FIELD TRIP REPORT

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MMO 319- FIELD TRIP/ STUDY TOUR- PRATICAL

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MSC. MARINE MICROBIOLOGY PART II

SEOAS GU

2022-2023

INTRODUCTION

On 10th march 2022 a field trip to Mallim jetty, offshore Miramar, near Chorao island and old goa was held for the students of MSc marine microbiology. We visited the above four stations on a trawler. From the teaching stuff Dr. Priya D'Costa and Dr. Nikita Lotlikar and from the non-teaching stuff Ms. Vaishali accompanied us along with our seniors. We left from Mallim jetty at 9:30am and headed towards the locations from where water sample was supposed to be collected. The main objective of our trip was to study the water and sediment parameters for which we had to collect water and sediment samples. Also, to get hands on training on the techniques learnt in theory. Surface water, near bottom waters and sediment samples were collected from each station. Surface water was collected by a bucket, near bottom water was collected by Niskin sampler and sediment samples were collected by Van Veen grab sampler.

The water sample collected was used for testing many parameters such as temperature, salinity, dissolved oxygen, pH, viable count, phytoplankton enumeration, suspended particulate matter (SPM), detection of bacteria by MPN (Most Probable Number), Chlorophyll and phaeo-pigments and water turbidity. All the necessary requirements like chemical preparation, media preparation and instruments required for the field trip was already prepared and kept ready in advance. Samples which needed to be fixed were fixed and kept safely after which they were analyzed in the lab. Also, latitude, longitude and depth of the 4 locations was noted down.

After all the sampling stations were completed, it was time for lunch. Lunch was specially prepared for us on the trawler by the cooks. On menu was brown rice, prawn curry, pickle, semolina fried prawns and mackerels. We had our delicious lunch and enjoyed the beautiful 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 below mentioned procedures.

MATERIAL AND METHODS

➢ LOCATION:

Water and sediment sample was collected from four location along the Mandovi river and its latitude, longitude and depth were noted as follows:

STATION	NAME	LATITUDE	LONGITUDE	DEPTH (meters)
1	MALLIM JETTY	15°28'20.8554"N	73°46'37.542"E	6
2	MIRAMAR OFF-SHORE	15°30'12.3192"N	73°49'55.5234"E	10
3	NEAR CHORAO ISLAND	15°30'19.3248"N	73°52'0.894"E	3.5
4	OLD GOA	15°30'31.248"N	73°54'50.9832"E	5.25

➢ SAMPLE COLLECTION

- Surface water sampling

The surface water was collected by the bucket method: a rope was tied on to a bucket and was gently lowered in the water, tilted, so that the water enters the bucket and gently pulled up with the help of a rope.

- Near bottom water sampling

The near bottom waters were collected by a Niskin bottle (fig 1). It is a cylinder made up of plastic, which eliminates chemical reaction between the bottle and the sample, it has two stoppers at each end and are held open by plastic cords attached to a release mechanism. The stoppers are connected by an elastic cord at the inside of the bottle. A weight is tied 1m below the niskin bottle so it touches the bottom and water sample is collected 1m above the bottom. Once the weight hits the bottom a messenger is send along the rope which strikes the release mechanism resulting in the two stoppers being pulled into the ends of the cylinder, as a result the water from that depth is trapped inside the niskin bottle.

- Sediment sampling

The sediment sample was collected by Van Veen grab sampler (fig 2). It comprises of two steel clamshells, which hits the seabed and grabs a sample by bringing the two clamshells together and cutting a bite from the soil. Unfortunately, we were able to collect sediment sample from just one location (station 2) as the other locations were rocky (station 1 & 4) and dredged (station 3). The collected sample (station 2) was then stored in polyethene bags in an ice-box.

