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FIELD TRIP REPORT

MMO-319

A report submitted in partial fulfilment of the requirements for the Award of Degree of

MASTER OF SCIENCE

In

MARINE MICROBIOLOGY

7.5/10

by

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2022-2023

INTRODUCTION

Marine microbiology department held a field trip on 11th March 2022. We were accompanied by our 2 Asst. professors Dr. Varada Damare & Dr. Nikita Lotlikar. We left from Malim jetty by 10 am on a trawler named 'Jesus King'. All the work was distributed and planned forehand by Dr. Nikita Lotlikar. We collected near surface, bottom water and sediment samples from 4 different station – Malim jetty, Miramar offshore, Near Chorao island and Old goa – marked as station 1, station 2, station 3, station 4. First we went to station 2 i.e. offshores of Miramar beach where the turbidity was recorded with the help of a Secchi disk. Surface waters was collected by clean bucket which was rinsed with the same seawater and bottom waters was collected by Niskin sampler. DO bottles were filled and fixed immediately after collection with Winkler A and Winkler B. Salinity was recorded on board with the help of a refractometer and temperature with thermometer. Phytoplankton sample bottles was filled and fixed with Lugols iodine. Sediment samples were collected by van Veen grab instrument and for chlorophyll estimation and SPM water was collected from surface and near bottom. Surface waters were collected for MPN analysis and pH was checked in lab under pH meter. Similarly this was done on all the stations by the students by following the work demonstrated by the Asst. professors. After finishing, the samples were then bought to Marine microbiology, SEOAS, Goa University lab for further processing. The processes further explained down below.

Locations:-

Stations	latitudes	Longitudes	Depth (mrt)
1	15°28'20.8554"N	73°46'37.542"E	6
2	15°30'12.3192"N	73°49'55.5234"E	10
3	15°30'19.3248"N	73°52'0.894"E	3.5
4	15°30'31.248"N	73°54'50.9832"E	5.25

(Table:-1 Location of sampling stations)

(Fig:-1Locations of sampling stations)

Analysis of following experiments were done :

- Turbidity
- pH
- Temperature
- Salinity
- DO
- Viable count
- MPN
- Analysis of phytoplankton
- Suspended particulate matter
- Chlorophyll estimation

Turbidity

PRINCIPLE: A Secchi disk is an 8-inch (20 cm) disk with alternating black and white quadrants. It is lowered into the water or a lake until it can no longer be seen by the observer. This depth of disappearance, called the Secchi depth, is a measure of the transparency of the water.

PROTOCOL:

- Secchi disk was tied on to a rope on which the measurement was marked and was gently lowered in the waters from the trawler.
- The disc was lowered until the observer could no longer differentiate between the quadrants of the disc.
- The depth was noted down known as Secchi depth.

pH

PRINCIPLE: 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.

PROTOCOL:

- 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	Secchi depth	pH
1	1.75	8.1
2	2	8
3	2.25	7.6
4	1.3	7.9

(Table 2. Secchi depth, pH)

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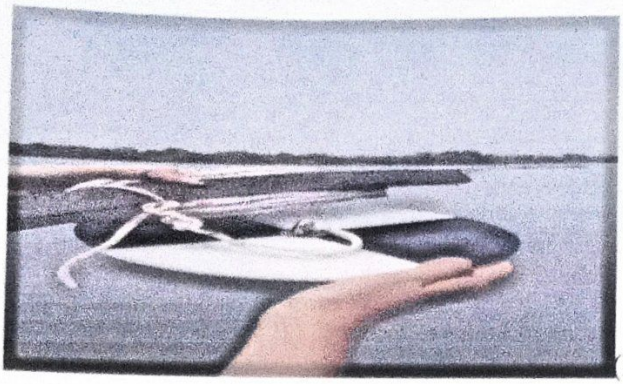
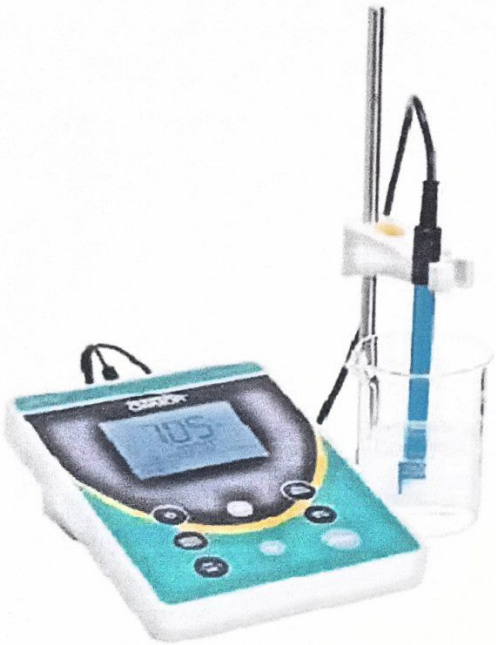


