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FIELD TRIP REPORT

MMO 319

A report submitted in partial fulfillment of the requirements for the Award of Degree of

MASTER OF SCIENCE

In

MARINE MICROBIOLOGY

PART II

For the academic year 2022-2023

Kambli
Seen
09/12/2022

By

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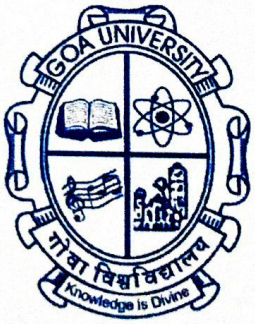

SCHOOL OF EARTH OCEAN AND ATMOSPHERIC SCIENCES

GOA UNIVERSITY, TALEIGAO-GOA

I had attended a field trip on 11th march 2022 organized by the department of Msc. Marine Microbiology, School of Earth ocean and atmospheric science, Goa. which was held in the Mandovi estuary where we learned about collecting seawater samples and collecting data for water parameters such as DO (Dissolved oxygen), MPN (Most probable number),Temperature ,salinity, phytoplankton fixation,, viable count, SPM (Suspend Particulate matter) and chlorophyll extraction and the longitude and latitude was measured at 4 sites in Mandovi Estuary , Panaji. All the necessary requirements like chemical preparation, media preparation and instruments required for the field trip was already prepared and kept ready in advance. The methods used or apparatus were bucket and rope (for surface sample), Niskin Sampler (Near bottom sample) Van Veen grab sampler (for sediment sample), digital pH meter (refractometer-for salinity), glass bottles and centrifuge tubes. The samples were collected from 4 different stations i.e., Station 1: Malim jetty, Station 2: Miramar, Station 3: Chorao, Station 4: Oldgoa .We started off in the morning at 9:00AM with station 2 i.e., Miramar since the water there was very turbulent, later followed by station 1, 3, 4. Once we reached the respective station, the seawater turbidity or seawater transparency was first measured by instrument called as secchi disk by which secchi depth was noted. The salinity and temperature of surface and near bottom was noted using a refractometer (for salinity) and a thermometer, Followed by the rest of the task to be performed. The samples were collected in 500ml and 1litre bottles and respective reagent were added and the bottles were in stored in shade or ice box. The samples were taken back to the lab and the analysis of each experiment was carried out. The following protocols are for the experiments performed.

Fig: location of different sampling station

Latitude, Longitude and Depth of sampling sites:

Stations	Latitude	Longitude	Depth in meters
1	15°30143"N	73°49.9076"S	6
2	15°475"N	73°773"S	10
3	15°30.438"N	73°51.9705"S	3.5
4	15°30.851"N	73°55.171"S	5.25

Table 1: Location of sampling

Analysis were done for the following experiments

- Analysis of Temperature
- Analysis of Salinity
- Analysis of Turbidity using secchi Disk
- Analysis of MPN
- Analysis of Total Viable Count
- DO
- Analysis of phytoplankton
- Analysis of chlorophyll
- SPM
- pH

ANALYSIS OF TEMPERATURE

PRINCIPLE:

As the temperature rises, mercury expands causing it to move upwards and depict the temperature.

PROTOCOL:

- Water samples from various stations were collected in a bucket from the surface, while water from the Niskin sampler was collected for near bottom waters.
- A mug of water was taken out and the thermometer was dipped into it.
- Readings were noted down.

OBSERVATIONS

Stations	Surface temperature	Near bottom temperature
1	30	31
2	31	30
3	30	30
4	31	31

Table: Temperature at surface and near bottom.

