{3}	-	1 km			10^{6} – 1 MHz

Figure (IX.1) : The electromagnetic spectrum, some band definitions and typical remote-sensing applications

The remote sensing methods could be divided also on three main groups: satellite, airborne and ground based. The used sensors could be active or passive. The optical passive remotely sensed sensors measure the reflected, or emitted radiance from the earth atmosphere system in ultra-violet, visible and infrared spectral range. The passive microwave radiometers measure the emitted radiance in microwave spectral range. The active microwave sensors generate and transmit a signal toward the target (in spectral range from 0.3 GHz (1 m, P band) to 1000 GHz (0.3 cm, W band) and receive and record the returned signal after its interaction with the target. The relationship between the transmitted and received signal is used to characterise the condition, or state of the target. The most important microwave spectral ranges are C band (3.9 - 5.75 GHz), sensitive to foliage, soil moisture and used in many space borne SAR's (ERS, Radarsat), L band (0.39 - 1.55 GHz), used by SEASAT and JERS.1. Table (IX.1) shows some of the satellite systems used for coastal zone and marine environment study.

Table (IX.2) outlines some of the marine applications performed by space-borne remote sensing.

IX.2 SENSORS

IX.2.1 Satellite Systems and Space Missions

The passive sensors detect and record the natural electromagnetic energy, reflected/or emitted from a target. The optical passive remotely sensed sensors measure the reflected radiance from the earth-atmosphere system in ultraviolet, visible and near infrared spectral range and the infrared thermal emitted radiance. The impact of the atmosphere on the remotely sensed measured spectral radiance in the optical spectral range is essential, which is requiring for marine and land surface environmental study atmospheric correction of satellite data. The main passive optical systems and missions with application in marine and coastal zone environmental study are:

NOAA

The AVHRR [Advanced Very High Resolution Radiometer, (NOAA User's Guide, 1997)] system is performing on NOAA polar orbiting satellites, the first one launched in 1978. These satellites are flying at altitude of 850 km in a nearly sun-synchronous orbit, with a cross track scanning mode of about 2700 km, covering a range of viewing angles up to 56o, with 1 km resolution. The AVHRR has five channels: CH1 (0.58-0.68 μm), CH2 (0.725-1.1 μm), CH3 (3.6-3.9 μm), CH4 (10.3-11.3 μm) and CH5 (11.5-12.5 μm). The spectral resolution and spectral ranges of the visible and near infrared bands of AVHRR makes AVHRR/ CH1 and CH2 / a strong indicators of green vegetation, clouds, atmospheric and water pollution. The essential influence of the atmosphere in visible and near infrared spectral range requires atmospheric correction of AVHRR data (Deschamps, 1980) in order to determine water surface bio-optical properties, land surface type, NDVI and albedo.

Table (IX.1) : Major satellite systems with application in marine and coastal zone

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
NOAA 11-14	AVHRR	0.58 - 0.68 0.725 - 1.1 3.55 - 3.3 10.5 - 11.5 11.5 - 12.5	1.1	3000	Near-polar sunsynchronous
DMSP	OLS	0.4 - 1.1 8 - 13	0.56 0.56		Polar Sunsynchronous
	SSM/I	19.35 GHz H, V 37.0 GHz H, V 85.0 GHz H, V 22.23 GHz V	56 - 13 15 13 50	1400	2 satellite's constellation
LANDSAT 4, 5 NASA	TM	0.45 - 0.52 0.52 - 0.60 0.63 - 0.69 0.76 - 0.90 1.55 - 1.75 2.08 - 2.35 10.4 - 12.5	30 X 30 m 120 x 120 m	185	Launched since 1986, with near-polar sun synchronous orbit, 16 days repeat cycle. Application in marine and land surface environmental study.
SPOT-3	HRV/XS	0.5 - 0.59 0.61 - 0.68 0.79 - 0.89	20 m spectral	117	With Near-polar sun-synchronous orbit, 26 days repeat cycle. Applied in marine and land surface environmental study. Steering platform, stereo capability
	PM	0.51 – 0.73	10m pan		

Satellite	Sensor	Spectral Range (μm)	Resolution (km)	Swath (km)	Characteristics and objectives
Nimbus7 Coastal Zone Colour Scanner (CZCS) NASA		443 ± 10 nm 520 ± 10 nm 550 ± 10 nm 670 ± 10 nm 700 – 800 nm	0.825 x 0.825	1636	Operated until 1986. Applied in marine and ocean study for surface chlorophyll concentration
Space Shuttle D2	MOMS-02 (Germany)	440 – 505 nm 530 – 575 nm 645 – 680 nm	12.8 m 12.8 m 12.8 m	78/43 78/43 78/43	Applied for atmospheric and land surface environmental study
	nadir	710 – 810 nm 520 – 760 nm	12.8 m 4.2 m	78/43 37/43	
Stereo +-21.4o		520 – 760 nm	12.8 m	78/43	
JERS1 / OPS Stereo 15.33o NASDA		0.56 ± 0.04 0.66 ± 0.03 0.81 ± 0.05 0.81 ± 0.05 1.655 ± 0.055 2.065 ±0.055 2.19 ± 0.06 2.335 ± 0.065	18 m	75	With Sun Synchronous orbit, launched 1992, applied for land and marine environmental study.
SAR		(LBand) H – H	1.275		
UARS Microwave Limb Sounder (MLS)		63.283 GHz 203.267 GHz 184.778 GHz			Limb sounder of Upper troposphere ClO, H_2O, O_3 stratosphere H_2O, O_3 pressure, temperature.

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
Seasat	SMMR	10 channels	30	150-840	Applied in meteorology
Nimbus 7 NASA		Between 6.6 and 37 GHz (5ch-H, 5ch-V)			
Geosat altimeter		13.5 GHz	Short-pulse 3.125 ns		For near sea surface wind speed.
Nadir-viewing radar					
IC/ID		0.52 - 0.59 0.62 - 0.68 0.77 - 0.86 1.55 - 1.7	23.5 m 23.5 m 23.5 m 71 m	148	
TOPEX/ POISEDON					With 10 days repeat non sun synchronous orbit, operating in ± 66°
SeaWiFS Orbview		402 nm - 422 nm 433 nm - 453 nm 480 nm - 500 nm 500 nm - 520 nm 545nm - 565 nm 600 nm - 680 nm 745 nm - 785 nm 845 nm - 885 nm	1.12		With 16 days repeat cycle, operating since 1997, applied for sea surface chlorophyll and sediments monitoring.
TRMM PR		13.8 GHz	4.3 km^2	220	Tropical rain fall mission
NASA/ TRMM		5 channels from 10 to 91 GHz			

Satellite	Sensor	Spectral Range (μm)	Resolution (km)	Swath (km)	Characteristics and objectives
NASA/VIRS		0.63 1.6 3.75 10.7 12.0	2	1500	
ADEOS					Sun synchoronous orbit.
AVNIR		0.42 - 0.5 0.52 - 0.6 0.61 - 0.69 0.76 - 0.89	16 m pan 8 m	80	
ILAS, ILAS-2		0.753 – 0.781 8.21 – 11.77			Improved infrared limb atmospheric spectrometer Stratospheric ozone and species
Occultation device		5.99 – 6.78			4 km vertical resolution from 10 to 60 km
IMG Nadir Forrier Spectrometer		3.3 – 14			With spectral resolution 0.1 cm^{-1} for CO_2, methane, greenhouse gases
NASA NSCAT Scatterometer		14 GHz	25	600	Ku band
NASDA OCTS		0.412 – 0.679			20 nm band width
TIR		3.7 – 12.0	0.700	1400	

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
POLDER CNES		8 bands in 0.443 – 0.91	5		In 3 bands all polarisation measurements. 10-20-40 nm band width.
RIS		Laser pulses in 0.4-14			Retroflector in space. For column density of ozone and trace species
NASA TOMS		6 bands in 0.309 – 0.36			With 1 nm bandwidth for daily global ozone
TERRA	ASTER	14 channels 0.5-12	0.015 0.03 0.09	60	ASTER (Advanced Spaceborne Thermal Emission and Reflection Radiometer) used to obtain detailed maps of land surface temperature.
	MISR	4 channels 0.443 -0.865	0.275	360	The Multi-angle Imaging SpectroRadiometer (MISR) viewing the sunlit Earth simultaneously at nine widely-spaced angles
	MODIS	36 channels 0.620-14.385	0.25,0.5,1	2300	The The Moderate Resolution Imaging Spectroradiometer (MODIS) measures physical properties of the atmosphere, and biological and physical properties of the oceans and land.
	CERES	0.3 - 5.0 8 - 12 Total: 0.3 to >100	20 at nadir	Limb to limb	The Clouds and the Earth s Radiant Energy System (CERES) to estimates the radiative fluxes at TOA and the Earth's surface and provide cloud property estimates.

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
	MOPITT	4.7 2.2-2.4	22	640	Measurement of Pollution in the Troposphere (MOPITT) focuses on the distribution, transport, sources, and sinks of carbon monoxide and methane in the troposphere.
AQUA	AIRS	0.4 - 1.0 3.7 - 15.4	13.5 at horizontal 1 vertical at nadir	1650	The Atmospheric Infrared Sounder (AIRS), an advanced sounder, aimed at obtaining highly accurate temperature profiles within the atmosphere plus a variety of additional Earth/ atmosphere products.
	AMSU-A	15 channel 15-90 GHz	40 horizontal at nadir	1650	The Advanced Microwave Sounding Unit (AMSU-A), a 15-channel microwave sounder designed primarily to obtain temperature profiles in the upper atmosphere (especially the stratosphere) and to provide a cloud-filtering capability for tropospheric temperature observations.
	HSB	4 channels: 1 at 150 GHz 3 at 183 GHz		1650	The Humidity Sounder for Brazil (HSB), a 4-channel microwave sounder provided by Brazil aimed at obtaining humidity profiles throughout the atmosphere.
	AMSR-E	89.0 GHz 36.5 GHz 23.8 GHz 18.7 GHz 10.65 GHz 6.925 GHz	6 x 4 14 x 8 32 x 18 27 x 16 51 x 29 75 x 43	1445	The Advanced Microwave Scanning Radiometer for EOS (AMSR-E) is a passive-microwave radiometer system to measure brightness temperatures.
	MODIS	36 channels 0.620-14.385	0.25, 0.5, 1	2300	The Moderate Resolution Imaging Spectroradiometer (MODIS) measures physical properties of the atmosphere, and biological and physical properties of the oceans and land.

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
	CERES	3 channels: 0.3 - 5.0 8.0 -12.0 0.3 - 100	20 at nadir	Limb to limb	The Cloud's and the Earth's Radiant Energy System (CERES) is a radiometer measuring the Earth's total thermal radiation budget and clouds
ICESAT	GLAS	infrared laser pulses 2 bands: 1.064, 0.532	0.07	0.17	The Geoscience Laser Altimeter System (GLAS) provides multi-year elevation data as well as cloud property information, in addition to topography and vegetation data.
AURA	MLS	1.5 x 1.9 x 1.8 m (GHz sensor); 0.8 x 1 x 1.1 m (THz sensor); 1.6 x 0.5 x 0.3 m (spectrometer)	5 (cross-track) x 500 (along-track) x 3 (vertical)		The Microwave Limb Sounder (MLS) measures thermal emission from the atmospheric limb and provides reliable measurements even in presence of dense cirrus and volcanic aerosol.
	HIRDLS	6 to 18 mm	500	10 x 300	The High Resolution Dynamic Limb Sounder (HIRDLS) observes global distribution of temperature and concentrations of O_3, H_2O, CH_4, N_2O, NO_2, HNO_3, N_2O_5, CFC_{11}, CFC_{12}, $ClONO_2$, and aerosols in the upper troposphere, stratosphere, and mesosphere
	OMI	0.0350 – 0.500 0.270 – 0.314 0.306 – 0.380	3		The Ozone Monitoring Instrument (OMI) measures key air quality components such as NO_2, SO_2, BrO, OClO, and aerosol characteristics and capable of mapping pollution products on urban-to-super-regional scales.
	TES	3.2–15.4	0.53 x 5.3	5.3 x 8.5	The Tropospheric Emission Spectrometer (TES) determins of long-term trends in radiative active minor constituents in the lower atmosphere to investigate effects on global radiative balance and atmospheric dynamics.

Satellite	Sensor	Spectral Range (μm)	Resolution (km)	Swath (km)	Characteristics and objectives
NOASS K.L.M		0.58 - 0.68	1.1	3000	Near polar sun synchoronous orbit
AVHRR/3		0.725 - 1.1 16 3.55 - 3.93 1.05 - 11.5 11.5 - 12.5			
Landsat 7 NASA		0.45 - 0.52 0.52 - 0.60 0.63 - 0.69 0.76 - 0.90 1.55 - 1.75 2.08 - 2.35 10.4 - 12.5	0.015 0.060	185	
ENVISAT/ESA	ASAR	Microwave: C-band, with choice of 5 polarisation modes (VV, HH, VV/HH, HV/HH, or VH/VV)	0.15,0.03,1	Image and alternating polarisation modes: up to 100, Wave mode: 5, Wide swath and global monitoring modes: 400 or more	Advanced Synthetic Aperture Radar (ASAR). For ocean currents and topography, landscape topography and snow and ice.
	MERIS	15 Channels 0.390 – 1.040	Ocean: 1.04 x 1.20 , Land & coast: 0.26 x 0.30m	1150	The Medium Resolution Imaging Spectrometer Instrument (MERIS). To investigate ocean colour biology, land vegetation and atmospheric clouds/precipitation.

Satellite	Sensor	Spectral Range (µm)	Resolution (km)	Swath (km)	Characteristics and objectives
	AATSR	7 Channels 0.555 – 12.0	1,500	500	The Advanced Along-Track Scanning Radiometer (AATSR) intended for atmospheric clouds/precipitation, land vegetation and sea surface temperature.
	RA-2	Microwave: 13.575Ghz (Ku-Band) & 3.2GHz (S-Band)			The Radar Altimeter 2 (RA-2) intended for sea ice, winds, land topography/mapping, and ocean waves, currents and topography.
	MWR	Microwave: 23.8 and 36.5GHz	20	20	The microwave radiometer (MWR) is intended to measure the atmospheric humidity and temperature.
	GOMOS	6 Channels 0.248-0.528			Atmosphere (chemistry, Ozone).
	MIPAS	4.15 , 14.6	Vertical resolution: 3km, vertical scan range 5-150km, Horizontal: 3km x 30k	3 x 30	The Michelson Interferometer for Passive Atmospheric Sounding (MIPAS) is a Fourier transform spectrometer for the measurement of high-resolution gaseous emission spectra at the Earth's limb.
	SCIAMA-CHY	8 Channels 0.240 - 2.380	Limb vertical 3 x 132 Nadir horizontal 32 x 215	Limb and nadir mode: 1000 (max)	The SCanning Imaging Absorption SpectroMeter for Atmospheric ChartographY (SCIAMACHY) primary mission objective is to perform global measurements of trace gases in the troposphere and in the stratosphere.
	DORIS	401.25MHz, 2036.25MHz			The Doppler Orbitography and Radio-positioning Integrated by Satellite (DORIS) utilized to determine the precise location of the ENVISAT satellite.
	LRR	0.350-0.800			The Laser Retro-Reflector (LRR) is mounted to support satellite ranging for precise orbit determination and measurement calibration

Table (IX.2) : Application of various space borne remote sensing systems for sea current, water quality and sediment transport analysis

Spaceborne remote sensing systems

Natural formation	Landsat		SPOT XS/PM	ERS-1/2		JERS-1	AVHRR	CZCS	Sea WiFs	Topex ALT	Envisat MERIS
	TM	MSS		ATSR	SAR						
Water classes :	G	S	S	S	S	S	G	G	G		G
Case 1	G	S	S	S	S	S	G	G	G		G
Ocean Waters											
Case 2	G	S	S	S		S	S	G	G		G
Coastal Zone											
Water quality,											
Suspended matter :											
chlorophyll	G	S	S	S		S	S	G	G		G
sediments	G	G	S	L		S	S	G	G		G
yellow substances	S	L	L	L			L	S	S		G
pollutants	S	L		S	S	S	S	S	S		G
Currents	S	L	L	S	G	S	S	G	G	S	G
wind waves	L	L	L	L	G	G	L	L	L	G	S
Tides	L	L	L	L	S	G	L	L	L	G	S
Wind speed					L	G				G	S
Sea level height						G				G	
Rivers	S	L	S		L	S	L	S	S		G

G = VERY USEFUL S = USEFUL L = LIMITED USEFUL

The infrared (IR) AVHRR channels could be used as indicator of cloud contaminated pixel (Goodman, 1988; Saunders, 1988 and 1986), oil spill on the sea surface, land surface properties and temperature monitoring. The AVHRR/3, which will fly on NOAA K, L, M (Kidder, 1995) will have six channels, where 1.6 μm channel will be used during daylight to gain information on the phase of clouds (ice, or water) and to discriminate between low clouds and snow cover and ice. The 3.7 μm channel will operate at night and the instrument is programmed to automatically switch channels at the terminator.

