# INTERNSHIP REPORT

# BY

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# M.Sc.

# MARINE MICROBIOLOGY PART 2

# MMO 319

# FIELD TRIP/STUDY TOUR-PRACTICAL

# 2022-2023

# SCHOOL OF EARTH, OCEAN AND ATMOSPHERIC SCIENCES GOA UNIVERSITY

# INTRODUCTION

The use of educational field trips has long been a major part of teaching and learning strategy. It shows that there is a significant increase in participant's factual knowledge and conceptual understanding. Field trips take students to locations that are unique and cannot be duplicated in the classroom. Our School of Earth, Ocean and Atmospheric Sciences had organized a field trip for part one's and part two's of the marine microbiology department on 10th and 11th of March 2022. We were allowed to come either on one of this two days, as the part two's were taken on 10th march we engaged for 11th march.

The destination place was Mandovi Estuary. Our reporting time was 8:00am and we were allotted with four different stations, they were as follows:

1. Malim Jetty

2. Miramar (off shore)

3. Chorao

4. Old Goa

First, our trawler took us to the second station Miramar (off shore) as a reason due to its high depth the trawler shakes more which makes us feel unconscious. Different batch of students were allotted to do different analysis on the four station for example measuring depth of sea water, salinity, temperature, dissolved oxygen, to check lat-long etc at respective stations. After collecting the samples while returning to our first location our teacher provided us refreshment.

On reaching to the first station that is Malim Jetty, from where we had left initially, same process was followed here with another batch of students. Completing this, then we moved

on to the third station repeated the same set of analysis and by then it was already time for lunch.

At station four same procedure as mentioned above was carried out and we completed all our four stations, all the analysis sample were returned to the lab to perform the different tests. and to make our field trip joyful and worth remembering delicious lip smacking fish curry, rice and fried fish was served for all of us.

# OBJECTIVES

- Analysis of the following parameters were carried out:

1. SEA WATER SAMPLE COLLECTION
2. TURBIDITY
3. TEMPERATURE
4. SALINITY
5. DISSOLVED OXYGEN (DO)
6. CHLOROPHYLL ESTIMATION
7. MPN (Most Probable Number)
8. ANALYSIS OF SPM (Suspended Particulate Matter)
9. PHYTOPLANKTON ANALYSIS
10. pH
11. VIABLE COUNT

## ❖ LATITUDE & LONGITUDE OF SAMPLING SITES

Latitude and longitude was measured in two ways i.e on phone (Google website or app) and from the trawlers monitor.

| STATIONS | LATITUDE | LONGITUDE |
|---|---|---|
| 1 | 15◦30143'N | 73◦49.9076'S |
| 2 | 15.475 | 73.773 |
| 3 | 15◦30.438'N | 73◦51.90705'S |
| 4 | 15◦30.851'N | 73◦55.171'S |

## 1. SEA WATER SAMPLE COLLECTION

Surface water was collected using the bucket and bottom water was collected using niskin sampler.

## 2. TURBIDITY

The secchi disk is an 8 inch diameter disk which consists of black and white quadrants that are lowered into water column until no longer be seen from the surface. It is used to measure Secchi depth.

- Take a secchi disk and place down into the water.
- Keep leaving the rope until the black and white quadrants are not seen.
- Look for transparency
- Measure the depth in meters

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FIG 1: SECCHI DISK

OBSERVATIONS:

| STATION | SECCHI DEPTH (mts) |
|---|---|
| 1 | 2 |
| 2 | 9 |
| 3 | 1.5 |
| 4 | 1.5 |

RESULTS: The turbidity measured the highest at station 2 and lowest at station 3 and 4.

## 3. TEMPERATURE

Temperature was measured using a thermometer. After removing sea water from the bottom and surface.

- Take a thermometer and place it into bucket containg sea water.
- Keep it for 1min.
- Measure the temperature.

