FIELD TRIP REPORT

08/10

MMO 319

ALONG MANDOVI RIVER VIA MALIM JETTY

Bagkar
seen 12/12/22

SHREYA SANTOSH BAGKAR

MSc MARINE MICROBIOLOGY

PART II

ROLL NO: 21P039003

SEOAS, GOA UNIVERSITY

ACADEMIC YEAR: 2021-2022

APRIL 2022

INTRODUCTION

A field trip was arranged for students of MSc Marine Microbiology Part 1 by Marine Microbiology Programme, SEOAS, Goa University as it is requirement for the completion of Mater's degree in Marine Microbiology. On 11th March 2022. Dr Nikita Lotlikar and Dr Varada Damare along with non-teaching staff accompanied 15 of students. A day in advance of field trip, proper planning and preparation of media, glassware, reagents, etc. for the sampling and experiments to be carried out was done. We boarded in the trawler at 10.00 in morning, it was meant to take across particular stretch of the river Mandovi. We went along the Mandovi river via Malim jetty and collected surface and depth water samples to carry out various tests. We went to other three stations via a trawler from Malim jetty. We collected water samples from 4 stations :- Malim jetty, Miramar, Chorao and Old Goa. Upon reaching at all stations, we noted latitude and longitude. We collected surface waters samples by using bucket, near bottom water sample was collected by Niskin water sampler, sediment sample was collected using Van Veen Grab. Turbidity was measured using Secchi disc. Temperature was noted at each station of each water sample using thermometer, while salinity of both water samples of each station was noted using Refractometer and pH of surface and near bottom waters was also recorded. Water samples for MPN were collected in glass stoppered 50ml test tubes and kept in ice containers to test for bacteria. Water samples for DO were collected in glass stoppered bottles and Wrinklers reagents were added and then proceeded with further testing in lab. Water samples were collected in 125ml bottles for phytoplankton analysis and for chlorophyll estimations to be carried out in laboratory. After finishing our work on all four stations all the students along with the teachers returned to the laboratory and carried out the processing of the samples collected. Each of the experiment were performed and obtained results were recorded.

Fig 1: Students and teachers along with equipment on trawler.

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DECLARATION

I completed my field trip on 11th March 2022.

I could do my field trip in guidance of:

Dr. Priya D'Costa

Programme Director

Marine Microbiology

SEOAS, Goa- University.

Also all other teachers and non-teaching staff.

The contents of this report are original and I'm reporting work carried by me.

(SHREYA BAGKAR)

DATE: 28th October 2022

OBJECTIVE

1. To learn about the different instruments and gain hands on experience on the various techniques employed to perform sampling of water.
2. To learn various physical parameters of water bodies.
3. Analysis of following parameters were carried out:
   - ❖ MPN
   - ❖ Viable count
   - ❖ Dissolved Oxygen
   - ❖ Temperature
   - ❖ Salinity
   - ❖ Analysis of phytoplankton
   - ❖ Chlorophyll estimation
   - ❖ Turbidity
   - ❖ pH

➢ **Latitude, Longitude and depth of sampling sites:**

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

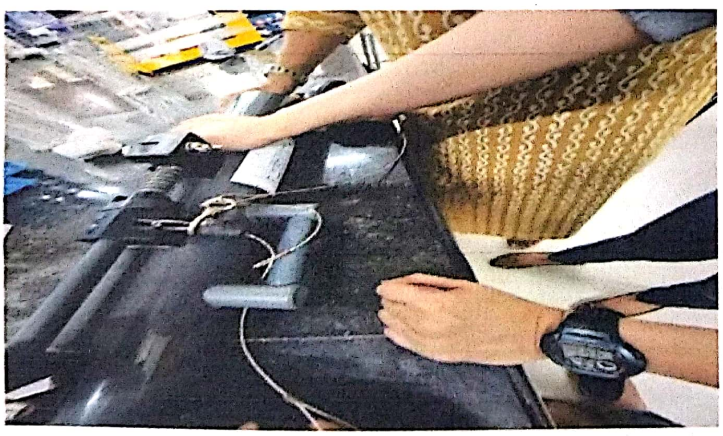

Fig 2: Niskin sampler

- **Analysis of Most Probable Number (MPN)**

  Principle:

  This test is carried out to detect coliforms and *E. coli*. Faecal coliforms are known to ferment lactose and produce acid and gas together, which can be detected by performing MPN where change in colour of the media i.e. Mac Conkey's indicates acid production and formation of gas bubble in the inverted Durham's tube indicates gas production. Together results in positive result indicating presence of faecal coliforms, and *E. coli* in water sample.

