

**MINISTRY OF TOURISM
TOURISM DEVELOPMENT AUTHORITY
USAID-EEPP**

***OBJECTIVE 8.2.1 DOCUMENTATION
VOLUME II***

***ENVIRONMENTAL MONITORING UNIT MANUALS
AND CHECKLISTS***



(RSSTI) Red Sea
Sustainable Tourism
Initiative



(TDA) Tourism
Development Authority



(USAID) United States
Agency for International

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Laboratory operational Guidelines

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Introduction

1- Introduction

Implementing a monitoring program requires access to resources including an equipped laboratory, office space, and equipment for field work, transport and trained personal.

This report will concentrate on the laboratory as one of the monitoring resources.

A number of options may be available for conducting analysis of water samples. The agency responsible for the monitoring program must have its own laboratory.

Some analytical work will inevitably be done in the field, using either field kits or a mobile laboratory. Regardless of the option chosen, the analytical services must be adequate for the volume of work expected. Some of the relevant considerations in this context are.

- Variables to be analyzed, If only a few simple tests are required, analyses can be under taken in the field using field kits. More complex testing programs may require the services of specialized laboratories.
- Sampling frequency and number sampling stations. The frequency with which samples must be taken and the number of sampling stations involved will obviously influence the volume of work necessary and hence, the staff and facilities required.
- On- Site testing. Some analyses must be performed in the field. Modern field kits are available that permit analyses of a wide range of variables. This makes it possible to run a monitoring program without the need for a fixed laboratory, but raises certain problems of analytical quality control.
- Temporary Laboratories—if a monitoring program is expected to be short duration, , It may be expedient to set up a temporary laboratory.
- Mobile laboratories. It is possible to set up a laboratory in a suitable motor vehicle, e.g. truck or van. In effect, this is a variant of on – site testing, but may provide better facilities than field kits.

In practice, the usual arrangement is for the agency responsible for water quality monitoring to establish its own central laboratory. However, if the monitoring area is large or transportation is difficult, regional laboratories may be set up or field kits used for certain analyses. Analyses that require only highly trained personnel can under take expensive and sophisticated equipment or that are performed only at the central laboratory.

(1-2)

Laboratory Safety

1-2 Laboratory Safety

The following points provide guidance to all laboratory workers in ensuring that analyses are performed safely.

- All employees must receive and understand safety instructions in the work place (Hazardous Materials Information System).
- Chemical should store according to color, coding and standard regulations.
- Good house keeping is extremely important to maintain a safe, clean work environment.
- Follow the safety precautions provided by the manufacturer when operating the instruments.
- Monitor instruments while they are operating.
- Avoid working alone. If you must work alone, have someone contact you periodically.
- Learn what to do in case of emergencies such as fire, chemical spill- etc.
- Learn emergency first aid.
- Report all accidents and near misses. Access to eyewash fountains and safety showers must not be blocked. Fountains and showers should be checked periodically for proper operation.
- Use forceps, tongs or heat – resistant gloves to remove containers from hot ovens or muffle furnaces.
- Do not eat, drink, or smoke in the laboratory.
- Do not use laboratory glassware for eating or drinking. Do not use food containers to hold chemicals
- Do not store food in the laboratory.
- All electrical, plumbing and instrument maintenance work should be done by qualified personnel.
- Use fume hoods when handling concentrated acids, bases and other hazardous chemicals. Fume hoods should be checked routinely for operating efficiency. Do not use them for storage.
- Muffle furnaces must be vented to the atmosphere.
- Use personal safety equipment as described below.
 - Body protection lab coat and chemical resistant apron.
 - Hand protection: gloves, particularly when handling concentrated acids, bases and other hazardous chemicals.
 - Dust mask when grinding soil samples- etc.
 - Eye protection safety glasses with side shields.
 - Full-face shield: wear face shields over safety glasses in experiments involving corrosive chemicals.

- Cylinders of compressed gases should be secured at all times.
- Never open a centrifuge cover until machine stops completely.
- Acid, hydroxides and other reagents should be in plastic. Coated bottles and carried in rubber bottle carries.
- Do not pipit by mouth.
- When diluting, always add acid to water. Not water to acid.
- Wash hands thoroughly after handling salts that might be toxic.
- Dispose of chipped or broken glassware in specially marked containers.
- Extreme care is required when using perchloric acid, otherwise fires or explosions may occur. Work must be performed in special fume hoods, certified as perchloric acid – safe with a duct wash-down system and no exposed organic coating, sealing compound or lubricant.

(1-3)

**Quality Assurance of analytical
Data**

Quality Assurance of Analytical Data

Quality Assurance principles are followed to ensure reliability of results. They consist of two parts:

- 1- Quality control: guidelines, procedures and practices developed and implemented to produce high quality results. These are implemented on a daily bases.
- 2- Quality assessment procedures and activities to verify the effectiveness of quality control procedures and to evaluate quality of data.

Quality control and Assessment procedures

- Good laboratory practices (e.g. house keeping, storage of chemicals, laboratory techniques) and good management practice (e.g. calibration, maintenance of equipments) are integral part of quality control. The laboratory should be maintained in a clean and organized manner. All chemicals should be dated on receipt and disposed of when shelf life is exceeded.
- Method of analyses are documented and followed.
- Specific conductance of distilled water is routinely checked. Double water is used for trace element analysis.
- Dilute working standard are prepared daily.
- Certain analysis such as Bacteriological Analysis and BOD are determined within 4 hours while some other e.g. extractable Fe and AL are determined within 48 hours. Of extraction.
- Glassware and plastic ware are rinsed with tap water immediately after use for most analyses rinsing with tap water followed by distilled water is sufficient. For certain analyses, however, washing with dilute HCl followed by thorough rinsing with distilled water is required.
- Glass ware stored in dust free cabinets.
- Care is exercised in sampling and sample handling. Sample integrity is ensured. Samples are stored according to their analytical requirements.
- Operations and service manual for all instrumentation are strictly followed.
- Balance are checked daily and serviced annually by trained service personnel.
- Records of downtime and service on equipment are maintained to assist in projecting repair and replacement needs.
- Sample should be collected in suitable containers, preserved in certain temperature and storage for a certain storage time. All these cautions and suggestion are listed in the following table.

Suggested preservative treatments and maximum permissible storage time

Variable	Recommended container 1	Preservative	Max permissible Storage time
Alkalinity	Polyethylene	Cool 4 °C	24h
Aluminum	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Arsenic	Polyethylene	Cool 4 °C	6 month
BOD	Polyethylene	Cool 4 °C	4h
Boron	Polyethylene	Cool 4 °C	6 month
Cadmium	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Calcium	Polyethylene	Cool 4 °C	7 days
Carbonates			
Pesticides	Glass	H ₂ SO ₄ to PH <4,10g Na ₂ SO ₄ l ⁻¹	Extract immediately
Carbon			
Inorganic/organic	Polyethylene	Cool 4 °C	24h
Particulate	Plastic Petri dish	Filter using G f /C filter: Cool 4 °C	6 month
Chloride	Polyethylene	Cool 4 °C	7 days
Chlorinated Hydrocarbon	Glass	Cool 4 °C	Extract immediately
Chlorophyll	Plastic Petri dish	Filter on GF/C filter Freeze – 20 °C	7 days
Chromium	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	Extract immediately
COD	Polyethylene	Cool 4 °C	24h
Copper	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Dissolved oxygen (Winkler)	Glass	Fix on site	6h
Fluoride	Polyethylene	Cool 4 °C	7 days
Iron	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Lead	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6month
Magnesium	Polyethylene	Cool 4 °C	7 days
Manganese	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Mercury	Glass or Teflon	1 ml Conc. H ₂ SO ₄ +aml 5% K ₂ Cr ₂ O ₇	1 month
Nickel	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month
Nitrogen			
Ammonia	Polyethylene	Cool 4 °C ,2ml 40%H ₂ SO ₄ ⁻¹	24h

