FIELD TRIP REPORT

MMO 319

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21P039016

Msc. Marine Microbiology Part II (2022-23)

SEOAS, Goa University

INDEX

INTRODUCTION

There is a proverb that, "Study and travel open the horizon of knowledge".No study related to marine is complete without a field trip to experience all kinds of wetlands at first hand. its important that lesson learned in classroom be put into practice in the field.

Recently we had a great opportunity to visit actual wetland. also we were able to learn the techniques of field studying. A field trip was organized by the department of Marine microbiology, SEOAS, Goa university, for the students as a requirement of master degree. on 10th of March 2022 from morning till evening. Two teachers (Dr. Priya D'costa and Dr. Nikita lotlikar) along with a non-teaching staff (Miss Vaishali) accompanied 13 students for the field trip on the trawler. Our meeting point was Malim jetty, where we boarded on trawler.

Basically our field trip had two major parts. They were:

(1) Field and Sample collection

(2) Sample Analysis (Day 2 in the laboratory).

We visited total four stations, which was station-1: Malim jetty, station-2: Miramar offshore, station-3 : near Chorao island, and station-4 : Old Goa. First we visited station two that is offshore because in the afternoon there would have been high tides as it is the sea. After that we visited station 1, 3 and 4 respectively.

LOCATION:

Station 1; Malim Jetty

Malim jetty is an area located in Panjim city in Goa state. There are about 300 boats that operate from the malim jetty, of which 10-15 boats bring fish to the jetty on an averagedaily. lately people have been dumping garbage in this area because jetty was isolated for past few months owing to the mandatory fishing ban.The debris has been lying there unattended.

latitude: 15.503

longitude: 73.83

depth in meters: 6m

✓

Station 2; Miramar offshore.

Miramar is a bustling area within the capital Panjim. a lovely golden beach of soft sand gridled with palm trees. this beach is the estuary of Mandovi river which opens in the Arabian Sea.it is one of the most visited beach in Goa by tourists.

latitude: 15.47

longitude: 73.77

depth: 10m

Station 3; near Chorao island.

Chorao also known as Choddnnem, was also called Chudamani meaning stunning precious stone in sanskrit. it is an island along the Mandovi river Ilhas Goa, India. At a distance of 24.5 km from Panjim. it is the largest among 17 other island in Goa. This area was one of the first to be conquered by portuguese. This island is famous for its Luso-Goan churches and houses it is also known for its bird sanctuary.

latitude: 15.505

longitude: 73.86

depth: 3.5m

Station 4; Old Goa

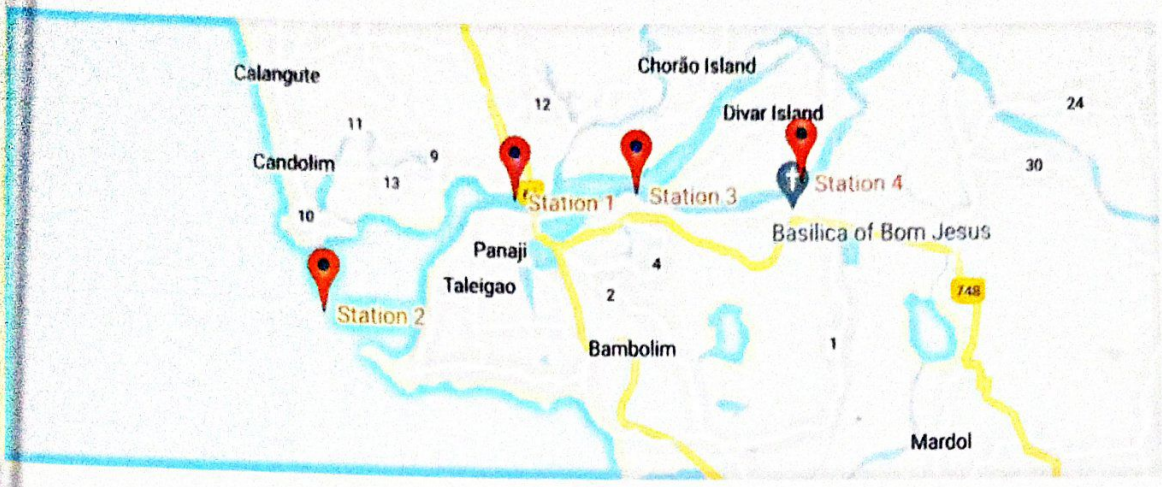
Old goa also known as Velha Goa ,City was founded in the 15th century as a port on the banks of the Mandovi river by the rulers of Bijapur sultanate. its known for colonial era monuments. Old goa was the second capital after Bijapur of the rule of Adil shahi dynastry. adding to its beauty it is also famous for churches constructed in portuguese era. the remains of city has been declared as World heritage site.

latitude: 15.508

longitude: 73.91

depth: 5.25m

LOCATIONS OF ALL 4 STATIONS.



