Abundance of Pollution Indicator Bacteria Along the Mandovi Estuary

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled **"Abundance of Pollution Indicator Bacteria Along the Mandovi Estuary"** is based on the results of investigations carried out by me in the **Marine Sciences** at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the Supervision of **Dr. Sheryl O. Fernandes.** 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 report **"Abundance of Pollution Indicator Bacteria Along the Mandovi Estuary"** is a bonafide work carried out by **Ms. Prachi Morje** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master of Science** in the Discipline of Marine Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

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CONTENTS

PREFACE

This dissertation marks a significant milestone in my academic journey in the field of Marine Sciences. It presents the results of my research on pollution indicator bacteria along the Mandovi estuary. The study was conducted at three different stations: Campal, Divar, and Volvoi.

The research aimed to understand the levels of bacterial contamination in these marine environments during two different seasons. The concentrations of various bacteria, including Total Fecal Coliforms, *Salmonella spp.*, *Shigella spp.*, andTotal Viable Counts, were enumerated to assess their abundance and provide insights on anthropogenic influence at the study sites.

The results presented in this dissertation provide valuable insights into the bacterial contamination levels at these marine stations. They highlight the need for continuous monitoring and effective measures to control bacterial contamination, thereby ensuring the health and safety of the marine ecosystem.

ACKNOWLEDGEMENT

I extend my sincere gratitude to the Almighty for providing me with the strength and guidance throughout the journey of this dissertation. I am also deeply thankful to my parents and family members for their unwavering support and belief in my capabilities.

It is with great pleasure and honor that I express my heartfelt thanks to my guide, Dr. Sheryl O. Fernandes, Assistant Professor, School of Earth, Ocean, and Atmospheric Sciences at Goa University. Her invaluable guidance, time, and consistent support were integral to the successful completion of this project.

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ABSTRACT

This study aimed to assess pertinent physico-chemical parameters and abundance of pollution indicator bacteria in water samples collected from three different locations along the Mandovi estuary during the post-monsoon and pre-monsoon seasons in 2024. Physico-chemical analysis including temperature, salinity, turbidity, pH, and dissolved oxygen levels were measured alongwith microbiological parameters viz., total viable counts, total fecal coliforms, *Salmonella spp.*, *Shigella spp.*, *Streptococcus faecalis*, and Enterococci. The results indicate variations in environmental parameters and bacterial concentrations among the stations and seasons. Campal consistently exhibited higher salinity, alkaline pH, and lower dissolved oxygen levels compared to Divar and Volvoi. Microbial counts showed higher abundance of most pollution indicator bacteria at Campal during the pre-monsoon period which experiences higher salinity conditions due its proximity to the mouth region. At Divar and Volvoi exhibited lower levels of contamination. At post-monsoon, significant decrease was observed in total fecal coliforms, *Shigella spp.*, total viable counts, and Enterococci counts, indicating improved water quality. Pearson's correlation analysis revealed temperature ($n=9$, $r=-0.84$, $p<0.001$) and dissolved oxygen ($n=9$, $r=-0.8$, $p<0.05$) as key factors influencing the pollution indicator bacteria, with higher temperatures associated with increased microbial abundance. These findings contribute to understanding the microbial dynamics and environmental factors affecting water quality in estuarine ecosystems, facilitating effective management and monitoring strategies for maintaining ecosystem health and public safety.

KEYWORDS

• Mandovi estuary • Water quality • Physicochemical analysis • Pre-monsoon

• Post-monsoon • Microbiological parameters

CHAPTER 1

1. INTRODUCTION

1.1 Background

Water pollution is a pressing global concern with significant implications for human health and the environment (Hicks, 1998). Pollution indicator bacteria serve as crucial markers for assessing water quality, particularly in water bodies used for drinking, recreational activities etc (Zaghloul et al., 2020). The Mandovi Estuary located in Goa, west coast of India, represents a critical nexus of ecological and socio-economic significance. As a vital water body supporting diverse aquatic life, providing livelihoods for local communities, and serving as a hub for recreational activities, the Mandovi Estuary's health is paramount (Toraskar et al., 2022). However, rapid urbanization, industrialization, agricultural activities, and tourism development have raised concerns about water quality degradation in this estuarine system (Kennish, 2002).

Pollution indicator bacteria are microorganisms used as indicators of the level of pollution in water bodies. They are typically harmless bacteria that are found in the intestines of warm-blooded animals, such as humans and other mammals (Zaghloul et al., 2020). The presence of these bacteria in water samples can indicate fecal contamination, which suggests the potential presence of harmful pathogens and pollutants (Jeanneau et al., 2012). Common types of pollution indicator bacteria include Escherichia coli (E. coli) and fecal coliforms. Testing for these bacteria is an important part of water quality monitoring to ensure the safety of water for human and environmental health (Zaghloul et al., 2020).

Water pollution, particularly bacterial contamination, poses significant threats to the Mandovi Estuary's ecological integrity and public health. The sources of pollution indicator bacteria along the Mandovi Estuary in Goa are multifaceted and can be attributed to various human activities and natural processes (Toraskar et al., 2022). Some of the primary sources include Urban Run-off, Sewage discharges, Agricultural Activities, Livestock Farming, Boat Traffic, Wildlife and Domestic Animals, Industrial Discharges, Seawater Intrusion, Anthropogenic Activities, and Atmospheric Deposition (Khatri & Tyagi, 2015). Understanding the sources of pollution indicator bacteria along the Mandovi Estuary is essential for implementing effective management strategies aimed at mitigating bacterial pollution and safeguarding the ecological and public health of this valuable coastal ecosystem (Toraskar et al., 2022).

Along the Mandovi Estuary in Goa, India, several types of pollution indicator bacteria are commonly found, reflecting the various sources of contamination and the dynamic nature of the estuarine environment (Toraskar et al., 2022). Some of the key types of pollution indicator bacteria include Fecal coliforms, *Escherichia coli (E. coli)*,Enterococci, and Total coliforms (Toraskar et al., 2022). In addition to the aforementioned bacteria, other microbial indicators may be monitored along the Mandovi Estuary to assess water quality and pollution levels (Toraskar et al., 2022). These may include specific pathogens such as *Salmonella spp.*, as well as microbial parameters indicative of organic pollution, nutrient enrichment, and other environmental stressors (Toraskar et al., 2022).

