FIELD TRIP REPORT

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MMO 319 - Field Trip/Study Tour - Practical

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M.Sc. MARINE MICROBIOLOGY PART II

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Seen
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A educational field trip was organised for us by department of MSc Marine Goa University for Part I and Part II students. The date was decided for the field trip was 10th and 11th March 2022, and both the classes were divide into two batches. We gathered near the Mallim jetty at around 8:00am and waited for our professors to arrive. Prof. Dr. Varada Damare and Prof. Dr. Nikita Lotlikar were with us as our guide and Ms. Vaishali was from Non teaching staff, along with us Part I (16) and from Part II one student was there, also one trawler along with two cooks we had completed our field trip in full enthusiasm. Thia was my first time to visit of a sea to the middle.

We were to visit 4 stations

STATION 1- Mallim jetty

STATION 2- Miramar off-shore

STATION 3- Chorao island

STATION 4- Old goa

We had started the sampling with station 2 as it was slightly into the deep waters, the tides in the morning were low as there was suitable environment for the sampling. As the trawaler stopped we experienced a lot of drifting. The aim of the field trip was to study the water and sediment parameters for which sample has been collected and also to get hands on training on the techniques learnt in theory.

To check the temperature, dissolved oxygen (DO), salinity, phytoplankton fixation and chlorophyll and phaeo pigment estimation from surface water, water sample was collected with the help of the bucket. To analyze the near bottom water parameters, we used the Niskin sampler. To collect sediment sample, using Van Veen Grab. Samples which needed to be fixed were fixed and kept safely after which they were analysed in the lab. Water turbidity was carried out using sacchi disk.

One by one we had headed to other stations and after all sampling stations were completed, we had lunch and we return back to Mallim jetty by 4;00pm, we had analysed the collected sample in the lab.

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➢ STATION DETAILS

Station number	Name	Latitude	Longitude	Depth
1	Malim Jetty	15º30.143'N	73º49.907'E	6 m
2	Offshore Miramar	15º28.264'N	73º46.228'E	10 m
3	Chorao	15º30.438'N	73º51.970'E	3.5 m
4	Old Goa	15º30.851'N	73º55.171'E	5 m

➢ SURFACE WATER SAMPLING

For all stations the surface water was collected by using a bucket and a rope. The bucket was lowered in the water and tilted so water is collected and bucket then pulled up with the help of a rope.

➢ BOTTOM WATER SAMPLING

For all the stations bottom water sampling was the help of Niskin sampler. The sampler was lowered to the depth till weight tide with it touches to depth. Then the messenger was set free which closed the Niskin sampler and trapping water inside it. The sampler was pulled up to the trawler and water sample has been taken for analysis

➢ SEDIMENT SAMPLING

To collect the sediment sample, we use the Van Veen Grab. We only able to collect sediment sample from the station to as the other stations were rocky (station I and IV) and dredged (station III). The sample collected was then stored in polythene bag in an ice-bo

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NISKIN SAMPLER VAN VEEN GRAB

➢ PARAMETERS TO ANALYSED

- Temperature
- Salinity
- Sacchi depth
- Dissolved Oxygen (DO)
- Viable Count
- Phytoplankton enumeration
- Suspended Particulate Matter (SPM)
- Detection of bacteria by MPN (Most Probable Number)
- Chlorophyll and phaeo-pigments
- Water turbidity

1. TEMPERATURE

Sea water temperature were measured using a thermometer, it is the indicator of productivity, pollution and global climate change. The temperature now is measured by various instruments which can be directly connected with satellites.