Variable	Recommended container 1	Preservative	Max permissible Storage time
Nitrate + Nitrite	Polyethylene	Cool 4 °C	24h
Organic nitrogen	Polyethylene	Cool 4 °C	24h
Organic particulates	Plastic Petri dish	Filter using Gf/Cfilter: Cool 4 °C	6 month
Organo-phosphorous	Glass	Cool, 4°C.10% HCl to Ph 4.4	No holding.
Pesticides			Extract-ion on site
Pentachlorophenol	Glass	H ₂ SO ₄ to PH <4,0.5g CuSO ₄ l ⁻¹	24h
PH	Polyethylene	None	6h
Phenolics	Glass	H ₃ PO ₄ toPH ₄ , 1.0g CuSO ₄ l ⁻¹ Sample: Cool 4 °C	24h
Herbicides	Glass	Cool 4 °C	Extract immediately
Phosphorus, Dissolved	Glass	Filter on site using 0.45 pm filter	24h
Phosphorus, Inorganic	Glass	Cool 4 °C	24 h
Phosphorus, Total	Glass	Cool 4 °C	1 month
Potassium	Polyethylene	Cool 4 °C	7days
Residue	Polyethylene	Cool 4 °C	7 days
Selenium	Polyethylene	1.5 ml Conc. HNO ₃ l ⁻¹ sample	6 month
Sodium	Polyethylene	Cool 4 °C	7 days
Silica	Polyethylene	Cool 4 °C	7 days
Electrical Conductivity	Polyethylene	Cool 4 °C	24h
Sulfate	Polyethylene	Cool 4 °C	7 days
Zinc	Polyethylene	2 ml Conc. HNO ₃ ⁻¹ sample	6 month

- Record of any stabilizing preservation treatment.
- Results of any measurements completed in the field.
- Sample bottle should be placed in a box for transport to the laboratory. Sturdy, insulated wooden or plastic boxed will protect sample from sunlight, prevent breakage of samples bottles, and should allow a temperature of 4 °C to be attained and maintained during transport.

Matrix match is important in calibration.

Calibration is important pH meters are calibrated against two buffers bracketing the expected pH of the samples. Atomic absorption spectrophotometers,

inductively coupled plasma spectrometers and other such meters are calibrated with standard solutions for every batch of samples. Standards are checked every 20-50 samples, and at the end of each batch. After standardization of the instrument, accepted deviation of analytical results must range within 0-4% of the true value.

All details of the analytical work (worksheets) are filed as permanent records.

Number of significant integers: Only the last figure reported should be in doubt.

Samples received for analysis are checked for acceptability (e.g., sample condition, appropriate documentation) and lab numbers are assigned and noted in a logbook. The logbook records names of submitters, consecutive serial (lab) numbers, date samples were received, date samples were analyzed, date of sample disposition, and name of analyst.

Method blanks are required to correct for contamination in reagents and other materials (e.g., filter paper, acids, water). Method blanks are run for each group of samples analyzed. This involves repetition of the entire procedure without including the sample. Blanks containing the matrix of the calibration standards are analyzed at the beginning of each batch, after every 20-50 samples, and at the end of each batch.

Duplicate samples are used to determine within-run precision. To duplicate means to repeat the whole procedure. If an analysis is repeated because the first result appears anomalous, this should not be considered a duplicate. For routine analysis one duplicate sample is run for every 20 samples to monitor the precision or reproductively of the method. All relative standard deviations calculated from duplicate sample analysis should be within acceptable limits (5-15%, depending upon parameter and an analyte concentration). No further samples are analyzed unless duplicate results are acceptable. The total within laboratory standard deviation includes between -run (between-batch) and -within-run (within-batch) variations.

Internal audits are performed using "blind" check samples. These are samples of known composition that are given to the analyst without his or her knowledge. Blind samples are intermingled with and indistinguishable from actual samples to ensure that they do not receive special treatment.

Recover (%) of added elements: Samples are "spiked" with a known amount of pure analyte. "Spikes" are added to unprocessed samples (e.g., soil, foliage), extracts, digests or other solutions. The level of spike should be approximately equal to the endogenous level or 10 times the instrumental Detection limit, whichever is greater. Percent recovery of the added element is calculated-as follows:

$$\% \text{Spike recovery} = \frac{\text{Concentration of Analyte in Spiked sample} - \text{concentration of analyte in unpicked sample}}{\text{concentration of spike}} \times 100$$

Recoveries should be within acceptable limits ($100 \pm 10\%$). high recoveries may indicate variable blank and contamination.

This is a useful procedure for "total" analysis but not for extractable on soil because the form of the spike addition (i.e., compound added) may be fully recovered, which does not necessarily indicate whether the extraction recovers 100% of the fraction (e.g., Fe^{++} by pyrophosphate) that it is thought to recover.

To ensure valid data, known **reference materials** are run with each batch of samples. If results are not acceptable corrective measures are taken before performing analysis on actual samples. Also, if the results are questionable, the analysis is repeated on those samples. Reference materials include the following:

- (i) **Internal reference materials:** Samples collected, prepared, and analyzed by several analyst within the analytical service. Analytical Northern Forestry Center.
- (ii) **External reference materials:** Samples analyzed by different, laboratories. To ensure that laboratories produce credible data, it is important to participate in inter laboratory comparison studies.

The results are reviewed and checked for calculation and transposition errors before they are released. The same care is exercised in checking data that is exercised in doing the analytical work. The calculation check includes the entire process and a check of arithmetic.

(1-4)

Lab. Techniques

Lab Techniques

Sampling Technique:

- **Sample flask cleansing:** Usually, the flasks are suitable for sampling. If the samples are to be kept for long period before testing, the flask should be sealed firmly to avoid evaporating (in case of volatility) and it should be well cleaned before usage. This includes washing by a detergent and rinsing by tap water, then to be rinsed by diluted Nitric acid solution 1:1, and again to be rinsed by water. Finally, it should be rinsed by desalinated/distilled water, dried and stored in a closed place.
- **Note:** If a white layer, similar to calcium scales, is formed on the flask or its cap, such layer is to be removed by strong sulfuric acid or other strong acid. Using water and sand can also clean the flask.
- **Sampling:** Sample should be taken carefully and never to be exposed to any pollution. Only clean flasks should be used after being rinsed several times by water before sampling. The tap or source of water should be left opened for a while to get rid of any line turbidity (sample line), and then the sample is to be taken. The flow of sample should be slow to avoid whirlpool and air bubbles.
- **Lab techniques:** Flask cleansing: a detergent must be used to clean all flasks of chemical tests like cones, glass, standard jar, and burette. They all must be rinsed with tap water several times and finally rinsed by desalinated water.
- **How to mix (add) samples and buffers:** there are two methods to add detectors to the sample, namely:
 - **First:** swirling method:
This method depends mainly on rotating movement and is used on adding buffers to the sample in quadrant-shaped flask. The flask is to be hold in a certain manner where the thumb is put on the flask neck and the forefinger is put on the flask mouth. The forefinger of the other hand is to be put under the bottom of the flask. The rotating direction is two ways direction, one from right to left and the opposite to mix the sample well. This method is used also on using titration flasks – graduated cylinder. In this case, the cylinder is hold firmly by three fingers (thumb, forefinger, and middle finger) and putting the cylinder at 45-degree angle. This makes the cylinder in a rotating movement and mixed the sample in few turns.
 - **Second method: Using Volumetric flasks:**
The chemicals are to be weighed accurately on a balance (in very small glass, e.g. 50 mm). Then the substance is to be

moved into the flask. If some remaining substance is left in the glass, it has to be washed by water or by other detergent if the substance is not dissolving in the water. The wash bottle is the best way for this. The sample flask is to be filled by water or a suitable solution up to the mark on the flask. The mixing process shall be done manually or by stirrer several times to ensure mixing.