  Procedure:

  - Water sample from surface was collected using bucket while near bottom water was collected using Niskin sampler.
  - The water samples were collected into sterile centrifuge tubes of 50mL and stored in ice box until further analysis in laboratory.
  - Water samples were inoculated into double strength and single strength MacConkey's broth containing inverted Durham's tube in the respective volumes.
  - Using sterile pipette 10mL of water was inoculated into 5 tubes containing 10mL double strength MacConkey's broth.
  - 1mL of water sample was inoculated into 5 tubes containing 10mL of single strength MacConkey's broth.
  - 0.1mL of water sample was inoculated into 5 tubes containing 10mL of single strength MacConkey's broth.
  - All the tubes were incubated at 37° for 24 hrs.

- Positive results were indicated by production of acid (pinkish colour to yellow colour) and gas (by production of gas bubble in Durham's tube).
- The number of tubes giving positive results were compared to the standard chart (MacCrady's table) and number of bacteria per 100mL of sample present in it was recorded.

Flow chart of Presumptive MPN

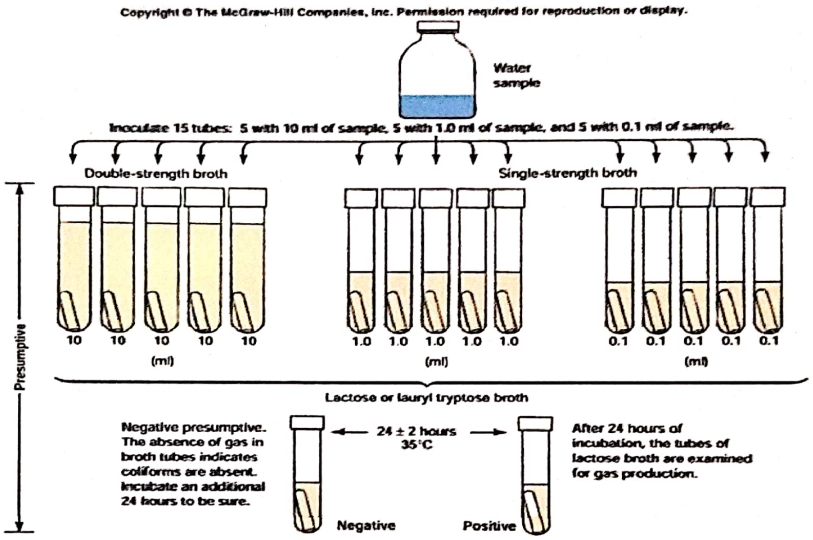

Fig 3: MPN Presumptive tests

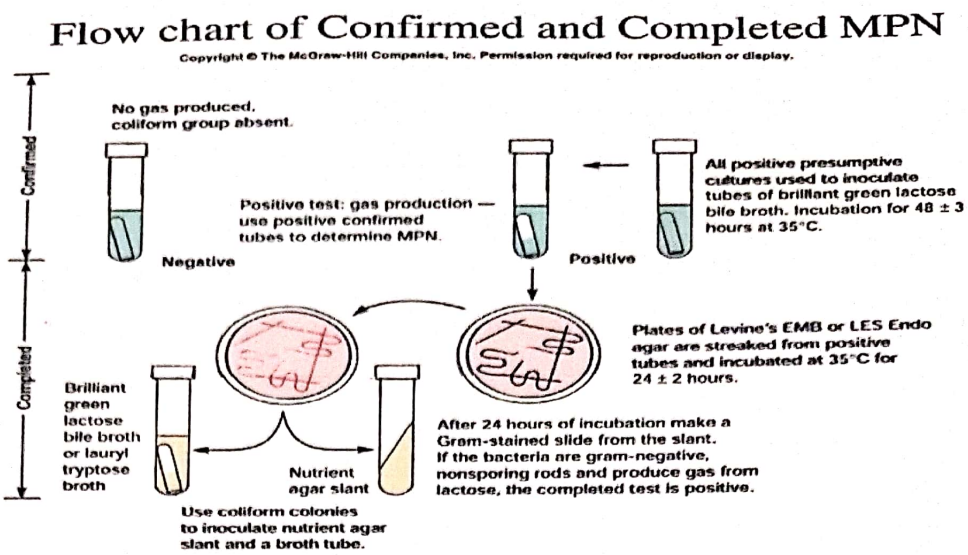

Fig 4: MPN confirmed and completed tests.

