# GEF GUINEA CURRENT LARGE MARINE ECOSYSTEM PROJECT

# MARINE POLLUTION MONITORING MANUAL

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

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## SECTION ONE

# POLLUTION MONITORING IN THE COASTAL AND MARINE ENVIRONMENT OF THE GCLME

## 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 Côte d'Ivoire and Ghana), as well as generally stable subsystems, depending on nutrient input originating from land drainage, river flood waters 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 marine 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 livelihood, whilst at the same time 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 in turn directly and indirectly impacted on the socio-economic success of the populations that inhabit these expansive but relatively fragile ecosystems (GOOS Report No. 99).

Over the years the use of nitrate and phosphate based chemical fertilizers 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 the pollution of both freshwater and marine ecosystems. Pollution from these nutrients, when coupled with sewage pollution, is seen as 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.*

In recognition of the fact that the coastal and marine ecosystem of the GCLME and its resources are stressed as a result of increasing socio-economic and unsustainable developmental activities, a regionally harmonized approach to monitoring the coastal water is being developed. This Marine Pollution Monitoring Manual is the first step in this process and it is expected that it will become a reference document for all institutions involved in monitoring coastal water quality in the GCLME.

# CHAPTER 1: INTRODUCTION

## 1.1 THE NEED FOR POLLUTION MONITORING IN THE GCLME COASTAL AND MARINE ENVIRONMENT

The Guinea Current Large Marine Ecosystem (GCLME) region has witnessed a substantial increase in industrial development mostly in the area of its coastal cities. Massive rural-urban migration of populations have overwhelmed infrastructure including water supplies and sewage systems resulting in wastewater run-off into coastal waters. Nutrient inputs to the coastal and marine area from human activities has lead to eutrophication, anoxia, and subsequent 'death' of many water bodies. As a consequence, there has been a loss of near-shore and estuarine fisheries, which has negatively affected 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 summarizes 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 levels of pollution as a result of 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 blooms. 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 the status of marine fishery resources in 1994 indicated that apart from off-shore demersal resources, all other fisheries in the sub-region were 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 increasing socio-economic and unsustainable developmental activities. From surveys conducted in the various countries as detailed in the various country reports, sectoral/thematic reviews and regional synthesis reviews as well as information obtained from some of the above mentioned donor funded programmes and projects, three broad issues related to the marine, coastal and associated freshwater environment pollution in the GCLME region have been identified. These are:

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

The socio-economic and cultural implications from the above can be significant 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. Therefore, there is 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, the perceived environmental problems in the GCLME area and the causes of these problems were listed and organized under the major concerns of the GIWA methodology (Guinea Current GIWA Regional assessment 42, 2003). The root cause of many of the environmental and resource problems in the GCLME area are related to inadequate policy provision, lack of legislation, and ineffective compliance monitoring and enforcement.

## 1.2 THE REGIONAL APPROACH TO TRANSBOUNDARY POLLUTION ISSUES

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

- Loss of critical habitats 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 discard of by-catch with consequent loss of marine resources, biodiversity and biomass.
- Various states of depletion of straddling and highly migratory fisheries stocks as result of over fishing and over exploitation.
- Unplanned development of coastal areas with incidence of erosion.
- Pollution from toxic chemicals and oil spills as well as insidious pollution from ballast waters.
- Exotic biological species from ship traffic.
- High rate of coastal erosion.

Socio-economic implications including loss of revenue, food security concerns, resource use conflicts and increasing poverty.

The environmental and socio-economic impacts from land- and sea-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 blooms on the shoreline areas in the western region of Ghana is believed to originate in Cote d'Ivoire.

In spite of the various sectoral national monitoring and assessment efforts, marine monitoring data in the region has limited scope to provide integrated transboundary and regional information upon which management actions and political decisions can be based.

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 stewardship 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 for preventing, reducing and combating pollution in the marine environment, and the coastal and related inland waters of Western Africa.

The current Global Environment Facility (GEF) GCLME Project aims to facilitate the development of a regional Strategic Action Plan (SAP) for the coastal States. This approach is designed to support and supplement the 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 applied for coastal and marine environmental management. The GEF funded a pilot phase project titled, "Water Pollution Control and Biodiversity Conservation in the Gulf of Guinea Large Marine Ecosystem" which 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 US NOAA (under the United States Department of Commerce) and the collaboration of a host of national, regional and international organizations. The Gulf of Guinea LME (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 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 of the Gulf of Guinea LME Project was to build a regionally co-ordinated and integrated programme of monitoring and assessment onto already existing national infrastructures, including:

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 marine ecosystems.

The 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 transboundary issues of fisheries, pollution, coastal erosion and habitat protection that affected 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 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 GCLME project.

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

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

- A 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 for effective assessment of the pollution status in the Region.
- Upgrading the technical capacity of the Regional Centres of Excellence. Under the GCLME project, UNIDO is providing laboratory and other facilities at the Regional Centre for Pollution Assessment and Management in Owerri, Nigeria, where regional capacity development training programmes as well as reference Laboratory investigations will be undertaken.
- The development of joint efforts amongst countries and laboratories in nutrient and pollution assessment in shared waters and degraded habitats (e.g. joint cruise/expeditions).

# CHAPTER 2: PRINCIPLES IN POLLUTION MONITORING

## 2.1 WATER POLLUTION MONITORING

### 2.1.1 Introduction and Definitions

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.

The pollution monitoring process has evolved into a set of assessment activities that 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. physiochemical 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.

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

1. The objectives of the assessment were not properly defined.
2. The monitoring system was installed with insufficient knowledge of the water body.
3. There was inadequate planning of sample collection, handling, storage and analysis.
4. Data were poorly treated, handled or archived.
5. Data were improperly interpreted and reported.

### 2.1.2 Principal Elements of a Pollution Monitoring Programme

Any pollution monitoring programme must include the following principal elements:

- 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.

### 2.1.3 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 in multi-purpose monitoring programmes 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.
6. The analytical quality of data must be regularly checked through internal and external control (e.g. inter-calibration exercises).
7. 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 action.
8. 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.

### 2.1.4 The Structure of a 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 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.

## 2.2 GCLME POLLUTION MONITORING PROGRAMME DESIGN

### 2.2.1 Key Components

The key components for designing the GCLME pollution monitoring programme are summarized as follows:

**(a) Objectives:** These should take into account hydrological factors, water uses, economic development and legislative policies. The objectives should also consider whether the emphasis should be put on concentrations or loads, or spatial or temporal distributions and the most appropriate monitoring media.

**(b) Preliminary Surveys** may need to be undertaken 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 the final monitoring programme.

**(c) Monitoring Programme Design** should include parameters to be measured, source and media to be sampled, station location, sampling frequency, sampling apparatus etc.

**(d) Resource Estimation** needs to take into account the laboratory capabilities and requirements, transport costs, staffing and training needs.

**(e) Field Monitoring Operations** include *In situ* measurements, the sampling of appropriate media (water, biota, and particulate matter), sample pretreatment and preservation processes, and identification and shipment of samples.

**(f) Hydrological Monitoring** data and information is crucial to any pollution monitoring programme but is often not given due weight. Hydrological monitoring includes water discharge measurements, wave/tide level measurements, river water levels, thermal profiles etc, and should always be related to the water pollution assessment activities.

**(g) Laboratory Activities** are concerned with physical and chemical analysis, and laboratory tests and procedures relating to microbiological, biological etc.

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

**(i) Data Management and Reporting** involves the quality control of data, storage (using standard database formats), statistical analysis, trend determination, etc. and the determination of results in appropriate forms (graphs, tabulated data, spread sheets etc).

**(j) Data Interpretation** 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 judgment 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.

**(k) Water Management Recommendations and 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, the outlay for any monitoring programme should be commensurate with the socio-economical and technical/scientific development of the country, since the monitoring programme will only succeed and remain sustainable as the level of these factors available.

### 2.2.2 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. For the purposes of the GCLME Pollution Monitoring Manual, the following parameters have been considered.

1. **Physical parameters:**
   a) Dissolved Oxygen
   b) Chemical Oxygen Demand (COD) Biochemical Oxygen Demand (BOD)
   c) Total Suspended Solids (suspended particulate matter)
   d) Turbidity
   e) Total Dissolved Solids (TDS)
   f) Temperature
   g) pH
   h) Salinity
   i) Conductivity

2. **Nutrients:**
   a) Inorganic nitrogen (Nitrates, Nitrites and Ammonia)
   b) Inorganic phosphorus (soluble reactive phosphorus)
   c) Silica

3. **Human Pathogens and Indicator Organisms:**
   a) Faecal contaminants
   b) Bacterial Pathogens

4. **Phytoplankton pigment (chlorophyll)**

5. **Heavy Metals**

   Including Arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), cobalt (Co), manganese (Mn), lead (Pb), selenium (Se), nickel (Ni), vanadium (V), zinc (Zn)

6. **Chlorinated mercury**

   Methyl Mercury

7. **Chlorinated hydrocarbons**

   Including Heptachlor, DDT, DDE DDD, alpha-HCB, alpha-HCH, Endosulfan, Lindane, Dieldrin, Endrin, Chlordane, Methoxychlor.

