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FIELD TRIP REPORT

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SEOAS, GOA UNIVERSITY

Submitted by

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I would also like to thank all my colleagues who worked along with me with their patience and openness that created an enjoyable working environment. It is indeed with a great sense of pleasure and immense sense of gratitude that I acknowledge the help of these individuals.

EXECUTIVE SUMMARY

This report encapsulates all the learning outcomes that resulted from the field trip in Mandovi Estuary. This study tried to explore the microbial community and measured the biological and physiological parameters of water from four sampling stations i.e., Miramar Offshore, Malim Jetty, Divar Island and Old Goa by using different methods and instruments. For this purpose, we collected and analyzed samples using different instruments and methods such as bucket, Niskin sampler, DO bottles, Plastic bottles, Centrifuge tubes, pH meter, refractometer, Niskin sampler, thermometer, etc. From this study, we identified two species of phytoplankton (such as *Coscinodiscus* sp, *Nitzchia* sp.), estimated MPN, Viable count, D.O, Temperature, Salinity, Turbidity, pH, Analysis of phytoplankton, Chlorophyll estimation and Suspended Particulate Matter.

INTRODUCTION

A field trip was organized by the Marine Microbiology SEOAS Department, Goa University on 11th March 2022. Sixteen students of Marine Microbiology were accompanied on this one-day trip on a trawler by professors, Dr. Nikita Lotlikar, Dr. Varada S. Damare and two Laboratory associates, Ms Sitam and Ms Vaishali. Sample Collection were done at four different Stations. They are: Station 1- Miramar off shore, Station 2- Malim jetty, Station 3- near Chorao Island and Station 4- Old Goa. This report is a detailed analysis of the sea water and sediment sample collected during the field visit in Mandovi estuary.

One day prior to the field trip, preparation of reagents, preparation of media, sterilization of glasswares required for analysis were done well in advance. Demo of the instruments, techniques for sample collection, jobs to be done for the field trip were explained distributed equally among the students accurately. In this field trip we learnt about the sampling of water and sediments. Sampling was carried out for surface water and near bottom waters and sediment. For surface water, the sampling was done using bucket and near bottom water was done using a Niskin Sampler. Sediment sampling was done using Van Veen Grab. Turbidity was also measured using Secchi disc. The report also gives an insight into the various objectives of the trip and how these were fulfilled by the students. It also provides a view of the learning outcomes of the field visit.

Fig.1. Students along with teaching and non-teaching staff

Fig.2. Demo of working of Niskin Sampler

OBJECTIVES

The study trip made to Various Stations had the main aim of collecting sediment and sea water from surface and near bottom. The students were thus exposed to the areas where the samples could be collected. The objectives of the field trip are mentioned as follows:

- The sample collection trip gave each and every student who were part of the trip, an idea about the places, techniques and instruments that can used to collect samples.
- Exposure to sample collecting techniques
- To gain some practical knowledge of Marine ecology and Marine resources
- Knowledge of preservation of the collected samples- The samples collected for bacteria in centrifuge tubes were to be kept in ice and the samples for phytoplankton fixed with iodine, SPM and chlorophyll were to be placed in dark after collection for future uses in the laboratory and for other research works. The preparation of preserving solutions was prepared by the students themselves under guidance of an expert. This gave knowledge about constituents of the solution. The trip therefore, was arranged with all these aims ahead and thus imparted all essential knowledge about all the parameters mentioned above.
- Following parameters were analyzed:
  - MPN
  - Viable count
  - D.O
  - Temperature
  - Salinity
  - Turbidity
  - pH
  - Analysis of phytoplankton
  - Chlorophyll estimation
  - Suspended Particulate Matter

Fig. 1. (a) Niskin Sampler

(b) sample collection using Niskin

Fig. 2. Van Veen Grab

Locations of all four stations along with latitude and longitude.

Sr. No.	Location	Station 1 Miramar Offshore	Station 2 Malim Jetty	Station 3 Chorao Island	Station 4 Old Goa
1.	Latitude	15°.475'N	15°30.143'N	15°30.438'N	15°30.851'N
2.	Longitude	73°.773'E	73°49.907'E	73°51.970'E	73°55.171'E

MPN ANALYSIS:

Principle: Water to be tested is diluted serially and inoculated in lactose 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 color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted 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 table.

MPN test is performed in 3 steps:

- ✓ Presumptive test
- ✓ Confirmatory test
- ✓ Completed test

PROCEDURE FOR MPN:

• Water sample collection was done from the surface of all four stations 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 (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.

