

## **APPENDIX 3**

# MARINE ENVIRONMENT SURVEY

## Methodology

### I- IN SITU MEASUREMENTS

Salinity (‰), pH and water temperature (°C) were measured with a multi probe sensor (Horiba, WATER CHECKER U-10). The Checker is always properly calibrated according to the manufacturer manual guide weekly and prior to the sampling process.

### II-WATER SAMPLES

#### 1- Sampling, and sample handling and preservation

5 liters of the water sample was collected at 1 meter under surface sea level at the selected 8 stations using 5 l Niskin water sampler. Water samples for dissolved oxygen (DO) determination were firstly collected into 300 ml DO bottles, in which the DO contents were fixed using Winkler Reagents. Four liters of the sample were emptied into a plastic container and saved into an ice box; the filtration step was commenced as soon as samples were retrieved in the laboratory. For total metals analysis, 500 ml of the raw seawater sample was emptied into an acid presoaked 500 ml plastic container, and preserved using 2.5 ml concentrated nitric to prevent metals adherence on the walls of the container.

#### a- Dissolved Oxygen

The classical Winkler method described by the UNEP (1991) for the dissolved Oxygen (DO) determination was used during the present work analysis. 2-3 aliquots (300 ml each) were treated with divalent manganese solution, followed by strong alkali solution, forming manganous hydroxide precipitate, which is rapidly oxidized by the dissolved oxygen present in the sample to form higher valency state basic hydroxide. Upon acidification in presence of iodide, the high valency state hydroxide

reverts to the divalent state, and equivalent iodine to the original DO in the sample is liberated. This iodine is titrated against standardized thiosulphate solution. The obtained results were expressed as ml O<sub>2</sub> l<sup>-1</sup>.

#### **b- Total Suspended Matter**

Filtration of the samples was commenced directly after collection. The water content in the plastic container was vigorously shaken to homogenize the suspended matter in the sample, and then 1 liter of the water sample was filtered through a pre-weighed 0.45 µm pore size membrane filter papers mounted on glass filtration system. The filter was transferred onto a clean glass Petri dish, and dried in desiccators. The filters then were weighed to the nearest four decimal figures to a constant weight. The results were expressed in mg l<sup>-1</sup> of the water sample.

#### **c- Chlorophyll-a**

The method agreed to by a SCOR/UNESCO working group and published by UNESCO in Monographs on Oceanographic Methodology; No. 1 and described in ROPME (1999) was used for chlorophyll pigments determination as a source for food for the transplanted bivalve during field bioassay experiment.

The method depends on the filtration of the seawater sample (1000 ml) through a nylon net of 300-µ mesh (to remove zooplanktons), and then the phytoplankters are filtered onto 0.45 µm membrane filter paper using about 10 mg MgCO<sub>3</sub>/cm<sup>2</sup> suspension of filter surface. The pigment was then extracted using 90 % acetone and the extinction measured spectrophotometrically at 750, 663, 645 and 630 nm against 90 % acetone blank. Extinctions were corrected using the measured extinction at 750 nm. µg / ml chlorophyll was calculated using the following equations:

$$\text{Chlorophyll-a} = 11.64 E_{663} - 2.16 E_{645} + 0.10 E_{630}$$

Where E refers to the extinction measured at the next number in nm.

The obtained results were expressed as µg l<sup>-1</sup> of seawater (≡ mg m<sup>-3</sup>).

#### **d- Nutrient Salts**

After filtration, 100 ml aliquots of the filtrate was emptied into 100 ml plastic containers (100 ml into a glass container for dissolved inorganic phosphorous) and refrigerated at -20 °C till analysis.

100 ml of the raw sample was deep frozen for total phosphorous (TP) and total nitrogen (TN) determination. The determined nutrient salts species were expressed as  $\mu\text{M l}^{-1}$ .

#### **Reactive Phosphate (Dissolved Inorganic Phosphorous, DIP)**

The method described by Strickland and Parsons (1972) was used for DIP determination. The phosphate ions in the sample were allowed to react in acidic solution with ammonium molybdate to yield a phosphomolybdate complex. This heteropoly acid is reduced by ascorbic acid with trivalent antimony ions as catalyst to a blue colored complex, the absorbance of which is then measured in a spectrophotometer at 882 nm. A series of standards of potassium dihydrogen phosphate was treated in the same way as the sample to construct a calibration curve. Reagent blank was carried out using distilled water.

#### **Nitrite**

The method described by Strickland and Parsons (1972) was used for DIP determination. The determination of nitrite is based on the reaction of nitrite with an aromatic amine (sulfanilamide) which leads (at pH 1.5 - 2.0) to the formation of diazonium compound. This diazo compound then couples with a second aromatic amine N-(1-naphthyl)-ethylenediamine to form the azo dye, in which its absorbance was measured at 540 nm using spectrophotometer. The calibration of the method was carried out using a series of standards prepared using sodium nitrite. Reagent blank was carried out using distilled water.