## 8. Petroleum Hydrocarbons

Petroleum hydrocarbons and PAHs

## SECTION TWO

## CHAPTER 3: SAMPLE COLLECTION

### 3.1 GENERAL PROCEDURES FOR SAMPLE COLLECTION.

#### 3.1.1 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. $4^0$C 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.

#### 3.1.2 Taking Samples for Microbiological Analysis

Use only sterile ($NaS_2O_3$-treated) containers reserved specifically for the purpose of microbiological sampling. All containers should be treated with $NaS_2O_3$ (sodium thiosulphate solution) to neutralize any residual chlorine, which might stop any bacterial action.

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

When sampling, 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 container top, grasp the bottle firmly and submerge the top to at least 20 cm below the surface of the water. If there is a current of water, the mouth of the container 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 waters, or off a boat, it may be necessary to weigh the sample container and lower it on a string or rope to obtain the sample. In such cases, great care must be taken to tie the container and weight firmly. The container should be lowered and raised with great care, avoiding any external contamination, e.g. direct contact with the side of the boat.

In all cases, sample containers should be firmly resealed after sampling and all relevant information either written on or attached to the container. If the container 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 be done immediately in the cleanest area available.

#### 3.1.3 Sample Information

All information pertinent to a sampling trip or a field survey should be recorded in a log book for each location sampled. To ensure retention of sample identity, indelible sample labels or seals should be used. A sample record sheet/chain of custody format is as follows:

**Sample Information**

1. Sampling location
2. Date and time of sampling
3. Medium sampled e.g. grab, composite, integrated replicate blank
4. Volume of sample collected.
5. Sampling method and/or apparatus
6. Depth of sampling
7. Preservation method, if any
8. Other field pretreatment, e.g. filtration, fixing
9. Name of collector
10. Identification of project

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

# CHAPTER 4: ANALYTICAL TECHNIQUES

## 4.1 ANALYSIS OF HEAVY METALS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

### 4.1.1 Background Information

The effects of metals in water and 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. However, in general, beneficial elements turn toxic when in excess concentrations.

### 4.1.2 Principles of Spectrophotometry

A common method used to determine metals in environmental samples is atomic absorption spectrophotometry (AAS). Flame atomic absorption spectrophotometry (FAAS) is generally applicable at moderate concentration levels in clean and complex matrix systems. In FAAS, 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, or 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.

The hydride generation (HG) 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 AAS. The hydrides are purged continuously by argon or nitrogen into an appropriate atomizer of an AAS 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” (CVG) 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.

### 4.1.3 Apparatus and Reagents

#### 4.1.3.1 General Apparatus/Equipment

The list below indicates the general apparatus and equipment needed to prepare samples for AAS. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories would also be required.

- Telfon® digestion block and crucibles
- Beakers
- Hot plate
- Micropipettes (0.1 ml) and pipettes (1, 2, 10 ml)
- Fume hood
- Drying oven ($105^0$C)
- Stainless steel spatula
- Desiccators
- Volumetric flasks of various capacities (25, 100, 500, 1000 ml)
- Analytical balance (100-200 g) with a precision of 0.001 g

- Atomic absorption spectrophotometer
- 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.
- Water distilling or deionising apparatus
- Graphite Furnace

#### 4.1.3.2 Chemicals and Reagents

The list below indicates primary chemicals and reagents needed to prepare samples for AAS. It is not comprehensive. Basic laboratory chemicals and reagents found in most well stocked laboratories would also be required.

- Concentrated nitric acid (d $20^0$C=1.4 g/ml).
- Concentrated hydrochloric acid (d $20^0$C = 1.19 g/ml)
- Concentrated sulphuric acid (d $20^0$C = 1.84 g/ml)
- Stock solutions for each element to be analyzed
- Distilled and/or deionized water.
- Stannous chloride/hydroxylamine sulphate solution for mercury determination: mix 10 ml $H_2SO_4$ and 60ml distilled water and allow to cool 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.

#### 4.1.3.3 Standard Solutions

**Stock solutions for metals: 1 g/l (1000ppm)**

Prepare preferably from commercially available stock solutions for each element. Alternatively, dissolve 100 mg 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.

**Stock solutions for organic Hg: 100 mg/l**

Dissolve 15 mg methyl mercuric dicyandiamide or an equivalent of another compound in 1 litre of 1% cysteine hydrochloride.

**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.

**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.

**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.

**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.

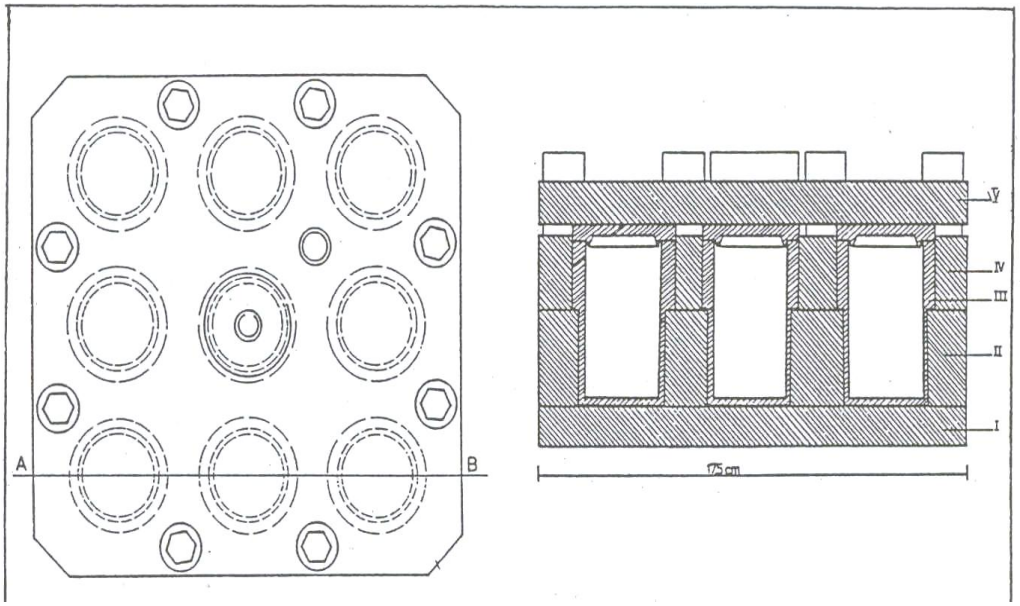
**NOTE**: For analysis of minerals in sea or lagoon water, make standard solutions in artificial sea water (with similar salinity to that of the samples) to compensate for chemical and marine interferences.

**4.1.4 Sample Digestion (Mineralization) and Extraction of Organic Mercury**

**4.1.4.1 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 solubilization of dry ash, is avoided.

Pressurized acid-digestion of biological material in Teflon-lined steel bombs 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 (See diagram below). The plates are kept together by 8 fastening screws during pressurized digestion which normally takes place in an oven or on a hotplate.




**~~Unit for 9 crucibles with 35 ml volume. Cutting A – B, ground plate;; II container plate; III, Teflon crucible 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 0.5 g dry weight or 2.0 g 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 6 ml) 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$^{o}$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).

#### 4.1.4.2 Extraction of Methylmercury

Homogenize about 6 g fresh weight or 1.5 g dry weight of sample in a glass centrifuge screwcap tube with 3 ml of distilled water Add 1 ml concentrated hydrochloric acid and 3 ml of toluene and shake vigorously for 5 minutes. Centrifuge and pipette 2 ml of the upper toluene phase (note total volume, Vt) into another tube. Add 4 ml of a 1 % cysteine chloride solution and shake vigorously for 2 minutes. Centrifuge and transfer 3 ml of the aqueous phase (note total volume, Vc) to a glass test tube. Dilute to about 10 ml with water, add 1.0 ml sulphuric acid diluted in equal volume of water and 2 ml of 10% v/v ammonium persulphate. Heat slowly to boiling point and continue for about 2 minutes in order to remove the excess persulphate. Cool, transfer to an appropriate volumetric flask and top up to the mark. Analyze by flameless or cold vapour AAS.

#### 4.1.4.3 Solubilization of Minerals in Water

Adjust an aliquot of each water sample to pH 2.5 and add 2 % v/v solution of ammonium pyrrolidine dithiocarbamate. Extract the complex formed with an equal volume of methylisobutyl-ketone and analyze the organic layer using AAS. If

necessary, concentrate the organic layer (by heating in a water bath) before AAS determination. Use the aqueous layer (considered free from metals) for blank determinations.

#### 4.1.5 Atomic Absorption Measurement

Trace elements to be monitored in the marine environment include arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), cobalt (Co), manganese (Mn), lead (Pb), selenium (Se), nickel (Ni), vanadium (V) and zinc (Zn), amongst others. The AAS techniques recommended are described below.