- **Analysis of Viable Count**

  Principle:

  It is a method which is used in cell culture to determine the number of living cells in a culture. Number of colonies can be counted. Colonies grows when incubated under suitable conditions for that specimen. Viable count unit is CFU/mL

  Procedure:

  - Water sample from surface was collected using bucket while near bottom water was collected using Niskin sampler.
  - The water samples were collected into sterile centrifuge tubes of 50mL and stored in ice box until further analysis in laboratory.
  - 0.1mL of water sample was spread on ZMA, MacConkey, TCBS and XLD agar plates.
  - The plates were incubated at 37°C for 24 hours.
  - Results were recorded after incubation period, wherein colonies were counted and recorded. Average of total number of colonies was taken and viable count was calculated.

- **Analysis of Dissolved oxygen by Winkler's method**

Principle:

The chemical determination of dissolved oxygen can be detected by Winkler's method. Dissolved oxygen in the seawater reacts with manganese hydroxide in strongly alkaline medium forming manganese (trivalent) hydroxide. When acidified to the pH less then 2.5, the manganese hydroxide dissolves to liberate manganese, which is strong oxidising agent in acidic media. Trivalent manganese reacts with iodine, which was previously added and liberate same amount of iodine. This is titrated against standard sodium thiosulphate solution using starch as indicator, which changes from blue to colourless and it marks end of titration.

Procedure:

- Determination of reagent blank
  - 50mL of distilled water was pipetted into conical flask, to that 1mL of 50% H_2SO_4 was added, 1ml of Winkler A and 1mL of Winkler B reagent was added. The mixture was swirled properly to avoid precipitation.
  - 1mL of starch solution was added.
- Standardization of thiosulphate solution
  - Solution was prepared in the same way as prepared for blank.
  - 10mL of 0.01N potassium iodate solution was added. Solution was mixed and kept in the dark for 3 minutes 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. This was carried out in triplicates to obtain the mean burette reading.
- D.O. estimation

- Samples was collected in 125mL D.O. bottles from different stations making sure no air bubble were formed during collection from surface and near bottom waters.
- 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 laboratory. 1mL of 50% of H_2SO_4 was added and swirled until precipitate dissolved.
- 50mL of the sample was pipetted and transferred in the conical flask and titrated against Sodium Thiosulphate solution until pale yellow colour appears.
- 1mL of starch was added and titration was continued until blue colour disappeared.
- Burette reading was noted. This was repeated three times to obtain mean burette reading. The required calculations were done.

- **Analysis of Temperature**

Principle:

Water temperature is measured as the degree or intensity of thermal energy in the water. 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 depicts the temperature.

Procedure:

- Water samples from all 4 stations were collected by bucket from surface waters while near bottom waters were collected using Niskin sampler.

- A mug of water sample was taken out and the thermometer was dipped into it.
- Readings were noted down.

- **Analysis of Salinity**

  Principle:

  Salinity refers to the concentration of salt present in the water. A refractometer is a sensor that measures the salinity of water sample. Salinity is determined by measuring how much light refracts when it passes through the sample. The more salt there is Placed one drop of water sample on the prism and secured the cover plate to ensure water to distribute evenly.

  Procedure:

  - Water sample was collected by a bucket from the surface while near bottom water was collected using Niskin sampler.
  - Using a dropper water was taken out and 2-3 drops were put onto the refractometer.
  - The lid was closed ensuring no air bubbles were trapped in and viewed through the eyepiece.
  - Salinity reading was noted for all the stations for surface and near bottom waters.

- **Analysis of Phytoplanktons** ✓

  Principle:

  In order to estimate phytoplanktons from water samples, the said sample is to be fixed in order to keep their cells intact. The cells are observed under microscope later to identify them.

Procedure:

- Water samples was collected by a bucket from the surface while near bottom waters, was collected using Niskin sampler.
- The water samples were collected into 500mL bottles, 15 drops of Lugol's iodine solution were added into it and stored into shade until further analysis.
- The bottles were brought back to laboratory and left for settling.
- Siphoning was done to concentrate the sample.
- Microscopy was done using an inverted microscope under 10x and 20x objective lens.

- **Analysis of Chlorophyll (Chlorophyll estimation)**

Principle:

Chlorophyll is light harvesting pigment integral to the photosynthesis process. The experiment is carried out to separate pigments from seawater sample containing phytoplankton. Acetone is used as a solvent since it allows to dissolve polar substances and allows greater resolution between pigments. Pigment analysis is done spectrophotometrically.

Procedure:

- Water sample were collected in 500mL bottles and then brought to laboratory for further analysis.
- A filter was placed in between filtration apparatus and sample was filtered using a pump.

