REPORT ON FIELD VISIT

Place visited	Mandovi estuary, Goa, India
Date and time	10.03.2022; 11.03.2022 (9.00am to 03.00pm)
Department/School/Directorate/ Section	Marine Microbiology; School of Earth, Ocean and Atmospheric Sciences (SEOAS), Goa University
Participants (Total)	31
Faculty attended	03
Students attended	28
The objectives / description of the activity (50 words)	The main objective of this course is to give students the hands-on experience in the field so as to understand the different sampling techniques and collection, on-board experiments and getting a thorough knowledge of bacterial and phytoplankton diversity in estuarine ecosystems which will make them accomplished field marine microbiologists.
Photos	attached
Benefit/Key outcome of the event in terms of learning/skills/knowledge	The students be able to analyse phytoplankton diversity and study the impact of pollution load on microbial diversity.

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Introduction

A field trip divided into two batches was organized by the Marine Microbiology programme, SEOAS, Goa university, for the students (M.Sc. Part I and II) as a requirement for the completion of master's degree. On the 10th of March 2022 Two teachers (Dr. Priya D'Costa and Dr. Nikita Lotlikar) along with a non-teaching staff (Ms Vaishali) accompanied 13 students for the trip on the trawler, while on 11th March 2022, Dr. Nikita Lotlikar and Dr. Varada S. Damare accompanied 15 students along with a non teaching staff (Ms. Sitam). Prior to the day of the field trip, proper planning and preparation of media, glassware etc. for all the sampling and experiments to be carried out was done well in advance. Use of the required instruments, techniques for sample collection, jobs to be done for the field trip were explained accurately a day before the field trip. The excursion began around 9:30 am in the morning wherein all the students, teachers and non-teaching staff boarded the trawler. The trawler was meant to take us across a particular stretch of the Mandovi estuary. Four sites were marked for sampling and analysis of other parameters such temperature, salinity etc. which were designated as station 1, station 2, station 3 and station 4. Our first sampling site was station 2 which was at offshore waters of Miramar. Upon reaching there, latitude and longitude of the location was noted. Sampling of water was done for surface waters using a bucket and for near bottom waters using Niskin sampler. Water was collected for D.O estimation, phytoplankton estimation, chlorophyll estimation and SPM from surface as well as near bottom. While for MPN and bacterial count water from only surface was collected. Other physical parameters like temperature, salinity, and pH of surface and near bottom waters was also recorded. Additionally, turbidity was also measured using Secchi disc. Furthermore, sediment was also collected using a van Veen grab. Similarly, this process was repeated for the other stations as well. The four different stations were Malim jetty, offshore Miramar, near Chorao island and Old goa. After finishing our work on all the four stations all the students along with the teachers returned to the laboratory and carried out the processing of all the samples collected. Each of the respective experiments were performed and the obtained results were recorded.

OBJECTIVES:

1. To learn about the different instruments and gain hands on experience on the various techniques employed to perform sampling of water. For the analysis of various parameters of water bodies.
2. Analysis of the following parameters were carried out:
    - MPN
    - Viable count
    - D.O.
    - Temperature
    - Salinity
    - Analysis of phytoplankton
    - Chlorophyll estimation
    - Suspended Particulate Matter

- Turbidity
- pH

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

Stations	Latitude	Longitude	Depth (meters)
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

Locations of all 4 stations.

❖ **Analysis of MPN:**

PRINCIPLE: This test is mainly carried out to detect *E. coli* and coliforms. Faecal coliforms are known to ferment lactose and produce both acid and gas. This 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.

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 from pinkish red to yellow) and gas. (by production of gas bubble in Durham's tube)
- 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)