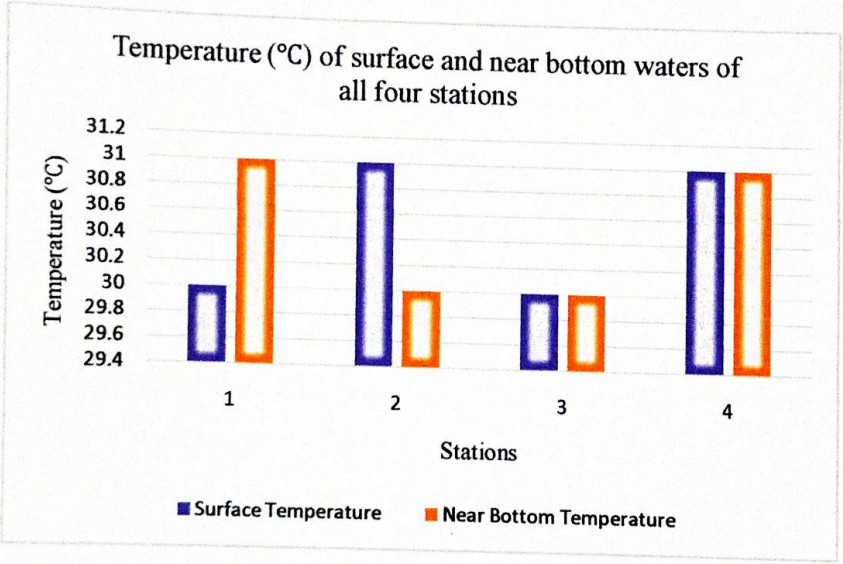

Fig: Graph of temperature (□) of all four stations (surface and near bottom).

ANALYSIS OF SALINITY

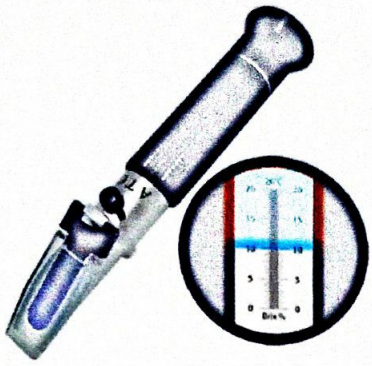

Fig: Refractometer

PRINCIPLE:

A Refractometer is a tool that can determine the concentration of a specific substance in a liquid solution. It employs the principle of refraction, which describes how light bends as it crosses the boundary between two mediums.

PROTOCOL

- Water sample from different stations was collected and using a dropper 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.

OBSERVATION

Stations	Surface salinity	Near bottom salinity
1	23	28
2	32	33
3	21	29
4	19	22

Table: Salinity at the surface and near bottom

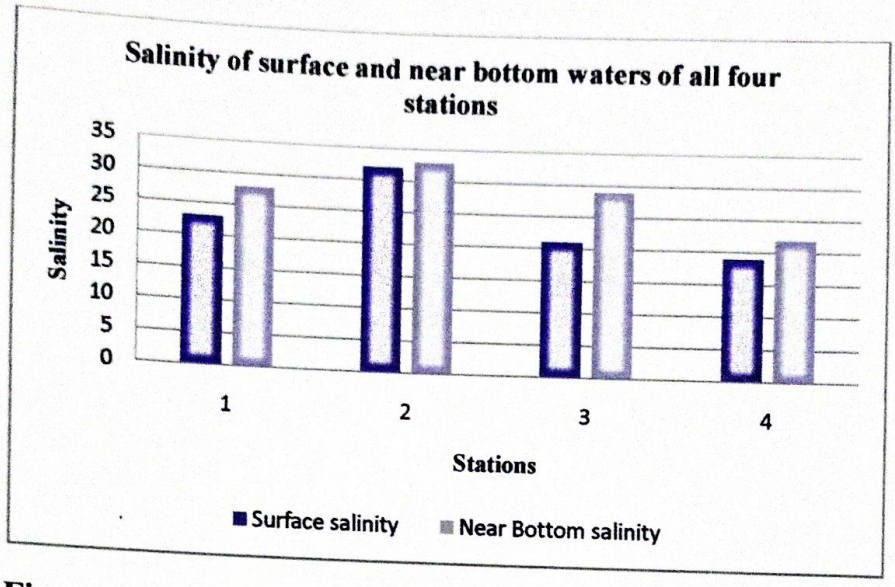

Fig: Graph of salinity of all four stations (surface and near bottom waters)

ANALYSIS OF TURBIDITY USING SECCHI DISK

The Secchi disk is a plain white, circular disk 30 cm (12 in) in diameter used to measure water transparency or turbidity in bodies of water. It has alternating black and white quadrants. The disc is mounted on a pole or line, and lowered slowly down in the water. The depth at which the disk is no longer visible is taken as a measure of the transparency of the water. This measure is known as the Secchi depth and is related to water turbidity.

