Role and contribution of heterotrophic bacteria in estuarine carbon cycle: a case study of tropical estuary- Mandovi estuary.

A Dissertation for

MID DISSERTATION

Credits: 08

Submitted in partial fulfilment of Master's Degree

M.Sc in Microbiology

By

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21P042019

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I hereby declare that the data presented in this Dissertation entitled, "**Role and contribution of heterotrophic bacteria in estuarine carbon cycle: a case study of tropical estuary-Mandovi estuary.**" is based on the results of investigations carried out by me in the Microbiology Program at the School of Biological Sciences and Biotechnology, Goa University under the Supervision/Mentorship of Dr. Lata Gawde and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation **"Role and contribution of heterotrophic bacteria in estuarine carbon cycle: a case study of tropical estuary- Mandovi estuary."** is a bonafide work carried out by Miss Esther Guadalupe Pires under my mentorship in partial fulfilment of the requirements for the award of the degree of **M.Sc in Microbiology** in the Discipline Microbiology Program at the School of Biological Sciences and Biotechnology , Goa University.

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ACKNOWLEDGMENTS

I would like to express my gratitude to my guide Dr. Lata Gawade, SBSB Microbiology, for proposing my topic to me and helping me with my monthly analysis.

I thank our programme director, Dr. Lakshangy Charya for providing me with the laboratory equipment, chemicals, and compounds required for my work.

I am also indebted to Dr. Hilda Joao, Scientist CSIR-NIO, and her Project staff all the help

and support.

I thank all the staff members from the Microbiology Programme specially Dr. Judith Noronha , Dr. Milind Naik , Dr. Bhakti Salgaonkar, Dr. Sandeep Garg and Dr. Trupti Asolkar and store attendants for always going out of their way to help me. I also thank the store attendants from the zoology and biotechnology programmes for allowing me to use their equipment.

I am also grateful to my friends Prachi, Kunjlata, Shruti, Sherley, Simran, Sharwani, Leanne etc for lending me a helping hand from time to time.

I would also like to thank my parents for taking me for sampling every month and for all their support and understanding.

List of abbreviations

ATP Adenosine triphosphate

BCP Bacterial carbon pump

BGE Bacterial growth efficiency

BP Bacterial production

BR Bacterial respiration

DO Dissolved oxygen

DOC Dissolved organic carbon

DOM Dissolved organic matter

EMB Eosin-methylene blue

EPS Extracellular polymeric substances

KI Potassium iodide

KOH Potassium hydroxide

MCP Microbial Carbon Pump

RDOC Rrefractory dissolved organic carbon

POC Particulate organic carbon

TEP Transparent exopolymer particles

TSM Total suspended matter

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AIM:

To study the role and contribution of heterotrophic bacteria in estuarine carbon cycle of tropical estuary- Mandovi estuary.

Objectives:

Objectives of this study include:

- Understanding the hydrography and culturable bacterial number in Mandovi river estuary.
- Estimation of bacterial respiration rates in Mandovi river estuary.
- Estimation of particulate organic carbon and its impact on bacterial respiration rates and abundance.

CONTENTS

Chapter I

INTRODUCTION &

REVIEW OF LITERATURE

As we all know the increase of CO2 in the atmosphere is a major problem because of its greenhouse effect which leads to global warming. Large water bodies like oceans, rivers and estuaries play an important role in carbon cycling as they can act as sinks of CO2 and also harbor microorganisms which help to reduce the amount of gaseous carbon dioxide by utilizing it as a source of energy. Therefore, it is important to study the role of the microorganisms from these water bodies and the extent of how much CO2 is mitigated by them. In this study, a monthly analysis of the Mandovi estuary was carried out and various parameters were studied.

Carbon cycle in general

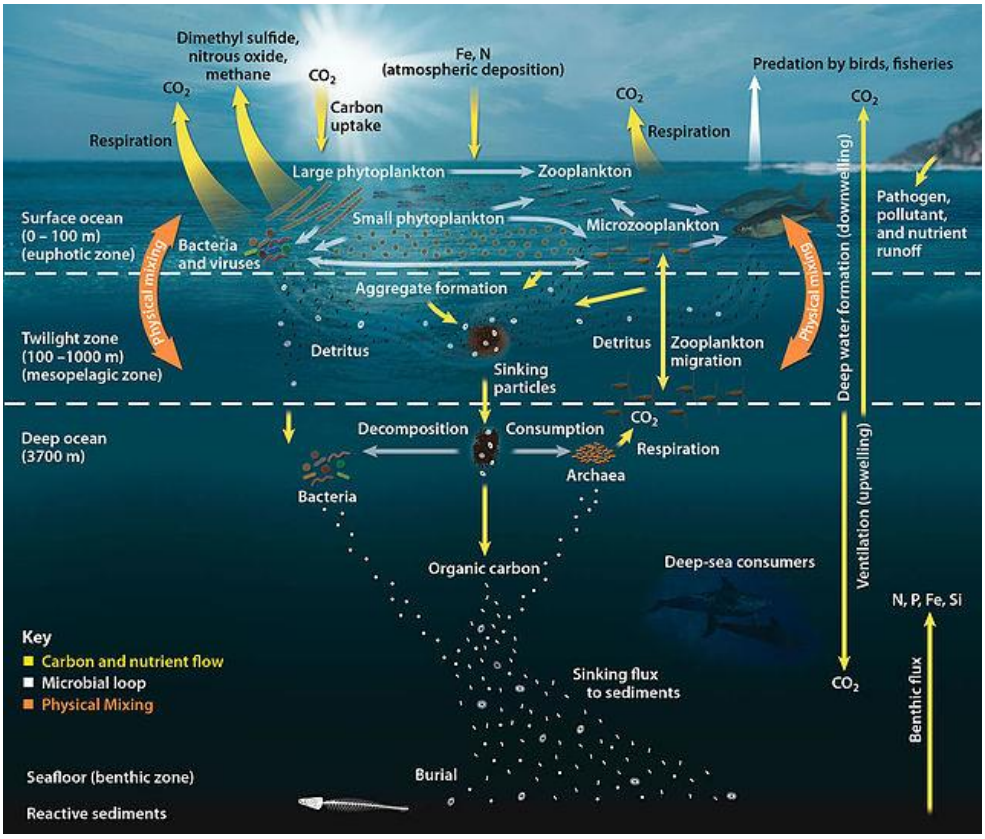


Figure 1. Types of Carbon cycles in Marine environments (Munn, 2020).

The carbon cycle in oceans takes place with the help of three types of pumps they are: (1) the solubility pump, (2) the biological pump, and (3) the carbonate pump.

In the solubility pump since gaseous CO2 is easily soluble in water, atmospheric CO2 dissolves in water and forms carbonic acid (H2CO3 -) most of this quickly dissociates to produce bicarbonate (HCO3-).

1) Several physical processes are responsible for facilitating the adsorption of CO2 from the atmosphere by the surface waters of estuaries and circulating it to deeper layers of

water which results from tilted density gradients brought on by varying surface temperature and salinity, wind pressure and mixing, and the Coriolis effect brought on by the rotation of Earth (Munn, 2020).

A cap of lighter overlying waters effectively prevents CO2 from re-equilibrating with the atmosphere when these masses of water descend into the depths of the ocean as they are carried laterally (Falkowski *et.al,*. 2000)

Upwelling of water to the top elsewhere occurs that replaces the masses of water sinking to the deep ocean. Re-equilibration only happens after decades to several hundred years when waters from the depths of the ocean are brought to the top by upwelling. (Falkowski *et.al.,* 2000) CO2 from the water escapes into the environment as the temperature of the water increases. (Munn, 2020).

2) <u>Biological Carbon Pump (BCP)</u>

Primary production is triggered by the integration of carbon into cellular components as a result of phytoplankton's fixation of CO2 in the upper water. Fish and zooplankton may eat phytoplankton cells, so some of that carbon is immediately assimilated into the food chain. Exudation and when phytoplankton cells split up and perish as a result of natural senescence or viral lysis, both of which cause significant quantities of fixed carbon to be released as DOC. Transparent exopolymer particles (TEP), Extracellular polymeric substances (EPS), and cell detritus from the photic zone combine to make POM, which combines with calcium carbonate and protist silica shells to produce larger marine snow particles. By means of gravitation, zooplankton vertical migration, and the subduction of water bodies, these sink into deeper seas. A significant portion of the fixed organic carbon is respired by microorganisms as it travels through the water column and will be released back into the atmosphere over a timeframe of 10–1000 years, depending on depth. A quarter of primary output makes it to the ocean bottom (Munn, 2020).

There, microbial fermentation and metabolism break down the majority of the carbon molecules, feeding sulphate-reducing bacteria and methanogenic archaea.
About 0.3% of substances from primary production will be trapped in deep sediments, wherethey will remain for many

millennia before being changed by rising heat and pressure to create enormous stores of hydrocarbons (Munn, 2020).

The focus of research in recent years has been on determining whether microbes should be viewed as producers or sinks of carbon for the pelagic food web.

Dissolved Organic Carbon (DOC)

One of the major active organic carbon reservoirs in the biosphere is the dissolved organic carbon (DOC) found in aquatic environments and is about the same amount as the amount of CO, -carbon which exists in the atmosphere (Farrington 1992).

According to (Pomeroy 1974), dissolved organic matter (DOM) is mainly consumed and re-mineralized by heterotrophic bacteria in the ocean, and they play a very active role in the biogeochemical cycles that occur worldwide.

The interactions between bacteria and DOM are crucial to the marine carbon cycle, hence the circumstances that control DOM formation and consumption have a significant impact on carbon fluxes. The biochemical make-up and molecular size of DOM, the quantities of inorganic nutrients, and other environmental elements like temperature all likely affect how readily available it is to heterotrophic bacteria (Rainer *et.al*,1996)

The percentage of high molecular weight fractions DOC decreases with increase in salinity of water, as found in (Rainer *et.al*,1996)

The amount of DOC influences bacterial respiration rates. High molecular weight DOC showed to have high amounts of BR rates and production of bacterial biomass (Rainer *et.al*,1996).

Microbial Loop

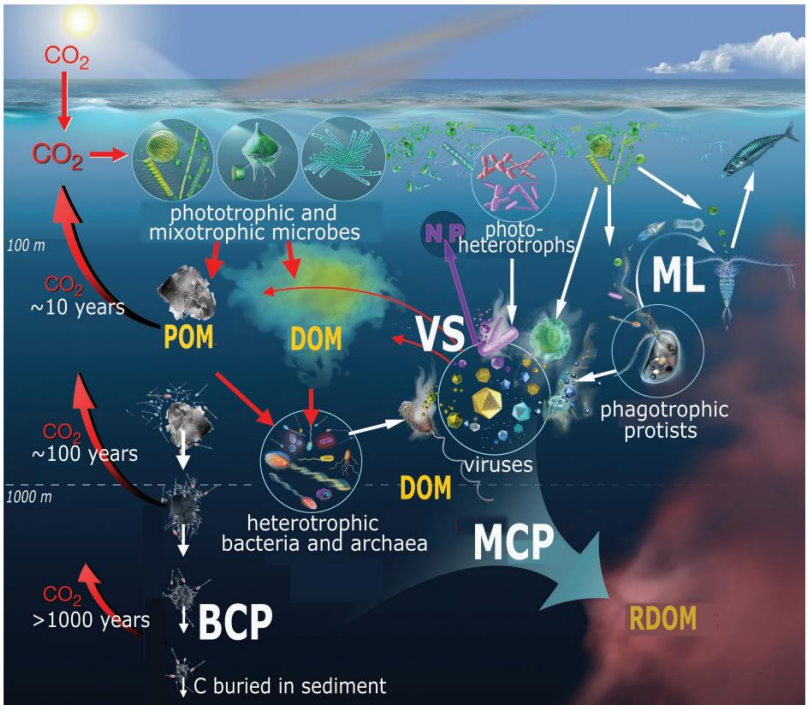


Figure 2. Microbial Loop and Microbial/Biological Carbon Pump.

The "microbial loop" theory proposes that bacteria serve as a crucial trophic connectio n between dissolved organic carbon (DOC) released from biota and upper trophic leve ls.

