Aniket Singh

2.25/10

FIELD TRIP REPORT

MMO 319

Aniket Singh

21P039002

MSc. Marine Microbiology Part II (2022-2023)

SEOAS, Goa University

A field trip was organized by the Department of Marine Microbiology, SEOAS, Goa University, for students as a prerequisite for completing the master's degree. On March 10, 2022.Two teachers (Dr.Priya D' Costa and Dr.NikitaLotlikar) together with a non-teaching staff (Ms. Vaishali) accompanied 13 students for the Field trip. Before the day of the field visit, proper planning and preparation of media, glassware, etc. was done well in advance for all sampling and experiments that would be carried out the very next day . The use of the instruments needed, the sampling techniques, the work to be carried out for the excursion were explained exactly one day before the excursion. The trip started around 9:30 am with all students, teachers and non-teaching staff boarded the trawler .The trawler was meant to guide us through a specific section of the Mandovi estuary. Four sites were selected for sampling and analysis of other parameters like temperature, salinity etc as Station 1, Station 2, Station 3 and Station 4. Our first sampling site was Station 2 which was located in waters off the coast of Miramar. Upon arrival there, the latitude and longitude of the location were noted. Sampling of water was performed for surface Water with a bucket and for near the ground water using a Niskin sampler. Water was collected from both the surface and near the bottom for DO estimation, phytoplankton estimation, chlorophyll estimation and SPM. While for the MPN and bacterial count, water was only collected from the surface. Other physical parameters such as temperature, salinity and pH of surface and bottom waters were also recorded. In addition, the turbidity was also measured with a Sacchi disk. Additionally, sediments were also collected with a van Veen grab. Similarly, this process was repeated for the other stations. The four different stations were Malim Jetty, Offshore Miramar, near Chorao Island, and Old goa. After ending our work on all the four Stations, all the students together with the teachers returned to the lab and processed all the collected samples. Each of the respective experiments was performed and the results obtained were recorded.

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

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 waterbodies.
2. Analysis of the following parameters were carriedout:
   - MPN
   - Viablecount
   - D.O.
   - Temperature
   - Salinity
   - Analysis of phytoplankton
   - Chlorophyllestimation
   - Suspended ParticulateMatter
   - Turbidity
   - pH

❖ **Latitude, Longitude, and Depth of Samplingsites:**

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

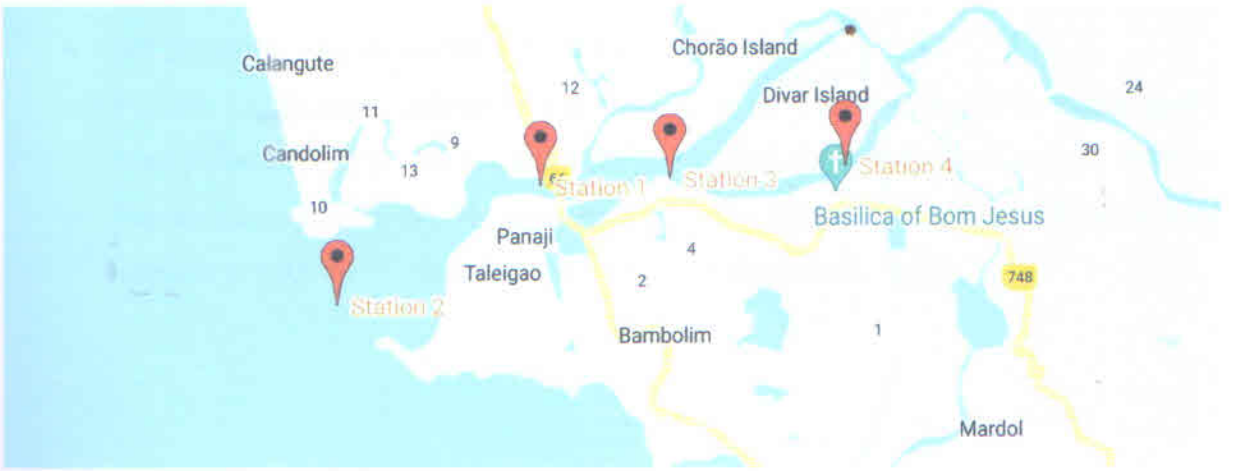

Fig 2: Locations of all 4 stations.