- **Analyzing techniques:**
- **Sample dilution:** titration method: in this method, a quantity of the sample is calibrated with a titrant. In some cases, the color of the sample is changed soon after adding few drops of the titration substance, so a big amount of the sample should be used in order to use bigger amount of the titrant to have accurate results. In this case, before calibrating the sample, a solid buffer should be added, or a liquid buffer if necessary.
- This dilution process shall be followed when the sample have a lot of elements to be assessed. Therefore, a big burette (50 mm) full of the titrant is to be used.
- For example, if the sample to be taken is 50 mm, it can be diluted to half or quarter, etc, by desalinated water.
- For instance, we can take 25 mm of the sample + 25 mm desalinated water, so the dilution rate is 50% or 1:1 (the result x 2) according to the dilution process.

Sampling sites:

First: Desalination Plant: The sample is to be taken form the outlet of the desalination units after adding the necessary chemicals or to be taken from storage tanks.

Second: Wastewater Plant: The sample is to be taken from the following places:

- 1- Raw water inlet.
- 2- Sedimentation water.
- 3- Filtration water (valid for agriculture purposes).

(1-5)

Data & Results Management

1-5 Data and Results Management

The management of the data and results starts from the beginning of sample collection till the sort of the results. The management are conducted by three teams, field, lab and QA / QC. Unit.

1-5-1 Field Team:

The tasks of the field team are concentrated in the following activities

- ↖ Water samples collection
- ↖ Coding the samples
- ↖ Record the field visual observations
- ↖ Field measurements such as Temp, EC, pH, D.O.
- ↖ Sample reservation and transportation according to QA / QC process.

The Field team manages the previous activities as follow: -

Water samples with their codes are sent to the lab
List of recommended analyses is also sent to the lab.

Field visual observations and field measurements to the QA / QC unit.

1-5-2 The laboratory

The tasks of the laboratory are summarized as follow

- ↖ Check the samples (Code and Temp) to be sure that the sample is accepted to be analyzed
- ↖ Conducting the analysis which are recommended
- ↖ Sending the results after carrying out the QA/QC process to the QA / QC unit.

1-5-3- Quality Assurance and Quality Control unit (QA / QC)

This unit is responsible for the following: -

- Receiving the field visual observations and measurements from The field team
- Receiving the results of the analysis from the lab team.
- Comparing the results with the field visual observations and other notes.
- Storing the final products of the results.

(1-6)

**Laboratory Analysis to be
assessed**

1-6 Laboratory Analysis to be Assessed

Chemical analyses to be assessed in the marine environment (seas , lakes , jetties, walk ways and marinas) are the elements identified in annex (a) of the annexes of the Executive Regulations of Law 4, 1994, concerning criteria and standards of some substances to be discharged in sea water, namely:

- Temperature.
- PH.
- Color.
- BOD.
- Total dissolved solids
- Suspended Particulate matters.
- Turbidity.
- Sulfates.
- Hydrocarbons Petroleum.
- Phosphate.
- Nitrates.
- Phenols.
- Fluorides.
- Aluminum.
- Ammonia.
- Mercury.
- Lead.
- Cadmium.
- Arsenic.
- Chrome.
- Copper.
- Nickel.
- Iron.
- Zinc.
- Silver.
- Barium.
- Cobalt.
- Pesticides of all kinds.
- Fecal coli-form MPN/100 cm³.

In addition to some in-site sample visual observations like:

- Alga presence.
- Feces presence.
- Oil spots.

1-6-1 Chemical solutions

The determination of the previous parameters differs from one to one. Many instruments such as ICP, AABS, can determine the heavy Metals. These instruments need only standard solutions.

There are some other parameters can be determined color-metry such NO₃, phenols & sulfate. These parameters can be determined by colorimeter or spectrophotometer and they need to prepare chemicals and solutions.

There are some other parameters such as Cl, SO₄, HCO₄, BOD and COD, which need chemicals and standard solutions to be determined. The preparations of the chemicals and solution will be explained as follow.

1-6-2 CHEMICAL AND SOLUTIONS PREPARATION

It is important to write down all the information concerning the solution on a label on the flask or bottle. That is:

- Solution name.
- The concentration.
- Date of preparation.
- Date of expiring.
- Who prepared it.
- How to store it.

1-6-2-1 Preparation of Titration Solutions

Water samples

0.05n AgNO₃ Dissolve 8.4935g AgNO₃ in 1 liter of distilled water (measuring Flask). Store in a dark bottle at 4°C.

0.1 n HCl: Dilute 8.3 ml of concentrated HCl till 1 liter with distilled water (Measuring flask).

0.01 m EDTA: Dissolve 3.72 g Na₂ EDTA. 2H₂O in 1 liter of distilled water (Measuring flask). Store in a plastic bottle at 4°C.

Soil Paste samples

0.005 nAgNO₃: Dissolve 0.8494 g Ag NO₃ in 1 liter of distilled water (measuring flask). Store in a plastic bottle at 4°C

0.01 in HCl: Dilute 0.83 ml of concentrated HCl till 1 liter with distilled water (measuring flask).

0.01 m EDTA: Dissolve 3.72 g Na₂ EDTA. 2H₂O in 1 liter of distilled water (Measuring flask). Store in a plastic bottle at 4°C.

1-6-2-2 Standard Solutions

0.05n NaCl: Dissolve 2.9225g NaCl 1 liter of distilled water (Measuring flask). The NaCl must be dried 2 hours at 300 °C.

0.01 m CaCl₂: Weight 1.000 g CaCO₃. Put this in a measuring flask of liter. Add 50ml in HCl. Dilute till 1 n liter with distilled water. The CaCO₃ must be dried during 2 hours at 400 °C. Store at 4°C.

0.05 n Na₂ CO₃: Dissolve 2.6500 g Na₂CO₃ in 1 liter of distilled water (Measuring flask). The Na₂CO₃ must be dried 2 hours at 300° C

1-6-2-3 Adjusting Normality

Ag NO₃

0.005n: Pipet 1 ml of 0.05n NaCl standard solution. Treat this as a normal sample. Note the amount necessary to reach the endpoint.

The normality is;

$$\frac{0.05}{\text{Amount.titrated}(ml)}$$

0.05n: Pipet 10 ml 0.5 n NaCl standard solution. Treat this as a normal sample. Note the amount necessary to reach the endpoint.

$$\frac{0.5}{\text{Amount.titrated}(ml)}$$

The normality is;

HCl

0.01n: Pipet 2 ml of 0.05 Na₂CO₃ standard solutions. Treat this as a normal sample. Note the amount necessary to reach the endpoint.

The normality is;

$$\frac{0.1}{\text{Amounttitrated}(ml)}$$

Or Titrate 20.00 ml HCl in a beaker of 100ml. Titrate this solution with 0.05 n Ag Na₃ to measure the chloride. Note the volume necessary to reach the endpoint.

Note the normality;

$$\frac{\text{Volume AgNO}_3 \times \text{normality AgNO}_3}{20.00}$$

0.1n: Pipet 20 ml of 0.05 n Na₂CO₃ standard solutions. Treat this as a normal sample. Note the volume necessary to reach the endpoint.
Note the normality is: -

$$\frac{20 \times 0.05}{\text{Amount titrated (ml)}}$$

Or Titrate 5.00 ml HCl in a beaker of 100 ml. Titrate this solution with 0.05 n Ag NO₃ to measure the chloride. Note the volume necessary to reach the endpoint.
Note the normality;

$$\frac{\text{Volume AgNO}_3 \times \text{normality AgNO}_3}{5.00}$$

Ferrous ammonium sulfate solution:

Pipet 10.0ml standard potassium dichromate solution 0.25 N and dilute to about 250ml. Add 20ml conc. H₂SO₄ and allow to cool. Titrate against the ferrous ammonium sulfate using 2or3 drops of ferrion indicator.
Note the normality;

pH

Prepare buffer solutions from standard tablets or solutions prepare fresh solutions every month and compare new solutions with old ones.