Particles that are an order of magnitude smaller than an organism likely to be used by it (Sheldon *et.al,* 1972). Photoautotrophic phytoplankton use the atmospheric CO2 which dissolves in the water for photosynthesis and Bacteria use DOM that is produced by phytoplankton and, to a much lesser amount, by aquatic fauna for their growth. These bacteria along with autotrophic cyanobacteria are consumed by flagellated heterotrophs, because they are in ingestible particle sizes, and keep them at densities of a density of 5 to 10×10^6 cells ml^-l. These flagellated whether heterotrophic or autotrophic are in-turn ingested by microzooplankton which are about the same size as the larger phytoplankton. Therefore, this loop of circulating the energy released by phytoplankton in the form of DOC is given back to the main food chain via the microbial loop consisting of Bacteria-flagellates-microzooplankton (Azam *et.al,* 1983).

Role of Bacteria in carbon cycle

A recent experiment by (Ducklow *et.al*.1992) has added a new support to the idea that microbes are carbon sinks.

The conversion of DOC into bacterial carbon is a significant stage in the microbial cycle. DOC is obtained from either the producers in the environment (autochthonous) or outside sources (allochthonous) (Tranvik *et.al*, 1987). Bacterial associations are involved in the conversion and mineralization of organic materials and are the most prevalent and crucial living elements in aquatic ecosystem. Heterotrophic bacteria play a significant role in the nutrient and carbon cycles by producing new bacterial cells (secondary production) and re-mineralizing nutrients and organic carbon which serves as a short-term carbon storing system and, through the remineralization of minerals and organic carbon, becomes a primary source of carbon dioxide. They are primarily responsible for the majority of the microbial growth and activity in water, and they obtain both their energy and carbon needs from organic substances (Giorgio *et.al* 1998;Zaccone *et.al* 2003).

The estuarine system's respiration of organic carbon depicts the reversion of CO2, back into the atmosphere, that had been fixed previously by terrestrial systems (Gawade *et.al* 2017).

The majority of bacterioplankton are heterotrophic, and play a significant role in the carbon cycle in aquatic systems.

These organisms are significant inorganic carbon (CO2) providers through the oxidation of organic matter, in addition to turning dissolved organic matter (DOM) into food supply for higher trophic levels. Therefore, simultaneous measurements of bacterial production (BP) and respiration (BR), which represent, respectively, the amount of carbon incorporated into bacterial biomass and that used for ATP generation and subsequently lost as CO2 must be used to estimate the magnitude of carbon flow through bacterioplankton (Guenther *et.al* 2008).

The overall quantity of organic carbon taken up by bacteria, or the bacterial carbon demand, as well as the percentage of carbon that is ultimately converted into bacterial biomass, or the bacterial growth efficiency (BGE), are both estimated by adding up BP and BR, (BGE).

A low BGE indicates that bacteria primarily function as a source of CO2 and other inorganic nutrients and a sink of organic matter. The latter measures the physiological state of bacterial cells and can also be used as an indicator of the bacterioplankton role in a system (Guenther *et.al* 2008).

Bacterial Respiration

In order to conserve energy, microorganisms employ a range of respiration systems, such as aerobic and anaerobic respiration with distinctly different energy transduction rates. Compared to aerobic respiration, anaerobic respiration typically produces less biological energy. Considering contrasting environments like oxic, suboxic/hypoxic, and anoxic marine waters and sediments that harbor different microbial communities with noticeably different energy conservation efficiencies may help us better understand the efficiency of the microbial carbon pump (MCP) for dissolved organic matter DOC transformation and sequestration (Burgin *et.al*. 2011).

A dominant role is played by heterotrophic bacteria and archea in the MCP (Jiao *et.al* 2010). In addition to directly producing respiratory refractory dissolved organic carbon (RDOC) products, the respiratory process also contributes to the MCP by providing metabolic energy to the environment, which in turn powers MCP processes (Dang *et.al* 2014).

Adenosine 5'-triphosphate (ATP) and reducing equivalents are produced by metabolism in all microbes, with the exception of obligate fermenters, which depend on substrate-level phosphorylation (Carlson *et.al* 2007).

The production of ATP molecules during energy conversion processes like respiration is essential for the cellular metabolism of carbon (Dang *et.al* 2014)

Dissolved Oxygen

In oxic waters the measurement of oxygen dissolved in water is called dissolved oxygen (DO) ie. the quantity of oxygen that aquatic organisms have access to. We can learn a lot about the biological status of a stream or lake by measuring the quantity of dissolved oxygen present.

All aerobic aquatic life uses dissolved oxygen in surface water, so it is usually tested to determine the "health" of lakes and rivers and lakes and other waterbodies. Both groundwater runoff and atmospheric oxygen infiltrate water bodies.

The main factor influencing the relationship between dissolved oxygen and temperature is photosynthesis; oxygen is absorbed by water when it is released by aquatic plants and photosynthetic bacteria during photosynthesis. The rate of photosynthesis is influenced by water transparency, sunlight intensity and its duration (Song *et.al* 2011).

Bacterial respiration can be found out by using filtered samples and analyzing the amount of dissolved oxygen present in the water samples collected in BOD bottles and analyzed using the Winkler's method (in duplicates). The samples were incubated and the DO was estimated before and after the incubation.

The total respiration rate is the difference of the remaining dissolved oxygen measured after the incubation period from the value of dissolved oxygen before the incubation period (Ram *et.al* 2003)

Bacterial Abundance

In marine environments, bacteria play an important role in the biogeochemical cycles and microbial food webs (Ducklow *et.al* 1992)

Bacteria in oligotrophic waters frequently use dissolved organic matter to take upto ha lf of the primary products, which are then eaten by protistan grazers (Azam *et.al* 1983).

Heterotrophic bacteria play a vital role in the cycles of nutrients and carbon by producing new bacterial biomass (secondary production) and re-mineralizing minerals and organic carbon. A fundamental concept in modern microbial ecology is that planktonic bacteria have a dual nature that must be understood (Giorgio *et.al* 1998). Planktonic bacteria in aquatic settings eventually digest a large portion of the primary products. A comparison between numerous natural aquatic systems has suggested that planktonic bacterial production is connected with and typically accounts for 30% of net primary production (Ducklow et.al 1992);(Giorgio *et.al* 1998).

The majority of microbial biomass and activity in the water column of lakes and seas is produced by aerobic, planktonic, heterotrophic bacteria that use organic substances to meet both their energy and carbon requirements (Giorgio *et.al* 1998).

Growth efficiency is, by definition, the amount of biomass produced for every unit of absorbed substrate. Diverse substances, elements, and minerals are transformed into

cell material throughout the development process at the price of the energy source (Giorgio *et.al* 1998).

Total Suspended matter

Total suspended matter refers to fine, extremely small particles with a diameter more than 1 μm which are found in aquatic environments (Fanela *et.al* 2018). TSM may be influenced by many factors such as rainfall, tides, winds, currents and waves (Fanela *et.al* 2018). Besides these factors the amount of suspended particulate matter in the estuary ecosystem can be influenced by mining, dredging, barges carrying mined products like coal as well as mechanized boats. Total Suspended Matter (TSM) along the Mandovi estuary area was observed monthly (Siraswar et.al 2012).

Measuring TSM is crucial for managing water quality, especially for murky inland water. TSM can sometimes also act a source of nutrients and a catalyst for photosynthetic activity, TSM content has an impact on marine ecosystems. TSM is frequently linked to total primary production micropollutants, and heavy metal flows. It is closely related to issues with sediment fluxes and the amount of light that is accessible for primary production in many turbid areas (Song et.al 2011).

Chlorophyll a

One of the primary pigments found in phytoplankton is chlorophyll-a. The quantity of phytoplankton in coastal and estuarine areas and the ocean, is a good indicator of how productive certain aquatic habitats are. This justifies checking why chlorophyll-a level may be utilised as an indicator of productivity of the estuary as phytoplankton play a significant role in the microbial loop (Fanela *et.al* 2018)

Application

The worldwide MCP capacity of organic carbon sequestration is likely to have a significant impact on the Earth's carbon cycle and possibly its climate (Wang *et.al* 2014).

Chapter II

MATERIALS AND METHODS

Sampling and analysis

SAMPLING SITES: Samples were collected from five different areas along the Mandovi estuary. Samples from Diwar ferry, Ribandar, Patto, Panjim ferry and Dona Paula jetty were collected in bottles 1L ,2L pretreated with 1%HCL and washed with soap. Surface river water from the above five stations were collected for six months from the month of July – December 2022.

Figure 3 Collection of water sample from the field.

1. **Temperature**

The temperature of the water was measured using a thermometer. The thermometer was dipped into the estuarine water body for about 1 minute. The thermometer was removed from the water and the temperature was noted for each site.

2. **Salinity**

The salinity of water samples was determined using a refractometer. The measuring prism of the refractometer was gently wiped with distilled water and soft tissue paper. A drop water sample was added on the measuring prism and the flap cover was placed. The eye piece was adjusted to achieve a sharply focused image of the scale. The instrument was held up to a light source to obtain a reading. The value of salinity was noted down.

Figure 4 A Refractometer.

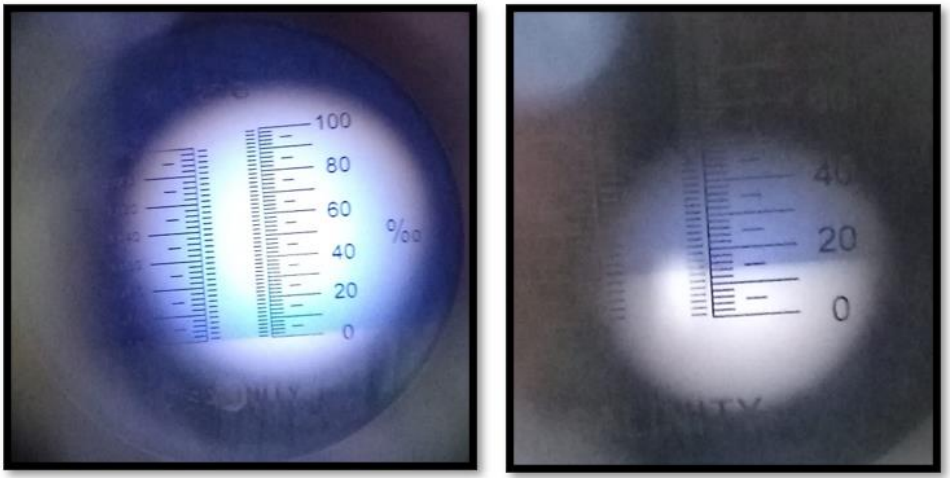


Figure 5 Refractometer Scale showing Salinity in parts per thousand.

3. **Dissolved oxygen (DO)**

Figure 6 Fixing of water samples with Winkler's reagents A and B in the field.

Figure 7 Water samples fixed with Winkler's A and B with precipitate settling down.

1. Preparation of Winkler's A and B solutions as follows:

Winkler A: Manganous sulfate

60g of Manganous sulphate monohydrate was dissolved in 100 ml distilled water and in a volumetric flask and store in an amber coloured bottle and labelled appropriately.

Winkler B: Alkaline iodine

60g of reagent grade KOH was dissolved in 30 ml distilled water and 32g reagent grade Ki (potassium iodide) was dissolved in 30 ml distilled water. The two solutions were mixed well and the volume was brought to 100ml in volumetric flask and store in an amber coloured bottle and labelled appropriately.

Note: do not mix droppers of the A & B solutions label droppers and bottles with stickers.

1. Stoppered bottles are labelled as 'zero-hour reading' and '24-hour reading' in duplicates for each station. The bottles are submerged into the water taking care that no air bubbles are trapped in the bottles, to remove bubbles the bottles are submerged under water and shaken vigorously. 1ml of Winkler's A& Winkler's B respectively was added in the 'zero reading' bottles in situ. The stopper was placed on the bottle and the bottle was agitated to mix everything well. The bottles were left undisturbed to let the precipitate form and settle down. The bottles were kept in the dark. The 24-hour bottles were incubated at room temperature for 24 hours. Winkler's A & B was added in 24 hour reading bottles after 24 hours of incubation.

   1 ml 50% H2SO4 (**Preparation :50 ml H2SO4 to 50 ml DW**) was added in all bottles after adding Winkler's reagents to give colour change of orange and shaken well to dissolve precipitate completely.

