

Implications of culturable bacterial community structure on mangrove carbon outwelling under variable physicochemical conditions

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By

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Declaration

As required under the Goa University Ordinance OA-19A, I state that the present thesis entitled “**Implications of culturable bacterial community structure on mangrove carbon outwelling under variable physicochemical conditions**” is my original contribution and that the same has not been submitted on any previous occasion for any degree. To the best of my knowledge, the present study is the first comprehensive work of its kind from the area mentioned. The literature related to the problem investigated has been cited. Due acknowledgments have been made, wherever facilities and suggestions have been availed.

Place: Taleigao Plateau

Seyieleno C Seleyi

Date:

Certificate

This is to certify that the thesis entitled “**Implications of culturable bacterial community structure on mangrove carbon outwelling under variable physicochemical conditions**” submitted by **Ms. Seyieleno C Seleyi**, for the award of the degree of Doctor of Philosophy in Biotechnology, is based on the original studies carried out by her under my supervision.

The thesis or any part thereof has not been submitted for any other degree or diploma in any University or Institution.

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Dedicated to my mother

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- Fig. 6.6. a.** Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial abundance (TBA), bacterial classes and enzymes in the Mandovi and Zuari estuaries during Premonsoon.
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Fig. 6.6. c. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial abundance (TBA), bacterial classes and enzymes in the Mandovi and Zuari estuaries during Postmonsoon.

Fig. 6.7. Principal component analysis (PCA) biplot of the mean PC scores of physicochemical and biological parameters as vectors, together with the PC scores of each season.

Fig. 7.1. Variations in $\delta^{13}\text{C}_{\text{mng}}$ and $\delta^{15}\text{N}_{\text{mng}}$ of mangrove leaves from the Mandovi and Zuari estuaries.

Fig. 7.2. Variations in $\delta^{13}\text{C}_{\text{sed}}$ and $\delta^{15}\text{N}_{\text{sed}}$ during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

List of Abbreviations

°C	Degree Celsius
δ	Delta
%	Percentage
~	Approximately
‰	Permil
δ ¹³ C	¹³ C/ ¹² C ratio
δ ¹⁵ N	¹⁵ N/ ¹⁴ N ratio
δ ¹³ C _{mng}	Carbon isotopic ratio in mangrove leaves
δ ¹³ C _{sed}	Carbon isotopic ratio in sediment
δ ¹⁵ N _{mng}	Nitrogen isotopic ratio in mangrove leaves
δ ¹⁵ N _{sed}	Nitrogen isotopic ratio in sediment
μ Ci ml ⁻¹	Micro Curie per millilitre
μg C L ⁻¹ h ⁻¹	Microgram carbon per litre per hour
μl	Microlitre
μmol/L	Micromole per litre
ACA	ε-Amino-n-Caproic Acid
AgNO ₃	Silver nitrate
ANOVA	Analysis of variance
C	Carbon
cells/g	Cells per gram
cells/ml	Cells per millilitre

cells mol ⁻¹	Cells per mole
CFU/g	Colony forming unit per gram
CFU/ml	Colony forming unit per millilitre
Chl <i>a</i>	Chlorophyll <i>a</i>
Ci mmol ⁻¹	Curie per millimole
Cl ⁻	Chloride
cm	Centimetre
CMC	Carboxymethyl cellulose
C:N	Elemental carbon and nitrogen ratio
C:N _{mng}	Elemental ratio in mangrove leaves
C:N _{sed}	Elemental ratio in sediment
CO ₂	Carbon dioxide
DAPI	40, 6-diamidino-2-phenylindole
dNTPs	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EDTA	Ethylenediaminetetraacetic acid
e.g.	For example
et al.	And others
EtBr	Ethidium bromide
Fig.	Figure

g	Gram
g C cell ⁻¹	Gram carbon per cell
GYP	Glucose yeast extract peptone
ha	Hectare
ha ⁻¹	Per hectare
HCl	Hydrochloric acid
hr	Hour
HT	High tide
KHP	Potassium hydrogen phthalate
km	Kilometre
km ²	Square kilometre
L	Litre
LT	Low tide
M	Mandovi
m	Meter
MgCl ₂	Magnesium chloride
mg/L	Milligram per litre
mg/g	Milligram per gram
ml	Millilitre
mm	Millimetre
mins	Minutes
Mo	Monsoon

N	Nitrogen
NA	Nutrient Agar
Na	Sodium
NaCl	Sodium chloride
NB	Nutrient Broth
Na ₂ S ₂ O ₃	Sodium thiosulphate
NCBI	National Centre for Biotechnology Information
NDIR	Non-dispersive infrared sensor
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
ng	Nanogram
NH ₄ ⁺	Ammonia
OM	Organic matter
OM _w	Organic matter in water
OM _s	Organic matter in sediment
<i>p</i>	Level of significance
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDB	Pee Dee Belemnite
PO ₄ ³⁻	Phosphate
POC	Particulate organic carbon
pM	Picomolar

PM	Premonsoon
PoM	Postmonsoon
ppt	Parts per thousand
psi	Pounds per square inch
PVC	Polyvinyl chloride
rRNA	Ribonucleic acid
rpm	Revolutions per minute
SiO_4^{4-}	Silicate
SD	Standard deviation
sp.	Species
TAE	Tris Acetate EDTA
TOC	Total organic carbon
TVC	Total viable counts
TVC_w	Total viable counts in water
TVC_s	Total viable counts in sediment
TBA	Total bacterial abundance
TBA_w	Total bacterial abundance in water
TBA_s	Total bacterial abundance in sediment
USD	United States Dollar
UV	Ultraviolet
vs.	Versus
YP	Yeast extract peptone

yr ⁻¹	Per year
Z	Zuari
ZMA	Zobell Marine Agar
ZMB	Zobell Marine Broth

ABSTRACT

The outwelling hypothesis states that blue carbon ecosystems such as mangroves export excess carbon to adjacent waters, thereby increasing their productivity. To test this hypothesis, the Mandovi and Zuari estuaries in Goa, India, known for their dense mangrove forests and rich fishery resources, were selected for the study. Investigations were conducted over two consecutive years during the premonsoon, monsoon, and postmonsoon seasons to assess the impact of variations in physicochemical parameters, bacterial abundance and diversity, trophic status, bacterial extracellular enzyme activity, and organic matter sources, on carbon outwelling. The hydrographic conditions in the mangrove and adjacent estuarine waters were influenced by monsoonal precipitation, freshwater input, tides, and seawater influx, leading to changes in their physicochemical parameters. Dissolved inorganic nutrients such as nitrite, nitrate, ammonia, phosphate, and silicate exhibited significant seasonal variability and were higher during low tide compared to high tide. *Proteobacteria* (58.71 %) was the predominant phylum, followed by *Firmicutes* (40.37 %) and *Actinobacteria* (0.92 %). The bacterial isolates were categorized into five classes: *Gammaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Bacilli*, and *Clostridia*. Our findings indicated that community respiration surpassed primary productivity, signifying a state of heterotrophy. Likewise, bacterial respiration exceeded bacterial productivity, suggesting that carbon is primarily used for respiration rather than assimilation. The decoupling of phytoplankton and bacteria during the monsoon and postmonsoon seasons suggested heterotrophic conditions in both the Mandovi and Zuari estuaries. Bacterial enzymatic activity, including amylase, protease, cellulase, and lipase, peaked during the monsoon, with *Oceanobacillus* sp. exhibiting notable poly-enzyme activity. Isotopic and elemental ratios of carbon and nitrogen indicated that productivity in the estuaries was enhanced by the outwelling of mangrove-derived organic matter. Multivariate statistical analyses revealed significant correlations between bacteria and enzyme activities, highlighting the role of environmental factors in shaping bacterial communities and their functional roles in organic matter degradation. Thus, this study highlights the significance of carbon outwelling from mangroves and its impact on productivity in adjacent estuarine waters, emphasizing the importance of these interactions in sustaining ecosystem resilience and functioning.

Chapter 1

Introduction

1.1. Mangrove ecosystems

Mangroves grow along tropical and subtropical shores and are typically woody plants that develop at the interface between the land and sea. Mangroves, as well as their associated bacteria, fungi, plants, and animals, constitute the mangrove forest communities or mangals. They are sometimes referred to as “tidal forests”, “coastal woodlands”, or “oceanic rain forests” (Kathiresan and Bingham 2001). Mangroves are found in high salinity conditions, extreme tides, strong winds, high temperatures, and muddy, anaerobic soils. No other group of plants with such highly developed morphological and physiological adaptations to extreme conditions exists. They are a complex and dynamic ecosystem depending on various environmental factors such as temperature, soil type, salinity, duration and frequency of inundation, accretion of silt, tidal and wave energy, and periodic factors such as cyclone or flood frequencies (Lugo and Snedaker 1974; Hutchings and Saenger 1987).

1.1.1. Distribution of mangroves

Mangroves occupy less than 1 % of the world's surface and are found mainly in all continents between the Tropic of Cancer and the Tropic of Capricorn, which covers approximately 75 % of the world's tropical coastline (Arunprasath and Gomathinayagam 2015). They are distributed in over 112 countries and territories comprising a total area of 1,81,000 km² in over one fourth of the world's coastline (Sahoo and Dhal 2009). Globally, mangrove distribution is distinguished into two zones: 1. West zone and 2. East zone. West zone embodies African coast of Atlantic, North and South American portions while the East zone embodies eastern part of African coast, South Asian countries viz. India and Pakistan, South East Asian countries like Indonesia, Australia and New Zealand (Basha 2018). There are about 6.9 million ha of mangroves in the Indo-Pacific region, 3.5 million ha in Africa, and 4.1 million ha in the Americas including the Caribbean (Sahoo and Dhal 2009). Mangroves

of South and Southeast Asia are the largest and most diverse mangrove systems in the world, accounting for 41.4 % of global mangroves.

India with a long coastline of about 7516.6 km, including the island territories has a mangrove cover of about 6749 km, the fourth largest mangrove area in the world (Naskar and Mandal 1999). The Indian mangroves account for about 3 % of global mangroves and 8 % of Asian mangroves (FAO 2007). These mangrove habitats comprise three distinct zones: East coast habitats having a coast line of about 2700 km, facing Bay of Bengal, West coast habitats with a coast line of about 3000 km, facing Arabian sea, and Island Territories with about 1816.6 km coastline. The East coast zone extends from West Bengal's Sundarban forest to Tamil Nadu's Cauvery estuary and includes 57 % of mangroves (Jagtap et al. 1993). The West coast region stretches from Bhavnagar estuary of Gujarat to Cochin estuary of Kerala and constitute 23 % mangroves (Mandal and Naskar 2008). The remaining 20 % is represented by the Andaman and Nicobar Islands (Ragavan et al. 2019). Around 57 % of the world's mangrove species are represented in India consisting of 46 true mangrove species belonging to 14 families and 22 genera, which includes 42 species and 4 natural hybrids. The East coast has 40 mangrove species belonging to 14 families and 22 genera. The West coast has 27 species belonging to 11 families and 16 genera and the Andaman and Nicobar Islands have 38 species belonging to 13 families and 19 genera (Ragavan et al. 2016).

1.1.2. Significance of mangrove ecosystem

Every ecosystem generally provides both life-sustaining and other valuable services, many of which are connected to human welfare (Farley et al. 2010). The significance of mangroves for coastal communities has been extensively studied across the tropics (Sandilyan and Kathiresan 2012). In general, the assessment of the importance of mangroves can be evaluated via their contributions to ecological sustainability (e.g., sediment control, retaining pollutants, nutrient cycling, and environmental security), environmental safety

(mitigating the effects of tsunamis, cyclones, floods, and greenhouse gas), and economic prosperity (supporting 80 % of the world's fish catch and providing wild seeds for the aquaculture industry) (Jennerjahn and Ittekkot 2002; Sandilyan 2007; Farley et al. 2010). The annual economic value of mangroves has been estimated to be USD 200,000 to 900,000 ha⁻¹ (Wells and Ravilious 2006). Besides aquaculture, the mangroves support a variety of wildlife such as the Bengal tiger, crocodiles, deer, sea turtles, monkeys, pigs, snakes, fishing cats, insects and birds.

Mangroves are one of the most carbon-rich forests in the tropics and form a significant part of the global carbon cycle (Breithaupt et al. 2012). Mangroves fix higher amounts of carbon dioxide than phytoplankton in the tropical oceans and serve as an effective climate change adaptation and mitigation strategy (Kathiresan and Bingham 2001; Bouillon et al. 2011). Besides, the carbon sequestration potential of mangroves is 50 times higher than that of other tropical forests (Sandilyan and Kathiresan 2012). They produce large amounts of litter in the form of falling leaves, branches and other debris. The decomposition of this litter contributes to the recycling of nutrients and production of dissolved organic matter (DOM) both in the mangroves and in adjacent habitats (Kristensen et al. 2008).

Mangroves also provide protection to other marine ecosystems by trapping sediments in their complex aerial root systems and acting as sinks for the suspended sediments (Furukawa et al. 1997). This mechanism helps in supply of clean and nutrient-rich water for the associated ecosystems like coral reefs, seaweeds and seagrass beds. However, when the mangroves are removed, the sediment becomes loose and gets deposited on those associated ecosystems and destroys them.

It is interesting to note that all the advantages offered by mangroves are permanent, incur no depreciation or maintenance costs, and are perpetually regenerated by solar energy

(Farley et al. 2010). Hence, conservation and restoration of mangrove ecosystems would be extremely beneficial to our planet and its inhabitants in innumerable ways.

1.2. Carbon outwelling in mangrove ecosystem

The "outwelling" characteristic of coastal wetlands was first postulated by Odum (1968). According to the "outwelling" hypothesis, coastal marshes and mangroves generate an excess of organic carbon and drive out these organic nutrients and detritus into the surrounding and coastal areas, increasing the productivity of local fisheries and marine plants. (Odum 1980). Carbon outwelling is most common in coastal wetlands, mangroves, seagrass meadows, and estuaries, where organic matter (OM) produced by plants is transported to the ocean by rivers and tidal currents. Climate, land use, nutrient inputs, and hydrology all have an impact on the magnitude and dynamics of carbon outwelling (Adame and Lovelock 2011). Changes in these variables can have a significant impact on the amount and quality of carbon exported to the ocean, affecting marine ecosystems.

Mangrove forests produce organic carbon well in excess of the requirements of the ecosystem. Although some of this OM simply accumulates in the sediments, large quantities may be transported offshore (Alongi 1990; Robertson et al. 1991; Lee 1995). The amount of material exported, however, depends strongly on local condition and varies enormously among mangrove habitats (Twilley et al. 1992). Regardless of the pathways and food webs involved, all mangrove OM which is not exported by tidal action enters the sediment and further degraded by microorganisms (Ylla et al. 2012; Logue et al. 2016). The exchange of materials between mangrove forests and coastal waters is very efficient due to regular tidal floods and mangrove drainage (Ayukai et al. 1998).

1.2.1. Carbon inputs in mangrove ecosystems

Essential organic carbon inputs in mangrove ecosystems are derived from several allochthonous and autochthonous sources which include:

- a. **Photosynthesis:** Mangroves, like other plants, use photosynthesis to convert atmospheric carbon dioxide into organic carbon, which is then stored in their biomass. Because of their unique adaptations to saline and anaerobic conditions, mangroves have a high primary productivity that allows them to accumulate large amounts of carbon in their above- and below-ground biomass (Alongi 2014).
- b. **Sedimentation:** Sedimentation is a process that contributes to the formation of mangrove soils, which can be highly organic and carbon-rich. There is both active and passive capture of carbon from tidal water containing fine particles from the nearby coastal ocean and from river water discharged from upstream. Mangrove forests trap and stabilize sediment, which can accumulate and bury organic carbon over time (Wolanski 1995).
- c. **Mangrove litter:** Leaves, propagules, branches, twigs and other plant waste from mangroves fall into the water and settle in the silt, where microbes break them down. (Alongi et al. 1989).
- d. **Upstream sources:** Upstream sources, such as rivers and estuaries, can also introduce carbon into mangrove ecosystems. This carbon can enter the mangrove ecosystem by freshwater flows from sources such as terrestrial vegetation, soils, and wastewater (Bouillon and Dehairs 2000).

1.2.2. Mechanism of carbon outwelling

The mechanism of carbon outwelling in mangrove ecosystems involves the transport of carbon from the mangrove forest to the adjacent waters, and ultimately to the open ocean (Odum 1968). This process is driven by a combination of physical and biological factors (Bouillon et al. 2003), which are described below:

- a. **Tidal flushing:** In mangrove ecosystems, tidal flushing is a crucial physical process that drives carbon outwelling (Wolanski et al. 1992). Mangroves tidally exchange

solutes and particulates, including inorganic and organic particles, with the coastal ocean (Santos et al. 2019). During high tide, seawater floods the soil in the mangrove forest resulting in sediment resuspension which mobilizes nutrients and OM. Thereafter, the receding seawater during low tide then flows back out along with the OM to adjacent waters, where it is decomposed and remineralized (Taillardat et al. 2019).

- b. **Microbial decomposition:** Mangrove OM typically contains a high proportion of labile organic compounds, such as simple sugars and amino acids (Kristensen et al. 2008). These compounds are readily metabolized by microorganisms, which break them down into smaller molecules and release carbon dioxide and other gases (Benner et al. 1986). The leaching of labile OM is a rapid process and can result in high rates of carbon outwelling (Kristensen and Pilgaard 2001). On the other hand, complex OM such as lignin and cellulose persist in the exported material after the initial leaching of labile organic matter (Bahri et al. 2008). These recalcitrant compounds are more resistant to microbial degradation. However, they are eventually broken down by microbes in the sediment and water column. This process is slower than leaching of labile OM taking months to years after the initial export of OM (Dittmar et al. 2001).
- c. **Export by fauna:** Fauna, such as crabs, shrimp, and fish, ingest organic material from the water and sediment of mangrove ecosystems before migrating to adjacent waters, where they are consumed by predators (Sousa and Dangremond 2011; Scharler 2012). This export of biomass and carbon via fauna is termed as “trophic transfer”, which involves the transfer of energy and nutrients from one organism to another, typically through predation (Sheaves and Molony 2000). For example, crabs and shrimp that feed on OM in the sediment within the mangrove forests are consumed by larger predators, such as fish. The rate of export by fauna can vary depending on a variety of

factors, including the density and diversity of the fauna within the mangrove ecosystem, the availability of OM, and the presence of predators in adjacent ecosystems (Walton et al. 2021).

1.2.3. Role of bacteria in carbon outwelling

In mangrove ecosystems, the study of heterotrophic bacteria is the fundamental backbone for understanding its overall ecological function. Highly productive and diverse bacterial communities are harboured within the unique mangrove ecosystem that continuously transforms nutrients from dead mangrove vegetation into sources of nitrogen, phosphorus, and other nutrients (Kamaleson and Gonsalves 2019). Microbial activity is responsible for the major transformation of nutrients in the mangrove ecosystem. The breakdown of mangrove vegetative material, resulting in detritus, represents OM undergoing active decomposition. This detritus is energy-rich and harbours a large, active microbial population, both attached and free-living (Odum and Heald 1975). Thus, bacteria are key players in facilitating carbon outwelling, driving the decomposition of OM and transforming it into forms that can be more easily transported by tidal flow.

Bacteria in mangrove sediments and water columns break down complex organic compounds, such as lignocellulose from fallen mangrove leaves and wood, into simpler molecules. This decomposition process is mediated by various extracellular enzymes produced by the bacteria, including cellulases, proteases, and lipases. As these enzymes break down OM, they release dissolved organic carbon (DOC) and particulate organic carbon (POC), which can be exported to nearby estuaries during tidal exchanges. This microbial activity ensures that a significant portion of the organic carbon stored in mangrove forests does not remain trapped but instead enters the coastal carbon cycle. **Fig. 1.1.** summarises the microbial processes and their link to higher trophic levels through the outwelling of OM (Ram 2002). Environmental factors such as salinity, temperature, and nutrient availability

significantly influence bacterial activity and, consequently, the rate of carbon outwelling. Salinity fluctuations, common in mangrove ecosystems, can alter bacterial community composition and enzymatic efficiency, impacting the breakdown of OM. Additionally, the presence of nutrients like nitrogen and phosphorus can enhance bacterial growth and metabolic rates, leading to increased decomposition and higher rates of carbon export. Understanding these dynamics is crucial for predicting how changes in environmental conditions, such as those brought about by climate change, might affect carbon outwelling processes.

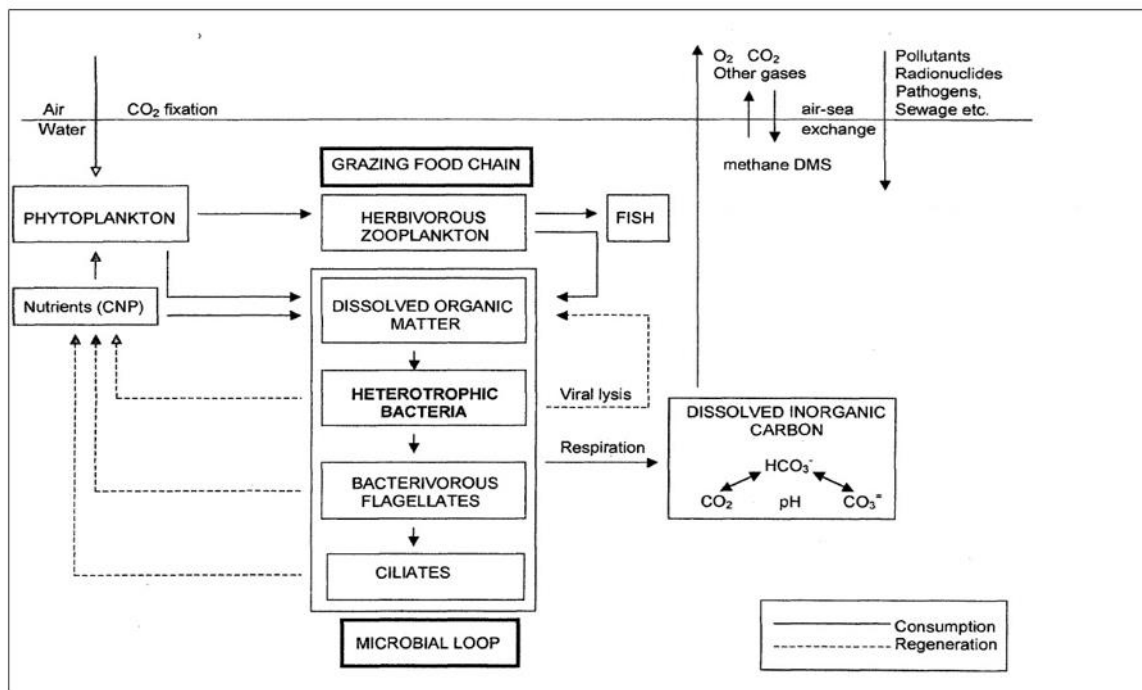


Fig. 1.1. Conceptual model for the microbial contribution to the carbon flow in the coastal waters (adapted from Ram 2002)

In summary, bacteria are central to the process of carbon outwelling from mangroves to adjacent estuaries. Through their decomposition activities, they transform complex OM into forms that can be readily transported by tidal flows, linking terrestrial and marine carbon cycles. The efficiency of these microbial processes is influenced by various environmental factors, highlighting the need for comprehensive studies to understand and predict the

impacts of environmental changes on carbon dynamics in mangrove ecosystems. This knowledge is essential for informing conservation and management strategies aimed at maintaining the ecological and biogeochemical functions of these critical coastal habitats.

1.2.4. Significance of carbon outwelling

The significance of carbon outwelling can be discussed based on the following points:

- a) **Ecological Role of Carbon Outwelling:** Carbon outwelling from mangrove ecosystems plays a crucial ecological role in coastal carbon cycles and the global carbon dynamics. Mangroves are highly productive coastal forests that sequester large amounts of carbon in their biomass and sediments. Through tidal movements and runoff, a significant portion of this stored carbon, in the form of detritus and DOC, is exported to adjacent coastal waters (Maher et al. 2013). This outwelling of carbon is vital for supporting the productivity of coastal food webs by providing an essential nutrient source for heterotrophic organisms, including bacteria, fungi, and marine invertebrates. These organisms, in turn, support higher trophic levels, thereby enhancing the biodiversity and productivity of coastal ecosystems (Nyunja et al. 2009). Moreover, the exported carbon contributes to the formation of organic-rich sediments in coastal zones, which play a crucial role in long-term carbon sequestration in marine environments.
- b) **Impact on Global Carbon Budget:** Mangroves act as a buffer in regulating atmospheric carbon dioxide levels by exporting carbon to the coastal ocean (Huxham et al. 2018). This function is particularly critical in the context of rising concentrations of greenhouse gases in the atmosphere. By sequestering carbon that would otherwise contribute to global warming, mangrove outwelling helps mitigate climate change. Additionally, understanding the dynamics of carbon outwelling is essential for predicting how changes in mangrove coverage - driven by factors such as deforestation, sea-level rise, and climate change - might impact global carbon cycles (Zakaria and Sharma 2020).

c) **Socio-Economic Implications:** Carbon outwelling from mangroves also has significant socio-economic implications, particularly for coastal communities. Coastal fisheries, which are vital for the livelihoods of many people, depend heavily on the productivity of coastal waters (Rönnbäck 1999). Healthy fish populations are crucial for food security and the economic well-being of coastal communities. Therefore, the preservation and restoration of mangrove ecosystems can directly enhance fishery yields and contribute to sustainable livelihoods. Recognizing the value of carbon outwelling underscores the importance of effective conservation and management strategies to protect these crucial ecosystems and the services they provide. Such strategies may include the implementation of marine protected areas, sustainable forestry practices, and community-based conservation initiatives that involve local stakeholders in the stewardship of mangrove resources.

1.3. Importance of the present study

The present study addresses a critical aspect of mangrove ecosystems that has far-reaching implications for global carbon cycling and coastal ecosystem health. Mangroves are known for their exceptional ability to sequester carbon, storing significant amounts of organic carbon in their biomass and sediments. However, the process of carbon outwelling, where organic carbon is exported from mangrove forests to adjacent estuarine and coastal waters, is less explored. This study is essential as it delves into the role of culturable bacterial communities in facilitating this carbon transfer, thereby filling a crucial knowledge gap. Understanding the structure and function of bacterial communities in mangroves under varying physicochemical conditions is vital because these microorganisms play a key role in the decomposition of organic matter. This study can provide insights into the active microbial players involved in outwelling by focusing on culturable bacteria. This is important for developing accurate models of carbon cycling and predicting the response of mangrove

ecosystems to environmental changes such as those caused by climate change or human activities.

Furthermore, the emphasis on variable physicochemical conditions is significant because mangrove ecosystems are highly dynamic environments. Factors such as salinity, pH, and nutrient availability fluctuate regularly due to tidal movements, freshwater input, and seasonal changes. These fluctuations can significantly influence bacterial community structure and function, and hence, the efficiency of carbon outwelling. By investigating how these environmental variables affect bacterial communities, this study identifies the conditions that enhance or inhibit carbon export. This knowledge is crucial for managing mangrove ecosystems to optimize their carbon sequestration and export functions.

Another critical aspect of this study is its potential implications for coastal and marine ecosystems. The OM exported from mangroves supports a range of ecological processes in estuarine and coastal waters, including nutrient cycling and primary production. By elucidating the mechanisms of carbon outwelling and the role of bacteria, this study can help predict the impacts of changes in mangrove health and management practices on coastal carbon budgets and ecosystem services. This is particularly relevant in the context of increasing coastal development and climate change, which threaten mangrove habitats worldwide.

In summary, the importance of this study lies in its comprehensive approach to understanding the microbial mediation of carbon outwelling from mangroves under variable environmental conditions. By focusing on culturable bacterial communities, it provides detailed insights into the microbial processes that drive carbon export. Besides, this study not only advances scientific knowledge of carbon cycling in mangrove ecosystems but also highlights the need for conservation and management strategies aimed at preserving these critical coastal habitats.

