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GEF GUINEA CURRENT LARGE MARINE ECOSYSTEM PROJECT

MARINE POLLUTION MONITORING MANUAL

(A TRAINING MANUAL FOR COASTAL AND MARINE POLLUTION MONITORING FOR THE GCLME REGION)

APRIL, 2006

VALIDATION

This Manual has been reviewed and validated by the Pollution Working Group drawn from the participating Countries of the Guinea Current Large Marine Ecosystem Project

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BACKGROUND

The Guinea Current Large Marine Ecosystem (GCLME) is an area of marine coastal space characterized by the Guinea current, an eastward flow that is fed by the North Equatorial Counter Current off the Liberian coast and subsumes the Gulf of Guinea region. The Guinea Current itself represents the dominant feature of this area of shallow ocean bordering countries in Western Africa. The region presents subsystems that are thermally unstable and characterized by intensive seasonal upwelling (around Cote dÍvoire-Ghana), as well as those generally stable depending on nutrient input originating from land drainage and river flood and oceanic turbulent diffusion, although periodic upwelling have been reported. These characteristics combine to make this region one of the world's most productive marine areas rich in fishery resources and an important reservoir of m arine biological diversity.

With most of the large cities of the region located along the coast, about 300 million inhabitants live in and around the coastal areas where they are dependent on the lagoons, estuaries,creeks, and inshore waters surrounding them for their sustenance and livelihood, while also contributing to the degradation of the coastal and marine environment. The health and productivity of such coastal and near-shore aquatic resources have thus been impacted and they in turn hold out direct impact on the socio-

economic successes of the populations that inhabit these expansive but relatively fragile ecosystems (GOOS Report No. 99

Over the years the use of chemical fertilizers (nitrate and phosphate based) and synthetic pesticides has increased with the advent of commercial agriculture. While this has helped enhance food production and protect human health against insect borne diseases, it has also contributed to pollution of the waters. Pollution from these nutrients, when coupled with sewage pollution, could be a serious threat to coastal waters, especially lagoons. *(Portmann et al. (1989), State of the Marine Environment in the West and Central Africa Region, UNEP Regional Seas Reports and Studies, No. 108.*

The full Guinea Current Large Marine Ecosystem (GCLME) Project is an ecosystem-based effort to assist countries adjacent to the Guinea Current ecosystem to achieve environmental and resource sustainability.

CHAPTER 1:

1.1 POLLUTION MONITORING IN THE GCLME COASTAL AND MARINE ENVIRONMENT

The GCLME region has witnessed a substantial increase in industrial development mostly in its coastal cities. Massive rural-urban migration of populations to the industrial cities take available infrastructure unawares, water supplies and sewage systems readily breakdown; urban run-off carry much of the spills, with its load of nutrient, into water bodies which ultimately end up at the coastal waters. Nutrient inputs to coastal and marine area from human activities thus leading to eutrophication, anoxia, and subsequent 'death' of water bodies. In consequence this often gives rise to the decimation of the near-shore and estuarine fisheries of the region and exerts negative impacts on the productivity patterns and economy of the region.

The State of Coastal and Marine Environment of the Gulf of Guinea report (UNIDO/UNDP/NOAA/UNEP, 1995) and the Coastal Areas Profiles of the GCLME coastal states summarises some of the studies that have been conducted on a limited number of the coastal wetlands particularly the Lagos lagoon in Nigeria, the Korle and the Chemu lagoons in Ghana, the Ebrie and the Grand Lahou lagoons in Cote d'Ivoire. The various studies indicate some levels of pollution regarding pathogens and micro-organisms in sewage, industrial effluents with high organic loading and hazardous chemicals, heavy metals, oils and hydrocarbons, tar balls in beaches, as well as serious problems of coastal erosion and coastal areas management. Other studies have also concentrated on weeds, water hyacinth and algal bloom. Similarly, studies have also been conducted on marine fishery resources of the Gulf of Guinea by CECAF, FAO and FRU-ORSTOM. Marine environmental and pollution monitoring programmes have also been carried out by WACAF in collaboration with UNEP/FAO/WHO/IAEA. A review of status of marine fishery resources in 1994 indicates that apart from off-shore demersal resources, all other fisheries in the sub-region are nearly fully exploited (Ajayi, 1995).

In summary, it is recognized that the coastal and marine ecosystem of the GCLME and its resources have witnessed various environmental stresses as a result of the increasing socioeconomic and unsustainable developmental activities. From surveys conducted in the various countries as contained in the various country reports, sectoral/thematic reviews and regional synthesis reviews as well as information obtained from some of the abovementioned donor funded programmes and projects, the three broad issues related to the marine, coastal and associated freshwater environment pollution in the GCLME region include:

- The decline of water quality, due to land-based and sea-based human activities, such as the introduction of sewage and waste water from industrial, domestic and agricultural run off, maritime activities as well as coastal urbanization;
- Physical degradation, alteration and modification of habitats/ecosystems; and
- Fishery resources depletion and the loss of marine biodiversity.

The socio-economic and cultural implications from the above broad issues can be tremendous in terms of income reduction arising from loss of fishery stocks and catches, recreation and tourism amenities, increase of water treatment and coastal protection costs. Because of the paucity of reliable, detailed and historic scientific data on coastal, marine and freshwater environment in the GCLME region, a certain degree of uncertainty still prevails in assessing the pollution load in general.

There is, therefore, an urgent need for a precise qualitative and quantitative assessment of the significant sources of land-based pollution as well as comprehensive assessments of the state of the fisheries and marine living resources and extent of ecosystem degradation (including status and trends analysis) in the region.

Generally, environmental stress from Land-based sources and activities are globally considered to contribute about 70% of the coastal and marine pollution, while maritime transport and dumping at-sea activities contribute about 10% each. From national reports, questionnaires and other published materials, perceived environmental problems in the Guinea Current area and causes of these problems were listed, organized under the major

concerns of the GIWA methodology. The root cause of many environmental and resource problems in the GCLME area has to do with inadequate policy, ineffective compliance monitoring and enforcement and lack of legislation.

1.2 THE REGONAL APPROACH TO TRANSBOUNDARY POLLUTION ISSUES

The major impacts which originate from individual coastal States and are invariably transboundary in nature in the sub-region include:

- Phenomenal rates of erosion of coastal lines
- Loss of critical habits particularly mangroves and wetlands that sustain biological diversity and provide spawning and nursery grounds of migratory fauna of commercial importance and endangered species.
- Wastage through transfer of discard by-catch with consequent loss of marine resources, biodiversity and biomass
- Various States of depletion of straddling and highly migratory fisheries stock including over exploited, declining, slow recovering as result of over fishing and over exploitation.
- Haphazard and over unrestrained development of the coastal areas with incidence of erosion.
- Toxic chemicals and oil spills as well as insidious pollution from oily and exotic biological species discharges from ship traffic,
- Socio-economic implications including loss of revenue, food security concerns, resources use conflicts and increasing poverty.

These environmental and socio-economic impacts from Land-based sources of pollution in the sub-region are of transboundary nature as a result of the movement of the Guinea Current from West to East, which transports pollutants along the coastal area from one country to another.

For instance, the seasonal occurrence of algae bloom shoreline areas in the Western Region of Ghana, currently being studied is believed to originate in Cote dÍvoire.

Inspite of the various sectoral national monitoring and assessment efforts, coastal areas and marine data and information in the region have limited scope to provide adequate, transboundary and integrated regional information upon which management actions and political decisions can be based at regional level negotiations.

The countries have recognized the environmental and socio-economic challenges facing their common marine, coastal and freshwater resources and have accepted the need for joint stewardships in managing the commonly shared resources of the GCLME in order to ensure its future sustainability.

The Abidjan Convention for Co-operation in the Protection, Management and Development of the Marine and Coastal Environment of the West and Central African Region was borne out of the need to undertake regional and common approaches to the prevention, reduction and combating of pollution in the marine environment, the coastal and related inland waters of Western Africa.

The current GEF LME Project Approach to integrated management, and sustainable development and use of the resources of the coastal areas and marine environment is a programme that facilitates the development of regional Strategic Action Plan (SAP) by coastal States towards long-term management through international co-operation within a subregional, inter-regional, or regional framework. This approach is designed to support and supplement national efforts of coastal states to promote integrated management and sustainable development of coastal and marine areas under the coastal states jurisdiction including their Exclusive Economic Zone (EEZ).

The Guinea Current region was one of the first regions where the LME concept was first applied for coastal and marine environmental management. The Global Environment Facility (GEF) funded pilot phase project titled, "Water Pollution Control and Biodiversity Conservation in the Gulf of Guinea Large Marine Ecosystem" was implemented between 1995 – 1999. The project, an initiative of five (later six with the participation of Togo) countries in the region namely Benin, Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo

was implemented with the technical assistance of UNIDO, UNDP, UNEP and the USNOAA (under the United States Department of Commerce) and the collaboration of a host of national, regional and international organizations. The GOG-LME project represented a regional effort to assess, monitor, restore and enhance the ecosystems capacity and productivity in order to sustain the socio-economic opportunities for the countries in the coming decades.

The development objective of the Gulf of Guinea LME (GOG-LME) project was "the restoration and sustenance of the health of the Gulf of Guinea LME and its natural resources, particularly as it concerns the conservation of its biological diversity and the control of water pollution".

The following specific strategic objectives were established for the project:

- Strengthening regional institutional capacities to prevent and remedy pollution of the Gulf of Guinea LME and associated degradation of critical habitats;
- Developing an integrated information management and decision making system for ecosystem management;
- Establishing a comprehensive programme for monitoring and assessing the living marine resources, health, and productivity of the Gulf of guinea LME
- Preventing and controlling land based sources of industrial and urban pollution;
- Developing national and regional strategies and policies for the long-term management and protection of the Gulf of Guinea LME.

An approach adopted in project implementation under the first phase Gulf of Guinea LME Project was to build onto already existing national infrastructures a regionally co-ordinated and integrated programme of monitoring and assessment and developing among others:

i. a structured regional monitoring programme to determine the quality of the coastal areas and the health of the Marine Ecosystem.

ii. a system of coastal and marine ecosystem measurements, information synthesis, and reporting for mitigation of coastal stress.

iii. indices of environmental quality assessment of coastal and the marine ecosystem.

The Guinea Current Large Marine Ecosystem Project (GCLME) brought 10 additional neighboring countries (Guinea-Bissau, Guinea, Sierra Leone, Liberia, Sao Tome & Principe, Equatorial Guinea, Gabon, Congo-Brazzaville, Congo-Kinshasa and Angola) into partnership activities for sustainable development of the GCLME with the original 6 countries of the Gulf of Guinea Project. This now meant that the countries bordered the full extent of the Guinea Current LME and were collectively ready to address the transbondary issues of fisheries, pollution, coastal erosion and habitat protection that pertained to the entire LME region.

1.3 CAPACITY BUILDING AS A KEY REGION-WIDE STRATEGY

Much effort is being invested in the identification and co-ordination of available expertise for region-wide investigations on pollution, nutrients and water quality using uniform laboratory protocols for the region, under the GCLME project.
The last and very successful Gulf of Guinea Large Marine Ecosystem (GOGLME) Project saw the establishment of the first ever Nutrient Activity Working Group (NAWG) in the Sub-region, drawn from the 6 countries that participated in that project. The present exercise seeks to bring on board experts from all sixteen countries now participating in the Guinea Current Large Marine Ecosystem Project .

Consequently, the NAWG has been expanded to include experts on nutrient analysis and water quality monitoring from the entire 16 countries of the GCLME Project who will ensure that uniform methodologies and protocols are adopted for comparability of data on a regional basis.

At the present the necessary tools for meaningful capacity development on a regional basis have been put in place. These include , but are not limited to:

- Nutrient and Water Quality Monitoring Manual, which details methodologies and procedures for regional adoption. This would ensure the inter-comparability of results among all participating countries – a strategy for effective Integrated Coastal Area policy implementation in the region.

- A Marine Pollution Monitoring Manual, is being put in place for effective assessment of the pollution status in the Region.

- Technical capacity upgrading for the Regional Centres of Excellence. UNIDO is providing under the Guinea Current Large Marine Ecosystem project, High-Tech Laboratory and other facilities at the Regional Centre for Pollution Assessment and Management in Owerri, Nigeria, where sustained regional capacity development training programmes as well as referral Laboratory investigations would hold.

- A key strategy in capacity development in the GCLME region is the encouragement of Joint efforts among countries, laboratories,(e.g. joint cruise/expeditions) in nutrient and pollution assessment in shared waters and degraded habitats.
  Generally, the issue of capacity development, both in manpower and instrumentation, is a priority agenda for the realisaion of the GCLME Project, and is being pursued with vigour.

CHAPTER 2

BASIC PRINCIPLES IN POLLUTION MONITORING.

A good water pollution monitoring programme enhances the chances of efficient decision making.

2.1 INFORMATION STATUS ON POLLUTION MONITORING:

Inadequate or unreliable information on water pollution monitoring may arise if:

(A) The objectives of the assessment were not properly defined.

(B) The monitoring system was installed with insufficient knowledge of the water body;

(C) There was inadequate planning of sample collection, handling, storage and analysis;

(D) Data were poorly treated, handled or achieved or worse still;

(E) Data were improperly interpreted and reported.

2.1.1 BASIC RULES FOR A SUCCESSFUL MONITORING PROGRAMME:

The following basic rules ensure a successful monitoring programme:

1- The objectives must be defined first and the programme adapted to them, and not vice versa (as was often the case for multi-purpose monitoring in the past). Adequate financial support must then be obtained.

2- The type and nature of the water body must be fully understood (most frequently through preliminary surveys), particularly the spatial and temporal variability within the water body.

3- The appropriate media (water, particulate matter of biota) must be chosen.

4- The variables, type of samples, sampling frequency and station location must be chosen carefully with respect to the objectives.

5- The field, analytical equipment and laboratory facilities must be selected in relation to the objectives and not vice versa.

6- A complete, and operational, data treatment scheme must be established.

7- The monitoring of the quality of the marine environment must be coupled with the appropriate hydrological information.

8- The analytical quality of data must be regularly checked through internal and external control (e.g. intercalibration exercises).

9- The data should be given to decision makers, not merely as a list of variables and their concentrations, but interpreted and assessed by experts with relevant recommendations for man action.

10- The programme must be evaluated periodically, especially if the general situation or any particular influence on the environment is changed. This is necessary to keep pace with emergent pollutants and thus sustain the relevance of the monitoring programme.

2.1.2 WATER POLLUTION MONITORING

The pollution monitoring process has evolved into a set of assessment activities which include the use of water chemistry, particulate materials and aquatic biota. At times the activity may consider only one type of water (e.g. lakes, lagoons, estuaries, seas,. etc.) or only one approach of monitoring (e.g. physio-chemical or biological methods). A combined use of water, particulate matter and biological monitoring produces comprehensive water quality assessments for most types of water body.

However economic constraints frequently mean that the variables to be monitored, and the methods to be used, must be chosen carefully to ensure water quality assessment objectives are met as efficiently as possible.

An efficient pollution monitoring programme for coastal and inshore waters should be designed or adopted to objectives set on the basis of impacting on environmental conditions, water uses (actual or future), prevailing water legislation and human health considerations etc.

2.1.3 THE STRUCTURE OF WATER POLLUTION MONITORING PROGRAMME

No monitoring programme should be started without critically scrutinizing the real needs for water pollution information. The several competing beneficial uses of the water resource necessitate that the monitoring should reflect the data needs of the various users involved. Fundamental to the exercise however is that baseline information on the parameters to be measured/assessed must be available for the water body, or national/international standards may be adopted.

The process of determining objectives should start with an in-depth investigation of all factors and activities which exert an influence, directly or indirectly, on water quality. Inventories have to be prepared on:

- the geographical features of the area (topography. land use, climate. hydrology. wave action, current direction and intensity).
- Water use (recreational, industrial, agricultural activities, navigational, fisheries, etc).
- pollution sources (present and expected) (domestic. industrial and agricultural etc.).

The benefits for an optimal monitoring operation drawn from careful preliminary planning and investigation by far outweigh the efforts spent during this initial phase. Indeed mistakes and oversights during this part of the programme may lead to costly deficiencies, or overspending during many years of routine monitoring.

Once the objectives have been set the monitoring design is determined by a review of existing water pollution data, sometimes supported by preliminary surveys. Implementation of water quality monitoring activities should by necessity include data interpretation followed by recommendations to relevant authorities for water management, water pollution control and eventually the adjustment or modification of monitoring activities.

2.1.4 WATER POLLUTION MONITORING OPERATION:

The key elements within the structure of water pollution monitoring operation are summarised as follows:

(a) **Objectives**:

These should take into account the hydrological factors, the water uses, the economic development, the legislative policies, necessary decisions involve whether the emphasis should be put on concentrations or loads, or spatial or temporal distributions and the most appropriate monitoring media.

(b) **Preliminary Surveys**:

Short-term limited activities to determine the water pollution variability, the type of monitoring media and pollutants to be considered, and the technical and financial feasibility of a complete monitoring programme. Preliminary surveys are also essential to enable the appropriate siting of baseline stations of effective monitoring programme.

(c) **Monitoring Design:**

This includes the selection of types of pollutants, station location, sampling frequency, sampling apparatus etc.

(d) **Field Monitoring Operations**

These are in-situ measurements, sampling of appropriate media (water, biota, and particulate matter), sample pretreatment and preservation, identification and shipment.

(e) **Hydrological Monitoring:**

This includes water discharge measurements, wave/tide level measurements water levels, thermal profiles etc, and should always be related to the water pollution assessment activities.

(f) **Laboratory Activities:** are concerned with concentration measurements, biological activities, etc.

(g).**Data Quality Control:** this must be undertaken by using analytical quality assurance within each laboratory and amongst all laboratories participating in the same programme, and by checking field operations and hydrological data.

(h) **Data Storage**

Treatment and Reporting: this is now widely computerized and involves the use of data-bases, statistical analysis, trend determination, etc. and determination of results in appropriate forms (graphs, tabulated data, diskettes, spread sheets etc).

(i) **Data Interpretation:** this involves comparison of water quality data between Stations (water quality descriptions, fluxes), analysis of water quality trends, development of cause- effect relationships between water quality data and environmental data (geology, hydrology. land use, pollution sources inventory), and judgement of the adequacy of inter quality for various uses, etc

For specific problems and the evaluation of the environmental significance of observed changes, external expertise may be needed. Publication and dissemination of water pollution reports to relevant authorities, to the public, and to the scientific community is the necessary final stage of water pollution assessment activities.

(j) **Water Management Recommendations:** decisions should be taken at various levels from local government to international bodies, by water and other environmental authorities. The redesign of assessment operations to improve the monitoring programme and to make it more cost effective is an important decision usually derived from the foregoing operations.

In general terms therefore, water pollution monitoring activities (i.e. long-term standardized measurement, observation, evaluation and reporting) must (i) generate the data, which are

essential for meaningful interpretation and management decisions, but (ii) must not lead to a vast collection of unnecessary data which are costly to obtain, but do not contribute to the required understanding of water quality.

Furthermore, monitoring programmes outlay should be commensurate with the socioeconomical and technical/scientific development of the country, since the monitoring programme can only so much succeed and remain sustainable as the level of these factors available.

2.2 PARAMETERS TO BE MEASURED

Water pollution monitoring must serve an explicit purpose, and since resource is always a constraint, only the most meaningful parameters should be selected for inclusion in the monitoring programme. The most common constituents of water pollution indices include:

a) **Hydrobiological parameters**:

Physical and chemical, namely-

Dissolved oxygen (DO), pH, temperature, BOD_5, turbidity, total solids, transparency, etc

Nutrients: nitrates, phosphates, silica, and other markers of eutrophication,

Human pathogens. In addition to indices which directly measure water quality, ecological indices are also sometimes used that measure the response of key species or groups of organisms to pollution.

Chlorophylls. Planktons, etc

Furthermore, estuarine and in-shore water samples arc expected to have widely varying concentration of total salt and organic compounds, and analytical techniques must accommodate these large matrix variations without loss in accuracy and precision.

The most important dissolved substances when considering water quality monitoring in estuaries and in—shore waters are the marine micronutrient (N ,P, Si); elements important in processes (particulate, Fe. Mn, Si); dissolved organic matter, heavy metals, and other specific pollutants.

However, the selection of variables for any water pollution monitoring programme depends

upon the objectives of the programme. Appropriate selection of variables will help these objectives to be met efficiently and in the most effective way.

For the purposes of this manual, the following parameters have been considered.

1. (a) Nutrients;

(i) inorganic nitrogen (Nitrates, Nitrites and Ammonia)

(ii) inorganic phosphorus (soluble reactive phosphorus)

(b) Dissolved Oxygen (and BOD_5)

(c) Human Pathogens and Indicator Organisms

(d) Total Suspended Solids (suspended particulate matter). Turbidity, Total Dissolved Solids (TDS).

(e) Phytoplankton pigment (chlorophyll)

(f) Temperature, pH

(g) Salinity. Conductivity.

Other parameters essential in Water Pollution Monitoring are:

2. Heavy Metals

3. Chlorinated mercury

4. Petroleum Hydrocarbons

5. Microorganisms

Faecal contaminants

Bacterial Pathogens

2.3 DESIGN OF A POLLUTION MONITORING PROGRAMME FOR THE GCLME

Monitoring is defined by the International Organization for Standardization (ISO) as: "the programmed process of sampling, measurement and subsequent recording or signaling, or both, of various water characteristics, often with the aim of assessing conformity to specific objectives". This general definition can be differentiated into three types of monitoring activities that distinguish between long-term, short-term and continuous monitoring programmes as follows:

- Monitoring is the long-term, standardized measurement and observation of the aquatic environment to define status and trends.
- Surveys are finite duration, intensive programmes to measure and observe the quality of the aquatic environment for a specific purpose.
- Surveillance is continuous, specific measurement and observation for the purpose of water quality management and operational activities.

2.3.1 RATIONALE FOR WATER POLLUTION MONITORING PROGRAMMES

The ultimate goal in pollution monitoring should be to maintain quality characteristics that protect the productivity and economic use of the water body. A reasonable first step in developing the program is to compile the data needed to reach sound decisions about its objectives – that is – uses to be protected.

Careful execution of these steps provides a solid foundation for implementing intervention programs.

Generally, monitoring provides the information that permit rational decisions to be made on the following:

- Describing water resources and identifying actual and emerging problems of water pollution
- Formulating plans and setting priorities for water pollution management
- Developing and implementing water pollution management programmes
- Evaluating the effectiveness of management actions

2.3.2 DESIGNING A POLLUTION MONITORING PROGRAMME

A monitoring programme document or study plan describes in details the objectives and possible limitations of a monitoring programme. If the objectives are vague and the information needs inadequately analyzed, the information gaps will be poorly identified and there will be a danger of the programme failing to produce useful data. A pollution monitoring programme can yield information that may serve as a basis for international agreements regarding the use of these waters, as well as for evaluation of compliance with

any such agreements and for pollution problem solving. The major elements of monitoring are therefore:

1. The Monitoring Plan: The process for designing a *monitoring plan, its* implementation and the interpretation of findings is guided by such factors as shown below:

- Objectives:
  - Information the monitoring programme should generate
- Monitoring Network Design
  - Parameters to be measured
  - Source to be sampled
  - *Frequency of sampling*
- Preliminary Survey
  - Test materials and methods
  - Obtain background information
  - Check the adequacy of the monitoring network
  - Check feasibility of the proposed monitoring strategy
- Resource Estimation
  - Laboratory requirements
  - Transport
  - Staffing and training
- Field Work
  - Field work and sampling
  - Field testing methods
- Analytical Quality Assurance
  - Production of reliable data
  - Quality Control: internal and external
- Laboratory Work
  - Physical and chemical analysis
  - Laboratory tests and procedures: microbiological, biological and other instrumentation
- Data Management and Reporting

- Quality control
- Storage
- Statistical analysis
- Interpretation and presentation

2. Principal Elements of a Pollution Monitoring Programme:

There must be:

- A clear statement of aims and objectives
- Information expectations and intended uses
- A description of the study areas concerned
- A description of the sampling sites
- A listing of the water quality variables that will be measured
- Proposed frequency and timing of sampling
- An estimate of the resources required to implement the design, and
- A plan for quality control and quality assurance

SECTION 2

CHAPTER 3

3.1 GENERAL PROCEDURES FOR SAMPLE COLLECTION.

(a) Sampling Procedures for Chemical Analysis.