OBSERVATIONS:

STATION 1	DS (10ml)		SS (1ml)		SS (0.1ml)	
	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 (0.1ml)	
	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

Fig 3. Single strength tubes after incubation

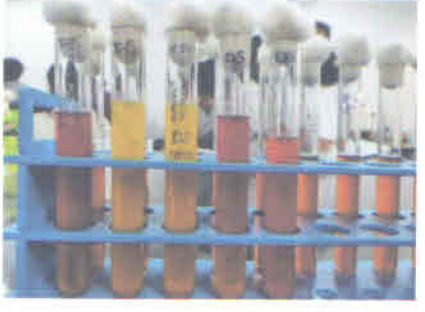


Fig 4. Double strength tubes after incubation

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

RESULTS: MPN analysis was performed for all four stations. 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.

ANALYSIS FOR VIABLE COUNT:

Principle: Total viable count (TVC), gives a quantitative estimate of the concentration of microorganisms such as bacteria, yeast or mould spores in a sample. The count represents the number of colony forming units (cfu) per g (or per ml) of the sample.

TVC is achieved by plating serial tenfold dilutions of the sample until between 30 and 300 colonies can be counted on a single plate. The reported count is the number of colonies counted multiplied by the dilution used for the counted plate

A high TVC count indicates a high concentration of micro-organisms which may indicate poor quality for drinking water or foodstuff.

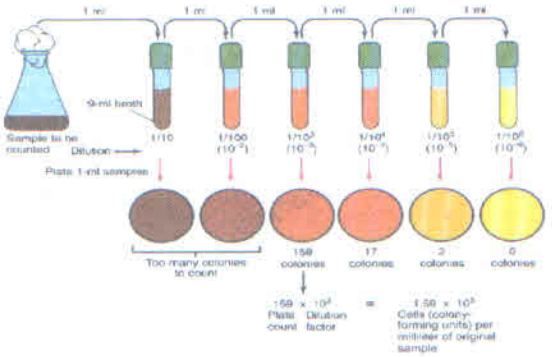


Fig. 5. Viable Count Technique

PROCEDURE:

• 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	No. Of Colonies	Average Number	Dilution Factor Cfu/Ml
Station 1	10^{-1}	6	7	1100
	10^{-1}	8		
	10^{-2}	2	1.5	
	10^{-2}	1		
Station 2	10^{-1}	10	12	1100
	10^{-1}	14		
	10^{-2}	1	1	
	10^{-2}	1		
Station 3	10^{-1}	6	3	2500
	10^{-1}	0		
	10^{-2}	4	4.75	
	10^{-2}	15		
Station 4	10^{-1}	23	8.25	4400
	10^{-1}	10		
	10^{-2}	16	8	
	10^{-2}	0		

RESULTS: Colonies were observed on the plates and the total viable count was calculated.

ANALYSIS FOR DO:

Principle: the concentration of DO in water varies in response to changes in atmospheric pressure and water temperature. The higher the atmospheric pressure, the higher the oxygen solubility in water and the higher the potential DO concentration. The opposite is true with temperature, where the higher the temperature, the lower the solubility and saturation concentration of oxygen in water. DO is one of the major factors that determines the types of biological communities that inhabit an aquatic system. The addition of organic or inorganic material that exerts an oxygen demand through respiration and biodegradation lowers the DO concentration and can facilitate the growth of nuisance organisms.

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.

PROCEDURE:

1. **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)

2. **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

3. **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% 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.

✓

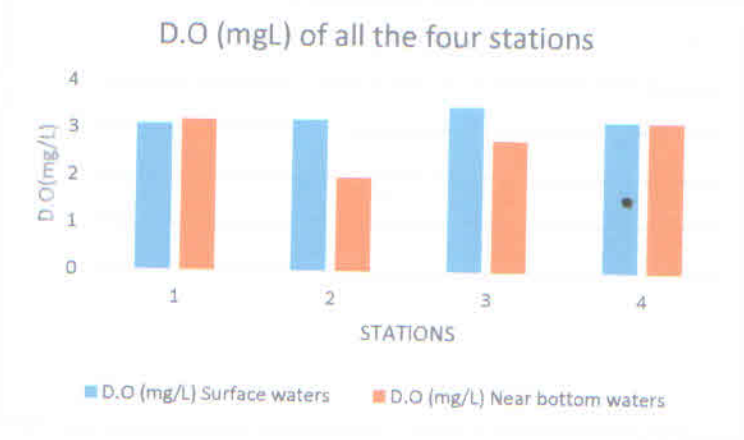
• 1mL starch was added and titration was continued until the blue colour disappeared. Burette reading was noted. This was repeated three times to obtain mean burette reading. The required calculations were done. (Winkler's Method).