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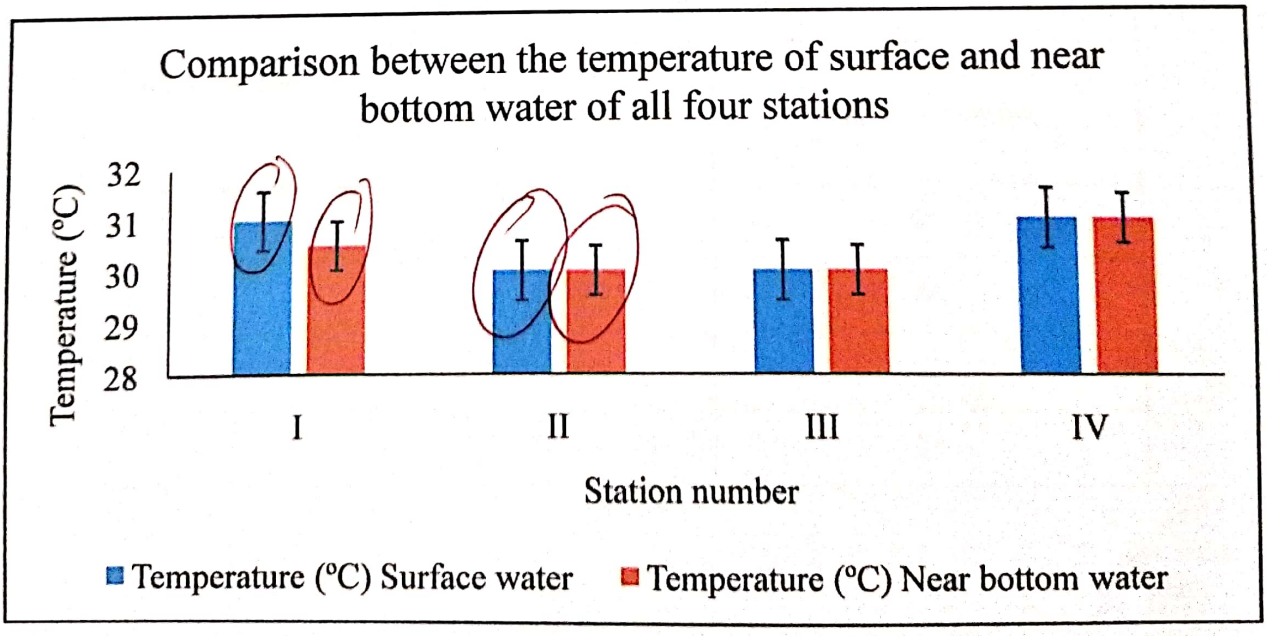
PROCEDURE

Some water is taken in the mug from niskin sampler or from bucket

The thermometer slowly dipped into mug

And reading was noted

Station number	Temperature (°C)	
	Surface water	Near bottom water
I	31	30.5
II	30	30
III	30	30
IV	31	31



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RESULT:It can be observed from the chart that there was no such temperature difference in surface water and in near bottom water, only there is difference at station I. Station I and IV have slightly higher temperature than station II and II.

2. SALINITY

Salinity was measured with help of a refractometer. It works on the principle of refraction of light.

PROCEDURE

To check the salinity the water of surface water and the near bottom water were taken in a mug

With help of dropper water sample was taken and 2-3 drops were placed on the refractometer

The refractometer stage covering was closed and viewed through the eyepiece and readings were noted.

Station number	Salinity (‰)	
	Surface water	Near bottom water
1	25	26
2	33	34
3	25	26
4	20	23