❖ **Analysis ofMPN:**

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 fecal coliforms, *E. coli* in the water sample.

PROTOCOL:

- Water sample collection was done from the surface of the station using abucket.
- The water was collected into sterile centrifuge tubes of 50 mL and stored in ice box until furtheranalysis.
- 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 respectivevolumes.
- 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'sBroth.
- 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 24-48hours
- 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'stube)
- The results were compared to a standard chart like McCrady's table and the numberof bacteria per 100ml of sample was determined. (Throndsen, 1978)

Flow chart of Presumptive MPN

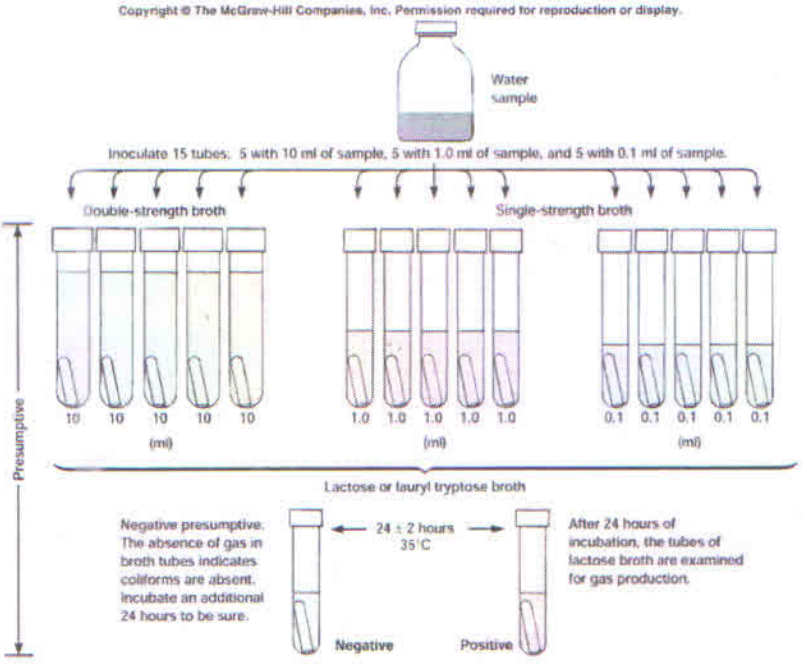

Fig 3: MPN Presumptive tests

Flow chart of Confirmed and Completed MPN

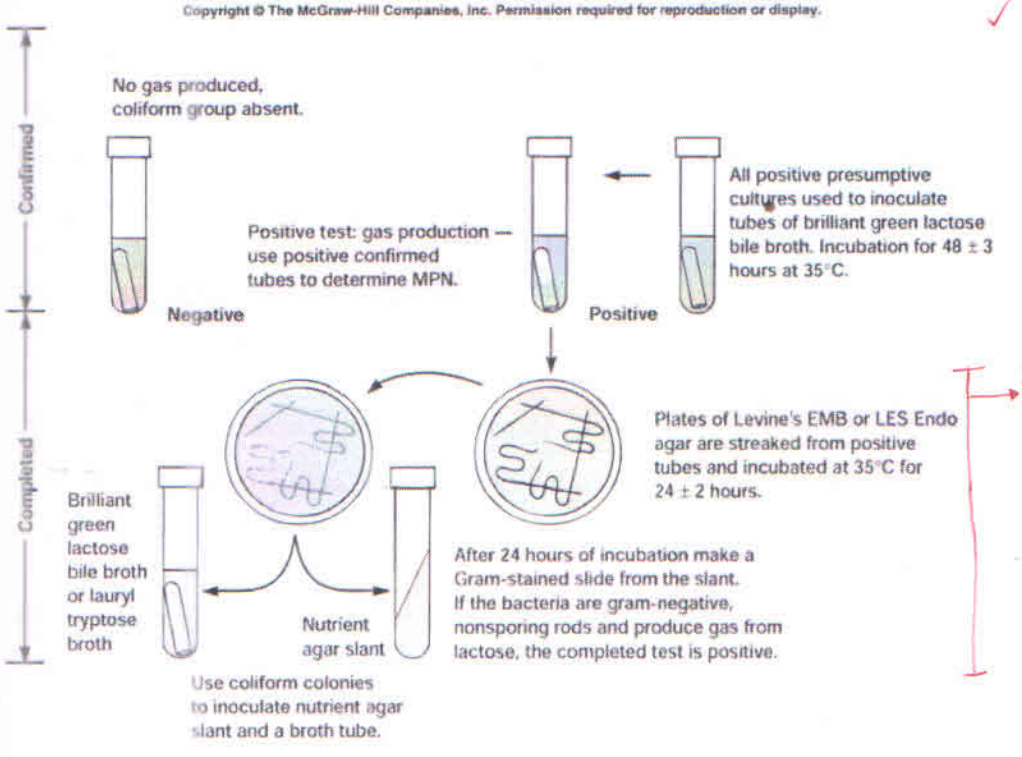

Fig 4: MPN confirmed and completed tests

❖ **Analysis of ViableCount:**

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 agarplates.
- The plates were incubated at 37°C for24-hours.
- Results were recorded after the incubation period. Wherein each colony was counted andwrittendown.Averageoftotalnumberofcolonieswastakenandviablecountwas calculated.

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

PRINCIPLE: The chemical determination of oxygen in water sample is carried out by using theWinklermethod.Thismethodisatypeofiodometrictitrationwhichoxidizesiodineionto 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 starchhelixandcanbereducedbythiosulphate.Thischangefrombluetocolourlessmarksthe end point of thetitration.

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

Standardization of thiosulphatesolution:

- 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 liberateiodine

- 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(remainedcolourlessfor30seconds).Thiswascarriedintriplicatestoobtain the mean burette reading

D.O. estimation:

- Samplewascollectedin125mLD.O.bottlesfromdifferentstationsmakingsurenoair 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 tosettle.
- 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'sMethod)

❖ **Analysis ofTemperature:**