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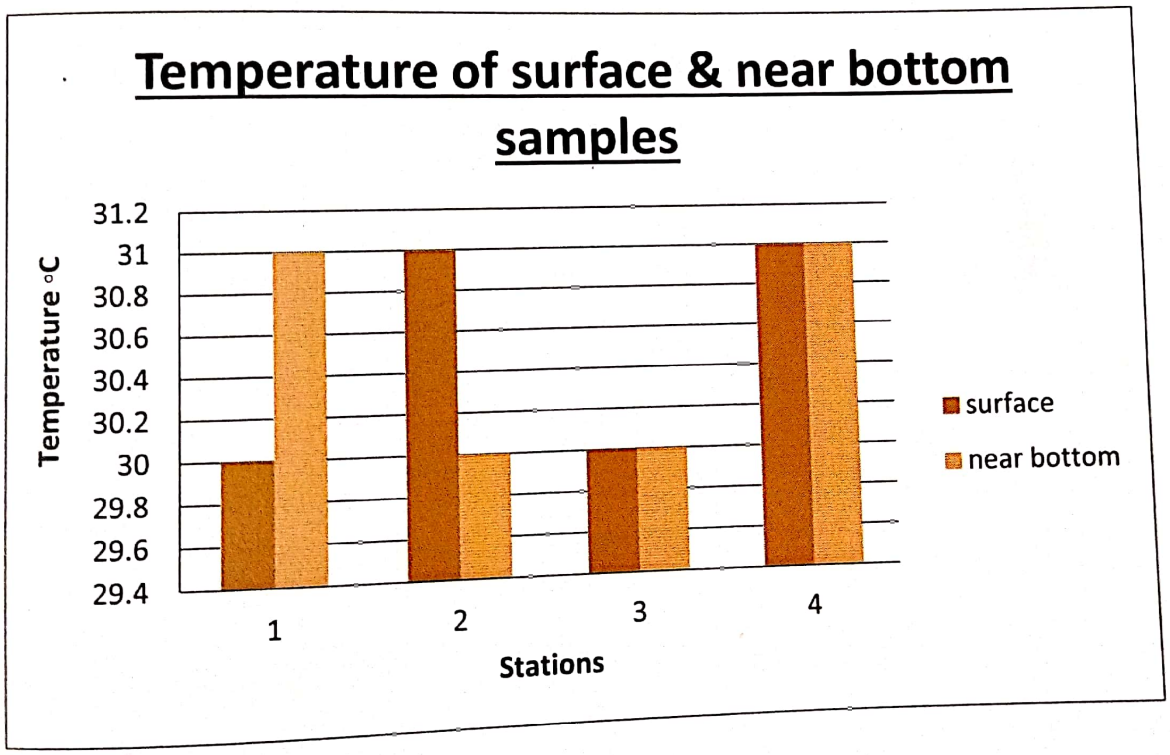
FIG 2: THERMOMETER

OBSERVATIONS:

| STATION | TEMPERTURE ◦C | |
|---|---|---|
| | SURFACE | NEAR BOTTOM |
| 1 | 30 | 31 |
| 2 | 31 | 30 |
| 3 | 30 | 30 |
| 4 | 31 | 31 |




RESULT: The temperature across all the stations in surface and near bottom waters varied by 1°C and was between 30-31°C.

## 4. SALINITY

Salinity was measured using refractometer.

- Take a drop of sea water.
- Place it on to the refractometer.
- Check the salinity.

