**Quantitative analyses of pathogenic bacteria along the Mandovi estuary, Goa**

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## Declaration by Student

I hereby declare that the data presented in this Dissertation report entitled "*Quantitative analysis of pathogenic bacteria along the Mandovi Estuary, Goa*" is based on the results of investigations carried out by me in the Discipline of Marine Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the Supervision of Dr. Sheryl Oliveira 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 in the dissertation.

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Place: Goa University, Taleigao Plateau, Goa

# Completion Certificate

This is to certify that the dissertation report "*Quantitative analysis of pathogenic bacteria along the Mandovi Estuary, Goa*" is a bonafide work carried out by Ms. Sweeta Rohidas Sawantodkar 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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# PREFACE

The intricate interplay between pathogenic bacteria and marine ecosystems is a topic of paramount importance, with far-reaching implications for both environmental sustainability and public health. In my endeavor to delve into this complex relationship, I turned my attention to the Mandovi estuary in Goa as the focal point for my research.

Sampling expeditions conducted during the post-monsoon and pre-monsoon seasons of 2024 across Campal, Divar, and Volvoi unveiled intriguing insights into the abundance and distribution patterns of pathogenic bacteria within these waters. Notably, Campal emerged as a hotspot for microbial activity, prompting further investigation into potential contributing factors such as the presence of casinos along the riverbanks.

This study transcends mere scientific curiosity; it underscores the urgent need to understand and mitigate the impacts of pathogenic bacteria on both marine ecosystems and human populations dependent on these waters. By unraveling the dynamics of pathogenic bacteria in the Mandovi estuary, we aim to shed light on their prevalence, identify potential sources of contamination, and elucidate the intricate relationships between environmental variables and microbial behavior.

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# CONTENTS

# LIST OF ABBREVIATIONS

| Entity | Abbreviations |
|---|---|
| Total Organic Carbon | TOC |
| Suspended Particulate Matter | SPM |
| *Shigella* like Organisms | SLO |
| *Pseudomonas aeruginosa* like Organisms | PALO |
| *Vibrio* like Organisms | VLO |
| *Salmonella* like Organisms | SLO |
| Inner estuary zone | IEZ |
| High Density Polyethylene | HDPE |
| Thiosulphate citrate Bile salts sucrose agar | TCBS |
| Species | Sp |
| *Vibrio species* | VB |

| | |
|---|---|
| *Vibrio Cholerae* | VC |
| *Vibrio Parahaemolyticus* | VP |
| Xylose Lactose Deoxycholate Agar | XLD |
| *Salmonella*sp | SA |
| *Shigella*sp | SH |
| Blood free Campylobacter selectivity agar base | BFCSAB |
| *Campylobacter*sp | CB |
| *Streptococcus faecalis* | SF |
| Bile esculinazide agar | BEAA |
| Campal | C |
| Divar | D |
| Volvoi | V |
| Most Probable Number | MPN |
| Millilitres | Ml |
| Meter | M |

| | |
|---|---|
| Standard Deviation | SD |
| Celcius | °C |
| Practical Salinity Unit | Psu |
| Total Colliforms | TC |
| *E coli*spp | EC |
| Total *Streptococci* | TS |

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## **ABSTRACT**

This study examines the seasonal variability and environmental factors influencing the presence of pathogenic bacteria in the Mandovi Estuary, focusing on three sampling stations: Volvoi, Divar, and Campal. Water samples collected in January and April 2024 underwent rigorous analysis for physico-chemical parameters and enumeration of pathogenic bacterial species including *Streptococcus faecalis*, *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.

Results indicate consistently higher pathogenic bacterial counts during the pre-monsoon season, with Campal exhibiting consistently elevated levels compared to Divar and Volvoi. Temperature, salinity, and dissolved oxygen emerged as significant influencers on bacterial abundance, with *Streptococcus faecalis*, *Campylobacter* spp., and *Vibrio* spp. favoring warmer temperatures and higher salinity, while *Salmonella* spp. and *Shigella* spp. showed nuanced responses.

Anthropogenic activities such as sewage discharge, notably at St. Inez Creek and Rio de Ourem Creek, pose a significant threat to water quality. Pathogen levels in water at all three stations surpassed the acceptable limit of less than 500 MPN/100 mL. They were observed to be at $\times 10^{6}$, far exceeding the observed limit of $\times 10^{6}$ and underscored the impact of untreated sewage on water quality and public health. Statistical analyses, including Pearson correlation coefficient, revealed intricate relationships between pathogenic bacteria and environmental factors, emphasizing the importance of continued monitoring and management to mitigate health risks associated with pathogenic bacteria in estuarine ecosystems.

# CHAPTER 1: INTRODUCTION

## 1.1BACKGROUND

The marine biome, comprising oceans, seas and estuaries, stands as the largest ecosystem on Earth spanning approximately 71% of the planet's surface. Within its vast expanse of saltwater, a diverse array of life flourishes, ranging from the smallest plankton to the largest whales. Its ever-changing dynamics, shaped by ocean currents and climate patterns, hold pivotal significance in regulating global climate systems and nurturing countless interconnected ecosystems. Yet, this critical biome faces mounting threats from human activities, including overfishing, pollution, and the looming specter of climate change. Safeguarding the integrity and sustainability of the marine biome demands urgent and concerted conservation efforts to ensure its continued vitality for generations to come.

Nestled amidst the captivating embrace of India's southwestern coast, Goa entices with its unique blend of mainland districts and picturesque offshore islands scattered along the coastline. Notable among these are the charming towns of Candolim, Palolem, and Chapora, each offering a distinct experience that adds to the allure of this vibrant state. Positioned on the north-central mainland, Goa's capital city, Panaji (also known as Panjim), gazes out over a coastline stretching approximately 105 kilometers (Shaikh et al., 2012). Celebrated for its abundant natural beauty and vibrant cultural heritage, Goa holds a special place in the hearts of tourists. Serving as the gateway to this coastal haven, Panaji (Panjim) provides travelers with a starting point to discover the region's captivating charms. One such attraction is an estuary located on the west coast of India in Goa, where freshwater from rivers and streams merges with the ever-changing coastal environment, creating a unique and mesmerizing landscape. A number of estuaries are found in Goa. The Mandovi and the Zuari rivers form an intricate estuarine network connected by interconnecting canals. From June to September, heavy rainfall and land runoff bring about dynamic changes in temperature, salinity, flow patterns, dissolved oxygen, and nutrient levels, transforming the estuary into a freshwater-dominated ecosystem. This monsoon season, followed by a recovery period from October to January and a stable pre-monsoon phase from February to May, witnesses the estuary shifting to a marine-dominated state. However, heightened concentrations of chemicals such as nitrogen and phosphorus in the coastal ocean promote the growth of algal blooms during this cycle. These blooms, potentially harmful to wildlife and humans, pose challenges to the local fishing and tourism industries, impacting both health and the environment.