(Fig 1)

As this field trip was all about testing the water samples, so in order to do that several tests were carried out. Like

(1) *Bacterial sampling*

(2) *Dissolved oxygen*

(3) *temperature of the water sample*

(4) *Salinity of the water sample*

(5) *Phyto-fixing*

(6) *Chlorophyll estimation (mg/m^3)*

(7) *Suspended particulate matter*

(8) *sediment collection*

OBJECTIVES OF THE FIELD VISIT

General objective

* To gather research experiences

* To study and extract information and try to make some findings

* To improve team work cooperation skills

* To familiarizing with the specialized tools and techniques

* To improve report writing skills

* How to collect the sample

Specified objective

To test water sample and carry out various experiments related to water.

FIELD METHODOLOGY

The methods and field techniques that are used in particular field work depends on the type of work to be undertaken.

Overall methodology at a glance:

FIRST STEP: Planning and preparation

- Site selection, which was done by our teachers
- Instrument taking: day before our field trip, we issued all the instruments from the store, that we were supposed to take with us during our trip.
- Plastic bottles of 500ml,1000ml and plastic cans of 2 litres was bought by students in order to collect the water sample during field trip.
- we were provided with the chart by our teachers where grouping of the students was done to carry out each experiments systematically during field trip.
- we were instructed about the workings of instrument, timing to report on the site and some safety precautions.

SECOND STEP: Data collection

Group wise we collected two samples on each stations that is surface water and the water near the bottom. to collect water from the surface we used a bucket which was tied to rope in order to pull it out after filling the water. We collected the near bottom water with the help of Niskin bottle. water sample was collected in bottles,cans and centrifugal tubes were properly labelled in order to carry out further exp. In laboratory without any confusion. sediment col-

lection was done with the help of Van-veen grab. temperature was checked with the thermometer, salinity was checked with the help of refractometer. latitude and longitude of the location was checked. and the depth of the water. Turbidity was checked with the help of secchi disc.

***THIRD STEP: Data manipulation** Analysis

This was done in the lab. which included finding the most probable number (MPN), amount of oxygen present in water sample, chlorophyll estimation was done, and suspended particulate matter was found out.

***FOURTH STEP: Analysis**

Which include report writing and submission.

INSTRUMENTS AND THEIR WORKINGS.

Niskin bottle/sampler: It is a cylindrical non-metallic usually made up of plastic, water collection device with stoppers at both the ends. This device is used to take water samples at a desired depth without the danger of mixing the water from other depths.

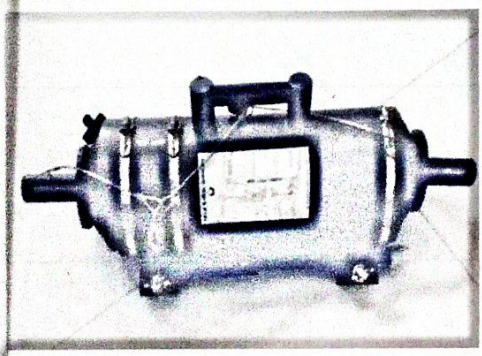


Fig 2: Niskin sampler

How does a Niskin bottle works?

The two stoppers at each end of the niskin bottle, are held opened by a plastic cords attached to a release mechanism, and stoppers are connected by an elastic cord at the inside of the bottle. and then attached to a cable, after opening the bottles it is lowered to a discreet depth in the water. when a small weight encircling the hydroghraphic line is released down the line, it strikes the release mechanism resulting in the two stoppers being pulled into the ends of the cylinder. As a result the water from the depth is trapped inside the niskin bottle.

Refractometer: It is a tool that can determine the concentration of a particular substance in a liquid solution. it uses the principle refraction.

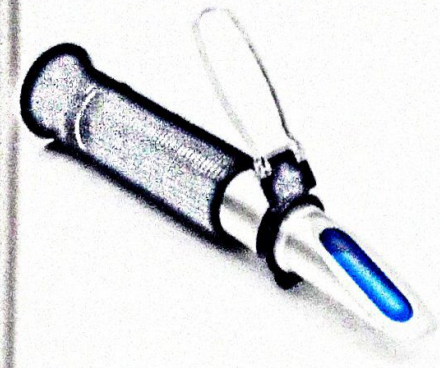


Fig 3: Refractometer

Working: As light passes from air into a liquid it slows down. this phenomenon gives a bent look to objects that are partially submerged in water

Van-veen grab sampler: instrument used to separate sample sediment in water environments, it is a clam shell bucket made of stainless steel. it is light weight and low tech. the smallest version even fits into it. It is used to for taking disturbed samples from the bottom of lakes, rivers, etc.