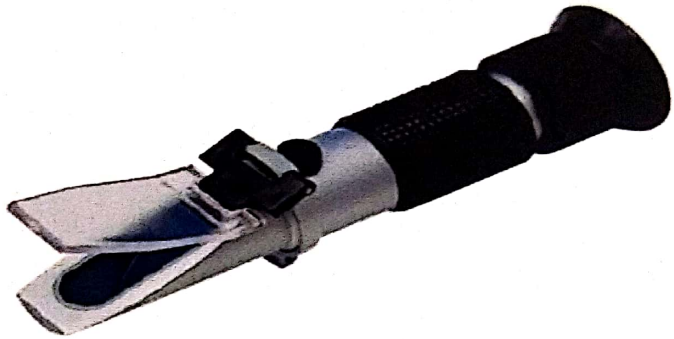
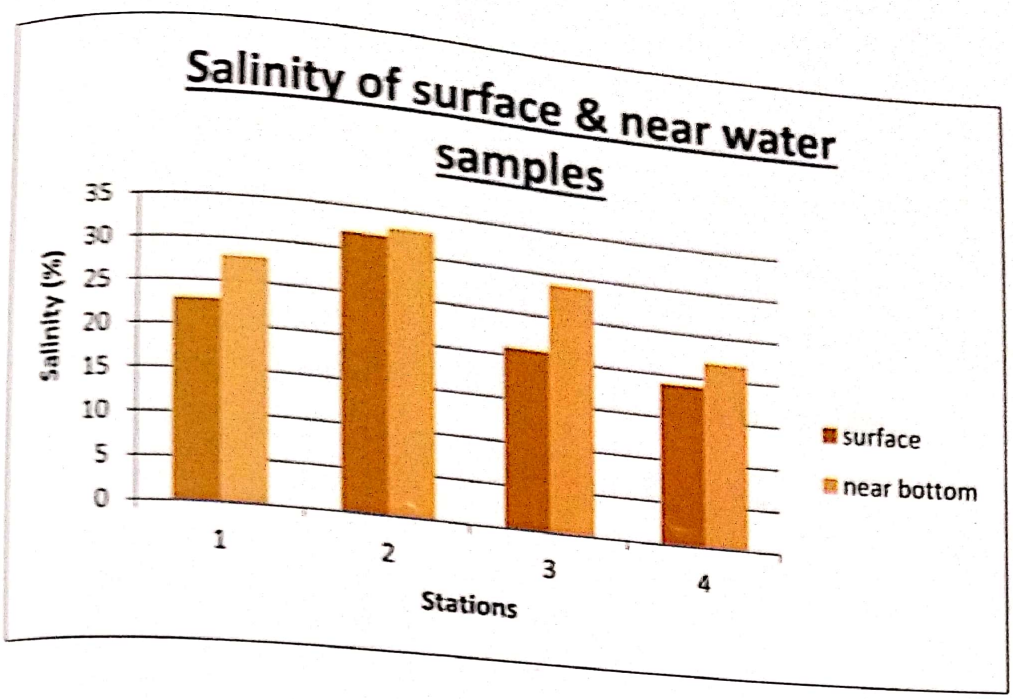


FIG 3; REFRACTOMETER

OBSERVATIONS:

| STATION | SALINITY (%) | |
|---|---|---|
| | SURFACE | NEAR BOTTOM |
| 1 | 23 | 28 |
| 2 | 32 | 33 |
| 3 | 21 | 29 |
| 4 | 19 | 22 |




RESULTS: Salinity was highest at station 2 and lowest at station 4 for both surface and near bottom.

## 5. DISSOLVED OXYGEN (DO)

DO is the amount of oxygen that is present in the water. It is an important parameter in assessing water quality because of its influence on the organisms living within water body. DO is a valuable tracer for water masses and is a sensitive indicator for biological and chemical processes occurring in the sea. Dissolved oxygen that is too high or too low can harm aquatic life and affect water quality.