#### 4.1.5.1 Flame atomisation

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.

#### 4.1.5.2 Flame Atomization After Hydride Generation

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

**Arsenic (As)**

Set up the AAS instrument with the hydride generator equipment for As analysis according to the manufacturer's instructions, and regulate to the optimum conditions as above. Pipette a predetermined volume of the test solution into the reaction vessel of the hydride generator and add 1 ml of diluted sulphuric acid in distilled water (1:3) Place the reaction vessel into the hydride generator and replace the atmosphere within the hydride generator with nitrogen. Add one pellet of $NaBH_4$ or 5 ml of the 5 % alkaline solution above, and mix using the device provided in the equipment. Ionic arsenic (III) is reduced in the reaction vessel to the hydride ($AsH_3$), which is swept by nitrogen into the open-ended heated quartz absorption cell ($900^0$C) where it is decomposed and the atomic As is determined. The cells 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 hydride generator equipment 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 hydride generator. Place the reaction vessel into the hydride generator equipment and replace the atmosphere within the hydride generator with nitrogen. Add one pellet of $NaBH_4$ or 5 ml of the 5% alkaline solution above, and mix using the device provided in the hydride generator equipment. 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 ($900^0$C) 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.

#### 4.1.5.3 Flameless or "Cold Vapour" Determination of Mercury

Set up the AAS instrument with the equipment 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 cold vapour generator. Use the Teflon bomb digest for total mercury determination, or the cysteine chloride extract for methylmercury determination. Place the reaction vessel in the cold vapour generator and replace the atmosphere within the cold vapour generator with nitrogen. Add 2 ml of the stannous chloride/hydroxylamine sulphate solution and mix using the device provided. 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.

### 4.1.6 Detection Limits

The detection limits of some elements using the above AAS techniques are given below.

| **AAS Technique** | **Element** | **Detection Limit** | |
|---|---|---|---|
| | | **In solution (ng/ml)** | **In marine organisms (ng/g FW)** |
| FAAS | Cu | 10 | 50 |
| FAAS | Cd | 2 | 5 |
| FAAS | Fe | 20 | |
| FAAS | Mn | 10 | |
| FAAS | Pb | 50 | 5 |
| FAAS | Zn | 5 | 250 |
| HG-AAS | As | 2 | 10 |
| HG-AAS | Se | 2 | 0.05 |
| CVG-AAS | Hg | 1 | 0.05 |

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 concentrations of four to five times the detection limits and extends to 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.

### 4.1.7 Standardization of Digestion and ASS Measurement

#### 4.1.7.1 Standardization of the 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 g dry weight) of the matrix. The last three serve as blanks. With a micropipette, add 0.1 ml of dilute nitric acid (about 0.25 % v/v) to the first crucible, and then 0.1, 0.2, 0.3 and 0.4 ml 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.

#### 4.1.7.2 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:

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

Where X ($X_0$, $X_1$, $X_2$, $X_3$, $X_4$) represents 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:

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

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

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

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

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

**4.1.8 Expression of Results**

**4.1.8.1 All Trace elements and Total Mercury**

From the AAS reading obtained on the test solution, determine the concentration of trace metal(s) by reference to the calibration curve (described above) and after making allowance for the blank determination. Calculate the metal concentration of the 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 25 ml be the volume (V) of the test solution 0.4 g be the dry weight (DW) of the matrix digested 5 ng/ml the concentration (C) of element read from the curve.

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

$$Q = \frac{C \times V}{DW} = \frac{5 \times 25}{0.4} = \frac{125}{0.4} = 312.5 \text{ ng/g DW}$$

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

$$Q1 = \frac{C \times V \times D}{DW} = \frac{5 \times 25 \times D}{0.4} = 312.5 \times D \text{ ng/g DW}$$

The amount of element in fresh sample is given by:

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

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 $105^0$C.

**4.1.8.2 Methylmercury**

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

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

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 was further extracted.

Vc: Volume in ml of the aqueous cystein chloride phase of which 3ml was solubilized 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 respect to sample fresh weight as above.

**4.1.9 Precision, Accuracy and Quality Control of Results**

**4.1.9.1 Precision**

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

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

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

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

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.

**4.1.9.2 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.

**4.1.9.3 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 glassware (clean them);
- Homogeneity of the sample (homogenize again);
- Contamination of the 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. Re-zero 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.

## 4.2 ANALYSIS OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY

### 4.2.1 Background Information

Organic compounds containing chlorine, fluorine, bromine or iodine differ from petroleum hydrocarbons because most of them are not readily degraded by chemical oxidation or bacterial action. These conservative pollutants are essentially permanent additions to the marine environment. Most of these persistent substances are artificial; they do not occur naturally and tend to accumulate in both biota and sediments. These substances are best classified in terms of their toxicity, persistence, tendency to bioaccumulate, and source functions (size and nature of land-based sources). Halogenated hydrocarbons include a wide range of compounds including: pesticides ( DDT congeners, dieldrin, lindane, HCB, Toxaphenes); PCB congeners; and Chlorinated dioxins and furans. The environmental half-life of these compounds varies from days (malathion) to decades (DDT congeners).

Pesticides are specifically designed to modify biological systems and there exists concern about their effects on marine ecosystems and human exposure to these compounds through the consumption of contaminated seafood products.

Other synthetic organic compounds such as PCBs and chlorinated dioxins result from industrial activity but their introduction to the environment may arise from point sources, discharges to municipal sewage systems or rivers and venting to the atmosphere.

### 4.2.2 Principles of Gas Chromatography

Gas chromatography (GC) is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined).

In gas chromatography, the moving phase (or "mobile phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chromatograph.

The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

### 4.2.3 Apparatus and Reagents

#### 4.2.3.1 General Apparatus/Equipment

The list below indicates the general apparatus and equipment needed to prepare samples for GC. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- Rotary evaporator
- Water bath
- Soxhlet extraction unit

- Vacuum pump
- Glassware
- Gas chromatography columns (1.8 m x 4 mm I.D.)
- Silica gel columns
- Separation funnels
- Centrifuge (at least 1000 rpm)
- Freezer-dryer and/or drying oven
- Analytical balance (100-200 g) with a precision of 0.001g or better, preferable top load
- Desiccators
- Glass wool (pre-cleaned by extraction in hexane or petroleum ether and oven baking)
- Gas chromatography with Ni-63 electron capture detector
- Stainless steel or glass blender
- Mechanical shaker
- Syringes (10 µl)
- Glass column Ø = 1 cm for open chromatography apparatus

#### 4.2.3.2 Chemicals and Reagents

The list below indicates primary chemicals and reagents needed to prepare samples for GC. It is not comprehensive. Basic laboratory chemicals and reagents found in most well stocked laboratories will also be required.

- 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=1.84 g/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 (0.040-0.063 mm size)
- Hexamethyldisilane, 10 % solution in toluene
- Potassium hydroxide pellets
- Florosil – 60-100 mesh.

#### 4.2.3.3 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 (0.01 µg/ml) and 2,5,2',6' – tetrachlorobiphenyl (0.025 ug/ml) in iso-octane to the sample before extraction, and to the standard solutions for instrument calibration.

a) Stock IS solution (1.0 and 2.5 mg/ml): Weigh 100 mg of 1,1-dichloro-2, diphenylethylene and 250 mg 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 25 µg/ml): Weigh 0.692 g (1 ml) 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 0.025 µg/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 100 mg of each reference substance in 100 ml 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 0.692 g (1.0 ml) of each reference stock solution 2 into a 100 ml volumetric flask and dilute with internal standard solution (IS dilution 2) to 69.2 g (100 ml). 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.

**4.2.4 Extraction and Clean-up of Samples**

**4.2.4.1 Dried Samples (Sediment, Plant, Fish, Shellfish and Molluscs)**

**$A_1$.Extraction**

Weigh about 5 g of ground freeze-dried material and place in an extraction thimble. Add 1 ml of internal standard solution 2 and extract with 200 ml of hexane, pentane or petroleum ether in a soxhlet apparatus for 8 hours. Concentrate the extract in a vacuum rotary evaporator to about 10 ml at about $50^0$C, and dry by passing through a small glass column plugged with glass wool and containing anhydrous sodium sulphate.

Collect and concentrate extract to about 2 ml, and record the volume. Determine solvent "extractable organic matter" (E.O.M.) by drying (evaporating with dry nitrogen) or heating (on a heating plate), a known volume of the extract in a clean pre-weighed beaker to a constant weight. (To remove water filter with $Na_2SO_4$). Note the weight of the residue and calculate the amount of E.O.M. (lipids) as follows:

$$\text{E.O.M. (mg/g)} = \frac{\text{Wt. of residue (mg)} \quad x \quad \text{Vol. of extract (µl)}}{\text{Vol. evaporated (µ1) x wt. of sample extracted (g)}}$$

**$A_2$ .Sulphuric Acid Cleanup**

This treatment is made when the lipid contents are higher than 100 mg/g of sample. For that: Transfer 1 ml of extract into a centrifuge tube with Teflon® screw cap containing 4 ml of concentrated sulphuric acid. Close the tube and turn it upside

down carefully 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 2 ml by evaporating the solvent with clean dry nitrogen. Never leave to run dry or it must be discarded.