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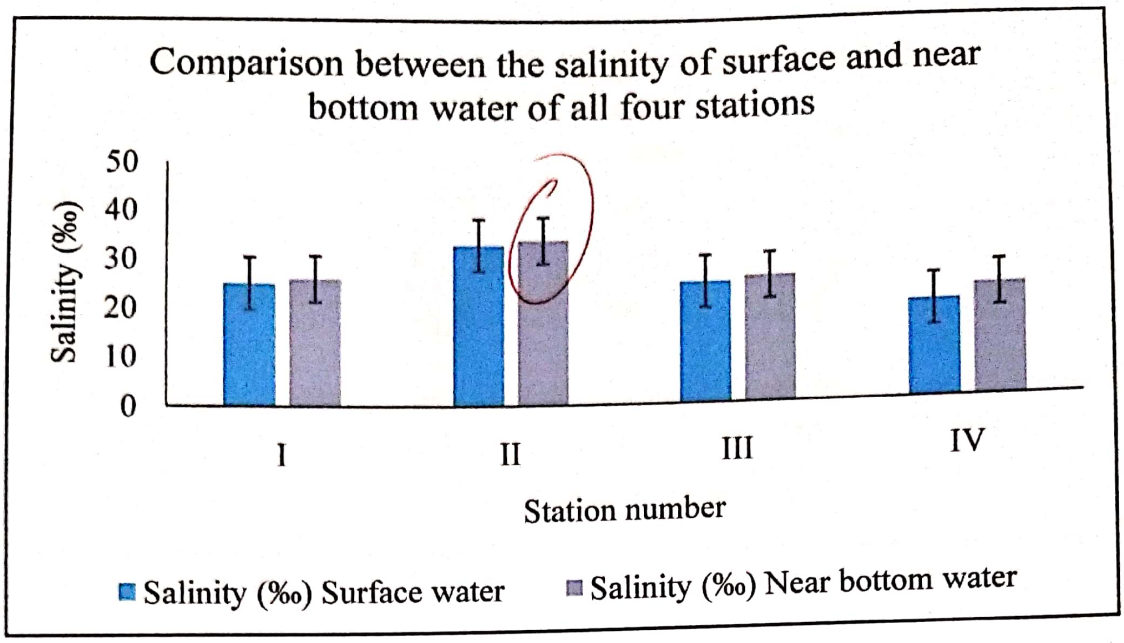

As we can observe from chart the salinity values vary slightly between each station. At every station near bottom water is slightly higher than surface waters.

3. Secchi disk

A secchi disk is an 8-inch (20) disk with alternating black and white quadrants. It is lowered into the water of a sea until it can no longer be seen by the observe.this depth of disappearance, called the secchi depth, is a measure of the transparency of the water.

Station number	Secchi depth (mts.)
I	1.75
II	2
III	2.25
IV	1.3

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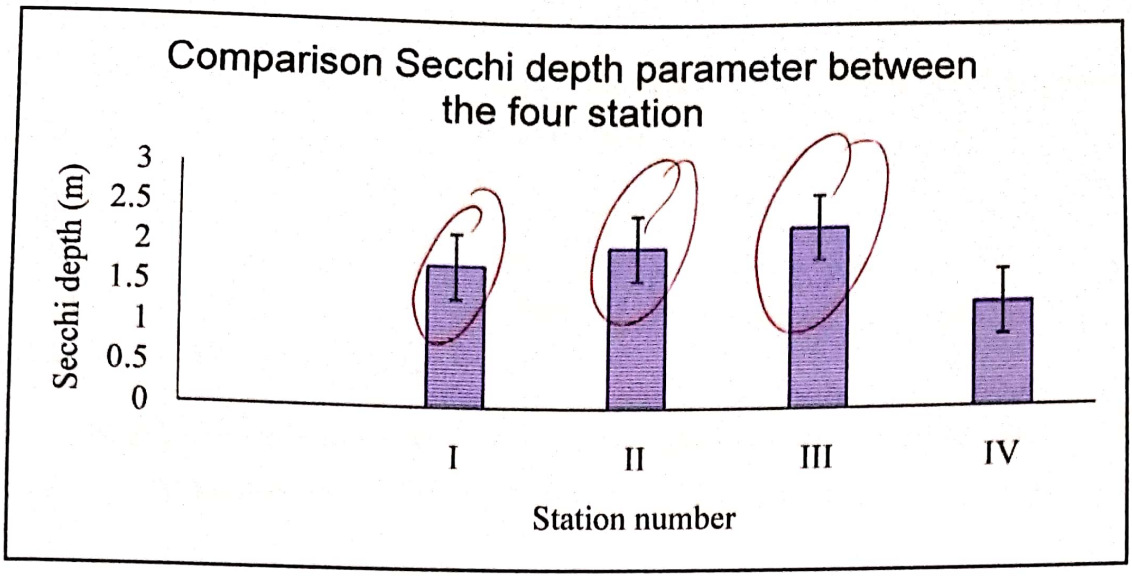

RESULT: The light can be goes deeper in the station III and not so deep in station IV

➢ DISSOLVED OXYGEN (DO)

Dissolved oxygen was measured by Winklers method, which was first proposed by Winkler (1888) and was later modified by Strickland and Parsons (1966).

