Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa

A Dissertation for

Course code and Course Title: ENV 651 & Discipline Specific Dissertation

Credits: 16

Submitted in partial fulfillment of Master's Degree

M.Sc. in Environmental Sciences

by

AYESHA RAMDAS KARMALI

Seat number: 22PO580003

ABC ID: 333-542-289-520

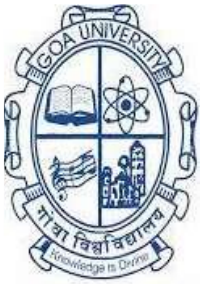
PRN: 201701777

Under the Supervision of

DR. CHANDA BERDE

Marine Microbiology

School of Earth, Ocean and Atmospheric Sciences



GOA UNIVERSITY

April 2024

Examined by: Seal of the School

Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa

A Dissertation for

Course code and Course Title: ENV 651 & Discipline Specific Dissertation

Credits: 16

Submitted in partial fulfillment of Master's Degree

M.Sc. in Environmental Sciences

by

AYESHA RAMDAS KARMALI

Seat number: 22PO580003

ABC ID: 333-542-289-520

PRN: 201701777

Under the Supervision of

DR. CHANDA BERDE

Marine Microbiology

School of Earth, Ocean and Atmospheric Sciences

GOA UNIVERSITY

April 2024

SOAS, GOA UNIVERSITY TALEIGAO PLATEAU GOA

Examined by:

Seal of the School

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa" is based on the results of investigations carried out by me in the Environmental Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the supervision of Dr. Chanda Berde 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 not be responsible for the correctness of observations / experimental or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation to the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Ayesha Ramdas Karmali

Seat No.: 22PO580003

M.Sc. Environmental Sciences

School of Earth, Ocean and Atmospheric Sciences

Date: 23/4/2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa**" is a bonafide work carried out by **Ms. Ayesha Ramdas Karmali** under my supervision in partial fulfillment of the requirements for the award of the degree of **Master of Science** in the Discipline Environmental Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

Date: 25/09/2024

chanda

Dr. Chanda Berde
Marine Microbiology
School of Earth, Ocean and Atmospheric Sciences

31/7/24

Sr. Prof. Sanjeev C. Ghadi
School of Earth, Ocean and Atmospheric Sciences
Date:
Place: Goa University

School Stamp

PREFACE

The research carried out for the dissertation titled "**Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa**" is centered on understanding the biodiversity associated with mangrove ecosystems along the Mandovi estuary in Goa in terms of the flora and fauna and the influences of physicochemical parameters. The study articulates a detailed analysis of physicochemical parameters, including temperature, salinity, and BOD, in estuarine water. By understanding the intricate relationship between these physicochemical parameters and water quality, an essential comprehension of the overall ecosystem health can be illustrated. Through the documentation of mangrove flora diversity across various sampling sites along the Mandovi estuary, the influence of water conditions on flora and associated fauna life can be examined. The documentation of associated macrofauna (avifauna, aquatic fauna, and terrestrial invertebrate species) and microflora (bacteria) within different sampling sites establishes a focus on their dependence on water ecosystems. Consequently, statistical correlations can provide a further understanding of the influence of physicochemical parameters, specifically water-related factors, on species richness and diversity.

The strategies employed in the observation and documentation of species diversity (Chapter 3), species observed (Chapter 4), and analysis of species diversity using the Shannon-Wiener Index and species richness using the Margalef Index (Chapter 4). The study highlights the influence of physicochemical parameters mainly salinity, temperature, and BOD, and its influence on biodiversity richness within these mangrove ecosystems (Chapter 3 and Chapter 4). The research methodology comprises

a holistic approach, considering the complex interplay between water and the ecosystem. It thus involves field studies, laboratory experimentation, and data analysis, all geared towards unraveling the intricate relationship within the mangrove ecosystems and the role of water in shaping them.

ACKNOWLEDGEMENT

In the successful accomplishment of this dissertation project, I would like to extend my earnest appreciation to all those who have helped me in the completion of my dissertation project titled "**Studies on biodiversity associated with the mangrove ecosystems along the Mandovi estuary, Goa**".

Firstly, I would like to express my sincere gratitude towards our current Dean Sr. Prof. Sanjeev C. Ghadi, and previous Dean Sr. Prof. C.U. Rivonker, for allowing me to carry out my dissertation and availing all facilities of the School. I am profoundly grateful to my guide, Dr. Chanda Berde, Marine Microbiology Program, School of Earth, Ocean, and Atmospheric Sciences, Goa University for her valuable guidance, immense encouragement, support, as well as her determined effort during the course of my dissertation.

I thank Dr. Varada Damare, Program Director, Marine Microbiology, for providing all the requirements for my dissertation work. Furthermore, I would like to express my sincere thanks and appreciation to my parents for their constant support and encouragement throughout the completion of this work and a sincere thanks to my friends for all their support too. I would also like to express my earnest gratitude towards Mr. Uday Mandrekar "Birdman of Goa" for his guidance on the avifauna coverage in the mangrove ecosystems along the Mandovi estuary. I would also like to express my sense of appreciation to the laboratory staff members for providing all the essential requirements in order to facilitate the successful completion of this project.

Ayesha R. Karmali

TABLES

TABLES

TABLES

TABLES

FIGURES

FIGURES

FIGURES

FIGURES

FIGURES

ABBREVIATIONS USED

Entity	Abbreviation
Biological Oxygen Demand	BOD
Colony Forming Units per Millilitres	CFU/mL
Concentrated	Conc.
Degree Centigrade	°C
Deoxyribonucleic acid	DNA
Distilled Water	D.W
Grams	g
Hectares	ha
Kilometers	km
Litres	L
Microlitres	µL
Milliliters	mL
Milligrams per litre	mg/L
Not Detected	ND
Parts per thousand	‰ or ppt
Potential of Hydrogen	pH
Room Temperature	RT
Seconds	Sec.
Species	Sp.
Volume	Vol
World Health Organization	WHO

ABSTRACT

The present study focuses on the documentation of the current status of biodiversity in terms of the mangrove flora and the associated macrofauna and microflora observed within the mangrove ecosystem sites along the Mandovi estuary in Goa. The primary aim of this study is to estimate the influence of physicochemical parameters, mainly, temperature, salinity, and BOD, and analyze its correlation with species richness of the documented biodiversity coverage using biostatistics. The six selected sampling sites estimated for the physicochemical characteristics as well as the documentation of biodiversity were Vagurbem, Saint Estevam Island, Divar Island, Chorao Island, Penhe de Franca-Britona, and Ponte de Linhares Causeway-Ribandar for the given study. The biodiversity in terms of the mangrove flora was documented and preserved using herbariums of the plant samples collected. The estuarine water samples were collected for the microflora estimation wherein bacterial colonies were isolated, quantified, and characterized morphologically by the Gram staining technique. The macrofauna associated with the mangrove ecosystems in terms of the avifauna species, aquatic fauna species, and terrestrial invertebrate species were observed, identified, and documented during each sampling visit. Further, correlation studies were utilized to estimate the influence of salinity and temperature on the total macrofauna species during the Monsoon and Post-monsoon seasons. Correlation studies were also utilized to assess the linkage between the influence of BOD on the bacterial colony count per site. Furthermore, the species diversity and species richness in terms of its flora and fauna species were documented and analyzed using the Shannon-Wiener Diversity Index and Margalef Index respectively. The outcome of this study aims to prove a correlative linkage between the influence of physicochemical parameters as a result of

climatic seasonal variations on the mangrove ecosystem biodiversity and the role of water in shaping these ecosystems.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Global marine ecosystems ranging from mangroves, seagrass beds, and coral reef ecosystems operate as a unifying network that monitors the health of coastal zones. Mangrove trees can trap sediments and pollutants that would otherwise flow out to sea while the seagrass beds provide a further barrier to mud and silt deposits that could otherwise smother the coral reefs. In return, the coral reefs protect the seagrass beds and mangroves from the impact of strong and turbulent ocean waves. Additionally, mangrove trees have the unique capacity to extract freshwater from the surrounding seawater source with the survival of many mangrove species like Red Mangroves dependent upon the filtration of approximately 90% of the salt found in seawater as it enters their roots while River Mangrove species can excrete out salts through glands in their leaves and bark as an exceptional means of adaptation (Kim et al.,2016). It is thus evident that without the presence of mangroves, these incredibly productive and intricate ecosystems would collapse.

Mangrove ecosystems constitute exclusive, highly productive coastal and intertidal estuarine habitats found in the tropical and sub-tropical regions around the world. They are characterized by the presence of mangrove trees, which are specialized halophytes adapted to thrive in the intertidal zones where the land and sea converge. The term ‘mangrove’ comes from the Portuguese word 'mangue' which describes a type of tree, while the term 'grove' refers to a group of trees. Thus, mangroves may refer to the habitat of trees and shrubs as a whole present in the mangrove swamp with their growth prominent in dense forests along tidal estuaries, salt marshes, and muddy coastal

habitats. Mangrove trees belong to a diverse group of plant species that have evolved to tolerate and adapt to high salinity, limited freshwater, strong tides, and muddy, oxygen-poor, loose substratum soils. These trees have several adaptations that enable them to survive in these challenging conditions. Diverse root adaptations such as pneumatophores are equipped with specialized functions that allow the uptake of oxygen from the atmosphere when the soil is waterlogged while stilt roots and prop roots serve as mechanical support in the marshy land substrate.

Mangrove forests provide numerous ecological benefits and play a critical role in coastal and marine ecosystems by serving as buffer zones, protecting the shoreline from erosion, storm damage, and cyclonic impacts through the reduction of wave force and by stabilizing the sediments. The intricate root systems of mangroves also trap and accumulate organic matter, creating a unique habitat for various organisms and providing essential habitats for thousands of terrestrial and aquatic species of fauna and a rich diversity of microbial life ranging from bacteria, fungi, actinomycetes, and plankton species. These ecosystems form supportive nursing and feeding grounds for numerous fish, crustaceans, and other marine invertebrate species. Many commercially important fish species rely on mangroves for their early life stages, making these ecosystems crucial for fisheries' productivity. Furthermore, mangroves provide a habitat for numerous species of birds, reptiles, amphibians, and mammals, including endangered species like the Bengal tiger, Eastern Indigo Snake, Brown Pelican, and the West Indian manatee.

One of the most scientifically evident features of mangrove trees is their vital ability to act as effective carbon sinks that sequester large amounts of atmospheric

carbon dioxide with approximately five times higher carbon sequestered in its organic form in comparison to the terrestrial upland forests. The organic matter accumulated in the soil in combination with the slow decomposition rate in waterlogged conditions allows mangroves to store carbon for long periods, making them significant agents at the forefront of climate change mitigation. Unfortunately, mangrove ecosystems face frequent threats due to human activities with deforestation and unsustainable land-use patterns mainly arising from aquaculture, agriculture, and coastal developmental needs as significant causes of concern. Pollution stemming from industrial activities, oil spills, landfills, water diversion, and improper municipal and sewage waste disposal also pose risks to these delicate and intricate ecosystems. Climate change, including rising sea levels and increased frequency of extreme weather events, further exacerbate the challenges faced by mangroves. Efforts are underway globally to conserve and restore mangrove ecosystems with conservation initiatives focused on protecting existing mangrove forests, establishing marine protected areas, and raising awareness about their ecological importance. Restoration projects aim to replant and rehabilitate degraded areas, helping to recover and expand mangrove habitats. Overall, mangrove ecosystems are vital for coastal protection, biodiversity conservation, fisheries productivity, and climate change mitigation. By recognizing their inherent value and implementing sustainable management practices, the long-term survival and health of these remarkable coastal ecosystems can be achieved.

1.1.1 Distribution of mangroves in India

According to UNESCO World Heritage Convention (2010) reports, satellite analysis by the US National Aeronautics and Space Administration and the US Geological

Survey, the global mangrove coverage is 1,37,760 km^2 in approximately 123 countries globally. This is approximately 12.3% lower than previous estimates and is shrinking (UNESCO World Heritage Convention, 2010). The greatest coverage was observed within 5° from the equator. In India, the current estimates suggest that the mangrove cover stands at 4,975 km^2, which constitutes 0.15% of the country's total geographical area (Global Mangrove Alliance, 2021).

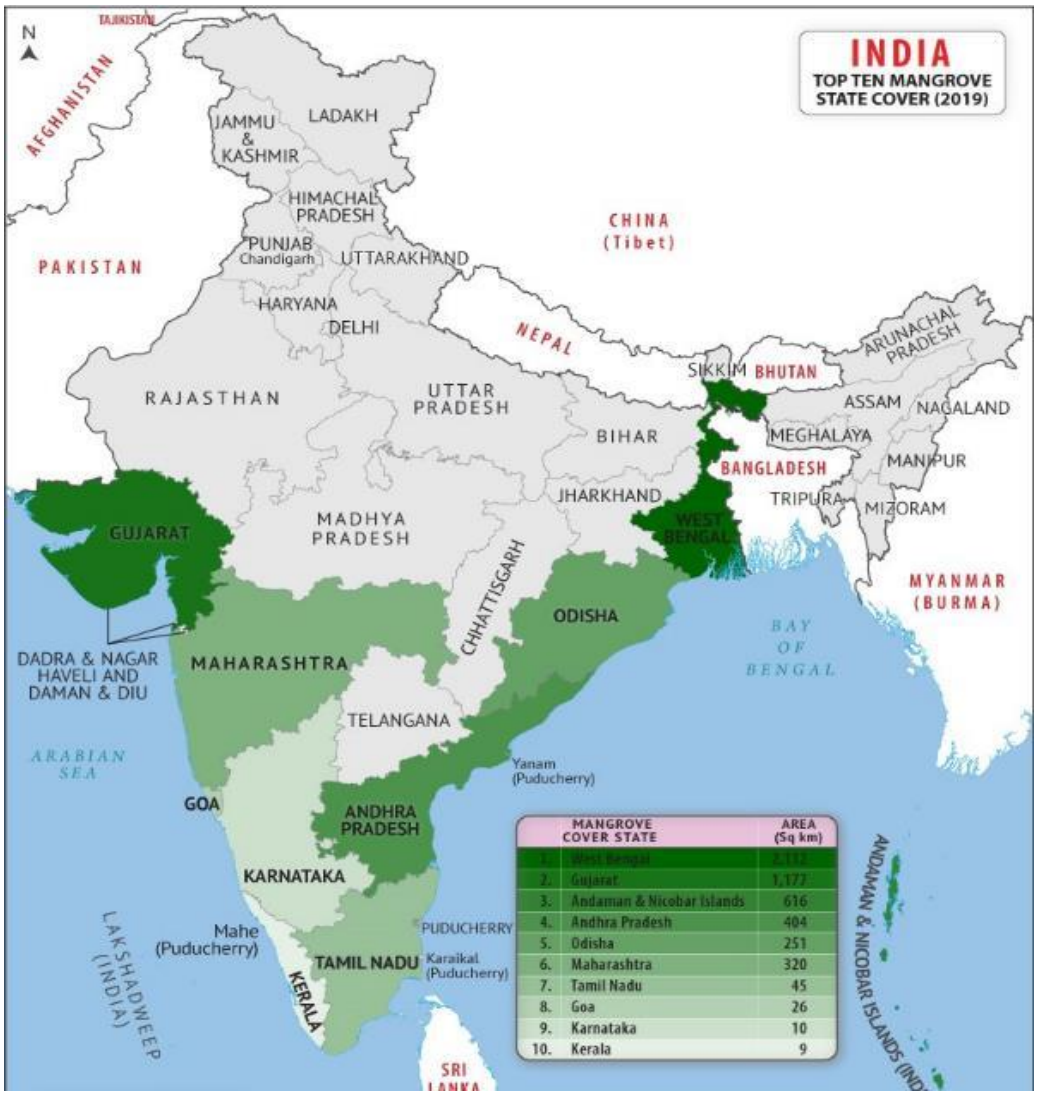


Figure 1.1: Geographical map depicting mangrove coverage in India

(Source: DrishtiIAS, 2021)

1.1.2 Distribution of mangroves in Goa

The state of Goa is located along the central western coast of India with a geographical area coverage of 3,702 km^2 and an altitude ranging from sea level to about 1,022 meters. Goa has an extended coastline of about 101 km and comprises seven major estuaries namely Mandovi, Zuari, Terekhol, Chapora, Talpona, Sal, and Galgibag. These estuarines originate from the Sahyadri ranges of the Western Ghats and flow westward forming an intersection with the Arabian Sea (Goa State Biodiversity Board, 2024).

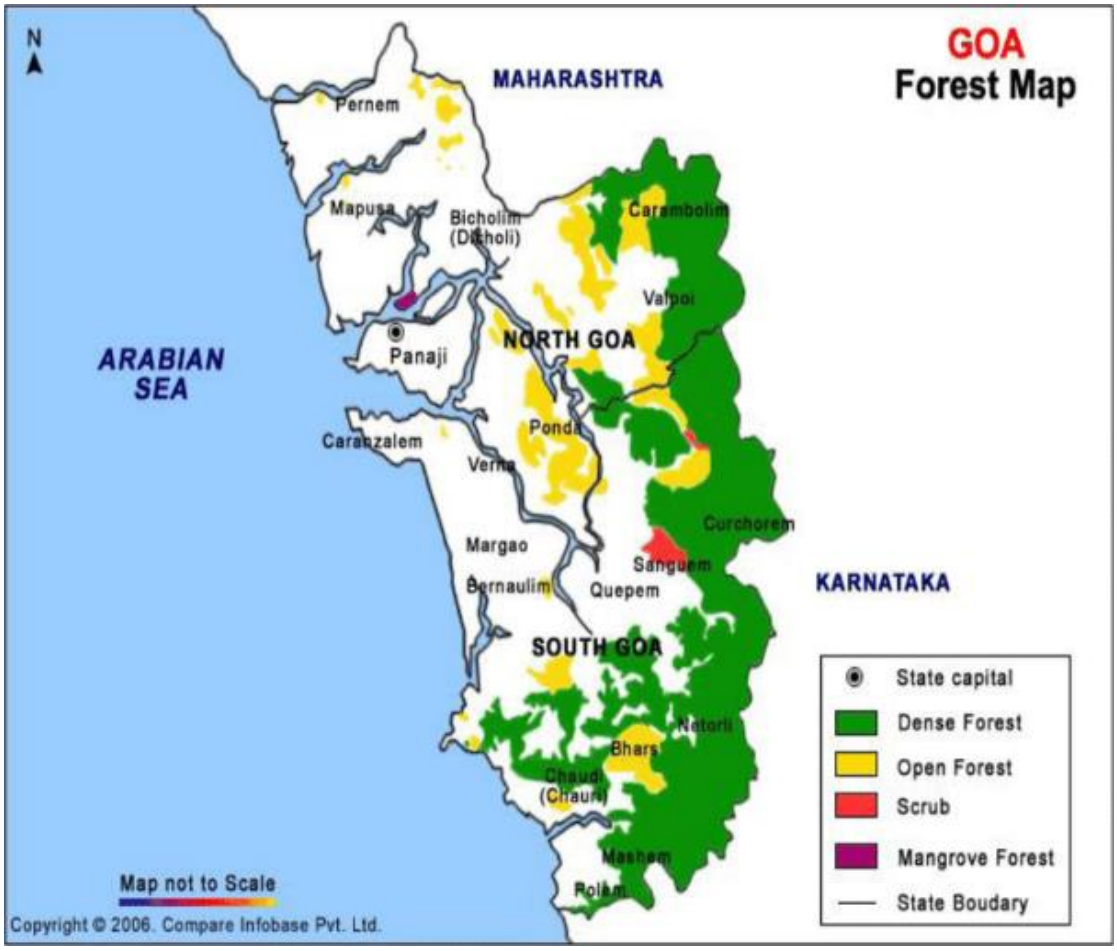


Figure 1.2: Geographical map depicting mangrove coverage in Goa

(Source: Oliveira et al., 2020)

Goa has been observed to house 16 major mangrove species and is known to be one of the best mangrove forests in the country with each estuary encapsulating the following coverage of mangrove habitat areas.

Table 1.1: Estimated mangrove area coverage in Goa

(Source: Kothari and Rao, 2002)

Estuaries/connecting canals and Patches	Estuarine Areas	Mangrove Areas	Percentage
1. Terekhol Estuary	349	30	8.6
2. Chapora Estuary	711	100	14.0
3. Mandovi Estuary	5564	700	12.6
4. Zuari Estuary	5790	900	15.5
5. Cumbarjua Canal (Connect Mandovi and Zuari Estuaries)	375	200	53.3
6. Sal / Estuary	302	30	9.9
7. Talpona Estuary	40	20	50.0
8. Galgibag Estuary	26	20	76.9
Total on Goa Territory	**13157**	**2000**	**15.2**

The riverine area in Goa is approximately 13000 ha. The extent of Khazan lands inundated by backwaters in Goa measures to approximately 18500 ha. Out of this, an estimated 14500 ha is for paddy cultivation while the remainder 4000 ha of land is fallow. The mangrove coverage found in these intertidal lands was reported to be 2000 ha of its total area coverage within the state of Goa (Kothari and Rao, 2002).

Table 1.2: Description of mangrove species coverage reported in Goa

(Source: Forest Department – Government of Goa, 2024)

Sr. No.	Species	Salinity gradient	Likely substratum
1.	*Rhizophora mucronata*	6% to 37%	Silt, sand & soil.
2.	*Rhizophora apiculata*	6% to 37%	Silt, Sand & soil
3.	*Bruguiera gymnorrhiza*	6% to 33%	Silt & sand
4.	*Bruguiera cylindrica*	11% to 35%	Silt & more sand
5.	*Ceriops tagal*	10% to 37%	Silty soil
6.	*Kandelia candel*	6% to 26%	Silty soil
7.	*Lumnizera racemosa*	6% to 30%	Silt, sand & silty soil
8.	*Avicennia marina*	6% to 40%	Silt, sand & silty soil
9.	*Avicennia alba*	7% to 35%	Silt.
10.	*Sonneratia alba*	10% to 37%	Silt & sand
11.	*Sonneratia caseolaris*	5% to 22%	Silt
12.	*Aegiceras corniculatum*	11% to 35%	Silt & soil
13.	*Acanthus illicifolius*	11% to 39%	Silt & soil
14.	*Excoecaria agallocha*	9% to 35%	Silt & more soil
15.	*Derris heterophylla*	5% to 30%	Silt & soil
16.	*Acrosticum aurum*	0% to 20%	Silt & soil

1.1.3 Biodiversity associated with mangrove ecosystems in Goa

Biodiversity generally defines the different levels of versatility that exist at the genetic level, species level, and ecosystem level. The component of biodiversity within tropical estuaries refers to the high diversity of species, habitats, food web links, and the diverse pathways of nutrient cycling and energy flow between the terrestrial and marine ecosystems that comprise the land-sea interface. The mangrove ecosystems are unique and wide-ranging with their richness in biodiversity with respect to the structural niches, breeding, feeding, and protection grounds of numerous vertebrate and invertebrate species associated with the mangrove habitats. A dominant ecological function of mangroves is the maintenance of near-shore marine habitats thus resulting in tremendously high primary and secondary productivity of tropical estuaries. Mangrove forest ecosystems support a total of 4011 species of flora and fauna home to 920 floral species and 3091 faunal species diversity (Kathiresan, 2010). Feeding, breeding, nesting, refuge, and nursing grounds to birds, fish, molluscs, crustaceans, mammals, shrimp, reptiles, amphibians and micro-organisms

The following evidence suggests the positive impact of biodiversity on the mangrove ecosystem:

1. **Species richness and diversity:** The presence of different species plays unique roles in the ecosystems' contributing to nutrient cycling, habitat formation, and providing food sources for other organisms.
2. **Habitat creation:** With the introduction of diverse plant species within the mangroves, a complex and structurally diverse habitat is created that helps

provide a wider range of niches and microhabitats for various organisms, such as birds, reptiles, crustaceans, and fish.

3. **Enhanced ecosystem services:** The diverse mangrove ecosystems offer enhanced ecosystem services, with a greater diversity of plant species that improve soil stability, erosion control, water quality regulation, enhance carbon sequestration, and act as a buffer against storm surges and coastal erosion.
4. **Food web dynamics:** Species diversity can strengthen the food web within mangrove ecosystems as different organisms occupy different trophic levels, ranging from primary producers (e.g., mangrove trees) to consumers (e.g., insects, crabs, fish, birds) forming diverse food webs that promote stability and resilience, ensuring that these ecosystems can withstand disturbances and changes in environmental conditions.
5. **Ecotourism and education:** Increased biodiversity can attract ecotourism activities, opportunities to observe a greater variety of flora and fauna as well as provide economic benefits to local communities through incentivizing conservation and protection of mangrove ecosystems. This can also provide educational opportunities for researchers, students, and the public to learn about the importance of biodiversity and ecosystem conservation.

a. *Floral biodiversity*

Mangrove vegetation majorly dominates the intertidal zones of tropical deltas, lagoons, and estuarine coastal systems essentially receiving significant terrigenous sediment inputs. The Coexisting Mangrove-Coral habitats are vital home grounds for other marine species and help maintain ocean health and coral protection as a refuge against

climate change impacts that include ocean acidification and thermal stress. About 56% of the world's mangrove species occur in India with 30 tree species, 18 herb species, 6 climber species, 4 grass species, and 4 epiphyte species. The prominent mangrove species include *Avicennia officinalis* & *Avicennia alba* (60%), *Rhizophora mucronata* and *Rhizophora apiculata* (30%), and *Acanthus illicifolius* & *Derris heterophylla* (10%). In India, the species diversity is highest in Orissa (101 species) followed by West Bengal (92 species), Andaman and Nicobar Islands (91 species), and Gujarat (40 species) (Goa Forest Department, 2021).

The mangrove habitats have three distinctive horizontal zones, sorted out according to their adaptability to saline water as unique zonations. They comprise special characteristics at each of the zones. Mangroves exhibit several different types of mechanisms for coping with highly saline conditions (0-90ppt) and oxygen-depleted soil (Goa Forest Department, 2021). All mangrove trees possess lenticular roots that consist of small pores that absorb oxygen that moves downwards through the spongy air passage of the aerenchyma tissue preventing salty water from entering the root cells. Mangroves can excrete excess salts through pores or salt glands located on the leaf surfaces. The old leaves storing excess salts subsequently fall off. Mangroves also produce a large number of viviparous seeds that are dispersed by water and can float for a retention period of a year in water till the right mud substratum is found to settle into for further propagation. Based on the zonation of mangroves, they can be classified as follows:

(a) ***Red Mangroves***: These commonly include species such as *Rhizophora* which are predominant near the water's edge growing seawards essentially possessing

stilt roots or prop roots that absorb oxygen, filter saline water, and protect against strong winds. The bark texture is square and smooth and the older leaves store excess salt and eventually fall off. Red mangroves produce elongated seeds that germinate by sprouting leaves and roots on the tree itself (viviparous seeds).

(b) ***Black Mangroves***: These commonly include species such as *Avicennia* which possess pneumatophores or breathing roots that are effective in oxygen absorption with a lesser capacity of filtering saline water. Predominantly present behind the red mangroves along the high-tide shore, consist of a small pebbly bark texture of black mangroves produce seeds that have a high rate of survival for over a year due to the ability to store large amounts of food reserved within the seeds. The salt glands present in black mangrove trees are located on the underside of the leaves to aid in the excretion of excess salts.

(c) ***White Mangroves***: These commonly include species such as *Ceriops* and *Bruguiera* presently found upland behind the red mangroves and black mangroves found in close association with Buttonwood mangroves. The bark texture is pale, columnar, and smooth. White mangroves crucially possess knee roots due to the lesser frequency of dealing with saline water. The leaves consist of two sugar glands called nectaries located at the base of the leaf that help regulate the salt content while the seeds possess an evident storage of abundant food reserves that allow for its increased survival rate for more than a year on average.

Herbariums are utilized to preserve the flora specimen for further reference. The herbarium refers to the collection of dried plant specimens that are arranged in terms of

a distinct classification based on their family, genus, and species, and preserved for future reference. The invention of the herbarium is credited to Luca Ghini, an Italian Physician and Botanist, in the early 16th century. The Herbarium serves as a quick referral to taxonomic studies. The preparation of a herbarium takes approximately 5 to 7 days on average. Initially, the plant sample is carefully collected and compressed between two flat surfaces and kept at a safe place for the specified 5 to 7-day period. Subsequently, the plant sample is carefully removed and placed onto an A4 size sheet of white paper, followed by which the plant specimen is placed and labeled precisely.

Advantages of Herbarium:

- Herbariums represent one of the best records of a plant's original distribution. This information can be further utilized to understand changes due to habitat loss, climate change, or other anthropogenic impacts.
- Herbariums provide detailed scientific information about plants for research and exhibition.
- Herbariums are a source of plant DNA for use in taxonomy and molecular systematics.
- Herbariums provide safety to important specimens and can be utilized to identify as well as classify unknown plant species.
- Herbariums serve as an educational tool for the public.
- Herbariums also offer several benefits to society by providing data or reference materials for critical endeavors in the fields of Agriculture, Biosecurity, Forensics, Control of invasive species, Conservation biology, Natural resources, Land Management, and Human health.

b. ***Faunal biodiversity***

Category 1: Microorganisms

- These primarily include fungi, free-living bacteria, actinomycetes, and yeast species.
- Biotechnology applications of microbial analysis include enzymatic, pigment, antitumor agents, immunosuppressants, immune modifiers, vitamins, bio-emulsifiers, and bioplastic applications.
- Mangrove-associated- microorganisms provide crucial significance in the formation of detritus in mangrove ecosystems.
- Bacterial and fungal species aid as nitrogen fixers, cellulose decomposers, phosphate solubilizers, nitrifiers and denitrifiers, iron oxidizers and iron reducers, and sulfur oxidizers in mangrove ecosystems.
- Mangrove-associated fungal species help decompose vegetative material.
- Mangrove-associated bacterial and yeast species colonize and carry out further organic material decomposition.

Category 2: Algae

- Algae plays a pivotal role in the maintenance of food web in mangrove ecosystems.
- Further classified into microalgal and macroalgal communities
- Microalgae include epiphytes that grow within the sediments and aerial roots of mangrove trees.
- Examples of microalgae commonly found include Diatoms and Cyanobacteria (blue-green algae)

- Diatoms comprise 73% of the total microalgal composition and include genera namely, *Gyrosigma, Navicula, Cyclotella, Nitzschia, Flagilaria*, and *Coscinodiscus* (Goa Forest Department, 2021).
- Cyanobacteria comprise photosynthetic prokaryotes (unicellular colonial or filamentous) that provide a crucial source of nitrogen to mangrove ecosystems with a high capacity of nitrogen fixation for future reforestation and rehabilitation of degraded mangroves.
- Division *Cyanophyta* (blue-green algae) comprise 17% of the microalgal population recorded 5 genera under this division with *Anabena* and *Oscillatoria* as crucial indicators of the health status of aquatic ecosystems with the estimation of eutrophication rate in polluted waters (Goa Forest Department, 2021).

Category 3: Seaweeds

- These include macroalgal communities that are specialized plant epiphytes on stems and roots of mangrove trees or have been associated with their growth on other substratum in mangrove ecosystems.
- Main food source for a variety of fish and invertebrate species like the genus *Bostrychia* in association with *Catenella* and *Caloglossa* found in mangrove habitats on the west coast of India, especially with *Bruigiera gymnorhiza* species most dominant association (Goa Forest Department, 2021).
- Biomass and habitat diversity are critical indicators for understanding ecosystem health.

- Red algae include genera *Hypnea, Laurencia, Polysiphonia* ,Green algae include genera *Enteromorpha, Cladophora, Rhizoclomium, Caulerpa*, Brown algae include genera *Ectocarpus, Padina, Hydroclathratus* (Goa Forest Department, 2021).

Category 4: Seagrasses

- These include flowering plants (angiosperms) submerged in saltwater habitats that resemble terrestrial grasses.
- Seagrass beds are crucial food sources and shelter grounds for crabs and fish, improve water quality by slowing down wave currents allowing sand particulate matter to settle down, and dense roots help shoreline stabilization.
- Patch distribution of seagrass beds include *Halophila beccari* in mudflats of Mandovi, Zuari, Chapora, and Terekhol estuaries, as well as *Halophila ovalis* growth in sub-littoral swamps of Chapora and Mandovi estuary (Goa Forest Department, 2021) .

Category 5: Zooplanktons

- These include 12 groups comprising 52 species majorly, Copepods (17 species), Protozoa (5 species), *Coelentrata* and *Cladocera* (2 species each), as well as Ctenophore (1 species).
- Variations in distribution and abundance patterns are determined by onset and prolonged southwest monsoons that cause hydrographical changes in mangrove ecosystems with high densities observed even in relatively low-nutrient conditions.

Category 6: Benthic invertebrate animals

- These include communities of organisms thriving in, on or near the seabed, mainly, filter feeders and deposit feeders.
- Filter feeders include Bivalves and Sponges that siphon particles from the water. Deposit feeders include Molluscs and Shrimp that ingest and sift sediments in water and consume organic matter from it.
- Critical for ecosystem functioning and food web maintenance by re-mineralization of nutrients in water column
- The 76 invertebrate taxa recorded in Goa include 22 crustaceans, 7 amphipods, 3 barnacles, 6 ploycheates, and 35 molluscs (21 gastropods and 16 bivalves) (Goa Forest Department, 2021).

Sub-category 6A: Crab species

- These include 6 families of 127 species found worldwide (Goa Forest Department, 2021).
- Among Brachyuran crabs documented consist of 12 genera, 5 families, and 16 species with *Portunidae* and *Ocipodidae* found to be the most dominant (Goa Forest Department, 2021) .
- Major quantities obtained commonly include *Thalamitta crenata* and *Portunus anguinolentus* (Goa Forest Department, 2021).
- Crab species commonly described as keystone species, contribute to higher biodiversity as they start their life cycle in mangrove ecosystems and crucially process leaf litter by consumption thereby decreasing sulphide levels in soils thus positively influencing soil and tree productivity.

- Other predominant crab species associated with mangrove habitats include *Uca* sp. (fiddler crab), *Thalassina anomala* (mud lobster), *Scylla serrata* (swimming crab), *Sesarma* sp., *Aratus pisonii* (Mangrove tree crab) and *Limulus polyphemus* (Horseshoe crab) (Goa Forest Department, 2021).

Sub-category 6B: Prawns (Shrimp)

- Freshwater prawns mainly include *Macrobrachium rosenbergii* while marine paneid prawns include *Metapeneaus brevicornis, Penaeus indicus, Paneaus monodon,* and *Penaeus merguiensis* (Goa Forest Department, 2021) .
- The biological life cycle dependency of shrimp species in mangrove ecosystems includes offshore spawning, inshore larval migration, estuarine juvenile stage, and offshore breeding.

Sub-category 6C: Molluscs

- Bivalves and oysters have been observed to encrust the pneumatophores and prop roots on mangrove tree species during the high tide levels.
- Barnacles and mussels are found to compete with oysters for space on roots.
- Gastropods are crucial for the turnover of organic material.
- Molluscs provide a source of high nutrition to predators with shellfish as an important

  protein source for coastal dwellers
- *Meretrix casta, Villorita crypinoides, Polymesoda erosa* are evidently present along the Mandovi-Cumbharjua Canal-Zuari estuarine system (Goa Forest Department, 2021).

Category 7: Fish

- Mainly comprised of marine, freshwater, estuarine, and backwater species.
- Mangrove ecosystems are well-established feeding, breeding, and nursing grounds for estuarine and marine fish species.
- Over 120 fish species are found in mangrove habitats, some of which primarily include fish species like Lactes, Polynemes, Sciana, Hilsa, Mugile and Liza associated with Indian mangroves (Goa Forest Department, 2021).
- Common commercially sold fish found among mangrove ecosystems include Mullets, Snappers, Sea bass, Milkfish, and Tilapia (Goa Forest Department, 2021) .
- Mudskipper fish (*Periophthalmus sp.*), one of the most conspicuous fish endemic to mangroves live along the mud flats within the mangrove habitats and adapted with the ability to survive alternating periods of exposure to air and submergence under high tide (Goa Forest Department, 2021).

Category 8: Birds

- Mangrove forests provide secure shelter grounds for feeding and breeding to terrestrial birds and wetland birds ranging from Kingfishers, Plovers, Herons, Storks, and Raptors (Goa Forest Department, 2021) .
- Approximately 121 species of resident and migratory birds have been found in mangrove forests in India (Goa Forest Department, 2021) .
- Species of Ducks (*Dendrocygna javanica*), Egrets (*Egretta gularis, Egretta garzetta*), Kingfishers (*Halcyon smyrensis, Halcyon pilenta, Halcoyn capnesis*), Kites (*Haliastur indicus, Milvas migrans*), and Cormorants

(*Phalacrocorax niger, Phalacrocorax carbo*) have been observed in mangrove habitats feeding on fish, invertebrates and plant materials (Goa Forest Department, 2021) .

Category 9: Reptiles and Amphibians

- Reptiles commonly found within the mangrove habitats include snakes, turtles, and crocodiles.
- The freshwater crocodile (*Crocodilus palustris*) is commonly found in mangrove habitats and is well-adapted to a wide range of saline conditions due to the presence of salt glands on their tongues (Goa Forest Department, 2021).
- Species of turtles such as the Loggerhead turtle (*Caretta caretta*), Ridley Sea turtle (*Lepidochelys kempii*), Hawksbill Sea turtle (*Eretmochelys imbricata*) Green Sea turtle (*Chelonia mydas*) utilize mangrove ecosystems as juvenile nursery grounds an feeding grounds while simultaneously receiving shelter from predators (Goa Forest Department, 2021).
- Amphibians such as large lizards like Iguana (*Iguana sp.),*Garrobo *(Ctensaura similis),* and the Indian monitor lizards are commonly found within the mangrove habitats (Goa Forest Department, 2021).
- Snake species like the Dog-faced water snake (*Cerberus rynchops*), Wart snake (*Acrochordus granulatus*), and Beaked sea snake (*Enhyrina schistose*) have been reportedly found within mangrove ecosystems (Goa Forest Department, 2021).

Category 10: Terrestrial animals

- Predominant terrestrial invertebrates include species of spiders, honeybees, ants, moths, termites, scorpions, dragonflies, mites, damselflies, and butterflies (Goa Forest Department, 2021).
- Predominant terrestrial vertebrates include langurs, wild boars, cattle, sheep, goats, wild cats, macaques, wild pigs, flying foxes, spotted deers, mouse deer, leopards, tigers, otters, mongooses (Goa Forest Department, 2021).
- These terrestrial animals inhabit the mangroves for feeding, breeding, and shelter needs.

1.1.4 Threats associated with mangrove ecosystems

As per the 2019 report from The Ministry of Environment, Forests, and Climate Change (MoEFCC), the mangrove cover in India has increased by 1.10% (54 km^2) (Global Mangrove Alliance, 2021). However, evidence suggests that for the past three decades, the state of Goa witnessed a sharp decline in mangrove ecosystems with mangrove cover declining from 20,000 ha in 1987 to 2,200 ha in 2015 (Rodrigues, 2020). The present mangrove cover in India in the year 2023 is 4,992 km^2 while the latest coverage by the Indian State of Forest Report (ISFR) 2021 suggests that the mangrove cover of Goa is presently 27 km^2. The primary drivers of mangrove loss include logging, agriculture, aquaculture, coastal development, pollution, and climate change impacts. Between the years of 1980 to 2000, approximately 35% of mangrove forest cover has been declined with a staggering 150000 loss annually, this marks a four times higher loss witnessed in comparison to the overall global terrestrial forest land (Forest Survey of India, 2021).

The impacts of climate change pose a critical threat to the loss of mangrove forests due to the rise in global temperatures and abrupt changes in rainfall regimes which greatly influence global mangrove distribution and associated loss of biodiversity due to rising sea levels causing local and regional extinction. Coastal development due to increasing urbanization is a massive driver of mangrove loss and degradation due to a rising human population density along the coastal regions found to be three times higher than the global average. Logging of mangrove forests for wood sources can cause alterations in species compositions, fragmentation, and total clearance of mangrove forests. Ever-increasing instances of pollution deteriorate mangrove aerial roots depriving the oxygen supply to the mangrove trees thereby smothering and clogging the roots through sedimentation, solid wastes, and oils. Aquaculture causes more than half of the global mangrove losses mostly stemming from shrimp cultures. Agriculture through its unsustainable practices leads to the conversion of land solely for rice paddy fields resulting in 88% of mangrove losses in developing countries.

On a global level, mangrove habitat loss has led to a 40% extinction rate risk for animal species as per the International Union for Conservation of Nature (IUCN) Red List (Polidoro et al., 2010). The two primary species of mangrove trees namely, *Heriteria fomes* (endangered) and *Sonneratia groffithii* (critically endangered) existing in India are under massive threat of extinction. *Kandelia candel* has also been reported to be on the verge of extinction in India (Kathiresan, 2010). Hence understanding the parameters that influence mangrove distribution is crucial along with appropriate localized conservation approaches to restore the mangrove forest coverage along the west and east coasts of India.

1.2 AIM AND OBJECTIVES

1.2.1 Aim

To estimate the link between species richness of biodiversity within the mangrove ecosystem in relation to the physicochemical parameters along the stretch of the Mandovi estuary in Goa.

1.2.2 Objectives

(1) To estimate the physicochemical parameters in terms of the salinity, temperature (sea-surface temperature), and Biological Oxygen Demand (BOD) of the estuarine water.

(2) To identify and document the different species of mangrove flora associated with the selected mangrove ecosystem sampling sites.

(3) To identify and document the associated microflora and macrofauna within selected mangrove ecosystem sampling sites.

(4) To carry out statistical correlation between species richness of biodiversity along mangrove ecosystems due to the influence of physicochemical parameters and estimate the Shannon-Wiener Diversity Index for species diversity as well as the Margalef Index for species richness using biostatistics.

1.3 HYPOTHESIS/ RESEARCH QUESTION

To estimate the presence of a correlative linkage between species richness within the mangrove ecosystem in relation to the influence of physicochemical parameters along the stretch of the Mandovi estuary in Goa.

1.4 SCOPE

Mangrove ecosystems are unique in terms of their rich grounds of biodiversity as well as their ability to regulate the climate in terms of their carbon sequestration capabilities. The level of resilience to natural calamities and exceptional adaptations to changing climatic and environmental conditions is a testament to the need for sustainable management and conservation of these intricate natural ecosystems.

The present study carried out in the monsoon and post-monsoon seasons aims to analyze the existence of a correlative linkage between the species richness of biodiversity within the mangrove ecosystems due to the influence of physicochemical parameters in terms of the salinity and temperature of the estuarine waters due to increasing climatic variations as a result of climate change as a major influencing factor. The present study provides up-to-date coverage of the documentation of mangrove flora and macrofauna present within the mangrove ecosystem location sites along the Mandovi estuary in Goa.

The isolated bacterial microflora viable count has also been found to provide a correlative linkage to the BOD. The Gram characteristics of the bacterial microflora isolated suggest a majority of Gram-positive bacteria were predominant. Further scope of the isolated bacterial microflora suggests understanding the various applications such as enzyme production, antimicrobial production, pigment production,bio-plastic production, and bio-fertilizer production.

Through the understanding of the influence of changing salinity, sea-surface temperature, and BOD of the estuarine water on the existing marine microflora, mangrove flora, and consequently the associated biodiversity of macrofauna further climate-related studies can provide ingenious solutions to minimize the consequences

linked to climate change on these natural ecosystems, raise awareness from the grassroot level, and aid in the restoration of mangrove habitats thereby conserving the associated biodiversity.

CHAPTER 2

LITERATURE REVIEW

On a global scale, mangrove ecosystems are categorized by their halophytic vegetation, adaptation to saline coastal environments, and ability to exhibit remarkable ecological diversity. A comprehensive assessment carried out by Alongi (2008) estimated the presence of approximately 150 species of true mangroves distributed across 123 countries worldwide. These mangroves belong to 20 different plant families, with amongst the largest areas present in Latin America, Africa, and Southeast Asia. The prominent biodiversity of global mangrove ecosystems extends beyond its plant diversity with its diverse array of faunal inhabitants in association with these mangrove ecosystems. These diverse macrofaunal inhabitants include various species of fish, crustaceans, mammals, insects, molluscs, and birds. In a study on the ecological role of mangroves by Nagelkerken et al. (2008), an emphasis on the importance of these mangrove habitats established that these mangrove habitats provide essential nurseries, breeding, and foraging grounds for abundant ecological and commercially valuable species.

Reports by Goa Forest Department (2021) suggests that global distribution of mangrove ecosystems is predominantly divided into the two hemispheres, mainly, the Atlantic East Pacific and the Indo-West Pacific. The Atlantic East Pacific as approximately 12 species of mangrove flora in comparison to the Indo-West Pacific which is comprised of approximately 58 species of mangrove flora. Out of a total of 82 mangrove species belonging to 52 genera and 36 families recorded from both these hemispheres, the Mangrove fern (*Acrostichum aureum*) has been associated in common with both these hemispheres. The most extensive coverage of mangrove ecosystems is

found in Asia, followed by Africa and South America. Approximately 41% of all mangroves in the world are mainly concentrated in Brazil, Indonesia, Nigeria, and Australia. The Gulf of Mexico and Caribbean regions are characterized with a relatively low species richness with the presence of four dominant species, namely, *Avicennia germinans, Laguncularia racemose, Conocarpus erectus,* and *Rhizophora mangle*.

According to reports by the Goa Forest Department, the mangrove coverage in India is spread over an area of 4,639 km^2 thereby occupying 0.14% of the total Asian mangrove coverage. Approximately 80% of mangroves are concentrated along the east coast while the remainder 20% are located along the west coast of India.

Along the east coast of India, the Sunderbans in West Bengal have a 46.39% mangrove coverage with the highest taxa diversity of 69 species, 49 genera, and 35 families of mangrove flora, including *Scyphiphora hydrophyllacea* and *Atalentia corea* as the first two reported mangrove flora species in the Sunderbans. Mangrove palms such as *Nypa fructicans* and *Pheonix paudosa* are largely restricted to the Sunderbans as well as Andaman and Nicobar groups of Islands; while the latter species has also been found to occur in Bhitarkanika mangrove forests. The Bhitarkanika mangrove forests comprise 57 species, 37 genera, and 29 families of mangrove flora with *Heriteria kanikensis* reported as recent occurrence. The Godavari and Krishna delta house over 36 species, 26 genera, and 21 families of mangrove flora while the Subarnarekha mangrove forest has been found to harbour 18 species, 14 genera, and 11 families of mangrove flora. The Pichavaram mangrove forests have over 35 species, 26 genera, and 20 families of mangrove flora including one additional new species *Rhizophora annamalayana* Kathir, a hybrid of *Rhizophora mucronata* and *Rhizophora*

apiculata. The Andaman and Nicobar group of Islands house 61 species, 39 genera, and 30 families of mangrove flora with additional two new species including *Rhizophora lamarkii* and *Rhizophora stylosa* and harbours the maximum species of Rhizophora.

Along the west coast of India, approximately 34 species, 25 genera, and 21 families have been reported. Out of these, 28 species have been reported from Maharashtra, 21 species from Gujarat,16 species from Goa, 18 species from Karnataka, 12 species in Kerala, and 1 species from the Lakshadweep group of Islands. Along the Gujarat coastline, the most dominant mangrove species *Avicennia marina* have been observed while along the coastline of Goa, the most predominant mangrove flora species include *Rhizophora mucronate, Avicennia officinalis, Aegiceras corniculatum, Sonneratia alba, Sonneratia caseolaris, Rhizophora apiculata, Bruguiera gymnorhiza*, and *Kandelia candel*.