1.4. Limitations of the present study

In spite of our earnest efforts, the present study has its limitations. Isotopic studies for tracking the extent of mangrove OM outwelling in the estuaries would have been beneficial. Testing for community-level physiological profiling of bacterial communities in mangrove water and sediment could have provided a clearer picture of OM utilization. The use of advanced sequencing techniques to study the bacterial whole genome through amplicon or nanopore sequencing could have provided a detailed insight into the metabolic pathways that are involved in OM degradation and carbon cycling.

1.5. Aims and objectives

The present study aims to test the outwelling hypothesis on the Mandovi (M) and Zuari (Z) estuaries and understand the impact of physicochemical parameters, OM sources, tidal patterns, and prevailing monsoon conditions on the variability of trophic status in the M and Z. By employing this comprehensive approach, our study seeks to elucidate the role of mangroves in enhancing estuarine productivity through the outwelling of OM and nutrients, thus contributing to a deeper understanding of tropical mangrove ecosystem carbon dynamics. Hence, to achieve this aim the following objectives have been formulated, with a corresponding work plan devised and implemented for each objective:

- **Objective 1 – To study the physicochemical parameters in the mangroves and its adjacent waters.**
 - Collection of water and sediment samples during low and high tides from the selected study locations during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons for two consecutive years.
 - Recording of field-based observations such as temperature, pH and salinity.
 - Laboratory-based analyses of dissolved oxygen, dissolved inorganic nutrients and organic carbon.
 - Impact of monsoonal and tidal patterns on the environmental conditions in mangrove and estuarine waters.

➤ **Objective 2 – To study the bacterial abundance, diversity and productivity in mangrove and adjacent waters.**

- Enumeration of phytoplankton abundance; total and viable counts of heterotrophic bacteria.
- Isolation of morphologically distinct bacterial isolates, DNA extraction, 16S rRNA polymerase chain reaction amplification, and identification by Sanger sequencing.
- Laboratory based analyses of community and bacterial respiration; primary and bacterial productivity using labelled isotopes.
- Assessment of bacterial growth efficiency and trophic status in the mangrove and estuarine waters.

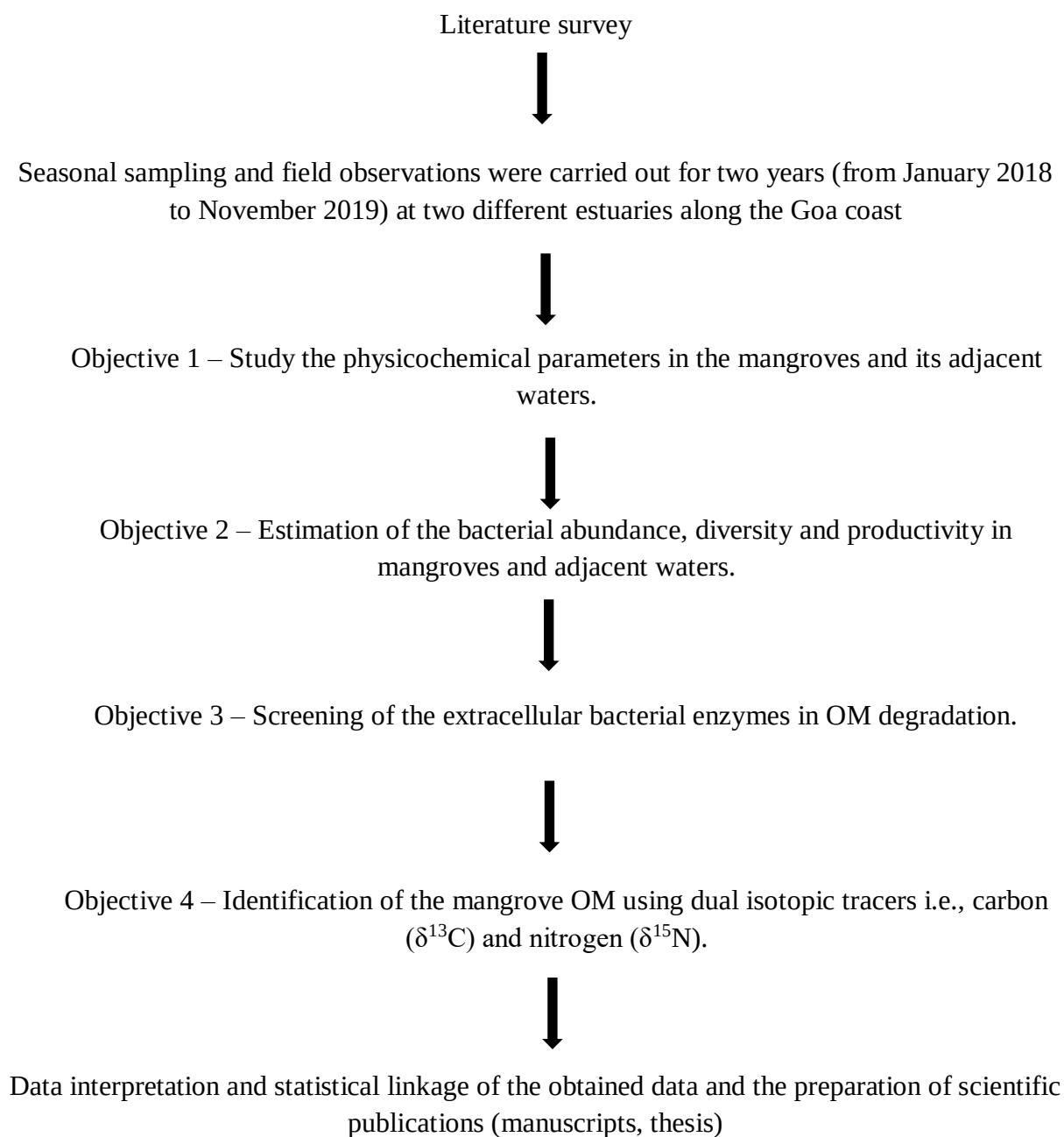
➤ **Objective 3 – To study the extracellular bacterial enzyme in OM degradation.**

- Screening of extracellular enzyme activity of amylase, lipase, cellulase and protease in selected bacterial isolates.
- Role of bacterial enzymes in OM degradation across variable seasons.

➤ **Objective 4 – To identify the mangrove OM using dual isotopic tracers i.e., carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$).**

- Collection of leaves of major mangrove species and sediments from the selected study locations.
- Laboratory based analyses of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and their elemental ratios (C:N).

1.6. General work plan



Chapter 2

Review of Literature

2.1. Studies on carbon outwelling in mangrove ecosystems

The concept of "outwelling" in coastal wetlands was initially proposed by E. P. Odum (1968) and was originally based on observations in salt marsh ecosystems. The term was introduced to describe organic carbon export from productive coastal wetlands, such as mangroves, to adjacent marine environments. However, it is important to note that little direct evidence was available to support the outwelling concept at that time. Nonetheless, his paper sparked numerous studies on the productivity of coastal salt marshes, ultimately developing many foundational paradigms in wetland ecology. The first study examining the tidal exchange of materials between a mangrove and an offshore community was conducted by Golley et al. (1962). Despite relying on a small data set to support their argument, their work was nonetheless seminal in the study of mangrove outwelling. Thereafter, various methods have been employed to study carbon outwelling, ranging from direct measurements to advanced modelling approaches. Early studies on carbon outwelling focused on direct methods including in-situ sampling of water, soil, and plant material measuring the flux of OM from mangrove creeks and estuaries (Dittmar et al. 2001; Bouillon et al. 2008; Maher et al. 2013). Techniques like tidal flux measurements, sediment traps, and stable isotope analysis were used to quantify the amount of carbon being exported. These studies generally found that mangrove ecosystems were net exporters of organic carbon, with estimates ranging from 10 to 70 % of the total mangrove net primary production being exported (Bouillon et al. 2008). More recently, the scope of carbon outwelling studies has expanded to include other pathways of carbon export, such as the movement of mobile fauna (e.g. fish, crabs) that feed in mangroves and transport carbon to other ecosystems (Granek et al. 2009; Walton et al. 2020). Studies have also looked at the role of groundwater discharge and porewater exchange in transporting dissolved inorganic carbon and alkalinity from mangroves to coastal waters (Maher et al. 2013). In addition to tidal flux measurements and

stable isotopes, techniques like radon tracing, eddy covariance, and mass balance approaches have also been employed to quantify carbon export (Maher et al. 2017; Adame et al. 2024). Remote sensing technologies, such as satellite imagery and aerial photography, have enabled large-scale assessments of mangrove productivity and carbon export (Sani et al. 2019; Araya-Lopez et al. 2023). Additionally, hydrodynamic and biogeochemical models have been developed to simulate carbon outwelling processes and predict their impacts on coastal ecosystems (Duarte et al. 2014; Baustian et al. 2018). These advanced methods have provided more comprehensive estimates of carbon outwelling from mangroves to the coastal ecosystems.

Subsequent studies across different geographic regions have contributed to an understanding of carbon outwelling in mangrove ecosystems. Studies in the Florida Everglades, the Brazilian coast, Southeast Asia, and the Australian mangroves have documented the export of both DOC and POC to adjacent estuarine and coastal waters (Dittmar et al. 2001; Santos et al. 2019; Zhao et al. 2021; Sharma et al. 2023). For example, a study in the Everglades quantified annual carbon outwelling rates, highlighting the significant contribution of mangroves to coastal carbon budgets (Zhao et al. 2021). Similarly, research in the Amazon estuary revealed that tidal exchanges facilitated the export of large quantities of organic carbon to the Atlantic Ocean (Serafim et al. 2023). One key finding is the substantial variability in outwelling rates, influenced by factors such as tidal amplitude, mangrove species composition, and seasonal changes.

Thus, the concept of carbon outwelling has evolved from early theoretical postulations to a well-established component of coastal biogeochemical research. Studies conducted worldwide have demonstrated the significant role of mangrove ecosystems in exporting organic carbon to coastal waters, influencing nutrient dynamics and carbon sequestration. The development of diverse methodological approaches has enriched our

understanding of carbon outwelling processes and their implications for coastal ecosystem health and sustainability. Furthermore, the export of carbon from mangroves contributes to long-term carbon sequestration in marine sediments, highlighting the role of mangroves as blue carbon ecosystems (Alongi 2014; Jennerjahn 2020).

2.2. Bacterial communities involved in carbon outwelling

Bacterial communities play a vital role in the outwelling process by breaking down OM, thereby facilitating the release and transformation of carbon. Understanding the composition and function of these bacterial communities is essential to comprehending the mechanisms behind carbon outwelling in mangroves. Globally, numerous studies have investigated the bacterial communities involved in the decomposition of OM within mangrove ecosystems. These studies typically employ molecular techniques such as 16S rRNA gene sequencing to identify and characterize the bacterial taxa present. For instance, research conducted in the mangrove forests of Southeast Asia revealed a diverse array of bacterial groups, including members of the phyla *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* (Thoetkiattikul et al. 2017; Lin et al. 2019; Muwawa et al. 2021). These bacteria are known for their ability to degrade complex organic compounds such as cellulose, lignin, and chitin, which are abundant in mangrove leaf litter and woody debris. The bacterial decomposition of OM in mangrove ecosystems involves a series of enzymatic processes. Different bacterial groups possess specific enzymes that enable them to break down various organic substrates. For example, bacteria belonging to the genus *Cellulomonas* are known for their cellulolytic activity, which helps decompose cellulose into simpler sugars (Kurniawan et al. 2018). Similarly, members of the genus *Bacillus* produce a range of hydrolytic enzymes, including amylases, proteases, and lipases, which contribute to the breakdown of starches, proteins, and lipids (Sengupta et al. 2020). A review by Kristensen et al. (2008) summarized the studies on organic carbon dynamics in mangrove ecosystems, highlighting the importance

of intertidal sediment processes; and porewater exchange in influencing the water column biogeochemistry in mangrove creeks. These sediment-water interactions mediated by microbes play a key role in the export and transformation of organic carbon from mangroves.

Extensive research on the Sundarbans, one of the largest mangrove forests in the world, has identified key bacterial taxa involved in OM decomposition. *Proteobacteria*, particularly the classes *Alphaproteobacteria* and *Gammaproteobacteria*, dominate these communities, alongside representatives from *Actinobacteria* and *Acidobacteria* (Ghose et al. 2024; Pramanik et al. 2024). These bacteria contribute to the degradation of OM through their diverse metabolic pathways, facilitating the export of DOC to coastal waters. In the Pichavaram mangroves of Tamil Nadu, studies have described that the presence of a diverse array of bacteria, including sulfate-reducing bacteria and sulfur-oxidizing bacteria, play significant roles in the mineralization of OM under anoxic conditions (Thatoi et al. 2013).

The mangrove ecosystems of Goa, though smaller in scale compared to the Sundarbans, also harbour diverse bacterial communities that play a crucial role in carbon cycling. Studies in these regions have employed metagenomic and metatranscriptomic approaches to elucidate the functional potential of mangrove-associated bacteria. A study from Goa by Sarma et al. (2001) measured the emission of carbon dioxide from the Zuari estuary, which receives inputs from the mangrove-fringed Mandovi estuary. The study found that the estuary acted as a source of CO₂ to the atmosphere, with the flux varying seasonally and spatially. While this study did not specifically focus on the role of bacteria, it highlights the importance of understanding greenhouse gas emissions from mangrove-estuarine systems in the context of carbon cycling. Another study by Fernandes et al. (2014) investigated the bacterial diversity in mangrove sediments along the Mandovi estuary in Goa. The authors found that the bacterial community composition varied with depth and was influenced by factors such as salinity, pH, and OM content. The study also recorded a significantly higher

denitrification potential at Divar, suggesting that the bacterial community may be contributing to modulating excess nitrate inputs from anthropogenic sources. Haldar and Nazareth (2018) investigated the bacterial diversity in mangrove sediments along the Mandovi and Zuari estuaries. The authors found that the bacterial community composition was dominated by the phyla *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. However, this study did not focus on the role of bacteria in carbon outwelling.

In summary, while research has been conducted on carbon cycling and bacterial communities in mangrove ecosystems globally and in India, there is a need for more targeted studies on the specific bacterial groups involved in breaking down and transforming the exported OM in the context of carbon outwelling. Understanding these microbial processes is crucial for advancing our knowledge of their ecological functions and quantifying the role of mangroves in the global carbon cycle.

2.3. Bacterial enzymes associated with OM breakdown in mangroves

Mangrove ecosystems are dynamic and productive environments where OM breakdown is a crucial process. Bacterial communities play a significant role in this process by producing various enzymes that degrade complex organic compounds. These bacterial enzymes are essential for nutrient cycling, carbon turnover, and maintaining the overall health and productivity of mangrove ecosystems. Understanding the specific bacterial species and their associated enzymes involved in OM breakdown provides valuable insights into the microbial ecology and functioning of these unique habitats.

A notable study conducted in North Sumatra, Indonesia, focused on culturable bacteria associated with *Bruguiera cylindrica* mangroves (Mamangkey et al. 2021). In this study, several bacterial strains were isolated, and enzymatic profiling was performed to assess their potential in producing extracellular enzymes. The identified strains were found to be capable of producing α -amylase, β -amylase, protease, cellulase, chitinase, and were

phosphate solubilizers. These enzymes are crucial for the degradation of OM, as they help break down polysaccharides, proteins, and phosphates, making nutrients available for mangrove plants and other organisms in the ecosystem. Another significant study focused on the microbial decomposition of soil OM in mangrove forests in East Asia, including regions like Hong Kong and Okinawa (Kim et al. 2021). Here, the study revealed that the enzymatic mechanisms involved in the breakdown of OM, particularly highlighting the role of phenol oxidase as a key enzyme in the decomposition process. This indicated that the accumulation of soil OM in mangroves is influenced by enzymatic activity, which is affected by environmental factors such as iron concentration and phenolic compounds. In mangroves of Brazil, Dias et al. (2009) identified key bacterial taxa belonging to *Vibrionales*, *Actinomycetales*, and *Bacillales* and enzymes such as amylases, proteases, esterases, and lipases involved in OM breakdown.

Gayathri et al. (2010) isolated endophytic bacteria and screened them for enzymatic activity from the Pichavaram mangroves in Tamil Nadu, India. Various enzymes, including amylase, lipase, protease, inulinase, invertase, chitinase, pectinase, cellulase, and urease, were tested among the endophytic strains. Thirty-six fast-growing isolates were selected for further screening. Among these 36 isolates, 94.4 % exhibited pectinase activity, 58.3 % showed protease activity, and 52.7 % demonstrated inulinase and invertase activities. A study conducted in the Sundarbans mangrove ecosystem revealed the presence of diverse bacterial communities belonging to *Bacillus* and *Pseudomonas* involved in OM decomposition (Biswas et al. 2020). This study emphasized the role of these bacteria in enhancing nutrient cycling and supporting the overall productivity of mangrove ecosystems. These findings suggest that the enzymatic activities of these bacteria are vital for maintaining the health and functionality of mangrove habitats. Another significant study conducted in the Sundarbans by Das et al. (2024) identified several dominant bacterial groups, including

Alphaproteobacteria, *Actinomycetota*, *Bacilli*, *Clostridia*, *Desulfobacterota*, *Gammaproteobacteria*, and *Nitrospira*, within the mangrove region. This study was the first to demonstrate that the Sundarbans, the largest mangrove ecosystem in the world, hosts a diverse array of methanogenic archaea, methanotrophs, pathogenic bacteria, salt-tolerant bacteria, probiotics, nitrifying bacteria, and bioremediation bacteria, all of which contribute to the breakdown of OM.

Regarding mangrove ecosystems in Goa, a study by Khandeparker et al. (2011) examined the abundance and phylogenetic diversity of culturable bacteria capable of producing complex carbohydrate-degrading enzymes in the Mandovi and Zuari estuaries. Notably, 65 % of the isolated bacteria were found to produce these enzymes which play a crucial role in the degradation of complex carbohydrates, proteins, and cellulose, supporting the detrital food web and nutrient cycling in the mangrove ecosystem. The majority of these bacteria belonged to the genus *Bacillus*, followed by *Vibrio*, *Marinobacter*, *Exiguobacterium*, *Alteromonas*, *Enterobacter*, and *Aeromonas*. Besides, a study by Haldar and Nazareth (2018) in the mangroves of Goa isolated bacteria, among which *Firmicutes*, were tested for their biofertilization capabilities and their production of various enzymes, including indole-acetic-acid, tannase, xylanase, glutaminase, amylase, cellulase, gelatinase, laccase, lipase, protease, and asparaginase enzymes. The study emphasized the significance of mangrove-associated bacteria in efficiently recycling OM and maintaining the ecological balance of Goa's mangrove ecosystems.

2.4. Enhancement of fisheries due to mangrove carbon outwelling

Mangrove ecosystems are recognized for their significant contributions to coastal fisheries due to their role in carbon outwelling, where organic carbon is exported to adjacent marine environments. The export of DOC and POC from mangroves provides a rich source of nutrients for primary producers such as phytoplankton, which form the base of the marine

food web. This increased primary production supports higher densities of zooplankton, which in turn provide food for juvenile and adult fish. Several studies have shown that carbon outwelling enhances the productivity of adjacent marine ecosystems by supplying OM and nutrients, supporting food web dynamics and fisheries (Ward et al. 2017; Nyunja et al. 2019).

A study conducted in the Gulf of California found that mangrove areas supported higher fishery yields compared to areas without mangroves (Mumby et al. 2004). The study estimated that mangroves contributed approximately 50 % of the total fish catch in the region, emphasizing their importance as nursery habitats for juvenile fish and crustaceans. The structural complexity of mangrove roots provides shelter and protection for juvenile species, enhancing their survival rates and contributing to higher adult populations in adjacent waters. In a comprehensive study by Nagelkerken et al. (2008), it was estimated that mangrove-derived organic carbon supports up to 32 % of the total fish biomass in adjacent coastal waters. This study, conducted across multiple sites in the Caribbean and Southeast Asia, highlighted the critical role of mangroves in sustaining fish populations. Similarly, offshore fisheries and fish stocks have been linked to the presence and extent of shoreline mangrove forests (Manson et al. 2005; Meynecke et al. 2008).

An economic analysis of secondary data on marine fish production in India revealed that mangroves significantly enhance fish output (Anneboina and Kumar 2017). The study found that each hectare of mangroves contributes 1.86 tonnes per year to marine fish production, accounting for 23 % of India's commercial marine fisheries output in 2011. Furthermore, the study emphasized the need for effective conservation and management strategies to ensure the sustainability of these vital ecosystems and the fisheries they support. The Sundarbans mangrove forest has been extensively studied for its impact on coastal fisheries. A study by Bhattacharya et al. (2018) revealed a total of 115 crab species and 312 brackish and marine fish species in the Sundarbans. These species were identified and

categorized, highlighting their predominant roles in 17 distinct clusters or survey areas within the localized mangrove-associated estuary. Additionally, the study highlighted that the productivity of the coastal waters was directly linked to the health and extent of the mangrove forests.

Thus, the enhancement of fisheries due to mangrove carbon outwelling is a globally recognized phenomenon, with significant implications for coastal ecosystems and local economies. Further research is needed to quantify the specific contributions of mangrove carbon outwelling to fisheries and to develop strategies for preserving these vital ecosystems against environmental changes.

Chapter 3

Seasonal Variations of Physicochemical Characteristics in Mangroves and Estuaries

3.1. General description of the study area

The state of Goa is located on the western coast of India and has seven large micro-tide estuaries with swamps consisting of laterite, loamy, and alluvial soils (Savoikar et al. 2021). Out of 130 km of coastal wetland in the state, 67.30 km is contributed by mudflats and mangroves. These mangroves are present in the narrow intertidal mudflats along the estuary banks and are of fringing nature which is said to be due to the rising topography of the coast (Singh et al. 2012). D'Souza and Rodrigues (2013) reported 17 species of mangroves in Goa, including 14 true and 3 associated species of mangroves, 80 % of which occur along the Mandovi-Zuari estuarine complex (Jagtap 1985).

The Mandovi and Zuari estuaries are a part of the Northern Indian Ocean (southeastern Arabian Sea), harboring a mangrove cover of 700 ha and 900 ha, respectively (Wakle et al. 2018). These regions experience distinct seasonal patterns: the south-west monsoon (June-September) brings heavy precipitation (250 to 300 cm yr⁻¹), while the dry season (November-May) sees a drastic reduction to less than 10 cm yr⁻¹ (Shetye and Murty 1987). During postmonsoon, significant OM transport occurs, potentially impacting the ecosystem's metabolism (Shynu et al. 2015; Wakle et al. 2018). These estuaries foster a thriving fish population (154 fish taxa in Mandovi; 176 fish taxa in Zuari), a source of food and livelihood for the local population (Rodrigues et al. 2011; Sreekanth et al. 2020).

3.2. Selection of sampling locations

Various sites along the Mandovi and Zuari estuaries on the coast of Goa were surveyed to select suitable sampling locations. The primary criteria for our study were to select mangrove forests directly adjacent to a flowing estuary and ensure the sites were accessible by boat for sample collection. Although most surveyed sites were lined with mangroves, many were inaccessible for effective sample collection. Two locations were ultimately selected due to their accessibility to both the mangroves and the adjacent estuary.

3.2.1. Description of the study locations

3.2.1.1. Mandovi estuary

Chorao Island in the Mandovi estuary boasts one of the most extensive mangrove covers in Goa. The island is 423.75 ha in size, of which 250 ha are covered with mangroves. Located at the confluence of the Mandovi and Mapusa rivers, it is also a well-known tourist destination due to the Salim Ali Bird Sanctuary. It is dotted with streams and backwaters and is subject to constant tidal oscillations (Sappal et al. 2014). The prominent mangrove species that make up the mangrove flora of the island are *Rhizophora mucronata*, *Avicennia marina*, *Sonneratia alba*, and *Excoecaria agallocha* (Gaonkar and Rodrigues 2020).

3.2.1.2. Zuari estuary

The Zuari estuary on the west coast of India is a dynamic tropical estuary. The upper reaches of its banks are lined with fringing mangroves, which are partially exposed during low tide. During high tide, these areas are covered by a water column ranging from 1 to 1.5 m, depending on the bank's gradient and the tidal range (Untawale and Parulekar 1976). Mangroves are represented mainly by two species – *Avicennia officinalis* and *A. alba*. Other mangroves here are *Acanthus ilicifolius*, *Sonneratia alba*, *Kandelia rheedei* and *Excoecaria agallocha* (<https://forest.goa.gov.in/mangroves>).

3.3. Strategy for sample collection

Sampling was carried out at two different estuaries, Mandovi (M) and Zuari (Z) (**Fig. 3.1.**). At each estuary, six sampling sites separated by a distance of ~ 400 m were chosen for sample collection. Locations M_m1 , M_m2 , M_m3 (M mangroves) and Z_m1 , Z_m2 , Z_m3 (Z mangroves) were selected within the mangrove swamps of M and Z, respectively. Similarly, locations M_w4 , M_w5 , M_w6 (M estuarine waters) and Z_w4 , Z_w5 , Z_w6 (Z estuarine waters) were stationed mid-river (~ 300-400 m away) opposite to locations of M_m and Z_m , respectively. The samples from M_m1 , M_m2 and M_m3 served as triplicates for the location M_m . Similarly,

the samples from M_w4, M_w5 and M_w6; Z_m1, Z_m2, Z_m3 and Z_w4, Z_w5 and Z_w6 represented M_w, Z_m and Z_w, respectively.

Water and sediment samples were obtained during the premonsoon (PM) (March-May), monsoon (Mo) (June-September) and postmonsoon (PoM) (October-November) seasons for two years (2018 - 2019). Surface water samples (depth < 0.5) from the mangrove and adjacent water sites were collected manually and using a Niskin sampler, respectively at low tide (LT) and high tide (HT). Sediment samples were collected using polyvinyl chloride (PVC) pipe corer (length 30 cm and diameter 7 cm) for the mangrove sites and Van Veen grab (area 1 m²) for adjacent water sites.

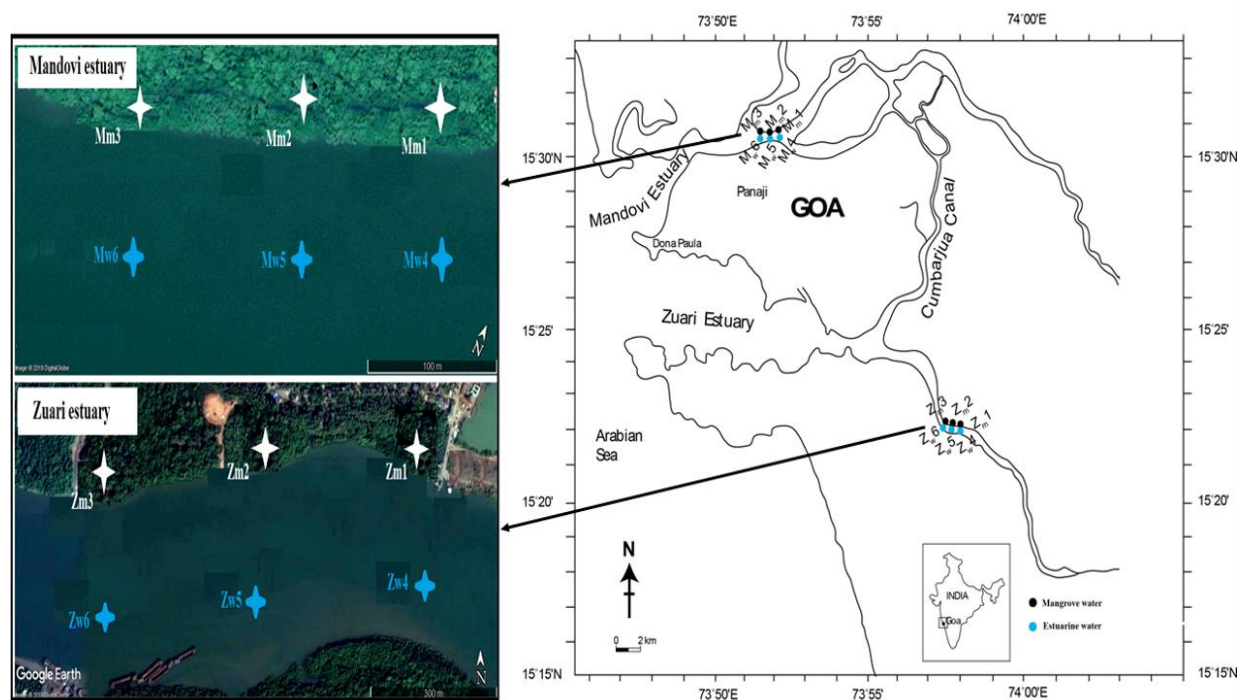


Fig. 3.1. Study area map of sampling sites in the Mandovi (M_m - Mandovi mangroves; M_w - Mandovi estuarine water) and Zuari (Z_m - Zuari mangroves; Z_w - Zuari estuarine water) estuaries, Goa, India

3.4. Materials and Methods

3.4.1. Determination of physicochemical parameters

Analyzing physicochemical parameters is essential for assessing the current environmental status of an estuarine system. Various environmental parameters, including temperature, pH, salinity, dissolved oxygen (DO), and OM, were measured using standard methods (Parsons et al. 2013)

3.4.1.1. Temperature

Surface water temperature (depth < 0.5 m) was measured in situ using a handheld mercury thermometer with a 0.1 °C graduation. Degrees Celsius (°C) were used to express the reported values.