Storms and sediment resuspension increase pathogen levels via surface runoff, while rainfall infiltrating sewage pipes through faults and unauthorized connections causes sewage overflow, compounding contaminant concentrations. Pathogenic bacteria, capable of causing diseases, are often transmitted through the fecal-oral route, utilizing various sources such as sewage, runoff, river discharge, groundwater seepage, and sewage sludge disposal to contaminate the marine environment. Significant health threats posed by pathogenic viruses in the marine realm, known as “enteric viruses,” also follow the fecal-oral transmission route. These marine pathogens contribute to a spectrum of ailments, including gastroenteritis, ocular and respiratory infections, hepatitis, myocarditis, meningitis, and neural paralysis, showcasing both acute and chronic impacts on human health. Survival of enteric microorganisms in the marine environment depends on factors such as salinity, microorganism type, temperature, sediments, nutrients, antagonistic elements, light, and dissolved oxygen. Temperature holds a pivotal role, with cooler temperatures below 10°C favoring enhanced survival of enteric pathogens;Highly pathogenic microorganisms can enter marine ecosystems directly or undergo amplification of their pathogenicity within these environments. Of particular concern is the threat posed by sewage, especially from hospitals and inadequately treated urban sewage, which serves as a significant source of introduction. Moreover, careful consideration must be given to ballast water management, not only due to the potential for triggering toxic algal blooms but also because of the risk of transferring highly pathogenic microorganisms. (Peperzak, 2005)

Microorganisms typically exhibit improved survival and infectivity when they adhere to particles (Brettar and Höfle, 1992; Griffin et al., 2003; Maugeri et al., 2004). Their attachment to plankton, both zooplankton and phytoplankton, is pivotal in marine environments, particularly for the sustenance and proliferation of pathogenic bacteria. This attachment provides a rich source of organic nutrients and a protective shelter. In the case of vibrios, their bonding with zooplankton holds significant implications, affecting their survival, propagation, and dispersion. This process involves transportation facilitated by the host animal and/or its fecal matter, contributing to the complex dynamics of microbial presence and dissemination (Baffone et al., 2006; Maugeri et al., 2004).

Sediments, especially at their surface, create conditions conducive to increased concentrations, prolonged survival, and enhanced infectivity of pathogenic microorganisms (Brettar and Höfle, 1992; Griffin et al., 2003; Fayer et al., 2004).Analyzing the domain of pathogenic bacteria in marine waters is crucial for several reasons. It provides insights into potential threats to aquatic ecosystems, impacting marine biodiversity and the human populations depending on these waters. Understanding the occurrence and behavior of pathogenic bacteria becomes essential for managing water quality, mitigating disease transmission, and ensuring the safety of seafood consumption. Therefore, research on pathogenic bacteria not only enhances our understanding of their dynamics in aquatic environments but also plays a vital role in devising strategies to mitigate their impact on both the ecosystem and public health.

This study conducted in an economically significant estuary in Goa, spanning post-monsoon and pre monsoon seasons, aims to accomplish several key objectives. Firstly, we seek to uncover the abundance and distribution patterns of pathogenic bacteria, providing insights into their prevalence within this crucial ecosystem. Secondly, our focus lies in pinpointing potential sources responsible for introducing pathogenic microbes into the estuary. Lastly, we strive to understand the intricate relationship between relevant environmental variables and the dynamics of pathogenic microorganisms, thereby enriching our comprehension of these microbial communities in the marine environment. This comprehensive approach not only advances scientific knowledge but also aids in the development of strategies to safeguard both the ecosystem and public health against emerging microbial threats.

## 1.2 AIMS AND OBJECTIVES

1. To assess the spatial variations in the abundance of pathogenic bacteria along the Mandovi estuary
2. To identify the probable sources of pathogenic microbes in the estuary
3. To understand the influences of pertinent environmental parameters on pathogenic bacteria

## 1.3 SCOPE

The research scope encompasses exploration of ballast water treatment strategies, longitudinal monitoring of bacterial dynamics, and socio-economic impact assessments to enhance water quality management along the Mandovi estuary, addressing public health concerns and promoting ecosystem resilience.

# CHAPTER 2: LITERATURE REVIEW

Pathogenic bacteria in marine waters

In a study conducted by Nagvenkar et al. (2008) along the Mandovi and Zuari estuaries, with seasonal sampling for pathogenic bacteria carried out at 22 different locations throughout the year 2005-2006. Notably, *Vibrio cholerae* emerged as the predominant bacterium in sewage discharges. Post-monsoon, particularly in November, saw consistently elevated counts of *E. coli*, *Vibrio cholerae*, and *Salmonella* across the entire region. Concurrently, the counts of *Aeromonas*, alongside other pathogenic groups, were notably higher during post monsoon(November) is contrasting to lower counts during the pre-monsoon period.

Similarly, Lee et al. (2019) conducted a study on 11 different coastal quarantine stations in South Korea during 2017-2018. Their research focused on estimating the prevalence of pathogenic *Vibrio* species. They observed a rapid increase in the isolation rate of these bacteria from 2017 to 2018. Interestingly, isolation rates of pathogenic *Vibrio* species positively correlated with sea water and atmospheric temperatures, but negatively correlated with salinity and turbidity.

Influence of anthropogenic activities on pathogenic bacteria

Singh et al. (2014) identified various human activities like urban runoff, wastewater discharge, and agricultural runoff as major sources of pathogenic bacteria in marine waters. They also noted the significance of malfunctioning septic systems and the direct deposition of bird droppings. Fayer and Trout (2005) highlighted the transport of pathogens like *Giardia* and *Cryptosporidium* in coastal environments. Sediment in seawater was found to enhance the survival of fecal coliforms like *E. coli* (Gerba and McLeod, 1976; Goyal et al., 1977). Additionally, Solo-Gabriele et al. (2000) emphasized the role of storms in influencing coastal water quality. Municipal point sources were identified as primary contributors to pathogen contamination in estuaries. Bed sediment was found to play a crucial role in the persistence and transport of pathogens (Smith et al., 1978; Desmarais et al., 2002). Chandran and Hatha (2005) demonstrated sunlight's impact on the survival of pathogens in estuarine waters.

Furthermore, Joseph et al. (2021) highlighted the critical role of bacterial pathogens in shellfish contamination, posing risks to both marine life and seafood consumers. Their findings underscored the importance of addressing microbial contamination to ensure the safety of marine ecosystems and seafood consumption.The research paper delved into the isolation of marine bacteria showcasing antagonistic properties against human pathogens.

Influence of environmental parameters on Pathogenic bacteria

Several studies conducted in the 1990s explored the diversity of *Vibrio* species, each contributing unique insights (Montilla et al., 1994; Hariharan et al., 1995; Arias et al., 1999; Pujalte et al., 1999; Maugeri et al., 2000; Caballo and Stabili, 2002; Castro et al., 2002; Guisande et al., 2004; Beaz-Hidalgo et al., 2008; Lafisca et al., 2008). These studies collectively concluded that environmental parameters, such as fluctuations in seawater temperature and salinity, play a significant role in influencing the diversity of *Vibrio* species.

Ramaiah et al. (2004) explored Mumbai Harbour's pollution zones with a detailed approach, assessing environmental health through various parameters like dissolved oxygen, biological oxygen demand, and nitrate-N concentrations. They also meticulously measured general microbial counts, including direct and viable counts, and specific indicators like total coliforms and pathogenic bacteria. Across three seasons, they found consistently high levels of pathogenic microorganisms, highlighting significant environmental health risks. Additionally, they conducted advanced serotyping of *E. coli* O157 and *Shigella* colonies, enhancing the specificity of their findings. This study emphasizes the urgent need for targeted interventions to protect public and environmental health in the region.

Kuchi et al.(2017) conducted a hypersaline water experiment by sampling sea water from Kandla Port, Gujarat, India, and collecting surface water from the Zuari Estuary, Goa, India. They also gathered samples from Paradip Port for saline water experiments and Kolkata Port for freshwater experiments. In all conditions, total bacterial count was highest in high saline water, negatively correlating with $NO_3$, and positively affected by $PO_4$. Total viable marine bacteria increased in high saline conditions, while total viable freshwater bacteria thrived in low saline conditions. *E. Coli* O157 was only found in low saline environments, while *Vibrio* species flourished in higher salinity, especially *V. parahaemolyticus* in mid-saline conditions. Total organic carbon (TOC) and suspended particulate matter (SPM) were identified as key factors positively influencing the populations of total viable freshwater bacteria, total viable marine bacteria, and pathogenic bacteria.