Analytical results are of no value if the sample tested is not representative. The time between sampling and analysis should be kept to a minimum. Storage in glass or polythene bottle at a low temperature (e.g. 4^0C in the dark) is recommended. Sample bottles must be clean and free from contamination. For chemical analysis sterile containers are not required, but special preservations may be needed for particular analytes. An appropriate sample collection form should accompany all samples.

(b) Taking Sample for Microbiological Analysis:

Use only sterile (NaS_2O_3-treated) bottles reserved specifically for the purpose of microbiological sampling. Thus bottles are treated with NaS_2O_3 (sodium thisulphate solution) to neutralize any residual chlorine, which might stop any bacterial action.

Open the sterilized bottle, and holding the cap face downwards to avoid contamination, fill the bottle leaving an air space at the top to facilitate sample mixing just before analysis. Avoid touching the neck of the bottle or the inside of the cap during sampling. Replace the cap and tighten firmly.

When sampling from a lake, reservoir or other watercourse, care must be taken to avoid personal injury. However, where there is adequate access, it may be possible to take samples by hand. Having removed the sample bottle top, grasp the bottle firmly and submerge the top to at least 20cm below the surface of the water. If there is a current of water, the mouth of the bottle should face the flow of water. When sampling in this fashion, samplers should take great care not to introduce external contamination or 'stir up sediment in the watercourse or reservoir.

When sampling from deeper reaches, or off a boat, it may be necessary to weigh the sample bottle and lower it on a string or rope to obtain the sample. In such cases, great care must be taken to tie the bottle and weight firmly. The bottle should be lowered and raised with great care, avoiding any external contamination, e.g. direct contact with the walls of the well.

In all cases, sample bottles should be firmly resealed after sampling and all relevant information either written on or attached to the bottle. If the bottle is to be returned to a laboratory for analysis. it should be transported in a cool, dark environment. e.g. a coldbox with ice-packs and processed within six hours. If the sample is to be processed on site, this should he done immediately in the cleanest area available.

A sample record sheet/chain of custody format is as follows:

Sample Information

All information pertinent to a sampling trip or a field survey should be recorded in a log book for each location so sampled.

To ensure retention of sample identity, indelible sample labels or seals may be used.

Information in each sample should include:

- sampling location
- date and time of sampling
- medium sampled e.g. grab, composite, integrated replicate blank
- sampling method and/or apparatus
- depth of sampling
- preservation method, if any
- other field pretreatment. e.g. Filtration, fixing
- name of collector
- identification of project
- volume of sample collected.

The signature of persons involved in the chain-of-custody, indicating date of possession should also accompany each sample or group of samples.

3.2 GENERAL HINTS ON WATER POLLUTION MONITORING PROGRAMME

A good water quality monitoring programme enhances the chances of efficient decision making. However, inadequate or unreliable information may arise if:

- the objectives of the assessment were not properly defined.
- the monitoring system was installed with insufficient knowledge of the water body;
- there was inadequate planning of sample collection, handling, storage and analysis;
- data were poorly treated, handled or achieved or worse still;
- data were improperly interpreted and reported.

The Following Basic Rules Ensure a Successful Monitoring Programme:

I. The objectives must be defined first and the programme adapted to them, and not vice versa (as was often the case for multi-purpose monitoring in the past). Adequate financial support must then be obtained.

2. The type and nature of the water body must be fully understood (most frequently through preliminary surveys), particularly the spatial and temporal variability within the water body.

3. The appropriate media (water, particulate matter of biota) must be chosen.

4. The variables, type of samples, sampling frequency and station location must be chosen carefully with respect to the objectives.

5. The field, analytical equipment and laboratory facilities must be selected in relation to the objectives and not vice versa.

6. A complete, and operational, data treatment scheme must be established.

7. The monitoring of the quality of the marine environment must be coupled with the appropriate hydrological information.

8. The analytical quality of data must be regularly checked through internal and external control (e.g. intercalibration exercises).

9. The data should be given to decision makers, not merely as a list of variables and their concentrations, but interpreted and assessed by experts with relevant recommendations for man action.

10. The programme must be evaluated periodically, especially if the general situation or any particular influence on the environment is changed. 'This is necessary to keep pace with emergent pollutants and thus sustain the relevance of the monitoringprogramme.

CHAPTER 4

4.0 ANALYTICAL TECHNIQUES:

4.1 ANALYSIS OF HEAVY METALS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

BACKGROUND INFORMATION

The effects of metals in water and industrial effluents (wastewaters) range from beneficial through adverse to dangerously toxic. Some metals are essential in small amounts but others may affect water quality and be deposited in sediments, which serve as the ultimate sink. Most metals may be either beneficial or toxic depending on their concentration. But generally, beneficial elements turn toxic when in excess concentrations.

PRINCIPLE

Metals may be determined satisfactorily by atomic absorption spectrophotometry. The flame technique is generally applicable at moderate concentration levels in clean and complex matrix systems. In flame atomic absorption spectrophotometry, a sample is aspirated into a flame and atomized. A light beam is directed through the flame into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element. Each metal has its own characteristic absorption wavelength. A source lamp, hollow cathode lamp, composed of each element to be measured, is therefore used. This eliminates to a large extent spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range.

Hydride generation method is applicable to the determination of arsenic and selenium and other metals by conversion to their hydrides using sodium borohydride ($NaBH_4$) reagent and aspiration into an atomic absorption atomizer. The hydrides are purged continuously by

argon or nitrogen into an appropriate atomizer of an atomic absorption spectrophotometer and converted to the gas-phase atoms. The sodium borohydride reducing agent, by rapid generation of the elemental hydrides in a reaction cell, minimizes dilution of the hydrides by the carrier gas and provides rapid and sensitive determinations of the metals.

The flameless or "cold vapour" technique is applicable to the determination of mercury. Ionic mercury is reduced with an excess of $SnCl_2$ or $NaBH_4$ in a reaction vessel to the metallic form, which is partitioned between the aqueous and gas phases. The metallic mercury is volatilized by aeration and swept into the absorption cell where it is detected.

APPARATUS/EQUIPMENT

Telfon digestion block and crucibles

Beakers

Hot plate

Micropipettes (0.1ml)

Pipettes (1, 2, 10 ml)

Fume hood

Drying oven (105^0C)

Stainless steel spatula

Desiccators

Volumetric flasks of various capacities (25, 100, 500, 1000 ml)

Analytical balance (100-200g) with a precision of 0.001 g, preferably a Metler's balance with data status

Atomic absorption spectrophotometer with recorder, if possible

Air compressor or high-pressure cylinder with compressed air, nitrous oxide, argon or nitrogen (for flame atomization)

High pressure acetylene cylinder (analytical grade) for flame atomization.

Glass water - distilling apparatus or deionising apparatus

Graphite Furnace

CHEMICALS AND REAGENTS

Concentrated nitric acid ($d20^0$ c=1.4 g/ml).

Concentrated hydrochloric acid ($d20^0C$ = 1.19g/ml)

Concentrated sulphuric acid ($d20^0$ C = 1.84g/ml)

Stannous chloride/hydroxylamine sulphate solution for mercury determination: mix 10 ml H_2SO_4 and 60ml distilled water and allow cooling to room temperature; dissolve 3g NaCl, 3 g hydroxylamine sulphate and 5g $SnCl_2$, and bring to 100 ml with distilled water.

1% cysteine chloride solution for methylmercury extraction

Toluene for methylmercury extraction

Ammonium sulphate for methylmercury extraction

Ammonium pyrrolidine dithiocarbamate for mineral complexation in water.

Methyl-isobutyl-ketone for mineral extraction in water.

Reducing agent for As and Se analyses: sodium tetrahydroborate ($NaBH_4$) in pellet form, or as a 5% solution in 0.2M NaOH, or other agent as specified by AAS supplier of As or Se analysis kit. Distilled water must be used in all cases.

Nitrogen carrier gas.

STANDARD SOLUTIONS

1. STOCK SOLUTIONS FOR METALS: 1 g/l (1000ppm)

Prepare preferably from commercially available stock solutions for each element. Alternatively, dissolve 100mg of purified metal or its salt equivalent (avoid salts that are hydrates) in a 100-ml volumetric flask in a minimum of distilled water. Dilute to the mark with 5% (v/v) nitric acid.

Individual or combined standard solutions may be prepared from the stock solutions.

2. STOCK SOLUTION FOR ORGANIC Hg: 100 mg/l

Dissolve 151mg methyl mercuric dicyandiamide or an equivalent of another compound in a litre of 1% cysteine hydrochloride.

3. INDIVIDUAL STANDARD SOLUTION

From above stock solutions prepare standard solution for each element, which in 0.1 ml contains twice the lowest concentration of the metal anticipated in the sample to be analyzed. The solution should be prepared frequently (at least weekly) using dilute nitric acid (about 0.25% v/v) as diluent. Avoid combinations of stock solutions which form a precipitate.

4. COMBINED STANDARD SOLUTION

From the respective stock solutions above prepare by appropriate dilutions, a standard solution in which 0.1ml contains twice the lowest concentration of each metal anticipated in the sample to be analyzed. This solution should be prepared fresh or frequently (at least weekly) using dilute nitric acid (about 0.25% v/v) as diluent. Avoid combinations of stock solutions which form a precipitate.

5. INDIVIDUAL CALIBRATION SOLUTION

From above stock solutions prepare by appropriate dilutions a calibration solution for each element of ng/ml concentration (say 2, 4, 6, 8 and 10) in order to cover the working range of AAS for the element. This solution should be prepared frequently (at least weekly) using dilute nitric acid (about 0.25% v/v) as diluent.

6. COMBINED CALIBRATION SOLUTION

From the respective stock solutions above prepare by appropriate dilutions a combined calibration solution containing ng/ml concentration of each element under study in order to cover the working range of AAS for the element. This solution should be prepared frequently (at least weekly) using dilute nitric acid (about 0.25% v/v) as diluents.
NOTE: For analysis of minerals in sea or lagoon water, make standard solutions in artificial sea water (with similar salinity to that of the samples) to compensate for chemical and marine interferences.

4.1.1 SAMPLE DIGESTION (MINERALISATION) AND EXTRACTION OF ORGANIC MERCURY

WET-ASHING OF SEDIMENT, PLANT, FISH AND SHELLFISH

It is preferable to do 'wet-ashing" rather than "dry-ashing". The number of steps and manipulations leading to the final test solution are fewer for the former, and the risk of contamination, particularly during solubilisation of dry ash, is avoided.

Pressurized acid-digestion of biological material in Teflon-lined steel bombs (units) is highly recommended. A typical digestion unit presently in use in many laboratories consists of three steel plates: a ground plate, an intermediate plate with containers for 9 steel-lined Teflon crucibles with caps (35 ml) and a top plate (fig. 4). The plates are kept together by 8 fastening screws during pressurized digestion which normally takes place in an oven or on a hotplate.

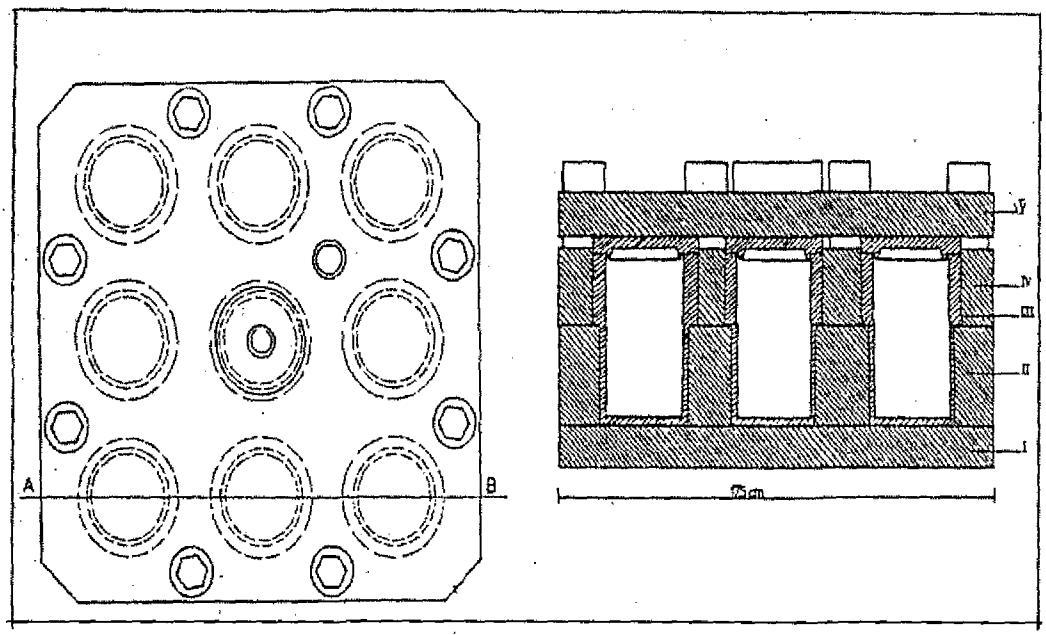


Figure 4. Unit for 9 cruscibles with 35 ml volume. Cutting A – B, ground plate;; II container plate; III, Teflon cruscible with cover; IV, intermediate plate; V, top plate.

The digestion procedure using this unit is as follows:

Weigh suitable amount of each sample (less than 0.5g dry weight or 2.0g fresh weight) into the teflon crucibles.

Place the crucibles into the holes in the container plate, and carefully add the necessary amount of nitric acid (not more than 6ml) required for complete destruction of organic material.

Close the crucibles and the unit and let the samples predigest at room temperature for at least one hour.

Place the unit in a preheated oven or on a hot plate at 120-150°C for at least 6 hours until the organic material is completely destroyed and the resulting solution is clear.

Transfer the content of each crucible into a 10-ml or 25-ml volumetric flask (as appropriate) and bring up to volume with glass-distilled water to obtain test solution.

Analyze test solutions immediately by AAS or store in plastic vials until analysis.

NOTE: The sample/nitric acid ratio as well as the digestion temperature and time should be predetermined for each organic matrix in order to avoid explosive reactions, and to obtain complete digestion and clear solution. In the case of sediment and other matrices, where it can be difficult to obtain a clear solution at the optimum sample/nitric acid ratio, it may be necessary to digest with nitric acid, and then if necessary, with an appropriate volume of a mixture of nitric acid/perchloric acid/hydrofluoric acid (3:2:1).

EXTRACTION OF METHYLMERCURY

Homogenize about 6g fresh weight or 1.5g dry weight of sample in a glass centrifuge screwcap tube with 3ml of distilled water

Add 1ml concentrated hydrochloric acid and 3ml of toluene and shake vigorously for 5 minutes.

Centrifuge and pipette 2ml of the upper toluene phase (note total volume, Vt) into another tube.

Add 4ml of a 1% cysteine chloride solution and shake vigorously for 2 minutes.

Centrifuge and transfer 3ml of the aqueous phase (note total volume, Vc) to a glass test tube.

Dilute to about 10ml with water, add 1.0ml sulphuric acid diluted in equal volume of water and 2ml of 10% v/v ammonium persulphate.

Heat slowly to boiling point and continue for about 2minutes in order to remove the excess persulphate.

Cool, transfer to an appropriate volumetric flask and top to mark. Analyze by flameless or cold vapour AAS.

SOLUBILISATION OF MINERALS IN WATER

Adjust an aliquot of each water sample to pH 2.5 and add 2% v/v solution ofammonium pyrrolidine dithiocarbamate.

Extract the complex formed with an equal volume of methylisobutyl-ketone and analyze organic layer using AAS. If necessary, concentrate the organic layer (by heating in a water bath) before AAS determination.

Use aqueous layer (considered free from metals) for blank determinations.

4.1.1.1 ATOMIC ABSORPTION MEASUREMENT

Trace elements to be monitored in the marine environment are As, Cd, Cu, Fe, Hg, Co, Mn, Pb, Se, Ni, V and Zn. The AAS techniques recommended are described below.

FLAME ATOMISAITON

For Cd, Cu, Fe, Mn, Pb, Ni, V and Zn. The procedure is as follows:

Set up the AAS instrument according to the manufacturer's instructions.

Regulate to optimum conditions for the element under study, particularly with respect to the wavelength of the corresponding hollow-cathode lamp, lamp current, slit width and air acetylene flame intensity (should be lean blue).

Determine the optimum flame atomization and calibrate the instrument with calibration solutions representing the linear range of the absorption curve. Calibration should be done on a daily basis.

Introduce a blank solution into the atomizer and record the absorption signal obtained. Do the same for the test solution, after rinsing the atomizer with acidified water diluent until the absorption signal returns to its base line. Rinse after every determination.

FLAME ATOMISATION AFTER HYDRIDE GENERATION

Applicable to As and Se, and those elements that form volatile hydrides.

ARSENIC (As)

Set up the AAS instrument with the kit for As analysis according to the manufacturer's instructions, and regulate to optimum conditions as above.

Pipette a predetermined volume of the test solution into the reaction vessel of the kit and add 1ml of diluted sulphuric acid in distilled water (1:3)

Place the reaction vessel in the kit and replace the air therein with nitrogen.

Add one pellet of $NaBH_4$ or 5ml of the 5% alkaline solution above, and mix using the device provided in the kit. Ionic arsenic (III) is reduced in the reaction vessel to the hydride (AsH_3), which is swept by nitrogen into the open-ended quartz absorption cell that is heated at about 900^0C over the flame. The hydride is decomposed and the atomic As is determined. The cell's alignment in the path is provided by the normal burner-adjustment mechanism. When the reaction is complete (i.e., the absorption signal has returned to its base line), flush the absorption cell with nitrogen, empty the reaction vessel and wash with distilled water. The cell is ready to receive a new sample.

NOTE:

The volume of the test solution necessary for analysis is predetermined using calibration solutions representing the linear range of the curve. Calibration should be done on a daily basis.

SELENIUM (Se)

Set up the AAS instrument with the kit for Se analysis according to the manufacturer's instructions, and regulate to optimum conditions as above.

Pipette an appropriate volume of the test solution into the reaction vessel of the kit.

Place the reaction vessel in the kit and replace the air therein with nitrogen.

Add one pellet of $NaBH_4$ or 5ml of the 5% alkaline solution above, and mix using the device provided in the kit. Ionic selenium is reduced in the reaction vessel to the hydride

form which is swept by nitrogen into the open-ended heated quartz absorption cell (900^0C) where it is decomposed and the atomic Se is determined.
When the reaction is complete (i.e., the absorption signal has returned to its base line), flush the absorption cell with nitrogen, empty the reaction vessel and wash with distilled water. The cell is ready to receive a new sample.

NOTE: The volume of the test solution necessary for analysis is predetermined using calibration solutions representing the linear range of the curve. Calibration should be done on a daily basis.

4.1.2 FLAMELESS OR "COLD VAPOUR" DETERMINATION OF MERCURY

Set up the AAS instrument with the kit for Hg analysis according to the manufacturer's instructions. With no flame, regulate to optimum conditions particularly with respect to the wavelength of the Hg hollow-cathode lamp and the slit width.
Pipette an appropriate volume of the test solution into the reaction vessel of the kit. Use the Teflon bomb digest for total mercury determination, or the cysteine chloride extract for methylmercury determination.

Place the reaction vessel in the kit and replace the air therein with nitrogen.
Add 2ml of the stannous chloride/hydroxylamine sulphate solution above (4e) and mix using the device provided in the kit. Ionic mercury is reduced by $NaBH_4$ in the reaction vessel to the metallic form which is partitioned between the aqueous and the gas phases in the reaction vessel. The nitrogen carrier gas sweeps the mercury in the gas phase into the absorption cell (aligned in the light path) where it is detected.
When the reaction is complete (i.e., the absorption signal has returned to its base line), flush the absorption cell with nitrogen, empty the reaction vessel and wash with distilled water. The cell is ready to receive a new sample.
NOTE: The volume of the test solution necessary for analysis is predetermined using calibration solutions representing the linear range of the curve. Calibration should be done on a daily basis.

4.1.2.1 DETECTION LIMITS

The detection limits of some elements using the above AAS techniques are given below (Table 2).

Sensitivity and detection limits vary with the instrument, the element determined, the nature of the matrix and the analytical technique. The sensitivity of flame atomic absorption spectrophotometry is defined as the metal concentration that produces an absorption of 1% (an absorbance of approximately 0.0044). The instrument detection limit is defined as the concentration that produces absorption equivalent to twice the magnitude of the background fluctuations.

The optimum concentration range usually starts from the concentrations of four to five times the detection limits and extends to the concentrations at which the calibration curves start to flatten. For best results, use concentrations of samples and standard within the optimum concentration range of the spectrometer. For further information, see APHA,(1998).

Table 2: AAS detection limits for some elements

AAS TECHNIQUE	ELEMENT	DETECTION LIMIT	
		IN SOLUTION (ng/ml)	IN MARINE ORGANISMS (ng/g FW)
Flame atomization	Cu	10	50
	Cd	2	5
	Fe	20	
	Mn	10	
	Pb	50	5
	Zn	5	250
Flame atomization after hydride	As	2	10
generation	Se	2	0.05
Flameless or "cold vapour" technique.	Hg	1	0.05

4.1.3. STANDARDISATION OF DIGESTION AND AAS MEASUREMENT

STANDARDISATION OF MATRIX

Before a new matrix is analyzed, standardize the digestion and AAS measurement procedures as follows:

Prepare appropriate individual or combined standard solutions of the elements(s) under study as above.

Prepare a series of 6 to 8 digestion crucibles with the first five containing each an equal amount (less than 0.5 dry weight) of the matrix. The last three serve as blanks.

With a micropipette, add 0.1ml of dilute nitric acid (about 0.25% v/v) to the first crucible, and then 0.1, 0.2, 0.3 and 0.4ml of the standard solution to the second, third, fourth and fifth crucibles, respectively.

Then add the predetermined amount of concentrated nitric acid to all the 8 crucibles, and carry out digestion, test solutions preparation and AAS measurement as above.

Determine the concentration of the elements in the 8 crucibles, and construct a calibration curve for each element by plotting the standard addition concentrations against their absorbance values.

NOTE: Likewise, the matrix can be calibrated for methylmercury determination by making standard additions of the methyl mercuric dicyanodiamide solution before toluene extraction.

CALIBRATION CURVE

The calibration curve should be a straight line. It is acceptable if the coefficient of correlation (r) between the standard addition concentrations and their absorbance values are at least 0.995. If not, rerun the matrix-standardization procedure by changing the amounts of matrix to be analyzed and the standard additions.

The coefficient of correlation (r) is given by the following equation:

$$r = \frac{\sum .XY - \frac{(\sum .X)(\sum .Y)}{n}}{\sqrt{(\sum X^2 - \frac{(\sum X)^2}{n}).(\sum Y^2 - \frac{(\sum Y)^2}{n})}}$$

where X (X_0, X_1, X_2, X_3, X_4) represent the standard addition concentrations of the element, Y (Y_o, Y_1, Y_2, Y_3, Y_4) their corresponding absorbance values, and n the number of values (5 in this case).