Fig 6. Water Collection for DO

Fig 7. D.O bottles after fixing with Winkler's A and B

OBSERVATIONS:

STATION	D.O (mg/L)	
	Surface waters	Near bottom waters
1	3.1	3.2
2	3.2	2
3	3.5	2.8
4	3.2	3.2



RESULTS:

- Among the surface waters in all the four stations, station 3 showed the highest and station 1 showed the lowest concentration of D.O.,
- Among the near bottom waters station 1 and 4 showed the highest and station 2 showed the lowest concentration of D.O.

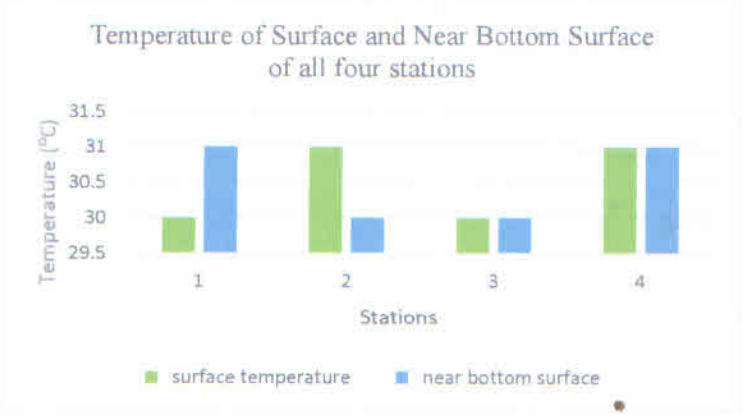
ANALYSIS OF TEMPERATURE:

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.

PROCEDURE: Water sample from different stations was collected in a bucket from the surface while for near bottom waters using Niskin sampler. From this, a mug of water was taken out and the thermometer was dipped into it and the temperature was determined.

OBSERVATION:

STATION	TEMPERATURE (°C)	
	Surface water	Near bottom water
1	30°C	31°C
2	31°C	30°C
3	30°C	30°C
4	31°C	31°C



RESULTS: The temperature across all the stations in surface waters and near bottom waters was between 30-31°C.

ANALYSIS OF SALINITY

Principle: The salinity of seawater is defined as the total amount by weight of dissolved salts in one kilogram of seawater. The average salinity of seawater is typically about 35. Salinity varies globally across the surface and with depth. Across the surface salinity varies with latitudes. At the poles, salinity is lowest, it is the highest at Tropic of Cancer and Tropic of

Capricorn and reduces near the equator. Several methods/instruments can be used to determine salinity, such as evaporation, salinometer, measurement of chlorinity and a rosette sampler coupled with CTD (Conductivity Temperature Depth profiler).

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.

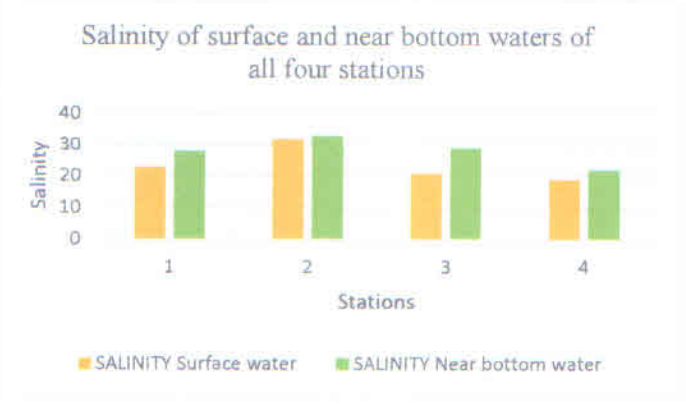
Fig.8. Refractometer

PROCEDURE:

- 2-3 drops of collected water sample was put using a dropper onto the refractometer.
- The lid of the refractometer was closed carefully taking care that no air bubbles were formed and the readings were viewed through the eyepiece.
- Similarly the salinity was noted for all the stations for surface and near bottom waters.

OBSERVATIONS:

STATIONS	SALINITY (%)	
	Surface water	Near bottom water
1	23	28
2	32	33
3	21	29
4	19	22



RESULTS: The salinity of all four stations were determined using refractometer. Salinity was found to be highest at station 2 and lowest at station 4.