- After filtering, placed the filter paper in vials, containing 10mL of 90% acetone.
- Crushed gently to ensure mixing occurred.
- Vials were kept undisturbed in refrigerator for 24hours.
- Blanked measured using 90% acetone in spectrophotometer, and kept at wavelength 665nm.
- Sample added in cuvette and absorbance noted.
- 2 drops of diluted HCl were added to cuvette containing sample and absorbance was noted again.
- Repeated same at 750nm wavelength.

- **Analysis of SPM (Suspended particulate matter)**

SPM are finely divided solids are dispersed in the water. When some particles retain on filters while filtering are called SPM. The particles which passes through thepore size of filter are called dissolved matter. The dry weight concentration of suspended particulate matter, is measured by passing a known volume of seawater throught a re-weighed filter and reweighing the filter after drying.

Procedure:

- Sample was collected in plastic bottles from surface waters as well as near bottom waters and stored in 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 sea water sample was filtered through the filter paper.

- After filtering the weight of the filter paper was again measured.
- 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.
- The readings were noted down and the calculations were carried out. (Parson et al.,1984) and (Stickland and Parson, 1965)

- **Analysis of turbidity using Secchi disc**

  Principle:

  A Secchi disc 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. The depth of disappearance called Secchi depth.

  Procedure:

  - The secchi disc was lowered slowly into the water column with the help of rope attached to it.
  - The disc was lowered until the observer could not differentiate
  - The depth at which this was observed was noted and designated as Secchi depth which indicates the turbidity of the water column.

- **Analysis of pH**

  Principle:

  The seawater pH is considered to be part of the carbon dioxide system. The potentiometric method is based on measurement of the cell emf in an electrochemical cell in which one of the electrode is selective for hydrogen ion and other electrode serves as reference.

  Procedure:

  - The pH meter was turned on and calibrate button was pressed.
  - The electrode was removed with distilled water and wiped gently with tissue paper into neutral pH buffer solution when the reading was stabilized it denoted as ready after which enter was pressed.
  - 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 and placed into the sample. The pH measurements were recorded.
- This was repeated for all the stations water samples.

- **SEDIMENT COLLECTION**

Procedure:

- Submerged the Van Veen Grab sampler to the bottom of water with the open position.
- Once the sampler touched the bottom the latch unhooked.
- When the latch was un hooked the sampler closed automatically and captured the sediment sample.
- The sediment sample was collected in the plastic bags.

Fig 5: Van Veen Grab (for sediment collection)

OBSERVATIONS

Station	Location
1	Malim Jetty
2	Offshore Miramar
3	Near Chorao Island
4	Old Goa

❖ **MPN**

Station 1	DS (10mL)		SS (1mL)		SS (0.1L)	
	Acid	Gas	Acid	Gas	Acid	Gas
1	+	+	-	-	-	-
2	+	+	-	-	-	-
3	+	+	-	-	-	-
4	+	+	-	-	-	-
5	-	+	-	-	-	-

Number of positive test tubes: 4-0-0 ≈ 13 cells/100mL

The most probable number of organisms present in 100mL of water sample is 13cells/100mL.

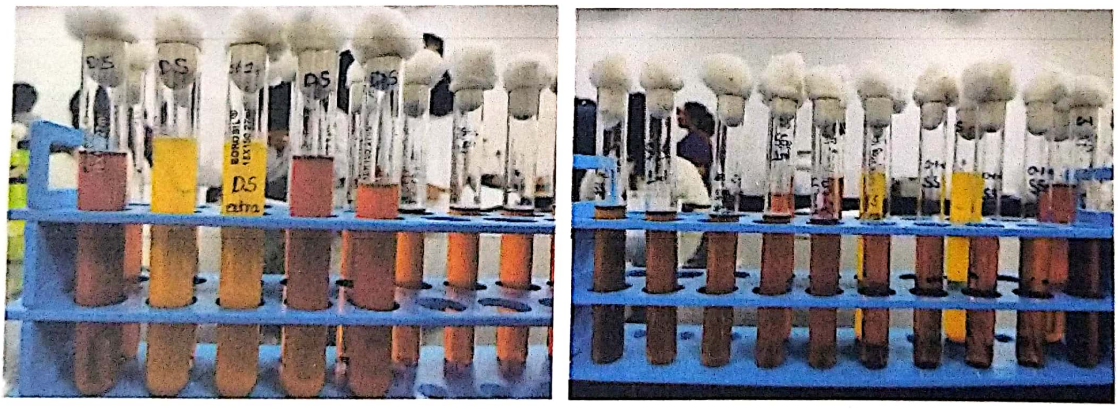

Fig 6 (a) and (b): MPN test tubes after the incubation period.

