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FIELD TRIP REPORT

MMO 319 - Field Trip/Study Tour - Practical

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21P039019

M.Sc. MARINE MICROBIOLOGY PART II

2022-2023

INTRODUCTION

A departmental educational trip was arranged for us, M.Sc, Marine Microbiology students. It was the most awaited trip. The date was decided for 11th March 2022. The sun rose and we were all excited for the trip. We gathered near theMalim jetty at around 8:30a.m and waited for our teachers to arrive. From the teaching staffDr. VaradaDamare and Dr. Nikita Lotlikar and from the non-teaching staff Ms. Vaishali accompanied us. We were 15 of us excluding 1 trawler master and 2 cooks. Full of enthusiasm and excitement we entered the trawler and our journey began. In total we were to visit 4 stations, the stations were:

STATION 1- Malim jetty

STATION 2- Miramar off-shore

STATION 3- Chorao island

STATION 4-Old goa

We began with station 2 as it was further ahead slightly into the deep waters, the tides in the morning were low enabling us a suitable environment for sampling. As we reached the station the trawler stopped, due to the wave current we experienced a lot of drifting although our teachers said that this was nothing much as compared to what they have experienced. The **main aim** of our trip was to study the water and sediment parameters for which we had to collect water and sediment sample. Also, to get hands on training on the techniques learnt in theory.

To check the temperature, dissolved oxygen (DO), salinity, phytoplankton fixation and chlorophyll from surface water, water sample was collected with the help of the bucket. To analyze the near bottom water parameters, we used the Niskin sampler. To collect sediment sample, we made use of a van Veen Grab. Samples which needed to be fixed were fixed and kept safely after which they were analyzed in the lab. Water turbidity was carried out using Secchi disk.

After collecting samples from station II, we headed to station I, collected samples, headed to station III followed by station IV. After all the sampling stations were completed, it was time for lunch. Lunch was specially prepared from us on the trawler by the cooks. On menu was brown rice, prawn, curry, pickle, semolina fried prawns and mackerels. We had our lunch and enjoyed the scene on our way back to the jetty.

We returned back to the jetty at around 4:00pm after which we headed to the university to analyze the samples collected. We performed the analysis by the following mentioned procedures.

PARAMETERS ANALYZED

- 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 in 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).

OBSERVATIONS

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

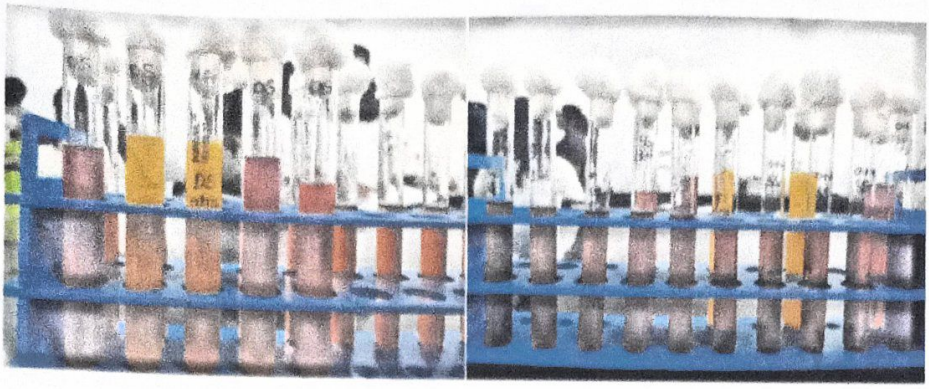
Station 3	DS (10ml)		SS (1ml)		SS (0.1)	
	Acid	Gas	Acid	Gas	Acid	Gas
1	✓	✓	✓	✓		
2	✓	✓				
3	✓	✓				
4	✓	✓				
5						

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 bacterial/100mL

✓



MPN tubes after the incubation period

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.

OBSERVATIONS:

Stations	Dilutions	Number of colonies	Average number	Dilution factor (CFU/mL
Station I	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
Station II	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
Station III	10^{-1}	6	3	2500
	10^{-1}	-		
	10^{-2}	4	4.75	
	10^{-2}	115		
Station IV	10^{-1}	23	8.25	4400

	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	-		

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% H2SO4, 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% H2SO4 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).

