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FIELD TRIP REPORT

MMO 319

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SUBMITTED BY

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MSC. MARINE MICROBIOLOGY

PART II

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To study the environmental variables related marine ,coastal and aquatic ecosystems, water quality and sediment characteristics a field trip was organized by Marine Microbiology SEOAS department, Goa University on 10th March 2022. Dr. Priya D'Costa and Dr. Nikita Lotlikar along with Ms. Vaishali and 13 students were taken on trawler for field trip. All the preparations for the field trip were done on the 8th and 9th of March 2022. The preparations included preparation of media like the Mac Conkey's broth for the MPN test, preparation of reagents like the winkler's reagents, conc. H_2SO_4, starch indicator solution, 90%acetone, etc. Cleaning and autoclaving of all the glassware and plastic wares . Weighing of the filter paper used for chlorophyll extraction and SPM .The media prepared were also autoclaved. How to go about with the sampling and other important things to be taken care during the field trip were also explained .

We started for the field trip from the Malim jetty at 8.45 am in the morning in a fishing trawler. We collected water samples from four different stations:

STATION 1: Malim jetty(09:41)

STATION 2: Offshore Miramar(11:17)

STATION 3: Near Chorao Island (11:59)

STATION 4: Old Goa (12:53)

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

The samples were collected from near bottom and the sea surface and sediment sample from the bottom of the sea floor.The station 1 for the sample collection was Malim jetty but due to the change in tides at offshore Miramar station 2 during the later hours of the morning we went to collect the samples at offshore Miramar station 2 first. A bucket of water was collected for DO estimation, chlorophyll estimation, phytoplankton estimation, SPM for surface water. For bottom water sample Niskin sampler was used. Also temperature, salinity, pH of both surface and bottom water was noted down. Turbidity of the water was measured using Secchi disc. The same was done on all four station. Once the samples were collected and fixed we returned back to the lab to carry bout further tests.

Following analysis was carried out

- Temperature
- Salinity

- pH
- Turbidity
- SPM
- DO
- MPN
- Viable Count
- Phytoplankton concentration
- Chlorophyll estimation

ANALYSIS OF TEMPERATURE

PRINCIPLE: It is very important to know the temperature of the sea water as it effects the distribution of life in the ocean, the exchange of gases, survival of organisms. It also controls the rate at organisms metabolize or break down food into usable nutrients.

The ocean surface temperatures range from 0-30degree C. The water temperature varies with the depth, geographical location, season, elevation and climatic conditions and is influenced by stream flow, stream side vegetation, ground water inputs, industrial effluents, etc. Temperature is measured by a simple thermometer.

PROTOCOL:

- The water sample was collected in a bucket and the temperature was measured using a simple mercury thermometer.

ANALYSIS OF SALINITY

PRINCIPLE: It is the term used to define the total amount of dissolved inorganic salts in the ocean. (Definition: - The salinity of seawater is defined as the total amount by weight of dissolved salts in one kilograms of sea water.) The average salinity of sea water is typically about 35%. Salinity varies globally across the surface and with the change in the depth. Evaporation, formation of sea ice increases the salinity of sea water while rainfall, run off, melting of sea ice decrease the salinity.Refractometer is one of the simplest and most common tool for obtaining a reasonably accurate measurement of salinity. They work on the principle that water bends light by slowing it down and salt water bends light more than pure water.

PROTOCOL:

- Water sample from different stations was collected and using a dropper 2-3 drops were put onto the refractometer.

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- 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 pH

PRINCIPLE: pH is often described as the 'MASTER VARIABLE' in sea water and other aquatic systems since many properties, processes and reactions are pH dependent. However the sea water pH is usually considered as a part of the carbon dioxide system which provides the major pH buffer in sea water. Sea water is normally slightly basic with a surface water pH of 8.1-8.2, but as the ocean continues to absorb more CO2, the pH decreases and the ocean becomes more towards the acidic side. It can be measured by a pH paper or a pH meter in the laboratory.