Pollution indicator bacteria are crucial for managing water quality along the Mandovi Estuary, informing risk assessments, regulatory compliance, pollution event response, source identification, and long-term monitoring (Odonkor & Ampofo, 2013; Wen et al., 2020). Various factors influence their presence, including environmental conditions like temperature, pH, salinity, dissolved oxygen, nutrient enrichment, and hydrological dynamics (Odonkor & Ampofo, 2013; Wen et al., 2020). Anthropogenic inputs from urban development, agriculture, sewage, and industrial discharges exacerbate contamination (Odonkor & Ampofo, 2013; Wen et al., 2020). Implications for public health include risks of waterborne diseases such as gastrointestinal, skin, and respiratory infections (Toraskar et al., 2022). Addressing these concerns requires collaborative efforts, integrated watershed management, and public awareness initiatives (Wen et al., 2020; Toraskar et al., 2022). Future research should focus on longitudinal studies, microbial source tracking, advanced molecular techniques, antibiotic resistance, and climate change impacts (Toraskar et al., 2022). By understanding pollution dynamics and implementing effective management strategies, stakeholders can safeguard both the ecological health and human well-being of the Mandovi Estuary (Toraskar et al., 2022).

The Goa State Pollution Control Board (GSPCB) has directed 30 village panchayats located along the river banks to survey residential and commercial establishments situated along the stretch involved in discharging sewage into the river or other source of river water (Herald Goa, 2016, March 4). The St Inez Creek in Panjim, Goa, is a major hotspot for sewage discharge (Herald Goa, December 9, 2023). This creek, which passes through Panjim, is filled with waste, raw sewage, decaying food, and filth. The creek has been heavily silted, and there is no free movement of water. The faecal coliform presence in the water, which has to be zero, was found to be as high as 9,20,000 per 100 mL of water. This bacterium occur due to the release of untreated sewage (Herald Goa, December 9, 2023).

Another creek located further upstream is the Rua de Ourem creek in Panjim. It is indeed a major hotspot for sewage discharge. Untreated sewage is being released into the Rua De Ourem creek by the sewage pump house at Patto. Instead of first treating the raw waste and then releasing it into the River Mandovi, untreated sewage is being dumped directly into the creek as reported by the Times of India (July 16, 2023).

The permissible limit of pollution indicator bacteria, specifically fecal coliform (FC), in estuarine water is set below a geometric mean (GM) of 88 MPN/100 mL with less than 10% of samples exceeding 260 MPN/100 mL for a five tube MPN (Udyavara et al., 2019). However, it’s important to note that there could be a chance of pathogenic bacteria even when the sewage pollution indicator bacteria may be within range or below the permissible limit

(Sharma et al., 2023). Also the Mandovi estuary receives ~ 5.21 x 10^{6}□^{3}of sewage and their effluents per year (Kessarkar et al., 2009).

This study focusses on assessing pollution indicator bacteria relating them to the water quality, identifying sources of contamination, and also gain some insights on the environmental factors likely to influence their abundance along the Mandovi Estuary. By addressing key findings and implementing evidence-based management measures, stakeholders can work collaboratively to safeguard the ecological integrity and socio-economic well-being of this vital estuarine ecosystem for current and future generations. By addressing these future directions and research needs, we can advance our understanding of pollution indicator bacterial dynamics in the Mandovi Estuary, improve water quality management practices, and promote the sustainable stewardship of this valuable estuarine ecosystem for future generations.

1.2. Objectives

- To compare the variation in the abundance of pollution indicator bacteria along Mandovi estuary during post-monsoon and pre-monsoon season.
- To understand the influence of pertinent environmental parameters on pollution indicator bacteria.
- To link the abundance of pollution indicator bacteria to sites of pollution in the estuary.

CHAPTER 2

2. LITERATURE REVIEW

The study by Ramaiah et al. (2005) focused on quantifying pollution-indicator and pathogenic bacteria in Mumbai waters from a ballast water exchange perspective. Sampling was conducted during different seasons, and various bacterial groups were quantified from water, sediment, marine plant, and animal samples. The study found that compared to similar studies from other harbors worldwide, Mumbai Harbor had exceptionally high levels of coliforms, over 100 times higher. Pathogenic bacteria such as Escherichia coli O157, Shigella-Alkaligens Dispar group, Vibrio cholerae, V. parahaemolyticus, *Salmonella spp.*, campylobacters, and aeromonads were present in large numbers throughout the year. The findings strongly suggested that both ballasting and deballasting activities should be avoided in the Mumbai Harbor region, and alternative procedures for treating or handling ballast water should be developed.

The study conducted by Nagvenkar et al. (2009) aimed to address the gap in literature concerning the abundance and types of pollution indicator bacterial populations in tropical estuaries. The study focused on the Mandovi and Zuari Rivers in the central west coast of India, the research spanned various seasons and locations, investigating the impact of land drainages, domestic sewage outfalls, and other discharges on microbial populations. The study found that the abundance of sewage pollution indicator bacteria such as total coliforms and total streptococci was generally lower compared to many other locations worldwide. This indicated potential differences in microbial ecology and pollution dynamics in tropical estuarine environments compared to other regions.

Sumampouw et al. (2014) discussed the role of bacteria as indicators of environmental pollution. The study highlighted the increasing degradation of water, soil, and air quality and emphasized the need for pollution prevention through environmental monitoring. Specific bacterial species such as Coliform, *Escherichia coli, Streptococcus spp., Pseudomonas spp.,* *Vibrio spp., Clostridia spp., Bifidobacterium pseudolongum, Pseudomonas spp., Vibrio spp., Clostridia spp., Bifidobacterium pseudolongum, Arcobacter spp.,* and *Thiobacillus spp.* were discussed as environmental indicators. These bacteria were utilized in monitoring various types of pollution, including household waste (human and animal feces, household waste), heavy metal pollution, crude oil contamination, and other forms of pollution.

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Sangodkar et al. (2020) investigated the prevalence of indicator and potential pathogenic bacterial groups in the Chapora bay-estuarine system along the central west coast of India, which was influenced by anthropogenic inputs. The study sampled water and sediments from different zones along the river Chapora, including offshore, inshore, inner estuary, and upper estuary. It analyzed the abundance of indicator bacterial groups such as total coliforms and Escherichia coli, as well as potential pathogenic bacteria. Certain bacterial groups, such as *Shigella* like organisms and *Pseudomonas aeruginosa* like organisms, exhibited increasing abundance from offshore to upper estuary zones, while others, like *Proteus/Klebsiella* like organisms, showed a reverse trend. The inner estuary zone was identified as the most contaminated, based on the higher abundance of indicator and potential pathogenic bacterial populations, as well as a significantly lower water quality index value.

The study by Toraskar et al. (2022) explored seasonal variations in water quality and antibiotic resistance of microbial pollution indicators in the Mandovi and Zuari estuaries, Goa, India. The study investigated changes in water quality and microbial pollution levels during pre-monsoon, monsoon, and post-monsoon seasons. The water quality index was assessed based on parameters like temperature, salinity, pH, dissolved oxygen, biochemical oxygen demand and nutrients. Results indicated poor water quality in pre-monsoon and post-monsoon seasons, while it improved to a good status during the monsoon. Pollution indicator organisms were assessed, showing the highest counts in the pre-monsoon season, decreasing during the monsoon, and further declining in the post-monsoon season. The study evaluated antibiotic resistance of bacterial isolates, revealing maximum resistance in monsoonal water and sediment samples. This suggested potential health risks due to the introduction of harmful pathogens during the monsoon, despite improved water quality.