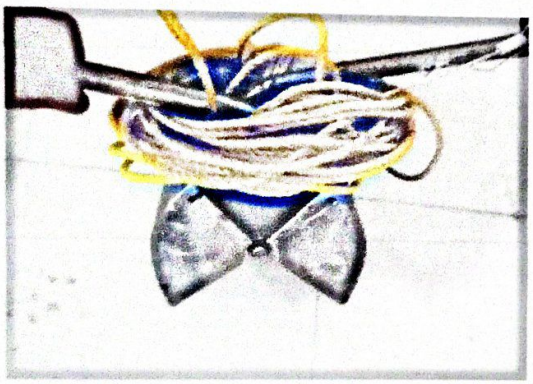


Fig 4: Van-veen grab

Working: While letting the instrument down into the water by cable, the two levers with buckets at their ends are spread like an open scissor. the levers are locked in this position by a little hook.once it hits the ground. the levers will be unlocked. once we pull, it will be locked again with the sediment collected in it. once it is pulled out the van veen grab is emptied in the vessel.

Secchi disc : it is used to measure water transparency turbidity. it is a plain white, circular disc 30 cm in diameter.

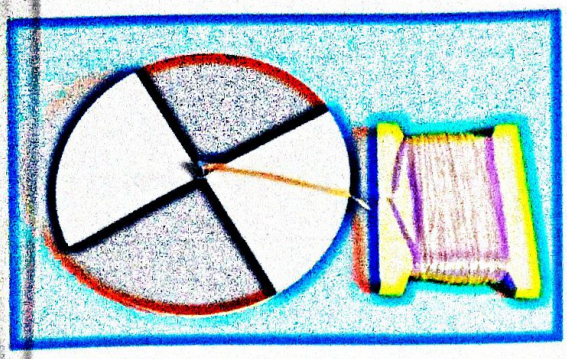


Fig 5: Secchi disc

Working: it is lowered into the water, until it can no longer be seen by the observer. this depth of disappearance is called the secchi depth.

PRINCIPLE ANDMETHODOLOGY OF THE EXPERIMENT:

(1) Analysis of MPN

PRINCIPLE: This test is done in order to detect E.coli and coliforms. Faecal coliforms are known to ferment lactose and produce both acid and gas. This can be detected by performing MPN where change in colour of the media (MacConkey's) indicates acid production, and forming of a gas bubble in the inverted Durham's tube indicates gas production. Both of which designate a positive result indicating presence of faecal coliforms, E.coli in the water sample.

PROTOCOL:

- Water was collected from surface and near bottom with the help of the bucket and Niskin sampler resp.
- Water was collected in the sterile centrifuge tubes of 50ml and stored in ice box until further analysis.
- Samples were taken to the lab and inoculated into double strength and single strength MacConkey's Broth containing inverted Durham's tube in resp. volumes.
- 10 ml of sample water sample was inoculated into 5 tubes containing 10 ml of Mac-Conkey's Broth
- 1ml of water sample was inoculated into 5 tubes containing 10 ml of single strength MacConkey's Broth
- 0.1ml of water sample was added to 5 tubes containing 10ml of single strength broth.
- All tubes were incubated at 37degree C for 24-48 hours.

- Positive results were indicated by production of acid (change of media colour from pinkish red to yellow) and gas. (by production of gas in Durham's tunbe)
- Results were compared to standard chart like McCrady's table and the number of bacteria per 100ml of sample was determined.