FIGURES:

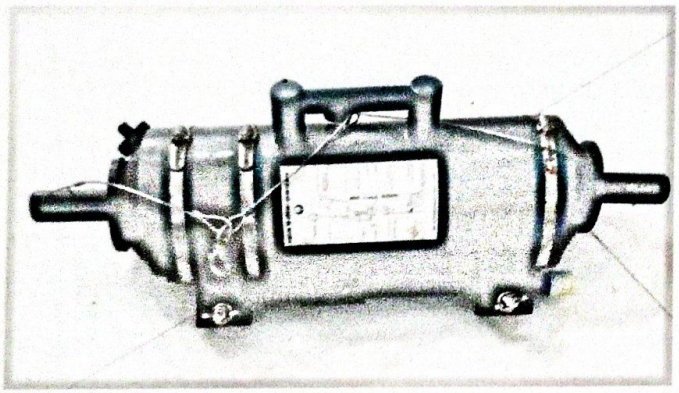

Fig 1: Niskin sampler

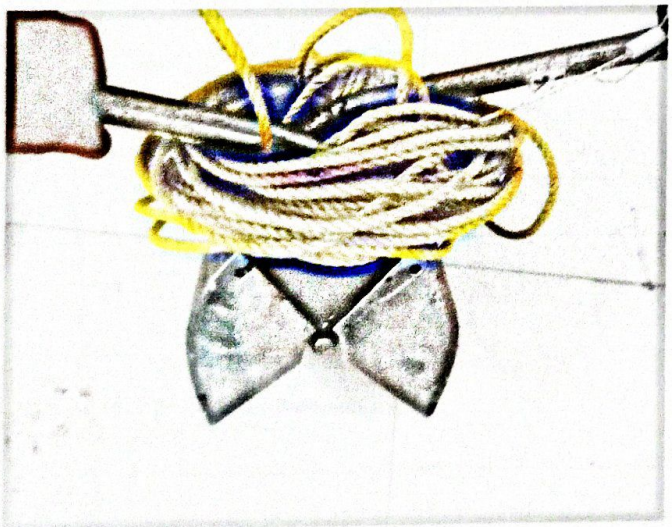

Fig 2: Van Veen Grab Sampler

➢ ANALYSIS

1. TEMPERATURE

PRINCIPLE: Sea temperature is a string indicator of productivity, pollution and global climate change. It was measured using a thermometer. As the temperature rises, mercury expands causing it to move upwards and depict the temperature.

PROCEDURE: for surface waters:

i. The thermometer was slowly dipped in the water collected and the reading was noted.

For near bottom water:

i. Some water was taken in a mug from the Niskin sampler and the thermometer was dipped into the mug. Reading was noted.

2. 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. Salinity defined by Kundsen is, the weight in grams of the dissolved inorganic matter in 1kg of seawater. Usually, sea water salinity should be 35.

PROCEDURE: To check salinity of surface water:

i. With the help of a dropper water sample was taken from the bucket and 1-2 drops were placed on the refractometer.
ii. The refractometer stage covering was closed and viewed through the eyepiece. The reading was noted.

To check salinity of bottom water:

i. Water was taken in the mug from the Niskin sampler.
ii. Procedure was same a performed to check surface water salinity.

3. pH

PRINCIPLE: pH is a measure of hydrogen ion concentration in a solution. The pH scale ranges from 0-14 (acidic to basic), pH 7 being the neutral pH. pH is often described as master variable in seawater and other aquatic systems since many processes, properties and reactions are dependent on pH. Seawater pH is also considered as a part of carbon-dioxide system which provides a major pH buffer in seawater.

PROCEDURE: Calibration

i. The pH meter was switched on after which the 'cal' button on the pH meter was pressed.
ii. The electrode was rinsed with distill water and wiped genteelly with tissue paper and placed in neutral pH buffer solution (pH7) and left for some time to attain stability.
iii. Once the machine was stable it was denoted as 'ready' on the screen.
iv. The electrode was then removed, rinsed with distill water and wiped genteelly.
v. Same steps were repeated for acid and alkali pH buffer.

Sample testing:

i. Once the calibration was complete the electrode was rinsed and placed in the sample and the reading was noted.

4. DISSOLVED OXYGEN

PRINCIPLE: Dissolved oxygen was measured by a method proposed by Winkler. This is an 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.

