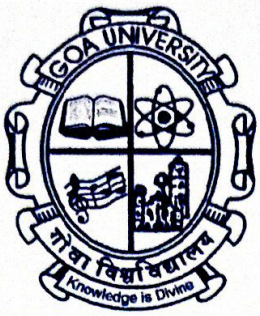

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FIELD TRIP REPORT BY

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M.Sc.

MARINE MICROBIOLOGY PART II

MMO 319

FIELD TRIP/STUDY TOUR- PRACTICAL

SCHOOL OF EARTH, OCEAN AND ATMOSPHERIC SCIENCES

GOA UNIVERSITY

2022 – 2023

INTRODUCTION

Field trips are a valuable tool in making learning more engaging and provide unique opportunities for learning certain concepts and putting them into a more realistic and relevant context. So, currently at Goa University, the School of Earth, Ocean and Atmospheric Sciences of Marine Microbiology Department had conducted a field trip for the post graduate students on 10th and 11th of March 2022. The students were taken in set of batches (I & II) and I was part of the II batch that visited on 11th March. The location of our field trip was Mandovi Estuary, wherein we had to visit 4 stations. The stations were as follows:

1. **MALIM JETTY**
2. **MIRAMAR OFFSHORE**
3. **CHORAO**
4. **OLD GOA**

Collecting sea water sample was the main aim of this field trip and performing different analysis with those samples. All the students were divided and allotted with work for various analyses. The reporting time was 8:00 am at station 1 Malim Jetty. A trawler was booked to travel these 4 stations. Though the Miramar offshore was our 2nd station we visited it at first due to huge waves which makes trawler shake even more and cause us feel dizzy and unconscious.

A part of students were assigned work at every station. As we reached, all got to their own respective work for example collecting sea water sample for various analysis like dissolved oxygen analysis, measuring sea water depth, temperature, salinity, latitude longitude and so on. On finishing with this work we moved on to station 1 and while travelling refreshments were allotted to maintain a good metabolism and to stay active at rest of the stations.

At station 1 same procedure was held to perform with another set of students. Subsequently same steps were carried at station 3 and station 4 and the samples were taken to the laboratory for further analysis. To bring this to an end the field trip was incomplete without the authentic goan food which was prepared itself in the trawler and was served to all.

OBJECTIVES

Analyses of following parameters were done:

- Collection of sea water sample
- Temperature
- Turbidity
- Salinity
- Chlorophyll estimation
- Dissolved Oxygen
- Most Probable Number (MPN)
- Phytoplankton analysis
- Suspended Particulate Matter (SPM)
- pH
- 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

PROCEDURE

➢ COLLECTION OF SEA WATER SAMPLE

In order to collect the sea water sample, a bucket was used to collect the water from the surface, and to collect water from the bottom niskin bottle was used.

➢ TEMPERATURE

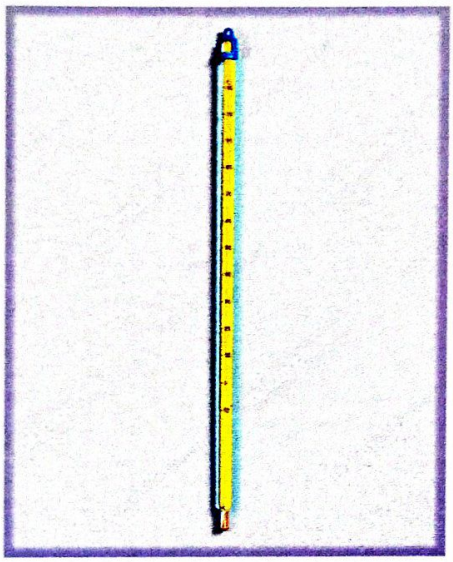


Fig 1: Thermometer

Temperature is the measurement of coldness or hotness of any substance. A thermometer was used to measure the temperature of sea water from the surface and bottom.

1. Place the thermometer into the collected sea water sample.
2. Keep it for a minute.
3. Note down the temperature.

➢ TURBIDITY

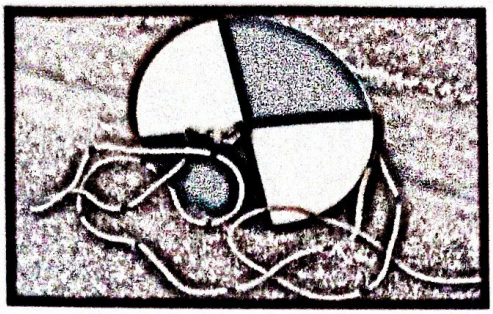


Fig 2: Secchi disk

Secchi disk is used to measure the depth of sea water. It refers to the depth at which a disk is lowered into the water can no longer seen from the surface. Secchi depth is related to water clarity and is a measure of how deep light can penetrate into the water.