ANALYSIS FOR TURBIDITY USING SECCHI DISC:

Principle:

The Secchi disk (or Secchi disc), as created in 1865 by Angelo Secchi, is a plain white, circular disk 30 cm (12 in) in diameter used to measure water transparency or turbidity in bodies of water. 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

Fig. 9. Secchi Disc

PROCEDURE:

- On all the stations Secchi disc was gently lowered into the water from the trawler with the help of a rope.
- It was lowered to a depth where the observer could no longer differentiate between the quadrants of the disc.
- The depth at which this was observed was noted as Secchi depth.

OBSERVATION:

STATIONS	SECCHI DEPTH (m)
1	2
2	9
3	1.5
4	1.5

RESULTS: Turbidity was highest at station 2 and lowest at station 3 and 4.

ANALYSIS FOR pH

Principle: A pH meter is an analytical instrument used to measure the activity of hydrogen ions in a solution which is then expressed as pH. The overall working principle of pH meter depends upon the exchange of ions from sample solution to the inner solution (pH 7 buffer) of glass electrode through the glass membrane.

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. 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.

Fig. 10. pH Meter

PROCEDURE:

- The pH meter was turned on and calibrated.
- The electrode was rinsed with distilled water and wiped gently with tissue paper.
- It was placed in neutral pH buffer solution, when the reading on the screen stabilized it denoted as 'ready', after which enter button was pressed.

- The electrode was washed and wiped again similarly and the same steps were repeated for acidic and alkaline pH buffer.
- After calibration, the electrode was rinsed and placed in the sample and the measurements were recorded.

OBSERVATION:

STATION	pH
1	8.1
2	8
3	7.6
4	7.9

RESULTS: The pH was found to be in the range of 7.5- 8.1 with the highest pH being found at station 1 and lowest at station 3.

ANALYSIS OF PHYTOPLANKTON

Principle: Phytoplanktons constitute the basis of nutrient cycle of an ecosystem; hence play an important role in maintaining equilibrium between living organisms and abiotic factors. Fixation of the phytoplankton sample is required to estimate the number of phytoplanktons in the sample to keep the cells intact and carry out microscopy later. Qualitative analysis can also be done using the same method. The samples are fixed to prevent the adverse effects of light, temperature and other microorganisms that might cause rapid decay of organisms.

PROCEDURE:

- Water samples from different stations were collected in a bucket from the surface and by using a niskin sampler from the near bottom waters.
- Samples were filled into 500 ml bottles and 15 drops of lugol's iodine solution was added and the bottles were stored in shade until further analysis.
- The bottles were brought back to the laboratory and was kept for settling.
- After settling siphoning was done to concentrate the sample.
- Microscopy was carried out using an inverted microscope under 10X and 20X objective lens.

Fig.11. Sample fixed with Lugols Iodine

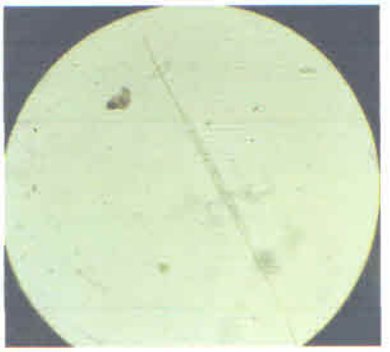


Fig. 12. *Nitzschia* sp.

Fig. 13. *Coscinodiscus* sp.

RESULTS:

Under microscopical examination, *Coscinodiscus* sp and *Nitzschia* sp. were observed.

CHLOROPHYLL ESTIMATION:

Principle: Pigment extraction is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is used as a solvent since its polarity aloows to dissolve polar substances and allows greater resolution between pigments.

The pigment chlorophyll a generally contributes 0.5-1 per cent of the ash-free dry weight of phytoplankton organisms. Although the pigment content may vary according to the physiological state of the organisms (e.g. it increases if light availability is low), chlorophyll a is a widely used and accepted measure of biomass. It is an especially useful measure during cyanobacterial blooms, when the phytoplankton chiefly consists of cyanobacteria, often of only one species. However, when chlorophyll a determination is used with mixed phytoplankton populations (cyanobacteria and other species), it gives an overestimation of cyanobacterial biomass. Rough microscopic estimations of the relative share of cyanobacterial cells among

the total phytoplankton may be used to correct the overestimate. Analysis of chlorophyll a requires relatively simple laboratory equipment, principally filtration apparatus, centrifuge and spectrophotometer. However, the main procedural steps in most methods are essentially the same: solvent extraction of chlorophyll a, determination of the concentration of the pigment by spectrophotometry, and adjustments to the result to reduce the interference by phaeophytin a which is a degradation product of chlorophyll a.