A study by Baheerathi et al. (2018) investigated the presence of fecal pollution indicator bacteria in Muttukadu backwaters, located on the east coast of Tamil Nadu, India. The study aimed to analyze the presence of fecal coliform bacteria in Muttukadu backwaters, which were used for harvesting various types of seafood for human consumption. Water samples were collected during pre-monsoon, monsoon, and post-monsoon seasons. The presence of fecal indicator bacteria was assessed using multiple tube tests to determine most probable number (MPN) values. Further confirmation of *Escherichia coli* presence was done through membrane filtration and biochemical tests. Analysis revealed higher numbers of fecal indicator bacteria during the monsoon season, followed by the post-monsoon season. The prevalence was comparatively lower during the pre-monsoon season. The findings suggested potential health risks associated with fecal contamination in Muttukadu backwaters, especially during the monsoon season when bacterial levels were highest. This highlighted the importance of monitoring and managing water quality in these areas to protect public health.

Price et al. (2017) explored the significance of *Escherichia coli* (*E. coli*) as an indicator of contamination and health risk in environmental waters. E. coli and Enterococci were identified as primary indicator organisms for contamination in fresh and marine water, respectively, which likely delved into similar studies examining the use of *E. coli* as an indicator organism in water quality monitoring, assessed the performance of different detection methods, and explored advancements in technology for field-based analysis. Additionally, it may have explored the regulatory frameworks governing acceptable levels of indicator organisms in various water sources and discussed the implications for public health and environmental management.

The importance of understanding pollution indicators and pathogenic microorganisms in wastewater treatment and their implications on receiving water bodies has been studied by Olaolu et al. (2014). The article emphasized the significant impact of water-borne diseases, with over five million deaths annually attributed to such diseases. Microbial intestinal infections, particularly cholera and diarrhea, were identified as major contributors to this burden. Various physical and chemical disinfection methods were discussed for removing biological contaminants from water, including ultraviolet radiation, solar radiation, boiling, chlorination, chloramination, and ozonation. It may have also delved into emerging technologies and approaches for improving water quality and reducing the transmission of water-borne pathogens.

CHAPTER 3

3. DATA AND METHODOLOGY

3.1. Study Area

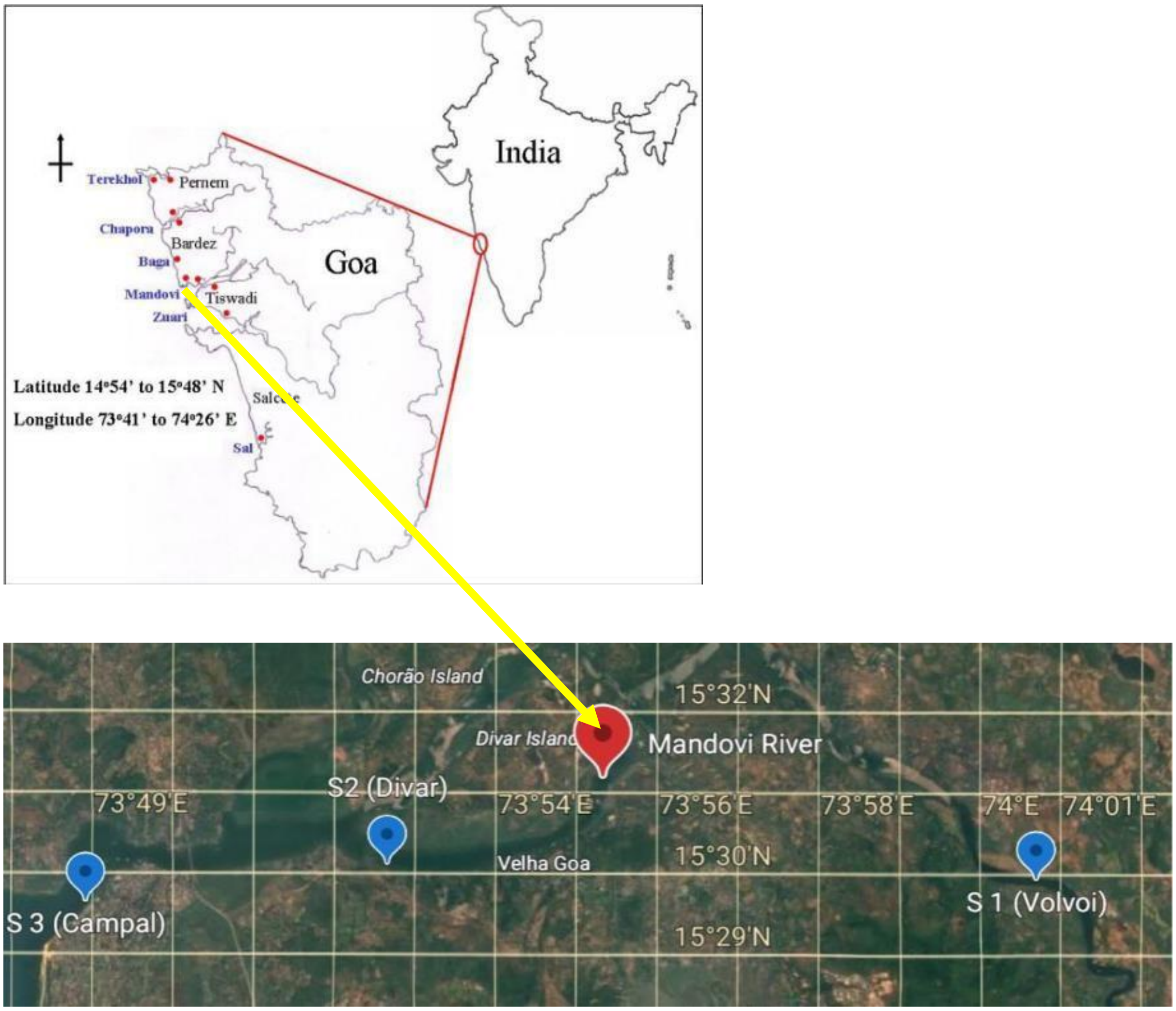


Fig. 3.1. Study area indicating sampling locations

The investigation was carried out along Mandovi estuary at three separate locations : Volvoi (upstream), Divar (mid- stream), and Campal (mouth region), during pre-monsoon and post-monsoon season.

Water samples were collected using a clean well-rinsed buckets, then transferred to sterile HDPE bottles of 250 mL capacity. Physical parameters were measured on site. The samples were transported to the laboratory in insulated boxes, and analysis commenced immediately upon reaching the laboratory. A 50 µL inoculum was spread plated on specific media plates in triplicates. The plates were sealed with parafilm and incubated for 3 weeks at room temperature. After the incubation period, colony forming units (CFU) were recorded and expressed as CFU L^{-1}.