Certain regions of the world, like the Sundarbans, located as a cluster of low-lying islands in the Bay of Bengal spread across India and Bangladesh, stand out as critical global biodiversity hotspots. As highlighted by Ellison et al. (2010), the Sunderbans mangrove forest is well-renowned for its exceptional richness in both plant and animal species diversity, including the iconic Bengal tiger (*Panthera tigris tigris*). Additionally, it is evident that mangrove forests provide a range of ecosystem services. As reviewed by Lee et al. (2019), these ecosystem services include ecologically regulatory features such as carbon sequestration, coastal protection, and nutrient cycling. Thus, the diverse flora and fauna associated with these mangrove ecosystems contribute to their resilience and provision of these ecosystem services. Nevertheless,

despite their ecological significance, global mangroves are under severe threat. As a review by Alongi (2014) articulates, habitat loss observed due to urbanization, industrial development, unsustainable agricultural patterns, and aquaculture, in combination with the adverse impacts of overharvesting of fish resources, pollution, and climate change, pose substantial challenges to the conservation of mangrove ecosystems and its associated biodiversity.

The extensive coastline of India spans approximately 7,500 km and hosts a varied range of mangrove ecosystems. According to a taxonomic analysis, India is home to around 46 species of true mangroves, belonging to 22 genera and 15 families. Among these species of true mangroves, noteworthy examples include *Rhizophora spp.*, *Avicennia spp.*, and *Sonneratia spp*. Several studies conducted in India have focused their attention on the biodiversity of mangroves, encircling surveys of taxonomic research of fauna and flora as well as ecological investigations.

In order to conserve and protect the mangrove biodiversity within India, the implementation of various initiatives and policies have been established. The Coastal Regulation Zone (CRZ) regulations restrict specific activities in coastal areas, thereby safeguarding and monitoring mangrove habitats from illegal logging and habitat destruction. Furthermore, the National Mangrove Action Plan outlines strategies for mangrove conservation and sustainable management (MOEFCC, 2019). The ever-increasing impact of climate change is an additionally crucial consequence affecting the Indian mangroves. Studies by Das and Vincent (2009) have estimated the vulnerability of these mangrove ecosystems to the impacts arising from continual sea-level rise and associated climate change-related stressors. Thus, conservation measures

that progressively incorporate climate adaptation approaches and initiative plans to mitigate potential impacts are a necessity.

The state of Goa located along the western coast of India is a coastal state well-known for its diverse and unique mangrove ecosystems. Extensive research has been carried out in order to understand the composition, distribution, and conservation status of mangroves within the state of Goa. Studies by Untawale et al. (1979) and Kulkarni et al. (2013) have recognized a range of mangrove species in Goa some of which include *Avicennia marina, Rhizophora mucronata,* and *Sonneratia alba are* most abundantly present. The mangroves of Goa display prominent biodiversity, with different species adapted to the distinct microenvironments within the intertidal zones and estuarine habitats.

Based on a study carried out by Silva and Bhat (2011), the mangrove area coverage in Goa extends to approximately 2,619 ha however mangrove cover has declined from 20,000 ha in 1987 to 2,200 ha in 2015 (Rodrigues, 2020). Common mangrove species such as *Kandeila candel, Sonneratia caseolaris,* and *Acanthus ilicifolius* while areas of increasing salinity have been associated with the presence of *Bruguiera gymnorhiza, Rhizophora mucronata,* and *Sonneratia alba*. Studies by Cajy and Bhat, (2010) suggest that few species of mangrove flora like *Ceriops tegal, Bruguiera cylindrica, Sonneratia caseolaries*, *Baringtonia racemosa* along with *Lumnitzera racemosa* and *Cynomitra iripa* were amongst the first reported in Goa, having lesser frequency, density, abundance and have been confined to certain sub-stations of estuaries. *Avicennia marina* have been reported as the most salt-tolerant mangrove species.

Research carried out by Giri et al. (2008), and D'Souza and Untawale (2004) have mapped the distribution of mangroves in Goa and have highlighted their status as critical habitats in the coastal landscape. The mangroves of Goa are primarily situated in the deltaic and estuarine regions evidently found to thrive along the Mandovi, Zuari, Sal, Chapora, Galgibag, Terekhol, Talpona estuarine rivers as well as the Cumbarjua Canal. A review by Shetye et al. (2015) underlines the significance of mangroves in supporting a variety of marine and avian species, including commercially and ecologically essential migratory birds and fish species. These ecosystems play an important role in Goa's birdwatching tourism industry and fisheries industry.

Despite their ecological importance, Goa's mangroves continue to face increasing threats arising due to urbanization, tourism, industrial, and coastal development which has resulted in severe cases of habitat fragmentation and degradation. Warranting the conservation of these ecosystems as a priority, highlighting the importance of the implementation of the Coastal Regulation Zone (CRZ) guidelines and promotion of community-based mangrove management initiatives are some conservation measures presently in need of being established. The influence of rising sea-levels and heightened temperatures within the state have also been associated with the impact on the distribution and health of mangrove species. The vulnerability of mangroves due to climate change in Goa highlights the need for critical adaptation strategies. Mangrove ecosystems provide crucial roles in carbon sequestration, nutrient cycling, as well as maintenance of the overall ecosystem health making them a subject of great importance to researchers and biologists. Studies by Lee and Chong (2009) stipulate that microflora in mangrove ecosystems encompass a diverse range of

taxonomic groups, including ciliates, diatoms, nematodes, copepods, and foraminifera species. Recent advances as articulated by Silva and Souza (2013) mention that molecular techniques such as DNA barcoding, have revealed previously unknown microfaunal diversity associated with mangrove ecosystems.

The diverse species of microflora associated with mangrove ecosystems reveal a treasure trove of research on the ecologically significant communities that occupy these unique coastal environments. Microflora present within mangrove ecosystems is comprised of an extensive array of microscopic organisms such as bacteria, archaea, fungi, cyanobacteria, yeast, protists, actinomycetes, microalgae, meiofauna, and microcrustaceans. Studies by Kathiresan and Bingham (2001) report that the common bacterial groups of the mangroves are sulfate--reducing (*Desulfovibrio*, *Desulfotomaculum*, *Desulfosarcina*, *Desulfococcus* sp.), Nitrogen-fixing (Azospirillum, Azotobacter, Rhizobium, Clostridium, Klebsiella sp., etc.), phosphate-solubilizing (*Bacillus, Paenibacillus, Xanthobacter, Vibrio proteolyticus, Enterobacter, Kluyvera, Chryseomonas, and Pseudomonas sp.*), photosynthetic anoxygenic (*Chloronema, Chromatium, Beggiatoa, Thiopedia, Leucothiobacteria sp.*) and methanogenic (*Methanoccoides methylutens sp.*) bacteria. Additionally, various groups of fungi, such as ligninolytic, cellulolytic, pectinolytic, amylolytic, and proteolytic fungi as well as actinomycetes have also been reported in mangrove ecosystems. Findings by Sen and Naskar (2003) suggest that among the various species of algae observed and documented in the mangrove ecosystems, *Chlorophyta, Chrysophyta, Phaeophyta, Rhodophyta,* and *Cyanophyta* are the most prominent.

A study on the taxonomic diversity of bacteria associated with the mangrove sediments of Goa reported by Haldar and Nazareth (2018) suggests that using paired-

end amplicon sequencing of 16S rDNA and culture-based analysis, the 16S rDNA (recombinant DNA) sequencing revealed that *Proteobacteria, Firmicutes*, and *Actinobacteria* as the dominant phyla in the mangrove sediments of Goa. *Bacteroidetes* from Mandovi sediment and *Acidobacteria* and *Gemmatimonadetes* from Zuari sediment were the other exclusive major phyla while *Chloroflex*i, *Cyanobacteria, Nitrospirae, Planctomycetes, and Verrucomicrobia*, were the minor phyla observed in both Mandovi and Zuari estuarine sediments.

Studies carried out by Alongi (1987) depict that mangrove sediments hold microfloral species in high densities with biomass levels that exceed those of macrofaunal species. Microflora are habitually more abundant in the upper sediment layers, where oxygen levels are higher (Hossain and Yusoff, 2008). The microflora associated with mangrove ecosystems play essential roles in nutrient cycling which include the decomposition of organic matter, sulfur cycling, and nitrogen fixation processes. These mangrove-associated microflora contribute to the stability of mangrove ecosystems by enhancing the integral soil structure and promoting the nutrient availability for mangrove vegetation (Boanglia and Meysman, 2018).

Studies by Gao et al. (2022) state that microflora in mangroves are critical components of food webs, serving as prey for various macrofaunal and avian species. Ecological interactions ranging from mutualism, predation, and parasitism, among microflora species commonly contribute to the ecosystem dynamics. Gao et al. (2022) articulate that microflora communities are influenced by environmental factors such as salinity, temperature, pH, and sediment characteristics with the anthropogenic impacts of pollution, climate change, and habitat degradation having the ability to alter the

microflora community composition and abundance. Understanding the importance of microflora communities is a necessity for the sustainable management and conservation of mangrove ecosystems. The implementation of potential restoration efforts should consider the role of microfauna in ecosystem resilience and recovery (McKee and Faulkner, 2000).

Alongi (2014) stipulates that despite substantial progress in the field of research, knowledge gaps continue to exist regarding the ecological and taxonomical features of microflora in mangrove ecosystems. Nagelkerken et al. (2008) suggest that long-term monitoring studies are required to assess the impact of environmental changes on these microflora communities.

Studies depicted by Bik et al. (2012) and Creer and Yu (2017) state that advances in the field of metagenomics and environmental DNA (eDNA) analysis have the potential to reform the study of microfauna in mangrove ecosystems by providing crucial insights into their functional roles and interactions. Research by Kristensen et al. (2008) and Alongi (2015) elaborate that integrated research that combines microbiological, ecological, and environmental approaches is vital for a comprehensive understanding of microflora species associated with global mangrove habitats.

The species of macrofauna associated with global mangrove ecosystems reveal a diverse and ecologically significant community that play a crucial role in the functioning of these unique coastal and estuarine habitats. The mangrove forests provide the presence of both hard and soft bottom habitats for the survival of a wide variety of bivalves, crustaceans (crabs and shrimps), clams, tunicates, barnacles, snails, isopods, amphipods, and polychaete worms that thrive in the bottom sediments. The

muddy bottom sediments, open waters, and mangrove root systems. These benthic invertebrates feed on leaf litter, detritus, microorganisms, and planktons. Categories of invertebrates such as marine wood borers (mollusks and crustaceans) have also been observed in mangrove ecosytems globally. Among the crustacean wood borers, the most commonly observed family is Sphaeromalidae while the mollusc family commonly includes Teredinidae. The Teredinidae family are highly specialized bivalves commonly referred to as pile worms or shipworms that possess lignimolytic and cellulolytic enzyme that help digest lignin and cellulose from wood. These shipworms have a unique ability to tolerate a wide range of salinity alterations. Out of the total 68 species of shipworms recorded worldwide, 25 species have been observed and documented in mangrove habitats. Among the 14 species of crustacean species of marine wood borers recorded in marine habitats, only approximately 5 species have been observed in mangrove habitats.

Studies reviewed by Kathiresan and Bingham (2001) report that different crab species respond differently to disturbance and thus this affects their species distribution. *Sesarma guttatum* observed in Kenya prefer shaded habitats and are most commonly found to inhabit the mangrove canopies. In the mangrove swamps of East Africa, *Sesarma leptosoma* are observed as active climbers of mangrove trees as a behavioural measure for protection against predators. Burrowing isopods such as *Sphaeroma terebrans* and *Sphaeroma peruvianum* have been observed in many regions of the Caribbean, Eastern Pacific, and the Atlantic. Mangrove meiofaunal communities including annelids (oligochaetes) and crustaceans. Among the meifaunal species of nematodes, *Parapinnanema ritae*, *Parapinnanema rhipsoides*, and *Parapinnanema alii* have been reported in Guadelope. In the Belgian coast of the North Sea,

Pseudochromadora interdigitatum, *Eubostrichus africanus* and *Chromaspirina okemwai* have been observed while mangrove sediments of Kenya report findings of *Papillonema clavatum* and *Papilonema danieli*. Polychaete worms have been observed as dominant macrobenthic invertebrates in the mangrove flats of Inhaca Islands, Mozambique.

Crabs are most frequently observed in mangrove ecosystems and are crucial keystone species contributing to high biodiversity within the mangrove habitats. The mangrove tree crabs that reside in mangrove flora canopies, feeding primarily on red mangrove leaves have been reported in India. Horseshoe crabs have observed in the mudflats of Godavari and Narmada rivers as well as Andaman and Nicobar group of Islands harbouring a large population of these crab species along the east coast of India. The common inhabitants of the intertidal mangrove zones throughout the Indo-Pacific regions include various species of Sesarmid crab (*Sesarma sp.)* and Fiddler crab (*Uca* sp.). Crab species such as *Ucides cordatus* and *Sesarma sp*. are abundantly present in mangrove habitats and serve as chief detritivores that influence the sediment structure and nutrient cycling process (Smith et al., 1991; Kristensen et al., 2008). Shrimp species such as *Palaemonidae* also contribute towards nutrient cycling process as well as aid as prey for various organisms (Lee, 1999).

Studies by Alongi (1987) and Kristensen et al. (2008) state that gastropods like *Cerithidea spp.* and bivalves like *Anadara spp.* commonly associated with mangrove ecosystems are filter-feeders that assist in maintaining water quality and sediment stability. Polychaete worms like *Marphysa spp*. and *Nereididae* play intrinsic roles in bioturbation and nutrient cycling and are abundantly found within mangrove habitats

(Kristensen et al., 2008; Kristensen et al., 2011). Studies depicted by Moens et al. (2013) state that nematode communities are highly diverse and influence the nutrient cycling and organic matter decomposition process within mangrove ecosystems. Amphipods such as *Gammarus spp.* are detritivores and significant prey for other organisms (Lee, 1999). Globally, approximately 147 species of clams (*Polymesoda)* have been reported. In India, *Polymesoda erosa* has been widely documented along the west coast most commonly in mangrove areas of Maharashtra, Goa, and Karnataka. Along the east coast of India, *Polymesoda bengalensis* has been observed.

According to studies reported by the Goa Forest Department, a total of 76 invertebrate taxa have been recorded within mangrove ecosystems along the estuaries of Goa. This includes 35 species of molluscs (16 bivalve species and 21 gastropod species), 22 species of crustaceans, 7 species of amphipods, 6 species of ploychaetes, 3 species of branacles and a oligochaete. Among the bivalve species, *Crassostrea madrasensis* is most dominant, followed by *Meretrix meretrix, Meretrix casta*, *Perna viridis* and *Anadara granosa*. Oysters are also common occurrences in mangrove ecosystems and most commonly commercially sold species include *Crassostrea madrasensis* and *Crassostrea cucullata*. Along the Mandovi-Cumbarjua Cannal-Zuari estuarine system *Meretrix casta, Villorita crypinoides, Polymesoda erosa* have been documented and commonly harvested. The four primary species of bivalves commercially exploited in Goa include *Paphia malabarica*, *Meretrix casta*, *Katelysia opima,* and *Villorita cyprinoids.* Among the crustacean species documented in Goa, the Giant mud crab (*Scylla serrata*), Mud lobster (*Thalassina anomala*), Fiddler crab (*Uca* sp.), and a variety of shrimp and prawn species have been documented. Within the species of prawns reported along the estuaries of Goa, the most commonly reported

species include Giant freshwater prawn (*Macrobachium rosenbergii*), and the marine penaeid prawns (*Penaeus indicus, Penaeus merguiensis, Penaeus mondon,* and *Metapenaeus brevicornis*). Along the Goa coast, the wood borer species of invertebrates observed in mangrove ecosystems include *Martesia* sp., *Nausilora hedleyi*, and *Sphaeroma terebrans*.

Research by Nagelkerken et al. (2008) suggest that juvenile fish species mainly gobies, grunts, and snappers utilize mangrove habitats as nursing grounds which offer protection from predator organisms. In a study by Liem (1978), Mudskipper fish such as *Periophthalmus spp.* are well-adapted and endemic to mangrove habitats with specialized adaptation features that help provide an amphibious life. Sea cucumbers like *Holothuria spp.* have been associated to play a role in the cycling of nutrient by processing detritus (Uthicke, 2001). Sponges and tunicates are filter-feeding organisms contribute towards maintaining nutrient cycling and water quality (Mariani et al., 2006).

Extensive findings on fish species in mangrove ecosystems comprehensively covered by Kathiresan and Bingham (2001) suggest that juvenile fish are present in high densities in mangrove waters due to enormous food supply and protection from predatory impacts. In the Solomon Islands, the mangrove estuaries that are clogged with woody debris house *Pomacentrids* as well as some species of *Gobiidae* and *Apogonidae.* Fish species residing in mangrove habitats have the ability to adjust both in terms of spatial and temporal variability under chemical and physical conditions, with some species possessing unique measures of adaptation. Globally, the widely distributed species of hermaphrodite Killfish (*Rivulus marmoratus*) is well adapted to the mangrove microhabitats with its specialized adaptability to survive in moist detrital

substrates during periods of low water supply or drought-like conditons as well as undergo reproduction through internal self-fertilization. Mudskippers also possess the ability to endure in extremely hypoxic conditions by digging extensive burrows into anoxic mangrove sediments. *Cyprinodon* species of fish possess the unique ability to withstand higher temperatures in comparison to other fish species. Juvenile snook (*Centropomus undecimalis*) present in mangrove estuaries worldwide have also been observed to move to oxygenated surface waters when deeper waters become anoxic as a survival strategy.

Studies reported by Saha et al. (2018) state that the fish fauna present in the estuarine waters in and around Indian Sundarbans has been classified into residents and transients (migrants). The species whose individuals of different sizes are present during all the months of the year in any zone of the estuary are referred to as resident species while the transient species enter and stay in the Bay of Bengal for shorter periods of time. Some of the important fish species include *Mugil parsia, Mugil tade, Polynemus paradiseus, Polydactylus indicus, Otolithoides biauritus, Lates calcarifer, Hilsa toli, Arius jella, Harpodon nehereus, Setipinna taty,Ilisha elongata, Setipinna phasa, Coilia ramcarati, Otolithoides pama* and *Sillaginopsis panijus*. According to findings by the Goa Forest Department, approximately 121 species of fish have been reported in the mangrove habitats. The common species of fish associated with mangrove ecosystems in Goa include Pearl Spot (*Etroplus suratensis*), Tilapia (*Oreochromis niloticus*), Milkfish (*Chanos Chanos*), Mangrove red snapper (*Lutjanus argentimaculatus*),Giant sea perch/Asian sea bass (*Lates calcarifer*), Butterfish (*Scatophagus argus*), Mullet (*Mugil cephalus*), Northern whiting (*Sillago sihama*), Long-finned herring (*Opisthopterus tardoore*), Whipfin-silver biddy (*Gerres*

filamentosus), Gibbous sweetlips (*Plectorhinchus gibbosus*), Anchovy (*Anchoviella commersonii*), Grouper (*Epinephalus malabaricus*), and Cat fish (*Osteogeneiosus militaris*).

Avifaunal species such as egrets, kingfishers, and herons depend on mangrove forests for nesting and foraging sites (Day et al., 2017). Studies reported by Kathiresan et al. (2001) suggest that mangrove ecosystems provide an essential habitat for shorebirds, landbirds and waterfowl. Mangrove habitats worldwide have been associative homegrounds to a number of threatened species, namely spoonbills (*Ajala ajala*), large snowy egrets (*Cosmorodium albus*), scarlet ibis (*Eudocimus ruber*), fish hawks (*Pandion haliaetus*), West-Indian whistling ducks (*Dendrocygna arborea*), royal terns (*Sterna hirundo*). Approximately 77 species of birds have been recorded in the Pacific mangroves of Colombia. In South-east Asian countries, kingfishers,sand pipers, white-bellied eagles, plovers, egrets and herons are regular visitors to the mangrove habitat. About 315 species of birds have been recorded from the Sunderbans and Bangladesh and a total of 121 species of migratory and residential birds recorded in India. The yellow warbler (*Dendroica petechia*) and Mangrove vireo (*Vireo pallens*) are endemic to mangrove habitats. Among the total avifaunal population associated with mangrove ecosystems, 43% are permanent residents, 22% are regular visitors, and 18% are temporary winter residents. In Florida Bay, U.S.A., bald eagles nest exclusively in mangrove trees, particularly, *Rhizophora mangle* and *Avicennia germinans*.

Studies by Kothari and Rao (2002) depict that, within the state of Goa, the most common avifaunal species recorded include, little cormorant,brahminy kite, white-

bellied kingfisher, small blue kingfisher, black-crowned night heron, Indian darter, common sandpiper,Indian pond heron, pin-tailed duck,Eurasian coot, and Great egret.

Insects associated with mangrove ecosystems are permanent residents and constitute a significant proportion of fauna in many mangrove communities globally. Findings reported by Kathiresan and Bingham (2001) suggest thatmangrove insects reveal a complex assemblage of species that fill an extensive variety of niches. In the mangrove habitats of Andaman and Nicobar Islands, approximately 276 insect species have been reported with over 197 species of herbivores, 43 species of parasites, and 36 species of predators. Recent findings on insects observed in the mangrove ecosystems suggest that over 28 species of dragonflies have been documented in India, a water strider (*Mesovelia polhemusi*) in Belize, termite species (*Nasutitermes nigriceps*) in Jamaica, and psyllid (*Telmapsylla* sp.) in Costa Rica and Florida. Studies by Araújo et al. (2006) articulate that terrestrial insects namely, mangrove tree-dwelling ants, contribute towards the cycling of nutrients and facilitate mutualistic ecological interactions.

Mosquitoes are amongst the most commonly occurring insects observed in mangrove habitats and have ben associated as vector reservoirs for several pathogenic viruses for diseases such as Ketpang, Bakau, Dengue, Malaria, and Haemorrhage fever. Other insect inhabitants, including honeybees, are most dominant in countries like India, the Caribbean, Southwest Florida, and Bangladesh. *Apis dorsata* has been reported as the most dominant bee species in India and has been found to construct its honeycombs most commonly on *Excoecaria* sp. among other mangrove flora species. Within the Brazilian mangrove habitats, 22 species of ants have been documented with

Camponotus and *Solenopsis* as the most common genera. The Australian mangrove habitat is home to 16 species of ants with *Polyrchachis sokolova* as the most common occurrence. Within the state of Goa, the most prominently documented insects include Mangrove Moth (*Hyblaea puera),* Mangrove Cricket (*Apteronemobius asahinai*), Mangrove Hopper (*Prokelisia marginata*),Mangrove Spider (*Lycosidae* family), as well as various species of butterflies,ants, mosquitoes, beetles (weevils and ground beetles), dragonflies, damselflies, and termites.

Thus, the complex interactions among the diverse macrofaunal species are necessary for the optimal functioning of mangrove ecosystems through its influence on sediment stability, nutrient cycling, and providing a food source for higher trophic levels. Additionally, the mangrove roots provide nursing grounds for various marine species, contributing to coastal fisheries. Sustainable management practices and the establishment of marine protected areas are essential for preserving mangrove biodiversity and the valuable provisional and regulatory ecosystem services they provide.

The impact of physicochemical parameters namely salinity and temperature on the mangrove flora, macrofaunal, and microflora diversity associated with mangrove ecosystems reveals an intricate interaction that exists between environmental conditions and the richness in species diversity surrounding coastal habitats. Mangrove ecosystems are among the most valuable, productive, and uniquely diverse biological ecosystems on Earth. However, these ecosystems are highly vulnerable and susceptible to environmental changes, including alterations in temperature and salinity. Understanding how environmental parameters such as salinity, temperature, and BOD

influence the biodiversity of flora and fauna associated with mangrove ecosystems can provide crucial insights into effective mangrove restoration, conservation, and management measures.

According to Smith et al. (2013), mangrove ecosystems are characterized by their salt-tolerant adaptability and are known for their richness in biodiversity as well as its associated numerous ecological functions. Thus, despite their highly adaptive abilities to salinity and temperature fluctuations, drastic alterations in environmental parameters like salinity and temperature have been found to have an impact on species diversity in association with mangrove habitats.

Alongi (2008) suggests that observed fluctuations in salinity levels due to tidal cycles is a definitive feature of mangrove ecosystems and shape the distribution of species within them. A study by Ball (2016) states that drastically increasing salinity levels can stress flora and fauna species while some mangrove species are uniquely adapted to remain acclimatized to higher levels of salinity due to the presence of salt excretion mechanisms to thrive in these existing conditions.

Temperature is a critical environmental factor that influences the distribution and physiology of mangrove species as seasonal variations in temperature can affect the growth rate and reproductive success of mangrove species diversity. Studies by Osland et al. (2017) depict that with the rising temperatures associated with climate change, not only will there be a profound impact on the mangrove species diversity but in addition to this, a higher temperature gradient will influence the species composition of microflora and macrofaunal species as well as the migration pattern of a variety of macrofaunal species associated with mangrove ecosystems. The tolerance of salinity

and inundation in mangroves has been associated with the efficient use of water for photosynthetic carbon gain, this fortifies the anticipated gains in productivity with increasing levels of carbon dioxide.

Feller et al. (2010) suggest that salinity and temperature play essential roles in shaping the composition and diversity of mangrove tree species, with some species exhibiting adaptations to specific salinity and temperature ranges. Biodiversity in mangrove ecosystems extends to a wide range of organisms, specifically fish, crabs, molluscs, and birds, all of which exhibit varying degrees of sensitivity to salinity and temperature (Dahdouh-Guebas et al., 2005). The impact of climate change, driven by rising global temperatures, can result in shifts in the distribution of mangrove species and alter the overall biodiversity of these ecosystems (Gilman et al., 2008). Increased temperatures have also been found to exacerbate salinity-related stress, impacting the health and abundance of mangrove flora species (Alongi et al., 2015).

Studies by Sari and Soeprobowati (2021) suggest that mangrove ecosystems are highly susceptible to changes in water quality. The impact of BOD on mangrove microflora is a crucial aspect to aid in the understanding of the ecological health surrounding mangrove ecosystems. Elevated levels in BOD have been found to influence the composition and structure of the microbial community in mangrove ecosystems. Mangrove ecosystems are characterized by anaerobic sediments, and the balance of oxygen availability is crucial for the survival and biological activity of microflora.

BOD has also been closely linked to nutrient cycling of mangrove ecosystems, with excessive organic matter decomposition possibly leading to increased nutrient

enrichment thereby altering the nutrient availability towards the microflora and subsequently causing a potential impact to the overall nutrient dynamics of the ecosystem. Increased levels in BOD have often been associated with anthropogenic activities and pollution. The health of mangrove microflora is therefore a crucial indicator of the overall health of the mangrove ecosystem with changes in microflora composition affecting the resilience and adaptability of mangrove ecosystems. Mangrove microflora including several symbiotic associations, such as mycorrhizae and nitrogen-fixing bacteria may be potentially influenced by changes in BOD levels thus influencing nutrient uptake and cycling capacities within the mangrove ecosystem.

Research by Duke et al. (2007) suggests that effective conservation and management strategies towards the protection and restoration of mangrove ecosystems should consider the combined effects of salinity and temperature on biodiversity as well as include habitat restoration and the establishment of protected areas as optimal solutions. Through monitoring and research efforts, essential changes in physicochemical parameters and their impacts on biodiversity in mangrove ecosystems can be tracked, especially in the context of climate change (Lovelock et al., 2015). Further interdisciplinary research is necessary to comprehensively assess and mitigate the impacts of environmental changes on these pivotal coastal ecosystems and their associated biodiversity.

CHAPTER 3

METHODOLOGY

3.1 SAMPLE COLLECTION

The six mangrove sampling sites were selected within the Mandovi estuary in Goa, India. These specific sites, namely, Site 1 (Control site) - Vagurbem (Lat. 15.459776°, Long. 74.034066°), Site 2 - Saint Estevam Island (Lat. 15.523854°, Long. 73.933171°), Site 3 - Divar Island (Lat. 15.505503°, Long. 73.878612°), Site 4 - Chorao Island (Lat. 15.513077°, Long. 73.87042°), Site 5 - Penhe de Franca - Britona (Lat. 15.517901°, Long.73.845683°), and Site 6 - Ponte de Linhares Causeway- Ribandar (Lat. 15.5011146°, Long.73.848788°) were chosen as sampling sites for the documentation of macrofaunal and macrofloral diversity and for the estimation of microflora diversity.

The water samples were collected from all six specified sampling sites using sterile plastic bottles of 250 mL capacity by grab sampling technique. The samples were then brought immediately to the lab for further processing. Samples were preserved for a short time period at 4°C. The parameters such as ambient temperature, sea-surface temperature, and tide level prevalent at that site during the time of sampling were recorded.

Figure 3.1: Google maps image of six selected sampling sites (up to scale)


GPS Map Camera
Vagurbem, Goa, India
F26M+28J, Vagurbem, Goa 403115, India
Lat 15.459776°
Long 74.034066°
04/12/23 12:18 PM GMT +05:30
Google


GPS Map Camera
Saint Estevam Island, Goa, India
Tolto Ferry Terminal, Betqui, St Estevam Island, Goa 403107, India
Lat 15.523854°
Long 73.933171°
03/12/23 10:49 AM GMT +05:30
Google


GPS Map Camera
Navelim, Goa, India
St. Pedro Ribandar Ferry, Piedade, Goltim-Navelim VP, Goa 403403, India
Lat 15.505503°
Long 73.878612°
20/09/23 11:05 AM GMT +05:30
Google

Figure 3.2: Geo-tag images of six selected sampling site locations

3.2 MEASUREMENT OF PHYSICOCHEMICAL PARAMETERS

The physicochemical parameters, mainly, sea-surface temperature, salinity, and BOD were analyzed for the water samples collected at all the six selected sampling sites, namely, Vagurbem, Divar Island, Chorao Island, Saint Estevam Island, Penhe de Franca - Britona, and Ponte de Linhares Causeway - Ribandar. The physicochemical parameters describe a crucial measure of water quality (presence of organic matter) as well as the influence of climatic variables such as temperature, relative humidity, precipitation, wind, waves, and tides.

3.2.1 Sea-surface temperature estimation

Material required: Labworld Glass Thermometer 300mm (Reading between -10℃ and 110℃), water samples, plastic vial bottles (5mL capacity).

Procedure: The water sample was collected in plastic vials from the sampling site. The sea-surface temperature was analyzed and noted in situ using the glass thermometer.

3.2.2 Salinity estimation

Materials required: MCP portable Handheld Refractometer (for salinity between 0-100‰), water samples, plastic vial bottles (5mL capacity).

Procedure: The water sample was collected from the sampling site and brought to the laboratory for salinity estimation using a Refractometer to analyze the salinity and the salinity value was subsequently noted.

Figure 3.3: Salinity measured using MCP portable Handheld Refractometer

3.3 BOD estimation (modified protocol) (Source: Grasshoff et al., 2009)

Materials required*:* Glassware, Burette, Glass funnel, Glass pipette (50mL capacity), Volumetric flasks (100mL capacity), Conical flasks (250mL capacity),Glass rods, Glass beakers, Burette stand (100mL capacity), BOD stoppered glass bottles (125mL capacity), Measuring cylinder (100mL capacity), distilled water.

Chemical requirements: Manganese chloride, Potassium iodide, Sodium hydroxide, conc. Sulphuric acid, Starch indicator, Potassium iodate, Sodium thiosulphate.

Procedure:

I. <u>Standardization of Sodium thiosulphate</u>

1. Approximately 10mL of 0.01N Potassium iodate is added to 1mL of 50% conc. Sulphuric acid, 1mL of Winkler A (Manganese chloride), and 1mL Winkler B (Potassium iodide and Sodium hydroxide) in a conical flask.
2. Mix well and store in the dark for 3 minutes.
3. Titrate against Sodium thiosulphate till solution in the conical flask turns from dark yellow to pale yellow colour.

4. To this, add 3 drops of starch indicator producing a blue colour solution in the conical flask.
5. Titrate once again to arrive at an endpoint colour change from blue to colourless. Repeat the process thrice to obtain a Constant Burette Reading value.
6. Take 50mL blank (distilled water) is also estimated alongside with this, along with the addition of 1mL of Winkler A (Manganese chloride), and 1mL Winkler B (Potassium iodide, Sodium hydroxide), followed by 1mL of concentrated Sulphuric acid and 3 drops of starch indicator.
7. The endpoint colour change from blue to colourless is observed when titrated against Sodium thiosulphate.

Calculation for standardization of Sodium thiosulphate

(Sodium thiosulphate) $N_1V_1 = N_2V_2$ (Potassium iodate)

$$N_1 = N_2V_2/V_1$$

To obtain the normality of Sodium thiosulphate

II. BOD estimation for Day Zero (D_0) and Day 5 (D_5)

1. The water sample (in triplicates) was collected from each sampling site.
2. Carefully fill a BOD bottle with sample water without making air bubbles.
3. Add 1ml of Winkler A (Manganese chloride) to the BOD stoppered bottle carefully by inserting the pipette just below the surface of water in order to avoid the formation of air bubbles.

4. Add 1mL of Winkler B (Potassium iodide and Sodium hydroxide) to the BOD stoppered bottle by carefully inserting the pipette just below the surface of the water in order to avoid the formation of air bubbles.
5. Then close the bottle and mix the sample by inverting many times. A brownish cloudy precipitate is found to appear in the solution as an indicator of the presence of dissolved oxygen in the water.
6. Allow the brown precipitate to settle out to the bottom of the bottle.
7. Add 1ml of concentrated Sulphuric acid carefully to the bottles making sure the formation of air bubbles is inhibited.
8. The bottle is then closed and the solution is mixed well to dissolve the precipitate.
9. Approximately 50mL of the above solution is added to a conical flask and titrated with standard Sodium thiosulphate to a pale yellow colour.
10. Followed by which, 1mL starch indicator added causing the solution to turn blue in colour.
11. The titration is continued till end point from blue to colourless is achieved. This process is repeated thrice to obtain the Constant Burette Reading value.
12. The bottle is further kept in BOD incubator/ dark place for 5 days of incubation.
13. After incubation, 50 ml of this sample is titrated with standard Sodium thiosulphate to a pale yellow colour.
14. Then add 1ml of starch indicator causing the sample solution to turn blue in colour.
15. The titration is continued till the sample solution produces an end point of colourless. This process is repeated thrice to obtain the value of the Constant Burette Reading thus noted.

16. The concentration of dissolved oxygen in the sample for the Day zero and Day five is equivalent to the number of millilitres of titrant used.

(NOTE: Sodium azide may be added to avoid the interference of nitrite in the water sample).

Calculations for BOD estimation for Day Zero (D_0) and Day 5 (D_5)

Dissolved Oxygen (D_0) = (Volume of Sodium thiosulphate consumed× Normality of Sodium thiosulphate ×1000×8) / (V_2 (V_1 – v)/V_1)

Dissolved Oxygen (D_5) = (Volume of Sodium thiosulphate consumed×Normality of Sodium thiosulphate×1000×8) / (V_2 (V_1 – v)/V_1)

V = 1 + 1 = 2 mL (Winkler A reagent + Winkler B reagent)

V_1 = Volume of BOD (Biochemical Oxygen Demand) bottle (mL) = 125 mL

V_2 = Volume of water sample for analysis = 50 mL

BOD = Dissolved Oxygen **(**D_0) - Dissolved Oxygen (D_5)

Figure 3.4: Collection of water sample on site for BOD testing

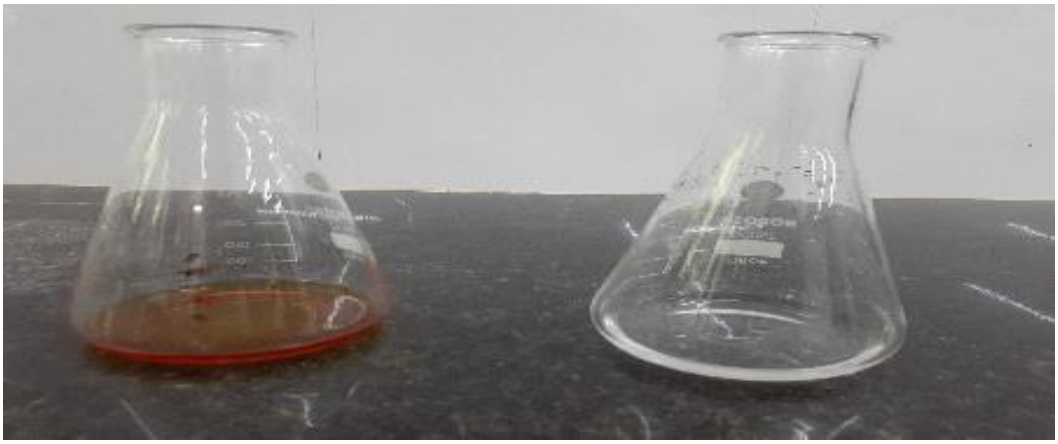


Figure 3.5: Water sample estimated for BOD colour change from initial colour (dark yellow) to end point (colourless)

3.3 DOCUMENTATION OF MACROFLORAL DIVERSITY

Visual documentation: For the observation and documentation using a Samsung Galaxy A20 13-megapixel (f/1.9) primary camera, and a 5-megapixel (f/2.2) camera, Godrej Mangrove Identifying application.

Identification key for mangrove flora: Godrej Mangrove Identifying application

Herbarium: For observation, documentation, and preservation of leaves and fruit/flower of mangrove floral species.

3.3.1 Herbarium Preparation (Source: Godrej and Boyce, 2021)

Procedure:

1. The different varieties of mangrove plant samples were collected from the five specified sampling sites, namely Divar Island, Chorao Island, Saint Estevam Island, Penhe de Franca- Britona, and Ponte de Linhares Causeway - Ribandar.
2. The plant specimens thus collected were arranged and kept in an appropriate manner making sure no damage was caused to the collected sample.
3. The plant specimen was compressed between two flat surfaces, that is, cardboard or plywood may be utilized.
4. The plant specimen was kept compressed for a period of 5 to 7 days.
5. The leaves, stem, and fruit/ flower (if present) are utilized to make the herbarium of the plant specimen.
6. This dried plant specimen was then adhered to on an A4 size sheet of white paper and labeled according to their kingdom, phylum, class, order, family, genus, species, location, coordinates, and date of collection.
7. A real-time image of the representative mangrove species is also added to showcase additional accuracy. To obtain precise results, the plant specimen should be compressed as soon as the plant sample is collected.

3.4 PREPARATION OF CULTURE MEDIA FOR ISOLATION OF MICROFLORA (Source: Chaudhari et al., 2017)

Bacterial Cultures: The bacteria were isolated from the estuarine water sample from the six specified sites, namely, Vagurbem, Divar Island, Chorao Island, Saint Estevam Island, Penhe de Franca - Britona, and Ponte de Linhares Causeway - Ribandar. These water samples were cultured on Zobell Marine Agar Media and dilutions were made for the water samples. For the water samples, the undiluted and 10^{-1} dilutions were plated by spread plating technique for all the specified six sampling sites. Approximately 0.1 mL (100 µL) of inoculum was added to each media plate and the plates were incubated at 37°C for 1 to 2 days.

3.4.1 Morphological Identification (Source: Microbiology Society, 2024)

Identification of microflora (bacteria) was carried out based on morphological characterization and microscopic observation. Morphological characterization included the study of the colony characteristics such as size, shape, colour, opacity, margin, elevation, and consistency. Microscopic observation was done by staining the colonies on slides by Gram staining.

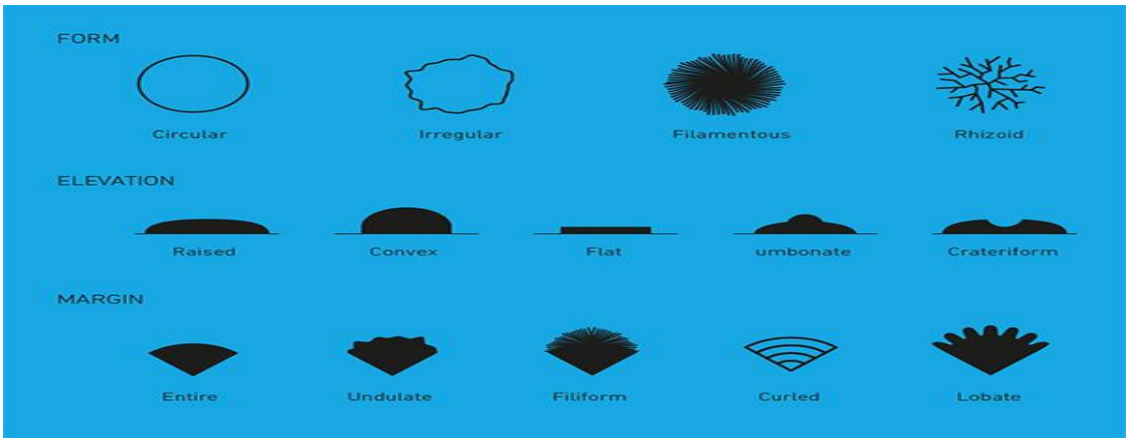


Figure 3.6: Colony characteristics morphology for bacterial species (Source: Microbiology Society, 2024)

3.4.2 Gram staining (Source: Smith and Hussey, 2005)

Materials Required: Gram's staining kit (Gram's Crystal Violet, Gram's Decolourizer, Gram's Iodine, Safranine), nichrome loop, dropper, microscopic glass slides, saline (0.85%), bacterial culture colonies, Phase Contrast Microscope (LABOMED).

Procedure: The smear of bacterial culture cells was prepared, dried, and heat-fixed on a clean microscopic glass slide. The smear was then flooded with Gram's Crystal Violet for 1 minute. The slide was then washed with distilled water and flooded with Gram's Iodine. The slide was then washed again with distilled water and flooded with Gram's Decolourizer for 45 seconds. The slide was washed again and a counter stain Safranin was poured over the slide and kept for 30 seconds. The slide was then washed with distilled water and air-dried. The slide was then observed under the oil immersion at 100X magnification using a Phase Contrast Microscope. The bacterial colonies that appeared purple were classified as Gram-positive and the bacterial colonies which appeared pink were classified as Gram-negative.

3.5 ESTIMATION OF MACROFAUNAL DIVERSITY (Source: Grewal and Fonseca, 2004; Goa Forest Department, 2021; Preston-Mafham, 2007)

The biodiversity of macrofauna was carried out at the six specific sampling sites, namely, Vagurbem, Saint Estevam Island, Divar Island, Chorao Island, Penhe de Franca - Britona, and Ponte de Linhares Causeway - Ribandar. The biodiversity of avifaunal species, aquatic vertebrate fauna species, and terrestrial invertebrate fauna species was primarily documented.

Visual documentation: The observation and documentation of macrofauna diversity was carried out using a Samsung Galaxy A20 13-megapixel (f/1.9) primary camera, and a 5-megapixel (f/2.2) camera, Google lens, Ebird application, Cason Binoculars Professional 10 X 60 HD Folding 10 X Zoom Binoculars.

Consultation with local fisherfolk communities*:* To obtain traditional knowledge with regards to fishing practices, local and commercially harvested fish, molluscs, and crustacean species.

Identification key for macrofauna: Avifauna identification by Birds of Goa (Grewal and Fonseca, 2004), Aquatic fauna identification (Goa Forest Department, 2021), Terrestrial invertebrate fauna identification by Insects and other invertebrates (Preston-Mafham, 2007).

3.6 BIOSTATISTICAL ANALYSIS

3.6.1 Correlation analysis (Source: Armitage et al., 2008)

Correlation studies were carried out using MS Excel – Analysis ToolPak to understand the influence of physicochemical parameters such as salinity and temperature (sea-surface temperature) on the species richness of macrofauna for the Monsoon and Post-monsoon seasons. Correlation studies were also carried out to understand the influence of BOD on the bacterial viable count of microflora. The correlation statistics were proved with R^2 level of significance.

3.6.2 Shannon-Wiener Diversity Index for species diversity estimation (Source: Albueajee et al., 2020)

The Shanno-Wiener Diversity Index or Shannon-Weaver Diversity Index is an widely

utilized measure of species diversity in an ecological community. It was developed by Claude Shannon and Warren Weaver in the 1940s, primarily in the field of information theory, but later adapted for use in ecology.

The Shannon-Wiener Diversity Index takes into account both the number of species present (species richness) and the evenness of the species abundances within a community. It quantifies the uncertainty associated with predicting the identity of a randomly selected individual from the community. The Shannon-Wiener Diversity Index for species diversity considers the number of species and the evenness of the species and helps determine the environmental and habitat diversity. The index increases with more unique species or greater

species evenness. By utilizing statistical software such as MS Excel – Analysis ToolPak, the Shannon-Wiener Diversity Index for species diversity was calculated per sampling site by utilizing the mathematical formula as follows,

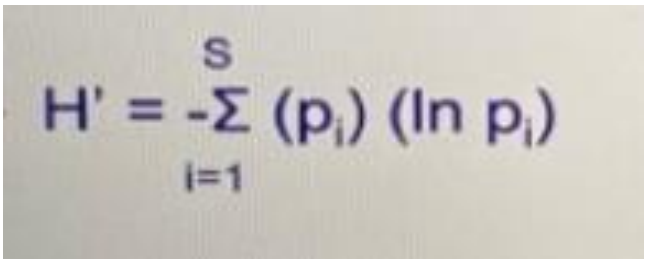


Figure 3.7: Mathematical formula for the Shannon-Wiener Diversity Index (Source: Aslam, 2009)

Here, H' specifies the Diversity Index, p denotes the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations, and S signifies the total number of species within the community (ecosystem).

Table 3.1: Pollution level based on the Shannon-Wiener Diversity Index

(Source: Albueajee et al., 2020)

Diversity level	Shannon Wiener index	Pollution level
High	3.0–4.5	Slight
Moderate	2.0–3.0	Light
Less	1.0–2.0	Moderate
Very less	0.0–1.0	Heavy pollution

The Shannon-Weiner index estimated per sampling site was obtained to estimate the consequential pollution levels per sampling site. The Shannon-Wiener Diversity Index increases with both species richness and evenness. Relatively higher values of the diversity index indicate greater diversity within the community.

The Shannon-Wiener Diversity Index is crucial in ecological studies to determine and compare the diversity of different ecosystems, assess the impact of disturbances on biodiversity, and monitor changes in diversity over time. Thus providing essential information for conservation efforts and ecosystem management strategies.

3.6.3 Margalef Index for species richness estimation (Source: Latumahina et al., 2020)

The Margalef Index is an ecological metric utilized to quantify the species richness within a given ecosystem. It was developed by the Spanish ecologist

Ramon Margalef in the 1950s and has since become a well-known measure in ecological studies.

$$d = \frac{S - 1}{\ln N}$$

Figure 3.8: Mathematical formula for the Margalef Index
(Source: Aslam, 2009)

Here, D signifies the Margalef Index, representing species richness, S is the total number of species observed in the ecosystem, and N is the total number of individuals (population size) in the ecosystem.

Table 3.2: Evaluation criteria for species richness using Margalef Index values
(Source: Latumahina et al., 2020)

Index Value	Category
R < 2,5	· Low species richness
2,5 > R < 4	· Medium species richness
R > 4	· High species richness

The Margalef Index accounts for both the total number of species present and the abundance of individuals within those species. It thus provides a measure of species richness relative to the size of the population suggesting that higher values of the

Margalef Index indicate greater diversity within the ecosystem. This index is predominantly useful for analyzing comparisons between the diversity of different ecosystems or monitoring changes in diversity over time within a single ecosystem. It facilitates ecologists to gain an understanding of the ecological health and stability of an ecosystem and can further assist as a tool for the management and conservation efforts of biodiversity.

3.6.4 Analysis of Variance (ANOVA) and Post-hoc Dunnet testing (Source: Armitage et al., 2008)

The total macrofauna abundance data associated with the mangrove ecosystems of the six selected sampling is analyzed to follow its Gaussian distribution. Further parametric testing using One-way Analysis of Variance (ANOVA) is utilized as a statistical technique to compare two or more samples and determine if significant difference exists between the total macrofaunal abundance among the sampling sites. The Post-hoc Dunnett Test provides further robust analysis of statistical comparison of the total macrofaunal abundance between the control site and the five other sampling sites.