PRINCIPLE:Athermometerworksontheprinciplethatsolidsandliquidsexpandonheating. 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 intoit
- Readings (temperature) were noteddown.

❖ **Analysis ofSalinity:**

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:

- Watersample(fromdifferentstations)wascollectedinabucketfromthesurfacewhile 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 therefractometer.
- 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 bottomwaters.

❖ **Analysis of phytoplankton:**

PRINCIPLE:Toestimatetheamountofphytoplanktonsineachwatersampleoneneedstofix thesample,tokeepthecellsintactandcarryoutmicroscopylater.Qualitativeanalysiscanalso be done using the samemethod.

PROTOCOL:

- Watersample(fromdifferentstations)wascollectedinabucketfromthesurfacewhile for near bottom waters, water was taken from the Niskinsampler.
- Filled into 500mL bottles, next 15 drops of Lugol's iodine solution were added and stored in shade until furtheranalysis.
- The bottles were brought back to laboratory and left forsettling.
- After the settling period siphoning was done to concentrate thesample.
- Microscopy was done using an inverted microscope under 10x and 20x objectivelens.

❖ **Analysis of chlorophyll (Chlorophyllestimation):**

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:

- Samplewascollectedinaplasticbottlefromsurfaceaswellasnearbottomwatersand stored in the shade. A filter paper of 0.75 µm was placed in the filtration unitattached to the vacuumpump.
- 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 thebottle
- Crushed and gently and capped. The bottle was kept undisturbed for 24 hours in the refrigerator
- Nextdaythesampleswereanalysedspectrophotometricallyat665nm,then2dropsof HCL acid wasadded.
- Absorbance was measured at 750nm. Readings were recorded and calculations were done. (Parsons et al.1984)

❖ **Analysis of SPM (suspended particulatematter):**

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 matterthatpassesthroughasmallporesizefilterisdefinedasdissolvedmatter(DM).Thedry weightconcentrationofsuspendedparticulatematerial,[SPM](units:mgL^{-1}),ismeasuredby passing a known volume of seawater through a pre-weighed filter and reweighing the filter afterdrying.