PROCEDURE: Determination of blank

i. 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.

ii. 1mL starch was added (blue color did not develop. If it had to develop then titration needs to be carried out)

Standardization of thiosulphate solution.

i. Solution was prepared in the same method as prepared for blank.

ii. 10mL of 0.01N potassium iodate solution was added. Solution was mixed and kept in dark for 3 mins to liberate iodine.

iii. Liberated iodine was titrated against sodium thiosulphate till the solution turned pale yellow.

iv. 1mL starch was added and titration was continued till the color changed from blue to colorless (remained colorless for 30 seconds). This was carried in triplicates to obtain the mean burette reading.

DO estimation

i. 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).

i. D.O. was fixed by adding 1mL of Winkler's A and 1mL of Winkler's B and the precipitate was left to settle.

ii. The samples were brought back to the laboratory. 1mL of 50% H_2SO_4 was added and shaken till the precipitate dissolved.

iii. 50mL of the sample was then transferred to a conical flask and titrated against thiosulphate solution until a pale yellow appeared.

iv. 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)

5. VIABLE COUNT

PRINCIPLE: The viable plate count, (plate count) is a count of viable or live cells. It is based on the principle that viable cells replicate and give rise to visible colonies when incubated under suitable conditions for the specimen.

PROCEDURE:

i. From water sample collected, 50ml was kept aside in a centrifuge tube.
ii. 0.1ml from that water was taken and spread plated on previously prepared ZMA, MacConkey's, TCBS and XLD agar plates.
iii. The plates were incubated at 37°C for 24-hours. Results were noted.

6. ENUMERATION OF PHYTOPLANKTON

PRINCIPLE: Phytoplankton are single celled microscopic marine organisms that prepare their own food with the help of sunlight through photosynthesis. They form the base of the food chain and are majorly responsible for primary productivity.

PROCEDURE:

i. From the collected surface and bottom water 500ml water was collected separately in bottles from each station.
ii. 15 drops of Lugol's iodine solution were added as a preservative.
iii. The bottles were kept in the lab for 15 days after which a drop of the sample was placed on a clean grease free slide, covered with a clean coverslip and observed under the microscope (5x, 20x and 45x).

7. SUSPENDED PARTICULATE MATTER (SPM)

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. The dry weight

concentration of suspended particulate material, (units: mg/L), is measured by passing a known volume of seawater through a pre-weighed filter and reweighing the filter after drying.

PROCEDURE:

i. From the bucket and Niskin sampler water was collected in the bottle and stored in shade.
ii. A filter paper of 0.45micron was placed in the filtration unit attached to the vacuum pump.
iii. Before filtering the water sample the weight of the filter paper was measured and noted.
iv. Around 250mL of seawater sample is filtered through the filter paper.
v. After filtering the weight of the filter paper was again measured (designated as wet weight).
vi. 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. (Dry weight).
vii. The readings were noted down and the calculations were carried out.

8. PRESCENCE OF BACTERIA BY MPN (Most Probable Number)

PRINCIPLE: This test is mainly carried out to detect E. coli and coliforms. Fecal coliforms are known to ferment lactose and produce both acid and gas. This can be detected by performing MPN where change in color 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.

PROCEDURE:

i. Water sample collection was done from the surface of the station using a bucket.
ii. The water was collected into sterile centrifuge tubes of 50 mL and stored in ice box until further analysis.
iii. 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.
iv. 10ml of water sample was inoculated into 5 tubes containing 10mL of MacConkey's Broth.

v. 1ml of water sample was inoculated into 5 tubes containing 10mL of single strength MacConkey's Broth.

vi. And 0.1 ml of water sample was added to 5 tubes containing 10mL of single strength broth.

vii. All the tubes were incubated at 37°C for 24-48 hours.

viii. Positive results were indicated by production of acid (change in color of the media) and gas.

ix. The results were compared to a standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined.

9. ANALYSIS OF CHOLOROPHYLL

PRINCIPLE: Pigment extraction (phaeopigments, chlorophyll) is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone being a polar solvent allows polar substances to dissolve and greater resolution between pigments, therefore it is suitable for pigment extraction. Pigment analysis is done spectrophotometrically.