Conductivity

0.05 m KCl: Dissolve 3.735g KCl in liter of distilled water (measuring flask)

0.01 m KCl: Dissolve 0.745g KCl in liter of distilled water (measuring flask)

Chloride

- Indicator Potassium chromate: dissolve 5 g Potassium chromate in 50 ml distilled water.
- 1 N HNO₃: dilute 7 ml conc. HNO₃ to 1 liter.

Bicarbonate

- Phenolphthalein indicator: dissolve 105 g Phenolphthalein in 100 ml ethanol 96%.
- Methyl orange indicator: dissolve 100 mg methyl orange in 1 liter distilled water.

Calcium and magnesium

- Buffer solution: dissolve 16.9 g ammonium chloride (NH₄CL) in 112 ml conc. Ammonium hydroxide (NH₄OH) dilute to 1 liter with distilled water.
- Indicator solution: dissolve 0.5 g Erie- chrome-black- T and 4.5 g of hydroxylamine hydrochloride in 100 ml of ethanol 96%.
- Hydrochloric acid (HCl) 6 mol/L
- Phosphate buffer: dissolve 8.5 g K₂HPO₄; 33.4 Na₃HPO₄. 7H₂O and 1.7 g NH₄Cl in about 500 ml distilled water and dilute to 1 liter. The pH of this buffer should be 7.2.
- Magnesium sulfate solution: dissolve 22.5 g Mg SO₄. & H₂O in distilled water and dilute to 1 liter.
- Calcium Chloride solution: dissolve 27.3 g anhydrous Ca Cl₂ in distilled water and dilute to 1 liter.
- Dilution solution: prepare fresh before use. Place the desired volume of distilled water in a suitable bottle and add 1 ml each of phosphate buffer, Magnesium sulfate, Calcium chloride and ferric chloride solutions for each liter of water. Aerate this solution for 5 hours.

Chemical oxygen Demand

- Standard Potassium dichromate solution 0.250N: dissolve 12.259 g K₂Cr₂O₇ dried at 105 C for 2 hours in distilled water and dilute to 100 ml (measuring flask).
- Conc. H₂SO₄ with silver: add 10 g Ag₂SO₄ to 1 liter conc. H₂SO₄. Leave it for a day to dissolve.
- Standards ferrous ammonium sulfate solution 0.25N: dissolve 98g Fe(NH₄)₂(SO₄)₂. 6H₂O in distilled water. Add 20ml conc. H₂SO₄, cool and dilute to 1000 ml (measuring flask). This solution must be standardized against the K₂Cr₂O₇ daily (see 6.3).
- Ferrion indicator: this indicator solution can be purchased already prepared (Merck)

1-6-2-4 Reference samples

- Nitrate: Dissolve 606,0mg NaNO₃ (dried 4 hours at 110C) in 1000ml of distilled water. Prepare this solution every 6-month and store at 4C. Dilute 20 times before use. This solution contains 5 mg-N/L or 0.36 meq-N/L. Prepare this solution every day before use.
- COD: Dissolve 2.125g potassium hydrogen phthalate acid (dried one hour at 103C) in 100 ml of distilled water. This solution contains 2500 mgO₂/L Store at 4C. Prepare a new solution every week.
- BOD: Dissolve 150 mg glucose and 150 mg glutamic acid (both dried one hour at 103C) in 1000 ml of distilled water. Store at 4°C. This solution contains 200 mg O₂ /l. Prepare a new solution every week.

(1-7)

Methods of analysis

WATER PARAMETERS MEASUREMENTS: -

1-Total Dissolved Solids Measurement

This experiment is considered the main issue to classify the water quality and identify the possibilities of using such water for different human requirements.

Apparatus

-Evaporating dishes , Dishes of 100 ml capacity made of the following material:

(a) Porcelain , 90mm diam.

(b) Platinum –generally satisfactory for all purposes

-wide pore pipets

-Graduated cylinder

-low form beaker

-Desiccators provided with desiccant

-Analytical balances

Procedure.

-Dry the glass beaker in the oven at 100-105°C for one hour.

-Put the glass beaker in the desiccators to cool. Then weigh it .Dry and weigh until the constant weight is obtained

Stir the sample with a magnetic stirrer and pipet a measured volume onto glass –fiber filter with applied vacuum wash with three successive 10-ml volumes of reagent grade water.

Transfer total filtrate to a weighed evaporating dish and evaporate to dryness in a drying oven –dry evaporated sample for at least one hour at 180 °C. Cool in a desiccators to balance temperature and weigh .

-Repeat drying cycle of drying, cooling desiccating and weighing until a constant weight is obtained.

Calculations

Filtrated Weight in 100cm³ of the sample = the weight of beaker with filtrated matter after evaporation –empty weight

$$\text{TDS (PPM)} = \frac{\text{Filtrated weight in 100 cm}^3 \times 10000}{\text{Total volume of the sample}}$$

2 Conductivity Measurements

Conductivity is the numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valiancy and relative concentrations, and on the temperature of the solutions.

Calibration

Be sure that on the right of the instrument only the lamp of 3 KHz is on.

Take the 0.05 m KCl solution.

Measure the temperature of the solution. Adjust the temperature on the meter.

Rinse the cell with some KCl solution.

Measure the conductivity. Adjust with CONST ADJ to read 6.66.

Note the value of CONST ADJ in the control table.

Measure the conductivity of the 0.01 m KCl solution. The reading must be 1.41 ± 0.05 . If not, make new solutions. Note the value in the control table.

3-pH Measurement

pH is considered one of the most important elements for conducting chemical experiment

- It is defined as minus log of Hydrogen Ion concentration ($\text{pH} = -\log H$)

The Range of pH values is ranged between 0- 14

The Reading of the pH can be measured colorimetric or by electrical instrument

Tools and Instruments

Nessler Tubes 50 cm³

pH meter

Procedure

Calibrate the pH –meter

Rinse the electrode with distilled water.

Take buffer pH-7.

Adjust the meter with pH to read 7.

Rinse the electrode with distilled water.

Take buffer pH-4 and adjust the meter to read 4 with mV/pH.

Rinse the electrode with distilled water.

Control the meter with buffer pH-7. If the reading is 7.0, the calibration was correct. If the deviation is smaller than 0.1 unit re-adjust and the calibration is also correct. If the deviation is more than 0.1 unit, start again.

Change the reading of the instrument to mV and measure the potential of buffer pH-7. Note this value in the buffer pH-4 and note this value in the control table.

Change the reading of the instrument to pH and start measurements.

Colorimetric Methods

Pipet 25 ml of the sample to Nessler tube

Add 0.1 BDH – universal indicator and shake

Put this tube in the right side of Nessler set

Pipet 25 ml of the sample in other tube

Compare between the colors of the two tubes and that of the standard colors of the pH

Note the accurate and suitable Reading

4-Chloride Measurement

Procedure iteration of chloride concentrations less than 100 mg/L: Use a 100-mL sample or smaller portion so that the chloride content is less than 10 mg.

Add 1.0 mL indicator –acidifier reagent. (The color of the solution should be green-blue at this point. A light green indicates pH less than 2.0; a pure blue indicates pH more than 8.3.) For 0.10 For most potable waters, the pH after this addition will be 2.5 ± 0.10 For highly alkaline or acid waters. Adjust pH to about 8 before adding indicator –acidifier reagent.

Titrate with 0.0141 N Hg (NO₃) titrate to a definite purple end point. The solution turns from green –blue to blue a few drops before the end point. Determine blank by titrating 100 mL distilled water containing 100 mg NaHCO₃.

b. Titration of chloride concentrations greater than 100 mg/L: Use a sample portion (5 to 50 mL) requiring less than 5 mL titrant to reach the end point. Measure into a 150-ml beaker. Add approximately 5.0 mL mixed indicator reagent and mix well. The colour should be purple. Add 0.1 N HNO₃ drop wise until the color just turns yellow. Titrate with strong Hg (NO₃)₂ titrant to first permanent dark purple. Titrate a distilled water blank using the same procedure.