(Fig 2. Secchi disk)



(Fig 3. pH meter)

Temperature

PRINCIPLE: A thermometer works on the principle that solids and liquids expand on heating. As the temperature rises, mercury expands causing it to move upwards and depict the temperature. ✓

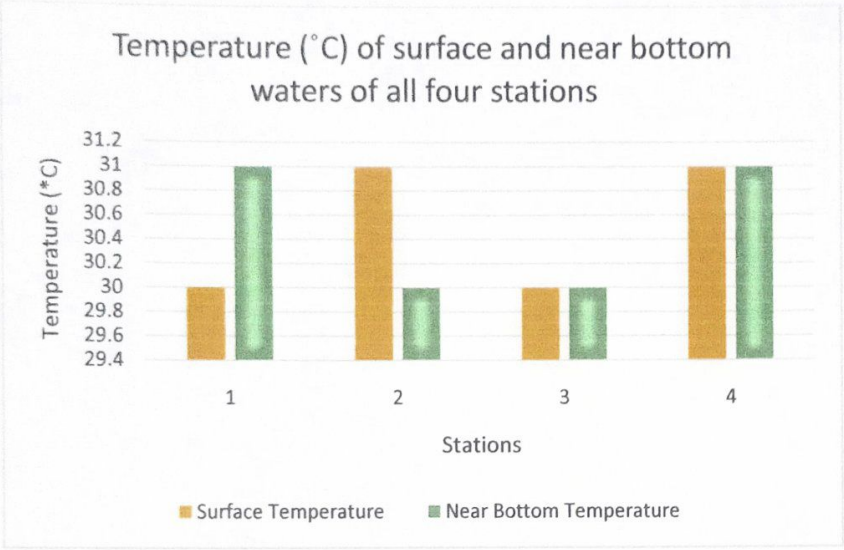
PROTOCOL:

- Water sample from different stations was collected in a bucket from the surface while for near bottom waters water from Niskin sampler was collected from this a mug of water was taken out and the thermometer was dipped into it.
- Readings were noted down

Observation:-

Station	Temperature (^{0}C)	Temperature (^{0}C)
	Surface	Near Bottom
1	30	31
2	31	30
3	30	30
4	31	31

(Table 3. Temperature at surface and near bottom)



Graph of temperature (*C) of all four stations (surface and near bottom)

Salinity

PRINCIPLE: A refractometer is a tool that can determine the concentration of a particular substance in a liquid solution. It uses the principle of refraction, which describes how light bends as it crosses the boundary between one medium and another. ✓