CHAPTER 4

RESULTS

4.1 RESULTS

4.1.1 Water analysis – salinity, sea-surface temperature, and BOD

In reference to the given result on the physicochemical parameters, Table 4.1 depicts the physicochemical parameters for Vagurbem site (Control site – Site 1), Table 4.2 depicts the physicochemical parameters for Saint Estevam Island site (Site 2), Table 4.3 depicts the physicochemical parameters for Divar Island site (Site 3), Table 4.4 depicts the physicochemical parameters for Chorao Island site (Site 4), Table 4.5 depicts the physicochemical parameters for Penhe de Franca – Britona site (Site 5), and Table 4.6 depicts the physicochemical parameters for Ponte de Linhares Causeway – Ribandar site (Site 6). Figures 4.1, 4.3,4.5, 4.7, 4.9, and 4.11 depict the Google map images for Vagurbem site (Control site – Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively. Figures 4.2, 4.4,4.6,4.8,4.10, and 4.12 depict the Global Positioning System (GPS) images of Vagurbem site (Control site - Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively.

From the preliminary studies carried out on water sample analysis of the estuarine water samples obtained from the six primarily selected sampling sites along the Mandovi estuary in Goa, a site-wise description of the physicochemical parameters was analysed and documented. The physicochemical parameters of the estuarine water

samples were analysed in the Monsoon season (August-November) and the Post-monsoon season (December-January-February). The physicochemical parameters in terms of the sea-surface temperature were monitored on field site while the salinity and BOD were analysed in the laboratory.

Table 4.1: Physicochemical parameters for Vagurbem site (Site 1 – Control site)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
02-07-2023	12:37 PM	4‰	27.5°C	13.1 mm	29°C /27°C	High tide	Monsoon season 0.698 mg/L
26-11-2023	11:25 AM	7‰	28°C	0 mm	29°C /27°C	High tide	
04-12-2023	12:30 PM	13‰	30.5°C	0 mm	35°C /27°C	Low tide	
21-01-2024	10:44 AM	9‰	30.5°C	0 mm	32°C /20°C	High tide	Post-monsoon season 2.602 mg/L
04-02-2024	11:45 AM	8‰	30°C	0 mm	33°C /24°C	Low tide	

Figure 4.1: Google map image for Vagurbem site (Site 1)

Figure 4.2: Vagurbem site (Control site – Site 1)

Table 4.2: Physicochemical parameters for Saint Estevam Island site (Site 2)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
02-07-2023	11:09 AM	3‰	27.5°C	13.1 mm	29°C /27°C	High tide	Monsoon season 0.787 mg/L
26-11-2023	10:20 AM	5‰	27.5°C	0 mm	29°C /27°C	High tide	
03-12-2023	10:45 AM	17‰	31.5°C	0 mm	28°C /27°C	High tide	
21-01-2024	11:45 AM	22‰	29°C	0 mm	32°C /20°C	High tide	Post-monsoon season 0.488 mg/L
04-02-2024	10:30 AM	9‰	28°C	0 mm	33°C /24°C	High tide	

Figure 4.3: Google map image for Saint Estevam Island site (Site 2)

Figure 4.4: Saint Estevam Island site (Site 2)

Table 4.3: Physicochemical parameters for Divar Island site (Site 3)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
21-07-2023	10:00 AM	4‰	29°C	11 mm	28°C/25°C	High tide	Monsoon season 1.092 mg/l
04-11-2023	10:20 AM	5‰	28.5°C	0 mm	27°C/26°C	High tide	
28-12-2023	9:30 AM	24‰	30.5°C	0 mm	27°C/26°C	High tide	
28-01-2023	10:45 AM	24‰	29°C	0 mm	33°C/25°C	Low tide	Post-monsoon season 0.813 mg/L
05-02-2024	10:25 AM	24‰	28.5°C	0 mm	33°C/25°C	High tide	

Figure 4.5: Google map image for Divar Island site (Site 3)

Figure 4.6: Divar Island site (Site 3)

Table 4.4: Physicochemical parameters for Chorao Island site (Site 4)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
22-07-2023	10:30 AM	1‰	26.5°C	97.9 mm	27°C /26°C	High tide	Monsoon season 2.987 mg/L
05-11-2023	10:45 AM	14‰	28.5°C	0 mm	28°C /27°C	High tide	
24-12-2023	9:50 AM	30‰	27°C	0 mm	28°C /25°C	Low tide	
26-01-2024	9:45 AM	26‰	29°C	0 mm	32°C /22°C	Low tide	Post-monsoon season 0.812 mg/L
06-02-2024	10:45 AM	28‰	29.5°C	0 mm	34°C /23°C	High tide	

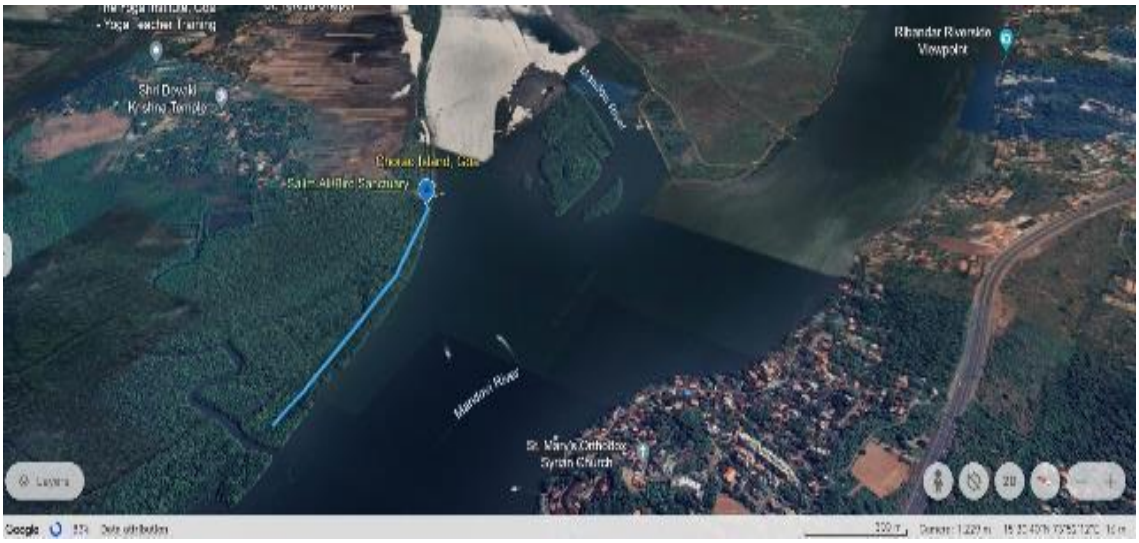


Figure 4.7: Google map image for Chorao Island site (Site 4)

Figure 4.8: Chorao Island site (Site 4)

Table 4.5: Physicochemical parameters for Penhe de Franca -Britona site (Site 5)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
22-07-2023	10:25 AM	4‰	28°C	97.9 mm	29°C /27°C	High tide	Monsoon season 1.001 mg/L
01-11-2023	11:30 PM	4‰	30.5°C	0 mm	33°C /32°C	Low tide	
23-12-2023	10:00 AM	21‰	29°C	0 mm	33°C /32°C	High tide	Post-monsoon season 0.813 mg/L
16-01-2024	11:10 AM	6‰	26°C	0 mm	26°C /24°C	Low tide	
05-02-2024	9:20 AM	26‰	26°C	0 mm	33°C /25°C	High tide	

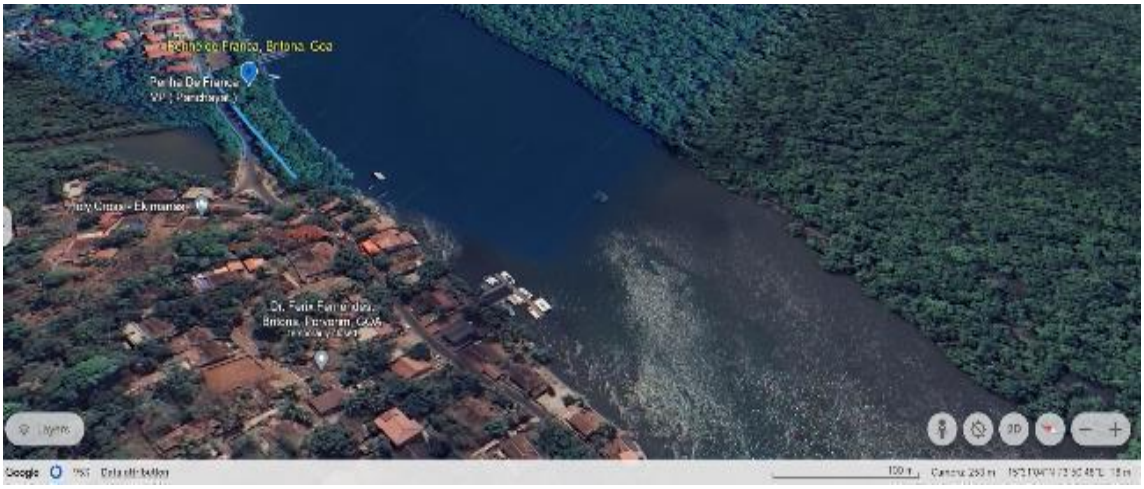


Figure 4.9: Google map image for Penhe de Franca - Britona site (Site 4)

Figure 4.10: Pehe de Franca - Britona site (Site 4)

Table 4.6: Physicochemical parameters for Ponte de Linhares Causeway-Ribandar site (Site 6)

Date of water sample collection	Time of water sample collection	Salinity (ppt/‰)	Temperature of water sample collected (°C)	Rainfall (in mm)	Ambient Temperature (°C)	Tide level	BOD (mg/L)
01-07-2023	11:30 PM	1‰	30°C	12.7 mm	30°C /27°C	High tide	Monsoon season 2.114 mg/L
01-11-2023	10:30 AM	5‰	28.5°C	89.2 mm	29°C /27°C	High tide	
17-12-2023	9:30 AM	30‰	27.5°C	0 mm	27°C /25°C	High tide	
16-01-2024	9:45 AM	31‰	26°C	0 mm	26°C /24°C	Low tide	Post-monsoon season 1.3 mg/L
06-02-2024	9:30 AM	29‰	27°C	0 mm	27°C /23°C	High tide	

Figure 4.11: Google map for Ponte de Linhares Causeway - Ribandar site (Site 6)

Figure 4.12: Ponte de Linhares Causeway - Ribandar site (Site 6)

Climatic seasonal variations suggest that the salinity in the Monsoon months (1‰ to 14 ‰) and Post-monsoon months (8‰ to 31‰); Sea-surface temperature in the Monsoon months (26.5°C to 30.5°C) and Post-monsoon months (26°C to 31.5°C). The BOD was observed to follow within the ranges between 0.6 mg/L to 2.9 mg/L (Monsoon season) and 0.4 mg/L to 2.6 mg/L (Post-monsoon season).

A higher degree of salinity and temperature variations were observed in the Post-monsoon season in comparison to the Monsoon season. The BOD findings suggest that moderately clean water with minimal organic matter content was observed in both, the Monsoon and Post-monsoon seasons.

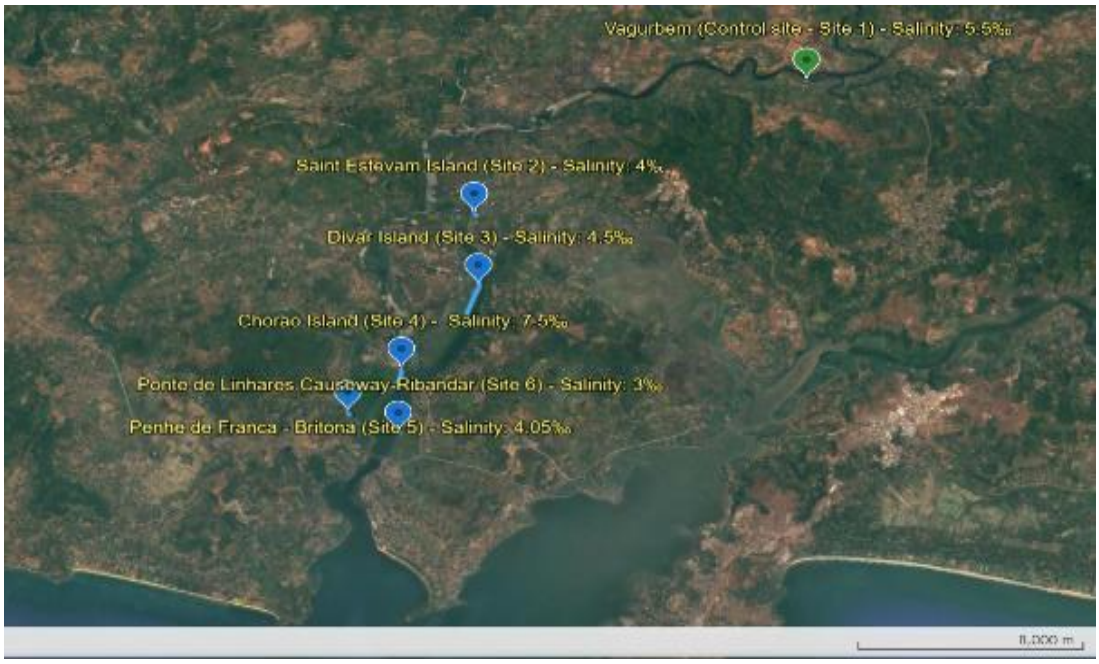


Figure 4.13: Google map image of salinity at selected sampling sites during monsoon season

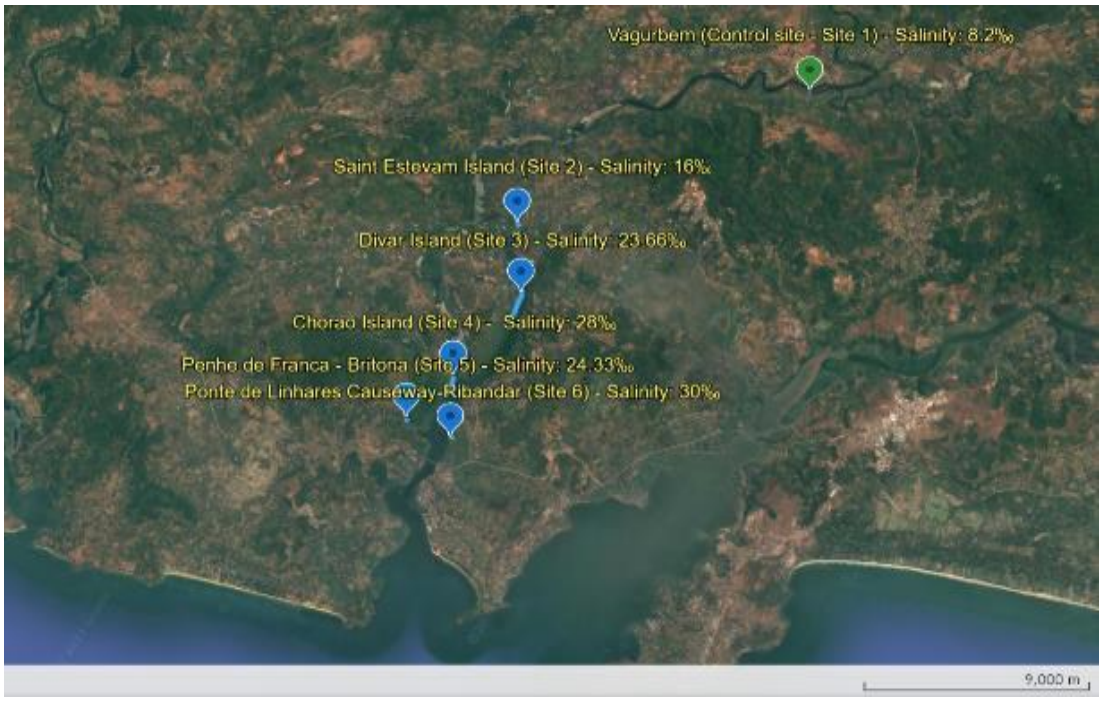


Figure 4.14: Google map image of salinity at selected sampling sites during post-monsoon season

4.1.2 Identification and documentation of mangrove flora

In reference to the given result on the coverage of mangrove flora, Table 4.7 depicts the mangrove flora species diversity along Saint Estevam Island site (Site 2), Table 4.8 depicts the mangrove flora species diversity along Divar Island site (Site 3), Table 4.9 depicts the mangrove flora species diversity along Chorao Island site (Site 4), Table 4.10 depicts the mangrove flora species diversity along Penhe de Franca – Britona site (Site 5), and Table 4.11 depicts the mangrove flora species diversity along Ponte de Linhares Causeway – Ribandar ste (Site 6). Figure 4.15 depicts the herbarium for *Aegiceras corniculatum* and *Clerodendrum inerme* both flora specimen from Saint Estevam Island site (Site 2), Figure 4.16 depicts the herbarium for *Avicennia officinalis* from Ponte de Linhares Causeway – Ribandar site (Site 5) and *Rhizophora apiculata* from Divar Island site (Site 3), Figure 4.17 depicts the herbarium for *Acrostichum aureum* and *Acanthus illicifolius* both flora specimen from Chorao Island (Site 4), Figure 4.18 depicts the herbarium for *Avicennia marina* and *Avicennia officinalis* both flora specimen collected from Saint Estevam Island (Site 2), and Figure 4.19 depicts the herbarium for *Bruguiera cylindrica* from Penhe de Franca – Britona site (Site 5) and *Rhizophora apiculate* from Ponte de Linhares Causeway – Ribandar site (Site 6). Figures 4.20, 4.22, 4.24, 4.26, and 4.28 depict the graphical representation of mangrove flora diversity count from Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively. Figures 4.21, 4.23, 4.25, 4.27, and 4.29 depict the images of different mangrove flora species documented along Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe

de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively.

The mangrove flora species were identified and documented for their diversity and count from the five selected sampling sites along the Mandovi estuary, mainly Saint Estevam Island site, Divar Island site, Chorao Island site, Penhe de Franca – Britona site, and Ponte de Linhares Causeway – Ribandar site respectively. The control site – Vagurbem site displayed no mangrove flora coverage as located closest to the mouth of Mandovi river. The flora specimens were collected and herbariums were prepared for the collected mangrove flora samples. Furthermore, the mangrove flora (macroflora) documented from each sampling site was characterized site-wise based on its diversity and abundance count within one kilometer of each sampling sites. Graphical representation of the mangrove flora diversity count in terms of bar graphs from each sampling site were also described.

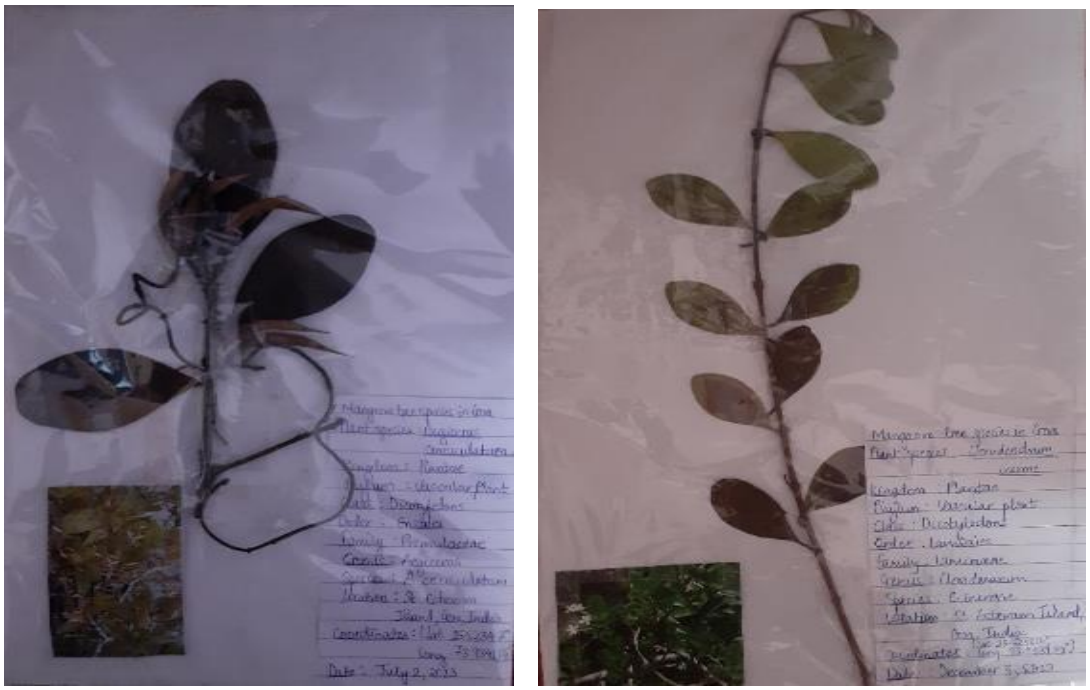


Figure 4.15: Herbarium of *Aegiceras corniculatum* and *Clerodendrum inerme*

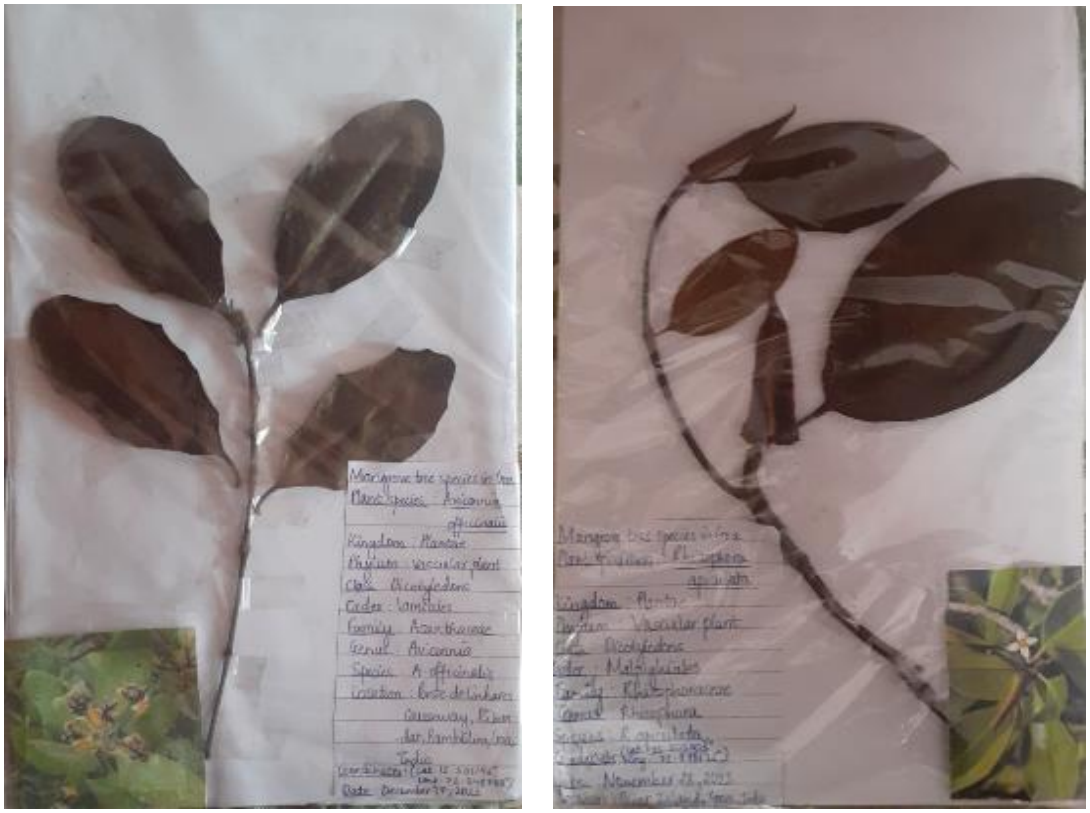


Figure 4.16: Herbarium of *Avicennia officinalis* and *Rhizophora apiculata*

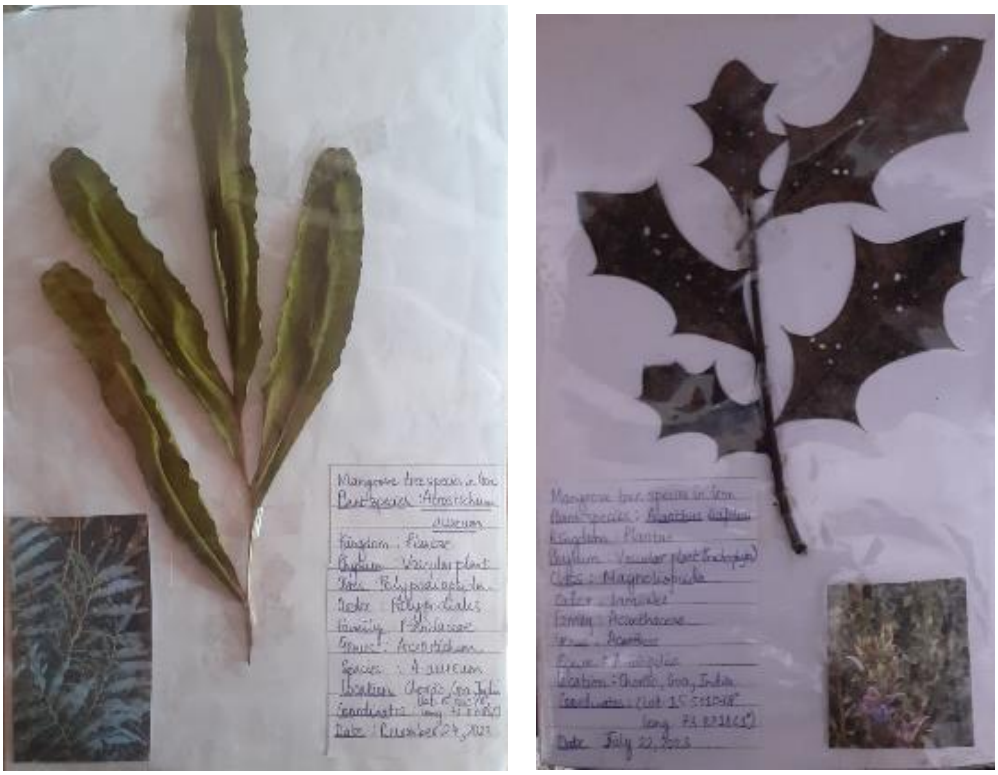


Figure 4.17: Herbarium of *Acrostichum aureum* and *Acanthus illicifolius*

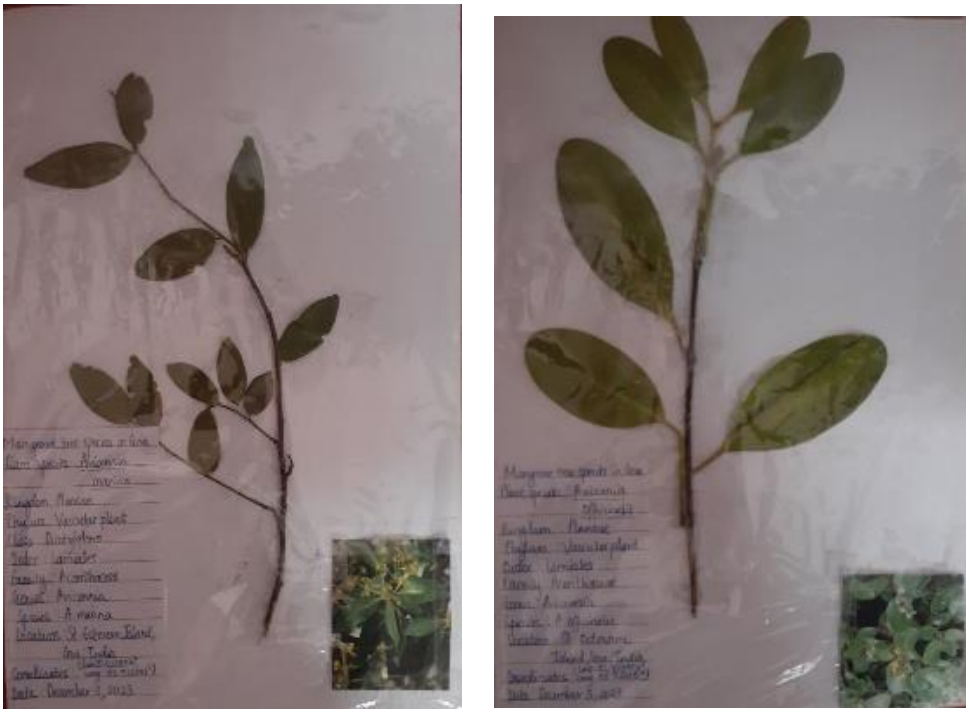


Figure 4.18: Herbarium of *Avicennia marina* and *Avicennia officinalis*

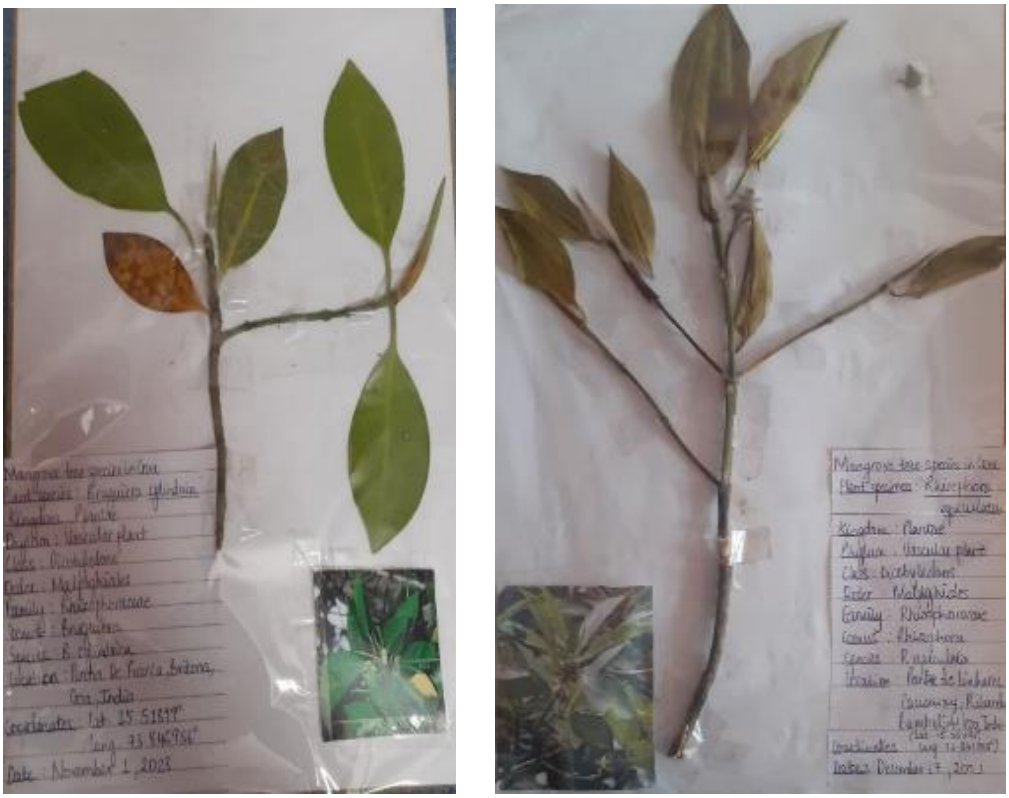


Figure 4.19: Herbarium of *Bruguiera cylindrica* and *Rhizophora apiculata*

Table 4.7: Macrofloral diversity at Saint Estevam Island site (Site 2)

Mangrove flora diversity	Macroflora count (within 1 km)
Aegiceras cornciculatum	**4**
Avicennia officinalis	**7**
Avicennia marina	**1**
Acanthus illicifolius	**2**
Clerodendrum inerme	**9**
Exocecaria agallocha	**1**
Avicennia alba	**5**
Derris heterophylla	**10**
Rhizophora mucronata	**3**
Rhizophora apiculata	**8**

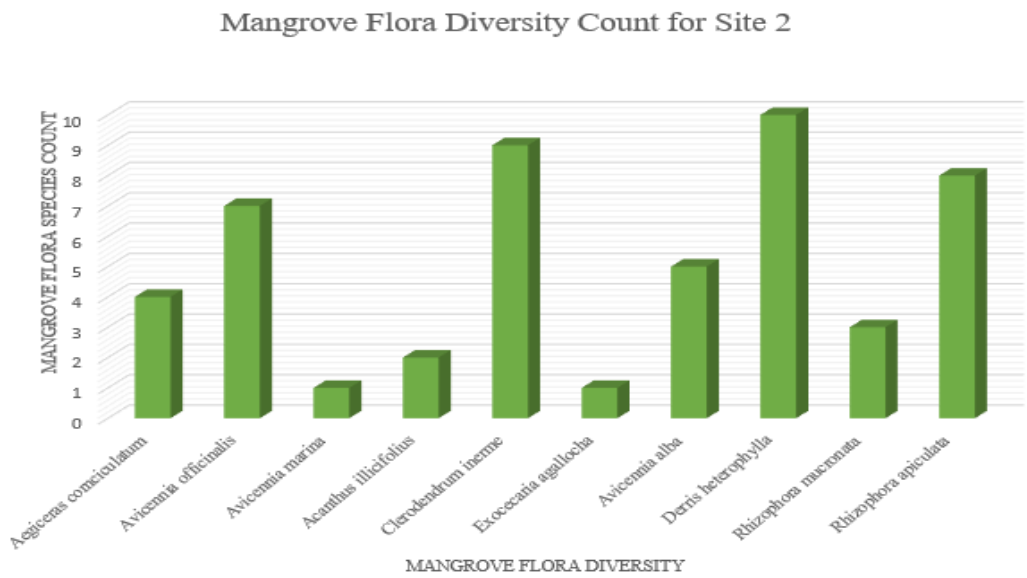


Figure 4.20: Graphical representation for mangrove flora diversity count for Saint Estevam Island site (Site 2)

Avicennia officinalis

Exocecaria agallocha

Aegiceras cornciculatum

Acanthus illicifolius

Avicennia marina

Clerodendrum inerme

Rhizophora mucronate

Derris heterophylla

Rhizophora apiculata

Figure 4.21: Mangrove flora diversity at Saint Estevam Island site (Site 2)

Table 4.8: Macrofloral diversity at Divar Island site (Site 3)

Mangrove flora diversity	Macroflora count (within 1 km)
Rhizophora apiculata	**11**
Avicennia officinalis	**15**
Avicennia marina	**26**
Acanthus illicifolius	**9**
Clerodendrum inerme	**18**
Avicennia alba	**7**
Rhizophora mucronata	**8**
Excoecaria agallocha	**5**
Sonneratia alba	**3**

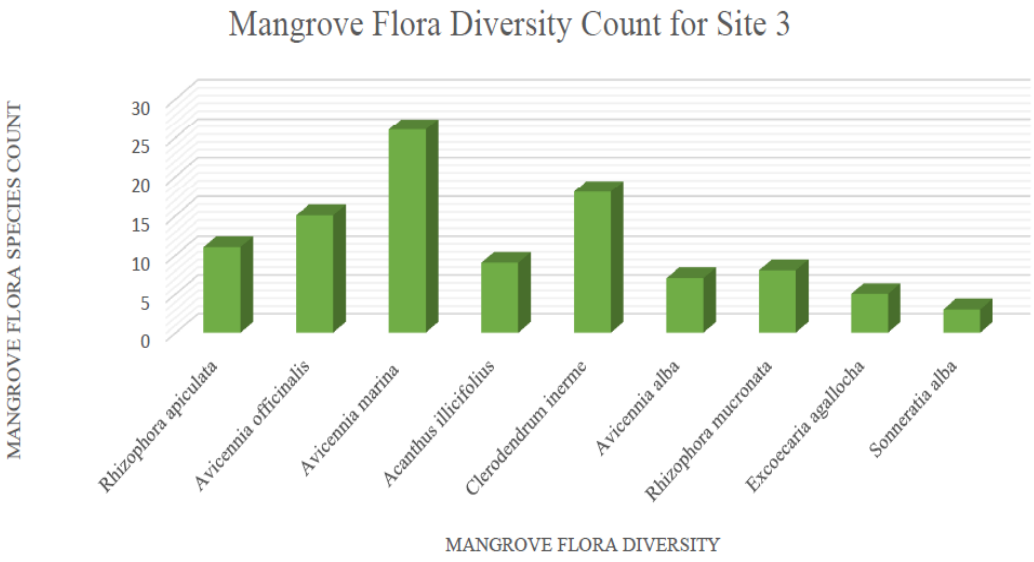


Figure 4.22: Graphical representation for mangrove flora diversity count for Divar Island site (Site 3)

Clerodendrum inerme

Avicennia marina

Acanthus illicifolius

Rhizophora mucronata

Excoecaria agallocha

Avicennia officinalis

\

Rhizophora apiculata

Sonneratia alba

Figure 4.23: Maangrove flora diversity at Divar Island site (Site 3)

Table 4.9: Macrofloral diversity at Chorao Island site (Site 4)

Mangrove flora diversity	Macroflora count (within 1 km)
Avicennia officinalis	**11**
Avicennia marina	**16**
Avicennia alba	**3**
Acanthus illicifolius	**12**
Aegiceras corniculatum	**7**
Excocecaria agallocha	**5**
Acrostichum aureum	**17**
Cleodendrum inerme	**12**
Rhizophora apiculata	**8**
Rhizophora mucronata	**36**
Bruguiera cylindrica	**7**
Derris heterophylla	**3**
Kandelia candel	**4**

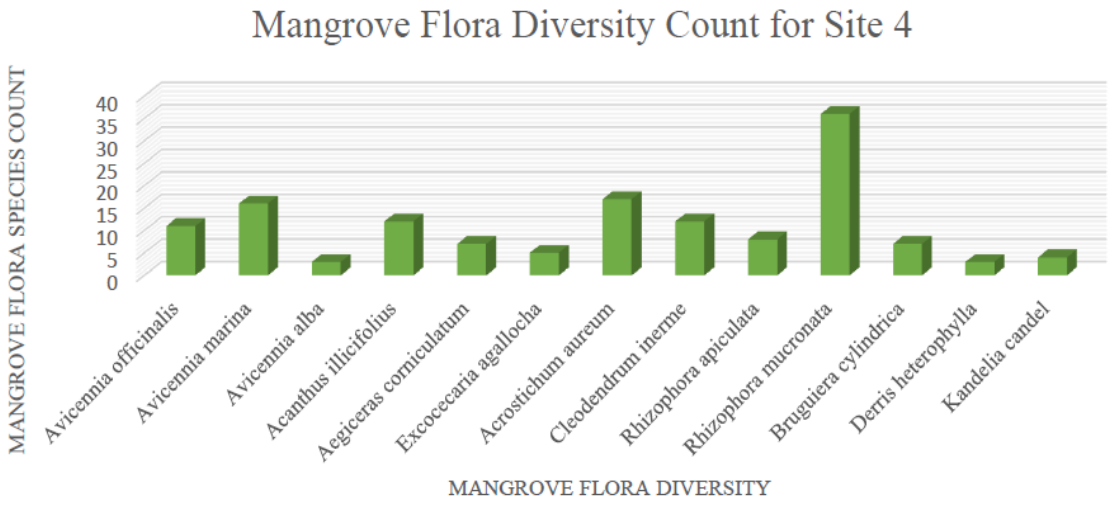


Figure 4.24: Graphical representation for mangrove flora diversity count for Chorao Island site (Site 4)

Acanthus illicifolius

Rhizophora mucronata

Rhizophora apiculata

Avicennia officinalis

Aegiceras corniculatum

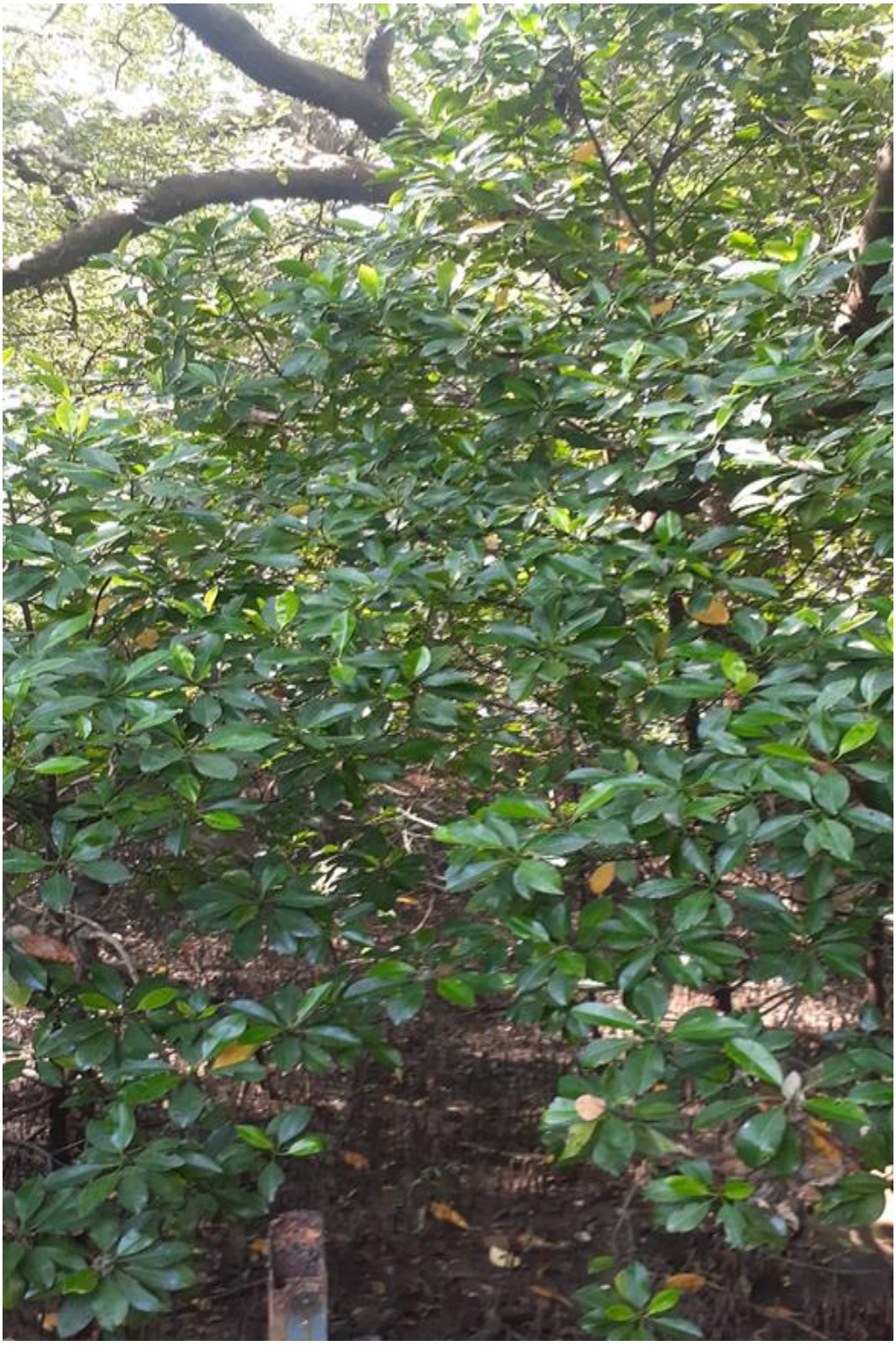
Acrostichum aureum

Excocecaria agallocha

Avicennia marina

Cleodendrum inerme

Derris heterophylla



Bruguiera cylindrica

Figure 4.25: Mangrove flora diversity at Chorao Island site (Site 4)

Table 4.10: Macrofloral diversity at Penhe de Franca - Britona site (Site 5)

Mangrove flora diversity	Macroflora count (within 1 km)
Rhizophora apiculata	**7**
Bruguiera cylindrica	**8**
Acanthus illicifolius	**11**
Avicennia officinalis	**3**
Avicennia marina	**13**
Rhizophora mucronata	**15**
Derris heterophylla	**9**
Avicennia alba	**7**

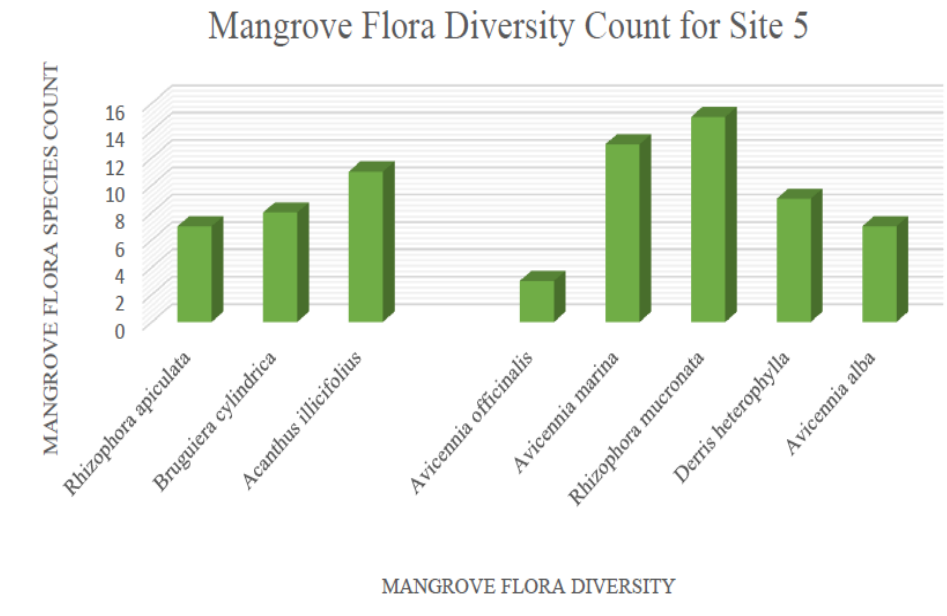


Figure 4.26: Graphical representation for mangrove flora diversity count for Penhe de Franca - Britona site (Site 5)

Acanthus illicifolius

Bruguiera cylindrica

Rhizophora apiculate

Avicennia marina

Derris heterophylla

Avicennia officinalis

Rhizophora mucronata

Figure 4.27: Mangrove flora diversity at Penhe de Franca – Britona site (Site 5)

Table 4.11: Macrofloral diversity at Ponte de Linhares Causeway - Ribandar site (Site 6)

Mangrove flora diversity	Macroflora count (within 1 km)
Rhizophora apiculata	**17**
Avicennia officinalis	**7**
Avicennia marina	**9**
Acanthus illicifolius	**12**
Clerodendrum inerme	**3**
Excocecaria agallocha	**5**
Avicennia alba	**6**
Rhizophora mucronata	**16**
Derris heterophylla	**3**

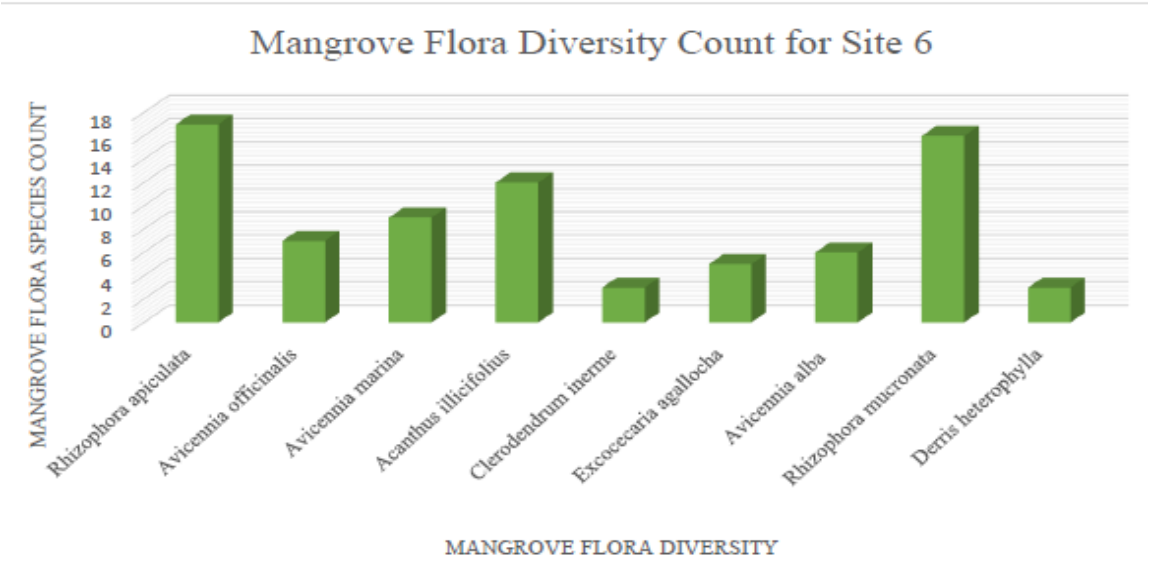


Figure 4.28: Graphical representation for mangrove flora diversity count for Ponte de Linhares Causeway - Ribandar site (Site 6)

Clerodendrum inerme

Excocecaria agallocha

Avicennia officinalis

Acanthus illicifolius

Rhizophora apiculata

Rhizophora apiculata

Avicennia marina

Avicennia marina

Derris heterophylla

Rhizophora mucronata

Figure 4.29: Mangrove flora diversity at Ponte de Linhares Causeway – Ribandar site (Site 6)

4.1.3 Identification and documentation of associated macrofauna and bacterial microflora

In reference to the given result, Table 4.12 depicts the common macrofaunal diversity observed at Vagurbem site (Site 1), Table 4.17 depicts the common macrofaunal diversity observed at Saint Estevam Island site (Site 2), Table 4.22 depicts the common macrofaunal diversity observed at Divar Island site (Site 3), Table 4.27 depicts the common macrofaunal diversity observed at Chorao Island site (Site 4), Table 4.32 depicts the common macrofaunal diversity observed at Penhe de Franca – Britona site (Site 5), Table 4.37 depicts the common macrofaunal diversity observed at Ponte de Linhares Causeway – Ribandar site (Site 6). Tables 4.13 and 4.14 depict the bacterial viable count for Vagurbem site (Site 1) during the Monsoon and Post-Monsoon seasons respectively. Tables 4.18 and 4.19 depict the bacterial viable count for Saint Estevam Island site (Site 2) during the Monsoon and Post-Monsoon seasons respectively. Tables4.23 and 4.24 depict the bacterial viable count for Divar Island site (Site 3) during the Monsoon and Post-Monsoon seasons respectively. Tables 4.28 and 4.29 depict the bacterial viable count for Chorao Island site (Site 4) during the Monsoon and Post-Monsoon seasons respectively. Tables 4.33 and 4.34 depict the bacterial viable count for Penhe de Franca – Britona site (Site 5) during the Monsoon and Post-Monsoon seasons respectively. Tables 4.38 and 4.39 depict the bacterial viable count for Ponte de Linhares Causeway – Ribandar site (Site 6) during the Monsoon and Post-Monsoon seasons respectively.