Place the extract on about 1.63 g florosil contained in a small column with an internal diameter of 1 cm, rinse the tube and add onto the column. Allow the column to equilibrate by letting the florosil absorb all the extract. Rinse and elute with hexane until about 50 ml 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 1 ml. The extract is ready for injection into the GC.

**$A_3$.Potassium Hydroxide Cleanup**

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-DDD, 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 1 ml of 95.5 % ethanol together with 0.05 ml of water, in a screw cap centrifuge tube. Transfer 1 ml of the extract to the tube, close tightly and place in a water bath at $50^0$C for 30 minutes, Add 2 ml of a solution of sodium chloride in orthophosphoric acid (11.7 g NaCl in 1 litre of 0.1 m 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 1 ml. The extract is ready for injection into the GC.

**B. Fresh Samples (Sediment, Plant, Fish and Shellfish)**

Place about 25 g 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 200 ml 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 the freeze-dried sample.

**C. Extraction and Cleanup of Water Samples**

Mix 1 litre of the water sample with about 500 ml or less of hexane in a 2 l separation funnel and shake for 30 minutes. Separate the organic phase from the aqueous phase and extract the latter three times, using 50 ml hexane each time. Concentrate the combined hexane phase to a volume of 10ml in a vacuum rotary evaporator at $50^0$C. Dissolve in 25 ml distilled acetonitrile and wash with 450 ml of 4 % (w/v) sodium sulphate. Extract again successively with 3 x 25 ml 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 1 ml. The extract is ready for injection into the GC.

**4.2.4.2 Separation of Compounds by Silica Gel Column Chromatography**

Gas chromatography of the hexane extracts of water or biota (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 $250^0$C 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 (6 mm I.D.) plugged with hexane-pre-cleaned 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 35 ml of hexane. Collect the hexane eluate (fraction 1). Elute the column again with about 45 ml of benzene and collect the eluate (fraction 2). Concentrate the above fractions separately to about 1 ml by evaporating the solvent with clean dry nitrogen gas. The established composition of each of the above fractions is given in the table below.

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

**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 |
| Heptachlor epoxide | |
| Dieldrin | |
| Endrin | |
| Chlordane | |
| Methoxychlor | |

### 4.2.5 Analysis Using Gas Chromatography

#### 4.2.5.1 Column Preparation

Chlorinated hydrocarbons are generally analyzed on a gas chromatograph equipped with an electron capture detector (ECD). The glass column (1.8 m x 4 mm I.D.) should be filled with acid-washed and dried chromosob W (80-100 mesh) coated with 5 % OV – 101 silicon oil. The column should preferably be a ready-made commercial item.. However, it is possible to 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 10 mm 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. When the column is full, put about 10 mm of 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 chromatograph (detector end unconnected), applying a carrier gas (flow rate of 60 ml/min) and heat the column to $250^0$C (allowed maximum temperature).

**4.2.5.2 Column Test**

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

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

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

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

Where L is the column length.

**4.2.5.2 Quantification**

**(a) DDT 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)

Calculate the result on fat weight basis as follows:

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

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

Where F is the fat content in gram.

c: concentration of standard (µg/ml)

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

#### 4.3.1.1 Introduction

The level of dissolved oxygen in surface and near surface water is an important measure of the state of the health of the aquatic environment. Dissolved oxygen levels become depressed as a result of the inability of natural processes to supply oxygen at the rate demanded for the oxidation of organic matter or reduced chemical substances. Dissolved oxygen deficiency may be particularly acute in the cases of eutrophication, discharge of sewage and the discharge of organic industrial, agricultural and aquacultural effluents. Extreme oxygen deficiencies (e.g., anoxia) can result in the elimination of all higher life forms. Anoxic conditions, especially in sediments, can also lead to the liberation of less reactive forms of metals from particles into aqueous phases.

Levels of dissolved oxygen of >7 mg/l in surface marine and freshwaters, depending upon temperature, represent essentially oxygen-saturated conditions. Levels below 4 mg/l represent serious oxygen depletion with some species exhibiting avoidance. Species mortalities can occur below these levels with severe prejudice to most aerobic organisms occurring below 3 mg/l.

Data on concentrations of dissolved oxygen (DO) in water are essential for documenting changes to the environment caused by natural phenomena and human activities.

Two field methods for determining concentrations of dissolved oxygen in surface and ground waters are the membrane electrode method and the spectrophotometric method: the membrane electrode method is the standard procedure for determination of DO concentrations; and the spectrophotometric method is recommended for determining concentrations of DO less than 1.0 mg/l. These methods are applicable to unfiltered surface and ground waters, from fresh to saline.

The iodometric (Winkler) method can be recommended for field determination of dissolved oxygen in the marine environment provided the method has been standardized and quality assured using appropriate inter laboratory performance studies.

#### 4.3.1.2 Materials and Chemicals

The list below indicates the general apparatus and equipment needed to prepare samples for dissolved oxygen determination. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- Oxymeter or multiparametric probe
- Amber glass bottles (300 ml) with glass stoppers
- Automatic pipettes
- Normal or automatic burette
- Magnetic stirrer
- 500 ml 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, $KH(1O_3)_2$
- Potassium fluoride

#### 4.3.1.3 *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 (e.g. 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 $l^{-1}$.

#### 4.3.1.4 Laboratory Determination: Modified Winkler Method (Using 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 determined according to the original Winkler's method.

**Preparation of Solutions**

**i) *Manganese* (II) sulphate solution (Reagent 1)**

Dissolve 480 g $MnSO_4$, $4H_2O$ or 400 g $MnSO_4.2H_2O$ or 364 g $MnSO_4H_2O$ in distilled water and dilute to 1 litre. 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 400 g of $MnCl_2.4H_2O$.

***ii) Alkaline azido-iodide (Reagent 2)***

a) For saturated samples: Dissolve 500 g, NaOH (or 700 g KOH) and 135 g Nal (or 150 g KI) in distilled water and diluted to 1 litre. Add 10 g $NaN_3$ dissolved in 40 ml distilled water. This reagent must not give any colour with starch solution when diluted and acidified.

b) For supersaturated samples: Dissolve 10 g of $NaN_3$ in 500 ml of distilled water. Add 480 g NaOH and 750 g 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 under any circumstance as toxic fumes of hydrazoic acid may be formed.

***iii) Starch***

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

***iv) Sodium thiosulphate standard solution***

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

***v) Postassium hydrogen diiodate standard solution, 0.0021M***

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

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

**Modified Winkler Method**

a) From the sampling bottle, fill a 300 ml 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) 1 ml of Reagent 1 and 1 ml 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 a number of times, place it in a closed container (away from light) and transport to the laboratory. A 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 1 ml 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 result).

g) Place an aliquot of the mixture (corresponding to 200 ml 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 2 ml of reagents R1 and R2 used, the volume of mixture to be taken should be 200 x 300/(300 – 2) = 201 ml.

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

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

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

or, 1 g 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/1) of $S_2O_3^{2-}$

$$\text{ie } X = \frac{Vt\ (ml) \times C}{1000}$$

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

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/I)

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***

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

Where % Sat: percentage saturation in oxygen ($O_2$)

mg/l: dissolved oxygen in water sample

Solubility C* is related to temperature by the formula:

$$\text{Ln } C^* = -139.34411 + (1.575701 \times 10^5/T) - (6.642308 \times 10^7/T^2) + (1.243800 \times 10^{10}/T^3) - (8.621949 \times 10^{11}/T^4) - \text{chl } [ (3.1929 \times 10\text{-}^2) - (1.9428 \times 10/T) + (3.8673 \times 103/T^2)]$$

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

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

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

**4.3.2 Chemical Oxygen Demand**

**4.3.2.1 Introduction**

The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water, making COD a useful measure of water quality.

COD is used as a measure of the oxygen equivalence of organic matter (in a 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 are 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 preferable to other methods using other oxidants because $K_2Cr_2O_7$ is a very powerful oxidizing agent, is easy to use and applicable to a wide variety of samples.