PROTOCOL

Taking the secchi Disk readings

- Dip the secchi Disk into the water from the shady side of the trawler.
- Keep lowering the disk until the observer could no longer differentiate between the quadrants of the disc.
- Note the depth on the cord
- The depth at which this was observed was noted and designated as Secchi depth, which indicates the water body's turbidity.

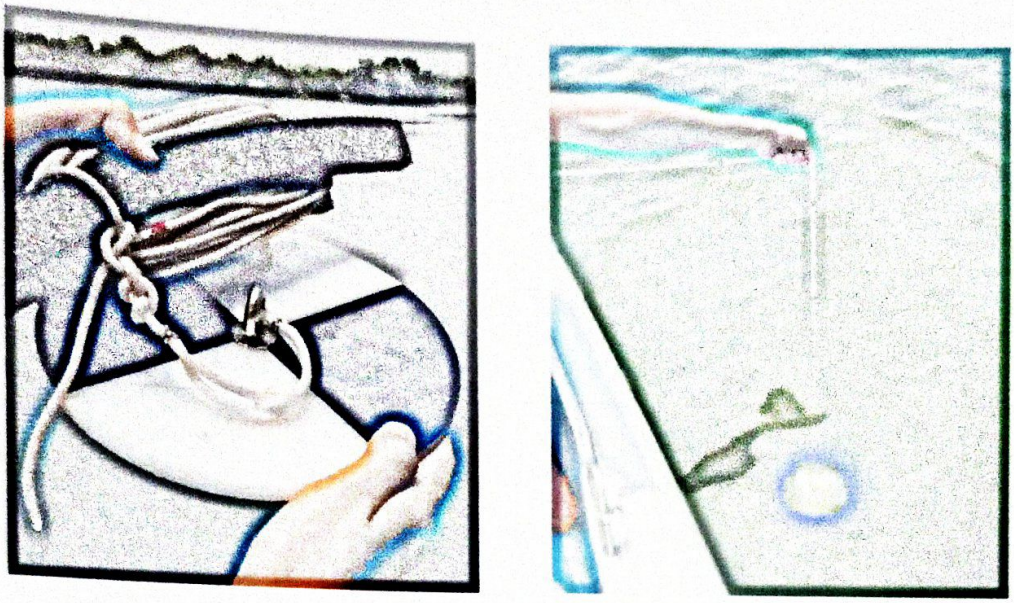

Fig: Secchi Disk

OBSERVATION

Station	Secchi depth (meters)
1	2
2	9
3	1.5
4	1..5

Table: secchi depth

ANALYSIS OF MPN

The Most Probable Number (MPN) method is used to estimate the concentration of viable microorganisms in a sample by replicating liquid broth growth in ten-fold dilutions. It is commonly used to estimate microbial populations in soils, waters, and agricultural products.

PRINCIPLE

This test is primarily used to detect E. coli and coliforms. Lactose fermentation by faecal coliforms is known to produce acid and gas. This is detectable through MPN, where a change in the colour of the media (MacConkey's) indicates acid production and the formation of a gas bubble in the inverted Durham's tube indicates gas production. Both indicate a positive result indicating the presence of faecal coliforms, E. coli, in the water sample.

PROTOCOL

- With the help of a bucket water sample was collected from the station surface.
- The water was then transferred into 50mL sterile centrifuge tubes and stored in ice box for further analysis
- The samples were brought to the laboratory and inoculated in the appropriate volumes of 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.
- 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).

OBSERVATION

Station 1	DS (10mL		SS (1mL		SS (0.1mL	
	Acid	Gas	Acid	Gas	Acid	Gas
1.	+	+				
2.	+	+	+	+	9	9
3.	+	+	+	+		
4.	+	+	+	+	.	.
5.	+	+	+	+		

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

Station 2	DS (10mL		SS (1mL		SS (0.1mL	
	Acid	Gas	Acid	Gas	Acid	Gas
1.						
2.			+	+	9	9
3.	+	+	+	+		
4.	+	+	+	+	.	.
5.	+	+	+	+		