3.4.1.2. Salinity

Salinity was measured using a handheld refractometer (ATAGO PAL-06S Pocket refractometer) and the values were expressed in ppt.

3.4.1.3. pH

The pH of the water samples was determined using a pH meter (Eutech pH 700).

3.4.1.4. Dissolved oxygen

Winkler's iodometric technique was used to measure the concentrations of dissolved oxygen (Carpenter 1965; Trivedi and Goel 1986). As part of the procedure, water samples were collected in a 125 ml glass stoppered bottle and the dissolved oxygen was immediately fixed using Winkler reagents (Winkler A and B). The fixed samples were titrated in the laboratory using a standard solution of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$). The obtained results were expressed in mg/L.

3.4.1.5. Dissolved inorganic nutrients

Water samples were collected in acid washed 50 ml polypropylene tubes. The samples were transported to the laboratory in an insulated icebox and immediately stored at

- 20 °C until further analysis. Concentrations of dissolved nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) were determined using an Autoanalyser (Skalar San Plus) following standard colorimetric methods (Grashoff et al. 1983). The results are expressed in $\mu\text{mol/L}$.

3.4.1.6. Organic matter

Total organic carbon (TOC) was estimated using a TOC analyzer (Shimadzu TOC LCPH) in which potassium hydrogen phthalate (KHP) was used as a quality control to check the performance of the TOC analyzer. The acidified samples ($\text{pH} < 2.0$) underwent catalytic oxidation at 680 °C in an oxygen-rich environment inside tubes filled with a platinum catalyst. The resulting CO_2 was detected and measured using a non-dispersive infrared sensor (NDIR). This method effectively oxidizes both easily decomposable low molecular weight organic compounds and insoluble, hard to decompose organic macromolecules. The instrument detection range spans from 0.004 mg/L to 30,000 mg/L. TOC concentrations were calculated using the formula $\text{TOC} = \text{TC} - \text{IC}$, where TC is Total Carbon and IC is Inorganic Carbon. The organic matter (OM) content was obtained by multiplying the TOC by a factor of 1.724 (Trask 1939). The results are expressed in mg/L of water and mg/g of sediment.

3.4.2. Statistical analysis

The statistical softwares PAST 4.03 (Hammer and Harper 2001) and IBM® SPSS 23.0 (IBM Corp., Armonk, NY, USA) were used to determine the statistical significance and each result was stated in mean $\pm$ standard deviation (SD). The relationship between the physicochemical parameters was understood using a two-tailed Pearson's correlation analysis ($p < 0.001$). One-way analysis of variance (ANOVA) was performed to understand the impact of seasonal variations on the observed physical and chemical parameters during LT and HT.

3.5. Results

3.5.1. Spatio-temporal variation of physical parameters

3.5.1.1. Temperature

During the sampling period, the average ambient water temperature ranged between 27 to 32 °C at LT and 27 to 30 °C at HT in both the M and Z estuaries (**Fig. 3.2. a; b**). Tidal variations in the surface water temperatures were not prominent. The highest temperatures were observed during the PM (average 30.59 °C), followed by PoM (average 28.75 °C) and Mo (average 28.11 °C). Irrespective of seasons, higher temperatures were observed in the estuarine waters (i.e., M_w and Z_w) compared to the mangrove waters (i.e., M_m and Z_m).

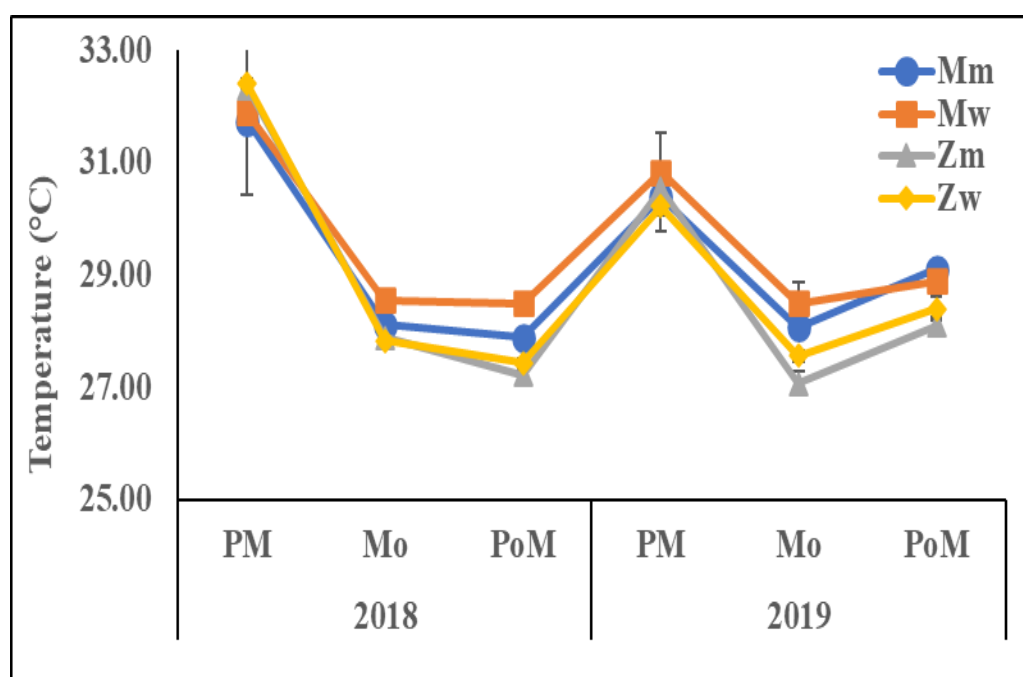


Fig. 3.2. a. Variation in temperature during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at low tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

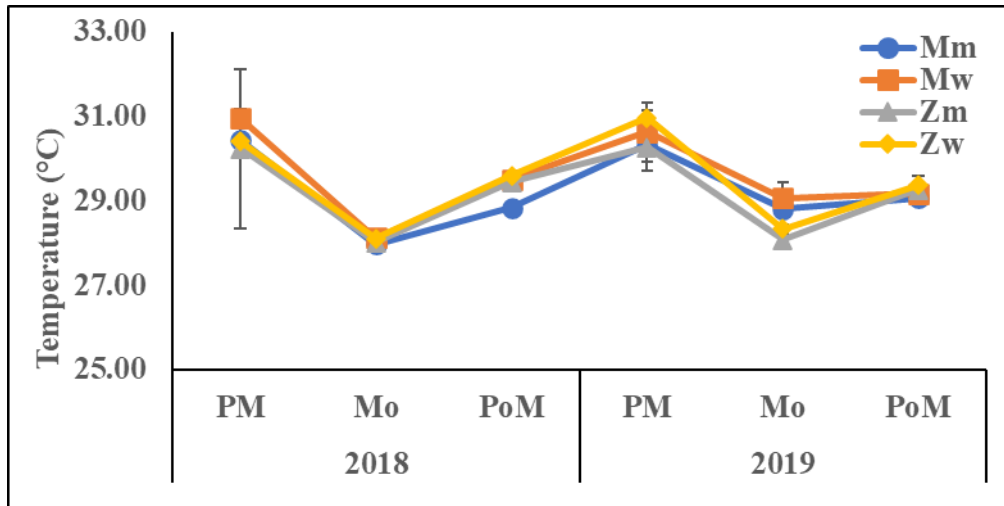


Fig. 3.2. b. Variation in temperature during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at high tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

3.5.1.2. Salinity

Distinct changes in the salinity were recorded varying from 2 to 6 ppt in Mo, 17 to 23 ppt in PoM and 27 to 31 ppt in PM at LT (**Fig. 3.3. a.**). During HT, salinities of 1 to 8 ppt in Mo, 21 to 29 ppt in PoM and 29 to 33 ppt in PM were observed (**Fig. 3.3. b.**). A decrease in salinity was observed during Mo followed by a steady increase from PoM to PM.

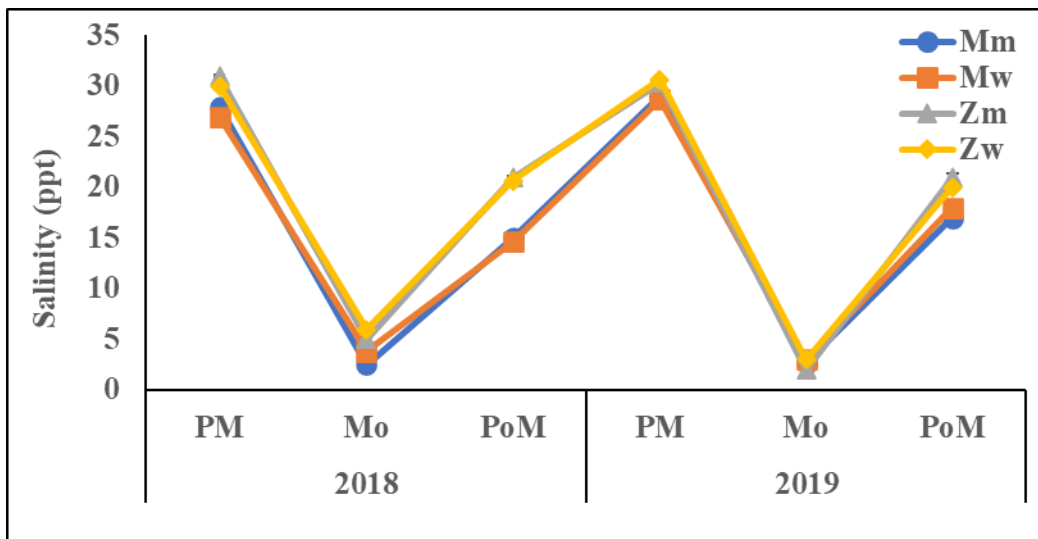


Fig. 3.3. a. Variation in salinity during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at low tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

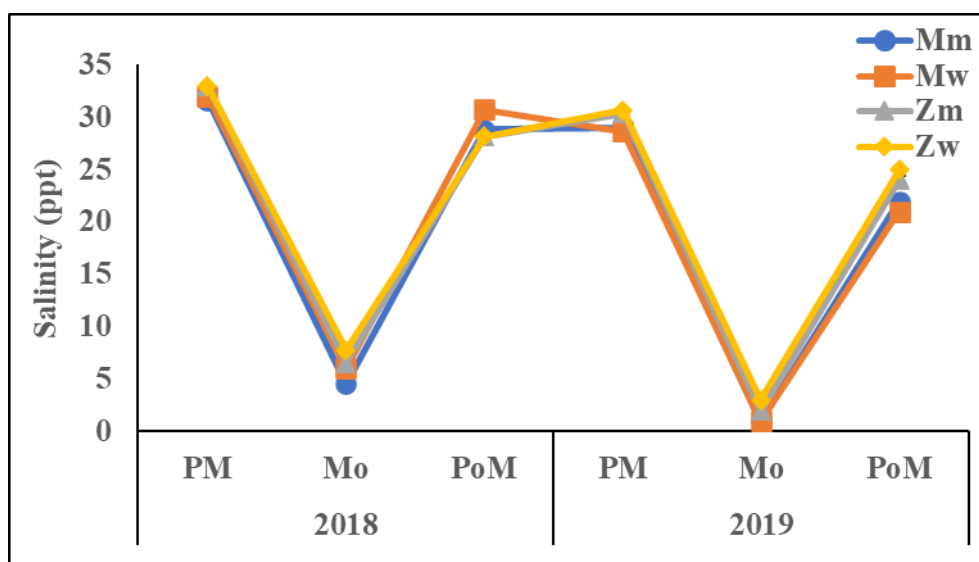


Fig. 3.3. b. Variation in salinity during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at high tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

3.5.1.3. pH

pH displayed variation over a very narrow range with the highest values during PM (7.50 to 7.69) and lowest during Mo in all our sampling sites (6.95 to 7.36). No variation was observed in the pH between LT (7.0 to 7.6) and HT (7.0 to 7.7) (**Fig. 3.4. a, b.**).

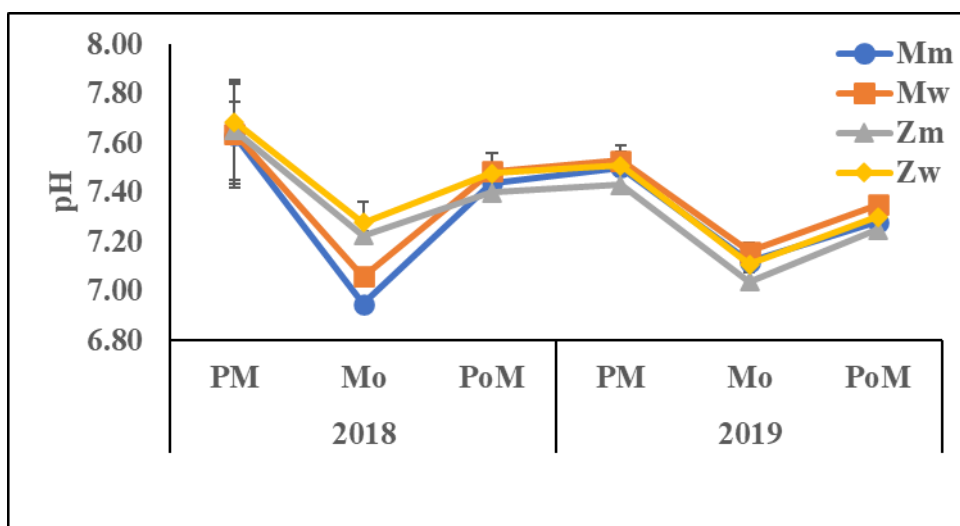


Fig. 3.4. a. Variation in pH during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at low tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

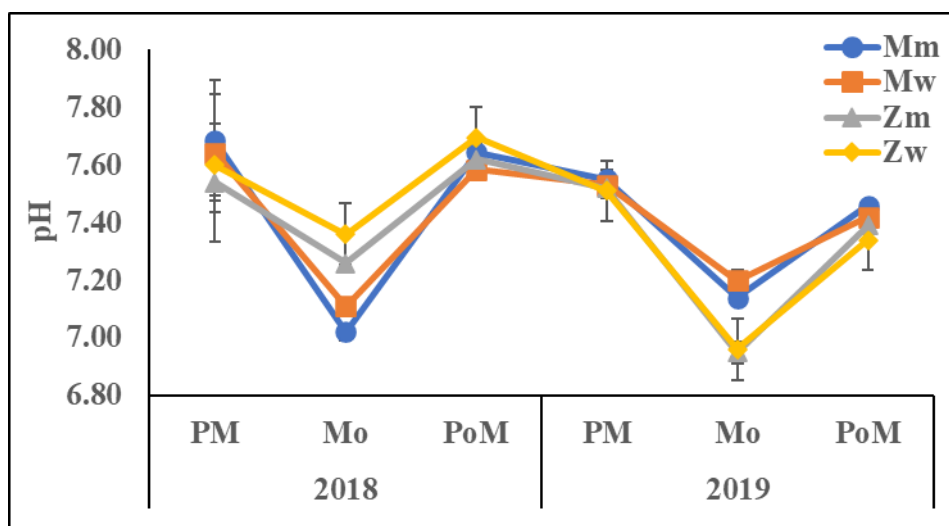


Fig. 3.4. b. Variation in pH during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at high tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

3.5.2. Spatio-temporal variation of chemical parameters

3.5.2.1. Dissolved oxygen

The average DO concentrations at LT were maximum during Mo (6.74 mg/L), followed by PoM (6.09 mg/L) and PM (4.92 mg/L) in both the estuaries (**Fig. 3.5. a.**). During HT, the highest DO concentrations were observed during Mo (6.29 mg/L) > PoM (5.39 mg/L) > PM (4.63 mg/L) (**Fig. 3.5. b.**).

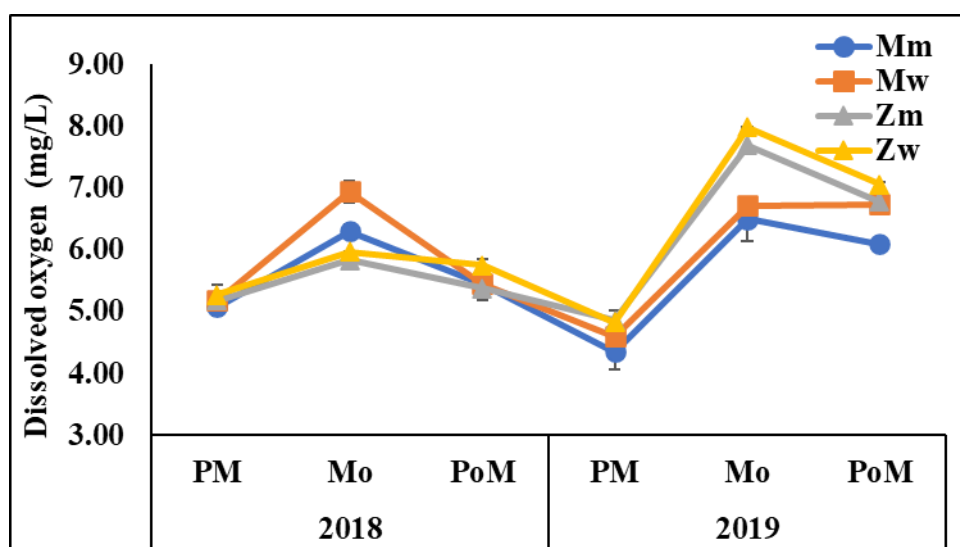


Fig. 3.5. a. Variation in dissolved oxygen during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at low tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

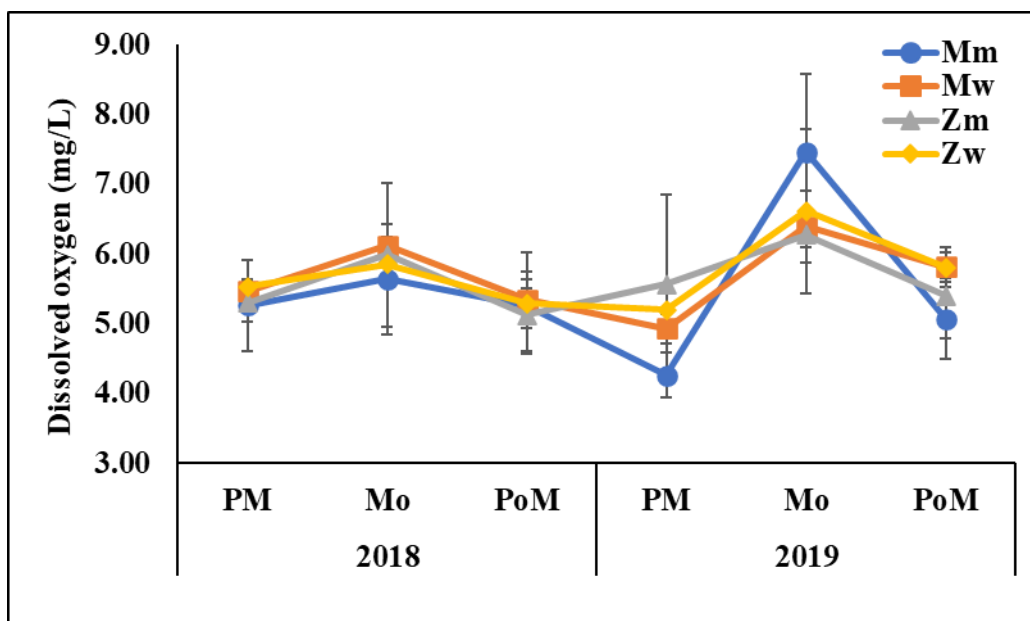


Fig. 3.5. b. Variation in dissolved oxygen during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons at high tide in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

3.5.2.2. Dissolved inorganic nutrients

During LT, concentrations of NO_2^- varied from 0.07 to 5.28 $\mu\text{mol/L}$; NO_3^- were between 2.17 $\mu\text{mol/L}$ to 24.23 $\mu\text{mol/L}$; NH_4^+ fluctuated between 0.32 to 8.05 $\mu\text{mol/L}$; PO_4^{3-} ranged from 0.06 to 3.64 $\mu\text{mol/L}$ and SiO_4^{4-} ranged between 14.75 to 74.42 $\mu\text{mol/L}$ (**Table 3.1.**). In case of HT, NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} , and SiO_4^{4-} concentrations varied between 0.03 to 4.25 $\mu\text{mol/L}$; 0.51 to 19.72 $\mu\text{mol/L}$; 0.11 to 37.39 $\mu\text{mol/L}$, 0.06 to 6.06 $\mu\text{mol/L}$ and 6.04 to 125.66 $\mu\text{mol/L}$, respectively.

Notably, highest concentrations of NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} and SiO_4^{4-} was observed during Mo, followed by PM and PoM in both the estuaries. Lower nutrient concentrations observed during PoM and PM coincided with increased salinity and lesser freshwater inflow. Also, nutrient concentrations were higher in M_m and Z_m in comparison to their corresponding adjacent sites i.e., M_w and Z_w.

Table 3.1. Variations (mean± SD; n=3) of nitrite (NO₂⁻), nitrate (NO₃⁻), ammonia (NH₄⁺), phosphate (PO₄³⁻), silicate (SiO₄⁴⁻) in µmol/L during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) at low tide (LT) and high tide (HT) in the Mandovi and Zuari estuaries during 2018 and 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters)

2018							
Nutrients	Location	PM		Mo		PoM	
		LT	HT	LT	HT	LT	HT
NO ₂ ⁻	M _m	1.32± 0.04	1.16± 0.05	3.28± 0.03	2.17± 0.01	0.07± 0.02	1.03± 0.02
	M _w	1.4± 0.05	1.25± 0.05	3.69± 0.04	3.6± 0.02	0.10± 0.02	1.04± 0.02
	Z _m	1.06± 0.01	1.02± 0.01	4.7± 0.03	2.38± 0.06	0.19± 0.04	0.14± 0.10
	Z _w	1.19± 0.04	0.93± 0.12	4.87± 0.01	2.36± 0.06	0.29± 0.01	0.16± 0.04
NO ₃ ⁻	M _m	3.35± 0.1	0.51± 0.12	14.79± 0.06	5.13± 0.07	2.57± 0.08	2.46± 0.10
	M _w	4.12± 0.02	0.94± 0.02	15.36± 0.16	4.91± 0.2	3.18± 0.08	2.26± 0.10
	Z _m	3.05± 0.04	2.61± 0.05	22.49± 0.02	12.24± 0.02	2.64± 0.05	3.00± 0.06
	Z _w	3.46± 0.05	2.96± 0.06	24.23± 0.25	14.62± 0.31	3.22± 0.02	2.23± 0.02
NH ₄ ⁺	M _m	0.75± 0.04	2.72± 0.05	1.84± 0.19	1.08± 0.2	0.68± 0.02	1.66± 0.37

	M _w	0.78± 0.02	1.69± 0.07	3.07± 0.03	2.08± 0.23	0.71± 0.01	1.4± 0.28
	Z _m	4.65± 0.15	0.4± 0.02	6.17± 0.06	6.66± 0.04	0.80± 0.01	1.13± 0.15
	Z _w	5.03± 0.03	1.15± 0.02	7.38± 0.32	10.36± 0.1	0.98± 0.02	0.92± 0.09
PO ₄ ³⁻	M _m	0.54± 0.04	0.18± 0.05	1.89± 0.01	0.55± 0.04	0.06± 0.02	0.05± 0.02
	M _w	0.81± 0.04	0.06± 0.04	1.62± 0.02	1.77± 0.05	0.12± 0.05	0.11± 0.02
	Z _m	1.15± 0.01	3.62± 0.01	3.55± 0.05	5.11± 0.04	0.29± 0.05	0.27± 0.05
	Z _w	1.49± 0.1	3.34± 0.05	3.64± 0.05	6.06± 0.01	0.36± 0.03	0.19± 0.01
SiO ₄ ⁴⁻	M _m	16.6± 0.04	20.75± 0.05	67.60± 0.16	122.62± 0.23	14.7± 0.30	23.29± 0.02
	M _w	18.52± 0.06	16.22± 0.02	72.61± 0.19	120.35± 0.04	17.98± 0.23	20.35± 0.01
	Z _m	32.74± 0.02	51.25± 0.18	55.19± 0.02	111.18± 0.07	26.87± 0.12	31.71± 0.01
	Z _w	37.64± 0.02	56.49± 0.04	57.04± 0.08	125.66± 0.39	25.79± 0.07	33.42± 0.02
2019							
NO ₂ ⁻	M _m	1.30± 0.02	0.13± 0.06	4.07± 0.02	3.81± 0.07	0.09± 0.04	0.09± 0.02
	M _w	1.54± 0.03	0.07± 0.06	5.04± 0.03	4.25± 0.02	0.12± 0.03	0.1± 0.04

	Z_m	1.01 ± 0.01	0.03 ± 0.01	4.28 ± 0.09	3.58 ± 0.43	0.06 ± 0	0.14 ± 0.02
	Z_w	2.09 ± 0.01	0.08 ± 0.01	5.28 ± 0.09	4.09 ± 0.02	0.09 ± 0.02	0.15 ± 0.02
NO_3^-	M_m	6.25 ± 0.02	4.67 ± 0.26	15.56 ± 0.31	15.8 ± 0.38	2.44 ± 0.14	6.95 ± 0.17
	M_w	8.90 ± 0.08	4.88 ± 0.1	16.35 ± 0.03	18.83 ± 0.04	2.59 ± 0.18	8.23 ± 0.22
	Z_m	7.96 ± 0.02	5.68 ± 0.02	22.19 ± 0.04	18.26 ± 0.05	2.17 ± 0.04	8.51 ± 0.05
	Z_w	8.85 ± 0.06	6.83 ± 0.07	23.34 ± 0.04	19.72 ± 0.05	2.92 ± 0.04	9.94 ± 0.05
NH_4^+	M_m	1.26 ± 0.02	5.57 ± 0.31	2.23 ± 0.02	0.42 ± 0.24	0.41 ± 0.07	3.94 ± 0.04
	M_w	1.46 ± 0.03	4.5 ± 0.06	2.50 ± 0.23	0.27 ± 0.1	0.32 ± 0.04	5.93 ± 0.2
	Z_m	4.49 ± 0.06	28.13 ± 0.21	7.74 ± 0.05	0.13 ± 0.02	0.91 ± 0.12	31.74 ± 0.07
	Z_w	5.04 ± 0.05	37.39 ± 0.07	8.05 ± 0.11	0.11 ± 0.02	0.72 ± 0.12	32.34 ± 0.18
PO_4^{3-}	M_m	0.12 ± 0.02	0.45 ± 0.02	1.19 ± 0.06	0.87 ± 0.02	0.16 ± 0.12	0.43 ± 0.05
	M_w	0.36 ± 0.08	0.06 ± 0.04	1.15 ± 0.02	0.83 ± 0.04	0.26 ± 0.03	0.63 ± 0.04
	Z_m	1.38 ± 0.09	1.24 ± 0.01	3.41 ± 0.35	0.61 ± 0.11	0.12 ± 0.02	3.08 ± 0.01
	Z_w	1.56 ± 0.02	1.41 ± 0.01	3.72 ± 0.02	0.77 ± 0.11	0.62 ± 0.02	0.49 ± 0.02

SiO_4^{4-}	M_m	18.73 ± 0.25	7.94 ± 0.04	69.29 ± 0.2	61.27 ± 0.02	15.89 ± 0.03	29.93 ± 0.09
	M_w	21.38 ± 0.05	6.04 ± 0.04	74.42 ± 0.08	60.16 ± 0.28	16.30 ± 0.16	31.79 ± 0.05
	Z_m	28.44 ± 0.17	6.91 ± 0.07	43.12 ± 0.02	17.99 ± 0.06	20.91 ± 0.06	23.29 ± 0.15
	Z_w	30.59 ± 0.06	6.43 ± 0.06	55.97 ± 0.02	18.31 ± 0.13	18.55 ± 0.15	23.26 ± 0.15

3.5.2.3. Organic matter

At LT, organic matter in water (OM_w) concentration varied from 8.48 to 69.87 mg/L in M and from 5.84 to 68.97 mg/L in Z (**Fig. 3.6. a.**). During HT, values of OM_w varied between 7.60 to 75.89 mg/L and 4.28 to 70.49 mg/L in M and Z, respectively (**Fig. 3.6. b.**). During LT, organic matter in sediment (OM_s) in M ranged between 2.29 to 62.61 mg/g and in Z from 4.71 to 57.36 mg/g (**Fig. 3.6. c.**). During HT, OM_s concentrations varied from 2.61 to 48.65 mg/g in M whereas in Z concentrations were recorded from 4.04 to 57.48 mg/g (**Fig. 3.6. d.**). Higher concentrations were witnessed mostly during the LT in comparison to the HT. The seasonal variation of OM_w and OM_s were in the order: Mo > PoM > PM in both the estuaries during both tides. The OM in the mangrove waters and sediment was higher than in its adjacent waters and sediments for both the estuaries.