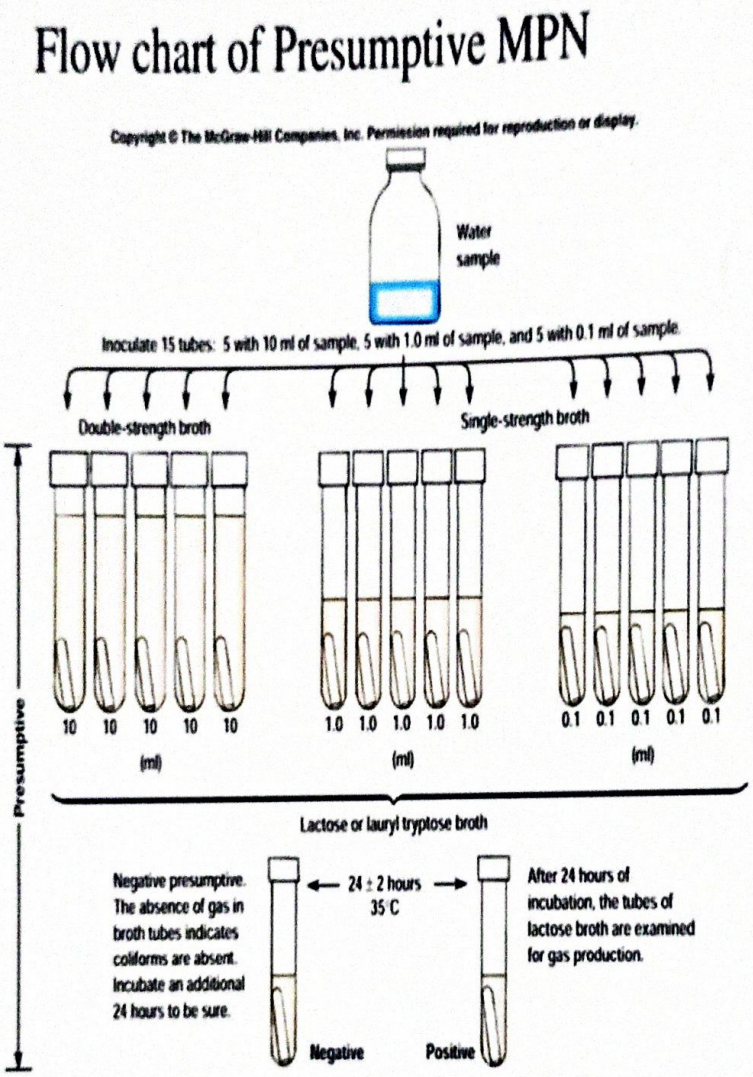


Fig 6: MPN presumptive tests

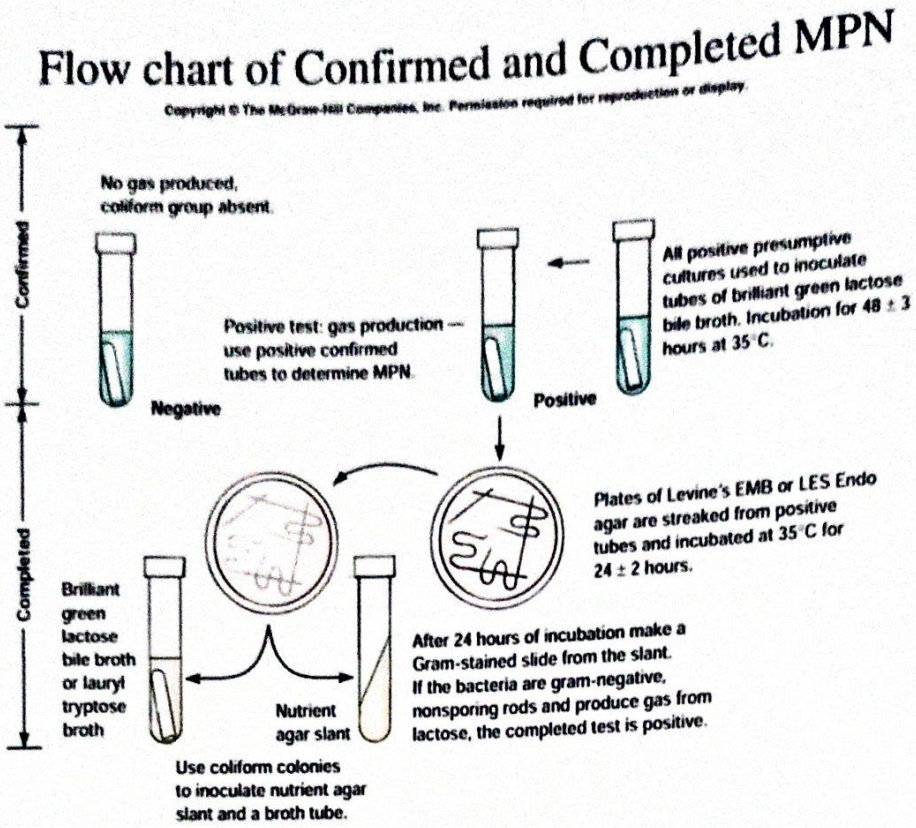


Fig 7: MPN confirmed and complete test.