2. **Preparation of Sodium Thiosulphate solution: -**

   2.5g sodium thiosulphate was dissolved in 1000ml volumetric flask with distilled water.

3. **Prepare starch solution: -**

   1g soluble starch powder was weighed and added in 100ml distilled water and kept in water bath to be digested to get a clear solution.

Titration

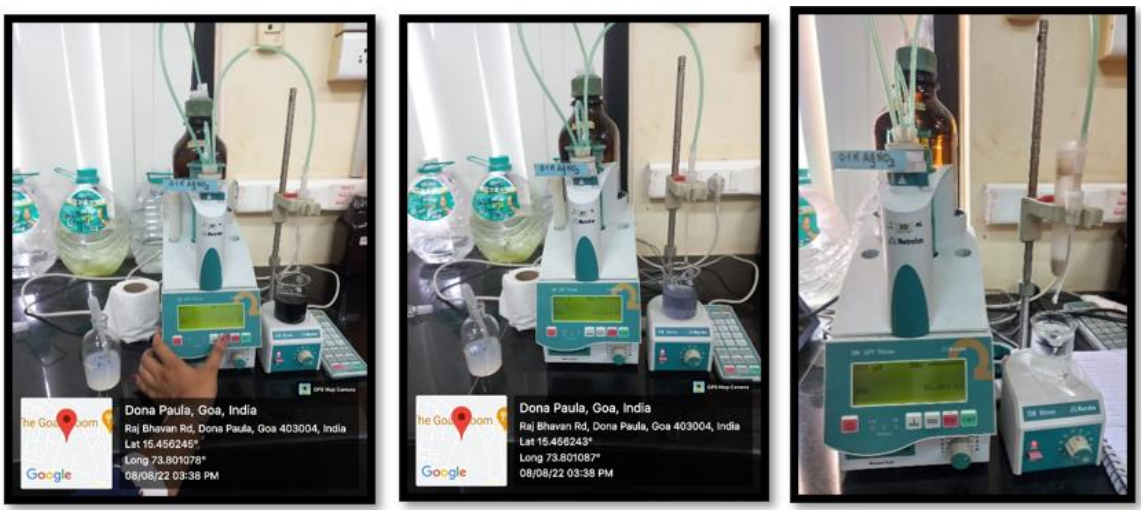


Figure 8 An Automated Titrator (Dosimat) used for titration. Titration id done until solution turns colourless.

For titration a 50ml bulb pipette was used to pipette out 50 ml of acidified sample and was put in a 200ml beaker .1ml starch was used as indicator and there was a colour change to dark bluish black. It was titrated against sodium thiosulphate until solution turns to colourless. The readings were recorded and DO was calculated (*JGOFS Protocols*).

4. Bacterial respiration (BR)

The BR rate was measured in two ways.

i. Monthly BR rates at room temperature

The water samples were collected in stoppered bottles and fixed with Winkler's A and B at 0 hour and 24 hours. The samples were acidified with 50% H_2SO_4 and agitated until precipitate dissolved completely and the solution turned pale yellow to orange in colour. 50ml of the acidified solution was pipette out with a bulb pipette and added in a beaker. 1ml of 1% starch solution was added as an indicator. the colour of the solution changes to bluish black and is titrated against 2.5 N Sodium thiosulphate solution. End point was when bluish-black solution turned colourless. DO was calculated at 0 hour and 24 hours. The DO values for 0 hour and 24 hours were measured and were used to calculate BR.

ii. BR rates at Elevated Temperature.

Figure 9 Filtered water samples fixed with Winkler's A and Winkler's B.

This experiment was carried out for filtered and unfiltered samples which were each incubated at room temperature and Elevated temperature (34°C).

A 0.77 µm GF filter was used to filter the water samples.

A set of unfiltered and filtered samples were incubated at room temperature and one set was incubated at elevated temperature for 24 hours.

The unfiltered and filtered samples were analyzed for Dissolved oxygen (DO) using the Winkler's titration method of Carritt and Carpenter (1966) using automated titration unit.

5. Total suspended matter (TSM)

Figure 10 TSM concentrated on the filter paper after filtration.

0.45 µm pore Millipore filters were weighed and the weight of the filter papers were noted down. 300 ml of the water sample from each station was filtered with 0.45 µm pore Millipore filters using a vacuum pump to retain planktons and other microscopic organisms that contribute to TSM. The filter papers were carefully folded and kept in aluminum foil and dried in the oven. The dry weight was noted down. The filter papers were weighted after filtration and the weight of the filters before and after filtration. The value obtained was then used to calculate TSM for 1liter of water sample by cross multiplication. The unit for TSM is mg/L.

6. Chlorophyll-a measurement

Figure 11 Filtration of water samples using a vacuum pump.

500ml of water sample from each station was filtered through a GF/F filter (Whatman)/0.47 µm acetate filters to retain phytoplankton biomass by the filter within 24 hours after sample collection. The filter papers were put in 20 ml centrifuge tubes containing 15ml 90% acetone and the tubes were incubated in at 4°C in the dark for 12hours. The tubes were vortexed for 30 seconds and left Chl-a concentrations were determined spectrophotometrically by taking the optical densities at wavelengths of 664, 647 and 630. the absorbance was noted and the Chl-a concentration was calculated by using the formula.

Chl a = 11.85*E664 – 1.54*E647 – 0.08*E630 *Ve/L*Vf (Jeffrey, 1975);(Aminot,2000)

Where:

E = extinction coefficient .

Vf = Filtered volume in litre.

Ve = Extraction volume in ml.

L = Light-path of the cuvette in centimetre.

Concentrations are in unit mg m−3

7. Particulate organic carbon (POC)

Figure 12 Filter papers were pre-combusted in a Muffle furnace.

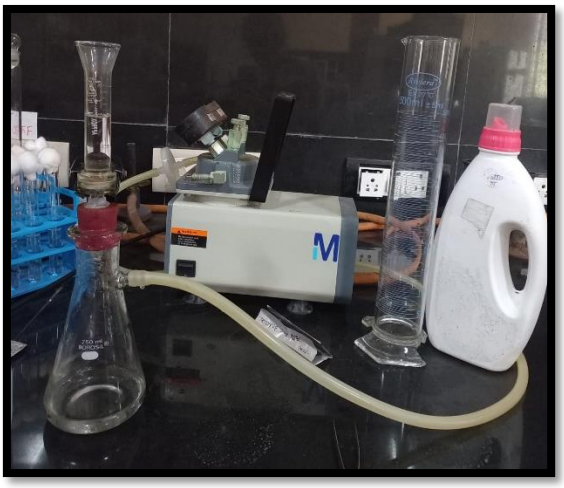


Figure 13 Filtration unit.

Figure 14 Acid fumigation of Filter papers in a Fumigator with concentrated HCl.

GF/F filters were pre-combusted at 450°C for 5 h in a muffle furnace. 200 ml of water sample was filtered through the GF/F filters. The filter papers were folded and wrapped in aluminum foil and labelled with the station name and date and stored in the freezer. Acid fumigation treatment was performed on the filter papers using concentrated HCl in a fumigator just before the analysis. The sample filter papers were to be analyzed using Elemental Analyzer.

8. **Bacterial abundance (BA)**

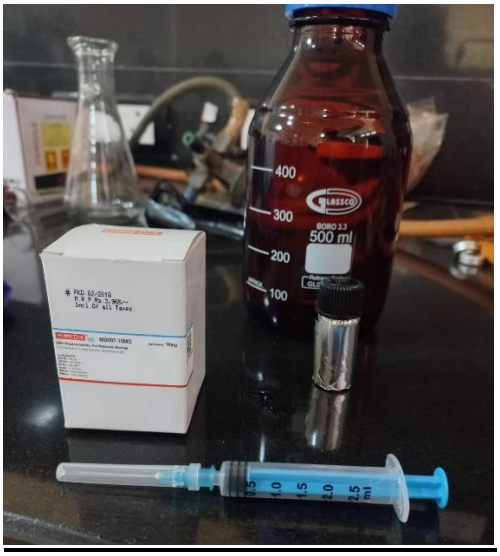


Figure 15 DAPI stain

500 ml of the water sample was filtered out using 0.45 μm Millipore filters. The filtered water was siphoned into 7ml vials (duplicates) and wrap in a black cloth. Incubate at room temperature. Collect samples at 0 and 24 h from incubated Nalgene bottles and preserve at 4°C with 5% formalin until analysis. Stain cells with 40-60 - Diamidino-2-phenylindole (DAPI) stain and count using epifluorescence microscope.

9. **Bacterial colony character**

MacConkey's agar, EMB agar, Nutrient agar and Listeria identification agar was weighed and the media were autoclaved and poured into sterile petri plates. Dilutions up to 10^-3 were prepared of water samples with 0.85 N sterile saline. 100 µL of 10^0 and 10^-3 dilutions of the water samples were spread plated on each media. The plates were wrapped, inverted and incubated for 2-4 days at 37°C. The plates were observed for growth and colony characteristics were noted down.

Chapter III

RESULTS AND DISCUSSION

Sampling

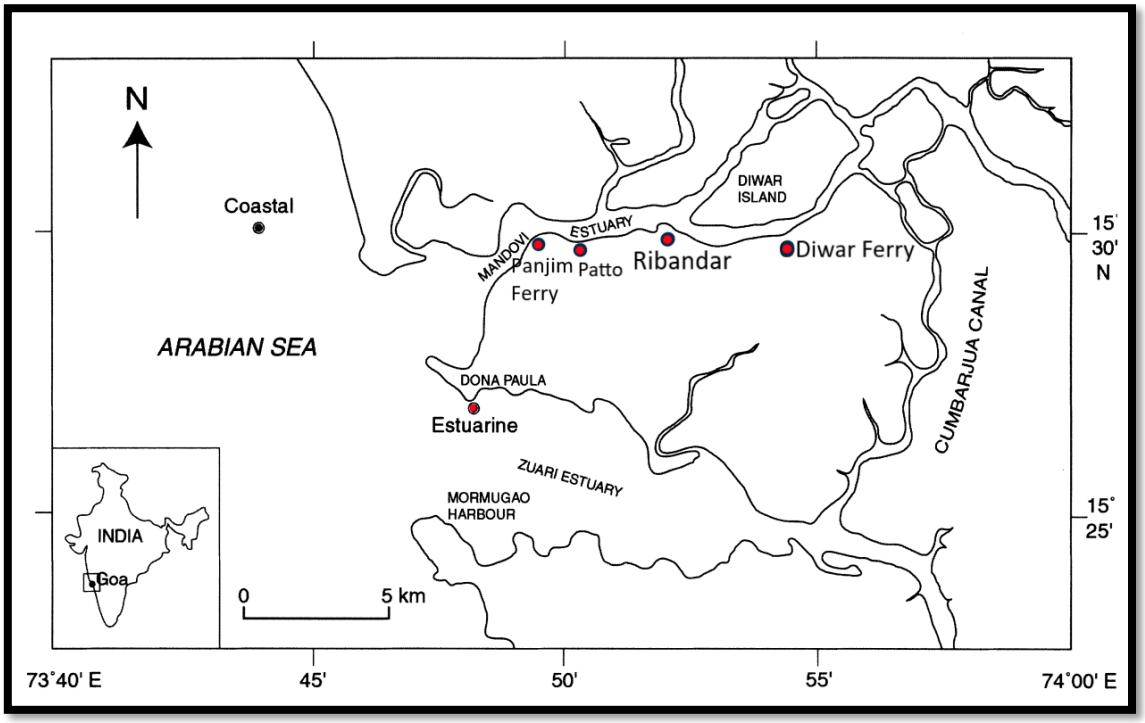


Figure 16 Map of Sampling Stations.

