FIELD TRIP REPORT

MMO 319

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21P039005

MSC MARINE MICROBIOLOGY PART II

SEOAS, GOA UNIVERSITY

(2022-2023)

08/10

For Pamila
seen
Amulya
(12/12/22)

SEOAS, Department of Marine Microbiology had organized a trawler trip for the students of Marine Microbiology from Goa University. It was carried out on 11th of March 2022. Our guides for the day were Dr. Nikita Lotlikar and Dr. Varada Damare along with Miss Vaishali and Miss Sitam and the students were 16 of us. All the requirements, apparatus, media and equipments were kept ready the day before the trip. A demo of the instruments were already given to us. The trip began at around 9:00 am in the morning. As decided four sites were selected for the sampling of the samples in Mandovi Estuary i.e Station 1: Malim Jetty, Station 2: Offshore waters of Miramar, Station 3: near Chorao Island& Station 4: Old Goa. We were taken to Station 12 at first followed by Station 1, 3 and 4. Firstly sampling of surface waters was done with the help of buckets, which was then filled in DO bottles, 500 ml plastic bottles for MPN analysis& SPM and chlorophyll estimation and also temperature & salinity was checked. Whereas Niskin samplers were used to collect the near bottom waters and then filled in DO bottles for DO estimation, SPM and chlorophyll estimation and also temperature & salinity was checked. Latitude and longitude of each station was also noted. Sediment sample was collected with Van Veen Grab. Secchi disc was used to measure the turbidity of the water at the locations. All the records were noted down in a notebook. The further analysis were done in the lab right after the samples were collected.

Figure 1. the students present for the field trip along with the teachers and all the materials

AIM : To study the working of the different instruments used for sampling of waters in the marine environment.

The following analysis were carried out:

- MPN
- Viable Count
- Temperature
- Salinity
- Turbidity
- DO estimation
- Suspended Particulate Matter (SPM)
- Chlorophyll estimation
- Analysis of Phytoplankton
- pH

Latitude & Longitude of Sampling sites:

Stations	Latitude	Longitude
1.	15°28'20.8554"N	73°46'37.542"E
2.	15°30'12.3192"N	73°49'55.5234"E
3.	15°30'19.3248"N	73°52'0.894"E
4.	15°30'31.248"N	73°54'50.9832"E

- **Analysis of MPN:**

PRINCIPLE: Most Probable Count (MPN) is used to estimate the concentration of viable microorganisms in a sample by means of replicating liquid broth growth in ten-fold dilutions. In this method, water to be tested is inoculated in lactose broth (i.e, MacConkey's broth), coliforms if present in water utilizes the lactose present in the medium to produce acid and gas. The presence of acid is indicated by the colour change of the medium and the presence of gas is detected by gas bubbles collected in Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e both color change and gas

production) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables.

PROTOCOL:

- water samples were collected from both the surface and near bottom surface of every station.
- The water was collected in sterile centrifuge tubes and then stored in ice box.
- The further analysis was done in the lab.
- The sample water (10 ml) inoculated into 5 tubes containing 10 ml of double strength MacConkey's broth whereas 1 ml of water sample inoculated into 5 tubes of 10 ml MacConkey's broth which was single strength and 0.1 ml of water sample added into 10 ml single strength MacConkey's broth.
- All the tubes contained Durham tubes in it.
- The inoculated tubes were incubated at 37°C for 24-48 hours.
- Positive results were indicated by production of acid (colour change from pink to yellow)and gas(collected in Durham Tube).
- The results were compared to the standard table of McCrady's and the number of bacteria in per 100 ml of sample was determined.