(2) Analysis of viable count:

PRINCIPLE: The viable count or plate count, is a count of viable or live cell. 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:

- 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□ for 24 hr.
- Results were recorded after the incubation period. Wherein each colony was counted and written down. Average of total numbers of colonies was taken and viable count was calculated.

(3) Analysis of Dissolved Oxygen (D.O)

PRINCIPLE:Chemical determination of oxygen in water sample is carried out byusing the Winkler method. It is a type of iodometric titration which oxidizes iodine ion to iodine using manganese as a transfer medium. It 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.

PROTOCOL:

*DETERMINATION OF REAGENT BLANK:

- 50ml of distilled water taken in conical flask to that 1ml of 50% H2SO4 + 1ml alkaline iodide (Winkler's B) + 1ml manganous chloride reagent (Winkler's A) was added. Was mixed thoroughly to avoid precipitation.
- 1ml of starch was added.(if blue colour was developed than titration needed to be carried out further, there was no blue colour.)

*STANDADIZATION OF THIOSULPHATE SOLUTION:

- Solution was prepared same as prepared for blank.
- 10ml of 0.01N potassium iodate sol. Was added. Solution was mixed and kept in dark for 3min to liberate iodine.
- Liberated iodine was titrated against thiosulphate till solution turned pale yellow.
- 1ml starch was added and titration was cont. till the colour changed from blue to colourless (should remain colourless for 3o sec approx..) this was carried to triplicate to obtain mean burette reading.

*D. O estimation:

Was done on field.

- sample collected from surface and bottom, was taken in the DO bottle, making sure that no air bubble was formed.
- immediately it was fixed with 1ml Winkler's A and 1ml Winkler's B, inserting the pipette tip at the bottom of the bottle, and precipitate was left to settle.

further experiment was carried out in the lab:

- 1ml of H_2SO_4 was added and shaken vigorously till the precipitate dissolved.
- 50 ml of sample was transferred to a conical flask and titrated against Thiosulphate solution until a pale yellow colour appeared

- 1ml of starch solution was added and titration was continued until the blue colour disappeared. Burette reading was noted, titration was repeated three times to find out the mean burette reading. (Winkler's method)

(4) 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.

PROTOCOL:

- Samples from different stations was collected in a bucket from surface and near bottom in Niskin sampler, was collected in a mug.
- By dipping the thermometer into the mug, temperature was observed and noted down.

(5) Analysis of Salinity:

PRINCIPLE: Refractometer is a tool that determines conc. Of a particular substance in a liquid solution.

PROTOCOL:

- several drops of the sample liquid was placed on the angled prism of the refractometer.
- clear plate was sealed on top of it (ensuring no air bubbles were trapped)
- than refractometer was pointed towards the direct light source, and observed through eyepiece, and reading was noted down

(6) Analysis of Phytoplankton:

PRINCIPLE: To estimate the amount of phytoplankton 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.

PROTOCOL:

- Water samples from different stations was filled in 500ml bottles, and 15 drops of lugols iodine was added and stored in shade until further analysis.
- Bottles was brought back to lab, 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.

(7) Analysis of Chlorophyll (Chlorophyll estimation):

PRINCIPLE: Pigment extraction is carried out in order to separate different pigments from seawater sample containing phytoplankton. Acetone is being used as a solvent since its polarity allows it to dissolve polar substances and also allows greater resolution between pigments. Pigment analysis is done spectrophotometrically.

PROTOCOL:

- Collected sample was taken to the lab.
- filter paper (0.75um GF/F) was placed between filtration equipment.
- slowly pour seawater onto the filter paper approx. 500ml (so the phytoplankton remain on the paper)
- after coat of phytoplankton was formed, filter paper was put in the vial (dark coloured) + 10 ml of 90% acetone was poured
- filter paper was crushed gently and kept undisturbed for 24 hours in refrigerator
- next day samples were analysed spectrophotometrically at 665nm, then 2 drops of HCL acid was added.
- Absorbance was measured at 750nm. Readings were recorded and calculations was done.

(8)Analysis of Suspended particulate matter (SPM):

PRINCIPLE: 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 pour size filter is defined as dissolve matter (DM). The dry weight concentration of suspended particulate material, (using: mg L^{-1}), is measured by passing a known volume of seawater through a pre-weighed filter and reweighing the filter after drying.

PROTOCOL:

- Collected sample was taken to the lab.
- 0.45micron filter paper was weighed before filtering and noted the weight.
- place it on the filtration unit and filter around 1-2L of water sample
- take out filter paper carefully and dry it in the oven at 30□.
- after drying take the weight of the filter paper again and note down

- then subtract filtered (kept on oven) filter paper, from unfiltered (which was measured before filtering)

(9) Analysis of turbidity using Secchi disc:

PRINCIPLE: A secchi disc is a disc which has a black and white quadrant on it and is used to measure the transparency of the water. By lowering it into the water until it can no longer be seen by the observer.