Figure 17 Station 1. Diwar ferry

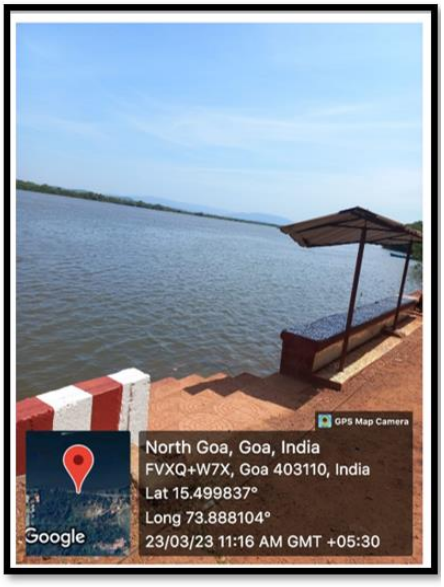


Figure 18 Station 2. Ribandar

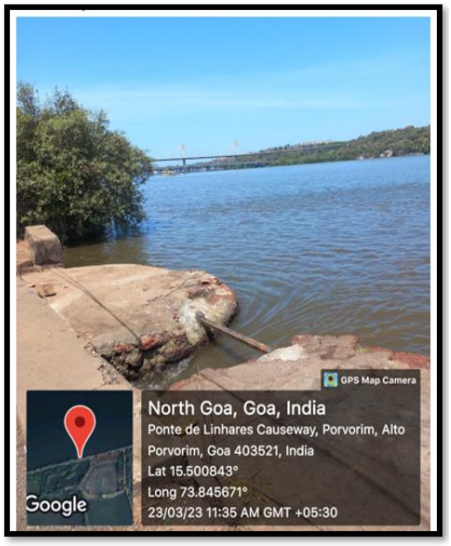


Figure 19 Station 3. Patto

Figure 20 Station 4. Panjim ferry

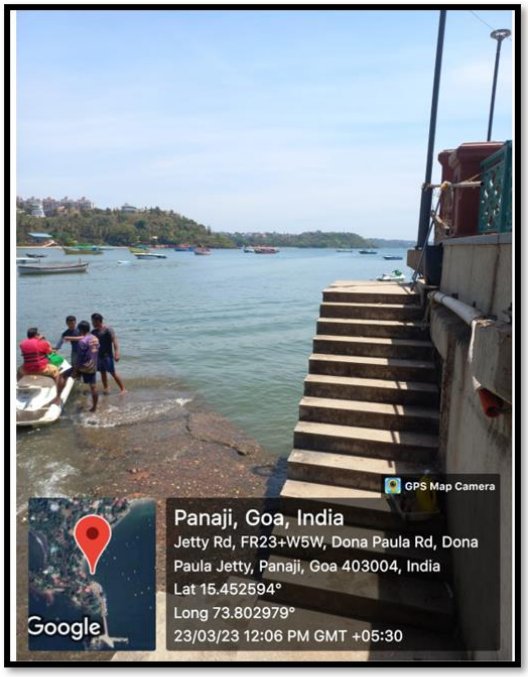
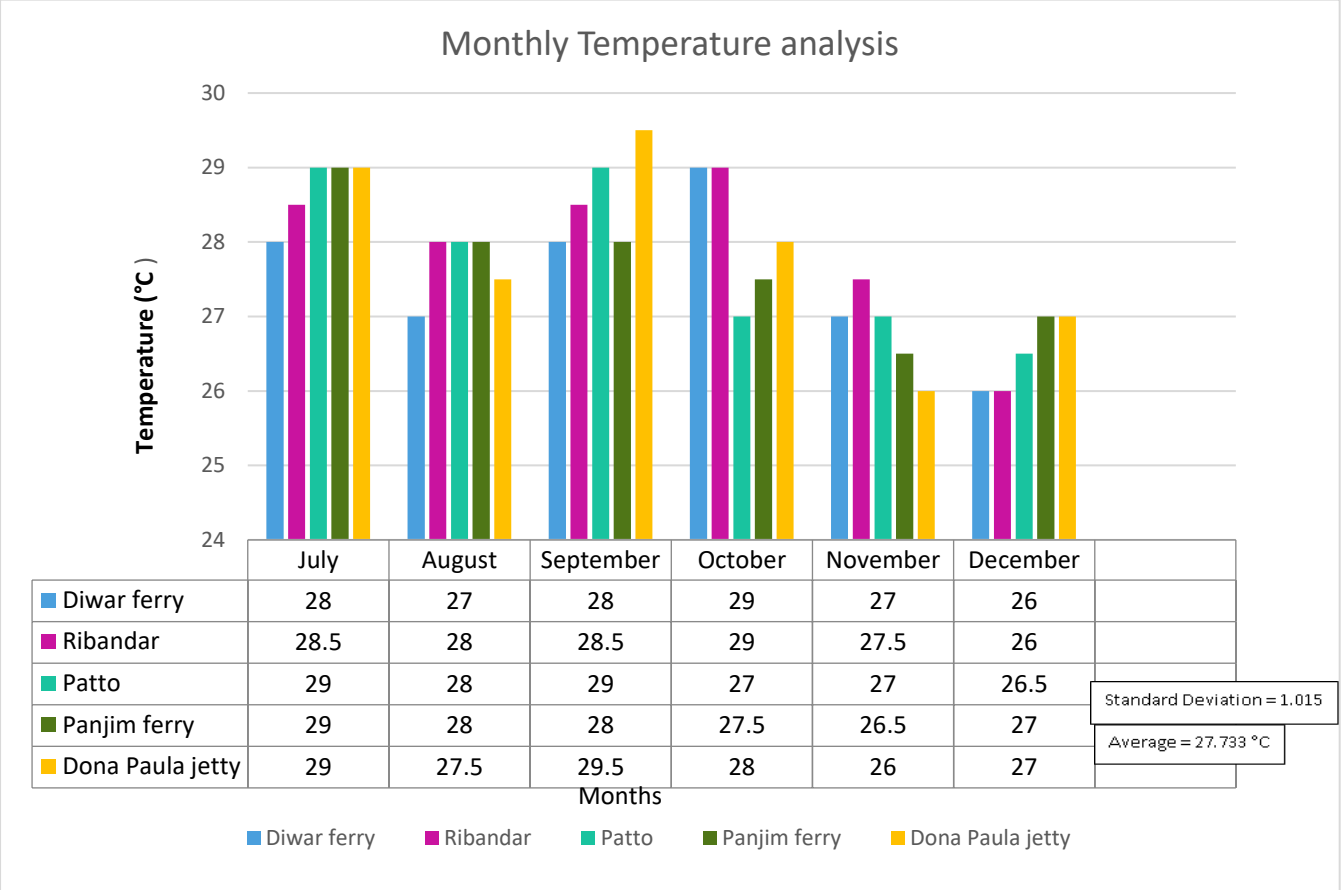


Figure 21 Station 5. Dona Paula Jetty

1. Temperature



	July	August	September	October	November	December
Diwar ferry	28	27	28	29	27	26
Ribandar	28.5	28	28.5	29	27.5	26
Patto	29	28	29	27	27	26.5
Panjim ferry	29	28	28	27.5	26.5	27
Dona Paula jetty	29	27.5	29.5	28	26	27

Figure 22. Graph showing the relationship between each Month and Temperature.

Temperature of the water in all five stations was measured using a thermometer in situ from the months of July to December 2022 during the day time from approximately 10 am to 12 noon.

The spatiotemporal variation of temperature varied between 26 – 29.5 °C (27.733 ± 1.0148).

As can be seen in figure 22, during the month of July the temperatures of the stations were higher followed by the month of September. The highest recorded temperature was 29.5°C at Diwar ferry in the month of September. The temperature of the water was lower in the months of November and December this is because of the beginning of winters in Goa. the temperatures if the waters were high during the months of July–September because the temperature was recorded on sunny days during the monsoon season.

In (Ram *et.al.* 2003), during the period of study, the temperature of the water at the sites within the Mandovi estuary ranged from 27.7-32°C .

2. Salinity

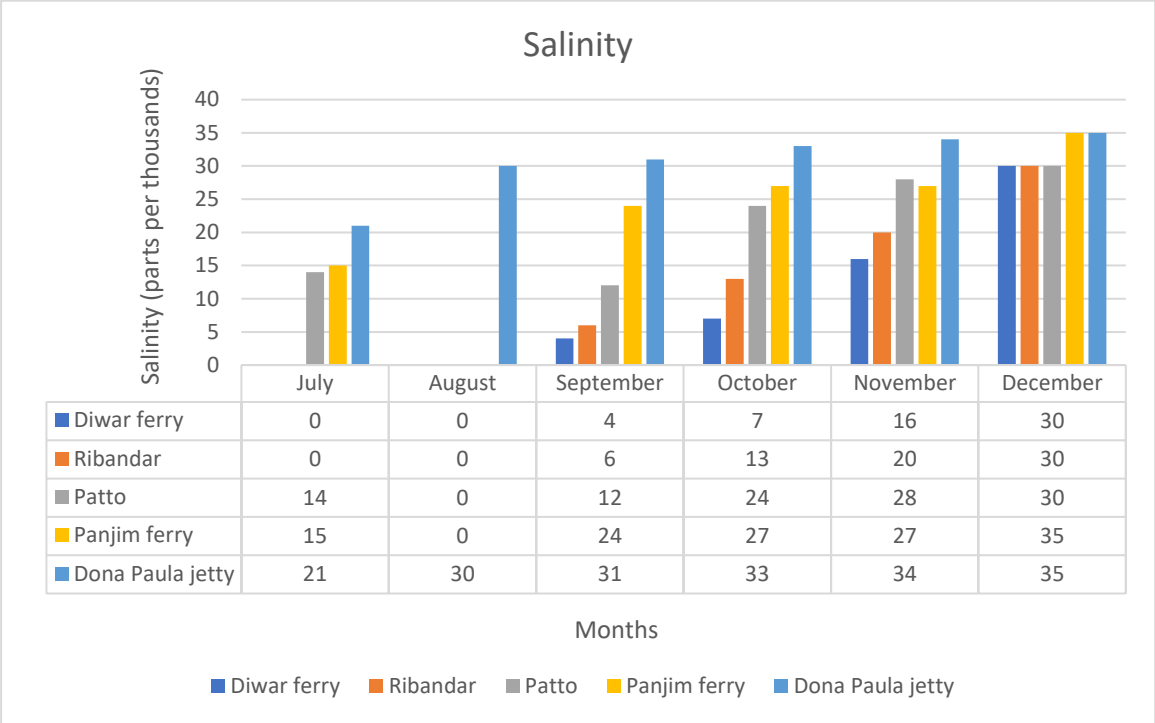


Figure 23 Graph of Month v/s Salinity.

The salinity of the water in all five stations was measured from July-December 2022 during the day time from approximately 10 am to 12noon.

The spatiotemporal variation of salinity ranged from 0-35 parts per thousands (18.2 ± 12.65).