❖ **Viable Count**

Station surface waters	Dilution	Number of colonies	Average number	Dilution factor CFU/mL
1	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
2	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
3	10^{-1}	6	3	2500
	10^{-1}	0		
	10^{-2}	4	4.75	
	10^{-2}	15		
4	10^{-1}	23	8.25	4400
	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	0		

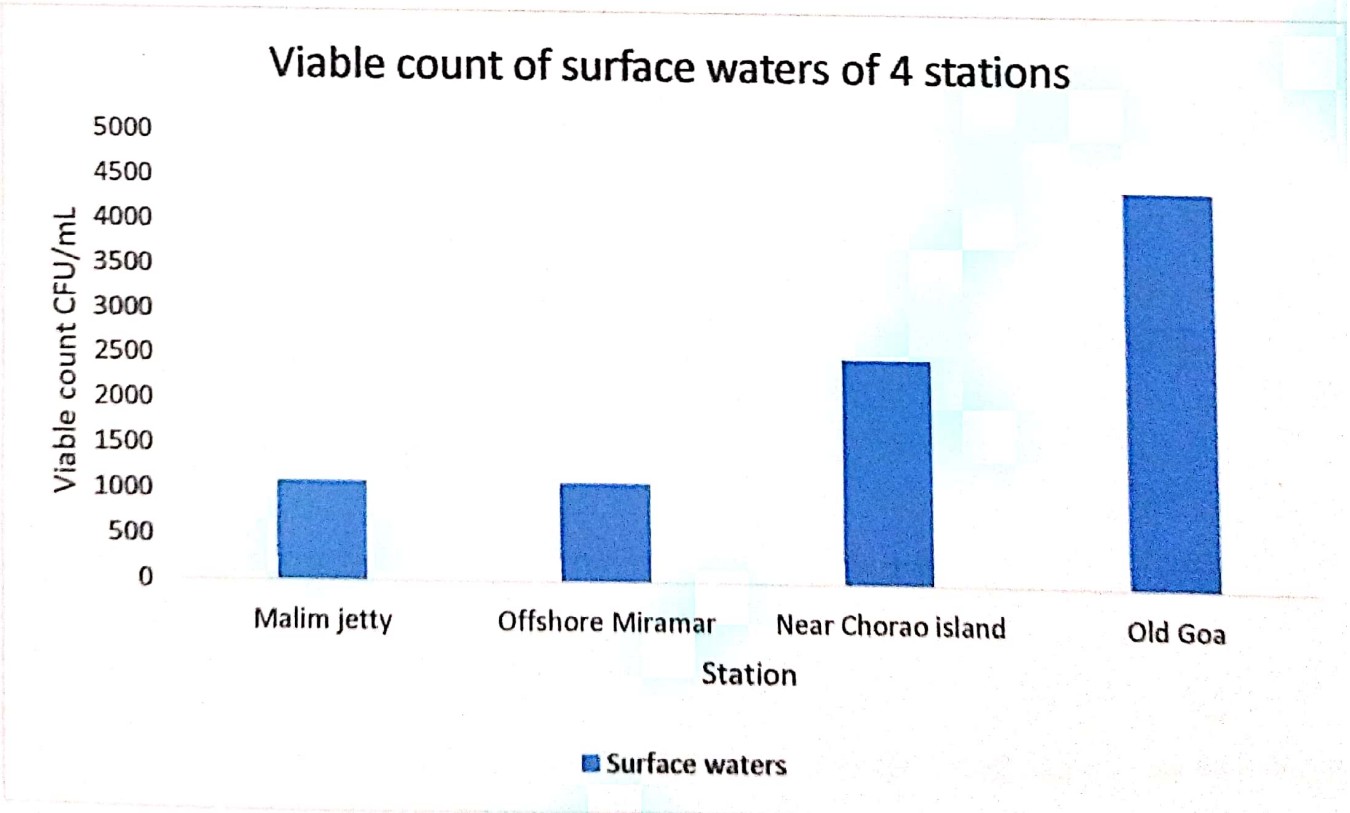

Fig 7: Graph of viable

❖ **Dissolved Oxygen**

Station	D.O. ml/L	
	Surface	Near bottom
1	4.37	4.47
2	4.47	2.89
3	4.89	3.92
4	4.47	4.47