PROTOCOL:

- Secchi disc was taken and gently lowered from the trawler into the water (for all the stations) with the help of the rope attached to it.
- Disc was lowered until the observer could no longer differentiate between the quadrants of the disc.
- The depth at which this was observed was noted and designated as secchi depth which indicates the turbidity of the water body.

(10) Analysis of PH:

PRINCIPLE: 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 serves a s a reference. An important consequence of this fact is that the change in potential on moving the electrodes from the buffer to the sample is the sum of changes that occur in the contributions to the cell potential.

PROTOCOL:

- pH meter was turned on and calibrate button was pressed.
- Electrodes was placed in 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.
- Electrodes was washed was wiped, and the above steps were repeated for acidic and alkaline pH buffer.
- After the calibration, electrodes were rinsed and placed into the sample. The pH measurement was recoded.
- This was done for all the stations water samples.blanked measured using spectophotometer, acetone added in cuvette 665nm wavelength set

- auto zero, sample added and absorbance measured, cuvette removed and 2 drops of diluted HCL added and then absorbance was measured

OBSERVATIONS:

(1) MPN:

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 bacteria/100mL

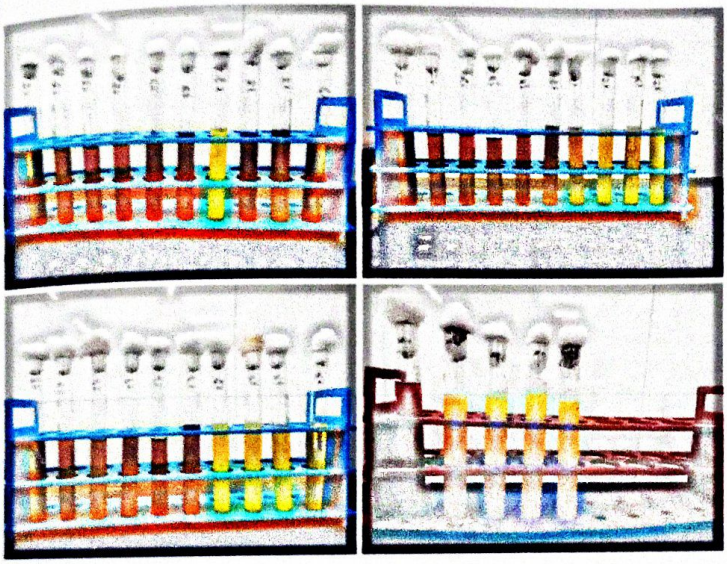


Fig 8: MPN tubes after the incubation period

(2) Viable count: No growth was observed on all the medias (ZMA, XLD, TCBS, MacConkey's agar)

Fig 9: Collection of water sampling for viable count and MPN analysis

(2) Dissolved Oxygen:

Station	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

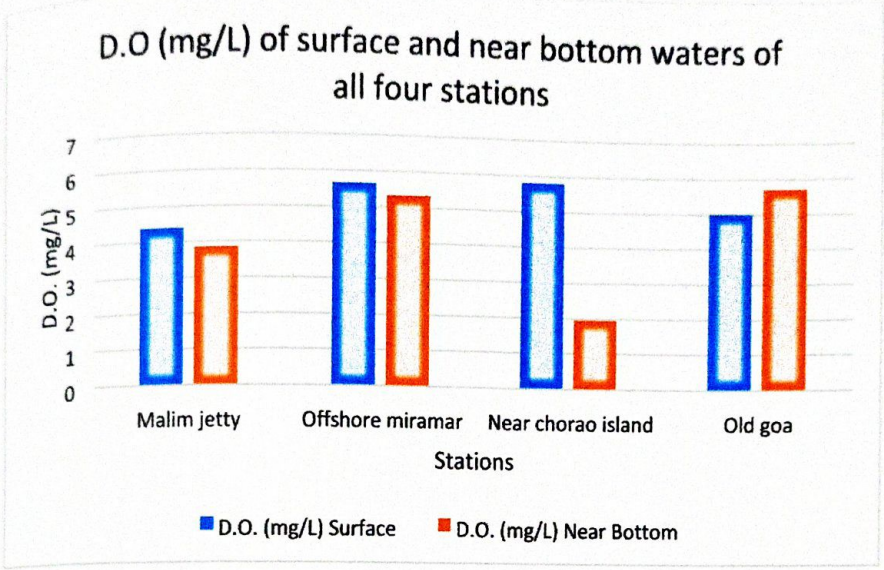


Fig: Graph of D.O. (mg/L) of surface and near bottom waters of all four stations.