HIRS/2 and HIRS/3 on board of NOAA K, L, M are the infrared sounding unit, designed for sounding atmospheric temperature profile (15 μm and 4.3 μm channels) moisture content (6.3 μm) and ozone (9.7μm), Table (IX.3).

Table (IX.3) : Spectral channels of HIRS/2 and HIRS/3 sensors

HIRS/2		Half Power		HIRS/3		GOES Sounder	
Channel	Description	Wave length (μm)	Band width (cm^{-1})	Wave length (μm)	Band width (cm^{-1})	Wave length (μm)	Band width (cm^{-1})
1	15 μm CO_2	14.95	3	14.95	3	14.71	13
2	15 μm CO_2	14.71	10	14.71	10	14.37	13
3	15 μm CO_2	14.49	12	14.49	12	14.06	13
4	15 μm CO_2	14.22	16	14.22	16	13.64	16
5	15 μm CO_2	13.97	16	13.97	16	13.37	16
6	15 μm CO_2	13.64	16	13.64	16	12.66	30
7	15 μm CO_2	13.35	16	13.35	16	12.02	50
8	Window	11.11	35	11.11	35	11.03	50
9	O_3	9.71	25	9.71	25	9.71	25
10	H_2O	8.16	60	12.47	16	7.43	55
11	H_2O	7.33	40	7.33	40	7.02	80
12	H_2O	6.72	80	6.52	55	6.51	60
13	4.3 μm CO_2	4.57	23	4.57	23	4.57	23
14	4.3 μm CO_2	4.52	23	4.52	23	4.52	23
15	4.3 μm CO_2	4.46	23	4.47	23	4.45	23
16	4.3 μm CO_2	4.4	23	4.45	23	4.13	40
17	4.3 μm CO_2	4.24	23	4.13	28	3.98	40
18	4.3 μm CO_2	4.0	35	4.0	35	3.74	100
19	Window	3.76	100	3.76	100	0.698	
20	Vis. Window	0.69	1000	0.69	100		

The Microwave sounding unit on NOAA K, L, M will be significantly improved and renamed as Advanced Microwave Sounding Unit (AMSU), Table (IX.4).

Table (IX.4) : Microwave sounding units on board NOAA/K,L,M and DMSP

Satellite Instrument Frequency	DMSP SSM/T GHz	NOAA MSU GHz	DMSP SSM/I GHz	NOAA K,L,M AMSU/A GHz	NOAA K,L,M AMSU/B GHz	DMSP SSM/T-2 GHz)
Channel 1	50.5 H	50.3 R	19.35 H	23.8 R	89.0 R	183.3+3 V
2	53.2 H	53.74 R	19.35 V	31.4 R	157.0 R	183.3+1 V
3	54.35 H	54.96 R	22.235	50.3 R	183.3+1 R	183.3+7 V
4	54.9 H	57.95 R	37.0 H	52.8 R	183.3+3 R	150 V
5	58.4 V		37.0 V	53.6 R	183.3+7 R	
6	58.825 V		85.5 H	54.4 R		
7	59.4 V			54.9 R		
8				55.5 R		
9				57.2 R		
10				57.29+0.217 R		
11				57.29+0.322+0.048 R		
12				57.29+0.322+0.0.022		
13				57.29+0.322+0.01		
14				57.29+0.322+0.0045 R		
15				89.0 R		
Ground Res (km)	204	110	12.5-50	48	16	50-100
Swath width (km)	2053	2347	1394	2179	2179	2053

DMSP

DMSP F7 was the second of the DMSP block 5D-2 series of satellites, and was launched on November 18, 1983, consisting of two satellites (one with ascending node in the early morning, the other at local noon), operating in 830 km, sun-synchronous polar orbits with following orbital parameters: inclination - 98.7°, perigee - 817 km, apogee - 839 km, period - 101.4 min. The objectives of the programme are : to provide cloud and specialised environmental images in support of weather analysis and forecasting.

The 6.4 meter long DMSP block 5D-2 satellites consists of four sections: 1) the Precision Mounting Platform (PMP) containing sensors and equipment requiring precise alignment; 2) the Equipment Support Module (ESM), which held the reaction wheels, electronics and some meteorological sensors; 3) a Reaction Control Equipment (RCE) support structure containing the rocket motor

and associated reaction control equipment; 4) the 9.29 square meter solar panel array for electrical generation. The primary instrument is the Operational Linescan System (OLS) which consists of a scanning optical telescope driven in an oscillating motion (with compensation for image motion) resulting in near constant resolution throughout the field of view. The radiometer which collects the image and operates in two spectral intervals: (1) visible and near infrared (0.4 to 1.1 μm) and (2) infrared (8 to 13 μm) with resolution 0.56 km. The Special Sensor Microwave Imager (SSM/I) is designed to provide day and night measurements of ocean surface wind speed, ice coverage and age, area and intensity of precipitation, cloud water content, and land surface moisture. Microwave brightness temperatures are obtained with a seven channel passive microwave radiometer operating at four frequencies: 19.35, 37.0 and 85.0 GHz at both horizontal and vertical polarisation, and 22.23 GHz works at vertical polarisation. Resolution varies from 13 to 50 km (depending on the frequency) over the 1400 km wide swath.

LANDSAT / EROS

The first remote sensing satellite images in digital form were available by the EROS Data Centre's Landsat (ERTS) program beginning in July 1972. LANDSAT/1 (ERTS), 2, 3 are launched in 1972, 1975, 1978 and continued by LANDSAT 4, 5. Landsat (Landsat Data User's Handbook, 1984) satellites are performing on altitude of 918 km (Landsats 1, 2, 3) and 705 km (Landsats 4, 5), with a 16 days, or 18 days repeat cycle. The sun-synchronous polar orbit of the LANDSAT allows to pass over mid-latitudes at the same mid-morning time every day. Landsats 1, 2 and 3 contained a Return Beam Vidicon (RBV) and Multispectral Scanner (MSS); Landsats 4 and 5 contained the MSS and Thematic Mapper (TM) imaging systems. The RBV on Landsats 1 and 2 has three television cameras, recording green, red and near-infrared bands with 40 m ground resolution. Landsat 3 contains two RBV cameras, each with a single panchromatic band, designed for stereo images, covering 99 x 99 km. In MSS the reflected light is recorded by solid state detector that convert it to a digital electric signal. The MSS image covers an area 176 x 176 km with a resolution of 79 m. The blue light band (CH1) has better water penetration than the green light band (CH2), allowing to generate bathymetric maps and true colour images. The spectral ranges of MSS and TM on board of LANDSAT 4, 5 are given in Table (IX.5). Advantages of TM is the combination of seven spectral channels with high spatial resolution over an area of 176 x 170 km, allowing better discrimination of surface materials and monitoring the turbulent heat exchange on the surface. The thermal band is useful in mapping shallow groundwater, springs, offshore currents, marine and land surface temperature.

SPOT / CNES

Beginning the 1986 the French Space Agency launched the System Probatoire d'Observation de la Terre (SPOT 1) satellite at sun-synchronous 832 km orbit with 26-day repeat cycle (SPOT User's Guide). Like Landsat, it crosses mid-latitudes at mid-morning and is designed to gather earth resources imagery. An innovation is the ability to tilt or point the sensor up to 27° from vertical in a cross-track direction, so that the same area can be imaged on successive orbits with the resulting overlap used for stereo viewing. The area covered in vertical mode is 60 x 60 km.

SPOT P acquires a single image in the range 0.51 - 0.73 microns, roughly green and red visible wavelengths with resolution 10 m. SPOT XS records three channels of data (0.50 - 0.59 microns, 0.61 - 0.68 microns, 0.79 - 0.89 microns). The resolution of the XS system is 20 m.

Table (IX.5) : Spectral characteristics of MSS and TM system on-board of LANDSAT

MSS				TM				
CH	Band Width (nm)	Sat Rad. mW/cm^2 µm sr	Resolu. (m)	CH	λ max (nm)	Δλ (nm)	Band Width (nm)	Resolu. (m)
4	500-600	24.8	79	1	486	66	450-520	30
5	600-700	24.4	79	2	570	82	520-600	30
6	700-800	20.0	79	3	660	67	630-690	30
7	800-1100	17.6	79	4	838	128	760-900	30
	58.825 V			5	1650	200	1550-1750	30
	59.4 V			6	11450	2100	10400-12500	120
				7	2220	270	2080-2350	30

ERS / ESA

ERS1 is a polar orbiting satellite with sun synchronous near polar orbit at altitude 777-785 km and is specially designed for climate research. It is launched on 16th July 1991 (ERS1, 1991) by ESA and the following ERS2 is launched in 1995 with on board passive and active systems, Table (IX.6).

Along Tracking Scanning Radiometer/ATSR

The three days repeat cycle of ERS-1 ensures ground track repeatability within 1 km. The ground speed of the sub-satellite point is 6.67 km/s and the distance of 905 km between the centres of the two scans is covered in 135s. In order to achieve the high accuracy needed for climate research ATSR is calibrated in flight by an on-board calibration sub-system with accuracy of 0.1°C. During the night ATSR works approximately at the same spectral intervals as AVHRR/NOAA, while during the day 3.55 - 3.93 µm of Channel 3 is switched into 1.58 - 1.64 µm.

Microwave sounder ATSR/M

The ATSR/M is working at 23.8 and 36.5 GHz at near nadir viewing direction. Each channel is with 200 MHz bandwidth. The output signal from the synchronous detector is integrated and sampled every 150 ms (synchronised with the ATSR scan rate) and transmitted to the ground as a numerical count, together with the reference load temperature and internal temperatures. The footprints of the two channels are not co-incident on the Earth's surface, as the 36.5 GHz channel view slightly forward of the nadir point with resolution 22.4 x 17.6 km and 23.8 GHz channel views slightly behind with resolution 21.2 x 21.2 km, but the both channels are on sub-satellite track. The main characteristics are given in Table (IX.7).

Table (IX.6) : ERS-1 satellite

Instrument	Specification range	Resolution (km)	Swath (km)
Active Microwave Instrument (AMI)	5.3 GHz (C-Band)		200
Synthetic Aperture Radar (SAR)	5.3 GHz	0.03	100
Wind Scatterometer	5.3 GHz	50	500
Radar Altimeter (RA)	13.8 GHz (Ku-band)		
Along Track Scanning Radiometer ATSR During the night During the day	11.5 - 12.7 μm 10.4 - 11.3 μm 3.55 - 3.93 μm 1.58 - 1.64 μm	1 1.5 x 2 forward	500
Microwave sounder	23.8 GHz 36.5 GHz	21.2 x 21.2 22.4 x 17.6	
Precise Range and Range-rate Equipment (PRARE) Laser Retroreflector	59.4 V		
ERS-2 all systems from ERS-1 and GOME	240-720 nm		
ATSR-2	0.55 μm 0.67 μm 0.87 μm	1 km	500 km

Table (IX.7) : Characteristics of MWS (ATSR/M) on board ERS-1

Frequency	23.8 GHz	36.5 GHz
Resolution	21.2 x 21.2 km	22.4 x 17.6 km
Pointing angle	-1.93°	2.5°

Coastal Zone Colour Scanner / NASA

The first satellite-flown instrument, designed for ocean colour monitoring and determination of pigment concentration in the ocean, successfully operating till 1986, is the Coastal Zone Colour Scanner (CZCS), launched on Nimbus 7 in October 1978. It is a scanning radiometer, viewing the ocean in 6 spectral intervals in: visible (Channel 1 (CH1) 433 - 453 nm; Channel 2 (CH2) 510 - 520 nm; Channel 3 (CH3) 540 - 560 nm; Channel 4 (CH4) 660 - 680 nm; near infrared (Channel 5 (CH5) 700 - 800 nm); and thermal spectral range (Channel 6 (CH6) 10.5 - 12.5 µm) (CZCS User's Guide, 1986). The sensor has an active scan of 78°, centred on nadir and an instance angular field of view 0.0495°, which from height 995 km produces a ground resolution 0.825 km at nadir. It is equipped with provision for tilting the scan plane +20° from nadir in 2° increments along the track to minimise the influence of sun-glint. Comparing with Landsat/MSS and SPOT, designed for remote sensing of the Earth surface in the optical spectral range, the CZCS has enhanced radiometric and spectral sensitivities, which enable to detect lower radiance changes, due to atmospheric and ocean influence. The first four channels of the CZCS are designed for remote sensing of the water and may saturate over some lands targets. CH5 has the same response as CH3 of the Landsat MSS.

MOMS-02

The Modular Opto-electronic Multi-spectral scanner (MOMS-02) was launched on board of the second German Spacelab Mission D2 on Space Shuttle flight / STS-52 / from 26 April to May 1993. The MOMS-02 panchromatic channel provides cartographic resolution for topographic mapping. The high spatial resolution and combination of multi-spectral with stereo imaging are great advantages of MOMS-02 for monitoring small scale textures in coastal zone. Stereo data set and high spatial resolution allow the derivation of high quality digital terrain models with an accuracy of up to 3m, important for detecting tectonic features and stratigraphic layers, suspended matter, transparency and depth of coastal waters, atmospheric spectral optical thickness with high spatial resolution, industrial plumes and sources of pollution.

SeaWiFS

SeaWiFS is launched on board of Sea Star satellite with Sun synchronous polar orbit with the height = 705 km, inclination = 92.8°, repeat time = 16 days, spatial resolution at nadir = 1.12 km.

MLS/UARS

The Upper Atmosphere Research Satellite (UARS) was launched on 12 September 1991. The primary measurement objectives of the Microwave Limb Sounder (MLS) are ClO, O_3, H_2O, temperature and pressure. The 57° inclination of UARS orbit allows MLS measurements from 34° latitude on one side of the equator to 80° on the other, allowing 10 time per year, with periodicity of about 36 days, to view northern and southern high latitudes. The MLS field of view (FOV) is directed 90° from the UARS orbital velocity and the main characteristics of MLS are listed in Table (IX.8).

Table (IX.8) : Main characteristics of Microwave Limb Sounder

Radiometer	Frequency (GHz)	Wave length (mm)	IF Range (MHz)	Primary Measurement
1	63.283	4.74	90-540	Pressure temperature
2	203.267	1.48	830-1340 1053-1563 2610-3120	ClO H_2O_2 O_3
3	184.778	1.62	1213-1723 145 -655	H_2O_2 O_3

IX.2.2 Active Remote Sensing Systems

The active sensors generate and transmit a signal toward the target and receive and record the returned signal after its interaction with the target. The relationship between the transmitted and received signal is used to characterise the condition, or state of the target.

