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REPORT ON FIELD TRIP

TO

MANDOVI ESTUARY

BY

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SCHOOL OF EARTH, OCEAN, AND ATMOSPHERIC SCIENCES,

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Paper Code: MM0 319 (Field Trip/Study tour- Practical)

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INTRODUCTION:

The Goa University Department of Marine Microbiology organised a field excursion on 11th March, 2022. Students went on the trawler field trip with two teachers (Dr. Varada Damare and Dr. Nikita Lotlikar) and non-teaching staff. Glassware, equipment, and other necessary preparations were made in a timely manner before the field trip. The day before the field trip, the tasks that needed to be completed were explained. The different locations were Malim jetty, Miramar, Chorao island, Old Goa (Station 1, Station 2, Station 3, Station 4). A trawler was was used to reach these locations. Different types of samplers were used to collect the sample. On the stations the sample for surface, near bottom and sediment was collected. For collecting the surface sample a simple bucket and a rope was used. The rope was tied to the bucket and it was slowely released in to the surface of the water. For collecting the near bottom sample, a Niskin sampler was used. For collecting the sediment sample, a Van Veen grab was used. The samples for DO (Dissolved Oxygen), MPN (Most Probable Number) , Phytoplankton fixation and SPM (Suspended Particulate Matter) chlorophyll were also taken. The latitude and the longitude was also noted simultaneously. For checking the temperature, the thermometer was directly dipped in to the water sample. For checking the salinity, the use of refractometer was done. All the students and teachers returned to the lab to conduct the various experiments and record the results after collecting various samples from all four stations.

Fig 1 : A group photograph of Students and Teachers with equipments on trawler.

OBJECTIVE:

- To obtain knowledge of the various tools and practical expertise with the various methods used to undertake water sampling for the investigation of several water body parameters.

- The following parameters underwent analysis:
  1) Dissolved Oxygen
  2) Most Probable Number
  3) Temperature
  4) Salinity
  5) Viable Count
  6) Analysis of phytoplankton
  7) Chlorophyll estimation
  8) Suspended Particulate Matter

- Latitude and Longitude :

Stations	Latitude	Longitude
1	15°30.143'N	73°49.907'E
2	15°47.143'N	73°77.907'E
3	15°30.438'N	73°51.907'E
4	15°30.143'N	73°49.907'E

COLLECTION OF THE SAMPLE :

1) Niskin bottle: To obtain a water sample at depth, a niskin sampler is slowly lowered into the water. Without running the risk of contaminating the water sample, it is used to collect water samples at a particular depth. There are valves on both ends of it. The bottle's valve is open and it is fastened to the wire. The weight (messenger) is then allowed to slide down the cable after being discharged into the sea. The valve then closes, collecting the water.

2) Van Veen grab : A Van Veen grab is made specifically for collecting sediment samples. It is made up of two buckets joined together by a hinge. The two buckets stay apart once it is dropped into the water. The buckets close when it reaches the bottom, enabling for the collecting of the water sample

TO CHECK THE CLARITY OF WATER :

1) Secchi disk : A Secchi disc is made of of quadrants that alternate between black and white. It is released into the water, where the viewer can no longer see it. The water's transparency is gauged by its depth of disappearance. The colour of the algae, water, and suspended particles can have an impact.