PROCEDURE:

i. Sample was collected in a plastic bottle from surface as well as near bottom waters and stored in the shade. A filter paper of 0.7 micron was placed in the filtration unit attached to the vacuum pump.

ii. Around 500Ml (mL) of seawater sample is filtered through the filter paper. After filtration the filter paper was picked using forceps and placed into a dark colored plastic bottle.

iii. Next 10ml of 90% acetone was put into the bottle.

iv. Crushed and gently and capped. The bottle was kept undisturbed for 24 hours in the refrigerator.

v. Next day the samples were analyzed spectrophotometrically at 665nm, then 2 drops of HCL acid was added.

vi. Absorbance was measured at 750nm. Readings were recorded and calculations were done. (Parsons et al. 1984)

10. TURBIDITY OF WATER

PRINCIPLE: Turbidity of the water was analyzed with the help of a Secchi disk (Fig- 3). Turbidity of the water will tell us up till what depth the sunlight penetrates as sunlight is essential for phytoplankton to carry out photosynthesis.

PROCEDURE: The disk was lowered in the water up till the depth were a clear distinction of the whiteblack quadrants were noticed.

FIGURE:

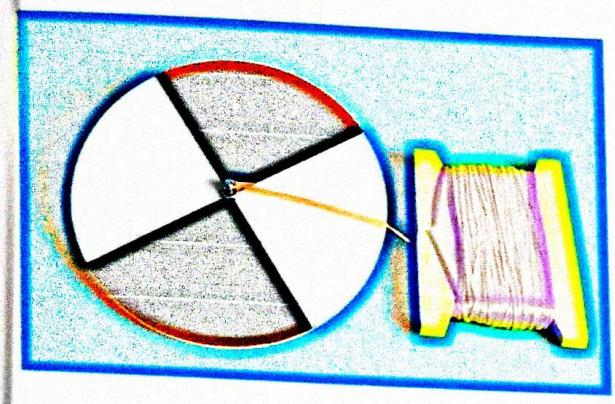

FIG 3: Secchi disk

OBSERVATIONS:

- TEMPERATURE, SALINITY, pH AND SECCHI DEPTH

Stations	Temperature(˚C)		Salinity		pH	Secchi depth (m)
	Surface	Near_bottom	Surface	Near_bottom		
1	31	30.5	25	26	8.1	1.75
2	30	30	33	34	8	2
3	30	30	25	26	7.6	2.25
4	31	31	20	23	7.9	1.3

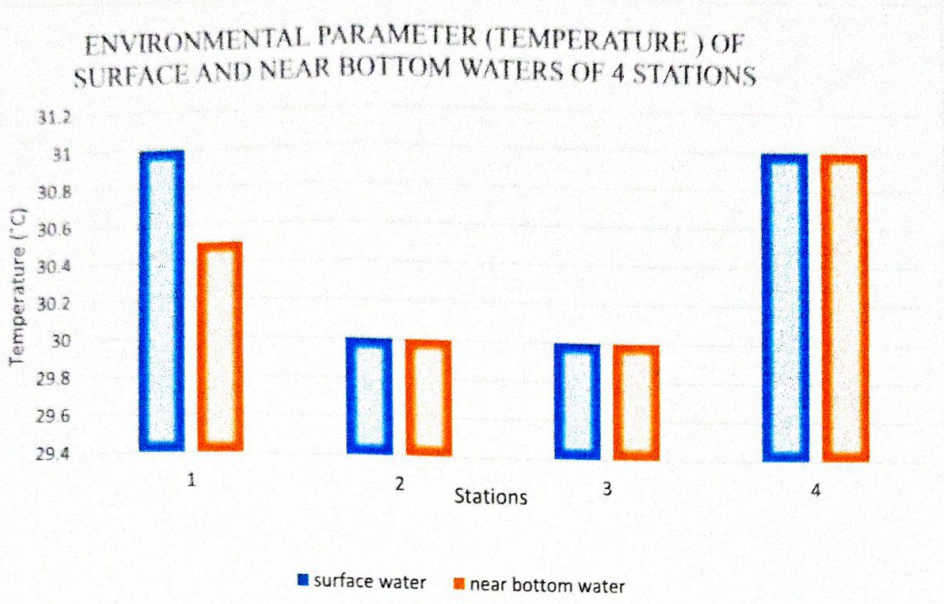

FIG 4: graph of temperature of 4 stations.