Let Y = A + BX be the general equation of the straight line (calibration curve), where B is the slope, and A the intercept on the y-axis. The most accurate straight line can be constructed using Lison's statistical method of least squares, so that the sum of the squares of the deviations of the experimental points (X_1, Y_1) to the line is as small as possible. The values of B and A are determined by this method as follows:

$$B = \frac{\text{Covariance } (X,Y)}{\text{Variance } (X)}$$

$$\frac{\sum XY - .\frac{\sum X .\sum Y}{n}}{n - 1}$$

$$= \frac{.\sum X^2 - \frac{(.\sum X)^2}{n}}{n - 1} = \frac{\sum XY - \frac{\sum X .\sum Y}{n}}{.\sum X^2 - \frac{(.\sum X)^2}{n}}$$

where n –1: degree of freedom (i.e. 5 – 1 = 4)

$$A = Y - Bx$$

Where y (mean) = $(Y_0 + Y_1 + Y_2 + Y_3 + Y_4)/5$

X (mean) = $(X_0 + X_1 + X_2 + X_3 + X_4)/5$

EXPRESSION OF RESULTS

1. ALL TRACE ELEMENTS AND TOTAL MERCURY

From the AAS reading obtained on the test solution, determine the concentration of trace metal (s) therein, by reference to the calibration curve above and after making allowance for the blank determination.

Calculate the metal concentration of sample, taking into account the exact weight of each sample placed in each digestion vessel. Express this concentration both in µg/kg DW (dry weight) and in ug/kg FW (fresh weight).

Let 25ml be the volume (V) of the test solution

0.4g be the dry weight (DW) of the matrix digested

5ng/ml the concentration (C) of element read from the curve

The quantity of trace element (Q) in the sample is given by

$$Q = \frac{C \times V}{DW} = \frac{5 \times 25}{0.4} = \frac{125}{0.4} = 312.6 \text{ng/g DW}$$

In case of dilution of the test solution (by a factor D) before AAS analysis, the quantity of element (Q1) in the sample is given by:

$$Q_1 = \frac{C \times V \times D}{Dw} = \frac{5 \times 25 \times D}{0.4} = 312.5 \times D \text{ng/g DW}$$

The amount of element in fresh sample is given by

$$Q_{fw} = \frac{312.5 \times 30}{100} = 93.75 \text{ng/f FW or } 93.75 \text{ug/kg FW}$$

Where the dry matter content of the fresh sample is 30%, determined as indicated above.

NOTE:

Even when stored in air-tight containers, dried powered samples can pick up moisture from the atmosphere due to frequent opening of containers during analysis. With time, the moisture content can reach 10% or more, especially in the humid tropical climate of the Gulf of Guinea. It is therefore recommended to make dry weight corrections during each analysis by drying separate aliquots of the sample to constant weight at 1050C.

2. METHYLMERCURY

The quantity (Q) of methylmercury in the sample can be computed as follows:

$$Q = \frac{C \times V}{DW} \times \frac{Vt}{2} \times \frac{Vc}{3} \quad ng/gDW$$

Where C: Concentration in ng/ml of methylmercury read from the calibration curve.

V: Volume in ml of the test solution.

Vt: Volume in ml of the toluene phase of which 2ml were further extracted.

Vc: Volume in ml of the aqueous cystein chloride phase of which 3ml were solubilised and diluted to give the test solution.

DW: Dry weight in g of sample analyzed.

Make necessary adjustments in case of dilution of the test solution, and express the results with respects to sample fresh weight as above.

4.1.4 PRECISION, ACCURACY AND QUALITY CONTROL OF RESULTS

PRECISION

Calculate the coefficient of variation (CV) of replicate results:

$$CV = \frac{S \times 100\%}{X}$$

Where S (standard deviation) = $\frac{1}{n-1\ (x_1 - X)^2}$

and X (mean) = $\frac{1\ Xi}{n}$

The CV should be less than 10%. If not check for possible errors such as homogeneity of the sample, digestion procedure and contamination.

Precision of the results can be improved by taking replicates of AAS instrument calibration solutions and blanks through the whole wet ashing process, and comparing the results with replicates read directly.

Accuracy

Test accuracy of results by analyzing a matrix similar to that under study for which others have reported known values using the same reference method as above. In addition, participate as often as possible in intercalibraiton exercises.

QUALITY CONTROL

To guarantee precision and accuracy of results, carry out standardization of the matrix as often as possible. If there is a fluctuation in the standard deviation or the accuracy of the results by more than 5%, check the following.

Stability of stock solutions (prepare new solutions);

Contamination of equipment and glass ware (clean them);

Homogeneity of sample (homogenize again);

Contamination of matrix (select suitable alternative for analysis).

Use replicates to establish precision and known additions recovery to determine bias. For example, add a known amount of metal and reanalyze to confirm recovery. The amount of

metal recovered should be approximately equal to the amount added. Recovery of added metal should be between 85 and 115%.

Analyze a blank between sample or standard readings to verify baseline stability. Rezero when necessary. Analyze an additional standard solution after every ten samples or with each batch of samples, to confirm that the test is in control. For recommended concentrations of standards to be run, limits of acceptability and reported single-operations precision data, see APHA, 1998.

4.1.5 ANALYSIS OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY

A. EQUIPMENTS AND MATERIALS

Rotary evaporator

Water bath

Soxhlet extraction unit

Vacuum pump

Glassware

Gas chromatography columns (1.8m x 4mm I.D)

Silica gel columns

Separatory funnels

Centrifuge (at least 10.00rpm)

Freezer-dryer and/or drying oven

Analytical balance (100-200g) with a precision of 0.001g or better, preferable top load.

Desiccators

Glass wool (precleaned by extraction in hexane or petroleum ether and oven baking)

Gas chromatography with Ni-63 electron capture detector

Stainless steel or glass blender.

Mecheenical shaker

Syringes(10µl)

Glass column Ø = 1cm for open chromatography apparatus

B. REAGENTS AND SOLVENTS

Hexane, pentane or petroleum ether (400 – 60^{0}C) (glass-distilled)

Iso-octane, acetone, diethyl ether, methanol, benzene, acetonitrile, dichloromethane and toluene (all glass-distilled)

Sodium chloride

Sodium sulphate (anhydrous)

Concentrated sulphuric acid (d20^{0}C=1.84g/l)

Orthophosphoric acid

Fuming sulphuric acid

Ethanol 95.5% (spectroscopic grade)

Purified nitrogen gas

Very high purity silica gel for chromatography such as Merck

Florine,60-100 mesh

Kieselgel 60 (0.040-0.063mm size)

Hexamethyldisilane, 10% solution in toluene

Potassium hydroxide pellets

Florosil – 60-100 mesh.

C. STANDARD SOLUTIONS

C_1 INTERNAL STANDARDS

In order to increase the accuracy of the analysis, it is necessary to add the internal standard (IS) of 1,1-dichloro-2,2-diphenylethylene (0.01µg/ml) and 2,5,2',6' – tetrachlorobiphenyl (0.025ug/ml) in iso-octane to the sample before extraction, and to the standard solutions for instrument calibration.

a. Stock IS solution (1.0 and 2.5mg/ml): Weigh 100mg of 1,1-dichloro-2, diphenylethylene and 250mg of 2,5,2', 6'-tetrachlorobiphenyl into a 100-ml volumetric flask and fill to the mark with iso-octane.

b. IS dilution 1 (10 and 25µg/ml): Weigh 0.692g (1ml) of the IS stock solution into a

100-ml volumetric flask and fill to the mark with iso-octane.

c. IS dilution 2 (0.01 and 0.025µg/ml): This is obtained by making a one-hundredth dilution of IS dilution 1 in the same way as above.

C_2 GC REFERENCE SOLUTIONS

a) Reference stock solutions 1: Prepare reference stock solutions (RS) of PCB's (aroclor 1254 and aroclor 1260), DDT's (p,p – DDT, o,p-DDT, p, p-DDE, o,p-DDE, p,p-DDD), HCB, gamma-HCH (lindane), beta-HCH, aldrin, dieldrin and other chlorinated hydrocarbons under study by dissolving 100mg of each reference substance in 100ml of the internal standard solution (IS dilution 1 above). Store in sealed glass ampoules.

b) Reference stock solution 2: Weigh aliquots of the different reference stock solutions 1 into a 100-ml flask and dilute to mark with the internal standard solution (IS dilution 2). Aliquots are calculated to result in concentrations 100 times higher than the values given by manufacturers for corresponding compounds in the commercial formulations used.

c) GC standard solution: Weigh 0.692g (1.0ml) of each reference stock solution 2 into a 100-ml volumetric flask and dilute with internal standard solution (IS dilution 2) to 69.2g (100ml). It may be necessary to have several mixtures so that analytes that have similar retention times are not in the same standard solution, thus enabling their retention order to be determined.

4.1.6 EXTRACTION AND CLEAN-UP OF SAMPLES:

A. DRIED SAMPLES (FISH, SHELLFISH,MOLLUSCS, PLANT, SEDIMENT

A_1.EXTRACTION

Weigh about 5g of ground freeze-dried material and place in an extraction thimble.

Add 1ml of internal standard solution 2 (2.1c) and extract with 200ml of hexane, pentane or petroleum ether in a soxhlet apparatus for 8 hours.

Concentrate the extract in a vacuum rotary evaporator to about 10ml at about 500C, and dry by passing through a small glass column plugged with glass wool and containing anhydrous sodium sulphate.

Collect and concentrate extract to about 2ml, and record the volume.

Determine solvent "extractable organic matter" (E.O.M.) by drying (evaporating with dry nitrogen) or on a heating plate, a known volume of the extract in a clean preweighed beaker to constant weight. (To remove water filter with Na2So4)

Note the weight of the residue and calculate the amount of E.O.M. (lipids) as follows:

$$\text{E.O.M. (mg/g)} = \frac{\text{Wt.of residue (mg)} \times \text{Vol.of extract (µl)}}{\text{Vol. Evaporated (µl)} \times \text{wt. Sample extracted (g)}}$$

A_2 .SULPHURIC ACID CLEAN-UP

This treatment is made when the lipid contents are higher than 100mg/g of sample. For that:

Transfer Iml of extract into a centrifuge tube with Teflon screw cap containing 4ml of concentrated sulphuric acid.

Close the tube and turn it upside down carefully for about twenty times (without shaking) to allow concentrated sulphuric acid to precipitate unwanted organic matter as salts.

Centrifuge to separate the phases and transfer the upper hexane layer quantitatively into another glass tube.

Reduce the volume of the extract to about 2ml by evaporating the solvent with clean dry nitrogen. Never leave to run dry or it must be discarded.

Place the extract on about 1.63g florosil contained in a small column with an internal diameter of 1cm, rinse the tube and add onto the column.

Allow the column to equilibrate by letting the florosil to absorb all the extract and rinsing, and elute with hexane until about 50ml of eluate is obtained.

Concentrate the eluate first in a vacuum rotary evaporator, then in a test tube (by evaporating the solvent with clean dry nitrogen), to a final volume of about 1ml. The extract is ready for injection into the GC.

A_3.POTASSIUM HYDROXIDE CLEAN-UP

This procedure is applicable if compounds such as dieldrin and heptachor epoxide which are unstable in sulphuric acid are to be analyzed. On the other hand, compounds such as lindane, beta-HCH, aldrin, p,p' – DDT, p,p-DD, and p,p-DDT are unstable in potassium hydroxide. The last three are transformed into o,p-DDE, p,p'-DDMU and p,p-DDE, respectively. The internal standards 1,1-dichloro-2,2-diphenylethene and 2,5,2,6-tetrachlorobiphenyl are stable in both sulphuric acid and potassium hydroxide.

The clean-up procedure is as follows:

Dissolve 1 pellet of potassium hydroxide in 1ml of 95.5% ethanol together with 0.05ml of water, in a screw cap centrifuge tube.

Transfer 1ml of the extract to the tube, close tightly and place in a water bath at 500C for 30 minutes,

Add 2ml of a solution of sodium chloride in orthophosphoric acid (11.7g NaCl in 1 litre of 0.1m orthophosphoric acid).

Shake the tube, centrifuge and collect the upper hexane layer quantitatively. Remove residual water by passing through anhydrous sodium sulphate in a small glass column. Rinse with hexane and concentrate to about 1ml. The extract is ready for injection into the GC.

B. FRESH SAMPLES (FISH, SHELLFISH, PLANT AND SEDIMENT)

Place about 25g of fresh weight sample in a blender, add 3 times the weight of anhydrous sodium sulphate and blend at high speed until well blended.

Transfer the mixture to an extraction thimble and extract with 200ml hexane or petroleum ether for 8 hours in a soxhlet apparatus.

Concentrate the extract, determine the E.O.M., and clean up with sulphuric acid or ethanolic potassium hydroxide in the same way as for freeze-dried sample.

C.EXTRACTION AND CLEAN-UP OF WATER SAMPLES

Mix 1 litre of water sample with about 500ml or less of hexane in a 2-litre separatory funnel and shake for 30 minutes.

Separate the organic phase from the aqueous phase and extract the latter three times, using 50ml hexane each time.

Concentrate the combined hexane phase to a volume of 10ml in a vacuum rotary evaporator at 50^0C.

Dissolve in 25ml distilled acetonitrile and wash with 450ml of 4% (w/v) sodium sulphate. Extract again successively with 3x25ml portions of hexane and remove residual water from the combined hexane extract by passing through anhydrous sodium sulphate in a small glass column. Concentrate the extract to about 1ml. It is ready for injection into the GC.

4.1.7 SEPARATION OF COMPOUNDS BY SILICA GEL COLUMN CHROMATOGRAGHY

Gas chromatography of the hexane extracts of water or of marine organism, plant or sediment (following sulphuric acid clean-up), may give numerous compounds with interferences and poor resolution. In particular, the analyses of PCBs and DDTs can be interfered with by two types of complex chlorinated hydrocarbons, namely chlorinated terpenes (campheclor, toxaphene) and chlordane-related compounds. The silica gel column chromatographic method described below will separate about 90% of toxaphene and chlordane from PCBs. To do this;

Extract silica gel in hexane in a Soxhlet apparatus and dry at low temperature in an oven. Activate the dried gel by heating at 250^0C for two hours and partially deactivate with 3% water by weight. Allow to equilibrate gently for a day with a mechanical shaker before use

Weigh out one gram of the gel in a beaker, cover with hexane and mix into a slurry. Pour into a glass column (6mm I.D) plugged with hexane-precleaned glass wool, allow the gel to settle into an even bed and drain the solvent to just above the gel bed.

Rinse down any gel adhering to the sides of the column with hexane, allow the gel to settle and drain the column as above.

Apply the cleaned-up and concentrated hexane extract obtained above to the silica gel and carefully elute with about 35ml of hexane. Collect the hexane eluate (fraction 1).

Elute the column again with about 45ml of benzene and collect the eluate (fraction 2).

Concentrate the above fractions separately to about 1ml by evaporating the solvent with clean dry nitrogen gas.

The established composition of each of the above fractions is given below (table 3).

NOTE: Calibrate the column with standard mixtures for the recovery of all compounds of interest.

Table 3 Composition of hexane and benzene eluates from silica gel column.

FRACTION 1: Hexane eluate	FRACTION 2: Benzene eluate
Heptachlor 70%	Heptachlor 30%
DDE 85%	DDE 15%
alpha-HCB	alpha-HCH
Endosulfan	Lindane
PCBs	DDT
	DDD
Heptachlor epoxide	
Dieldrin	
Endrin	
Chlordane	
Methoxychlor	

4.2. GAS CHROMATOGRAPHY

4.2.1. COLUMN PREPARATION

Chlorinated hydrocarbons are generally analyzed on a gas chromatography equipped with an electron capture detector (ECD). The glass column (1.8m x 4 mm I.D.) should be filled with acid-washed and dried chromosob W (80-100mesh) coated with 5% OV – 101 silicon oil. Preferably, use a ready-made commercial column. Otherwise, prepare one in the laboratory as indicated below:

Fill the column with concentrated HCl and leave it for one hour.

Wash with distilled water, then with acetone and finally with toluene.

Fill the column with a toluene solution of hexamethyldisilane (HMDS, 10%). Warning: use a fume hood and do not touch the HMDS reagent.

Wash with distilled water, then with methanol and finally with acetone.

Dry the column either with air, or in an oven

Put about 10mm of glass wool in the outlet end of the column and weigh the empty column.

Attach a funnel to the inlet of the column and fill at least half a coil with the filling material before the other end is connected to a water jet air pump.

Fill the column by gentle tapping. If the filling material gets stuck before the column is filled, disconnect the pump and put the continue wit the warm column.

When the column is full, put about 10mm glass wool in the inlet end.

Weigh the filled column, label it and store.

Before use, condition column overnight by connecting the inlet to the injector in the chromatography (detector end unconnected), applying a carrier gas (flow rate of 60ml/min) and heating the column to 250^{0}C (allowed maximum temperature).

COLUMN TEST

After conditioning the column, connect the other end to the detector, and set the gas flow to 30ml/min (for a column with 4mm 1.D).

Set injector and detector (ECD Ni-63) temperatures at 200 and 320^{0}C respectively, and the column oven temperature at 180^{0}C.

Inject p,p –DDT or the methyl mercury standard, respectively, and measure their retention times Tr. Adjust the column temperature to get a retention time relative to aldrin of 3.03.

Measure the width of the DDT-peak or the methyl mercury peak respectively at its half height ($b_{1/2}$) in minutes and the retention time (Tr) also in minutes.

The performance of the column is measured by the "number of theoretical plates" for each of the above standards, which is calculated with the formula

$$N = 5.54\left[\frac{Tr}{b_{1/2}}\right]^2$$

A measure independent from the column length is then the height equivalent to a theoretical Plate (HETP)

$$HETP = \frac{L}{N}$$

Where L is the column length

4.2.2.QUANTIFICATION

(a). DDTs AND OTHER CHLORINATED HYDROCARBONS

Set up gas chromatograph as indicated above.

Inject microlitre quantities of the sample (eluate concentrate from silica gel column) and corresponding standards and measure the peak heights of the compound under study with similar retention times.

Calculate the concentration of the compound in the sample using the following formula:

$$C\ (\mu g/g\ DW) = \frac{V}{DW} \times \frac{h}{his} \times \frac{his}{h'} \times e$$

Where V: total extract volume (ml)

DW: dry weight of the sample (g)

h: peak height of the compound in the sample (mm)

h': peak height of the compound in the standard (mm)

his: peak height of the internal standard in the sample (mm)

h'is: peak height of the internal standard in the sample (mm)

c: concentration of standard (µg/ml)

. Calculate the result on fat weight basis as follows:

$$C\ (ug/g\ fat\ wt.) = \frac{V}{DW} \times \frac{h}{his.} \cdot \frac{his}{h} \cdot c \cdot \frac{Dw}{F}$$

$$C\ (ug/g\ fat\ wt.) = V \times \frac{h}{his.} \cdot \frac{his}{h} \times \frac{c}{F}$$

where F is the fat content in gram.

e. Express the above result per gram fresh weight by taking into account the dry matter content of the original fresh sample.

b) QUANTIFICATION OF PCBs

PCBs are a complex mixture of compounds that cannot all be resolved on a packed column. Also, there is no single standard available for their quantification. Each peak in a sample chromatogram might correspond to a mixture of more than one individual compound. The usual method to quantify PCBs is to compare packed column chromatograms of commercially available technical formulations with the sample chromatogram. Aroclor 1254 and 1260 are the commercial preparations frequently used.

The quantification procedure is as follows:

Set up gas chromatograph as indicated above.

Inject microlitre quantities of the sample and the standards (Aroclor 1254 and 1260) and note relative heights of corresponding peaks with similar retention times which elute after DDE.

Calculate the contribution (on weight basis) of the individual peaks matched with the appropriate commercial standard as follows:

$$\text{Peak. Contribution of sample} = \text{Wt of std. injected} \times \frac{\text{Sample peak ht.}}{\text{std. Peak ht.}} \times \frac{\text{Mean wt.\%}}{100}$$

These values may be further corrected for recoveries based on the use of an internal standard.

NOTE: The contribution of each peak to the total commercial formulation in terms of mean weight % of the various constituents is provided by the manufacture of the formulation.

Summation of the individual weight contributions of each sample peak matched with one in the standard provides an estimate of the PCB content.

However,values persistently below 6 or above 9 reflect disturbances in the ecosystem.

4.3 MARKERS OF EUTROPHICATION

4.3.1 DISSOLVED OXYGEN

Concentration of dissolved oxygen can be measured in situ (using an oxymeter or a multiparametric probe) or in the laboratory (by titration) i.e. Winkler's method

MATERIALS AND REAGENTS

Oxymeter or multiparametric probe

Amber glass bottles (300 ml) with glass stoppers

Automatic pipettes

Normal or automatic burette

Magnetic stirrer

500ml Erlenmeyer flask

Manganese sulphate solution ($MnSO_4.4H_2O$, $MnSO_4.H_2O$)

Sodium or potassium hydroxide

Sodium or potassium iodide

Sodium azide

Sodium carbonate

Concentrated sulphuric acid (d=1.84)

Salicylic acid sodium thiosulphate

Potassium hydrogen diiodate, $KH(IO_3)_2$

Potassium fluoride

IN SITU MEASUREMENT

Calibrate oxymeter according to manufacturer's instructions.

Immerse the probe in water to the required depth. Read the value displayed (dissolved oxygen) in $mg.l^{-1}$ or percentage saturation.

NOTE: In situ measurement has the advantage of giving immediate results, and numerous and closely-spaced values (case of a vertical profile). But precision of measurement varies considerably with the quality of the probe. This type of measurement is only suitable for water with dissolved oxygen values greater than 1 $mg.l^{-1}$.

4.3.1.1 LABORATORY DETERMINATION: MODIFIED WINKLER'S METHOD (USE OF AZIDE)

The modified Winkler's method using an azide has the advantage of eliminating interferences (frequently encountered) due to the presence of nitrite ions, especially in factory effluents, sewage, river water and in samples incubated for BOD determination.

Once these interferences are eliminated, dissolved oxygen is determine according to the original Winkler's method.

PREPARATION OF SOLUTIONS

i) ***Manganese*** (II) sulphate solution (Reagent 1) Dissolve 480g $MnSO_4$, $4H_2O$ or 400g $MnSO_4.2H_2O$ or 364g $MnSO_4H_2O$ in distilled water and dilute to 11. The manganese sulphate solution must not give any colour with starch solution when acidified potassium iodide solution is added. Manganese sulphate can be replaced by 400g of $MnCl_2.4H_2O$.

ii) Alkaline azido-iodide (Reagent 2)

a) Form more or less saturated samples: Dissolve 500g, NaOH (or 700g KOH) and 135g Nal (or 150g KI) in distilled water and diluted to one litre. Add 10g NaN_3 dissolved in 40ml distilled water. This reagent must not give any colour with starch solution when diluted and acidified.

b) For supersaturated samples: Dissolve 10g of NaN_3 in 500ml of distilled water. Add 480g NaOH and 750g Nal and stir to dissolution. A white precipitate (due to sodium carbonate) will be formed, but should be ignored as it is of no consequence. This solution should not be acidified as it is of no consequence. This solution should not be acidified under any circumstance as toxic fumes of hydrazoic acid may be formed.

iii) Starch

Dissolve 2g of soluble laboratory starch and 0.2g of salicylic acid in 100ml boiled distilled water.

iv) Sodium thiosulphate standard solution

Dissolve 6.205g $Na_2S_2O_3$. $5H_2O$ in distilled water. Add 1.5ml NaOH 6N or 0.4g solid NaOH and dilute to 1000ml. Standardise with diiodate solution.

v) Postassium hydrogen diiodate standard solution, 0.0021M

a) Dissolve 812.4mg KH $(1O_3)_2$ in distilled water and dilute to 1000ml.

b) Standardisation: dissolve approximately 2g KI in 100 to 150ml distilled water. Add 1 ml H_2SO_4 6N and 20ml of the standard diiodate solution. Dilute to 200ml and titrate the iodine liberated with the sodium thiosulphate solution using starch solution as end point indicator (appearance of palm yellow colour). The volume of sodium thiosulphate (0.025M) added must be 20ml at end point.

