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FIELD TRIP REPORT

MMO 319

Submitted by

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MSc Marine Microbiology Part II (2022-23)

SEOAS, Goa University

INTRODUCTION

The Department of Marine Microbiology, SEOAS, Goa University had organized a field trip for the students of MSc Part I and Part II in Mandovi estuary. The students were taken in two different batches on 10th and 11th March 2022 on the trawler. Dr. Varada Damare and Dr. Nikita Lotlikar alongwith non teaching staff Ms Vaishali and Ms Sitam accompanied the students for the field trip. All the required equipments, instruments for sampling were kept ready before the trip. Also preparation of media, glasswares and other requirements to be used for further analysis of the samples were done a day before the trip. The methodology of sampling, various instruments and jobs to be done on the field trip were properly planned and explained to the students. The students were told to be present at the destination i.e. Mallem jetty sharp at 9.30 a.m. The excursion started exactly at the given time. Four different sites across the stretch of Mandovi estuary were marked as sampling stations (station 1, station 2, station 3 and station 4) where different analysis like temperature, salinity, etc. were to be carried out on board alongwith sampling procedures. The first sampling site was station 2(offshore waters, Miramar). Latitude and longitude of the station was noted. Sampling of surface waters and near bottom waters was done using a bucket and Niskin sampler respectively. The water collected from surface and near bottom was used for D.O estimation, phytoplankton estimation, chlorophyll estimation and SPM. For the MPN and bacterial count only the surface water collected was used. Other physical parameters like temperature, salinity, pH of surface and near bottom waters was also recorded. Turbidity was also measured using Secchi disc. Using a van Veen grab sediment was also collected. The same procedures were repeated for other stations as well. The 4 different stations were Malim jetty, offshore Miramar, near Chorao island and Old Goa. After finishing all the procedures on all the marked four stations the students alongwith the teachers returned to the laboratory for carrying out the further processing and analysis of the collected samples.

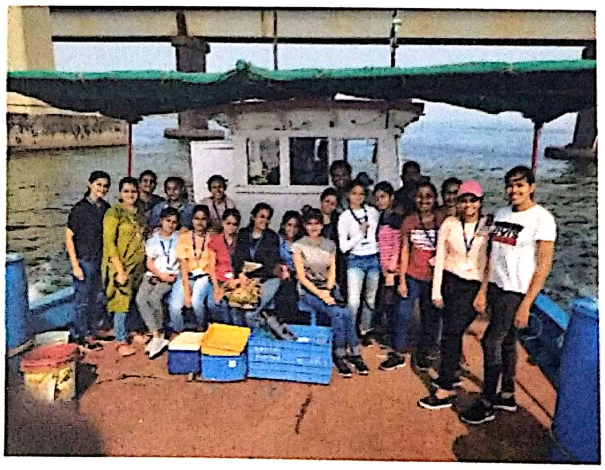

Fig 1. Students along with teachers and non teaching staff

OBJECTIVES:

1. To learn about the different instruments and various techniques employed to perform sampling of water and for the analysis of various parameters of water bodies.

2. Analysis of the following parameters of collected samples:

- ✓ MPN
- ✓ Viable count
- ✓ D.O
- ✓ Temperature
- ✓ Salinity
- ✓ Analysis of phytoplankton
- ✓ Chlorophyll estimation
- ✓ Suspended Particulate Matter
- ✓ Turbidity
- ✓ pH

Fig 2. Niskin Sampler

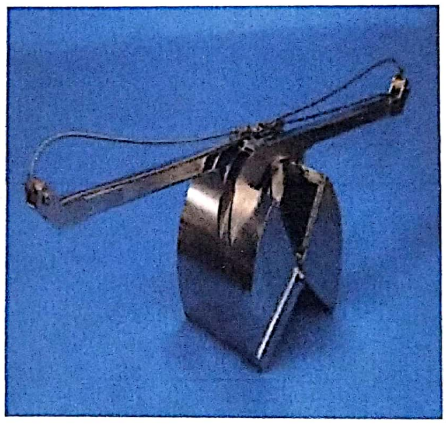

Fig 3. van Veen grab

- **Latitude, Longitude and Depth of Sampling sites:**

STATIONS	LATITUDE	LONGITUDE
1 (Malim Jetty)	15°301.43'N	73°49.9076 S
2 (Miramar)	15.475	73.773
3 (Chorao)	15°30.438'N	73°51.9705'S
4 (Old Goa)	15°30.85'N	73°55.171'S