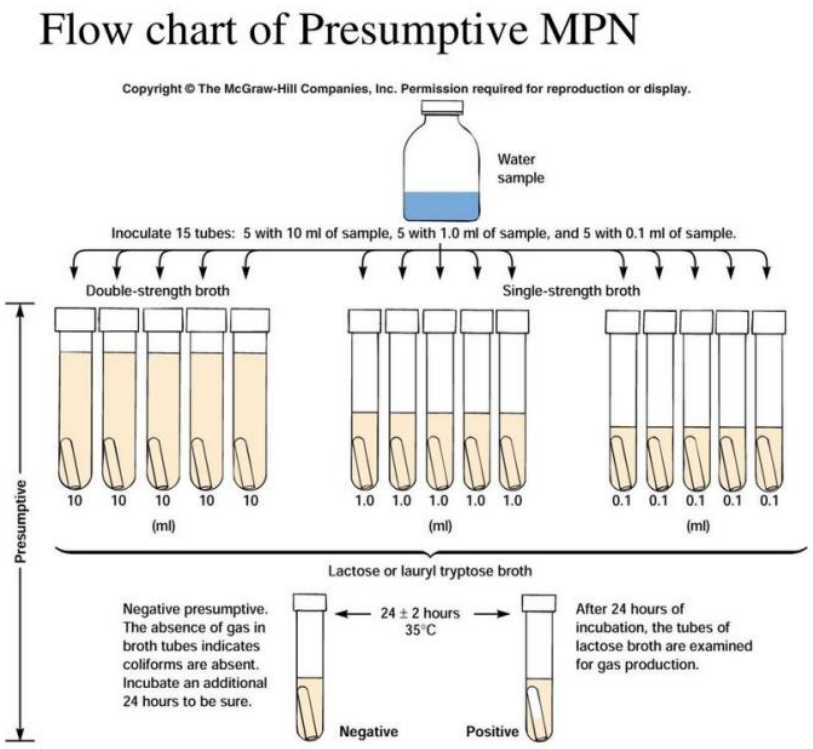

Fig 3: MPN Presumptive tests

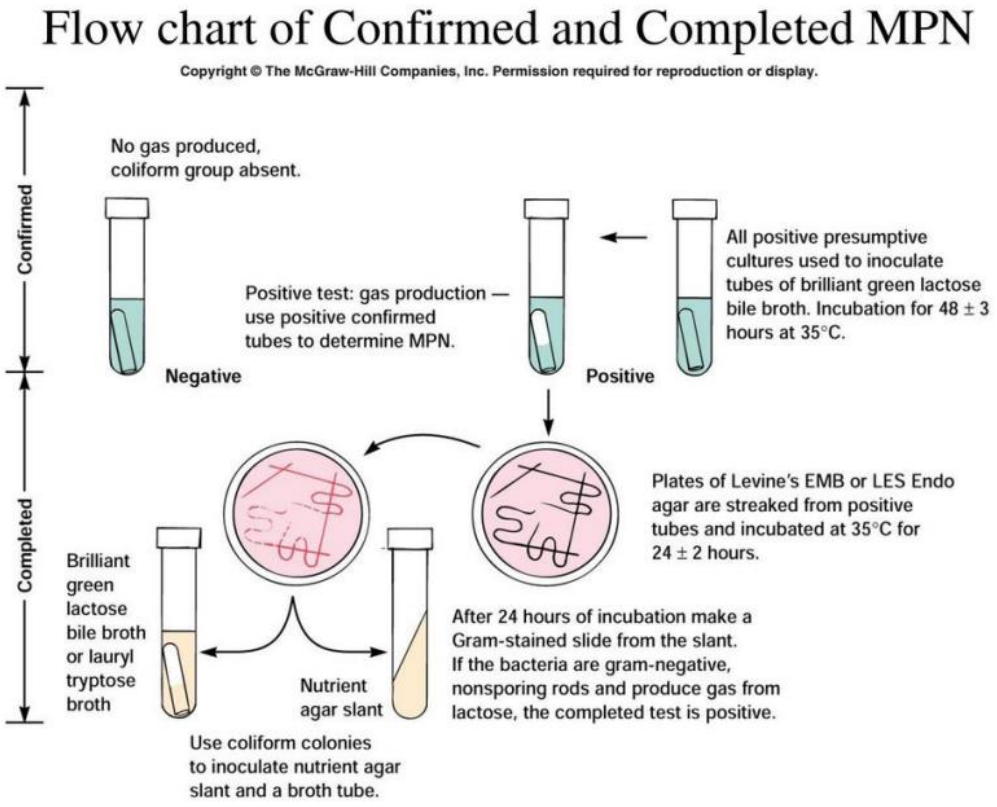

Fig 4: MPN confirmed and completed tests

- **Analysis of 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. Wherein each colony was counted and written down. Average of total number of colonies was taken and viable count was calculated.

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

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% H_2SO_4, 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 from surface (using a bucket) and near bottom waters (using a Niskin sampler)
- 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% H_2SO_4 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. The required calculations were done. (Winkler's Method)

❖ **Analysis of 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 (temperature) were noted down.

❖ **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 and another.

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. 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 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.