In a study conducted by Sangodkar et al. (2020), researchers investigated the prevalence of indicator and pathogenic bacterial groups in various water and sediment locations along the Chapora Estuary during March 2017. They collected surface and bottom water samples from 22 stations across different saline zones, including offshore, inshore, inner estuary, and upper estuary. Interestingly, *Shigella*-like organisms (SHLO) were identified as the dominant species across all four zones, ranging from 12% in inshore bottom waters to 84% in offshore sediments. *Pseudomonas aeruginosa*-like organisms (PALO) showed a gradual increase in abundance from offshore to upper estuary, contributing up to 35% and 25% in surface and bottom waters, respectively. *Vibrio*-like organisms (VLO) and *Salmonella*-like organisms (SLO) were notably scarce in sediment samples, while a minor fraction of *Streptococcus faecalis*-like organisms (up to 9%) and *Vibrio*-like organisms (up to 19%) were present in both water and sediment samples across all zones. Notably, the inner estuary (IEZ) exhibited the highest contamination levels, with elevated abundances of both indicator and potential pathogenic bacterial populations compared to the other zones.

Hofle et al. (2004) underscore the critical role of effective sewage treatment in minimizing bacterial contamination. They highlight how heavy rain periods or floods can lead to the overflow of untreated or poorly treated sewage into rivers and coastal areas, exacerbating the presence of pathogenic microorganisms. This issue persists even in developed countries with advanced sewage treatment systems. Despite efforts to treat wastewater, a significant proportion still carries pathogens. These pathogens, including bacteria, viruses, and protozoa, can either be directly introduced into the marine environment or become more virulent in such settings. Sewage, particularly from hospitals and densely populated urban areas, emerges as a major source of contamination. Furthermore, rivers, influenced by runoff, sewage, and groundwater, often experience spikes in pathogen levels following heavy rainfall events. Human and animal feces constitute the primary reservoirs of these pathogens, with sewage, runoff, groundwater, and river discharge acting as key conduits for their transmission. Moreover, these same transmission routes also transport nutrients like nitrogen and phosphorus, contributing to eutrophication. Eutrophication, in turn, fosters the proliferation of algae, thereby creating favorable conditions for the growth of zooplankton and indigenous pathogenic bacteria such as *vibrios*.

Cabral et al. (2010) underscore the primary habitat of *Salmonella* as the intestinal tracts of both humans and animals. This bacterium is frequently encountered in environmental samples due to excretion by humans, pets, farm animals, and wildlife. Municipal sewage, agricultural pollution, and stormwater runoff emerge as the major contributors of *Salmonella* contamination in natural water bodies. Although *Salmonella* do not exhibit significant multiplication in the natural environment, they can survive for several weeks in water and soil under favorable conditions of temperature, humidity, and pH.

*Salmonella* strains isolated from environmental sources predominantly belong to non-Typhi or *Paratyphi serovars*. A study conducted in Tunisia from 1994 to 2004 revealed that *serovars Anatum, Enteritidis,* and *Corvallis* were the most commonly isolated types from food sources. These strains were predominantly found in poultry, red meat, milk, dairy products, vegetables, and fruits. Among environmental sources, tap water accounted for 73% of isolates. *Serovars Corvallis, Enteritidis,* and *Anatum* were among the most prevalent in these environmental samples.

Yusof et al. (2021) conducted a study where they examined ballast water samples taken from eight ocean-going vessels of various origins. In 2017, samples were collected from a ship cargo berthed at TanjungPelepas Port in Johor and analyzed for bacterial diversity. Ballast water 5-Yangon and Ballast water 7-South Africa exhibited the highest bacterial diversity, with an average Shannon index value of 5. These values slightly exceeded those reported by Brinkmeyer (2016) for ballast water undergoing open ocean Ballast water exchange. The study revealed a concerning finding: a total of 33 bacterial pathogens with more than 10% operational taxonomic unit  prevalence were identified. These pathogens pose a potential threat to both marine ecosystems and human health.

Xue et al. conducted a study in the fall of 2016 where they sampled the ballast water of four vessels at Yangshan Port in Shanghai, China. The original source of this ballast water was the South China Sea. Each cabin collected 1 L of mixed water sample in sterilized glass bottles, which were then placed in ice boxes and transported back to the laboratory within 2 hours for microbial analysis.In their research, they detected 16 potential pathogens in the ballast water, which belonged to the phyla *Proteobacteria, Bacteroidetes*, and *Firmicutes*, with a notable abundance of *Gammaproteobacteria*. This class of bacteria was found to be the most abundant and diverse in various sea areas, including many important pathogens. Notably, species such as *Arcobacter*, *Aeromonas*, and *Acinetobacter* were identified as anaerobic bacteria, capable of surviving in diverse environments, possibly explaining their presence in ballast tank environments after extended voyages.

# CHAPTER 3: METHODOLOGY

## 3.1 STUDY AREA

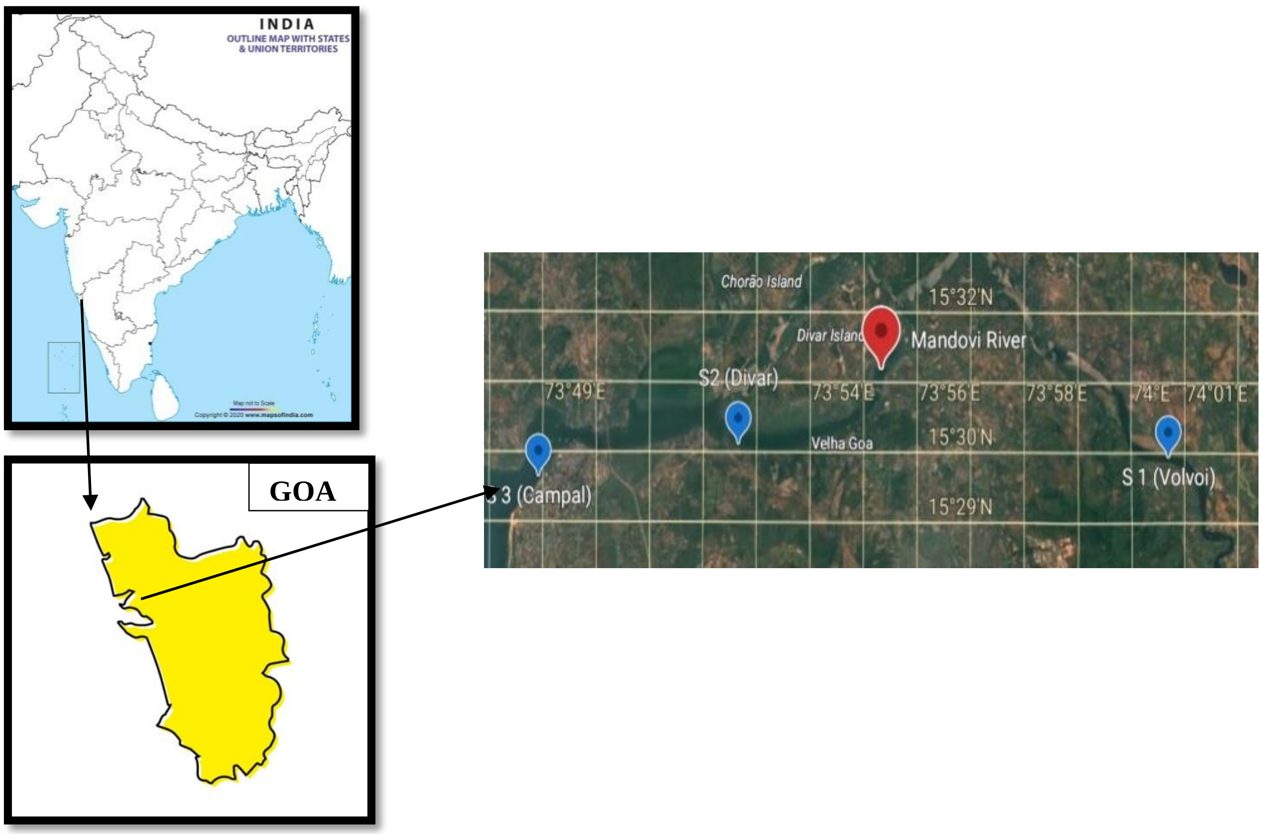



**Fig.3.1**: Study area showing Station 1 (Volvoi) , Station 2 ( Divar) and Station 3 ( Campal) along the Mandovi estuary.