Winkler method is an iodometric titration which oxidises iodine ion to iodine using manganese as a transfer medium. Manganese (II) chloride and potassium iodide solution are to be added to the sample, manganese gets precipitate as hydroxide and oxidised to manganese (II) hydroxide.

PROCEDURE

Determination of blank

50 ml distilled water +1ml 50% H_2SO_4 + 1ml alkaline iodide (winklers B) + 1ml manganese chloride reagent (winklers A)mixed in a conical flask

Mixed thoroughly to avoid any precipitation of manganese hydroxide

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1ml strach indicator added (if blue color did not develop). if it is developed titration is to be carriewd out

Standardization of thiosulphate solution

50 ml distilled water + 50%H_2SO_4 + 1ml alkaline iodide (winklers B) + 1ml manganese chloride reagent (winklers A) mixed in a conical flask

10ml 0.01N potassium iodate solution (kIo)

Mixed well (keep in dark)

DO estimation: Sample in DO bottle filled upto brim

Fixed with 1ml wrinkler A + wrinkler B

After precipitate settled add 1ml 50% H_2SO_4

Mixed well till precipitate dissolved

50ml sample in conical flask

Titration with sodium thiosulphate till solution turns pale yellow

1ml starch indicator added (blue color develops)

Titration with sodium thiosulphate

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Color change from blue to colorless (End point)

Repeat 3 times and mean burette reading was found

Calculations:

Normality of thiosulphate soln. = $\frac{\text{Normality of } KIO_3 \text{ (0.01 N) x Volume of } KIO_3 \text{ (10 ml)}}{\text{Volume of thiosulphate used in standardization}}$

$$= \frac{0.01 \times 10}{11.6}$$

$$= 0.0086 \sim 0.01 \text{ N}$$

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

BR - Burette reading (Volume of thiosulphate used in titration)

N - Normality of thiosulphate soln.

E - Equivalent weight of oxygen = 8

1000 - To express in per litre

V/v - $\frac{\text{Volume of bottle}}{\text{Volume of bottle-volume of reagent}} = \frac{125}{125-2} = 1.016$

Dissolved oxygen (mg/l) = $\frac{BR \times V/v \times N \times E \times 1000}{\text{Volume of sample titrated}} = \frac{BR \times 1.016 \times 0.01 \times 8 \times 1000}{50}$

$$= BR \times 1.6256$$

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OBSERVATION TABLE

Station number	Sample	Burette reading (ml)			Constant burette reading (ml)	Dissolved oxygen (mg/L) [BR x 1.6256]
		I	II	III		
I	Surface water	3.4	3.1	3.1	3.1	5.03936
	Near bottom water	3.2	3.2	3.0	3.2	5.20192
II	Surface water	3.2	3.3	3.2	3.2	5.20192
	Near bottom water	2	2.4	2	2	3.2512
III	Surface water	3.2	3.5	3.5	3.5	5.6896
	Near bottom water	2.3	2.8	2.8	2.8	4.55168
IV	Surface water	3.2	3.2	3.4	3.2	5.20192
	Near bottom water	3.2	3.2	3.5	3.2	5.20192