❖ **Analysis of chlorophyll (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 0.75 µm was placed in the filtration unit attached to the vacuum pump.
- 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.
- 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)

❖ **Analysis of SPM (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 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 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))

❖ **Analysis of turbidity using Secchi disc:**

PRINCIPLE: A Secchi disk is an 8-inch (20 cm) disk with alternating black and white quadrants. It is lowered into the water of 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 disc was taken and gently lowered from the trawler into the water (for all the stations) with the help of the rope attached to it.
- The disc was lowered until the observer could no longer differentiate between the quadrants of the disc.
- The depth at which this was observed was noted and designated as Secchi depth which indicates the turbidity of the water body.

❖ **Analysis of pH:**

PRINCIPLE: The potentiometric method is based on measurement of the cell emf in an electrochemical cell in which one of the electrodes is selective for hydrogen ions and the other electrode serves as a reference. An important consequence of this fact is that the change in potential on moving the electrodes from the buffer to the sample is the sum of all changes that occur in the contributions to the cell potential.
PROTOCOL:

- The pH meter was turned on and calibrate button was pressed.
- The electrode was removed with distilled water and wiped gently with tissue paper and then placed 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 measurement was recorded
- This was repeated for all the stations water samples.

OBSERVATIONS:

- **MPN:**

Station 1	DS (10ml)		SS (1ml)		SS (0.1)	
	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 (0.1)	
	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 (0.1)	
	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 (0.1)	
	Acid	Gas	Acid	Gas	Acid	Gas
1	✓	✓	✓	✓		
2	✓	✓	✓	✓		
3	✓	✓	✓	✓		
4	✓	✓				
5						

Number of positive tubes: 4-3-0 ≈ 27 bacteria/100mL

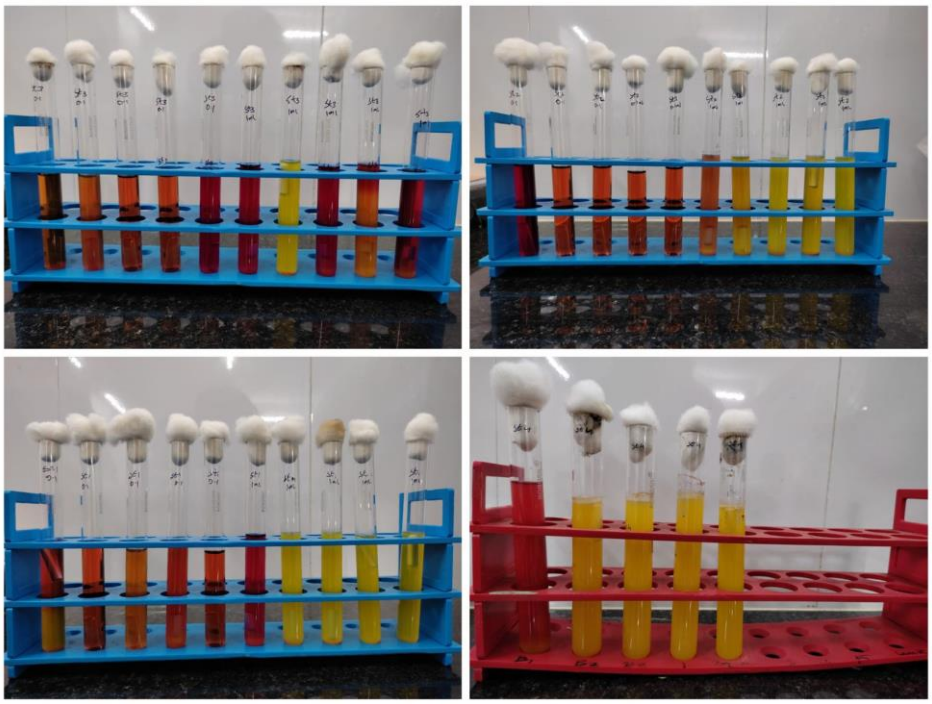

Fig 5: MPN tubes after the incubation period.