Calculations:

$$\text{Dissolved oxgyen, mg L}^{-1} = \frac{BR * \frac{V}{v} * N * E * 1000}{\text{Volume of sample titrated}}$$

BR = Burette reading (volume of thiosulphate used in titration)

N = Normality of thiosulphate solution

E = Equivalent weight of Oxygen = 8

1000 = To express per liter

Note: The factor V/v was correction for displacement of oxygen in the sample when reagents were added.

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

** Use factor (0.698) to convert parts per million (mg L^{-1}) to (ml L^{-1}) of oxygen

Fig 10: D.O. bottles after fixing the sample with Winkler's A and Winkler's B

(3) Temperature and salinity:

Station	Temperature (°C)		Salinity	
	Surface	Near Bot-tom	Surface	Near Bot-tom
1	31	30.5	25	26
2	30	30	33	34
3	30	30	25	26
4	31	31	20	23

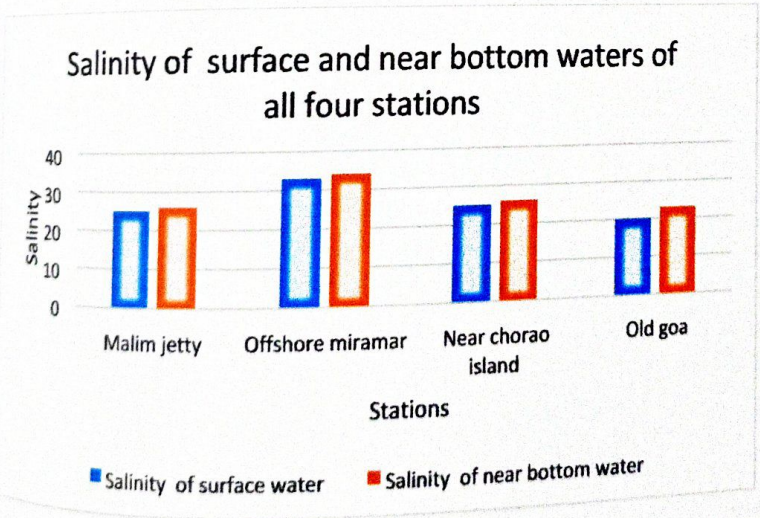


Fig: Graph of salinity of all four stations (surface and near bottom waters)

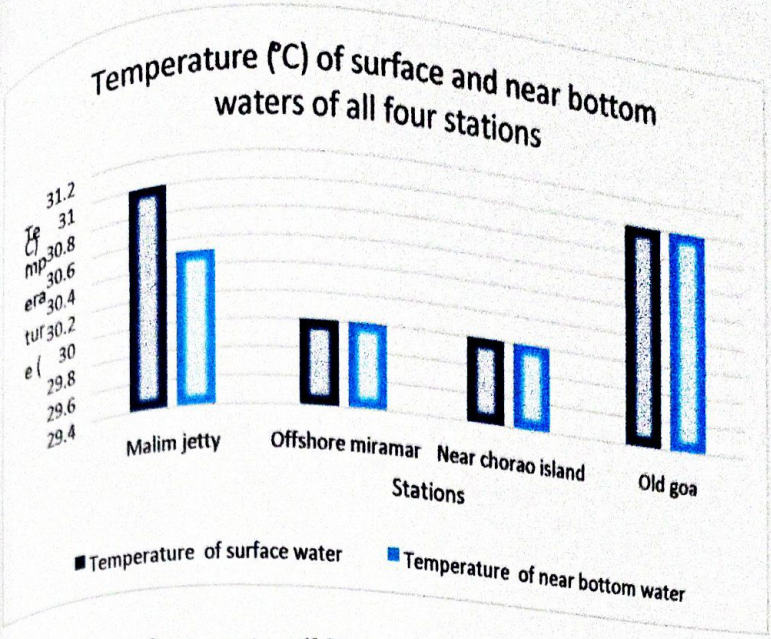


Fig 11: Graph of temperature (°C) of all four stations (surface and near bottom waters)

(4) Estimation of phytoplankton: Qualitative analysis of phytoplankton was carried out

Fig 12: a) *Coscinodiscus sp.* b) Fixed sample bottles after siphoning.