➢ **Analysis of MPN:**

PRINCIPLE: Most Probable Number is a statistical method used to estimate the viable numbers of bacterial in a sample by inoculating broth in 10 fold dilutions. MPN test is used to detect *E. coli* and coliforms. Coliforms if present in water utilizes the lactose present in the medium to produce acid (colour change) and gas(gas bubbles in inverted Durham's tube).

PROCEDURE:

- Water was collected from the surface using a bucket.
- 50 ml of the collected sample was stored in 50 ml centrifuge tubes and kept in ice box until further analysis.
- The samples were brought back to the laboratory and were inoculated into double strength and single strength MacConkey's broth containing Durham's tubes in the respective volumes.
- 10 ml of sample was inoculated into 5 tubes containing 10 ml of double strength MacConkey's broth.
- 1 ml of sample was inoculated into 5 tubes containing 10 ml of single strength MacConkey's broth.
- 0.1 ml of sample was inoculated into 5 tubes containing 10 ml of single strength MacConkey's broth.
- All the tubes were incubated at 37°C for 24-48 hours.
- Production of acid (change in colour of the media from pink to yellow) and gas production indicated positive results.
- The results were compared to a standard chart like McCrady's table and the number of bacteria per 100 ml of sample was determined.

OBSERVATION:

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 bacteria/100ml

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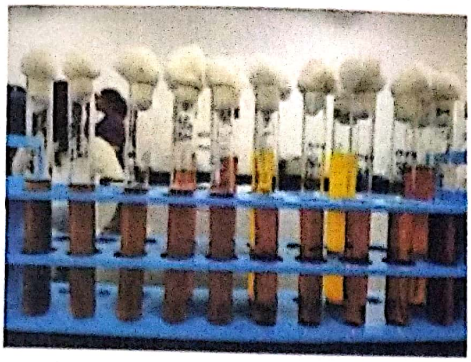

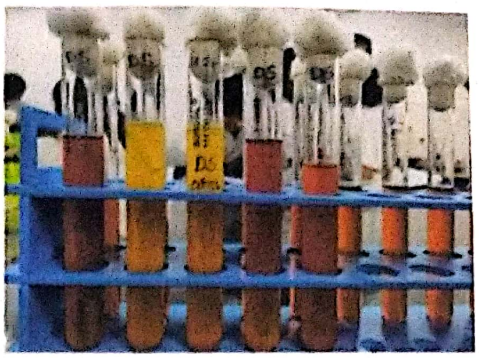

Fig 4. Single strength tubes after incubation Fig 5. Double strength tubes after incubation

RESULTS: 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.

- **Analysis of viable count:**

PRINCIPLE: The viable count or 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.

PROCEDURE:

- 0.1 ml from the collected water sample in the centrifuge tube was taken and spread plated on ZMA, MacConkey's, TCBS and XLD agar.
- All the plates were incubated at 37˚C for 24 hours.
- After the incubation period colonies on the plates were counted and the results were noted down. An average of total number of colonies were taken and viable count was calculated.

OBSERVATION:

STATIONS	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		

RESULTS: Colonies were observed on the plates.

- **Analysis of Dissolved Oxygen (D.O):**

PRINCIPLE: Dissolved Oxygen determination measures the amount of dissolved or free oxygen present in water or wastewater. In the Winkler method titration is carried out to determine dissolved oxygen in the water sample. The dissolved oxygen in the sample is fixed by adding a series of reagents that form an acid compound that is then titrated with a neutralizing compound that results in color change.