Fig 2: Niskin bottle

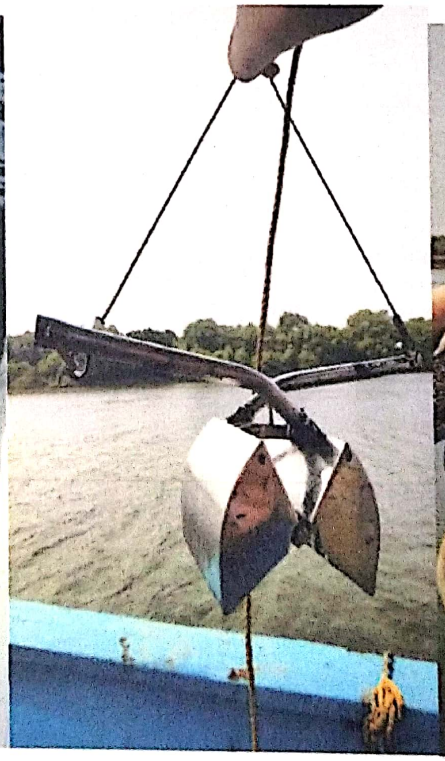

Fig 3: Van Veen Grab

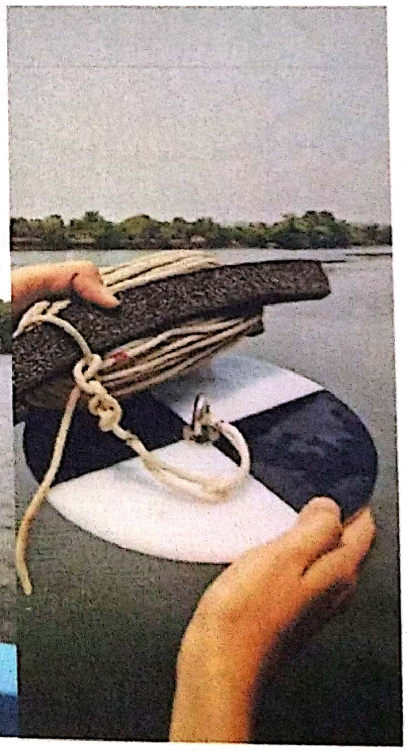

Fig 4: Secchi disk

1)DISSOLVED OXYGEN

PRINIPLE: Dissolved oxygen (DO) refers to the concentration of the oxygen gas incorporated in the water. Dissolved oxygen is a valuable tracer for water masses and is a sensitive indicator for biological and chemical processes occurring in the sea. Water has the ability to dissolve atmospheric gases such as oxygen , nitrogen, carbon dioxide and noble gases. The Winkler method is an iodometric titration, in this the oxygen in the seawater sample is made to oxidize, iodine ion to iodine quantitatively. The amount of iodine generated is determined by titration with a standard thiosulfate solution. The DO is chemically bound to Mn(II)OH in a strongly alkaline medium which results in a brown precipitate, which is maganic hydroxide ($MnO(OH)_2$). The change from blue to colourless marks the end of the titration.

METHODOLOGY :

Determination of reagent blank :

1)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

2) 1mL starch was added (if blue colour develops titration needs to be carried out)

Standardization of thiosulphate solution:

1)Solution was prepared in the same method as prepared for blank .

2) 10mL of 0.01N potassium iodate solution was added. Solution was mixed and kept in dark for 3 mins to liberate iodine .

3) Liberated iodine was titrated against sodium thiosulphate till the solution turned pale yellow.

4) 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:

1)The DO sample was collected in the DO bottle (make sure there are no air bubbles)

2)DO was fixed by adding 1ml of wrinklers A and 1ml of wrinklers B, by inserting the pipette tip at the bottom of the bottle, in a bottle containing the sample.

3)The precipitate was left to settle.

4)1ml of 50% H_2SO_4 was added and shaken vigorously till the precipitate dissolved.

5)50ml of the sample was transferred to a conical flask and titrated against thiosulphate solution until a pale yellow colour appeared.

6)1ml starch solution was added and titration was continued until the blue colour disappeared.

7)Burette reading was noted. Titration was repeated three times to find out the mean burette reading.

OBSERVATION : Standard value : 11.6

Station 1: Surface

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.4	3.1	3.1	3.1

Station 1: Bottom

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.2	3.2	3.0	3.2

Station 2: Surface

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.2	3.3	3.2	3.2

Station 2: Bottom

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		2	2.4	2	2

Station 3: Surface

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.2	3.5	3.5	3.5

Station 3: Bottom

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		2.8	3.8	2.8	2.8

Station 4: Surface

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.2	3.2	3.4	3.2

Station 4: Bottom

Pilot reading	Burette reading	I (ml)	II (ml)	III (ml)	Average burette reading
Analysis		3.2	3.2	3.5	3.2

CALCULATIONS:

$N_1V_1=N_2V_2$

N_1 = Normality of KIO_3 (0.01) V_1= Volume of KIO_3 (10ml)