(5) Chlorophyll estimation:

Station	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

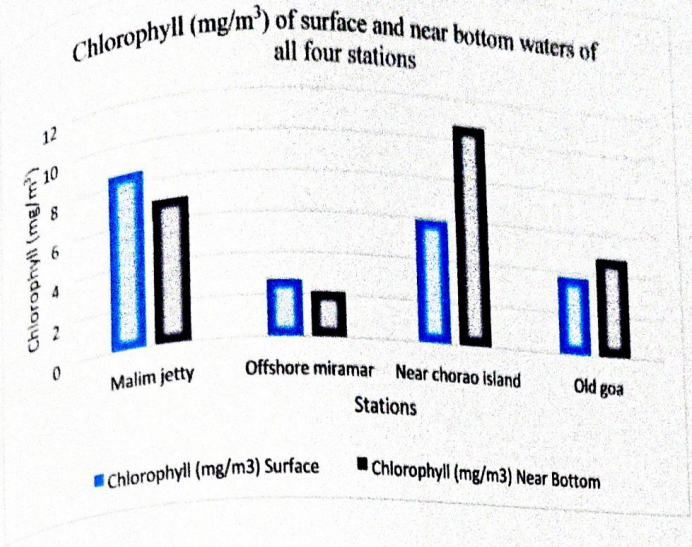


Fig 13: Graph of Chlorophyll (mg/m3) estimation of all four stations (surface and near bottom waters)

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).

Fig 14: Collection of water sample from Niskin sampler for phytoplankton fixing.

(6) 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

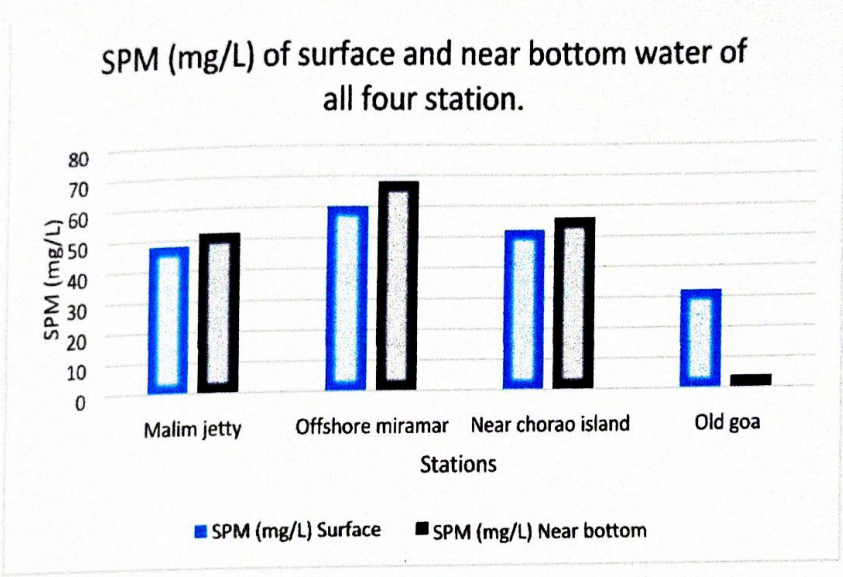


Fig 14: Graph of SPM (mg/L) of all four stations (surface and near bottom waters)

SPM = X-Y

Volume of water filtered in litres

(7) Turbidity And pH:

Station	Secchi (me- depth ters)	pH
1	1.75	8.1
2	2	8
3	2.25	7.6
4	1.3	7.9

RESULTS:

(1) 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.

(2) Viable count: No growth was observed on any plate.

(3) D.O.: Amongthe 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.

(4) 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.

(5) Estimation of Phytoplankton: The following phytoplankton were observed – Diatoms: *Rhizosolenia sp., Coscinodiscus sp., Gyrosigma sp., Chaetoceros sp.*, and an unidentified pennate diatom. Dinoflagellates were not observed.

(6) 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.

(7) 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.

PERSPECTIVE:

Overall it was a great experience. We were able to learn many good and new things like how to use and handle different instruments such as Niskin sampler, Van Veen grab etc. We also gained experience on proper sample collection and storage to get accurate results for analysis of various parameters of water like D.O., phytoplankton fixing, etc.

On the plus side we also had fun while working. Relishing the lunch that was served which we ate on the trawler parked in the middle of the estuary, watching the mesmerizing view was one of the pleasures that we had on this trip. Having this trip amidst the pandemic, refreshed our minds and brought back our interest into academics as we learned a lot through this opportunity given to us.

Fig 15: Food served at the trawler

Fig 16: Students of MSc. Part I and Part II

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