1. Take a secchi disk and tie a long rope along the disk, and make sure that the disc is marked to note the measurements.
2. Place the disc in the sea water.
3. Lower the disc to the bottom until the black and white colour of the disc disappears.
4. Look for transparency and measure the depth in meters labelled on the rope by gently removing the disc from water.

➢ SALINITY

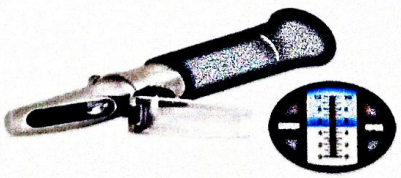


Fig 3: Refractometer

Salinity is the saltiness or amount of salt dissolved in water body. It is measured using refractometer.

1. Take a drop of sea water.
2. Place it onto the refractometer.
3. Measure its salinity.

➢ CHLOROPHYLL ESTIMATION

It is a process of extracting chlorophyll from marine ecosystem which is a most useful method for determining the total quantity of phytoplankton in a sea water and estimate the amount of chlorophyll.

1. Place the Whatman no.42 filter paper in the filter equipment.
2. Collect the sea water sample in 1 litre bottle.
3. Filter the sea water sample.
4. Make an aluminium foil pouch and place the filter paper in it.
5. Refrigerate for 24 hours. ✓
6. Take an amber coloured bottle and add 10mL of 90% acetone.
7. Place the sample into the bottle and crush gently.
8. Keep the sample undisturbed for 24 hours in the refrigerator.
9. Blank measured using spectrophotometer, acetone added in cuvette.
10. Measure the absorbance at 665 nm and 750nm spectrophotometrically and calculate using the formula.

➢ DISSOLVED OXYGEN (DO)

Fig 4: DO

Dissolved oxygen is a valuable tracer for water masses and a sensitive indicator for biological and chemical processes occurring in the sea. It is an important parameter in assessing water quality control because of its influence on the organism living within water body.

1. Take DO bottles and fill with sea water sample avoiding air the bubbles.
2. Add 1mL of Winkler A and Winkler B solution.
3. Close it, drain off the excess and keep the bottle in dark.
4. Estimation of blank and standardization of thiosulphate.
5. Add 1mL of 50% of DO sample into the conical flask and titrate against thiosulphate solution until pale yellow colour is obtained. ✓
6. Add 1mL of starch solution and titrate it until the blue colour disappears.
7. Note down the readings and repeat the titration for 3 times.
8. Calculate the amount of DO present by using the formula.

➢ **MOST PROBABLE NUMBER (MPN)**

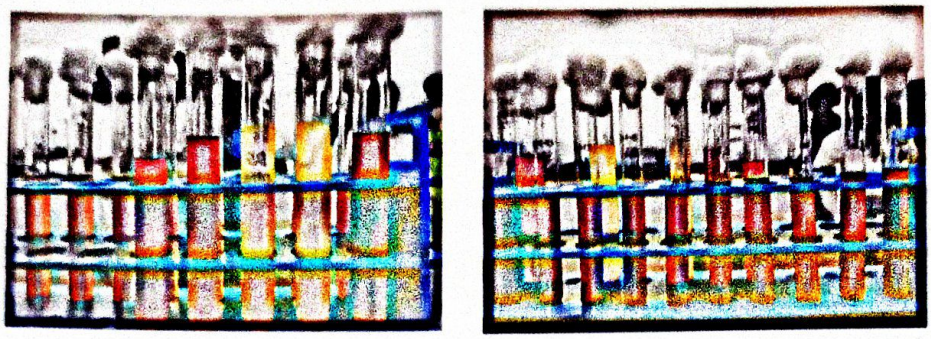


Fig 5: MPN

A most probable number method is practised for the qualitative and quantitative water to determine microorganism's presence e.g. *E. coli* and coliforms. Faecal coliforms are known to ferment lactose and produce both acid and gas and can be detected by performing MPN where change in colour of the media (MacConkey's) indicates acid production and formation of a gas bubble in the inverted Durham's tube indicates gas production. Both of which designate a positive result indicating presence of faecal coliforms, *E. coli* in the water sample.