In figure 3.1, situated along the west coast of Goa, the study area encompasses the Mandovi Estuary, a significant water body formed by the convergence of the Mandovi River and the Arabian Sea. Renowned for its ecological importance, the estuary serves as a vital habitat for diverse marine life, enriched by the surrounding mangrove forests. Its scenic beauty adds to its ecological significance, attracting attention from conservationists and nature enthusiasts alike.

Beyond its ecological value, the Mandovi Estuary holds economic importance, sustaining local fisheries and providing livelihoods for nearby communities. However, this pristine ecosystem faces numerous pollution sources, threatening its ecological health. Urban areas contribute to pollution through the discharge of untreated sewage and stormwater runoff, introducing contaminants into the river.

Adding to the pollution burden are the floating casinos operating within the Mandovi Estuary. These establishments discharge various pollutants, including untreated sewage and waste materials, further compromising water quality and endangering aquatic life. Addressing these pollution sources is imperative to safeguard the Mandovi Estuary's ecological integrity and ensure the sustainability of its resources for future generations.

## 3.2 WATER SAMPLES FOR PHYSICO-CHEMICAL PARAMETERS

Water samples were collected from three stations (Figure 3.1) in January and April, 2024 to analyse physico-chemical parameters.

### Environmental parameters

Environmental parameters, viz. seawater temperature, salinity, pH,dissolved oxygen and total suspended solids were analyzed during the study.

**Temperature**

Water temperature readings were recorded with a mercury thermometer at the field, and the readings were recorded.

**Salinity**

Water samples collected were analyzed for salinity using a hand-held refractometer (ATAGO 2491) and the readings were recorded.

**pH**

Water samples collected were analyzed in the laboratory using a calibrated pH meter on the same sampling day. The pH meter measures a solutions electric potential and converts it into the respective pH values.

**Turbidity**

Water samples were collected using Secchi disk, and the readings were recorded.

**Dissolved oxygen**

Dissolved oxygen in the seawater was analyzed by the Winkler's titration method described by Parsons et al. (1969) and modified by Carpenter et al.(1965) on theday of samplingwater samples (250 mL) collected were fixed with Winkler A and Winkler B on the field and were analyzed in the laboratory. When treated with an acid, the dissolved oxygen binds with manganese and liberates manganic ions, which reacts with potassium iodide to liberate free iodine. This free iodine when treated with sodium thiosulphate, gives a layer of light yellow colour. Then 1ml of starch solution is added to the solution and the titration is continued until the blue colour disappears. This gives the end point.

**Total suspended solids**

Gravimetric analysis was employed for the determination of total suspended solids in a seawater sample. A well mixed water sample was filtered through a weighed standard glass fibre filter, and the residue retained on the filter was dried to a constant weight at 103-105 °C. The increase in weight of the filter represented the total suspended solids.

## 3.3 SAMPLING AND STORAGE FOR MICROBIAL ANALYSIS

Water samples were collected in triplicates from Volvoi (Station 1), Divar (Station 2) and Campal (Station 3).The samples were collected by using a clean, well-rinsed bucket. The procedure involved collecting 500 milliliters of sample from each location in pre-sterilized High-Density Polyethylene (HDPE) bottles. All samples were gathered with necessary precautions for microbiological analysis, kept in an ice in an icebox during transportation to the laboratory, and stored in a refrigerator at 2°C until further anaylsis.

## 3.4 MEDIA PREPARATION

To ensure a minimum salinity of 15 PSU, aged seawater was added to all media. Plates were made 5–7 days before sampling. The water was filtered using a cotton plug and filter paper before autoclaving. Media-containing flasks were sterilized using a pressure cooker. The laminar airflow was cleaned with isopropyl and benzalkonium chloride surface disinfectant. Pre-sterilized plastic petri plates were used. The flasks were allowed to cool until lukewarm, then the media was poured into petri plates. After pouring, the plates were closed and flipped upside down to prevent condensation droplets from falling onto the agar surface. The media plates were stacked upside down and wrapped in cling film and stored at room temperature until spread plating. The pH was adjusted to 7.8-8.0 before adding agar.

Specific media were used for measuring bacterial groups: Xylose Lactose Deoxycholate (XLD) agar for *Salmonella* spp. (SA) and Shigella spp. (SH); Blood-free Campylobacter-selective agar base (BFCSAB) for *Campylobacter* spp. (CB); Bile esculinazide agar for *Streptococcus faecalis* (SF). All the agars were prepared using a required amount of agar and aged seawater, then autoclaved at 121 °C and 15 lbs pressure. The media plates were prepared under sterile conditions.

## 3.5 SAMPLE INOCULATION

To inoculate the samples, 50 µL of the collected sample was carefully dispensed into thepetri plate. Using an 'L' shaped glass spreader, the sample was evenly spread onto the plates by moving the spreader in a circular motion while simultaneously rotating the Petri plates. Subsequently, the plates were sealed with Parafilm to prevent contamination. They were then stacked and wrapped with cling film before being placed in an incubator at room temperature for approximately a week, with the plateskept upside down to minimize contamination risk. All procedures were conducted in a laminar airflow chamber under sterile conditions. After the incubation period, each colony-forming unit was counted.

The collected samples were inoculated onto four agar media types: TCBS agar for enumerating Vibrio species, XLD agar for detecting Salmonella and Shigella species, BFCSAB agar for identifying Campylobacter species, and BEA agar for enumerating Streptococci species.

The assessment of microbial presence and diversity within the collected samples was based on directly recorded bacterial growth on agar media plates.

$$\text{Bacterial plate count (CFU } L^{-1}) = \frac{\text{Number of bacterial colonies counted}}{\text{Volume used as inoculum (mL)}}$$

Sampling sites were selected to encompass a broad spectrum of marine based on the manufacturer's instructions for the media used and extensive experience gained from numerous prior analyses, the enumeration of pathogenic bacteria was carried out. Environments, chosen to reflect a gradient extending from coastal areas with minimal sewage and industrial effluent impact to locations more directly influenced by these anthropogenic factors. The counts were expressed as CFU $L^{-1}$.

## 3.6 SEWAGE DISCHARGE POINTS

Sewage discharge points along the study area are present notably at St. Inez Creek, where upstream and downstream assessments indicated total coliform levels surpassing permissible limits throughout the year (GSPCB 2020). Another site of concern is Rua de Ourem Creek, where untreated sewage from the sewage pump house at Patto is released. This influx of raw sewage has led to the accumulation of pollutants under the Mala-Patto Bridge and even threatens the integrity of essential water pipelines supplying drinking water to residents near the Sinari petrol pump. Such unregulated sewage discharge poses a grave threat to water quality and fosters the proliferation of pathogenic bacteria within these aquatic environments. The acceptable threshold for pathogen levels is set at less than 500 MPN/100 mL, with a maximum permissible limit of 2500 MPN/100mL.Exceeding these limits poses significant risks to water quality and public health, indicating the presence of potentially harmful levels of bacterial contamination. The Mandovi receives ~5.21 × $10^6$ $m^3$ of sewage and their effluents per year (Kessarkar et al., 2009).