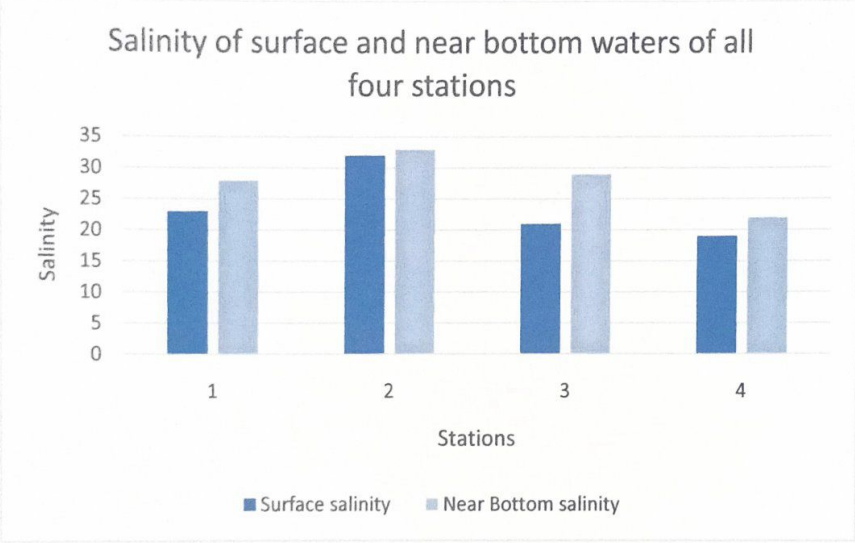
PROTOCOL:

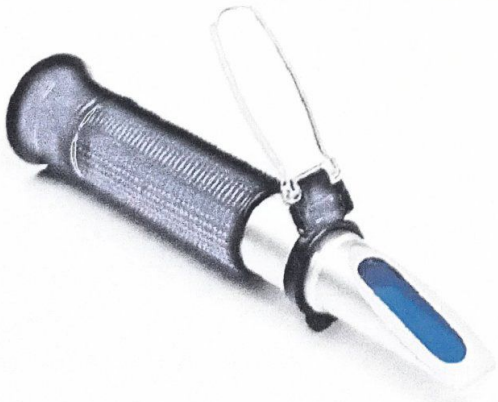
- Water sample was collected in a bucket from the surface while for near bottom waters, water was taken from the Niskin sampler.
- Using a dropper water was taken out and 2-3 drops were put onto the refractometer.

- The lid was closed making sure no air bubbles were trapped and viewed through the eyepiece
- Salinity reading was noted for all the stations.

Station	Salinity	Salinity
	Surface	Near Bottom
1	23	28
2	32	33
3	21	29
4	19	22

(Table 4. Salinity at surface and near bottom)





(Fig 4. Refractometer)

Dissolved Oxygen

PRINCIPLE: The chemical determination of oxygen in water sample is carried out by using the Winkler method. This method is a type of iodometric titration which oxidizes iodine ion to iodine using manganese as a transfer medium. This iodine is then titrated against sodium thiosulphate, the end point of redox titration is indicated with starch as it forms a complex compound with iodine resulting in a blue colour. The iodine molecules can further leave the starch helix and can be reduced by thiosulphate. This change from blue to colourless marks the end point of the titration.

PROTOCOL: Determination of reagent blank

- 50mL of distilled water was pipetted out into a conical flask, to that 1mL of 50% H2SO4, 1mL alkaline iodide (Winkler B) and 1mL manganous chloride reagent (Winkler A) was added. The solution was mixed thoroughly to avoid precipitation
- 1mL starch was added (if blue colour develops titration needs to be carried out)

Standardization of thiosulphate solution:

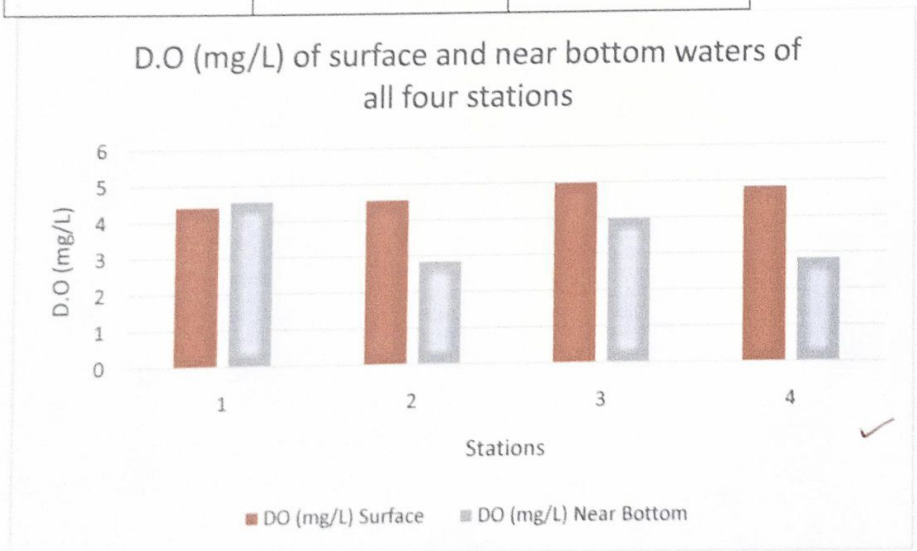
- Solution was prepared in the same method as prepared for blank
- 10mL of 0.01N potassium iodate solution was added. Solution was mixed and kept in dark for 3 mins to liberate iodine ✓
- Liberated iodine was titrated against sodium thiosulphate till the solution turned pale yellow.
- 1mL starch was added and titration was continued till the colour changed from blue to colourless (remained colourless for 30 seconds). This was carried in triplicates to obtain the mean burette reading