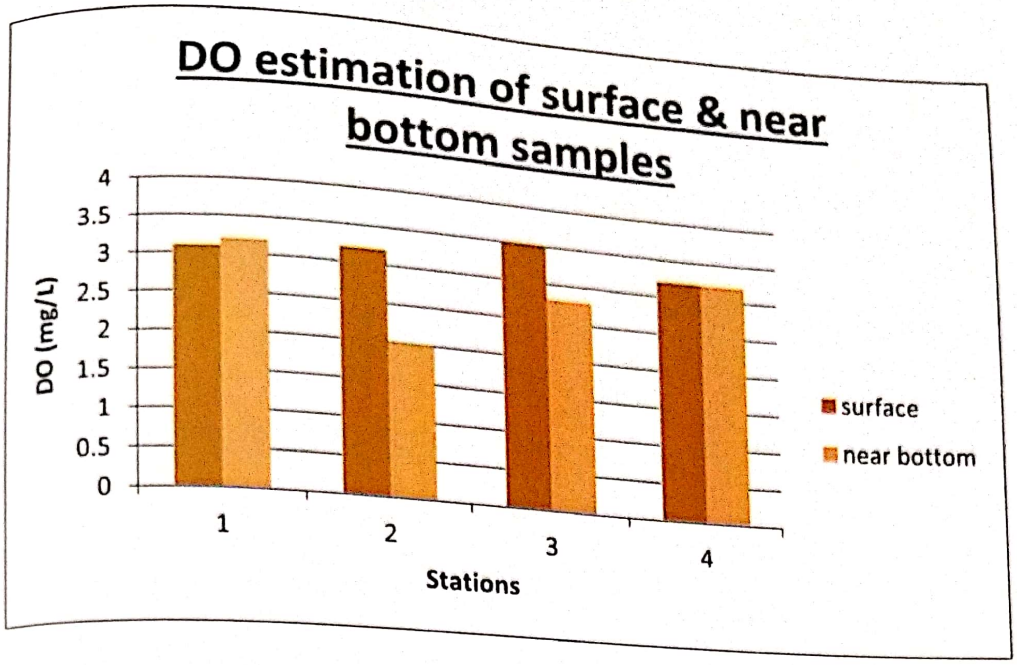
- Take a DO bottles and fill it with sea water without forming air bubbles.
- Then add 1 ml of Winkler A and Winkler B.
- Close the bottle and drain off the excess, and keep the bottle in dark.
- Estimation of blank and standardization of thiosulphate.
- Add 1ml of 50% Sulphuric acid to the DO bottles to dissolve the precipitate.

- Take 50ml of DO sample into the conical flask and titrate against thiosulphate solution until pale yellow colour is obtained.
- Add 1ml of starch solution and titrate it until the blue colour dissapers.
- Note down the readings and repeat the titration for 3 times.
- Calculate the amount of DO present by using the formula.

FIG 4: DO BOTTLES

OBSERVATIONS:

| STATION | DISSOLVED OXYGEN (mg/L) | |
|---|---|---|
| | SURFACE | NEAR BOTTOM |
| 1 | 3.1 | 3.2 |
| 2 | 3.2 | 2 |
| 3 | 3.5 | 2.8 |
| 4 | 3.2 | 3.2 |




$$\text{Dissolved oxgyen, mg L}^{-1} = \frac{BR \cdot \frac{V}{v} \cdot N \cdot E \cdot 1000}{\text{Volume of sample titrated}}$$

BR = Burette reading (volume of thiosulphate used in titration)
N = Normality of thiosulphate solution
E = Equivalent weight of Oxygen = 8
1000 = To express per liter
**Note:** The factor V/v was correction for displacement of oxygen in the sample when reagents were added.

$$V/v = \frac{\text{Volume of bottle}}{\text{Vol. of bottle} - \text{Vol. of reagents}}$$

** Use factor (0.698) to convert parts per million (mg $L^{-1}$) to (ml $L^{-1}$) of oxygen

RESULTS: Among the surface waters station 3 showed the highest and station 1 showed the lowest conc. of DO, while among the near bottom station 1 showed the highest and station 2 showed the lowest conc. of DO.

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## 6. CHLOROPHYLL ESTIMATION

It is a most useful method of extracting chlorophyll from marine ecosystem and to estimate amount of chlorophyll present.