Tables 4.15 and 4.16 depict the Gram characteristics of the bacterial colonies from Vagurbem site (Site 1) during the Monsoon and Post-monsoon seasons respectively. Tables 4.20 and 4.21 depict the Gram characteristics of the bacterial

colonies from Saint Estevam Island site (Site 2) during the Monsoon and Post-monsoon seasons respectively. Tables 4.25 and 4.26 depict the Gram characteristics of the bacterial colonies from Divar Island site (Site 3) during the Monsoon and Post-monsoon seasons respectively. Tables 4.30 and 4.31 depict the Gram characteristics of the bacterial colonies from Chorao Island site (Site 4) during the Monsoon and Post-monsoon seasons respectively. Tables 4.30 and 4.31 depict the Gram characteristics of the bacterial colonies from Chorao Island site (Site 4) during the Monsoon and Post-monsoon seasons respectively. Tables 4.35 and 4.36 depict the Gram characteristics of the bacterial colonies from Penhe de Franca – Britona site (Site 5) during the Monsoon and Post-monsoon seasons respectively. Tables 4.40 and 4.41 depict the Gram characteristics of the bacterial colonies from Ponte de Linhares Causeway – Ribandar site (Site 6) during the Monsoon and Post-monsoon seasons respectively. Figures 4.30, 4.34, 4.38, 4.42, 4.46, and 4.50 depict the macrofauna diversity observed at Vagurbem site (Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively. Figures 4.31, 4.35, 4.39, 4.43, 4.47, and 4.51 depict the graphical representation of macrofaunal diversity count from at Vagurbem site (Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively.

Figures 4.32 and 4.33 depict the Gram-stained isolated bacterial colonies for Vagurbem site (Site 1) during the Monsoon and Post-Monsoon seasons respectively. Figures 4.36 and 4.37 depict the Gram-stained isolated bacterial colonies

for Saint Estevam Island site (Site 2) during the Monsoon and Post-Monsoon seasons respectively. Figures 4.40 and 4.41 depict the Gram-stained isolated bacterial colonies for Divar Island site (Site 3) during the Monsoon and Post-Monsoon seasons respectively. Figures 4.44 and 4.45 depict the Gram-stained isolated bacterial colonies for Chorao Island site (Site 4) during the Monsoon and Post-Monsoon seasons respectively. Figures 4.44 and 4.45 depict the Gram-stained isolated bacterial colonies for Penhe de Franca – Britona site (Site 5) during the Monsoon and Post-Monsoon seasons respectively. . Figures 4.52 and 4.53 depict the Gram-stained isolated bacterial colonies for Ponte de Linhares Causeway – Ribandar site (Site 6) during the Monsoon and Post-Monsoon seasons respectively.

From the selected six sampling sites, the macrofaunal diversity and abundance count was documented. The prevalent macrofauna associated within the mangrove ecosystems observed were further classified into the categories, that is, avifauna species, terrestrial invertebrate fauna species, and aquatic fauna species respectively. The coverage of macrofauna in terms of its diversity and count during the Monsoon and Post-monsoon season have been described site-wise. The bacterial microflora was isolated on Zobell Marine Agar from water samples obtained from estuarine water samples obtained from each sampling site in the Monsoon and Post-monsoon season. Further, the colony characteristics and bacterial viable count of the isolated bacterial microflora were observed and subsequently the identification of bacterial microflora was carried out by Gram staining characteristics.

Table 4.12: Common macrofaunal diversity at Vagurbem site (Site 1)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
25.44 m	385 m^2	*Corvus* (Crow)	32	*Castalius rosimon* (Common Pierrot butterfly)	1	*Rasbora dandia* (Broad-striped rasbora)	>3
		Abroscopus superciliaris (Yellow-bellied Warbler)	1	*Pieris rapae* (Cabbage white butterfly)	1		
		Aegithina tiphia (Common Iora)	2	*Neptis hylas* (Common sailor butterfly)	2		
		Phalacrocorax auratus (Double-crested Cormorant)	1	*Eurema hecabe* (Common grass yellow butterfly)	9		
		Haliastur indus (Brahminy Kite)	1	*Camponotus compressus* (Indian black ant)	5		
		Motacilla cinerea (Grey wagtail)	1	*Enallagma cyathigerum* (Common blue damselfly)	1		
		Egretta garzetta (Little Egret)	1	*Heteronympha merope* (Common brown butterfly)	3		

Corvus

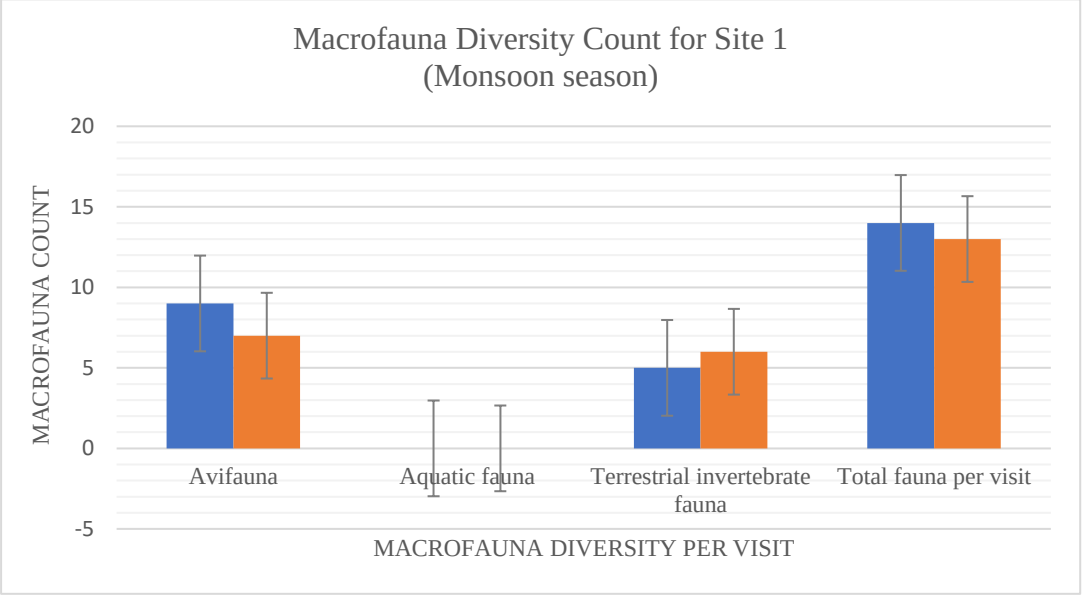
Haliastur indus

Egretta garzetta

Castalius rosimon

Enallagma cyathigerum

Figure 4.30: Macrofauna observed at Vagurbem site (Site 1)



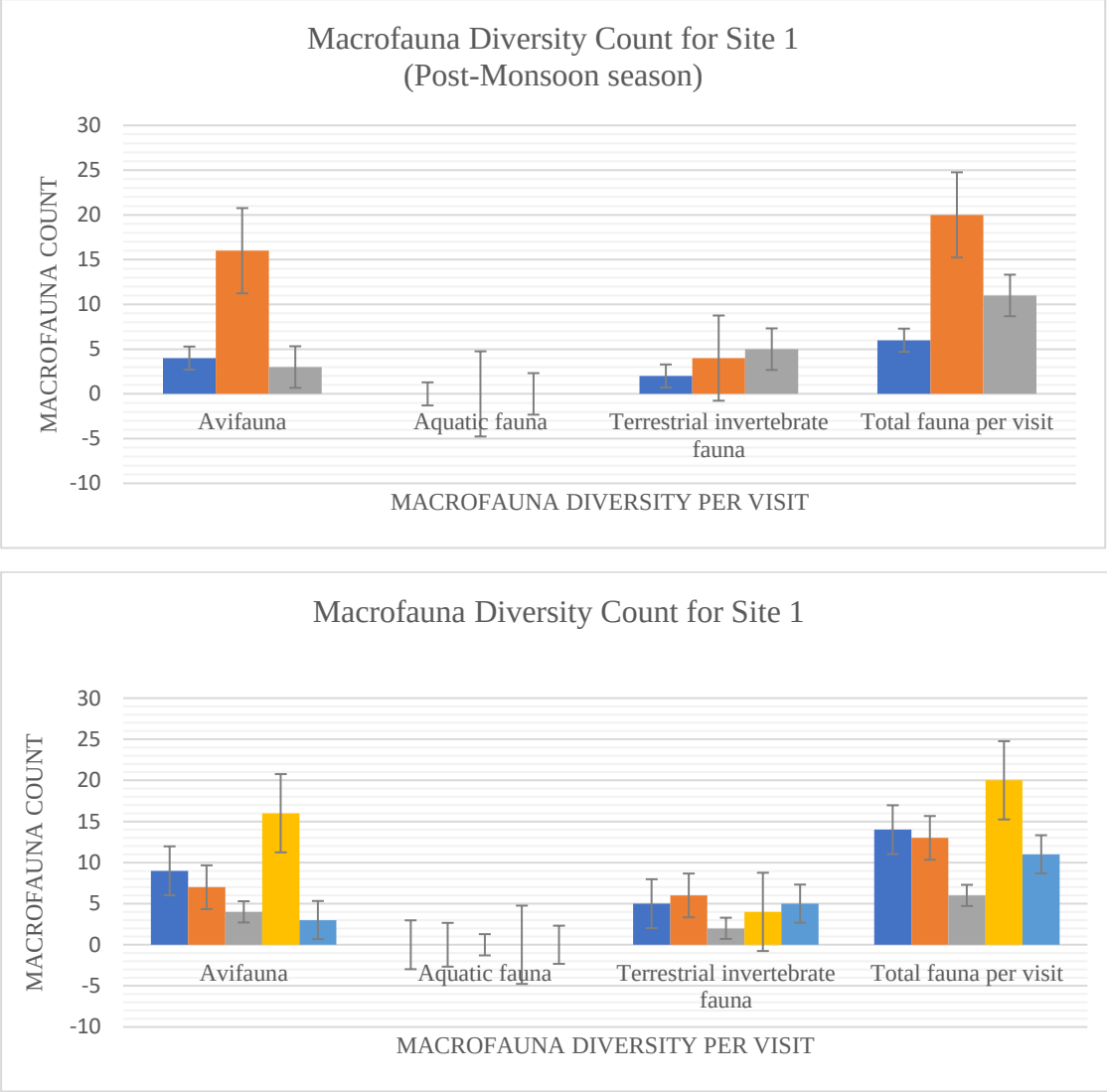


Figure 4.31: Graphical representation for macrofauna diversity count for Vagurbem site (Site 1)

Table 4.13: Bacterial Viable Count for Vagurbem site (Site 1) water sample for Monsoon season

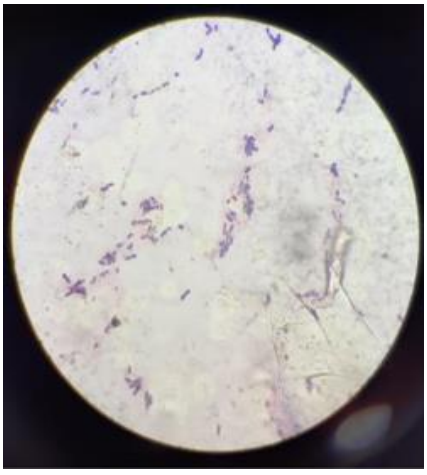
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Vagurbem	**11 colonies**	**0.011× 10^4 CFU/mL**
Vagurbem	**9 colonies**	**0.09× 10^4 CFU/mL**

Table 4.14: Bacterial Viable Count for Vagurbem site (Site 1) water sample for Post-monsoon season

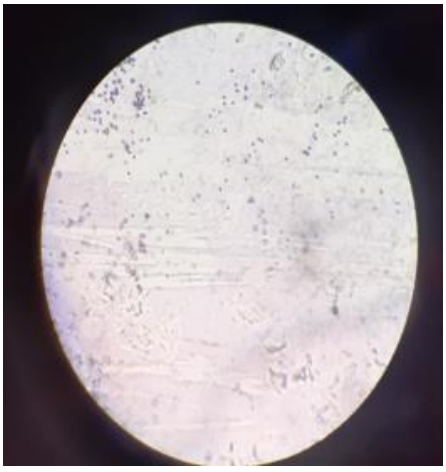
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Vagurbem	**204 colonies**	**0.204× 10^4 CFU/mL**
Vagurbem	**184 colonies**	**1.84 × 10^4 CFU/mL**

Table 4.15: Gram characteristics of the three isolated colonies from Vagurbem site (Site 1) water sample for Monsoon season

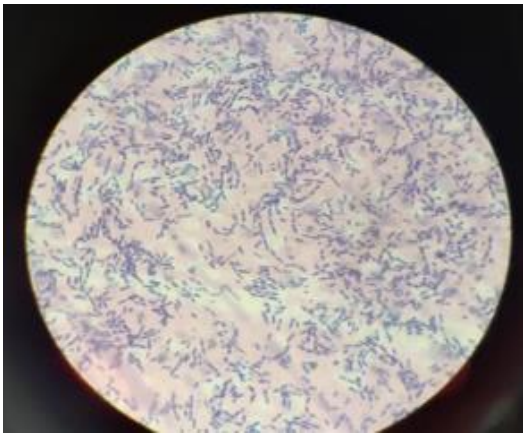
Colonies	**VW1**	**VW2**	**VW4**
Gram positive/ Gram negative	**Gram positive**	**Gram positive**	**Gram positive**
Shape	**Short rods**	**Cocci**	**Rods**



VW1



VW2

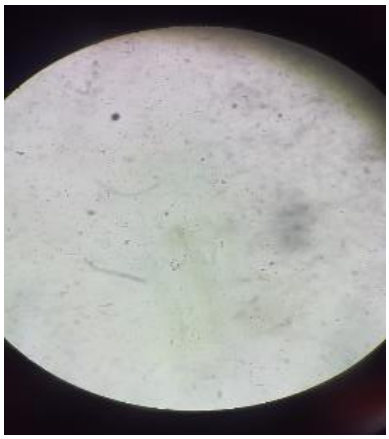


VW4

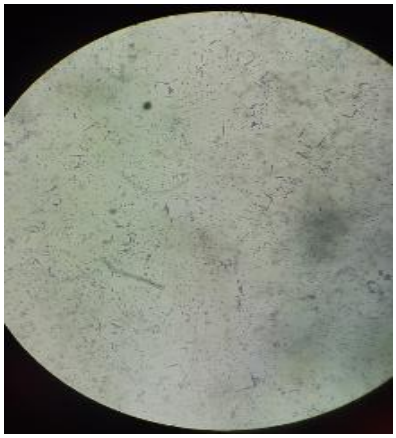
Figure 4.32: Gram-stained isolated colonies from Vagurbem site (Site 1) water sample for Monsoon season

Table 4.16: Gram characteristics of the four isolated colonies from Vagurbem site (Site 1) water sample for Post-monsoon season

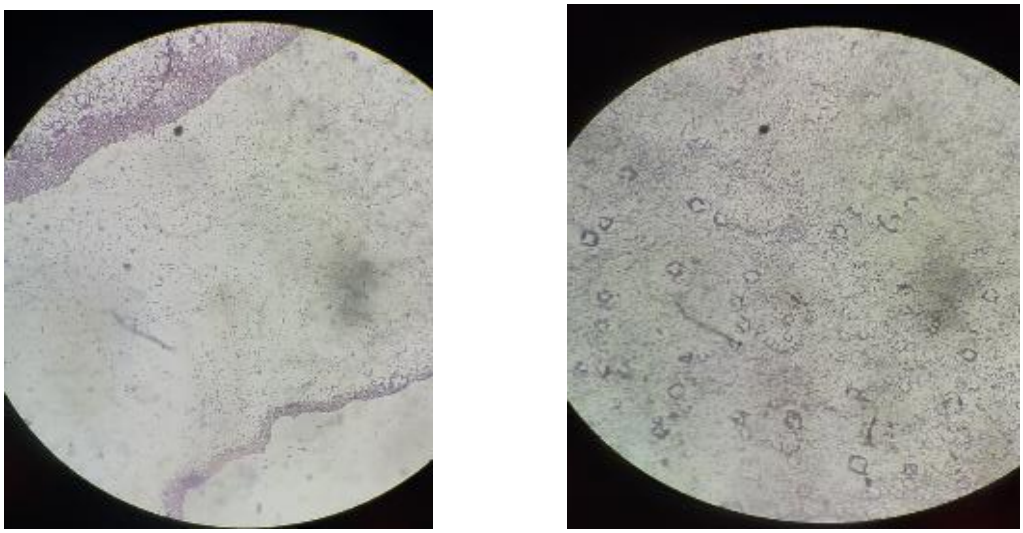
Colonies	**VW1**	**VW2**	**VW3**	**VW4**
Gram positive/ Gram negative	**Gram positive**	**Gram positive**	**Gram positive**	**Gram positive**
Shape	**Cocci**	**Short rods and cocci**	**Short rods and cocci**	**Short rods and cocci**



VW1



VW2



VW3 VW4

Figure 4.33: Gram-stained isolated colonies from Vagurbem site (Site 1) water sample for Post-monsoon season

Table 4.17: Common macrofaunal diversity at Saint Estevam Island site (Site 2)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
135.72 m	5630 m^2	*Ardea alba modesta* (Eastern Great Egret)	5	Troides minos (Southern birdwing butterfly)	2	*Anguilliformes* (Eel)	> 6
		Haliastur indus (Brahminy Kite)	16	*Pieris rapae* (Cabbage white butterfly)	2	*Megalops atlanticus* (Tarpon)	> 20-30
		Alcedo attis (Common Kingfisher)	2	*Neptis hylas* (Common sailor butterfly)	5	*Penaeus monodon* (Asian tiger shrimp)	<600
		Corvus (Crow)	11	*Eurema hecabe* (Common grass yellow butterfly)	16	*Mugil cephalus* (Flathead grey mullet)	> 5
		Anas zonorhyncha (Eastern spot-billed Duck)	3	*Junonia atlites* (Grey pansy butterfly)	10	*Epinephelus chlorostigma* (Reef cod)	> 45
		Dendrocygna javanica (Lesser Whistling Duck)	3	*Celastrina argiolus* (Holly blue butterfly)	13	*Lutjanus argentimaculatus* (Mangrove red snapper)	> 50-60
		Spizella pusilla (Field Sparrow)	1			*Engraulidae* (Anchovies)	> 50
		Merops orientalis (Asian green bee-eater)	1			*Parambassis ranga* (Indian glassy perch)	> 15
		Pycnonotus jocosus (Red-whiskered bulbul)	3			*Lates calcifer* (Asian sea bass)	> 15
		Leptocoma zeylonica (Purple-rumped sunbird)	5			*Leiognathus equulus* (Common ponyfish)	> 10
		Aegithina tiphia (Common Iora)	2			*Sphyraena barracuda* (Great Barracuda)	>15
		Ficedula zanthopygia (Yellow -rumped flycatcher)	3			*Etroplus suratensis* (Pearl Spot fish)	>20-30
						Scylla serrata (Mud crab)	18
						Uca rapax (Fiddler crab)	3

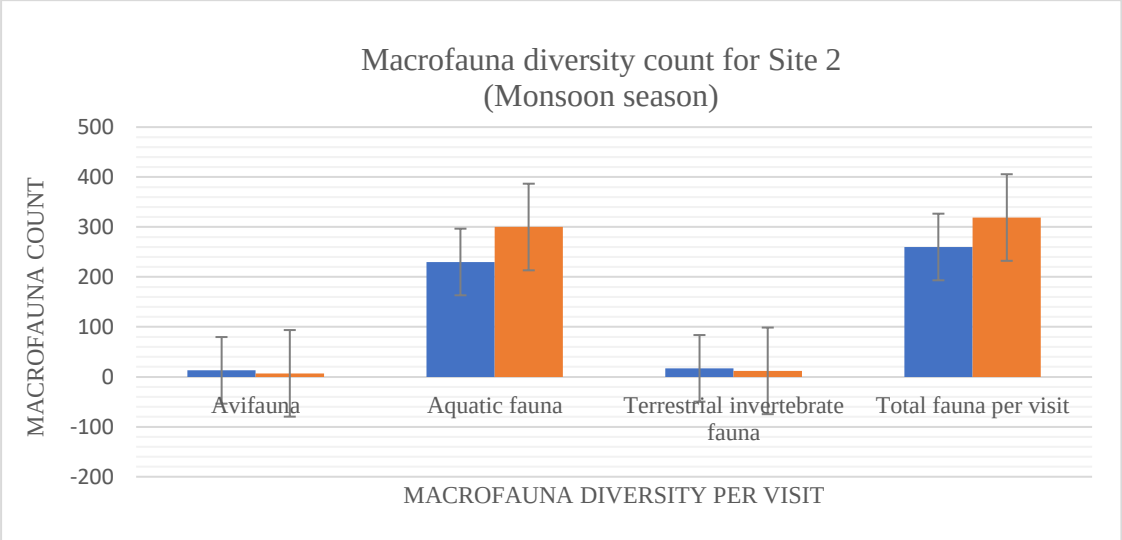
Ardea alba modesta

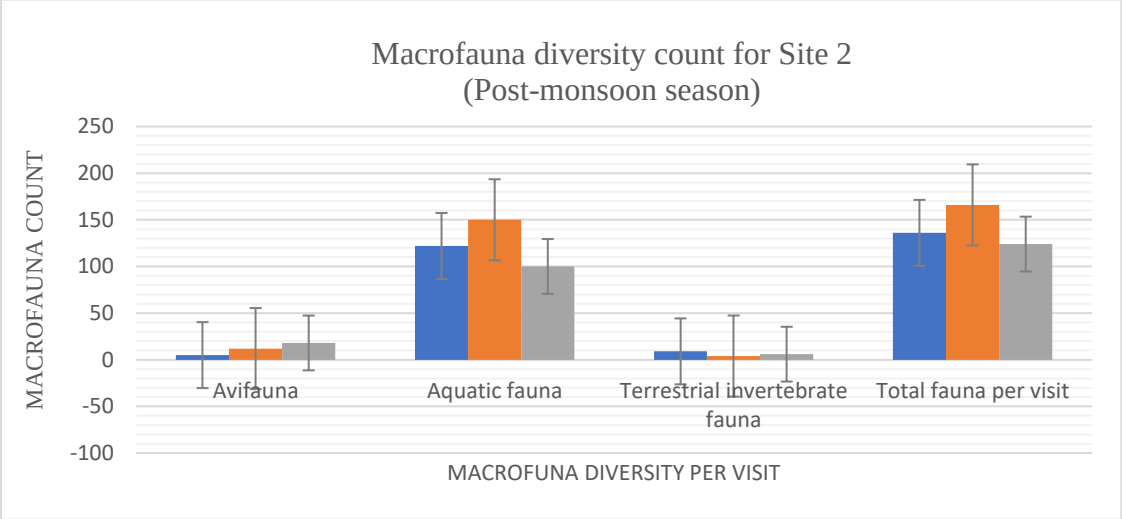
Haliastur indus

Uca rapax

Scylla serrata

Figure 4.34: Macrofauna observed at Saint Estevam Island site (Site 2)





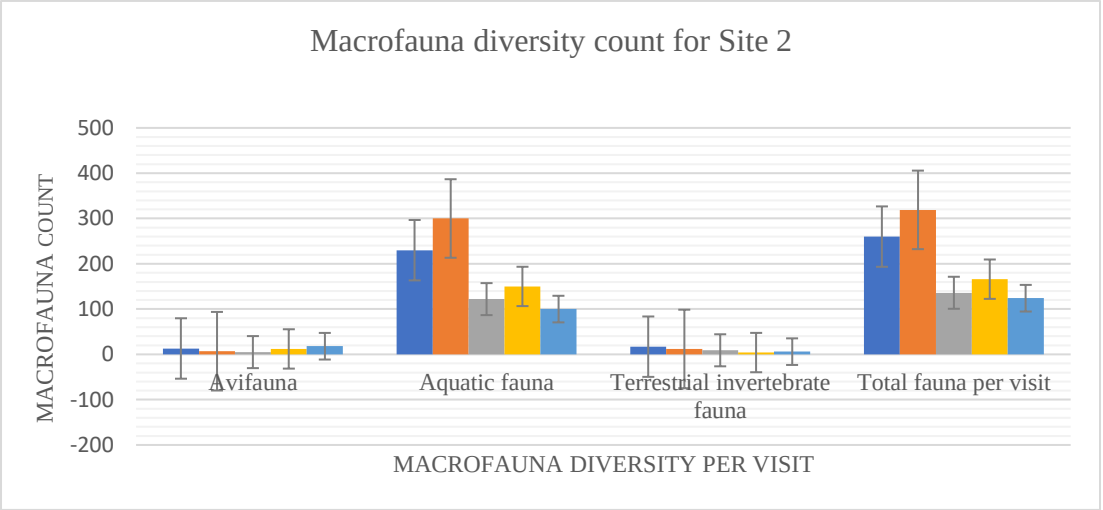


Figure 4.35: Graphical representation for macrofauna diversity count for Saint Estevam Island site (Site 2)

Table 4.18: Bacterial Viable Count for Saint Estevam Island site (Site 2) water sample for Monsoon season

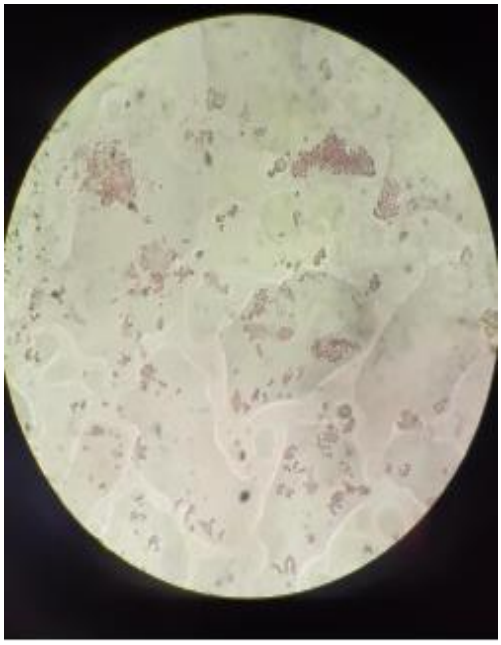
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Saint Estevam Island	**27 colonies**	**0.027× 10^4 CFU/mL**
Saint Estevam Island	**8 colonies**	**0.08× 10^4 CFU/mL**

Table 4.19: Bacterial Viable Count for Saint Estevam Island site (Site 2) water sample for Post-monsoon season

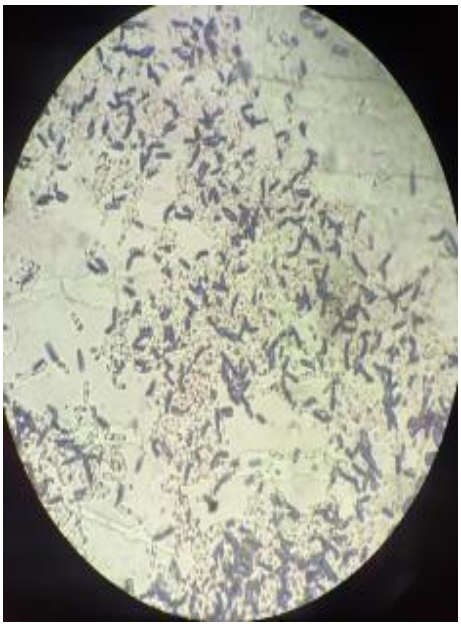
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Saint Estevam Island	**128 colonies**	**0.128 × 10^4 CFU/mL**
Saint Estevam Island	**92 colonies**	**0.92 × 10^4 CFU/mL**

Table 4.20: Gram characteristics of the three isolated colonies from Saint Estevam Island site (Site 2) water sample for Monsoon season

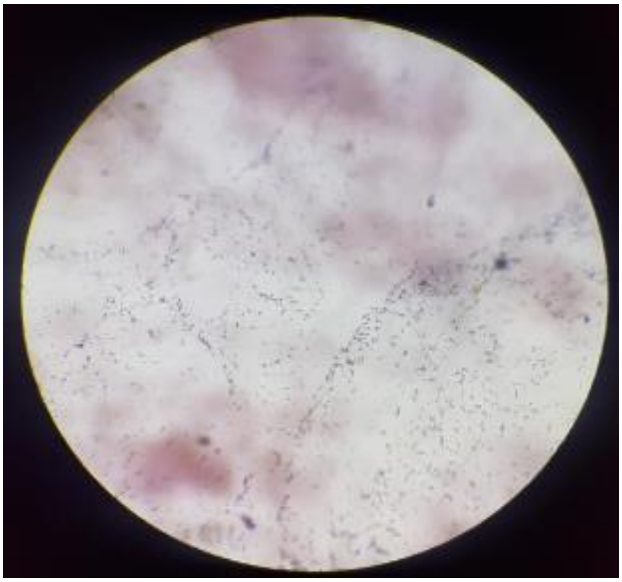
Colonies	SW1	SW2	SW4
Gram positive/ Gram negative	**Gram negative**	**Gram positive**	**Gram positive**
Shape	**Cocci**	**Rods**	**Short rods**



SW1



SW2

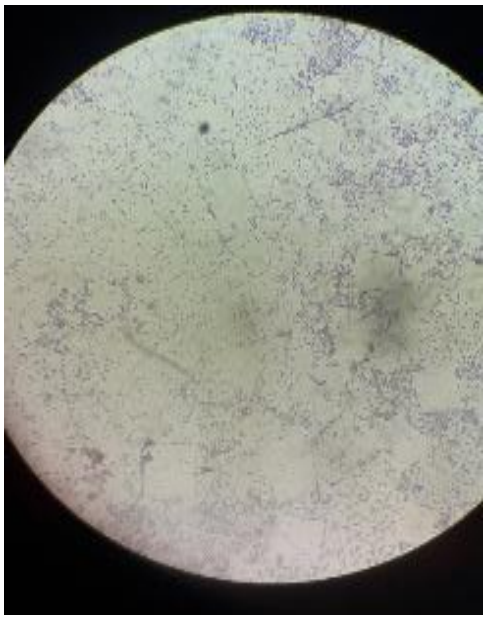


SW4

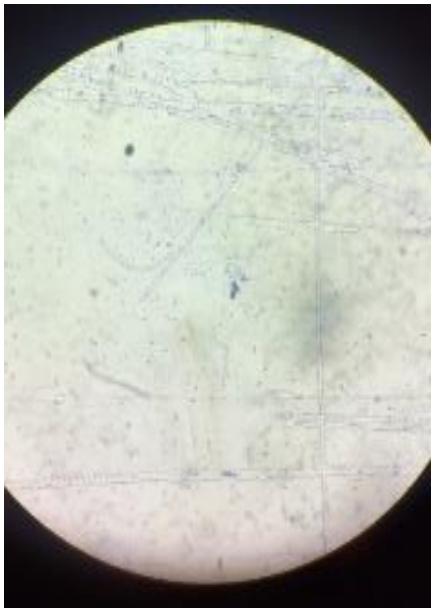
Figure 4.36: Gram-stained isolated colonies from Saint Estevam Island site (Site 2) water sample for Monsoon season

Table 4.21: Gram characteristics of the four isolated colonies from Saint Estevam Island site (Site 2) water sample for Post-monsoon season

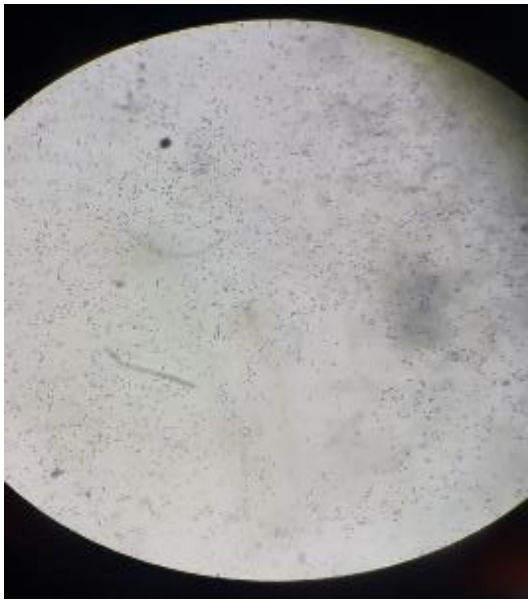
Colonies	SW1	SW2	SW3	SW4
Gram positive/ Gram negative	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Cocci	Cocci	Short rods and cocci	Short rods and cocci



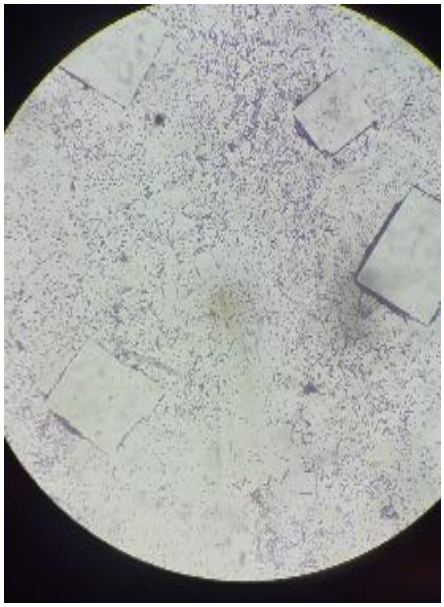
SW1



SW2



SW3



SW4

Figure 4.37: Gram-stained isolated colonies from Saint Estevam Island site (Site 2) water sample for Post-monsoon season

Table 4.22: Common macrofaunal diversity at Divar Island site (Site 3)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
1740 m	283531 m^2	*Ardea alba modesta* (Eastern Great Egret)	11	*Junonia atlites* (Grey pansy butterfly)	21	*Scylla serrata* (Mud crab)	60
		Aegithina tiphia (Common Iora)	1	*Eurema hecabe* (Common grass yellow butterfly)	10	*Sphyraena barracuda* (Great Barracuda)	15
		Haliastur indus (Brahminy Kite)	17			*Lutjanus argentimaculatus* (Mangrove red snapper)	>15
		Pycnonotus jocosus (Red-whiskered bulbul)	2	*Pieris napi* (Green-veined white butterfly)	2	*Thalassina anomala* (Mud lobster)	3
		Corvus (Crow)	8	*Heteronympha merope* (Common brown butterfly)	2	*Oxudercinae* (Mudskipper)	3
		Halcyon smyrnensis (White-throated Kingfisher)	40	*Agraulis vanilla* (Gulf fritillary butterfly	10	*Geosesarma* sp. (Vampire crab)	1
		Alcedo meninting (Blue-eared Kingfisher)	1	*Neurothemis tullia* (Pied paddy skimmer dragonfly)	5		
		Phalacrocorax auratus (Double-crested Cormorant)	2	*Colias cesonia* (Southern dogface butterfly)	5		
		Alcedo attis (Common Kingfisher)	3	*Dryas iulia* (Julia Heliconian butterfly)	2		
		Leptocoma zeylonica (Purple-rumped sunbird)	2				
		Threskiornis melanocephalus (Black-headed Ibis)	2				
		Merops orientalis (Asian green bee-eater)	2				
		Egretta garzetta (Little Egret)	1				
		Pelargopsis capensis (Stork-billed Kingfisher)	2				
		Microcarbo niger (Little Cormorant)	2				

Phalacrocorax auratus

Halcyon smyrnensis

Threskiornis melanocephalus

Microcarbo niger

Ardea alba modesta

Pycnonotus jocosus

Alcedo attis

Heteronympha merope

Pieris napi

Neurothemis tullia

Figure 4.38: Macrofauna observed at Divar Island site (Site 3)

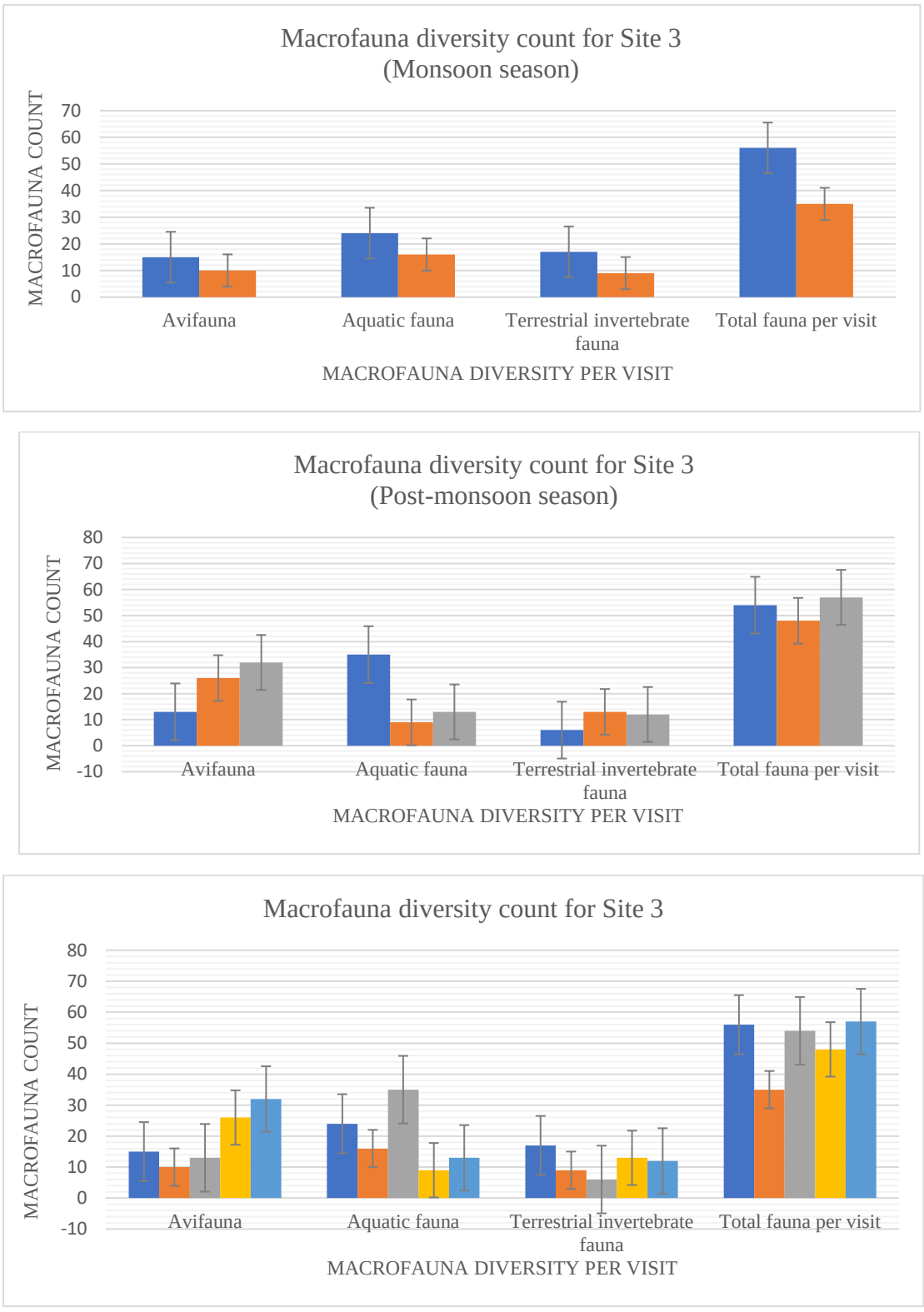


Figure 4.39: Graphical representation for macrofauna diversity count for Divar Island site (Site 3)

Table 4.23: Bacterial Viable Count for Divar Island site (Site 3) water sample for Monsoon season

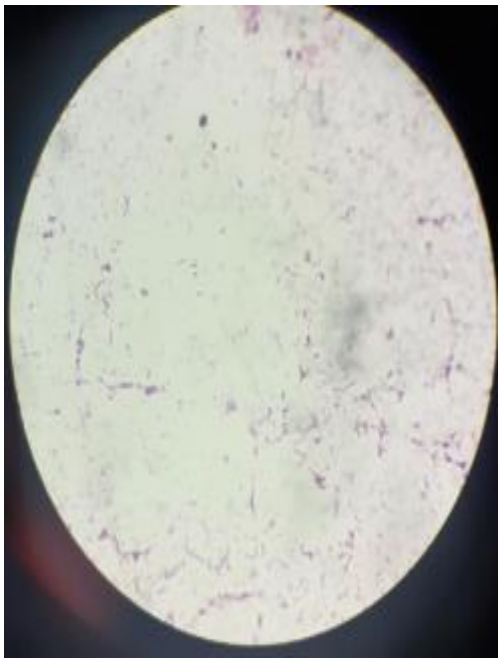
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Divar Island	**15 colonies**	**0.015×10^4 CFU/mL**
Divar Island	**48 colonies**	**0.48×10^4 CFU/mL**

Table 4.24: Bacterial Viable Count for Divar Island site (Site 3) water sample for Post-monsoon season

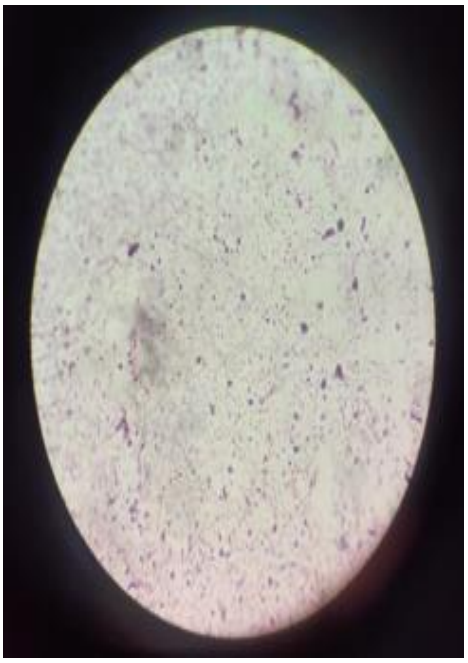
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Divar Island	**368 colonies**	**0.368×10^4 CFU/mL**
Divar Island	**38 colonies**	**0.38×10^4 CFU/mL**

Table 4.25: Gram characteristics of the five isolated colonies from Divar Island site (Site 3) water sample for Monsoon season

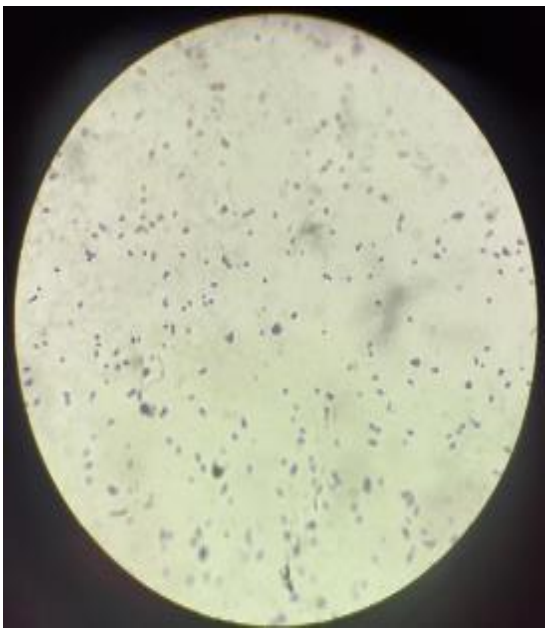
Colonies	DW1	DW2	DW3	DW4	DW5
Gram positive/ Gram negative	**Gram positive**	**Gram positive**	**Gram positive**	**Gram positive**	**Gram positive**
Shape	**Rods**	**Cocci and short rods**	**Cocci**	**Cocci**	**Cocci and rods**