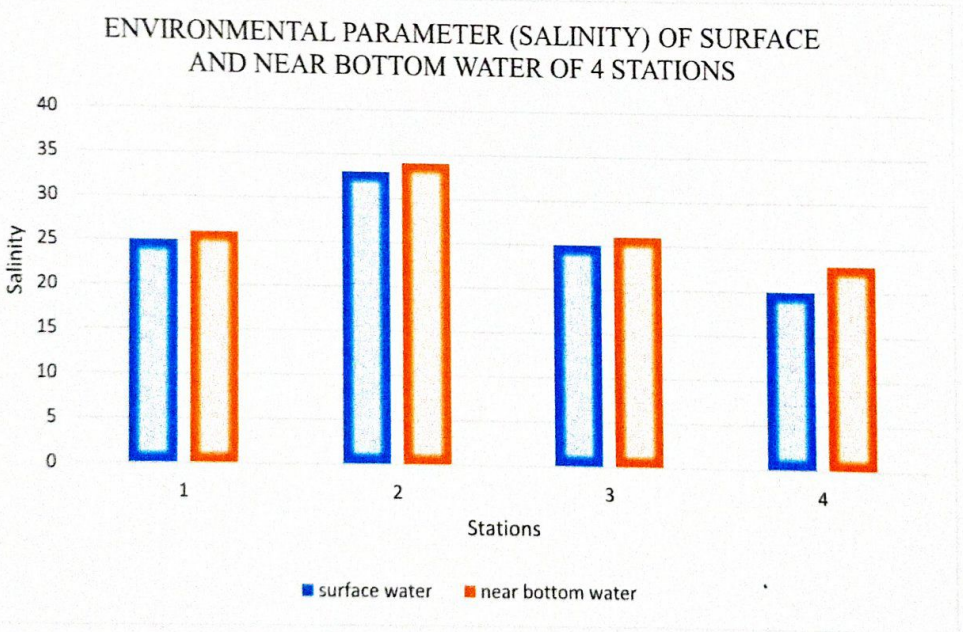

FIG 5: Graph of salinity of 4 stations.

- DISSOLVED OXYGEN (DO)

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

Calculation:

$$DO\ (mg/L) = \frac{BR \times V/v \times N \times E \times 1000}{\text{Volume of sample titrated}}$$

Where: BR = burette reading, N= normality of thiosulphate solution, E= equivalent weight of oxygen = 8, 1000= to express per liter, V/v= volume of bottle/ vol. of bottle – vol. of reagent.

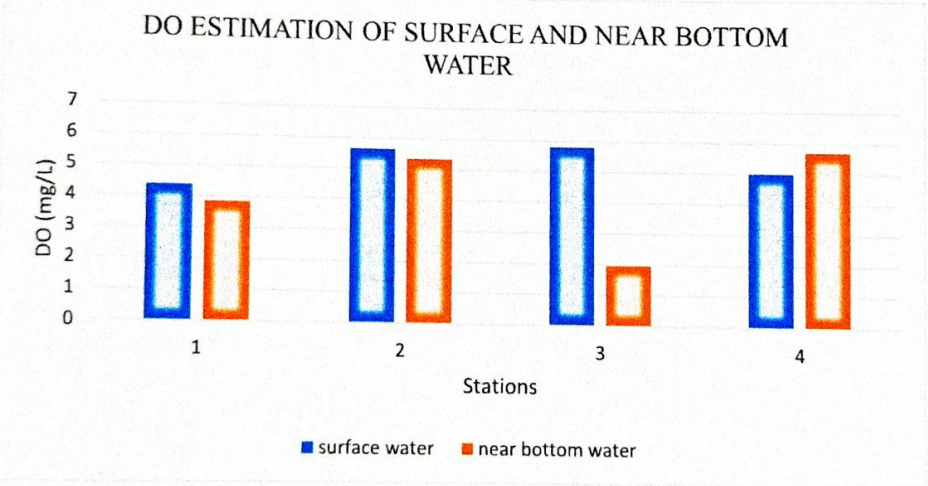

FIG 6: Graph of DO estimation

FIG 7: D.O. bottles after fixing the sample with Winkler's A and Winkler's B

- ENUMERATION OF PHYTOPLANKTON

Qualitative analysis of phytoplankton was carried out

FIG 8: Fixed sample bottles after siphoning

FIG 9: *Coscinodiscus sp.*

- SPM

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

Calculation:

$$\text{SPM (mg/L)} = \frac{\text{X-Y}}{\text{Volume of water filtered in liters}}$$