#### 4.3.2.2 Materials and Chemicals

The list below indicates the general apparatus and equipment needed to prepare samples for COD. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- Reflux system comprising a 500 ml 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
- Pumice stones (antibumping granules)
- Spectrophotometer HACH DR
- Potassium dichromate solution

#### 4.3.2.3 Reagents

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

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

c) Orthophenanthroline iron sulphate indicator (Ferroin): Dissolve 1.485g) of 1,10 – ortho phenanthroline monohydrate and 0.695 g of $FeSO_4.7H_2O$ in 100 ml 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 98 g of ammonium iron sulphate (AFS) in deionized water and dilute to 1000 ml.

e) Standardization of ASF solution: Add 20 ml of concentrated sulphuric acid (d =1.84) to 250 ml 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 6.6 g of $Ag_2SO_4$ in 1000 ml of concentrated $H_2SO_4$

**4.3.2.4 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 $4^oC$.

c) Store sample at $4^oC$ wrapped in aluminum foil until analysis (within 6 h 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 a 50 ml aliquot of sample in a 500 ml round bottomed flask: samples rich in organic matter (COD > 900 mg/l) should first be diluted appropriately before taking the 50 ml. Verify this concentration before making the analysis. If the COD is greater than 900 mg/l, the mixture will turn green, so dilute.

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

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

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

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

j) Slowly add 70 ml 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.

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

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

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

n) Cool down to room temperature and titrate excess potassium dichromate with ammonium iron sulphate solution No1 using 0.1 to 0.15 ml 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.

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

**Approach when sample contains 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 10 mg of sulfamic acid to the dichromate standard solution for every milligram of nitrite present in the reflux flask.

In this case, accurately determine chloride content in the sample by Mohr's method, as follows: Pipette 50 ml of water sample into a 500 ml round bottomed flask: sample rich in organic matter (COD > 900 mg/1) should first be diluted appropriately before taking the 50 ml. 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 20000 mg/l. Pipette 50ml of each solution into a 500ml round bottom flask. Add 10 mg $HgSO_4$ to the water sample and to each of the NaCl dilutions, for each milligramme Cl present in 50 ml.

Determine COD as indicated above (General Application) for water samples 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.

**Approach when samples have low COD (<50 mg/l)**

Place at least 50 ml of water sample in a 500 ml round bottomed flask. Add all reagents as indicated above (General Application). Reduce volume to 150 ml by heating (without refrigerant), after adding 10 mg of mercury sulphate ($HgSO_4$) for every 1 mg 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 hydrogen phtalate and dry to constant weight at $120^0$C.

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

c) Take 50 ml of this solution in a bottomed flask, treat and determine its COD as indicated above (General Application).

d) Calculate the efficiency of the method as follows:

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

Where CODexp is the result from step c), above

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

**Expression of Results**

a) For natural waters and industrial wastewaters not requiring correction for chlorides:

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

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

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

Where B: quantity of ammonium iron sulphate 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

1.20: empirical compensation factor

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

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

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

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

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

T: water content of sample (in %)

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

**4.3.3 Biochemical Oxygen Demand**

**4.3.3.1 Introduction**

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.

The BOD test is widely used for determining the polluting potential of domestic and industrial wastewaters. It is also 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 practice, BOD (or $BOD_5$.) measures the amount of oxygen consumed in a sample of water (or wastewater) over a period of 5 or 7 days at 20$^{o}$C under laboratory conditions).

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.

**4.3.3.2 Materials and Chemicals**

The list below indicates the general apparatus and equipment needed to prepare samples for $BOD_5$. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- 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
- 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

**4.3.3.3 Reagents**

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

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

c) $CaCl_2$ solution: 27.5 g/l in distilled water.

d) $FeCl_3.6H_2O$ solution: 0.25 g/l in distilled water.

e) $NH_4Cl$ Solution: 2 g/l in distilled water.

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 5 ml phosphate buffer, 1 ml $MgSO_4.2H_2O$ solution, 1 ml $CaCl_2$ solution and 1 ml $FeCl_3.6H_2O$ 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.

**4.3.3.4 Method**

**General Application (Dilution Method0**

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 $4^0$C to the laboratory and store in the refrigerator until analysis (within 24 h 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 8 mg/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 $H_2SO_4$ 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.

   1 to 5 % dilution for urban wastewaters.

   25 to 100 % dilution for polluted river waters.

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

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

i) 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), and another BOD bottle with 2% glucose-glutamic acid solution in inoculated dilution water.

j) Incubate BOD bottles at $20^oC$ in the dark for 120 hours.

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

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

$$BOD_5 \text{ (mg/l)} = F(To - Ts) - (F - 1)(Do - Ds)$$

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 $0.40 < To - Ts < 0.6\ To$

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

$$T = \frac{BOD_{exp} \times 100}{BOD_{th}}$$

Where BOD exp: BOD measured for the glucose-glutamic acid solution

$BOD_{th}$: BOD reference value in literature for the said solution

**Samples of Low $BOD_5$ (Direct Method)**

For samples with $BOD_5$ lower than 7 mg/l, it is not necessary to dilute. It is assumed 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) 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, other oxygen – consuming processes may occur which disturb BOD measurement. These include:

- Nitrification by bacteria.

- Presence of reducing chemical substances such as sulphides, sulphites, ferrous salts, etc., which have a depressive effect on the oxygen balance in the water body.
- Presence of bactericidal or bacteriostatic substances (chlorides, etc.) which inhibit the activity of bacteria, that 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 many inputs, it is unnecessary to inoculate the dilution water with microorganisms.

### 4.3.3 Nutrients

#### 4.3.3.1 Introduction

Nutrients include all the bioavailable forms of nitrogen, phosphorous and silicon introduced to the fresh and marine waters partly as a result of human activities. Although the major nutrients are relatively simple variables to quantify, it is difficult to distinguish between natural and anthropogenic sources and also to identify and quantify non-point sources of these substances.

Point sources include sewage, plant outfalls and fertiliser factories. Non-point sources comprise run-off from agriculture, deposition of atmospheric contaminants, upland municipal sources, etc. Nutrients recycled from sedimentary reservoirs may also have anthropogenic origins. The major effect of excessive nutrient inputs is the promotion of eutrophication and disturbance of the structure of aquatic ecosystems.

Changes in nutrient fluxes affecting the inter-relationships among the relative supplies of nitrogen compounds, phosphate and silicate can alter the types of planktonic organisms that occur and bloom in an area. Reductions in silicate supplies in some rivers has resulted in a shift from diatoms to flagellates as the predominant primary organisms in seasonal blooms. There is also concern that excessive influxes of nitrogen compounds over supplies of phosphorus compounds can result in phosphorus limitation in receiving areas with associated changes in algal communities and the possible stimulation of toxic algae (i.e., algae that contain or produce natural toxins).

Analysis of nutrients consists in determining both total concentrations of salts and the dissolved fraction alone. It is therefore necessary to collect at least two aliquots of the sample. The first, to be used for determining dissolved and undissolved nutrients. The second (intended for dissolved nutrient determination alone), will undergo a second filtration through a Whatman GF/F filter (about 0.7µm pore size). Nutrients to be determined are ammonium ions ($N\text{-}NH_4$), nitrites ($N\text{-}NO_2$), nitrates ($N\text{-}NO_3$) and orthophosphates ($P\text{-}PO_4$).

#### 4.3.4.2 Determination of Ammonium Ions

**Materials and Chemicals**

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of ammonium ions. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- 50 ml tubes with Teflon® screw-caps
- Dark box
- Assorted pipettes
- Automatic burettes with reagent bottles
- UV/visible spectrophotometer
- Phenol
- Sodium nitroprusside ($Na_2[Fe\ (CN)_{\ 5}\ NO].2H_2O$

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

b) Reagent R2: Dissolve 140 g sodium citrate and 11 g sodium hydroxide in 400 ml of deionized water and mix. Add 0.7 g camel water (about 4.6 ml) and complete to 500 ml. Camel water can be replaced by 2.5 g 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 7 g. This reagent is stable for about 2 months. Store at $4^0$C.

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

d) Ammonium chloride calibration solutions: Prepare, by appropriate dilutions of the stock solution, calibration solutions containing 1000,100,10,1 and 0.1 mg N-$NH_3$ per litre.

**Method**

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

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

c) Take 50 ml of each fraction into 250 ml.

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

e) Place the flasks in a dark box (away from light) at room temperature for colour development, and transport to the laboratory.

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

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

The concentration of unionized ammonia N-$NH_3$ (toxic at high dose) can be calculated from that of N-$NH_4$, given the temperature and pH of the water sample, by the following formula:

$$[\text{N-NH}_3] = \frac{[\text{N} - \text{Nh}_4]}{[1 + 10\,(10\text{-pH} - 0.03\text{T})]}$$

It should be noted that a N-$NH_3$ content higher than 0.025 mg/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 100 m deep and stored many months in the dark) or artificial seawater conveniently diluted to the same salinity as the sample.

### 4.3.4.3 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**

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of nitrites and nitrates. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- 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 50 ml concentrated HCl in 300 ml distilled water, add 5 g sulfanilamide and dilute to 500ml. This solution is stable for long periods in the refrigerator.

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

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

***Stock nitrite solution:*** Dry anhydrous sodium nitrite at $110^0$C for several hours. Dissolve about 0.345 g in distilled water, dilute to a litre and add 1 ml chloroform. 1ml of this solution (stable for 1 to 2 months) contains 5 µmoles of $N\text{-}NO_2$.