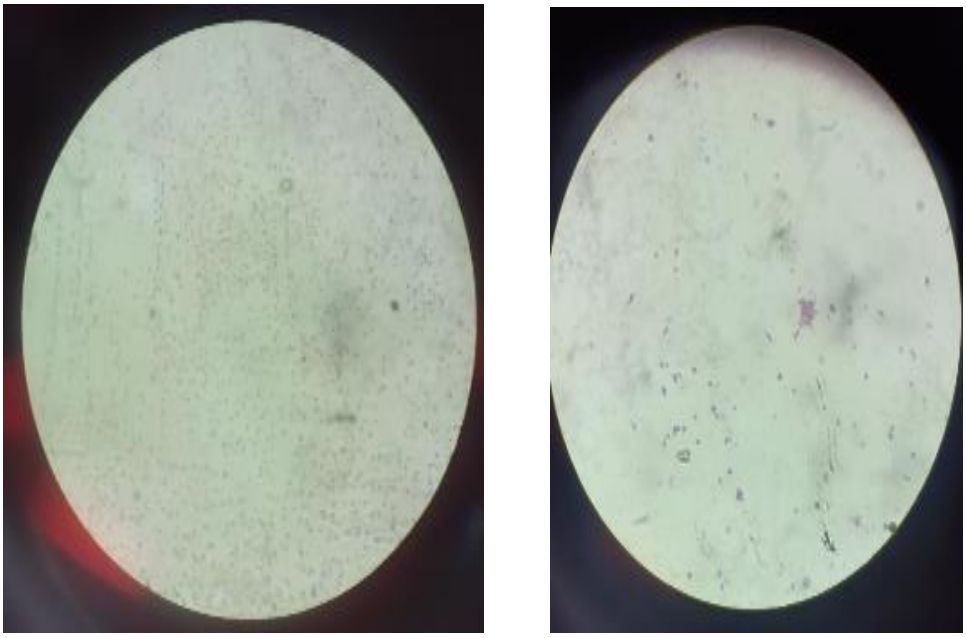
DW1



DW2



DW3

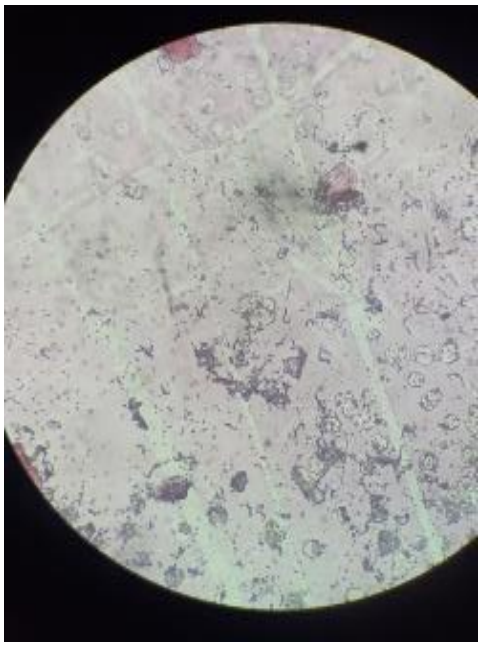


DW4 DW5

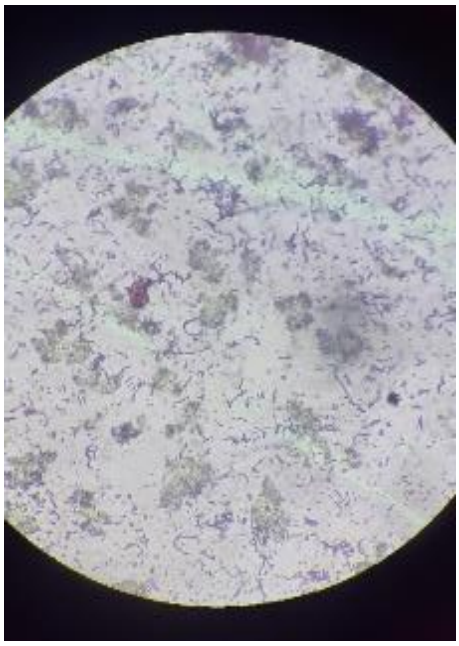
Figure 4.40: Gram-stained isolated colonies from Divar Island site (Site 3) water sample for Monsoon season

Table 4.26: Gram characteristics of the seven isolated colonies from Divar Island site (Site 3) water sample for Post-monsoon season

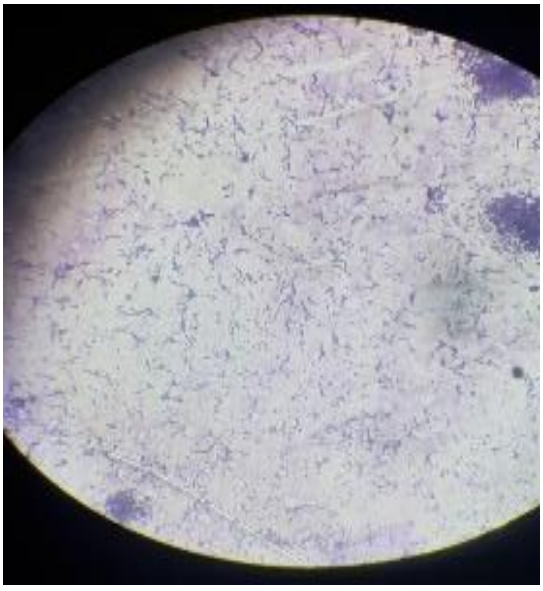
Colonies	DW1	DW2	DW3	DW4	DW5	DW6	DW7
Gram positive/ Gram negative	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Rods and cocci	Rods and cocci	Rods and cocci	Short rods and cocci	Short rods and cocci	Rods	Rods and cocci



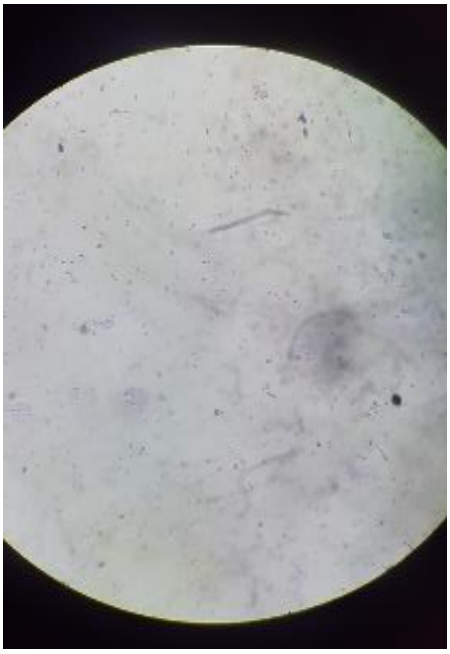
DW1



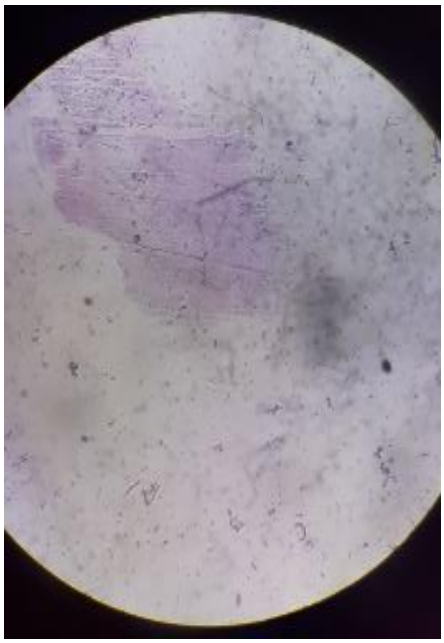
DW2



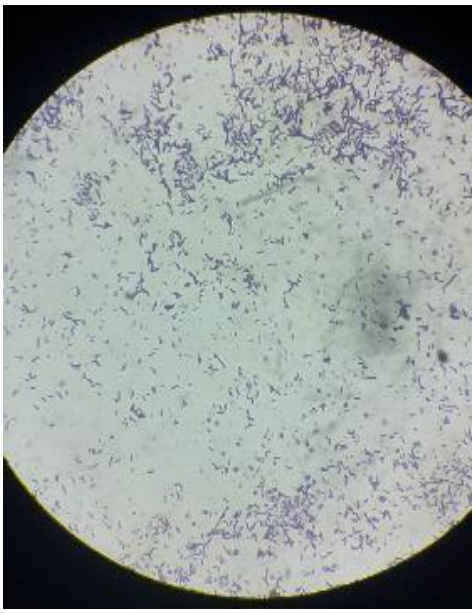
DW3



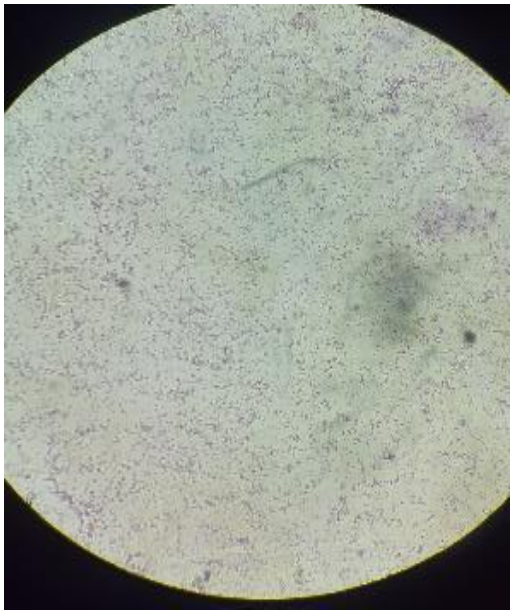
DW4



DW5



DW6



DW7

Figure 4.41: Gram-stained isolated colonies from Divar Island site (Site 3) water sample for Post-monsoon season

Table 4.27: Common macrofaunal diversity at Chorao Island site (Site 4)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
923 m	1780 m^2	*Ardea alba modesta* (Eastern Great Egret)	32	*Oxyopes salticus* (Striped lynx spider)	2	*Thalassina anomala* (Mud lobster)	35
		Pelargopsis capensis (Stork-billed kingfisher)	4	*Phocides pigmalion* (Mangrove Skipper moth)	4	*Scylla serrata* (Mud crab)	84
		Haliastur indus (Brahminy Kite)	13	*Idea leuconoe chersonesia* (Mangrove tree nymph butterfly)	2	*Littoraria angulifera* (Mangrove Periwinkle Snail)	6
		Butorides striata (Striated Heron)	2			*Uca pugnax* (Blue Fiddler crab)	36
		Coracias benghalensis (Indian Roller)	1	*Hygronemobius alleni* (Mangrove ground cricket)	1	*Megalops atlanticus* (Tarpon)	16
		Phalacrocorax auratus (Double-crested Cormorant)	3	*Neptis hylas* (Common sailor butterfly)	5	*Ostreidae* (True Oyster)	>50
		Pandion haliaetus (Osprey)	2	*Solenopsis* (Fire Ant)	15	*Polymesoda erosa* (Mud clams)	60
		Corvus (Crow)	23	*Desidae* (Intertidal spider)	2	*Taxotes jaculatrix* (Banded archerfish)	3
		Ardeola grayii (Indian Pond Heron)	3	*Heteronympha merope* (Common Brown butterfly)	4	*Gecarcinus quadratus* (Red land crab)	15
		Tringa totanus (Common Redshank)	4	*Eurema hecabe* (Common grass yellow butterfly)	2	*Oxudercinae* (Mudskipper)	3
		Merops orientalis (Asian green bee-eater)	2			*Penaeus monodon* (Juvenile Shrimp)	17
		Microcarbo niger (Little Cormorant)	1			*Austruca annulipes* (Porcelain Fiddler crab)	3
		Dendrocygna javanica (Lesser Whistling Duck)	1				7
		Acridotheres tristis (Common Myna)	2				
		Eudynamys scolopaceus (Asian Koel)	5				
		Dicrurus macrocercus (Black Drongo)	2				
		Threskiornis melanocephalus (Black-headed Ibis)	4				
		Egretta garzetta (Little Egret)	6				

Pandion haliaetus

Pelargopsis capensis

Coracias benghalensis

Haliastur indus

Butorides striata

Threskiornis melanocephalus

Ardea alba modesta

Ardeola grayii

Phalacrocorax auritus

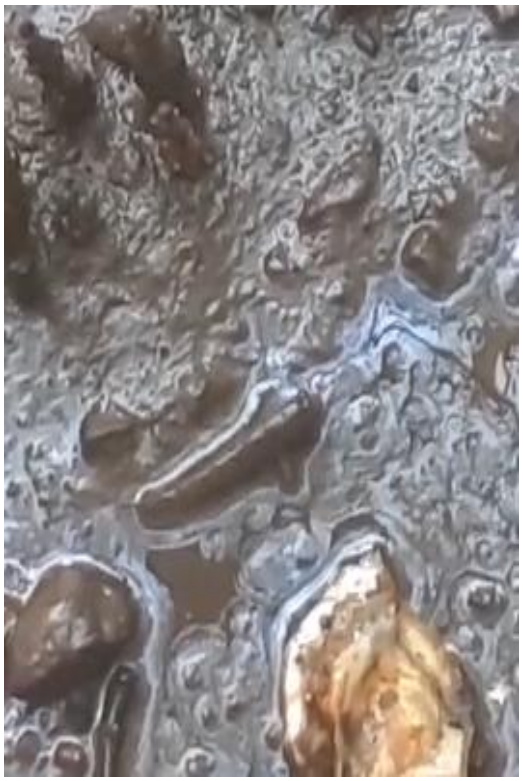
Uca pugnax

Hygronemobius alleni

Scylla serrata

Solenopsis

Ostreidae



Oxudercinae

Austruca annulipes

Thalassina anomala mound

Oxyopes salticus

Littoraria angulifera

Figure 4.42: Macrofauna observed at Chorao Island site (Site 4)

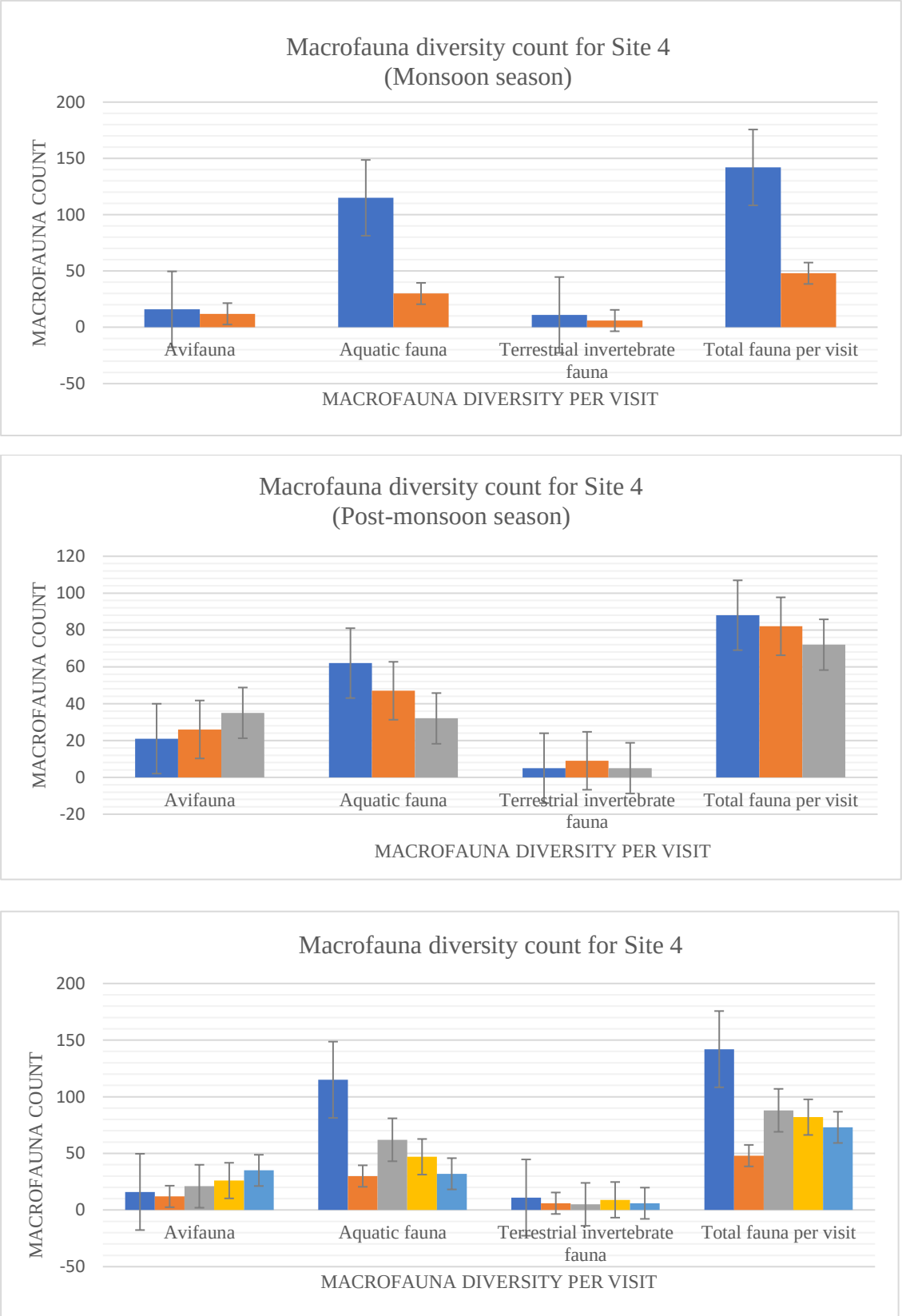


Figure 4.43: Graphical representation for macrofauna diversity count for Chorao Island site (Site 4)

Table 4.28: Bacterial Viable Count for Chorao Island site (Site 4) water sample for Monsoon season

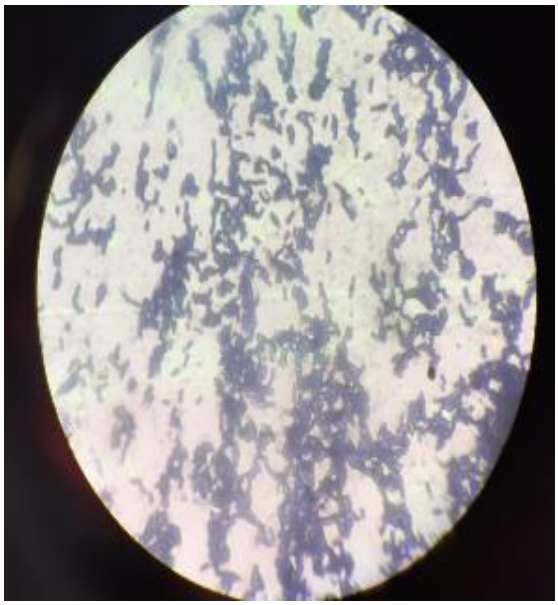
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Chorao Island	7 colonies	0.007×10^4 CFU/mL
Chorao Island	9 colonies	0.09×10^4 CFU/mL

Table 4.29: Bacterial Viable Count for Chorao Island site (Site 4) water sample for Post-monsoon season

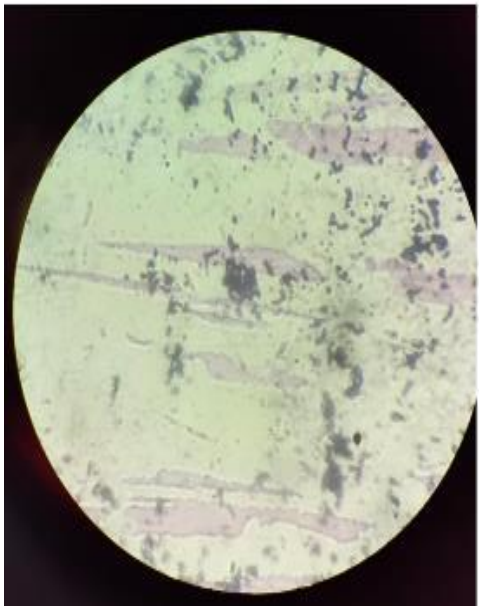
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Chorao Island	212 colonies	0.212×10^4 CFU/mL
Chorao Island	20 colonies	0.20×10^4 CFU/mL

Table 4.30: Gram characteristics of the three isolated colonies from Chorao Island site (Site 4) water sample for Monsoon season

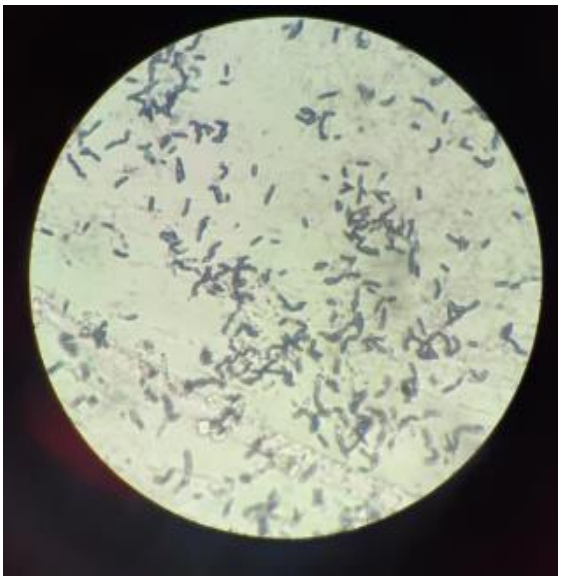
Colonies	**CW1**	**CW2**	**CW3**
Gram positive/ Gram negative	**Gram positive**	**Gram positive**	**Gram positive**
Shape	**Rods**	**Rods and cocci**	**Cocci**



CW1



CW2

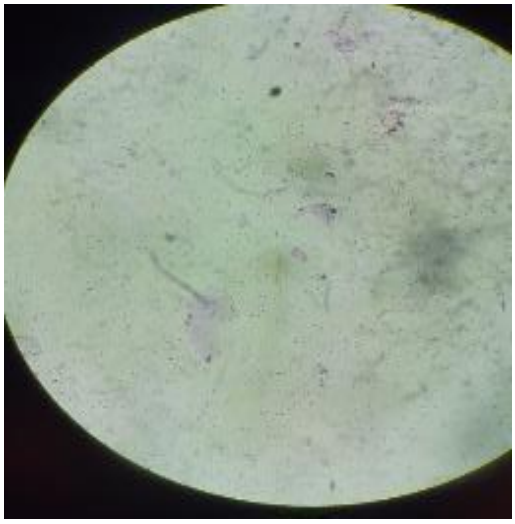


CW3

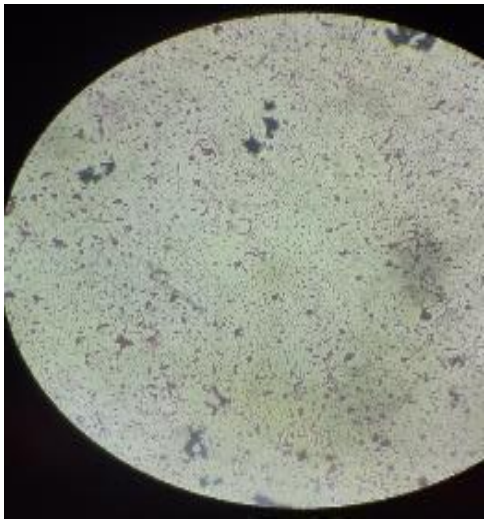
Figure 4.44: Gram-stained isolated colonies from Chorao Island site (Site 4) water sample for Monsoon season

Table 4.31: Gram characteristics of the five isolated colonies from Chorao Island site (Site 4) water sample for Post-monsoon season

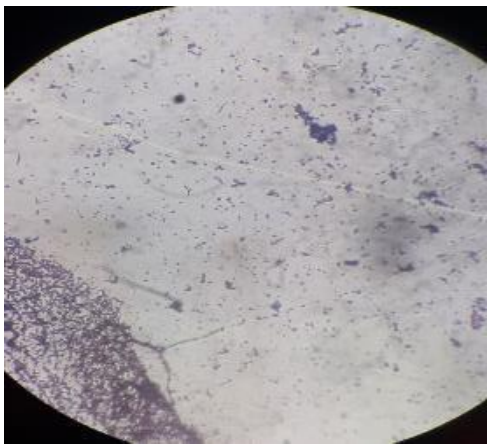
Colonies	CW1	CW2	CW3	CW4	CW5
Gram positive/ Gram negative	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Cocci	Short rods and cocci	Short rods and cocci	Rods and cocci	Rods and cocci



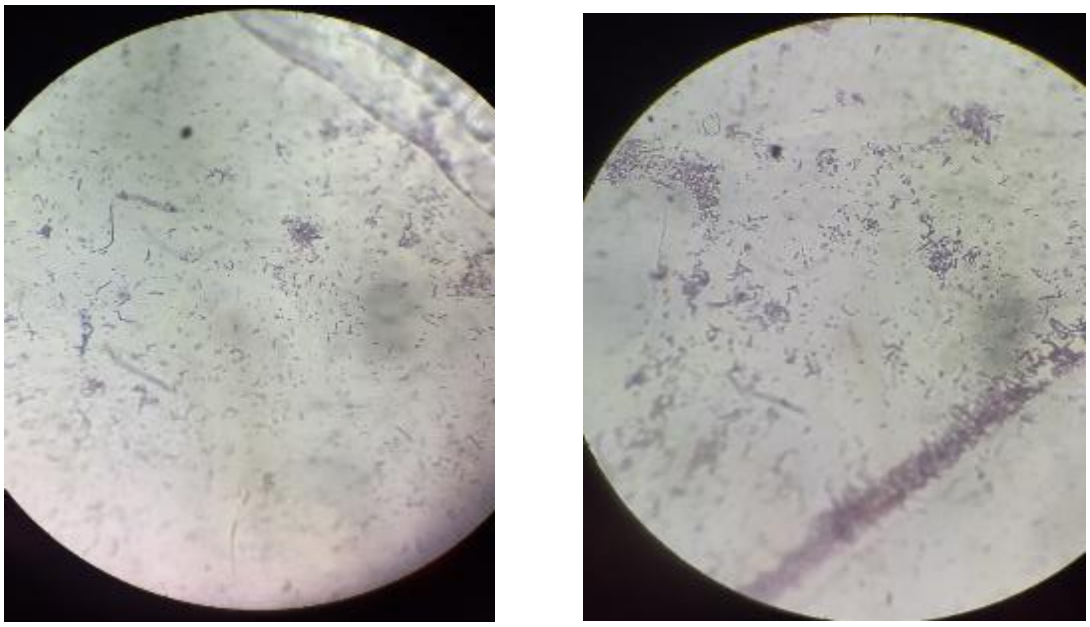
CW1



CW2



CW3

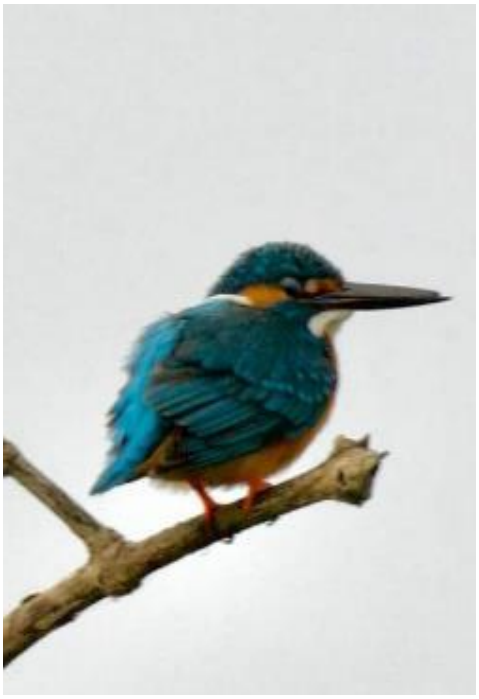


CW4 CW5

Figure 4.45: Gram-stained isolated colonies from Chorao Island site (Site 4) water sample for Post-monsoon season

Table 4.32: Common macrofaunal diversity at Penhe de Franca - Britona site (Site 5)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
115.83 m	1693 m^2	*Corvus* (Crow)	35	*Eurema hecabe*	2	*Scylla serrata* (Mud crab)	125
		Alcedininae (River Kingfisher)	4	(Common grass yellow butterfly)	5	*Thalassina anomala* (Mud lobster)	10
		Haliastur indus (Brahminy Kite)	4	*Papilio indra* (Indra Swallowtail butterfly)	8	*Oxudercinae* (Mudskipper)	30
		Ardea alba modesta (Eastern Great Egret)	6	*Heteronympha merope* (Common brown butterfly)		*Ostreidae* (True Oyster)	>250
		Anas zonorhyncha (Eastern spot-billed duck)	3			*Uca rapax* (Fiddler crab)	25
		Ardeola grayii (Indian Pond Heron)	2				
		Aegithina tiphia (Common Iora)	3				
		Phalacrocorax auratus (Double-crested Cormorant)	4				
		Leptocoma zeylonica (Purple-rumped sunbird)	7				



Alcedininae

Haliastur indus

Ardea alba modesta

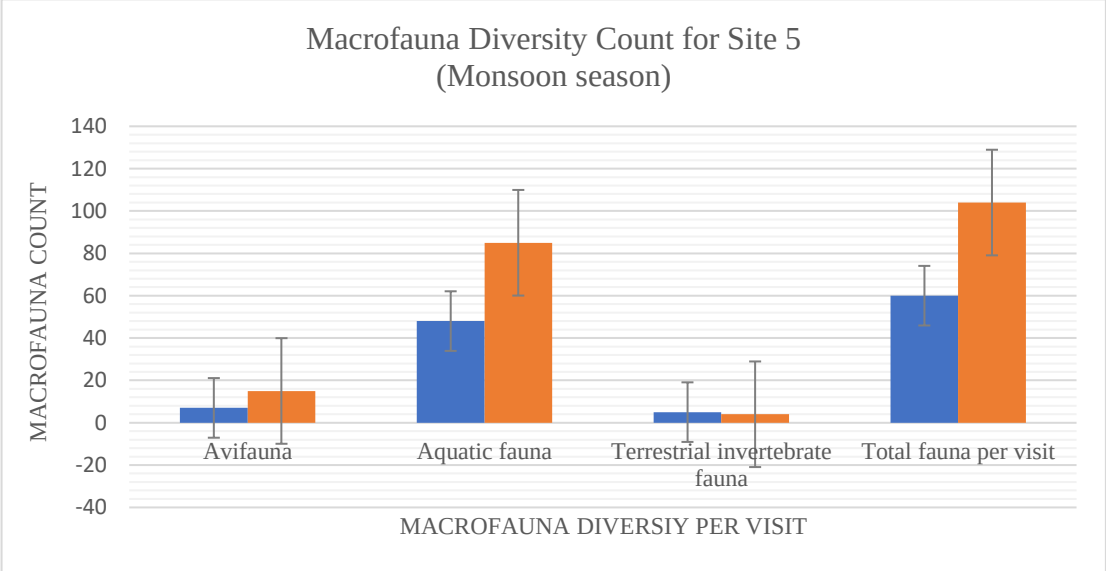
Scylla serrata

Oxudercinae

Thalassina anomala mound

Ostreida

Figure 4.46: Macrofauna observed at Penhe De Franca – Britona site (Site 5)



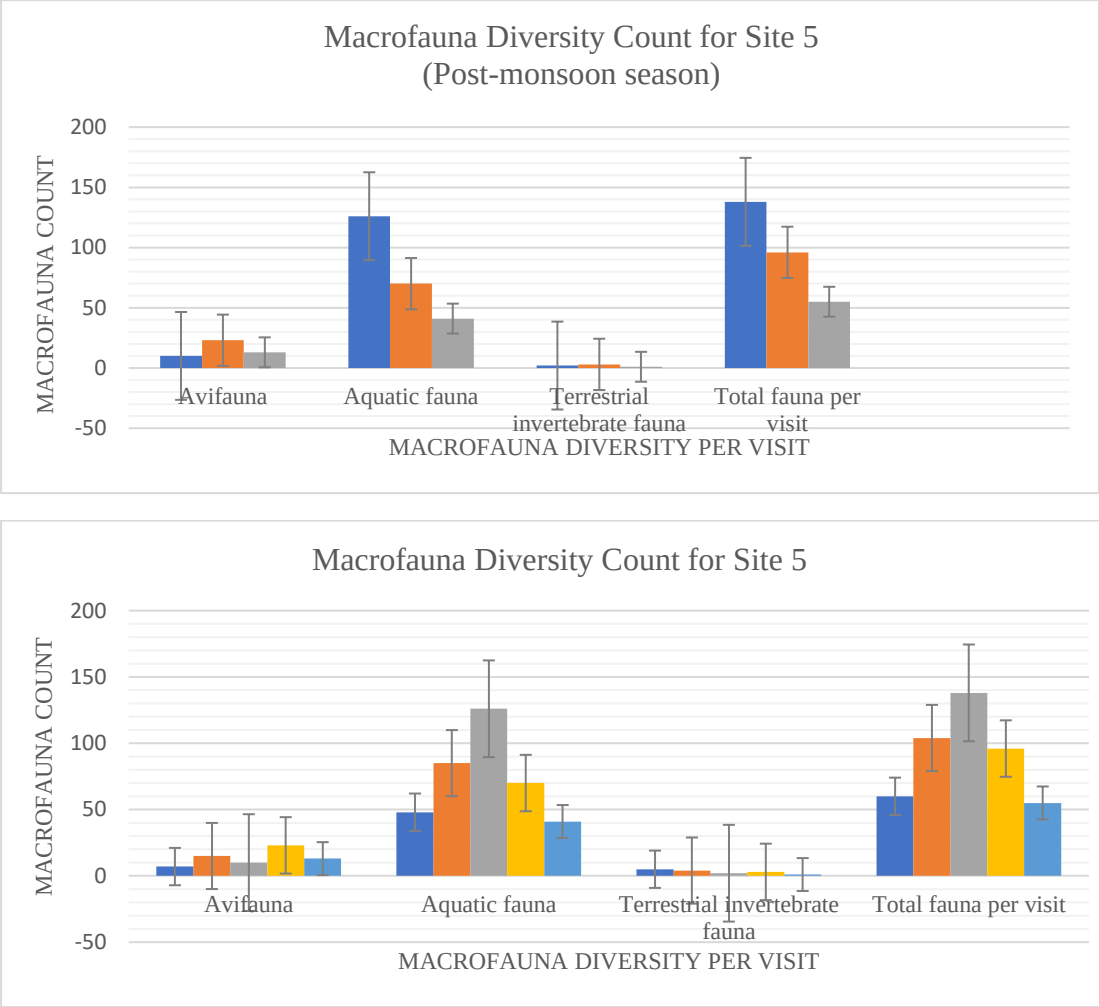


Figure 4.47: Graphical representation for macrofauna diversity count for Penhe de Franca – Britona site (Site 5)

Table 4.33: Bacterial Viable Count for Penhe de Franca-Britona site (Site 5) water sample for Monsoon season

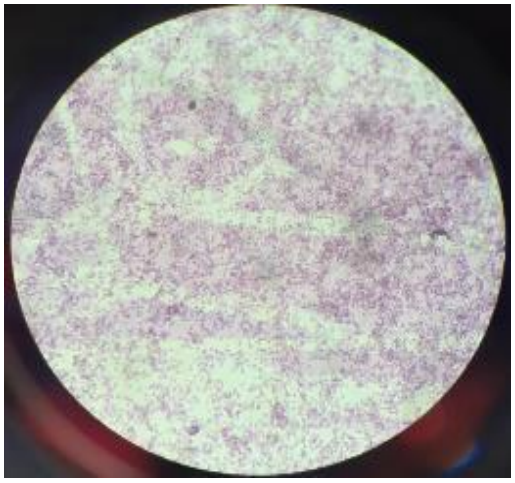
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Penhe de Franca, Britona	94 colonies	0.094×10^4 CFU/mL
Penhe de Franca, Britona	226 colonies	2.26×10^4 CFU/mL

Table 4.34: Bacterial Viable Count for Penhe de Franca-Britonasite (Site 5) water sample for Post-monsoon season

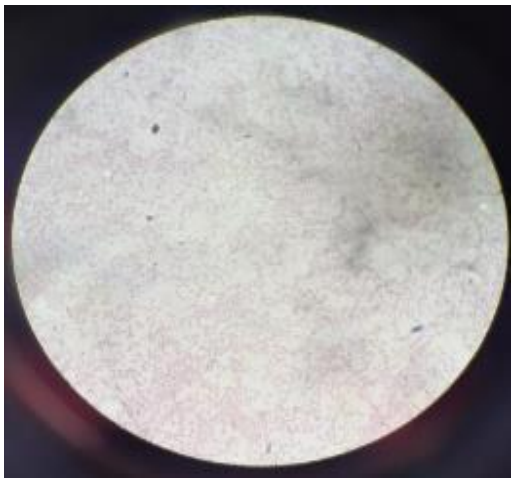
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Penhe de Franca, Britona	18 colonies	0.018×10^4 CFU/mL
Penhe de Franca, Britona	34 colonies	0.34×10^4 CFU/mL

Table 4.35: Gram characteristics of the five isolated colonies from Penhe de Franca-Britona site (Site 5) water sample for Monsoon season

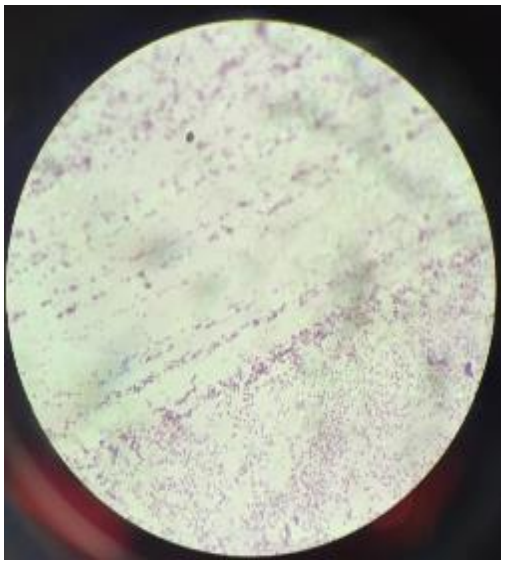
Colonies	**PFW1**	**PFW2**	**PFW3**	**PFW4**	**PFW5**
Gram positive/ Gram negative	**Gram positive**	**Gram positive**	**Gram positive**	**Gram positive**	**Gram positive**
Shape	**Short rods**	**Cocci**	**Short rods**	**Cocci and rods**	**Cocci and rods**



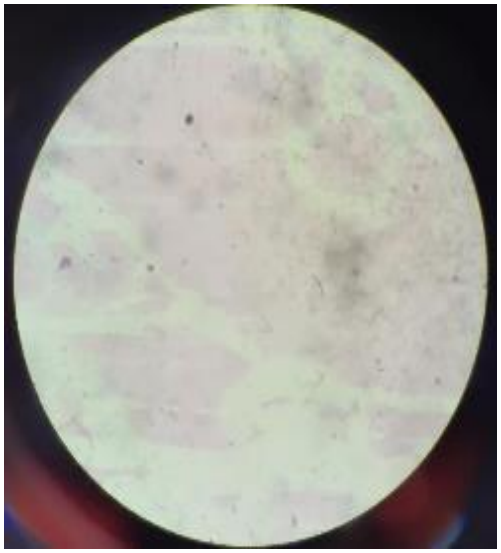
PFW1



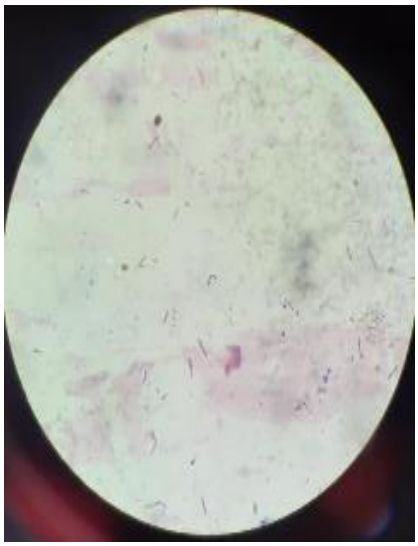
PFW2



PFW3



PFW4

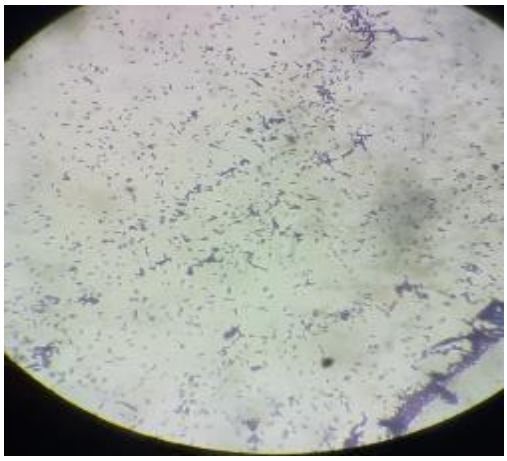


PFW5

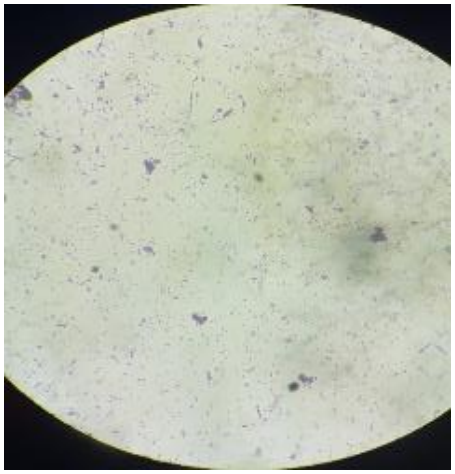
Figure 4.48: Gram-stained isolated colonies from Penhe de Franca – Britona site (Site 5) water sample for Monsoon season

Table 4.36: Gram characteristics of the three isolated colonies from Penhe de Franca-Britona water sample for Post-monsoon season

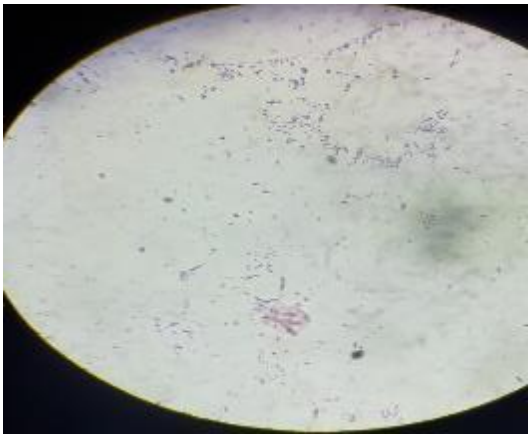
Colonies	PFW1	PFW2	PFW3
Gram positive/ Gram negative	Gram positive	Gram positive	Gram positive
Shape	Rods	Rods and cocci	Rods and cocci



PFW1



PFW2



PFW3

Figure 4.49: Gram-stained isolated colonies from Penhe de Franca – Britona site (Site 5) water sample for Post-monsoon season

Table 4.37: Common macrofaunal diversity at Ponte de Linhares Causeway – Ribandar site (Site 6)

Site distance	Site area	Avifauna diversity	Avifauna count	Terrestrial invertebrate fauna	Terrestrial invertebrate fauna count	Aquatic fauna diversity	Aquatic fauna count
131.75 m	1181 m^2	*Tringa totanus* (Common Redshank)	7	*Desidae* (Intertidal spider)	1	*Geosesarma hagen* (Red Devil Vampire Crab)	1
		Phalacrocorax auritus (Double-crested Cormorant)	8	*Tramea onusta* (Red-mantled saddlebags dragonfly)	2	*Toxotes jaculatrix* (Banded archerfish)	3
		Haliastur indus (Brahminy Kite)	14	*Phyllotreta nemorum* (Yellow-striped flea beetle)	4	*Sardinella longiceps* (Sardine)	< 50
		Alcedo atthis (Common Kingfisher)	7	*Eurema hecabe* (Common grass yellow butterfly)	9	*Lutjanus argentimaculatus* (Mangrove Red Snapper)	>12
		Corvus (Crow)	19	*Heteronympha merope* (Common brown butterfly)	7	*Mugil cephalis* (Mullet)	>25
		Dicrurus macrocercus (Black Drongo)	3	*Pieris rapae* (Cabbage white butterfly)	5	*Sphyraena barracuda* (Great Barracuda)	>10
		Haematopus ostralegus (Eurasian Oystercatcher)	9	*Belenois java* (Caper white butterfly)	3	*Scylla serrata* (Mud crab)	7
		Ardea alba modesta (Eastern Great Egret)	2	*Neptis sappho* (Common glider butterfly)	4	*Gecarcinus quadratus* (Red land crab)	3
		Spizella pusilla (Field Sparrow)	5	*Phasmatodea* (Walking Stick Insect)	1		
		Anas poecilorhyncha (Indian Spot-billed Duck)	3	*Neptis hylas* (Common Sailor butterfly)	8		
		Ardeola grayii (Indian Pond Heron)	3	*Discolampa ethion* (Banded Blue Pierrot butterfly)	2		
		Dendrocygna javanica (Lesser Whistling Duck)	5				
		Larinae (Seagull)	7				
		Leptocoma zeylonica (Purple-rumped sunbird)	4				

Haematopus ostralegus

Haliastur indus

Alcedo atthis

Phalacrocorax auritus

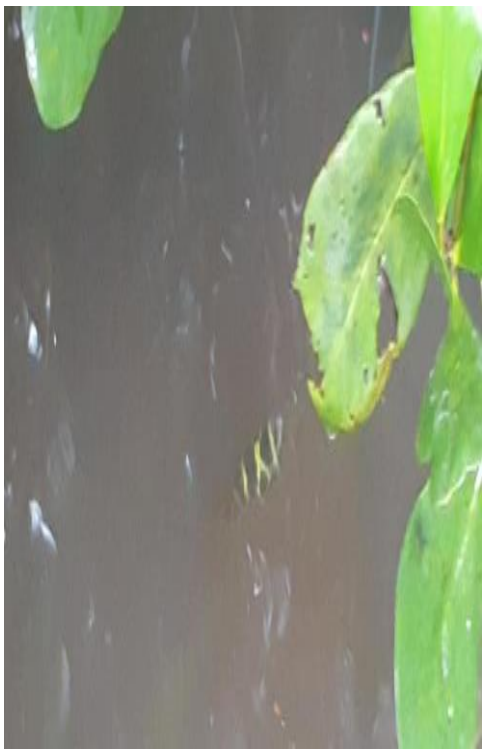
Belenois java

Neptis hylas

Discolampa ethion

Phyllotreta nemorum

Tramea onusta

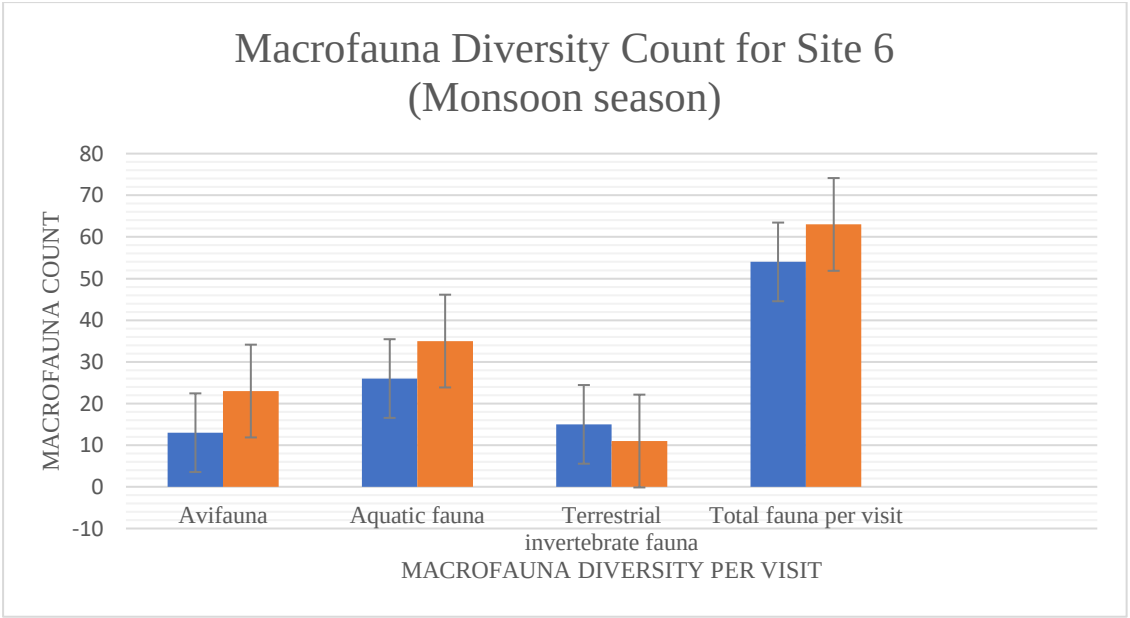


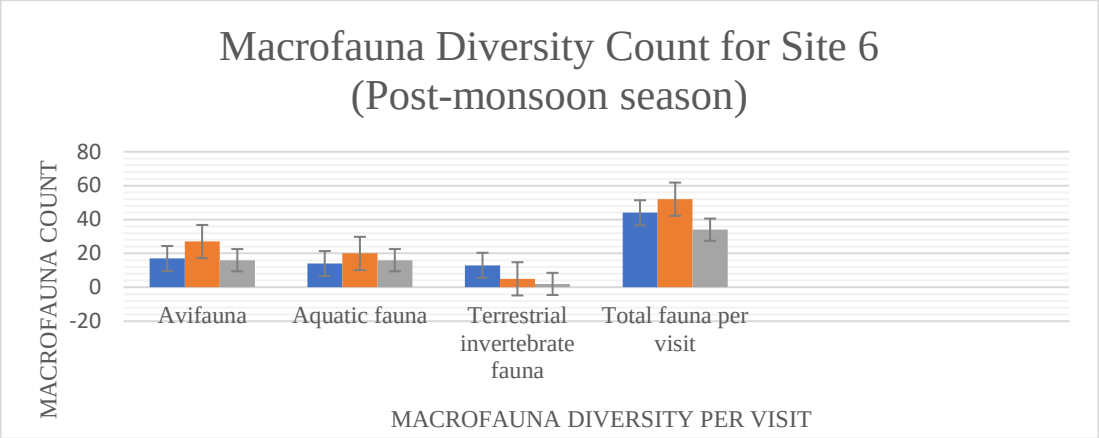
Toxotes jaculatrix

Geosesarma hagen

Gecarcinus quadratus

Figure 4.50: Macrofauna observed at Ponte de Linhares Causeway – Ribandar site (Site 6)