3.2. Physico-chemical analysis

The temperature of water samples was monitored using a mercury thermometer. A secchi disks were used to measure turbidity. The Secchi disk was submerged in the water until its visibility was lost and the depth was recorded. A digital pH meter was used in the lab to determine the pH of the water samples.

3.3. Dissolved Oxygen (DO)

The analysis of dissolved oxygen in seawater was conducted using the Winkler's titration method as outlined by Parson et al. (1996) and further adapted by Carpenter et al. (1965) on the day of sample collection. Water samples of 250 mL capacity were collected in the field and treated with Winkler A and Winkler B reagents. In this process, dissolved oxygen reacts with manganese in the presence of acid, releasing manganic ions which then react with potassium ions to produce free iodine. This free iodine is subsequently titrated with sodium thiosulphate to determine the endpoint.

3.4. Microbiological Parameters

Specific media were used for the enumeration of total viable counts (TVC), total fecal colifirms (TFC), *Streptococcus faecalis* (SF), Enterococci, *Salmonella spp.*, and *Shigella spp.*

were in order to identify pollution indicator bacteria from seawater samples. The plates were inoculated using the spread plate technique. The viable count method was employed to quantify the total number of cells in a sample capable of forming colonies. Microorganisms from seawater samples were cultured on nutrient agar to facilitate their growth and enable their counting.

3.5. Prepration of media

The procedure begins by dissolving the media in estuarine water and sterilizing it in an autoclave. Once cooled, the media is poured into sterile Petri dishes within a laminar flow hood to ensure sterility. After the media solidifies, the sample is inoculated using a micropipette and spread on the Petri plate using a glass spreader ensuring spread plate method. This is done under sterile conditions to prevent contamination. The inoculated Petri plates are then sealed with parafilm and incubated at a temperature typically between 35-37 °C. After sufficient growth, the colonies are enumerated. It's crucial to maintain sterile conditions throughout the process to ensure accurate results.

3.6. Detection of *Salmonella spp.* and *Shigella spp.*

The Xylose Lysine Deoxycholate (XLD) Agar is a specialized medium for isolating and distinguishing gram-negative enteric microorganisms. Most gram-negative enteric bacteria, including *Salmonella spp.*, ferment xylose rapidly, turning the phenol red indicator yellow due to acidification. However, *Shigella spp.* does not utilize xylose, resulting in red colonies. This feature aids in distinguishing *Shigella spp.* Once the xylose is depleted, *Salmonella spp.* decarboxylates lysine, raising the pH and producing red colonies similar to *Shigella spp.* However, *Salmonella spp.* also metabolizes thiosulfate, generating hydrogen sulfide, leading to colonies with black centers, distinguishing them from *Shigella*. After incubation, colonies of various colors develop, aiding in differentiation. Organisms fermenting lactose, sucrose, and xylose but lacking lysine decarboxylase activity create an acidic pH and yield yellow colonies.

3.7. Detection of Total Fecal Coliforms

Detection of total fecal coliforms in water samples is pivotal for assessing water quality and identifying potential health hazards linked to fecal contamination. The methodologies employed for this detection are crucial for ensuring public health and environmental safety. *M-Enterococcus* agar is commonly utilized to detect total fecal coliforms, with the total number of colonies grown on this medium serving as the fecal coliform count. Notably, *M-Enterococcus* agar exhibits selectivity for fecal coliforms, rendering it an effective tool for water quality assessment.

3.8. Detection of Enterococci

Enterococci are Gram-positive bacteria commonly found in the gastrointestinal tracts of humans and animals. They are known for their ability to survive in harsh environments and can be indicators of fecal contamination in water and food samples. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar is a selective and differential medium commonly used for the isolation and identification of *Vibrio* species, particularly *Vibrio cholerae* and V*ibrio parahaemolyticus*. However, TCBS agar can also support the growth of Enterococci under certain conditions.Colonies of Enterococci typically appear as small, round, yellow colonies on TCBS agar.Yellow colonies on TCBS agar may indicate the presence of Enterococci. Also the presence of non-yellow colonies may indicate the growth of other bacteria or contaminants.

3.9. Statistical Analysis

To understand the influence of physico-chemical parameters on the microbiological variables, Pearson's correlation analysis was carried using *Microsoft Excel*.

CHAPTER 4

4. ANALYSIS AND CONCLUSIONS

4.1. Results and Discussions

4.1.1. Physico-chemical Parameters

Environmental parameters recorded at the sampling locations during the post-monsoon period are in Table 4.1.

Table 4.1. Environmental parameters recorded at different locations along the Mandovi estuary during the post-monsoon period

Environmental parameters	Parameters		
	Campal	**Divar**	**Volvoi**
Temperature (°C)	28.33 ± 0.5774	27.33 ± 0.5774	28 ± 0.5774
Salinity	35 ± 1	32.66 ± 0.57	15 ± 1
pH	7.96	7.79	7.36
Dissolved Oxygen (mL L^{-1})	0.87 ± 0.14	1.12 ± 0.05	1.54 ± 0.20
Suspended Particulate Matter (gL^{-1})	0.0118	0.0263	0.0172
Turbidity (m)	Not done	3.39	3.39

The temperatures remain consistent across all three locations, with minor fluctuations. These values fall within the typical range for coastal environments. Campal had the highest salinity, followed by Divar and then Volvoi. The decreasing trend suggests a transition from marine to freshwater influence. All three locations have pH values close to neutral (around 7). Campal remained slightly alkaline (7.96), while Volvoi is more neutral (7.36). These pH levels are conducive to various aquatic organisms. Divar had the lowest dissolved oxygen content, followed by Volvoi and Campal. Adequate dissolved oxygen is essential for aquatic life, and these values warrant attention. Suspended particulate matter levels are low across all three locations. Clear water conditions are favorable for aquatic ecosystems. Campal shows no detectable turbidity, while Divar and Volvoi exhibit moderate turbidity. Turbidity affects light penetration and influences ecological dynamics.

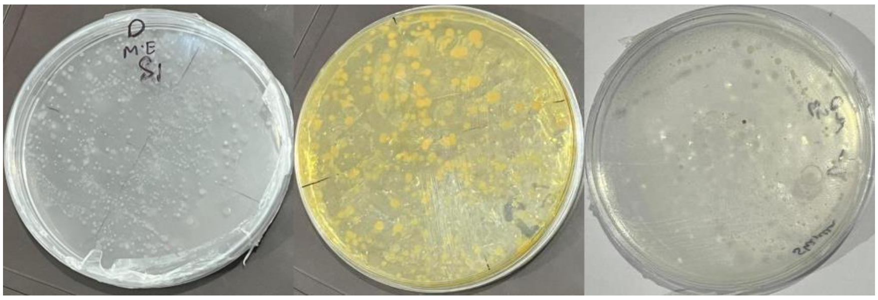
Environmental parameters recorded at the three sampling locations during the sampling during the pre-monsoon period have been tabulated in Table 4.2.