PRECAUTIONS:

- Do not allow air to trap while sampling water during analysis.
- Dip the tip of the pipette just at the bottom of the bottle and gently release the reagents.

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PROCEDURE: Determination of reagent blank

- In a conical flask, 50ml of distilled water was pipette out, to that 1 ml of 50% H_2SO_4, 1 ml of alkaline iodide (Winkler B) and 1 ml of manganous chloride (Winkler A) was added. The solution was mixed thoroughly to avoid precipitation.
- 1 ml of starch solution was added in the flask (if after this step blue colour develops then titration needs to be carried out).

Standardization of Thiosulphate solution

- Same method as for the blank was used to prepare the solution.
- 10 ml of 0.01N potassium iodate solution was added, mixed and kept in dark for 3 minutes to liberate iodine.
- Liberated iodine was titrated against sodium thiosulphate till the solution turned pale yellow.
- 1 ml starch was added to it that turned the solution to blue colour and titration was continued till the solution turned colourless. This was carried out in triplicates to get the mean burette reading.

D.O estimation

- Samples from different stations were collected in 125 ml D.O bottles making sure no air bubbles were formed during collection.
- D.O was fixed by adding 1 ml of Winkler's A and 1 ml of Winkler's B and the precipitate was left to settle.
- The fixed samples were brought to the laboratory for futher analysis.
- 1 ml of 50% H_2SO_4 was added and shaken to dissolve the precipitate.
- 50 ml of these was then transferred to a conical flask and was titrated against thiosulphate solution until a pale yellow colour appeared.
- 1 ml starch was added and titration was continued until the blue colour disappeared.
- Burette reading was noted when the colour of solution changed from blue to colourless.
- Same steps were repeated 3 times to obtain the mean burette reading.

OBSERVATION:

STATION	D.O (mg/L)	D.O (mg/L)
	Surface waters	Near bottom waters
1	3.1	3.2
2	3.2	2
3	3.5	2.8
4	3.2	3.2

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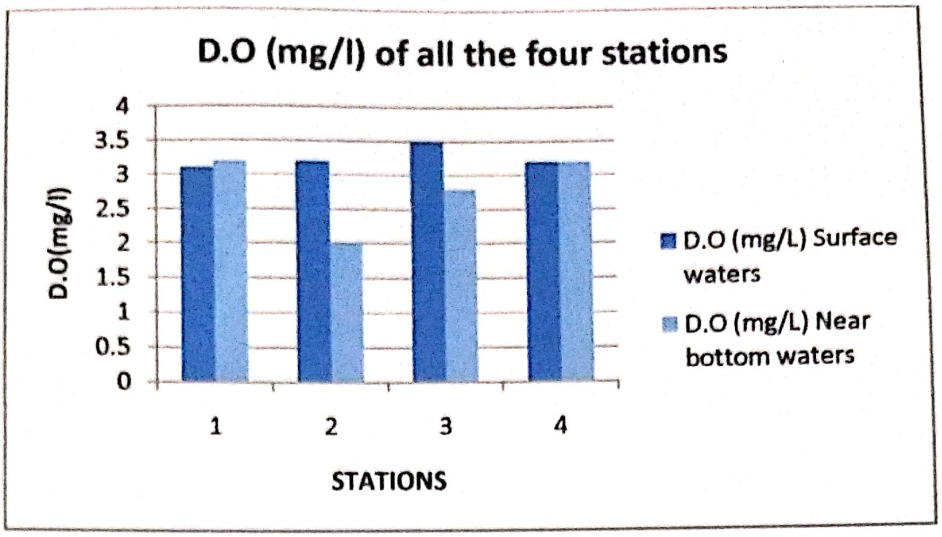


Fig 6. D.O bottles after fixing with Winkler's A and B

RESULTS:

- Surface water of station 3 showed the highest concentration of D.O and station 1 showed the lowest concentration.
- Near bottom water of station 1 and station 4 showed the highest D.O concentration and station 2 showed lowest concentration of D.O.