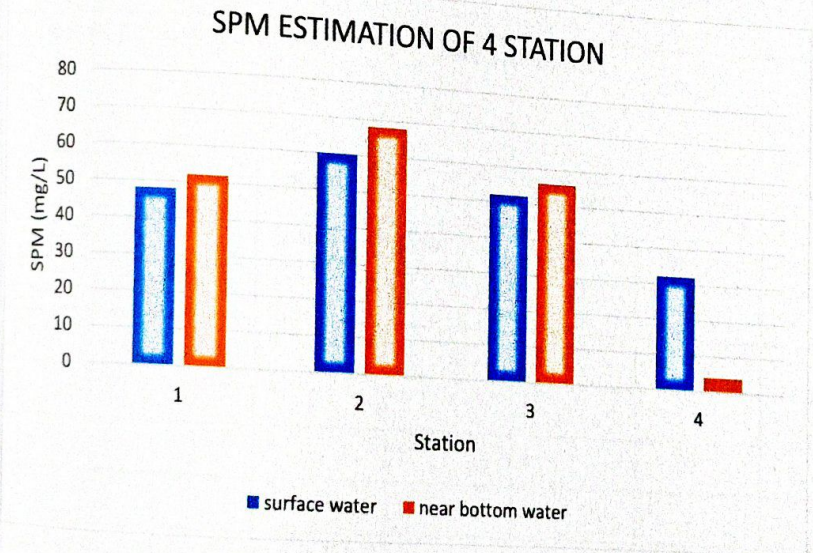

FIG 10: graph of SPM estimation.

- MPN

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

Number of positive tubes: 3-4-0 ≈ 13-17 bacteria/ 100ml

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

Number of positive tubes: 4-1-0 ≈ 17 bacteria/100ml

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

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

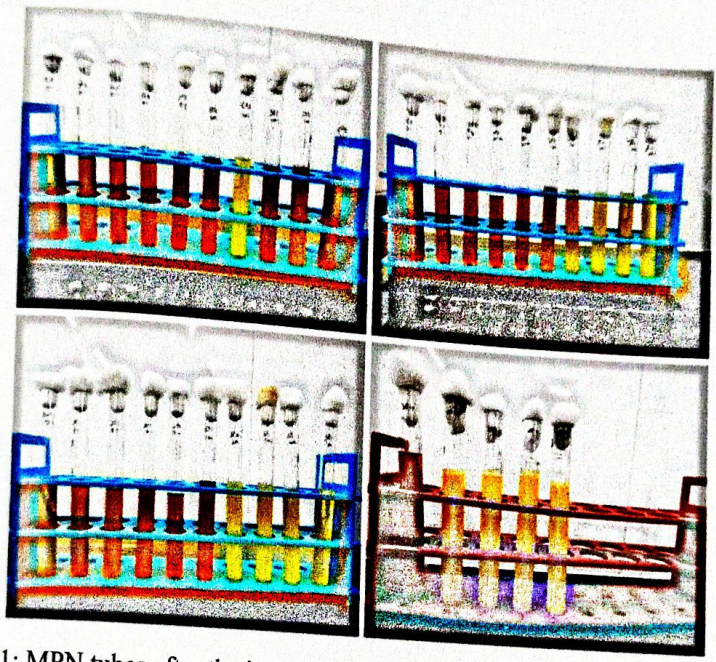

FIG 11: MPN tubes after the incubation period.

- CHLOROPHYLL ESTIMATION

STATION	CHLOROPHYLL (mg/m^3)	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

Calculations:

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

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

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).