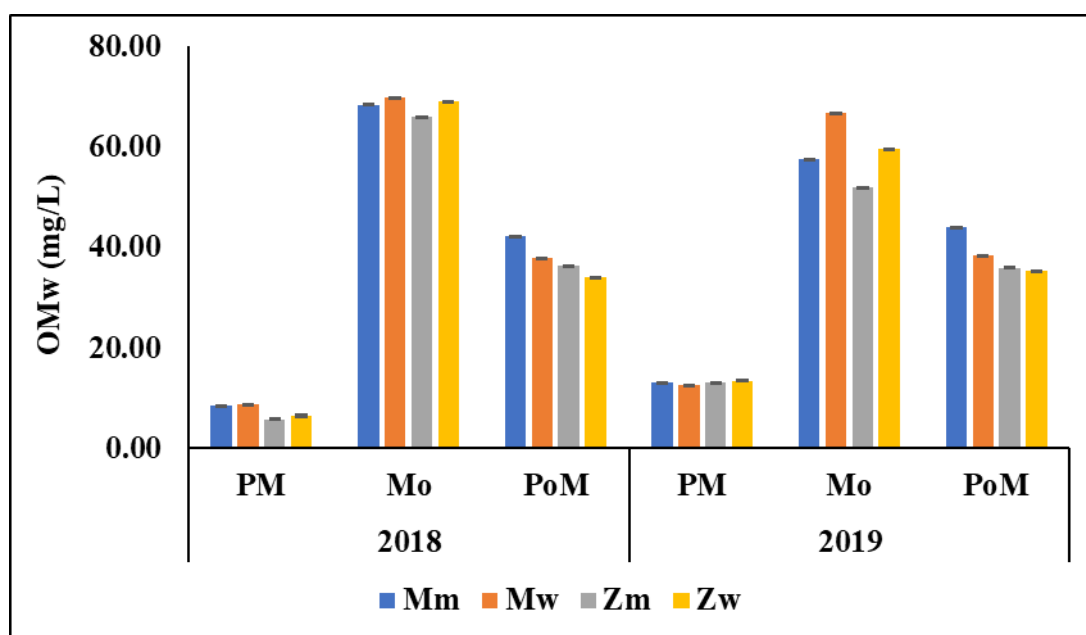


Fig. 3.6. a. Variation of organic matter in water (OM_w) during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) at low tide in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

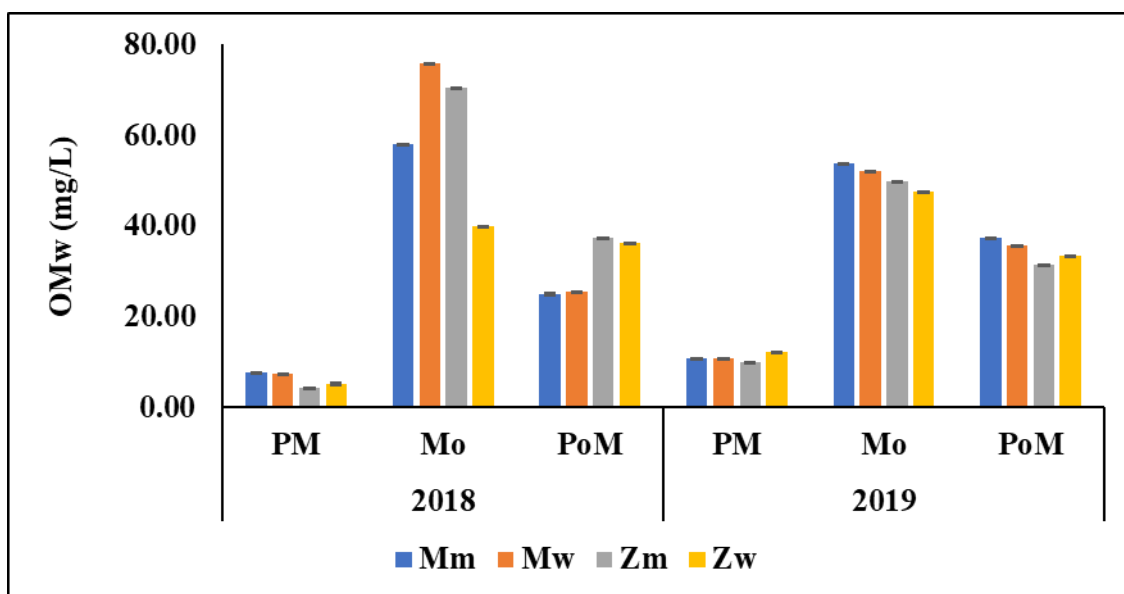


Fig. 3.6. b. Variation of organic matter in water (OM_w) during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) at high tide in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

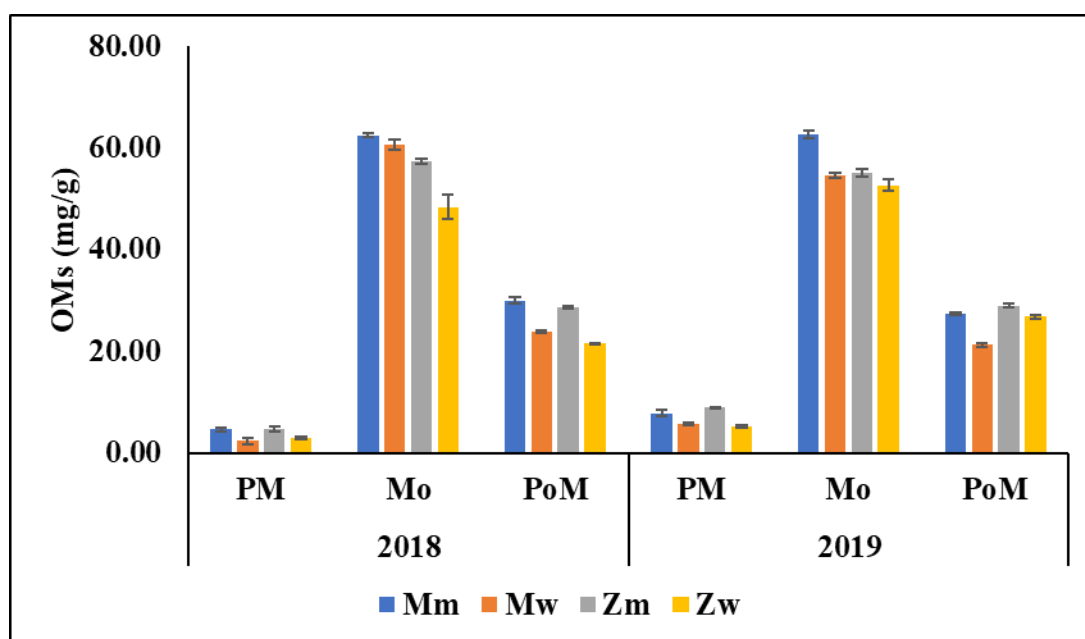


Fig. 3.6. c. Variation of organic matter in sediment (OM_s) during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) at low tide in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

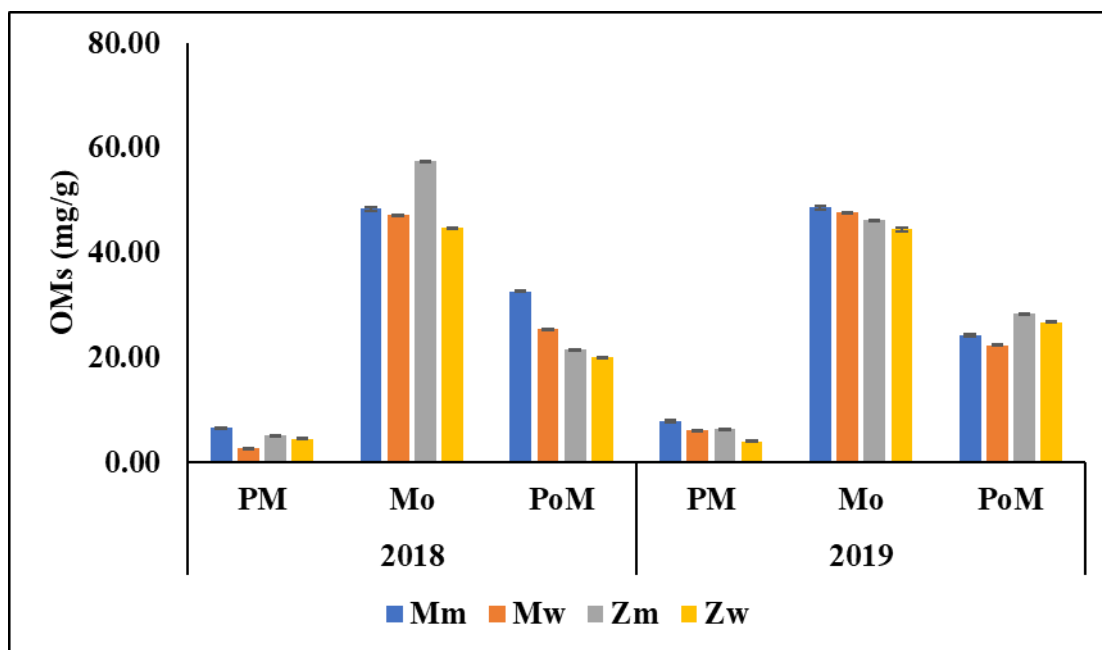


Fig. 3.6. d. Variation of organic matter in sediment (OM_s) during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) at high tide in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

3.5.3. Relationship between physicochemical parameters

Pearson's correlation analysis revealed interesting correlations between the physicochemical parameters. Irrespective of the tides, temperature was positively correlated to salinity, pH, and DO; whereas it was negatively correlated to DO, nutrients, and OM. Salinity and pH also negatively correlated with DO, nutrients, and OM (**Fig. 3.7. a, b**). DO was positively correlated to nutrients and OM. Nutrients and OM were negatively correlated to temperature, pH, and salinity while being positively correlated to DO. One-way ANOVA aided us in illustrating the impact of the seasonal variations on the observed parameters during LT and HT (**Table 3.2. a., b.**). The spatiotemporal variations of temperature, salinity, pH, DO, NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} , SiO_4^{4-} , OM_w and OM_s were found to be significantly different ($p < 0.001$).

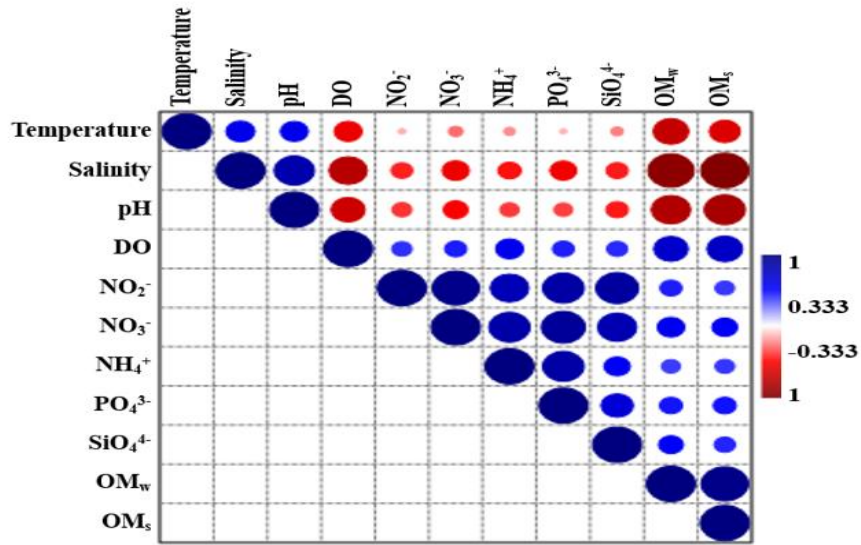


Fig. 3.7. a. Pearson's correlation coefficients between temperature, salinity, pH, dissolved oxygen (DO), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), organic matter in water (OM_w) and organic matter in sediment (OM_s), during low tide in the Mandovi and Zuari estuaries.

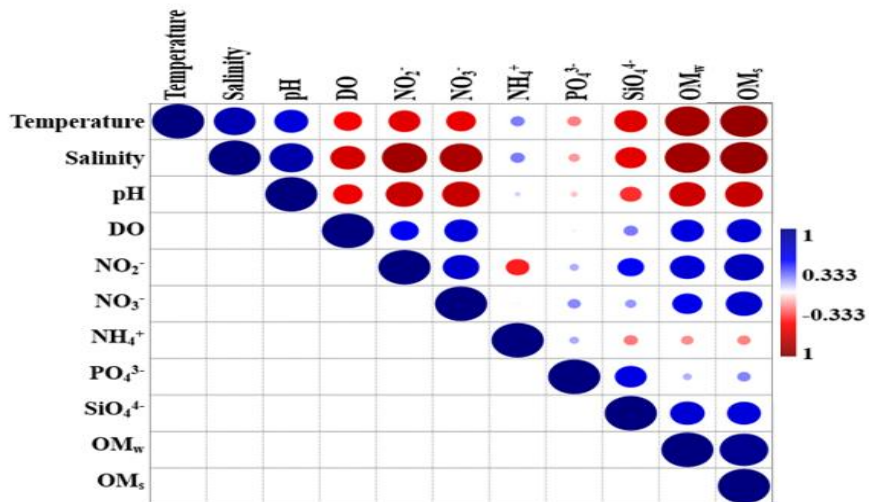


Fig. 3.7. b. Pearson's correlation coefficients between temperature, salinity, pH, dissolved oxygen (DO), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), organic matter in water (OM_w) and organic matter in sediment (OM_s), during high tide in the Mandovi and Zuari estuaries.

Table 3.2. a. Spatio-temporal variations of physicochemical parameters analyzed through one-way ANOVA in the Mandovi and Zuari estuaries during low tide and their significance ($p < 0.001$). (Dissolved oxygen (DO), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), organic matter in water (OM_w) and organic matter in sediment (OM_s))

		Sum of				
		Squares	df	Mean Square	F	<i>p</i>
Temperature	Between Groups	204.196	23	8.878	143.914	.000
	Within Groups	2.961	48	.062		
	Total	207.158	71			
pH	Between Groups	8373.284	23	364.056	3960.596	.000
	Within Groups	4.412	48	.092		
	Total	8377.696	71			
Salinity	Between Groups	3.080	23	.134	9.099	.000
	Within Groups	.707	48	.015		
	Total	3.787	71			
DO	Between Groups	65.613	23	2.853	110.464	.000
	Within Groups	1.240	48	.026		
	Total	66.852	71			
NO_2^-	Between Groups	244.973	23	10.651	8004.939	.000
	Within Groups	.064	48	.001		
	Total	245.037	71			
NO_3^-	Between Groups	20727.415	23	901.192	1.042	.438
	Within Groups	41520.778	48	865.016		
	Total	62248.193	71			
NH_4^+	Between Groups	17792.996	23	773.609	66475.491	.000
	Within Groups	.559	48	.012		
	Total	17793.555	71			
PO_4^{3-}	Between Groups	101.133	23	4.397	395.590	.000
	Within Groups	.534	48	.011		
	Total	101.667	71			
SiO_4^{4-}	Between Groups	29293.246	23	1273.619	69840.515	.000

	Within Groups	.875	48	.018		
	Total	29294.121	71			
OM _w	Between Groups	11986.209	23	521.140	100568.334	.000
	Within Groups	.249	48	.005		
	Total	11986.458	71			
OM _s	Between Groups	11117.166	23	483.355	962.425	.000
	Within Groups	24.107	48	.052		
	Total	11141.273	71			

Table 3.2. b. Spatio-temporal variations of physicochemical parameters analyzed through one-way ANOVA in the Mandovi and Zuari estuaries during high tide and their significance ($p < 0.001$). (Dissolved oxygen (DO), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), organic matter in water (OM_w) and organic matter in sediment (OM_s))

		Sum of				
		Squares	df	Mean Square	F	<i>p</i>
Temperature	Between Groups	65.839	23	2.863	22.216	.000
	Within Groups	6.185	48	.129		
	Total	72.024	71			
pH	Between Groups	10351.953	23	450.085	1383.103	.000
	Within Groups	15.620	48	.325		
	Total	10367.573	71			
Salinity	Between Groups	3.776	23	.164	10.563	.000
	Within Groups	.746	48	.016		
	Total	4.522	71			
DO	Between Groups	47.723	23	2.075	3.671	.000
	Within Groups	27.129	48	.565		
	Total	74.852	71			
NO_2^-	Between Groups	151.812	23	6.601	659.413	.000
	Within Groups	.480	48	.010		
	Total	152.293	71			

NO ₃ ⁻	Between Groups	2434.579	23	105.851	5075.785	.000
	Within Groups	1.001	48	.021		
	Total	2435.580	71			
NH ₄ ⁺	Between Groups	9433.715	23	410.162	15101.830	.000
	Within Groups	1.304	48	.027		
	Total	9435.018	71			
PO ₄ ³⁻	Between Groups	192.115	23	8.353	3956.612	.000
	Within Groups	.101	48	.002		
	Total	192.217	71			
SiO ₄ ⁴⁻	Between Groups	103675.060	23	4507.611	256154.707	.000
	Within Groups	.845	48	.018		
	Total	103675.905	71			
OM _w	Between Groups	10310.631	23	448.288	54660.047	.000
	Within Groups	.394	48	.008		
	Total	10311.025	71			
OM _s	Between Groups	7636.516	23	332.022	11167.717	.000
	Within Groups	1.427	48	.030		
	Total	7637.943	71			

3.6. Discussion

It is interesting to note that the M and Z estuaries are highly susceptible, yet remain remarkably adaptive to the continuous and dynamic changes observed throughout the study period. The surface water temperatures recorded were higher in M_w and Z_w than M_m and Z_m as a result of the dense mangrove canopy over the latter thereby, restricting the entry of sunlight. The seasonal variation in rainfall is an important contributor of freshwater flux exerting a considerable influence over the salinity patterns. These estuaries receive the highest amount of rainfall (460 to 1160 mm) during Mo (July to September), lesser rainfall (6.44 to 210 mm) during PoM (October to December) and almost no rainfall during the driest PM months of January and February (0.2 mm) (https://disc.gsfc.nasa.gov/datasets/GPM_3IMERGGH_06/ summary). Additionally,

freshwater discharge from rivers upstream regulates the salinities in these estuaries. It has been estimated that the freshwater supply increases by $\sim 64\%$ during Mo, $\sim 35\%$ during PoM and $\sim 7\%$ during PM (Ghosh et al. 2021). While it is known that both these estuaries originate from the Western ghats and share a common point of discharge, Z maintains more saline conditions in comparison to M almost throughout the year. This phenomenon is attributed to the wider mouth of Z connecting to the sea and lower number of tributaries resulting in higher salinities recorded at Z (Padmavati and Goswami 1997). In contrast, M receives a considerable amount of runoff through a larger tributary system (Qasim and Sengupta 1981) attaining an almost freshwater salinity range during Mo. This pattern of increasing salinity from Mo, PoM to PM seasons has also been documented in the tropical estuaries of Pondicherry, Sundarbans and Tamil Nadu (Prabu et al. 2008; Satheeshkumar and Khan 2009; Basu et al. 2021). The observed alterations in pH were not significant, consistent with findings reported by Basu et al. (2021) in the mangrove estuary of Tamil Nadu. The slightly elevated pH recorded during PM is caused by seawater intrusion which goes as far as 65 km into the estuarine zone, while lower pH observed during Mo is due to limited seawater extending only upto 10 km upstream in M and 12 km upstream in Z (Qasim and Sengupta 1981). The DO concentrations suggested well aerated surface waters in all the sites during the sampling period. Relatively higher DO concentrations during the Mo and PoM were due to the higher solubility of oxygen in freshwater (Mitra et al. 2018).

The nutrient profiles in the mangrove and estuarine waters are shaped by dual forces of the monsoonal pattern and the tidal inundation. The elevated concentrations of nutrients during LT in the mangrove swamps and its adjacent estuarine waters suggest a complex interplay of ecological dynamics. Unlike HT, where dilution effects might occur due to greater water volumes, the reduced water levels during LT concentrate nutrients within a smaller volume, leading to higher observed concentrations. This phenomenon could signify

localized nutrient accumulation, possibly from sediment resuspension or the retention of nutrients within the confined area of the mangrove swamps (Hasan et al. 2022). Moreover, it emphasizes the ecological importance of mangroves during tidal fluctuations, highlighting their role as nutrient reservoirs or hotspots within the larger estuarine ecosystem. It was consistently observed that the nutrient concentrations in the mangrove waters i.e., M_m and Z_m were higher than those of the estuarine waters i.e., M_w and Z_w , suggesting the outwelling of nutrients from the mangrove swamps. The nutrient outwelling arising from OM decomposition or root exudates within mangroves elucidates the role of mangroves as crucial sources of essential nutrients. The highest nutrient concentrations in the M and Z estuaries were observed during Mo when freshwater input was the maximum which has been recorded previously by Mochamadkar et al. (2013). This trend aligns with previous observations by Prabu et al. (2008) and Basu et al. (2021). Apart from the freshwater inputs, the surge in NO_2^- concentration during Mo arises due to heightened biological activity such as phytoplankton excretion, ammonia oxidation, nitrate reduction, N recycling and bacterial decomposition of planktonic detritus (Govindasamy et al. 2000). The increased concentrations of NO_3^- in Z_m and Z_w during Mo has been reported previously by Maya et al. (2011), despite receiving lower freshwater input compared to M. This could also be a result of the greater expanse of mangrove forests in Z. Mangroves act as significant contributors of nitrogen compounds through biological processes like nitrogen fixation and decomposition of OM. The larger coverage of mangroves in Z likely enhances biological activities, such as microbial nitrogen transformations and nutrient recycling, resulting in elevated NO_3^- concentrations despite relatively lower freshwater input compared to M. Similarly, the higher NH_4^+ concentrations in Z might be attributed to the greater expanse of mangroves, leading to an accelerated rate of bacterial decomposition, mineralization, and ammonification of dissolved organic nitrogen originating from plant debris (Kristensen et al. 1988). Our results indicated a decrease in

PO_4^{3-} concentrations as salinity increased, attributed to the tendency of higher salinity to cause phosphorus precipitation. This process results in the removal of phosphorus from the water column through sedimentation or its binding with other particles (De Jonge and Villerius 1989; Fang 2000). Reduced rainfall during PM and PoM led to a considerable decrease in SiO_4^{4-} concentrations as the SiO_4^{4-} supply becomes limited from natural weathering of rocks and erosion (Hessen 1999). Overall, the hydrographical conditions of the estuaries in our study were dictated primarily by the monsoon conditions, the freshwater input, nutrient outwelling and the proximity to the sea that regulates the ebb and flow of water.

Higher OM observed in M than in Z can be attributed to the higher river runoffs in the former (Qasim and Sengupta 1981). The Mo season is characterized by maximum freshwater inputs and land runoffs; consequently, maximum OM concentrations were recorded during this season. Gireeshkumar et al. (2013) reported a similar seasonal peak in OM concentrations during the monsoon (Mo) period in the Cochin estuary. Higher OM in M_m and Z_m than in M_w and Z_w may have resulted from the breakdown of mangrove leaf litter as reported by Kristensen et al. (2008), indicative of the outwelling of OM from the mangroves. This phenomenon of elevated OM in surface sediments was also documented in similar mangrove-dominated aquatic systems (Kristensen et al. 2008; Prasad and Ramanathan 2009).

The negative correlations found between nutrients and OM with temperature, pH, and salinity reflect the monsoonal impact on the concentrations of the nutrients and OM during both LT and HT. Additionally, the positive correlations observed between temperature, salinity and pH signify strong interdependencies among these environmental factors. Collectively, Pearson's correlations suggest that physicochemical parameters are intricately linked to the availability of nutrients and OM and are influenced by temperature, salinity, pH, and DO. Moreover, the significance of the impact of monsoonal variations on all the

observed parameters i.e., during LT as well as HT was established by one-way ANOVA, affirming that the monsoonal precipitation exerts a noteworthy influence on the estuarine hydrography and productivity.

3.7. Summary

Seasonal variations in temperature, salinity, pH, DO, dissolved inorganic nutrients, and OM are considerably influenced by the southwest monsoon in the Mandovi and Zuari estuaries. Heavy precipitation and land runoff during the Mo season cause significant changes in the physicochemical conditions, making the estuaries freshwater-dominated. Dynamic conditions prevail due to the continuous ebb and flow of water, resulting in well-oxygenated waters, as indicated by high DO concentrations. Among the observed physicochemical parameters, salinity is a defining factor influencing the conditions in both the mangrove and adjacent estuarine waters. Dissolved inorganic nutrients such as NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} , and SiO_4^{4-} were highest during Mo, correlating with lower salinity and higher freshwater inflow. Notably, nutrient profiles revealed that mangrove waters consistently exhibit higher concentrations of nutrients than estuarine waters, suggesting nutrient outwelling from mangrove swamps. The major contributors of OM are mangroves and terrestrial runoff which is heightened during the Mo period. Elevated OM concentrations highlight the complex relationship between monsoonal precipitation, nutrient dynamics, and estuarine productivity. Pearson's correlations and ANOVA analyses confirmed that physicochemical parameters such as temperature, salinity, pH, DO, nutrients and OM are strongly interlinked and significantly impacted by monsoonal variations. Overall, our study highlights the dynamic interplay of tidal and seasonal influences on the physicochemical environment of these estuaries.