TOPEX / POSEIDON Mission NASA/CNES

TOPEX/POSEIDON was launched on August 10, 1992 by an Ariane 42P from ESA Guina Space Centre. The main characteristics of the orbit are given in Table (IX.9). The main instruments on board TOPEX/POSEIDON are divided on: a) operational sensors: dual frequency radar altimeter (ALT/NASA), TOPEX microwave radiometer (TMR/NASA), laser retroreflector array (LRA/NASA) and Doppler orbitography and radiopositioning, integrated by satellite (DORIS) and Doppler tracking system receiver (CNES); b) experimental sensors: single frequency solid state radar altimeter (SSALT/CNES) and global positioning system (GPS/NASA). The ALT, which is the first spaceborne dual-frequency altimeter is performing at 5.3 and 13.6 GHz. The TOPEX/TMR measures the sea surface microwave emissivity at 19, 21 and 37 GHz for total water vapour content in the atmosphere. The NRA TOPEX altimeter operates at 13.6 GHz (Ku band) and 5.3 GHz (C band) simultaneously and is the first space borne altimeter, that uses two-channel measurements to compute the effect of the ionosphere free electrons in the satellite range measurements. The NRA/TOPEX ground track has at the equator a spacing of 2.835° (314 km), an along track sampling rate of once per second and the orbit accuracy is 10 cm.

Table (IX.9) : Characteristics of TOPEX/POSEIDON orbit

Reference equatorial altitude	1336 km
Inclination	66°
Ground track equatorial spacing	316 km

Satellite altimeters

Since the first altimeter on board Skylab (1973), altimeter data have been received from Geoss-3, launched in 1975, followed by Seasat in 1978, Geosat in 1985, ERS-1 in 1991 and by TOPEX-POSEIDON in 1992. The satellite altimeter is radar, which transmits short pulses towards the Earth. The height of the satellite is calculated by measurement of the returned time of the pulse after reflection at the Earth surface, providing that the radar wave speed is known. To achieve 1 cm accuracy is required the return time to be measured within 30 picoseconds.

SAR

The civilian operation of radar satellites in space started with the launch of Seasat, followed by ERS-1, Almas, the Japanese radar satellite JERS-1, launched in 1992, followed by currently operating ERS-2 and RADARSAT. Synthetic aperture radar (SAR) sensors send and receive waves in the microwave region, made up of the P, L, S, C, X, K, Q, V and W band. X band is used for military reconnaissance and commercially for terrain surveys. The C band is used in many space borne SAR's like ERS-1 and RADARSAT. The X band is used in Almaz. The L band is used by SEASAT and JERS-1. Experimental airborne SAR may carry up to three band radars (P, C, X bands) as well as multipolarization capabilities. Different bands penetrate materials to varying degrees, producing different back scatter information from the respective layers. The penetration depth of the natural targets by band X, C and L depends from the wavelength and the moisture of the target. Clouds are penetrated by wavelengths of 2 cm or greater. To penetrate rain the wavelength must be longer than 4 cm. The various radar bands (starting from the X band) result in back scatter mainly depending on canopy leaf geometry and orientation. The C-band is sensitive to foliage, L and P bands result in back scatter from branches, trunks, soil layer and soil-trunk interactions, The back scatter coefficient determines the tone: water produces a relatively dark tone, due to little back scatter towards the radar, trees give a medium tone and the building makes a light tone. The surface scattering pattern depends on the surface roughness. Whether the target is rough, or smooth depends on the height of the surface variations, the wavelength and the angle of incident. A surface is considered "rough" if its surface structure has dimensions comparable to the incident wavelength. As greater the roughness is, the more back scatter towards the radar antenna and is translated into brighter images (diffuse reflection). The smooth water, or paved highways appears dark on a radar image (called specula reflectance).

The most promising types of SAR image products today are ERS low resolution images, ENVISAT's Wide Swath, and RADARSAT's Narrow-ScanSAR modes. ENVISAT's Polarisation mode can offer good detection capability both against soft targets (e.g. oil slicks) and against hard targets (e.g. ships), but the swath width is more narrow. The wind scatterometer, used on ERS, ERS-2 and later on ENVISAT, measures the echo power of a signal transmitted by the satellite and returned from the ocean surface, which depends on the ocean roughness, closely related to the surface wind conditions and the radar beam angle. For marine and ocean observations, SAR, wind scatterometer and altimeter find grate applications.

RADARSAT

RADARSAT is the first Canadian remote sensing satellite (RADARSAT, 1995), launched in 1995, developed by the Canadian Space Agency in cooperation with United States. The orbit is sun-

synchronous at altitude at 798 km and inclination of 98.6°. The orbit period is 100.7 min and the repeat cycle is 24 days. The RADARSAT SAR is a C-band (5.6 cm), Table (IX.10), operating with HH polarisation, in various look angle (20 - 59°), swath width (5 - 500 km) and resolution (10 - 100 m).

Table (IX.10) : Some parameters for present and future radar satellites

	ERS1/2	JERS-1	Radarsat	ENVISAT
Frequency	C-band	L-band	C-band	C-band
Polarization	VV	HH	HH	VV or HH or alternating
Modes	One	One	Many	Many
Incidence angle range	27°	35°	10-50°	15-45°
Resolution	30 m	18 m	10-1000 m	30-150 m
Swath width	100 km	75 km	50-100 km	56-405 km

SIR - C / NASA

The Shuttle Imaging Radar-C, launched in 1994, is a joint effort between NASA, the German Space Agency, and the Italian Space Agency. There were two instruments on board: a dual-frequency radar with L-band (23 cm) and C-band (6 cm), and X-SAR with an X-band (3 cm) wavelength. The ground swath is variable (15 - 90 km) and the resolution is 10 - 200 m. The incidence angles vary between 15 and 55° off nadir and flight altitude is 225 km.

Lidar

Lidar is active remote sensing system. It can operate in a profiling, or scanning mode. One of the applications of lidar data is the accurate profiling of water depth by transmitting pulses coherent laser light beneath an aircraft such that a strong reflected return is recorded from the water surface, followed closely by weaker return from the bottom of the water. The depth of the water is determined from the time delay of the pulse. Another applications are related with measuring heights within timber, estimating forest biomass, laser induced fluorescence properties of earth surface features, etc. Because different materials tend to fluoresce at different wavelengths, it can be used to discriminate among material types. A single channel laser source to illuminate objects and multichannel receivers to record the spectral characteristics of the resulting fluorescence, emitted by the objects have been used operationally to detect oil slicks and other water pollutants and to map the chlorophyll concentration. In atmospheric remote sensing lasers have found application to measure profiles of atmospheric constituents (water vapour, aerosols, gas constituents).

IX.2.3 Earth Observing System /EOS Era

The Earth Observing System is designed for multidisciplinary study of Earth's critical, interrelated processes, involving the atmosphere, oceans, land surface, polar regions and solid Earth and dynamic and energetic interactions among them. The most important units, planned for monitoring of hydrological, biogeochemical, climatological, ecological and geophysical processes of marine, ocean and coastal regions are :

EOS-COLOUR satellite

The EOS Ocean Colour Instrument (EOS-COLOUR) is a follow-on to the Nimbus7 CZCS and SeaWiFS. It was planned for launch 1998 and is designed primarily to measure marine phytoplankton, aerosol and global carbon cycle.

EOS-AM satellite (Terra)

The main objectives are: Clouds, aerosols and radiation balance, characterisation of the terrestrial ecosystems, land use, soils, terrestrial energy and moisture, troposphere chemical compositions, contribution of ocean to climate and primary productivity. It is including the following instruments and missions:

MISR

The Multi Angle Imaging Spectro Radiometer (MISR) will make images at nine fixed angles in the along track direction: one in nadir and four in both for and after up to 70.5^{o} at four spectral bands: 0.443, 0.555, 0.67, 0.865 µm with ground resolution selectable between 240 m and 1.92 km. It will be used for clouds, aerosols and radiation budget studies over land and ocean.

MODIS

The Moderate Resolution Imaging Spectrometer (MODIS) is designed with 0.25-1 km resolution in 36 visible and infrared bands. The orbit and scan geometry are such that each point on the Earth will be sensed every day, or two. It could find various applications in land, ocean and atmospheric processes study.

MOPIT

Measurements of Pollution in the Troposphere (MOPIT) is a four-channel gas-correlated spectrometer for measuring profiles of CO concentration and column integrated CH_4 amounts.

ASTER

The Advanced Space-borne Thermal Emission and reflection Radiometer (ASTER) will have 14 channels between 0.5 and 12 µm and spatial resolution between 15 and 90 m. ASTER will have after pointing channel, which will allow stereo images.

EOS-PM satellite (Aqua)

The main objectives are: cloud formation, precipitation and radiative properties, air-sea fluxes of energy and moisture and sea-ice extent, including the following main instruments:

AIRS

The Atmospheric Infrared Sounder (AIRS) is a high resolution sounder, which will measure radiance in 2300 spectral channels between 0.4 and 15.4 µm and is designed for atmospheric sounding.

MIMR

The Multifrequency Imaging Microwave Radiometer (MIMR) will sense horizontally and vertically polarised radiation at six frequencies between 6.8 and 90 GHz in a 50^{o} conical scan with ground resolution from 5 to 60 km.

EOS-ALT satellite (ICESAT)

The main objectives are ocean circulation and ice sheet mass balance, including the following main instruments.

DORIS, SSALT, TMR

The Solid State Altimeter (SSALT) is a radar altimeter that measures ocean wave height, wind speed and ocean surface current velocity, topography of the sea surface and polar ice sheets. Doppler Orbitography and Radiopositioning Integrated by Satellite (DORIS) precisely locates the satellite in orbit to correct the SSALT measurements. TOPEX Microwave Radiometer (TMR) provides atmospheric water vapour measurements for SSALT corrections.

GLAS

The Geoscience Laser Altimeter System (GLAS) will measure the clouds height, vertical aerosol structure, land and water topography.

EOS-CHEM (Aura)

The main objectives of EOS Chemistry mission are atmospheric chemical composition, chemistry-climate interactions, air-sea exchange of chemicals and energy and is planned for launch 2002, including the following main instruments:

HIRDLS

The High Resolution Dynamic Limb Sounder (HIRDLS) measures gas and aerosol concentration in the upper troposphere, stratosphere and mesosphere in the spectral range 6-18 μm

MLS

Microwave Limb Sounder (MLS) will measure temperature, moisture and variety of gases in the stratosphere, including chlorine compounds.

ADEOS II

Advanced Earth Observing Satellite IIa and IIb is Japan satellite, whose main objectives are: measurements of visible, near-infrared and microwave radiance, infrared and laser atmospheric sounding, troposphere and stratospheric atmospheric chemistry, altimetry and scatterometry.

NSCAT

The NASA Scatterometer II (NSCAT) flew on Japan's on ADEOS II to measure ocean surface wind speed under all weather conditions.

ENVISAT

The main objectives are : environmental study of atmospheric chemistry, marine biology and land surface processes, including the following instruments :

AATSR - Advanced Along Track Scanning Radiometer
ASAR - Advanced Synthetic Aperture Radar
GOMOS - Global Ozone Monitoring by Occultation of Stars
IASI - Infrared Atmospheric Sounding Interferometer
IRTS - Infrared Temperature Saunder
MERIS - Medium Resolution Imaging Spectrometer
MIPAS - Michelson Interferometer for Passive Atmospheric Sounding
MWS (ATSRM) - Microwave Sounder
RA-2 - Radar Altimeter-2
SCHIAMACHY - Scanning Imaging Absorption Spectrometer for Atmospheric Cartography

IX.2.4 Airborne Remote Sensing Systems

The airborne systems could find application for remote sensing of phenomenon, or processes in the coastal region and lower troposphere, requiring higher spatial and spectral resolution and time of measurements not available through satellite observations, or for testing new scientific equipment for satellite remote sensing, based on new technical and physical principles. The airborne systems are passive : cameras (film and digital), scanners, imaging spectrometers, microwave radiometers, and active: SAR, radar, altimeters, scatterometers, lidars. In Table (IX.11) are given specifications of some of the airborne imaging spectrometers.

Table (IX.11): Specifications of some airborne imaging spectrometers

Name	Spec. band	Spec. range (nm)	Band width (nm)	Quant. (bits)	FOV (degr.)	IFOV (mrad)
AVIRIS	224	400-2450	9.4-16	10	30	1
DAIS-7915	80	400-12300	10-2000	15	39	3.3
VTVHSI	1024	300-1100	0.6		35	0.6
VIFIS	576	420-1060	10-14	8/12	48	0.3
CASI	288	400-1000	2.2	12	37.8-44.7	0.069

Synergistic use of airborne measurements from imaging spectrometers, as CASI-Canada, AVIRIS-NASA, DAIS 7915 developed under ERSEC programme, or high spectral resolution spectrometer as VTVHSI, with satellite observations particular in polluted coastal regions will help for calibration and validation of satellite information, considering the chemical, physical and biological processes in the atmospheric and coastal zone waters.

The main disadvantage is that for aircraft survey could be used much time and costs, than the cost for satellite data. Some specific applications of airborne remote sensing concerning marine application are given in Table (IX.12)

IX.2.5 Ground Based System

Ground (ship) based measurements are applicable mostly for regions with high pollution, or with extreme environmental changes, allowing to receive an information for atmospheric or marine properties in depth (optical thickness, gas concentration, etc.), important for physical, chemical or biological modelling, calibration of high spectral resolution satellite measurements, atmospheric corrections, etc.

Side-scan Sonar

Imaging sonar is similar to radar imagery. It is used for survey varying water depths (from 15 m to 1.000 m) below and behind the survey vessel, and can have adjustable swath widths. Unlike radar, this system uses acoustic waves instead of microwaves. Overlapping swaths are required for properly mosaic and stereo images. Sonar imagery can be used at any depth and can locate not only ship wrecks but has been used to map slop stability for oil platforms, pipeline and cable routes, etc.

For oceanography, marine and coastal zone environment monitoring is required synergistic use of different remotely sensed measurements.

Table (IX.12) : Application of various airborne remote sensing systems for sea current, water quality and sediment transport analysis

Airborne Remote Sensing Techniques							
Natural Formations	**Spectral Range Sensors**						
	UV Visible	**Visible NIR**	**Scanners**				
			Visible NIR	**TIR**	**Passive Microwave**	**Active Sensors**	
						Radars	**Lidar**
Water Classes							
Case 1, clean water		G	G	L	L		L
Case 2, coastal zone	L	G	G	S	L	L	L
Suspended materials Water quality :							
chlorophyll		G	G	G			G
sediments		G	G	S			S
yellow substances		S	S	L			S
pollutants		S	G	S	S	G	G
Currents							
wind waves/currents		G	S		S	G	
Tides		S	S	L	L	S	
Rivers		G	G	S	S	S	S
G = very useful		S = useful			L = limited useful		

IX.3. PROCESSING AND ANALYSES OF REMOTELY SENSED DATA

The processing of satellite images is based on two main steps: pre-processing and retrieval of geophysical parameters.

IX.3.1 Pre-processing

Processing of the raw satellite data includes calibration, geometric corrections, enhancement, smoothing, multispectral statistical analyses and classification.

IX.3.1.1 Calibration

The calibration methods are based on:

- Reference target with known radiance or reflectance
- Second calibrated instrument

The basic approaches, used for absolute calibration of sensors are shown on Figure (IX.2). The first approach is based on use of a reference target with known radiance or reflectance (black body, stars, sun, surface target). The second approach is based on simultaneous measurements of emitted or reflected radiance from a reference target, using ground based, airborne, or satellite absolute calibrated instrument.