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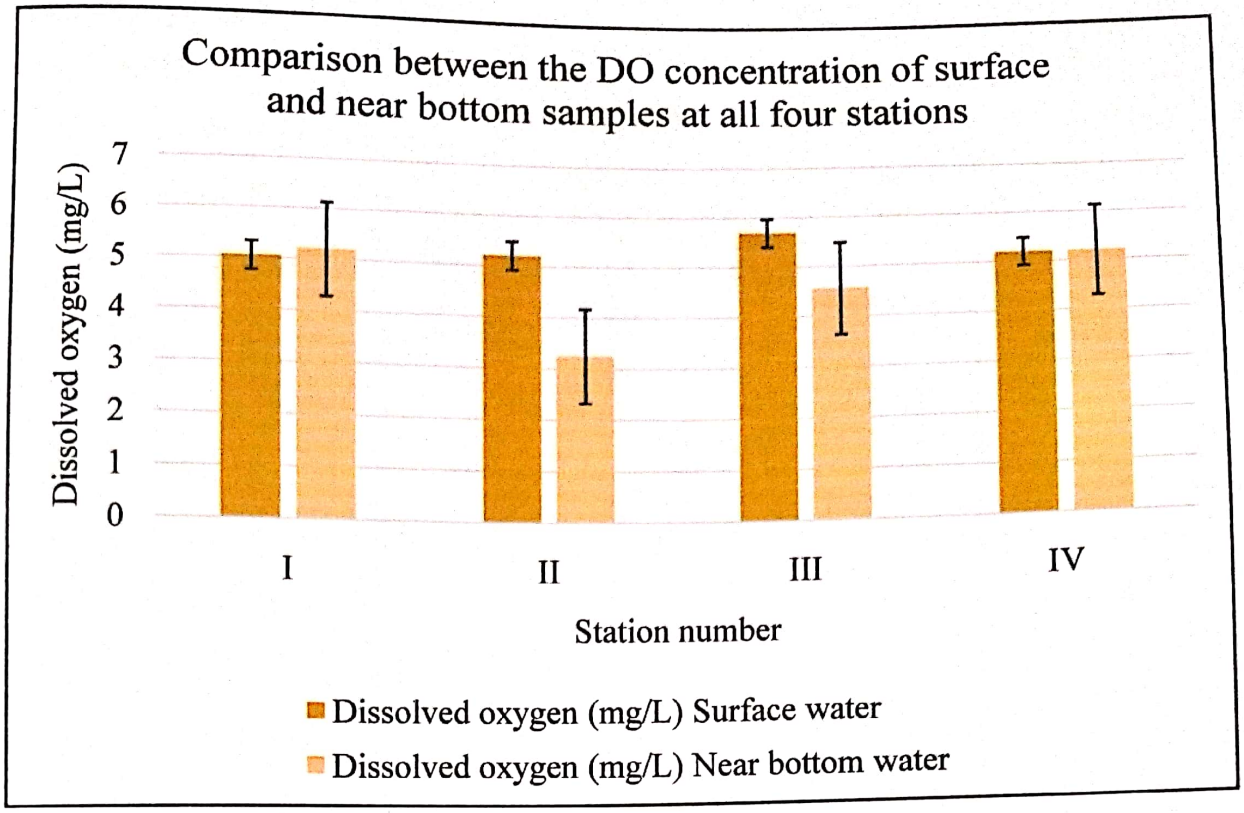

RESULT: At all the four stations, station III showed highest and station I showed lowest concentration of DO at the surface water. And at near bottom waters station IV showed highest and station III showed the lowest concentration of DO.

➢ VIABLE COUNT

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:

From water sample collected, 50ml was kept aside in a centrifuge tube.

0.1ml from that water was taken and spread plated on previously prepared ZMA, MacConkey's, TCBS and XLD agar plates.

The plates were incubated at 37°C for 24-hours. Results were noted.