Table 4.2. Environmental parameters recorded at different locations along the Mandovi estuary during the pre-monsoon period

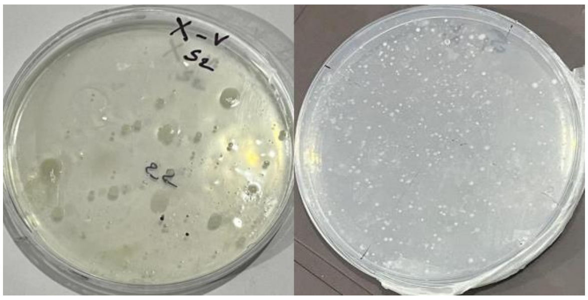
Environmental parameters	**Parameters**		
	Campal	**Divar**	**Volvoi**
Temperature (°C)	30.33 ± 0.5774	31.66 ± 0.5774	32.33 ± 0.5774
Salinity	35 ± 1	25.66 ± 0.57	21.66 ± 0.57
pH	8.22	7.75	7.67
Dissolved Oxygen (mL L^{-1})	0.72 ± 0.02	0.52 ± 0.10	0.65 ± 0.13
Suspended Particulate Matter (g L^{-1})	0.0359	0.0224	0.0286
Turbidity (m)	Not done	4.52	3.96

The temperatures across all three locations are quite similar, with only slight variations. Campal has the highest salinity, followed by Divar, and then Volvoi. These values fall within the general trend observed in estuaries with higher values towrads the mouth and lower salinity towards the head region. The variation suggests differences in water sources and mixing patterns. All three locations had pH values close to neutral (around 7). Campal has the highest pH of 8.22, indicating slightly alkaline conditions, while Volvoi has the lowest pH of 7.67, suggesting a more acidic environment. Divar had the highest dissolved oxygen content, followed by Campal and then Volvoi (Table 4.1). Adequate dissolved oxygen is crucial for aquatic organisms, and these values seem reasonable. The suspended particulate matter levels were low across all three locations. This suggests relatively clear water, which is beneficial for aquatic life. Though turbidity values at Campal could not be recorded due to logistic reasons, Divar and Volvoi had moderate turbidity levels. Turbidity can affect light penetration and influence ecosystem dynamics.

4.1.2. Microbiological Analysis



a) **b)** **c)**



d) **e)**

Fig. 4.1. Bacterial Colony Growth of: a)Total Fecal Coliforms b)Enterococci c)*Shigella spp.* d)*Salmonella spp.* e)Total Viable Counts

Growth of bacterial colonies on specific media were observed as shown in above figures.

4.1.3. Variations in the abundance of pollution indicator

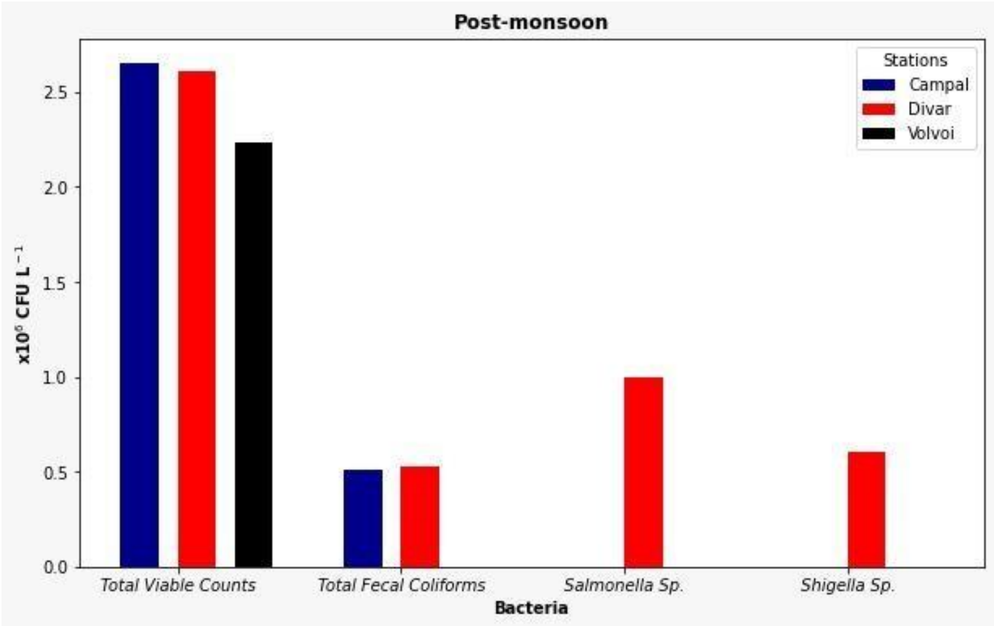


Fig. 4.2. Variations in the abundance of pollution indicator bacteria during the post-monsoon

The abundance of various bacteria at Campal, Divar and Volvoi during the pre-monsoon season are shown in Fig. 4.2.Total Viable Counts, representing the overall microbial load including both harmful and harmless bacteria, provide insights into the microbial diversity of each location. Campal records the highest count at 2.65x*10*^{6}CFU L^{-1}, followed closely by Divar at 2.61x*10*^{6}CFU L^{-1}, and Volvoi at 2.23x*10*^{6}CFU L^{-1}. This suggests that Campal and Divar have relatively similar levels of microbial diversity, while Volvoi exhibits a slightly lower microbial load.

For Total Fecal Coliforms, Campal has the highest count at 0.51x*10*^{6}CFU L^{-1}. This suggests a presence of fecal coliforms in the water of Campal, albeit at a relatively low level. On the other hand, no presence of Total Fecal Coliforms was observed in Divar or Volvoi, indicating cleaner conditions regarding fecal contamination in these locations.

Salmonella spp. has highest count at Divar, which is about 1x*10*^{6}CFU L^{-1}. Conversely, neither Campal nor Volvoi show any presence of *Salmonella spp.* Similarly, *Shigella spp.*

exhibits the highest count in Divar at 0.6x10^6CFU L^{-1}. Campal and Volvoi, however, showed no presence of *Shigella spp.*, indicating a lower risk of gastrointestinal infections associated with this bacterium in these locations.

The Campal station has high concentrations of Total Fecal Coliforms and moderate levels of Total Viable Counts. Divar showed equal levels of *Salmonella spp.* and *Shigella spp.*, and low levels of Total Fecal Coliforms and Total Viable Counts. Volvoi has a very high concentration of Total Viable Counts but no growth for the other bacteria types were observed.