Number of positive tubes: 3-4-0=13-17 bacteria/ 100mL

Station 3	DS (10mL		SS (1mL		SS(O.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

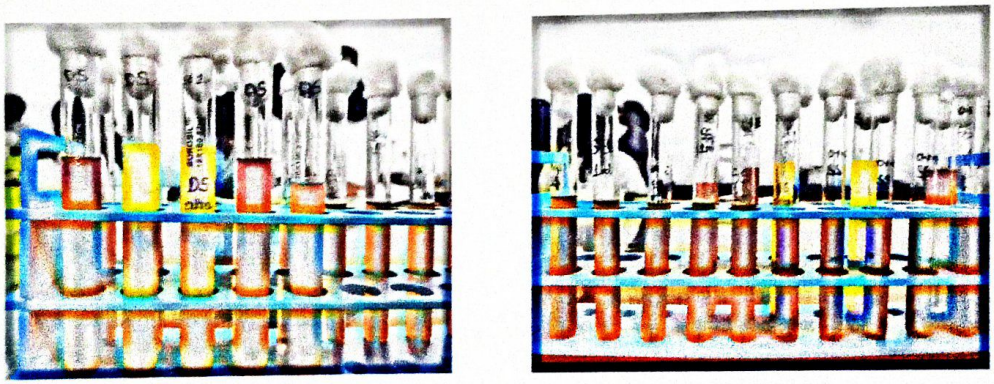

Fig: MPN tubes after the incubation period

ANALYSIS OF VIABLE COUNT

Total viable count (TVC) is a test that estimates the total number of microorganisms in a water sample, such as bacteria, yeast, or mould species.

PRINCIPLE

The viable plate count, also known as the plate count, is a tally of viable or live cells. It is based on the principle that when viable cells are incubated in appropriate conditions for the specimen,

they replicate and form visible colonies. The results are usually expressed as colony-forming units per milliliter (CFU/mL).

PROTOCOL

- 0.1ml sample of the water collected in the centrifuge tubes in the preceding method was taken and spread plated onto ZMA, MacConkey's, TCBS, and XLD agar plates.
- The plates were incubated at 37°C for 24-hours.
- Following the incubation period, the results were recorded. In which each colony was counted and recorded. The total number of colonies was averaged, and the viable count was computed.

OBSERVATION

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)

The dissolved oxygen concentration of seawater if defined as the number of milliliters of dioxygen gas (O_2) per liter of seawater (mL L^{-1}).

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

PRINCIPLE

The Winkler method is used to determine the chemical concentration of oxygen in a water sample. This method is an iodometric titration that uses manganese as a transfer medium to oxidize iodine ion to iodine. This iodine is then titrated against sodium thiosulphate; the end point of redox titration is indicated with starch because it forms a complex compound with iodine, resulting in a blue colour. Iodine molecules can then exit the starch helix and be reduced by thiosulphate. The transition from blue to colourless denotes the end of the titration.

PROTOCOL

I. Determination of reagent blank:

- Pipette out 50mL of distilled water in a conical flask.
- To that add 1mL of 50% H_2SO_4, 1mL alkaline iodide (Winkler B) and 1mL manganous chloride reagent (Winkler A).
- Mix the solution thoroughly to avoid precipitation.
- Add 1mL starch (if blue colour develops titration needs to be carried out).

II. Standardization of thiosulphate solution:

- Prepare the solution in the same method as prepared for blank.
- To that add 10mL of 0.01N potassium iodate solution. Mix the Solution and keep in dark for 3 mins to liberate iodine.

- The solution of liberated iodine is titrated against sodium thiosulphate until it turned pale yellow.
- Add 1mL starch and titration is continued till the colour changes from blue to colourless (remained colourless for 30 seconds).
- This was carried in triplicates to obtain the mean burette reading.