#### **Nitrate**

The colorimetric method described by Strickland and Parsons (1972) was used for nitrate determination. Nitrate is reduced to nitrite in a reduction column filled with copper-coated cadmium granules. The yield of the reduction depends on the pH of the solution and on the activity of the metal surface. The conditions of the reduction described in the method are adjusted to a pH of about 8.5, so that nitrate is converted

to nitrite almost quantitatively (90-95%) and not reduced further. Ammonium chloride buffer is used to control the pH and to complex the liberated cadmium ions. The nitrite formed is then determined colorimetrically (at 540 nm) according to the reaction already described above.

### **Total Nitrogen and Total Phosphorous**

The method described by the UNEP (1991) was used for the simultaneous determination of total nitrogen and total phosphorous.

**The method of total nitrogen (TN)** determination exhibits the possibility of simultaneous analysis of the total concentrations of nitrogen and phosphorus in the same seawater sample. TN was determined in unfiltered sea water samples which were oxidized in screw capped Teflon bottles (ca. 100 ml). The digestion was performed in a pressure cooker at about 110°C for 30 minutes. Wet oxidizable, organic nitrogen compounds were quantitatively converted into nitrate with peroxodisulphate in an alkaline medium. In the simultaneous wet oxidation, the reaction started at pH 9.7 and finished at pH 4-5. These conditions were established by a boric acid-sodium hydroxide buffer system. The free chlorine formed in seawater samples was reduced by adding ascorbic acid before the analysis is started. The precipitate, which is formed at elevated temperatures, dissolves as oxidation proceeds.

After completion of the oxidation step, the nitrogen compounds were determined as nitrate according to the above outlined procedure.

A standard solution made of glycine ( $\text{NH}_2\text{CH}_2\text{COOH}$ , a.g.), previously dried at 110°C and kept in a desiccator, was used and analyzed as samples for obtaining the calibration curve.

**Total phosphorus (TP)** was determined using the same method described for total nitrogen. In this method organically bound phosphorus was quantitatively converted into phosphate which was determined as inorganic phosphate according to the procedure outlined above.

The same standard for DIP was used and analyzed like TP samples for obtaining the calibration curve.

### **2- Total Trace Metals**

100 ml of the acidified sample was transferred into an acid presoaked Teflon container, and 5 ml of concentrated nitric acid was added according to the method described by standard methods (APHA/AWWA/WPCF, 1989).

The container was heated near dryness avoiding boiling. The digest was cooled down, and another 5 ml of concentrated nitric acid was added. The sample was allowed to reflux till the digestion complete. The residue was dissolved by warming in 2 ml concentrated nitric acid. The final volume was adjusted to 20 ml using de-ionized distilled water. Samples then were analyzed using FAAS using the specific wavelength of each metal.

### **3- Total Petroleum Hydrocarbons (Dissolved Dispersed Petroleum Hydrocarbons, DDPH)**

Following the recommended device and method by IOC (1984), water samples were collected from one meter depth in amber glass bottle (2.8 l) supported with a weighted bottle holder. The bottle was allowed to sink to 1 meter depth to fill with water. Upon retrieval, 50 ml of DCM were added, and then the bottle was shaken vigorously for 5 minutes to disperse the contents.

In this technique, the type of solvent used (n-hexane) was replaced by DCM, which was recommended by several authors (Law *et al.*, 1987 and Burns and Villeneuve, 1983). Practically, the DDPH extracted yield in usage of DCM was found to be double folds higher than in using n-hexane or n-pentane (Law *et al.*, 1987).

As soon as the sample arrives to the laboratory, the sampling bottle cap was loosened slowly to allow the DCM vapor to escape. The extract was then collected using a clean pipette into a clean flask through anhydrous sodium sulfate for dryness. Extraction of DDPH in water samples was completed using another 50 ml DCM on two successions each with 25 ml. Finally, the extracts are combined into a dry, clean glass container.

Petroleum hydrocarbon contents in sediments were separated from the total extractable materials using column chromatography as described in UNEP (1992). A column of 1cm diameter was packed with slurry of 8 g silica gel in hexane, followed by 8 g of alumina and 1g of anhydrous sodium sulfate. Total extract was applied in a minimum amount of hexane to the surface of the column. The saturated hydrocarbon fraction was eluted with a total of 20 ml hexane (F1), unsaturated and aromatic hydrocarbons were eluted with 20ml 20% DCM in hexane (F2), followed by 20ml

50% DCM in hexane (F3) to elute the poly-aromatic hydrocarbons. The recovered clean hydrocarbon fractions were air-dried and stored in dark in 10ml glass vials enveloped by aluminum foil for detection.

All samples were measured against chrysene standards at excitation wavelength of 310 nm and emission of 381 nm. Measurements were commenced by pipette aliquots of the hexane solution of blanks, standards and samples into a Teflon-capped 1 cm quartz fluorescence cell.