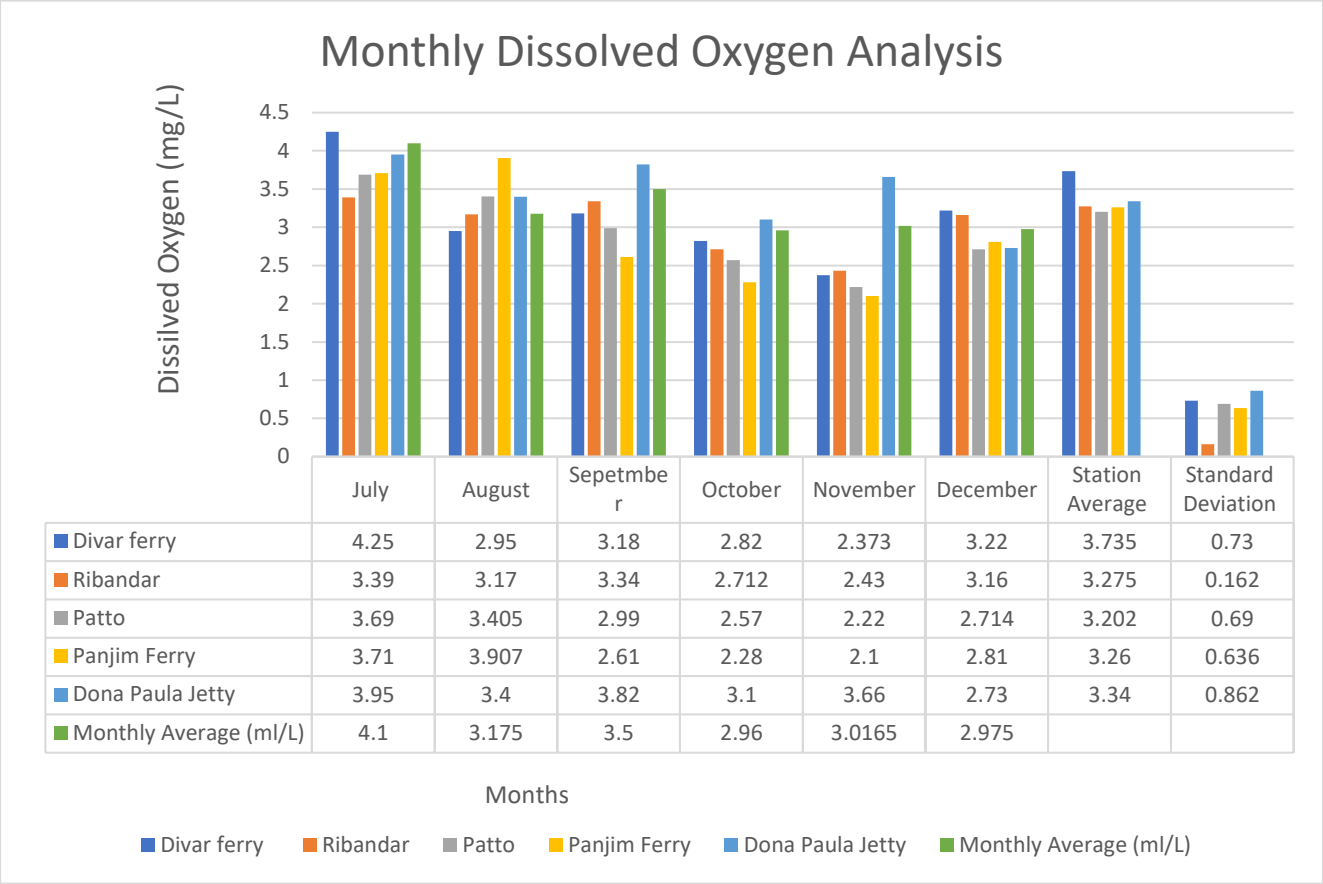
As can be seen in figure 23, the overall salinity of the estuary in the months of July -August are the lowest and the overall salinity of the estuary during the month of December is the highest. The salinity of the water in Diwar ferry and Ribandar in the month of July and the salinity of water in Diwar ferry, Ribandar, Patto and Panjim in the month of August were found to be zero. This is most likely because of the heavy rains during the monsoon season.

A gradual increase of salinity is seen in the water of all the stations after the month of July as the winter approached.

It is seen that the Dona Paula jetty holds the record for having the highest salinity in all cases, this is because there is maximum mixing of sea water directly at this point near the Dona Paula jetty while the stations upstream receive water from various streams originating from Bhimgad in the Western Ghats in the Belagavi district of Karnataka and a lot of rain water runoff from land during the monsoons and hence are more dilute.

In (Ram *et.al.*,2003) the average of the salinity was 25.70 (±6.50) within the Mandovi estuary.

3. Dissolved Oxygen



	July	August	Sepetmber	October	November	December	Station Average	Standard Deviation
Divar ferry	4.25	2.95	3.18	2.82	2.373	3.22	3.735	0.73
Ribandar	3.39	3.17	3.34	2.712	2.43	3.16	3.275	0.162
Patto	3.69	3.405	2.99	2.57	2.22	2.714	3.202	0.69
Panjim Ferry	3.71	3.907	2.61	2.28	2.1	2.81	3.26	0.636
Dona Paula Jetty	3.95	3.4	3.82	3.1	3.66	2.73	3.34	0.862
Monthly Average (ml/L)	4.1	3.175	3.5	2.96	3.0165	2.975		

Figure 24. Graph of Month v/s Dissolved Oxygen.

The dissolved oxygen in the water samples in all the five stations was analyzed from the months of July to December 2022 using an automated titrator.

The spatiotemporal variation of DO was found to be between 2.1- 4.25 mg/L (3.12 ± 0.53).

As can be seen in Figure 24, the dissolved oxygen of the water , in the month of July, in the Diwar Ferry is the highest with 4.247 mg/L and the DO of the water ,in the month of November, in Panjim is the lowest with 2.1 mg/L.

The standard deviation of DO was minimum in Ribandar from July–December it was 0.162 mg/L and was maximum in Dona Paula Jetty which was 0.86 mg/L.

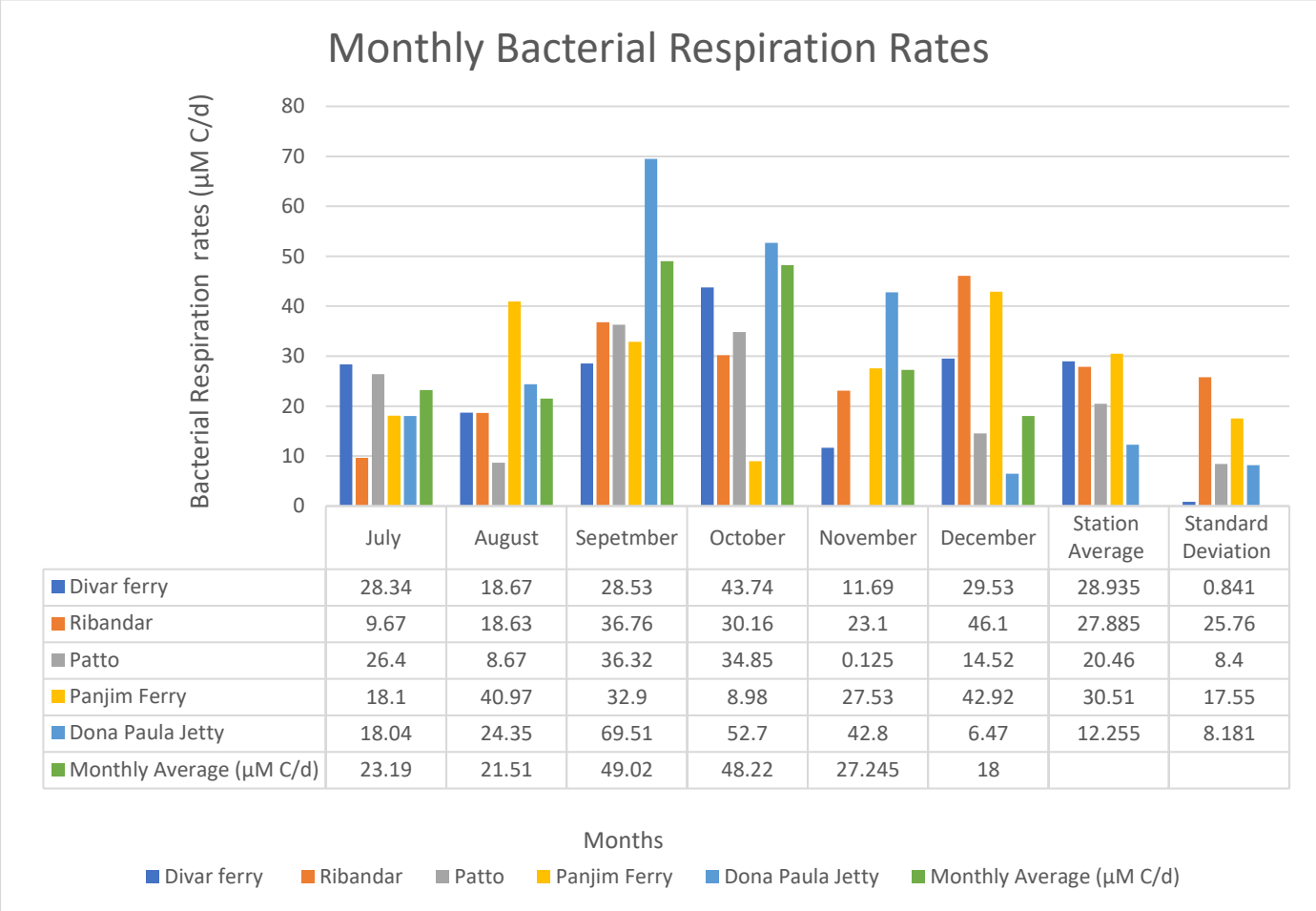
The average DO value calculated for the month of July was the maximum (4.1 mg/L) and was minimum in the month of October (2.96mg/L).

The DO levels in water can be influenced by many factors one of them may be physical aeration by rain water from surface runoff or by waves when there is a high tide.

The DO during the monsoon months July–September may be higher because of this reason. Due to lack of rain during the winters there is less aeration of water hence the DO values during the months of October–December seems to be lower.

In (Ram *et.al.* 2003) the Dissolved oxygen level was found to be (4.0 to 6.0 mg/L).

4. Bacterial Respiration (BR)



	July	August	Sepetmber	October	November	December	Station Average	Standard Deviation
Divar ferry	28.34	18.67	28.53	43.74	11.69	29.53	28.935	0.841
Ribandar	9.67	18.63	36.76	30.16	23.1	46.1	27.885	25.76
Patto	26.4	8.67	36.32	34.85	0.125	14.52	20.46	8.4
Panjim Ferry	18.1	40.97	32.9	8.98	27.53	42.92	30.51	17.55
Dona Paula Jetty	18.04	24.35	69.51	52.7	42.8	6.47	12.255	8.181
Monthly Average (µM C/d)	23.19	21.51	49.02	48.22	27.245	18		

Figure 25. Graph of Month v/s Bacterial Respiration.

The BR of all the five stations was calculated from July- December using the DO values.

The BR value was the highest in Dona Paula Jetty in the month of September with a value of 69.51 µM C/d and was the lowest in December with a value of 6.47 µM C/d.

As seen in figure 25, the average BR rate of the Mandovi estuary was found to be the highest in the month of September with a value of 49.02 µM C/d. This could be a result of large amounts of DOM that is introduced in the water during the monsoon season which acts as food for heterotrophic microorganisms therefore they use up a large quantity of dissolved oxygen from the water to utilize the food source.

The month of December showed the lowest average BR rate for the Mandovi estuary with a value of 18.00 µM C/d. This is because during the winter since the levels of organic matter in the water are lower, microorganisms have less available organic matter to feed on and their number decreases hence utilize lesser dissolved oxygen.

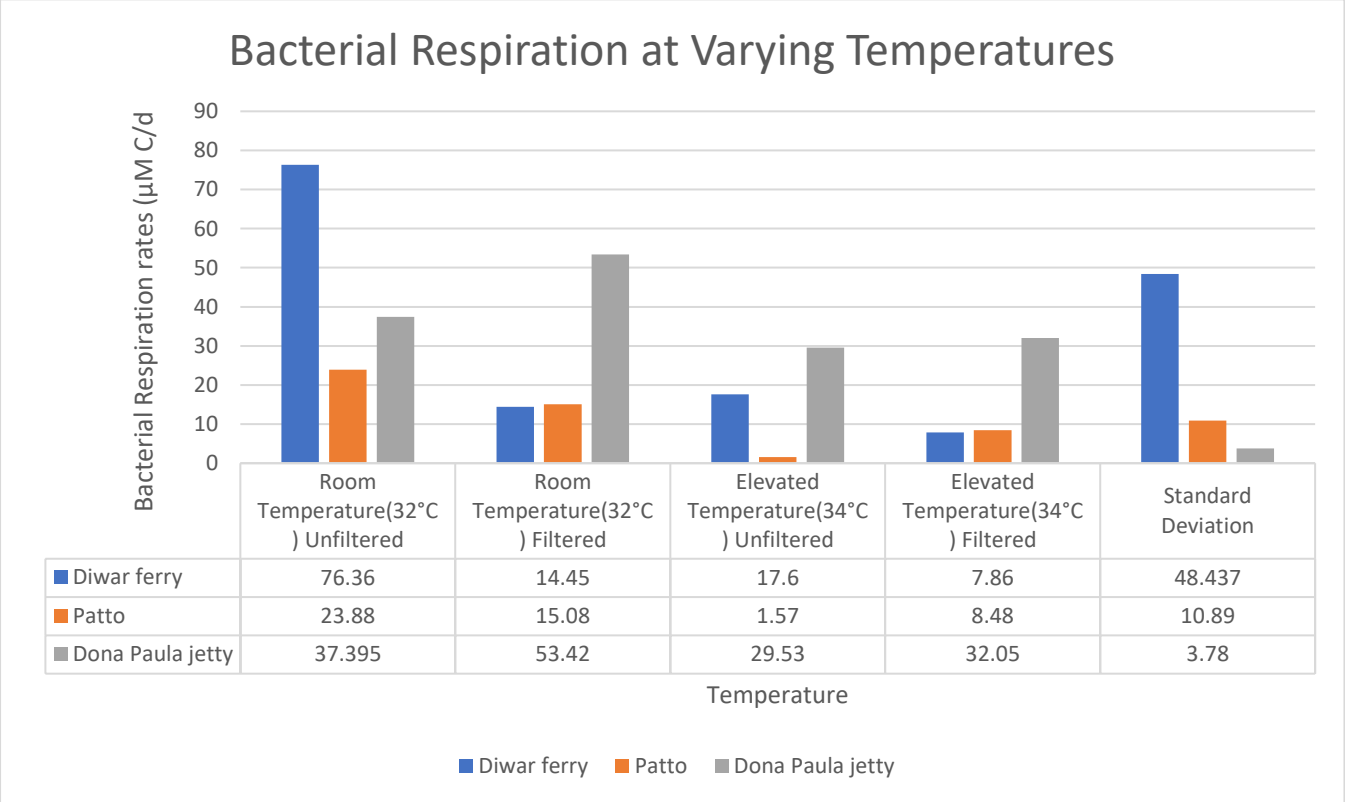


Figure 26. Graph of Varying Temperature v/s Bacterial Respiration rates.