III. **D.O Estimation:**

- Collect the Sample in 125mL D.O. bottles from different stations making sure no air bubbles are formed during collection from surface (using a bucket) and near bottom waters (using a Niskin sampler).
- Fix the D.O bottles by adding 1mL of Winkler's A and 1mL of Winkler's B and the precipitate is left to settle.
- The samples are brought back to the laboratory.
- Add 1mL of 50% H_2SO_4 and shake till the precipitate dissolves.
- Take 50mL of the sample and transfer to a conical flask and titrate against thiosulphate solution until a pale yellow appears.
- Add 1mL starch and titration is continued till the colour changes from blue to colourless (remained colourless for 30 seconds).
- This was repeated three times to obtain mean burette reading. The required calculations were done. (Winkler's Method).
- The amount of DO present in the sample was calculated by using the formula

$$DO = \frac{\text{burette reading} \times V/v \times N \times E \times 1000}{\text{Volume of sample titrated}}$$

OBSERVATION

Blank: 0

Standardization of sodiumthiosulphate: 11.6

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 KIO3 (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.

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

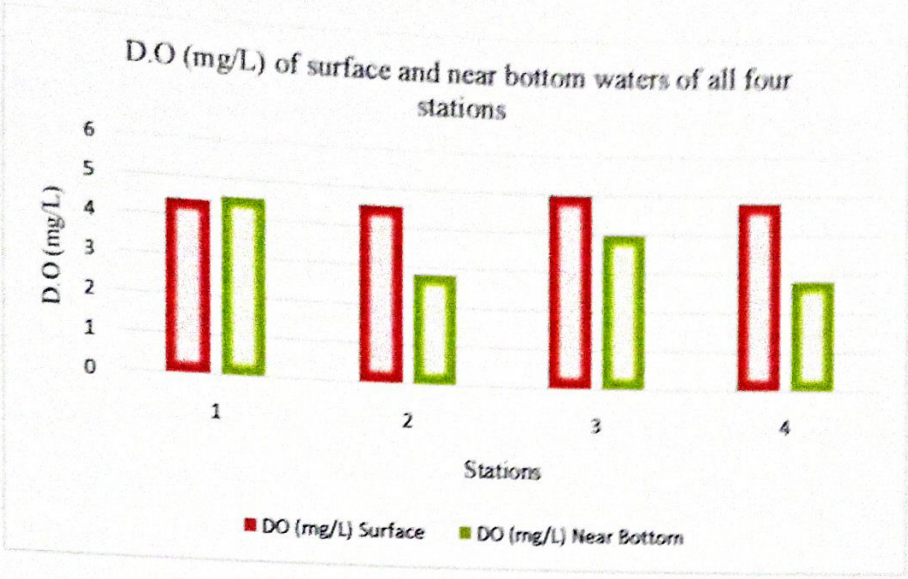

Fig: Graph of D.O. (mg/L) of surface and near bottom waters of all four stations.

ANALYSIS OF PHYTOPLANKTON

PRINCIPLE:

To estimate the amount of phytoplankton's 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.

A) B)

Fig: A) Sample collected and fixed with Lugol's Iodine B) fixed sample bottles after siphoning

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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.

OBSERVATION

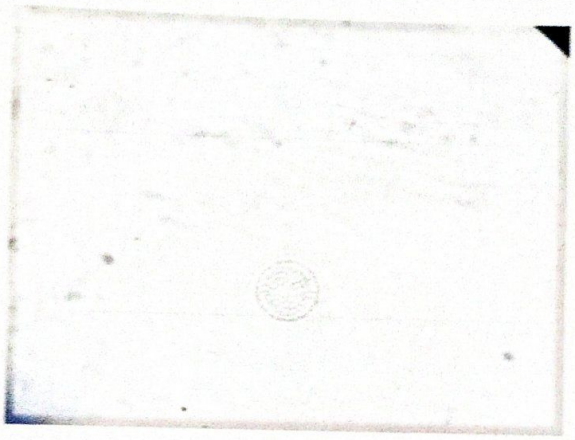

Fig: *Coscinodiscus sp.*

ANALYSIS OF CHLOROPHYLL

PRINCIPLE:

Pigment extraction is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is used as a solvent in this method. It is done by spectrophotometrically.

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 analyzed spectrophotometrically at 665nm, and then 2 drops of HCL acid was added.
- Absorbance was measured at 750nm.
- Readings were recorded and calculations were done.