## 3.7 STATISTICAL ANALYSIS AND INFLUENCE OF ENVIRONMENTAL PARAMETERS ON PATHOGENIC BACTERIA

In investigating the impact of environmental parameters on pathogenic bacteria in the Mandovi estuary, a comprehensive analysis was conducted using a combination of statistical methods. Significant seasonal variations in pathogenic bacteria were examined through two-way Analysis of Variance (ANOVA) utilizing the Analysis Tool Pack in Microsoft Excel. Specifically, bacterial numbers were log10 transformed before analysis to ensure robust statistical assessment.To further elucidate the relationship between pathogenic bacteria and environmental factors, Pearson's correlation coefficient was employed to analyze associations within a depth range of 3-5 cm. This correlation analysis, conducted using STATISTICA software (version 6), provided valuable insights into the interplay between pathogenic bacteria and other abiotic/biotic factors present in the estuarine ecosystem

# CHAPTER 4: RESULTS

Physico-chemical parameters:

Maximum depth at sampling locations ranged from 3 to 5 m. Temperature of ambient estuarine water varied from 27°C to 29°C during January 2024 whereas in April it increased further ranging from 30–33°C (Table 4.1). Salinity was generally lower during January ranging from 14 to 16 at Volvoi , 34 to 36 psu at Campal and 32 to 34 at Divar. In the pre-monsoon period, the pH levels were 8.22 at Campal, 7.95 at Divar, and 7.67 at Volvoi. In the post-monsoon period, Campal exhibited a pH of 7.96, while Divar had a pH of 7.79, and Volvoi measured 7.36. In the pre-monsoon period, the turbidity levels were 4.5 m at Divar and 3.96 m at Volvoi. Turbidity measurement at Campal was not feasible due to the inability to access the middle of the water body. In the post-monsoon period, both Divar and Volvoi exhibited the same turbidity level, which was 3.39 m (Table 4.1).The concentration of dissolved oxygen showed a slight increase across the sampling locations. Specifically, at Campal, it was recorded at 0.72 ± 0.02 $mL^{-1}$, while at Divar and Volvoi, it was 0.52 ± 0.10 $mL^{-1}$ and 0.65 ± 0.13 $mL^{-1}$, during the pre-monsoon season, whereas in the post-monsoon period, the concentration of dissolved oxygen varied. Specifically, at Campal, it was measured at 0.87 ± 0.14 mL $L^{-1}$, while at Divar, it was 1.12 mL $L^{-1}$, and at Volvoi, it was recorded as 1.54 ± 0.20 mL $L^{-1}$ (Table 4.1).Additionally, total suspended solids were observed to be 0.035 g $L^{-1}$ at Campal, 0.022 g $L^{-1}$ at Divar, and 0.028 g $L^{-1}$ at Volvoi during the pre-monsoon season. In the post-monsoon period, it was higher at Divar, measuring 0.026 g $L^{-1}$, while at Campal it was 0.011 g $L^{-1}$, and at Volvoi, it was 0.017 g $L^{-1}$ (Table 4.1).These variations in water quality parameters highlight the dynamic nature of the Mandovi estuary and underscore the importance of understanding seasonal fluctuations for effective ecosystem management.

**TABLE 4.1**.Average with standard deviation (S.D.) for various environmental parameters during the post-monsoon (January 2024) and pre-monsoon (April 2024) seasons measured at sampling locations along the Mandovi Estuary. These mean values obtained arefor triplicates samples collected during respective season.

| Environmental parameters | Pre monsoon (results) | | | Post monsoon (results) | | |
|---|---|---|---|---|---|---|
| | Campal | Divar | Volvoi | Campal | Divar | Volvoi |
| Temperature (°C ) | 30.33 ± 0.57 | 31.66 ± 0.57 | 32.33 ± 0.57 | 28.33 ± 0.57 | 27.33 ± 0.57 | 27 ± 0.57 |
| pH | 8.22 | 7.95 | 7.67 | 7.96 | 7.79 | 7.36 |
| Salinity | 35 ± 1 | 25.66 ± 0.57 | 21.66 ± 0.57 | 35 ± 1 | 32.66 ± 1.15 | 15 ± 1 |
| Turbidity (m) | Not done | 4.52 | 3.96 | Not done | 3.39 | 3.39 |
| Dissolved oxygen ($mL\ L^{-1}$) | 0.72 ± 0.02 | 0.52 ± 0.10 | 0.65 ± 0.13 | 0.87 ± 0.14 | 1.12 ± 0.05 | 1.54 ± 0.20 |
| Suspended particulate matter ($g\ L^{-1}$) | 0.0359 | 0.0224 | 0.0286 | 0.0118 | 0.0263 | 0.0172 |

Note:  Single Observations for Suspended particulate matter and Turbidity.

Abundance of *Streptococcus faecalis* (SF) was the highest during pre-monsoon (2450.66 ± 329.21 $\times 10^6$ CFU L-1) and the least during post monsoon (1248 ± 825.47$\times 10^6$ CFU $L^{-1)}$.In the Campal area, the presence of SF bacteria was notably abundant during the pre monsoon period, ranging from 935 ± 401.9$\times 10^6$ CFU $L^{-1}$. However, as we moved upstream, there was a noticeable decline in their abundance. Conversly, at the Volvoi location, SF bacteria were found to have a slightly higher abundance range of 718 ± 167.20$\times 10^6$CFU $L^{-1}$ (fig.4.2 and 4.3) during the post monsoon period, there was a slight decrease in the abundance of SF bacteria compared to the pre-monsoon phase, averaging at 712 ± 210.79 $\times 10^6$CFU $L^{-1}$. Conversely, at the Volvoi site, SF were present at a abundance of 168± 78.61$\times 10^6$CFU $L^{-1}$. Divar exhibited moderate abundance of 368 ± 221.24$\times 10^6$ CFU $L^{-1}$, falling between the levels observed at Campal and Volvoi. *Streptococcus faecalis* thrives under specific environmental conditions, including a temperature range of 42°C, salinity levels between 14 to 16 practical salinity units (psu), and a pH range of 7 to 8. During the sampling period, the salinity and pH conditions at Volvoi aligned with these optimal growth parameters, contributing to the observed abundance of *Streptococcus faecalis* during the post monsoon period.

In the pre-monsoon period, the abundance of *Campylobacter* bacteria was relatively higher at Campal, ranging around 823.33 ± 185.40$\times 10^6$ CFU $L^{-1}$, while at Volvoi, it was lower, at 388 ± 256.90$\times 10^6$ CFU $L^{-1}$. At post monsoon, there was a decrease in *Campylobacter*abundance, with Campal recording an abundance of 746.33 ± 285 $\times 10^6$ CFU $L^{-1}$, and while at upstream location (Volvoi) showing a decrease to 164.5± 191.55$\times 10^6$ CFU $L-^{-1}$, respectively (fig.4.2 and 4.3). Divar exhibited a moderate level of abundance, falling between Campal and Volvoi, at 32.33 ± 30.33$\times 10^6$ CFU $L^{-1}$. These findings indicate fluctuations in *Campylobacter* abundance across different locations during both pre and post-monsoon periods.*Campylobacter* species thrive under specific environmental conditions, with optimal growth parameters including a temperature of 42°C, salinity levels ranging from 14 to 16 practical salinity units (psu), and a pH range of 7 to 8. Throughout the sampling period, the observed pH and salinity conditions at Volvoi were conducive to the proliferation of *Campylobacter*, thus explaining its abundance during the post-monsoon period.