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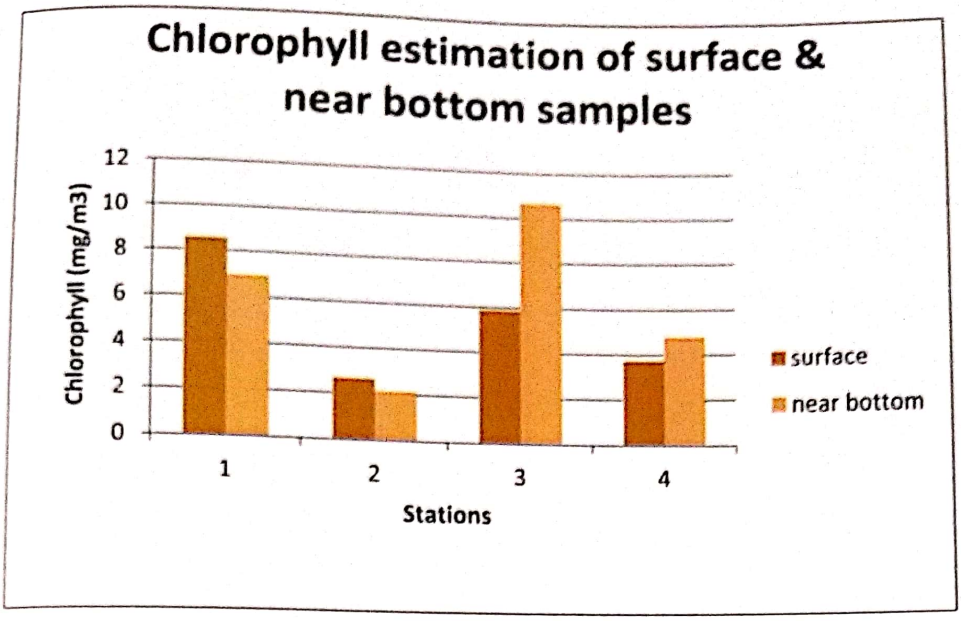
- Collect the sea water sample in 1liter bottle.
- Place the whatman no.42 filter paper in the filter equipment.
- Filter the sea water sample.
- Make aluminium foil pouch and keep the filter paper in it.
- Refrigerate for 24 hours.
- Take an amber coloured bottle and add 10ml of 90% acetone.
- Place the filter paper into the bottle and crush gently.
- Keep the sample undisturbed for 24 hours in the refrigerator.
- Measure the blank using spectrophotometer, add acetone in cuvette.
- Measure the absorbance at 665nm spectrophotometrically and calculate using formula.

OBSERVATIONS:

| STATION | CHLOROPHYLL ($mg/m^3$) | |
|---|---|---|
| | SURFACE | NEAR BOTTOM |
| 1 | 8.544 | 6.947 |
| 2 | 2.67 | 2.136 |
| 3 | 5.874 | 10.68 |
| 4 | 3.738 | 4.806 |




$$\text{chlorophyll } a \text{ (mg/m}^3\text{)} = \frac{26.7(665_o - 665_a) \times v}{V \times l}$$

$$\text{phaeo-pigments (mg/m}^3\text{)} = \frac{26.7(1.7[665_a] - 665_o) \times v}{V \times l}$$

**where $665_o$ is the extinction at 665 nm before acidification, $665_a$ is the extinction at 665 nm after acidification, $v$ is the volume of acetone extract(ml), $V$ is the volume of water filtered (liters) and l is the path length of the cuvette (cm).**

RESULT: The highest concentration of chlorophyll was found to be at station 3 at near bottom waters and lowest was found at station 2 at near bottom waters.

## 7. MPN (Most probable number)

MPN analysis is a statistical method based on the random dispersion of microorganism per volume in a given sample.

- Water sample collection was done from the surface of the station using a bucket.

- The water was collected into sterile centrifuge tubes of 50 mL and stored in ice box until further analysis.
- The samples were brought back to the laboratory and inoculated into double strength and single strength MacConkey's Broth containing inverted Durham's tube in the respective volumes.
- 10ml of water sample was inoculated into 5 tubes containing 10mL of MacConkey's Broth.
- 1ml of water sample was inoculated into 5 tubes containing 10mL of single strength MacConkey's Broth.
- And 0.1 ml of water sample was added to 5 tubes containing 10mL of single strength broth.
- All the tubes were incubated at 37°C for 24-48 hours.
- Positive results were indicated by production of acid (change in colour of the media from pinkish red to yellow) and gas. (By production of gas bubble in Durham's tube)
- The results were compared to a standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined.