PROTOCOL:

- The pH meter was turned on and calibrated
- 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

ANALYSIS OF TURBIDITY

PRINCIPLE: To check the water turbidity a Secchi disk is a simple standard tool used to measure water clarity. I t is an 8 inches (20cm) diameter black and white disk which is attached to a rope. Inch/ centimetre or meter intervals are marked on the rope with permanent ink or paint. The Secchi disk is lowered into the water until it is no longer visible and that depth is measured. Secchi depth values that are high indicate clearer water and low Secchi depths indicate high turbidity.

PROTOCOL:

- Secchi disc was lowered slowly from the trawler into the water
- The disc was lowered until there is no clear vision
- The depth measurement was noted down.

ANALYSIS OF SPM (SUSPENDED PARTICULATE MATTER)

PRINCIPLE: The SPM is determined by filtering a known volume of water sample through 0.45micron pre-weighed filter paper drying and then weighing again. The difference between the initial weight and the final weight in mg divided by the amount of water filtered in litres is given as the SPM(mg/L).

PROTOCOL:

- Sample was collected in a plastic bottle. A filter paper of 0.75 microns was placed in the filtration unit attached to the vacuum pump.
- The weight of the filter paper was measured and noted.
- 250mL of seawater sample is filtered through the filter paper.
- After filtering the weight of the filter paper was again measured
- The filter paper was then kept for drying in the oven at 30°C till it completely dried after which the weight of the filter paper was again measured.
- The readings were noted down and the calculations were done.

ANALYSIS OF DO (DISSOLVED OXYGEN)

PRINCIPLE: It is the measure of how much oxygen is dissolved in the water. The amount of dissolved in sea water can tell us a lot about its water quality. The concentration of dissolved oxygen in ocean water is typically between 7 to 8 milligrams per litre (mg/L). Water temperature and biological processes are the major factors affecting the dissolved oxygen concentration of sea water. Photosynthesis, stream flow, aeration increase the DO of sea water, whereas respiration, decomposition decrease the DO of the sea. The DO of sea water is estimated by Winkler's 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. 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.

D.O. estimation:

- Sample was collected in D.O. bottles from different stations without any air bubbles formation during collection
- D.O. was fixed by adding 1mL of Winkler's A and 1mL of Winkler's B and the precipitate was allowed to settle down.
- 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 changes to colourless. Burette reading was noted.

ANALYSIS OF MPN : PRESENCE OF BACTERIA BY MPN (most probable number)

PRINCIPLE: The presence of bacteria in the sea water is tested by doing the MPN test. MacConkey's broth is used for the MPN test in double strength and single strength. The water sample is inoculated in the broth test tubes containing the inverted Durham's tube and incubated at 37degree for 24 hours in the incubator. The change of the broth from red to yellow and formation of gas bubble inside the inverted Durham's tube indicates the presence of coliforms in the collected sea water sample.

PROTOCOL:

- Water sample collection was done from the surface using a bucket.
- The water was collected into sterile centrifuge tubes of 50 mL and stored in ice box.
- The samples were brought back to the laboratory and inoculated into double strength and single strength MacConkey's Broth containing inverted Durham's tube.
- 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 and gas.
- The results were compared to a standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined.

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:

- 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.

ANALYSIS OF CHLOROPHYLL AND PHAEOPIGMENTS

PRINCIPLE: The concentration of chlorophyll is an indicator for the amount of photosynthetic plankton or phytoplankton present in the ocean. Individual samples of

chlorophyll are measured by filtering a known amount of sample through a glass fibre filter. The filter paper itself is used for the analysis. The chlorophyll is extracted using 90% acetone and then a spectrophotometric analysis is carried out to measure the absorbance at 665 and 750 nm wavelengths.

PROTOCOL:

- Sample was collected in a plastic bottle and stored in the shade.
- A filter paper of 0.45 microns was placed in the filtration unit attached to the vacuum pump.
- Around 500mL of seawater sample is filtered through the filter paper.
- After filtration the filter paper was picked using forceps and placed into a dark coloured plastic bottle.
- 10ml of 90% acetone was put into the bottle
- Filter paper was crushed.
- The bottle was kept undisturbed for 24 hours in the refrigerator
- 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.

ANALYSIS OF PHYTOPLANKTON

PRINCIPLE: To estimate the amount of phytoplanktons in each water sample, the sample has to be fixed.