METHOD

a) From the sampling bottle, fill a 300ml glass amber bottle with water until it overflows, using a PVC tube, which delivers the water right at the bottom of the bottle.

b) Let water overflow (about 3 times the volume of the bottle) while gently withdrawing the PVC tube to avoid formation of air bubbles.

c) Put on the glass stopper to displace excess water and then remove it again.

d) Quickly pipette (with a propipette) 1ml of Reagent 1 and 1ml of Reagent 2 to the bottom of the bottle, and cork immediately to stop the reagent from climbing to the surface; the propipette stops water from climbing in the pipette. This is known as 'Fixing'.

e) Mix by turning the bottle upside down many times, place it in a closed container (away from light) and transport to the laboratory.

Sample so treated can be stored in darkness for up to one month before analysis. (At the time of analysis, make sure that the precipitate formed is abundant, and fills about half the bottle.)

f) Add 1ml concentrated H_2SO_4, recork and mix by turning the bottle upside down several times until the precipitate completely dissolves (loss of liquid during corking of bottle is normal and does not affect the results much).

g) Place an aliquot of the mixture (corresponding to 200ml of original water sample) in an Erlenmeyer flask, after correcting for loss incurred during corking of bottle and addition of reagents: for a total of 2ml of reagents R1 and R2 used, the volume of mixture to be taken should be 200x300/(300 –2) = 201ml.

h) Carefully mix on a magnetic stirrer and titrate with Na_2SO_3 0.025M solution until colour turns pale yellow.

k) Add a few drops of starch solution and continue titrating until the blue colour disappears.

l) Note the volume Vt of thiosulphate used.

CALCULATIONS AND RESULTS

i) Expression of results in mg/l

According to the equation of the reaction:

1 mole of O_2 requires 4 moles of $S_2O_3^{2-}$

ie., 32g of O_2 ------> 4 moles of $S_2O_3^{2-}$

or, 1g of O_2 ---> 1/8 mole of $S_2O_3^{2-}$

Let Vml of water sample contain Xg oxygen.

The Xg O_2 will require X/8 mole of $S_2O_3^{2-}$

But, X/8 mole of $S_2O_3^{2-}$ = Vt (L) x C (mol/l) of $S_2O_3^{2-}$

ie., $x = \frac{Vt\ (ml) \times C}{1000}$

$X = Vt\ (ml) \times C \times 8$

Let Y be the amount of oxygen (in g) in a litre of sample:

$$Y = \frac{X}{Ve(ml)} = \frac{1000.X}{Ve(ml)} = 8 \times \frac{Vt\ (ml)}{Ve\ (ml)} \times C$$

Dissolved oxygen content in mg/l is given by:

$$[O_2]\ mg/l = \frac{8000 \times Vt \times C}{Ve}$$

Where Vt: volume of sodium thiosulphate solution used for titration of iodine (in ml)

C: concentration of sodium thiosulphate solution (in mol/l)

For Ve = 200ml and C = O.025M

$$\frac{8000 \times O.025 \times Vt\ mg/l}{200}$$

Dissolved oxygen content (in mg/l) is therefore given by the same number as the volume of $Na_2S_2O_3$ expressed in ml.

Example: For Ve = 8.5.ml

$[O_2]$ = 8.5mg/l

ii) Expression of results in percentage saturation

$$\%\ Sat = \frac{[O_2]\ mg/l \times 100}{C^*}$$

Where % Sat: percentage saturation in oxygen (O_2) mg/l: dissolved oxygen in water

sample Solubility C* is related to temperature by the formula:

Ln C* = - 139.34411 + (1.575701 x 10^5/T) – (6.642308 x 10^7/T^2)

+ (1.243800 x 10^{10}/T^3) – (8.621949 x 10^{11}/T^4) – chl [(3.1929 x 10-^{2}) - (1.9428 x 10/T) + (3.8673 x 103/T^2)]

Chlorinity (chl) can be deduced from salinity measurement of sample (see protocol for salinity determination above) using the formula:

Salinity = 1.80655 x chl

Knowing the temperature and chlorinity, the solubility of oxygen in the water sample can be determined. A total based on the above formula gives Ln C* directly at known temperature and chlorinity.

Example: Let $[O_2]$ = 5mg/l

At 20^0C, and for chlorinity of 0.000mg/l

Ln C* (from table) = 2.207 and C* = 7.457

% Sat = $\frac{5}{7.457} \times 100 = 67.05\%$ = 67.05%

4.3.2 CHEMICAL OXYGEN DEMAND (COD)

(Oxidative reflux titration using dichromate)

Chemical oxygen demand (COD) is used as a measure of the oxygen equivalence of organic matter (in sample) susceptible to oxidation by a powerful chemical oxidant. The COD tests permits monitoring and control of organic material flux in a receiving water body.

Organic matter and other substances oxidized by refluxing with a standard solution of dichromic acid in the presence of silver sulphate (Ag_2SO_4) and mercury sulphate ($HgSO_4$) as catalysts.

Excess dichromate is then titrated with ammonium iron sulphate standard solution using orthophenanthroline iron complex (or ferroin) indicator. The dichromate reflux method is prefer able to methods using other oxidants because $K_2Cr_2O_7$ is a very powerful oxidizing agent, and it is easy to use and applicable to a wide variety of samples.

MATERIALS AND REAGENTS

Common laboratory material

Reflux system comprising a 500ml round bottomed flask and condenser

Magnetic stirrer/heater – a heating mantle

Potassium dichromate $K_2Cr_2O_7$ (PDC)

Ammonium iron sulphate Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ (AFS)

1,10- Phenanthroline

mercury (II) sulphate (crystallized)

silver sulphate (ground)

concentrated sulphuric acid (d = 1.84)

deionized water (Distilled H_2O)

potassium hydrogen phtalate, $HOOC_6H_4COOK$ (reference compound: KHP)

Sodium chloride

Punice stones (antibumping granules)

Spectrophotometer HACH DR

Potassium dichromate solution

SOLUTIONS

a) Potassium dichromate standard solution No1 (0.25 N or 0.0417 M): Dissolve 12.2588g of dried (2h at 110°C) $K_2Cr_2O_7$ in deionized water and dilute to 1000ml.

b) Potassium dichromate standard solution No2 (0.025 N or 0.00417 M): Dilute 100ml of solution No 1 to 1000ml with deionised water.

c) Orthophenanthroline iron sulphate indicator (Ferroin): Dissolve 1.485g) of 1,10 –

orthophenanthroline monohydrate and 0.695g of $FeSO_4.7H_2O$ in 100ml of deionized water. This indicator is also commercially available and could be used.

d) Ammonium iron sulphate solution No1 (0.25 N or 0.25 M): Dissolve 98g of ammonium iron sulphate (AFS) in deionized water and dilute to 1000ml.

e) Standardization of ASF solution: Add 20ml of concentrated sulphuric acid (d =1.84) to 250ml of potassium dichromate solution No 2 and allow to cool. Titrate with the ASF solution using 8 to 10 drops of ferroin solution as indicated. Calculate the normality of the ammonium iron sulphate standard solution to the nearest 1/10000. Standardization of this solution should be done daily or before use

.

f) Silver Sulphate, Ag_2SO_4 : Add 6.6g of Ag_2SO_4 in 1000ml of concentrated H_2SO_4

METHOD

GENERAL APPLICATION

a) Collect water (from sampling bottle) in a clean glass or Teflon bottle previously rinsed with distilled water and then abundantly with water sample.

b) Cork the bottle immediately and transport to the laboratory in a cooler at 4OC.

c) Store sample at 4^0C wrapped in aluminum foil until analysis (within 6h at most).

d) Before analysis, remove sample from the refrigerator and let it warm up to room temperature, still wrapped in aluminums foil (to avoid light).

e) Take 50ml aliquot of sample in a 500ml round bottomed flask: samples rich in organic matter (COD > 900mg/l) should first be diluted appropriately before taking the 50ml.Verify this concentration before making the analysis. If the COD is greater than 900mg/l, the mixture will turn green, so dilute.

f) Add 1g $HgSO_4$ and a few pumice-stones (previously heated in a furnace at 600^0C for 1h).

g) Slowly add 5ml concentrated H_2SO_4 and mix under cooling in an ice bath until completely dissolved. Cooling prevents loss of volatile material.

h) Slowly add 5ml concentrated H_2SO_4 and mix under cooling in an ice bath until completely dissolved. Cooling prevents loss of volatile material.

i) Add 25ml of potassium dichromate solution No1 and mix thoroughly.

j) Fit refrigerant on the round bottom flask, with gentle rotation or the latter in an ice bath to avoid over-heating.

k) Slowly add 70ml concentrated sulphuric acid containing Ag_2SO_4 (d = 1,84) through the top of the refrigerant with continuous rotation and shaking of the flask.

CAUTION: Mix well to avoid over-heating and projection of the mixture through the refrigerant.

l) Cover the top of the condenser with a beaker to avoid introduction of foreign particles, adjust refrigerant water flow and boil for 2 hours.

m) Cool the flask and rinse inside of condenser with 25ml deionized water (into the flask).

n) Remove refrigerant and dilute content of flask with distilled water to 300ml.

o) Cool down to room temperature and titrate excess potassium dichromate with ammonium iron sulphate solution No1 using 0.1 to 0.15ml of ferroin indicator (2 to 3 drops).

Note volume (A) of AFS solution used at end point when colour changes from blue-green to red-brown.

p) Run an analytical blank with 50ml distilled water and note volume (B) of AFS solution used.

CASE OF SAMPLES CONTAINING MORE THAN 2000 mg/1 OF CHLORIDE

Reducing substances such as iron, nitrites and chlorides, interfere with COD determination because they are oxidized. Chlorides are by far the most important and common substances, which interfere with organic matter as they are quantitatively oxidized by dichromic acid. Chlorides are therefore complexed by adding mercury sulphate to the sample to form soluble mercury chloride. Nitrites are eliminated by adding 10mg of sulfamic acid to the dichromate standard solution for every milligram of nitrite present in the reflux flask.

In this case, the method of determination is as follows:

a) Accurately determine chloride content in the sample by Mohr's method, as follows:

b) Pipette 50ml of water sample into a 500ml round bottomed flask: sample rich in organic matter (COD > 900mg/1) should first be diluted appropriately before taking the 50ml.

From the stock solution of sodium chloride (NaCl), make a series of dilutions with deionized water containing 2000 to 20000 mg/l Cl -: 2500,5000,7500,10000,12500,17500 and 20000mg/l. Pipette 50ml of each solution into a 500ml round bottom flask.

Add 10mg $HgSO_4$ to the water sample and to each of the NaCl dilutions, for each milligramme Cl^- present in 50ml.

Determine COD as indicated above (general application) for water sample and each of the NaCl dilutions. From the graph, determine COD value C (in mg/l) corresponding to the Cl^- content of the water sample. This value should be subtracted from the COD of the sample to correct for chloride interference.

CASE OF SAMPLES WITH LOW COD (<50mg/l)

Place at least 50ml of water sample in a 500ml round bottomed flask.

Add all reagents as indicated above (general application).

Reduce volume to 150ml by heating (without refrigerant), after adding 10mg of mercury sulphate ($HgSO_4$) for every 1mg of Cl^-originally present in the water sample.

Proceed with determination of COD as indicated above (general application) using the following solutions: 0.025 N $K2Cr_2O_7$ standard solution (instead of 0.25 N) ; 0.25 N ammonium iron sulphate standard solution (instead of 025 N).

NOTE: Analyze samples with care as traces of organic matter on the glassware or from the air can lead to false results.

DETERMINATION OF EFFICIENCY OF THE METHOD

a) Roughly grind potassium hydrogenphtalate and dry to constant weight at 120^0C.

b) Dissolve 425mg in distilled water and dilute to 100ml.

c) Take 50ml of this solution in a bottomed flask, treat and determine its COD as indicated above (general application), say CODexp.

d) Calculate the efficiency of the method as follows:

$$\text{Efficiency (in\%)} = \frac{\text{CODEXP X 100}}{\text{CODth}}$$

where COD_{th} is the theoretical COD value of potassium hydrogen phthalate standard solution, taken as 500µg O_2 per ml

EXPRESSION OF RESULTS

From natural waters and industrial wastewaters not requiring correction for chlorides:

$$COD\ (mg/l) = \frac{(B-A)\ N \times 8000}{\text{Vol. of sample (ml)}} \times \frac{100}{Eff}$$

b) For natural waters and industrial wastewaters requiring correction for chlorides:

$$COD\ (mg/l) = \frac{[(B-A)\ N \times 800 - C] \times 1.20}{\text{Vol. of sample (ml)}} \times \frac{100}{Eff}$$

Where COD: chemical oxygen demand from dichromate

B: quantity of ammonium iron suplhate for blank (ml)

A: quantity of ammonium iron sulphate for sample (ml)

N: normality of ammonium iron sulphate solution

C: Correction for chloride from graph of chloride concentration versus COD (see 4:2.3.2. g above)

1.20: empirical compensation factor

c) COD can also be expressed in mg of O_2 per kg sample dry weight by the relationship:

$$COD\ (mg/kg) = \frac{COD\ (mg/l)}{DW(kg/l)}$$

$$\text{with } Dw = \frac{W(100-T)}{100}$$

Dw: dry weight of 1L of water sample (in kg)

W: weight of 1L of water sample (in kg)

T: water content of sample (in %)

$$T = \frac{\text{weight of water} - \text{dry weight}}{\text{weight of water}} \times 100$$

4.3.3 MEASUREMENT OF BIOLOGICAL OXYGEN DEMAND (BOD)

(Dilution method)

Biochemical oxygen demand (BOD) is the amount of oxygen necessary for the aerobic decomposition of organic matter by bacteria. In this process, the organic matter represents food for the bacteria, and the energy required is derived from oxidation of the organic material.

BOD test is widely used for determining the polluting potential of domestic and industrial wastewaters, of used waters in general. It is a good means of studying the natural phenomena of organic matter destruction in the aquatic ecosystem. It gives an appreciation of the potential effect of organic matter on the oxygen balance of the receiving water body.

In pr actice, BOD test consists in the consumption of oxygen by water sample kept in the dark, and at constant temperature (20^0C),for a determined duration (5 days). It is termed BOD_5.

In general, the dilution method is used, and dissolved oxygen is measured by the modified Winkler's method. The dilution method is based on the fundamental concept that the rate of biochemical degradation of organic matter is always directly proportional to the amount of existing unoxidized material. According to this concept, the rate of oxygen consumption in diluted water is directly related to the percentage of waste present, all the other factors being constant.

MATERIALS AND CHEMICALS

-BOD oxymeter

-BOD incubator (or temperature-controlled cupboard)

-Amber BOD bottles (neck wide enough to receive oxymeter probe) with glass - stopper

-Sodium sulphate dipotassium hydrogen phosphate

-Common laboratory glassware

-Disodium hydrogen phosphate, $Na_2HPO_4.2H_2O$

-Potassium dihydrogen phosphate, KH_2PO_4

-Magnesium sulphate, $MgSO_4.2H_2O$

-Calcium chloride, $CaCl_2$

-Ferric chloride, $FeCl_3.\ 6H_2O$

-Ammonium chloride, NH_4Cl

-Distilled water (free from chlorine and chloramines)

-Inoculation water (fresh urban wastewater, sample in the collector of a residential area with very low industrial contamination, and filtered)

-Glucose, $C_6H_{12}O_6$

-Glutamic acid

REAGENTS AND SOLUTIONS

a) Phosphate buffer: Dissolve, 8.5g of KH_2PO_4, $Na_2HPO_4.2H_2O$ and 21.75g KH_2PO_4 in 500ml-distilled water and complete to 1litre

b) $MgSO_4.2H_2O$ solution: 20g/l in distilled water.

c) $CaCl_2$ solution: 27.5g/l

d) $FeCl_3.\ 6H_2O$ solution: 0.25g/l.

e) NH_4Cl Solution: 2g/l

f) Glucose-glutamic acid mixture: Dissolve 150 mg glucose and 150 mg glutamic acid in distilled water and complete to 1 litre.

g) Dilution water: Pipette 5ml phosphate buffer, 1ml $MgSO_4.2H_2O$ solution, 1 ml $CaCl_2$ solution and 1ml $FeCl_3$. $6H_2O$ solution into a 1 litre volumetric flask, and complete to the mark with distilled water. This mixture should not be stored for more the 24 hours.

METHOD

GENERAL APPLICATION (DILUTION METHOD)

a) Collect water (from sampling vessel) in a clean glass bottle (amber or wrapped in aluminum foil) without any trace of organic material.

b) Transport in a cooler at 4^0C to the laboratory and store in the refrigerator until analysis (within 24h at most).

c) Before analysis, remove sample from refrigerator and let it warm up to room temperature.

d) Prepare dilution water and pour into a big glass container. Aerate by gently tilting the container until dissolved oxygen content measured with an oxymeter) reaches 8mg/l. Allow to equilibrate for at least one hour, and then add one volume of inoculation of dilution water.

(e) Verify that the pH of water sample is between 6.5 and 7.5. if not, adjust pH with dilute H2SO4 or NaOH, without increasing the volume of water by more than 0.5%.

(f) Place a known volume of water sample in a 1 litre volumetric flask and fill to the mark with inoculated dilution water. Mix gently and make several dilutions:

- 0 to 1% dilution for industrial effluents.
- 1to5% dilution for urban wastewaters.
- 25 to 100% dilution for polluted river waters.

The dilution factor should be chosen according to the dominant source of pollution in the lagoon or marine environment.

(g) Measures dissolved oxygen content (by Winkler's method or by oxymeter) just after dilution, and note the value, say, Do.

(h) Gently fill BOD bottles with water sample (diluted or not) without creating air bubbles until it overflows, and put the stopper in place. At the same time, fill a BOD bottle with dilution water inoculated with a strain of microorganism for the blank or control), water inoculated with a strain of microorganism (for the blank or control), and another BOD bottle with 2% glucose-glutamic acid solution in inoculated dilution water.

(i) Incubate BOD bottles at 20OC in the dark.

(j) After 120 hours, measure dissolved oxygen for each dilution, the control and glucose-glutamic acid solution, by Winkler's method.

(k) The biochemical oxygen demand after five days (BOD_5) is calculated by the formula:

BOD_5 (mg/l) = F(To – Ts) – (F – 1) (Do – Ds)

Where DO average O_2 content of dilution water at the beginning of the assay (in mg/l)

Ds: average O_2 content of dilution water after 5 days incubation

To: initial O_2 content of one of the sample dilutions

Ts: O_2 content of one of the sample dilutions after 5 days incubation

F: dilution factor such that $0.40 < To - Ts < 0.6\ To$

l) The efficiency of the method is determined by the measurement for the glucose – glutamic acid solution, and is given by the formula:

$$\text{Eff}(\%) = \frac{\text{BOD}_{exp} \times 100}{\text{BOD}_{th}}$$

Where BOD exp: BOD measured for the glucose-glutamic acid solution
BOD: BOD reference value in literature for the said solution

CASE OF SAMPLES OF LOW BOD_5 (DIRECT METHOD)

For samples with BOD_5 lower than 7mg/l, it is not necessary to dilute. It is esteemed that they are sufficiently aerated to have saturated levels of oxygen at the beginning of BOD test. Only some river waters fall within this category. In this case, the test procedure is direct and as follows:

a) Maintain sample (in bottles brought from the field) at 20^0C and aerate to increase or reduce dissolved oxygen content to above saturation point.

b) Then fill two or several BOD bottles with sample; quickly determine dissolved oxygen content in at least one of them, and incubate the others for 5 days (120 hours) at 20^0C.

c) After five days, determine remaining dissolved oxygen in the incubated samples and calculate the BOD by subtracting the values obtained from those before incubation.

Direct BOD measurement does not involve any modification of the sample, and as such gives results quite close to natural conditions of the environment. Unfortunately, very few samples have BOD values with the range of dissolved oxygen content given above.

NOTE:

During decomposition of organic matter (hydrocarbons), other oxygen – consuming processes may occur which disturb BOD measurement. They are:

-nitrification by bacteria

-presence of reducing chemical substances such as sulphides, sulphites, ferrous salts, etc., has a depressive effect on the oxygen balance in the water body.

-presence of bactericidal or bacteriostatic substances (chlorides, etc.) inhibits the activity of bacteria, which otherwise would proliferate and cause rapid exhaustion of dissolved oxygen and disturbance of the BOD test.

For the control, oxygen consumption at the end of 5 days should be between 0 and 1.5 mg/l. if not, then the dilution water culture is unsuitable, and needs modification. For lagoon samples, which contain all sorts of inputs, it is unnecessary to inculcate the dilution water with microorganisms.

4.3.4 NUTRIENTS

Analysis of nutrients consists in determining dissolved and undissolved salts on the one hand, and dissolved salts alone on the other hand. It is therefore necessary to collect at least two lots of the sample. The first, to be used for determining dissolved and undissolved nutrients, intended for dissolved nutrients determination alone, will undergo a second filtration through Whatman GF/F filter (about 0.7µm pore size).

Nutrients to be determined are ammonum ions ($N\text{-}NH_4$), nitrites ($N\text{-}NO_2$), nitrates ($N\text{-}NO_3$) and orthophosphates ($P\text{-}PO_4$).

4.3.4.1 DETERMINATION OF AMMONIUM IONS

MATERIAL AND CHEMICALS

50ml tubes with Teflon screw-caps

Dark box

Assorted pipettes

Automatic burettes with reagent bottles

UV/visible spectrophotometer

Phenol

Sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H_2O$

Sodium citrate

Potassium dichlorocyanurate ($C_3Cl_2KN_3O_3$)

Sodium dichlorocyanurate ($C_3Cl_2NaN_3O_3$)

Sodium citrate, Camel water , Sodium hydroxide

Ammonium chloride

Ammonium sulphate

Fresh deionized or distilled water.

PREPARATION OF REAGENTS

a) Reagent R1: Dissolve 17.5g phenol and 200mg sodium nitroprussate in deionized water and complete to 500ml. This reagent is stable for weeks, and should be replaced when it turns green. It should be stored in brown glass bottle at 4^0C, away from direct sunlight

b) Reagent R2: Dissolve 140g sodium citrate and 11g sodium hydroxide in 400ml of deionized water and mix. Add 0.7g camel water (about 4.6ml) and complete to 500ml.

Camel water can be replaced by 2.5g potassium dichlorocyanurate. For analysis of water samples with salinity lower than 5 psu, the amount of sodium hydroxide in the reagent can be reduced by 7g. This reagent is stable for about 2 months. Store at 4^0C

c) Ammonium chloride stock solution: Dissolve 3.819g NH_4Cl (dried at 100^0C) in freshly prepared distilled water and dilute to 1l. This solution contains 1mg N or 1.22g NH_3 per ml.

d) Ammonium chloride calibration solutions: Prepare, by appropriate dilutions of the stock solution, calibration solutions containing 1000,100,10,1 and 0.1mg N-NH3 per litre.

METHOD

a) From the sampling bottle, collect water sample (already filtered through a 200µm nylon mesh) in a 500ml flask.

b) Filter about 250ml of the water through a Watman GF/F filter (0.7µm pore size) for determination of dissolved NH_4, and keep the rest for determination of total ammonium ions (dissolved and particulate).

c) Take 50ml of each fraction in a 250ml.

d) Add 3ml of reagent R1 cork the flask and homogenize.

f) place the flasks in dark box (away from light) at room temperature for colour development, and transport to the laboratory

g) Treat 50ml of a blank deionised water) and each of the NH_4Cl calibrating solutions in the same way as the samples.

h) After 6 to 12 hours, read the optical density (OD) with a spectrophotometer at 630 nm.

j) The concentration of unionized ammonia N-NH_3 (toxic at high dose) can be

calculated from that of $N\text{-}NH_4$, given the temperature and pH of the water sample, by the following formula:

$$[N\text{-}NH_3] = \frac{[N - NH_4]}{[1+10\,(10\text{-}pH\text{-}0.03T)]}$$

It should be noted that $N\text{-}NH_3$ content higher than 0.025mg/l reflects high nitrogen pollution.

NOTE: To refine the analysis, it is advisable to make calibration solutions with water of the same salinity as the sample; use either stale seawater (i.e., sea water collected at more than 100m deep and stored many months in the dark) or artificial seawater conveniently diluted to the same salinity as the sample.