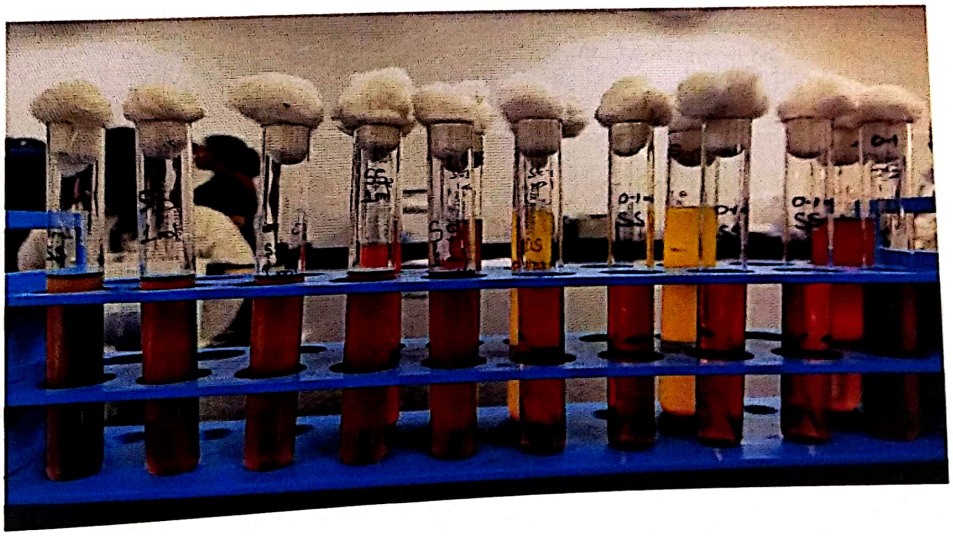


FIG 5: MPN TUBES

OBSERVATIONS:

| STATION 1 | DS (10mL) | | SS (1mL) | | SS (0.1mL) | |
|---|---|---|---|---|---|---|
| | ACID | GAS | ACID | GAS | ACID | GAS |
| 1 | ✓ | ✓ | | | | |
| 2 | ✓ | ✓ | ✓ | ✓ | ? | |
| 3 | ✓ | ✓ | ✓ | ✓ | | |
| 4 | ✓ | ✓ | ✓ | ✓ | | |
| 5 | ✓ | ✓ | ✓ | ✓ | | |

Number of positive tubes: 5-4-0 ≈ 130 bacteria/ 100mL

| STATION 2 | DS (10mL) | | SS (1mL) | | SS (0.1mL) | |
|---|---|---|---|---|---|---|
| | ACID | GAS | ACID | GAS | ACID | GAS |
| 1 | | | | | | |
| 2 | | | ✓ | ✓ | | ? |
| 3 | ✓ | ✓ | ✓ | ✓ | | |
| 4 | ✓ | ✓ | ✓ | ✓ | | |
| 5 | ✓ | ✓ | ✓ | ✓ | | |

Number of positive tubes: 3-4-0 ≈ 13-17 bacteria/100mL

| STATION 3 | DS (10mL) | | SS (1mL) | | SS (0.1mL) | |
|---|---|---|---|---|---|---|
| | ACID | GAS | ACID | GAS | ACID | GAS |
| 1 | ✓ | ✓ | ✓ | ✓ | | |
| 2 | ✓ | ✓ | | | | |
| 3 | ✓ | ✓ | | | | |
| 4 | ✓ | ✓ | | | | |
| 5 | | | | | | |