V_2= Volume of sodium thiosulphate used in standardization N_2= Normality of Sodium thiosulphate

$$N_2 = \frac{\text{Normality of } KIO_3 \text{ X } 10}{BR(std) - BR(b)} = \frac{0.01 \text{ X} 10}{11.6-0} = 0.0086$$

$$v/v = \frac{\text{Volume of the bottle}}{\text{Volume of the bottle} - \text{Volume of the reagents}} = \frac{125}{125-2} = 1.016$$

$$DO, mgL^{-1} = \frac{BR \text{ X } v/v \text{ X } N \text{ X } E \text{ X } 1000}{\text{Volume of the sample titrated}} = \frac{3.1 \text{ X } 1.016 \text{ X } 0.0086 \text{ X } 8 \text{ X } 1000}{50}$$

$= 4.33mg/L$

(Substitute the rest of the values and do similar calculations)

Station 1 : 1) Surface : 4.33mg/L 2)Bottom : 4.47 mg/L

Station 2 : 1) Surface : 4.47mg/L 2)Bottom : 2.79 mg/L

Station 3 : 1) Surface : 4.89mg/L 2)Bottom : 3.91 mg/L

Station 4 : 1) Surface : 4.47mg/L 2)Bottom : 4.47 mg/L

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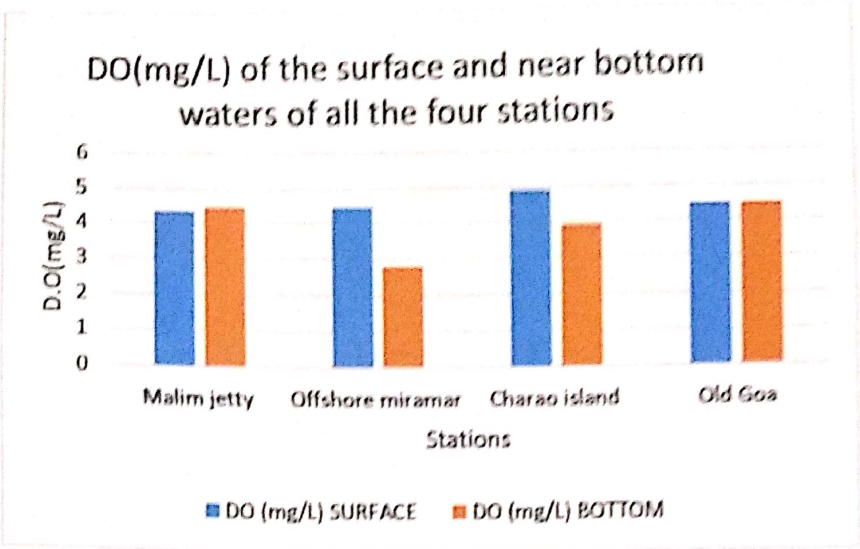

Fig 5: Graph of D.O. (mg/L) of surface and near bottom waters of all four stations

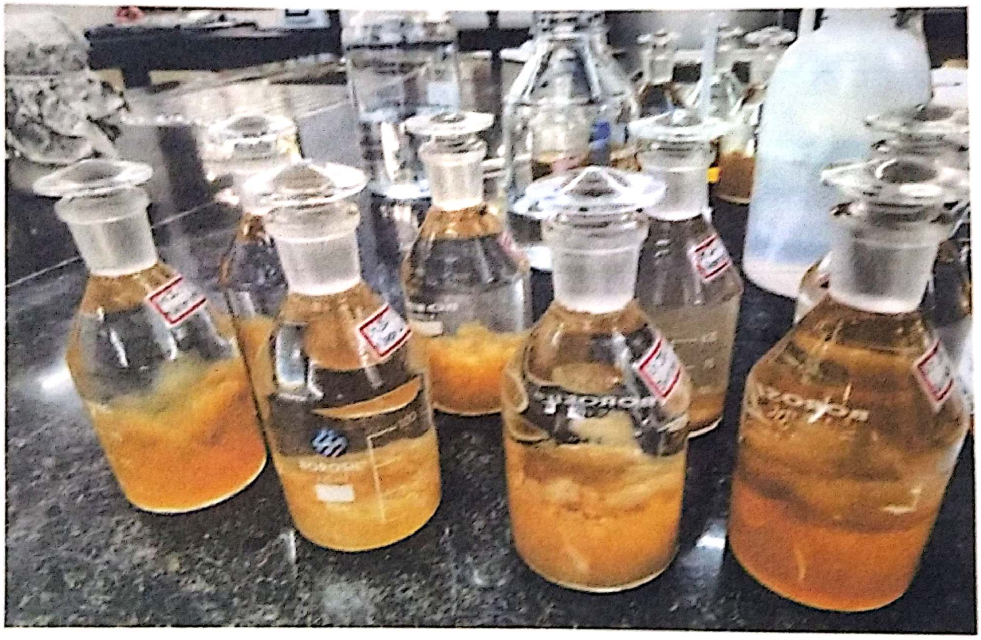

Fig 6: D.O. bottles after fixing the sample with Winkler's A and Winkler's B

RESULT : Different concentrations of DO was present in the different seawater sample.