$N - NH_4$ values usually found in lagoons vary from 2-to 20-$\mu g\ l^{-1}$ (0.14 to 1.4 mg/l). Higher values reflect pollution. In the open seas, the values are generally lower.

4.3.4.2 DETERMINATION OF NITRITES ($N\text{-}NO_2$) AND NITRATES ($N - NO_3$)

PRINCIPLE

In the presence of hydrochloric acid and N – (1-naphtyl)- ethylenediamine dichlorate, sulfanilamide reacts with nitrites to give a yellow colour proportional to nitrite concentration.

Nitrates are determined by differences after reduction in a Cd-Cu column and quantification of the total resulting nitrites.

MATERIALS AND CHEMICALS

Glass or plastic flasks

Chloroform

Graduated tubes

UV/visible spectrophotometer

Cd-Cu columns

Concentrated hydrochloric acid (d=1.18)

Sulfanilamide

N-(1-naphtyl)-ethylenediamine dihydrochloride

Ammonium chloride, NH_4Cl

Anhydrous sodium nitrite, $NaNO_2$

Anhydrous potassium nitrate, KNO_3

Methyl chloride, CH_3Cl

Spectrometric arc

REAGENTS AND SOLUTIONS

Reagent R1: Dilute 50ml concentrated HCl in 300ml distilled water, add 5g sulfanilamide and dilute to 500ml. This solution is stable for very long in the refrigerator.

Reagent R2: Dilute 0.5g N-(1-naphytyl)-ethylenediamine dichlorate in 500ml distilled water. Renew this solution every month, or as soon as it turns brown.

Concentrated buffer: Dilute 250g of ammonium chloride in a litre of distilled water. Dilute this stock solution 40 times (25ml in a litre) before use.

Stock nitrite solution: Dry anhydrous sodium nitrite at 110_0C for several hours. Dissolve about 0.345g in distilled water, dilute to a litre and add 1ml chloroform. 1ml of this solution (stable for 1 to 2 months) contains 5 µmoles of $N\text{-}NO_2$.

Stock nitrate solution: Dissolve 0.506g of dried anhydrous potassium nitrate in a litre of distilled water and add 1ml of chloroform. 1ml of this solution (stable for 1 to 2 months) contains 5 μmoles of $N - NO_3$.

METHOD

From the sampling bottle, collect 500ml of water sample (already filtered through a 200 μm nylon mesh) in a glass or plastic container.

Keep sample in a cooler (away from light), transport to the laboratory and store in the refrigerator. Analyze within 24 hours at most.

Before analysis, filter about 250ml of sample through Whatman GF/F filter (0.7μm pore size) for determination of dissolved nitrites and nitrates. Keep the rest of unfiltered sample for determination of total nitrite and nitrate (dissolved + particulate).

DETERMINATION OF NITRITES

Take 50ml of sample, add 1ml of R1 and shake.

After 5 minutes, add 1ml of R2 and shake.

Allow 10 minutes (at least) and 60 minutes (at most) for full colour development measure the optical density at 543nm.

Also prepare a series of nitrite calibration solutions by appropriate dilutions of the stock standard solution. Treat like the sample, and measure the optical densities. Plot a graph of OD versus $N\text{-}NO_3$ concentration.

From the graph, readout the concentration of $N - NO_3$ in the sample corresponding to its OD. If the OD of sample is out of the linear range of the graph, make appropriate dilutions before the 50ml are taken for analysis.

DETERMINATION OF NITRATES

It is necessary to reduce the NO_3 present to NO_2 by passing through a Cd – Cu column. Total nitrite is then determined, and NO_3 concentration is deduced by subtracting the initial nitrite content. Precision of the analysis depends on the efficiency of the column, which must be controlled periodically.

Take 100ml of water sample, add 2ml of concentrated buffer and shake.

Rinse the column with 5ml of water sample, and pour the rest into the column. Let the first 30ml flow out of the column to waste, and collect the next 50ml.

Add 1ml of reagent R1, shake and wait for 5 minutes. Add 1ml of reagent R2, and shake again.

Read the OD at 543 nm after allowing 10 to 60 minutes for full colour development

NOTE: Nitrate concentrations normally found in lagoons vary from 0 to 20 µg1-1. Higher values reflect pollution. Nitrite concentrations are generally much lower (0 to 2 µg 1-1), except in cases of pollution.

4.3.4.3 DETERMINATION OF ORTHOPHOSPHATE ($P - PO_4$)

PRINCIPLE

In acid solution, phosphate and molybdate form a complex, which is reduced by ascorbic acid to give a blue colour of intensity proportional to the phosphate ions.

MATERIALS AND CHEMICALS

UV/visible spectrophotometer

Glass or plastic flasks of 250 and 500ml

GF/F filter paper of 0.7 µm pore size

Nylon cloth of 200 µm mesh size

Graduated tubes

Ammonium paramolybdate, $(NH_4)_6M0_7O_{24}.4H_2O$

Concentrated sulphuric acid (d=1.84)

Ascorbic acid

Potassium antimony oxytartrate, $K(SbO)C_4H_4O_6.\ ½\ H_2O$

Anhydrous potassium dihydrogen phosphate, KH_2PO_4

Chloroform

REAGENTS AND SOLUTIONS

a) Reagent R1: Dissolve 15g of ammonium paramolybdate in 500ml-distilled water. This solution is stable in the refrigerator for long. Store in a plastic bottle.

b) Reagent R2: Carefully add 140ml of concentrated sulphuric acid to 900ml water. Cool and store in the refrigerator.

c) Reagent R3: Dissolve 8.8g ascorbic acid in 500ml-distilled water. This solution can be stored several weeks in the refrigerator and several months in the freezer.

d) Reagent R4: Dissolve 4.3888g potassium antimony oxytartrate in 200ml distilled water (heat if necessary). This solution can be store several months in the refrigerator. Store in a dark bottle

e) Composite reagent: Mix the reagents in the following proportions: R1 75ml, R2, 250ml, R3, 150ml, R4, 25ml, total 500ml. This mixture cannot be stored for more than 6 hours, and should be made just before analysis (500 ml of the mixture can be used for 50 samples). A more stable mixture (several months) can be prepared by committing R3. The latter is added just before use.

f) Stock standard solution: Dry anhydrous potassium dihydrogen phosphate at 100^0C, dissolve 0.6805g in 1l distilled water and add 1ml chloroform. 1 ml of this solution (stable for several months), contains 5 µmoles PO_4 ions.

METHOD

Collect water from sampling bottle and store under the same conditions as for nitrites and nitrates analysis.

Measure 100ml of sample (at room temperature) into a flask and add 10ml of the composite reagent.

Allow the colour to develop, and read the OD at 885nm after 10 to 60 minutes.

Also prepare a range of calibration solutions of potassium dihydrogen phosphate from the stock solution using appropriate dilutions. Treat as for samples and read the OD at 885nm.

Plot a calibration curve of OD versus PO_4 concentration.

From the graph, determine the concentration of PO_4 in the sample corresponding to its OD.

If the OD of sample is out of the linear range of the graph, make appropriate dilutions before taking the 100ml for analysis.

NOTE: Values normally obtained in lagoons vary from 0.2 to 5µg l^{-1}.

Higher values reflect pollution.

4.4 SUSPENDED PARTICLES

PRINCIPLE

Suspended particles are determined by gravimetric method after filtration on ashless filter paper and drying in the oven. The amount of organic material is determined after ashing by difference between the dry weight of suspended particles and weight of ash..

MATERIAL

Filtration rack, tweezers and vacuum pump

GF/F filter of 0.7 µm pore size (e.g. Whatman)

Precision balance

Desiccators

Oven

Borosilicate weighing crucibles with lids

Furnace

METHOD

Put filter paper in a crucible and dry in an oven at 105^0C for 3 hours.

Cool in a desiccator for at least 30 minutes, and weigh the crucible empty and with the filter; let Mo and M_1 be the weights, respectively.

Filter water sample through the dried filter paper and rinse abundantly with distilled water.

Carefully replace the filter paper in the crucible, dry in the oven at 105^0C for at least 24 hours and cool in a desiccator.

Weigh and note the weight M_2 (crucible + filter + suspended particles).

Calculate the weight Ps of suspended particles from the formula:

$$Ps\ (mg/l) = \frac{M_2 - M_1 \times 1000}{V}$$

Where V: volume of filtered water (in ml)

If the weight of particulate organic matter is also required proceed as follows:

Place the crucible (+ filter + particles) in a furnace at 550^0C for at least one hour.

Cool in a desiccator and determine the weight M_s

The organic content Po of the suspended particles is estimated by the formula:

$$Po\ (mg/l) = \frac{(M_2 - M_1) - (M_3 - Mo) \times 1000}{V}$$

Note: The volume of water filtered must be adequate: neither too much to clog the filter, nor too little to contain sufficient amount of suspended particles and organic matter for analysis. Use vacuum of 20 to 30mm Hg for filtration.

The concentration of suspended particles usually encountered in the sea ranges from 1 to 10mg/l. In the lagoon, the range is either (5 to 50mg/1) with possibility of higher values (> 200mg/1) during floods.

4.5 DETERMINATION OF SILICA BY MOLYBDOSILICATE METHOD

4.5.1 PRINCIPLE

Ammonium molybdate at pH approximately 1.2 reacts with silicate and any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicic acid. Even if phosphate is known to be absent, the addition of oxalic acid is highly desirable and mandatory in this method. The intensity of the yellow colour developed is proportional to the concentration of " molybdate- reactive" silica.

4.5.2 INTERFERENCE

(i) Both glassware and reagents may contribute to silica. Avoid using glassware as much as possible and use reagents of low silica. Also make blank determinations to correct for silica introduced.

(ii) Tannin, large amounts of iron, colour, turbidity, sulphide, and phosphate interfere. Treatment with oxalic acid eliminates interference from phosphate and decreases interference from tannin. Photometric compensation is used to cancel interference from colour or turbidity.

4.5.3 CHEMICALS AND SOLUTIONS

4.5.3.1Chemicals

Sodium bicarbonate ($NaHCO_3$)
Purity: Analar
Producer: BDH Chemical Ltd.

Sulphuric acid (HCl)
Purity: Analar
Producer: BDH Chemical Ltd.

Hydrochloric acid (HCl)
Purity: Analar
Producer: BDH Chemical Ltd.

Ammonium molybdate $(NH4)_6Mo_7O_{24}.4H_2O$
Purity: Analar
Producer: BDH Chemical Ltd.
Oxalic acid ($H_2C_2O_4.H_2O$)
Purity: Analar
Producer: BDH Chemical Ltd.

Sodium hexafluorosilicate (Na_2SiF_6)
Purity: Analar
Producer: BDH Chemical Ltd.

Sodium metasilicate ($Na_2SiO_3.9H_2O$)
Purity: Analar
Producer: BDH Chemical Ltd.

4.5.3.2 Solutions

(a) Ammonia molybdate reagent

Dissolve 10 g $(NH4)_6Mo_7O_{24}.4H_2O$ in distilled water with stirring and gentle warming and dilute to 100 ml. Filter if necessary. Adjust to pH 7 to 8 with silica- free NH4OH or NaOH and store in a polyethylene bottle to stabilise.

(b) Oxalic acid solution

Dissolve 7.5 g $H_2C_2O_4.H_2O$ in distilled water and dilute to 100 ml.

(c) Hydrochloric acid, 1+1

(d) Stock silica solution (1000 mg/l)

Dissolve 3.1303 g Na_2SiF_6 (previously dried at 105°C for 1 hr) in a distilled water and dilute to one litre in a volumetric flask. Transfer solution to a polyethylene container for storage.

OR

Dissolve 4.73 g $Na_2SiO_3.9H_2O$, in distilled water and dilute to 1000 ml.

(e) Intermediate silica standard solution (50 mg/l)

Transfer 5 ml of the stock silica solution into 100 ml volumetric flask and dilute to the mark with distilled water.

(f) Control standard (10 mg/l SiO_2)

Transfer 20 ml of the intermediate standard solution into a 100 ml volumetric flask and dilute to the mark with distilled water.

4.5.3.3 INSTRUMENTS AND LABORATORY EQUIPMENT

Spectrophotometer

Volumetric flask (100 ml)

Pipette(5 ml, 10 ml)

Volumetric flask (1L)

Cuvette (1 cm path length)

4.5.3.4 CALIBRATION CURVE

From the intermediate silica standard solution, prepare silica standards within the range 1- 30 mg/l . Add the colour developing reagents, read the absorbances and plot a graph.

4.5.3.5 DETERMINATION

(i) To 50 ml sample add in rapid sucession 1.0 ml 1-1 HCl and 2.0 ml ammonium molybdate reagent.

(ii) Mix by inverting at least six times and let it stand for 5 to 10 min.

(iii) Add 2 ml oxalic acid solution and mix thouroughly.

(iv) Read colour after 2 min but before 15 min at 410 nm, measuring time from addition of oxalic acid.

(v) To correct for colour and turbidity, prepare a blank by adding HCl and oxalic acid but no molybdate reagent. Adjust the photometer to zero absorbance with the blank containing no molybdate before reading absorbance of molybdate-treated samples.

4.5.3.6 CALCULATIONS AND EXPRESSION OF RESULTS

Silica concentration is determined directly from the calibration curve
Results are expressed in mg/l SiO2 to two decimal

4.6 CHLOROPHYLLS

The phytoplankton biomass is estimated by fluorimetry and spectrophotometry. The first technique permits detection of chlorophyll by measurement of the concentration of chlorophyll a.This measurement can be done by fluorimetry and spectophotometry. The first technique permits detection of chlorophyll a only, even at low concentrations. The latter permits measurement of chlorophylls a, b and c, but with much les sensitivity.

MATERIAL AND CHEMICALS

UV/visible fluorimeter

UV/visible spectrophotometer

Cryotubes

Vacuum pump

11 amber glass bottles

GF/F filter of 0.7 µm pore size and 25mm diameter (fluorimetric measurment) or 47mm diameter (spectrophotometric measurement)

Centrifuge

Methanol/ethanol , absolute

Acetone

Hydrochloric acid, 1 N

METHOD

Collect water samples in 11 amber glass bottles and transport to the laboratory in coolers (away from light).

Filter through GF/F filters (0.7 µm pore size) immediately upon arrival, envelope each filter in aluminum foil and store in the refrigerator until analysis.

Introduce filter paper into a centrifuge tube, and add 10ml of extraction solvent; methanol/ethanol for spectrophotometry, and 90% acetone for fluorimetry.

Disintegrate filter to bits and pieces using a glass rod or a glass tube with sharp edge, cork the centrifuge tube and shake.

Allow extraction in acetone for about 20 hours in the refrigerator, or in methanol for an hour at room temperature.

Warm up to room temperature, if necessary, adjust volume to 10ml, cork the centrifuge tube and shake

Centrifuge for a minute at 3000-4000rpm.

Remove tubes from centrifuge and gently shake off pieces of filter paper on the wall of the tube into the solvent.

Centrifuge again for 5 to 10 minutes at 3000 – 4000 rpm; cork tubes to avoid evaporation.

Collect supernatant in a tube, cork and store for analysis.

4.6.1. FLUORIMETRIC DETERMINATION

CALIBRATION OF FLOURIMETER

Extract chlorophyll from fresh spinach or from pure chlorophyll (e.g. Sigma C5753) in 97% or pure methanol.

Measure the OD at 750nm (turbidity blank) and at 665nm using a spectrophotometer.

Acidify extract with 0.5 N HCl and measure its OD at 665nm.

Chlorophyll concentration (mg/1) = 12.98 x (OD665 – OD730), for 1cm cuvette.

To verify that chlorophyll extract is pure, calculate the ratio t = OD665/OD650, which must be between 1.93 and 2.0.

If extraction is done in 90% acetone, OD readings of extract are taken at 730nm (turbidity blank) and at 663nm, and that of acidified extract at 665nm. In this case, chlorophyll concentration (mg/1) = 11.23 x ($OD_{665} - OD_{730}$), and the ratio t = OD_{665} (for purity of chlorophyll) must be between 1.75 and 1.80.

DETERMINATION OF CHLOROPHYLL IN SAMPLE

Read the fluorescence before (Fo) and after (Fa) acidification (addition of 2 drops of HCL 1N to sample extract). Note the value of Fa at the same time interval for each determination (e.g. 2 minutes).

Calculate chlorophyll concentration without pheopigments as follows:

[Chl] (µg/l)= k x 10^3 x Fo x (v/V) x (1/D)

where k: slope of graph of concentration versus fluorescence

v: volume of extract (ml)

V: volume of filtered water sample (ml)

D: dilution factor

Calculate chlorophyll concentration with pheopigments as follows:

[Chl] in µg/L = k x 103 x (t/{t-1)) x (Fo – Fa) x (v/V) x (1/D)

4.6.3 SPECTROPHOTOMETRIC MEASUREMENT

Read OD of extracts at 730, 630,645 and 663nm, acidify with 2 drops HC1 1 N, wait for a given time (to be standardized), and read OD at 650nm (methanol as solvent) or 663nm (acetone as solvent).

Calculate chlorophyll concentration from a set of equations using net values (ODpic – OD730), such as the trichromatic equations:

[Chl a](µg/l)= (11.43 x OD663 – 2.16 x OD645 – 0.11 x OD630) x F

[Chl b] (µg/l)= (20.97 x OD645 – 3.94 x OD663 – 3.66 x OD630) x F

[Chl c] (µg/l)= (54.22 x OD630 – 14.81 x OD645 – 5.53 x OD663) x F

Where F: v/(1 x V) ratio calculated from the volume v of extract (ml)

V: volume of filtered water sample (ml)

The optical path of the cuvette is 1cm.

NOTE: In the open sea, chlorophyll values are situated between 2 and 10 µg/l at water surface, and between 0.2 and 1 µg/1 at profound depth. In the lagoon, surface concentrations are usually between 5 and 75 µg/l. Higher values are indicative of eutrophication in the area.

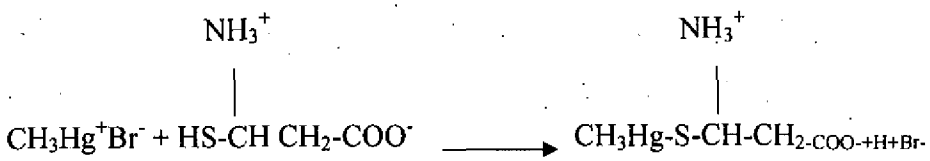
It should be noted that in both sea and lagoon, water "coloration" phenomenon (algal bloom) can occur, reflecting a temporarily rich medium. The life span of the algae population hardly exceeds a tidal cycle.

4.7 ANALYSIS OF MERCURY BY GAS CHROMATOGRAPHY

Methylmercury accumulates in marine organism because it is bound to sulphur-containing amino-acids (such as cystein) in the protein. This bond can be broken by means of a strong acid such as bromic acid (HBr), which forms methymercury bromide.

$CH3Hg\text{-}S\text{-protein} + H^+Br^- \; CH_3Hg^+Br^- + HS\text{-protein}$

The methymercury bromide is extracted with benzene and purified by conversion into a water-soluble complex by means of cystein hydrochloride.



The complex is dissociated with HBr, and the resulting methymercury bromide is extracted with benzene and analyzed by gas chromatography.

6.5.1. CHEMICALS

Concentrated hydrochloric acid (d20°C=1. 191 g/ml)

Sodium bromide

Anhydrous sodium sulphate

Benzene (glass-distilled)

Teramilic alcohol

4.7.1. STANDARD SOLUTIONS

Stock Solution: (100 mg Hg/ml|). Weigh 0.1254 g of methyl mercury chloride (CH_3HgCl) and make into a solution in 1000ml of distilled water. This solution is stable for years.

Calibration solution: (1.00 mg Hg/ml): Pipette 1 ml of the above stock solution into a 100 ml volumetric flask, and bring up to mark with distilled water. This solution should not be kept for more than two days.

Stock solutions for GC. 100 mg Hg/ml. Weigh 0.1254 g CH_3HgCI and make into a solution in 1000 ml of benzene. This solution is stable for years.

Calibration solutions for GC. Make appropriate dilutions of the stock solution for GC (0.05, 0.1, 0.2, 0.3 ml in 1000ml of benzene) to obtain calibrations solutions containing 0.05, 0.10, 0.20, 0.3 ng Hg/ml. These solutions are stable for several months.

Cystein solution 1%: dissolve 1.00 g cystein hydrochloride ($1.H_2O$), 0.775 g sodium acetate ($3.H_2O$) and 12.5 g anhydrous sodium sulphate in 50 ml distilled water. Dilute to 100ml with ethanol.

4.7.2. SAMPLE EXTRACTION AND CLEAN-UP

Homogenize about 1.0 g dry weight of tissue sample of marine organism with 60 ml of water in a big centrifuge bottle.

Add 14 ml concentrated HCl and 10 g sodium bromide (NaBr), and mix.

Add 70ml benzene, shake for about 10 minutes vigorously by hand, and centrifuge.
Recover the entire benzene phase (note the volume Vb), and transfer 50 ml therefore into a separatory funnel.
E) Add 6 ml of the 1% cystein solution and shake vigorously for about 2 minutes. If there is too much emulsion or foaming present, add an antifoam agent such as teramilic alcohol.
Centrifuge again, recover the clear water layer (note the volume Vw), and transfer 2 ml thereof into a test tube.
Acidify with 1.2 ml 6N HCl, add 0.5 g NaBr, and extract with 4 ml benzene by shaking for about 2 minutes.
Recover the benzene phase, dry by passing through a small glass column containing anhydrous sodium sulphate, and note the volume (Vt). This extract constitutes the test solution.
Make a blank extraction by replacing the tissue sample with 5 ml distilled water.
Also make extractions of 1,2,3,4 and 5ml of the calibration solution (see 4.5.2.above) in place of the tissue sample.

4.7.3. GAS CHROMATOGRAPHY

COLUMN PREPARATION

Analysis of methylmercury is done on gas chromatography equipped with an electron capture detector ($^3H_1$) the glass column (1.8 x 4 mm I.D) acid contain lithium chloride and 5% phenyldiethanolamine succinate on acid-washed silanised Chromosorb w (60-80 mesh).
Preferably, ready-made commercial columns should be used. Otherwise, prepare a column as described below:
dissolve 0.5 g lithium chloride and 1.5 g phenyl diethanol diethylamine succinate in 2.5 ml ethanol and 50 ml acetone in a round bottom flask.
Add 10g acid-washed and silanised (hexamethyl-disilane, 10%) Chromosorb W, and evacuate the flask with a vacuum pump until all air bubbles have disappeared
Transfer the mixture into a glass filter funnel, remove the liquid by suction, and air-dry the solid on filter paper.
Prepare a glass column as indicated above
Attach a funnel to the inlet and fill the column with the dry Chromosorb W by gently tapping.

Connect the inlet to the injector and heat the column under low gas flow rate (25-30 ml N2/min) at 210^0C for 18 hours without attaching the outlet to the detector.

Allow the column to cool, weigh, label and store.

COLUMN TESTING

inlet and the outlet of the column (ready-made or laboratory-made) to the injector and detector of the gas Chromatography, respectively. Set the apparatus to normal operating conditions as follows: gas flow, 60-70 ml N_2/min; column tem Connect the perature, 175^0C; njection temperature, 200^0C; detector temperature, 205^0C.

Saturate the column with methylmercury by injecting repeatedly large amounts (about 10 ng in 5 ml in benzene) of an appropriate dilution of the CH_3HgCl stock solution in benzene.

Check the efficiency of the column by injecting 0.10 ng Hg as CHgHgCl from an

appropriate dilution of the above stock solution. The peak height should be at least 20% of the maximal observable height at saturation.

If not, improve the column by injecting repeatedly about 40 ng Hg as benzene solution of methymercury iodide or methoxyethyl-mercury. These solutions are prepared by adding sodium iodide to a water solution of CH_3HgCl or metoxyethyl-mercury hydroxide, extracting with benzene, and drying the benzene layer with anhydrous sodium sulphate.