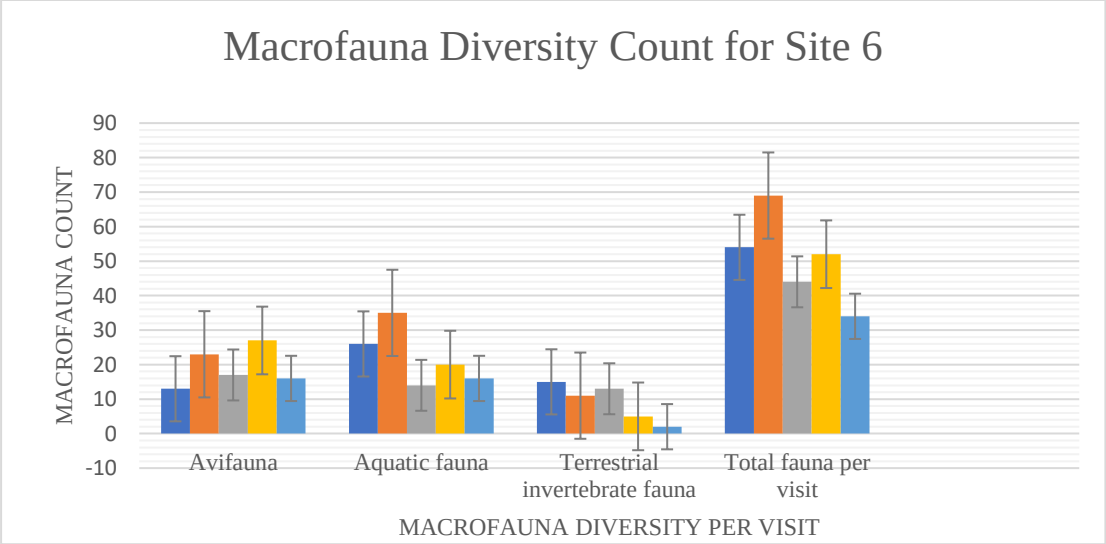


Figure 4.51: Graphical representation for macrofauna diversity count for Ponte de Linhhares Causeway – Ribandar site (Site 6)

Table 4.38: Bacterial Viable Count for Ponte de Linhares Causeway – Ribandar site (Site 6) water sample for Monsoon season

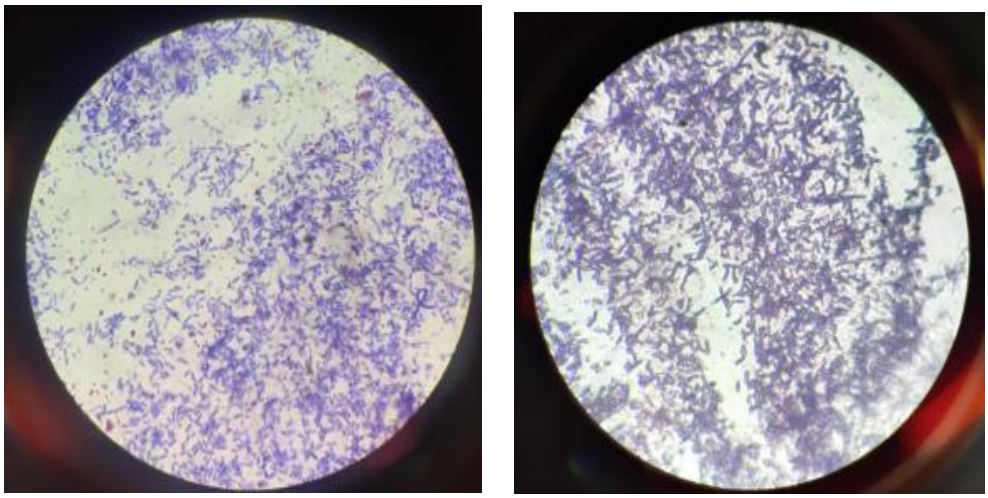
Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Ponte de Linhares Causeway, Ribandar	48 colonies	0.048×10^4 CFU/mL
Ponte de Linhares Causeway, Ribandar	25 colonies	0.25×10^4 CFU/mL

TABLE 4.39: Bacterial Viable Count for Ponte de Linhares Causeway – Ribandar site (Site 6) water sample for Post-monsoon season

Sampling Site	Bacterial Colony Count	Bacterial Viable Count
Ponte de Linhares Causeway, Ribandar	61 colonies	0.061×10^4 CFU/mL
Ponte de Linhares Causeway, Ribandar	3 colonies	0.03×10^4 CFU/mL

Table 4.40: Gram characteristics of the two isolated colonies from Ponte de Linhares Causeway - Ribandar site (Site 6) water sample for Monsoon seaso

Colonies	**PLW1**	**PLW2**
Gram positive/ Gram negative	**Gram positive**	**Gram positive**
Shape	**Rods**	**Rods**

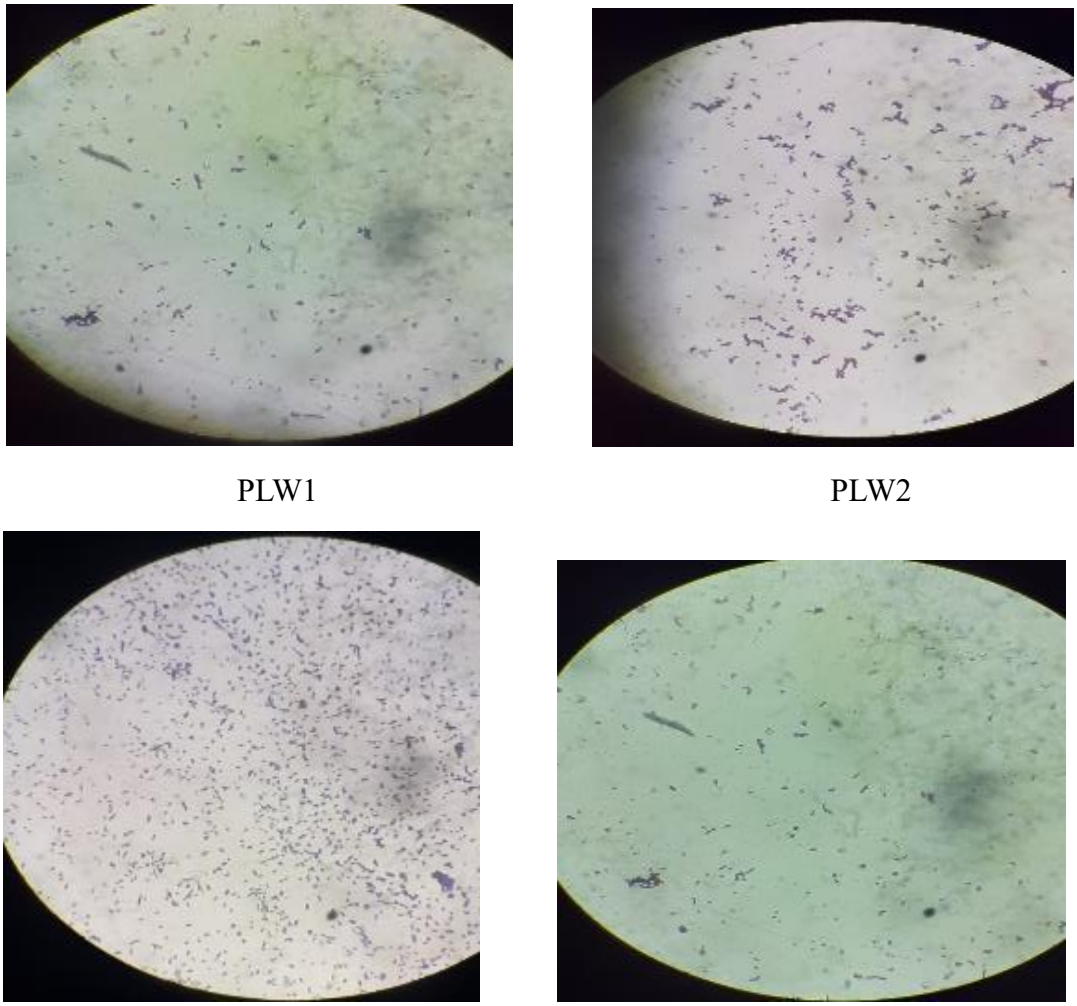


PLW1 PLW2

Figure 4.52: Gram-stained isolated colonies from Ponte de Linhares Causeway – Ribandar site (Site 6) water sample for Monsoon season

Table 4.41: Gram characteristics of the four isolated colonies from Ponte de Linhares Causeway - Ribandar site (Site 6) water sample for Post-monsoon season

Colonies	PLW1	PLW2	PLW3	PLW4
Gram positive/ Gram negative	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Rods and cocci	Rods and cocci	Short rods and cocci	Rods and cocci



PLW1 PLW2

PLW3 PLW4

Figure 4.53: Gram-stained isolated colonies from Ponte de Linhares Causeway – Ribandar site (Site 6) water sample for Post-monsoon season

Table 4.42: Colony characteristics for estuarine water sample for all six selected sampling sites for Monsoon season

Sites	Colony number	Size	Colour	Margin	Elevation	Shape	Texture
Vagurbem (Site 1)	VW1	18 mm	White (Opaque)	Erose	Flat	Filamentous	Translucent
Vagurbem (Site 1)	VW2	4 mm	White (Opaque)	Undulate	Flat	Irregular	Slimy, moist
Vagurbem (Site 1)	VW4	5 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist
Saint Estevam Island (Site 2)	SW1	3 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Slimy, moist
Saint Estevam Island (Site 2)	SW2	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist
Saint Estevam Island (Site 2)	SW3	7 mm	White (Opaque)	Entire (Smooth)	Flat	Irregular	Translucent
Divar Island (Site 3)	DW1	7 mm	White (Opaque)	Curled	Flat	Round	Slimy, moist
Divar Island (Site 3)	DW2	4 mm	Milky (Yellow)	Entire (Smooth)	Flat	Round	Dry, mucoid
Divar Island (Site 3)	DW3	4 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist
Divar Island (Site 3)	DW4	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Divar Island (Site 3)	DW5	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Chorao Island (Site 4)	CW1	8 mm	White (Opaque)	Lobate	Convex	Round	Slimy, moist
Chorao Island (Site 4)	CW2	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist
Chorao Island (Site 4)	CW3	3 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Slimy, moist
Penhe de Franca - Britona (Site 5)	PFW1	4 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist
Penhe de Franca - Britona (Site 5)	PFW2	2 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Penhe de Franca - Britona (Site 5)	PFW3	8 mm	White (Opaque)	Erose (Serrated)	Flat	Irregular	Slimy, moist
Penhe de Franca - Britona (Site 5)	PFW4	2 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Penhe de Franca - Britona (Site 5)	PFW5	7 mm	White (Opaque)	Undulate (Wavy)	Flat	Irregular	Dry, mucoid
Ponte de Linhares Causeway - Ribandar (Site	PLW1	3 mm	White (Opaque)	Curled	Flat	Punctiform	Slimy, moist
Ponte de Linhares Causeway - Ribandar (Ste 6	PLW2	6 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Slimy, moist

Table 4.43: Colony characteristics for estuarine water sample for all six selected sampling sites for Post- monsoon season

Sites	Colony number	Size	Colour	Margin	Elevation	Shape	Texture
Vagurbem (Site 1)	VW1	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Vagurbem (Site 1)	VW2	6 mm	White (Opaque)	Undulate	Flat	Irregular	Dry, mucoid
Vagurbem (Site 1)	VW4	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Vagurbem (Site 1)	VW5	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Saint Estevam Island (Site 2)	SW1	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Saint Estevam Island (Site 2)	SW2	2 mm	White (Opaque)	Undulate	Flat	Round	Dry, mucoid
Saint Estevam Island (Site 2)	SW3	6 mm	White (Opaque)	Entire (Smooth)	Flat	Irregular	Dry, mucoid
Saint Estevam Island (Site 2)	SW4	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Divar Island (Site 3)	DW1	9 mm	White (Opaque)	Undulate (Wavy)	Flat	Irregular	Dry, mucoid
Divar Island (Site 3)	DW2	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Divar Island (Site 3)	DW3	2 mm	White (Opaque)	Erose (Serrated)	Flat	Filamentous	Dry, mucoid
Divar Island (Site 3)	DW4	5 mm	White (Opaque)	Undulate	Flat	Irregular	Dry, mucoid
Divar Island (Site 3)	DW5	4 mm	White (Opaque)	Undulate (Wavy)	Flat	Punctiform	Slimy, moist
Divar Island (Site 3)	DW6	5 mm	Brown	Curled	Flat	Round	Dry, mucoid
Divar Island (Site 3)	DW7	9 mm	Pink	Entire (Smooth)	Flat	Irregular	Slimy, moist
Chorao Island (Site 4)	CW1	7 mm	White (Opaque)	Lobate	Flat	Irregular	Dry, mucoid
Chorao Island (Site 4)	CW2	3 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Chorao Island (Site 4)	CW3	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Dry, mucoid
Chorao Island (Site 4)	CW4	6 mm	White (Opaque)	Lobate	Flat	Irregular	Dry, mucoid
Chorao Island (Site 4)	CW5	1 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Penhe de Franca - Britona (Site 5)	PFW1	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Penhe de Franca - Britona (Site 5)	PFW2	3 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid
Penhe de Franca - Britona (Site 5)	PW3	1 mm	White	Entire (Smooth)	Flat	Punctiform	Translucent
Ponte de Linhares Causeway - Ribandar (Site 6)	PLW1	2 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Translucent
Ponte de Linhares Causeway - Ribandar (Site 6)	PLW2	5 mm	White (Opaque)	Entire (Smooth)	Convex	Irregular	Dry, mucoid
Ponte de Linhares Causeway - Ribandar (Site 6)	PLW3	1 mm	White (Opaque)	Entire (Smooth)	Flat	Punctiform	Translucent
Ponte de Linhares Causeway - Ribandar (Site 6)	PLW4	4 mm	White (Opaque)	Entire (Smooth)	Flat	Round	Dry, mucoid

4.1.4 Biostatistics – Correlation studies, Shannon-Wiener Diversity Index, Margalef Index and Analysis of Variance Test

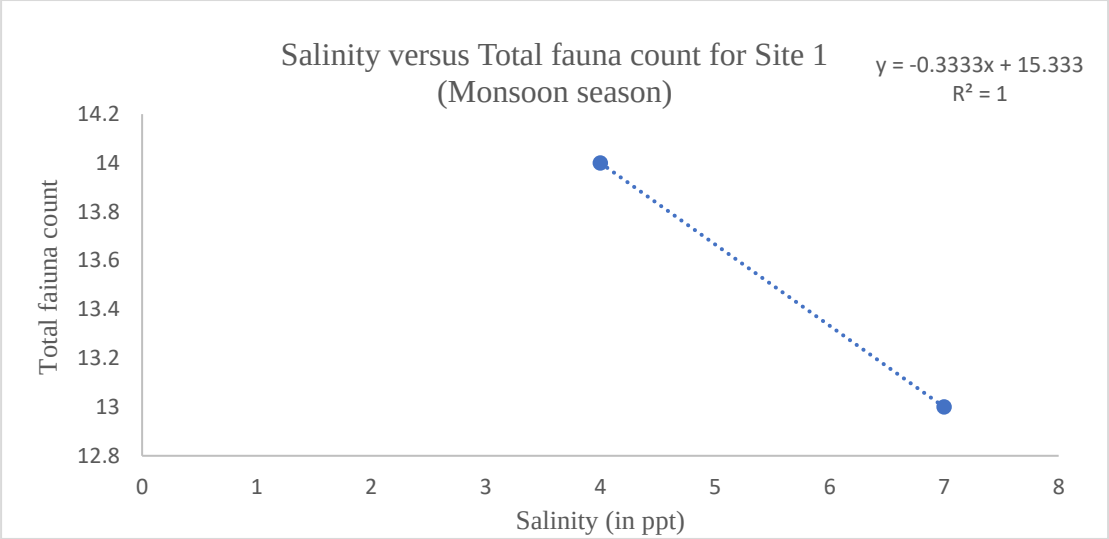
In reference to the given result, Figure 4.54, 4.55, 4.56, 4.57, 4.58, and 4.59 depict the graphical correlation between salinity and temperature influence on total fauna in the Monsoon and Post-monsoon seasons for Vagurbem site (Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6). Figure 4.60 depicts the BOD for the six selected sampling sites in Monsoon and Post-monsoon seasons. Figure 4.61 depicts the bacterial viable count for the six selected sampling sites in Monsoon and Post-monsoon seasons. Figure 4.62 depicts the correlation between BOD and Bacterial Viable Count for six selected sampling sites in Monsoon and Post-monsoon seasons.

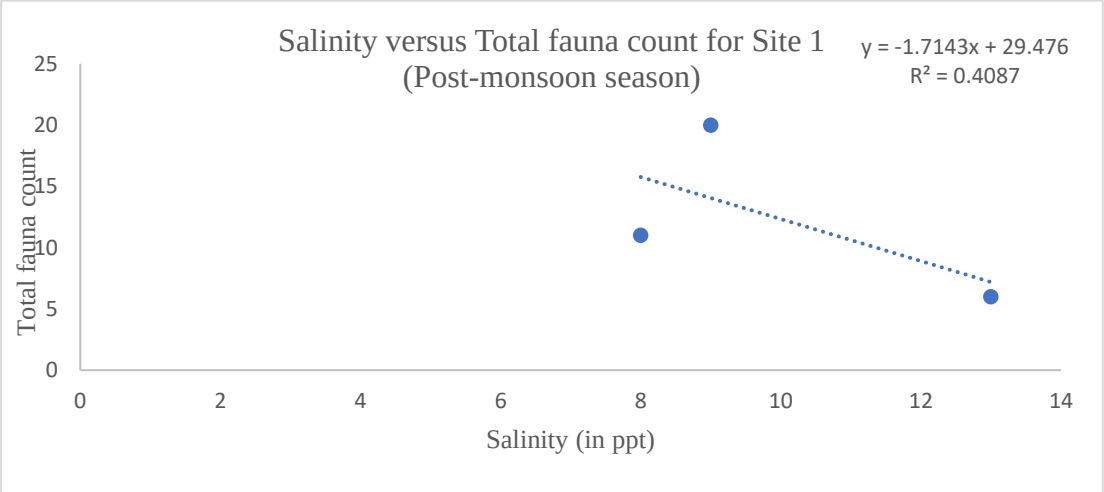
Table 4.44 decribes the estimated pollution level based on Shannon-Wiener Diversity Index. Tables 4.45, 4.46, 4.47, and 4.48 describe the estimated pollution level based on Shannon-Wiener Diversity Index for mangrove flora diversity, avifauna diversity, terrestrial invertebrate fauna diversity, and aquatic fauna diversity respectively for selected sampling sites. Table 4.49 describes the estimated species richness level based on Margalef Index values. Tables 4.50, 4.51, 4.52, and 4.53 describe the estimated species richness level based on Margalef Index values for mangrove flora, avifauna, terrestrial invertebrate fauna, and aquatic fauna respectively within selected sampling sites. Table 4.54 describes the summarized variance for dataset of selected sampling sites, Table 4.55 describes the One-way Analysis of Variance (ANOVA) for dataset of selected sampling sites, Table 4.56 describes the

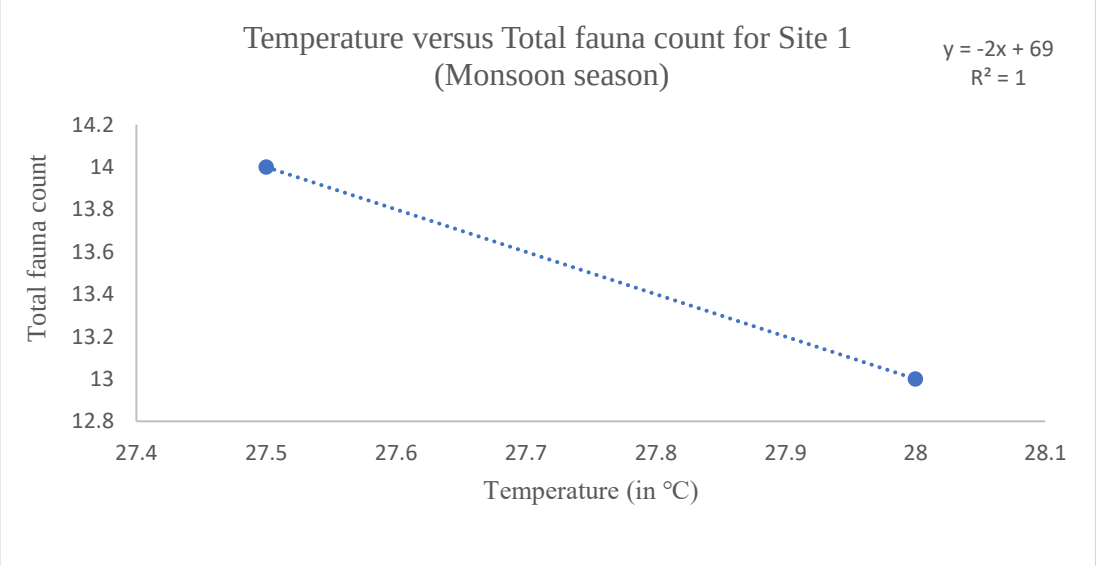
Alpha estimation to prove significance of Pot-hoc test (Bonferroni Correction), and Table 4.57 describes the estimation of Post-hoc test by Dunnett Test.

Figures 4.63, 4.65, 4.67, and 4.69 depict the graphical representation of Shannon-Wiener Diversity Index for mangrove flora diversity, avifauna diversity, terrestrial invertebrate fauna diversity, and aquatic fauna diversity respectively for selected sampling sites. Figures 4.64, 4.66, 4.68, and 4.70 depict the pie-chart representation of Shannon-Wiener Diversity Index based percentage diversity for mangrove flora, avifauna, terrestrial invertebrate fauna, and aquatic fauna respectively at selected sampling sites. Figures 4.71, 4.72, 4.73, and 4.74 depict the graphical representation of Margalef Index for mangrove flora, avifauna, terrestrial invertebrate fauna, and aquatic fauna species richness respectively at selected sampling sites. Figure 4.75 depicts the graphical representation of Post-hoc testing using Dunnett Test.

Correlation studies were carried out to understand the influence of physicochemical parameters such as salinity and temperature (sea-surface temperature) on the species richness of macrofauna for the Monsoon and Post-monsoon season. Correlation studies were also carried out to through scatter plot graphs understand the influence of BOD on the bacterial viable count of microflora. The correlation statistics were proved with R^2 level of significance.


Salinity versus Total fauna count for Site 1
(Monsoon season)
y = -0.3333x + 15.333
R² = 1
Total faiuna count
14.2
14
13.8
13.6
13.4
13.2
13
12.8
0
1
2
3
4
5
6
7
8
Salinity (in ppt)


Salinity versus Total fauna count for Site 1
(Post-monsoon season)
y = -1.7143x + 29.476
R² = 0.4087
Total fauna count
25
20
15
10
5
0
0
2
4
6
8
10
12
14
Salinity (in ppt)


Temperature versus Total fauna count for Site 1
(Monsoon season)
y = -2x + 69
R² = 1
Total fauna count
14.2
14
13.8
13.6
13.4
13.2
13
12.8
27.4
27.5
27.6
27.7
27.8
27.9
28
28.1
Temperature (in ℃)

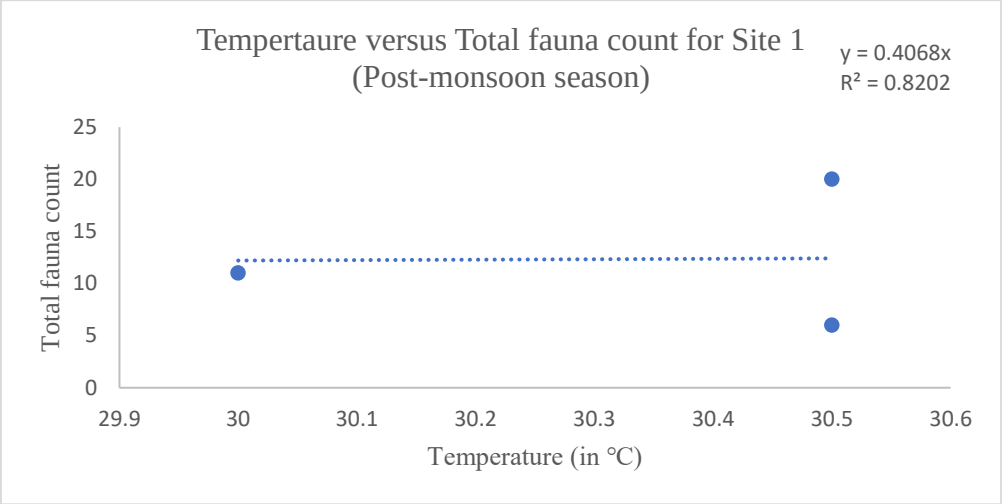
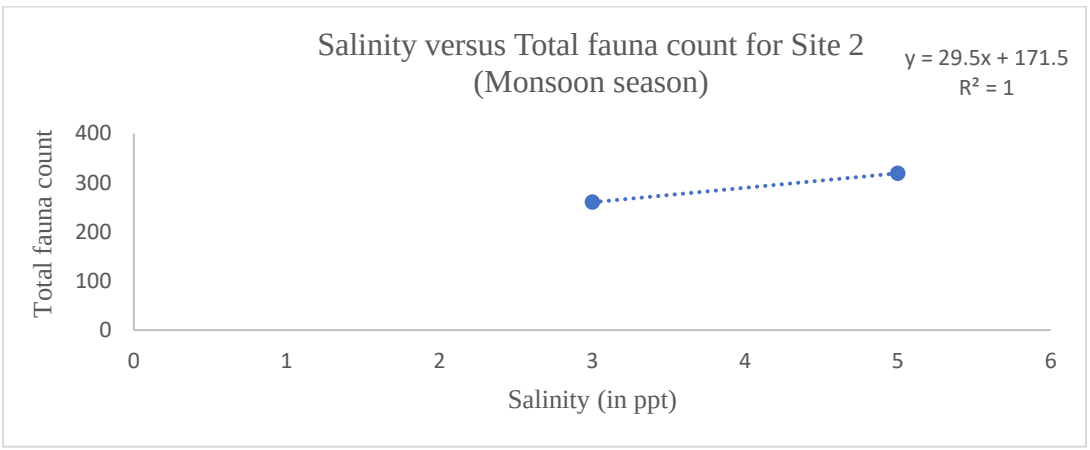
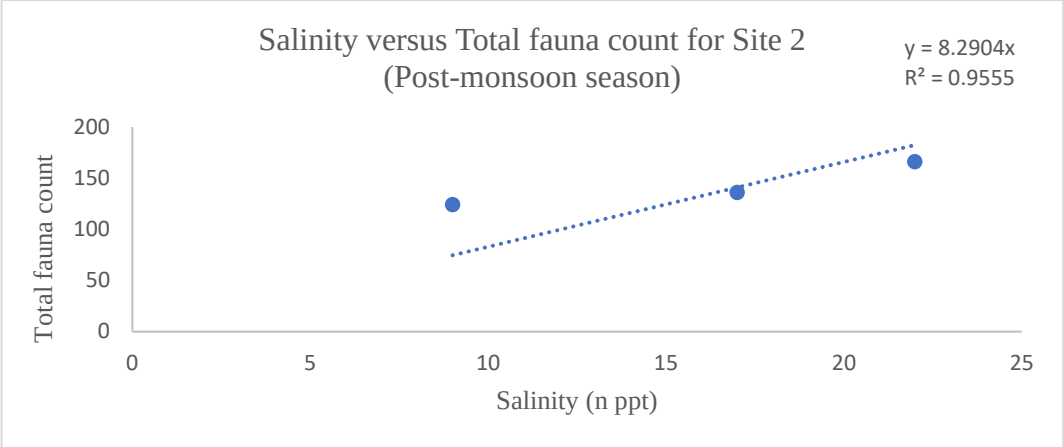
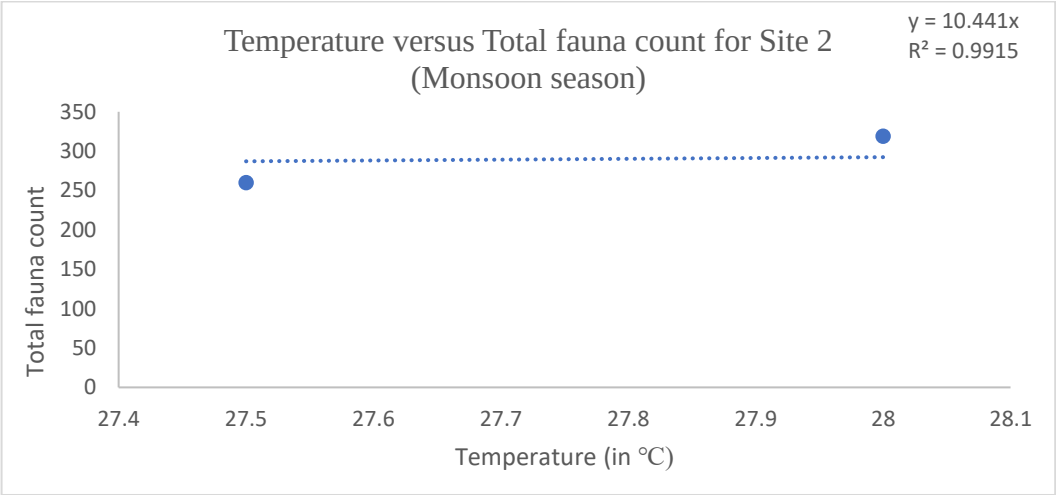


Figure 4.54: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Vagurbem site (Site 1)

The data suggests a strong negative correlation of -1 and -1 respectively for salinity and temperature influence on total fauna in monsoon season while a moderately negative correlation of -0.6 for salinity and a weak positive correlation of 0.16 for temperature influence on total fauna in post-monsoon season was observed at Vagurbem site (Site 1). Thus, the salinity and temperature have a robust inversely proportional influence to the total fauna predominantly in the monsoon season.







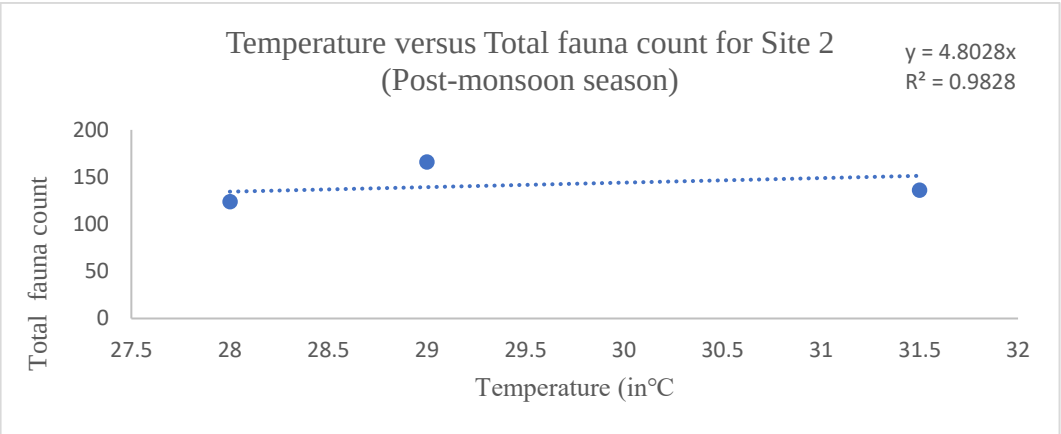
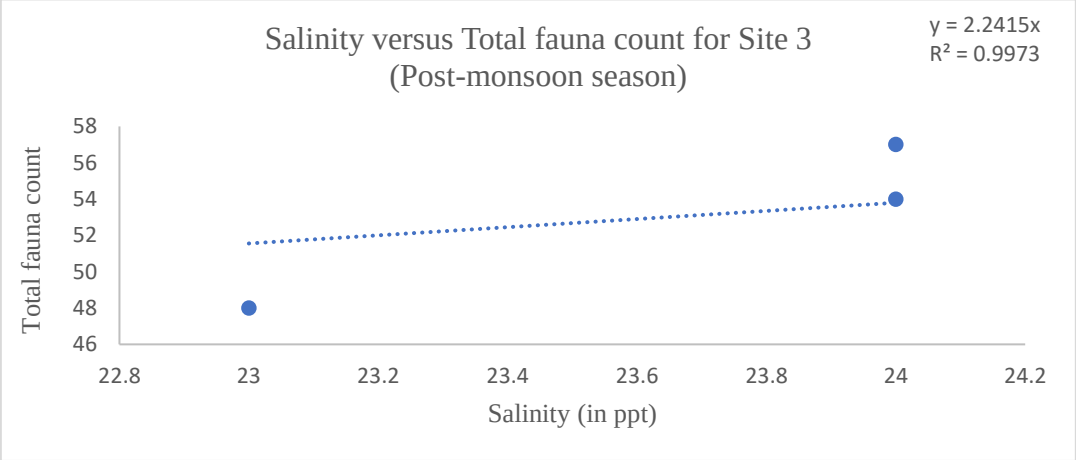
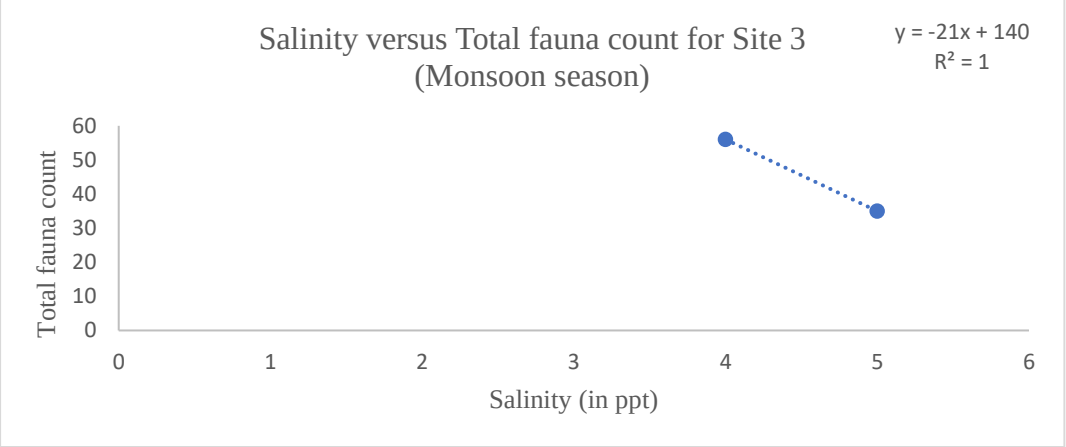
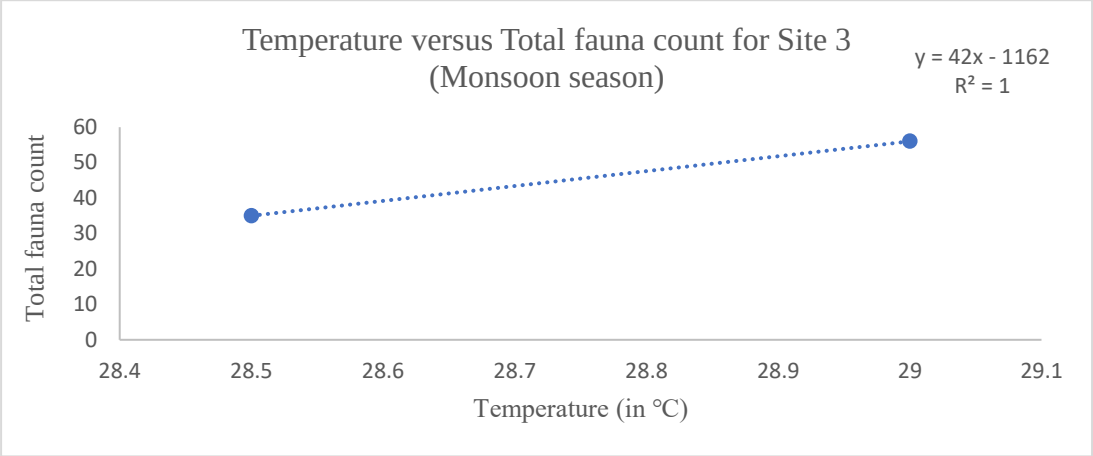


Figure 4.55: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Saint Estevam Island site (Site 2)

The data suggests that a strong positive and negative correlation of 1 and -1 respectively for salinity and temperature influence on total fauna respectively during the monsoon season while a moderate and weak positive correlation of 0.9 and 0.03 respectively were observed for salinity and temperature influence on total fauna respectively in the post-monsoon season was observed at Saint Estevam site (Site 2). Predominantly, a robust inversely proportional influence between temperature and total fauna as well as a directly proportional influence was observed between salinity and total fauna in the monsoon season.







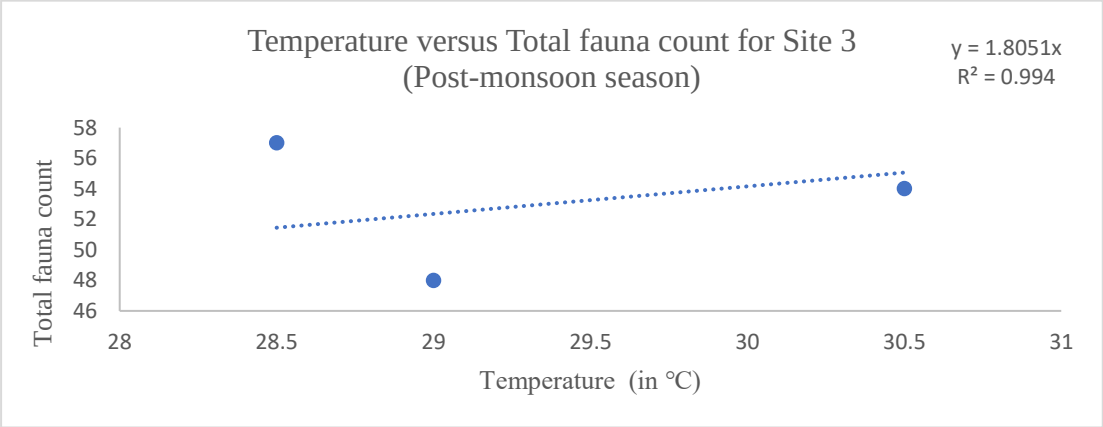
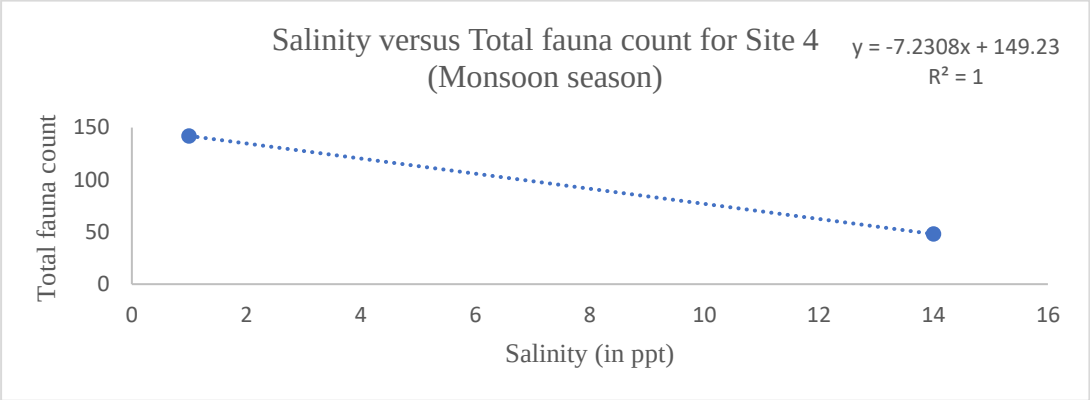
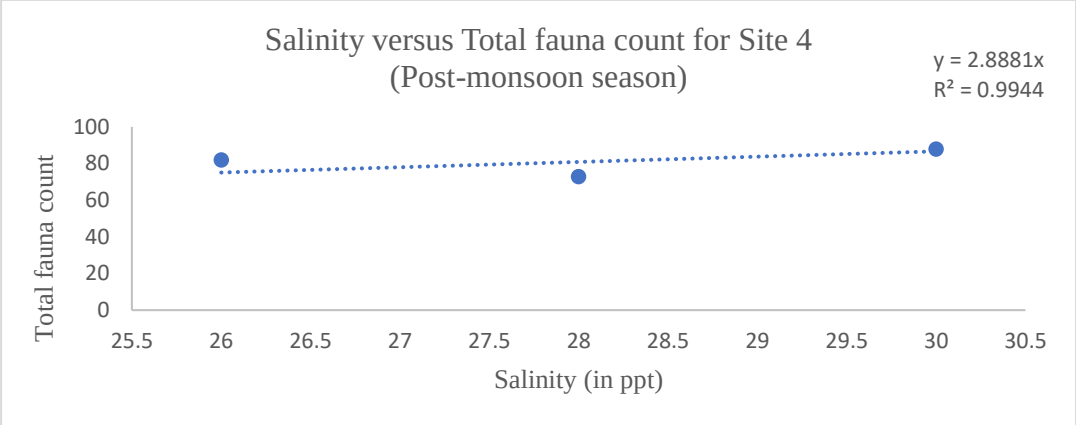


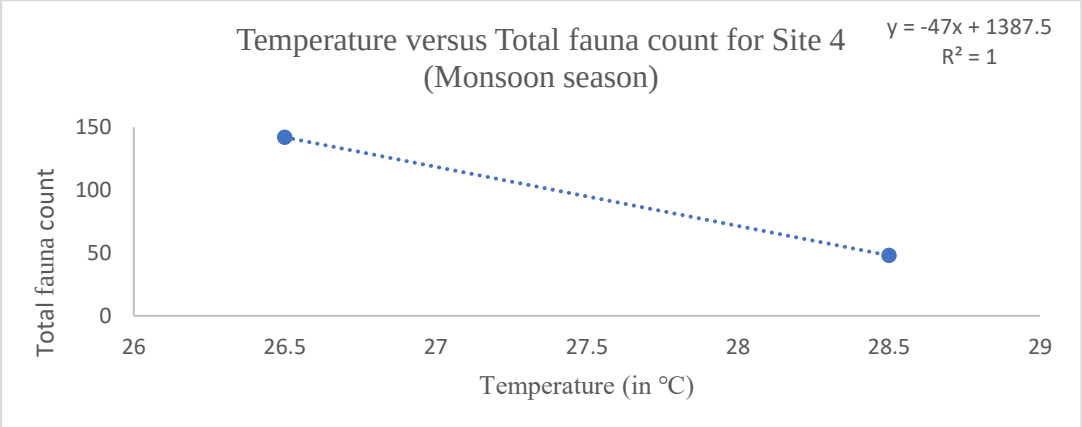
Figure 4.56: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Divar Island site (Site 3)

The data suggests that a strong correlation of -1 and 1 for salinity and temperature influence on total fauna respectively during the monsoon season while a moderate positive correlation of 0.9 for salinity influence on total fauna and weak negative correlation of -0.05 was observed for temperature influence on total fauna respectively for post-monsoon season was observed at Divar Island site (Site 3). Predominantly, a robust inversely proportional influence between salinity and total fauna as well as a

directly proportional influence was observed between temperature and total fauna in the monsoon season.







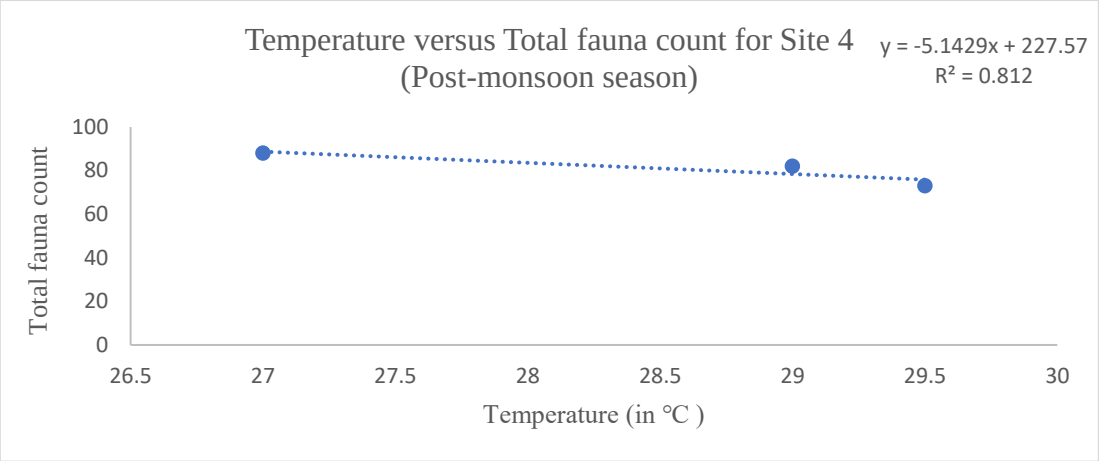
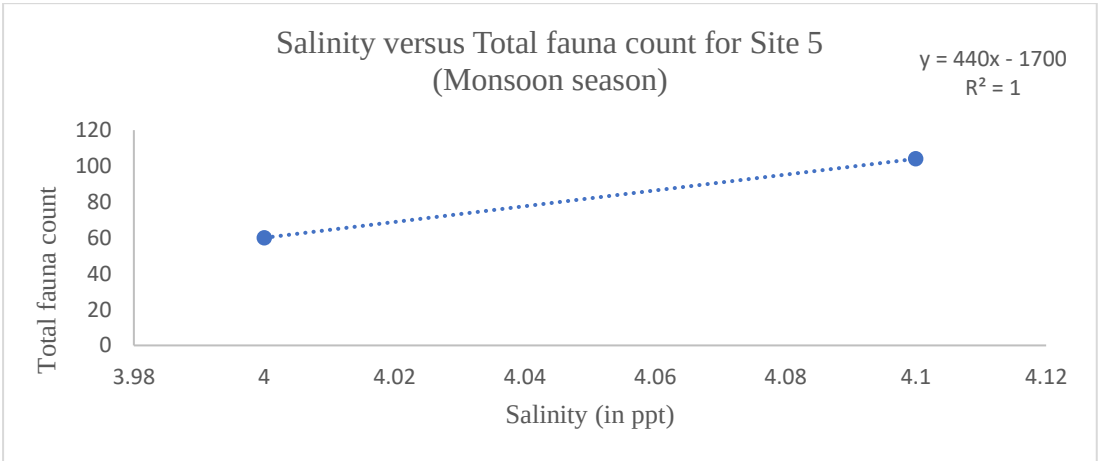
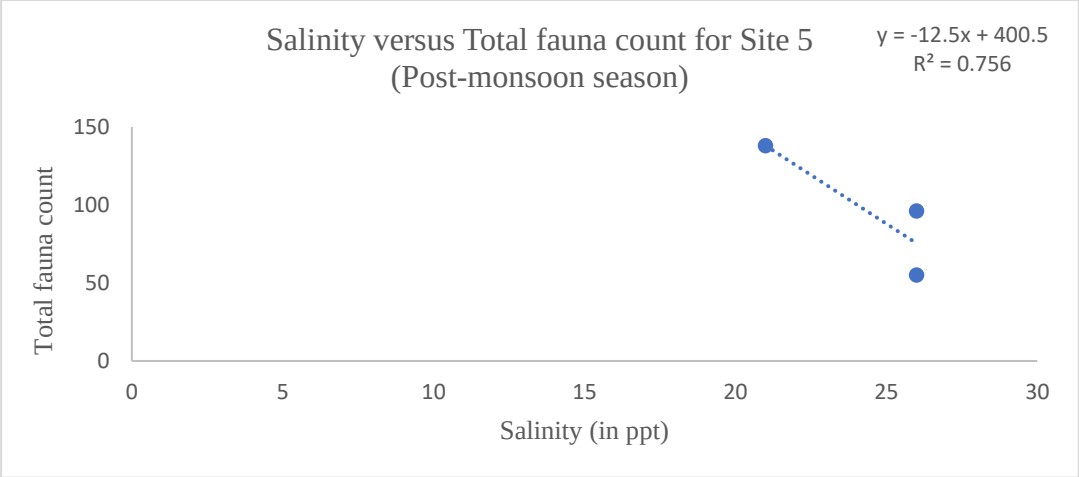
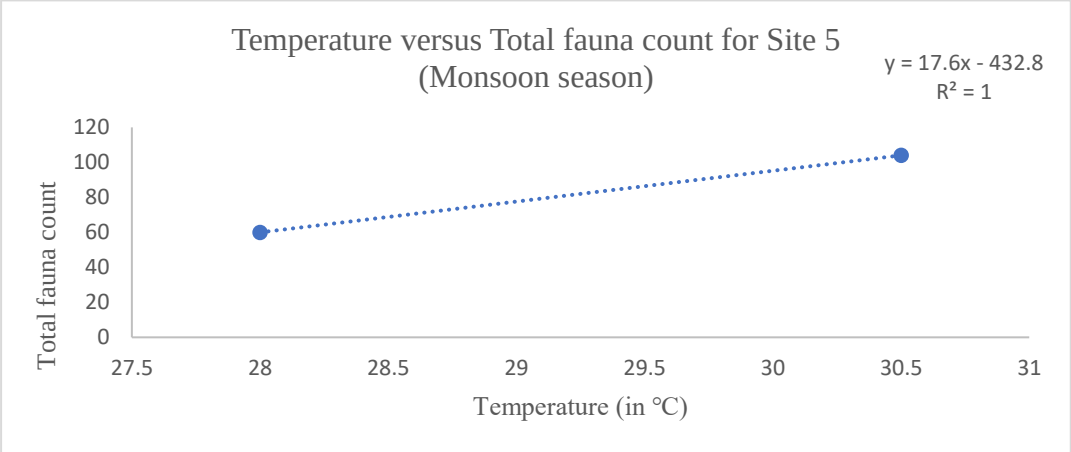


Figure 4.57: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Chorao Island site (Site 4)

The data suggests that a strong negative correlation of -1 and -1 for salinity and temperature influence on total fauna respectively during the monsoon season while a weak positive correlation of 0.3 for salinity influence on total fauna and moderate negative correlation of -0.9 was observed for temperature influence on total fauna respectively for the post-monsoon season was observed at Chorao Island site (Site 4). Predominantly, a robust inversely proportional influence between salinity as well as temperature on total fauna was observed in the monsoon season.