Viable count: No growth was observed on all the medias (ZMA, XLD, TCBS, MacConkey's agar)

Fig 6: Collection of water sampling for viable count and MPN analysis

- **Dissolved Oxygen:**

Station	D.O. (mg/L)	
	Surface	Near Bottom
1	4.37	3.85
2	5.64	5.33
3	5.8	1.95
4	5	5.71

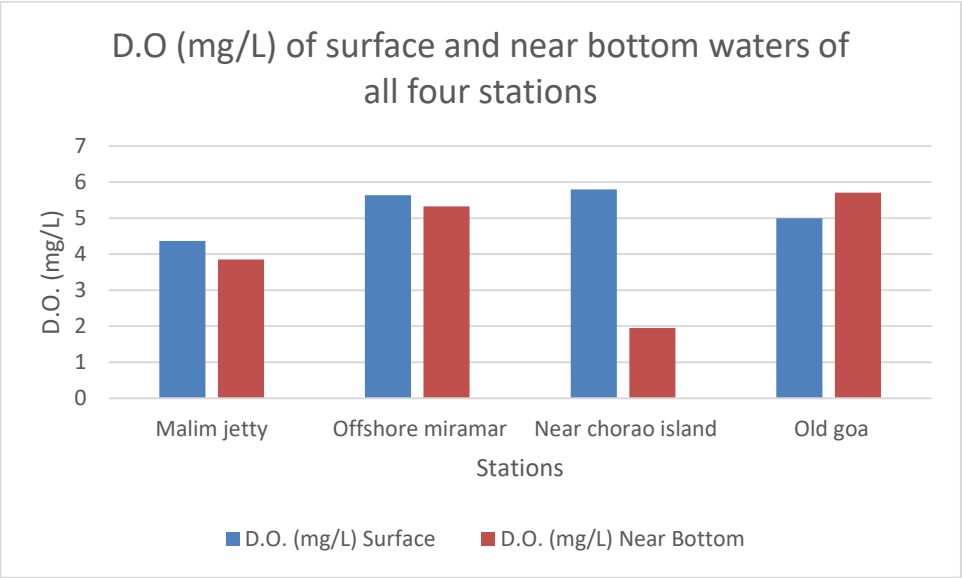

Fig: 7: Graph of D.O. (mg/L) of surface and near bottom waters of all four stations

CALCULATION:

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

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

Fig 8: D.O. bottles after fixing the sample with Winkler's A and Winkler's B

Temperature and salinity:

Station	Temperature (°C)		Salinity	
	Surface	Near Bottom	Surface	Near Bottom
1	31	30.5	25	26
2	30	30	33	34
3	30	30	25	26
4	31	31	20	23

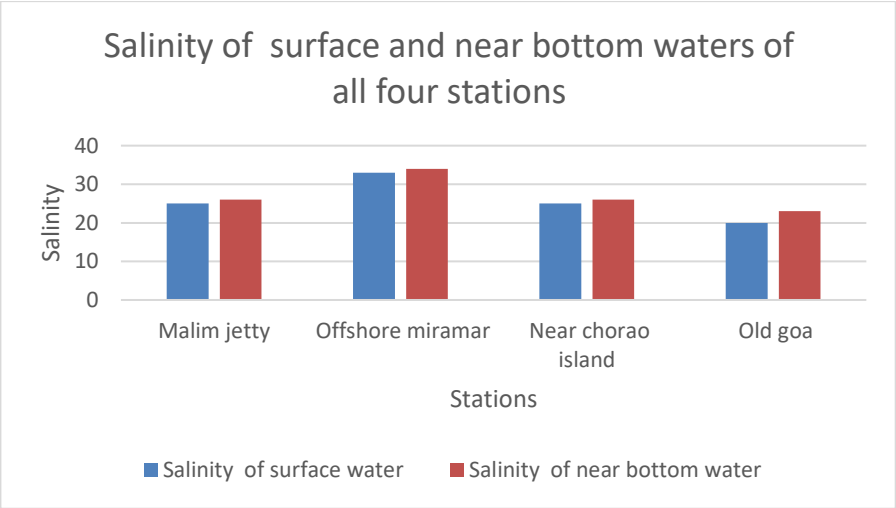

Fig 9: Graph of salinity of all four stations (surface and near bottom waters)