D.O. estimation:

- Sample was collected in 125mL D.O. bottles from different stations making sure no air bubbles were formed during collection.
- D.O. was fixed by adding 1mL of Winkler's A and 1mL of Winkler's B and the precipitate was left to settle.

- The samples were brought back to the laboratory.
- 1mL of 50% H2SO4 was added and shaken till the precipitate dissolved.
- 50mL of the sample was then transferred to a conical flask and titrated against thiosulphate solution until a pale yellow appeared.
- 1mL starch was added and titration was continued until the blue colour disappeared. Burette reading was noted. This was repeated three times to obtain mean burette reading.

Station	D.O. (mg/L)	D.O. (mg/L)
	Surface	Near Bottom
1	4.412	4.554
2	4.554	2.846
3	4.981	3.985
4	4.839	2.846



Graph of D.O. (mg/L) of surface and near bottom waters of all four stations.

(Fig 5. DO bottles after fixing with Winklers A&B)

Calculations:-

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

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

Viable count

PRINCIPLE: 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.

PROTOCOL:

- 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.
- The plates were incubated at 37°C for 24-hours.
- Results were recorded after the incubation period.

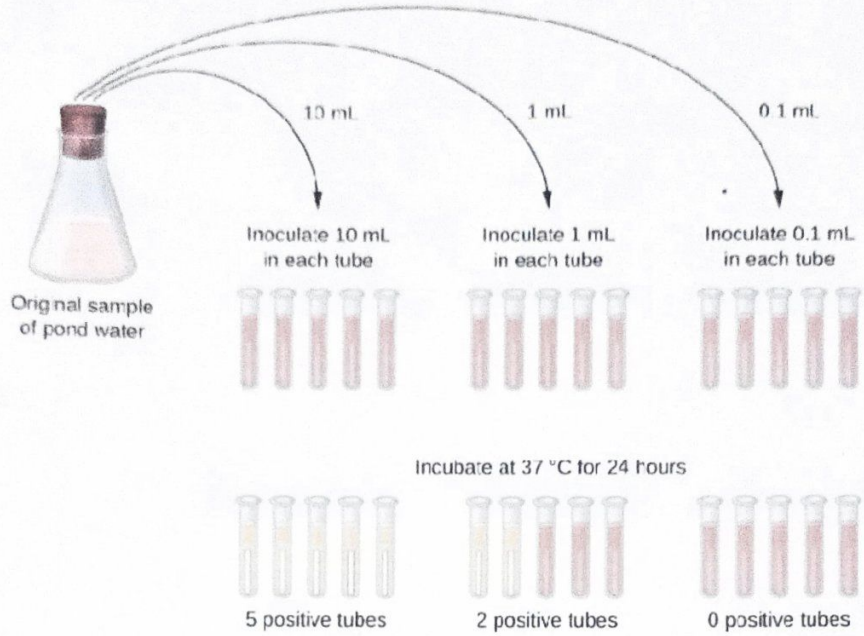
Stations	Dilutions	Number of colonies	Average number	Dilution factor (CFU/mL
Station I	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
Station II	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
Station III	10^{-1}	6	3	2500
	10^{-1}	-		
	10^{-2}	4	4.75	
	10^{-2}	115		
Station IV	10^{-1}	23	8.25	4400
	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	-		

MPN

PRINCIPLE: Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilizes the lactose present in the medium to produce acid and gas. The presence of acid is indicated by the color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e both color change and gas production) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables.