1. Water sample collection was done from the surface of the station using a bucket.
2. The water was collected into sterile centrifuge tubes of 50 mL and stored in ice box until further analysis.
3. 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.
4. 10ml of water sample was inoculated into 5 tubes containing 10mL of MacConkey's Broth
5. 1ml of water sample was inoculated into 5 tubes containing 10mL of single strength MacConkey's Broth.

6. And 0.1 ml of water sample was added to 5 tubes containing 10mL of single strength broth.
7. All the tubes were incubated at 37°C for 24-48 hours
8. 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)
9. The results were compared to a standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined.

➢ PHYTOPLANKTON ANALYSIS

Fig 6: Phytoplankton & identification (*Coscinodiscus*)

In order to estimate the amount of phytoplankton's in each water sample one needs to fix the sample, to keep the cells intact and carry out microscopy later. Qualitative analysis can also be done using the same method.

1. 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.
2. Filled into 500mL bottles, next 15 drops of Lugol's iodine solution were added and stored in shade until further analysis.
3. The bottles were brought back to laboratory and left for settling.

4. After the settling period siphoning was done to concentrate the sample.
5. Microscopy was done using an inverted microscope under 10x and 20x objective lens.

➢ SUSPENDED PARTICULATE MATTER (SPM)

Suspended particulate matter (SPM) is finely divided solids or liquids that are operationally defined via filtration of seawater as the material retained on a certain type of filter with certain pore size. The dry weight concentration of suspended particulate material (mg L–1), is measured by passing a known volume of seawater through a pre-weighed filter and reweighing the filter after drying.

1. Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade.
2. A filter paper of 0.45 microns was placed in the filtration unit attached to the vacuum pump.
3. Before filtering the water sample the weight of the filter paper was measured and noted.
4. Around 250mL of seawater sample is filtered through the filter paper.
5. After filtering the weight of the filter paper was again measured (designated as wet weight)
6. 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)
7. The readings were noted down and the calculations were carried out.

➢ 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.

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

➢ 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. □ Results were recorded after the incubation period.