OBSERVATIONS:

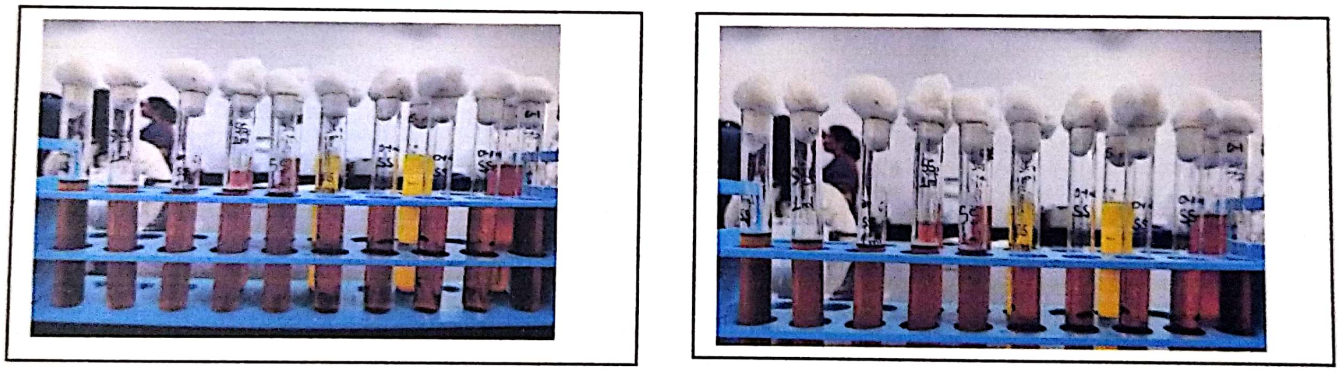

Fig 2. MPN tubes after incubating for 24-48 hours

STATION 1	DS (10 mL)		SS (1 mL)		SS (0.1 mL)	
	Acid	Gas	Acid	Gas	Acid	Gas
1	+	+			-	-
2	+	+	+	+	-	-
3	+	+	+	+	-	-
4	+	+	+	+	-	-
5	+	+	+	+	-	-

Number of positive tubes: 5-4-0=130 bacteria/100 ml

STATION 2	DS (10 mL)		SS(1 mL)		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/ml

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

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

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

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

RESULTS:

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- **Analysis of Viable Count**

PRINCIPLE: A viable count is used to determine the number of cells in the sample capable of forming colonies on a suitable agar medium, with the assumption that each viable cell can yield one colony. For this reason, the viable count is often called the colony plate count or plate count.

PROTOCOL:

- From the samples collected in the centrifuge tubes 0.1 ml of sample was spread plated on 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. Each colony was counted and written down. Average of total number of colonies was taken and viable count was calculated.

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OBSERVATION TABLE:

Surface waters		Colonies	Number	CFU/Ml
1	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
2	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
3	10^{-1}	6	3	2500
	10^{-1}	0		
	10^{-2}	4	4.75	
	10^{-2}	15		
4	10^{-1}	23	8.25	4400
	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	0		

- **Analysis of Temperature**

PRINCIPLE: Done with the help of Thermometer. Thermometer is the oldest form of a temperature sensor and are based on the principle of thermal expansion. It states that liquid expands on heating and contracts on cooling.

PROTOCOL :

- Water sample from surface water and near bottom surface was collected with the help of bucket and niskin sampler respectively. A jug of water taken out from it and temperature was measured by dipping the thermometer into it.
- Readings were noted in the table.

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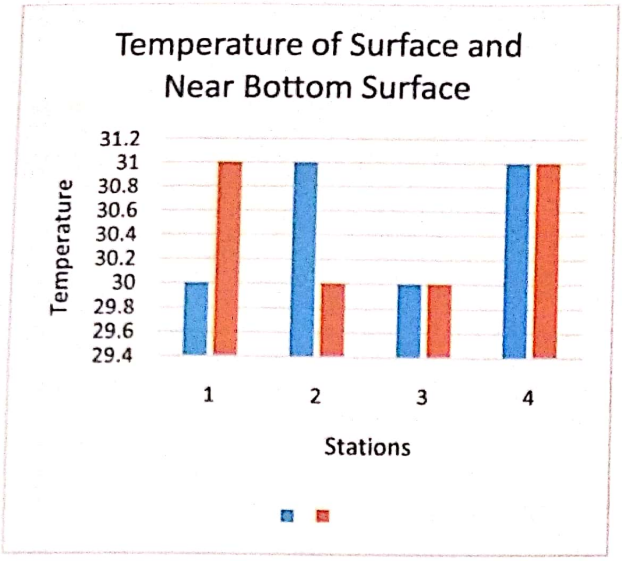
OBSERVATIONS:

STATIONS	TEMPERATURE	
	Surface	Near Bottom
1	30°C	31°C
2	31°C	30°C
3	30°C	30°C
4	31°C	31°C



RESULTS: the temperature across stations was between 30-31°C.