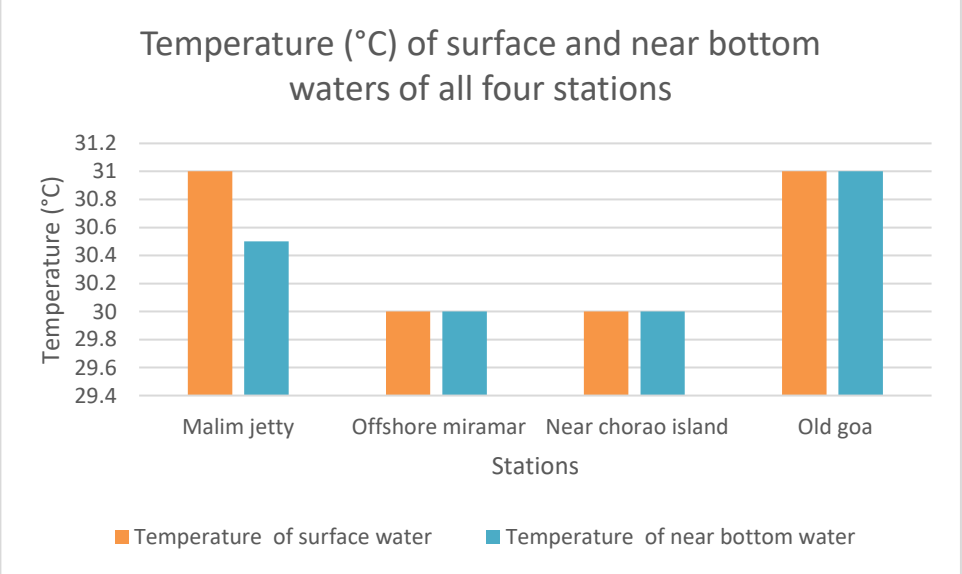

Fig 10: Graph of temperature (°C) of all four stations (surface and near bottom waters)

- **Estimation of phytoplankton:** Qualitative analysis of phytoplankton was carried out

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

- **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

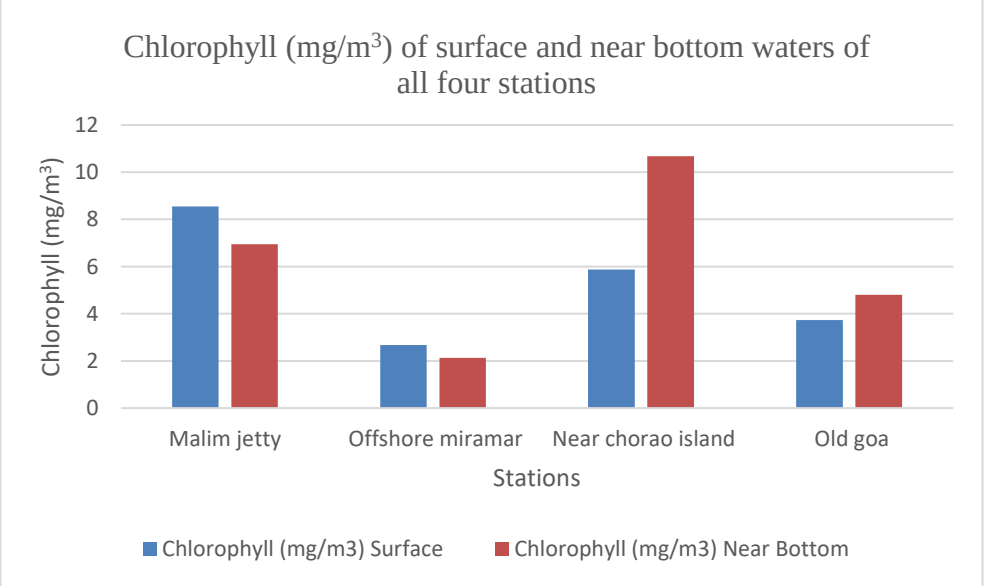

Fig 12: Graph of Chlorophyll (mg/m^3) estimation of all four stations (surface and near bottom waters)

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).

Fig 13: Collection of water sample from Niskin sampler for phytoplankton fixing.

- **SPM:**

Station		Filter paper weight (g) (x)	Dry weight (g) (y)	Difference (g) (x-y)	SPM (mg/L)
Station 1	Surface	0.085	0.097	0.012	48
	Bottom	0.082	0.095	0.013	52
Station 2	Surface	0.088	0.103	0.015	60
	Bottom	0.084	0.101	0.017	68
Station 3	Surface	0.086	0.099	0.013	52
	Bottom	0.08	0.094	0.014	56
Station 4	Surface	0.085	0.093	0.008	32
	Bottom	0.081	0.091	0.01	4