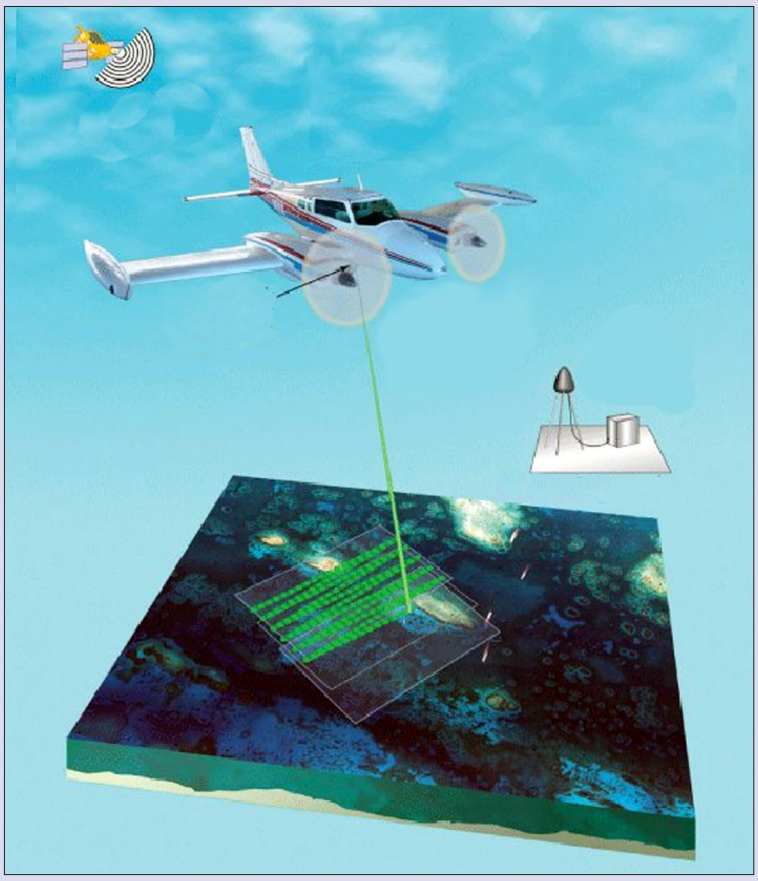


Figure (IX.2) : Calibration methodologies

IX.3.1.2. Image Analyses

Satellite imagery could be photo (such as photograph or laser print copy), or digital (stored on electronic, magnetic, or optical media) images. Depending on the spectral interval in which the radiation is measured by satellite sensor the images are visible, infrared, or microwave.

The purpose of image analyses is to relate significant features in the image to physical processes, or variables in the atmosphere, or on the surface.

Some atmospheric, land, or marine variables are easily identified by imagery from more than one spectral range. Then the **multispectral analyses** is applied. For example low level stratus is often difficult to identify on an infrared image because the cloud may have a radiative temperature that is near to that of the surface. On a visible image, stratus clouds appear bright in contrast to the darker background of land or water. But the detection of thin cirrus clouds can be difficult on a visible image because they are almost transparent to visible light. These clouds are cold and mostly opaque to infrared radiation from the warmer surface below and thus present a cold signal on the infrared image. Deep convection, or thick cirrus is both cold and optically thick and therefore is bright in either a visible or an infrared image.

For land, marine and cloud study colour composition of images in different spectral regions is used with, obtained in March 1997 and on Figure (IX.3a) is a colour composition of MODIS channels (CH7, CH2 and CH1). In Figure (IX.3b) showing the same region with different thermal bands composition of MODIS channels (CH20, CH6 and CH7)

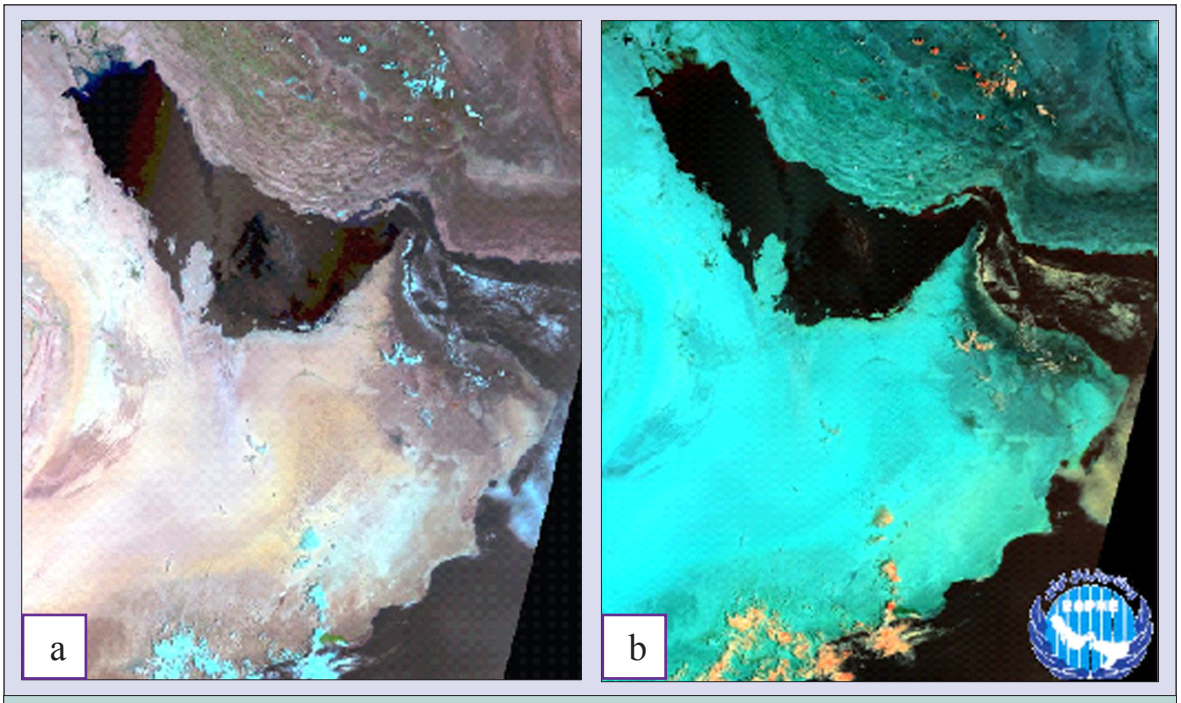


Figure (IX.3) : Different colour composition of MODIS data over RSA, March 1997 with false colour composition (7,2,1) (a) and (20,6,7) (b) © ROPME

IX.3.1.3. Image Enhancement

The image enhancement involves highlighting certain values or regions within an image to emphasise and identify atmospheric, marine, or land surface parameters, or features. There are three primary methods of enhancement: colour enhancement, physical enhancement and statistical enhancement. Colour enhancement involves the assignment of different colours to each pixel of the image based on the value of the pixel, applying either a look up table, or generation of (red-green-blue) RGB image, using multi-spectral data.

Two other common types of physical enhancement are: looping and three dimensional image display. Both require specialised hardware, or software. Looping of satellite imagery is used for identifying features on the image, for graphically displaying the cycle of synoptic and mesoscale meteorological features. Three dimensional display is used for topographical, coastal zone clouds, or land surface purposes.

IX.3.1.4. Classification

Different methods for classification of land cover, clouds, or marine constituents have been applied. Wide application for land cover has found statistical methods Maximum Likelihood (ML) classification, Maximum Distance (MD), or Neural Network (NN) (Parlow, 1995). The bases of the statistical algorithms is the assumption that pixel with similar spectral statistics belong to the same landuse class. The NN is applied especially in those fields, where the mathematical modelling is difficult and the application of statistical algorithms requires some simplifying hypothesis. If there are topography controlled illumination effects, the digital elevation models are required to improve the accuracy of classification. Supervised, or unsupervised classification are applied, depending on a-priory information for training set. High resolution spectral measurements from TM and SPOT have found wide applications in land cover, crop and forest monitoring. On Figure (IX.4) is an image of TM land cover classification of Ontario during the period 1989-1991. Nine classes of trees are discriminated on the studied area, using ML classifier.

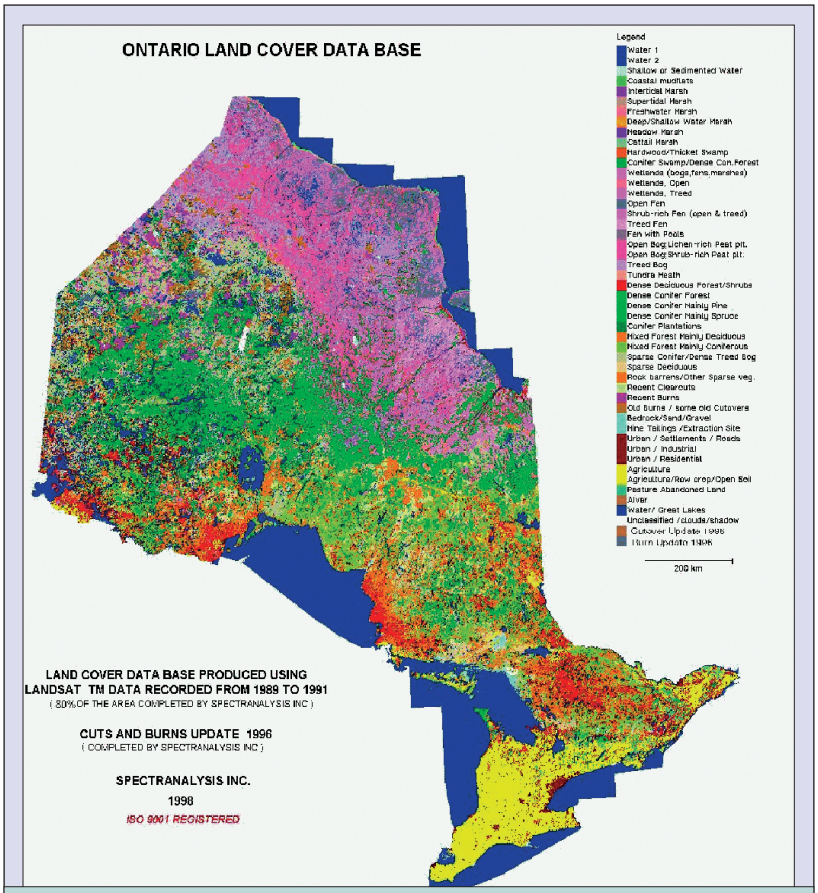


Figure (IX.4) : TM land cover classification of Ontario during the period 1989-1991

The next step of processing remotely sensed data is physical retrieval of atmospheric and surface variables and processes and their application in marine, atmospheric, land surface environment and climate research. The basic principles and methods for retrieval geophysical variables and processes by remotely sensed measurements depend on the spectral range, viewing direction and resolution of the satellite system.

IX.3.2 Retrieval of Geophysical Variables

Currently flying satellite missions and the planned ones allow to control a large number of surface and atmospheric variables and processes. The most important coastal zone, marine and atmospheric variables, affecting environmental and climate change and retrieved from remotely sensed measurements are:

- coastal zone (topography, erosion, land surface temperature albedo, NDVI, etc.)
- marine/ocean (currents, temperature, water quality (sediments, bio-optical properties, chlorophyll), pollution (oil), near-shore bottom topography, etc.)
- atmosphere (aerosols, gases, meteorological variables (clouds, wind speed, water content, precipitation), etc.)

The coastal zone, marine and atmospheric variables interact with global climate system (Raval, 1989; Chess, 1990; Mantoura, 1991) through atmospheric aerosol, greenhouse impact, cloud radiative influence and turbulent heat exchange. On other hand the global heating, or cooling could lead to regional weather, or climatic changes, affecting the following processes of interaction in the coastal zone:

- land sea interaction (tidal currents, the flows and circulation, sedimentation patterns, sand waves, beach erosion processes, the distribution of the suspended solids, mixing and dispersion processes)
- land-atmospheric interaction (release of anthropogenic gases, acting as catalysts for ozone destruction, greenhouse gases and aerosols and their impact on the radiative and chemical processes in the atmosphere, sensible and latent heat fluxes, etc.)
- atmosphere-sea interaction (through precipitation and heat fluxes, influenced from clouds and atmospheric constituents - gases, aerosols)
- sea-atmosphere interaction through turbulent heat exchange. The sensible and latent heat fluxes, arising from the surface regulate the regional changes of the boundary conditions, affecting weather and climate system

The accuracy of the retrieved surface variables (temperature, phytoplankton, etc.) and processes depends on the used technique for retrieval, spectral range, distribution and amount of atmospheric constituents and processes. The presence of clouds, variations of water vapour, gas constituents and aerosols affects surface variables (temperature, albedo) and processes (turbulent heat exchange on the surface, primary production and evaporation from the surface). To evaluate the key atmospheric and surface variables, their interaction, feedback and impact on environmental changes and climate, simultaneous information for atmospheric and surface variables and processes is required, which is possible to obtain in global scale from satellite observations.

Different techniques have been developed for retrieval atmospheric and surface variables from remotely sensed data. The main approaches are classified as: physical, statistical and hybrid.

The physical retrieval (Chahine, 1970) is applied usually for retrieval vertical profiles of atmospheric temperature and constituents (water vapour, gas) and Earth surface variables (albedo, temperature). The physical retrieval of atmospheric constituents is based on the following iterative process: i) a first guess temperature profile is chosen; ii) the weighting functions are calculated; iii) the forward problem is solved to estimate the radiance in each channel of the radiometer; iv) if the computed radiance match the observed radiance, within the noise level of the radiometer, the current profile is accepted as a solution; v) if convergence has not been achieved, steps ii to v are repeated until a solution is found.

In statistical retrievals of atmospheric variables (Smith and Woolf, 1972) a training data set (from nearly coincident in time and space radiosonde data) are used to calculate a statistical relationship between observed radiance and atmospheric temperatures. These relationships are then applied to other observed radiance to retrieve temperatures.

Hybrid methods are known as inverse matrix methods (Fritz *et al*., 1972). They use weighting functions like physical retrievals, but do not involve integration of the radiative transfer equation and do not require large training data set and the main advantage is that they are easier to put into operation than the physical or statistical retrievals. The retrieval technique depends also on the type of the remotely sensed measurements: passive, or active. There are two basic types of passive atmospheric sounding: **vertical sounding**, (in which the sounding instrument senses radiation coming from the atmosphere and the Earth's surface) and limb sounding. The active atmospheric sounding includes the use of lidar and radar. The vertical sounding theory starts from the integrated form of Schwarzschild's equation (Kidder, 1995) and has found application in retrieval vertical profiles of atmospheric temperatures, trace-gas concentrations, especially these of water vapour and ozone. Physical retrieval of surface variables is based on solving the radiative transfer equation in the spectral range of remotely sensed data. Visible, thermal infrared and microwave spectral ranges are the most frequently used in satellite remote sensing.

Visible and near infrared spectral range

The main processes, affecting the remotely sensed spectral radiation 1 (λ,Θ,z) reflected from the surface in VIS and NIR spectral range are scattering by molecules and aerosols (Van de Hulst, 1957; Ishimaro, 1978) and absorption by gas constituents and aerosol particles in the atmosphere (Handbook of Geophysics, 1960, Goody, 1964). Absorption is due to O_2, mixed gases, NO_x, and water vapour. For the spectral interval of 200-1000 nm the absorption is due to the permanent gases O_2 (210-250 nm, 760 nm) and O_3 (200-300 nm, 312, 332 nm and 450-740 nm), SO_2 (250 nm-320nm), where the O_2 is uniformly mixed in the atmosphere. There are weak absorption lines of CO_2 at 0.78-1 μm and NO_2 (for $\lambda<350$ nm, $\lambda>400$ nm (445, 448, 488 nm) (McEsvan, 1975; Rasool, 1973; Valley, 1985). The impact of O_3, H_2O, O_2 and molecular scattering on vertical transmission of the atmosphere in spectral range 0.25-1 mm is shown on Figure (IX.5). Rayleigh scattering by air molecules is the chief limitation to the transfer of ultraviolet and visible radiation through the clear atmosphere.