- **Analysis of Salinity**

PRINCIPLE: Refractometers are instruments used to measure substances dissolved in water and certain oils. The refractometer works using the principle of light refraction through liquids. Used to determine the amount of dissolved solids in liquids by passing light through a sample and showing the refracted angle on a scale.

PROTOCOL:

- The water sample were collected from both surface and near bottom surface and a drop of it was added onto the refractometer and the lid was closed.
- The reading was measured by looking at the line that corresponded to the scale.

OBSERVATIONS:

STATIONS	SALINITY	
	Surface	Near Bottom
1	23	28
2	32	33
3	21	29
4	19	22

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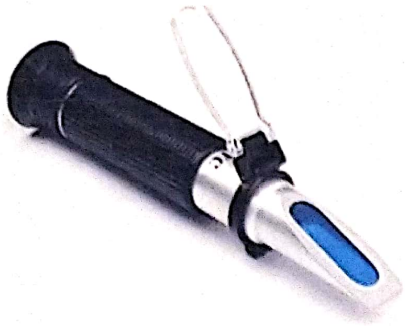
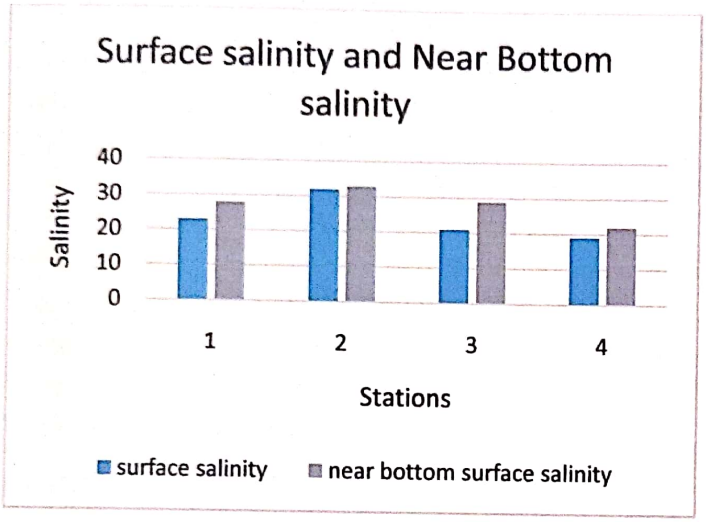


Figure 3. refractometer



RESULTS: Salinity was the highest at station 2 and lowest at station 4 for both surface and near bottom.

- **Turbidity using Secchi disc**

PRINCIPLE: It is an 8 inch disk with alternating black and white quadrants used to measure water clarity. It is lowered into the water until it can no longer be seen by the observer. This depth of disappearance, called Secchi depth is a measure of transparency of the water.

PROTOCOL:

- Secchi disc tied with a rope was lowered into the water until no white or black part of it was visible.
- The rope already had the markings in meters.
- Secchi disc was taken out from water and it was measured.
- The records were noted down at every location.

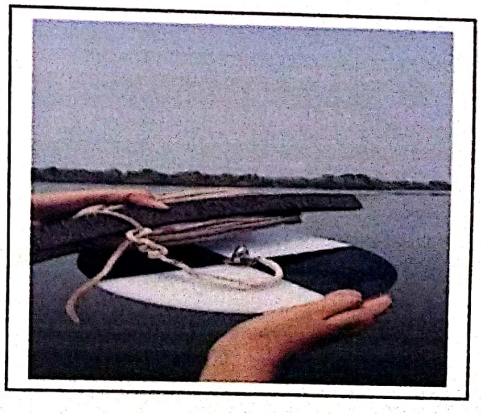


Figure 4. secchi disc

OBSERVATIONS:

STATIONS	Secchi Depth (m)
1	2
2	9
3	1.5
4	1.5

RESULT: The turbidity measured the highest at station 2 and lowest at station 3 & 4.