PROTOCOL:

- Samplewascollectedinaplasticbottlefromsurfaceaswellasnearbottomwatersand stored in the shade. A filter paper of 0.45 microns was placed in the filtration unit attached to the vacuumpump.
- Beforefilteringthewatersampletheweightofthefilterpaperwasmeasuredandnoted.

- Around 250mL of seawater sample is filtered through the filterpaper.
- 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 Sacchidisc:**

PRINCIPLE: A Sacchi 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 Sacchi depth, is a measure of the transparency of the water.

PROTOCOL:

- Sacchi disc was taken and gently lowered from the trawler into the water (for all the stations) with the help of the rope attached toit.
- The disc was lowered until the observer could no longer differentiate between the quadrants of thedisc.
- The depth at which this was observed was noted and designated as Secchi depthwhich indicates the turbidity of the waterbody.

❖ **Analysis of pH:**

PRINCIPLE: The potentiometric method is based on measurement of the cell emf in an electrochemicalcellinwhichoneoftheelectrodesisselectiveforhydrogenionsandtheother 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 cellpotential.

PROTOCOL:

- The pH meter was turned on and calibrate button waspressed.

- Theelectrodewasremovedwithdistilledwaterandwipedgentlywithtissuepaperand then placed into neutral pH buffer solution when the reading was stabilized it denoted as ready after which enter waspressed
- Theelectrodewaswashedandwiped,andtheabovestepswererepeatedforacidicand alkaline pHbuffer.
- When the pH meter calibration was done the electrode was rinsed and placed into the sample. The pH measurement wasrecorded
- This was repeated for all the stations watersamples.

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 ≈ 17bacteria/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 ≈ 27bacteria/100mL

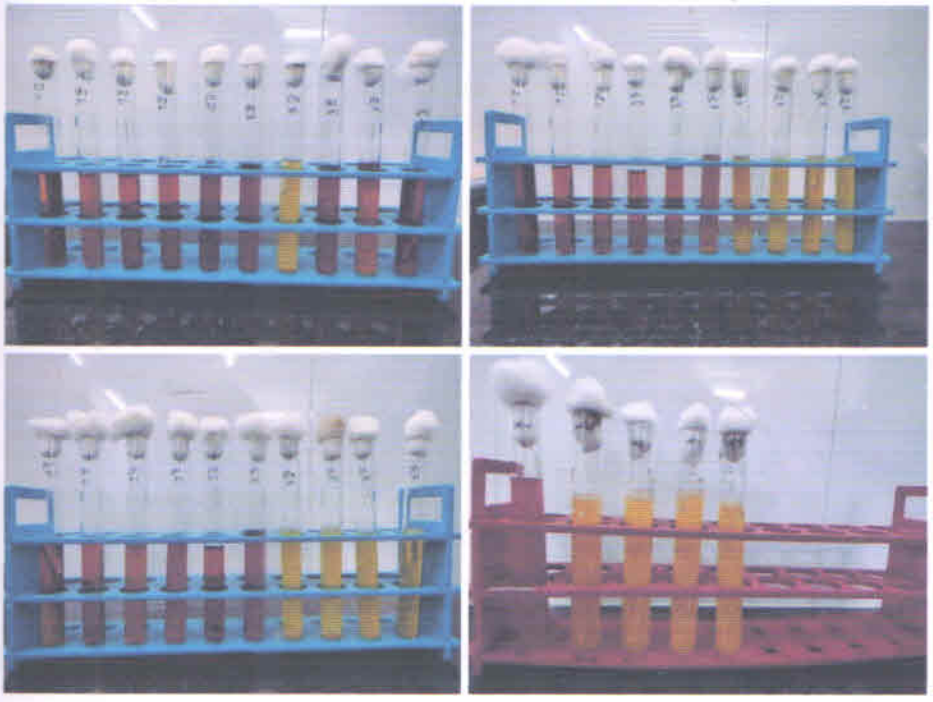

Fig 5: MPN tubes after the incubation period.