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

**Method**

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

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

c) Before analysis, filter about 250 ml of sample through Whatman GF/F filter (0.7 µm 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***

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

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

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

d) 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.

e) 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.

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

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

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

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

**4.3.4.4 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**

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of orthophosphate. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- UV/visible spectrophotometer
- Glass or plastic flasks of 250 and 500 ml
- 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 15 g of ammonium paramolybdate in 500 ml-distilled water. This solution is stable in the refrigerator for long periods. Store in a plastic bottle.

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

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

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

e) Composite reagent: Mix the reagents in the following proportions: R1 75 ml, R2, 250 ml, R3, 150 ml, R4, 25 ml, total 500 ml. 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).

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

**Method**

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

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

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

d) 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.

e) Plot a calibration curve of OD versus $PO_4$ concentration.

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

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

### 4.3.5 Suspended Particles

#### 4.3.5.1 Introduction

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.

#### 4.3.5.2 Materials

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of suspended particles. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- 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

#### 4.3.5.3 Method

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

b) 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.

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

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

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

f) Calculate the weight (Ps) of suspended particles from the formula:

$$Ps\ (mg/1) = \frac{M_2 - M_1 \times 1000}{V}$$

Where V: volume of filtered water (in ml)

g) If the weight of particulate organic matter is also required proceed as follows: Place the crucible (+ filter + particles) in a furnace at $550^0$C for at least one hour. Cool in a desiccator and determine the weight Ms. 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 30 mm Hg for filtration.

### 4.3.6 Determination of Silica by the Molybdosilicate Method

#### 4.3.6.1 Introduction

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.

#### 4.3.6.2 Interference

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.

Tannin, large amounts of iron, colour, turbidity, sulphide, and phosphate all 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.

#### 4.3.6.3 Materials

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of silica. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- Spectrophotometer
- Volumetric flask (100 ml)
- Pipette (5 ml, 10 ml)
- Volumetric flask (1 litre)
- Cuvette (1 cm path length)

#### 4.3.6.4 Chemicals and Solutions

***Chemicals***

- Sodium bicarbonate (NaHCO3)
- Sulphuric acid (HCl)
- Hydrochloric acid (HCl)
- Ammonium molybdate (NH4)6Mo7O24.4H2O
- Oxalic acid (H2C2O4.H2O)
- Sodium hexafluorosilicate (Na2SiF6)
- Sodium metasilicate (Na2SiO3.9H2O)

All chemicals should be BDH Analar Grade purity

***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 $NH_4OH$ or NaOH and store in a polyethylene bottle to stabilize.

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 $^oC$ for 1 hr) in 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 a 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.

#### 4.3.6.5 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.

#### 4.3.6.5 Determination

a) To 50 ml sample add in rapid succession 1.0 ml 1-1 HCl and 2.0 ml ammonium molybdate reagent.

b) Mix by inverting at least six times and let the sample stand for 5 to 10 min.

c) Add 2 ml oxalic acid solution and mix thoroughly.

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

e) 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.

#### 4.3.6.6 Calculations and Expression of results

Silica concentration is determined directly from the calibration curve. Results are expressed in mg/l $SiO_2$ to two decimal.

### 4.3.7 Chlorophylls

#### 4.3.7.1 Introduction

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 spectrophotometry. 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.

#### 4.3.7.2 Materials and Chemicals

The list below indicates the general apparatus and equipment needed to prepare samples for the determination of chlorophylls. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- UV/visible fluorimeter
- UV/visible spectrophotometer
- Cryotubes
- Vacuum pump
- 11itre amber glass bottles
- GF/F filter of 0.7 µm pore size and 25 mm diameter (fluorimetric measurement) or 47 mm diameter (spectrophotometric measurement)
- Centrifuge
- Methanol/ethanol (absolute)

- Acetone
- Hydrochloric acid, 1 N

#### 4.3.7.3 Extraction Process

a) Collect water samples in 11itre amber glass bottles and transport to the laboratory in a cool box (away from light).

b) 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.

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

d) Disintegrate filter using a glass rod or a glass tube with sharp edge, cork the centrifuge tube and shake.

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

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

g) Centrifuge for a minute at 3000 - 4000 rpm.

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

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

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

#### 4.3.7.4 Fluorimetric Determination

***Calibration of Fluorimeter***

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

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

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

d) Chlorophyll concentration (mg/1) = 12.98 x ($OD_{665} - OD_{730}$), for 1cm cuvette.

e) To verify that chlorophyll extract is pure, calculate the ratio t = $OD_{665}/OD_{650}$, which must be between 1.93 and 2.0.

f) 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:

$$[Chl]\ (\mu g/l) = k \times 10^3 \times Fo \times (v/V) \times (1/D)$$

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)

**4.3.7.5 Spectrometric Measurement**

Read OD of extracts at 730, 630, 645 and 663 nm, 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 – $OD_{730}$), such as the trichromatic equations:

[Chl a](µg/l)= (11.43 x $OD_{663}$ – 2.16 x $OD_{645}$ – 0.11 x $OD_{630}$) x F

[Chl b] (µg/l)= (20.97 x $OD_{645}$ – 3.94 x $OD_{663}$ – 3.66 x $OD_{630}$) x F

[Chl c] (µg/l)= (54.22 x $OD_{630}$ – 14.81 x $OD_{645}$ – 5.53 x $OD_{663}$) 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.

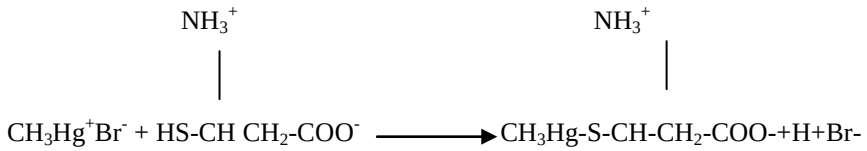
## 4.4 ANALYSIS OF METHYLMERCURY BY GAS CHROMATOGRAPHY

### 4.4.1 Introduction

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

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

The methylmercury 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 methylmercury bromide is extracted with benzene and analyzed by gas chromatography.

### 4.4.2 Materials and Chemicals

The list below indicates the general apparatus and equipment needed to prepare samples for the analysis of mercury by gas chromatography. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- Concentrated hydrochloric acid (d20$^o$C=1. 191 g/ml)
- Sodium bromide
- Anhydrous sodium sulphate
- Benzene (glass-distilled)
- Teramilic alcohol

### 4.4.3 Standard Solutions

**(a) Stock Solution (100 mg Hg/ml|):** Weigh 0.1254 g of methylmercury chloride ($CH_3HgCl$) and make into a solution in 1000 ml of distilled water. This solution is very stable.

**(b) 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.

**(c) 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 very stable.

**(d) 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, 0.30 ng Hg/ml. These solutions are stable for several months.

**(e) 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.

### 4.4.4 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 70 ml benzene, shake for about 10 minutes

vigorously by hand, and centrifuge. Recover the entire benzene phase (note the volume Vb), and transfer 50 ml into a separatory funnel. 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 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 4.3.7.3 above) in place of the tissue sample.

**4.4.5 Gas Chromatography**

**4.4.5.1 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. 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 $N_2$/min) at $210^0$C for 18 hours without attaching the outlet to the detector. Allow the column to cool, weigh, label and store.

**4.4.5.2 Column Testing**

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

**4.4.5.3 Quantification**

Set up the chromatography to normal operating conditions as indicated above. Inject 5 ml benzene as a 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. 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. Determine the concentration of methylmercury (Q) in the sample as follows:

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

Where C: amount in ng of methylmercury 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.

#### 4.4.5.4 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 mg/kg FW.

#### 4.4.5.5 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).

## 4.5 ANALYSIS OF PETROLEUM HYDROCARBONS BY GAS CHROMATOGRAPHY

### 4.5.1 Introduction

Hydrocarbons form a very complex mixture in the aquatic environment. 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 the means available and the set objectives.

Gas chromatography is one of the more rapid methods for determining hydrocarbons. With the right column and detector it is possible to determine total hydrocarbons and the 18 PAH's recommended by the US-EPA for monitoring the quality of the environment.

### 4.5.2 Sampling and Sample Treatment

Prescribed procedures for sampling, sample transport and treatment of petroleum hydrocarbon samples are generally the same as those for particulates. However, attention should be paid to the materials used (e.g. knife, scalpel, packaging, containers, etc.). It is important that all equipment is metal, glass or Teflon. Samples should be freeze-dried (in preference) or dried in an oven at $60^0$C, 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.

### 4.5.3 General Apparatus/Equipment

The list below indicates the general apparatus and equipment needed to prepare samples for the analysis of mercury by gas chromatography. It is not comprehensive. Basic laboratory equipment, found in most well stocked laboratories will also be required.