Chapter 4

Spatio-temporal variation

of Bacterial Abundance and

Diversity

4.1. Introduction

The aquatic environment generally contains the largest and most diverse pool of microbial species (Slapeta et al. 2005; Wilhelm and Matteson 2008; Auguet et al. 2010). Microbes are key components of the mangrove environment, playing a crucial role in nutrient cycling and enhancing the chemical environment of this ecosystem (Alongi et al. 1993; Thatoi et al. 2013). Despite the rich microbial diversity in mangrove ecosystems, less than 5 % of species are well-known for their ecological and biological roles (Thatoi et al. 2013). Total heterotrophic prokaryotes have been identified as major contributors to organic matter (OM) utilization and nutrient recycling (Azam et al. 1983; Pernthaler 2005; Pomeroy et al. 2007). The microbial diversity in mangrove ecosystems has not been studied to the same extent as in other environments. Hence, a comprehensive methodological description of bacterial diversity and distribution in mangrove plants and their rhizosphere sediment would enhance our understanding of their functionality and microbial interactions within this ecosystem (Kathiresan and Selvam 2006).

4.2. Materials and Methods

4.2.1. Determination of bacterial abundance

4.2.1.1. Total viable counts of bacteria

About 1 g of sediment was transferred to a 50 ml sterile falcon tube containing 9 ml sterile saline (0.85 % NaCl). The suspension was mixed with vigorous shaking and kept on a shaker at 125 rpm for 15 mins for proper homogenization. The soil particles were then allowed to settle for 20 mins. The resulting solution was serially diluted up to 10^{-5} using sterile saline. Aliquots of 100 μ l were then taken from dilutions 10^{-3} , 10^{-4} , 10^{-5} and spread plated on autoclaved Zobell Marine Agar (ZMA) (HiMedia) or Nutrient Agar (NA) (HiMedia), depending on the salinity of the sampling sites. Similarly, water samples were serially diluted up to 10^{-3} , and 100 μ l aliquots were spread plated from each dilution.

Following a 28 °C incubation for 24 hrs, total viable counts (TVC) were recorded and expressed in CFU (colony forming unit)/ml or CFU/g.

4.2.1.2. Total bacterial abundance

To obtain the total bacterial abundance (TBA), water and sediment samples were fixed with buffered formaldehyde (2 % final concentration). Following the method of Porter and Feig (1980), 2 ml subsamples were incubated for 20 mins with 40, 6-diamidino-2-phenylindole (DAPI; 20 µl of 1 mg/ml working solution) and filtered through black polycarbonate membrane filters (0.22 µm, Millipore). The bacterial cell counts were determined by counting 20 observed fields to obtain a reliable mean using an epifluorescence microscope (Olympus BX-51, Japan). The mean obtained was expressed in cells/ml or cells/g.

4.2.2. Isolation and identification of bacteria

4.2.2.1. Isolation of pure bacterial cultures

Colonies with distinct morphological characteristics were streaked on Zobell Marine Agar (ZMA) or Nutrient Agar (NA) plates to obtain isolated pure colonies. Each pure culture was then transferred to Zobell Marine Broth (ZMB) (HiMedia) or Nutrient Broth (NB) (HiMedia) and incubated at 28 °C for 24 hrs in a shaker incubator (125 rpm).

4.2.2.2. Maintenance of pure cultures

Storage of cultures was done by suspending 500 µl of broth cultures in 500 µl of 50 % glycerol in a cryovial for the long-term storage of bacterial cultures in - 80 °C. All precautions were taken to minimize contamination of media, samples, and glassware, and to maintain microbiological laboratory standards. All the media and glasswares were autoclaved at 15 psi for 20 mins (Sakhia et al. 2016).

4.2.2.3. Genomic DNA extraction of bacteria

The bacterial cultures were subjected to genomic DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega, USA). 1 ml of an overnight culture was added to a

1.5 ml microcentrifuge tube. The tube was centrifuged at 15000 rpm for 2 mins to pellet the cells, and the supernatant was discarded. 10 mg/ml of lysozyme was added to the resuspended cell pellet in a total volume of 120 μ l and gently pipetted to mix, to weaken the cell wall for efficient lysis. The sample was incubated at 37 °C for 45 mins, centrifuged for 2 mins at 15000 rpm, and the supernatant was removed. Next, 600 μ l of nuclei lysis solution was added, and the cells were gently resuspended. The sample was incubated at 80 °C for 5 mins to lyse the cells, then cooled to room temperature. 3 μ l of RNase solution was added to the cell lysate, and the tube was inverted 2 - 5 times to mix. The sample was incubated at 37 °C for 45 mins and then cooled to room temperature. 200 μ l of protein precipitation solution was added to the RNase-treated cell lysate, and the mixture was vortexed vigorously at high speed for 20 sec. The sample was then incubated on ice for 5 mins and centrifuged at 15000 rpm for 3 mins. The supernatant containing the DNA was transferred to a clean 1.5 ml microcentrifuge tube containing 600 μ l of room temperature isopropanol, leaving any residual liquid in the original tube to avoid contaminating the DNA solution with precipitated protein. The mixture was gently inverted until thread-like strands of DNA formed a visible mass. It was then centrifuged at 15000 rpm for 2 mins. The supernatant was carefully poured off, and the tube was drained on clean absorbent paper. 600 μ l of room temperature 70 % ethanol was added, and the tube was gently inverted several times to wash the DNA pellet. After centrifuging at 15000 rpm for 2 mins, the ethanol was carefully aspirated. The tube was drained on clean absorbent paper and allowed to air-dry for 10 to 15 mins. 100 μ l of DNA rehydration solution was added to the tube, and the DNA was rehydrated by incubating at 65 °C for 1 hr, periodically mixing the solution by gently tapping the tube. Finally, the DNA was stored at 4 °C.

4.2.2.4. Agarose gel electrophoresis

The open ends of a clean and dry gel bed (casting tray) were closed off using tape. A well-formed comb was placed in the middle set of notches, ensuring it sat firmly and evenly across the bed. A 250 ml conical flask was used to prepare the 0.8 % agarose gel solution. The mixture was swirled to disperse clumps of agarose powder and heated in an oven to dissolve the agarose. The final solution appeared clear, like water, without any undissolved particles. The agarose solution was cooled to 55 °C, and 2 µl of ethidium bromide (EtBr) was added. The cooled agarose solution was then poured into the tray and allowed to solidify completely, becoming firm and cool to the touch after approximately 20 mins. Once the gel was solidified, the tape was carefully and slowly removed from the tray. The comb was removed by slowly pulling it straight up. The gel was placed into the electrophoresis chamber, properly oriented, centered, and leveled on the platform. The electrophoresis apparatus chamber was filled with the required volume of diluted (1x) Tris Acetate EDTA (TAE) buffer, ensuring the gel was completely covered with buffer. The sample, mixed with 6x loading buffer (2 µl dye + 5 µl sample), was slowly loaded into the slots of the gel using a micropipette. A voltage of 80 volts was applied, and the gel was run until the bromophenol blue and xylene cyanol fast flow had migrated an appropriate distance through the gel. The gel tray was then removed and placed directly in a gel documentation system. When the UV lamp was switched on, the bands were visualized.

4.2.2.5. Polymerase chain reaction (PCR) amplification

Extracted DNA was then utilized for PCR amplification using the ReadyMix™ Taq PCR Reaction mix (Sigma-Aldrich) which included DNA polymerase, deoxynucleotide triphosphate (dNTPs), Taq DNA polymerase, nuclease free water, 10x reaction buffer, and MgCl₂. The bacterial 16S rRNA gene was amplified using the primers 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTTACGACTT-3'

(Bioserve) (Lane 1991). In an autoclaved PCR tube, 25 µl of ReadyMix™ Taq PCR reaction mix was added to 10 pM of each primer in a volume of 2 µl with 4 µl of genomic DNA (~ 50 ng), and finally made the volume upto 50 µl with nuclease free water. Thermal cycling conditions included an initial denaturation step at 94 °C for 4 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 10 min (Sambrook et al. 1989). PCR was conducted in a Veriti™ 96-well thermal cycler, and PCR amplification was confirmed by 1 % agarose gel electrophoresis. DNA concentration was assessed by using Nanodrop (Thermo Fisher Scientific).

4.2.2.6. PCR product purification

PCR products were purified using Wizard SV Gel and PCR Cleanup system (Promega, USA). An equal volume of membrane binding solution was added to the PCR product. The minicolumn was inserted into the collection tube, and the prepared PCR product was transferred to the minicolumn assembly. After incubating at room temperature for 1 min, the assembly was centrifuged at 15000 rpm for 1 min. The flowthrough was discarded, and the minicolumn was reinserted into the collection tube. Next, 700 µl of membrane wash solution (with ethanol) was added, and the assembly was centrifuged at 15000 rpm for 1 min. The flowthrough was discarded, and the minicolumn was reinserted into the collection tube. This step was repeated with 500 µl of membrane wash solution, followed by centrifugation at 15000 rpm for 5 mins. The collection tube was emptied, and the column assembly was recentrifuged for 1 min with the microcentrifuge lid open to allow any residual ethanol to evaporate. The minicolumn was carefully transferred to a clean 1.5 ml microcentrifuge tube, and 50 µl of nuclease-free water was added to the minicolumn. After incubating at room temperature for 1 min, the assembly was centrifuged at 15000 rpm for 1 min. The minicolumn was discarded, and the purified DNA was stored at 4 °C until further analysis.

4.2.2.7. 16S rRNA gene sequencing and sequence analysis

PCR products were sequenced in an ABI 3130XL genetic analyzer (Applied Biosystems, USA) using primers, 27F and 1492R (Lane 1991). Non-chimeric consensus sequences devoid of vector or primer residues and a quality score of 20 (which translates to more than 99.5 % correct bases) were used for further analyses. The sequences were classified using 1000 pseudo bootstrap replications with a 95 % bootstrap value, resulting in a standard error of only 1.3 %. Sequences were assembled and compared to the NCBI database. Trimming of alignments was done automatically using Gblocks to remove badly aligned locations and divergent sections (Castresana 2000). If these sequences had > 80 % of their identity with database sequences, they were assigned to a phylum.

4.2.3. Statistical analysis

The statistical analyses were carried out using the software PAST 4.03 (Hammer and Harper 2001). A two-tailed Pearson's correlation analysis ($p < 0.001$) was conducted to examine the relationship between physicochemical parameters, bacterial abundance, and diversity during PM, Mo, and PoM. To further reduce intercorrelated variability, a principal component analysis (PCA) biplot was created, combining the sample scores plot and the vector loading plots to elucidate the relationships between these parameters.

4.3. Results

4.3.1. Spatio-temporal variation in total viable counts of bacteria

The TVC_w (TVC in water) in M ranged from $1.46 (\pm 1.5) \times 10^4$ CFU/ml to $18.76 (\pm 0.8) \times 10^4$ CFU/ml (**Fig. 4.1.a.**). The TVC_w in Z ranged from $1.16 (\pm 2.1) \times 10^4$ CFU/ml to $18.00 (\pm 1.1) \times 10^4$ CFU/ml. In the case of TVC_s (TVC in sediment), a variation of $0.23 (\pm 2.2) \times 10^6$ CFU/g to $21.66 (\pm 3.3) \times 10^6$ CFU/g was recorded in M and $0.07 (\pm 2.2) \times 10^6$ CFU/g to $19.33 (\pm 0.8) \times 10^6$ CFU/g in Z (**Fig. 4.1. b.**). In both M and Z, the highest abundance of TVC_w as well as TVC_s was observed during the Mo, followed by PoM and PM.

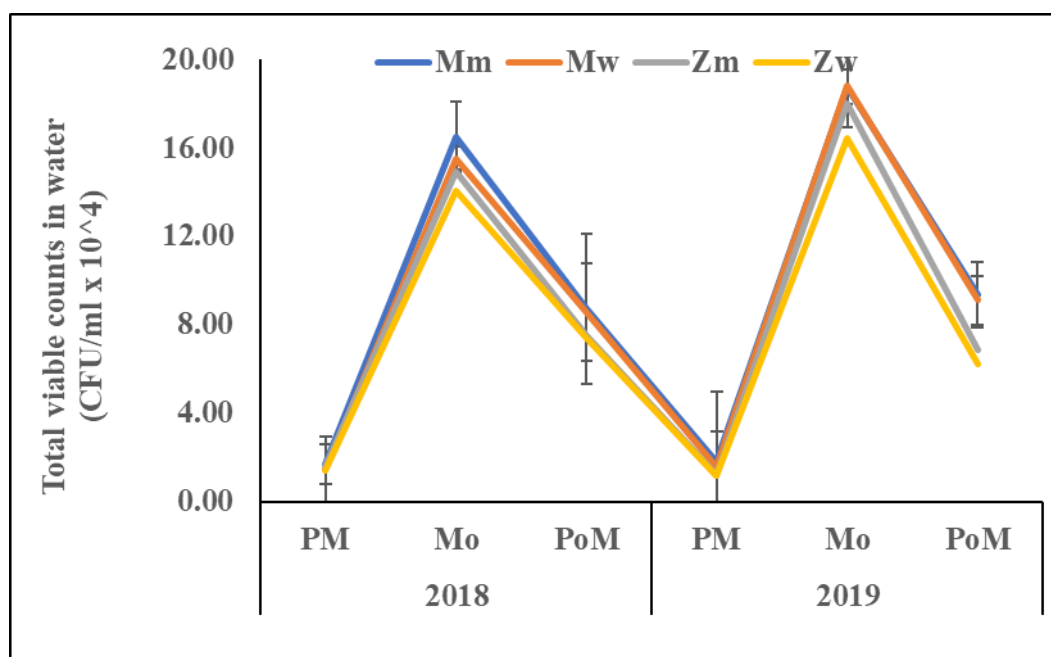


Fig. 4.1. a. Variations in total viable counts of bacteria in water during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

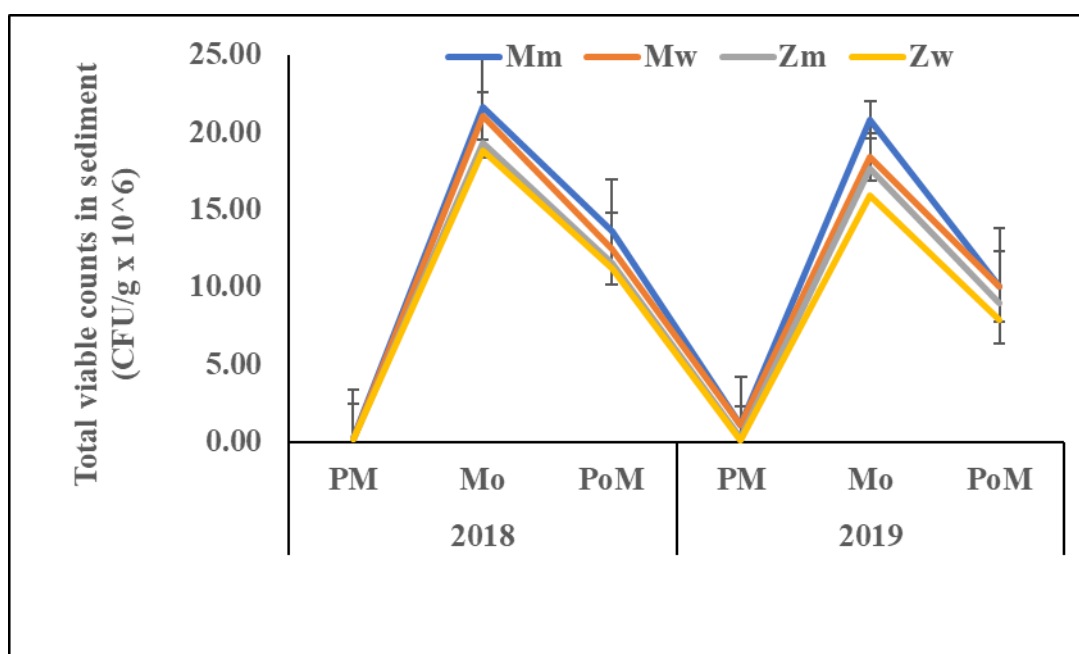


Fig. 4.1. b. Variations in total viable counts of bacteria in sediment during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

4.3.2. Spatio-temporal variation of total bacterial abundance

The TBA_w (TBA in water) in M, ranged from $3.84 (\pm 2.1)$ to $9.47 (\pm 1.2) \times 10^6$ cells/ml and in Z it varied from $3.49 (\pm 2.0)$ to $8.32 (\pm 1.0) \times 10^6$ cells/ml (**Fig. 4.2. a.**). While TBA_s (TBA in sediment) varied from $8.21 (\pm 3.3)$ to $21.89 (\pm 3.1) \times 10^9$ cells/g in M and $7.91 (\pm 2.0)$ to $18.38 (\pm 3.1) \times 10^9$ cells/g in Z (**Fig. 4.2. b.**). In both the estuaries, mangrove sites (M_m , Z_m) showed higher TBA than estuarine sites (M_w , Z_w). Season-wise comparison revealed that TBA_w and TBA_s was highest in the Mo followed by PoM and PM in both M and Z. Overall, higher TBA was observed in M in comparison to Z.

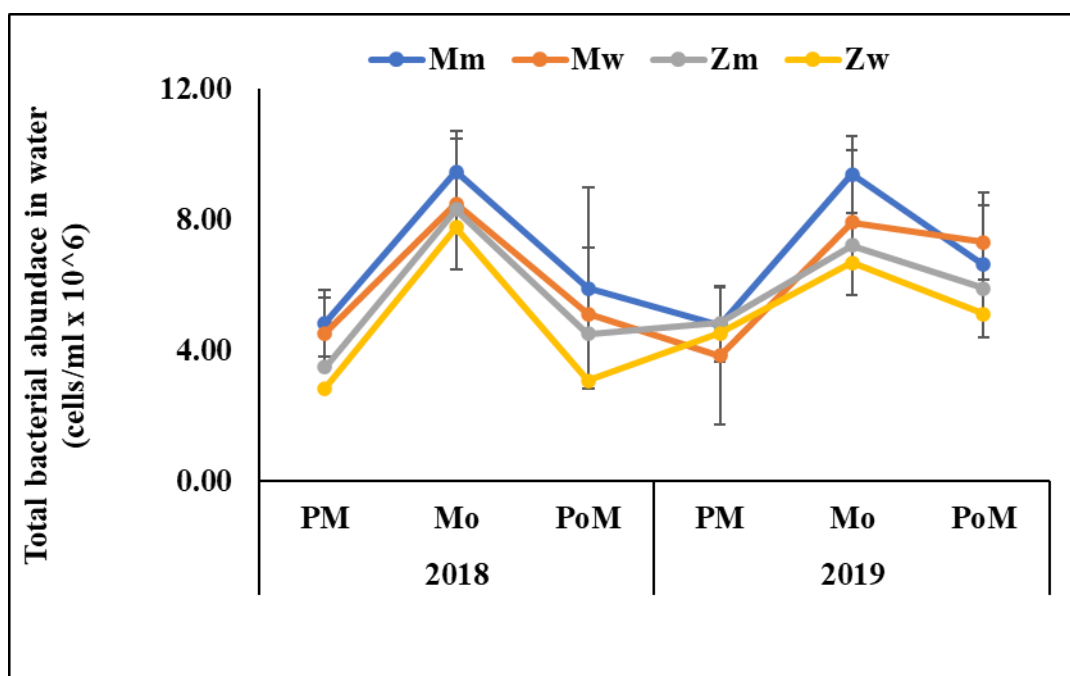


Fig. 4.2. a. Variations in total bacterial abundance of water during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

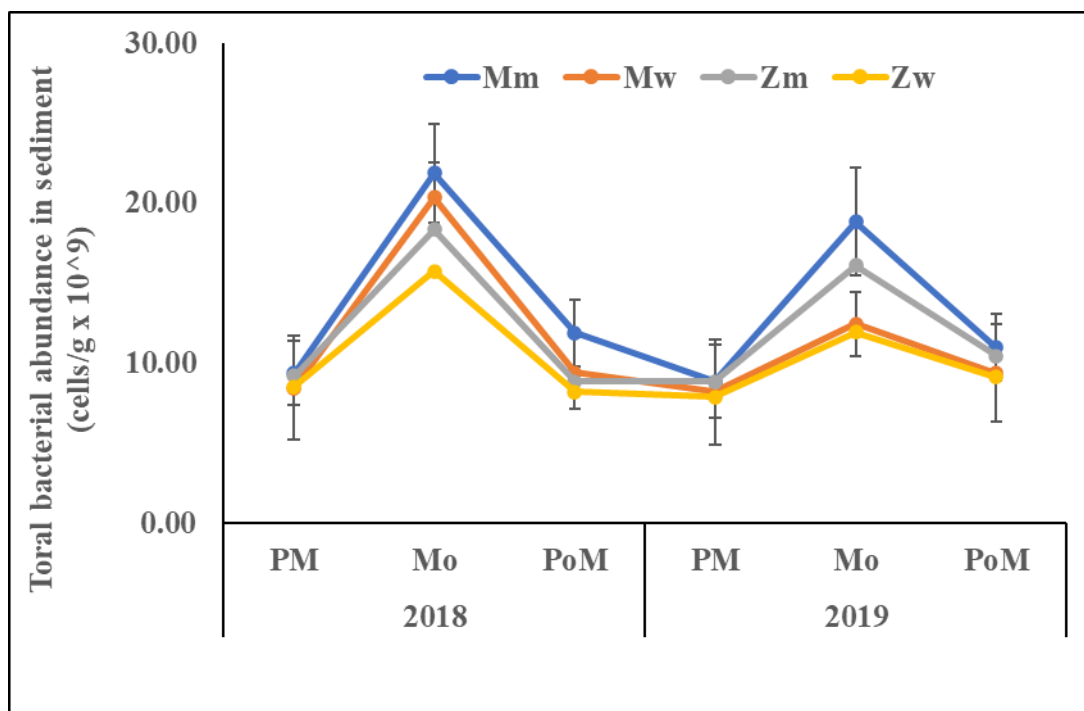


Fig. 4.2. b. Variations in total bacterial abundance of sediment during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) seasons in the Mandovi and Zuari estuaries (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

4.3.3. Spatio-temporal variation of bacterial diversity

Based on morphological characteristics, 649 bacteria were isolated from M and Z belonging to three major phyla i.e., *Proteobacteria*, *Actinobacteria*, and *Firmicutes*. Phylum *Proteobacteria* (58.71 %) was predominant followed by *Firmicutes* (40.37 %) and *Actinobacteria* (0.92 %). All the isolates were categorized under five classes namely, *Gammaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Bacilli* and *Clostridia*. *Gammaproteobacteria* was the most abundant ranging from 27.27 % - 75.86 % in M_m and 33.33 % - 65.38 % in M_w (**Fig. 4.3. a.**). Its seasonal abundance followed the order PM > Mo > PoM in M_m and M_w. In Z, *Gammaproteobacteria* was consistently found throughout all sampling seasons, comprising 38.46 % to 87.10 % in Z_m and 30.77 % to 81.82 % in Z_w, with the highest abundance in PM, followed by PoM and Mo (**Fig. 4.3. b.**). The abundance of *Bacilli* ranged between 22.22 % - 59.09 % in M_m (Mo > PoM > PM) and 34.62 % - 66.67 %

in M_w ($PoM > Mo > PM$). In Z_m , the abundance of *Bacilli* was between 58.97 % - 51.85 % ($Mo > PoM > PM$) while in Z_w it was 69.23 % - 18.18 % ($Mo > PM > PoM$).

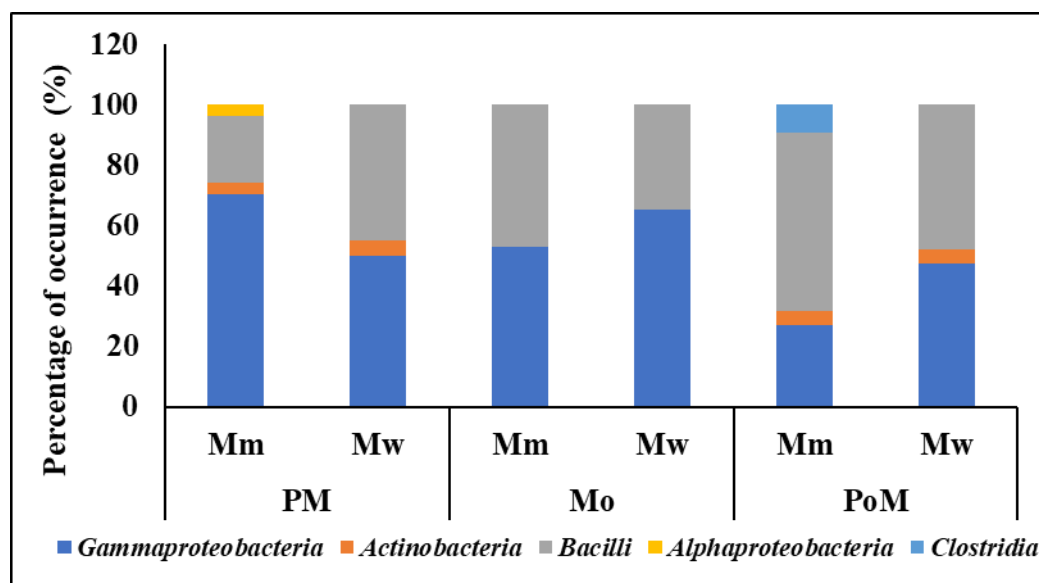


Fig. 4.3. a. Variation in the percentage occurrence of bacterial classes in Mandovi during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water)

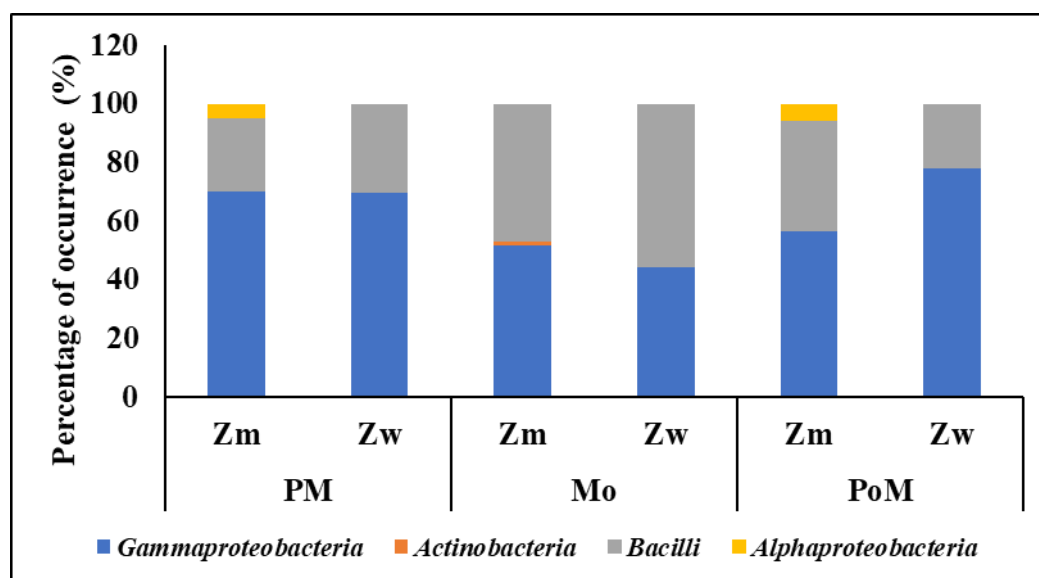


Fig. 4.3. b. Variation in the percentage occurrence of bacterial classes in Zuari during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) in the Mandovi and Zuari estuaries. (Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

In M_m , *Actinobacteria* ranged from 3.70 % to 4.55 %, whereas in M_w it ranged from 4.76 % to 5.00 %. *Actinobacteria* was solely present in Z_m during Mo (2.56 %). *Alphaproteobacteria* were observed exclusively during PM in M_w , constituting 4%, and *Clostridia* was only detected during PoM in M_m , accounting for 9.09 %. In Z_m , other classes of bacteria like *Alphaproteobacteria* were found during PM (9.68 %) and PoM (11.54 %), while *Clostridia* were absent in Z.