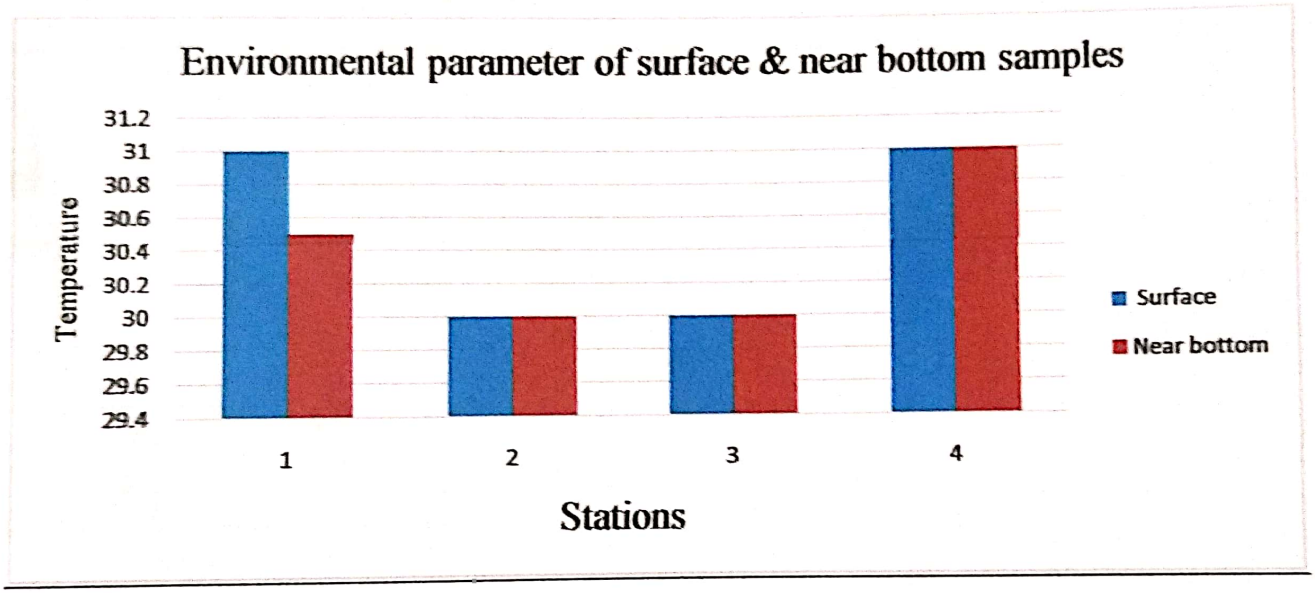
PROTOCOL:

- Water sample from different stations was collected in 500ml bottles.
- 15 drops of Lugol's iodine solution were added and stored in shade.
- 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

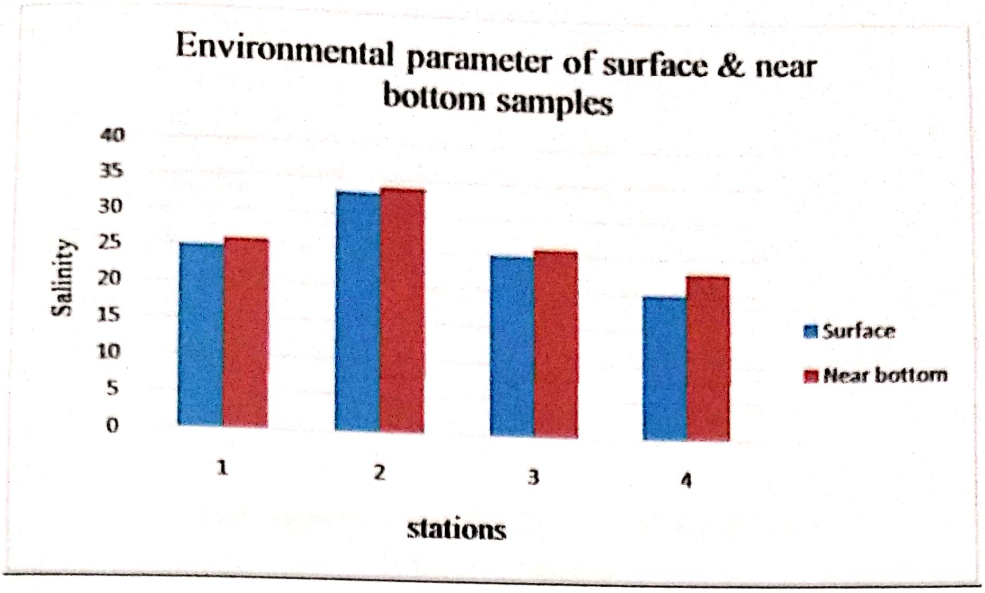
TEMPERATURE:

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



SALINITY:

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



pH :

Station	pH
1	8.1
2	8
3	7.6
4	7.9

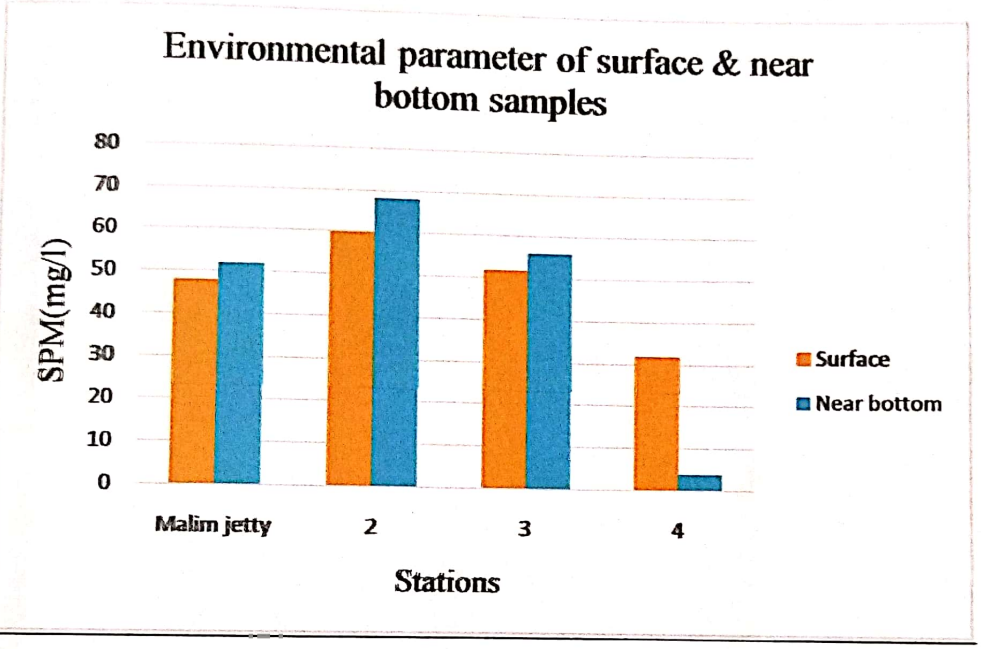
TURBIDITY:

Station	Secchi depth (meters)
1	1.75
2	2
3	2.25
4	1.3

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



DISSOLVED OXYGEN

Station	D.O. (mg/L)	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

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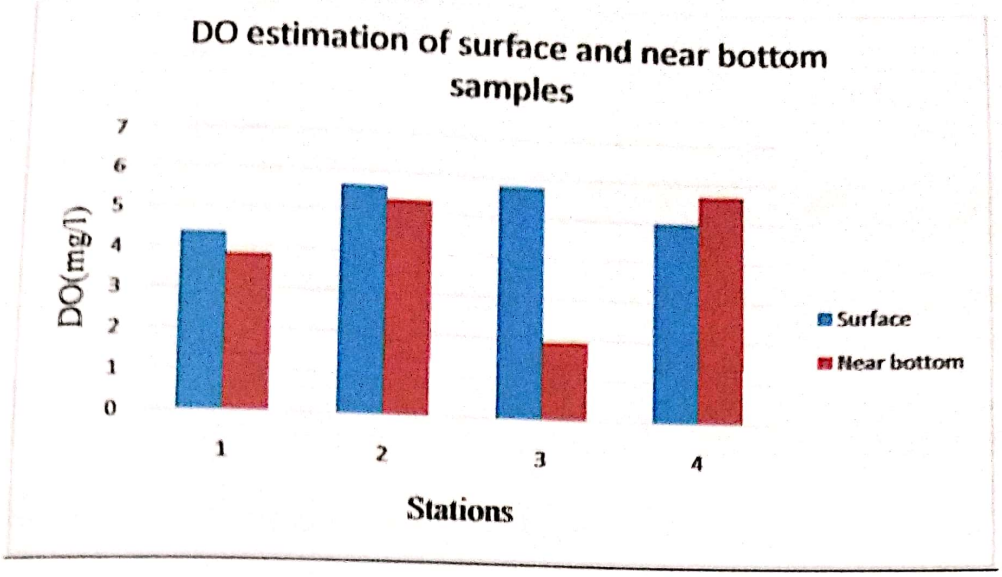