QUANTIFICATION

Set up the chromatography to normal operating conditions as indicated above.

Inject 5 ml benzene as solvent blank followed by 5 ml each of the GC calibrating solutions containing 0.05, 0.1, 0.2 and 0.3 ng Hg/ml (equivalent to 0.25, 1.0 and 1.5 ng Hg).

Inject 5 ml each of the extracts of 1,2,3,4 and 5 ml of the calibrations.

Inject 5 ml of the test solution and measure the height of the peak obtained.

Deduce the concentration of methyl mercury from the above plot

Compute the amount (Q) of methyl mercury in the sample as follows:

$$Q\ (\mu g\ Hg/kg\ DW) = \frac{C}{5} \times \frac{1000 \times VB2}{DW} \times \frac{Vw}{2} \times \frac{VB1}{50}$$

Where C: amount in ng of methyl mercury in 5ml of the test solution

VB1: volume in ml of the first benzene phase

Vw: volume in ml of the water layer

VB2: volume in ml of the second benzene phase

DW = dry weight in g of sample analyzed

Express Q per g fresh weight by taking into account the dry matter content of the original fresh sample.

DETECTION LIMIT

For the determination of methylmercury in biological material after liberation with a strong acid from a homogenized sample, the detection limit is 0.001 $mgkg^{-1}$ FW.

QUALITY CONTROL

Estimate the precision of the entire analytical procedure by analyzing 5 subsamples from one original sample. If the coefficient of variation is greater than 20%, check the procedure for possible errors and contamination. A standardization test can also be used for the estimation of the precision of the gas chromatographic analysis.

If the quality control checks reveal a fluctuation in the standard deviation for the accuracy by more than 5%, check the following factors: stability of stock solutions (prepare new solutions); instrumental drift or inadvertent changes in operational parameters; contamination of the working matrix (select alternative reference material for analysis); contamination of equipment (e.g. glassware) and operator error (s).

4. 8. PETROLEUM HYDROCARBONS

4.8.1.INTRODUCTION

Hydrocarbons form a very complex mixture in the aquatic environment (lagoon and sea). They originate from diverse and varied sources: either from chronic or accidental discharge of petroleum products (alkanes mixed with simple, alkylated or sulphur-containing aromatic compounds, etc.), from atmospheric fallout due to complete combustion of organic matter (predominance of non alkylated PAH), or sometimes from the biosynthesis of organic matter. The qualitative and quantitative study of these compounds in sedimentary or bio-organic matrices,

where they are generally more abundant because of their fat solubility, poses a problem, especially when it concerns individuals molecules.

In the last two decades, many studies have been done on hydrocarbons, especially polycyclic aromatic hydrocarbons (PAH), because of their carcinogenic and mutagenic effects in man. Many analytical methods have been developed, according to means available and set objectives. Among these can be mentioned the method for total hydrocarbondetermination by infrared spectrometry, and that for the estimate of aromatic hydrocarbons by UV-spectrofluorimetry alone or coupled to high performance liquid chromatograph.

Although spectrofluorimetry appears to be a very sensitive method, and less prone to interference's for the analysis of PAHs, it has the inconveniences of relatively long analytical time, high solvent consumption and frequent bubble formation in chromatographic columns.

4.8.2. PRINCIPLE

Under the present project "Large Marine Ecosystem of the Gulf of Guinea", gas chromatography seems to be one of the rapid methods for control of pollution by hydrocarbons. Its system of detection by flame ionization, and a judicious choice of column, permits the determination of total hydrocarbons and the 18 PAH's particularly recommended by the US-EPA for monitoring the quality of the environment. Good indicator matrice for monitoring this of pollution are living organisms and sediment.

4.8.3 SAMPLING AND SAMPLE TREATMENT.

Prescribed procedures for sampling, sample transport and treatment for petroleum hydrocarbon analysis are generally the same as those for the particulate, attention should be paid to the following:

Material used (knife, scalpel, packaging, containers, etc.) should be metallic, glass or Teflon Samples should be freeze-dried (in preference) or dried in an oven at 60^0C, in the absence of a freeze-[dryer. However, it should be noted that drying in an oven causes volatilization of light hydrocarbons such as alkanes and naphthalenics.

All contact of sample with oil, grease or plastic material should be

avoided. Aluminum foil and containers for wrapping and conservation of sample should be cleaned with he hexane before use.

Living organisms must be rinsed with alcohol immediately after sampling on the field. During sampling of muscle tissue on the dorsal (lateral) inside of fish, the fatty part of the abdomen should be avoided.

4.8.4 EQUIPMENT

-Soxhlet apparatus

-Kuderna-Danish tubes

-25 or 500 ml burette

-500 and 1000 ml amber bottles

-200 to 500 ml separation funnels

-Assorted glassware (with telfon caps)

-Rotary evaporator

-Furnace

-Corer

-Freeze-dryer

-Oven

-Vortex mixer or ultrasonic bath

-Gas chromatograph with flame ionization detector, "split / splitless" Injector and integrator.

-Molten silica capillary column (type WCOT) filled with CpSil 8 CB (reticular phase), with length 50 m, diameter 0.32 mm, film thickness 0.11mm and efficiency of about 160,000 theoretical plates

-High-pressure cylinder with nitrogen

-Nitrogen-purification kit

-High-pressure cylinder with oxygen

-High-pressure cylinder with hydrogen

-Micro syringes

4.8.5.ABSORBANTS

Silica gel

Alumina

Florosil

4.8.6. SOLVENTS AND REAGENTS

Hexane

Isopropane

Dichloromethane

Methanol

Pentane

Iso-octane

Carbon tetrachloride

Sulphuric acid

Anhydrous sodium sulphate

Standard alkane compounds, from C15 to C32

Standard PAH compounds, from Naphthalene to Indeno-(1,2,3. cd)- pyrene.

Internal standards: perdeuterised pyrene, perdueterised tetracosane

Rack for evaporation under nitrogen

NOTE: Reagents and solvents used should be for "residue analysis"

4.8.7 PREPARATION OF CALIBRATION SOLUTIONS

PREPARATION OF STOCK SOLUTIONS OF 10^{-4} mol/l

Make tiny weighing dishes from hexane-cleaned aluminum foil.

Weigh about M ng of standard compound in a dish (M being the molecular weight number).

Carefully introduce the dish into a 10 ml vial using a tweezers.

Add 10 ml of isopropane, cork the vial and stir on a vortex mixer or in an ultrasonic bath until dissolution of the compound.

Store at 4^0C in the refrigerator.

PREPARATIOPN OF CALIBRATION SOLUTIONS OF 10^{-5} mol/l

Take 1 ml of each stock solution in a 10 ml volumetric flask, complete to the mark with hexane, cork and mix.

NOTE: Calibration solutions are prepared before each analysis.

PREPARATION OF DISTILLED WATER

Use glass-bidistilled water free from organic material. It can be prepared by two successive distillations as follows:

Add $KMnO_4$ to tap water (0.1 g/l) and redistill.

Add $KMnO_4$ to the distilled water (0.1 g/l) and redistill.

Extract the bidistilled water with hexane in a funnel, and store in a container previously washed with hexane.

4.8.8. EXTRACTION AND FRACTIONS OF HYDROCARBONS

a) EXTRACTION

Weigh about 5 g of dried powdered sample (organism or sediment) and place in an extraction thimble.

Add 50 ml each of the following internal standard solutions (0.1 ng/ml in hexane); perdueterised tetracosane ($C_{24}D_{50}$), perdeuterised pyrene ($C_{16}D_{10}$) and Dotriacontane (n-C_{32}).

Plug the thimble with hexane-precleaned glass wool and place in Soxhlet apparatus.

Extract with 200 ml methanol for 8 hours (regulate heat to obtain 20 to 50 extraction cycles in all)

Add 20 ml of KOH (0.7 mol/l 39.2 g of KOH per litre) and 30 ml bidistilled water in the sox let flask, and extract further until complete saponification of liquids.

Transfer the extract to a separatory funnel, add 90 ml hexane and shake for about 3 minutes.

Collect the upper hexane fraction, and rinse the methanolic fraction twice with hexane, using 50ml hexane each time.

Pool the three hexane fractions together and filter through hexane-precleaned glass wool.

Add 5 g of anhydrous sodium sulphate to the filtered hexane extract to eliminate residual water.

Recover water-free hexane extract and concentrate to 15 ml at 300C (at most) in a rotary evaporator under light vacuum.

Concentrate further in a Kuderna-danish evaporator (graduated conical tube) to 5ml (i.e.1 ml/g of sample extracted).

Concentrate even further to 1 ml under clean nitrogen gas current and store in an airtight glass vial until chromatography on silica/alumina column.

b) DETERMINATION OF EXTRACTABLE ORGANIC MATERIAL (E.O.M)

Acidify the methanolic phase obtained above with 1 M sulphuric acid, extract thrice with hexane (30 ml each time) and concentrate to a known volume.

Take an aliquot of the concentrate, evaporate in a water bath and determine the weight of the resulting residue. Express the weight of residue (in the whole concentrate) as a percentage of the dry weight of sample extracted.

c) TREATMENT OF ABSORBANTS

Extract the absorbants (Silica gel, alumina and florosil 60-100 mesh) with methanol in a Soxhlet for 8 hours and then with hexane for the same duration.

Dry them in an oven at 60°C and then at 200°c for 8 hours. Store in an amber bottle (away from light) until needed.

Before use, activate a required amount of the absorbant at 200°C for 4 hours in an oven, and deactivate partially with 5% distilled water. Florosil is activated at 300°C.

4.8:9. ABSORPTION CHROMATOGRAPGHY ON FLOROSIL COLUMN

Plug a glass column (1 cm I.D.) or a 50 ml burette with hexane-precleaned glass wool. Fill with pentane, pour in 10 g of florosil and allow the gel to settle into an oven bed. Overlay the gel with a 1 cm layer thick of sodium sulphate previously cleaned with hexane, avoiding air bubbles. Drain the pentane.

Wash the column immediately with 50 ml hexane and drain to about 0.5 cm above the sodium sulphate.

Apply the hexane extract obtained above (1 ml at the top of the column, complete to 2 ml with 1 ml hexane and elute the hydrocarbons with 60 ml of pentane.

4.8.10. SEPARATION OF SATURATED AND AROMATIC HYDROCARBONS BY SILICA/ALUMINA GEL COLUMN CHROMATOGRAPHY

Plug a glass column (6 mm ID) or a 50 ml burette with hexane-precleaned glass wool and pour in 10 ml of silica gel hexane (minimum amount). Let the gel settle by gently tapping on the column. Then pour 10 ml of an alumina slurry (in hexane) and allow to settle.

Drain the solvent to just about 0.5 cm hexane above the alumina bed, close the tap and begin chromatography of the pentane extract.

4.8.11. GAS CHROMATOGRAPHY

a) REGULATION OF GAS FLOW-RATE

The operational conditions depend on the type of chromatograph and column. For determination of hydrocarbons on capillary columns, the conditions are as follows:

Flow-rate of carrier gas = 2 ml/mn

Hydrogen pressure = 0,7 bar

Oxygen pressure = 1,2 bar

b) TEMPERATURE PROGRAMMING

To elute heavy products (such as petroleum hydrocarbons) fast and with sharp peaks, the temperature programme should be set as follows:

T1 = 50^0C for 2mn

T2 = 180^0C for 2mn

T3 = 290^0C for 30mn

R1 = 10^0C per minute (passage from T1 to T2)

R2 = 2^0C per minute (passage from T2 to T3)

Time for splitless: 30s

NOTE: When setting the temperature programme, it should be remembered that thecapillary column does not withstand temperatures higher than 300^0C in isothermal regime, and 325^0C under programmed regime. In addition, it is advisable to start with a temperature at least 200C below boiling point of the solvent.

c) TESTING OF COLUMN EFFICIENCY

Before analysis, test the efficiency of each column given by the formula:

$$N = 5.54 \times \frac{[Tr]^2}{[b_{1/2}]}$$

Where Tr: retention time of standard (in minutes)

$b_{1/2}$: peak width of standard at half height (in minutes)

The efficiency is tested using standards with relatively short retention times. For capillary columns 50 meters long, the efficiency is about 160,000 theoretical plates.

d) RETENTION TIME AND RESPONSE FACTOR OF STANDARDS.

Inject 1 to 2ml (m ng) of each standard and note the retention time and area of peak in minutes on the integrator printout. The response factor of the standard is given by the relationship:

$$RF_{st} = \frac{\text{Peak area}}{\text{Wt. Sample injected}} = \frac{A_{st}}{m_a} = \frac{A_{st}}{m_{at} \times C_{at} \times V_{inj} \times 10^{-6} \times 10^{9}}$$

$$RF_{st} = \frac{A_{st}}{M_{st} \times C_{st} \times V_{inj} \times 10^{3}}$$

Where M_{st} : molecular weight of standard (in grammes)

A_{st} : peak area of standard (in absolute units)

W_s = sample weight injected (in ng)

C_{st} = concentration of standard solution (mol/l)

V_{inj} = volume of standard solution injected (in ml)

RF_{st} is expressed in ng-1

The mean response factor (RF_m) is obtained by calculating the arithmetic means of aliphatic and aromatics separately.

$$\mathbf{RF_m} = \frac{\sum \mathbf{RF_i}}{ni}$$

where Rfi : response factor of standard I

ni : number of standards used for determination of total hydrocarbons.

4.8.12 DETERMINATION OF TOTAL SATURATED ALIPHATIC COMPOUNDS (FRACTION 1)

Inject 1 to 2 ml of fraction 1 (F1) and note total area of the peaks. (At).

Calculate overall content of aliphatic (CALT) according to the following

Formula:

$$CALT = \frac{At}{RF_m} \times \frac{Vt}{V_{inj}} \times \frac{1}{DW_s} \times \frac{100}{P_r}$$

Where CALT: total content of aliphatic (ng/g sample dry weight)

At: total area of peaks of fraction F1 injected (in integrator unit)

Vt: *total volume of* F1 injected (nl)

RF_m: mean response factor of aliphatics (in area unit/ng standard)

V_{inj} : volume of F1 injected (nl)

Dw_s: total dry weight of sample extracted (g)

Pr: Stands for percentage recovery, given by:

$$P_r = \frac{m_f}{m_o} \times 100$$

4.8.13 DETERMINATION OF INDIVIDUAL PAH's

Inject 1 to 2 nl (m ng) of each standard, note the retention time and then calculate the response factor RF for each.

Inject 1 to 2 nl of sample extract (fraction 2) under the same experimental conditions as for the standard. Assign the chromatographic peaks to the compounds under study by comparing with those of the standards. The connection Ci of each PAH in the sample is given by:

$$C_{PAH\,I} = \frac{Ai}{RF_i} \times \frac{Vt}{V_{inj}} \times \frac{1}{DW_s} \times \frac{100}{P_r}$$

Where $C_{PAH\,I}$: PAH I content (ng/g sample dry weight)

Ai : area of PAH I peak in fraction F2 injected (in integrator unit)

Vt : total volume of fraction F2 (in µl)

RF_i: response factor of PAH I

V_{inj}: volume of F2 Injected (µl)

DW_s: total dry weight of sample extracted (g)

Pr: percentage recovery

NOTE:

It is also possible to, express hydrocarbon content with respect to fat content. This is done by dividing the hydrocarbon content by the extraction organic material of the sample.

4.8.14. ANALYTICAL BLANK

It is necessary to include blanks in each series of analysis. It permits evaluation of the level of contamination associated with the analytical procedure. Proceed as follows:

a) Add the same amount of internal standards in an empty soxhlet extraction thimble as for the sample.

b) Extract for the same duration, reconcentrate, purify and separate the different fractions.

c) Analyze by gas chromatography.

The amount of pollutant in the blank is deduced from that determined in the sample.

Table 4: ***List of standard compounds***

Aliphatic standards

Aliphatic standards		Aromatic Standards	
Saturated Aliphatic Standards	Molecular Weight (g/mol)	Aromatic Standards	Molecular Weight (g/mol)
C_{15}	212	Naphtalene*	128
C_{16}	226	Biphenyle*	154
C_{17}	240	Acenaphlene*	154
C_{18}	254	Acenaphtlene*	154
C_{19}	268	Fluorene*	166
C_{20}	282	Anthrancene*	178
C_{21}	296	Phenanthrene*	178
C_{22}	310	Pyrene*	202
C_{23}	324	perdeuteriosed pyrene*	212
C_{24}	**338**	**Fluoranthene***	**202**
C_{25}	352	Benzo(a)anthracene*	228
C_{26}	366	Triphenylene	228
C_{27}	380	Chrysene*	228
C_{28}	**394**	**Naphtacene**	**228**
C_{29}	408	Benzo(b)fluranthene*	252
C_{30}	542	Benzo(i)Fluoranthene*	252
C_{31}	560	Benzo(k)fluoranthene*	252
C_{32}	578	Benzo(a)pyrene*	252
C_{33}	596	Benzo(e)pyrene	252
C_{34}	614	Perylene	252
$C_{24}D_{50}$	388	Chroranthrene	254
		7,12 Dimethy l benzo(a)anthracene	256
		Dibenzo (a,h) anthracene*	278
		Dibenzo(a,j)anthracene	278
		Dibenzo(a,c)anthracene	278
		Benzo(g,h,j)perylene*	278
		Ideno(1,2,3,cd)pyrene*	278
		Anthanthrene	278
		Benzo(h)thiophene	134
		Dibenzo(b,d)thiophene	184
		Naphto(1,2,b)thiophene	184

4.9 BACTERIOLOGICAL ANALYSES OF WATER AND SEDIMENTS

SAMPLING METHODS:

SURFACE WATER SAMPLING

Plunge sterile sampling bottle 5 to 20 cm below water surface and allow to fill up.
Pull to the surface, cork and store in a cooler at 4^0C.

DEEP WATER SAMPLING (AT WATER-SEDIMENT INTERFACE)

Plung deep-water sampler (e.g. Zobell J-Z type), to the required water depth and allow the sterile bottle to fill up.
Pull sampler to the surface, remove the bottle and keep in a cooler at 4^0C.

SAMPLING OF SEDIMENT

Lower sediment sampler (van Donsel and Geldreich type) or a corer to the water bed and collect sediment.
Raise the device to the surface, sample sediment in two parts (0-2 and 2-5 cm), transfer subsamples to individual sterile containers and keep in a cooler 4^oC.

NOTE:

All samples must be kept in the cold and analyzed as soon as possible, not later than 12 hours after sampling.

4.9.1 ENUMERATION OF INDICATOR MICROORGANISMS OF FAECAL CONTAMINATION

ENUMERATION IN WATER

METHOD: MEMBRANE FILTRATION

Filter 1 to 100 ml of sample (according to the degree of pollution and turbidity of water body) under vacuum on sterile nitrocellulose filter (0.45 nm) using the filtration rack.

Rinse filter with about 20 ml phosphate buffer or physiological serum.

Shut off the vacuum and remove the filter aseptically with a flame-sterilized metallic tweezer.

Place filter on appropriate agar culture medium (in a Petri dish), avoiding the formation of air bubbles between the filter ad agar.

Incubate the Petri dish inverted under conditions (temperature and duration) specified for each microorganism.

SPREAD PLATE TECHNIQUE

Pipette 0.1 ml of water sample (diluted or not) on appropriate agar culture medium and spread aseptically with a flame-sterilization spreader.

Incubate the Petri dish inverted under conditions (temperature) specified for each microorganism.

REMARKS: The dilution factor of samples in phosphate buffer depends on the degree of pollution: 10^{-2} to 10^{-4} for untreated wastewater; 1, 10^{-1},10^{-2} for polluted lagoon water; 100, 10, 1ml (no dilutions) for sea water.

ENUMERATION OF *E. coli*

PRESUMPTION TEST

It is done on desoxycholate lactose agar or any other media available in 90 mm Petri dishes.

After placing the filter (containing sample) or spreading the sample on the agar culture medium (see 4.1.1.1. and 4.1.1.2, respectively), incubate Petri dishes inverted at 44^0C for 24h.Count *E. Coli* bacteria, which form characteristic shiny red circular colonies of 1 to 2 mm diameter. Note number of colonies for Petri dishes containing 20 to 200 only.

CONFIRMATION TEST:

Do a confirmation test for acid and gas production by aseptically inoculating a loopful of suspicious colonies in tubes of brilliant-green lactose bile broth containing Durham tubes. Incubate tubes at 44^0C for 24h and note those (positive) with bacterial growth and formation of gas in the Durham tubes.

Alternatively, do a confirmation test for indole production by inoculating suspicious colonies in tubes of peptone water containing 1 ml indole reagent. Incubate tubes at 37^0C for 24h and note positive tubes with red ring formation.

NOTE: Confirmation test is done on suspicious colonies (not quite shiny red) when they constitute more than 10% of the total colony count for the presumption test.

EXPRESSION OF RESULTS

Considering that each colony is produce by one microorganism, the number of presumptive indicator is calculated as follows:

$Np = Cp \times 100/V \times D = CFU/100$

where Np: number of presumptive colonies in 100 ml sample

Cp: dilution factor of cultured sample

D: dilution factor of cultured sample

V: volume of cultured sample (ml)

For the confirmation test, the number of indicator micro-organisms is given by the formula: $N_c = (C_p - C_n) \times 100/v \times D$

Where N_c: number of confirmed colonies in 100 ml of sample

C_m: number of confirmed presumption colonies

4.9.2 ENUMERATION OF ENTEROCOCCI

PRESUMPTION TEST

It is done on D-cocossel agar or any other available media in 90 mm diameter Petri dishes. Lay membrane (after filtration), or plate sample on agar culture medium and incubate the Petri dish (inverted) at 37^0C for 24h.

Count Enterococci appearing as small red colonies with dark peripheries. Record number of colonies for dishes containing 20 to 200 only.

Determine the number of presumptive bacterial count in 100 ml of sample as for E. coli.

CONFIRMATION TEST

It consists in differentiating (by a catalase test) between Enterococci and Listeria monocytogenes which all have the same growth characteristics on D-cocossel agar.

Streak at least 10% of presumptive colonies on fresh D-cocossel agar and incubate at 37^0C for 24h.

For hydrogen peroxide solution (3.5%) into each dish to just cover the agar surface.

Colonies wth formation of bubbles (catalase positive) are Listeria. Enterococci and catalase negative.

EXPRESSION OF RESULTS

The number of Enterococci is calculated as follows:

$Np = Cp \times 100/V \times D = CFU/100ml$

Where Np : number of presumptive colonies in 100 ml of sample

Cp: number of presumptive colonies(Enterococci+ Listeria)

D: dilution factor of cultured sample

V: volume of cultured sample (ml)

For the confirmation test, the number of indicator micro-organisms is given by the formula:

$$N = \frac{N_p N_c \times 100}{N_t} == CFU/100ml$$

Where Np : number of presumptive colonies

Nc : number of confirmed colonies

Nt : number of tested colonies

4.9.3 ENUMERATION OF MICROORGANISMS IN SEDIMENT

Indicator microorganisms are estimated in sediments by the Most Probable Number (MPN) method after cultured decimal dilutions of the sample in the appropriate broth (3 culture tubes per dilution). Dilutions are done according to the degree of pollution of the area and possible microbial load of the sample (e.g. 1, 10^{-1} and 10^{-2}).

FAECAL COLIFORMS

i) PRESUMPTION TEST

a) Make appropriate decimal dilutions of sample in sterile phosphate buffer.
b) Pipette 1.0 ml each dilution in a tube (3 per dilution containing 10 ml of lactose broth and an inverted Durham tube.
c) Incubate tubes for 24 to 48 h and at 44^0C for faecal coliform determinations.
d) Note positive tubes with bacterial growth and gas production in the Durham tubes.