- 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), 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 cylinders of oxygen and hydrogen
- Micro syringes

### 4.5.4 Chemicals and Reagents

The list below indicates primary chemicals and reagents needed to prepare samples for GC. It is not comprehensive. Basic laboratory chemicals and reagents found in most well stocked laboratories will also be required.

- Silica gel
- Alumina
- Florosil
- 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"

### 4.5.5 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 $4^0$C in the refrigerator.

**Preparation of Calibration Solutions of** $10^{-5}$ mol/l

Take 1 ml of each stock solution in a 10 ml volumetric flask, fill 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.

### 4.5.6 Extraction and Fractionation of Hydrocarbons

#### 4.5.6.1 Extraction

Weigh about 5 g of dried powdered sample (biota 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-pre-cleaned 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 soxhlet flask, and extract further until complete saponification of liquids.

Transfer the extract to a separation 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-pre-cleaned 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 $30^0$C (at most) in a rotary evaporator under light vacuum. Concentrate further in a Kuderna-danish evaporator (graduated conical tube) to 5 ml (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.

#### 4.5.6.2 Determination of Extractable Organic Material (EOM)

Acidify the methanolic phase obtained above with 1 M sulphuric acid, extract three times 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.

#### 4.5.6.3 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$^{o}$C and then at 200$^{o}$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$^{o}$C for 4 hours in an oven, and deactivate partially with 5 % distilled water. Florosil is activated at 300$^{o}$C.

### 4.5.7 Absorption Chromatography on a Florosil Column

Plug a glass column (1 cm I.D.) or a 50 ml burette with hexane-pre-cleaned 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.

### 4.5.8 Separation of Saturated and Aromatic Hydrocarbons by Silica Gel Column Chromatography

Plug a glass column (6 mm ID) or a 50 ml burette with hexane-pre-cleaned 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.

### 4.5.9 Gas Chromatography

#### 4.5.9.1 Regulation of Gas Flow Rate

The operational conditions depend on the type of chromatograph and column. For The 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

#### 4.5.9.2 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$^{0}$C for 2mn

T2 = 180$^{0}$C for 2mn

T3 = 290$^{0}$C for 30mn

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

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

Time for splitless: 30 seconds

**NOTE:** When setting the temperature programme, it should be remembered that the capillary column does not withstand temperatures higher than $300^0$C in an isothermal regime, and $325^0$C under a programmed regime. In addition, it is advisable to start with a temperature at least $20^0$C below the boiling point of the solvent.

#### 4.5.9.3 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.

#### 4.5.9.4 Retention Time and Response Factor of Standards

Inject 1 to 2 ml 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:

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

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

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:

$$Rf_m = \frac{\sum Rf_i}{ni}$$

Where Rfi : response factor of standard I

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

**4.5.10 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: percentage recovery, given by:

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

**4.5.11 Determination of Individual PAH's**

Inject 1 to 2 nl 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 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.

### 4.5.12 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: Add the same amount of internal standards in an empty soxhlet extraction thimble as for the sample. Extract for the same duration, reconcentrate, purify and separate the different fractions. Analyze by gas chromatography. The amount of pollutant in the blank is calculated from that determined in the sample.

***List of standard compounds***

| **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 |

## 4.6 BACTERIOLOGICAL ANALYSES OF WATER AND SEDIMENT

### 4.6.1 Introduction

Pathogens are organisms that can cause disease in higher organisms including humans. These include bacterial pathogens (e.g., *Streptococcus*, *Vibrio*), viruses, (e.g., Hepatitis virus) and enteric parasites. There is currently considerable concern and uncertainty regarding the persistence and particularly the viability of pathogens and viruses and their passage through the marine food chain. Major sources of such materials are human sewage, inappropriate disposal of hospital wastes, uncontrolled discarding of wastes from pathology and microbiology research laboratories and indigenous reservoirs of pathogens.

In 1990, GESAMP placed sewage discharges to the sea at the top of its list of concerns. This is not because of the damage sewage causes to the marine environment but because of associated public health risks.

Faecal contamination is measured by a count of the bacterium *E. coli* (faecal coliform count) or another group of intestinal bacteria, *faecal streptococci*, which survive longer in the sea. These indicators are surrogates as their counts do not reflect the level of contamination by pathogens directly, merely the level of faecal contamination.

### 4.6.2 Sampling Methods

#### 4.6.2.1 Surface Water Sampling

Plunge a 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 $4^0$C.

#### 4.6.2.2 Deep Water Sampling (at Water Sediment Interface)

Plunge a 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 $4^0$C.

#### 4.6.2.3 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 $4^o$C.

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

### 4.6.3 Enumeration of Microorganisms in Water

#### 4.6.3.1 Membrane Filtration

Filter 1 to 100 ml of sample (according to the degree of pollution and turbidity of water body) under vacuum on a sterile nitrocellulose filter (0.45 nm) using a 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 and agar. Incubate the Petri dish inverted under conditions (temperature and duration) specified for each microorganism.

**NOTE**: 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.

#### 4.6.3.2 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.

#### 4.6.3.3 *Escherichia coli*

***Presumption Test***

The presumption test is carried out 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 above), incubate Petri dishes inverted at $44^0$C 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.

***Conformation 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 $^0$C 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 $37^0$C 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***

For the presumptive test, the number of indicator microorganisms is given by the formula:

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

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 microorganisms 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
$C_n$: number of confirmed presumption colonies

**4.6.3.4 *Enterococci***

***Presumption Test***

The presumption test is carried out 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 $37^0$C 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*.

***Conformation Test***

The conformation test 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 $37^0$C for 24h. Add hydrogen peroxide solution (3.5 %) into each dish to just cover the agar surface. Colonies with formation of bubbles (catalase positive) are Listeria. *Enterococci* are 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:

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

Where $N_p$ : number of presumptive colonies

$N_c$ : number of confirmed colonies

$N_t$ : number of tested colonies

**4.6.4 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 $10^{-2}$).

**4.6.4.1 Faecal Coliforms**

***Presumption Test***

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

***Conformation Test***

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. Incubate tubes for 24 to 48h at $37^0$C for total coliform, and at $44^0$C for faecal coliform determinations. Note positive tubes with bacterial growth and gas production in the Durham tubes. Express results as most probable number of microorganism per milliliter of sample.

**4.6.4.2 Faecal *Streptococci***

***Presumption Test***

Make appropriate decimal dilution of the sample in sterile phosphate buffer. Pipette 1.0 ml of each dilution in a tube (3 per dilution) containing 10 ml of Roche broth. Incubate for 24 to 48h at $37^0$C and note positive (cloudy) tubes with bacterial growth.

***Conformation 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 $37^0$C and note positive (cloudy) tubes with bacterial growth. Express results as most probable number of microorganism per milliliter of sample.

**4.6.4.3 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 | ------ |

**4.6.5 Enumeration of Bacteria Pathogens**

**4.6.5.1 Search for *Vibrio cholerae***

*Vibrio Cholerae* are straight or curved rod-shaped gram-bacteria with monotrichous polar flagellum. They are aerobic, oxydase-, more or less basophilic (pH 8.6), and ferment glucose without gas production. The estimation of *V. cholerae* in water is done by filteration and then the 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 g/l NaCl) nutritive broth or alkaline peptone water (pH 8.6) for 24h at $37^0$C.

***Isolation of Microorganisms***

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

This process consists of an agglutination test on previously isolated bacteria with *V. cholerae* biochemical characteristics, using *V. cholerae* 01 antiserum. Deposit a drop of serum 01 on a clean slide. Add the bacterial culture (from Mueller-Hinton agar) to the serum and homogenize. 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.

**4.6.5.2 Search for *Pseudomonas 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.

**i) Determination in Water by Membrane Filtration**

***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.

***Conformation 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

**ii) Determination in Sediment by the Most Probable Number Method**

***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.

***Conformation 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.

**4.6.6 Preparation of Culture Media Solutions and Buffers**

**4.6.6.1 Double Concentrated Lactose Broth**

This 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 |

Dissolve by heating, cool and adjust pH to 6.9 with phosphoric acid. Dispense in 220 x 22 ml (10 ml) tubes with Durham tubes. Sterilize for 20 minutes at $120^0$C in an autoclave. For analysis, add equal volumes of sample to double concentrated broth.

**4.6.6.2 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 220 x 22 ml (10 ml) tubes with Durham tubes. Sterilize for 20 minutes at $120^0$C. For analysis, add equal volumes of sample to broth.

**4.6.6.3 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 22 0x 22 ml (10 ml) tubes with Durham tubes. Sterilize for 20 minutes at $120^0$C in an autoclave.

**4.6.6.4 Rothe Broth**

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| 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 | | |

Mix until completely dissolved. Adjust pH to 6.8-7.0, if necessary, and dispense 10 ml in tubes. Sterilize in an autoclave at $115^0$C for 20 minutes.

**4.6.6.5 Litsky Broth**

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| 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 $^0$C for 20 minutes.