The Bacterial Respiration Rate of the water samples from Diwar ferry, Patto and Dona Paula jetty were calculated in the month of February for unfiltered and filtered samples kept at room temperature and elevated temperature.

The spatiotemporal variation of the BR was found to be between 1.57-76.36 µM C/d (26.47 ±21.37).

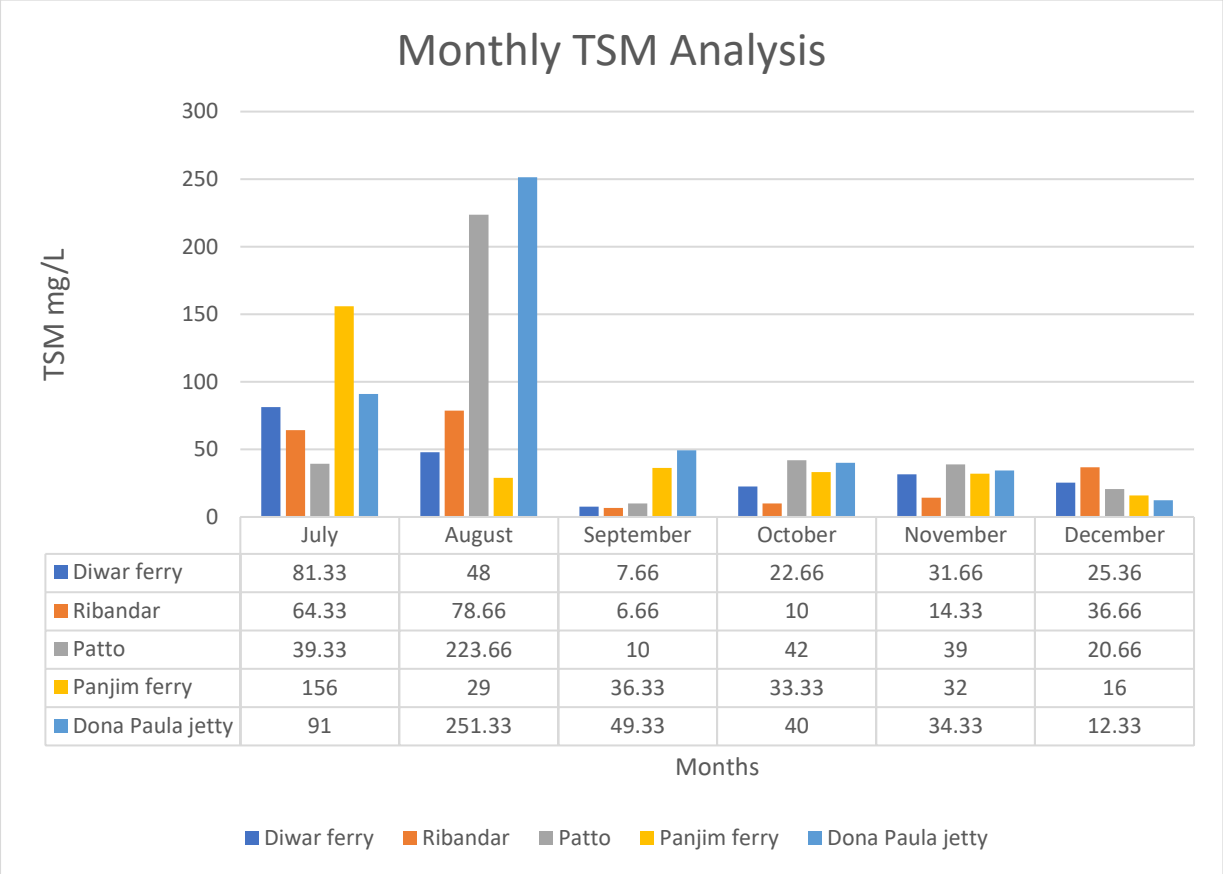
As can be seen in figure 26, BR rate was maximum in Diwar ferry's unfiltered water sample at room temperature which was found to be 76.36 µM C/d followed by filtered water sample from Dona Paula jetty which was found to be 53.42µM C/d.

The maximum BR rate of water samples kept at elevated temperature was found to be 32.05 µM C/d in the Dona Paula Jetty water sample and the minimum was found to be 1.57 µM C/d in the Patto water sample.

It can be seen that increased temperature negatively affects the BR rates in water. In (Apple *et.al*. 2006) it can be seen how higher temperatures lower the bacterial production rates and BR rates.

The BR rates were higher in the room temperature water rather than elevated temperature. This could indicate that if the temperatures of water keep increasing due to Global warming then it could have a negative impact on BR rates which would affect the carbon cycles in marine environments.

5. Total Suspended Matter (TSM)



	July	August	September	October	November	December
Diwar ferry	81.33	48	7.66	22.66	31.66	25.36
Ribandar	64.33	78.66	6.66	10	14.33	36.66
Patto	39.33	223.66	10	42	39	20.66
Panjim ferry	156	29	36.33	33.33	32	16
Dona Paula jetty	91	251.33	49.33	40	34.33	12.33

Figure 27. Graph of Month v/s TSM

The TSM of the water in all five stations was analyzed by filtration using a 0.45 µm pore filters from the months of July to December 2022.

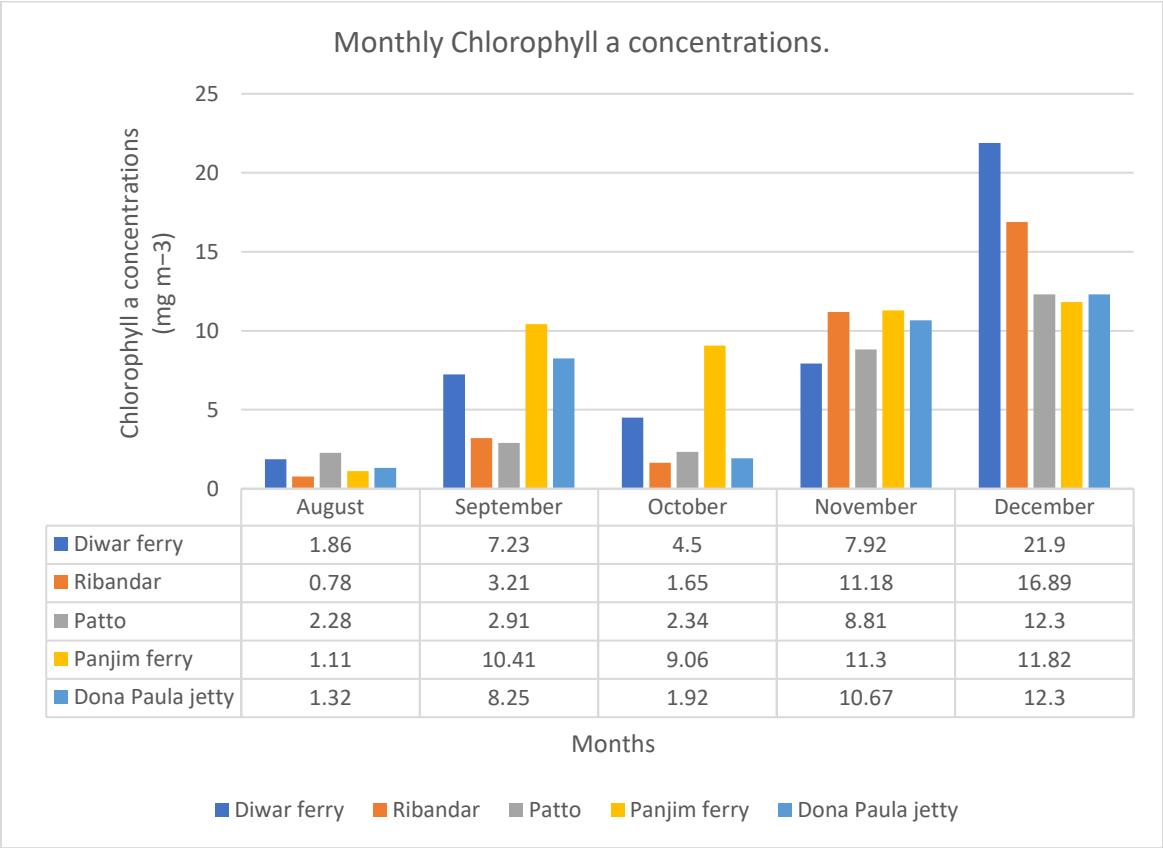
The spatiotemporal variation of TSM was found to be between 6.66 - 251.33 mg/L (52.76 ± 58.931).

As can be seen in figure 27, the TSM during the months of July and August seem to be comparatively higher than the rest of the months. The possible reason for this could be that since those were the months of monsoons a lot of rain water and surface runoff could have contributed to the amount of suspended matter in the water bodies.

The TSM during the remaining months is lower most likely because the water bodies are more calmer during the winter seasons which lead to the suspended particles to settle down and hence the waters are more clearer with less suspended particles during this time. Many factors like moving barges and water boats can contribute to the amount of TSM during the

winter months as their movement in the water causes ripples and formation of small waves that stirs up the water and leads to the water becoming turbid.

6. Chlorophyll a



	August	September	October	November	December
Diwar ferry	1.86	7.23	4.5	7.92	21.9
Ribandar	0.78	3.21	1.65	11.18	16.89
Patto	2.28	2.91	2.34	8.81	12.3
Panjim ferry	1.11	10.41	9.06	11.3	11.82
Dona Paula jetty	1.32	8.25	1.92	10.67	12.3

Figure 28. Graph of Month v/s Chlorophyll a concentration.

The chlorophyll a in the water in all five stations was analyzed by filtration using 0.77µm pore GF filters from the months of July to December 2022.

The spatiotemporal variation of chlorophyll a was found to be between 0.78-21.9 mg m−3 (7.36 ± 5.53).

As can be seen in figure 28, the chlorophyll a in the months of August, September and October are comparatively lower than November and December. The possible reason for this could be that the amount of chlorophyll a is inversely proportional to TSM. The more the TSM means that the water will be turbid and hence the sunlight available for photosynthesis for photosynthetic bacteria and plankton will be lower and hence the amount of chlorophyll a is lower. These results are similar to the results found in (Fanela *et.al* 2018) which shows that high levels of TSM has a negative impact on chlorophyll a levels. Hence the number of photosynthesizing microorganisms that contain chlorophyll a will be lower. Since the water is

comparatively clearer during the winter season i.e. November and December, it is seen that the chlorophyll a levels have increased.

7. **Particulate Organic Carbon**

Analysis of POC samples is awaited due to some technical issues of the elemental analyzer.

8. **Bacterial abundance**

Analysis of the samples is awaited due to a shortage of black nucleopore polycarbonate filters.