A total of 31 different bacterial genera were recorded from M and Z estuaries. During PM, M exhibited the highest number of 20 genera, whereas in Z, this peak was observed during Mo, with 25 genera (**Fig. 4.4. a, b, c.**). Within *Gammaproteobacteria*, a multitude of genera such as *Acinetobacter* sp., *Ferrimonas* sp., *Marinobacter* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Shewanella* sp., *Kangiella* sp., *Aeromonas* sp., *Alteromonas* sp., *Klebsiella* sp., *Idiomarina* sp., *Kosakonia* sp., *Enterobacter* sp., *Psychrobacter* sp., *Pantoea* sp., and *Vibrio* sp. were found from this study. *Alphaproteobacteria* was represented by the genera *Marimonas* sp. and *Dermacoccus* sp.. *Microbacterium* sp., and *Curtobacterium* sp. were the genera belonging to *Actinobacteria* found in this study. *Bacillus* sp., *Paenibacillus* sp., *Staphylococcus* sp., *Exiguobacterium* sp., *Bhargavaea* sp., *Virgibacillus* sp., *Oceanobacillus* sp., *Solibacillus* sp., and *Lysinibacillus* sp. were among the genera represented by *Bacilli*. *Clostridia* was represented by a single genus, *Paraclostridium* sp., which was found only in M_m . Across all three seasons, *Vibrio* sp., *Pseudomonas* sp., and *Bacillus* sp. were consistently found in both M and Z. Other genera found exclusively in M were *Paracoccus* sp., *Dermacoccus* sp., *Paenibacillus* sp., *Microbacterium* sp., *Idiomarina* sp., *Virgibacillus* sp., *Pantoea* sp. and *Paraclostridium* spp while *Kosakonia* sp., *Psychrobacter* sp., *Oceanobacillus* sp. and *Enterobacter* sp. were restricted to Z. During the Mo season, *Alteromonas* sp. and *Bhargavaea* sp. were exclusively identified in M_w and Z_m , *Klebsiella* sp. in M_w and Z_w , and *Solibacillus* sp. in Z_m and M_m .

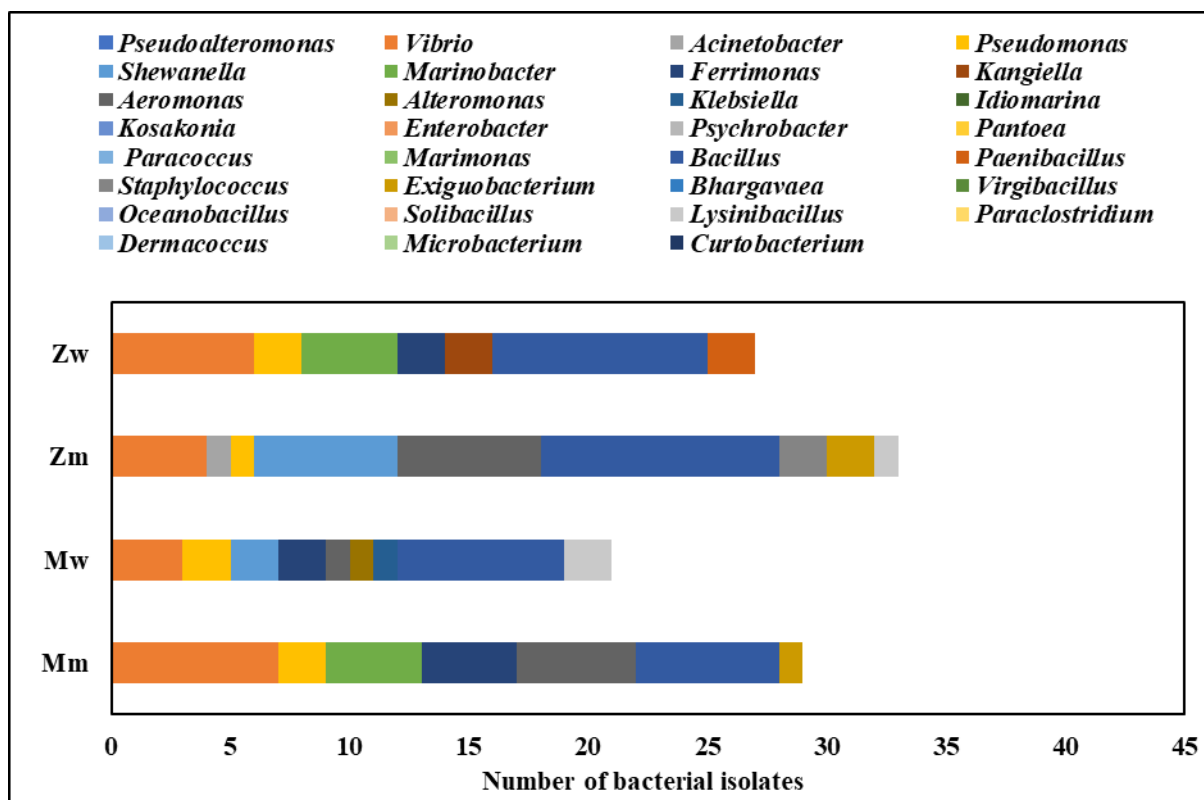


Fig. 4.4. a. Variation in the number of bacterial genera during Premonsoon (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

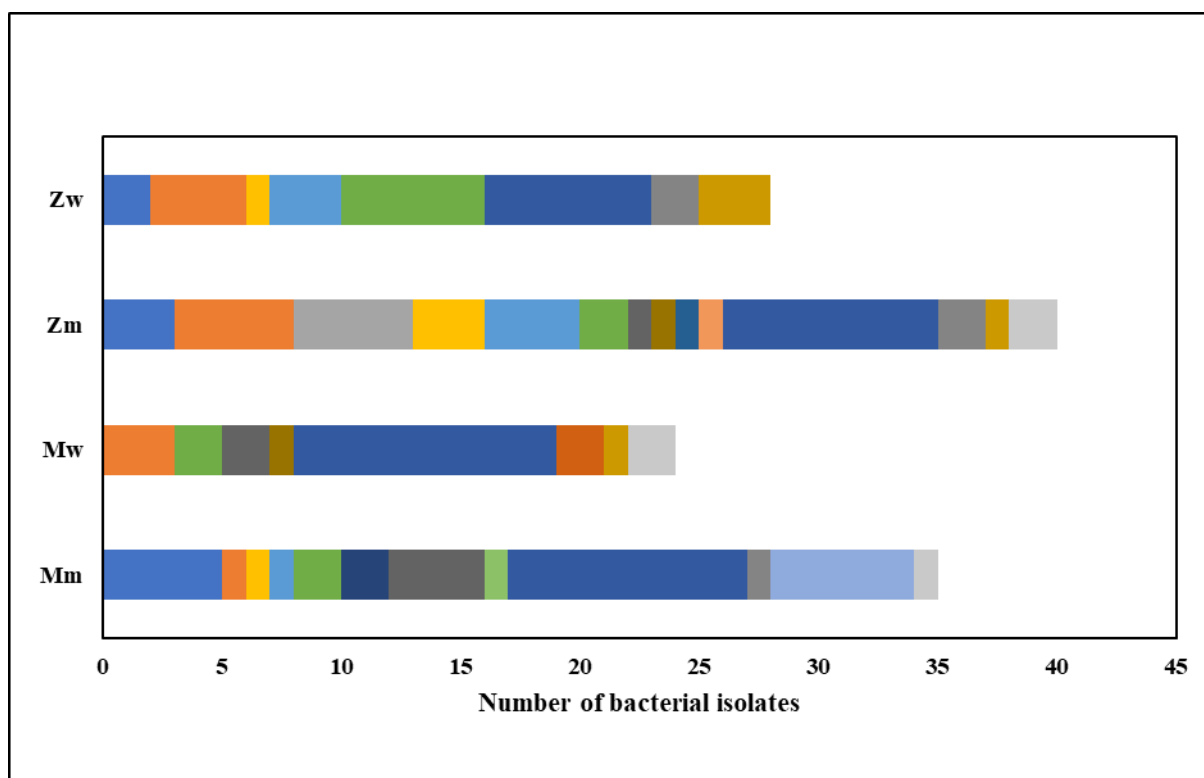


Fig. 4.4. b. Variation in the number of bacterial genera during Monsoon (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

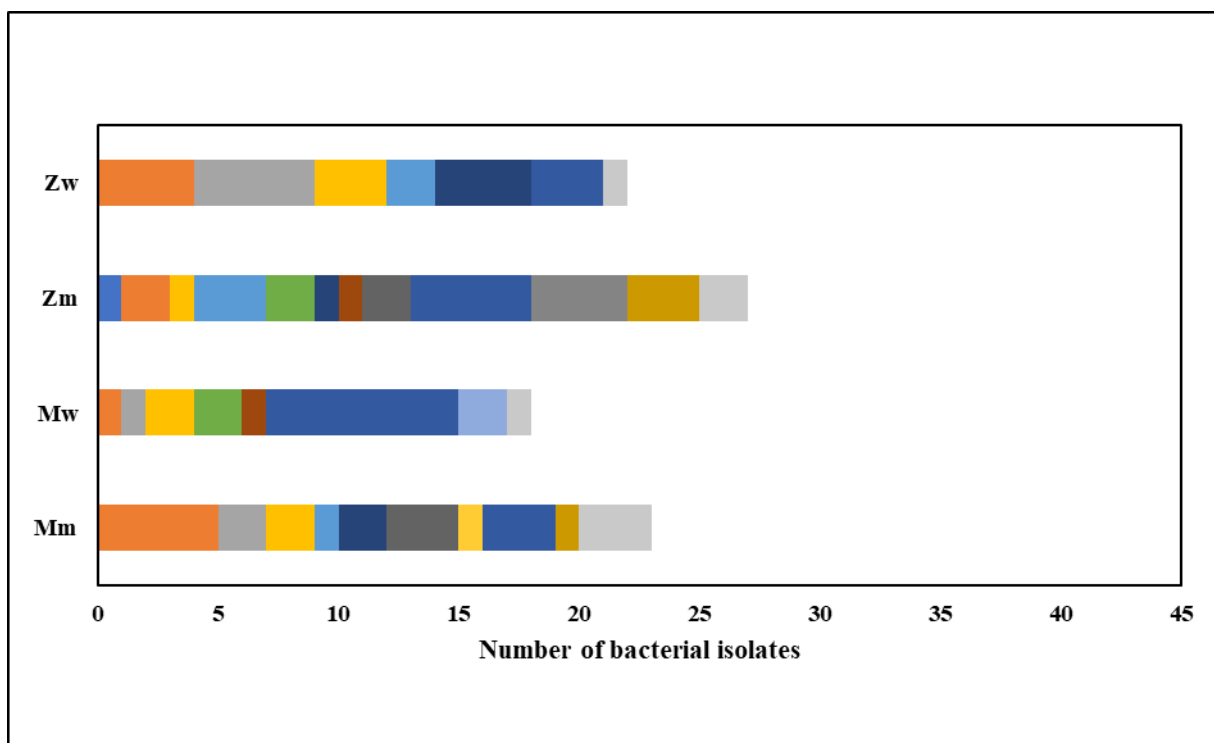


Fig. 4.4. c. Variation in the number of bacterial genera during Postmonsoon (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

4.3.4. NCBI Accession number

The acquired sequences are entered into the GenBank database with the accession numbers - MT585804-MT585812, MT588819-MT588844, MT588889-MT588907, MT588852-MT588862, MT590509-MT590525, MT590605-MT590615, MT590634-MT590655, MT590714-MT590727, MT591282-MT591298, MT591300-MT591305, MT591344-MT591358, MT597962-MT598003, MT597921-MT597961, MW534922-MW534984, OL310256-OL310287, OL314813-OL314837, OL314841-OL314877, OL321137-OL321171, OL336539-OL336604, OL336796-OL336852, OL339350-OL339369, OL357751-OL357776, OL377998-OL378023 and OL372635-OL372646.

4.3.5. Relationship between physicochemical parameters, bacterial abundance and diversity

Pearson's correlation coefficients revealed that during the PM season TVC_w , TVC_s , TBA_w , and TBA_s were negatively correlated to almost all the physicochemical parameters

except OM_w and OM_s (**Fig. 4.5. a.**). *Gammaproteobacteria* exhibited moderate positive correlations with temperature, salinity, pH, DO, NH₄⁺, PO₄³⁻, SiO₄⁴⁻, OM_s, TVC_w, and TBA_s. *Actinobacteria* showed strong negative correlations with salinity, NO₃⁻, NH₄⁺, SiO₄⁴⁻ and OM_s. *Bacilli* was strongly correlated to NO₃⁻ and OM_w. *Alphaproteobacteria* was strongly correlated to temperature and OM_s. The negative correlations of TVC_w, TVC_s, TBA_w, and TBA_s to all the physicochemical parameters were contrasted by strong positive correlations to OM_w, and OM_s were retained during the Mo season (**Fig. 4.5. b.**). *Gammaproteobacteria* showed a significant negative correlation to pH, salinity, SiO₄⁴⁻ and OM_w; *Actinobacteria* did not exhibit significant correlations to most of the parameters, and *Bacilli* was positively associated with pH and salinity while being negatively associated with DO. During PoM, TVC_w, TVC_s, TBA_w, and TBA_s showed either positive or no correlation to temperature; negative correlations to the remaining physicochemical parameters; and positive correlation with OM_w and OM_s (**Fig. 4.5. c.**). *Gammaproteobacteria* was positively influenced by salinity, NO₂⁻, NH₄⁺ and SiO₄⁴⁻ whereas it was negatively influenced by temperature and DO. *Actinobacteria* was positively correlated to salinity, NO₃⁻ and OM_s; negatively correlated to DO, NO₂⁻, PO₄³⁻ and SiO₄⁴⁻. *Bacilli* exhibited significant negative correlations to NO₂⁻, NO₃⁻, PO₄³⁻ and SiO₄⁴⁻; *Alphaproteobacteria* was positively correlated to PO₄³⁻; and *Clostridia* showed mild positive correlations with PO₄³⁻ and OM_s.

The PCA biplot reveals the relationships between different environmental parameters and bacterial groups across the monsoonal seasons (**Fig. 4.6.**). The distinct clustering of sample points by season indicates distinct seasonal patterns in bacterial community composition and environmental conditions. Notably, *Gammaproteobacteria* showed a strong positive correlation with NO₃⁻ and SiO₄⁴⁻, indicating their preference for these conditions. *Alphaproteobacteria* align closely with temperature and PO₄³⁻, while *Actinobacteria*, *Bacilli*,

Clostridia are clustered in the center, suggesting niche differentiation among these bacterial groups.

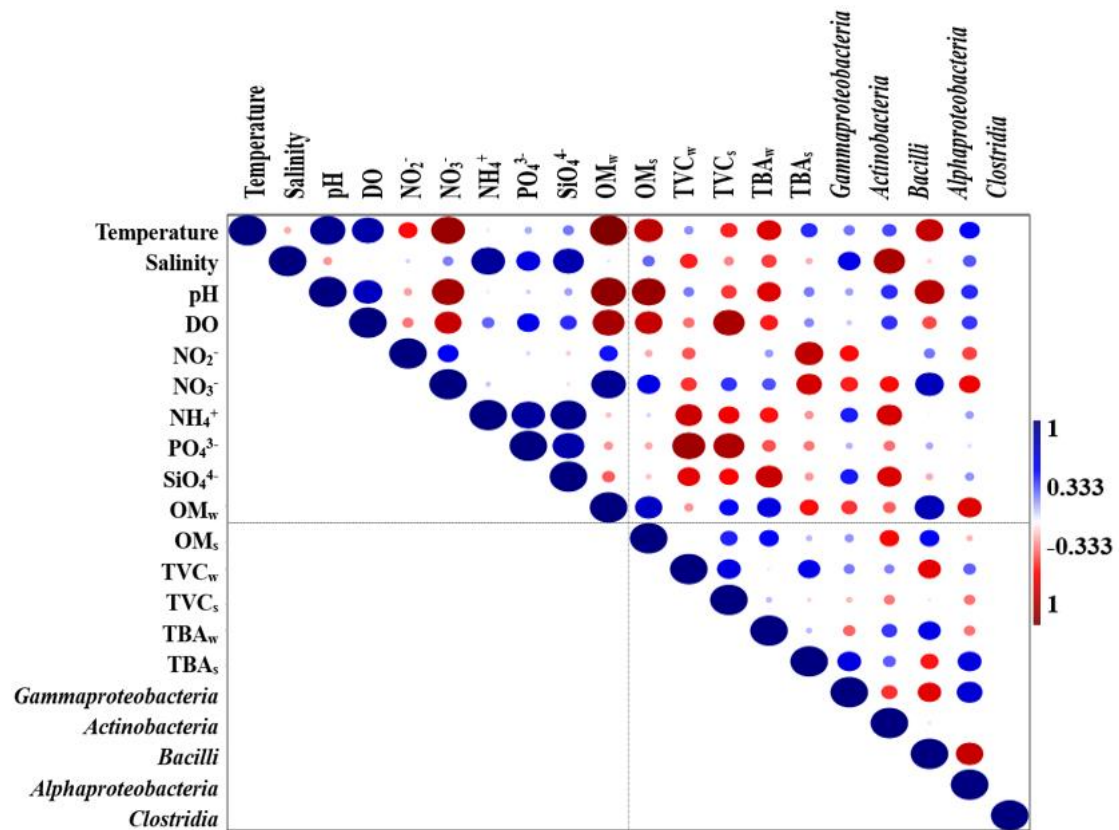


Fig. 4.5. a. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial counts (TBC) and bacterial classes in the Mandovi and Zuari estuaries during Premonsoon.

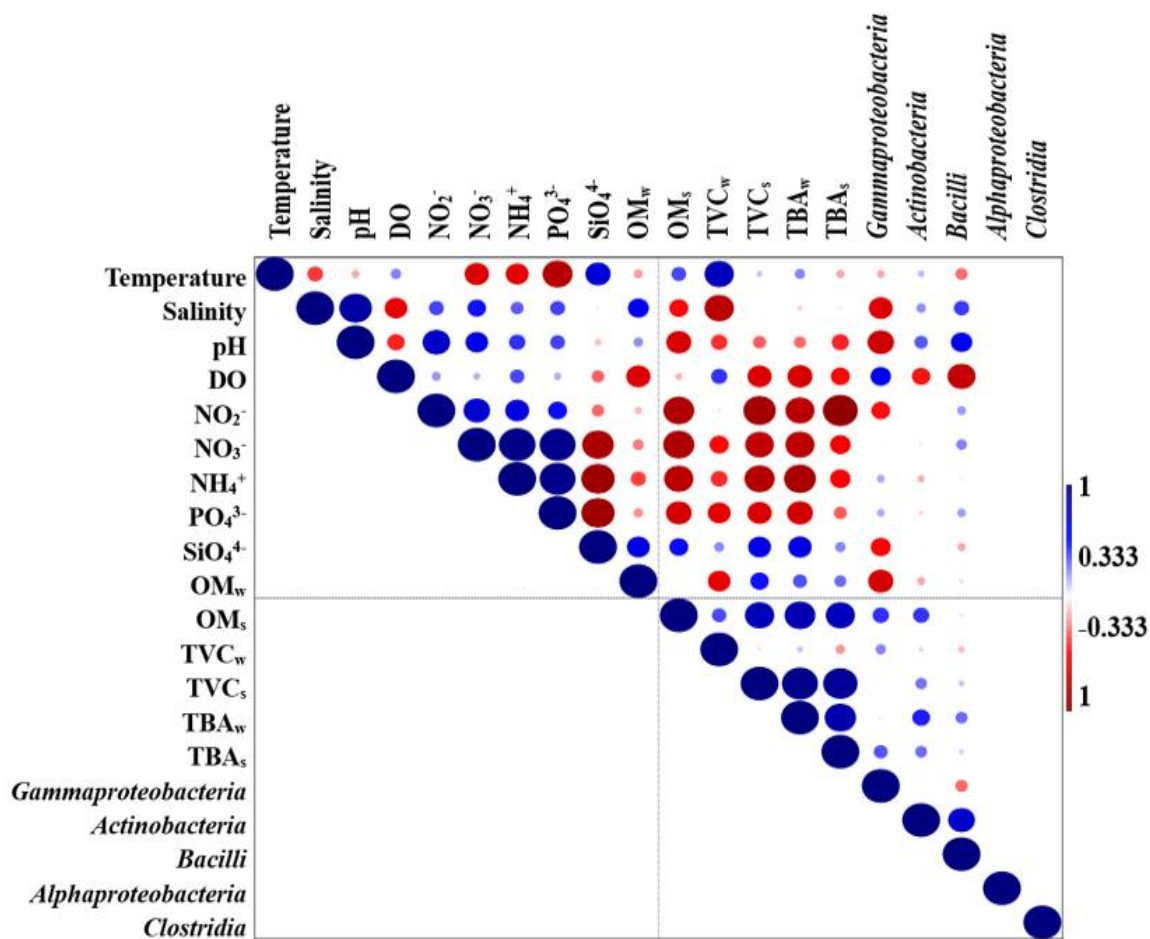


Fig. 4.5. b. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial counts (TBC) and bacterial classes in the Mandovi and Zuari estuaries during Monsoon.

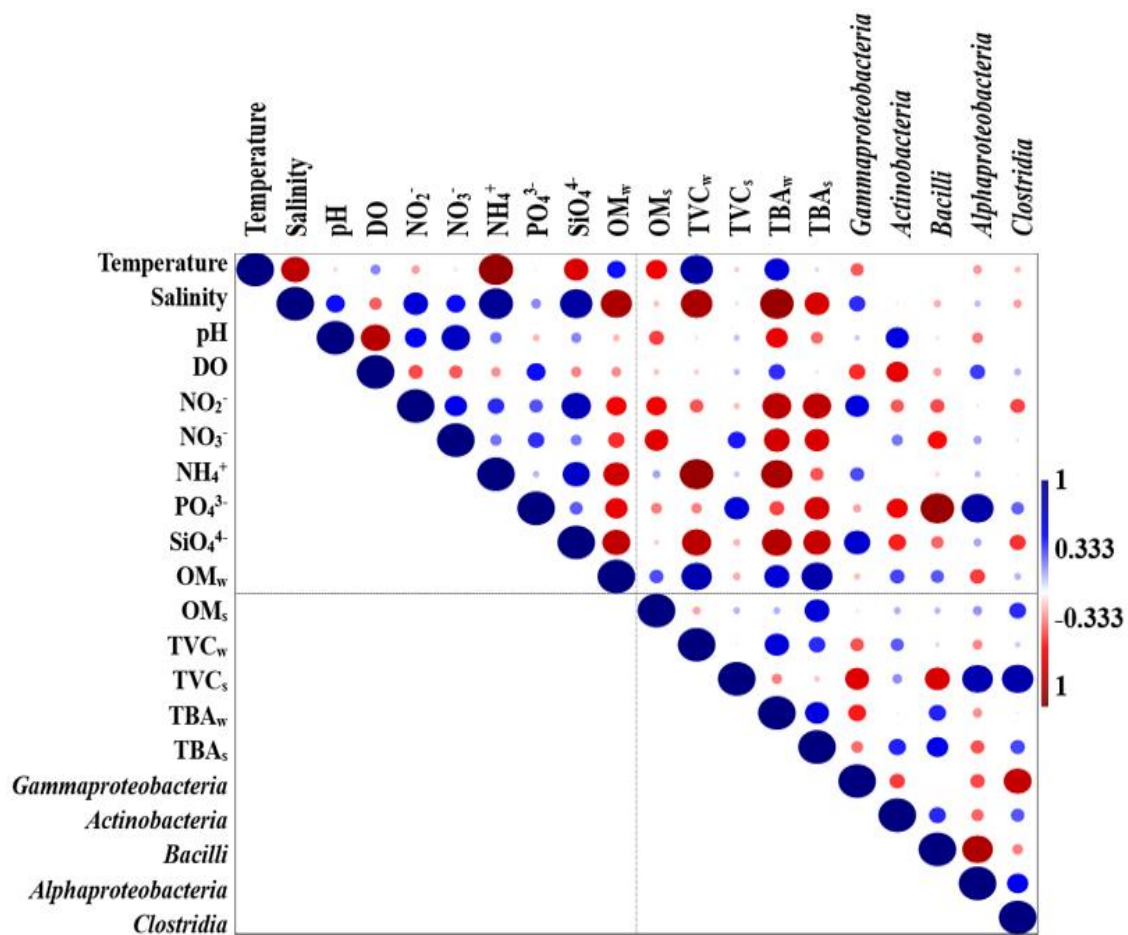


Fig. 4.5. c. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial counts (TBC) and bacterial classes in the Mandovi and Zuari estuaries during Postmonsoon.

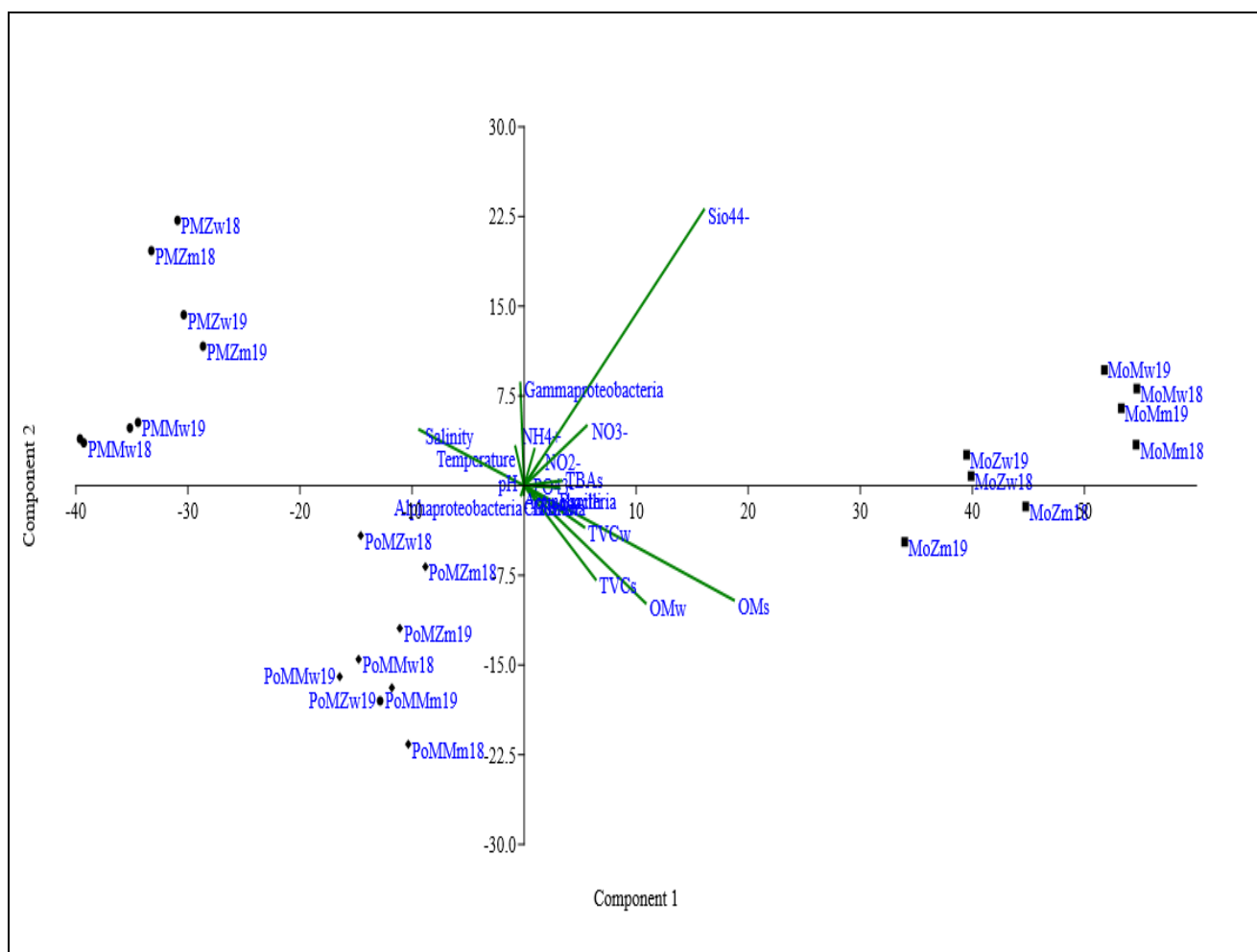


Fig. 4.6. Principal component analysis (PCA) biplot of the mean PC scores of each physicochemical and biological parameter as vectors, together with the PC scores of each season.