PROTOCOL:

- 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) and gas.
- The results were compared to a standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined. (Throndsen, 1978)



(Fig 6. MPN flowchart)

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.	+	+	+	+		

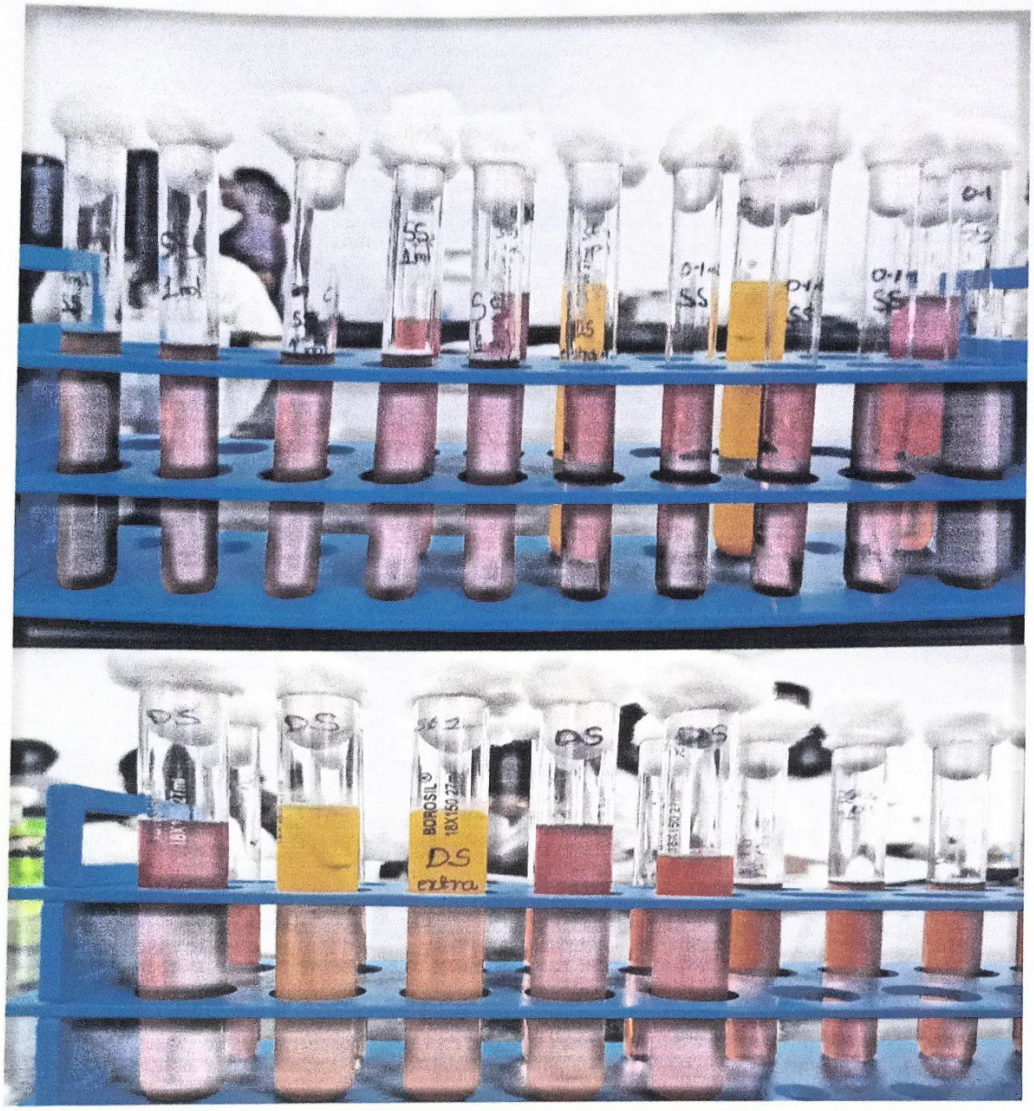
Number of positive tubes: 3-4-0=13-17 bacteria/ 100mL