During the pre-monsoon phase, *Vibrio* species exhibited higher abundance levels at Campal, with readings averaging around 975. ± 250.57×$10^6$ CFU $L^{-1}$. Conversely, Volvoi showed lower levels, of 222.33 ± 34.64×$10^6$ CFU $L^{-1}$. After the monsoon, *Vibrio* levels decreased, with Campal recording 717.66 ±160.72 ×$10^6$ CFU $L^{-1}$and upstream Volvoi showing a decline to 201.33 ± 67.11×$10^6$ CFU $L^{-1}$ respectively (fig.4.2 and 4.3). Divar displayed a moderate abundance level, ranging between Campal and Volvoi, at 267.33 ± 148.97×$10^6$CFU $L^{-1}$. *Vibrio* species are highly adaptable, thriving best in environments with salinity values of 30 practical salinity units (psu) or higher and temperatures ranging from 36°C to 40°C. During the post-monsoon period, Campal exhibited temperatures ranging from 30°C to 31°C, coupled with optimal salinity levels of 34 to 36 psu, creating favorable conditions for *Vibrio* proliferation. Despite slightly lower temperatures, *Vibrio* species can still thrive within the range of 30°C to 33°C, especially when accompanied by optimal salinity conditions. Divar also experienced conditions closely aligned with *Vibrio* growth parameters during this period, contributing to the observed abundance of *Vibrio* species.

However, Campal demonstrated even greater abundance of *Vibrio* species throughout both seasons. This heightened abundance may be attributed to environmental conditions more conducive to *Vibrio* growth, potentially influenced by higher levels of human activities or anthropogenic impacts compared to Divar. Factors such as pollution, urban runoff, and other human-related influences can introduce nutrients and organic matter into the water, creating an environment favorable for bacterial proliferation.

During the pre-monsoon period, Divar exhibited temperatures favorable for *Vibrio* growth, ranging from 30°C to 31°C. However, salinity levels ranged from 25 psu to 26 psu, slightly lower than the optimal range for *Vibrio* proliferation. Consequently, despite favorable temperatures, the suboptimal salinity range may have contributed to a less pronounced abundance of *Vibrio* species at Divar compared to Campal. This highlights the significance of both temperature and salinity in influencing the abundance and distribution of *Vibrio* species in aquatic ecosystems.

Salmonella species exhibited higher levels at Campal during the pre-monsoon period, ranging around 712 $\times 10^6$ CFU $L^{-1}$ compared to Volvoi at 556.66$\times 10^6$ CFU $L^{-1}$ and Divar at 634 CFU $L^{-1}$. Following the monsoon, Campal recorded 746.33$\times 10^6$ CFU $L^{-1}$, while Volvoi and Divar showed lower levels at 285 $\times 10^6$ CFU $L^{-1}$and 32.33 $\times 10^6$ CFU $L^{-1}$, respectively (fig.4.2 and 4.3). During the pre-monsoon season, Campal likely harbored higher levels of Salmonella species compared to Divar and Volvoi. The temperatures at Campal ranged from 30°C to 31°C, with salinity levels between 34 and 36 psu, providing optimal conditions for Salmonella growth. In contrast, Divar and Volvoi experienced lower temperatures and salinities, potentially leading to lower Salmonella abundance.

In the post-monsoon season, both Campal and Divar exhibited temperatures ranging from 28°C to 29°C, along with suitable salinity levels (32 to 36 psu), conducive for Salmonella proliferation. While Divar showed favorable conditions, the observed CFU count was lower compared to Campal, indicating potential environmental factors influencing *Salmonella* abundance.

Similarly, *Shigella* species had greater abundance at Campal during the pre-monsoon period, with levels at $227\times10^6$ CFU $L^{-1}$, decreasing to $133.66\times10^6$ CFU $L^{-1}$ after the monsoon. Volvoi and Divar displayed ranges of 173.66 CFU $L^{-1}$and 193.66 $\times10^6$ CFU $L^{-1}$ respectively during pre-monsoon, and decreased to $33.33\times10^6$ CFU $L^{-1}$ and $101\times10^6$ CFU $L^{-1}$ post-monsoon. Based on the temperature and salinity conditions observed during both the pre-monsoon and post-monsoon seasons, the abundance of *Shigella* species would likely be higher at Campal compared to Divar and Volvoi.

During the pre-monsoon season, Campal exhibited temperatures ranging from 30°C to 31°C, which are within the favorable range for *Shigella* growth. Additionally, the salinity levels recorded at Campal (ranging from 34 to 36 practical salinity units) are suitable for *Shigella* proliferation. In contrast, Divar and Volvoi experienced lower temperatures and salinities, potentially creating less favorable conditions for *Shigella* species abundance.

Similarly, during the post-monsoon season, Campal exhibited temperatures ranging from 28°C to 29°C, which are still within the favorable range for *Shigella* growth. Additionally, the salinity levels recorded at Campal remained suitable for *Shigella* proliferation. In contrast, Divar and Volvoi continued to experience lower temperatures and salinities, which may have contributed to less favorable conditions for *Shigella* species abundance compared to Campal.

Overall, the pre-monsoon period showed elevated levels of pathogenic bacteria along the Mandovi estuary compared to the post-monsoon period. Seasonal variations were evident across all pathogenic bacteria during the pre-monsoon period (Fig. 4.4 and 4.5). Both preand post monsoon seasons, Campal consistently exhibited a higher abundance of pathogenic bacteria compared to Divar and Volvoi. This disparity in bacterial abundance at Campal may be attributed to factors such as sewage discharge, casino discharges, fishing activities, and agricultural runoff. The introduction of human and animal feces significantly contributes to the presence of these pathogens in water bodies. Conversely, Volvoi demonstrated lower pathogenic bacteria abundance, likely due to fewer anthropogenic activities in the river, as evidenced by the absence of casinos.