CONFIRMATION TEST

a) Shake each tube and inoculate a drop of its content (with a pasteur pipette) in another tube containing 10 ml of brilliant-green lactose bile broth and an inverted Durham tube.
b) Incubate tubes for 24 to 48h at 37^0C for total coliform, and at 44^0C for faecal coliform determinations.
c) Note positive tubes with bacterial growth and gas production in the Durham tubes.
d) Express results as most probable number of microorganism per milliliter of sample

4.9.4 FAECAL STREPTOCOCCI

i) PRESUMPTIVE TEST

Make appropriate decimal dilution of sample in sterile phosphate buffer

Pipet 1.0 ml pf each dilution in a tube (3 per dilution) containing 10 ml of Roche broth.

Incubate for 24 to 48h at 37^0C and note positive (cloudy) tubes with bacterial growth.

ii) ***CONFIRMATION TEST***

Shake each tube and inoculate a drop of its content (with a Pasteur pipette) in another tube containing 10 ml 0f Litsky broth.

Incubate for 24 to 48h at 37^0C and note positive (cloudy) tubes with bacterial growth.

Express results as most probable number of microorganism per milliliter of sample.

4.9.5 CHARACTERISTIC VALUES FOR INDICATOR MICROORGANISMS

There are norms on the presence of coliforms in clean natural waters, established for temperate regions. These values should be applied with caution in the tropics where the situation is different. However, natural tropical waters should be free of pathogens (WHO guidelines, 1983)

Micro-organism	Guide number (/100ml)	Maximum number (/100ml)
Total coliforms	500	10000
Faecal coliforms	100	2000
Faecal streptococci	100	------

4.9.6 ENUMERATION OF BACTERIA PATHOGENS

4.9.6.1 SEARCH FOR *Vibrio cholerae*

Vibrio Cholerae are straight or curved rod-shaped gram-bacteria with monotrichous polar flagellum. They aerobic, oxydase-, more or less basophilic (pH 8,6), and ferment glucose without gas production.

Estimate of *V. cholerae* in water is done by filteration and then culture of filter

membrane on thiousuiphate-citrate-bille-sucrose agar (TCBS). It is estimated in sediment by the most probable number method after enrichment of decimal dilutions of the sample (3 tubes per dilution).

ENRICHMENT CULTURE

Make two successive enrichment cultures of each decimal sample dilution in biotryticase alkaline and saline (10 gl^{-1} NaCl) nutritive broth or alkaline peptone water (pH 8,6) for 24h at 37^0C.

ISOLATION OF MICROORGANISMS

Plate the resulting surface growth of the enrichment culture on TCBS suspicious colonies on Mueller-Hinton agar, and incubate at 37^0C for 24h. Repeat the operation twice to check purity of colonies.

Check colonies belonging to *V. cholerae* species by standard biochemical tests such as: oxydase, urease, indole production, glucose and manitol fermentation, lysine and ornithine decarboxylase, arginine dihydrolase and tryptophan deaminase or use identification kits.

SEROLOGICAL IDENTIFICATION

It consists in an agglutination test on previously isolated bacteria with *V. cholerae* biochemical characteristics, using *V. cholerae* 01 antiserum.

a) Deposit a drop of serum 01 on a clean slide.

b) Add the bacterial culture (form Mueller-Hinton agar) to the serum and homogenize.

c) Observe the slide against a dark background or on a concave mirror; occurrence of agglutination is a positive test for *V. cholerae*.

NOTE: Germs isolated on selective media could auto-agglutinate. This should be verified by running a control in physiological water.

4.9.6.2 SEARCH FOR PESUDOMONAS AERUGINOSA

Pseudomonas aeruginosa present in water can be estimated by membrane filtration and culture on M-PA agar. These bacteria are determined in sediment by the most probable number method after dilution and enrichment.

4.9.7 DETERMINATION IN WATER BY MEMBRANE FILTRATION

i) PRESUMPTION TEST

Filter an appropriate aliquot of water as indicated

Place the filter on m-PA agar and incubate at 41.5 ^{0}C for 72h.

Count typical *P. aeruginosa* colonies: flat, with a slightly transparent edge, a brown or greenish-black center, and 0.8 to 2.2.mm diameter.

ii) CONFIRMATION TEST

Streak a proportion (at least 10%) of the typical *P. aeruginosa* on milk agar and incubate at 37^{0}C for 24h.

Note typical *P. aeruginosa* colonies with yellowish-green colour.

Calculate the number of bacteria in 100 ml as indicated above

4.9.8 DETERMINATION IN SEDIMENT BY THE MOST PROBABLE NUMBER METHOD

i) PRESUMPTION TEST

Make decimal dilutions of sediment sample and culture in tubes containing 10 ml aspraragine broth (3 tubes per dilution).

Incubate at 35-37^{0}C for 24h, then for 48h. Examine tubes under ultra violet light in a dark room: production of fluorescence constitutes a positive test.

ii) ***CONFIRMATION TEST***

Incubate at least 10% of the positive tubes (with Pasteur pipette) in tubes containing acetamide broth.

Incubate 35-37^{0}C for 24 to 36h: change of colour from medium to purple (alkaline pH) is a positive test.

Determination number of bacteria in 100 g of sample as indicated above.

4.9.9 PREPARATION OF CULTURE MEDIA SOLUTIONS AND BUFFERS

DOUBLE CONCERNTRATED LACTOSE BROTH

It is used for analyzing samples of 10 to 100 ml volume, and is composed of:

Beef extract	6 g
Peptone	10 g
Lactose	10 g
Distilled water	1000 ml

a) Dissolve by heating, cool and adjust pH to 6.9 with phosphoric acid

b) Dispense in 220x22 ml (10 ml tubes with Durham tubes.

c) Sterilize for 20 minutes at 120^{0}C in an autoclave.

For analysis, add equal volume of sample to double concentrated broth.

NORMAL LACTOSE BROTH

This formula is used for analyzing samples of 0.1 to 1 ml volume, and the composition is as follows:

Beef extract	3 g
Peptone	5 g
Lactose	5 g
Distilled water	1000 ml

Dissolved by heating, cool and adjust pH to 6.9 with phosphoric acid.

Dispense in 220x22 ml (10 ml) tubes with Durham tubes.

Sterilize for 20 minutes at 120^{0}C.

For analysis, add equal volume of sample to broth.

LACTOSE BILE BRILLIANT-GREEN BROTH.

Exists commercially in dehydrated form. It can be prepared in the laboratory as follows:

Peptone	10 g
Lactose	10 g
Ox bile salt	20 g
Brilliant-green (0.1% aqueous soln)	13.3 ml
Distilled water	1000 ml

Dissolve peptone and lactose in 500 ml distilled water and add bile salt (dissolved in 200 ml distilled water).

Mix and adjust volume to 950 ml and pH to 7.

Add 13.3 ml of 0.1% aqueous brilliant-green solution and dilute to 1000 ml with distilled.

Dispense in 220x22 ml (10 ml) tubes with Durham tubes.

Sterilize for 20 minutes at 120^0C in an autoclave.

ROTHE BROTH

Peptone	20. 0 g	Potassium phosphate	2. 7 g
Glucose	5. 0 g	Potassium hydrogen phosphate	2. 7 g
Sodium chloride	5. 0 g	Sodium azohydrate	0.2g
Distilled water	1000 ml		

a) Mix until completely dissolved.

b) Adjust pH to 6.8-7.0, if necessary, and dispense 10 ml in tubes.

c) Sterilize in an autoclave at 115^0C for 20 mn.

LITSKY BROTH

Peptone	20. 0 g	K hydrogen phosphate	2.7 g
Glucose	5. 0 g	Sodium Azohydrate	0.3 g
Sodium chloride	5.0 g	Ethyl-violet	0.0005 g

Potassium Phosphate 2. 7 g Distilled water 1000 ml

Mix until completely dissolved.

Adjust pH to 6.8-7.0, if necessary, and dispense 10ml in tubes.

Sterilize in an autoclave at 115^0C for 20 minutes.

BIO-TRYPTIPCASE ALKALINE AND SALINE NUTRITIVE BROTH

Trypticase peptone	17.0g	K hydrogen phosphate	2.5g
Phytone peptone	3.0g	Glucose	2.5g
Sodium chloride	10.0g	Distilled water	1000ml

a) Adjust pH to 8.6 and dispense as appropriate in round-bottomed flasks.

b) Sterilize 15min at 121^0C.

THIOSULPHATE – CITRATE – BILE – SUCROSE AGAR (TCBS)

It comprises the following ingredients:

Yeast extract	5g	Ox bile	8.0g
Peptone	10g	Sodium chloride	10.0g
Sucrose	20g	Ferric citrate	1.0g
Sodium thiosulphate	10g	Thymol blue	0.05g
Sodium citrate	10g	Bromothymol blue	0.04g
Agar – No 1	10.0g		

a) Stir ingredients in all distilled water and bill until completely dissolved.

Do not autoclave.

b) Cool to 45^0C and dispense 15 to 20ml in 90mm (diameter) Petri dishes.

MUELLER – HINTON BROTH

Beef maceration	300g	Starch	1.5g
Acid casein hydrolysate	7.5g	Agar – No 1	10.0g.

Complete to 1 l with distilled water and wait for 5mn.

Mix to a homogeneous suspension.

Heat under gentle and frequent shaking, and boil until complete dissolution.

Adjust pH to 7.4, if necessary, and dispense in tubes.

Sterilize in an autoclave at 121^{0}C for at most 15min.

M-PA AGAR

Its composition is as follows:

L-lysine HCl	5.0g	Lactose	1.25g
Sodium chloride	5.0g	Phenol red	0.08g
Yeast extract	2.0g	Fe ammonium citrate	0.08g
Xylose	2.5g	Sodium thiosulphate	6.8g
Sucrose	1.25g	Agar	15.0g
Distilled water	1000ml		

a) Adjust pH to 6.5 and sterilize in an autoclave at 120^{0}C for 20min.

b) Cool to 45-65^{0}C, adjust pH to 7.1 and add the following antibiotics per litre: sulfapyridin, 176mg; kanamycine, 8.5mg; Nalidixic acid, 37mg; cycloheximide, 50mg.

c) Mix and dispense 3ml portions in Petri dishes (55mm diameter) and store at 2 to 10^{0}C (at most before use).

MILK AGAR

It is made of two parts:

Medium A: Skimmed milk	100.0g	Distilled water	500ml
Medium B: Nutritive broth	1 2.5g	Sodium chloride	2.5g
Agar	15.0g	Distilled water	500ml

a) Sterilize the two media separately and cool quickly to 55^{0}C.

b) Mix both and pour into Petri dishes.

The alternative: Milk Agar can also be used

Formula:	Yeast Extract	3g
	Peptone	5g
	Milk solids (equiv to 10ml fresh milk)	1g
	Agar No.	3 20g
	pH	7.2 approx

Suspend 24g in 1l of distilled water. bring to boil to dissolve completely. Sterilize at 121^{0}C for 15 min

ASPARAGINE BROTH.

Its composition is as follows:

DL – asparagines	3.0g	Anhydrous K phosphate	1g
Magnesium sulphate	0.5g	Distilled water	1000ml

Adjust pH to 6.9-7.2, sterilize and cool.

ACETAMIDE BROTH

It is composed of:

Magnesium sulphate	0.5g	Acetamide	10g
Anhyd. K dihydrogen phosphate	0.73g	Sodium chloride	5g
Anhyd. K dihydrogen phosphate	1.39g	Phenol red	0.012g

Adjust pH to 6.9-7.2, sterilize and cool.

PEPTONE WATER

Peptone	10.0g	Sodium chloride	5.0g
Distilled water	1000ml		

a) Adjust pH to 7.2 or 8.6 for normal or alkaline peptone water. Peptone. 10g, NaCl-10g, Distilled water, 1000ml (for the ten-fold strength required; only 100ml of distilled water is used for preparation.

b) Dispense 9ml in 220x22ml (10ml) tubes or an appropriate volume in 500ml round bottom flasks.

c) Sterilize in an autoclave at 121^{0}C for 15 mn.

KOVAC'S REAGENT FOR INDOLE

Para-dimethy/ amino bezaldehyde	1g.
Iso-amyl alcohol,	15 ml
Concentrated H_2SO_4,	5 ml

The aldehyde is dissolved in the alcohol and the acid added slowly. Regent is stored in the refrigerator.

PHYSIOLOGICAL SERUM

A) Dissolve 9g NaCl in distilled water and complete to 1 litre.

B) Sterilize by filtration on cellulose nitrate or acetate membrane (0.45 µm) or in an autoclave at 121^{0}C for 15 mn.

PHOSPHATE BUFFER

A) Dissolve 34g potassium dihydrogen phosphate in 500 ml distilled water.

B) Adjust pH to 7 with NaOH solution, dilute to 1000ml and store (stock solution).

C) Before use, add 1.25ml of the stock buffer solution to 1l distilled water and sterilize at 121^{0}C for 15mn.

Table 5 most probable number (MPN) Index for three tubes and three dilutions (10^{-1}, 10^{-2} et 10^{-3}).

Number of positive tubes for dilutions			MPN per gramme or millilitre	95% confidence limits	
10^{-1}	10^{-2}	10^{-3}		lower	upper
0	0	0	<3	-	-
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	389
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800
3	3	3	>2400		

SECTION THREE

TREATMENT OF RESULTS

CHAPTER 5

5.1 DATA MANAGEMENT IN WATER POLLUTION MONITORING:

Data analysis and presentation, together with interpretation of the results and report writing, form the last steps in the water monitoring process. It is this phase that shows how the monitoring operation has been in attaining the objectives of the assessment.

It is also these steps that provide the information needed for decision making, such as choosing the most appropriate solution to a water quality problem, assessing the state of the environment or refining the water quality monitoring process itself.

Although computers now help the process of data handling and presentation considerably, these activities are still very labour intensive, involving a good understanding of statistics as it applies to the science of water and sediments analyses and assessment.

Data Handling and Presentation

Before the data from any monitoring programme are used, it is important to ensure that the confidence limits be established and reported. This is to ensure that the confidence with which recorded numbers handled and interpreted are not misplaced. It is also necessary to decide how the data should be handled for future reference and use.

Errors of Accuracy and Precision:

The results from any monitoring programme can be subject to errors of accuracy and precision. Precision may be high and accuracy poor in which case, all results for a set of

analyses of the same sample will be very close together, for example, differing by no more than one percent (1 %), though they may differ from the true result by much more. Some errors will arise from the nature of the samples. These can be minimised by proper statistical design of the sampling procedures and attention given to the collection of uncontaminated sample.

All analytical procedures have inherent errors in precision and accuracy. To a greater or lesser extent either or both types of error can be compounded by operator or laboratory errors, some of which are often not recognised.

However, by using good analytical equipment and methods and following a rigorous analytical quality assurance scheme, it should be possible to achieve high accuracy and precision for all analytical data, and allow quantification of the scale of errors.

Intercomparability Requirements:

In most cases where monitoring programmes are operated on multilateral basis it is essential that the results obtained by all contributors are truly comparable. Establishing comparable monitoring programmes may prove difficult. However, it is desirable that targets be set for comparability of data. Analytical comparability is also one method of monitoring data and methods of analysis.

The actual programmes run by different countries must also be comparable. One may be on water analysis, another on fish species and sediments. Even when agreement is reached on whether to sample water, biota or sediments it will be necessary to agree, for example, which species of fish should be used, whether the water should be filtered before analysis or whether whole sediment should be analysed or only a particular size fraction.

Requirement for Analytical Quality Control:

It may be impossible to arrange that all contributors use identical analytical procedures. Even if they do, for the reasons given previously, intercomparability is not guaranteed. To establish whether differences do exist and to minimize them, a programme of

intercalibration is essential. Each laboratory should assure the quality of its data by participating in intercalibration exercises and analysing at intervals reference materials containing certified concentrations of the pollutants of interest in appropriate matrices and concentrations. Data quality control is a complex and time consuming activity which must be undertaken continuously to ensure meaningful water quality assessments. This is particularly crucial for some of the chemical analyses undertaken on water samples, such as dissolved trace elements, pesticides or even ammonia and phosphates.

5.2 DATA STORAGE, RETRIEVAL AND EXCHANGE:

Designing a water quality data storage system needs careful consideration to ensure that all the relevant information is stored such that it maintains data accuracy and allows easy access, retrieval and manipulation of the data.

Depending on the scale of the monitoring programmes various methods of data storage and transfer may be appropriate. It is essential that the design of the storage/retrieval system be carefully worked out to reflect the end use of the data in both raw and interpreted form.

The most efficient method is in many respects to use a computer. It is essential that the limitations of any set of data be instantly recognized when it is achieved.
To this end, information such as performance in a recognised intercalibration exercise, analysis or reference materials, etc. should be retrievable with the data. Ideally the data should be freely accessible by all contributors and the scientific community in general. However, if a country or groups of contributors wish certain types of data to be available only to a limited audience that wish must be safeguarded.
Regions may exhibit different natural backgrounds on baseline concentration, have different resources to be protected and exposed to different pollutants. As a consequence their monitoring programmes may differ - for example, different fish species may be used as indicators, permissible limits differ according to exposure patterns and different targets may be set for sampling and analytical accuracy. Therefore it will probably be more practical and effective, at least initially, to organise monitoring programmes and data storage on a regional rather than a global basis.

Once a satisfactory level of regional comparability has been achieved, inter regional comparability should follow on a logical progression.

INFORMATION RETRIEVAL:

The primary purpose of a database is to make data rapidly and conveniently available to users. The data may be available to users interactively in a computer database, through a number of customised routines or by means of standard tables and graphs.

The various codes used to identify the data enable fast and easy retrieval of the stored information. Tables provide an easy and convenient way of presenting the information generated. Graphs, including maps, also facilitate data presentation, analysis and understanding.

CHAPTER 6

QUALITY ASSURANCE / QUALITY CONTROL

INTRODUCTION

The broad objective of the pollution monitoring programme is the acquisition of reliable and relevant data for the purpose of protection of the Gulf of Guinea coastal zone against land-based and other sources of pollution. This will constitute the anchor identifying polluted sites and recognizing trends for planning and implementation purposes. For the success of this programme therefore, an awareness of the usefulness of a sound Quality Assurance/Quality Control (QA/QC) and Good Laboratory Practice is paramount.

This section therefore attempts to summarize the important steps for ensuring a QA/QC and Good Laboratory practice for use by participating laboratories.

6.1 QUALITY ASSURANCE (QA)

DEFINITION

Quality Assurance is a set of principles that, if strictly followed during sample collection and analysis, will produce reliable, relevant and defensible data. It is a system of activities that ensures that resultant data are derived from dependable and reliable procedur.es

QUALITY ASSURANCE PLANNING

This ensures the establishment of a set of principles that will constitute a QA programme .

Specifically, the following are contained in a QA plan:

-Cover sheet for QA plan title and plan approval signature .

-Staff organization and responsibilities

-Sample control and documentation procedures

-Standard operating procedures for each analytical method

-Analyst training requirements

-Equipment preventive maintenance procedures

-Calibration procedures

-Corrective actions

-Internal quality control activities

-Performance actions

-Data assessment procedures for bias and precision

-Data reduction, validation and reporting

Stock acceptance

Training (Refresher course) for laboratory staff

6.2 QUALITY CONTROL (QC)

DEFINITION

Quality control is a set of steps taken to minimize error .It is an integral component of QA and it could be external or internal. When it is external it is described as Quality Assessment.

STEPS FOR GOOD QC

They include the following :

-Certificate of operator competence

-Recovery of known additions (internal standards)

-Analysis of externally supplied standards

-Analysis of reagent blanks

-Calibration with standards

-Analysis of duplicates

-Maintenance of control chart.

CERTIFICATE OF OPERATOR COMPETENCE

The analyst must be certified by a competent person. Usually an operator is deemed competent if an acceptable precision is demonstrated after analysis of a minimum of four replicate of an independently prepared check sample having concentrations between 5 to 50 times the method detection level (MDL)for the analysis in that laboratory.

6.2.1. RECOVERY OF KNOWN ADDITONS (INTERNAL STANDARDS)

Use the recovery of known additions (internal standards) to eliminate matrix effect. Always use the recovery factor in the calculation of the final concentrations.

6.2.2. ANALYSIS OF EXTERNALLY SUPPLIED STANDARDS

Use externally supplied standards whenever the recovery of an internal standard or know addition is not acceptable. An acceptable recovery level should not be lower than 50%.

6.2.3 ANALYSIS OF REAGENT BLANKS

Always analyse a reagent blank whenever new reagent are used . 5 % of the sample load is the minimum requirement for analysis.

6.2.4. CALIBRATION WITH STANDARBS

When using standards to calibrate :

a) Measure three different dilutions of the standards .

b) Verify daily the standard curve by analyzing one more standard within the linear range.

c) Report only results within the range of standard dilutions used.

d) Do not report values above the highest standard unless an initial demonstration of greater linear range has been made, no instrument parameter has been changed, and the value is less than 1.5 times the highest standard.

e) Lowest reportable is the MDL, provided the lowest calibration standard is less than 10 x the MDL.

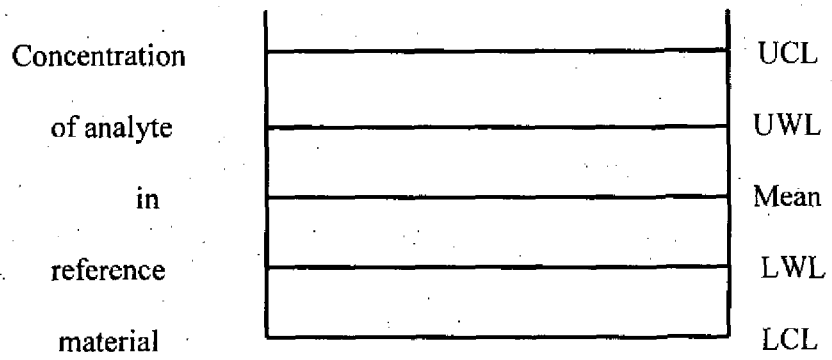
f) If blank is subtracted, report its value.

6.2.5. ANALYSIS OF DUPLICATE

Analyse 5 % or more of the sample in duplicate to assess precision .

6.2.6. CONTROL CHARTS

Control chart enables objective evaluation of quality of data .To construct a control chart proceed as follows :



Periodic measurement of reference material

Figure 1. Blank control chart

UCL = Upper Control Level

LCL = Lower Control Level

UWL = Upper Warning Level

LWL = Lower Warning Level

a) Select the reference material (RM) to be analyzed with samples on a regular basis.

b) Analyze the RM at least 10 times for the analyte under examination

These analyses should not be done on the same day but spread over a period of time in an attempt to ensure that the full range of random errors within and between batch analyses are covered.

c)Calculate the mean (x),and the standard deviation (r) and then plot the following values on a blank control chart:

x,

$x + 2r = UWL$

$x + 3r = UCL$

$x - 2r = LWL$

$x - 3r = LCL$

6.2.7 USING A QUALITY CONTROL CHART

Figure 1 represents a typical use of the Quality Control chart . To use, the following guidelines are important to note :

a) A single result falling outside the warning limit may not require any action provided the next result falls within the warning limit.

b) Assess source of systematic error if results fall outside warning limit too frequently, particularly if the same warning limit has been more than once on consecutive results.

c) Check the analytical procedure to determine source of error if on more than one successive occasion result fall on the same side of x line (either between x and UWL or x and LWL).

d) Check the analytical procedure to determine the cause of error if results fall outside the UCL and LCL.

e) Results of particular batch of samples should be rejected if any of a to d occurs , and no further analysis until source of error is identified and corrected .

6.3 QUALITY ASSESSMENT

Quality Assessment is the process of using external and internal control measures to evaluate the quality of the data being processed by the laboratory

This includes :

-Performance evaluation samples

- Laboratory inter comparison samples

-Performance audits

-Internal QC

6.3.1 PEREFORMANCE EVALUATION SAMPLES

Use known amount of constituents of interest for determination to evaluate analyte recovery .Recovery should be within the range of acceptable and predetermined recovery levels.