The instrument used for measuring fluorescence intensity in the present work was Shimadzo (RF \_ 510) spectrofluorometer using the following program setup:

<b><i>Excitation wavelength:</i></b>	310 nm.
<b><i>Emission wavelength:</i></b>	381 nm.
<b><i>Average measurement time:</i></b>	1 sec.
<b><i>Band width:</i></b>	10 nm.

### III- SEDIMENTS

#### 1- Sampling

Surface sediment samples were collected using a Peterson grab sampler, sediments for trace metals analysis was collected from the utmost top 1-2 cm of the surface sediments were carefully collected using a pre-cleaned plastic spoon and transferred into a pre-cleaned and acid soaked glass containers, the samples then, were stored freeze until drying step. Sediments for total petroleum hydrocarbons (TPH) were collected into foil bags using a stainless steel spatula. The whole contents of the grab was emptied into plastic bags for grain size and TOC analysis.

#### 2- Methods

### **a- Grain Size Analysis**

The samples, which are left to air-dried, are then disaggregated by rubbing with fingers, and split by the cone and quarter technique. After splitting, 25 represented gm were subjected to grain size analysis by sieving for coarse fraction (more than  $4\Phi$ ) and pipette analysis for finer fractions less than  $4\Phi$  (1/16mm). It is noteworthy that the biogenic clastics were not removed in case they are broken and mixed with samples. In fact, represented sub samples were sieved through a standard set of sieves (Geyer Type) mounted on an electric shaker machine (Labortechnik-Ilmenau). The standard time of sieving was 15 min. The sieves were arranged in a 1  $\Phi$  class interval from top to bottom. The sieving technique was only applied, as the sediments subjected to the grain size analysis were lacking fraction smaller than 4  $\Phi$ . But, for analysis of finer fractions (silt and clay), the technique described by Krumbein and Pettijohn (1938), which is based on settling velocity of the particles, was used.

The graphic measures given by Folk and Ward (1957) were employed for the results of the grain size analysis using the  $\Phi$  notation, from which Mean size ( $Mz$ ), Sorting ( $\sigma I$ ) and Skewness ( $SK_I$ ) were calculated.

### **b- Organic Carbon**

The content of Total Organic Carbon was determined by the direct method described by El Wakeel and Riley (1957). The method is based on the oxidation of the sediment with chromic acid, and then the unconsumed oxidant was titrated against a reducing agent (ferrous ammonium sulfate) using ferrous phenanthroline as indicator. Potassium dichromate primary standard solution was used to standardize ferrous ammonium sulfate. Total organic carbon was expressed as percentage to dry sample weight.

### **c- Trace Metals**

The method of total sediments decomposition described by ROPME (1999) was used for total metal decomposition in surface sediment samples.

Sediment samples were thawed, and dried in a drying oven at 60 °C. After grinding the sediments in agate mortar, 0.3 g was weighed into a teflon tubes, 1ml aqua regia and 6 ml concentrated hydrofluoric acid were added. Sample were left at

room temperature for one hour. Then heated on a hot plate at 120 °C for 2.5 hours. If the digest is not clear, another suitable volume of hydrofluoric acid was added and digestion process was presumed. Once the digest attains clarity, after cooling, samples were quantitatively transferred into 50 ml polypropylene vials containing 3.7 g of boric acid. Then, samples were completed to 50 ml with de-ionized distilled water.

Three blanks were run with every batch of samples in the same steps used for samples decomposition without involving a sample. In addition, three certified reference materials samples (MESS2 and HESS1, NRCC) were run and treated in the same way as the samples. Chemical analysis was tested against standard sediments certified by the NRCC (National Research Council of Canada). Samples then were analyzed using FAAS using the specific wavelength of each metal.

#### **d- Total Petroleum Hydrocarbons (TPH)**

The foil bags containing the collected sediments were thawed in the laboratory, and dried in clean Petri dishes at 50 °C in a drying oven. Sediments were grinded in agate mortar, and 10 gram of the dry sediments was weighed into a clean glass flasks.

The ultra-sonic method described by the UNEP (1992) was used for the determination of TPH in sediments. In this method 100 ml of 1:1 v/v dichloromethane/acetone was added to the sediments. The sediments were sonicated in an ultra-sonic bath for about 3 min at settings appropriate to yield efficient extraction. After the extraction, the solvent was decanted into a clean flask. The extraction was repeated two more times, and all extracts were combined into the flask. The extract was filtered through glass wool and dried over anhydrous sodium sulfate. The extract was air dried in dark and then dissolved in 10 ml hexane.

The separation of the petroleum hydrocarbons from other organic contents in the extract was carried out in column chromatography containing silica gel and alumina as chromatographic support as described above for water samples extracts. Also the detection was done as the same equipment used for the water samples detection using spectrofluorometer.