9. **Colony Characteristics**

The colony characteristics for 10^0 and 10^-3 dilutions of the water samples were checked on different agar media. This experiment was done to check the diversity of microorganisms present in the water samples. Various kinds of colonies grew on the different media and their colony characters were noted down. The colonies which grew ranged from *Pseudomonas, E.coli, S.aureus* etc.

Colonies of *E.coli* were found growing on EMB media in October in Panjim ferry (figure 40) and in the month of December in Patto (figure 48).

The overall types of colonies from the sampling sites remained the same.

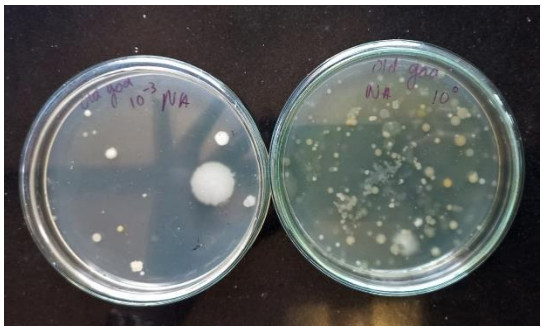


Figure 29

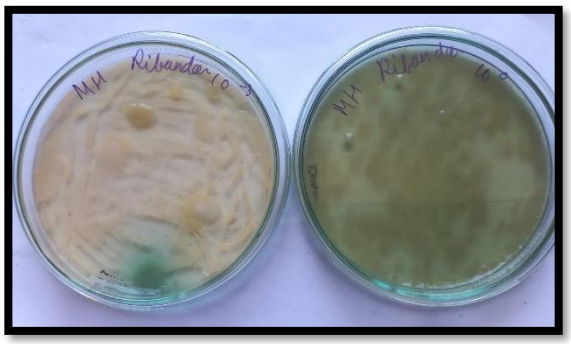


Figure 30

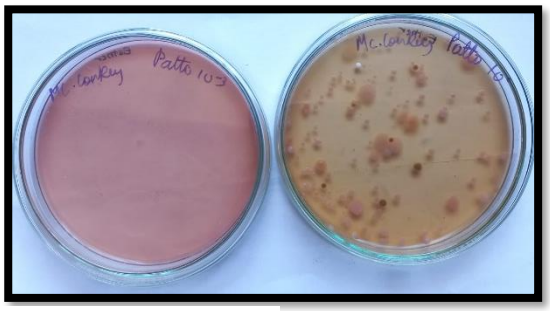


Figure 31

Figure 32

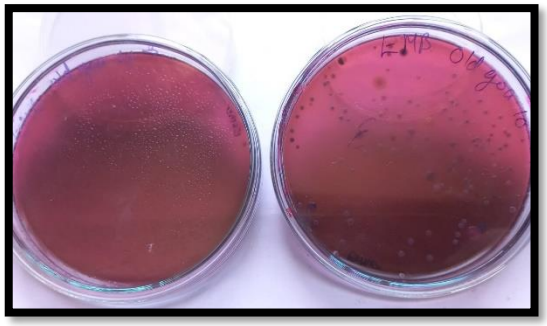


Figure 33

Figure 34

Water samples spread plated on various media in the month of <u>September.</u>

Figure 29. *Water sample from Diwar ferry spread plated on Nutrient agar with 10^-3 and 10^0 dilutions respectively.*

Figure 30*. Water samples from Ribandar showing the presence of Pseudomonas colonies on Mulher Hinton Agar for 10^0 and 10^-3 dilutions in the month of September.*

Figure 31. *Water sample from Patto spread plated on MacConkey's agar with 10^-3 and 10^0 dilutions respectively.*

Figure 32. *Water sample from Dona Paula jetty spread plated on MacConkey's agar with 10^-3 and 10^0 dilutions respectively.*

Figure 33. *Water sample from Diwar ferry spread plated on EMB agar with 10^-3 and 10^0 dilutions respectively.*

Figure 34. *Water sample from Dona Paula jetty spread plated on EMB agar with 10^-3 and 10^0 dilutions respectively.*

Figure 35

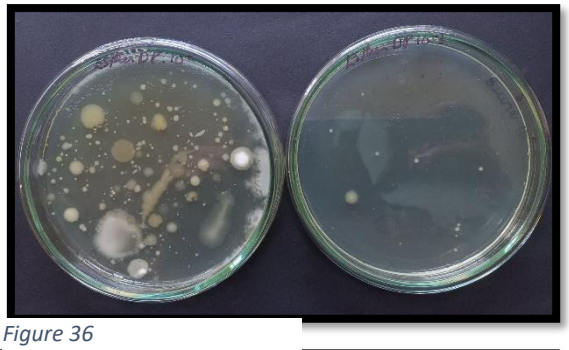


Figure 36

Figure 37

Figure 38

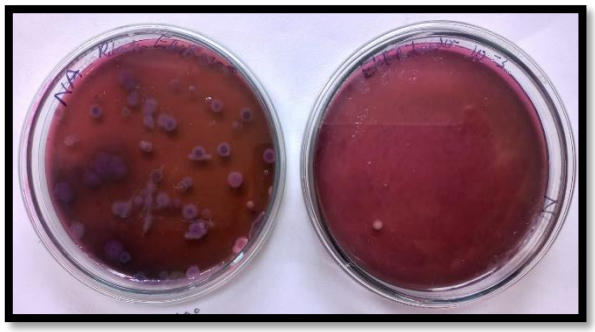


Figure 39

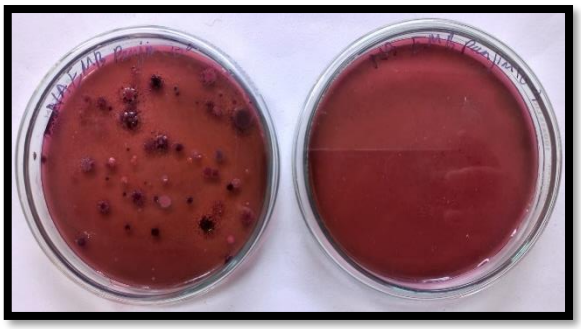


Figure 40

Water samples spread plated on various media in the month of <u>October.</u>

Figure 35. *Water sample from Patto spread plated on Nutrient agar with 10^-3 and 10^0 dilutions respectively.*

Figure 36*. Water sample from Dona Paula spread plated on Nutrient agar with 10^0 and 10^-3 dilutions respectively.*

Figure 37. *Water sample from Patto spread plated on MacConkey's agar with 10^0 and 10^-3 dilutions respectively.*

Figure 38. *Water sample from Patto spread plated on Listeria Identification Agar Base (PALCAM) agar with 10^0 and 10^-3 dilutions respectively.*

Figure 39. *Water sample from Ribandar spread plated on EMB agar with 10^0 and 10^-3 dilutions respectively.*

Figure 40. *Water sample from Panjim ferry spread plated on EMB agar with 10^0 dilutions and 10^-3 dilution showing growth of <u>E.coli</u>.*

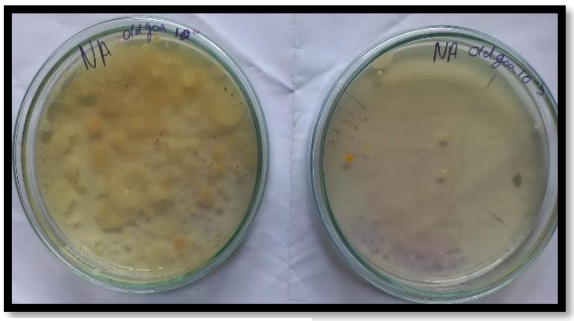


Figure 41

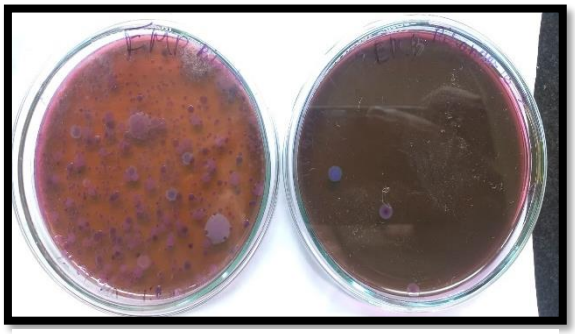


Figure 42

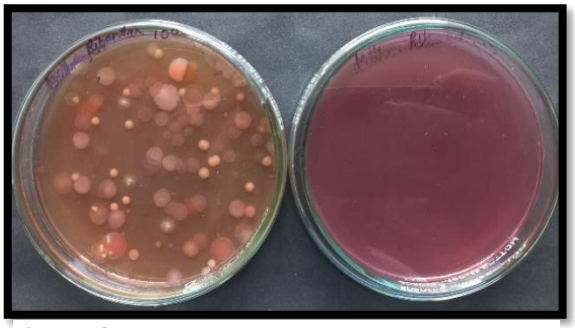


Figure 43

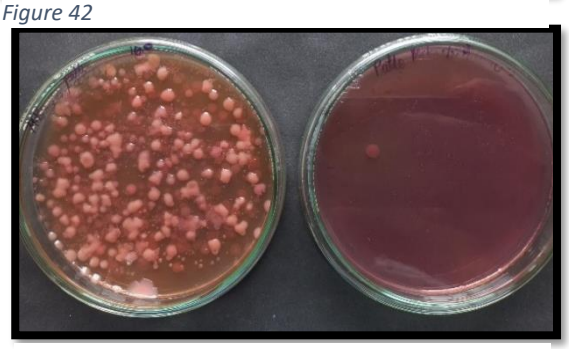


Figure 44

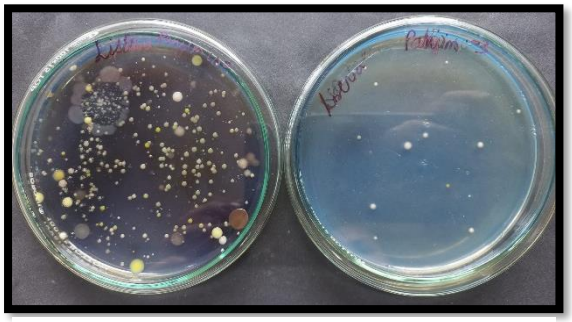


Figure 45

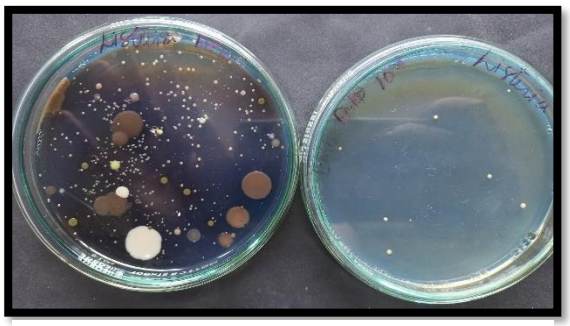


Figure 46

Water samples spread plated on various media in the month of <u>November.</u>

Figure 41. *Water sample from Diwar ferry spread plated on Nutrient agar with 10^-3 and 10^0 dilutions respectively.*

Figure 42*. Water sample from Ribandar spread plated on EMB agar with 10^0 and 10^-3 dilutions respectively.*

Figure 43. *Water sample from Ribandar spread plated on MacConkey's agar with 10^0 and 10^-3 dilutions respectively.*

Figure 44. *Water sample from Patto spread plated on MacConkey's agar with 10^0 and 10^-3 dilutions respectively.*

Figure 45. *Water sample from Panjim ferry spread plated on Listeria Identification Agar Base (PALCAM) agar with 10^0 and 10^-3 dilutions respectively.*

Figure 46. *Water sample from Dona Paula jetty spread plated on Listeria Identification Agar Base (PALCAM) agar with 10^0 and 10^-3 dilutions respectively.*