Among the surface waters in all the four stations, station 3 showed the highest and station 1 showed the lowest concentration of D.O., while among the near bottom waters station 1 and station 4 showed the highest and station 2 showed the lowest concentration of D.O

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2) MOST PROBABLE NUMBER

PRINCIPLE: Most Probable Number is used to estimate the concentration of viable microorganisms in a sample. It is used to check if the water is safe or not in terms of bacteria present in it. A group of bacteria commonly called as fecal coliforms acts as an indicator of fecal contamination of water. If there are few fecal coliform bacteria, it would indicate that water probably contains no disease causing organisms, if there are large number of fecal coliform bacteria, it would indicate that the water could contain disease producing organisms, which will make water unfit for consumption.

METHODOLOGY:

1)Take 5 tubes of double strength and 10 tubes of single strength, for the water sample to be tested.

2)Add 10ml of the water to 5 tubes containing 10ml of double strength medium.

3)Similarly add 1ml of water to 5 tubes containing 10ml single strength medium and 0.1ml water to the remaining 5 tubes containing 10ml single strength medium. Add inverted durhams tube.

4)Incubate all the tubes at 37^0C for 24 hours.

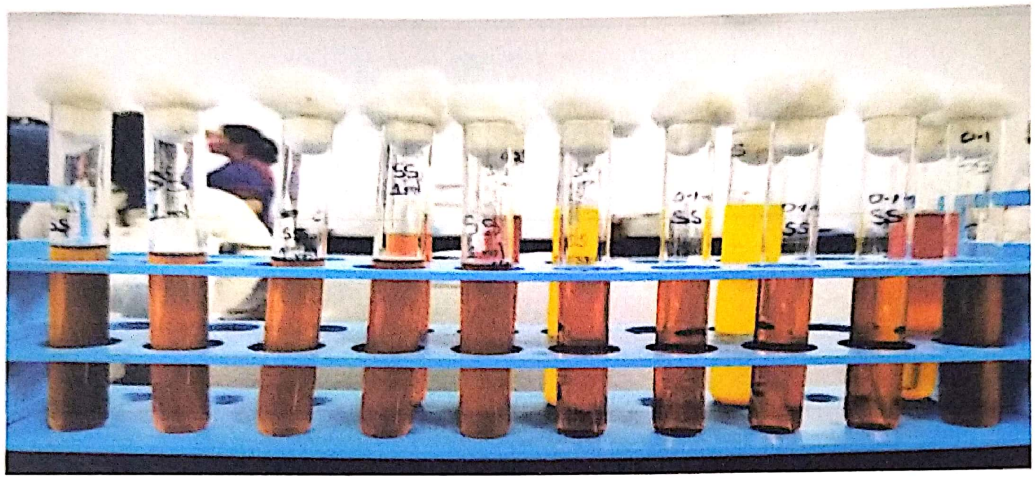
5)Check the tubes for colour change and gas production.

OBSERVATIONS : (Only for station 2)

	ACID	GAS
Single strength (0.1)		
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
Single strenght (1ml)		
1	-	-
2	-	-