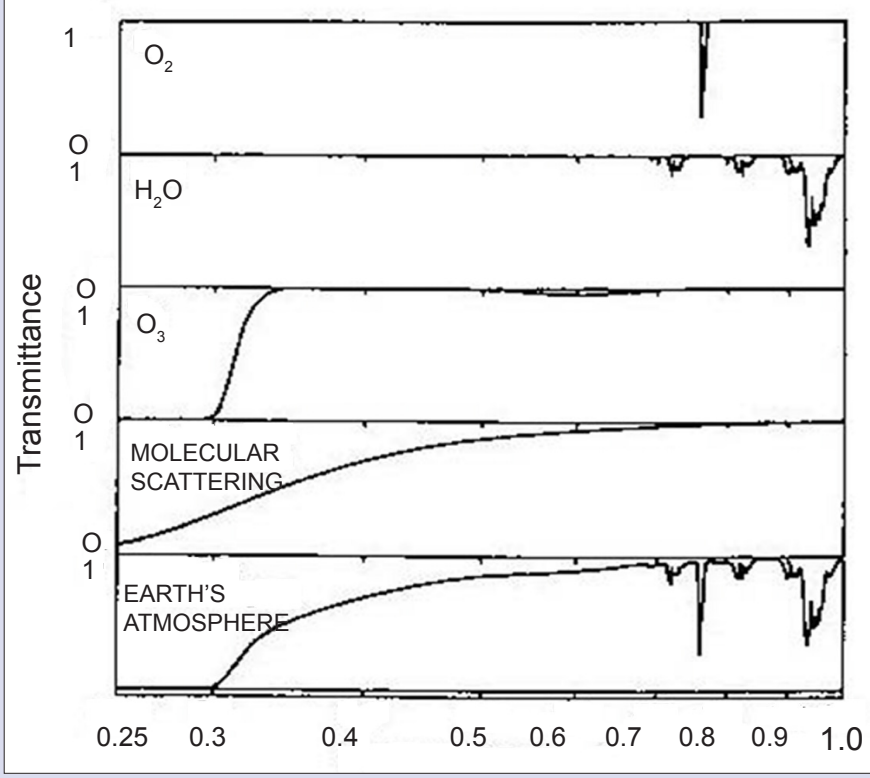


Figure (IX.5) : Vertical transmittance of the earth's atmosphere in spectral range 0.25-1 μm, calculated by Lowtran 6 for : O_2, H_2O, O_3; molecular scattering and total, not considering the impact of aerosols

The distribution of solar radiance at the Earth's surface, considering the scattering by molecules, aerosols and absorption of gas constituents is shown in Figure (IX.6).

The radiance in visible (VS) and near infrared spectral range (NIR), which reaches the Satellite sensor consists of four parts: 1) target reflected diffuse radiance; 2) direct sunlight reflected by the target; 3) molecular and aerosol particles back scattered sunlight; 4) a contribution from "background" outside the target (Vermote, 1995). If we denote $\rho(\lambda)$, $\rho_a(\lambda)$, $\rho_o(\lambda)$, $\rho_e(\lambda)$ - respectively planetary , atmospheric, surface and environmental (surrounding the observed target) spectral reflectance, φ-sensor view angle, φ_s - solar zenith angle, $\mu=\cos\varphi$, $\mu_s=\cos\varphi_s$, the spectral albedo, $\rho(\lambda)$ observed on the top of the atmosphere is defined by:

Equation IX.1 :

$$\rho(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s) = t_g(\lambda, \varphi_s, \Phi_s)\, t_g(\lambda, \varphi, \Phi) \{\rho_a(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s) + t(\lambda, \varphi, \varphi_s)\, [\rho_O(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s) e^{-t/\mu} + \rho_\varepsilon(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s)\, t_d(\lambda, \varphi, \Phi_v)]\} \; 1-\rho_e(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s)\; S(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s)$$

where $t(\lambda, z, \Phi_s)$ is the total transmission, given as a sum of direct $e^{-t/\mu}$ and diffuse $td(\lambda, \varphi, \Phi_v)$ transmission and $S(\lambda, \varphi, \varphi_s, \Phi_v, \Phi_s)$ is the spherical albedo of the atmosphere. From Equation IX.1,

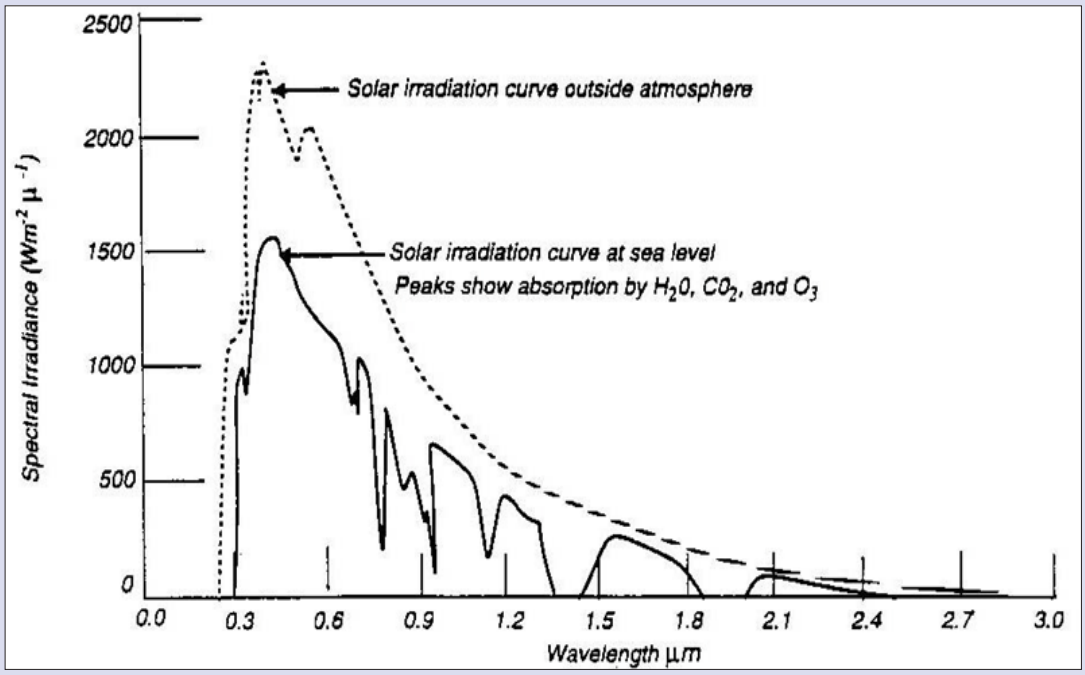


Figure (IX.6) : Solar spectral irradiance at the Earth's surface

Modified from Chahine *et al*., 1983, Erdas

the bi-directional spectral surface albedo ρ_o (λ, φ, φ_s, Φ_v, Φ_s) is possible to calculate. For marine environment, or homogeneous land surface (desert), or for very large pixels $\rho_o = \rho_\varepsilon$ could be considered. The atmospheric and surface variables in Equation IX.1 is possible to calculate either for known distribution of atmospheric constituents, or using standard atmospheric model and applying MODTRAN, or 6s radiative code (Vermote, 1995). The problem is that the optical properties, amount and distribution of atmospheric aerosols and gas constituents are usually unknown and different methods have been applied to retrieve the aerosol optical properties over water (Gordon, 1978, 1980, 1990), land (Igbal, 1983; Saunders, 1990), or the amount of gas constituents, using ground based multispectral measurements, or satellite observations (Prigent, 1994; Smith *et al*., 1989). The bi-directional surface albedo is indicator of the crop state, forest type and inventory, soil moisture, hydrological modelling, geological study of rocks and minerals (Parlow, 1995), marine bio-optical properties (pollution, chlorophyll and sediments) (Morel, 1989). The reflectance spectra of some natural formations are shown on Figure (IX.7). Combining classification of atmospheric corrected spectral surface albedo, retrieved from satellite data (SPOT, TM, AVHRR, ATSR) with digital elevation model the obtained land classification maps have fount different applications in agriculture, forest, geology, coastal zone monitoring (Hilton, 1993; Askne, 1995). For turbulent heat exchange, NWP and GCM is important the broad band surface albedo, which is possible to calculate from bi-directional spectral surface albedo, retrieved from satellite spectral measurements (AVHRR, ATSR, TM, SPOT) in visible and near infrared spectral range (Saunders, 1990). The methods for retrieval atmospheric constituents and surface albedo depend on the used spectral ranges, viewing direction and underlying surface.

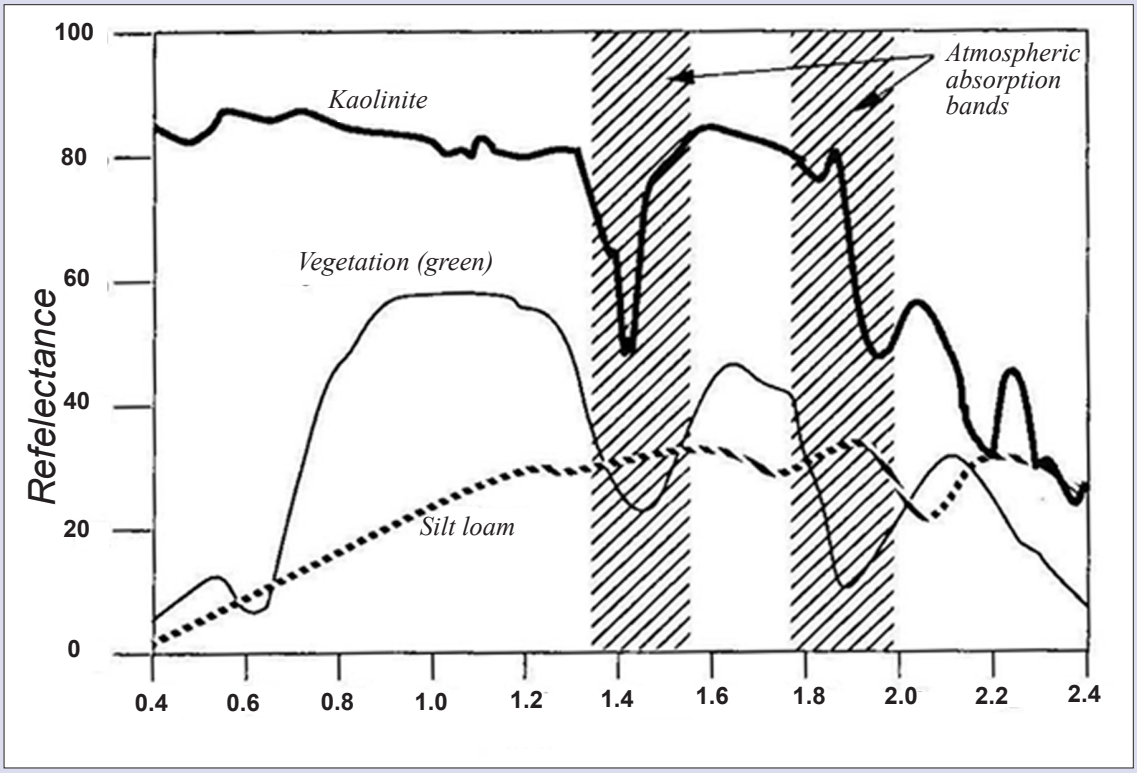


Figure (IX.7) : Reflectance spectra of natural formations

Thermal infrared

For a cloud - free atmosphere the radiance measured at the top of the atmosphere, $I_H(\chi, \varphi, T_\lambda)$, in spectral range χ and zenith observation angle Φ is the sum of three terms (Becker, 1995; Djepa, 1998): 1) the radiation emitted from surface, $I_o\uparrow(\chi, \varphi, T_0)$, attenuated by the atmosphere; 2) the down welling radiation, $I_a\downarrow(\chi, \varphi, T_a)$, emitted by the atmosphere, reflected from the Earth surface towards the sensor and attenuated in the atmosphere; 3) the up welling radiation, $I_a\uparrow(\chi, \varphi, T_o)$, emitted by the atmosphere towards the sensor:

Equation (IX.2.):

$$I_H(\chi, \varphi, T_\lambda) = I_o\uparrow(\chi, \varphi, T_{0\lambda\varphi})\, t(\chi, \varphi) + I_a\downarrow(\chi, \varphi, T_a)\, [1- \varepsilon\, (\chi, \varphi)]\, t(\chi, \varphi) + I_a\uparrow(\chi, \varphi, T_a)$$

where $t(\chi, \varphi)$ is the transmission function of the atmosphere, $\varepsilon(\chi, \varphi)$ is the surface emissivity, $T_{0\lambda\varphi}$ is the directional radiometric surface temperature, which depends on the wavelength χ and viewing angle, T_a is the atmospheric temperature. For marine and land surface processes study are used usually spectral intervals in atmospheric windows, free from gas absorption lines. The upward and downward spectral atmosphere radiance $I_a\uparrow(\chi, \varphi, T_a)$, $I_a\downarrow(\chi, \varphi, T_a)$, and transmission function, $t(\chi, \varphi)$ is possible to calculate , using MODTRAN radiative transfer code (Djepa, 1998) for given standard atmospheric model and from Equation IX.2. to estimate the atmospheric corrected radiance, $I_o\uparrow(\chi, \varphi, T_{0\lambda\varphi})$, emitted from the surface with emissivity $\varepsilon\, (\chi, \varphi)$. The surface radiometric temperature is calculated, inverting the Planck function for given spectral surface emissivity. The problem is

that spectral surface emissivity is usually unknown and for water it depends on the spectral range, viewing angle, pollution (oil), wind speed and over land the variations of the surface emissivity are greater (Rubio, 1997). The land surface spectral emissivity depends on the viewing angle, heterogeneity of and physical properties of the surface constituents, (canopy, soil, rocks, etc.). For n spectral ranges and single viewing angle the task is underestimated, there are n+1 unknowns, n spectral emissivities and a surface temperature. Different approaches for retrieval spectral surface emissivity and temperature from multi-spectral satellite observations have been applied (Becker, 1995; Deschamps, 1980 and 1983), usually with limited, or regional application. The multiangle viewing systems (currently ATSR-2/ERS2 and future AATSR/ENVISAT and PRISM) allow from directional spectral satellite observations, corrected with atmospheric effects, to estimate the spectral emissivity and aerodynamic temperature of different (canopy, soil) components of heterogeneous land surface (Djepa, 1998). The retrieval of surface radiometric temperature becomes more complicated when the used spectral intervals are affected from gas absorption. Then for accurate atmospheric correction is required a-priory information for amount and vertical distribution of temperature and atmospheric constituents absorbing in the used spectral range. The EOS , planned for marine, coastal and land surface monitoring are performing usually in atmospheric windows (3.5-4 μm, 8-9.3 μm, 10-12 μm), which are slightly affected by water vapour, or ozone absorption.