Using Potassium Chromate as indicator

Water samples

Pipet 20 ml sample in an erlenmeyer flask of 100 ml.

Add 10 drops (about 0.2 ml) indicator solution.

Titrate with 0.05 m AgNO₃ to a pinkish /yellow endpoint.

Note the amount of AgNO₃ used (a ml).

Do a blank titration using 20 ml distilled water.

Note also this amount used (b ml).

Note the normality of the solution.

Calculation

$$mCl - / L = \frac{(A - B) \times N \times 35450}{mL_{sample}}$$

$$- \{Cl\} = 50 \times n \times a \text{ (meq/l)}$$

Where n = normality of AgNO₃ solution

a = amount of AgNO₃ used for sample.

5-Hardness Measurement

Originally, water hardness was understood to be a measure of the capacity of water to precipitated soap is precipitated chiefly by calcium and magnesium ions present. Other polyvalent cations also may be precipitate soap, but they often are in complex form, frequently with

organic matter constituents, and their role in water hardness may be difficult to define. In practice the total hardness is defined as the sum of Ca and Mg, both expressed as calcium carbonate, in milligram per liter.

Procedure

a. Pretreatment of polluted water and wastewater samples: Use nitric acid-sulfuric acid-perchloric acid digestion (section 3030).

b. Titration of sample: Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from time of buffer addition.

Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point color change in the titration usually means that an inhibitor has deteriorated.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation (2c3). Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears. Add the last few drops at 3- to 5-s intervals. At the end point the solution normally is blue. Daylight or a daylight fluorescent lamp is recommended highly because ordinary incandescent lights tend to produce a reddish tinge in the blue at the end point.

C. Low-hardness sample: For ion-exchanger effluent or other softened water and for natural waters of low hardness (less than 5 mg/L), take a larger sample, 100 to 1000 mL, for titration and add proportionately larger amounts of buffer, inhibitor, and indicator. Add standard EDTA titrant slowly from a microburet and run a blank, using redistilled, distilled, or deionized water of the same volume as the sample, to which identical amounts of buffer, inhibitor, and indicator have been added. Subtract volume of EDTA used for blank from volume of EDTA used for example.

Calculation

$$\text{Hardness (EDTA) as mg CaCO}_3\text{/L} = \frac{A \times B \times 1000}{\text{mL sample}}$$

Where:

A = mL titration for sample and

B = mg CaCO₃ equivalent to 1.00 EDTA titrant.

$$\text{Hardness by calculations} = 2.497[\text{Ca mg/l}] + 4.118[\text{Mg mg/l}]$$

6- Biological Oxygen Demand Measurement

The biological oxygen demand is the measure for the oxygen necessary to degrade organic matter by micro-organisms. In the method the water

is allowed to stand for 5 days and the oxygen concentration is measured at the beginning and after 5 days. The difference is the BOD. Because the maximum oxygen concentration in water is about 10 mg/l, water samples with high BOD values need a dilution.

Standard Solution

Phosphate buffer: dissolve 8.5 g KH_2PO_4 ; 21.75 g K_2HPO_4 ; 33.4 Na $\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 1.7 g NH_4Cl in about 500 ml distilled water and dilute to 1 liter. The pH of this buffer should be 7.2.

Magnesium sulphate solution: dissolve 22.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water and dilute to 1 liter.

Calcium chloride solution: dissolve 27.3 g anhydrous CaCl_2 in distilled water and till 1 liter.

Ferric chloride solution: dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water and dilute till 1 liter.

Dilution solution: prepare fresh before use. Place the desired volume of distilled water in a suitable bottle and add 1 ml each of phosphate buffer, magnesium sulphate, and calcium chloride and ferric chloride solutions for each liter of water. Aerate this solution for 2 hours.

Method

Prepare a fresh dilution solutions

Calibrate the oxygen –electrode

If the sample have a $\text{pH} > 9$ or < 5 neutralized with 1 N NaNO_3 or H_2SO_4 .

Make 2 dilutions for every sample.

Aerate the diluted sample for one minute.

Immediate fill the BOD bottle.

Measure the oxygen concentration in the bottle and note the value.

Close the bottle and be sure that is completely filled.

Store the bottles during 5 days in dark incubator at the temperature of 20 $^{\circ}\text{C}$.

After 5 days measure the O_2 contents again.

Measure also the O_2 content of the dilution –water at start and after 5 days.

Calculation

Dilution water:

$\text{BOD} = \text{O}_2 \text{ <start> } - \text{O}_2 \text{ <5 days > (mg/l)}$

7- Inorganic Elements Measurement

Instrument: Spectrophotometer

Solution –: ready made standard solution & calibration

Procedure –: calibration according to manufactures calibration procedure

8-EC Measurement

Instrument : EC meter with a rang 0.00 to 1. 99uS/cm
Chemicals : KCl standard solution 0.01 and 0.05 N
Procedure : calibrate the instrument with the standard solution ,.01 gives a reading of 1.41 and 0.05 gives a reading of 6.65
Immerse the electrode in a water sample and find and record the reading, there is an automatic adjustment for the reading to be at 25° C

9-Salinity Measurement

Instrument : EC meter
Solution : KCl 0.01 n and 0.05 n
Procedure : measure the EC of the water sample
Salinity = EC x 640 mg

10- WATER TURBIDITY MEASUREMENT

Instrument : Turbidity Meter
Solution : standard solution of known NTU concentration
Procedure : calibrate the instrument with the standard solution
Immerse the electrode in the sample, Record the results

11-TEMPERATURE MEASUREMENT

Instrument: Temperature Meter
thermometer should have a scale marked for every 1 °C

(1-8)

Bacteriological analyses

1-8-1 Sampling

Samples must be collected into clean sterilized glass bottles of min. 100 ml capacity, in such a way to guarantee that they are representative of the water being examined. It is important to prevent contamination of the water sample. Sample bottles should not be filled to the brim, since enough space should be left to shake the contents thoroughly before cultures are prepared.

Microbiological testing should take place as soon as possible after the samples have been collected. If the water cannot be tested within one hour they should be well cooled, especially if any lengthy transportation is involved. Samples should be transported in insulated containers and stored in a refrigerator. Even with carefully cooling, no more than 30 hours (Standard Methods) elapse between sample collection and analysis.

1-8-2 Bacteriological Count

There are two ways of establishing the bacterial count in water:

- The MPN (most probable number) method;
- The membrane filtration method.

1-8-2-1 Decimal dilution Series

Fill a series of test tubes or conical flasks with 9 ml or 99 ml of water or buffer solution, close with cotton wool plugs or caps and sterilize.

Pipet 1 ml of water sample into a tube or flask and shake to mix. Pipet 1 ml from the first tube or flask into a second tube or flask and again shake to mix.

Prepare the subsequent stages in a similar manner. Thereby producing dilutions, which differ progressively by one (1+9 ml) or two (1+99ml) powers of 10.

Counting methods

MPN METHOD:

Implementation of the MPN (most probable number) method requires that the sample be divided into several (min.3) parallel dilutions by mixing with a liquid medium. The number of tubes per dilution series exhibiting growth following incubation is matched against table 11 and the MPN, i.e. the most probable bacterial count referred to 100 ml of water sample, is read off. In the case of more than three dilution stages, the highest stages at which all the tubes are still positive as well as the next two highest are used for the evaluation.

If the quantity of sample per for the three dilution stages under scrutiny differs by a factor (e.g. 0.1,10) from the table, the MPN should be multiplied by the reciprocal of the factor (e.g. 10,0.1).

Most probable number per 100 ml for 3 or 5 parallel cultures per dilution stage.