✓

3	-	-
4	-	-
5	-	-
Double strength		
1	+	+
2	+	+
3	+	+
4	+	+
5	-	+



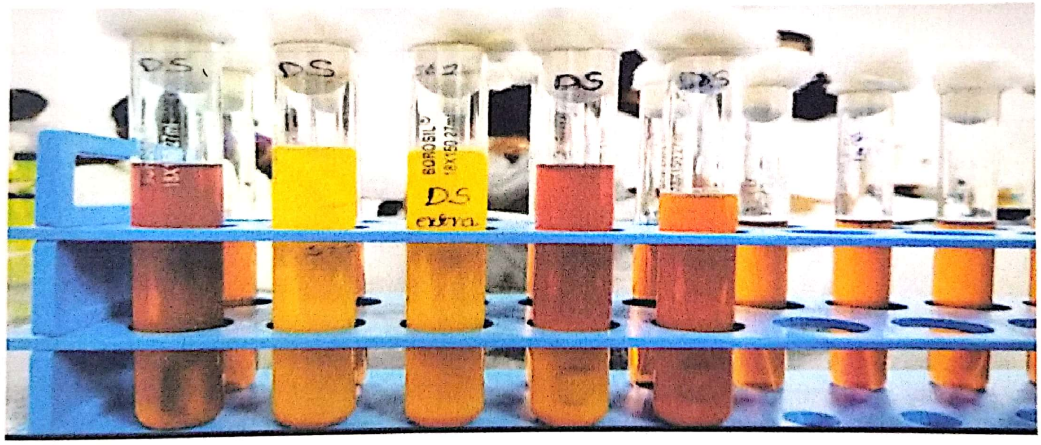


Fig 7: MPN tubes after the incubation period

RESULT :

Acid: If there is color change of MacConkeys broth from red to yellow it indicates a positive test.(+)

Gas: If there is gas production inside the Durhams tube it indicates a positive test.(+)

If the broth colour remains same (red and no gas formation) it will give a negative result.(-)

3)TEMPEATURE

PRINCIPLE:

The glass tube of a thermometer is sealed at both ends. It is partly filled with a liquid such as mercury. When mercury gets hotter, it expands by an amount that's directly related to the temperature. As the temperature raises, mercury expands causing it to move in upward direction and show the temperature on thermometer.

METHODOLOGY:

1)Before using a thermometer give a few jerks. These jerks help to bring the level of mercury down. (Or keep in ice).

2)Submerge the thermometer inside the water.

3)Keep the thermometer inside the water for around 1min .

4) Note down the temperature.

OBSERVATION :

Station 1 -	1) Surface : 30^0C	2)Near bottom : 31^0C
Station 2 -	1) Surface : 31^0C	2)Near bottom : 30^0C
Station 3 -	1) Surface : 30^0C	2)Near bottom : 30^0C
Station 4 -	1) Surface : 31^0C	2)Near bottom : 31^0C

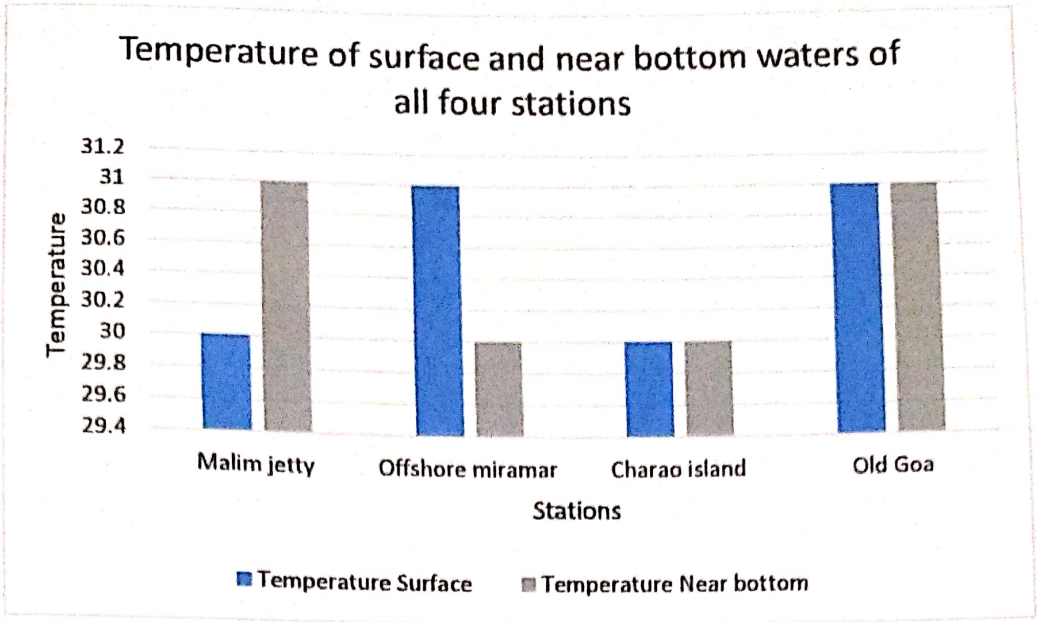


Fig 8: Graph of temperature (°C) of all four stations (surface and near bottom waters)

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

PRINCIPLE:

Salinity can be measured using refractometer. A water sample with more salinity will cause the light to refract more than a sample with less salinity. The instrument works on the critical angle principle, it uses lenses and prisms to project a shadow line on to a small glass reticle inside the refractometer, which is than viewed through a eyepiece.