Streptococcus feacalis and Enterococci was absent in all three stations. This data could be useful for understanding the bacterial contamination levels at these stations during the post-monsoon season.

In summary, the significant decreases observed in Total Fecal Coliforms, *Shigella spp.*, Total Viable Counts, and Enterococci at post-monsoon highlight the positive impact of the rainy season on water quality and microbial populations.

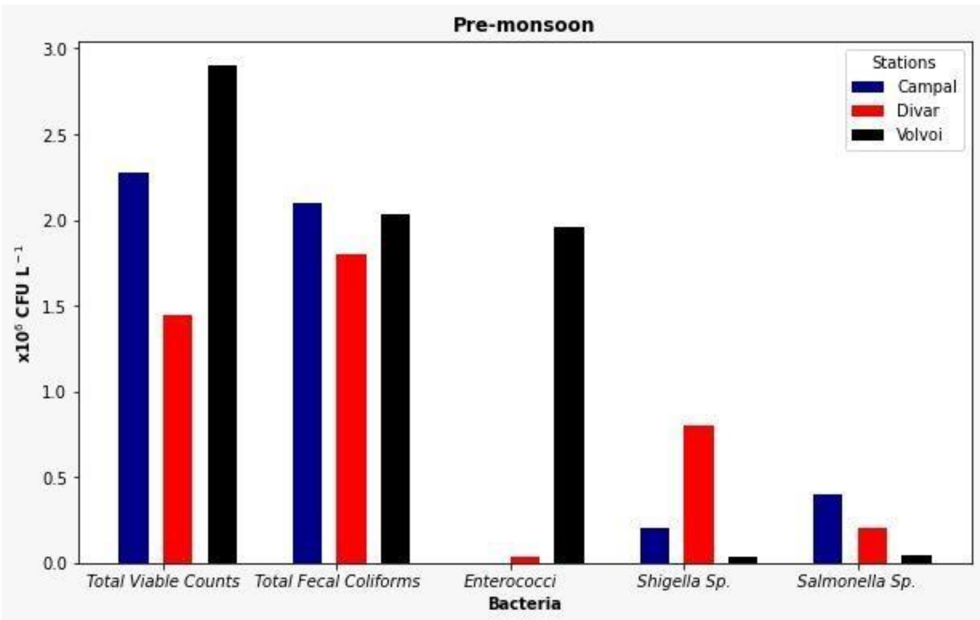


Fig. 4.3. Variations in the abundance of pollution indicator bacteria during the pre-monsoon

Abundance of various bacterial groups at Campal, Divar and Volvoi during the pre-monsoon season are shown in Fig. 4.3. Total Viable Counts, representing the overall microbial load including both harmful and harmless bacteria, reveal a slightly different trend. Volvoi records the highest count at 2.9x10^6CFU L^{-1}, suggesting presence of a rich microbial community. Campal followed closely with a count of 2.275x10^6CFU L^{-1}, indicating a substantial microbial presence, while Divar showed a comparatively lower count at 1.442x10^6CFU L^{-1}.Total fecal coliforms, serve as indicators of fecal contamination and potential health risks. Campal exhibited the highest abundance of Total fecal coliforms at 2.1x10^6CFU L^{-1}, suggesting a comparatively higher level of fecal contamination in this area. Divar followed with an abundance of 0.4x10^6CFU L^{-1}, indicating a lower presence of fecal coliforms. At Volvoi, lowest count were recorded at 0.2x10^6CFU L^{-1} signifying relatively cleaner conditions w.r.t. fecal contamination.

For *Salmonella spp.*, Campal again showed the highest abundance at 1.803x10^6CFU L^{-1}, compared to Divar (0.2x10^6CFU L^{-1}) and Volvoi (0.8x10^6CFU L^{-1}). *Shigella spp.*, demonstrated the highest count in Campal (2.035x10^6CFU L^{-1}), followed by Divar (0.046x10^6CFU L^{-1}) and Volvoi (0.04x10^6CFU L^{-1}).

For Enterococci, Volvoi exhibited the highest abundance of these at 1.955x10^6CFU L^{-1}, suggesting a significant presence of Enterococci in this area. Campal recorded no presence of Enterococci, indicating cleaner conditions in this regard, while Divar shows a minimal count of 0.04x10^6CFU L^{-1}.

4.1.4 Influence of environmental parameters on bacterial groups

A correlation matrix of environmental parameters and bacterial abundance in the pre-monsoon season has been shown in Table 4.3.. Temperature has a strong positive correlation with Dissolved oxygen($n=9$; $r=0.81$, $p<0.001$) and a moderate positive correlation with Total viable counts. This suggests that as the temperature increases, the Dissolved oxygen and Total viable counts also tend to increase. However, it has a strong negative correlation with Salinity, *Shigella*, and *Salmonella*, indicating that these parameters decrease as the temperature increases.

Table 4.3. Correlation of environmental and microbiological parameters during the post-monsoon

	Temp	Salinity	DO	TVC	TFC	SHI	SAL
Temp	1						
Salinity	0.228	1					
DO	**0.585**	-0.054	1				
TVC	-0.081	-0.023	-0.019	1			
TFC	-0.009	**0.538**	-0.293	0.064	1		
SHI	**-0.703**	**-0.489**	-0.441	0.228	0.092	1	
SAL	**-0.608**	0.355	**-0.728**	0.141	0.617	0.599	1

Note: Total Viable Count = TVC; Total Fecal Count =TFC; *Shigella* = SHI; *Salmonella* =SAL; DO = Dissolved Oxygen.

Correlation matrix of environmental parameters and bacterial colony types in the post-monsoon season are in Table 4.2. Temperature has a moderate positive correlation with Dissolved Oxygen (Dissolved oxygen). However, it has a strong negative correlation with *Shigella* (SHI) and *Salmonella* (SAL), indicating that these parameters decrease as the temperature increases.

Salinity has a weak positive correlation with Temperature and a moderate positive correlation with Total Fecal Count (Total fecal coliforms) and *Salmonella spp.* This suggests that as salinity increases, the temperature, Total fecal coliforms, and *Salmonella spp.* also tend to increase.

Dissolved Oxygen has a moderate positive correlation with Temperature. However, it has a moderate negative correlation with Total fecal coliforms and a strong negative correlation with *Salmonella spp.*. This suggests that as Dissolved oxygen increases, the temperature also increases, but Total fecal coliforms and *Salmonella spp.* decrease.

Total viable counts has a weak positive correlation with *Shigella spp.* and *Salmonella spp.* This suggests that as Total viable counts increases, *Shigella spp.* and *Salmonella spp.* also tend to increase.

Total fecal coliforms has a moderate positive correlation with Salinity and*Salmonella spp.*, but a moderate negative correlation with Dissolved oxygen. This suggests that as Total fecal coliforms increases, Salinity and *Salmonella spp.* also increase, but Dissolved oxygen decreases.