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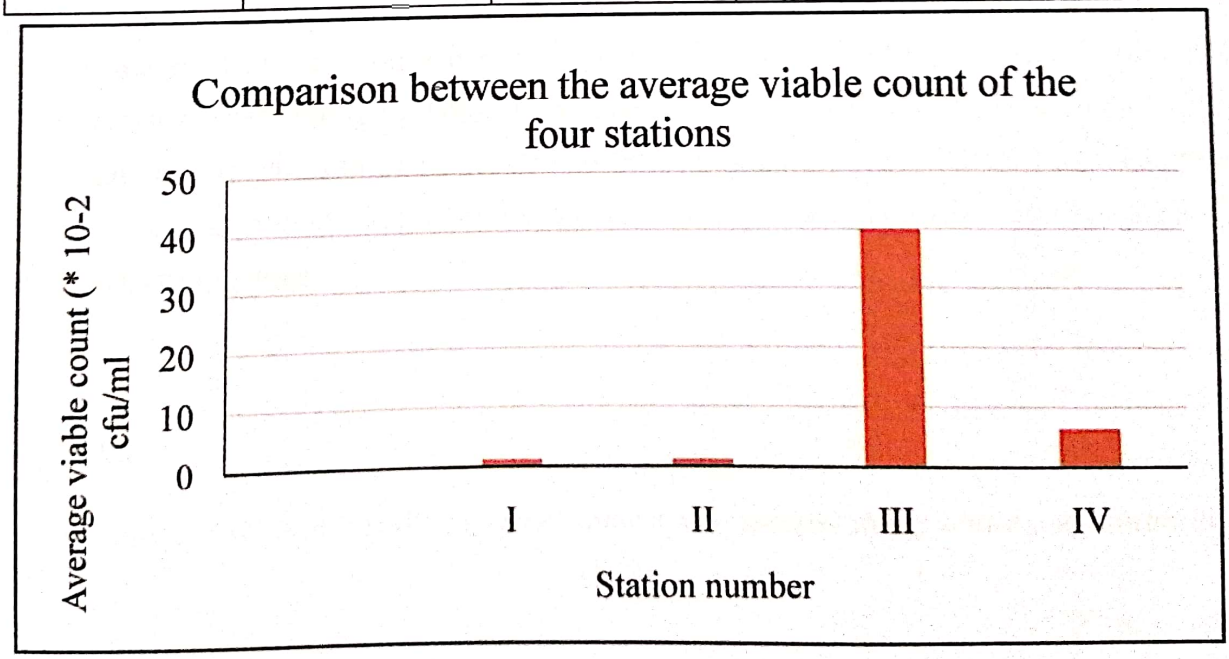
Observation table

Station number	Dilution	Number of colonies	Viable count (cfu/ml)	Average viable count (cfu/ml)
I	10^{-1}	6	0.6×10^{-2}	1.1×10^{-2}
	10^{-1}	8	0.8×10^{-2}	
	10^{-2}	2	2×10^{-2}	
	10^{-2}	1	1×10^{-2}	
II	10^{-1}	10	1.0×10^{-2}	1.1×10^{-2}
	10^{-1}	14	1.4×10^{-2}	
	10^{-2}	1	1×10^{-2}	
	10^{-2}	1	1×10^{-2}	
III	10^{-1}	6	0.6×10^{-2}	39.86×10^{-2}
	10^{-1}	-	-	
	10^{-2}	4	4×10^{-2}	
	10^{-2}	115	115×10^{-2}	
IV	10^{-1}	23	2.3×10^{-2}	6.43×10^{-2}
	10^{-1}	10	1.0×10^{-2}	
	10^{-2}	16	16×10^{-2}	
	10^{-2}	-	-	



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➢ ENUMERATION OF PHYTOPLANKTON

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:

From the collected surface and bottom water 500ml water was collected separately in bottles from each station.

15 drops of Lugol's iodine solution were added as a preservative.

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)

RESULT:

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.

➢ SUSPENDED PARTICULATE MATTER (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. 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:

From the bucket and Niskin sampler water was collected in the bottle and stored in shade.

A filter paper of 0.45micron was placed in the filtration unit attached to the vacuum

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pump.

Before filtering the water sample the weight of the filter paper was measured and noted.

Around 250mL of seawater sample is filtered through the filter paper.