METHODOLOGY:

1)Open the cover of the prism and put few drops of the collected water sample on it.

2)Than close the cover.

3)Look through the eyepiece and adjust the focus.

4)Check for the scale and take the reading.

OBSERVATION:

Station 1 -	1) Surface : 23%	2)Near bottom : 28%
Station 2 -	1) Surface : 32%	2)Near bottom : 33%
Station 3 -	1) Surface : 21%	2)Near bottom : 29%
Station 4 -	1) Surface : 19%	2)Near bottom : 22%

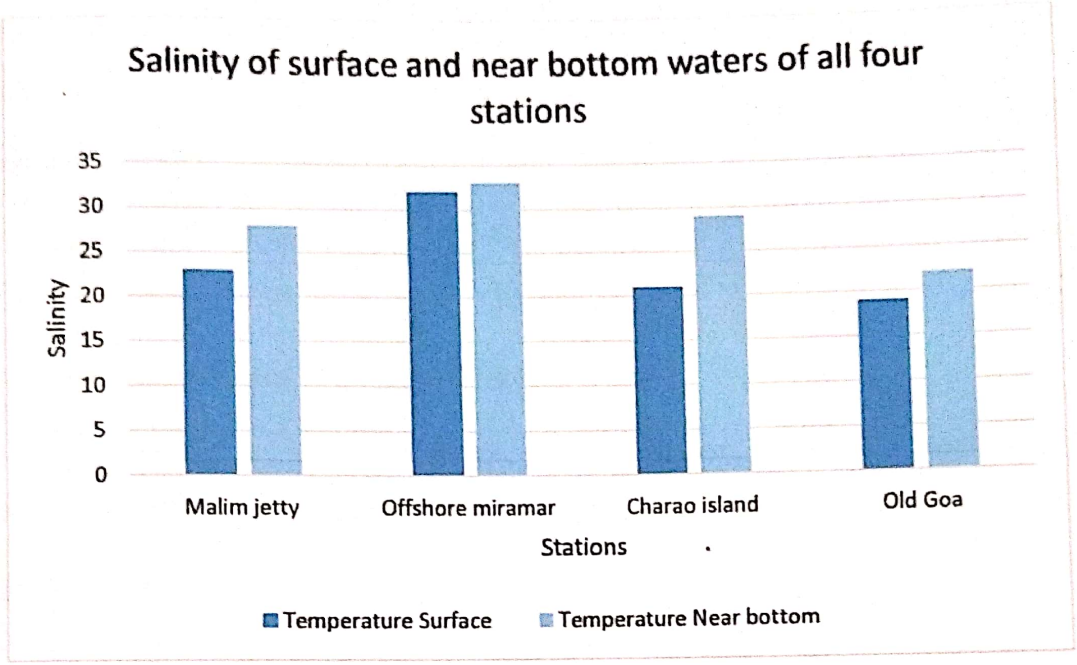


Fig 9 : Graph of salinity of all four stations (surface and near bottom waters)

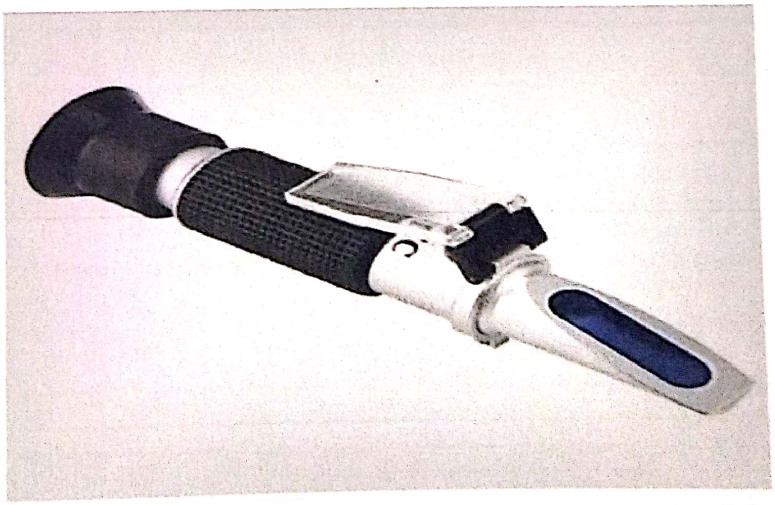


Fig 10 : Refractometer

RESULT: While salinity was the highest at station 2 and lowest at station 4 for both surface and near bottom waters.