Number of positive tubes: 4-1-0 ≈ 17 bacteria/100mL

| STATION 4 | DS (10mL) | | SS (1mL) | | SS (0.1ML) | |
|---|---|---|---|---|---|---|
| | ACID | GAS | ACID | GAS | ACID | GAS |
| 1 | ✓ | ✓ | ✓ | ✓ | | |
| 2 | ✓ | ✓ | ✓ | ✓ | | |
| 3 | ✓ | ✓ | ✓ | ✓ | | |
| 4 | ✓ | ✓ | | | | |
| 5 | | | | | | |

Number of positive tubes: 4-3-0 ≈ 27 bacterial/100mL

RESULT: As observed from the readings station 1 has 130 bacteria/100mL, station 2 has 13-17 bacteria/100mL, station 3 has 17 bacteria/100mL and station 4 has 27 bacteria /100ml. Indicating station 1 has the highest bacterial count while station 2 and station 3 has lowest bacteria/100ml.

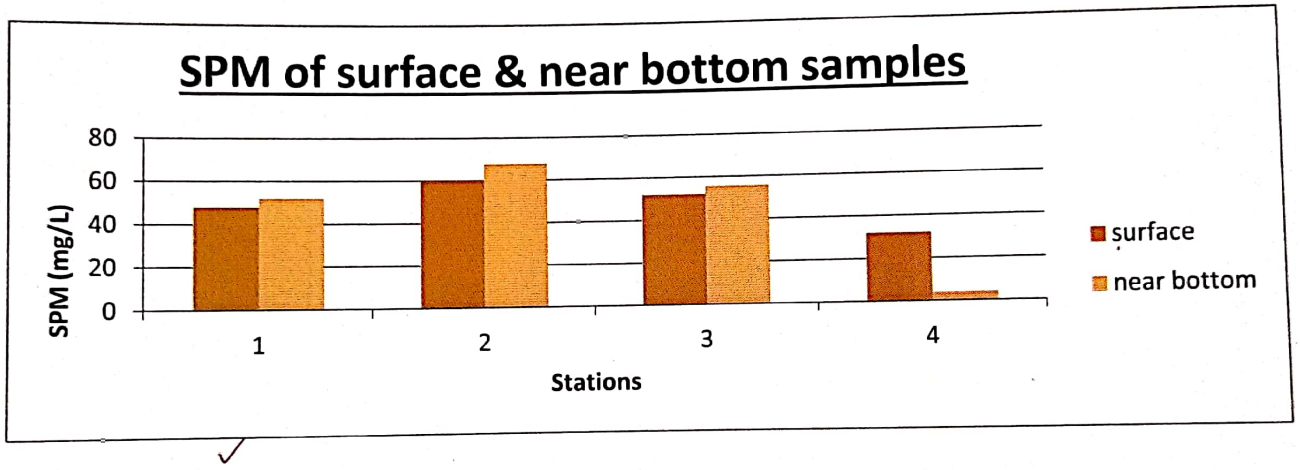
## 8. ANALYSIS OF SPM (Suspended Particulate Matter)

Suspended particulate matter (SPM) is operationally defined via filtration of seawater as the material retained on a certain type of filter with certain pore size, while the matter that passes through a small pore size filter is defined as dissolved matter (DM).

- Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of 0.45 microns was placed in the filtration unit attached to the vacuum pump.
- Before filtering the water sample the weight of the filter paper was measured and noted. Around 250mL of seawater sample is filtered through the filter paper.
- After filtering the weight of the filter paper was again measured (designated as wet weight)
- The filter paper was then kept for drying in the oven at 30°C till it completely dried after which the weight of the filter paper was again measured. (Designated as dry weight)
- The readings were noted down and the calculations were carried out.