Viable count: No growth was observed on all the media's (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

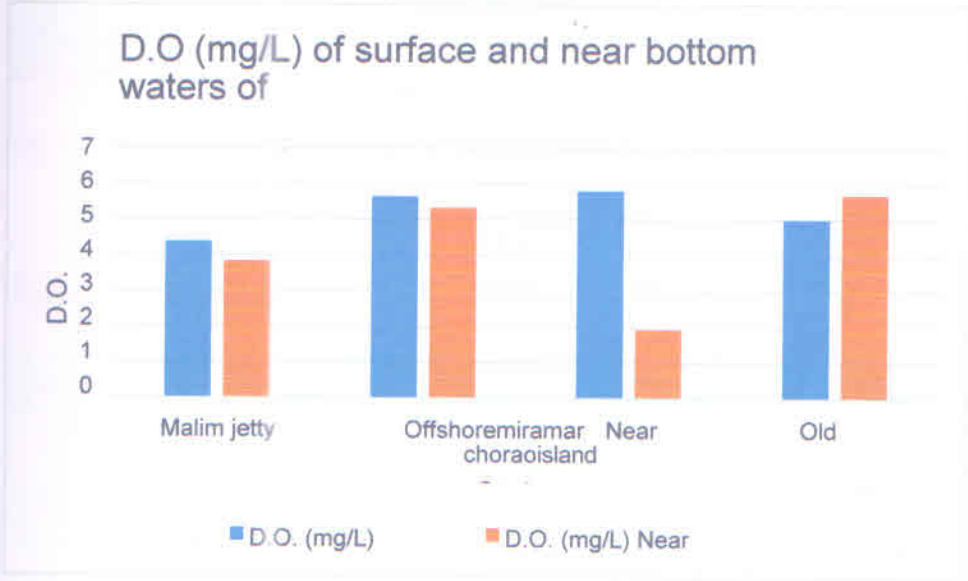

Fig: 7: Graph of D.O. (mg/L) of surface and near bottom waters of all four stations

CALCULATION:

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

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

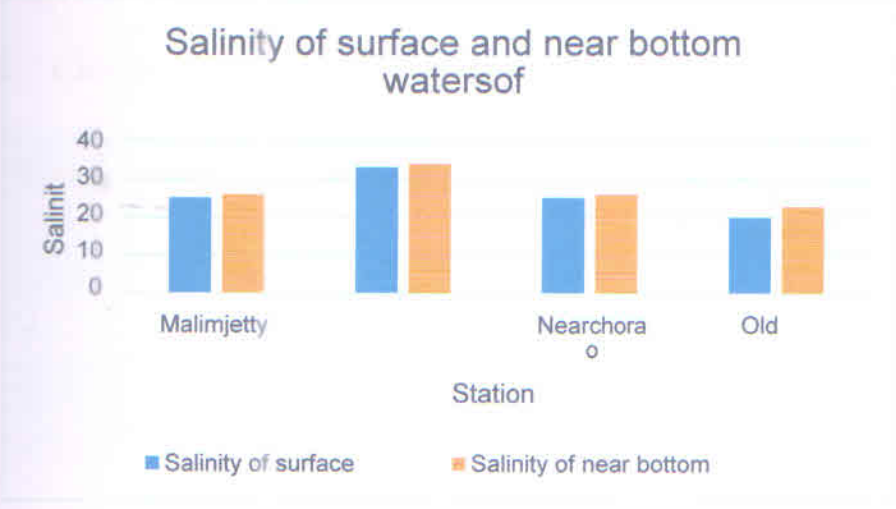

Fig 9: Graph of salinity of all four stations (surface and near bottom waters)