OBSERVATION

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

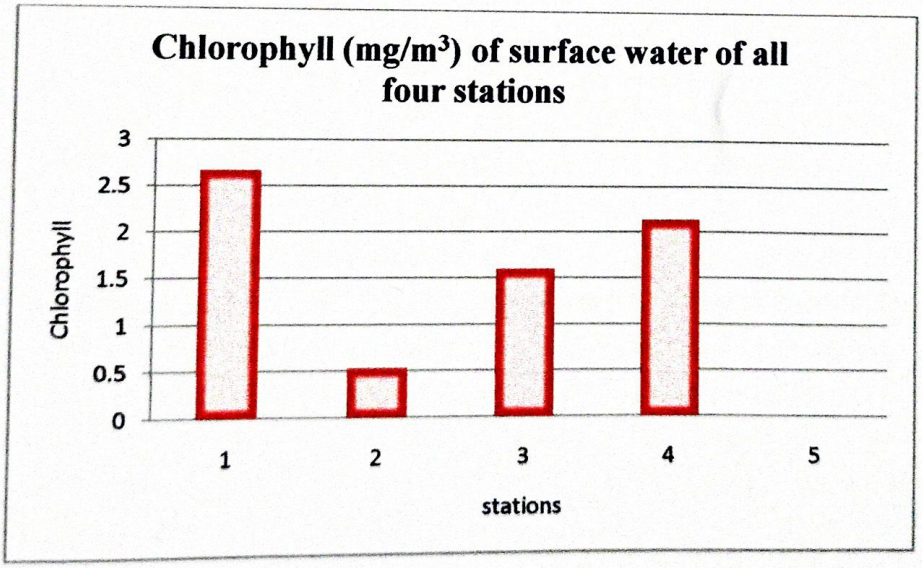

Fig: Graph of chlorophyll (mg/m^3) estimation of all four stations (surface and near bottom)

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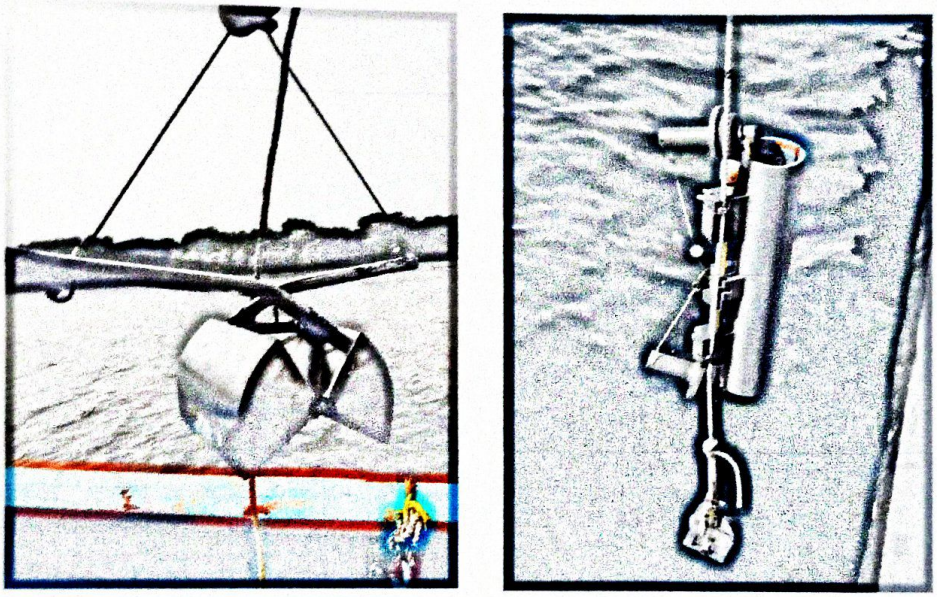
CALCULATION

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

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

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).



A) B)

Fig: A) Van Veen grab for sediment collection B) Niskin sampler for near bottom sample collection

ANALYSIS OF SPM

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. 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.