- **Analysis of DO**

PRINCIPLE: DO estimation is carried out by Winkler Method. It is a technique used to measure the dissolved oxygen in the water sample. Two methods are commonly used to determine DO concentration: (1) The iodometric method which is a titration-based method and depends on oxidizing property of DO and (2) The membrane electrode procedure, which works based on the rate of diffusion of molecular oxygen across a membrane.In the Iodometric method, divalent manganese solution is added to the solution, followed by addition of strong alkali in a glass-stopper bottle. DO rapidly oxidize an equivalent amount of the dispersed divalent manganese hydroxide precipitates to hydroxides of higher valence states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent of the original DO content. The iodine is then titrated with a stranded solution of thiosulfate. The titration end point can be detected visually with a starch indicator.

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.
- 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.
- 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.

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- 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 & repeated three times to obtain mean burette reading.

OBSERVATIONS:

STATION	DO (mg/ml) Surface waters	DO (mg/ml) Near Bottom waters
1	3.1	3.2
2	3.2	2
3	3.5	2.8
4	3.2	3.2

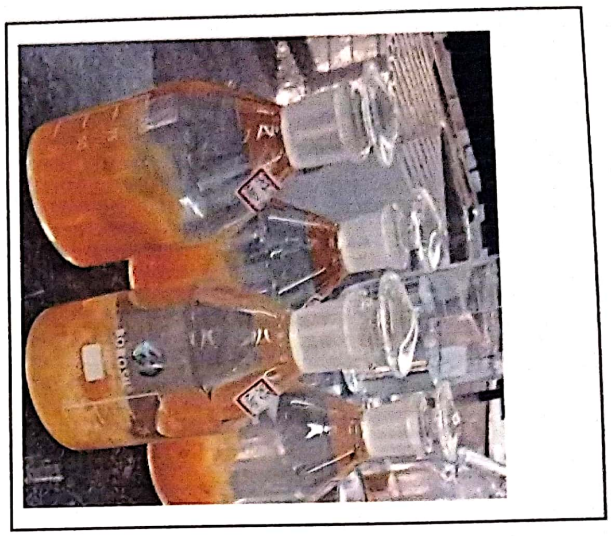
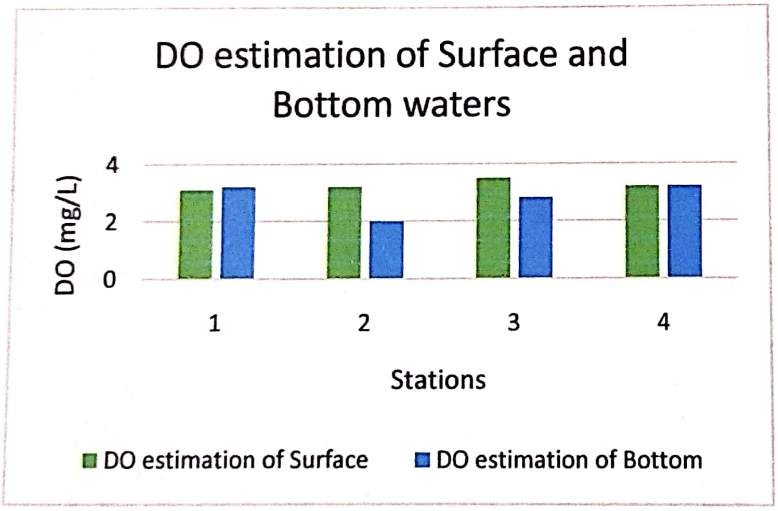


Fig DO bottles Fixed with Winklers A & B



Calculations:

D.O. in mg/lit = 8*100*N/V * v

Where: V = Volume of sample taken (ml)

v = Volume of used titrant (ml)

N = Normality of titrant

8 is the constant since 1ml of 0.025N Sodium thiosulphate solution is equivalent to 0.2mg oxygen.