OBSERVATIONS

Table 1: TEMPERATURE

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

1. a. GRAPH OF TEMPERATURE

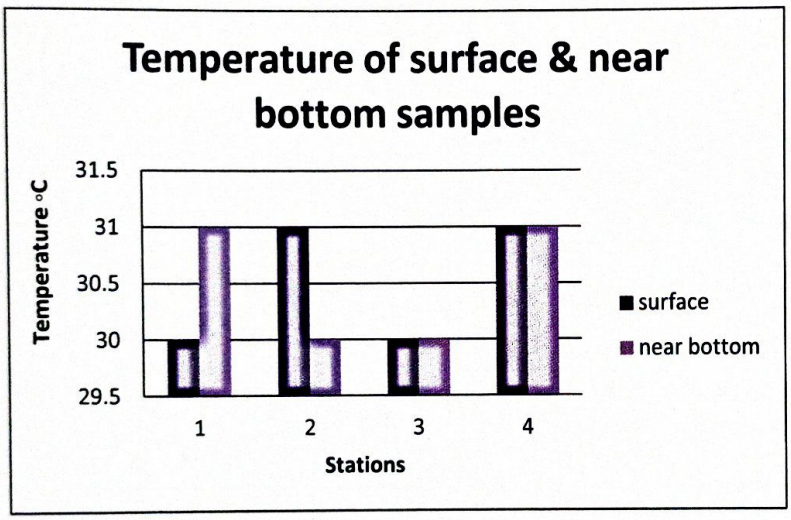


Table 2: SECCHI DISK

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

Table 3: SALINITY

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

3. a. GRAPH OF SALINITY

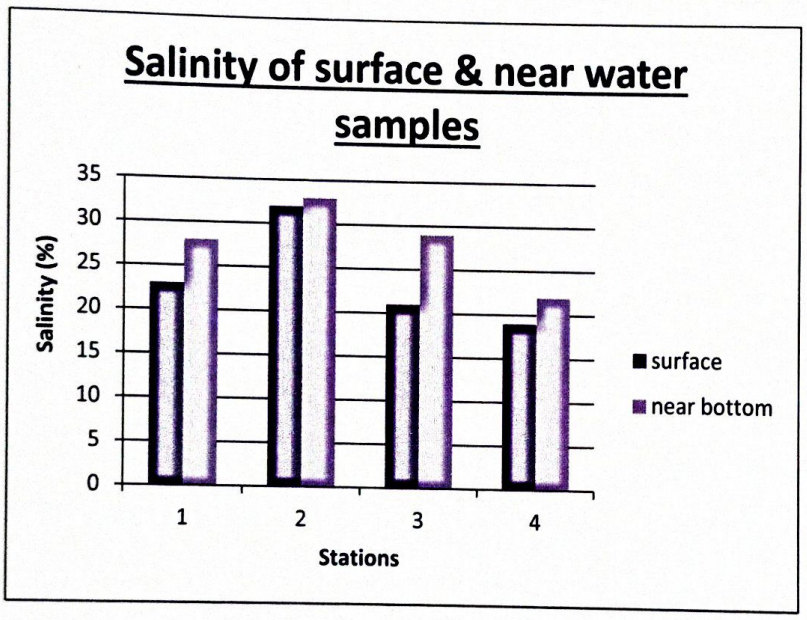
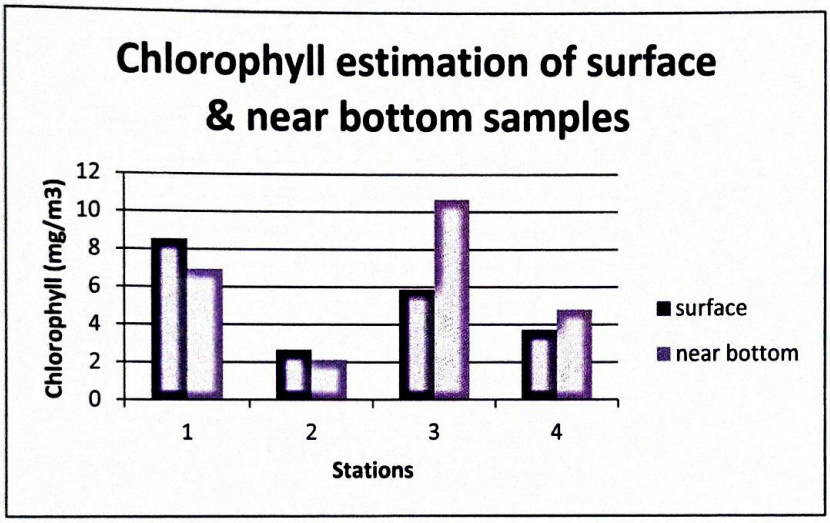


Table 4: CHLOROPHYLL ESTIMATION

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

4. a. GRAPH OF CHLOROPHYLL ESTIMATION



Calculation:

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

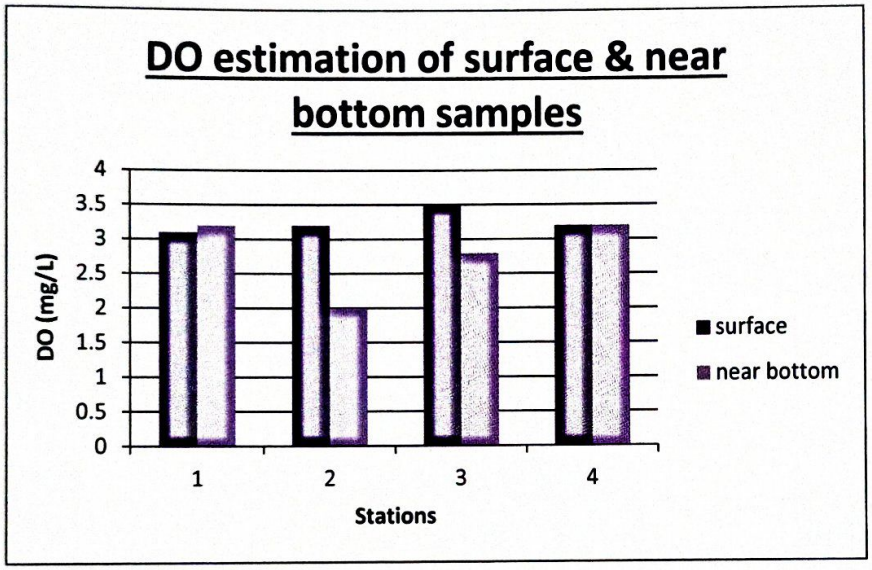
$$\text{phaeo-pigments (mg/m}^3) = \frac{26.7(1.7[665_a] - 665_o) \times v}{V \times l}$$

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).