OBSERVATION

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

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

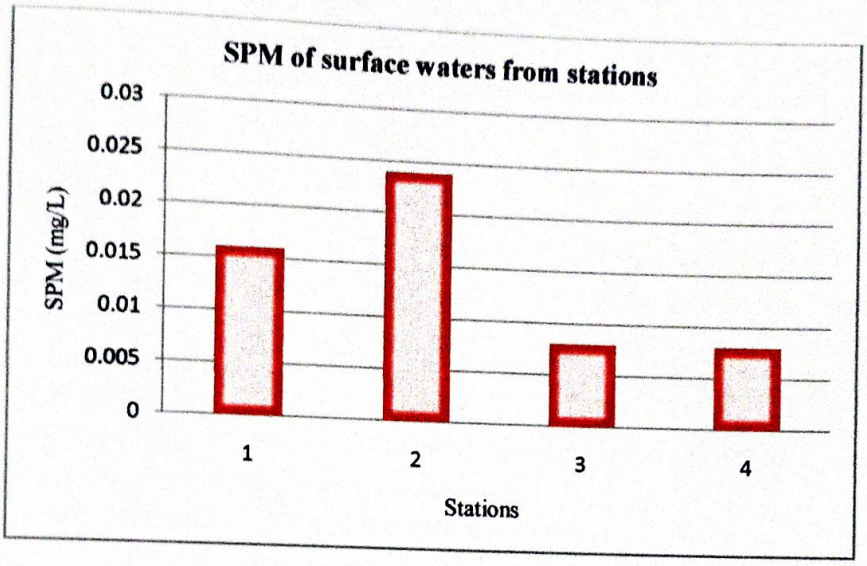


Fig: Graph of SPM (mg/L) of all four stations

ANALYSIS OF pH

pH is a measure of the amount of free hydrogen ions in water. A pH of 7 is considered to be neutral. Acidity increases as pH values decrease, and alkalinity increases as pH values increase.

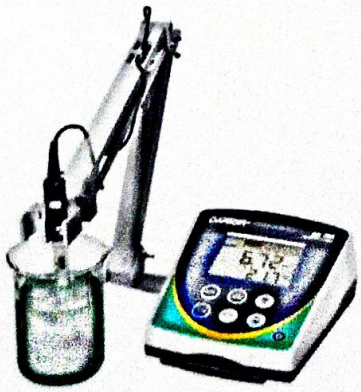


Fig: pH meter

PRINCIPLE

The potentiometric method is based on measuring the cell emf in an electrochemical cell with one electrode selective for hydrogen ions and the other serving as a reference. One important

implication of this fact is that the change in potential caused by moving the electrodes from the buffer to the sample is the sum of all changes 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:

Station	pH
1	8.1
2	8
3	7.6
4	7.9

Table: pH

RESULTS:

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 1 showed the highest and station 2 and 4 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.

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 1 and lowest at station 3.

MPN: As observed from the readings station 1 has 130 bacteria/100m L and station 2 has 13-17 bacteria/100mL and 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 bacterial/100mL.

Estimation of phytoplankton: The following phytoplankton were observed – Diatoms: *Rhizosolenia* sp., *Coscinodiscus* sp. ,*Gyrosigma* sp., *Chaetoceros* sp., and an unidentifiedpennate diatom,

SPM: SPM was highest at station 2 and lowest at station 3 and 4.

Chlorophyll: Chlorophyll concentration was found to be highest at station 1 and lowest at station 2.

CONCLUSION

It was a mesmerizing experience we all had during this field trip. I have gained a lot of working experience new knowledge and skills. We learned a variety of sample collection techniques. We learned how to use and handle various instruments such as the Niskin sampler and the Van Veen grab. We also gained experience in proper sample collection and storage in order to obtain accurate results for analysis of various water parameters such as D.O., phytoplankton fixing, and so on. We witnessed the difficulties of sampling in offshore waters, and how turbulence caused by strong wave action can cause problems with sample collection. We also had fun while working plus we ate food that was cooked and served in the middle of estuary. It was marvelous experience.

We now have a clear understanding of how things work practically and how to obtain results. I learnt many theories and analysis methods in class, but when I had the chance to see and how to apply these lessons in real life, it expanded my horizons.

Fig: Food served and students along with teaching and non teaching staff.

REFERENCE:

- Strickland, J. D. H., and Parsons, T. R. (1965). 'A Manual of Seawater Analysis', 2nd edn. (Queen's Printer and Controller of Stationery: Ottawa.)
- 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., and Lalli, C. M. (1984). 'Manual of Chemical and Biological Methods for Seawater Analysis.' (Pergamon Press: New York.)

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