OBSERVATIONS:

Blank:0
Standardization of sodiumthiosulphate: 11.6

Station 1 (surface water)

Burette reading(mL)	Pilot reading(mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	2.6 – 3.6	3.4	3.1	3.1	
Initial	0	0	0	0	3.1mL
Difference	2.6 – 3.6	3.4	3.1	3.1	

Station 1 (bottom water)

Burette reading(mL)	Pilot reading(mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	2.7 – 3.7	3.2	3.2	3.0	
Initial	0	0	0	0	3.2 mL
Difference	2.7 – 3.7	3.2	3.2	3.0	

Station 2 (surface water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	2.7 – 3.7	3.2	3.3	3.2	
Initial	0	0	0	0	3.2 mL
Difference	2.7 – 3.7	3.2	3.3	3.2	

Station 2 (bottom water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	1.5 – 2.5	2	2.4	2	
Initial	0	0	0	0	2 mL
Difference	1.5 – 2.5	2	2.4	2	

Station3 (surface water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	3 - 4	3.2	3.5	3.5	
Initial	0	0	0	0	3.5mL
Difference	3 - 4	3.2	3.5	3.5	

Station 3 (bottom water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	2.3 – 3.3	2.3	2.8	2.8	
Initial	0	0	0	0	2.8 mL
Difference	2.3 – 3.3	2.3	2.8	2.8	

Station 4 (surface water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	2.9 – 3.n	3.2	3.4	3.4	
Initial	0	0	0	0	3.4 mL
Difference	2.9 – 3.9	3.2	3.4	3.4	

Station 4 (bottom water)

Burette reading(mL)	Pilot reading (mL)	I (mL)	II (mL)	III (mL)	Constant reading
Final	1 - 2	2	2.4	2	
Initial	0	0	0	0	2 mL
Difference	1 - 2	2	2.4	2	

Station	**D.O (mg/L)**	**D.O (mg/L)**
	Surface	**Near Bottom**
1	4.412	4.554
2	4.554	2.846
3	4.981	3.985
4	4.839	2.846

✓

Calculations:

N_1 = normality of KIO_3(0.01N)

V_1 = volume of KIO_3 (10mL)

V_2 = volume of sodium thiosulphate used

N_2 = normality of thiosulphate

$$N_2 = \frac{\text{normality of } KIO_3 \times 10}{\text{BR (St.)} - \text{BR (b)}}$$

$$= \frac{0.01 \times 10}{11.6}$$

$$= 0.0086N$$

$$\textbf{Dissolved Oxygen(mg/L)} = \frac{\text{BR x V/v x N x E x 1000}}{\text{Volume of sample titrated}}$$

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

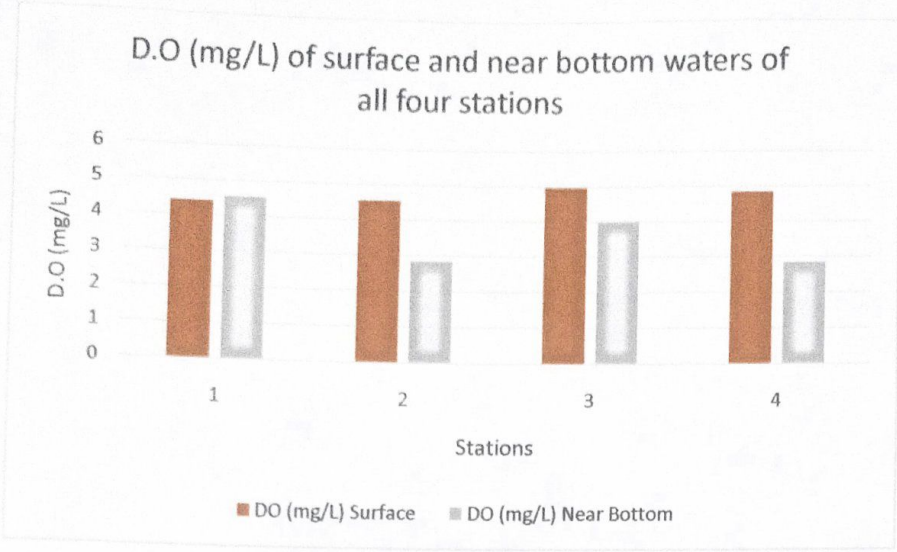
N= Normality of thiosulphate solution

E= Equivalent weight of oxygen= 8

1000= To express per litre

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

$$V/v = \frac{\text{Volume of bottle}}{\text{Volume of bottle- Volume of reagents}}$$