RESULTS: Among the surface waters, Station 3 showed the highest and Station 1 showed the lowest. Whereas among the near bottom waters, Station 1 & 3 showed the highest and station 2 showed the lowest DO consumption.

➢ **Analysis of SPM (Suspended Particulate Matter)**

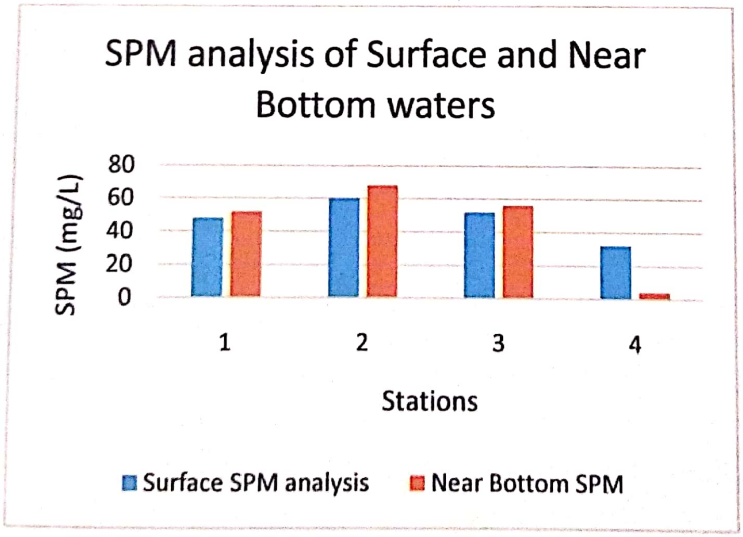
PRINCIPLE: Suspended Particulate Matter is operationally defined via filtration of sample as the material retained on certain type of filter paper of certain pore size, while the matter that passes through filter paper is defined as the dissolved matter. The dry weight concentration of suspended particulate material is measured by passing a known volume of sea water sample through a pre weighed filter paper and reweighing it after drying.

PROTOCOL:

- Water samples were collected from both surface and near bottom surface in 500 ml of water bottles.
- Filtration was done using 0.45 micron pore size filter paper (preweighed).
- After filtration, filter paper with the retained collection was then dried in oven, after which it was measured again.
- The readings were noted down.

OBSERVATIONS:

STATIONS		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
Station3	Surface	0.086	0.099	0.013	52
	Bottom	0.08	0.094	0.014	56
Station4	Surface	0.085	0.093	0.008	32
	Bottom	0.081	0.091	0.01	4



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

RESULTS: SPM was found to be highest at station 2 bottom waters and lowest at station 4 bottom waters.

➢ **Analysis of chlorophyll**

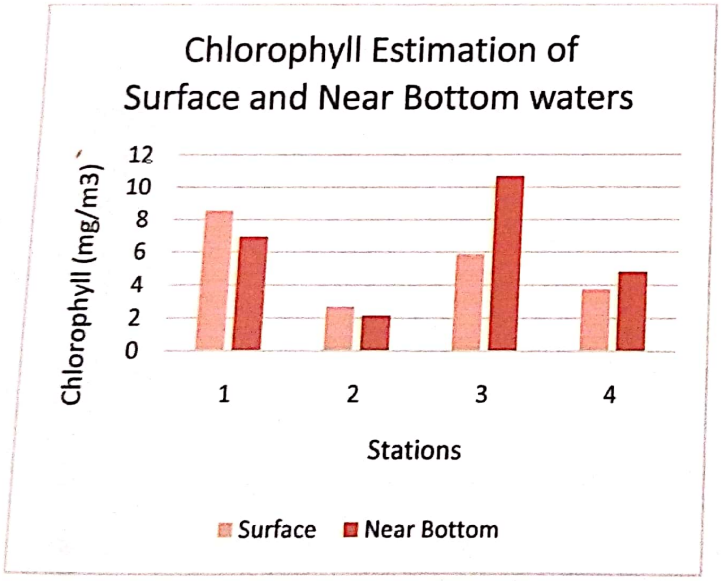
PRINCIPLE: Pigment extraction (phaeopigments, chlorophyll) is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is usedas a solvent in this method since its slight polarity allows it to dissolve polar substances and also allows greater resolution between pigments. Pigment analysis is done spectrophotometrically.