MPN for 100 ml

Positive tubes

10 ml	1.0 ml	0.1 ml	3 tubes per dilution stage	5 tubes per dilution stage
0	0	0	<3	>2
0	0	1	3	2
0	1	0	3	2
0	2	0	6	4
1	0	0	4	2
1	0	1	7	4
1	1	0	7	4
1	1	1	11	6
1	2	0	11	6
2	0	0	9	5
2	0	1	14	7
2	1	0	15	7
2	1	1	20	9
2	2	0	21	9
2	2	1	28	-
2	3	0	30	12
3	0	0	23	8
3	0	1	39	11
3	0	2	64	-
3	1	0	43	11
3	1	1	75	14
3	1	2	120	-
3	2	0	93	14
3	2	1	150	17
3	2	2	210	-
3	3	0	240	-
3	3	1	460	-
3	3	2	110	-
3	3	3	>=2400	-
4	0	0		13
4	0	1		17
4	1	0		17
4	1	1		21
4	1	2		26
4	2	0		22
4	2	1		26
4	3	0		27
4	3	1		33
4	4	0		34
5	0	0		23
5	0	1		31
5	0	2		43
5	1	0		33
5	1	1		46
5	1	2		63
5	2	0		49
5	2	1		70
5	2	2		94
5	3	0		79
5	3	1		110
5	3	2		140
5	3	3		180
5	4	0		130
5	4	1		170
5	4	2		220
5	4	3		280
5	4	4		350
5	5	0		240
5	5	4		350
5	5	2		540
5	5	3		920
5	5	4		1600
5	5	5		>=2400

1-8-2-2 MEMBRANE FILTRATION METHOD

The microorganisms present in a defined quantity of water are collected on an impervious membrane filter, transferred to a culture medium, incubated, and counted. First sterilize the equipment. Close the tap; place a sterile membrane filter on the screen using sterile tweezers with the printed matrix (if present) of the filter facing upwards. Attach the funnel and fasten it by bayonet catch. Pour the water sample into the funnel, open the tap on the filter base and draw the water through the membrane filter into the suction flask. Remove the funnel and maintain the suction for 5-10 seconds in order to remove completely and liquid remaining on the filter. Use sterilized tweezers to place the membrane filter on to the surface of the plate (with the underside of the filter in contact with the medium) and incubate. The active substances contained in the medium diffuse through the filter and enable the bacteria present on the upper surface to develop into visible colonies. For quantitative determinations, colonies are counted to the groups to which they are belonged.

E. Coli and coli form detection and count

DETECTION:

Place 100 ml of water sample into a bottle containing 100 ml of double-strength lactose peptone broth and incubate for 44±4 hours at 37-1.5 C. to be sure of detecting Escherichia coli, a parallel culture should be incubated at 42±0.5C. after 20±4 hours check for gas formation, color change (bromocresol purple: from purple to yellow) and turbidity caused by growth. If this check should prove negative, continue incubating for a further 24 hours. If, after this time, there is still no apparent change, this means that no coliform bacteria are detectable in 100 ml water sample. The test is thus concluded.

COUNT:

MPN Determination

Presumptive test: From the water sample, prepare several parallel dilutions in a decimal series in or, when a large number of secondary organisms is to be anticipated, in lauryl sulfate broth. Each test tube used, should contain a DURHAM fermentation tube. Incubate for 24±2 hours or for 48±3 hours at 35±0.5C. Tubes showing gas formation in the exploratory test are to be regarded as being a positive indication of the presence of coliforms.

Confirmatory test: From all tubes in the pre-emptive test where a gas bubble has collected in the Durham tube transfer a small quantity of material with a loop into test tubes of MacConky broth into which a Durham tube has again been inserted. Incubate for 48±3 hours at 35±0.5°C. For tubes where gas has collected the confirmatory test for the presence of coli form bacteria is positive. Inoculate streak wise on to ENDO C agar or LEVINE EMB agar for identification of the bacteria.

Bacterial counting: The NMPN is calculated for coli form taking the tubes from the 3 highest dilution stages in the exploratory test in which coli form bacteria were detected.

Test for faecal: Material from tubes which have proven to be positive in the test should be re-inoculated on to EC broth and incubated for 24±2 hours at 44.5±0.2°C. Under these conditions, any gas formation in EC broth indicates the faecal origin of the coli form bacteria.

Membrane Filtration

Filter the water sample, place the filter on to ENDO agar and incubate for 20±4 hours at 37±1.5°C. Coli forms grow as moist, red colonies and are counted according to the visual different groups to which they are belonged. In order to obtain pure cultures, at least one colony from each of the red types is transferred to the respective number of ENDO agar plates to thin as to enable individual colonies to develop.

Filter should be placed following filtration on to a cardboard disc saturated with membrane-filtration ENDO broth and incubated for 22 to 24 hours at 35±0.5°C. Improved selective cultivation of the coli forms can be achieved if the filter is first incubated for 2 hours on a cardboard disc with lauryl sulphate broth and then for 20 to 22 hours on a cardboard disc with membrane-filtration ENDO broth. The typical coliform colonies (dark red with metallic sheen) can then be counted.

Detection and count of enterococci

DETECTION

1. Presumptive Test

Inoculate vessels containing 100 ml of azide dextrose broth with quantities of water sample decreasing each time by a factor of 10. For sample quantities of

10 ml and more use double- strength broth, which is then diluted with the water sample to single strength. Incubate for up to 48+3 hours at 35+0.5C.

Positive reaction: Turbidity.

Indicates enterococci!

Negative reaction: No turbidity.

No enterococci present.

The test is thus concluded

2. Confirmatory

From each of the vessels, which yielded a positive result in the exploratory test, transfer 3 loop fills of material to a tube containing 10 ml bromocresol purple azide broth. Incubate for 24 hours.

Positive reaction: Color change to yellow, sometimes accompanied by turbidity. Enterococci present!

Negative reaction: No yellow color.

No enterococci present.

COUNT

1. MPN Determination

Prepare at least 3 dilutions of water sample in azide dextrose broth in the decimal series. Work out the MPN based on the positive tubes resulting from subsequent confirmatory test.

2. Membrane Filtration

Filter various quantities of water sample in azide filter. Then place the filter on KF streptococci agar and incubate for 48 hours at 35+0.5C. Enterococci grow as dark red to light red colonies. Take the total enterococci count, with preference being given to those plates with 20-100 colonies. Refer the count to 100 water samples.

(2)

Natural Resource Monitoring

(2-1)

**Environmental Monitoring
Guidelines Coral Reef**

2-1 Coral reef monitoring program

Introduction

To set up an effective coral reef monitoring program, we'll need to determine the following criteria:

- 1- to detect changes in abundance of a particular group of organisms
- 2- To discover possible cause-and-effect relationships
- 3- To determine if a specific management action is working (e.g., prohibition of spear fishing); and/or
- 4- To measure the effect of both natural and human-induced stresses

Some stresses or disturbances, like hurricanes or dredging, are of such magnitude as to cause immediately visible effects, while others, like over-fishing or pollution, may slowly undermine the health of the coral reef system and not be readily apparent through casual observation. An established monitoring program can help provide the necessary information to examine the effects of different stresses.

Coral reefs are the most complex marine ecosystems on earth, and it is not practical to monitor all of the reef's animals and plants and their many interactions. However, here are some basic principles to keep in mind regardless of a specific monitoring objectives.

1-To obtain in formations on

- Basic environmental parameters such as temperatures, salinity, and turbidity
- The abundance of stony corals, octocorals, algae, sponges and reef fish

2- to have both qualitative and quantitative records as well-documented site locations, use a combination of photographic and transect or quadrat method

3- To establish procedures for long-term monitoring that are as free as possible from observer bias, and easily repeated by people who may be assigned the task in the future, and make sure the procedures are well-documented

4- Make sure the monitoring site is clearly defined and easy to locate, not only by the first observer but by others who have never visited the site

Choosing a monitoring method

Choosing a monitoring method depends on what are the objectives, where are we going to monitor, how often should we collect data, for how long should we continue collecting data, what methods will give us the best data, who will conduct monitoring, what methods are realistic for us, given the available time, money, equipment, people, and skills and how will we analyze the data we collect?