**Fig.4.2**:- Pathogenic bacteria in water samples collected during pre monsoon seasons.

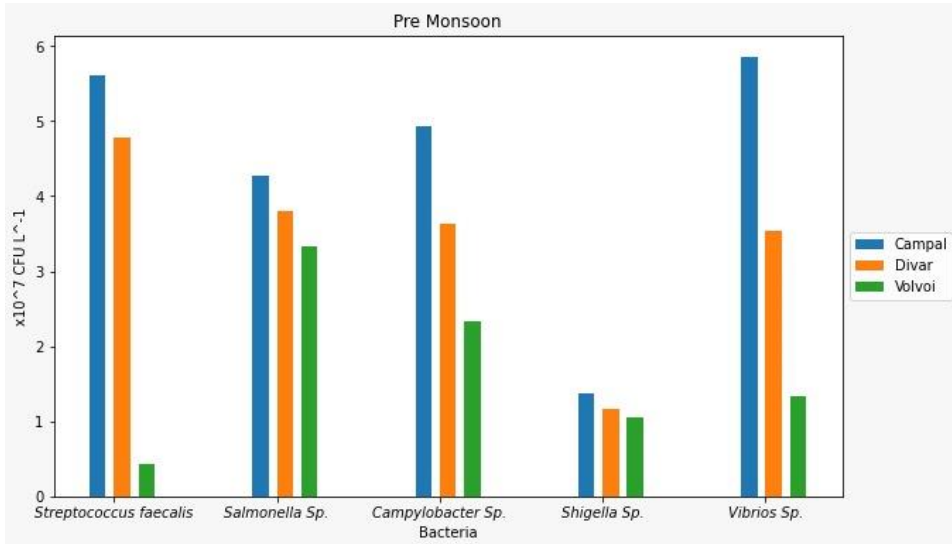
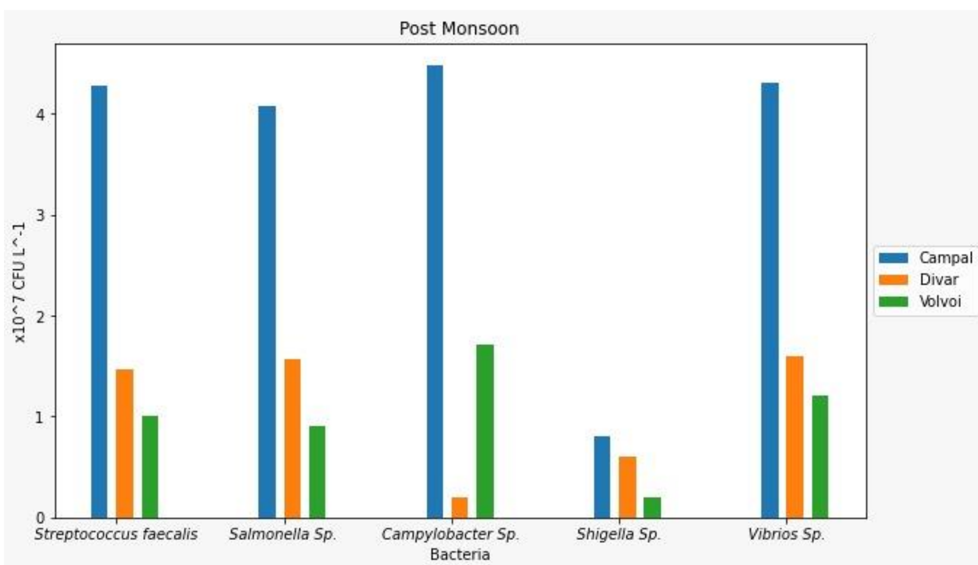



**Fig.4.3**:- Pathogenic bacteria in water samples collected during post monsoon seasons



**<u>Table: 4.4</u>** Correlation analysis of physico-chemical parameters and pathogenic bacteria during the post monsoon season (January,2024).

| | **Temp** | **Salinity** | **DO** | **SF** | **CA** | **SA** | **SH** | **VB** |
|---|---|---|---|---|---|---|---|---|
| **Temp** | 1 | | | | | | | |
| **Salinity** | 0.228341054 | 1 | | | | | | |
| **DO** | 0.585381237 | -0.05373365 | 1 | | | | | |
| **SF** | 0.521382437 | **0.733478791** | 0.389581063 | 1 | | | | |
| **CA** | **0.680801512** | -0.09992183 | **0.829499514** | 0.411615234 | 1 | | | |
| **SA** | 0.516192808 | **0.780423395** | 0.434250116 | 0.894254105 | 0.513367474 | 1 | | |
| **SH** | 0.23744079 | **0.871114409** | 0.192333019 | 0.904776471 | 0.130643819 | 0.871361828 | 1 | |
| **VB** | 0.462808266 | 0.548949973 | **0.617781311** | 0.857195659 | 0.672854619 | 0.929998121 | 0.769863006 | 1 |

DO: Dissolved oxygen, SF: *Streptococcus faecalis*, CA: *Campylobacter*, SA: *Salmonella*, SH: *Shigella*, VB:*Vibrio*

In the post-monsoon period, *Salmonella* species did not reach statistical significance at the $p < 0.05$ level, although its proximity to the threshold suggests marginal significance or an approach to significance. Salmonella species displayed strong positive correlations with salinity ($n=9$, $r= 0.78$, $p < 0.05$) and temperature, with weaker negative correlations with dissolved oxygen.

Conversely, *Campylobacter* species exhibited a robust positive correlation with temperature ($n= 9$, $r = 0.68$, $p < 0.05$), indicating a significant their abundance with rising temperatures, highlighting pivotal role of temperature in fostering their growth. *Campylobacter* species also showed strong positive correlations with salinity (Table 4.4), and a strong positive correlation with dissolved oxygen (n=9, r= 0.8, $p < 0.01$), suggesting that higher dissolved oxygen levels corresponded to increased *Campylobacter* abundance, favoring their growth due to improved water quality.

Additionally, *Shigella* species demonstrated a strong positive relationship with salinity (n = 9, r = 0.87 $p < 0.01$), indicating their adaptation to saline environments and enhanced survival in estuarine conditions. *Shigella* species also showed a strong positive correlation with salinity (n= 9, r= 0.87, $p < 0.01$) with a weaker positive correlation with dissolved oxygen (n=9, r= 0.19, $p < 0.01$), suggesting potential promotion of their growth by higher dissolved oxygen concentrations.

*Streptococcus faecalis* exhibited a moderate positive correlation with salinity (n=9, r=0.73, $p< 0.05$), while showing a weaker positive correlation with dissolved oxygen, suggesting its preference for warmer and saltier environments, albeit with some inhibition by higher dissolved oxygen levels.

*Vibrio* species exhibited a weak correlation with salinity, but weaker negative correlations with temperature and dissolved oxygen, suggesting potential inhibition by warmer temperatures and higher dissolved oxygen levels.

Analyzing the correlations between *Streptococcus faecalis* (SF), *Campylobacter* species (CA), *Salmonella* species (SA), *Shigella* species (SH), and *Vibrio* species (VB) with environmental factors revealed distinct patterns

**<u>Table 4.5</u>**: Correlation analysis of physico-chemical parameters and pathogenic bacteria during the pre monsoon season ( April,2024).

| | **Temp** | **Salinity** | **DO** | **SF** | **CA** | **SA** | **SH** | **VB** |
|---|---|---|---|---|---|---|---|---|
| **Temp** | 1 | | | | | | | |
| **Salinity** | -0.717350301 | 1 | | | | | | |
| **DO** | 0.815493206 | -0.803631068 | 1 | | | | | |
| **SF** | 0.12686003 | 0.347201268 | -0.152551549 | 1 | | | | |
| **CA** | -0.369694827 | **0.637417712** | -0.453008636 | 0.740490697 | 1 | | | |
| **SA** | 0.081524657 | 0.293033685 | -0.166924039 | 0.890344199 | 0.810516306 | 1 | | |
| **SH** | 0.287172995 | 0.257533045 | 0.145823661 | 0.724597171 | 0.545115526 | 0.502934505 | 1 | |
| **VB** | -0.626800867 | **0.857698246** | **-0.712011712** | 0.645630607 | 0.851722657 | 0.593412475 | 0.451131305 | 1 |

DO: Dissolved oxygen, SF: *Streptococcus faecalis*, CA: *Campylobacter*, SA: *Salmonella*, SH: *Shigella*, VB:*Vibrio*

The environmental conditions in the Mandovi Estuary at Campal, Divar, and Volvoi play a crucial role in shaping the abundance of various bacterial species, including *Streptococcus faecalis*, *Campylobacter* species, *Salmonella* species, *Shigella* species, and *Vibrio* species. *Streptococcus faecalis* tends to thrive in areas with higher temperature and salinity levels (weak positive correlation with temperature and salinity, Table 4.5).

Conversely, *Campylobacter* species show a moderate increase in abundance with elevated salinity levels ($n= 9$, $r= -0.63$, $p < 0.05$) and a negative correlation with dissolved oxygen levels.