Thermal emission in the microwave spectral range

In the microwave spectral range the spectral emission $I_H(v)$ at height H over the Earth's surface, and spectral frequency v is a sum of three components (Tsang, 1985; Jansen, 1993; Ulaby, 1981 and 1982): the upward atmospheric emission, the Earth's surface emission, and the downward atmospheric emission, reflected from the Earth's surface and attenuated in the atmospheric layer. From 1 to 300 GHz the absorption by atmospheric gases is dominated by water vapour lines at 22 GHz and 183 GHz, oxygen lines near 60 GHz and at 118 GHz and relatively narrow and weaker ozone lines above 100 GHz (Ulaby, 1981 and 1982; Liebe, 1985, 1989 and 1992; Waters, 1976). For Earth surface and marine study are used atmospheric windows, up to 20 GHz, and 30-45 GHz. The strong absorption lines of H_2O, O_2 are used for retrieval water vapour, temperature profiles and concentration of some chlorophlorocarbons. In the spectral ranges 36.5GHz and 23.8GHz (ATSR/M/ERS-1) the absorption in the atmosphere is determined mainly from oxygen, water vapour and liquid water content in clouds. The reflection coefficient R(v) depends on the zenith angle and the complex relative dielectric constant $e_{(v)}$ (Allan, 1983), which is function of land surface moisture and roughness. For water surface should be considered the impact of wind speed, roughness, oil pollution, etc. (Stogryn, 1971). Depending on the used spectral range, the satellite microwave observations are applied for estimation of atmospheric water content, cloud liquid water content, surface temperature, emissivity, wind speed, etc. The passive microwave observations are used for atmospheric constituents (water content, temperature, etc.), or surface wind speed estimation, but due to the bad spatial resolution they have not found great application for land surface temperature.

IX.4. REMOTE SENSING APPLICATIONS

The remote sensing applications in marine, coastal zone and oceanography are:

- marine atmosphere and oceanography (wind speed, clouds, precipitation, atmospheric aerosols and gas constituents)
- physical oceanography and biological processes (sea surface temperature, wind speed, topography, waves, tides, currents, algae blooms, chlorophyll concentration and primary production, ice parameters, heat fluxes)
- water quality (transport of nutrients and pollutants)
- coastal zone survey (erosion, coast and bottom topography, ship monitoring, surface temperature and vegetation state, heat fluxes)

The wide range of applications is based on information, obtained from atmospheric sounders, meteorological, or Earth observing satellites (EOS) (NOAA, 1980, ERS-1, 1991, Barach, 1993) and models. The atmospheric sounders are passive, high spectral resolution spectrometers, or radiometers (UARS, TOVS), (Carr, 1995), or active (lasers) performing in spectral ranges of gas absorption. The meteorological, polar orbiting, or geostationar satellites (METEOR, NOAA, NIMBUS 7, GOES, etc.) (Ghassem, 1989) are with on board active and passive radiometers, with low, or medium spectral and spatial resolution, but sensitive to meteorological variables, (clouds, temperature, wind speed, precipitation). The Earth observing systems include active (SAR), or passive (spectrometers, radiometers) with high (SAR, SPOT, TM), or medium (CZCS, SeaWiFs, ATSR, AVHRR) spatial resolution and are performing in atmospheric windows, sensitive to marine, or land surface variables. As the presence of clouds, aerosols, gas constituents are interfering with the reflected, or emitted radiance from the Earth, the EOS sensors could be applied also for retrieval some atmospheric variables (Olesen, 1985).

Aerosols

In coastal zone the composition of the aerosol particles varies from aluminum silicates (Al, Ca, Si and O), originating from coastal shelf (soil, rocks) to solid NaCl particles due to water evaporation over the coastal sea, or sulphate and nitrogen oxides aerosols in polluted regions. Only satellite remote sensing gives global distribution of aerosols and their optical properties. The aerosol measurements rely chiefly on the scattering of the sunlight, utilizing nadir viewing technique for tropospheric aerosols, or limb scarming for stratospheric aerosols. The dust storm shown in Figure (IX.8) was taken from ROPME MODIS station in March 2009.

Prediction of severe storms

An important application of satellite remote sensing, which has a great impact on weather forecasting is the prediction of mesoscale weather phenomena as tornadoes, thunderstorms, hailstorms, hurricanes, or typhoons. Wide application has found thermal infrared satellite observations from GOES, AVHRR/NOAA and microwave observations (at 19.35GHz) in tornadoes prediction and rainfall intensity measurements (Lo, 1991).

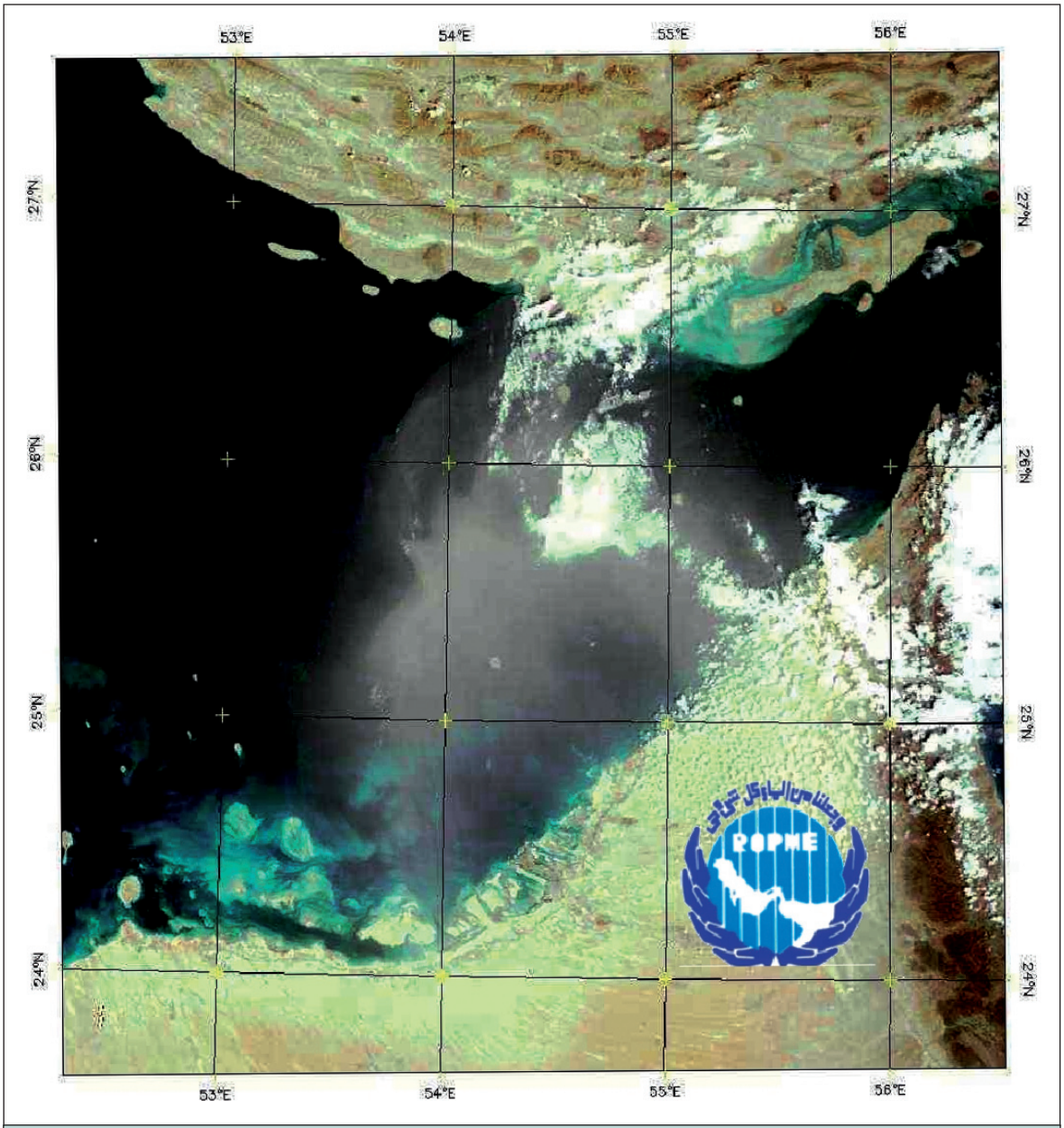


Figure (IX.8) : A dust storm in the RSA. The dust layers appear as greyish and the cloud coverage appear as white patches (Terra1 – MODIS 23 Mar 2009 10h LT – resolution 0.5 Km/pxl) © ROPME

Physical Oceanography

Marine and physical oceanography applications of satellite remote sensing could be divided on: i) circulation (both geostrophyc and wind driven), heat content of the ocean (temperature, heat fluxes) and how they are influenced by the atmosphere; ii) primary productivity and how it is influenced by the physical and chemical environment; iii) the grow and movement of the sea ice and how it is influenced by the atmosphere and ocean.

Sea Surface Temperature

Sea surface temperature is important variable for marine physical oceanography, weather and climate system. Satellite observations in atmospheric windows in microwave and infrared spectral range are used for sea surface temperature retrieval. Infrared spectral measurements are with better resolution (s<1km), but are affected by atmospheric constituents and clouds. Microwave measurements in spectral range λ>4cm are transparent for clouds, but are with very bad resolution. The retrieval of sea surface temperature in IR spectral range is based on the following approaches :

- physical, inverting the Planck function of surface radiance, obtained from satellite measured spectral radiance, corrected with the atmospheric effects
- split window, based on spectral measurements in two nearby spectral ranges in atmospheric IR window
- regression analyses, using in-situ (ship) and satellite measurements

The SST retrieved by MCSSTs scheme are called multichannel sea surface temperatures and are archived by NESDIS on a variety of space and time scales. The MCSST retrieval scheme could be in error by several Kelvins, due to impact of aerosols and water vapour. The accuracy of the retrieved sea surface temperature is possible to improve, employing radiative transfer code MODTRAN for atmospheric correction of thermal IR AVHRRR data and temperature and water vapour profile from TOVS. SSTs have been retrieved also by HIRS/2 and MSU data and by VAS data (Kidder,1995) and since 1991 SST is retrieved by ATSR/ERS1, followed by ATSR-2/ERS2. The above techniques only retrieve SSTs under cloudless conditions, utilizing thermal IR satellite spectral observations. In cloudy conditions the Scanning Multichannel Microwave Radiometer (SMMR) on board Nimbus 7 satellite is used for sea surface temperature retrieval. The sea surface temperature distribution (SST4) of RSA, retrieved from ROPME MODIS station, is shown in Figure (IX.9), taken on 30 August 2009 at 22:29 UTC.

Dynamical Oceanography

The satellite images (CZCS, SeaWiFS, LANDSAT/TM, AVHRR/NOAA, ERS1, 2, Radarsat, etc.) reveal eddies, frontal instabilities, or mixing processes (Robinson, 1991), using the plankton, or suspended sediments as a passive tracer.

Thermal infrared and radar satellite images can be used to detect surface currents. Enhanced infrared images of sea surface temperature differences delineate the warm streams in contrast to the colder shelf water, which found application for tankers and ship navigation and significant saving of fuel resources (Shnapf, 1985; Apel, 1987; Robinson, 1985). SAR data have been used to monitor coastal eddies, produced by mixing of waters of different temperatures.

Salinity

Salinity and sea surface temperature are two major factors, controlling the circulation of water in the sea. The dependence of microwave emission of sea water on temperature and salinity is used for remote sensing of salinity. Passive microwave radiometer at L band (1.43 GHz) and dual frequency microwave radiometer L band (1.43 GHz) and S band (2.65 GHz) have been used for remote sensing of the water salinity (Lo, 1991) and temperature with accuracy 0.91‰ and 0.56°C for salinity and surface temperature. LANDSAT MSS data have been used also for measure salinity of the sea,

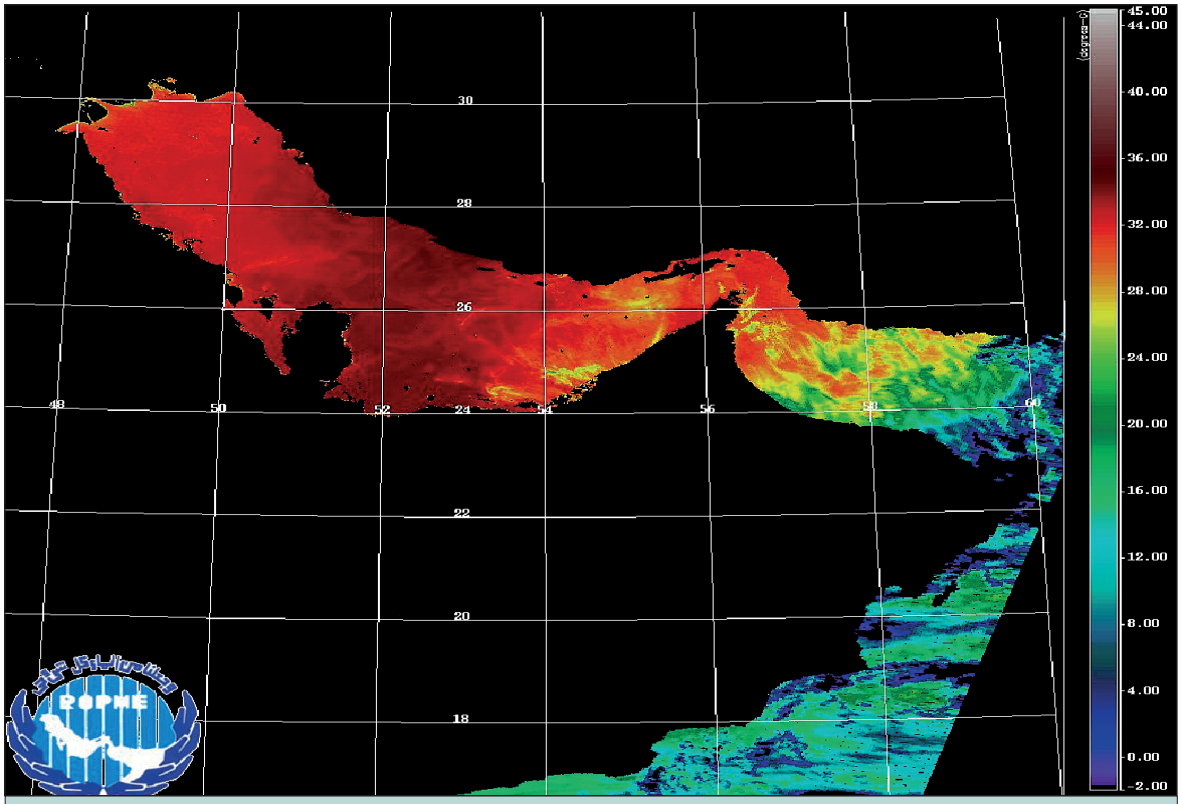


Figure (IX.9) : Sea surface temperature (SST4) in RSA, Aqua 30 August 2009 at 22:29 UTC © ROPME

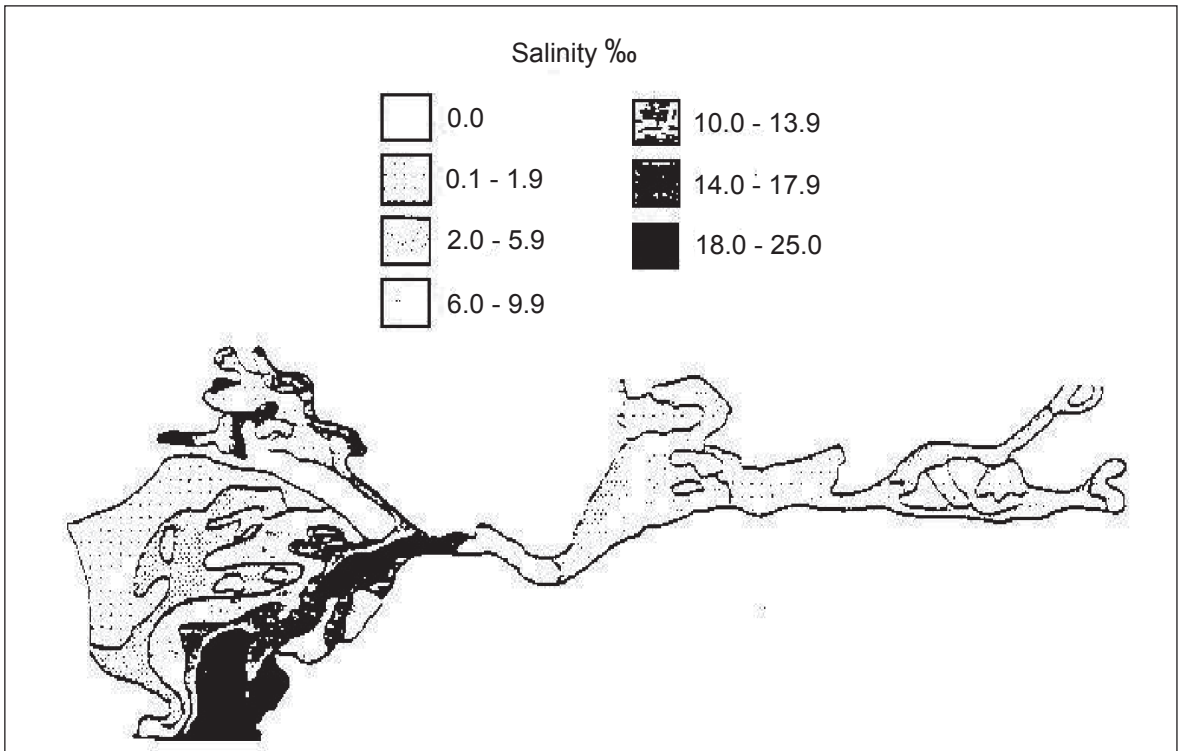


Figure (IX.10): Salinity distribution of the San Francisco Bay delta, derived from LANDSAT/MSS

using the regression models (Lo, 1991). The salinity distribution of the San Francisco Bay delta, derived from LANDSAT/MSS is shown on Figure (IX.10). It confirms the salinity distribution patterns, obtained by conventional measurements, but the accuracy of the retrieved salinity is not as high as that from passive microwave observations.