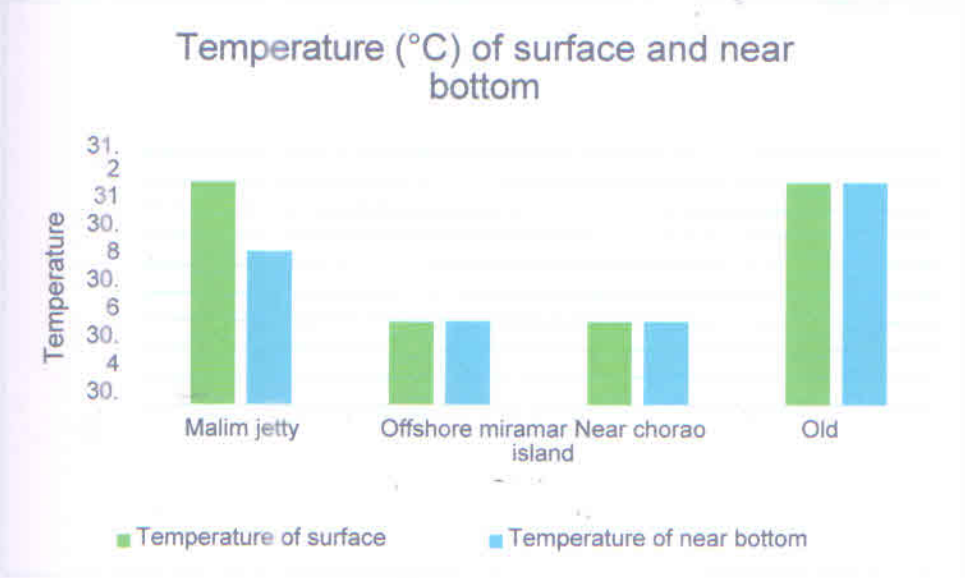

Fig 10: Graph of temperature (°C) of all four stations (surface and near bottom waters)

Estimationofphytoplankton: Qualitativeanalysisofphytoplanktonwascarriedout

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

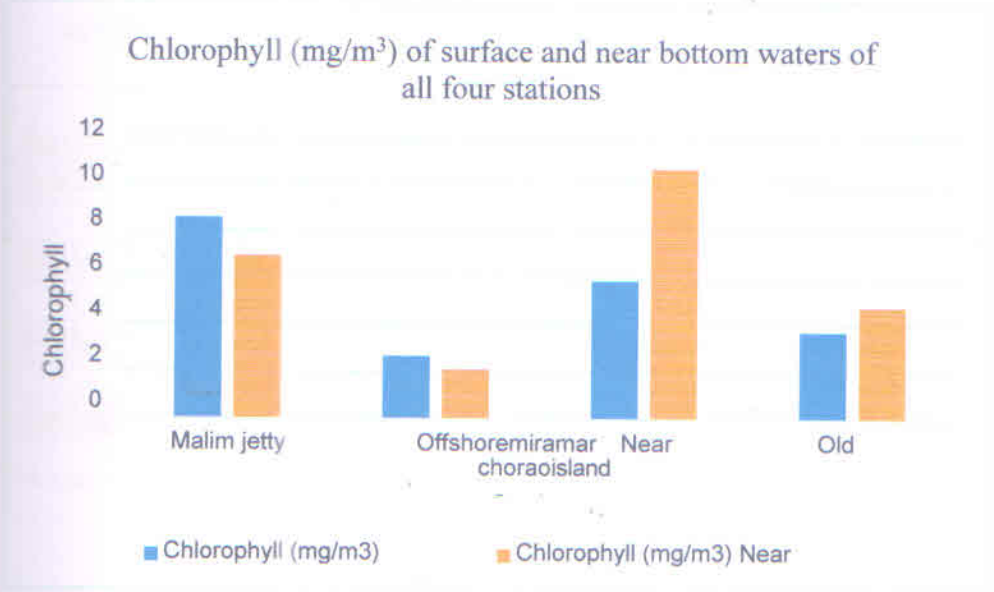

Fig 12: Graph of Chlorophyll (mg/m^3) estimation of all four stations (surface and near bottom waters)

Calculation:

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

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

where 665_o is the extinction at 665 nm before acidification, 665_a is the extinction at 665 nm after acidification, v is the volume of acetone extract(ml). V is the volume of water filtered (liters) and l is the path length of the cuvette (cm).