Station 3	DS (10mL		SS (1mL		SS(O.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 bacteria/100mL



(Fig 7. MPN tubes after incubation period)

Analysis of Phytoplankton

PRINCIPLE: To estimate the amount of phytoplanktons 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

PROTOCOL:

- 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.
- The 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 9. a) *Coscinodiscus sp.* b) Fixed sample bottles after siphoning.)

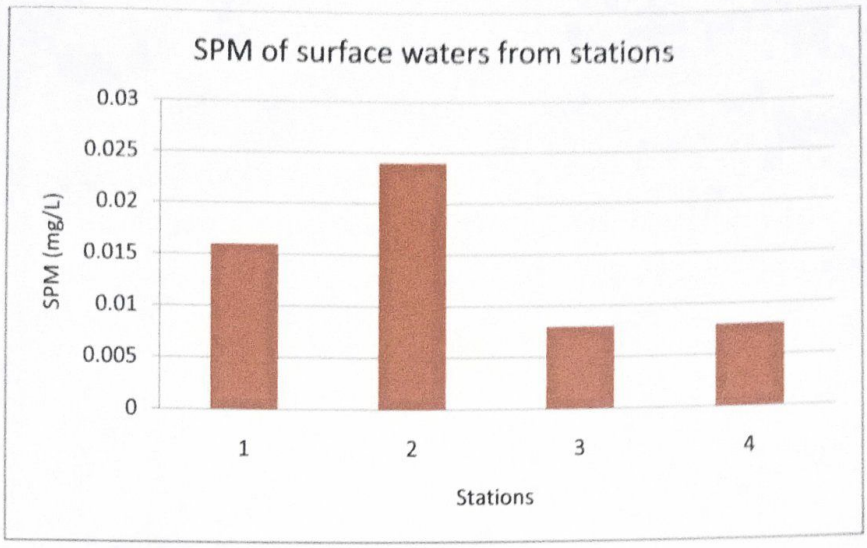
Suspended particulate matter ✓

PRINCIPLE: 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). The dry weight concentration of suspended particulate material, [SPM] (units: mg L–1), is measured by passing a known volume of seawater through a pre-weighed filter and reweighing the filter after drying.

PROTOCOL:

- Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of 41 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. (Parsons et al. (1984) and Strickland and Parsons (1965).

Stations	**Filter paper weight (g) (x)**	**Dry weight (g) (y)**	**Difference (g) (x-y)**	**SPM (mg/L)**
Station 1	0.080	0.084	0.004	0.016
Station 2	0,082	0.088	0.006	0.024
Station 3	0.078	0.080	0.002	0.008
Station 4	0.090	0.092	0.002	0.008