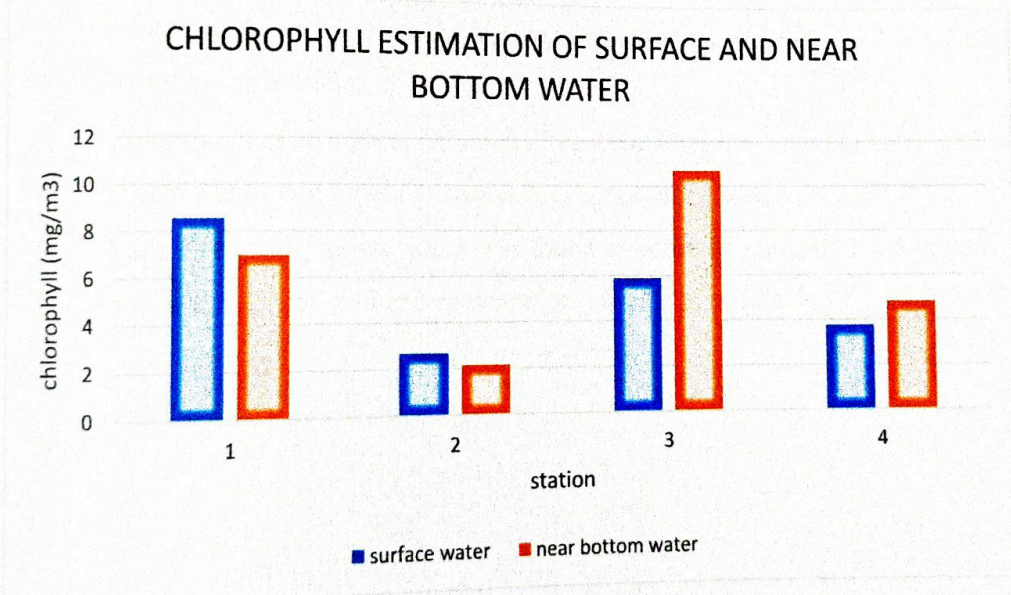

FIG 12: Graph of chlorophyll estimation.

RESULT

- Temperature: Temperature of the surface as well as bottom waters was between the range 30-31°C.
- 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.
- Salinity: for surface and bottom water salinity was the highest at station 2 and was the lowest at station 4.
- pH: pH was found to be towards the acidic range (7.5-8) with the highest pH at station 1 and lowest at station 3.
- Viable count: No growth was observed on any plate.
- Estimation of Phytoplankton: Diatoms *Rhizosolenia sp.*, *Coscinodiscus sp.*, *Gyrosigma sp.* and *Chaetoceros sp.* were observed along with an unidentified pennate diatom. Dinoflagellates were not observed.
- SPM: Station 2 bottom waters had the had the highest content of SPM whereas station 4 bottom waters had the lowest.
- MPN: Station 1 was found to have 130 bacteria/100mL, station 2 had 13-17 bacteria/100mL, station 3 had 17 bacteria/100mL and station 4 had 27 bacteria/100ml. Indicating station 1 to have the highest bacterial count while station 2 and station 3 had the lowest bacteria/100ml.
- Chlorophyll: The highest chlorophyll concentration was found to be at station 3, in near bottom waters and lowest at station 2, near bottom waters.
- Turbidity: clarity of the water was found to be in the range of 1-2.3 meters. Water was most turbid at station 3 and comparatively clearer at station 4.

PROSPECTIVE

It was a great learning experience. We get to know how to handle different instrumentation required for collection of water sample as well as sediment sample eg; Niskin sampler, Van Veen grab. Also, we learnt how to fix water sample which was collected from surface as well as bottom water and how to carry out biochemical test for the same. we got an idea on the obstacles which actually occur on the field and how we can avoid and overcome these obstacles without getting our work affected. Also, how to store the samples and get the samples to lab and do the further experiment. We also did the compilation of the results observed and all the data was represented in tabular form and graphically. Apart from doing our work and getting the required knowledge we had a lot of fun on the trawler, the food was delicious and the much-needed watermelon that we got on a hot sunny day. Indeed, a trip to remember. Grateful to our teachers and our dean who initiated and took all the trouble in making necessary arrangements to organize this trip.

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