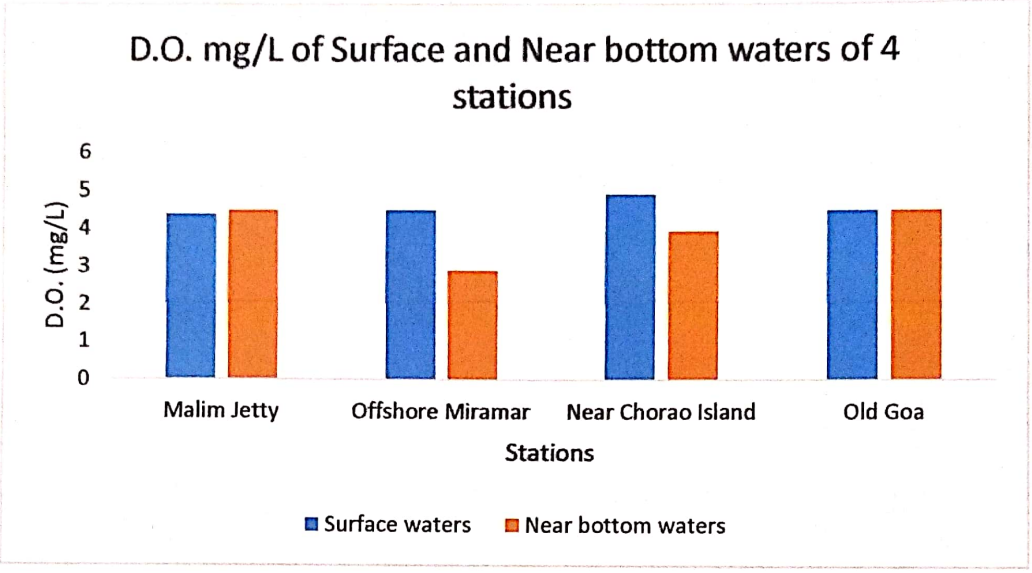

Fig 8: Graph of D.O. (mg/L) of surface waters and near bottom waters of 4 stations.

Calculations:

$$\text{Dissolved oxygen, mg/L} = \frac{BR \times (V/v) \times E \times 1000}{\text{Vol of sample titrated}}$$

BR= Burette reading (volume of thiosulphate used in titration)

N= Normality of thiosulphate solution

E= Equivalent weight of Oxygen

1000= To express per litre

Note: The factor V/v was correction for displacement of oxygen in the sample when reagents were added.

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V/v = Volume of bottle

Vol of bottle – vol of reagents

❖ **Temperature and Salinity**

Stations	Temperature (°C)		Salinity	
	Surface	Near bottom	Surface	Near bottom
1	30	31	23	28
2	31	30	32	33
3	30	30	21	29
4	31	31	19	22

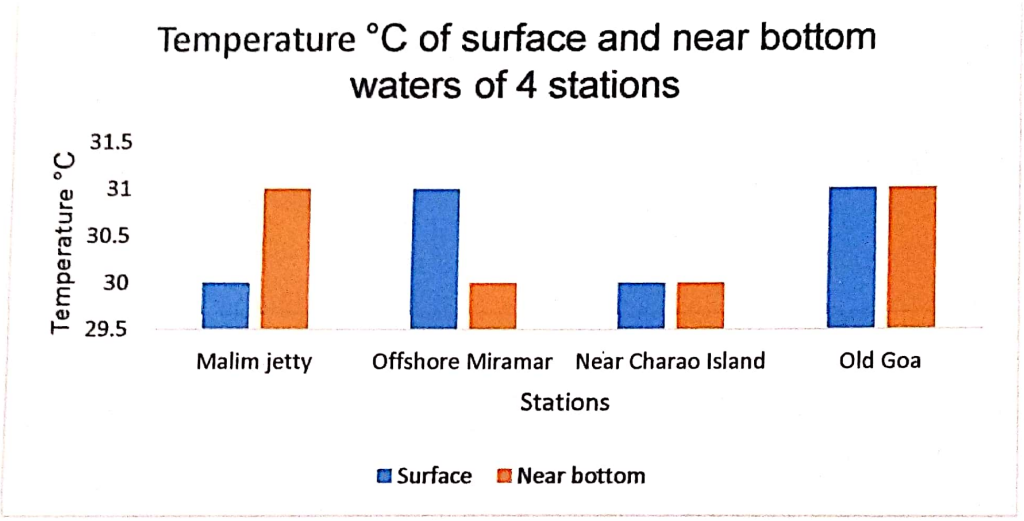

Fig 9: Graph Temperature of surface waters and near bottom waters of 4 stations.