➢ **Analysis of temperature**

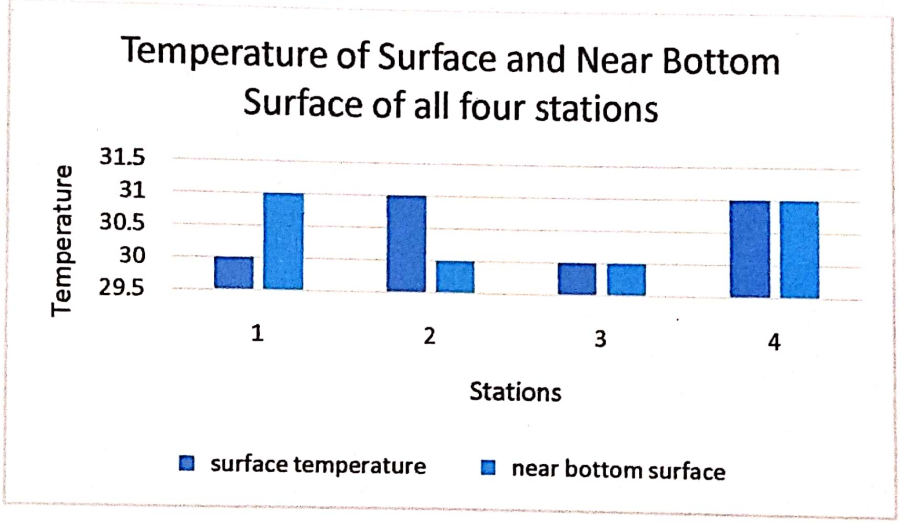
PRINCIPLE: A thermometer works on principle that solids and liquids expand on heating. As the temperature rises, mercury expands causing it to move upwards and depict the temperature.

PROCEDURE:

- Water samples from different stations were collected in a bucket from the surface and by using a niskin sampler from the near bottom waters.
- Some amount of water was transferred into a mug and a thermometer was dipped in it.
- Readings were noted down on each station.

OBSERVATION:

STATION	TEMPERATURE (°C)	TEMPERATURE (°C)
	Surface water	Near bottom water
1	30°C	31°C
2	31°C	30°C
3	30°C	30°C
4	31°C	31°C



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

➢ **Analysis of salinity**

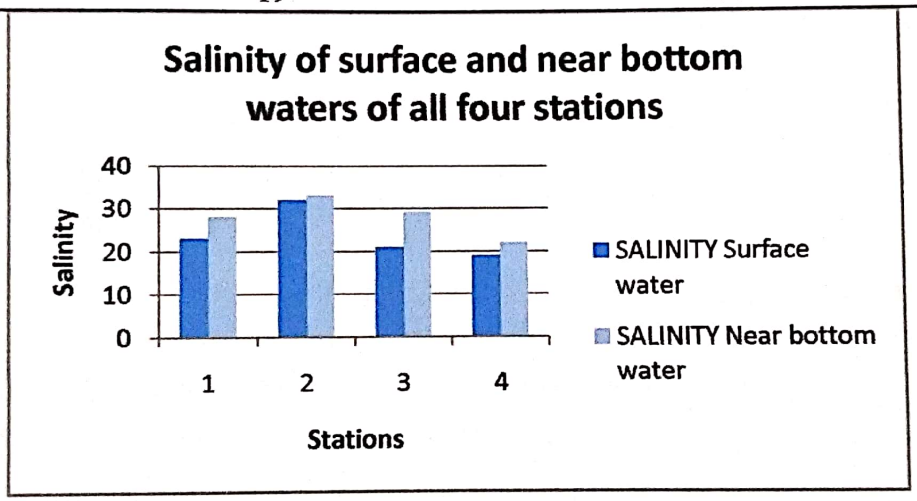
PRINCIPLE: A refractometer is a tool that can determine the concentration of a particular substance in a liquid solution. It uses the principle of refraction, which describes how light bends as it crosses the boundary between one medium to another. A few drops of liquid are placed on the prism of refractometer.The lid is closed creating a thin layer of water across the prism. When light enters the liquid layer, it changes the direction. The refractometer measures the amount of light shift and this is converted to a unit of measure.