#### 4.6.6.6 Bio-Tryptipcase Alkaline and Saline Nutrative Broth

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| Trypticase peptone1 | 7.0g | K hydrogen phosphate | 2.5g |
| Phytone peptone | 3.0g | Glucose | 2.5g |
| Sodium chloride | 10.0g | Distilled water | 1000ml |

Adjust pH to 8.6 and dispense as appropriate in round-bottomed flasks. Sterilize for 15min at at $121^0$C.

#### 4.6.6.7 Thiosulphate – Citrate – Bile – Sucrose Agar (TCBS)

This 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 | | |

Stir ingredients in all distilled water and bill until completely dissolved. Do not autoclave. Cool to $45^0$C and dispense 15 to 20 ml in 90 mm (diameter) Petri dishes.

#### 4.6.6.8 Mueller – Hinton Broth

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| Beef maceration | 300g | Starch | 1.5g |
| Acid casein hydrolysate | 7.5g | Agar – No l | 10.0g. |

Complete to 1 1itre with distilled water and wait for 5 mn. Mix to a homogeneous suspension. Heat gently with frequent shaking. 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.

#### 4.6.6.9 M-PA Agar

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| 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 |

Adjust pH to 6.5 and sterilize in an autoclave at 120$^0$C for 20 min. Cool to 45-65 $^0$C, adjust pH to 7.1 and add the following antibiotics per litre: sulfapyridin, 176 mg; kanamycine, 8.5 mg; Nalidixic acid, 37 mg; cycloheximide, 50 mg. Mix and dispense 3ml portions in Petri dishes (55 mm diameter) and store at 2 to 10$^0$C (at most before use).

#### 4.6.6.10 Milk Agar

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| Medium A: Skimmed milk | 100.0g | Distilled water | 500ml |
| Medium B: Nutritive broth | 12.5g | Sodium chloride | 2.5g |
| Agar | 15.0g | Distilled water | 500ml |

Sterilize the two media separately and cool quickly to 55$^0$C. Mix both and pour into Petri dishes.

An alternative Milk Agar can also be used:

| | |
|---|---|
| Yeast Extract | 3g |
| Peptone | 5g |
| Milk solids (equiv to 10ml fresh milk) | 1g |
| Agar No.3 | 20g |
| pH | 7.2 approx |

Suspend 24 g in 1l of distilled water. Bring to boil to dissolve completely. Sterilize at 121$^0$C for 15 min.

#### 4.6.6.11 Asparagine Broth

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| 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.

#### 4.6.6.12 Acetamide Broth

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| 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.

**4.6.6.13 Peptone Water**

This comprises the following ingredients:

| | | | |
|---|---|---|---|
| Peptone | 10.0g | Sodium chloride | 5.0g |
| Distilled water | 1000ml | | |

Adjust pH to 7.2 or 8.6 for normal or alkaline peptone water. Dispense 9ml in 220 x 22ml (10ml) tubes or an appropriate volume in 500ml round bottom flasks. Sterilize in an autoclave at $121^0$C for 15 min.

**4.6.6.14 Kovac's Reagent for Indole**

This comprises the following ingredients:

| | |
|---|---|
| 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.

**4.6.6.15 Physiological Serum**

Dissolve 9 g NaCl in distilled water and complete to 1 litre. Sterilize by filtration on cellulose nitrate or acetate membrane (0.45 µm) or in an autoclave at $121^0$C for 15 mn.

**4.6.6.16 Phosphate Buffer**

Dissolve 34 g potassium dihydrogen phosphate in 500 ml distilled water. Adjust pH to 7 with NaOH solution, dilute to 1000 ml and store (stock solution). Before use, add 1.25 ml of the stock buffer solution to 1 1itre distilled water and sterilize at $121^0$C for 15mn.

**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** | **lower** |
| 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

# CHAPTER 5: TREATMENT OF RESULTS

## 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 sediment analyses and assessment.

### 5.1.1 Data Handling and Presentation

Before the data from any monitoring programme are used, it is important to ensure that the confidence limits are 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.

### 5.1.2 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 minimized by proper statistical design of the sampling procedures and attention given to the collection of uncontaminated samples.

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 recognized.

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.

### 5.1.3 Intercomparability Requirements

In most cases where monitoring programmes are operated on a 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 analyzed or only a particular size fraction.

### 5.1.4 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 recognized 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.

### 5.2.1 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 customized 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 AND QUALITY CONTROL

## 6.1 INTRODUCTION

The broad objective of the GCLME Pollution Monitoring Programme is the acquisition of reliable and relevant data for the purpose of protection of the coastal zone against land-based and other sources of pollution. This process will identify polluted sites and recognize trends for planning and implementation purposes. For the success of this programme, an awareness of the usefulness of sound Quality Assurance/Quality Control (QA/QC) and Good Laboratory Practice is paramount. This section therefore attempts to summarize the important steps for ensuring QA/QC and Good Laboratory practice for use by participating laboratories.

## 6.2 QUALITY ASSURANCE (QA)

### 6.2.1 Definition

Quality Assurance (QA) 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 procedures.

### 6.2.2 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

## 6.3 QUALITY CONTROL (QC)

### 6.3.1 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.

### 6.3.2 Steps for Good Quality Control

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.

### 6.3.3 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 analysis in that laboratory.

### 6.3.4 Recovery of Known Additions (Internal Standards)

The recovery of known additions (internal standards) is used to eliminate matrix effect. Always use the recovery factor in the calculation of the final concentrations.

### 6.3.5 Analysis of Externally supplied Standards

Externally supplied standards are used whenever the recovery of an internal standard or know addition is not acceptable. An acceptable recovery level should not be lower than 50%.

### 6.3.6 Analysis of Reagent Blanks

Always analyse a reagent blank whenever new reagent are used. The minimum requirement for analysis is 5 % of the sample load.

### 6.3.7 Calibration with Standards

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.

### 6.3.8 Analysis of Duplicates

Analyze 5 % or more of the sample in duplicate to assess precision.

### 6.3.9 Control Charts

Control charts enable objective evaluation of the quality of data. An example of a control chart is given below:

| | | |
|---|---|---|
| Concentration of Analyte in Reference Material | | UCL |
| | | UWL |
| | | Mean |
| | | LWL |
| | | LCL |

UCL = Upper Control Level

LCL = Lower Control Level

UWL = Upper Warning Level

LWL = Lower Warning Level

The Figure above represents a typical use of a 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 results 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 the above occurs, and no further analysis should be carried out until the source of error is identified and corrected .

### 6.3.10 Measurement of Reference Materials

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

c) 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.

d) Calculate the mean (x) and the standard deviation (r) and then plot the following values on a blank control chart:

x,

$x + 2r = UWL$

$x + 3r = UCL$

$x – 2r = LWL$

$x – 3r = LCL$

## 6.4 QUALITY ASSESSMENT

### 6.4.1 Definition

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

### 6.4.2 Performance Evaluation Samples

Known amount of constituents of interest are used for determination to evaluate analyte recovery. Recovery should be within the range of acceptable and predetermined recovery levels.

### 6.4.3 Laboratory Inter-comparison Samples

Each laboratory should participate in inter-laboratory calibration exercises. A quarterly participation is acceptable.

### 6.4.4 Performance Audits

A checklist for standard operation procedure should be developed together with unscheduled visits to detect deviation from standard operation procedures.

### 6.4.5 Field Sample Collection

A field sample should be representative of the point of collection . In order to achieve this objective, a sampling design strategy is required. 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 the 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 in the field.

### 6.4.6 Chain of Custody Procedures

Chain of custody creates the ability to trace the possession and handling of a sample from the time of collection through analysis to final disposal. 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 the 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

### 6.4.7 Chain of Custody Record

A chain of custody record should be filed out to accompany each sample or group of samples. The records should 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 .

### 6.4.8 Sample Analysis and Request Sheet

The sample collector must complete the field portion of the request sheet, which includes most of the 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

### 6.4.9 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.

### 6.4.10 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.

### 6.4.11 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.

### 6.4.12 Supervision

The laboratory analysis is closely supervised by a competent person designated as the laboratory supervisor.

### 6.4.13 Determining Numbers 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.

# CHAPTER 7: SUMMARY

## 7.1 FROM LABORATORY DATA TO POLICY TOOL

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 coastal zone development programmes.

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, water pollution monitoring exercises provide the most reliable empirical information available to the policy maker.

In the course of the 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 generated by the water pollution monitoring exercise enables appropriate placement of the social, economic and political factors in the decision-making matrix.

In the long run, water pollution data generated should provide the necessary information to help decision makers draft policies and development strategies that respond to both national and international needs in the region.

In summary, the systematic collection and analysis of data yields vital information, including quantification of existing conditions, identification of information gaps and projection of future trends. Routine monitoring also provides feedback to the system, making it possible to adjust 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.

## 7.2 POLICY IMPLEMENTATION

The timing of intervention strategies as a response to the 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 to ensure 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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