PROTOCOL:

- Samples were collected in 500 ml bottles from both the surface and near bottom surface.
- This was them filtered using 0.75 micron pore size filter paper.
- The filter paper was then removed and cut and placed in vials containing 90% acetone.
- Crush it gently with the help of glass rod and then keep it overnight in refrigerator.
- Next day check for the absorbance at 750 nm.
- Readings were noted down.

OBSERVATIONS:

STATIONS	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



RESULTS: The highest Chlorophyll concentration was found at Station 3, and lowest at station 2 , at near Bottom waters. Whereas highest concentration was found that Station 1 and lowest at station 2 at Surface waters.

➢ **Analysis of Phytoplankton**

PRINCIPLE: the main objective to estimate the phytoplankton count in which the sample first needs to be fixed using Lugol's iodine, to keep the cells intact and to carry the microscopy later.

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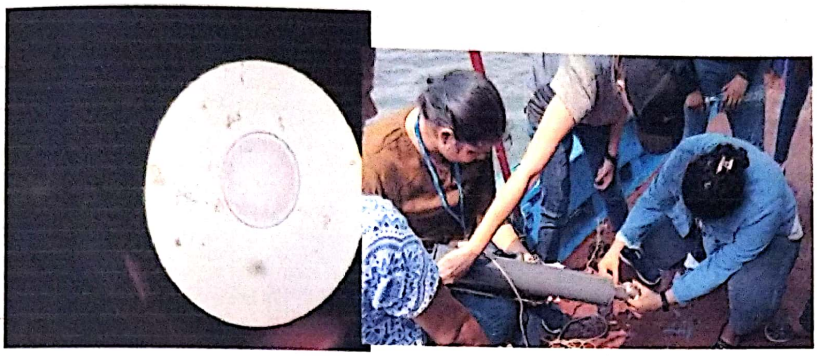
PROTOCOL:

- Water sample from different stations were collected using bucket and niskin sampler.
- 500 ml water bottles were filled and 10-15 drops of Lugol's iodine was used to fix the sample and stored in shade.
- The further analysis were carried in Lab.
- After the settling period, siphoning was done to concentrate the sample.
- Microscopy was done using microscope under 10X & 20X objective lens.

OBSERVATIONS:



Results:

Different types of phytoplankton species were observed under microscope.

➢ **Analysis of pH**

PRINCIPLE: The basic principle of pH meter is to measure the concentration of hydrogen ions. Acids dissolve in water forming positively charged hydrogen ions (H+). The greater the concentration of hydrogen ions, the stronger the acid is.

PROTOCOL:

- The pH meter was turned on and calibrated by placing into neutral pH buffer solution.
- The electrode was washed and wiped with the tissue and the above steps were repeated for acidic and alkaline pH buffer.
- When the pH meter was calibrated, the electrode was washed with distilled water and placed into the sample. The pH readings were measured.
- This experiment was performed for all the samples of different stations.

OBSERVATIONS:

STATIONS	pH
1	8.1
2	8
3	7.6
4	7.9

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Figure 4 pH meter

RESULTS: The pH was found to be in alkaline range, highest at station 1 and lowest at station 3.

PERSPECTIVE

Our filed trip was a great success with a good learning experience. We learnt something new which was really interesting to us. Everything went good as planned. We learned handling of instruments which can useful to us in future. I would like to thank our guides for the day for all the knowledge that they provided us. We fully enjoyed the trip and the lunch offcourse. It was one of the best experiences in life.

Those Happy Faces

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