Marine Bio Optical Properties

As the ocean colour is indirectly related to the chlorophyll concentration, which is an index of phytoplankton amount, the satellite colour images (CZCS, SeaWiFS) are used for estimation of sea surface phytoplankton, suspended particles and primary production (Gordon, 1978, 1980 and 1990; Morel, 1991). Due to essential contribution of the atmosphere in visible spectral range, different algorithms have been developed for atmospheric correction of the measured radiance on the top of the atmosphere (Gordon, 1978 and 1980). Since the launch of the CZCS, the atmospheric correction of ocean colour data has relied mainly on the approach based on assumption that the water leaving reflectance in near infrared spectral rage is zero and this approach has been continued to be applied to new sensors, such as SeaWiFs.

This approach is valid in Case 1 waters, particularly in longer wavelengths of SeaWiFS, but becomes invalid in Case 2 waters, due to presence of suspended inorganic sediments, which backskatter strongly in visible and near infrared spectral range. The approach of Land and Heigh, 1995 for atmospheric correction of SeaWiFS data for Case 2 waters is based on modelling the ocean and atmosphere to predict the reflectance, observed at each band. The predictions are compared with the observed radiance and the sum over all bands of the square difference is minimized by itteratively changing the ocean and atmosphere models radiance. The water constituents, phytoplankton, represented by chlorophyll concentration, gelbstoff and suspended sediments (SS) are retrieved from the atmospheric corrected spectral water leaving radiance and are linked to primary productivity (Platt *et al*., 1991) and optical quality of the surface waters (Gordon, 1983). The phytoplankton distribution, could be linked to important environmental variables such as primary productivity, some water constituents (phosphorus content) and the optical quality of the water. An indirect relationship exist between sea surface temperature (SST) and pigment or nutrient concentrations. In upwelling situations cool water is associated usually with higher nutrient concentrations and increased phytoplankton growth, and SST can be used as a tracer for nutrient rich water. In the warm pool the main processes, which regulate the SST are: surface heat fluxes, vertical entertainment of cooler subsurface water and phytoplankton absorption of solar radiation. Following Gordon (1983), Morel (1989), the first optical depth, Dw, the depth to which the sensor ‘seas’ into the water, is possible to calculate from the sea surface chlorophyll concentration.

The interpretation of ocean colour data in terms of the rate of primary production is one of the most challenging problems in ocean remote sensing. Initial approaches to the problem were empirical and more recent work is based on explanation of physiological principles of the nature in order to find results of a more general validity (Plat, 1988).

Wide application for monitoring primary production have found CZCS (Morel, 1991; Gordon, 1990) and SeaWiFs (Land and Heigh, 1995). CZCS in Level 1 (raw data), Level 2 (processed data) and Level 3 (geophysical parameters: phytoplankton pigment concentration, diffuse attenuation coefficient, normalised water leaving radiance 440 nm, normalised water leaving radiance 520 nm, normalised water leaving radiance 550 nm, and aerosol radiance 670 nm) are available from Godard Space Flight Centre (NASA GSFC Archive). The increase of the chlorophyll content increases the spectral

attenuation coefficient, decreases the penetration in the water the radiance in visible spectral range and hence the heating rate, on the surface (Morel, 1994). Wide application for monitoring sea surface bio-optical properties (chlorophyll, sediments concentration) have found AVHRR and TM (Lo, 1991).

Phytoplankton chlorophyll concentration, retrieved from ROPME MODIS station in January 2007, is shown in Figure (IX.11).

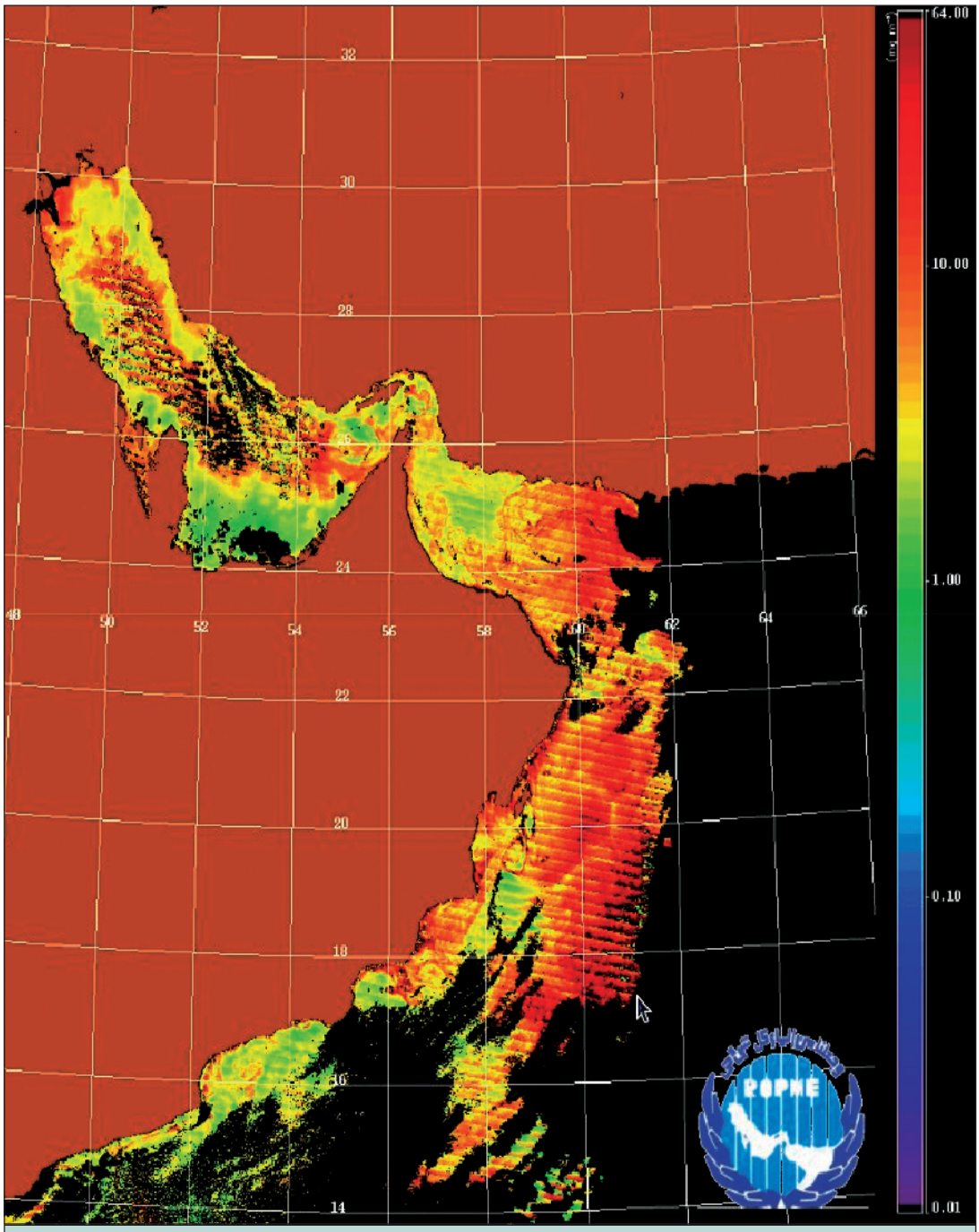


Figure (IX.11) : Chlorophyll-a concentration is presented in RSA. The high chlorophyll concentration is depicted in the northern and southern areas. The clouds are masking the map in the middle and in the South (Terra satellite at 10h LT on 29 January 2007. Resolution is 1 km/pxl) © ROPME

Detection of Fish stock

Another area of application of satellite remote sensing is the identification of productive regions and fish stock. Sea surface temperature, wind speed, salinity, phytoplankton and chlorophyll-a concentration are used as indicators for fish stock. LANDSAT have been used for menhaden and sardine distribution mapping, marine mammals have been tracked, using the Global Radionavigation System (GRS) (Lo, 1991). Using satellite information, the research vessels can be directed to places for fishing or collecting detailed vertical profile sampling, required for physical and biological parameters.

Water quality

The water quality refers not only to the chemical, but also physical and biological characteristics. The chemical characteristics include the organic and inorganic substances, such as heavy metals, pesticides and petroleum. LANDSAT images have been used for monitoring plume drift speeds and spreading. To differentiate acid waste plumes from other pollutants and clouds, principal component analyses was performed, using all four LANDSAT bands (Lo, 1991).

Oil spills

The increased contamination of oil slicks in the world's ocean and some regions as RSA, North Sea, etc. makes identification and monitoring of oil slicks important ecological task. Regular monitoring of oil slicks is necessary for optimal exploitation and management of national resources and protection of marine environment from the oil pollution. Due that the thermal infrared emittance of water is higher (during the night) than that of oil, the sea surface temperature, retrieved from AVHRR, NOAA instrument has been used for oil spills detection (Tseng, 1995). The lower emissivity of oil could cause higher surface temperatures. Thus, the temperature gradient, retrieved from daytime and night-time AVHRR data have been used as indicator of oil slicks. Figure (IX.12) shows oil spills detected by AVHRR in January 1991 in the northern part of the ROPME Sea Area (during the 1991 War) (Petrov, 1998).

A floating oil slick can lose oil both by the formation of a fine dispersion of oil droplets by solution into the sea water, microbiological degradation of crude oil, photochemical oxidation, spreading and evaporation of the oil. If the sea is rough at the time of the spill, the oil can be dispersed in very small droplets throughout a considerable depth of sea. The microbiological degradation of crude oil is an important factor contributing to its disappearance at sea after physical and chemical processes.

More detailed analyses of oil spills is possible based on satellite observations and modelling the oil spread on the marine surface, considering inertial, gravity-viscose surface tension, impact of wind speed and evaporation. While the oil slicks detection by TM satellite images requires cloudless conditions with the launch of ERS1/SAR/C (July,1990), followed by ERS-2/SAR C band, Japanese JERS-1 (launched in 1992) L band SAR and followed by RADARSAT / C band HH, the oil slicks and ship tracks is possible to detect in any weather conditions with high spatial resolution, (Parlow,1995). Figure (IX.13) shows RADARSAT SAR image of the north-western part of the ROPME Sea Area, March 2007 (ROPME Archive). The dark patches are caused by a surface oil film (Upper right side). The analyses of the potential of current and future SAR satellites for oil spills detection show that the RADARSAT Scan SAR Narrow Near Mode (Parlow, 1995) (incident angle range 20-40°, resolution 50x50m) is quite optimal for oil slicks detection and Scan Sar Narrow Far Mode (Incident angle range 32-40°, resolution 50x50m) is optimal for ship tracking.

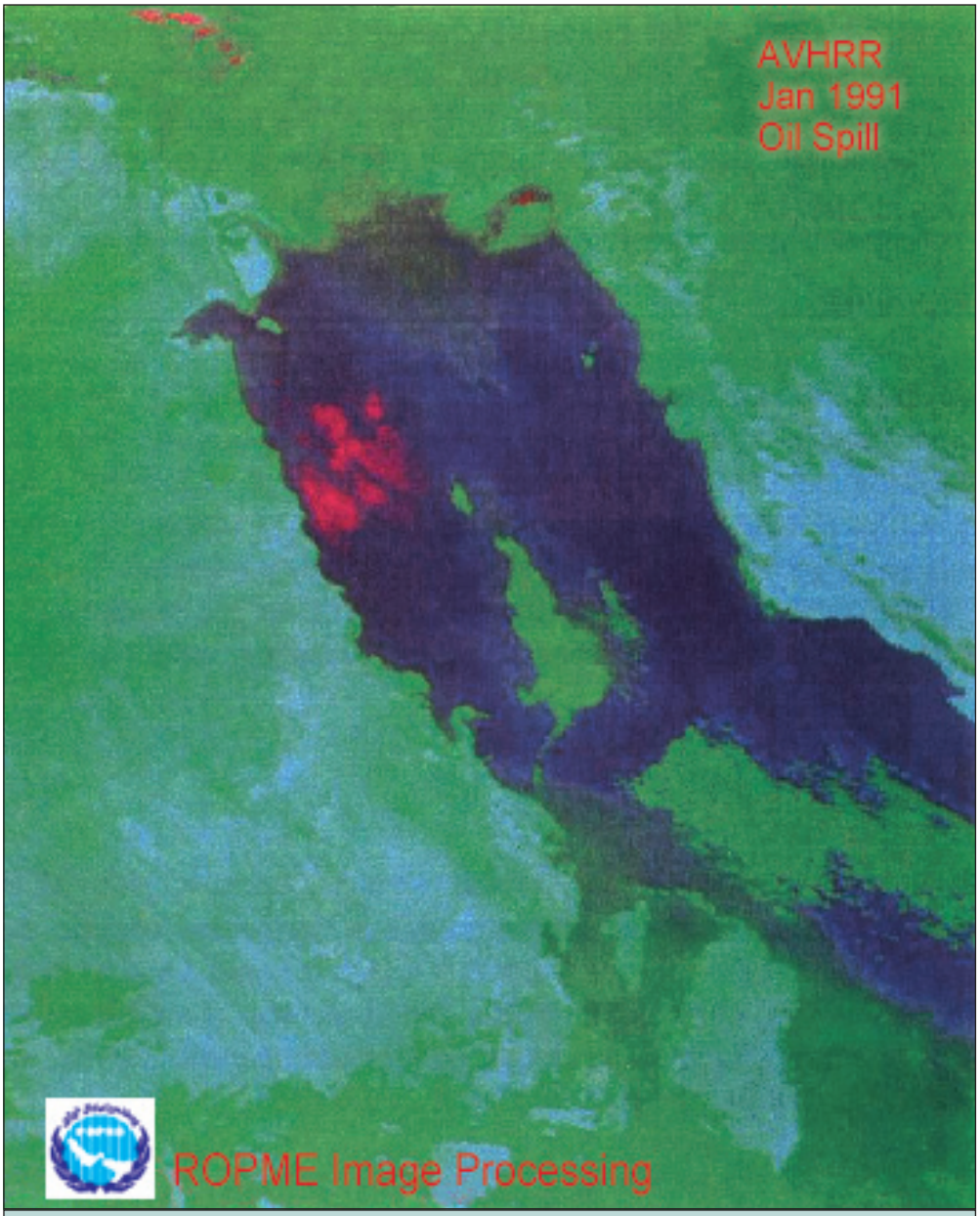


Figure (IX.12) : Massive oil spill (red dark) in the northern part of RSA during the 1991 War, January 1991 © ROPME

The following ENVISAT/SAR gives wide possibilities for oil spills monitoring. The accuracy of remote sensing of the oil spills depends also on wind speed, which requires a-priori information for wind speed from synergistic use of different satellite systems (ERS-2/SAR and Wind scatterometer and ATSR-2), or from ECMWF forecast.