After filtering the weight of the filter paper was again measured (designated as wet weight)

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) ✓The readings were noted down and the calculations were carried out.

OBSERVATION TABLE

Station number	Initial weight of filter paper (g)	Final weight of filtered paper (g)	Mass of SPM (g)
I	0.080	0.080	0
II	0.080	0.080	0
III	0.078	0.080	0.02
IV	0.090	0.092	0.02

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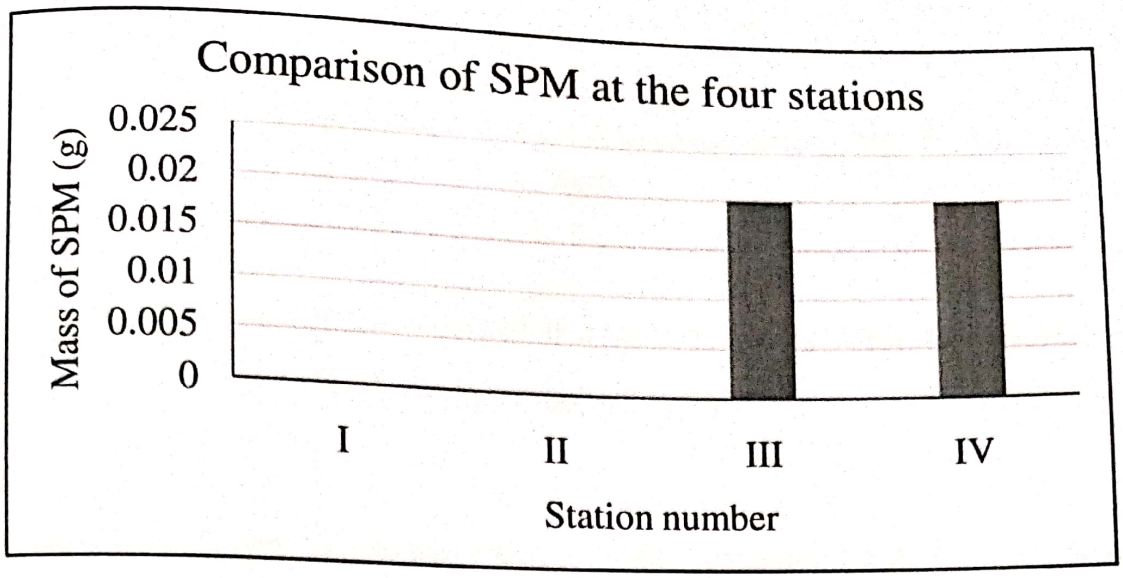

RESULT

Station III and Station IV have showed more number of spm.

➢ PRESCENCE OF BACTERIA BY MPN (Most Probable Number)

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:

Water sample collection was done from the surface of the station 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.

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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 color of the media) 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.

Concentration	Test tube number	Acid	Gas
Double strength (10 ml broth + 10 ml sample)	1	+	+
	2	+	+
	3	+	+
	4	+	+
	5	-	+
Single strength (9 ml broth + 1 ml sample)	1	-	-
	2	-	-
	3	-	-
	4	-	-
	5	-	-
Single strength	1	-	-

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(9.9 ml broth + 0.1 ml sample)	2	-	-
	3	-	-
	4	-	-
	5	-	-

MPN is carried out only from 1 station (station I)

➢ CHOLOROPHYLL AND PHAEO PIGMENTS

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:

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.

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 colored 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

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

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

station	Surface water	Near bottom water
1	8.544	6.947
2	2.67	2.136
3	5.874	10.68
4	3.738	4.806

Chlorophyll (mg/m^3)

RESULT

Chlorophyll and phaeo pigments: The highest chlorophyll concentration was found to be at station III, in near bottom waters and lowest at station II, near bottom waters.

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CONCLUSION

The water from surface and near bottom had sampled and sediment sample was collected. And all the parameters has be analysed in the lab. observations results has be noted.

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