PROCEDURE:

- Water samples from different stations were collected in a bucket from the surface and by using a niskin sampler from the near bottom waters.
- A dropper was used to put 2-3 drops of the collected sample onto the refractometer.
- The lid of the refractometer was closed carefully taking care that no air bubbles were formed and the readings were viewed through the eyepiece.
- Similarly the salinity was noted for all the stations for surface and near bottom waters.

OBSERVATION:

STATION	SALINITY	SALINITY
	Surface water	Near bottom water
1	23	28
2	32	33
3	21	29
4	19	22



RESULTS: The salinity of surface water as well as near bottom water was highest at station 2 and lowest at station 4.

- **Analysis of phytoplankton**

PRINCIPLE: Fixation of the phytoplankton sample is required to estimate the number of phytoplanktons in the sample. The samples are fixed to prevent the adverse effects of light, temperature and other microorganisms that might cause rapid decay of organisms.

PROCEDURE:

- Water samples from different stations were collected in a bucket from the surface and by using a niskin sampler from the near bottom waters.
- Samples were filled into 500 ml bottles and 15 drops of lugol's iodine solution was added and the bottles were stored in shade until further analysis.

- The bottles were brought back to the laboratory and was kept for settling.
- After settling siphoning was done to concentrate the sample.
- Microscopy was carried out using an inverted microscope under 10X and 20X objective lens.

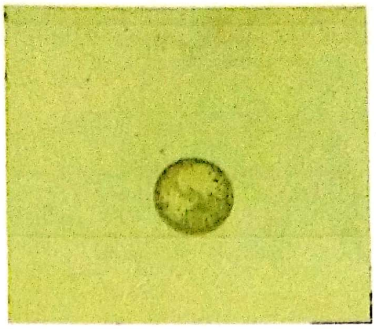



Fig 7. (a)fixed samples (b)*Coscinodiscus sp.*

RESULTS:

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

- **Analysis of chlorophyll**

PRINCIPLE: Pigment extraction is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is used as a solvent since its polarity aloows to dissolve polar substances and allows greater resolution between pigments. Further pigment analysis is done spectrophotometrically.

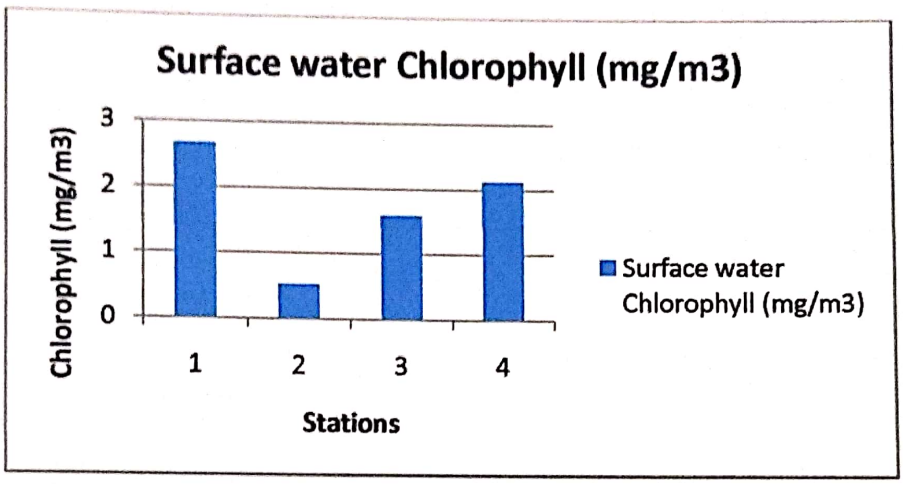
PROCEDURE:

- Samples from the surface water as well as the near bottom water was collected in plastic bottles and kept in shade.
- A 0.75µm filter paper was placed in the filtration unit attached to a vacuum pump.
- Around 500 ml of sample was filtered through the filter paper and the filter paper was picked using forceps and placed into a dark coloured bottle after filtration.
- 10 ml of 90% acetone was added into the dark coloured bottle containing the filter paper.
- The filter paper inside was crushed using a glass rod and the bottle was capped properly and kept undisturbed for 24 hours in the refrigerator.
- After 24 hours the samples were analyzed spectrophotometrically at 665 nm.
- 2 drops of HCL was added to acidify the samples and absorbance was measured at 750 nm.

- Readings were recorded and the calculations were done.

OBSERVATION:

STATION	Surface water Chlorophyll (mg/m^3)
1	2.67
2	0.53
3	1.60
4	2.14



RESULTS: The highest chlorophyll concentration was found at station 1 and lowest at station 2.

➢ **Analysis of Suspended Particulate Matter**

PRINCIPLE: Suspended Particulate Matter is operationally defines 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. The dry weight concentration of suspended particulate matter (units: mg L^{-1}), is measured by passing a known volume of seawater through a pre-weighed filter and reweighing the filter after drying.

PROCEDURE:

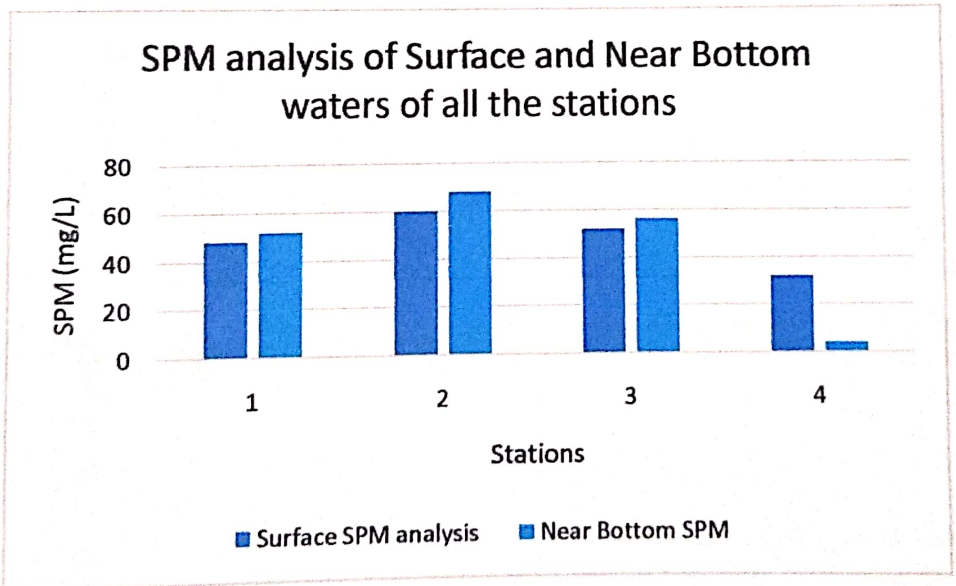
- Samples from the surface water as well as the near bottom water was collected in plastic bottles and kept in shade.
- 0.45µ filter paper was placed in the filtration unit attached to the vacuum pump.
- The weight of the filter paper was measured before filtering the water sample through it.
- Around 250 ml of sample was filtered through the filter paper.
- The weight of the filter paper was measured again after filtration. (Wet weight).

✓

- The filter paper was then completely dried in the oven at 30°C and the weight was measured again. (Dry weight)
- The readings were noted down and calculations were done.