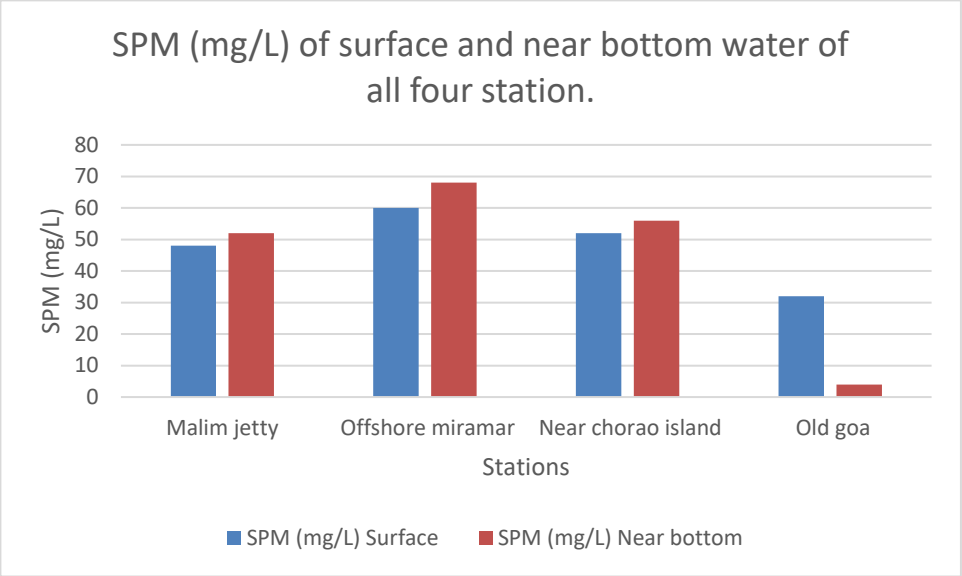

Fig 14: Graph of SPM (mg/L) of all four stations (surface and near bottom waters)

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

- **Turbidity And pH:**

Station	Secchi depth (meters)	pH
1	1.75	8.1
2	2	8
3	2.25	7.6
4	1.3	7.9

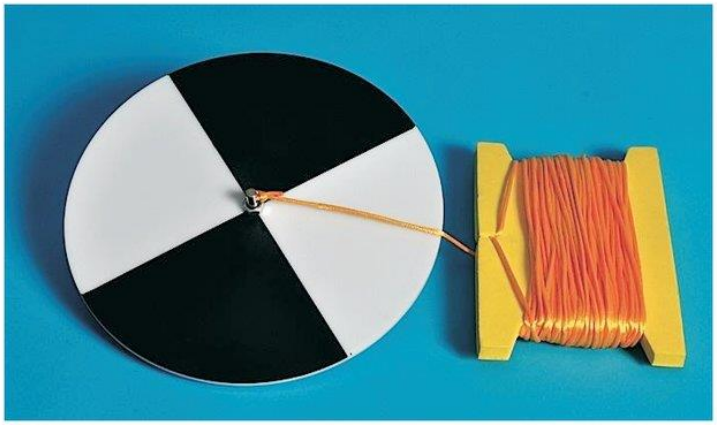

Fig 15: Secchi Disc

Fig 16: Niskin sampler

Fig 17: van Veen grab (for sediment collection)

RESULTS:

MPN: 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.

Viable count: No growth was observed on any plate.

D.O.: 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 4 showed the highest and station 3 showed the lowest concentration of 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 was the highest at station 2 and lowest at station 4 for both surface and near bottom waters.

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

Chlorophyll and Suspended Particulate estimation: The highest chlorophyll concentration was found to be at station 3, at near bottom waters and lowest was found to be at station 2, at near bottom waters. While SPM was found to be highest at station 2 bottom waters and lowest at station 4 bottom waters.

Turbidity and pH: The turbidity measured in terms 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 station 1 and lowest at station 3.

PERSPECTIVE:

Overall, it was a very enriching experience. We learnt about the use and handling of different instruments such as Niskin sampler, Van Veen grab etc. We also gained experience on proper sample collection and storage to get accurate results for analysis of various parameters of water like D.O., phytoplankton fixing etc. 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. Additionally, we also learnt about the problems associated with sampling in different sites such as failure of sample collection (sediment) due to rocky bottom etc. On the plus side we also had fun while working. Relishing the lunch that was served which we ate on the trawler parked in the middle of the estuary, watching the mesmerizing view was one of the pleasures that we had on this trip. Having this trip amidst the pandemic, refreshed our minds and brought back our interest into academics as we learned a lot through this opportunity given to us.

Fig 18: Food served at the trawler

Fig 19: Students of MSc. Part I and Part II

Fig 20: Students of MSc Part II along with the teachers and non-teaching staff.

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.)

Photographs of the fieldtrip

Students and Teachers along with equipment on trawler.