Graph of SPM (mg/L) of all four stations

$$SPM = \frac{X - Y}{\text{Volume of water filtered in litres}}$$

Chlorophyll estimation

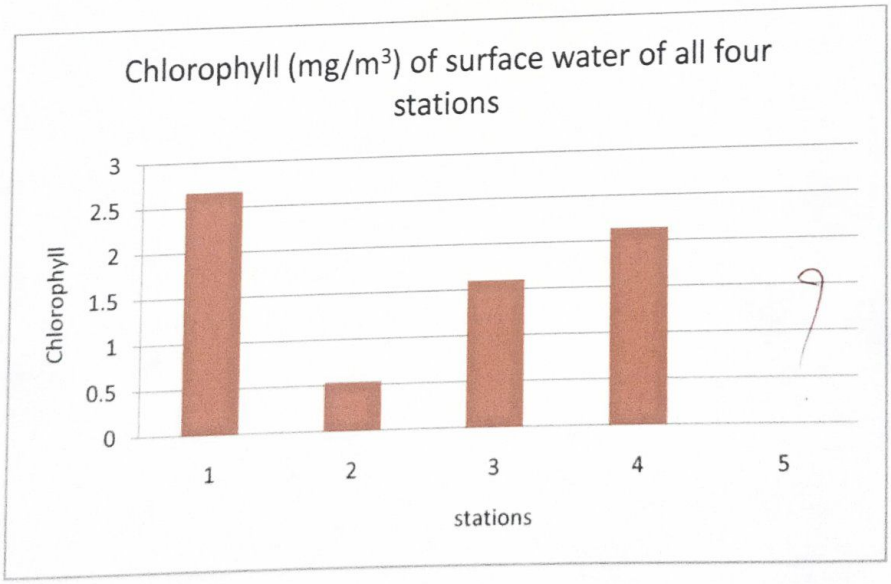
PRINCIPLE: Pigment extraction (phaeopigments, chlorophyll) is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is used as a solvent in this method since its slight polarity allows it to dissolve polar substances and also allows greater resolution between pigments. Pigment analysis is done spectrophotometrically.

PROTOCOL:

- Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of 41 microns was placed in the filtration unit attached to the vacuum pump.
- Around 500mL of seawater sample is filtered through the filter paper. After filtration the filter paper was picked using forceps and placed into a dark coloured plastic bottle.

- Next 10ml of 90% acetone was put into the bottle
- Crushed and gently and capped. The bottle was kept undisturbed for 24 hours in the refrigerator
- Next day the samples were analysed spectrophotometrically at 665nm, then 2 drops of HCL acid was added.
- Absorbance was measured at 750nm. Readings were recorded and calculations were done. (Parsons et al. 1984)

Stations	**Chlorophyll (mg/m^3)**
1	2.67
2	0.53
3	1.60
4	2.14



Calculations:-

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

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

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).

RESULTS:-

Turbidity and pH:- The turbidity measured interms of secchi depth was in the range of 1-2.3 meters with the highest being at station 3 and lowest at station 4. While the pH was found to be in the range of 7.5-8 with the highest being at 1 and lowest at station 3.

Temperature and Salinity :-The temperature across all the stations in surface and near bottom water varied by 1°C and was between 30-31°C. While salinity was the highest at station 2 and lowest at station 4 for both surface and near bottom waters.

D.O.:- Among the surface water in all the 4 stations, station 3 is the highest and station 1 showed the lowest concentration of D.O., While among the near bottom water station 1 showed the highest and station 2 and 4 showed the lowest concentration of D.O.

MPN:- As observed from the readings station 1 has 130 bacteria/100Ml and station 2 has 13-17 bacteria/100mL and 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.

Estimation of Phytoplankton:- the following phytoplankton were observed – Diatoms: Rhizosolenia sp., Coscinodiscus sp., Gyrosigma sp., Chaetoceros sp., and an unidentified pinnate diatom.

SPM:- SPM was highest at station2 and lowest at station3 and 4.

Chlorophyll:- chlorophyll concentration was found to be highest at station1 and lowest at station 2.

CONCLUSION

It was a great experience. We learnt many techniques regarding sample collections on board. This trip made us more interested in our academics and further experiments. We got a clear cut idea how things works practically and how to obtain results. We just didn't work or collect samples we had a lot of fun too. Lunch in between the estuary was a mesmerising experience and we had lot of fun while returning from the last station to the jetty. It was a very nice experience.

(STUDENTS OF MSC. MARINE MICROBIOLOGY PART-2)

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References:

- Throndsen, J. (1978). The dilution-culture method. In 'UNESCO Monographs on Oceanographic Methodology, Vol. 6, Phytoplankton Manual.' (Ed. A. Sournia.) pp. 218–224. (UNESCO Publishing: Paris.)
- Parsons, T. R., Maita, Y., and Lalli, C. M. (1984). 'Manual of Chemical and Biological Methods for Seawater Analysis.' (Pergamon Press: New York.)
- Strickland, J. D. H., and Parsons, T. R. (1965). 'A Manual of Seawater Analysis', 2nd edn. (Queen's Printer and Controller of Stationery: Ottawa.)

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