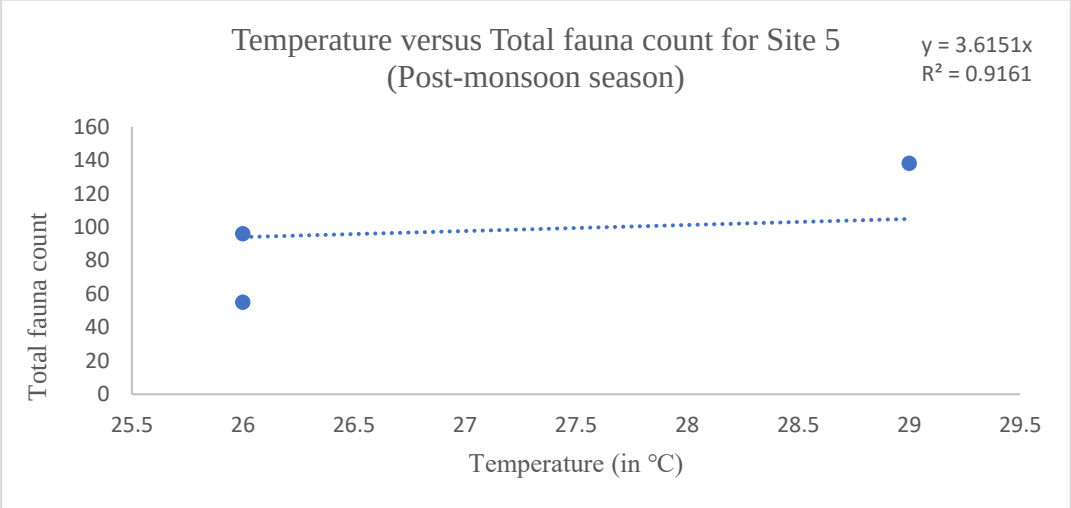
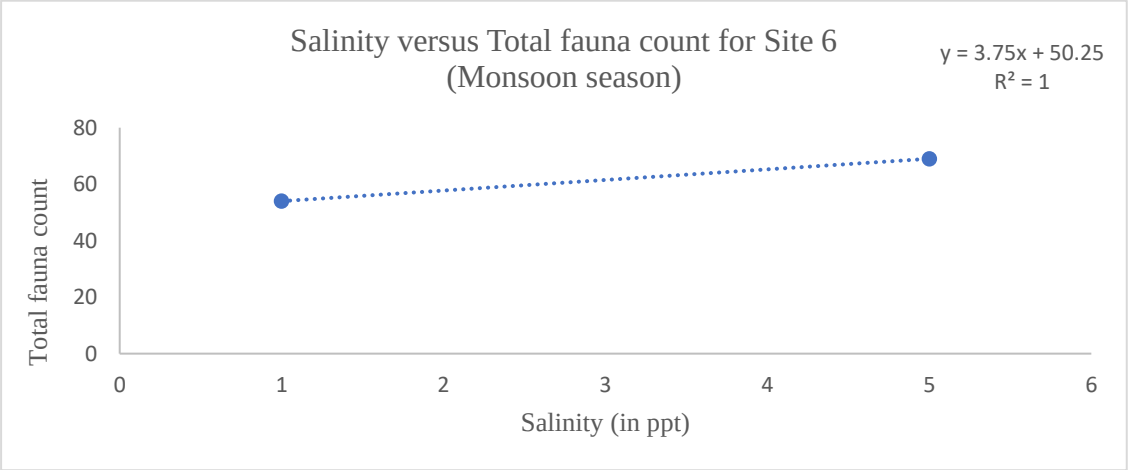
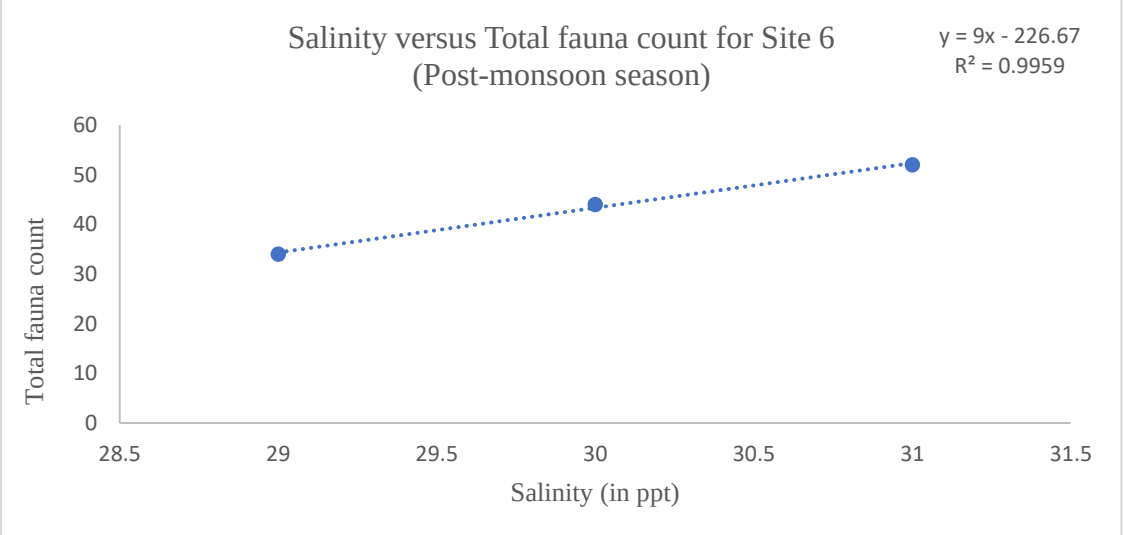


Figure 4.58: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Penhe de Franca - Britona site (Site 5)

The data suggests that a strong positive correlation of 1 and 1 for salinity and temperature influence on total fauna respectively during the monsoon season while a moderate negative correlation of -0.8 for salinity influence on total fauna and moderate positive correlation of 0.8 was observed for temperature influence on total fauna respectively in the post-monsoon season was observed at Penhe de Franca -Britona site (Site 5). Thus, a robust directly proportional influence between salinity as well as temperature on total fauna was observed predominantly in the monsoon season.





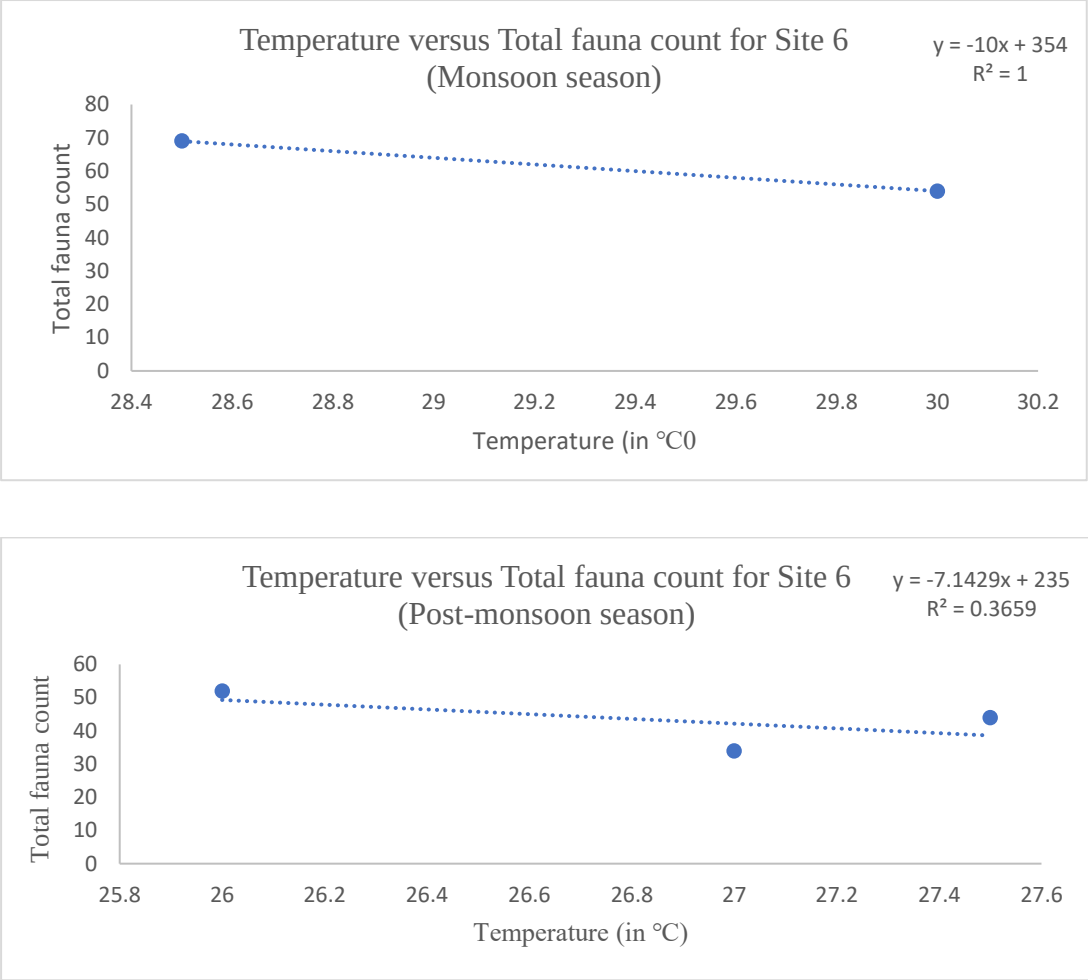


Figure 4.59: Salinity and temperature influence on total fauna in Monsoon and Post-monsoon season for Ponte de Linhares Causeway - Ribandar site (Site 6)

The data suggests that a strong positive and negative correlation of 1 and -1 respectively for salinity and temperature influence on total fauna respectively during the monsoon season while a moderate positive correlation of 0.9 for salinity influence on total fauna and moderate negative correlation of -0.6 was observed for temperature influence on total fauna respectively in the post-monsoon season was observed at Ponte de Linhares Causeway - Ribandar site (Site 6). Thus, a robust directly proportional influence between salinity and total fauna as well as a robust inversely proportional influence

between temperature and total fauna were observed predominantly in the monsoon season.

An overall analysis suggests that a predominant robust correlation between the total fauna and the influence of physicochemical parameters, mainly, salinity and temperature (sea-surface temperature) was observed in the monsoon season in contrast to the post-monsoon season. On average, an approximate inverse correlation was observed between the influence of physicochemical parameters on the total fauna suggesting that an increase in the salinity and temperature may result in a consequent decrease in total fauna species richness within the given sampling locations.

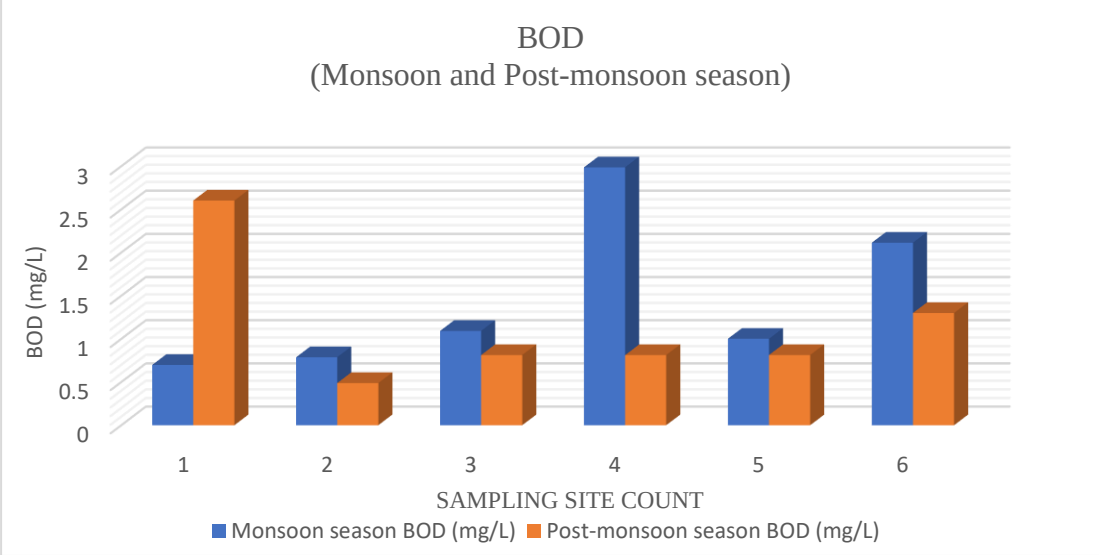


Figure 4.60: BOD for six selected sampling sites in Monsoon and Post-monsoon season

The BOD, on average, was observed to be overall higher in the monsoon season in comparison to the post-monsoon season. This can be attributed to the higher demand for dissolved oxygen by supporting life forms and monsoon showers bring nutrients from allochthonous sources into the estuarine sources thereby increasing the phosphate,

nitrate, and silicate concentrations in the water. A higher BOD may also be attributed due to the decay of vegetation and organic matter decomposition in rivers that mix with seawater during the rainy season.

The resulting findings of the BOD analyzed in the monsoon and post-monsoon season were found to exist under the permissible limits of 5 mg/L as set by the World Health Organization (WHO). In the monsoon season, the BOD was observed to be highest at Chorao Island site (Site 4) at 2.987 mg/L while the BOD was observed to be the lowest at Vagurbem site (Site 1) at 0.698 mg/L. In the post-monsoon season, the BOD was found to be highest at Vagurbem site (Site 1) at 2.602 mg/L while the BOD was found to be lowest at Saint Estevam Island site (Site 2) at 0.488 mg/L.

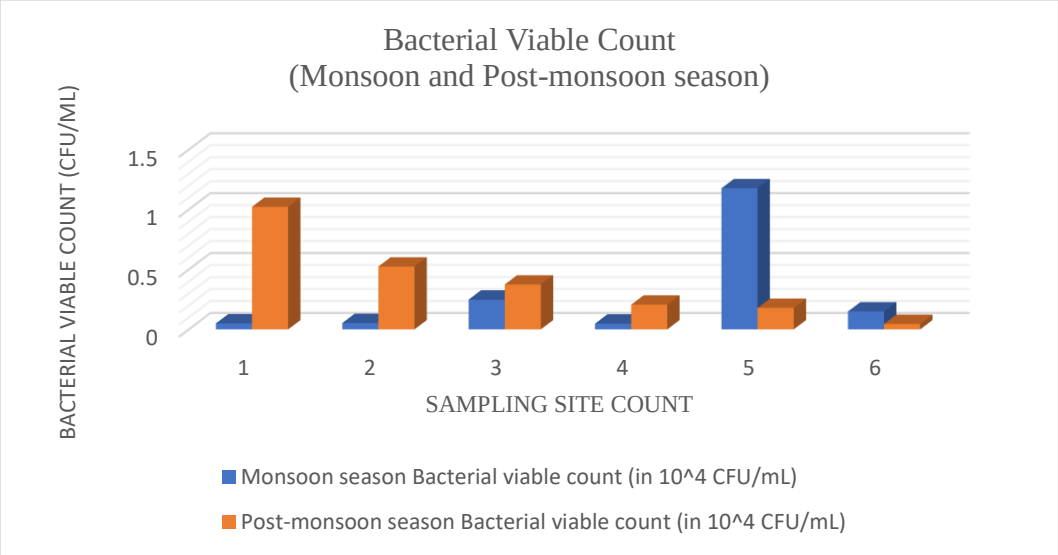


Figure 4.61: Bacterial Viable Count for six selected sampling sites in Monsoon and Post-monsoon season

The bacterial viable count (bacterial colony count) was observed to witness a steady gradual decline in the post-monsoon season in comparison to the monsoon season. In

the monsoon season, the bacterial viable count was observed to be highest at Penhe de Franca – Britona site (Site 5) at 0.094 × 10^{-4} CFU/mL to 2.26 × 10^{-4} CFU/mL and lowest at Chorao Island site (Site 4) at 0.007 × 10^{-4} CFU/mLto 0.09 × 10^{-4} CFU/mL. In the post-monsoon season, the bacterial viable count was observed to be highest at Vagurbem site (Site 1) at 0.204 × 10^{-4} CFU/mL to 1.84 × 10^{-4} CFU/mL and lowest at Ponte de Linhares Causeway – Ribandar site (Site 6) at 0.061 × 10^{-4} CFU/mL to 0.03 × 10^{-4} CFU/mL.

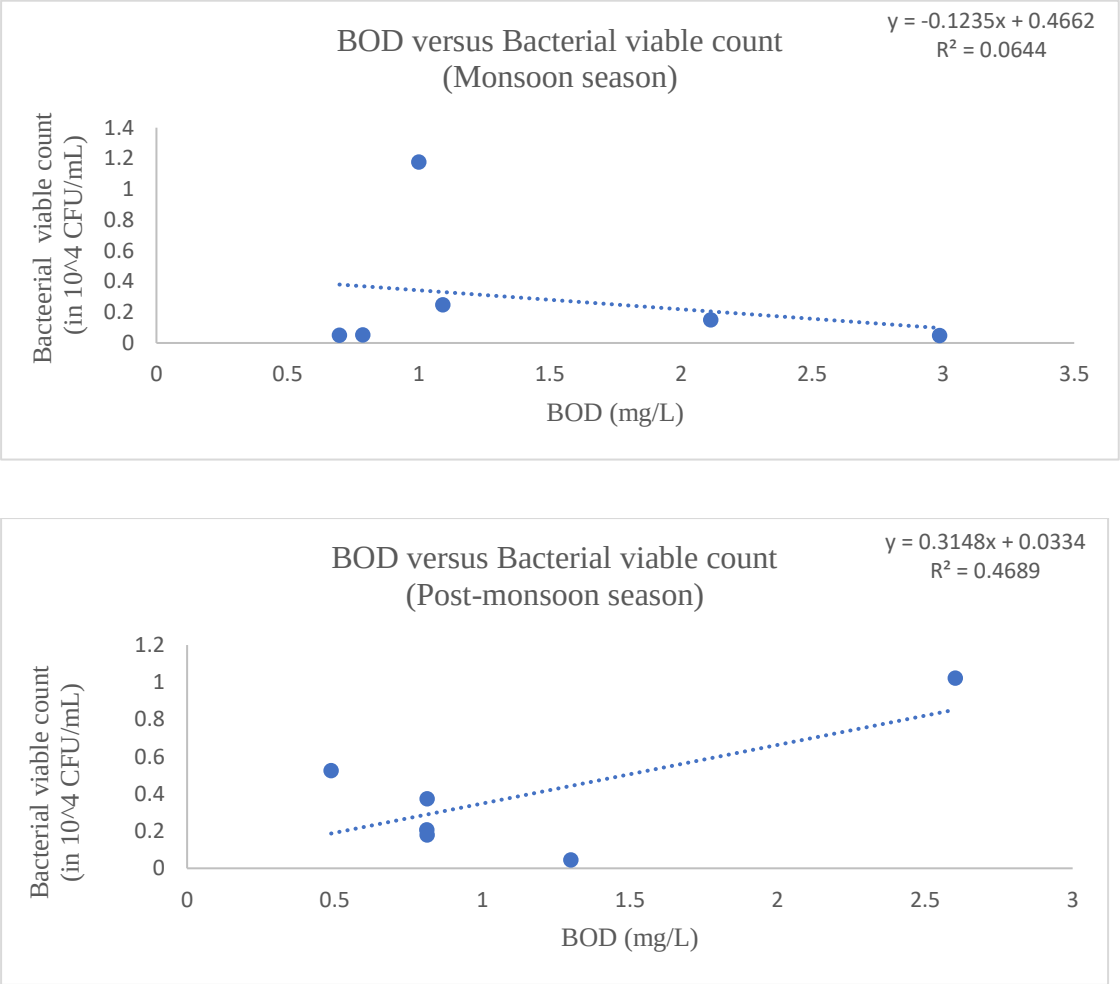


Figure 4.62: Correlation between BOD and Bacterial Viable Count for six selected sampling sites in Monsoon and Post-monsoon season

The data suggests that the influence of BOD on the Bacterial Viable Count is -0.25 (weak negative correlation) for the monsoon season and 0.68 (moderate positive correlation) for the post-monsoon season respectively. In the monsoon season, a weak inverse correlation between the BOD and bacterial viable count was observed suggesting that an increase in the BOD may result in a consequent decrease in the bacterial viable count. In the post-monsoon season, a moderately directly proportional correlation between the BOD and bacterial viable count suggests that an increase in the BOD may result in a consequent increase in the bacterial viable count.

The Shannon-Wiener Diversity Index (denoted as H') was utilized to calculate the diversity of species within each selected sampling location. The Shannon-Wiener Diversity Index also measures the level of habitat diversity in relation to its influence on pollution levels in a given location.

Table 4.44: Estimated pollution level based on Shannon-Wiener Diversity Index (Source: Albueajee et al., 2020)

Range of H' (Shannon-Wiener Index)	**Diversity Level**	**Pollution Level**
0.0 - 1.0	Very less	Heavy pollution
1.0 - 2.0	Less	Moderate pollution
2.0 - 3.0	Moderate	Light pollution
3.0 - 4.5	High	Slight pollution

Table 4.45: Estimated pollution level based on Shannon-Wiener Diversity Index for mangrove flora diversity for selected sampling sites

Sampling Sites	H' value - Shannon-Weiner Diversity Index (Mangrove flora diversity)	Diversity Level	Pollution Level
Site 1 (Control Site)	**No Mangrove flora**	**ND**	**ND**
Site 2	**2.085**	**Moderate diversity level**	**Light pollution level**
Site 3	**2.025**	**Moderate diversity level**	**Light pollution level**
Site 4	**2.313**	**Moderate diversity level**	**Light pollution level**
Site 5	**1.998**	**Less diversity level**	**Moderate pollution level**
Site 6	**2.034**	**Moderate diversity level**	**Light pollution level**

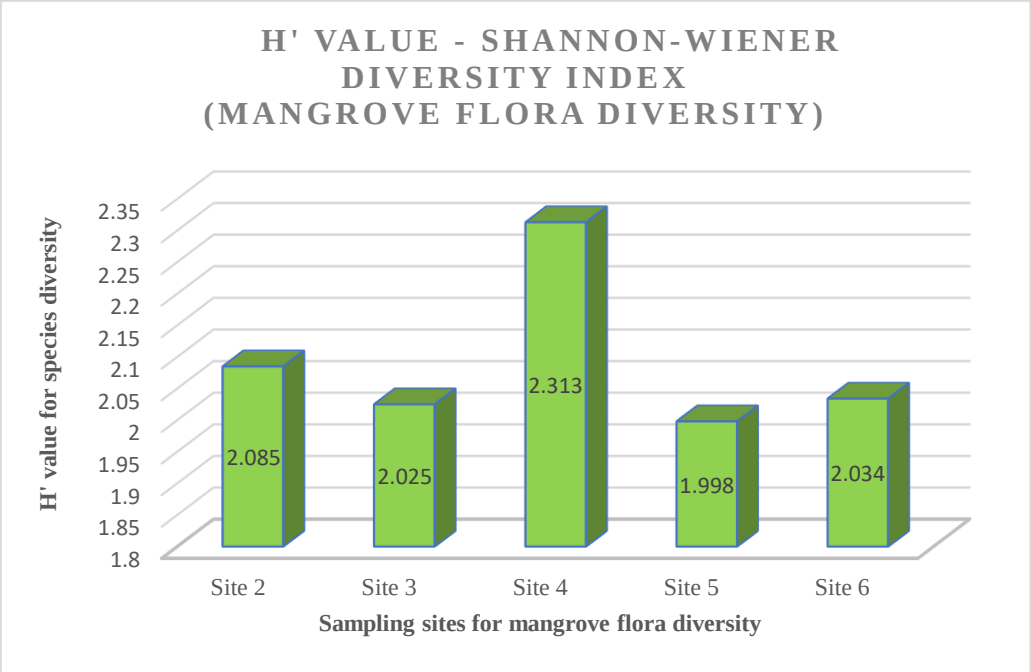


Figure 4.63: Graphical representation of Shannon-Wiener Diversity Index for mangrove flora diversity for selected sampling sites

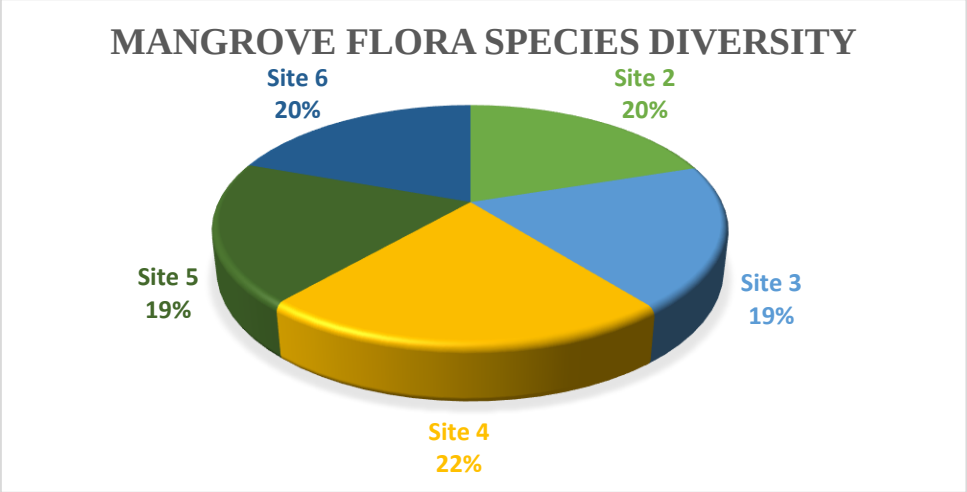


Figure 4.64: Pie-chart representation of Shannon-Wiener Diversity Index based percentage diversity for mangrove flora at selected sampling sites

The percentage diversity of mangrove flora was found to be highest at Chorao Island site (Site 4) and lowest at Divar Island site (Site 3) and Penhe de Franca – Britona site (Site 5). The overall mangrove floral diversity as per the Shannon-Wiener Diversity Index was observed to be on average of less to moderate levels of mangrove flora species diversity with the influence of light to moderate pollution levels within all the selected sampling sites.

Table 4.46: Estimated pollution level based on Shannon-Wiener Diversity Index for avifauna diversity for selected sampling site

Sampling Sites	H' value - Shannon-Weiner Diversity Index (Mangrove flora diversity)	Diversity Level	Pollution Level
Site 1 (Control Site)	0.784	Very less diversity level	Heavy pollution level
Site 2	2.138	Moderate diversity level	Light pollution level
Site 3	1.956	Less diversity level	Moderate pollution level
Site 4	2.333	Moderate diversity level	Light pollution level
Site 5	1.669	Less diversity level	Moderate pollution level
Site 6	2.472	Moderate diversity level	Light pollution level

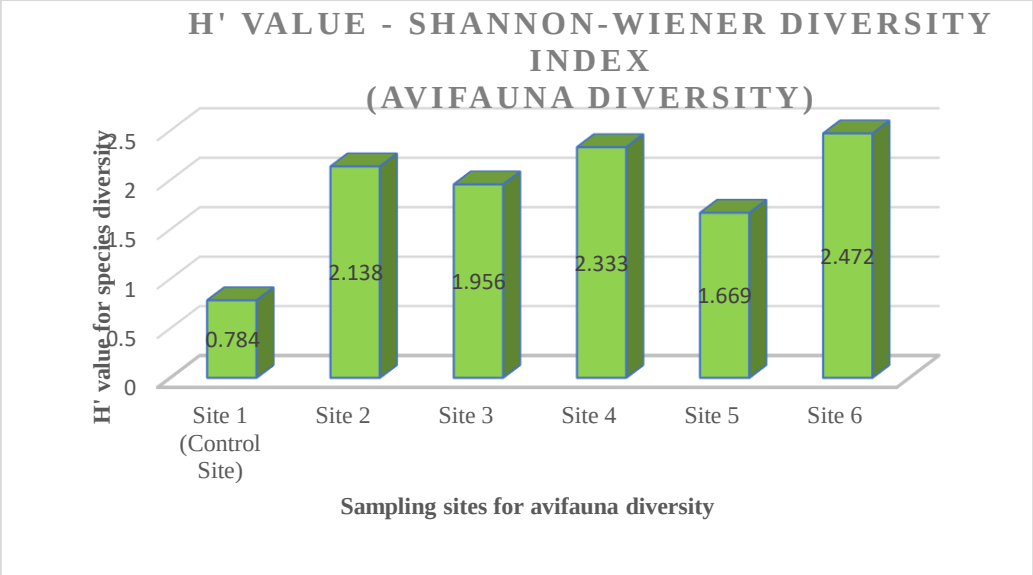


Figure 4.65: Graphical representation of Shannon-Wiener Diversity Index for avifauna diversity for selected sampling sites

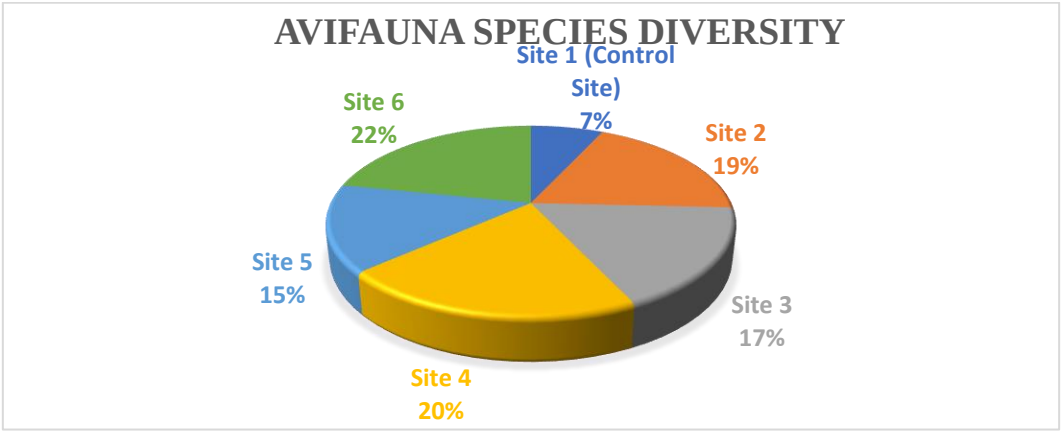


Figure 4.66: Pie-chart representation of Shannon-Wiener Diversity Index based percentage diversity for avifauna at selected sampling sites

The percentage diversity of avifauna was found to be highest at Ponte de Linhares Causeway – Ribandar site (Site 6) and lowest at Vagurbem site (Site 1). The overall avifaunal diversity as per the Shannon-Wiener Diversity Index was observed to be on average of less to moderate levels of avifaunal species diversity with the influence of light to moderate pollution levels within all the selected sampling sites.

Table 4.47: Estimated pollution level based on Shannon-Wiener Diversity Index for terrestrial invertebrate fauna diversity for selected sampling sites

Sampling Sites	H' value - Shannon-Weiner Diversity Index (Mangrove flora diversity)	Diversity Level	Pollution Level
Site 1 (Control Site)	**1.613**	**Less diversity level**	**Moderate pollution level**
Site 2	**1.662**	**Less diversity level**	**Moderate pollution level**
Site 3	**1.758**	**Less diversity level**	**Moderate pollution level**
Site 4	**1.883**	**Less diversity level**	**Moderate pollution level**
Site 5	**0.97**	**Very less diversity level**	**Heavy pollution level**
Site 6	**2.308**	**Moderate diversity level**	**Light pollution level**

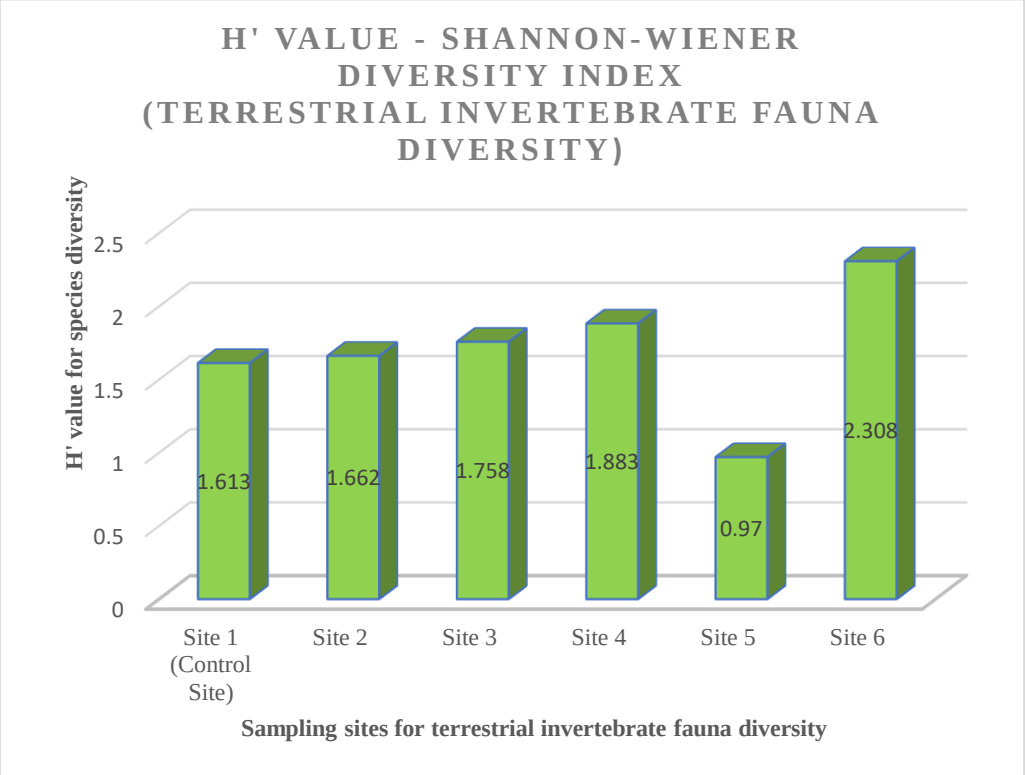


Figure 4.67: Graphical representation of Shannon-Wiener Diversity Index for terrestrial invertebrate fauna diversity for selected sampling sites

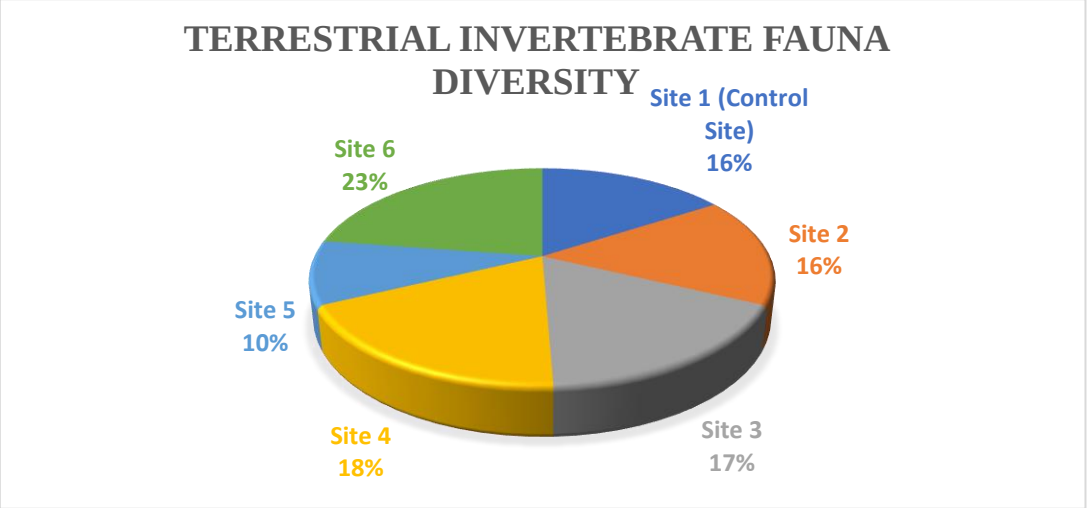


Figure 4.68: Pie-chart representation of Shannon-Wiener Diversity Index based percentage diversity for terrestrial invertebrate fauna at selected sampling sites

The percentage diversity of terrestrial invertebrate fauna was found to be highest at Ponte de Linhares Causeway – Ribandar site (Site 6) and lowest at Penhe de Franca - Britona site (Site 5). The overall terrestrial invertebrate fauna diversity as per the Shannon-Wiener Diversity Index was observed to be on average of less levels of terrestrial invertebrate fauna species diversity with the influence of moderate pollution levels within all the selected sampling sites.

Table 4.48: Estimated pollution level based on Shannon-Wiener Diversity Index for aquatic fauna diversity for selected sampling sites

Sampling Sites	H' value - Shannon-Weiner Diversity Index (Mangrove flora diversity)	Diversity Level	Pollution Level
Site 1 (Control Site)	0	Very less diversity level	Heavy pollution level
Site 2	1.862	Less diversity level	Moderate pollution level
Site 3	1.136	Less diversity level	Moderate pollution level
Site 4	2.007	Moderate diversity level	Light pollution level
Site 5	1.11	Less diversity level	Moderate pollution level
Site 6	1.564	Less diversity level	Moderate pollution level

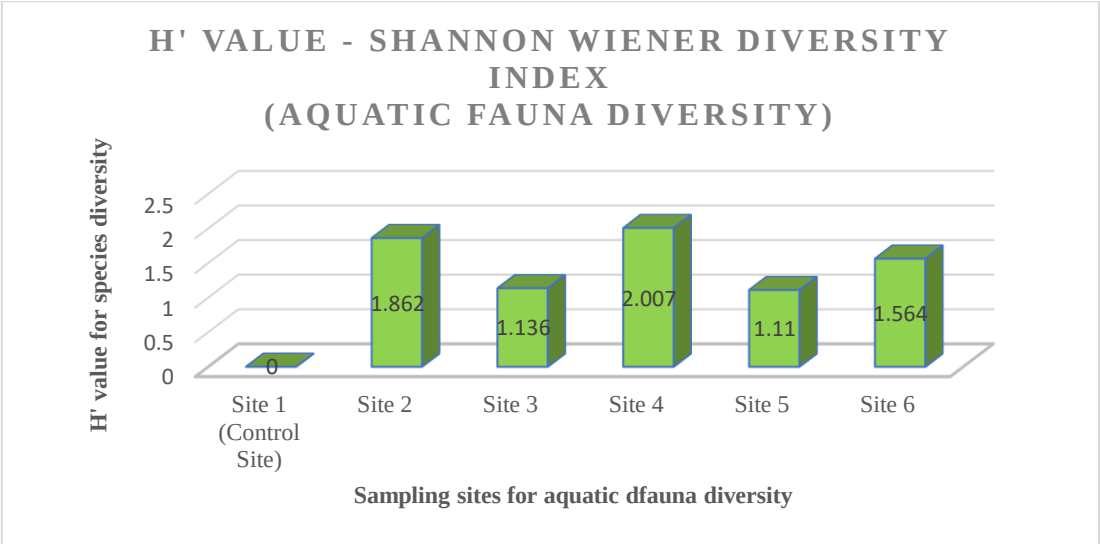


Figure 4.69: Graphical representation of Shannon-Wiener Diversity Index for aquatic fauna diversity for selected sampling sites

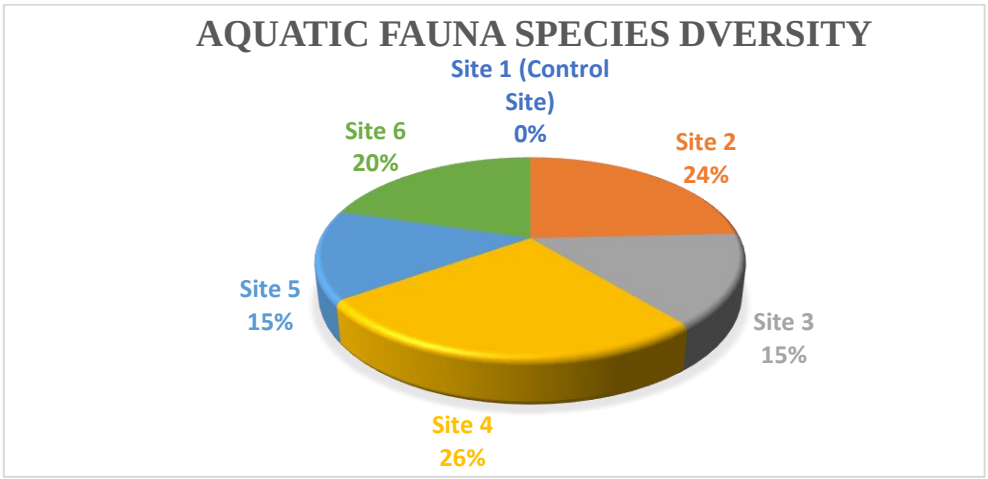


Figure 4.70: Pie-chart representation of Shannon-Wiener Diversity Index based percentage diversity for aquatic fauna at selected sampling sites

The percentage diversity of aquatic fauna was found to be highest at Chorao site (Site 4) and lowest at Vagurbem site (Site 1). The overall aquatic fauna diversity as per the Shannon-Wiener Diversity Index was observed to be on average of less levels of aquatic fauna species diversity with the influence of moderate pollution levels within all the selected sampling sites.

The Margalef Index (denoted as d) was utilized to calculate the species richness for the selected sampling sites.

Table 4.49: Estimated species richness level based on Margalef Index values (Source: Latumahina et al., 2020)

Index Value	Category
R < 2.5	Low species richness
2.5 > R < 4	Medium species richness
R > 4	High species richness

Table 4.50: Estimated species richness level based on Margalef Index values for mangrove flora within selected sampling sites

Sampling Sites	Margalef Index (d)	Species Richness Level
Site 1 (Control Site)	**No mangroves found**	**NA**
Site 2	**0.676**	**Low species richness**
Site 3	**0.396**	**Low species richness**
Site 4	**0.432**	**Low species richness**
Site 5	**0.413**	**Low species richness**
Site 6	**0.448**	**Low species richness**

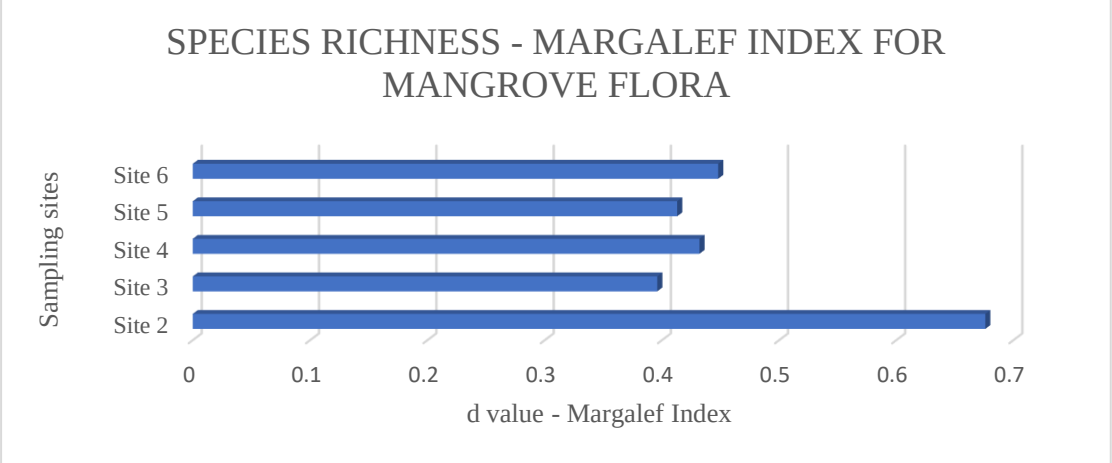


Figure 4.71: Graphical representation of Margalef Index for mangrove flora species richness at selected sampling sites

According to the Margalef Index for species richness for mangrove flora suggests the highest species richness was observed at Saint Estevam Island site (Site 2) and the lowest species richness was observed at Divar Island site (Site 3). An overall low level of species richness for mangrove flora was observed within the selected sampling sites.