5)VIABLE COUNT :

PRINCIPLE: A count of viable or live cells is known as the viable plate count, often known as the plate count. It is based on the idea that when viable cells are cultured in appropriate conditions , they reproduce and form visible colonies. It allows one to identify the number of actively growing cells in a sample. The plate count method relies on bacteria growing a colony on nutrient medium. The colony can be seen with naked eyes and the numbers on the plate can be counted.

METHODOLOGY:

1) From water sample collected in the centrifuge tubes, 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. Wherein each colony was counted and written down. Average of total number of colonies was taken and viable count was calculated.

OBSERVATION:

	DILUTIONS	NO OF COLONIES	AVERAGE NUMBER	DILUTION FACTOR CFU/mL
Station 1	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
Station 2	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
Station 3	10^{-1}	6	3	2500
	10^{-1}	0		
	10^{-2}	4	4.75	
	10^{-2}	15		

Station 4	10^{-1}	23	8.25	4400
	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	0		

RESULT: Growth was observed on the plate.

6)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.

METHODOLOGY:

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.

OBSERVATION:

Fig 11: a) Coscinodiscus sp. b) Fixed sample bottles after siphoning.

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

7)ANALYSIS OF CHLOROPHYLL:

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.

METHODOLOGY:

1) Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of 0.75 µm was placed in the filtration unit attached to the vacuum pump.

2) Around 500mL of seawater sample is filtered through 0.75µ GF/F filter paper. After filtration the filter paper was picked using forceps and placed into a dark coloured plastic bottle.

3) Next 10ml of 90% acetone was put into the bottle

4) Crushed and gently and capped. The bottle was kept undisturbed for 24 hours in the refrigerator

5) Next day the samples were analysed spectrophotometrically at 665nm, then 2 drops of HCL acid was added.

6) Absorbance was measured at 750nm. Readings were recorded and calculations were done. (Parsons et al. 1984)

OBERVATIONS :

Station	Surface waters Chlorophyll (mg/ m3)
1	2.67
2	0.53
3	1.60
4	2.14

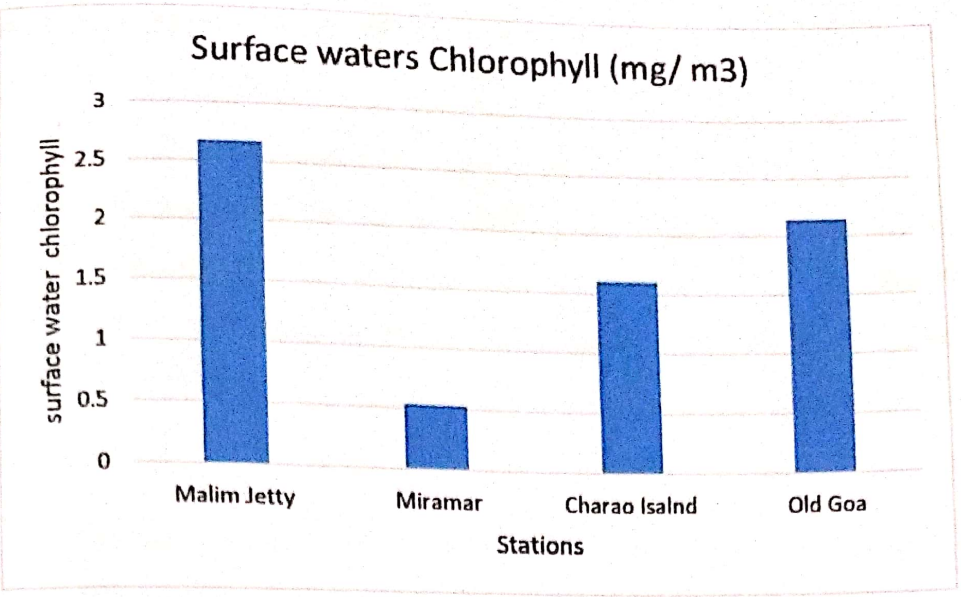


Fig 12: Graph of Chlorophyll (mg/m3) estimation

$$\text{chlorophyll } a\ (\text{mg/m}^3) = \frac{26.7(665_0 - 665_a) \times v}{V \times l}$$