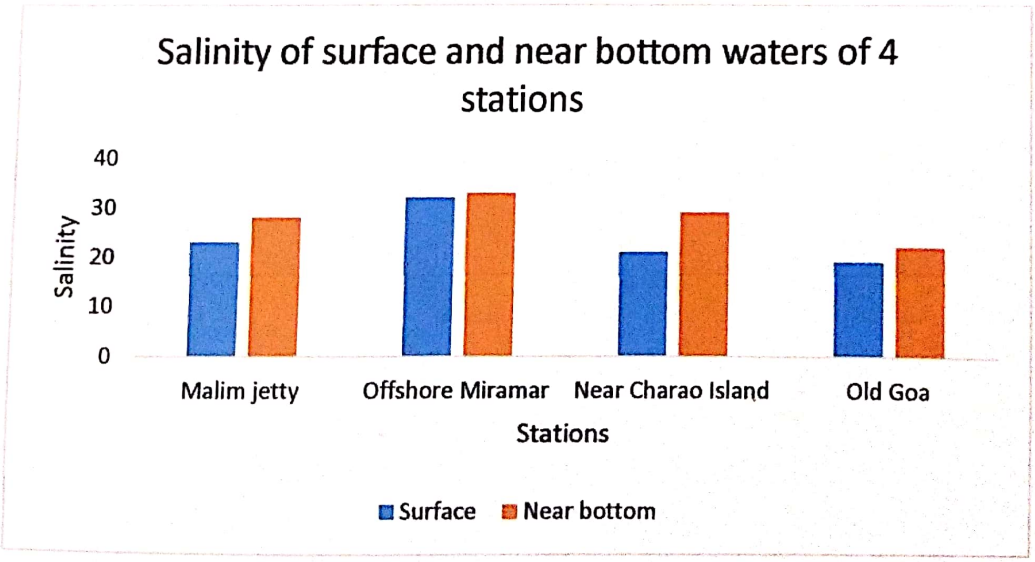

Fig 10: Graph Salinity of surface and near bottom waters of 4 stations.

❖ **Analysis of Phytoplankton**

Fig 11: (a) Water samples fixed with Lugol's iodine. (b) *Coscinodiscus* sp

❖ **Chlorophyll estimation**

Calculation

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

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).

Fig 12: Formula for chlorophyll estimation.

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

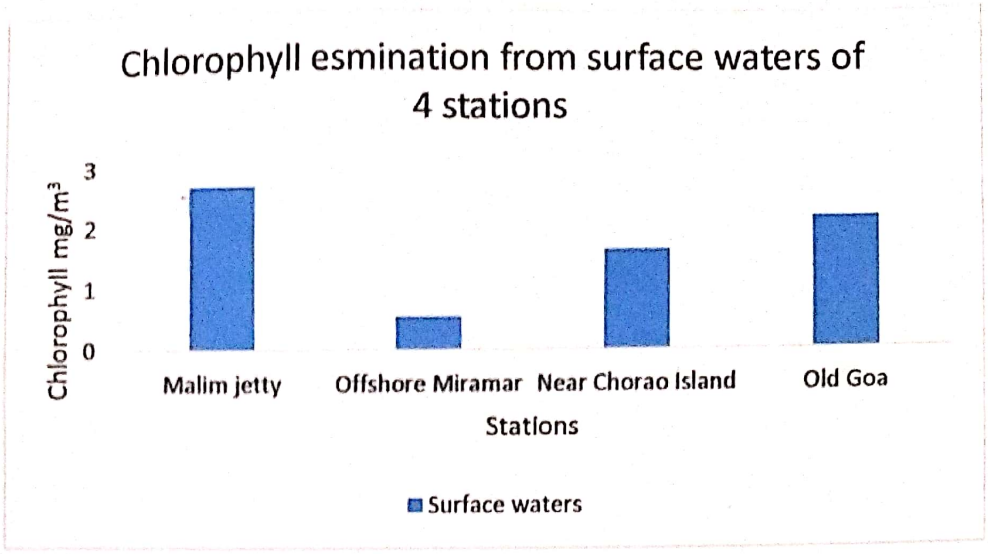

Fig 13: Graph of chlorophyll estimation from surface waters.