Table 4.51: Estimated species richness level based on Margalef Index values for avifauna within selected sampling sites

Sampling Sites	Margalef Index (d)	Species Richness Level
Site 1 (Control Site)	**1.442**	**Low species richness**
Site 2	**0.776**	**Low species richness**
Site 3	**0.922**	**Low species richness**
Site 4	**0.866**	**Low species richness**
Site 5	**0.557**	**Low species richness**
Site 6	**0.291**	**Low species richness**

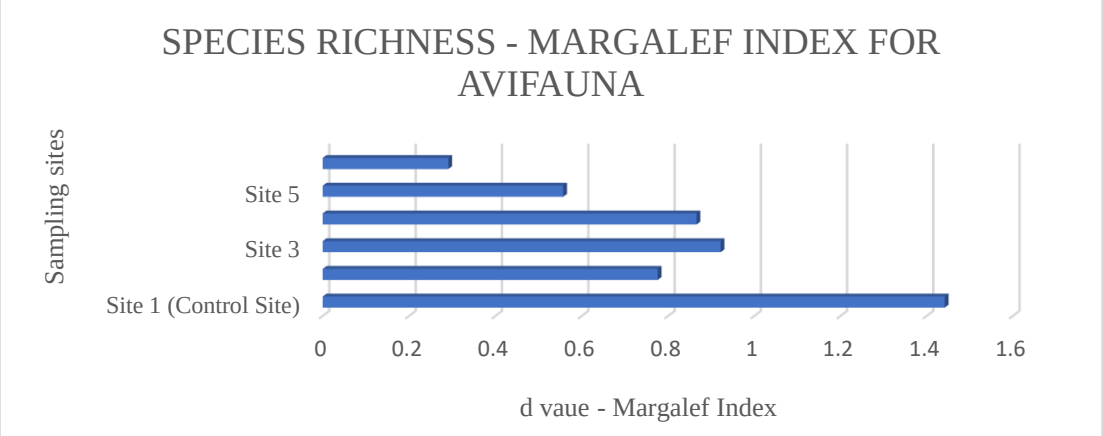


Figure 4.72: Graphical representation of Margalef Index for avifauna species richness at selected sampling sites

According to the Margalef Index for species richness for avifauna suggests the highest species richness was observed at Vagurbem site (Site 1) and the lowest species richness was observed at Ponte de Linhares Causeway – Ribandar site (Site 6). An overall low level of species richness for avifauna was observed within the selected sampling sites.

Table 4.52: Estimated species richness level based on Margalef Index values for terrestrial invertebrate fauna within selected sampling sites

Sampling Sites	Margalef Index (d)	Species Richness Level
Site 1 (Control Site)	1.071	Low species richness
Site 2	0.426	Low species richness
Site 3	0.54	Low species richness
Site 4	0.981	Low species richness
Site 5	0.456	Low species richness
Site 6	0.801	Low species richness

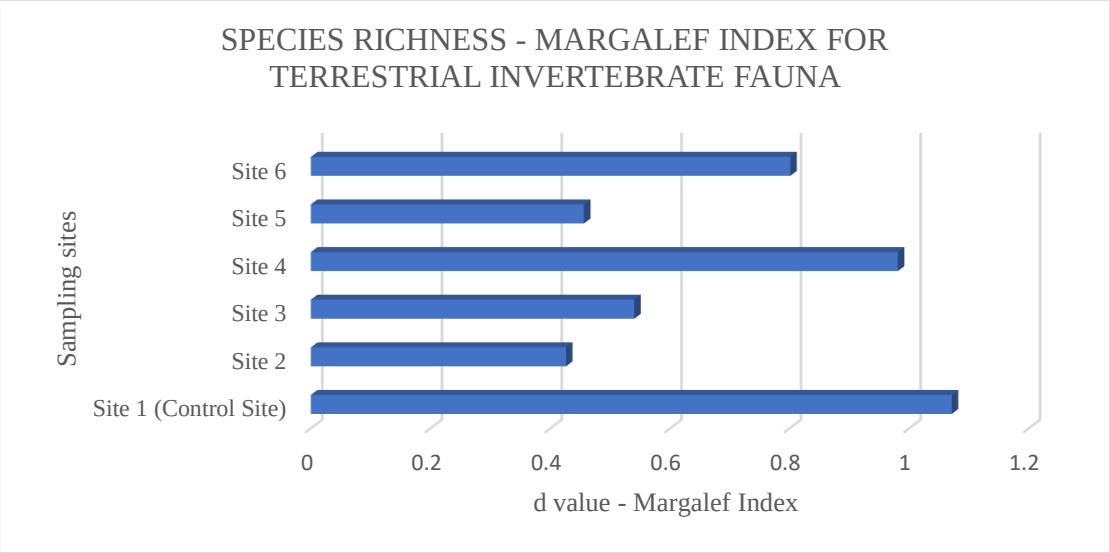


Figure 4.73: Graphical representation of Margalef Index for terrestrial invertebrate fauna species richness at selected sampling sites

According to the Margalef Index for species richness for terrestrial invertebrate fauna suggests the highest species richness was observed at Vagurbem site (Site 1) and the lowest species richness was observed at Saint Estevam Island site (Site 2). An overall low level of species richness for avifauna was observed within the selected sampling sites.

Table 4.53: Estimated species richness level based on Margalef Index values for aquatic fauna within selected sampling sites

Sampling Sites	Margalef Index (d)	Species Richness Level
Site 1 (Control Site)	0	Low species richness
Site 2	0.292	Low species richness
Site 3	0.427	Low species richness
Site 4	0.354	Low species richness
Site 5	0.207	Low species richness
Site 6	0.498	Low species richness

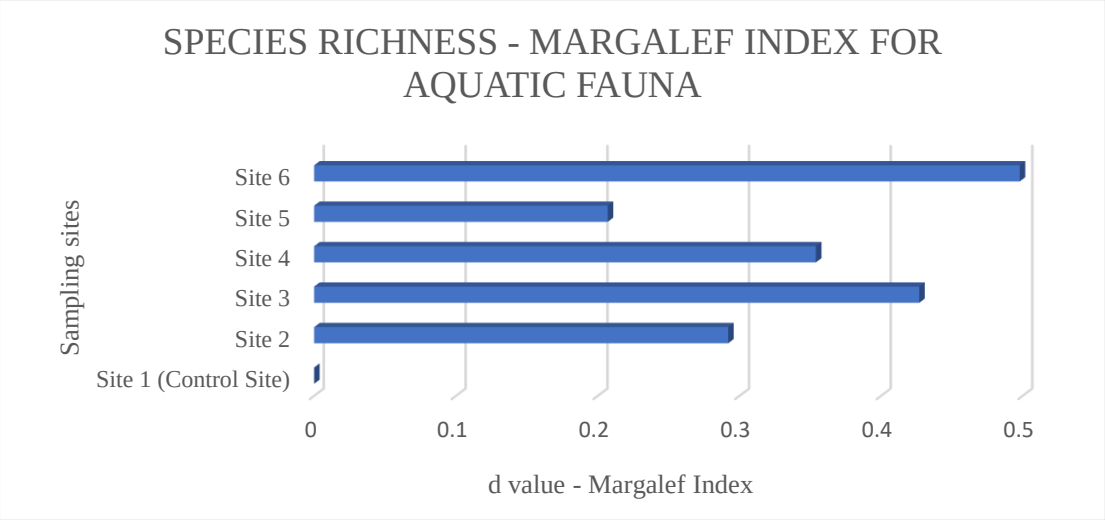


Figure 4.74: Graphical representation of Margalef Index for aquatic fauna species richness at selected sampling sites

According to the Margalef Index for species richness for aquatic fauna suggests the highest species richness was observed at Ponte de Linhares Causeway - Ribandar site (Site 6) and the lowest species richness was observed at Vagurbem site (Site 1). An overall low level of species richness for avifauna was observed within the selected sampling sites.

One-way Analysis of Variance (ANOVA) was carried out for estimating the macrofauna data of the selected sampling sites in comparison with the Control Site (Site 1 – Vagurbem site) by Post-hoc Testing (Bonferroni Correction) using Dunnett Test. The total macrofauna data for the selected sampling sites followed Gaussian Distribution thus following the parametric test through one-way Analysis of Variance (ANOVA) was carried out.

Table 4.54: Summarized variance for dataset of selected sampling sites

Groups	*Count*	*Sum*	*Average*	*Variance*
SITE 1 (CONTROL)	**5**	**64**	**12.8**	**25.7**
SITE 2	**5**	**1005**	**201**	**7196**
SITE 3	**5**	**250**	**50**	**82.5**
SITE 4	**5**	**433**	**86.6**	**1191.8**
SITE 5	**5**	**453**	**90.6**	**1164.8**
SITE 6	**5**	**253**	**50.6**	**167.8**

Table 4.55: One-way Analysis of Variance (ANOVA) for dataset of selected sampling sites

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	105273	5	21054.7	12.8531	3.84035E-06	2.62065
Within Groups	39314.4	24	1638.1			
Total	144588	29				

The p-value calculated was observed to be 3.84×10^{-6}, hence $p < 0.05$ supports the alternative hypothesis suggesting a significant difference between total macrofauna observed within all six selected sampling sites.

Table 4.56: Alpha estimation to prove significance of Pot-hoc test (Bonferroni Correction)

Test	*Alpha*
ANOVA	0.05
Post-hoc test (Bonferroni Correction)	0.008333333

Post-hoc test (Bonferroni Correction) p-value calculated was observed to be 0.0083 ($p<0.05$), hence Post-hoc Dunnett Test is significant.

Table 4.57: Estimation of Post-hoc test by Dunnett Test

Groups/Sites	*p-value (T-test)*	*Significant*
Site 1 (Control) v Site 2	0.001118003	Yes
Site 1 (Control) v Site 3	4.37956E-05	Yes
Site 1 (Control) v Site 4	0.001484056	Yes
Site 1 (Control) v Site 5	0.000999179	Yes
Site 1 (Control) v Site 6	0.000297154	Yes

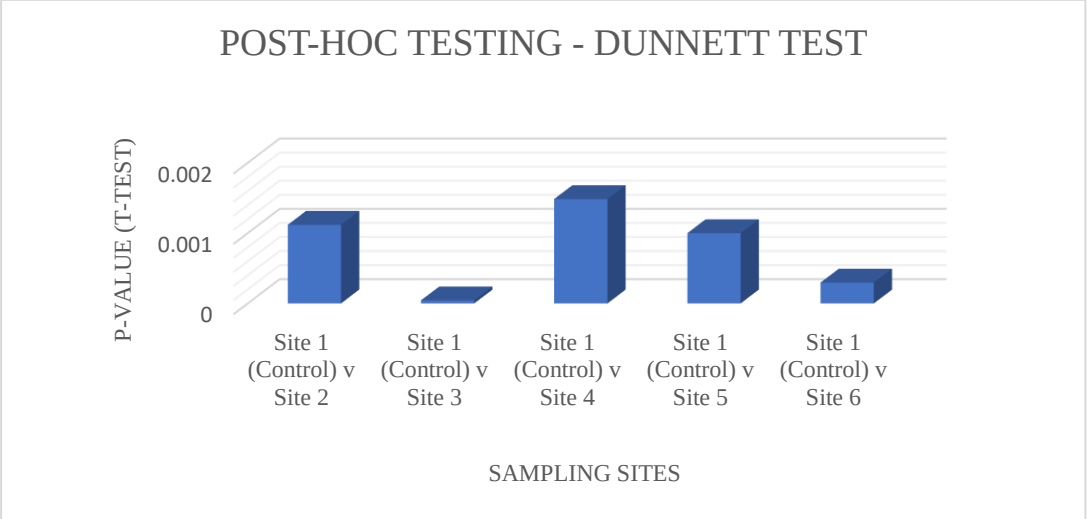


Figure 4.75: Graphical representation of Post-hoc testing using Dunnett Test

The given dataset evidently suggests a significant difference between Vagurbem site – Control Site (Site 1) versus Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively at significance level $p<0.001$. Thus, the dataset proves a significant difference in the total fauna observed and calculated between each selected sampling site in comparison with the Control Site (Site 1).

CHAPTER 5

DISCUSSION

AND

CONCLUSIONS

5.1 DISCUSSION

For the present study on the biodiversity associated with the mangrove ecosystems along the six selected sampling sites, mainly, Vagurbem, Saint Estevam Island, Divar Island, Chorao Island, Penhe de Franca – Britona, and Ponte de Linhares Causeway – Ribandar along the Mandovi estuary in Goa, the coverage of mangrove flora species as well as the associated macrofauna species and bacterial microflora species were documented and analyzed. According to the Goa Forest Department (2024) reports, Goa houses 16 species of mangrove flora along the seven estuaries of Goa.

As per the findings of the present study, the coverage of mangrove flora observed include an approximate of 14 species of mangrove flora, mainly, *Avicennia officinalis, Avicennia marina, Avicennia alba, Acanthus illicifolius, Sonneratia alba, Clerodendrum inerme, Excoecaria agallocha, Rhizophora apiculata, Rhizophora mucronata, Derris heterophylla, Kandelia candel, Aegiceras corniculatum, Acrostichum aureum*, and *Bruguiera cylindrica* observed along the five sampling sites. These include Saint Estevam Island site, Divar Island site, Chorao Island site, Penhe de Franca -Britona site, and Ponte de Linhares Causeway – Ribandar site. In comparison to the findings reported by the Goa Forest Department, approximately 13 mangrove flora species have been reported and documented along along Britona, Chorao Island, Divar Island, and Ribandar sites along the Mandovi estuary. These 13 species of mangrove flora include which include *Sonneratia alba, Bruguiera cylindrica, Avicennia officinalis, Avicennia alba, Rhizophira mucronata, Rhizophora apiculata, Kandelia candel, Kandelia rheedii, Acanthus illicifolius, Derris heterophylla Acrostichum aureum, Excoecaria agallocha*, and *Aegiceras cornmculatum* (Goa Forest Department, 2024)

As per reports by the Goa Forest Department (2024), the island of Saint Estevam has not been previously documented for its mangrove flora coverage, hence this study provided a new documentation of mangrove flora species observed along the Saint Estevam Island site. The mangrove flora species *Clerodendrum inerme* was observed to be a new finding documented at Saint Estevam Island, Divar Island, Chorao Island, and Ponte de Linhares Causeway – Ribandar site along the Mandovi estuary in comparison to previous reports by the Goa Forest Department (2024), and Kothari et al. (2002).

The analysis of physicochemical parameters in terms of the salinity, temperature, and BOD were carried out in the Monsoon and Post-monsoon seasons respectively. Climatic seasonal variations suggest that in the Monsoon season salinity ranged between 1‰ to 14‰, sea-surface temperature ranged between 26.5°C to 30.5°C, and the BOD ranged between 0.6 mg/L to 2.9 mg/L. For the Post-monsoon season, the salinity ranged between 8‰ to 31‰, the sea-surface temperature ranged between 26°C to 31.5°C, and the BOD ranged between 0.4 mg/L to 2.6 mg/L. A higher degree of fluctuations in salinity and temperature variations were more predominant in the Post-monsoon season in comparison to the Monsoon season.

The findings reported for the bacterial microflora suggest a predominantly extensive presence of Gram-positive rods and cocci bacterial species within the estuarine water source. This suggests an increasing dominance of anaerobic and aerobic bacterial species such as *Clostridium,* and *Actinobacteria* as well dominance in phylum Proteobacteria as reportedly found along the Mandovi estuary (Gose et al., 2024).The BOD during the Monsoon and Post-monsoon seasons were observed to be present within the World Health Organization's permissible limits of 5 mg/L (Singh et al.,2013).

The BOD was found to be relatively higher in the Monsoon season in comparison to the Post-monsoon season suggesting a possible attribution to the increasing presence of organic matter, phosphates, nitrates, and silicates accumulated from allochthonous sources in higher concentration due to the monsoon showers. The bacterial viable count was observed to witness a steady decline in the Post-Monsoon season in comparison to the Monsoon season. During the Monsoon season, the bacteria viable count ranged between the highest at Penhe de Franca – Britona site (Site 5) at 0.094×10^{-4} CFU/mL to 2.26×10^{-4} CFU/mL and lowest at Chorao Island site (Site 4) at 0.007×10^{-4} CFU/mLto 0.09×10^{-4} CFU/mL. In the Post-monsoon season, the bacterial viable count was observed to be highest at Vagurbem site (Site 1) at 0.204×10^{-4} CFU/mL to 1.84×10^{-4} CFU/mL and lowest at Ponte de Linhares Causeway – Ribandar site (Site 6) at 0.061×10^{-4} CFU/mL to 0.03×10^{-4} CFU/mL. The increase in the bacterial viable count in the Monsoon season as compared to the Post-monsoon season may be attributed to the increase in the cycling of organic matter by the estuarine microbiome due to the allochthonous sources in higher concentration during the monsoon showers.

Correlation studies carried out between the bacterial viable count and BOD suggest that a weak negative correlation (inverse correlation) was observed for the Monsoon season while a moderate positive correlation (direct correlation) was observed during the Post-monsoon season. Due to the increasing correlative robustness observed in the Post-monsoon season in comparison to the Monsoon season, this suggests that predominantly a direct correlative linkage was apparent illustrating that with an increase in the bacterial viable count a consequent increase is observed in the BOD.

An overall analysis of the correlative linkage between the total macrofauna present and the influence of physicochemical parameters, mainly, salinity and temperature suggests that increasing robustness in correlation was observed in the Monsoon season in comparison to the Post-monsoon season. An approximate strong negative correlation (inverse correlation) was more predominantly present especially during the Monsoon season thus suggesting an inverse correlative linkage stating that with an increase in the salinity and temperature, a consequent decline in species richness of fauna is a predicted outcome.This suggests that as stated by Ball (2016), a drastic increasing change in salinity levels can stress the mangrove flora and fauna species while some mangrove species are uniquely adapted to remain acclimatized to higher levels of salinity due to the presence of salt excretion mechanisms to thrive in these existing conditions. Consequently, this also suggests that as per studies by Osland et al. (2017), with rising temperatures associated with climate change, not only is there a profound impact on the mangrove species diversity but in addition to this, a higher temperature gradient will influence the species composition of microflora and macrofaunal species as well as the migration pattern of a variety of macrofaunal species associated with mangrove ecosystems.

The Shannon-Wiener Diversity Index was utilized to estimate the species diversity of mangrove flora and macrofauna within the six selected sampling sites. The percentage diversity of mangrove flora was estimated to be highest at Chorao Island site (Site 4) and lowest at Divar Island site(Site 3). The data suggest an overall less to moderate level of mangrove flora diversity with light to moderate pollution level influences within all sampling sites. The percentage diversity of avifauna was estimated to be highest at Ponte de Linhares Causeway – Ribandar site (Site 6) and lowest at

Vagurbem site (Site 1). The data suggests an overall less to moderate level of avifaunal diversity with light to moderate levels of pollution influences within all the sampling sites. The percentage diversity of terrestrial invertebrate fauna was estimated to be highest at Ponte de Linhares Causeway – Ribandar site (Site 6) and lowest at Penhe de Franca – Britona site (Site 5). The data suggests an overall less level of terrestrial invertebrate fauna diversity with moderate pollution level influences within all sampling sites. The percentage diversity of aquatic fauna was estimated to be highest at Chorao Island site (Site 4) and lowest at Vagurbem site (Site 1). The data suggests an overall less level of aquatic fauna diversity with moderate pollution level influences within all sampling sites.

The Margalef Index was utilized to estimate the species richness of mangrove flora and macrofauna within the six selected sampling sites. The species richness of mangrove flora was estimated to be highest at Saint Estevam Island site (Site 2) and the lowest at Divar Island site (Site 3). The data suggests an overall low level of species richness for mangrove flora was observed within all the sampling sites. The species richness of avifauna was estimated to be highest at Vagurbem site (Site 1) and lowest at Ponte de Linhares Causeway – Ribandar site (Site 6). The data suggests an overall low level of species richness for avifauna was observed within all the sampling sites. The species richness of terrestrial invertebrate fauna was estimated to be highest at Vagurbem site (Site 1) and lowest at Saint Estevam Island site (Site 2). The data suggests an overall low level of species richness for terrestrial invertebrate fauna was observed within all the sampling sites. The species richness of aquatic fauna was estimated to be highest at Ponte de Linhares Causeway – Ribandar site (Site 6) and lowest at Vagurbem site (Site 1). The data suggests an overall low level of species

richness for aquatic fauna was observed within all the sampling sites. This could possibly be attributed to the increasing pollution levels and drastic fluctuations in seasonal variations and altering weather patterns.

The parametric data on the total macrofauna coverage within the six selected sampling sites followed Gaussian distribution, hence One-Way Analysis of Variance (ANOVA) was carried out followed by Post-hoc test (Bonferroni Correction) using Dunnett Test to compare total macrofauna of five selected sampling sites with the Control site – Vagurbem site (Site 1 respectively). The dataset of the total macrofauna proved a significant difference (at $p<0.001$) between each selected sampling site in comparison with the Control site. Thus, suggesting that the Control site present closest in proximity to the mouth of the Mandovi river lacking the presence of mangrove flora coverage due to lower salinity conditions in comparison to the other five sampling sites along Mandovi estuary displaying increasing levels of salinity in connection with the Arabian sea, provides a significant comparison in the associated macrofauna coverage due to salinity variations shaping the mangrove flora coverage along the Mandovi estuary.

5.2 CONCLUSIONS

The present study's key focus encompasses the documentation of the present status of biodiversity in terms of the mangrove flora as well as the the associated macrofauna and microflora observed within the mangrove ecosystem sites along the Mandovi estuary in Goa. The six selected sampling sites along the Mandovi estuary include Vagurbem site (Control site – Site 1), Saint Estevam Island site (Site 2), Divar Island site (Site 3), Chorao Island site (Site 4), Penhe de Franca – Britona site (Site 5), and Ponte de Linhares Causeway – Ribandar site (Site 6) respectively. The given study provides an updated list of mangrove flora coverage suggesting that 13 species of mangrove flora were observed and documented within the selected sampling sites along the Mandovi estuary. The mangrove flora species *Clerodendrum inerme* was observed to be a new finding documented at Saint Estevam Island, Divar Island, Chorao Island, and Ponte de Linhares Causeway – Ribandar site along the Mandovi estuary.

The primary aim of this study was to estimate the influence of physicochemical parameters, mainly, temperature, salinity, and BOD, and analyze its correlation with species richness in terms of the flora and fauna coverage using biostatistics. The six selected sampling sites were estimated for the physicochemical characteristics in the Monsoon and Post-monsoon season with findings suggesting a higher degree of fluctuations in salinity and temperature observed in the Post-monsoon season while a higher degree of BOD was observed in the Monsoon season. The biodiversity in terms of the mangrove flora was documented and preserved using herbariums of the plant samples collected. The estuarine water samples were collected for the microflora estimation wherein bacterial colonies were isolated, quantified, and characterized morphologically by the Gram staining technique.The findings reported a predominant

abundance of Gram-positive rods and cocci bacterial microflora species found in the estuarine waters. The bacterial viable count displayed a steady decline in the Post-monsoon season in comparison to the Monsoon season. Correlation studies between bacterial viable count and BOD showcased a positive correlation (direct correlation) suggesting an increase in bacterial viable count may lead to an increase in the BOD. Consequently,the levels of BOD and bacterial viable count were observed to be higher in the Monsoon season in comparison to the Post-monsoon season.

The macrofauna associated with the mangrove ecosystems in terms of the avifauna species, aquatic fauna species, and terrestrial invertebrate species were observed, identified, and documented during each sampling visit. Further, correlation studies were utilized to estimate the influence of salinity and temperature on the total macrofauna species during the Monsoon and Post-monsoon seasons. The findings reported suggest a negative correlation (inverse correlation) predominantly in the Monsoon season exists between the total macrofauna due to the influence of the physicochemical parameters suggesting an increase in the temperature and salinity may result in the decrease in species richness of the total macrofauna within the sampling sites. Furthermore, the species diversity and species richness in terms of its flora and fauna species were documented and analyzed using the Shannon-Wiener Diversity Index and Margalef Index respectively. The species diversity of mangrove flora and macrofauna calculated using the Shannon-Wiener Diversity Index for the six sampling sites displayed that an approximate of less to moderate diversity was observed on average as a result of light to moderate pollution level influences. The Margalef Index utilized for calculating the species richness for the mangrove flora and macrofauna within the six sampling sites suggests that an overall low level of species richness was

observed. Lastly, one-way Analysis of Variance (ANOVA) followed by Post-hoc test (Bonferroni Correction) using Dunnett test showcased that a significant difference (at $p<0.001$) in the total macrofauna species richness between the Control site and the other five sampling sites were observed. Thus suggesting the influence of changing salinity from the mouth of the river (Control site) to the subsequent sampling sites further along the Mandovi estuary displaying an increase in salinity levels and hence the role of salinity in shaping the species diversity and species richness of mangrove flora and its associated macrofuna within the mangrove ecosystems.

BIBLIOGRAPHY

- Albueajee, A. I., Hassan, F. M., & Douabul, A. A. Z. (2020). PHYTOPLANKTON SPECIES COMPOSITION AND BIODIVERSITY INDICES IN AUDA MARSH-SOUTHERN IRAQ. *Iraqi Journal of Agricultural Sciences*, *51*. https://www.researchgate.net/publication/338804944_PHYTOPLANKTON_SPECIES_COMPOSITION_AND_BIODIVERSITY_INDICES_IN_AUDA_MARSH-SOUTHERN_IRAQ?enrichId=rgreq-8e14a4d7e3e42c657e30c87fa528b253-XXX&enrichSource=Y292ZXJQYWdlOzMzODgwNDk0NDtBUzo4NTA5NDc4MDI2NjQ5NjBAMTU3OTg5MzE3NDQ4MQ%3D%3D&el=1_x_3&_esc=publicationCoverPdf
  (Reference for Table 3.1: Pollution level based on the Shannon-Wiener Diversity Index,Table 4.44: Estimated pollution level based on Shannon-Wiener Diversity Index)

- Alongi, D. M. (1987). The ecology of tropical soft-bottom benthic ecosystems. Oceanography and Marine Biology: An Annual Review, 25, 281-345. https://revistas.ucr.ac.cr/index.php/rbt/article/view/23625/23825

- Alongi, D. M. (2008). Mangrove forests: Resilience, protection from tsunamis, and responses to global climate change. Estuarine, Coastal and Shelf Science, 76(1), 1-13. https://doi.org/10.1016/j.ecss.2007.08.024

- Alongi, D. M. (2014). Carbon cycling and storage in mangrove forests. Annual Review of Marine Science, 6, 195-219. https://doi.org/10.1146/annurev-marine-010213-135020

- Alongi, D. M. (2015). The impact of climate change on mangrove forests. Current Climate Change Reports, 1(1), 30-39. https://doi.org/10.1007/s40641-015-0002-x

- Alongi, D. M., Murdiyarso, D., Fourqurean, J. W., Kauffman, J. B., Hutahaean, A., Crooks, S., & Wagey, T. (2016). Indonesia's blue carbon: a globally significant and vulnerable sink for seagrass and mangrove carbon. Wetlands ecology and management, 24, 3-13. https://doi.org/10.1007/s11273-015-9446-y

- Araújo, A. S., et al. (2006). Ant-hemipteran mutualism in a Brazilian mangrove: The role of litter-feeding termites (Isoptera: Rhinotermitidae) in the viviparous reproduction of the mangrove ant Pachycondyla marginata (Formicidae: Ponerinae). Sociobiology, 47(3), 685-695.

- Armitage, P., Berry, G., & Matthews, J. N. S. (2008). *Statistical methods in medical research*. John Wiley & Sons. https://www.96.188.170.250:8080/jspui/bitstream/123456789/2964/1/Peter Armitage%2C Geoffrey Berry%2C J. N. S. Matthews - Statistical Methods in Medical Research 4th Edition-Blackwell (2002).pdf

- Aslam, M. (2009). Diversity, species richness and evenness of moth fauna of Peshawar. *Pak. Entomol*, *31*(2), 99-102. https://indiabiodiversity.org/biodiv/content/documents/635.pdf

- (2024). *About Goa Ecosystems*. Goa State Biodiversity Board. https://gsbb.goa.gov.in/about-goa-ecosystems/

- Ball, M. C. (2016). Ecophysiology of mangroves. Trees, 30(3), 335-345. https://doi.org/10.1007/978-3-319-27422-5_7

- Beutler, M., Wiltshire, K. H., Meyer, B., Moldaenke, C., Luring, C., Meyerhofer, M., & Hansen, U. P. (2014). APHA (2005), Standard Methods for the Examination of Water and Wastewater, Washington DC: American Public Health Association. Ahmad, SR, and DM Reynolds (1999), Monitoring of water quality using fluorescence technique: Prospect of on-line process control. Dissolved Oxyg. Dyn. Model. Case Study A Subtrop. Shallow Lake, 217, 95. https://www.proquest.com/openview/fb344e796503f232a1a8efb76a1fb9ae/1.pdf?pq-origsite=gscholar&cbl=18750&diss=y#page=106
-
- Bik, H. M., Halanych, K. M., Sharma, J., & Thomas, W. K. (2012). Dramatic shifts in benthic microbial eukaryote communities following the Deepwater Horizon oil spill. PLoS ONE, 7(6), e38550. https://doi.org/10.1371/journal.pone.0038550

- Bonaglia, S., & Meysman, F. J. (2018). Individual-based modeling of oxygen and sulfide dynamics in the rhizosphere of the seagrass Zostera noltii: Model development and sensitivity analysis. *Frontiers in Marine Science, 5*, 233.

- (2021).*Chapter 2: Distribution of Mangroves*. Goa Forest Department. https://forest.goa.gov.in/sites/default/files/2022-06/ch2_man.pdf

- (2021). *Chapter 3: Mangrove Cover – India State of Forest Report 2021*. Forest Survey of India. https://fsi.nic.in/isfr-2021/chapter-3.pdf

- (2021).*Chapter 4: Biodiversity*. Goa Forest Department. https://forest.goa.gov.in/sites/default/files/2022-06/ch4_man.pdf

- Chaudhari, R., Hajoori, M., Suthar, M., Desai, S., & Bharthana-Vesu, S. (2017). Isolation, screening and characterization of marine bacteria for exopolysaccharide production. *Biosci Discov*, *8*, 643-9. https://d1wqtxts1xzle7.cloudfront.net/69470949/Rahul643-649libre.pdf

- Creer, S., & Yu, D. W. (2017). Polygenic traits in ecological studies. *Trends in Ecology & Evolution, 32*(12), 919-932. https://doi.org/10.1111/evo.13385

- Dahdouh-Guebas, F., Jayatissa, L. P., Di Nitto, D., Bosire, J. O., Lo Seen, D., & Koedam, N. (2005). How effective were mangroves as a defense against the recent tsunami? *Current Biology, 15*(12), R443-R447. https://doi.org/10.1016/j.cub.2005.06.008

- Das, S., & Vincent, J. R. (2009). Mangroves protected villages and reduced death toll during Indian super cyclone. *Proceedings of the National Academy of Sciences, 106*(18), 7357-7360. http://dx.doi.org/10.1073/pnas.0810440106

- Day, J. W., et al. (2017). Impacts of sea-level rise on mangrove ecosystems: A region by region overview. *Ecosystem Health and Sustainability, 3*(4), e01211.

- D'Souza, E., & Untawale, A. G. (2004). Distribution of mangroves in Goa: an update. *Indian Journal of Marine Sciences, 33*(3), 224-226.

- Duke, N. C., Meynecke, J. O., Dittmann, S., Ellison, A. M., Anger, K., Berger, U.,& Wood, A. (2007). A world without mangroves? *Science, 317*(5834), 41-42. http://dx.doi.org/10.1126/science.317.5834.41b

- Ellison, A. M., Farnsworth, E. J., & Merkt, R. E. (1999). Origins of mangrove ecosystems and the mangrove biodiversity anomaly. *Global Ecology and Biogeography, 8*(2), 95-115. https://doi.org/10.1046/j.1466-822X.1999.00126.x

- Feller, I. C., Whigham, D. F., McKee, K. L., & Lovelock, C. E. (2010). Nitrogen limitation of growth and nutrient dynamics in a disturbed mangrove forest, Indian River Lagoon, Florida. *Oecologia, 162*(3), 731-741. https://doi.org/10.1007/s00442-002-1117-z

- Gao, F., Zhao, H., Zhao, P., Zhang, C., Xu, G., Liu, G., & Guo, X. (2022). Benthic Microbial Communities and Environmental Parameters of Estuary and Hypoxic Zone in the Bohai Sea, China. *Journal of Marine Science and Engineering, 10*(12), 1862. MDPI AG. http://dx.doi.org/10.3390/jmse10121862

- Ghose, M., Parab, A. S., Manohar, C. S., Mohanan, D., & Toraskar, A. (2024). Unraveling the role of bacterial communities in mangrove habitats under the urban influence, using a next-generation sequencing approach. *Journal of Sea Research*, *198*, 102469. https://doi.org/10.1016/j.seares.2024.102469

- Gilman, E. L., Ellison, J., Duke, N. C., & Field, C. (2008). Threats to mangroves from climate change and adaptation options: A review. *Aquatic Botany, 89*(2), 237-250. https://doi.org/10.1016/j.aquabot.2007.12.009

- Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., & Duke, N. (2011). Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography, 20*(1), 154-159.

- Godrej and Boyce. (2021). *Handbook on protocol for mangrove herbarium*. World Wildlife Fund (WWF). https://wwfin.awsassets.panda.org/downloads/handbook_on_protocol_for_mangrove_herbarium.pdf

- Govindasamy, C., Ruban, P., & Sekar, S. (2012). Seasonal distribution of halophilic bacterial population in water and sediment at Muthupet mangrove, Tamil Nadu. *Elixir Pollution*, *44*,7320-7323. https://www.researchgate.net/publication/228089796_Seasonal_Distribution_of_Halophilic_Bacterial_Population_in_Water_andSediment_at_Muthupet_Mangrove_Tamilnadu?_sg=g7QevlCzhYx_vS2wRMf1wHMCxndfY4K6y6QbbrrLAc0K5BTuqbEskv2XkXURRh6fqYvtlv3jQn4wSk&_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6Il9kaXJlY3QifX0

- Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (2009). Methods of seawater analysis. John Wiley & Sons. https://gradocienciasdelmar.files.wordpress.com/2012/09/methods-of-seawater analysis.pdf

- Grewal, B & Fonseca, S. (2004). *Birds of Goa*. Bird Institute of Goa.

- Haldar, S., & Nazareth, S. W. (2018). Taxonomic diversity of bacteria from mangrove sediments of Goa: metagenomic and functional analysis. *3 Biotech*, *8*, 1-10. https://doi.org/10.1007%2Fs13205-018-1441-6

- Hossain, M. S., & Yusoff, F. M. (2008). Abundance and distribution of meiobenthos in the mangrove intertidal sediment of Sungai Pulai Estuary, Malaysia. *Journal of Biological Sciences, 8(*2), 401-408.

- Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Adv Mar Biol* 40:81–251. https://doi.org/10.1016/S0065-2881(01)40003-4

- Kathiresan, K. (2010). Importance of mangrove forests of India. *Journal of Coastal Environment*, *1*(1), 11-26.

- Kathiresan, K. (2010). Globally threatened mangrove species in India. *Current Science*, *98*(12), 15-51.
http://indiaenvironmentportal.org.in/files/Globally%20threatened%20mangrove%20species%20in%20India.pdf

- Kim, K., Seo, E., Chang, S. K., Park, T. J., & Lee, S. J. (2016). Novel water filtration of saline water in the outermost layer of mangrove roots. *Scientific reports*, *6*(1), 20426.
https://doi.org/10.1038/srep20426

- Kothari,M.J. and Rao,K.M,(2002). Mangroves of Goa.*Botanical Survey of India*.
https://bsi.gov.in/uploads/documents/Public_Information/publication/books/miscellaneous/Mangroves%20of%20Goa.pdf
(Reference for Table 1.1: Estimated mangrove area coverage in Goa)

- Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. O., & Banta, G. T. (2012). What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine ecology progress series*, *446*, 285-302.
http://dx.doi.org/10.3354/meps09506

- Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008). Organic carbon dynamics in mangrove ecosystems: A review. *Aquatic Botany, 89*(2), 201-219.
https://doi.org/10.1016/j.aquabot.2007.12.005

- Kristensen, E., et al. (2011). Response of benthic metabolism and nutrient cycling to long-term nutrient enrichment in a tropical mangrove-dominated estuary. Aquatic Microbial Ecology, 62(2), 175-192.

- Kulkarni, C. S., Prabhudesai, R. G., & Wafar, M. V. M. (2013). Status of mangroves in Goa: a remote sensing perspective. *Environmental Monitoring and Assessment, 185*(9), 7775-7788.
https://doi.org/10.1111/j.1466-8238.2010.00584.x

- Latumahina, F. S., Mardiatmoko, G., & Sahusilawane, J. (2020). Richness, diversity and evenness of birds in small island. In *Journal of Physics: Conference Series* (Vol. 1463, No. 1, p. 012023). IOP Publishing.
https://iopscience.iop.org/article/10.1088/1742-6596/1463/1/012023/meta
(Reference for Table 3.2: Evaluation criteria for species richness using Margalef Index values, Table 4.49: Estimated species richness level based on Margalef Index values)

- Lee, S. Y. (1999). The ecological role of grapsid crabs in mangrove ecosystems: Consequences of claw removal on prey community structure and ecosystem processes. *Journal of Experimental Marine Biology and Ecology, 236*(2), 165-186. https://doi.org/10.1071/MF97179

- Lee, S. Y., & Chong, V. C. (2009). The role of benthic macrofauna in intertidal seagrass beds of a tropical estuary: A food web approach. *Estuarine, Coastal and Shelf Science, 81*(1), 81-88.

- Lee, S. Y., Hamilton, S., Barbier, E. B., Primavera, J., Lewis III, R. R., & Jani, D. (2019). Better restoration policies are needed to conserve mangrove ecosystems. *Nature Ecology & Evolution, 3*(6), 870-872. https://doi.org/10.1038/s41559-019-0861-y

- Liem, K. F. (1978). Modulatory multiplicity in the functional repertoire of the feeding mechanism in the mudskipper (Periophthalmus sobrinus). Copeia, 1978(4), 616-628.

- Lovelock, C. E., Cahoon, D. R., Friess, D. A., Guntenspergen, G. R., Krauss, K. W., Reef, R.,& Schile-Beers, L. M. (2015). The vulnerability of Indo-Pacific mangrove forests to sea-level rise. *Nature, 526*(7574), 559-563. https://doi.org/10.1038/nature15538

- Maiti, S. K. (2001). Handbook of methods in environmental studies: vol 1: water and wastewater analysis. ABD Publishers. https://www.researchgate.net/publication/257555649_Handbook_of_Methods_in_Environmental_Studies_Vol_I_Water_and_Wastewater

- (2021).*Mangrove Forests*.DrishtiIAS. https://www.drishtiias.com/to-the-points/paper3/mangrove-forests
(Reference for Figure 1.1: Geographical map depicting mangrove coverage in India)

- (2021). *Mangrove cover is on the rise in India*. Global Mangrove Alliance. https://www.mangrovealliance.org/news/mangrove-cover-is-on-the-rise-in-india/

- (2024).*Mangroves in Goa*. Goa Forest Department – Government of Goa. https://forest.goa.gov.in/mangroves
(Reference for Table 1.2: Description of mangrove species coverage reported in Goa)

- Mariani, S., et al. (2006). Diversity and spatial distribution of soft-bottom benthos in a tropical coastal lagoon, S.W. Thailand. *Estuarine, Coastal and Shelf Science, 66*(1-2), 197-208.

- McKee, K. L., & Faulkner, P. L. (2000). Restoration of biogeochemical function in mangrove forests. *Restoration Ecology, 8*(3), 247-259. https://doi.org/10.1046/j.1526-100x.2000.80036.x

- Ministry of Environment, Forest and Climate Change (MOEFCC). (2019). National mangrove action plan. *Government of India*. http://nbaindia.org/uploaded/pdf/IndiaNationalBiodiversityActionPlan2019.pdf

- Moens, T., Braeckman, U., Derycke, S., Fonseca, G., Gallucci, F., Gingold, R., & Vincx, M. (2013). Ecology of free-living marine nematodes. *Nematoda, 2*, 109-152. http://dx.doi.org/10.1515/9783110274257.109

- Nagelkerken, I., et al. (2008). Benefits of marine protected areas for tropical coastal fishers: A global synthesis. *Fish and Fisheries, 9*(4), 379-399.

- Nagelkerken, I., Blaber, S. J., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., & Withington, D. J. (2008). The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquatic Botany, 89*(2), 155-185. http://dx.doi.org/10.1016/j.aquabot.2007.12.007

- (2024). *Observing bacteria in a petri dish*. Microbiology Society. https://microbiologysociety.org/why-microbiology-matters/what-is-microbiology/bacteria/observing-bacteria-in-a-petri-dish.html

- Oliveira, A., Selvaraj, K., Tripathy, J. P., Betodkar, U., Cacodcar, J., Quadros, N., & Wadkar, A. (2020). Geospatial clustering, seasonal trend and forecasting of Kyasanur Forest Disease in the state of Goa, India, 2015–2018. *Tropical Medicine and Health*, *48*, 1-14. http://dx.doi.org/10.1186/s41182-020-00213-y
(Reference for Figure 1.2: Geographical map depicting mangrove coverage in Goa)

- Osland, M. J., Feher, L. C., Griffith, K. T., Cavanaugh, K. C., Enwright, N. M., Day, R. H., & Stagg, C. L. (2017). Climatic controls on the global distribution, abundance, and species richness of mangrove forests. *Ecological Monographs, 87*(2), 341-359. https://doi.org/10.5066/F78C9TDM

- Parsons, T. R. (2013). *A manual of chemical & biological methods for seawater analysis*. Elsevier. https://repository.oceanbestpractices.org/

- Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., & Yong, J. W. H. (2010). The loss of species: mangrove extinction risk and geographic areas of global concern. *PloS one*, *5*(4), e10095. https://doi.org/10.1371%2Fjournal.pone.0010095

- Preston-Mafham. (2007). *Insects and Other Invertebrates*. Brown Bear Books.

- Rodrigues, T. (2020). Mangroves in Goa.
Retrieved from ActforGoa: https://actforgoa.org/resource/mangroves-in-goa/

- Rönnbäck, P., Kautsky, N., Pihl, L., Troell, M., Söderqvist, T., & Wennhage, H. (2007). Ecosystem goods and services from Swedish coastal habitats: Identification, valuation, and implications of ecosystem shifts. *Ambio, 36*(7), 534-544.
https://doi.org/10.1579/0044-7447(2007)36[534:egasfs]2.0.co;2

- Saha, A., Pramanick, P., Zaman, S., & Mitra, A. (2018). Indian Sundarbans: An abode of brackish water fishes. *Techno International Journal of Health, Engineering, Management & Science*, *2*(3), 45-51.
https://www.researchgate.net/publication/325698095_Indian_Sundarbans_An_abode_of_brackish_water_fishes

- Sari, K., & Soeprobowati, T. R. (2021). The impact of water quality detorioration in mangrove forest in semarang coastal area. *Indonesian Journal of Limnology*, *2*(2), 37-48.
http://dx.doi.org/10.51264/inajl.v2i2.20

- Sen, N., & Naskar, K. (2003). *Algal flora of Sundarbans mangals*. Daya Books.

- Silva, J. B. B., & Souza, W. R. (2013). Structure of the benthic microfauna from mangrove sediments in southeastern Brazil. *Journal of the Marine Biological Association of the United Kingdom, 93*(6), 1557-1563.

- Silva, C., & Bhat, U. G. (2011). Diversity Status of Mangrove Species in Estuarine Regions of Goa, Central West Coast, India. *Nature, Environment and Pollution Technology*, *10*(4), 651-654.
https://www.researchgate.net/journal/Nature-Environment-and-Pollution-Technology23953454?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InB1YmxpY2F0aW9uIn19

- Singh, G., Ramanathan, A. L., & Prasad, M. B. K. (2005). Nutrient cycling in mangrove ecosystem: a brief overview. *Int J Ecol Environ Sci*, *30*, 231-244.
https://www.researchgate.net/journal/International-Journal-of-Ecology-and-Environmental-Sciences

- Singh, U. B., Thakur, R. K., Verma, R., & Ahluwalia, A. S. (2013). Nutritional studies of Chara corallina. *Recent Res Sci Technol*, *5*(3), 10-14.
https://www.researchgate.net/publication/261983205_Nutritional_studies_of_Chara_corallina

- Smith, T. J., et al. (1991). Effects of short-term salinity fluctuations on the survival, growth, and respiration of young-of-the-year red drum (Sciaenops ocellatus). *Bulletin of Marine Science, 48*(2), 228-239.

- Smith, A. C., & Hussey, M. A. (2005). Gram stain protocols. American Society for Microbiology, 1(14), 113-144.
https://asm.org/getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3/gram-stain-protocol-2886.pdf

- Smith, T. J., Foster, A. M., Tiling-Range, G., & Jones, J. W. (2013). Dynamics of mangrove-marsh ecotones in subtropical coastal wetlands: Fire, sea-level rise, and water levels. *Fire Ecology*, 9(1), 66-77.
https://doi.org/10.4996/fireecology.0901066

- (2010). *Satellite analysis of global mangrove forests released*. UNESCO World Heritage Convention.
https://whc.unesco.org/en/news/692

- Untawale, A. G., Jagtap, T. G., & Wafar, M. V. M. (1979). Ecology of mangroves in Goa. *Indian Journal of Marine Sciences, 8*(2), 89-95.

- Uthicke, S. (2001). Nutrient regeneration by abundant coral reef holothurians. *Journal of Experimental Marine Biology and Ecology*, *265*(2), 153-170.
http://dx.doi.org/10.1016/S0022-0981(01)00329-X

APPENDIX

Chemicals and Reagents

REAGENT	SOURCE
Gram's crystal violet	Hi Media Laboratories Pvt.
Gram's decolouriser	Hi Media Laboratories Pvt.
Gram's iodine	Hi Media Laboratories Pvt.
Gram's safranine	Hi Media Laboratories Pvt.
Sodium chloride	Hi Media Laboratories Pvt.
Agar powder (Bacteriological grade)	Hi Media Laboratories Pvt.
Starch soluble	Hi Media Laboratories Pvt.
Manganese chloride	Hi Media Laboratories Pvt.
Potassium iodide	Hi Media Laboratories Pvt.
Potassium iodate	Hi Media Laboratories Pvt.
Sodium hydroxide	Hi Media Laboratories Pvt.
Sodium thiosulphate	Hi Media Laboratories Pvt.
Concentrated Sulfuric acid	Hi Media Laboratories Pvt.

Reagent and Media Composition

Reagent Composition

- **For Gram Staining Reagents:**

1. **Gram's crystal violet** – Solution A and Solution B were mixed together and kept for 24 hours and filtered before its use.
   (a) **Solution A** – 2 g of crystal violet is dissolved in 10 mL of 95% ethanol and the total volume was brought up to 20 mL with distilled water.
   (b) **Solution B** – 0.8 g of ammonium oxalate was dissolved in 10 mL of distilled water and the total volume was brought up to 80 mL with distilled water.
2. **Gram's decolouriser** – 70% ethanol
3. **Gram's iodine** – 1 g of iodine and 2 g of Potassium iodide were dissolved in distilled water and the total volume was brought up to 300 mL with distilled water.
4. **Gram's safranine** – 0.25 g of safranine was dissolved in 10 mL of 95% ethanol and the total volume was brought up to 100 mL with distilled water.

Media Composition

- **For Saline solution (0.85%)**: Dissolve 0.85 g Sodium chloride in 100 mL distilled water
- **For Zobell Marine Agar**:Add Peptone – 5 g, Sodium chloride – 19.45 g, Yeast Extraxt – 1 g, Mangesium Chloride – 8.8 g, Ferric citrate – 0.1 g, Sodium sulfate – 3.24 g, Calcium chloride – 1.8 g, Potassium chloride – 0.55 g, Sodium

bicarbonate – 0.16 g, Potassium bromide – 0.08 g, Strontium chloride – 0.034 g, Disodium phosphate – 0.008 g, Boric acid – 0.22 g, Sodium silicate – 0.004 g, Sodium fluorate – 0.0024 g, Ammonium nitrate – 0.0016 g, Agar – 15 g to 1000 mL distilled water. The final pH is adjusted to 7.6 at 25℃.

To prepare Zobell Marine Agar Media plates, 55.25 g of Zobell Marine Agar powder in 1000 mL purified/ distilled water.