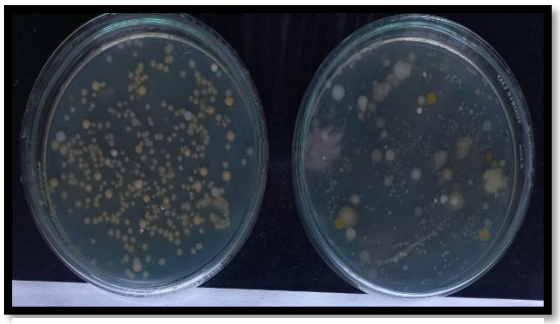


Figure 47

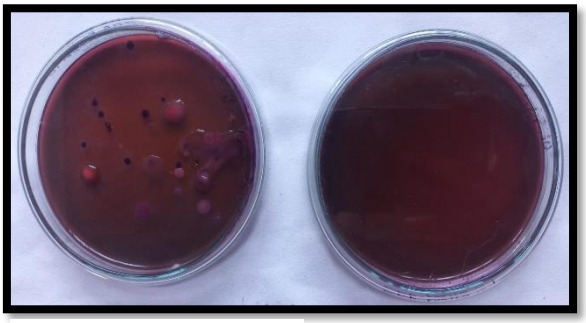
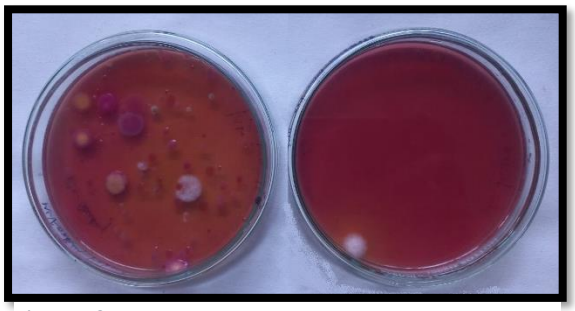


Figure 48



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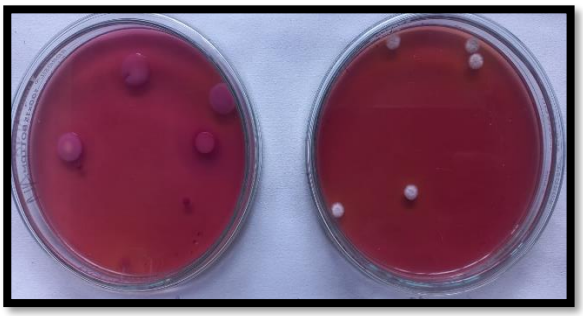

Figure 50

Figure 51

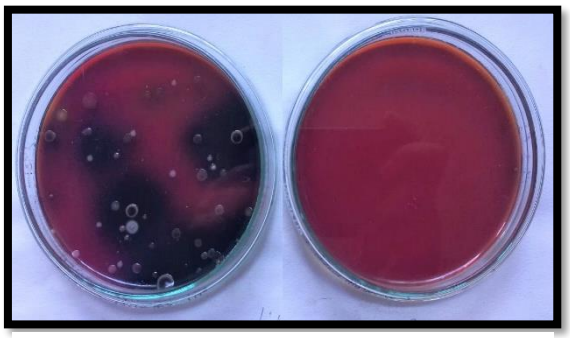

Figure 52

Water samples spread plated on various media in the month of December.

Figure 47. *Water sample from Diwar ferry spread plated on Nutrient agar with 10^-3 and 10^0 dilutions respectively.*

Figure 48*. Water sample from Patto spread plated on EMB agar with 10^0 dilution showing growth of E.coli and 10^-3 dilution showing no growth.*

Figure 49. *Water sample from Ribandar spread plated on MacConkey's agar with 10^0 and 10^-3 dilutions respectively.*

Figure 50. *Water sample from Dona Paula spread plated on MacConkey's agar with 10^0 and 10^-3 dilutions respectively.*

Figure 51. *Water sample from Panjim ferry spread plated on Listeria Identification Agar Base (PALCAM) agar with 10^0 and 10^-3 dilutions respectively.*

Figure 52. *Water sample from Dona Paula jetty spread plated on Listeria Identification Agar Base (PALCAM) agar with 10^0 and 10^-3 dilutions respectively.*

Colony Characteristics

September	MacConkey Agar				Nutrient agar				EMB			
	Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3	
Time	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.
Temperature	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 C	37 C	37 C	37 C
Size	3mm	2mm	Pinpoint	4mm	5mm	Pinpoint	Pinpoint	2mm	2mm	2mm	4mm	6mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Brown	Colorless	Light pink	Cream	Off white	Colorless	Off white	Yellow	Colorless	Wine color	Magenta	Pink with purple center
Margin	Entire	Serrated	Entire	Entire	Serrated	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Raised	Convex	Convex	Flat	Convex	Raised	Raised	Convex	Raised	Raised	Raised
Opacity	Transparent	Transparent	Opaque	Opaque	Opaque	Transparent	Opaque	Opaque	Transparent	Opaque	Opaque	Opaque
Surface texture	Smooth	Rough	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Powdery	Butyrous	Butyrous	Butyrous	Butyrous

September	Muller Hinton Agar			
	Ribandar 10^0		Ribandar 10^-3	
Time	48 hrs.	48 hrs.	48 hrs.	48 hrs.
Temperature	37 °C	37 °C	37 °C	37 °C
Size	3mm	2mm	pinpoint	2mm
Shape	Circular	Circular	Circular	Circular
Colour	Colorless	Cream	Cream	Orange
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Raised
Opacity	Transparent	Opacity	Opaque	Opaque
Surface texture	Matte	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous

Table 1 Characteristics of colonies growing on various media from Ribandar water sample in September.

Colony Characteristics

October	MacConkey Agar				Nutrient agar				EMB			
	Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C
Size	2mm	4mm	1mm	Pin point	3mm	4mm	2mm	3mm	2.5mm	4mm	3mm	3mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	filamentous	Circular	Circular	Circular	Circular
Colour	Pink	Colorless	Light brown	Brown	Off white	Colorless	Off white	White with blue centre	Pink	Purple	Purple	Light pink
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Raised	Convex	Convex	Convex	Raised	Convex	Convex	Convex	Convex	Raised	Convex
Opacity	Opaque	Transparent	Transparent	Opaque	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth	Matte	Smooth	Smooth	Brittle	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Powdery	Butyrous	Butyrous	Butyrous	Butyrous

October	Listeria Identification Base agar			
	Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C
Size	1mm	1.5mm	Pinpoint	1mm
Shape	Circular	Circular	Circular	Circular
Colour	Grey	Brown	White	Pink
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Raised
Opacity	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous

Table 2 Characteristics of colonies growing on various media from Ribandar water sample in October.

Colony Characteristics

November	MacConkey Agar				Nutrient agar				EMB			
	Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C
Size	1mm	1mm	1mm	Pin point	1mm	2mm	2.5mm	3mm	1.5mm	1mm	3mm	3mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Brown	Pink	Colorless	Brown	Yellow	Orange	White	Light pink	Wine	Purple	Pink	Violet
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Raised	flat	Convex	Convex	Raised	Convex	Convex	Convex	Convex	Convex	Convex
Opacity	Opaque	Opaque	Transparent	Transparent	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth	smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous

November	Listeria Identification Base agar			
	Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C
Size	4mm	1mm	1mm	1mm
Shape	Circular	Circular	Circular	Circular
Colour	Brown	Cream	White	Yellow
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Raised
Opacity	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous

Table 3 Characteristics of colonies growing on various media from Ribandar water sample in November.

Colony Characteristics

December	MacConkey Agar				Nutrient agar				EMB			
	Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3		Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C	35.5°C
Size	3.5mm	1.5mm	4mm	Pin point	1mm	1mm	1mm	1mm	1.5mm	1.5mm	1mm	2mm
Shape	Circular	Circular	Filamentous	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Peach	Grey	White	Colorless	Cream	Orange	White	Yellow	Purple	Pink	Brown	White
Margin	Entire	Entire	Serrated	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Serrated
Elevation	Convex	Raised	Raised	Convex	Convex	Raised	Convex	Convex	Convex	Convex	Convex	Raised
Opacity	Opaque	Opaque	Opaque	Transparent	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth	smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Rough
Consistency	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Brittle

December	Listeria Identification Base agar			
	Ribandar 10^0		Ribandar 10^-3	
Time	36 hrs.	36 hrs.	36 hrs.	36 hrs.
Temperature	35.5°C	35.5°C	35.5°C	35.5°C
Size	3mm	2.5 mm	1mm	4mm
Shape	Circular	Circular	Circular	Circular
Colour	Brown	Black with grey halo	Cream	Brown
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Raised
Opacity	Opaque	Opaque	Opaque	Opaque
Surface texture	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous

Table 4 Characteristics of colonies growing on various media from Ribandar water sample in December.

Chapter IV

SUMMARY

As the concern about Global warming increases new ways to mitigate the excess amount of CO2 in the atmosphere have to be developed. One way to do this is by carrying out an experimental study on the role of microorganisms in aquatic systems. A case study was carried out on the estuarine belt of river Mandovi, Goa.

Samples of water were collected from five different areas along the Mandovi river estuary those are Diwar Ferry, Ribandar, Patto, Panjim ferry and Dona Paula jetty. This sampling was done monthly from the months of July- December 2022 and a separate experiment to study the role of temperature on BR rates was carried out in the month of February for this experiment samples were collected from Diwar ferry , Patto and Dona Paula jetty.

Various parameters were studied like Temperature, Salinity, TSM, Chlorophyll a, Dissolved Oxygen, Bacterial Respiration and Colony characteristics and it was seen how they varied monthly. The procedure for Particulate Organic Carbon and Bacterial Abundance were carried out partially and the work is ongoing.

The salinity of the water was found to be more in the months of October, November and December.

The TSM was found to be more during the months of July and August.

Chlorophyll was seen to be almost inversely proportional to TSM and was more when TSM was less.

Dissolved oxygen of the water samples during the monsoon months of July – September was higher than the rest of the months.

The Bacterial Respiration was found to be the maximum during the month of September .

It was also seen that high temperature negatively affects Bacterial Respiration rates which denotes that if the atmospheric temperatures of the Earth increase due to global warming then Bacterial Respiration rates in water bodies may get negatively impacted due to rise in the water temperature which can also affect the marine carbon cycle as CO2 is less soluble in warmer waters.

Water samples were spread plated on different media and the bacterial diversity was observed colony characteristics were noted down Coliforms were identified in Patto in the month of December on EMB agar.

It is important to study these various parameters and their link to the marine carbon cycle due the rise in global warming. By learning and understanding the role of each parameter the knowledge can be applied to reduce the amount of atmospheric CO2 by using photosynthetic bacteria and plankton.

Bacteria play an important role in carbon cycle as they utilize organic carbon and give off CO2 thus incorporating it into bacterial calls to make biomass which is utilized by other organisms in the marine food web.

Chapter V

APPENDIX

Appendix 1

1. EOSINE METHYLENE BLUE (E.M.B) AGAR

Composition:

Ingredients	Gms/liter
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin	0.400
Methylene blue	0.065
Agar	13.500

2. MacConkey Agar Base

Ingredients	Gms / Liter
Peptic digest of animal tissue	17.000
Proteose peptone	3.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
Final pH (at 25°C)	7.1±0.2

3. Nutrient Agar

Ingredients	Gms / Liter
Peptone	5.000
HM Peptone B #	3.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

4. Listeria Identification Agar Base (PALCAM)

Ingredients	Gms / Liter
Peptone	23.000
Starch	1.000
Sodium chloride	5.000
Mannitol	10.000
Ammonium ferric citrate	0.500
Esculin	0.800
Dextrose (Glucose)	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	13.000
Final pH (at 25°C)	7.0±0.2

Appendix 2

Reeagents

1) Winkler A

Manganous sulphate monohydrate	60g
Distilled water	100 ml

2) Winkler B

Reagent grade KOH	60g
Reagent grade Ki	30gm
Distilled water	100 ml

3) 2.5 N Sodium thiosulphate

Sodium thiosulphate	2.5g
Distilled water	1000ml

4) 1% Starch indicator

Soluble starch	1g
Distilled water	99ml

Chapter VI

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Role and contribution of heterotrophic bacteria in estuarine carbon cycle: a case study of tropical estuary- Mandovi estuary.

As we all know the increase of CO2 in the atmosphere is a major problem because of its greenhouse effect which leads to global warming. Large water bodies like oceans, rivers and estuaries play an important role in carbon cycling as they can act as sinks of CO2 and also harbor microorganisms which help to reduce the amount of gaseous carbon dioxide by utilizing it as a source of energy. Therefore it is important to study the role of the microorganisms from these water bodies and the extent of how much CO2 is mitigated by them. In this study, a monthly analysis of the Mandovi estuary was carried out and various parameters were studied.