6.3.2 LABORATORY INTERCOMPARISON SAMPLES

Laboratory should participate in inter laboratory calibration exercises.A quarterly participation is acceptable.

6.3.3 PERFORMANCE AUDITS

Develop a checklist for standard operation procedure and make unscheduled visit to detect deviation from standard operation procedure .

6.3.4 FIELD SAMPLE COLLECTION

A sample collected is at best representative of the point of collection . In order to achieve the Objective, this project which among others requires analyzing samples, which are representative of the body of the water of interest , a sampling design strategy is therefore an essential component of this programme . The following guidelines are provided for sampling design.

a) Visit site to determine accessibility, point sources and extent of water.

b) If accessible, select all point sources and determine the coordinate using GPS. Describe the coordinates and write the level of the point source.

c) Move to the centre of body of water if possible and randomly sample along determined grids having the centre of body of water as the focus.

d) For in-situ parameters, determine immediately on location, and for sample to be analyzed elsewhere follow recommended sample collection containers and preservation methods.

e) For microbiological samples, it is preferred that inoculation and incubation start as soon as collected, if possible on the field.

6.3.5 CHAIN-OF-CUSTODY PROCEDURES

This procedure is the process, which creates the ability to trace possession and handling of sample from the time of collection through analysis to final disposition. This is an essential component of the programme which will ensure sample handling from analysis to data reporting.

The following are the required guidelines:

a) Sample labels: use indelible labeling

b) Sample seals: seal sample if required

c) Field log book: Record all information pertinent to a field survey or sampling in a bound log book. Minimum information included:

- Purpose of sampling

- Location of sampling point
- Name and address of field contact
- Procedure of material being handled
- Type of sample
- Method of preservation (if applicable)
- Number of samples taken
- Volume of sample taken
- Description of sampling point
- Description of sampling method
- Date and time of collection
- Collector'name and identification number(s)
- Sample distribution and transport
- References such as maps and photographs of sampling site
- Field observations and measurements
- Signature of personnel responsible for observation
- Protect logbook and keep it in safe place

6.3.6 CHAIN- OF-CUSTODY RECORD

Fill out a chain-of –custody record to accompany each sample or group of samples.

The records include :

Sample number

- Signature of collector
- Date, time and address of collector
- Sample type
- Signature of persons involved in the chain of possession, including

date of possession .

6.3.7 SAMPLE ANALYSIS AND REQUEST SHEET

Sample collector completes the field portion of request sheet, which includes most of pertinent information noted in the log book. The laboratory portion of the form is completed by laboratory personnel and includes :

- Name of person receiving sample
- Laboratory sample number
- Date of sample receipt
- Determinations to be performed

6.3.8 SAMPLE DELIVERY TO THE LABORATORY

a) Deliver sample to the laboratory as soon as practicable

b) Ensure that samples are accompanied by a chain-of –custody record and a sample analysis request sheet.

c) Deliver sample to sample-custodian.

6.3.9 RECEIPT AND LOGGING OF SAMPLE

In the laboratory the sample custodian inspects the condition of sample, reconciles information against the chain-of-custody record, assigns a laboratory number, logs sample in the laboratory log book and stores in a secured storage room/cabinet until it is assigned to an analyst .

6.3.10 ASSIGNMENT OF SAMPLES FOR ANALYSIS

The laboratory supervisor usually assigns samples for analysis. Once a sample is in the laboratory , the laboratory supervisor or analyst is responsible for its care and custody.

6.3.11. SUPERVISION

The laboratory analysis is closely supervised by a competent person designated as the laboratory supervisor

6.3.12 DETERMINING NUMBER OF SAMPLES

In view of the random variations in both analytical procedures and the occurrence of a constituent at a point of sampling , a single sample may not be sufficient for a desired level of uncertainty.

In that case , if an overall standard deviation is known , the required number of samples will be established by the following relationship:

$$N = \frac{(tr)^2}{u^2}$$

Where N= number of samples

t = student- t statistic for a given confidence level

r = overall standard deviation

u = acceptable level of uncertainty.

EPILOGUE

From Laboratory Data to Policy Tool:

(i) The ultimate objective of water pollution monitoring is to generate data which, when interpreted, should provide information for the decision making process. The data from water pollution monitoring programmes should not merely serve as a list of variables and their concentrations, but should be assembled and interpreted by experts with relevant recommendations, and forwarded for management action. A mechanism for regional co-ordination of water pollution data would enable a comprehensive assessment and understanding of the water quality trends in the inshore waters of the GCLME region. This is the tool required for efficient management and protection of the in-shore waters of the region vis-a-vis her coastal zone development programmes.

(ii) The usefulness of the information obtained from monitoring is severely limited unless an administrative and legal framework (together with an institutional and financial commitment to appropriate follow-up action) *exists* at local regional, or even international level.

Once decision-makers have determined the desired present interim and long term uses and associated objectives for a water body, a number of control strategies may be employed to achieve those objectives. To a very large extent, information derived from laboratory and field data generated in water quality monitoring exercises are the most reliable empirical pieces of information tool in the hands of the policy maker.

In the course of decision - making process to determine the appropriate line of action for the use, development or protection of a particular sector of the water environment, the laboratory data so generated by the water quality monitoring exercise enables appropriate placement of the social, economic and political factors in the decision-making matrix.

Moreover decision-makers need to be convinced that investments in enhancing the quality of the nation's water resource will directly contribute to social, economic and even political benefits.

In the long run also, water quality data generated should provide the necessary information to help the decision makers draft policies and development strategies that respond to international needs in the sector.

In summary, the systematic collection and analysis of data yields vital information to the resource manager, including quantification of existing conditions, identification of information gaps and projection of future trends. Routine monitoring also provides feedback to the manager, making possible the adjustment of management actions. Ultimately, data collection and analysis should result in an understanding of the "carrying capacity" or limits for sustainable use of the system and an ability to predict the effects of changes to the system.

Policy Implementation

Timing of intervention strategies sequel to responsive policy/decision-making process could make the difference between effective application of results from the monitoring programme and a wasted economic investment.

To justify the effort invested in a pollution-monitoring programme, the policy makers must summon the political will to apply the necessary resources to remedial action in good time for optimum impact. The water pollution scenario in the GCLME region deserves nothing less than prompt and effective intervention measures,if the deterioration of coastal and marine resources in the region must be halted.

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GEF GUINEA CURRENT LARGE MARINE ECOSYSTEM PROJECT

MARINE POLLUTION MONITORING MANUAL (VOLUME II)

POLLUTION MONITORING USING MARINE WATER, SEDIMENTS AND ORGANISMS

(A TRAINING MANUAL FOR COASTAL AND MARINE POLLUTION MONITORING FOR THE GCLME REGION)

APRIL, 2006

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SAMPLING PROCEDURE FOR DETERMINATION OF HEAVY METALS, METHYLMERCURY, CHLORINATED AND PETROLEUM HYDRPOCARBONS

1. SELECTION OF MATERIAL:

Monitoring of pollution in the marine environment, such as the Guinea Current Large Marine Ecosystem (GCLME), essentially employs the use of sediments and living organisms as the matrices of choice.

Water and suspended particles could also be used, as options, depending on the interest areas of laboratories conducting the monitoring.

1.1 MANDATORY MATERIALS

Generally, sediments and living organisms fall under the mandatory category of materials for marine pollution monitoring

1.1.1 SEDIMENTS

Samples to be analysed shall be those collected at 0-2 cm and 2-5 cm below the water bed.

1.1.2. LIVING ORGANISMS

In general, organisms for marine pollution monitoring should be selected according to the following criteria:

a) They should be easy to identify.

b) They should be sedentary and/or abundant in the area of study.

c) They should be sufficiently long-lived to allow sampling of more than one year class.

d) They should be geographically widespread to allow comparison of contamination levels in several sampling sites.

e) They should tolerate brackish water

f) If possible, there should exist a simple relationship between contaminant levels in the material and those in the surrounding seawater.

g) Where possible, the organisms studied should represent different levels in the food chain and different systematic groups with low, as well as with high, fat content.

The organisms recommended for marine pollution monitoring in the Gulf of Guinea are listed below.

1.1.2.1 **Plants**

Plants such as water hyacinth, moss, etc., which are known to be good indicators of aquatic pollution should be used.

1.1.2.2 **Fish**

- *Sphyraena sphyraena* or barracuda, pelagic
- *Cynglossus* or tonguesole, benthic.
- *Pseudotolithus senegalensis* or cassava croaker, lives in shallow waters, on muddy, sandy or rocky bottoms.
- *Sardinella* maderensis or madeiran sardinella, lives in warm coastal waters, sometimes in lagoons and estuaries.
- *Liza facipinnis* or sicklefin mullet, lives in coastal marine and brackish waters.
- *Boops boops* or bogue, semi-pelagic species found normally at 0-100 meters deep.
- *Sardina pilchardus* or European pilchard.

1.2.1.3 **Molluses**

They are good filter feeders. The following species shall be sampled:

- *Ostrea edulis* or flat oyster
- *Ostrea denticullata* or sea-rock oyster
- *Gryphea gasar* or mangrove oyster

1.2.1.4 **Crustaceans**

- *Penaeus notialis* or the pink shrimp

2. FIELD SAMPLING, TRANSPORT AND SAMPLE TREATMENT

The following points should be taken into consideration for field sampling and for transport and preparation of samples.

a) Sampling sites should be selected according to the expected presence or absence of pollution.

b) They should be clearly identified (geographical coordinates) and positioned on a map, and their depths and general characteristics (including pollution) described.

c) Preferably, field sampling should be done by experienced staff who are aware of the precautions to be taken to avoid further contamination. They should accompany new staff on their first field visit and on later visits to monitor their work.

d) It I of advantage to do sampling from a research or chartered vessel. However, fish and shellfish specimens could be purchased from local fishermen.

e) All specimens should be labelled and properly packaged immediately after collection. Labelling should include essential information, such as date, sampling site and code number.

f) Log books should be available on the field to record details of essential information on samples and sampling site characteristics.

g) Use plastic bags, jars, screw-cap bottles, knives, spatula, etc. for samples to be analysed for heavy metals, and similar equipment made of glass for samples required for analyses of pesticide residue and organic compounds. Stainless steel or teflon sampling and packaging gear can also be used for both types of samples. Plastic screw-caps of bottles containing samples for pesticide residue analyses should be lined with aluminum foil.

2.1 **SEDIMENT**

a) Collect core sample from water bed using a gravity or piston corer or devices. It is recommended to use a Van Veen Grab ($0.1m^2$) provided with two doors at the top which are closed during sampling.

b) Pull the grab with content on board the vessel, and allow water to drain.

c) Open the doors and drive in a clean glass tube with open ends (1. D. > 1 cm) into the sediment. Close the free end of the tube hermetically with a stopper and pull it out with its content. Extrude sediment from the glass tube and sample into two portions of 0-2 cm and 2-5 cm from the top using a plastic stainless steel spatula.

Collect 25-50 g fresh weight of each portion by repeating extraction with glass tube several times. Put each specimen in a screw-cap glass bottle precleaned with 6.5% nitric acid (analytical grade) and glass-distilled water and transport to the laboratory at 4°C in thermo-insulated boxes containing coolers (ice in heavy duty plastic bags or commercially available cooling bags).

Alternatively, transport the corer with its content to the laboratory at 4°C, and freeze. Extrude frozen sediment from the corer and cut into two portions of 0-2 cm and 2-5 cm from the top. Collect 25-50 g fresh weight of each portion and put in a pre-cleaned screw-cap glass bottle.

d) Remove extraneous material from the sediment, note the weight and dry to constant weight by one of the following methods (in descending order of preference):

- Freeze-drying.
- Drying in a vacuum oven at 50-60°C.
- Drying in an ordinary oven at about 80°C.

NOTE: Never dry any material intended for analysis at 105°C, as there may be loss of volatile substances.

e) Determine the weight of the dried sediment and calculate the dry weight to fresh weight ratio (R). Express as percentage dry matter by multiplying by a hundred (Rx100%).

f) Homogenise the dried sediment with a stainless steel homogeniser, or with a mortar and pestle (glass, quartz or teflon).

g) Sieve through a 63 um screen and analyse the silt-size fraction (< 63 um). The fraction of larger particles may be analysed, if necessary.

h) Store sample in an airtight plastic/glass container until analysis.

2.2 SEA WATER

a) Collect water sample using a bottle that can be opened and closed at the required water depth.

b) Homogenise by shaking bottle and pour aliquots of 100 ml in appropriate 200 ml bottles with screw-caps.

c) Transport samples to the laboratory at about 4°C in thermo-insulated boxes containing coolers, and store in refrigerator at 4°C until treatment time.

d) First filter water through a 200 um sieve to remove coarse particles, and then through a GF/F 0.7 um filter. Acidify the filtrate with concentrated HNO_3 (3.6 ml analytical grade/litre water) to convert all the metals into the inorganic form, and store at room temperature until analysis.

e) Store water samples for analysis of organic compounds in amber bottles to avoid exposure to sunlight.

Note: Filter and acidify water within 3 to 4 hours after sampling to avoid possible absorption of the metals on the containers' walls.

2.3 **ORGANISMS**

2.3.1 **PLANTS**

a) Collect samples and wash rapidly (about 10 minutes) in clean sea water or glass distilled water to remove surface contaminants. Let to drain, package, label and transport specimens to the laboratory as indicated below (see 2.3.2 b and c).

b) Upon arrival in the laboratory, dry to constant weight as indicated above (2.1 d). Determine the dry weight to fresh weight ratio and percentage dry matter.

c) Grind the dried plant in a stainless steel homogenizer and store in an airtight plastic/glass container until analysis.

d) If analysed fresh, determine the dry weight using another portion of the sample and heating to constant weight at 105c in an ordinary oven.

2.3.2 **FISH**

a) Put each large-size individual specimen in a plastic bag (for metal analyses) or aluminum foil (for chlorinated hydrocarbon analyses). Be careful that the fins do not make holes in the bag or foil. Squeeze out the air and close the plastic bag airtight.

For small – to medium-size specimens (which give less than 10 g muscle tissue sample) put 10-15 animals in the plastic bag/aluminum foil.

b) Put the plastic bag\foil with the animal(s) into another plastic bag and put a label in the outer bag. Roll to squeeze out air and close the bag airtight.

c) Put all the plastic bags containing specimens from the same sample into a big plastic bag, with a label inside. Transport to the laboratory in thermo-insulated boxes containing coolers.

d) Freeze specimens upon arrival in the laboratory. Remove later as required and prepare tissue samples for analysis as indicated below.

e) Remove fish from freezer and leave at room temperature until partially thawed. It is easier to cut partially thawed tissues than completely thawed ones.

f) Unrap and put the fih in a perweighed plastic/glass plate and determine the weight.. Place the specimen on a sheet of plastic/aluminum foil with the left side upward, and determine the fork-length (from tip of snout when the mouth is closed to the apex of the fork of the tail). Also note the sex (Fig. 2).

g) Cut off the pectoral fin and remove the skin from the lower part of the dorso-lateral muscle, in the middle of the fish, with plastic/stainless steel knife tweezers.

h) Cut a subsample of fillet of more than 10 g (for large-size fish) and put it in a preweighed plastic/glass container. Record and weight with a precision of 0.001 g or better.

For small- to medium-size fish, cut out pieces of fillet of approximately the same weight from several specimens. Pool them together, cut into smaller pieces, mix until there is a homogeneous mass, and put in an appropriate screw-cap bottle for immediate analysis or drying, as indicated above (2.1 d

i) Dry to constant weight, and determine the dry weight to fresh weight ratio and the percentage dry matter as indicated above.

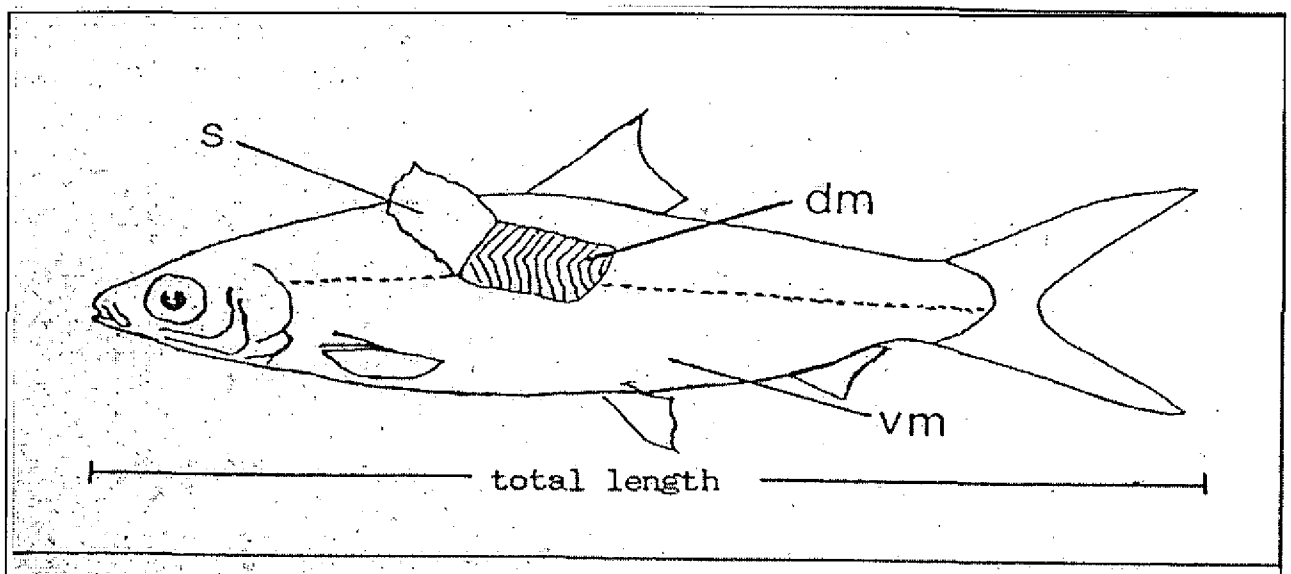

Figure 2. Dissection of a fish
S: skin being removed; dm: dorsolateral muscle; vm: ventrolateral muscle.

j) Grind the dried fish fillet into a fine powder and store in an airtight plastic/glass container until analysis.

k) If analysed fresh, determine the dry weight using another portion of the sample and heating to constant weight at 105°C in an ordinary oven.

NOTE: Remove fillet subsample from the left side of fish for heavy metal analyses, and from the right side for chlorinated hydrocarbon analyses, or vice versa.

It is recommended to also take individual or composite subsamples of the internal organs (gills, liver, brain, stomach, gonads, etc.) of predator organisms such as the barracuda.

2.3.3 **CRUSTACEANS (PINK SHRIMP)**

a) Sample as for small- to medium-size fish. Package, label, transport and freeze as above (2.3.2 a to d).

b) Remove specimens from the freezer and leave at room temperature until almost entirely thawed. Unrap them.

c) Put each shrimp in a preweighed plastic/glass plate and weigh. Place the specimen on a sheet of plastic/aluminum foil, and determine its length from rostrum to uropod (fig. 3).

d) Separate the abdomen from the cephalothorax and the tail with a plastic/stainless steel knife taking care that no viscera remain in the abdomen. Turn the abdomen with the ventral side up and remove the abdominal plates with a second knife and appropriate tweezers.

Determine and note the sex by examining the gonads.

e) Transfer the muscle into a preweighed plastic/glass container and determine its weight.

For composite sample, pool pieces of abdomen muscle from at least 6 different specimens of the same sex and size, and homogenize.

The weight of each piece should be the same as that from the smallest abdomen muscle.

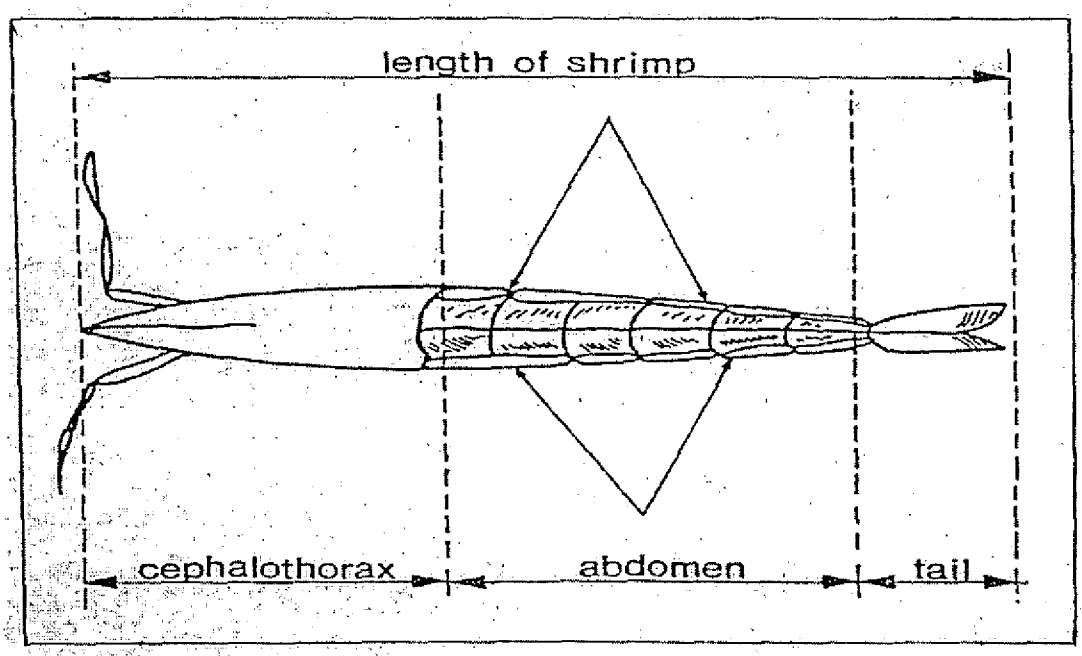

Figure 3. Schematic diagram of a shrimp (arrow indicate where to cut after the legs have b removed)

f) Dry to constant weight, and determine the dry weight to fresh weight ratio and the percentage dry matter, as indicated above (2.1 d).

g) Grind the dried muscle into a fine powder and store in an airtight plastic/glass container until analysis.

h) If analysed fresh, determine the dry weight using another portion of the sample and heating to constant weight at 105°C in an ordinary oven.

2.3.4 **MOLLUSCS (MUSSEL AND OYSTER)**

a) When possible, mussels and oysters should be carefully sampled (removed from their attachment) by a diver to avoid that particles get inside the shell.

b) Place the specimens in clean sea water for about 24 hours to allow defecation.

c) Remove them from water, let to drain and package with their valves closed. Label and freeze until analysis.

d) Take the animal out of the freezer, let it thaw completely and unrap it.

e) Scrape off all foreign material attached to the outer surface of the shell with a strong plastic/metal brush, rinse with clean sea water or glass distilled water, and let the water drain off.

f) Insert a plastic/stainless steel knife into the opening from where the byssus extrudes (without breaking it), and put the animal on a clean linen with the opening downward to let the excess water drain out. Weight the whole animal and note the weight.

g) Open the shell as wide as possible by cutting the adductor muscles and rinse the soft part in the shell with glass distilled water. Remove the soft part with a pair of plastic/stainless steel tweezers, let to drain and place in a pre-weighed plastic/glass plate.

h) Remove pieces of shell, etc. (if any) from the soft parts under a stereo microscope by means of plastic/stainless steel tweezers, and weigh.

i) Cut the soft part of big animals into smaller pieces, m ix them until there is a homogeneous mass, and put in a plastic/glass tube or jar.

For small- to medium-size animals pool 10-15 soft parts of specimens with similar size (length and weight), homogenise and put the composite sample into a plastic/glass tube or jar.

j) Dry to constant weight, and determine the dry weight to fresh weight ratio and the percentage dry matter, as indicated above.

k) Grind and dried muscle into a fine powder and store in an airtight plastic/glass container until analysis.

l) If analysed fresh, determine the dry weight using another portion of the sample and heating to constant weight at 105°C in an ordinary oven.

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