Monitoring a large coral reef area

The best way to monitor a large coral reef area is to use the manta tow survey where a snorkeler is typically towed over the reef by a small outboard motor boat, stopping periodically to record data, however, a sheet like the following one can be established and monitored periodically:

Mnta Tow Survey Data Sheet				
Location			Date:	
Observer:			Time	
Tow	Percent Cover			Other features
	Stony Corals	Octocorals	Algae	
1				
2				
3				
4				
5				
6				

Permanent sites monitoring:

Some sites are to be marked as permanent sites to be monitored periodically (monthly or seasonally), like the sites encountering possible stresses like the presence of marina, tourist constructions e.g. hotels, villages, desalinating pipes etc. In that case, two types of monitoring should be carried out:

1-Physical and chemical monitoring

Physical and chemical properties of the water at the study site should be measured regularly for possible correlation with any changes observed on the reef since any changes in these parameters may affect the growth and

survival of reef organisms. Examples of physical and chemical parameters to be monitored are temperatures, dissolved oxygen, salinity, pH, light transmission, sedimentation, nutrients, current speed and direction.

2- Biological monitoring

Biological monitoring can be carried out by using any of the following sampling units depending on the objectives and site characteristics:

Sampling Unit	Monitoring Uses
Coral colony	Monitor general condition of specific stony corals, including growth, bleaching, diseases, algal overgrowth
Quadrat	Measure percent cover, species diversity, relative abundance, density and size; and monitor corals, octocorals, sponges, sea grasses and algae
Linear transect	Measure percent cover, species diversity and relative abundance in zones dominated by head corals, estimate spatial index; unsuited to Elkhorn zones, octocoral dominated pavement areas, or areas where colonies are small and scattered

Monitoring frequency

Monthly observations are generally best for monitoring individual coral colonies. Quadrat and transect surveys done every six months provide sufficient data for assessing changes in percent cover and species diversity, and reduce the risk of damaging reef organisms during the survey process. Of course, in the event of a storm, oil spill or other disturbance, it's important to assess the effects as soon as possible, survey permanent quadrats or permanent transects for which data were obtained before the disturbance, and continue to monitor the aftermath and recovery.

(2-2)

**Environmental Monitoring
Guidelines for Mangrove Sit**

Background:

Mangrove forests are one of the wetland ecosystems having high importance in economy and ecology. Mangroves have been shown to create a sheltered environment for many immature forms of marine fauna, including commercially important species of fish, molluscs, and crustaceans, thereby creating an important ecological niche as the “the nurseries of the sea”. Traditional uses of mangroves are: firewood and charcoal; forage; medicine; purification of polluted water; and mangrove regions used as natural fish fry area. Also, the mangrove coasts are wonderful landscapes for tourism.

From Hurghada southward along the Red Sea coast the mangrove is a notable and common feature of the vegetation of the littoral landscape. *Avicennia marina* (Shora) grows in pure stands. The usual habitat of the mangrove is shallow water lagoons, bays, sharms, corals or sand bar parallel to the shore. In some localities *Avicennia marina* grows on the terrestrial side of the shore line e.g. delta of Wadi El-Jimal and Marsa Shagra. The bushes are either growing in salt marsh or partly covered by sand hillocks. This situation is apparently due to the silting of shore-line zone originally occupied by mangroves.

The ecology of mangroves along the Egyptian Red Sea coast was the subject of very few studies. This report updates our knowledge on the status of mangrove vegetation in the development areas (Hurghada – Hamata sector). The following objectives are considered: 1) identification of the mangrove status and the potential environmental problems associated with

Mangrove sites e.g. grazing, trampling, cutting, etc., 2) providing an inventory of plant species associated mangroves e.g. seagrasses, macro algae and halophytes, and 3) description of the distribution pattern, density, crown cover and height of mangrove species.

These results are useful in the development of monitoring data sheet to assess the effects of these activities on growth and productivity of mangrove species. This information will be used by TDA in the development of their environmental monitoring program.

Methods

The distribution and abundance status of fourteen mangrove sites along the Red Sea coast (Hurghada-Hamata) were recorded. The sites are presented using Latitude and Longitude obtained from GPS and stored in decimal format (i.e. degrees with minutes and seconds appended in their decimal form). The cover values of mangrove and the associated flowering plants, sea grasses and seaweeds were assessed in 10m x 10m quadrates using five points DAFOR-scale (Brodie 1985). Identification of species was according to Tackholm (1974) and Boulos (1995).

In each site, the height and canopy cover of 5 mangrove trees are recorded. Also, five branches were collected to study the morphological attributes in relation to a set of measured environmental factors. These attributes are: number of leaves, leaf area and number of fruits. The number of pneumatophores and the number juvenile plants were recorded in each site. Information on the phenology, regeneration, grazing, trampling and human impact is collected about each site.

A soil sample at depth 0-10 cm was taken from each stand dominated by *Avicennia marina*. Electrical conductivity of 1:5 soil extract (EC: mS/cm) was measured with an YSI Incorporated Model 33 Conductivity Meter. The extract reaction (pH) was determined using a combined pH Meter digital ion analyzer Model 5986-60 with a glass electrode. Organic matter was determined by the Walkely-Black method (Wilde et al., 1979).

Results

Fourteen sites of mangrove growth are recorded in the development areas (Hurghada – Hamata sector). Three of these sites are new records for the mangrove growth: 1) South Sharm El-Bahry (25 51 03 N, 34 25 30 E), 2) North Shams Alam 24 41 54 N, 35 05 13 E), and 3) Sharm Luliah (24 36 16N, 35 07 19E), where *Avicennia marina* grows on the terrestrial side of the shore line, and the bushes are either growing in salt marsh or partly covered by sand hillocks.

Table 1 shows the abundance status of *Avicennia marina* in the study sites. Four sites (1, 2, 5 & 13) were dominated by *Avicennia marina* (cover > 75 %); six sites (3, 6, 9, 11, 12 & 14) showed abundant growth of mangrove (cover 50-75%); two sites showed frequent growth (cover <50 %); and the other two sites namely: Marsa Shagara and Sharm Luliah have few bushes of *Avicennia* with vegetative cover less than 10 %.

The associated species

Three groups of associated plants are recorded in the 14 study sites:

- a. The flowering plants associated with mangrove in dry habitat are 11 species. These species are recorded with mangrove in dry habitat. *Zygophyllum album* is the most common associate (10 sites), followed by *Timonium axillaries* (7 sites) and *Nit aria reuse* (4 sites).
- b. Sea grass “bed or meadows” are grass-like flowering plants with extensive root system. Four species are recorded as an associated with mangrove in shallow water habitat. *Halodule uninervis* was recorded in 4 sites.
- c. Seaweeds: The muddy substrate under mangle vegetation is not favorable habitat for algal growth, only a few species (e.g. *Enteromorpha compressa* and *Laurantia obtusa*), and mats attached to *pneumatophores* and the basis of the mangrove trees.

Habitat type

The development of mangrove vegetation is found in the Red Sea coast of Egypt on saline mud deposited in the deltas of wadis, quiet bays and sharms where a relatively stable land surface have been developed. Mangles are also found on coral reef platforms in the lagoon like seawater. In the study area, *Avicennia marina* grows in eight sites of mud flats with shallow water (sharms and lagoons); and in six terrestrial sites of the shore line (Table 2). The mangrove bushes are highly developed in shallow water compared with the terrestrial habitat.

Mangrove height

The maximum height of *Avicennia* was recorded in mud flat habitat; at low soil salinity (4.1 meter south Sharm El-Kebly, 3.9 meter Lahmy). Stunted growth forms of mangroves are recorded in the salt marsh habitat and along the marginal zones of mud flat habitats (height is less than 1 meter), this may be attributed to the effect of soil salinity and camel grazing.