OBSERVATIONS:

| STATION | | FILTER PAPER WEIGHT (g) (x) | DRY WEIGHT (g) (y) | DIFFERENCE (g) (x-y) | SPM (mg/L) |
|---|---|---|---|---|---|
| 1 | SURFACE | 0.085 | 0.097 | 0.012 | 48 |
| | BOTTOM | 0.082 | 0.095 | 0.013 | 52 |
| 2 | SURFACE | 0.088 | 0.103 | 0.015 | 60 |
| | BOTTOM | 0.084 | 0.101 | 0.017 | 68 |
| 3 | SURFACE | 0.086 | 0.099 | 0.013 | 52 |
| | BOTTOM | 0.08 | 0.094 | 0.014 | 56 |
| 4 | SURFACE | 0.085 | 0.093 | 0.008 | 32 |
| | BOTTOM | 0.081 | 0.091 | 0.01 | 4 |




SPM = X-Y ÷ Volume of water filtered in litre

RESULT: SPM was found to be highest at station 2 bottom waters and lowest at station 4 bottom waters.

## 9. PHYTOPLANKTON ANALYSIS

To estimate the amount of phytoplankton in each water sample, one needs to fix the sample to keep the cells intact and carry out microscopy later.

- Water sample (from different stations) was collected in a bucket from the surface while for near bottom waters, water was taken from the Niskin sampler.
- Filled into 500mL bottles, next 15 drops of Lugol's iodine solution were added and stored in shade until further analysis.
- Bottles were brought back to laboratory and left for settling.
- After the settling period siphoning was done to concentrate the sample.
- Microscopy was done using an inverted microscope under 10x and 20x objective lens.

FIG 6: a. FIXED BOTTLES USING LUGOL'S IODINE

b. IDENTIFICATION (*Coscinodiscus sp.*)

RESULT: The following phytoplanktons were observed – Diatoms: *Rhizosolenia sp., Coscinodiscus sp., Gyrosigma sp., Chaetoceros sp*., and an unidentified pennate diatom. Dinoflagellates were not observed.

## 10. pH

A pH meter provides a value as to how acidic or alkaline a liquid is. The basic principle of the pH meter is to measure the concentration of hydrogen ions. Acids dissolve in water forming positively charged hydrogen ions (H+). The greater this concentration of hydrogen ions, the stronger the acid is.

- The pH meter was turned on and calibrated by placing into neutral pH buffer solution when the reading was stabilized it denoted as ready
- The electrode was washed and wiped, and the above steps were repeated for acidic and alkaline pH buffer.
- When the pH meter calibration was done the electrode was rinsed with d/w and placed into the sample. The pH measurement was recorded.
- This was repeated for all the stations water samples.

OBSERVATIONS:

| STATIONS | pH |
|---|---|
| 1 | 8.1 |
| 2 | 8 |
| 3 | 7.6 |
| 4 | 7.9 |

RESULT: The pH was found to be in the range of 7.5-8 with the highest being at station 1 and lowest at station 3.

## 11. VIABLE COUNT

The viable plate count, or simply plate count, is a count of viable or live cells. It is based on the principle that viable cells replicate and give rise to visible colonies when incubated under suitable conditions for the specimen.

1. From water sample collected in the centrifuge tubes in the above method 0.1ml was taken and spread plated onto ZMA, MacConkey's, TCBS and XLD agar plates.
2. The plates were incubated at 37°C for 24-hours.

3. Results were recorded after the incubation period.

RESULT: No growth was observed on any plate.

# SUMMARY

It was a very enriching experience. We learnt about the use and handling of different instruments such as Niskin sampler, Van Veen grab etc. We also gained experience on proper sample collection and storage to get accurate results for analysis of various parameters of water like D.O., phytoplankton fixing etc. We experienced the hardships involved in sampling on offshore waters, how the turbulence caused due to strong wave action can cause problems in sample collection. Additionally, we also learnt about the problems associated with sampling in different sites such as failure of sample collection (sediment) due to rocky bottom etc. On the plus side we also had fun while working. Relishing the lunch that was served which we ate on the trawler parked in the middle of the estuary, watching the mesmerizing view was one of the pleasures that we had on this trip. Having this trip amidst the pandemic, refreshed our minds and brought back our interest into academics as we learned a lot through this opportunity given.

Fig 7: STUDENTS AND TEACHERS ALONG WITH EQUIPMENT ON TRAWLER

No Refs/Appendix/conclusion

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15/11/22