❖ **SPM**

Calculation

$$SPM = \frac{x - b}{\text{Vol of water filtered}}$$

Station Surface waters	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

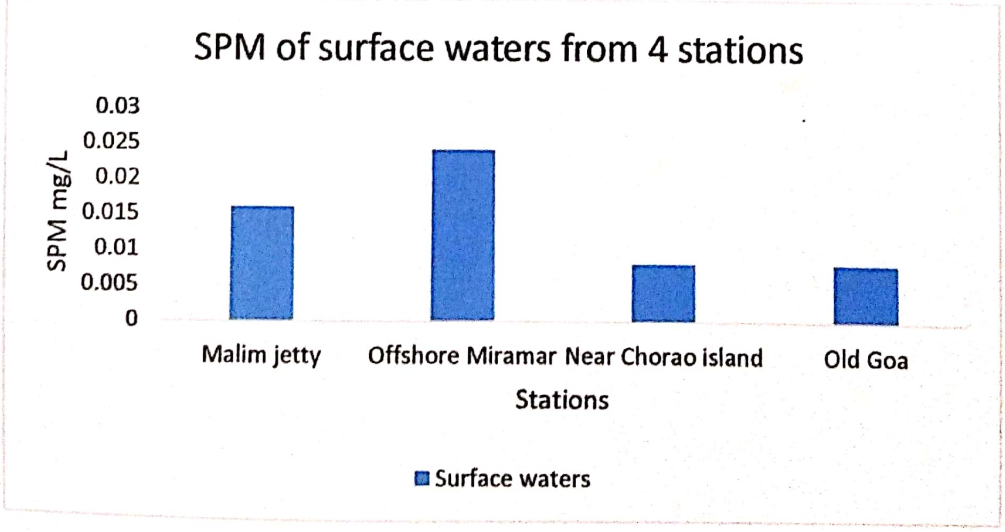

Fig 14: Graph of SPM of surface waters of 4 stations.

❖ **Turbidity and pH**

Station	Secchi depth (m)	pH
1	2	8.1
2	9	8
3	1.5	7.6
4	1.5	7.9

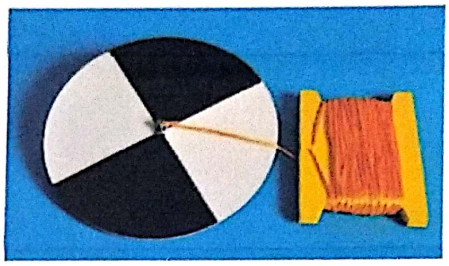

Fig 15: Secchi disc

RESULTS

MPN: As observed from the observation station 1, surface waters has 13 cells/100mL of water sample.

Viable count: As observed from reading. The viable count of surface waters of station 1 was 1100CFU/ml, station 2 was 1100CFU/ml, Station 3 was 2500CFU/ml and station 4 was 4400CFU/ml. Indicating station 1 and station 2 had lowest viable count while station 4 had highest.

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

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

Estimation of chlorophyll: The following phytoplankton were observed- Diatoms; *Rhizosolenia sp, Coscinodiscus sp, Gyrossigma sp, Chaetoceros sp,* and an unidentified pennate diatom. Dinoflagellate were not observed.

Chlorophyll estimation: The highest chlorophyll pigment was estimated from station 1from surface waters while lowest chlorophyll pigment lowest was found at station 2 from surface waters.

SPM: The highest particulate matter was present at station 2 in surface waters while lowest particulate matter was present in station 3 and station 4 surface waters.

Turbidity and Depth: The turbidity measured in terms of secchi disc was in range of 1.5-9m with the highest at station 2 and lowest being at station 3 and station 4. While pH was found to be in the range of 7.6-8.1 with the highest being at station 1 and lowest being at station 4.

PERSPECTIVE

Overall, it was really enriching fruitful field trip. We learned a lot about new instruments to be used. We learned to use and handle various instruments and learned got refresh our techniques and analysis to be done thereafter. We also learned to collect samples properly and to store them properly to get accurate results for analysis of various parameters of waters. We experienced the hardships involved while sampling from offshore waters, which caused turbulence due to strong wave actions. We also experienced failure in collecting samples as sometimes garbage waste was getting collected. The best part of the field trip was that we enjoyed a lot with our teachers while working on trawler. One of the best part of the trip was eating fresh served food in middle of the estuary and watching the nature and birds arounds. The chery on the cake moments were when we saw dolphins, jellyfishes and lots of fishes in the offshore waters. Last not the least the views which we could see was sun set which was relaxing after the tireding day. We enjoyed a lot as the trip was post pandemic which created a impact to refresh our minds and brought back interest in our academics.

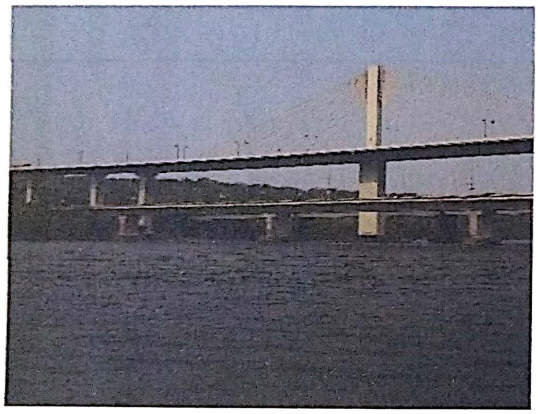

Fig 16: View from Trawler

Fig 17: Tea served to us

Fig 18: Students of MSc Part 2

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19/11/22