4.4. Discussion

In the present study, the TVC and TBA obtained from the M and Z revealed significant variations in bacterial abundance across different seasons, with peak abundances occurring during the Mo period. This finding aligns with the previous studies conducted in the M and Z by Rodrigues et al. (2011) and Khandeparkar et al. (2017). The variations in TVC and TBA may be attributed to factors such as changes in rainfall patterns, temperature, humidity, and nutrient availability, highlighting the dynamic response of bacterial communities to seasonal environmental changes. In addition, mangrove sites (M_m , Z_m) exhibited higher TVC and TBA than estuarine sites (M_w , Z_w) suggesting that the elevated bacterial populations in mangrove

ecosystems result from the abundance of OM, facilitating their robust growth and reproduction (Kristensen et al. 2008).

Our findings further illustrate that *Proteobacteria* was the predominant phylum, consistent with earlier studies conducted in mangrove areas such as M and Z (Halder and Nazareth 2018; Khandeparker et al. 2017), the Sunderbans (Basak et al. 2016), Bhitarkanika mangroves (Ranjan et al. 2012), Paranaguá Bay, Brazil (Ceccon et al. 2019), and Beilun estuary, China (Wu et al. 2016). Previously, Ghosh et al. (2010) reported the dominance of different members of *Proteobacteria*, with special reference to *Gammaproteobacteria* from the Sundarbans mangrove. *Gammaproteobacteria* are reportedly the most active bacteria during the degradation of fresh particulate OM (Moitinho et al. 2018), actively participating in C and N cycling (Lin et al. 2019). The genera belonging to *Gammaproteobacteria* from our study have been reported to utilize hydrocarbons and non-hydrocarbon substrates as a source of energy playing an important role in OM mineralization in mangrove ecosystems (McGenity 2019). Additionally, they partake in atmospheric nitrogen fixation, phosphate solubilization, sulphur reduction and oxidation (Pester et al. 2012, Yousuf et al. 2014, Noguiera et al. 2015). *Alphaproteobacteria* was represented by the genera *Marimonas* sp. and *Dermacoccus* sp. which are known to utilise low-molecular weight dissolved OM such as amino acids, protein, glucose and acetyl-glucosamine (Cottrell and Kirchman 2000; Malmstrom et al. 2005; Yokokawa and Nagata 2010).

Firmicutes, known for their role in decomposing complex OM in mangrove environments, have been consistently reported in various studies (Ghosh and Bhadury 2018; Tong et al. 2019; Parab et al. 2020). *Firmicutes* are known to play a role in the reduction of sulphate and nitrogen, as well as in the production of methane (Alzubaidy et al. 2016; Rampadarath et al. 2018). *Bacilli* play a critical role in the transfer of OM from mangrove detritus to higher consumers via the transformation of lignocellulosic detritus (Behera et al.

2014). Members of the class *Bacilli* are also reported to play a vital role in cellulose-based plant litter decomposition, resulting in the production of simple-sugar derivatives (Soares-Júnior et al. 2013); phosphate solubilization (Vazquez et al. 2000); and basic metabolic processes such as photosynthesis, respiration, and ion transport (Nathan and Ammini 2019). An interesting observation was that *Vibrio* sp. which are involved in nutrient cycling was previously reported to be limited only to the M mangroves (Haldar and Nazareth 2018). However, in our study *Vibrio* sp. were isolated from Z mangroves during all three seasons. Higher number of *Vibrio* sp. were detected in M (> 60 %) than in Z, indicating greater anthropogenic input and pollution in M. Another indication of greater human impact in M from our study was the exclusive presence of *Paraclostridium* sp., which has been associated with urban mangrove forests exposed to high levels of anthropogenic hydrocarbon inputs (Marcial Gomes et al. 2008). This genus representing the class *Clostridia* has been reported to be responsible for the anaerobic breakdown of OM (Lu et al. 2006).

Actinobacteria have been documented in numerous mangrove environments (Lee et al. 2014), aligning with the results of our study. *Actinobacteria* are involved in the production of biologically active chemicals and play a key role in the turnover of complex biopolymers such as lignocellulose, hemicellulose, pectin, keratin, and chitin (Govindasamy et al. 2011). Besides, they are important in the breakdown of hydrocarbons and cellulose (Harwati et al. 2007), metal oxidation (Johnson et al. 2009), and nitrate reduction (Van Keulen et al. 2005).

The observed correlations confirmed that the bacterial communities are influenced by seasonal environmental conditions. PM conditions, with higher temperatures and salinity, favor bacteria adapted to these environments, while increased OM and nutrient availability during Mo season promote specific bacterial communities. PoM conditions, characterized by lower temperatures and reduced nutrients, create selective pressures favoring resilient bacteria. The PCA biplot highlights these interactions, showing strong associations of

Gammaproteobacteria with nutrient-rich conditions, *Alphaproteobacteria* with warmer temperatures and phosphate-rich environments, and *Actinobacteria*, *Bacilli*, and *Clostridia* with roles in OM decomposition and nutrient cycling. Seasonal clustering of samples highlights the dynamic nature of mangrove ecosystems, where bacterial composition shifts with environmental changes, providing insights into their ecological roles and responses. Thus, understanding the specific environmental factors driving bacterial community composition can help predict how mangrove ecosystems will respond to future changes, such as climate variability or human disturbances. This knowledge is crucial for conservation efforts and sustainable management of these valuable coastal habitats.

4.5. Summary

The present study emphasizes the critical role of bacterial diversity in driving enzymatic activities responsible for OM degradation in mangrove ecosystems, demonstrating the profound effects of seasonal variability in rainfall, temperature, and salinity. Seasonal variations significantly influenced bacterial abundance, with peak levels observed during the Mo period, demonstrating the dynamic response of bacterial communities to environmental changes. Mangrove sites (M_m , Z_m) exhibited higher TVC and TBA compared to estuarine sites (M_w , Z_w), attributed to the abundance of OM facilitating bacterial growth and activity. *Proteobacteria* emerged as the predominant phylum, actively participating in carbon and nitrogen cycling, while *Firmicutes* and *Actinobacteria* also play essential roles in the decomposition of complex OM. The bacterial communities were classified into five classes: *Gammaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Bacilli*, and *Clostridia*. Seasonal clustering and PCA biplots highlighted the dynamic nature of mangrove ecosystems, where bacterial composition shifts with environmental changes, providing insights into their ecological roles and responses. Understanding these environmental drivers is crucial for the conservation and sustainable management of mangrove ecosystems amidst

climate variability and human disturbances. The functional diversity and adaptability of these bacterial communities play crucial roles in nutrient cycling, carbon sequestration, and overall ecosystem functioning, enhancing our understanding of bacterial-mediated OM cycling in coastal ecosystems.

Chapter 5

Productivity and Respiration Dynamics:

Phytoplankton vs. Bacteria

in Varied Monsoonal Conditions

1. Introduction

Tropical mangroves are carbon (C) rich ecosystems that play a pivotal role in enhancing productivity in adjacent waters by facilitating the transfer of organic C and nutrients to higher trophic levels (Odum 1968; Abrantes et al. 2015). This transfer is mediated by heterotrophic bacteria which are the main consumers of organic C and nutrients responsible for the majority of the global marine metabolism by serving as the link between dissolved organic carbon (DOC) and higher trophic levels (Azam et al. 1983; Pomeroy 1984). This link is sustained by bacteria through utilization of DOC for biomass production (i.e., bacterial production, BP) or by respiration of organic C to CO₂ (i.e., bacterial respiration, BR). The amount of C used for BP versus the total amount consumed for metabolism (both BP and BR) is referred to as bacterial growth efficiency (BGE) (del Giorgio and Cole 1998). BGE directly impacts the microbial food web which in turn, influences the phytoplankton-derived organic matter (Danger et al. 2007). The CO₂ production, the transfer of C to higher trophic levels as well as the export and storage of C, are all significantly impacted by this (García-Martín et al. 2019). Thus, the relationship between bacterial parameters (BR and BP) and phytoplankton productivity are prerequisite to determine the trophic status of the system (Thottathil et al. 2008a).

5.2. Materials and Methods

5.2.1. Determination of productivity and respiration

5.2.1.1. Dissolved organic carbon

Water samples were filtered using precombusted 0.45 µm GF/F filter papers, acidified with 0.2 M HCl until the pH reached 2, and stored in precombusted amber glass bottles at 4°C (Ray et al. 2018).

5.2.1.2. Phytoplankton abundance

Chlorophyll *a* (Chl *a*) was measured using the Fluorometer (Turner 7200-000). Water sample (1 L) was filtered through 0.7 μm GF/F Whatman filter paper, pigments were extracted in 90 % acetone (UNESCO 1994), and fluorescence was measured using a fluorometer (Turner 7200-000). A standard, Chl *a* (Sigma) was used to calibrate the fluorometer.

5.2.1.3. Primary productivity

Primary productivity (PP) was measured using the ^{14}C assimilation method (Strickland and Parsons 1972). Light and dark bottles (in duplicate) for each study location, were washed with 10 % HCl and filled with 300 ml water samples from every site. Labeled $\text{NaH}^{14}\text{CO}_3$ (Specific activity: 5 $\mu\text{Ci ml}^{-1}$; Bhabha Atomic Research Centre, Mumbai, India) was added to each bottle and incubated at *in situ* temperature for 12 hrs. After incubation, water samples were immediately filtered through a 0.7 μm filter (Whatman) under diffused light and low pumping pressure (< 50 mm Hg). The filters were then exposed to conc. HCl for 1 min [to remove radiolabeled dissolved inorganic carbon (DI^{14}C)] and placed in scintillation vials. Scintillation cocktail in dioxane (Spectrochem, Mumbai, India) was added right before the radioactivity was measured in a liquid scintillation counter (PerkinElmer Wallac 1409 DSA, USA).

5.2.1.4. Bacterial productivity

Bacterial productivity was determined by the incorporation of titrated thymidine (^3H -TdR) into bacterial DNA (Fuhrman and Azam 1982). Water samples were filtered through a 0.7 μm GF/F filter (Whatman) at a low differential pressure (< 50 mm Hg) to avoid rupture of fragile cells. Control (in duplicate) and water samples (in duplicate) of 20 ml were set up for each study site. Water samples were incubated with ^3H thymidine (^3H -TdR) (Specific activity: 18 Ci mmol^{-1} , Bhabha Atomic Research Centre) in situ conditions for 1 h (dark) and

arrested by adding 2 % formalin. The activity of the control was immediately terminated after the addition of ^3H thymidine (^3H -TdR) with 2 % formalin. The samples were filtered through a 0.22 μm cellulose nitrate filter [presoaked with 5 % trichloroacetic acid (TCA)], extracted in cold 5 % TCA, and rinsed with 80 % ethanol. The dried filters were placed in scintillation vials with dioxane-based scintillation cocktail (Spectrochem, Mumbai, India) and radioactivity was measured using a liquid scintillation counter (PerkinElmer Wallac 1409 DSA, USA). BP, calculated as mol TdR incorporated into DNA, was converted into the number of bacterial cells produced by applying a conversion factor (1.96×10^{18} cells mol^{-1}) (Pradeep Ram et al. 2007) and to carbon by using a conversion factor of 2×10^{-14} g C cell^{-1} (Lee and Fuhrman 1987).

5.2.1.5. Community respiration

Community respiration is the consumption of oxygen by the planktonic population when incubated in the dark for 24 hrs and is estimated by Winkler's titration method (Carignan et al. 2000). Water sample (125 ml) was collected in duplicates in biological oxygen demand bottles, fixed immediately with Winkler's reagents and the initial DO (D1) was measured. Another duplicate set of BOD bottles (125 ml) containing water samples was incubated at in situ temperature in the dark for 24 hrs, fixed with Winkler's reagent, and DO (D2) was measured. The total respiration was calculated as, $D1 - D2$. The oxygen consumed was converted to carbon respired by assuming a respiratory quotient of 1 (Biddanda et al. 1994).

5.2.1.6. Bacterial respiration

Bacterial respiration was estimated by filtering water samples through 0.7 μm GF/F Whatman filter paper, following which the samples were siphoned carefully into the BOD bottles (125 ml) to avoid the formation of air bubbles. For each site, controls were performed in duplicates and the initial DO (D1) concentration was measured by Winkler's method.

Another set of duplicate bottles was incubated at in situ temperature in the dark for 24 hrs, fixed with Winkler's reagent, and DO (D2) was measured. The calculations for bacterial respiration are described in section 5.2.1.5.

5.2.1.7. Bacterial growth efficiency

Bacterial growth efficiency (BGE) was derived as the slope of bacterial production vs the sum of bacterial production and bacterial respiration, and expressed as a percentage (Moran and Hodson 1990).

$$\text{BGE (\%)} = [\text{BP} / (\text{BP} + \text{BR})] \times 100$$

5.2.2. Statistical analysis

The statistical software IBM® SPSS 23.0 was used to determine the statistical significance and each result was stated in mean $\pm$ standard deviation (SD). For reduction in the number of inter-correlated variables, principal components analysis (PCA) was performed with nutrients, DOC, Chl *a*, TBA, PP, BP, CR, BR, and BGE. A PCA biplot based on correlation was obtained by combining the sample scores plot and the vector loading plots. One-way analysis of variance (ANOVA) was used to identify significant variations between the physicochemical and biological parameters. Subsequently, post hoc Tukey's honestly significant difference (HSD) analysis compared estuarine and mangrove waters within Mandovi (M) and Zuari (Z) for each season to determine the mutual significance of interactions between the variables.

5.3. Results

5.3.1. Spatio-temporal variation of Dissolved organic carbon (DOC)

DOC concentration in M varied from 4.92 ($\pm$ 0.04) to 40.53 ($\pm$ 0.03) mg/L and in Z it ranged from 3.39 ($\pm$ 0.01) to 40.01 ($\pm$ 0.01) mg/L (**Fig. 5.1.**). Higher concentrations of DOC were observed in mangrove waters than in estuarine waters in both M and Z estuaries. The maximum DOC concentrations were recorded during Mo, followed by PoM and PM in both

the estuaries. DOC concentrations were consistently higher in M than in Z, during all seasons.

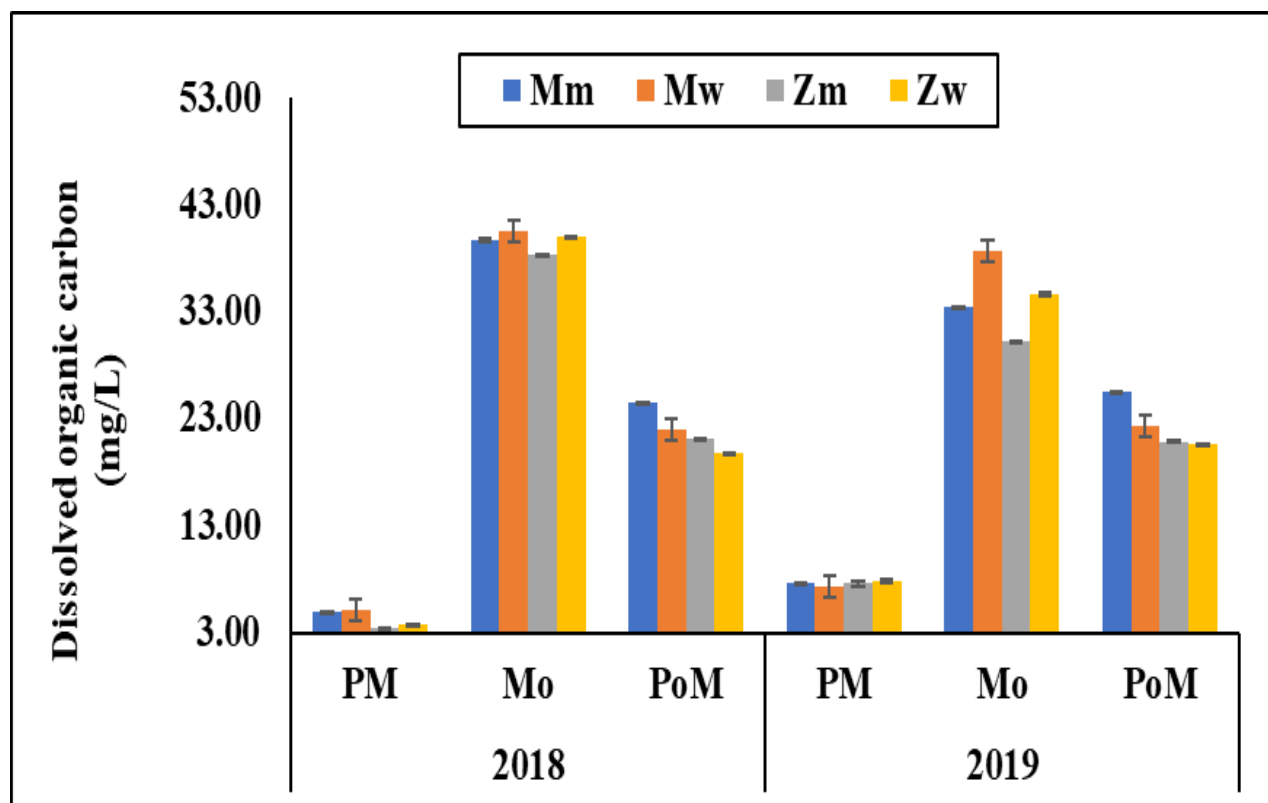


Fig. 5.1. Spatio-temporal variation of Dissolved organic carbon during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters)

5.3.2. Spatio-temporal variation of Phytoplankton abundance

Chl *a* concentration ranged between 1.19 (± 0.02) to 5.08 (± 0.09) $\mu\text{g/L}$ in M and 1.10 (± 0.02) to 9.34 (± 0.02) $\mu\text{g/L}$ in Z (**Fig. 5.2.**). In both the estuaries, the highest Chl *a* concentration, were recorded during PM, followed by Mo and PoM. The difference in Chl *a* concentration between the mangrove and estuarine waters of both M and Z was insignificant.

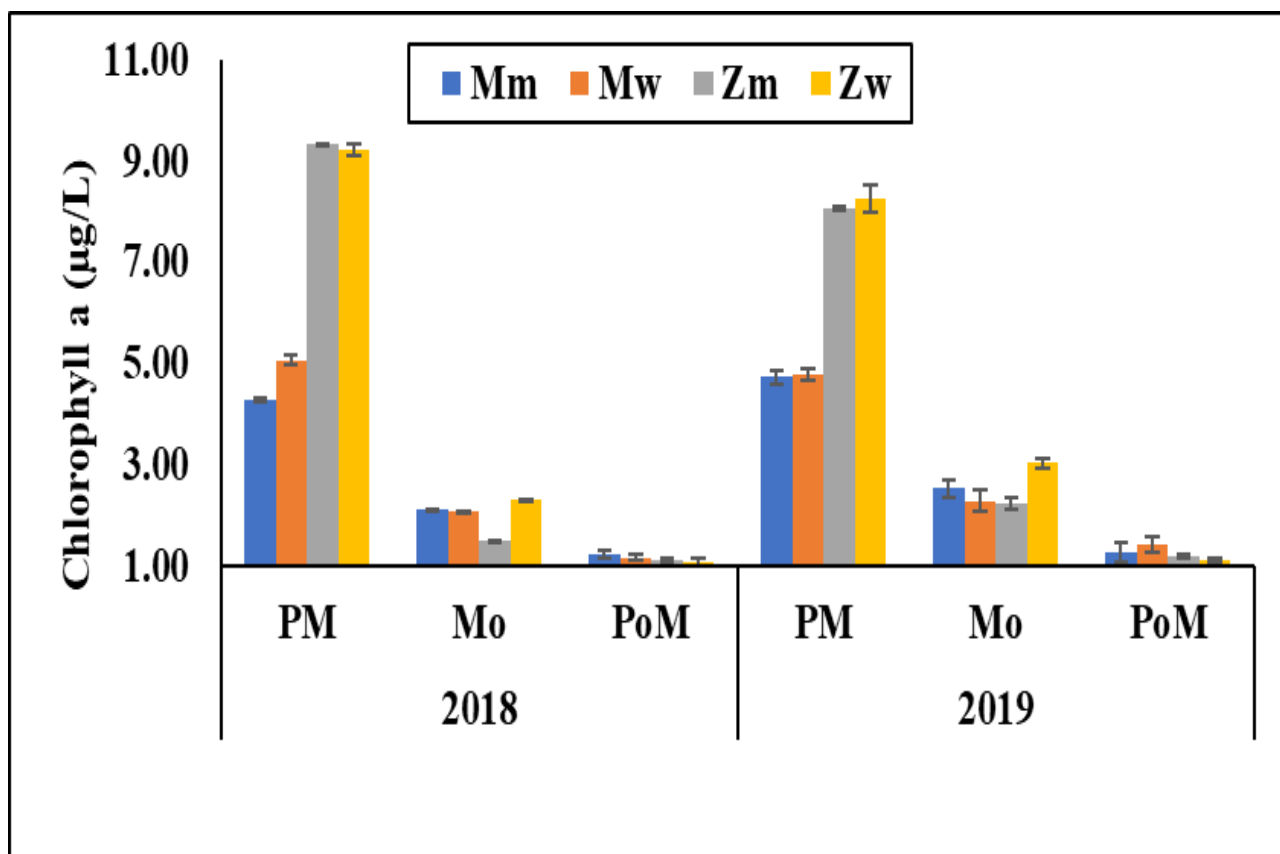


Fig. 5.2. Spatio-temporal variation of Chlorophyll *a* during premonsoon (PM), monsoon (Mo) and postmonsoon (PoM) (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters)

5.3.3. Spatio-temporal variation of Primary productivity (PP) and Bacterial productivity (BP)

Within M, PP fluctuated between $2.55 (\pm 1.22)$ to $17.64 (\pm 8.92) \mu\text{g C L}^{-1} \text{h}^{-1}$ while in Z it ranged from $2.56 (\pm 2.22)$ to $17.33 (\pm 5.66) \mu\text{g C L}^{-1} \text{h}^{-1}$ (**Fig. 5.3. a., b.**). In both M and Z, the highest PP was recorded during PM and the lowest during PoM.

In M, BP ranged from $5.79 (\pm 1.90)$ to $32.54 (\pm 8.22) \mu\text{g C L}^{-1} \text{h}^{-1}$ whereas in Z it varied from $8.62 (\pm 6.34)$ to $32.82 (\pm 2.87) \mu\text{g C L}^{-1} \text{h}^{-1}$ (**Fig. 5.4. a., b.**). In general, BP in estuarine waters (M_w, Z_w) was higher than mangrove waters (M_m, Z_m). Across both M and Z (mangrove and estuarine) waters, BP was the maximum during PM and minimum during PoM.

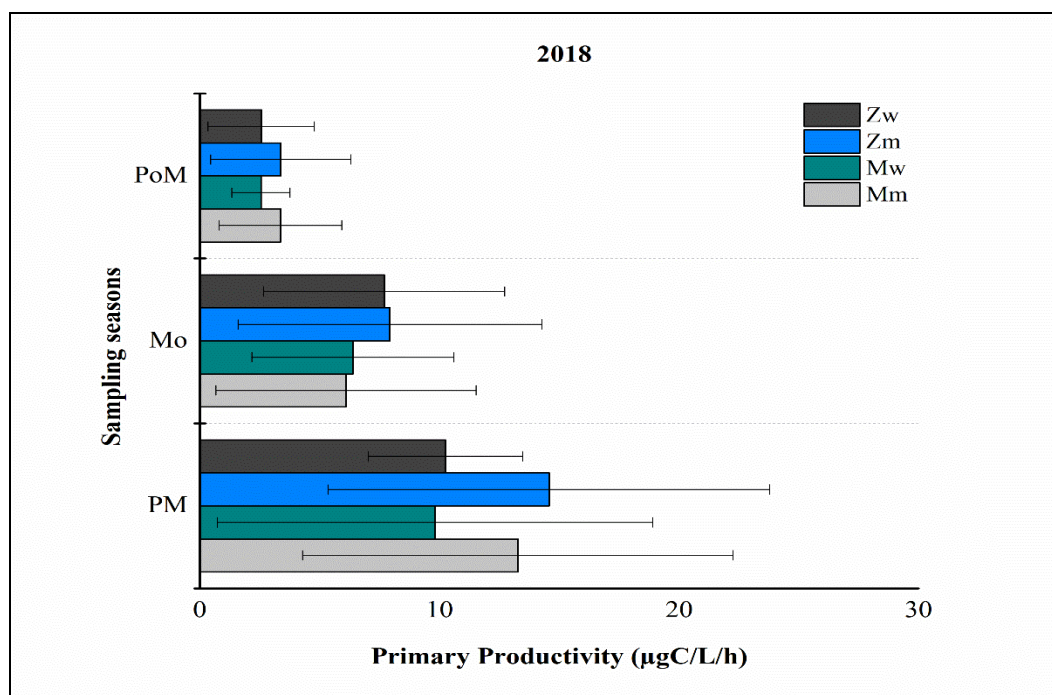


Fig. 5.3. a. Spatio-temporal variation of Primary productivity in the Mandovi and Zuari estuaries during 2018. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

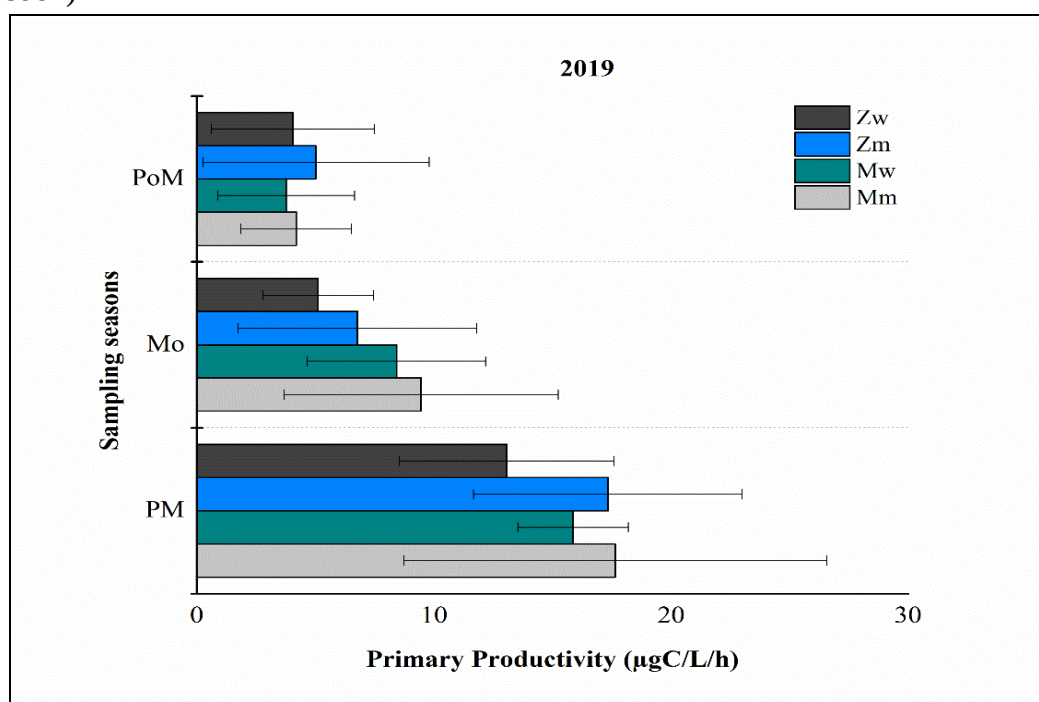


Fig. 5.3. b. Spatio-temporal variation of Primary productivity in the Mandovi and Zuari estuaries during 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