Coastal and Land Surface Area

The currently flying Earth Observing Systems (EOS) (LANDSAT/TM, SPOT, XS/PM, ERS-2/ATSR-2, NOAA/AVHRR, ERS1,2, RADARSAT, etc.) allow regional and global monitoring of important variables and processes (shore coast and bottom topography, erosion, temperature, albedo, heat fluxes, etc.) in coastal area.

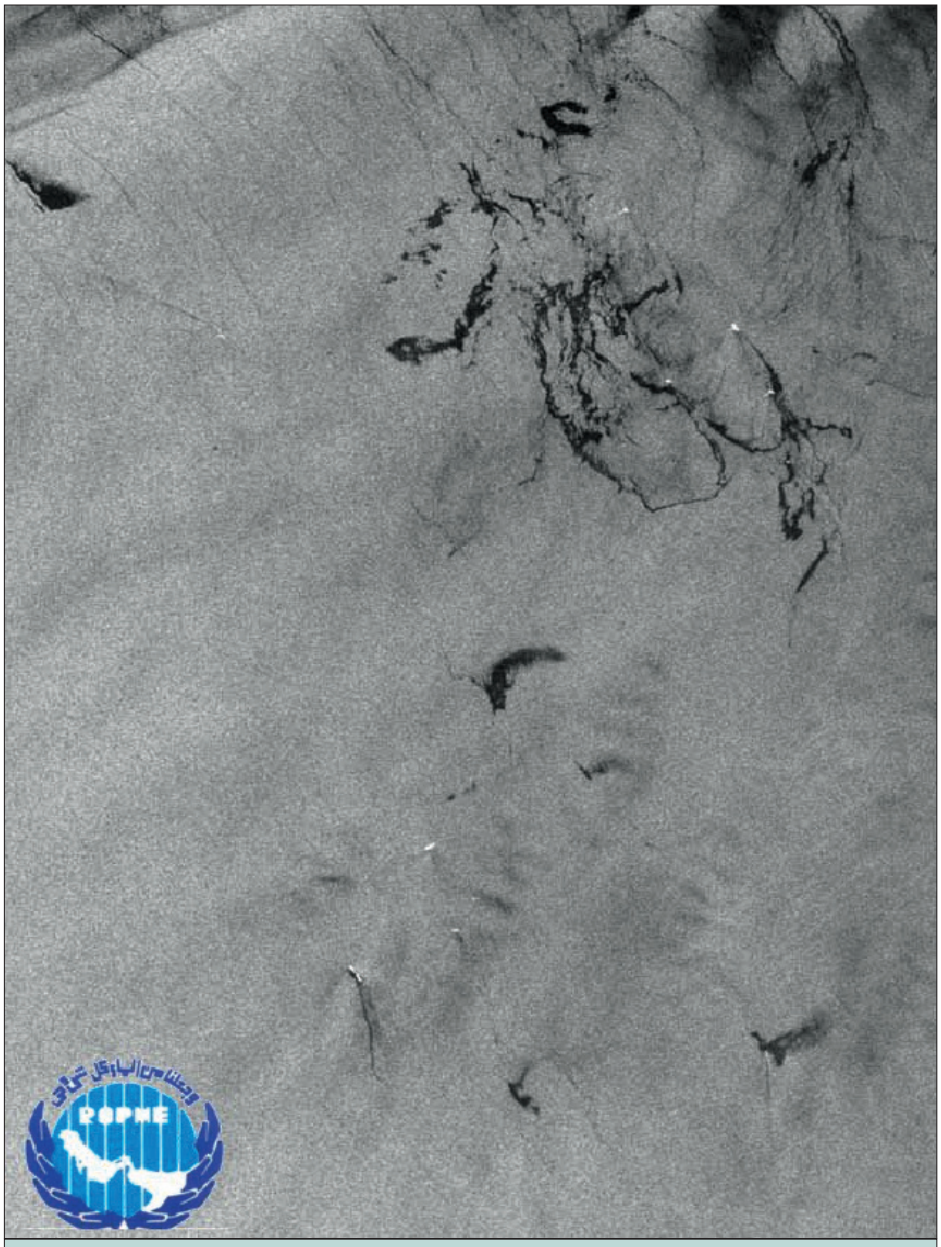


Figure (IX.13) : RADARSAT image of the northern-western part of RSA, March 2007. The black patches are caused by surface oil film © ROPME

Coastal bathymetry

Bathymetry refers to the measurement of water depth. Unlike turbidity detection, it depends on the degree of radiance energy penetration in water. Usually the bathymetry survey is done by means of shipborne echo sounding techniques. With remote sensing it is possible to determine the depth of water up to 30m. LANDSAT/MSS is generally preferred for water depth detection. A laser depth sounding is more accurate than passive remote sensing (Lo, 1991). The satellite bathymetry based on SAR remote sensing is related to shallow waters, for which the imaging mechanism is a sequence of three physical processes: interaction between tidal, currents and bottom topography produces modulations in the surface current velocity. These modulations cause variations in the wave spectrum, and the radar backscatter.

Many kinds of activities: as offshore operations, pipeline laying, platform constructions, etc. require precise information on bottom topography. This information should be up dated regularly, which is very time and money consuming.

Ship traffic monitoring

On a calm sea surface with 1-2 m/s wind speed, most ships, even small ones are detectable by SAR/ ERS, RADARSAT, but is impossible to detect a long ship in a stormy sea (15 m/s wind speed), which requires a priory information for wind speed, either from synergistic use of different satellite systems (ERS-2/Wind scatterometer, ATSR-2), or from ECMWF weather forecast (Parlow, 1995).

Coastal water turbidity and suspended sediments

The water turbidity study is important for coastal environment. LANDSAT images are ideal for mapping turbidity, or suspended sediments distribution. Band 5/TM correlates with turbidity and sediments. On the band 7 all hydrologic features are registered black, which is used to map area extend of flood. Applying supervised classification and using LANDSAT multi-spectral images, different types of sediments is possible to distinguish (Lo, 1991).

Vegetation

Wide applicability has found the vegetation index (NDVI), obtained from multispectral optical sensors /AVHRR, TM, SPOT, ATSR-2/: NDVI=(IR-R)/(IR+R), where IR and R are satellite measurements in near infrared (>0.7 μm) and red spectral range (0.6-0.7 μm). The vegetation index is related with leaf area index (LAI). LAI is a measure of amount of green vegetation, which permits crop growth and crop yield to be predicted (Lo, l991). The value of NDVI, calculated by MODIS Terra captured by ROPME MODIS station on 28 August 2009 have been calculated to show the distribution of vegetation in the ROPME Region, Figure (IX.14). Those pixels in which the NDVI has low dark tone values are considered to correspond to bared soil, water, clouds, etc.

Wide application has found LANDSAT, SPOT in inventory, monitoring and providing information for the management of renewable resources on a country, state and regional bases. The basic applications are in agriculture (irrigated lands, yield estimates of food production, multicrop inventory for estimating fertilizer, crop types, plant health and condition, based on the leaf water content, soil erosion, deforestation) forest (timber harvest, grazing, fire hazard) wetlands, urban (environmental impact of industrial development), etc. The high spatial resolution (30 m) and the seven spectral charnels in visible and infrared spectral range allow wide application of TM in crop and forest discrimination. In classification of LANDSAT/TM data the problem often occurs that during the vegetation period, different land use show similar spectral signatures. This is especially true for grassland of different use and different stages. Multitemporal TM images are used to improve the accuracy of classification, but due to cloud impact, often there are not more than one LANDSAT/TM cloud free images.

Land surface classification of LANDSAT/TM could be improved, involving Maximum-Likelihood classifier and data bases including digital terrain model and geoinformation (soil type, rain fall, temperature, topographic maps). For accurate classification, first absolute calibration, geolocation and atmospheric correction of satellite data is required. The analyses in Parlow, 1995 show that misclassification appear at higher elevation, steeper slopes and between grassland and cereals as

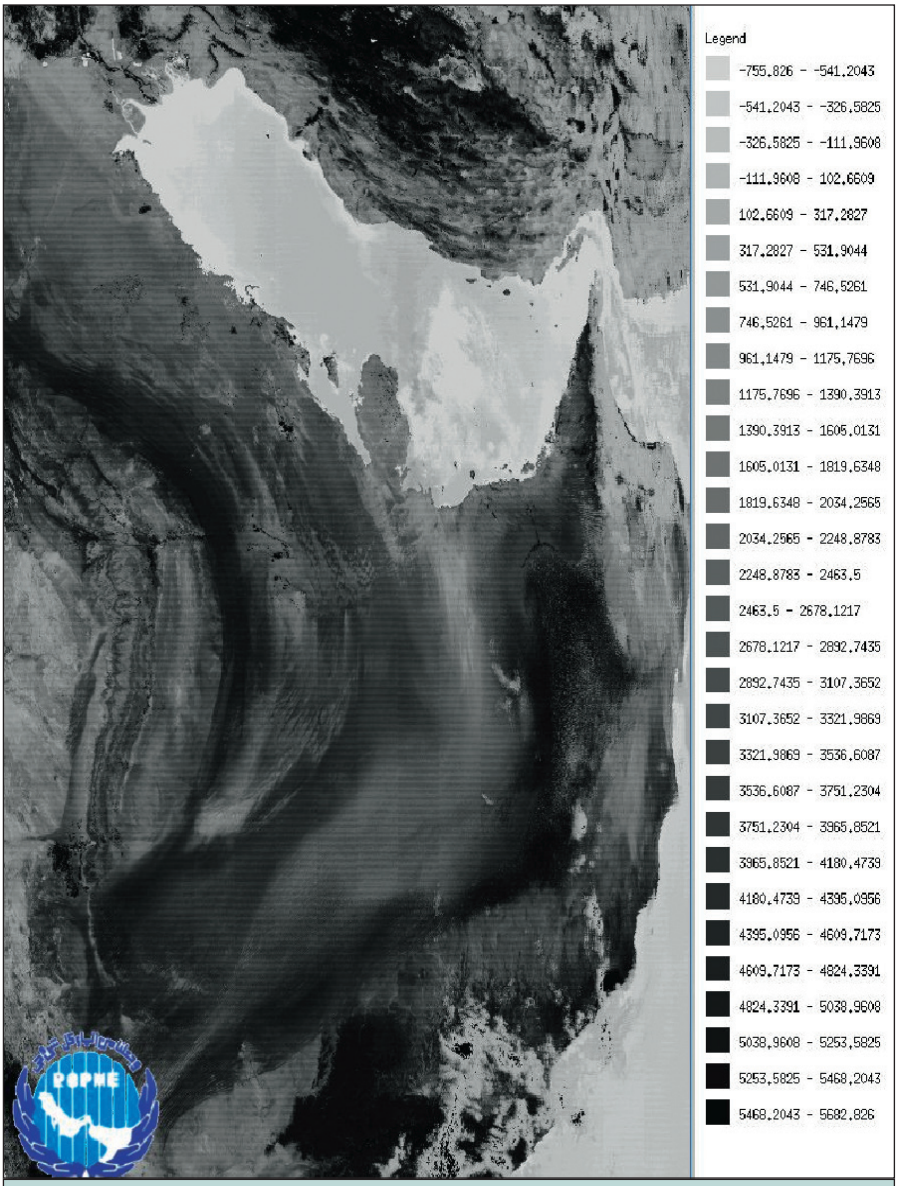


Figure (IX.14) : NDVI as shown by MODIS Terra on 28 August 2009 at 07:20 UTC with km/pxl resolution. The distribution of vegetation (the darker tone the higher the vegetation) in the ROPME Region © ROPME

well as between open soils and built-up areas, due to different land use patterns. When adding the geofactor, topographic height and slope, the misclassified fields at elevations above 700 m and steep slopes are drastically reduced. The incorporation of digital terrain model improves the classification accuracy in areas of heterogeneous terrain. Incorporating the geoinformation for soil properties improves the classification accuracy between meadows and cereals. The most important geofactors to distinguish between grasslands and cereals, open soils and build up areas are elevation, slope and soil fertility. To evaluate the applicability of ERS/SAR data into an Advance Agriculture Information System, developed into the framework of the European MARS project it was examined and compared the accuracy of SPOT-XS and ERS-1/SAR data classification of rice and cotton fields (Askne, 1995) in an area south of Seville. The main results are: ERS-1 data allow accurate discrimination (better than SPOT) between built-up and natural (forest, agriculture) vegetated surfaces, principle land use

components, bare soil/grass lands versus agricultural crops and forested areas. Landsat data have found application for yield prediction and crop forecasting (Lo, 1991).

Imaging radars have been used for vegetation mapping and crop classification and the frequencies above 8GHz have been suggested as best for crop discrimination (Ulaby, 1980).

Cartographic applications

Wide applications in topographic mapping have found LANDSAT/TM and SPOT/PM images, (Lo, l991), but the greatest potential for topographic mapping and as a source of valuable cartographic data have been used the synthetic aperture radar.

CONCLUSIONS

The great number of meteorological (GOES, Meteosat, NOAA), atmospheric sounders (TOVS, DMSP, UARS), environmental (Terra, Aqua, ERS-2, RADARSAT, ENVISAT) and resources (LANDSAT, SPOT) satellites and sensors allow global monitoring of atmospheric, ocean, coastal zone and land surface variables, processes and resources. Considering the interactive ocean-atmosphere-land surface processes, often it is required synergistic use of retrieved information from different sensors, employing physical, biological and numerical models. Important tasks of the current and future EOS are to evaluate the impact of the increased spatial resolution on accuracy of the retrieved Surface variables as well as synergistic use of sensors with different spatial resolution. Especially important for RSA are development of synergistic models based on optical (hyperspectral) and SAR data. Of great importance is also employment of new sensors and technique in satellite remote sensing (directional, high spectral and spatial resolution (AATSR/ENVISAT, PRISM) hyperspectral, laser and SAR measurements, as well as development of new algorithms for retrieval land/ocean and atmospheric variables, considering the physical and biological interactive processes. Synergistic use of remotely sensed data with different spectral and spatial resolution and modelling will allow to evaluate key climatic variables, affecting environmental changes, weather and climate system. The increased resolution of satellite systems will help to evaluate ocean/atmospheric environmental changes (pollution), or land (irrigation, drought, desertification) resources, important for optimal management and planning, especially of ROPME Region. Coordinated and collaborative efforts of scientific community will allow operational use of satellite remote sensing in different areas of climatology, meteorology, weather forecasting, ocean, marine and land surface environment.

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