PROCEDURE

• 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 μm was placed in the filtration unit attached to the vacuum pump.

• Around 500mL of seawater sample is filtered through 0.75μ 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.

OBSERVATION:

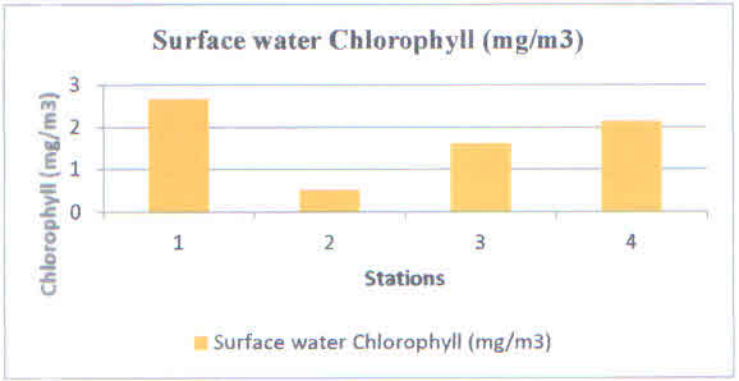
STATION	Surface water Chlorophyll (mg/m^3)
1	2.67
2	0.53
3	1.60
4	2.14

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).



RESULTS:

The highest chlorophyll concentration was found at station 1 and lowest at station 2.

ANALYSIS FOR SUSPENDED PARTICULATE MATTER (SPM):

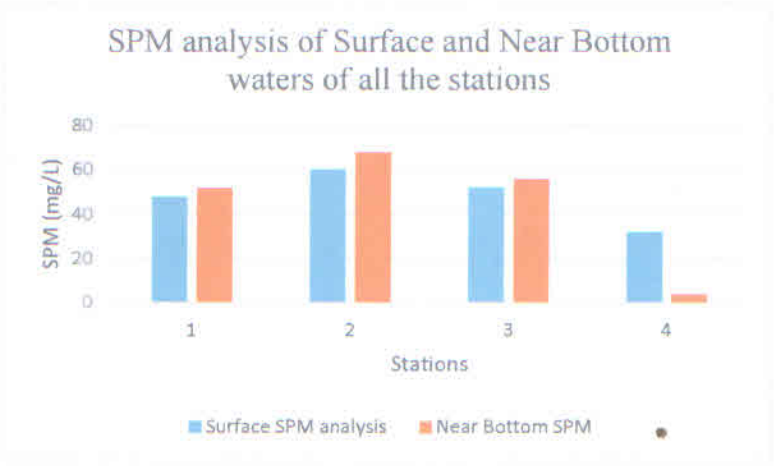
Principle: Suspended particulate matter (SPM) is operationally defined via filtration of seawater as the material retained on a certain type of filter with certain pore size, while the matter that passes through a small pore size filter is defined as dissolved matter (DM). The dry weight concentration 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.

PROCEDURE:

- Samples from the surface water as well as the near bottom water was collected in plastic bottles and kept in shade.
- 0.45µ filter paper was placed in the filtration unit attached to the vacuum pump.
- The weight of the filter paper was measured before filtering the water sample through it.
- Around 250 ml of sample was filtered through the filter paper.
- The weight of the filter paper was measured again after filtration. (Wet weight).
- The filter paper was then completely dried in the oven at 30°C and the weight was measured again. (Dry weight)
- The readings were noted down and calculations were done.

BSERVATION:

STATION	WATER SAMPLE	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
	Near bottom	0.082	0.095	0.013	52
2	Surface	0.088	0.103	0.015	60
	Near bottom	0.084	0.101	0.017	68
3	Surface	0.086	0.099	0.013	52
	Near bottom	0.08	0.094	0.014	56
4	Surface	0.085	0.093	0.008	32
	Near bottom	0.081	0.091	0.01	4



CALCULATION:

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

RESULTS:

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

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. 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. We also learnt the techniques of field studying. The food was delicious and everyone enjoyed it The trip was a great success and a memorable one.

PHOTO GALLERY

Fig. 14. Students of MSc MM Part II

Fig. 15. Malim Jetty

Fig. 16. Food served on the Trawler

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