Shigella spp. has a weak positive correlation with Total viable counts, but a moderate negative correlation with Temperature, Salinity, and Dissolved oxygen. This suggests that as *Shigella spp.* increases, Total viable counts also increases, but Temperature, Salinity, and Dissolved oxygen decrease.

Salmonella spp. has a moderate positive correlation with Salinity, Total fecal coliforms, and *Shigella spp.*, but a strong negative correlation with Temperature and Dissolved oxygen. This suggests that as *Salmonella spp.* increases, Salinity, Total fecal coliforms, and *Shigella spp.* also increase, but Temperature and Dissolved oxygen decrease.

In summary, temperature and Dissolved oxygen are key factors influencing the bacterial colony types in the post-monsoon season. As the temperature and Dissolved oxygen increase, the*Shigella spp.*and *Salmonella spp.* tend to decrease. Conversely, as Salinity and Total fecal coliforms increase, *Salmonella spp.* also tends to increase, but Dissolved oxygen tends to decrease. These correlations can be useful in predicting the bacterial colony types based on the environmental parameters. However, correlation Dissolved oxygenes not imply causation, and further research would be needed to determine the causal relationships between these parameters.

Table 4.4. Correlation of environmental and microbiological parameters during the pre-monsoon

	Temp	Salinity	DO	TVC	TFC	SHI	SAL
Temp	1						
Salinity	-0.717	1					
DO	0.815	-0.803	1				
TVC	**0.546**	-0.141	0.404	1			
TFC	**-0.493**	0.369	**-0.611**	-0.022	1		
SHI	**-0.756**	0.379	**-0.564**	-0.928	0.114	1	
SAL	**-0.845**	**0.912**	**-0.806**	-0.475	0.318	0.707	1

Note: Total Viable Count = TVC; Total Fecal Count =TFC; *Shigella* = SHI; *Salmonella* =SAL; DO = Dissolved Oxygen.

Salinity has a strong negative correlation with Temperature and Dissolved oxygen, and a strong positive correlation with *Salmonella* sp. This suggests that as salinity increases, the temperature and Dissolved oxygen decrease, but *Salmonella* sp. increases.

Dissolved oxygen has a strong positive correlation with temperature and a moderate positive correlation with Total viable counts. However, it has a strong negative correlation with salinity, Total Fecal Count, *Shigella* spp., and *Salmonella* spp.. This suggests that as Dissolved oxygen increases, the temperature and Total viable counts also increase, but salinity, Total fecal coliforms, *Shigella spp*., and *Salmonella* spp. decrease.

Total viable counts has a moderate positive correlation with Temperature and Dissolved oxygen, but a strong negative correlation with *Shigella* spp.. This suggests that as Total viable counts increases, the temperature and Dissolved oxygen also increase, but *Shigella* spp. decreases.

Total fecal coliforms has a moderate positive correlation with Salinity, but a strong negative correlation with Dissolved oxygen. This suggests that as Total fecal coliforms increases, Salinity also increases, but Dissolved oxygen decreases.

*Shigella spp.*has a moderate positive correlation with Salinity and *Salmonella spp*., but a strong negative correlation with Temperature, Dissolved oxygen, and Total viable counts. This suggests that as Shigella spp. increases, Salinity and *Salmonella spp.*also increase, but Temperature, Dissolved oxygen, and Total viable counts decrease.

Salmonella spp. has a strong positive correlation with Salinity and *Shigella spp.*, but a strong negative correlation with Temperature and Dissolved oxygen. This suggests that as *Salmonella spp.* increases, Salinity and *Shigella spp*. also increase, but Temperature and Dissolved oxygen decrease.

Overall, during pre-monsoon period, temperature and Dissolved oxygen are key factors influencing the bacterial colony types. As the temperature and Dissolved oxygen increase, the Total viable counts also tends to increase, but Salinity, Total fecal coliforms, *Shigella spp.*, and *Salmonella spp.* tend to decrease. Conversely, as Salinity increases,*Salmonella spp*. and *Shigella spp.*also tend to increase, but Temperature, Dissolved oxygen, and Total viable counts tend to decrease.

The post-monsoon period reveals a weakening of the correlation between salinity and bacterial counts, indicating a less pronounced impact compared to the pre-monsoon phase. Additionally, dissolved oxygen's influence remains relatively weak, though it displays a stronger negative correlation with SAL ($n=9$, $r=-0.6$, $p>0.05$) post-monsoon, suggesting potential inhibitory effects on Salmonella species during this period.

During the pre-monsoon period, salinity demonstrates a consistent positive correlation with TFC ($n=9$, $r=0.369$, $p<0.05$), SHI ($n=9$, $r=0.379, p<0.05$), and SAL ($n=9$, $r=0.912$, $p<0.05$), while exhibiting a negative correlation with TVC ($n=9$, $r=-0.141$, $p<0.05$). This suggests that higher salinity levels are conducive to the proliferation of microbial populations, particularly TFC, *Shigella* and *Salmonella* species, but inhibit the growth of TVC. Dissolved oxygen, albeit weakly correlated, tends to have a positive influence on TVC (n=9, r=0.404, p<0.05), while showing varied effects on SAL (n=9, r=-0.806, p<0.05), SHI (n=9, r=-0.564, p<0.05) and TFC (n=9, r=-0.611, p<0.05)counts.

Temperature emerges as a significant factor influencing microbial dynamics, particularly in the pre-monsoon period. Higher temperatures are strongly correlated with increased TVC (n=9, r=0.546, p<0.05)counts, indicating a favorable environment for microbial growth. Conversly, TFC, *Salmonella* and *Shigella* species exhibit a negative correlation with temperature, implying a reduction in their counts with rising temperatures (SAL: n=9, r=-0.845, p<0.05; SHI: n=9, r=-0.756, p<0.05; TFC: n=9, r=-0.493, p<0.05). In the post-monsoon phase, while the correlation between temperature and microbial counts persists, it appears less pronounced compared to the pre-monsoon period, particularly for Salmonella (n=9, r=-0.61, p<0.05) and Shigella (n=9, r=-0.7, p<0.05) species.

4.2. Summary

- Research conducted along the Mandovi estuary encompassed three distinct sampling locations: Volvoi (upstream), Divar (mid-stream), and Campal (mouth).
- Data was collected during both pre-monsoon and post-monsoon seasons to capture seasonal abundance of pollution indicator bacteria and seasonal variation of pertient environmental variables were also measured at Various bacterial types including total viable counts (TVC), total fecal coliforms (TFC), *Salmonella, Shigella,* and Enterococci were enumerated.