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

D.O. bottles

Analysis of temperature

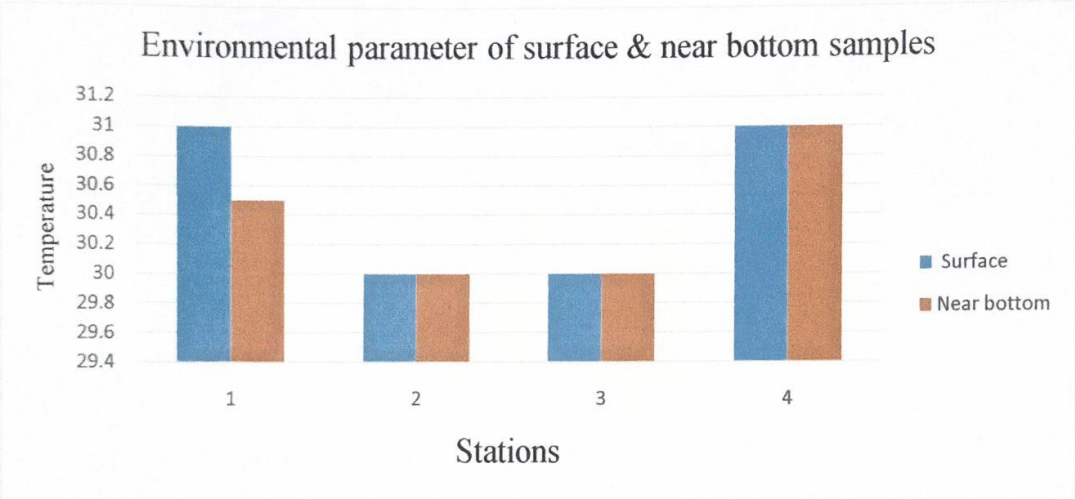
PRINCIPLE: A thermometer works on the principle that solids and liquids expand on heating. As the temperature rises, mercury expands causing it to move upwards and depict the temperature.

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.

OBSERVATIONS:

Station	Temperature (^{0}C)	Temperature (^{0}C)
	Surface	Near Bottom
1	31	30.5
2	30	30
3	30	30
4	31	31



<u>**Analysis of Salinity**</u>

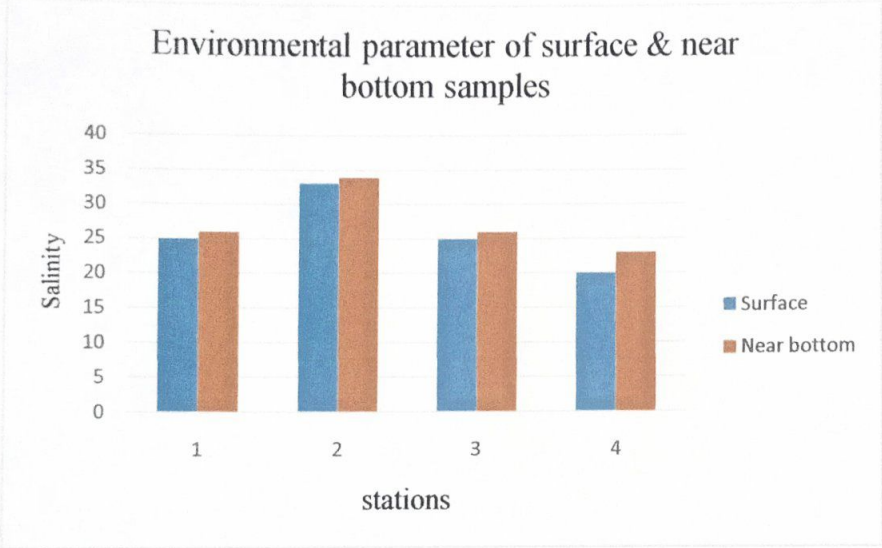
PRINCIPLE: A refractometer is a tool that can determine the concentration of a particular substance in a liquid solution. It uses the principle of refraction, which describes how light bends as it crosses the boundary between one medium and another.

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.