$$\text{phaeo-pigments } (\text{mg/m}^3) = \frac{26.7(1.7[665_a] - 665_0) \times v}{V \times l}$$

where 665_0 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 chlorophyll concentration was found to be at station 1, and lowest was found to be at station 2.

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8) SUSPENDED PARTICULATE MATTER

PRINCIPLE: The material held on a specific type of filter with a specific pore size is operationally characterised as suspended particulate matter (SPM) via seawater filtering, whereas the substance that flows through a small pore size filter is defined as dissolved matter (DM). A known volume of seawater is run through a pre-weighed filter to determine the dry weight concentration of suspended particulate material, [SPM] (units: mg L-1), and the filter is then reweighed after drying.

METHODOLOGY:

1)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.

2) Before filtering the water sample the weight of the filter paper was measured and noted.

3) Around 250mL of seawater sample is filtered through the filter paper.

4) After filtering the weight of the filter paper was again measured (designated as wet weight)

5) 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)

6)The readings were noted down and the calculations were carried out. (Parsons et al. (1984) and Strickland and Parsons (1965))

OBSERVATION:

Station Surface Water	Filter paper weight (g) x	Dry weight(g) y	Difference(g) x-y	SPM (mg/L)
1	0.080	0.084	0.004	0.016
2	0.082	0.088	0.006	0.024
3	0.078	0.080	0.002	0.008
4	0.090	0.092	0.002	0.008

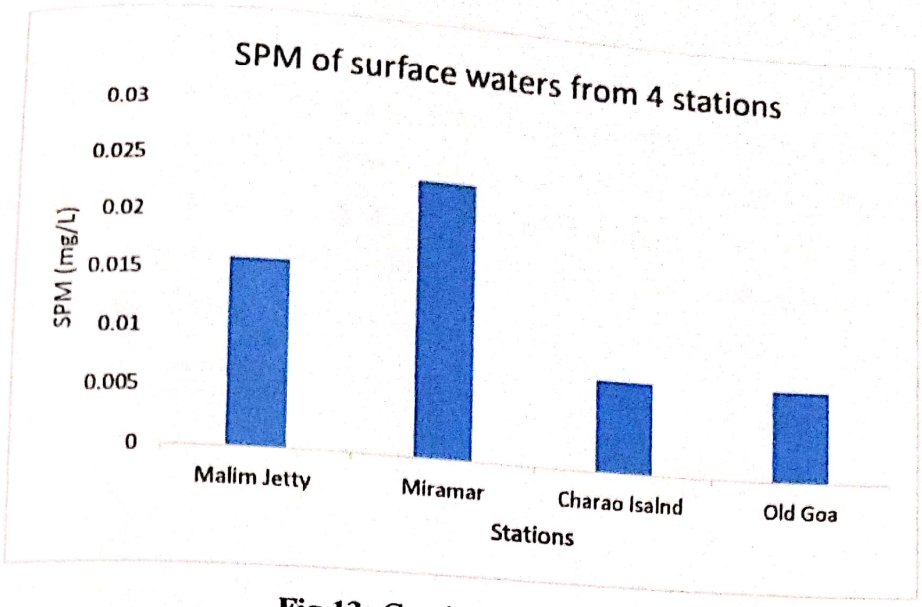

Fig 13: Graph of SPM (mg/L)

RESULT: SPM was found to be highest at station 2 and lowest at station 3.

CONCLUSION:

The whole trip was awesome. We learnt about the use and handling of different instruments like a Niskin sampler, Van Veen grab etc. We had a fantastic time and it was very much helpful for us, specially for developing co-operative behaviour in the students. We also had our lunch that was served, which we ate on the trawler parked in the middle of the estuary.To conclude the trip was very informative, effective and at the same time memorable.

Fig14 : Food served at the trawler

Fig15 : Students of MSc Marine Microbiology Part II

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