Mangrove density

The density of *Avicennia marina* in 10 x 10 m quadrats was low in salt marsh habitat ranging from 1 to 3 bushes (Marsa Shagra). The number increases upto 15 individual trees and shrubs in mud flat habitat (Sharm Kebly and Sharm Bahry).

Crown cover

The crown cover of mature *Avicennia* varied greatly according the habitat. Generally, salt marsh and sand dunes sites (Marsa Shagara and North Shams Alam) are covered by widely spaced mangrove plants with crown cover ranged from 1.3 to 2.7 m² per individual. The mud flat sites (South Safaga, South Abu Ghsun, Hamata, etc) have extensive growth of mangrove. The crown cover ranged from 1.3 to

32.9 m² per individual. A huge mangrove bush was recorded in Sharm Luliah with crown cover 88 m².

Leaf number

There was a relatively lower number of leaves in stressed sites (Marsa Shagra, 6-13 per branch), compared with mud flat sites (Sharm El-Bahry, 17-24 per branch).

Fruit number

The number of fruits was relatively high in less disturbed sites. The highest number of fruits per branch (8-14) was recorded in Site 1 (south Safaga). The mangrove growing in salt marsh habitat showed no fruits or very few fruits 1 or 3 per bush (delta of Wadi el-Jimal).

Regeneration

The regeneration of mangrove was recorded in 4 sites namely: South Safaga, North Quseir, Sharm Bahry and Sharm kebly. The number of juveniles ranged from 4 to 12 per square meter. No seedlings or juveniles are recorded in salt marsh and sand dune habitat. The physical environment seems to affect the regeneration of mangrove in the study area.

Environmental Impacts

Damages and Grazing

Human impacts were identified and qualitatively assessed. Over utilization of mangroves by wood cutting and livestock grazing are abundant in the sites south Marsa Alam. Intense grazing was recorded in Lahmy, Hamata, and Wadi El-Jimal. Trampling and grazing make stunted growth form of mangrove due to the destruction of respiratory roots and the reduction of vegetative growth (Table 2).

Soil characteristics

a. Soil Salinity: The electrical conductivity (EC) of 1:5 soil extract ranged from 4.4 mmohs/cm (Sharm El-Kebly) to 32.5 mmohs/cm (Marsa Shagra).

b. Soil reaction: The soil reaction of the soil samples collected from the studied mangrove sites was slightly acidic to neutral. It varied from 6.51 (Marsa Shagra) to 7.81 (the delta of Wadi Lahmy).

c. Soil organic matter: The percentage of organic matter in the mangrove soil showed a wide range of variation. It varied from 0.2 % (North Quseir) to 2.6% (Marsa Shagra).

Recommendations

- There are significant differences in the size and health of the surveyed mangrove and its biodiversity. Furthermore, it is important to monitor the mangrove growth as well as the environmental changes along the Red Sea coast.
- Monitoring the gradual changes of mangrove areas using geographical information system (GIS) techniques.
- Tagging individuals for continuous monitoring of survival, growth and associated physical factors like salinity and soil characteristics of naturally dispersed propagules in and out tree fall gaps and various distances from the shore.
- A large scale plantation of mangroves in the damaged sites e.g. Marsa Shagra is an immediate need.

Monitoring of the natural mangrove growth

This report presents information on the May 2003 field survey of the mangrove distribution and status in the development areas along the Red Sea coast (Sector from Hurghada to Hamata). It is also, to select long term monitoring sites. These monitoring sites will be monitored to determine the extent and cause of natural variation (e.g. inter-annual variability) of key ecological attributes.

Monitoring is a system of repeated investigation of defining biological processes to detect changes over time. It is essential to detect the health of mangrove and its environment and to gain an understanding of factors which influence the stability of mangrove along the coast.

It also includes an investigation of the gain in species richness under certain circumstances such as the faunistic and floristic recruitment of species new to a nonspecific natural mangrove stand.

Procedures

A-Site selection:

Two sites are selected for monitoring *Avicennia marina*:

- Site 1 is El-Sharm El-Bahry (25 52 08N, 34 25 30E), the mangrove is growing in water and mud flat soil. The site is not disturbed, the plant is healthy and many juveniles are growing.

- Site 2 is Ras Baghdady (the delta of wadi El-Jimal, 24 40 47N, 35 05 06E). The mangrove is growing landward on salt marsh and is forming sand hillocks along the shore. The site is subjected to heavy grazing and human activities. No regeneration recorded.

B- Tools used for assessment:

- Measuring density in permanent quadrates from water zone to landward zone (Site 1).
- Measuring canopy cover of individuals growing landward
- Measuring species composition.
- Calculation of species diversity.

C- Estimation of reproductive success:

Procedures

1. Tagging 10 branches on 5 individuals using plastic threads.
2. Counting the number of flower buds per branch per month.
3. Counting the number of flower per branch per month.

4. Counting the number of fruits per branch per month.
5. Determine litter fall gram per meter per month.

Calculations

$$\% \text{ of flower success} = \left\{ \frac{\text{no. of flowers}}{\sum \text{no. of buds} + \text{no. of flowers} + \text{no. of fruits}} \right\} \times 100$$

$$\% \text{ of fruit success} = \left\{ \frac{\text{no. of fruits}}{\sum \text{no. fls.} + \text{no. of frs.}} \right\} \times 100$$

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**Environmental Monitoring
Guidelines for Wild Animals**

Regulations for the monitoring of the wild animals

The result of the field survey showed that the study area from Qusier southward to Ras Banas is inhabited by a variety of animals some of which are either endangered or vulnerable to immediate extinction. These species as well as habitats that preferred by the animal species are must be conserved and then monitored.

Threatened species of wild animals include only one reptilian species called Egyptian Dabb Lizard, *Uromastyx ocellatus*. It also includes seven bird species namely: *Phaetons aethereus*, *Ardea goliath*, *Aquila verreauxii*, *Torgos tracheliotus*, *Gypaetus barbatus*, *Falco pelegrinoides* and *Larus leucophthalmus*. The mammalian species that inhabiting the area and categorized as threatened species includes common genet, wild cat, caracal, wild ass, dorcas gazelle and Nubian ibex. On the other hand, the marine animals in the area and also considered according to IUCN as threatened species are four species. These species include three turtles: Green, Hawksbill and leatherback, and one important mammal species Dugong dugon.

Other important comes from the use of the area as breeding site to eighteen bird species. The locality of the bird nest includes mountainous slopes, wadis, and mangrove trees as well as islands especially Wadi El-Gimal Island.

The monitoring of the animals in the study area composed of two major steps followed by other regulations:

1. Field training of the local people to identify the habitats, animal tracks and signs Certain animals to give a professional field guide.
2. Public awareness to the local people to the endangered species and their harvest Season.
3. No hunting to the animal species will be allowed in the different areas neither core or restricted areas.
4. Visitors should never be allowed to surround any animal the large animal as well as the small ones.
5. Visitors must remain alert never to get between animal parents and their young or isolate any individual from its group.
6. Visitors should never violate the escape distances of the wild animals, including birds. These distances vary among species, individuals and environmental circumstances as well as the transport machine. All visitors stay

on the periphery of the animal assemblages. Approaching wild animals should be slowly, quietly and avoid sudden movements.

7. Photography of any wild animal must be occurring in the natural habitat without any disturbance. Removal of the animal from their microhabitats, dens caves and nest, must be prohibited at all times.
8. Nesting birds should be viewed only through binoculars or/and appropriate telescopes at considerable distances from the nest site.
9. The group size that visits the area not exceeds ten persons in addition to your field guide.
10. The natural trails must be marked by painting rocks along the way in the designated number of the path and with intervals a visible guide on the column (**DO NOT LEAVE THE TWAIL**).