Station	Salinity	Salinity
	Surface	Near Bottom
1	25	26
2	33	34
3	25	26
4	20	23



Analysis of phytoplankton

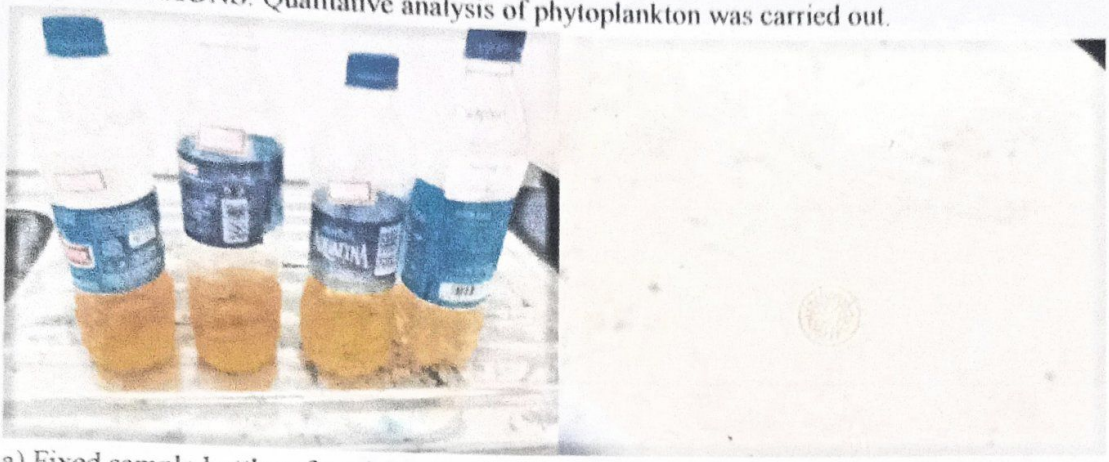
PRINCIPLE: To estimate the amount of phytoplanktons in each water sample one needs to fix the sample, to keep the cells intact and carry out microscopy later. Qualitative analysis can also be done using the same method. ✓

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.

OBSERVATIONS: Qualitative analysis of phytoplankton was carried out.



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

Analysis of chlorophyll (Chlorophyll estimation):

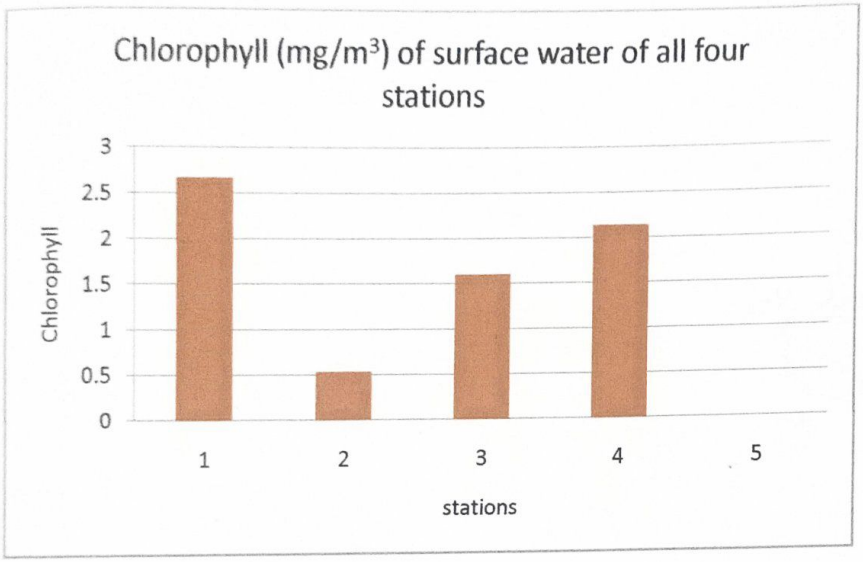
PRINCIPLE: Pigment extraction (phaeopigments, chlorophyll) is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is used as a solvent in this method since its slight polarity allows it to dissolve polar substances and also allows greater resolution 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 μ m was placed in the filtration unit attached to the vacuum pump.
- Around 500mL of seawater sample is filtered through 0.75 μ 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)

OBSERVATIONS:

Stations	Chlorophyll (mg/m^3)
1	2.67
2	0.53
3	1.60
4	2.14



Graph of chlorophyll (mg/m^3) estimation of all four stations

Calculations:

$$\text{Chlorophyll a } (mg/m^3) = \frac{26.7\,(665_o - 665_a) \times \upsilon}{V \times 1}$$

Where 665_o is the extinction at 665 nm before acidification, 665_a is the extinction at 665 nm after acidification, υ is the volume of acetone extract (mL), V is the volume of water filtered (litres) and 1 is the path length of the cuvette (cm).

Van veen grab for sediment collection

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:

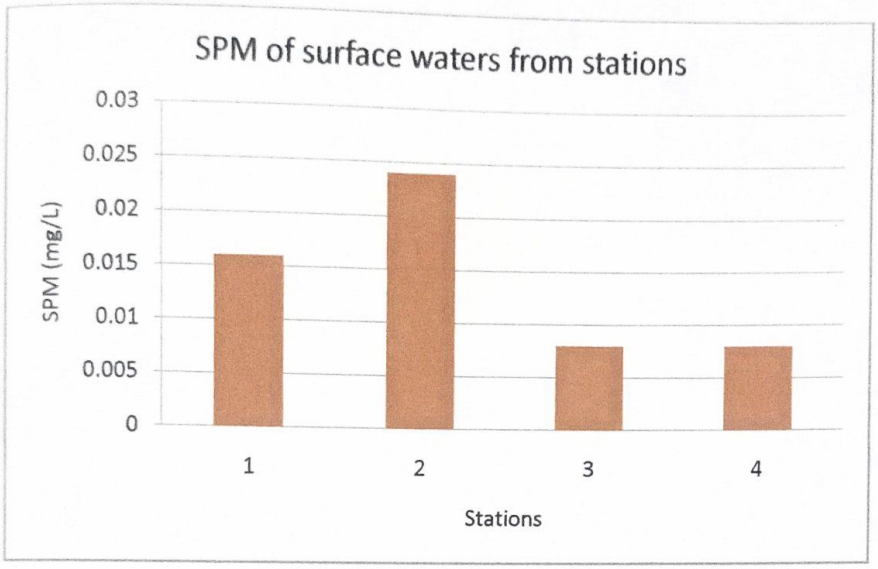
- Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of0.45 microns was placed in the filtration unit attached to the vacuum pump.
- Before filtering the water sample the weight of the filter paper was measured and noted.
- Around 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).

OBSERVATIONS:

Stations	Filter paper weight (g) (x)	Dry weight (g) (y)	Difference (g) (x-y)	SPM (mg/L)
Station 1	0.080	0.084	0.004	0.016
Station 2	0,082	0.088	0.006	0.024

Station 3	0.078	0.080	0.002	0.008
Station 4	0.090	0.092	0.002	0.008

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



Graph of SPM (mg/L) of all four stations

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.

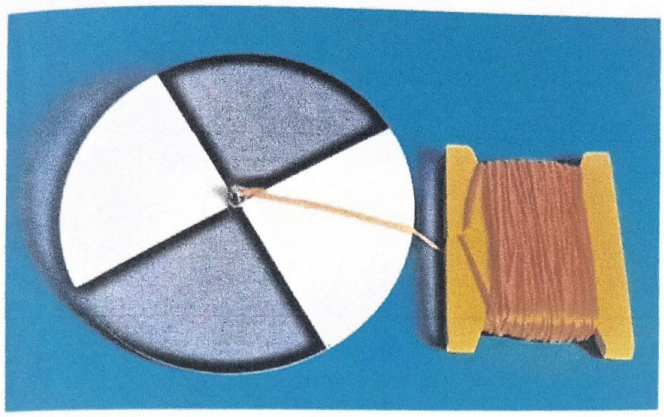
PROTOCOL:

- 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.

OBSERVATIONS: **Turbidity**

Station	Secchi depth (meters)

1	1.75
2	2
3	2.25
4	1.3



a) Secchi Disc

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: pH

Station	pH

1	8.1
2	8
3	7.6
4	7.9

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: Growth was observed on the plates and the number of colonies were counted.

D.O.: Amongthe 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 1showed the highest and station 2 and 4 showed the lowest concentration of D.O.

Temperature and Salinity: The temperature across all the stations is surface and near bottom waters varied from 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: Rhizosoleniasp., Coscinodiscus sp., Gyrosigma sp., Chaetoceros sp., and an unidentified pennate diatom. Dinoflagellates were not observed.

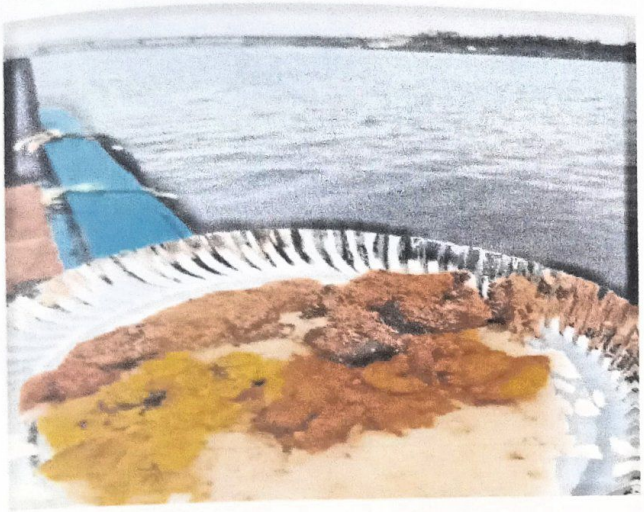
Chlorophyll and Suspended Particulate estimation: The highest chlorophyll concentration was found to be at station 1, at near bottom waters and lowest was found to be at station 2, at near bottom waters. While SPM was found to be highest at station 2 bottom waters and lowest at station 3 and 4 bottom 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.

PROSPECTIVE:

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.



Food served

Students along with teaching and non-teaching staff

18/11/22