OBSERVATION:

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
	Near bottom	0.082	0.095	0.013	52
2	Surface	0.088	0.103	0.015	60
	Near bottom	0.084	0.101	0.017	68
3	Surface	0.086	0.099	0.013	52
	Near bottom	0.08	0.094	0.014	56
4	Surface	0.085	0.093	0.008	32
	Near bottom	0.081	0.091	0.01	4



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

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

➢ **Analysis of turbidity using Secchi disc**

PRINCIPLE: A secchi disc is a simple, standard tool to measure water clarity. It is an 8 inch disc with alternating black and white quadrants. It is lowered into the water until it can no longer be seen by the observer. This depth where the disc disappears is called the Secchi depth that is the measure of the transparency of the water.

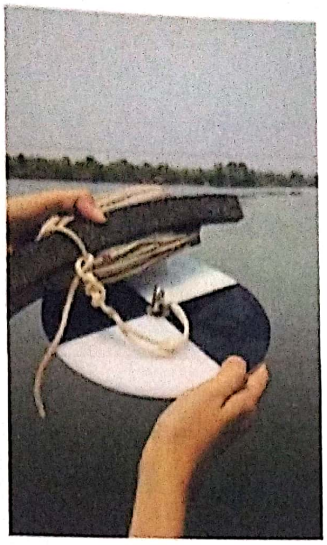


Fig 8. Secchi Disc

PROCEDURE:

- On all the stations Secchi disc was gently lowered into the water from the trawler with the help of a rope.
- It was lowered to a depth where the observer could no longer differentiate between the quadrants of the disc.
- The depth at which this was observed was noted as Secchi depth.

OBSERVATION:

STATIONS	SECCHI DEPTH
1	2 m
2	9 m
3	1.5 m
4	1.5 m

RESULTS: Turbidity was highest at station 2 and lowest at station 3 and 4.

✓

➢ Analysis of pH

PRINCIPLE: The overall working principle of pH meter depends upon the exchange of ions from sample solution to the inner solution (pH 7 buffer) of glass electrode through the glass membrane.

PROCEDURE:

- The pH meter was turned on and calibrated.
- The electrode was rinsed with distilled water and wiped gently with tissue paper.
- It was placed in neutral pH buffer solution, when the reading on the screen stabilized it denoted as 'ready', after which enter button was pressed.
- The electrode was washed and wiped again similarly and the same steps were repeated for acidic and alkaline pH buffer.
- After calibration, the electrode was rinsed and placed in the sample and the measurements were recorded.

STATIONS	pH
1	8.1
2	8
3	7.6
4	7.9

RESULTS: The pH was found to be in the range of 7.5- 8.1 with the highest pH being found at station 1 and lowest at station 3.

➢ **Perspective**

It was a great experience. We got an idea of how things work practically. We were able to experience the handling of various instruments like Niskin sampler, van Veen grab, etc on board. We experienced the difficulties faced during sampling. Overall we also had a lot of fun on the trawler. We enjoyed lunch and fruits on the trawler with the amazing view around us and also clicked a lot of pictures. We learned a lot of things from this opportunity given to us. The trip was a memorable experience.

Fig 9. Students of MSc Part II

Fig 10. Teacher giving instructions

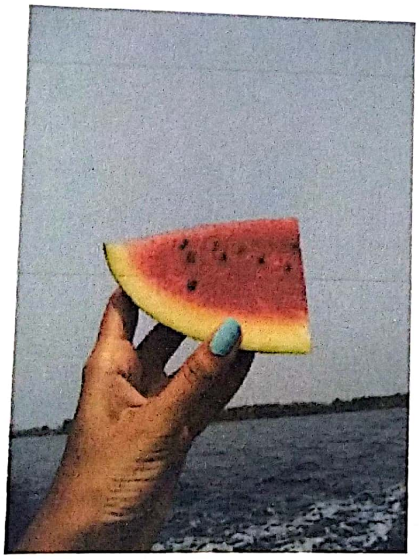


Fig 11. Watermelon served on trawler

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- Parsons, T. R., Maita, Y., and Lalli, C. M. (1984). 'Manual of Chemical and Biological Methods for Seawater Analysis.' (Pergamon Press: New York.)