*Salmonella* species exhibit weak correlations with temperature and salinity, as well as dissolved oxygen (Table 4.5).

*Shigella*species display a slight increase with elevated temperature and salinity levels but also exhibit a weak positive correlation with dissolved oxygen.

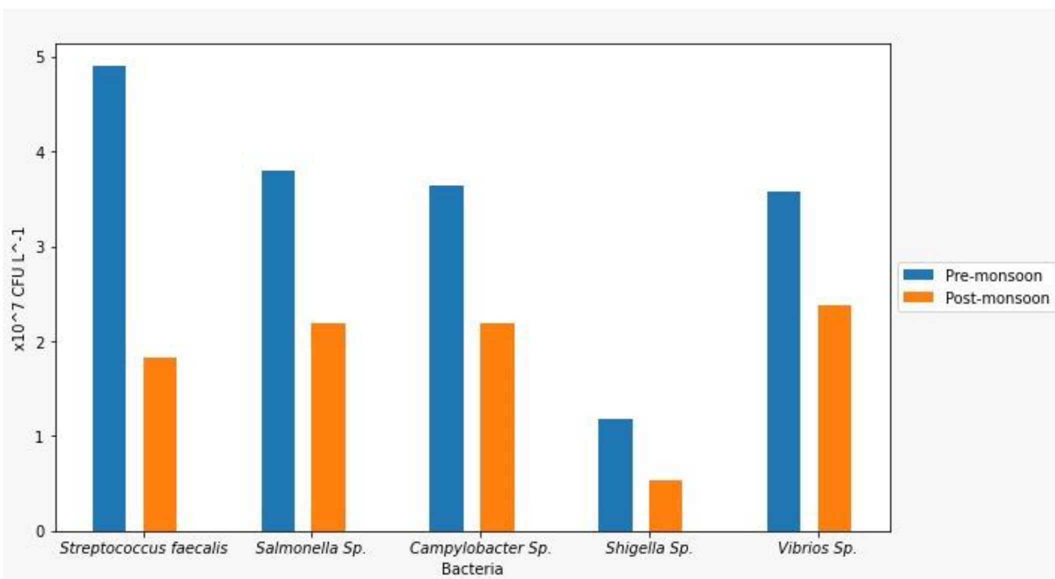
In contrast, *Vibrio* species thrive in higher salinity conditions ($n=9$, $r= 0.85$, $p < 0.01$) but decline with increasing temperatures (moderate negative correlation with temperature) and dissolved oxygen levels (strong negative correlation with dissolved oxygen). The study highlights a significant positive correlation between *Vibrio* species and salinity during the pre-monsoon period ($n=9$, $r=0.85$, $p < 0.01$), indicating favorable growth conditions. Conversely, an inverse relationship was observed between *Vibrio* species and dissolved oxygen levels during the pre-monsoon period ($n=9$, $r=0.71$, $p < 0.05$), possibly due to untreated sewage discharge and recreational activities in the estuary.

These findings underscore the intricate relationship between environmental variables and bacterial abundance in estuarine ecosystems, where factors like organic pollution and nutrient availability significantly impact microbial communities.

# CHAPTER 5: DISCUSSION

Fecal pollution indicator bacteria such as TC, EC, TS are routinely studied to gauge the prevalence of human pathogenic bacteria (APHA, 1980; Bordner and Winter, 1978). It's widely understood that higher counts of these organisms are linked to a greater incidence of human pathogenic bacterial species. While my selection of sampling locations aimed to capture the varying pollution levels in the area, I found significant reduction or seasonal differences in the numbers of pathogenic bacteria from high- to low-pollution zones. These observations over different seasons provide insights into the annual variations of both indicator (total coliforms) and many human pathogenic bacteria. This suggests that the waters of the Mandovi estuary may be unsuitablethroughout the year.

**Fig.5.1**:- Pathogenic bacteria in water samples collected during different seasons.



Pathogens such as *Vibrio cholera* (causing cholera), *V. parahaemolyticus* (causing gastroenteritis), *Salmonella* spp. (causing food poisoning), *Shigella spp.* (causing dysentery), *Streptococcus* spp. (associated with meningitis and skin infections), *Campylobacter* spp. (causing campylobacteriosis), and *aeromonads* (associated with septicaemic conditions) are potent human pathogens. Despite varying pollution levels within the Mandovi River, these pathogens were found abundantly across most locations. Characterizing different pathogens biochemically can be a laborious process, leading to uncertainties in speciation. Therefore, I utilized a combination of highly reliable specific media to quantify similar organisms.

The continued discharge of sewage and other pollutants into the Mandovi estuary introduces fresh stocks of coliforms and numerous other pathogenic bacteria. Many of these bacteria can proliferate substantially in the adjacent creeks. My study on different pathogenic bacterial groups provides valuable insights into wastewater management. For example, the Campal region experiences higher sewage discharge from sewage discharge points and other anthropogenic sources, resulting in a higher abundance of pathogens observed there compared to Divar and Volvoi. Additionally, *Streptococcus faecalis* was observed to be more abundant at Volvoi, with a concentration of $43.12 \times 10^6$ mL $L^{-1}$, compared to other species.

In aquatic environments, pathogenic bacteria associate with living organisms such as amoebae, shellfish larvae, and dinoflagellate cysts. Their attachment to the exoskeletons of copepods, other plankton, and seaweeds, as well as their ability to survive unattached in ballast water, significantly contribute to their survival and distribution in marine environments. Several studies have examined the distribution of human pathogenic bacteria and viruses in coastal waters to understand their relationship with environmental factors.It's Important to note that ships deballasting into these already compromised waters may introduce additional pathogens. Microfaunal and macrofaunal invasions pose imminent threats to natural ecosystems, possibly outweighing the risks posed by pathogenic bacteria. Therefore, ballasting in the presence of high bacterial loads should be avoided whenever possible.

# CHAPTER 6: CONCLUSIONS

Our investigation into the physico-chemical parameters and abundance of pathogenic bacteria in the Mandovi River has revealed significant seasonal and spatial variations, underscoring the urgent need for comprehensive management strategies to mitigate bacterial contamination and protect public health and aquatic ecosystems. The presence of unregulated sewage discharge points, particularly at St. Inez Creek and Rua de Ourem Creek, poses a serious threat to water quality, consistently exceeding permissible limits for total coliform levels throughout the year. Moreover, the higher concentrations of pathogenic bacteria during the pre-monsoon season, including *Streptococcus faecalis*, *Campylobacter* species, *Vibrio*-like organisms, *Salmonella* species, and *Shigella* species, highlight the influence of warmer temperatures and lower salinity levels on contamination levels.

Among all the stations analyzed, Campal emerges as a hotspot for elevated pathogenic bacteria abundance compared to other areas, due to factors like high sewage discharge, urbanization, and inadequate treatment infrastructure.This underscores the urgent need for targeted interventions in high-risk zones to prevent further deterioration of water quality. To address these challenges effectively, it is imperative to implement improved sewage treatment measures and enhance water quality monitoring programs. Additionally, further research is essential to gain a deeper understanding of the ecological drivers influencing bacterial abundance and distribution in the Mandovi River.

Moving forward, ongoing monitoring efforts and proactive management interventions are crucial to ensure the long-term sustainability of the Mandovi River and its surrounding communities. By implementing comprehensive management strategies, such as improved sewage treatment measures and enhanced monitoring programs, we can work towards mitigating bacterial contamination and safeguarding public health and aquatic ecosystems. It is imperative that stakeholders collaborate to develop and implement sustainable solutions to protect this vital natural resource for future generations.

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