Fig.01 :D.O.bottles with water sample fixed with Winkler's reagents.

MPN (MOST PROBABLE NUMBER)

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

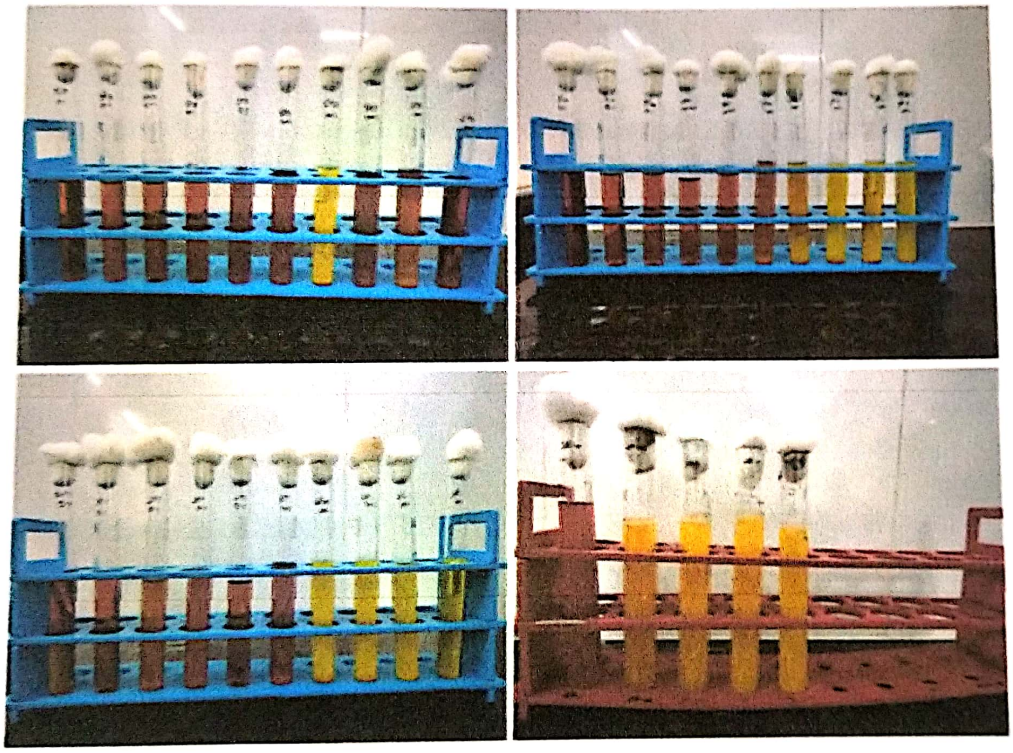

FIG.02:MPN TUBES AFTER 24 HOURS INCUBATION.

VIABLE COUNT :No growth was seen.

PHYTOPLANKTON ANALYSIS

FIG.03:PHYTOPLANKTON SAMPLE FIXED WITH LUGOLS IODINE

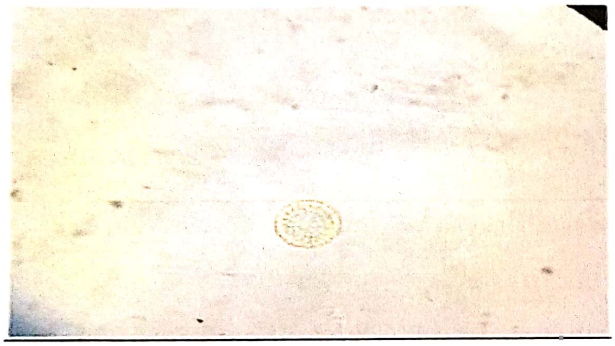

FIG.04:) Coscinodiscus sp.