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

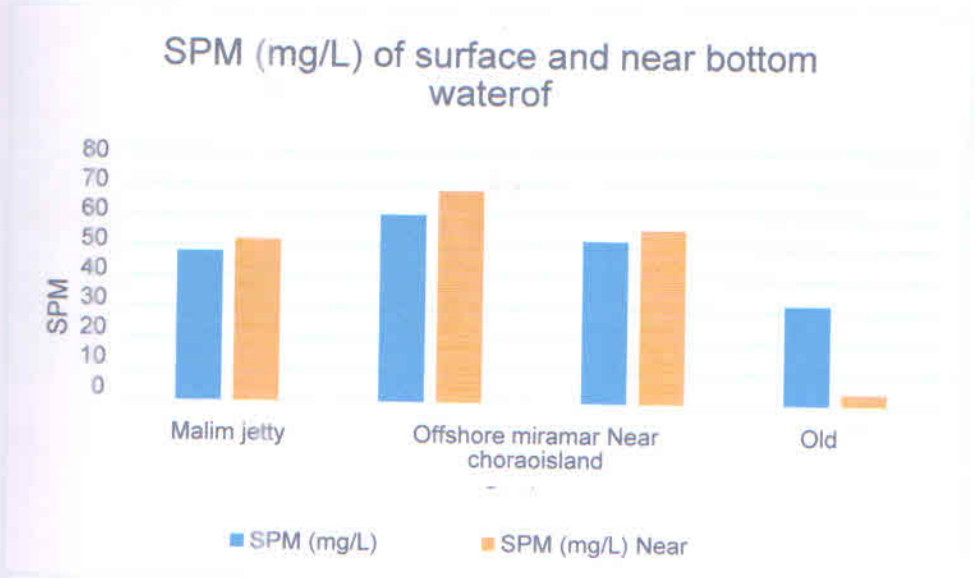

Fig 14: Graph of SPM (mg/L) of all four stations (surface and near bottom waters)

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

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

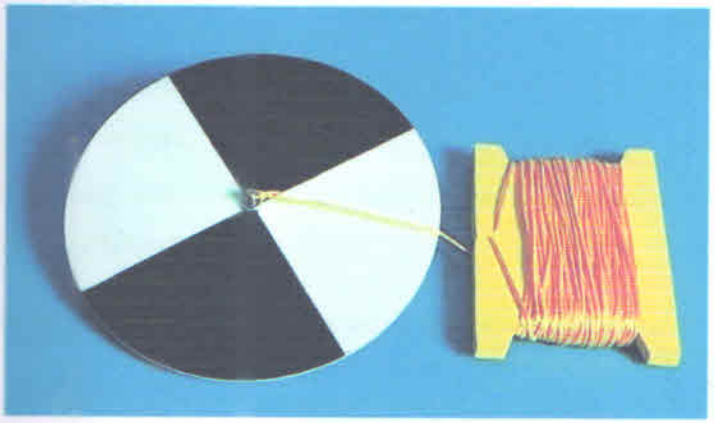

Fig 15: Secchi Disc

Fig 16:Niskinsampler

Fig 17: van Veen grab (for sedimentcollection)

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: ?. You never attended the fieldtrip!

Overall, it was a very enlightening 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 samplecollectionandstoragetogetaccurateresultsforanalysisofvariousparameterslike D.O, Phytoplankton fixing, MPN etc. We experienced the hardships involved in sampling on offshore waters, how the turbulence caused problems in samplecollection due to strong wavemotion .Additionally,wealsolearntabouttheproblemsassociatedwithsamplingin different sites such as failure of sample collection (sediment) due to rocky bottom , Motion and Sea sickness etc. On the plus side we also had fun while working. Enjoying the lunch that was served on thetrawlerparkedinthemiddleoftheestuary,watchingthemesmerizingviewwasoneofthe pleasures that we had on this trip. Having this trip amidst the pandemic, refreshed our minds andbroughtbackourinterestintoacademicsaswelearnedalotthroughthisopportunitygiven to us.

Fig 18: Food served at the trawler Fig 19: Students of MSc.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.,andLalli,C.M.(1984).'ManualofChemicalandBiological Methods for Seawater Analysis.' (Pergamon Press: NewYork.)
- Strickland, J. D. H., and Parsons, T. R. (1965). 'A Manual of Seawater Analysis', 2nd edn. (Queen's Printer and Controller of Stationery:Ottawa.)

12/11/2022