Table 5: DISSOLVED OXYGEN

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

5. a. GRAPH OF DISSOLVED OXYGEN



Calculation:

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

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

Table 6: MOST PROBABLE NUMBER

STATION 1	DS (10mL)		SS (1mL)		SS (0.1mL)	
	ACID	GAS	ACID	GAS	ACID	GAS
1	✓	✓				
2	✓	✓	✓	✓		
3	✓	✓	✓	✓	7	7
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

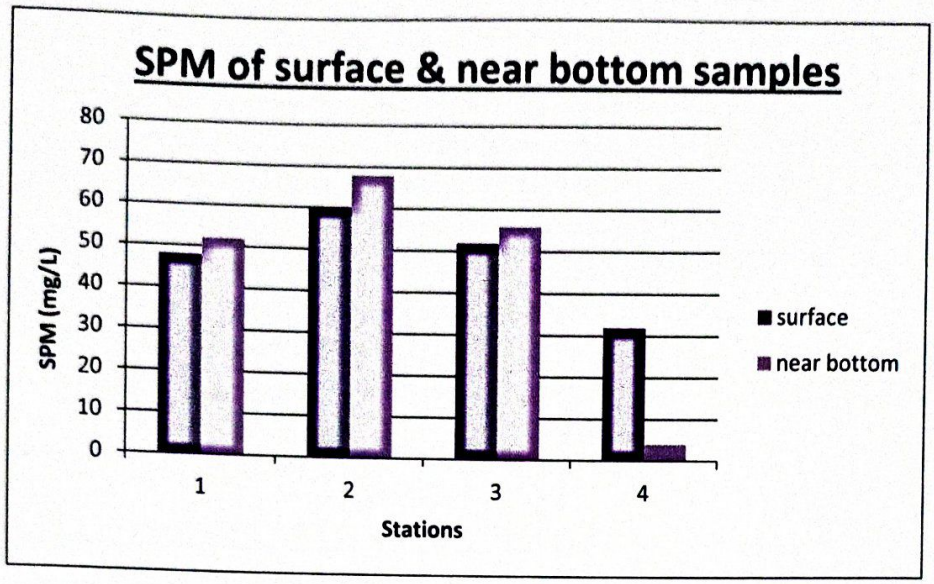
STATION 4	DS (10mL)		SS (1mL)		SS (0.1ML)	
	ACID	GAS	ACID	GAS	ACID	GAS
1	✓	✓	✓	✓		
2	✓	✓	✓	✓		
3	✓	✓	✓	✓		
4	✓	✓				
5						

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

Table 7: SUSPENDED PARTICULATE MATTER

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

7. a. GRAPH OF SPM



Calculation:

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

Table 8: pH

STATIONS	pH
1	8.1
2	8
3	7.6
4	7.9

RESULTS

- **TEMPERATURE**: The temperature across all the stations in surface and near bottom waters varied by 1°C and was between 30-31°C.
- **TURBIDITY**: The turbidity measured the highest at station 2 and lowest at station 3 and 4.
- **SALINITY**: Salinity was the highest at station 2 and lowest at station 4 for both surface and near bottom waters.
- **CHLOROPHYLL ESTIMATION**: 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.
- **DISSOLVED OXYGEN**: 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.
- **MOST PROBABLE NUMBER**: 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.
- **PHYTOPLANKTON ANALYSIS**: The following phytoplanktons were observed – Diatoms: *Rhizosolenia sp., Coscinodiscus sp., Gyrosigma sp., Chaetoceros sp*., and an unidentified pennate diatom. Dinoflagellates were not observed.
- **SUSPENDED PARTICULATE MATTER**: SPM was found to be highest at station 2 bottom waters and lowest at station 4 bottom waters.
- **pH**: 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.

- **VIABLE COUNT**: No growth was observed on any plate.

SUMMARY

It was wonderful experience. We learnt many techniques regarding sample collections on board. This trip made us more interested in our academics and further experiments. 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. We got an idea how things work practically and how to obtain results. Although, we just didn't work or collect samples we also had great fun. Lunch in between the estuary was a mesmerising experience and we enjoyed a whole lot while returning from the last station to the jetty. This field trip was commendable and worth remembering.

Fig 7: Students and Teachers along with equipment on trawler