OBSERVED FROM THE FIXED SAMPLE UNDER MICROSCOPE

CHLOROPHYLL AND PHAEOPIGMENTS :

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

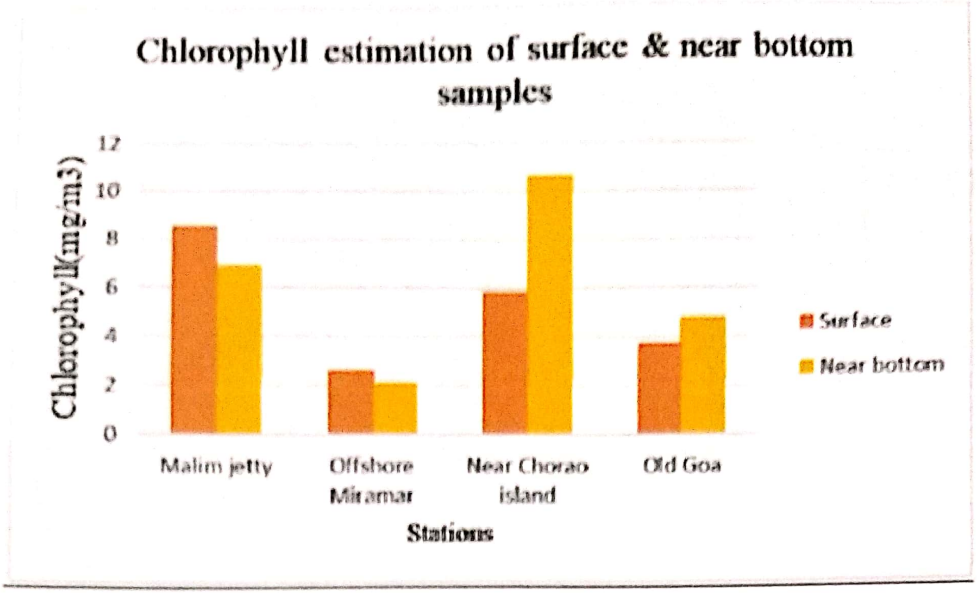

FIG.05 :NISKIN SAMPLER

FIG.06: VAN VEEN GRAB (FOR SEDIMENT COLLECTION)

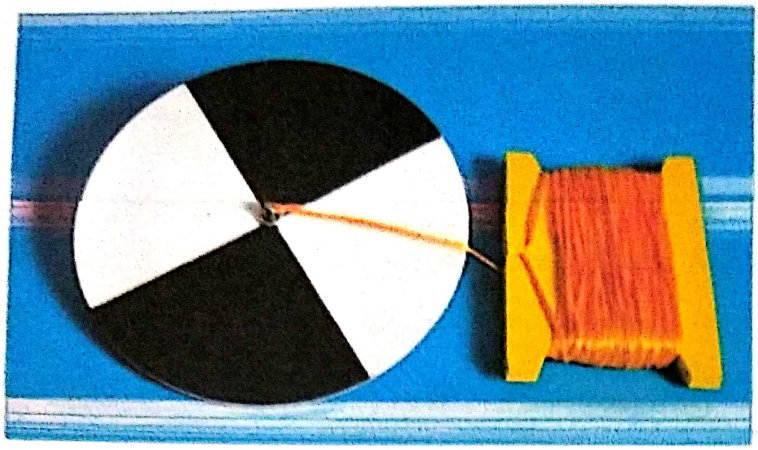

FIG.07: SECCHI DISC

RESULTS:-

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.

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.

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.

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.

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.

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.

PROSPECTIVE :

Going for the field trip has been a great learning experience. We learnt about many new things during the field trip like handling of the water sampling equipments like the niskin sampler ,the van veen grab which we had never seen or used before. We also learnt the different techniques of water sampling ,storage of water sample during the travel and fixing it for further tests to carry out in the lab. In the lab we did different tests to check the parameters and the results were than compiled together.

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