- Water samples were meticulously collected using clean well-rinsed buckets and transferred to sterile HDPE bottles.
- Physical parameters such as temperature and turbidity were measured on-site, with samples then transported to the laboratory in insulated boxes for further analysis.
- Microbial analysis involved spreading 50 µL inoculum on specific media plates in triplicates and incubating them for 3 weeks at room temperature.
- Temperature, turbidity, pH, and dissolved oxygen levels were monitored.
- Salinity was highest at Campal, indicating its proximity to the marine zone.
- The pH levels varied across locations, with Campal exhibiting a slightly alkaline environment and Volvoi tending towards acidity.
- Dissolved oxygen levels were highest at Divar (1.12±0.05), which is crucial for supporting aquatic life.
- Turbidity levels varied in the sampling sites.
- Campal consistently exhibited higher concentrations of most bacterial groups compared to Divar and Volvoi.
- At post-monsoon, there was an overall decrease in bacterial counts, indicating improved water quality.
- Notable reductions were observed in total fecal coliforms and Enterococci at post-monsoon.
- Pre-monsoon correlations revealed:

  - Positive correlation between salinity and TVC, TFC, and *Salmonella*.

  - Negative correlation between salinity and *Shigella*.

- Positive correlation between temperature and TVC ($n=9$, $r=0.546$, $p<0.05$) but negative correlation with TFC, *Salmonella* and *Shigella* (SAL: $n=9$, $r=-0.845$, $p<0.05$; SHI: $n=9$, $r=-0.756$, $p<0.05$; TFC: $n=9$, $r=-0.493$, $p<0.05$), particularly pronounced in the pre-monsoon period.

➢ Post-monsoon correlations showed:

- Weaker correlation between salinity with TVC and *Shigella spp*.

- Temperature had negative correlation with all the bacterial abundance.

4.3. Conclusions

Research conducted in the Mandovi estuary encompassing three distinct sampling locations: Volvoi (upstream), Divar (mid-stream), and Campal (mouth region) during post-monsoon and pre-monsoon season in 2024. Physico-chemical parameters such as temperture, pH, turbidity and suspended particulate matter and dissolved oxygen in ambient water were measured along with enumeration of total viable counts (TVC) and pollution indication bacteria viz., total fecal coliforms (TFC), *Salmonella*, *Shigella*, and Enterococci to gain insights on water quality.

At post-monsoon, there was an overall decrease in bacterial abundance, indicating improved water quality. Notable reductions were observed in total fecal coliforms and Enterococci. Salinity was highest at Campal indicating its proximity to the marine zone. The pH levels varied across locations, with Campal exhibiting slightly alkaline conditions wheeras those at Volvoi were more acidic. Dissolved oxygen levels were highest at Divar, which is crucial for supporting aquatic life. Turbidity levels varied, with Campal showing no detectable turbidity, suggesting relatively clear water conditions. Campal consistently exhibited higher abundance of most bacterial types compared to Divar and Volvoi.

At pre-monsoon, correlation analysis revealed positive relationship between salinity, and abundance of TFC, *Shigella spp.*, and *Salmonella spp.* (TFC: $n=9$, $r=0.369$, $p<0.05$); SHI:$n=9$, $r=0.379$,$p<0.05$; SAL:$n=9$, $r=0.912$, $p<0.05$). Negative correlation between salinity and abundance of TVCwas also observed($n=9$, $r=-0.141$, $p<0.05$). Temperature positively correlated with TVC($n=9$, $r=0.546$, $p<0.05$) but negatively correlated with TFC, *Salmonella* and *Shigella* species(SAL: $n=9$, $r=-0.845$, $p<0.05$; SHI: $n=9$, $r=-0.756$, $p<0.05$; TFC: $n=9$, $r=-0.493$, $p<0.05$).

At post-monsoon weaker correlation between temperature and bacterial groups was observed. Positive correlation between salinity and TFCbut negative correlation with TVC, *Salmonella* and *Shigella* wereparticularly pronounced in the post-monsoon period.

The examination of bacterial concentrations across different stations during the pre-monsoon period illuminates varying levels of microbial presence and fecal contamination along the Mandovi estuary. Campal emerges as a hotspot for bacterial abundance, exhibiting notably higher counts of Total Fecal Coliforms, *Salmonella spp.*, and *Shigella spp.* compared to Divar and Volvoi. These findings underscore Campal's susceptibility to fecal contamination and potential health risks, likely attributed to localized sources such as sewage and agricultural runoff.

Conversely, Divar and Volvoi demonstrate comparatively cleaner conditions, with lower counts of Total Fecal Coliforms and *Salmonella spp.* Furthermore, Volvoi stands out for its elevated Total Viable Counts, indicating a diverse microbial community in this area. Notably, *Streptococcus feacalis* and Enterococci are notably absent across all stations during this period, suggesting a relatively low presence of fecal indicator bacteria.

Transitioning to the post-monsoon phase, a notable decrease in bacterial concentrations is observed, particularly in Total Fecal Coliforms, *Salmonella spp.*, and *Shigella spp.* This reduction reflects improved water quality following the rainy season, highlighting the natural cleansing effect of increased rainfall and dilution of microbial populations. Interestingly, Divar records the highest count of *Salmonella spp.* during this period, emphasizing the importance of continued monitoring and management practices to mitigate potential health risks associated with this pathogen.

The correlation analysis between environmental parameters and bacterial colony types provides valuable insights into the complex interplay between physicochemical factors and microbial dynamics. Salinity emerges as a key influencer, positively correlating with Total Viable Counts, Total Fecal Counts, and *Salmonella species*, while negatively impacting *Shigella species*. Dissolved oxygen exhibits a weaker yet discernible influence, particularly on Total Viable Counts and Total Fecal Counts.

Temperature emerges as a significant driver of microbial dynamics, with higher temperatures correlating strongly with increased microbial abundance but inversely impacting *Salmonella* and *Shigella species*. These correlations underscore the multifaceted nature of microbial ecosystems and highlight the importance of considering environmental variables in understanding bacterial contamination levels along aquatic ecosystems like the Mandovi estuary.

Future research for Pollution Indicator Bacteria (PIB) could be directed towards several key areas. One such area is Microbial Source Tracking, which involves predicting and identifying sources of fecal pollution in the environment. This includes developing testing methods and analysis techniques that can define specific sources of these organisms. Another area of focus is on biological indicators, where research is being conducted on the use of biotas to perceive ecosystem pollutants. The integration between biological, chemical, and physical pollutant indicators is also a significant area of interest. Research is also being conducted on understanding the factors that control Fecal Indicator Bacteria (FIB) dynamics in tropical systems. Lastly, the development of new techniques based on the metabolism and physiological responses of traditional bioindicators is a promising area of research. These techniques can facilitate the development of suitable monitoring environmental systems according to different pollutant agents. These research areas are crucial for understanding the impact of pollution on our ecosystems and developing effective strategies for pollution control and remediation.

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