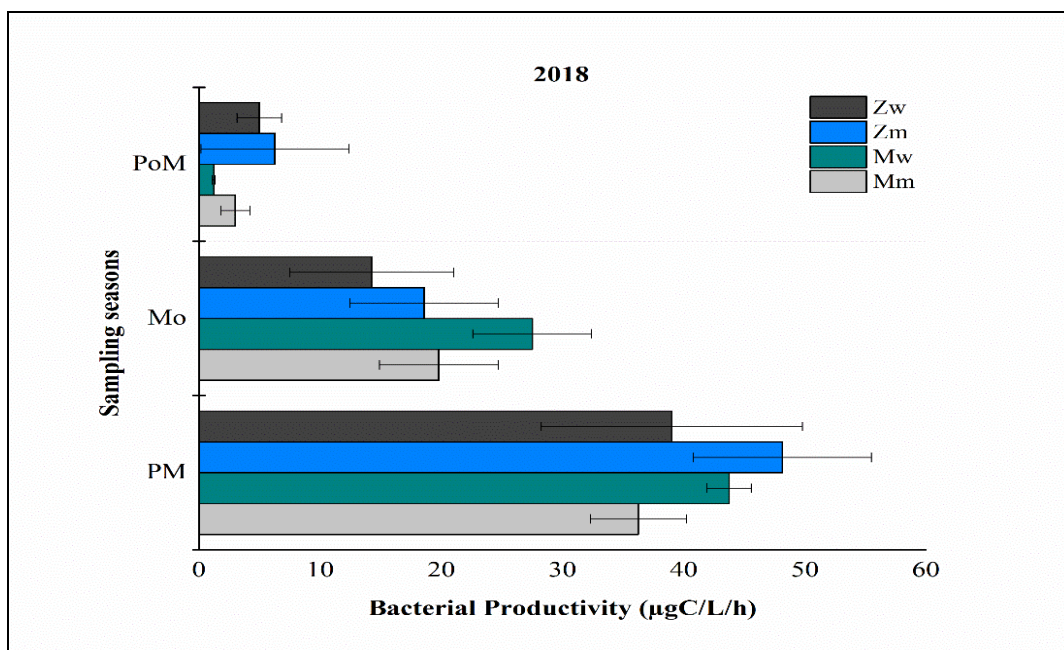


Fig. 5.4. a. Spatio-temporal variation of Bacterial productivity in the Mandovi and Zuari estuaries during 2018. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

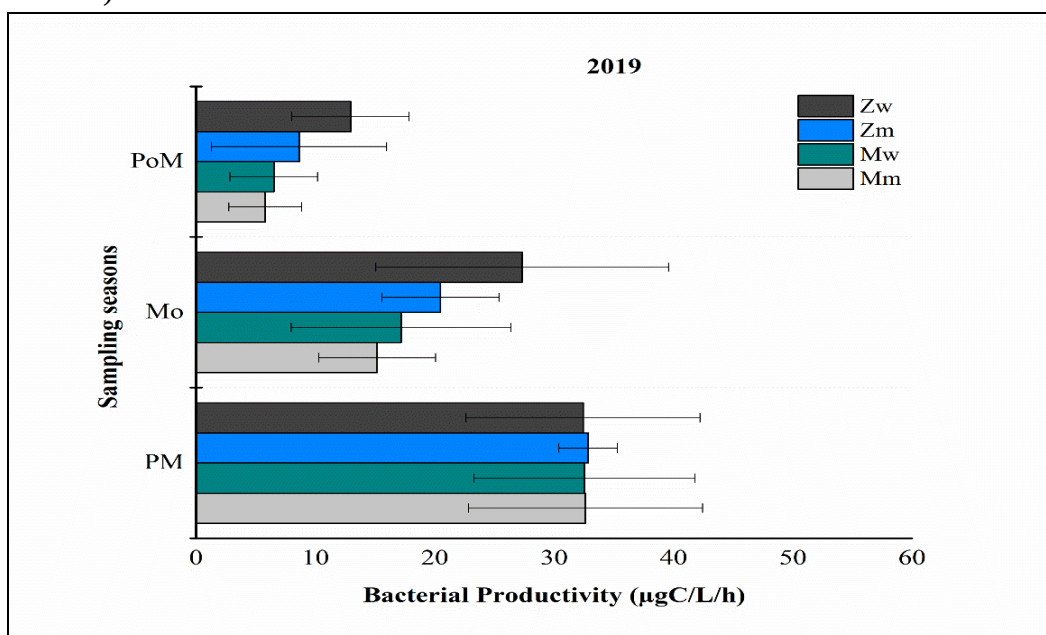


Fig. 5.4. b. Spatio-temporal variation of Bacterial productivity in the Mandovi and Zuari estuaries during 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

5.3.4. Spatio-temporal variation of Community respiration (CR) and Bacterial respiration (BR)

In M and Z, CR ranged from $16.95 (\pm 1.06)$ to $129.73 (\pm 2.37) \mu\text{g C L}^{-1} \text{h}^{-1}$ to $17.22 (\pm 0.95)$ to $207.72 (\pm 4.27) \mu\text{g C L}^{-1} \text{h}^{-1}$, respectively (**Fig. 5.5. a., b.**). The CR in the mangrove waters (M_m and Z_m) were higher than their respective estuarine waters (M_w and Z_w). In M, maximum values of CR were observed during PM and minimum during Mo. A similar pattern was observed in Z.

BR varied between $12.39 (\pm 0.82)$ to $159.62 (\pm 1.63) \mu\text{g C L}^{-1} \text{h}^{-1}$ in M and $13.00 (\pm 0.85)$ to $53.09 (\pm 2.69) \mu\text{g C L}^{-1} \text{h}^{-1}$ in Z (**Fig. 5.6. a., b.**). Higher BR were predominantly observed within M_m and Z_m in contrast to their corresponding estuarine sites (M_w and Z_w). Seasonally, BR in both M and Z was maximum during the PoM, followed by Mo and PM.

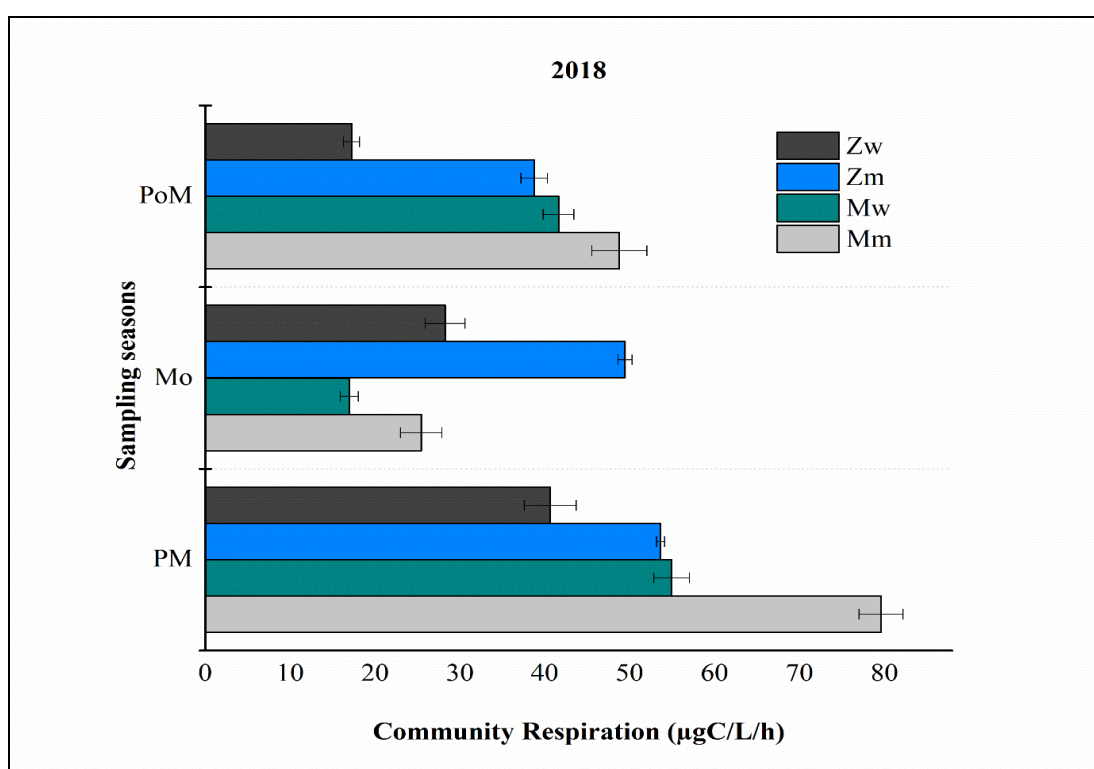


Fig. 5.5. a. Spatio-temporal variation of Community respiration in the Mandovi and Zuari estuaries during 2018. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM - Postmonsoon)

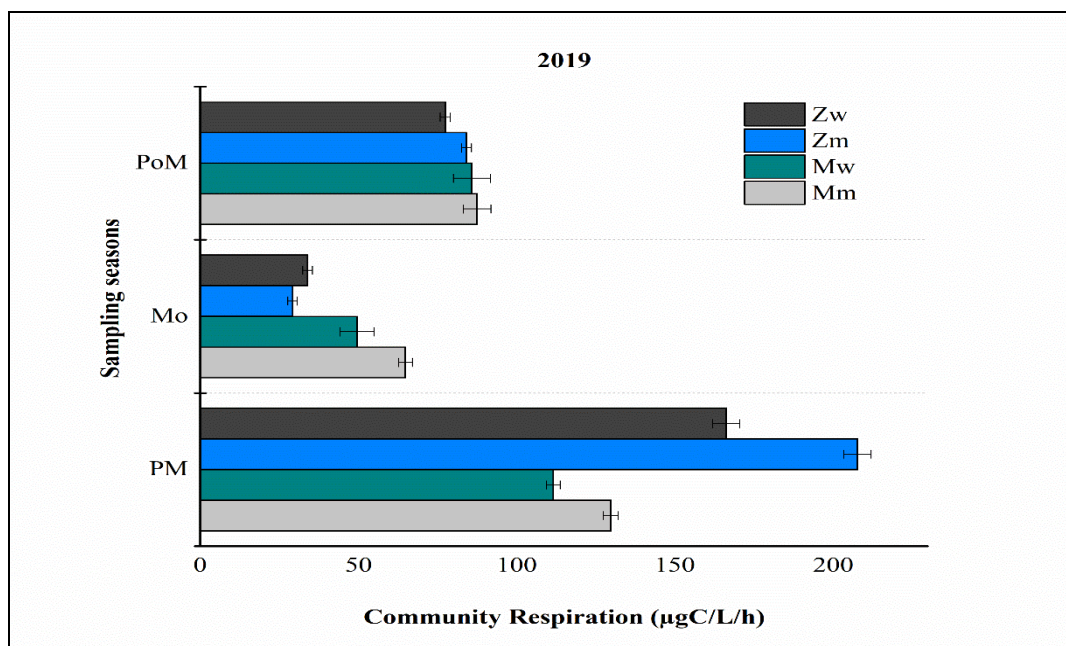


Fig. 5.5. b. Spatio-temporal variation of Community respiration in the Mandovi and Zuari estuaries during 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

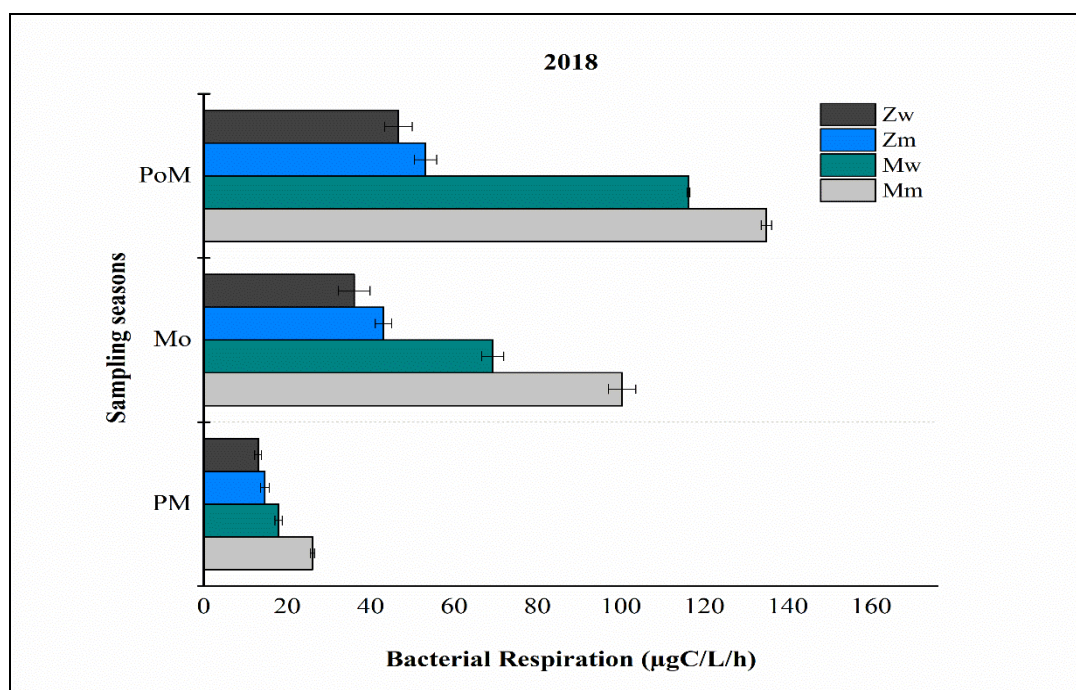


Fig. 5.6. a. Spatio-temporal variation of Bacterial respiration in the Mandovi and Zuari estuaries during 2018. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

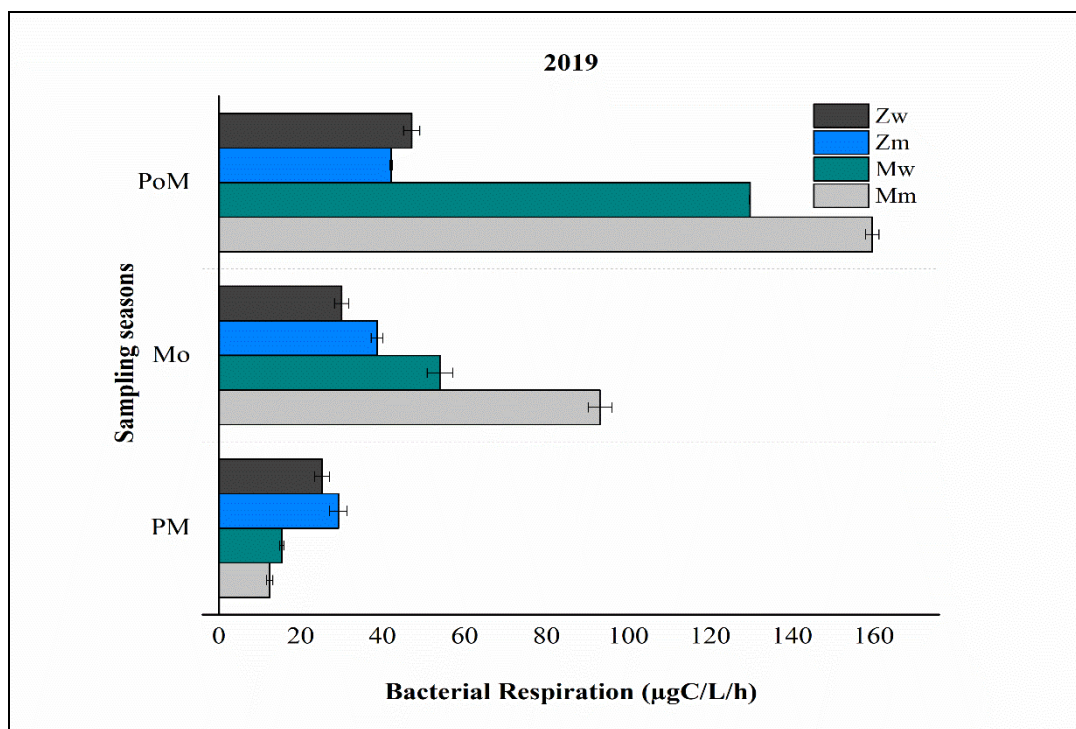


Fig. 5.6. b. Spatio-temporal variation of Bacterial respiration in the Mandovi and Zuari estuaries during 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM – Postmonsoon)

5.3.5. Spatio-temporal variation of BP/PP and PP/BR ratio

BP/PP ratios were > 1 across all sampling locations throughout the three seasons, except, during PoM (2018) in M_m (0.89) and M_w (0.48) (**Table 5.1.**). Higher ratios of BP/PP were observed in the M_w (average 2.51) and Z_w (average 3.10) than in M_m (average 1.95) and Z_m (average 2.36). Both the estuaries displayed their lowest BP/PP ratio was observed during PoM (average M – 1.21, Z – 2.18), with a steady increase in PM (average M – 2.77, Z – 2.87) and peaking during Mo (average M – 2.80, Z – 3.14).

With few exceptions, such as Z_m (PM, 2018) and M_m, M_w (PM, 2019), the PP/BR ratios recorded in all sampling sites were < 1 (**Table 5.1.**). The ratios of M_m (average 0.36), M_w (average 0.31), Z_m (average 0.36), and Z_w (average 0.31) across all seasons demonstrated a comparable trend. In both the estuaries, PP/BR ratios were lowest during PoM (average M – 0.03, Z – 0.08), with a gradual increase observed in Mo (average M – 0.10, Z – 0.19) and reaching maximum values during PM (average M – 0.88, Z – 0.72).

Table 5.1. Variation in BP/PP and PP/BR ratios in the Mandovi and Zuari estuaries during 2018 and 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM - Postmonsoon, PP - Primary productivity, BP - Bacterial productivity, BR - Bacterial respiration)

	2018				2019			
	BP/PP							
	M _m	M _w	Z _m	Z _w	M _m	M _w	Z _m	Z _w
PM	2.73	4.46	3.30	3.81	1.85	2.05	1.89	2.48
Mo	3.24	4.30	2.34	1.85	1.60	2.04	3.02	5.34
PoM	0.89	0.48	1.86	1.94	1.38	1.73	1.72	3.20
	PP/BR							
	M _m	M _w	Z _m	Z _w	M _m	M _w	Z _m	Z _w
PM	0.51	0.55	1.00	0.79	1.42	1.04	0.59	0.52
Mo	0.06	0.09	0.18	0.21	0.10	0.16	0.18	0.17
PoM	0.02	0.02	0.06	0.05	0.03	0.03	0.12	0.09

5.3.6. Spatio-temporal variation of Bacterial growth efficiency (BGE)

BGE displayed a wide range of variation from 1.04 % to 72.47 % in M and 9.62 % to 76.69 % in Z (**Table 5.2.**). In M, the BGE values in M_m (average 27.81 %) were typically lower than M_w (average 32.90 %) and in Z estuary, Z_w (average 39.75 %) displayed higher values than in Z_m (average 37.00 %). Seasonal fluctuations were observed, with the highest values of BGE recorded during the PM (average M – 68.11 %, Z – 65.24 %) and the lowest during the PoM (average M – 2.87 %, Z – 14.68 %) in both M and Z.

Table 5.2. Variation in BGE in the Mandovi and Zuari estuaries during 2018 and 2019. (M_m - Mandovi mangroves, M_w - Mandovi estuarine waters, Z_m - Zuari mangroves, Z_w - Zuari estuarine waters, PM - Premonsoon, Mo - Monsoon, PoM - Postmonsoon)

	2018				2019			
	BGE							
	M _m	M _w	Z _m	Z _w	M _m	M _w	Z _m	Z _w
PM	58.22	70.98	76.69	75.00	72.47	68.01	52.96	56.32
Mo	16.48	28.42	30.16	28.33	14.01	24.14	34.67	47.69
PoM	2.17	1.04	10.57	9.62	3.50	4.77	17.02	21.55

5.3.7. Relationship between physicochemical parameters and trophic status

Principal Component Analysis (PCA) was employed to discern the influence of seasonal variability on the estuarine and mangrove waters of M and Z. PCA analysis revealed that principal components 1 and 2 cumulatively represented 68.39 % of the total variability with eigenvalue >1 (**Fig. 5.7.**). Based on component 1 and 2, the PCA biplot was constructed. PCA projections revealed distinct clusters of the PM, Mo, and PoM seasons confirming that the seasonal variability had a significant influence on the estuarine and mangrove waters of Mandovi and Zuari. Notably, the primary drivers of seasonal variability were identified as fluctuations in salinity, dissolved inorganic nutrients, and DOC. The PM season exhibited heightened levels of temperature, salinity, pH, Chl *a*, PP, BP, and BGE. Conversely, the Mo season depicted elevated nutrient concentrations, DOC, BA, and BR, in contrast, the PoM season and CR displayed minimal susceptibility to variations among the different vectors. Analysis via one-way ANOVA revealed significant differences in physicochemical and biological parameters across the study locations, except for NO₃⁻ (**Table 5.3.**). Post hoc Tukey's HSD tests showed that nutrients, DOC, Chl *a*, and BA varied significantly ($p < 0.001$) between the estuarine and mangrove waters of both M and Z. However, the metabolic parameters such as PP, BP, BR, CR, and BGE did not exhibit significant variations (**Table 5.4.**).

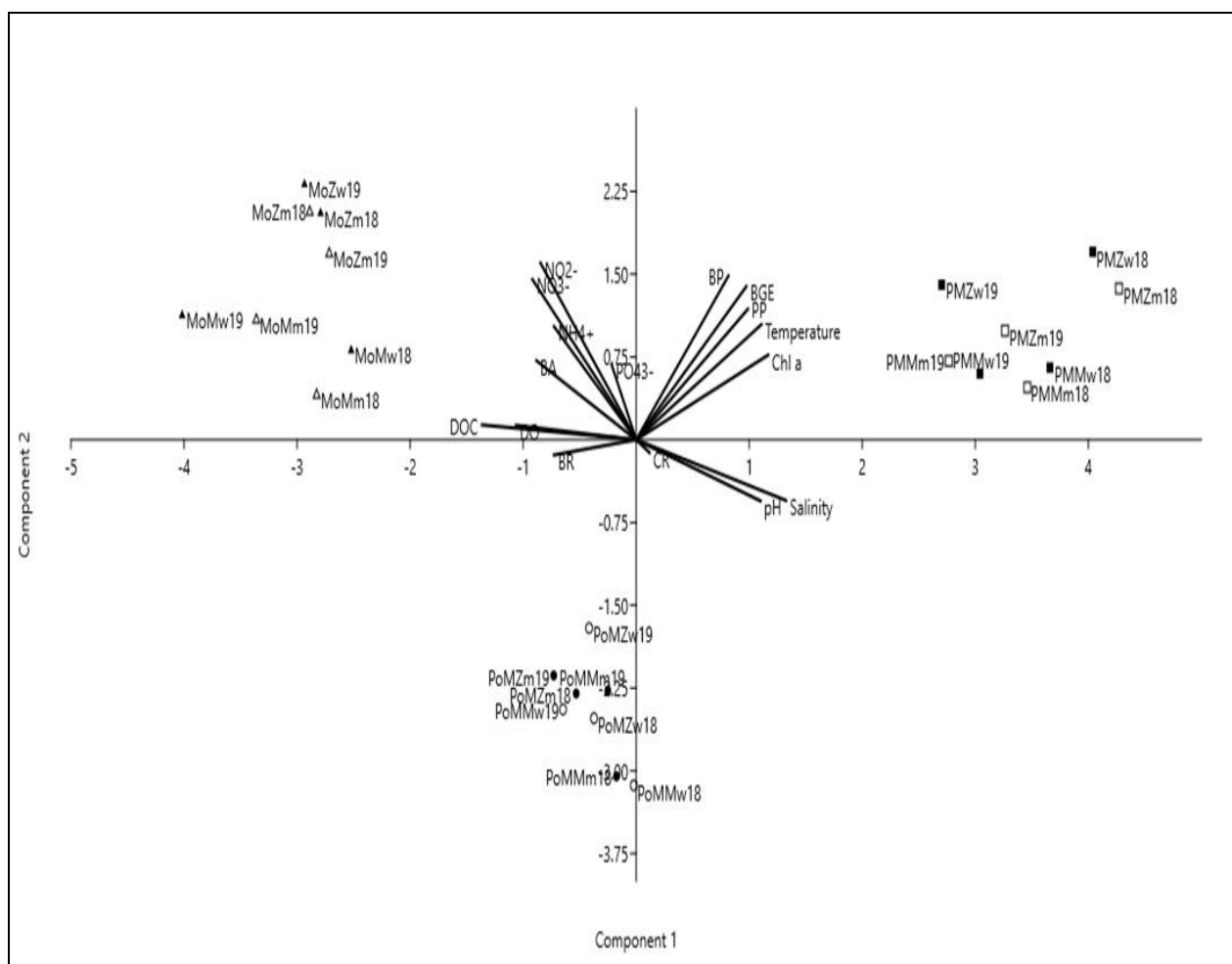


Fig. 5.7. Principal component analysis (PCA) biplot of the mean PC scores of each physicochemical and biological parameter as vectors, together with the PC scores of each season.

Table 5.3. Variation in physicochemical and biological parameters across the study sites and their significance ($p < 0.001$), analyzed by one-way ANOVA. (DO - Dissolved oxygen, Nitrite - NO_2^- , Nitrate - NO_3^- , Ammonia - NH_4^+ , Phosphate - PO_4^{3-} , DOC - Dissolved organic carbon, Chl *a* - Chlorophyll *a*, BA - Bacterial abundance, PP - Primary productivity, BP - Bacterial productivity, CR - Community respiration, BR - Bacterial respiration, BGE - Bacterial growth efficiency)

	F value	<i>p</i>
Temperature	143.91	<0.0001
Salinity	3960.59	<0.0001
pH	9.09	<0.0001
DO	118.24	<0.0001
NO_2^-	8004.93	<0.0001
NO_3^-	1.04	0.438
NH_4^+	12356.57	<0.0001
PO_4^{3-}	158842.70	<0.0001
DOC	100183.42	<0.0001
Chl <i>a</i>	1713.35	<0.0001
BA	2.32	0.007
PP	8.54	<0.0001
BP	13.44	<0.0001
CR	5.80	<0.0001
BR	4.99	<0.0001
BGE	4.953	<0.0001

Table 5.4. Post hoc Tukey's HSD test for physicochemical and biological parameters during premonsoon (PM), monsoon (M) and postmonsoon (PoM) seasons. Mean difference is significant at $p < 0.001$ level.

Dependent variables	Seasons				
	Location	Location	p (PM)	p (Mo)	p (PoM)
Temperature	M _m	M _w	0.99	0.99	0.13
	Z _m	Z _w	0.99	0.92	0.95
Salinity	M _m	M _w	<0.0001	0.12	1
	Z _m	Z _w	1	<0.001	<0.0001
pH	M _m	M _w	1	0.99	1
	Z _m	Z _w	0.99	0.99	0.99
DO	M _m	M _w	0.75	0.004	0.93
	Z _m	Z _w	1	0.50	0.93
NO ₂ ⁻	M _m	M _w	<0.0001	<0.0001	0.87
	Z _m	Z _w	<0.0001	<0.0001	0.27
NO ₃ ⁻	M _m	M _w	<0.0001	<0.0001	0.002
	Z _m	Z _w	<0.0001	<0.0001	<0.0001
NH ₄ ⁺	M _m	M _w	0.92	<0.0001	1
	Z _m	Z _w	<0.0001	<0.0001	0.006
PO ₄ ⁻³	M _m	M _w	<0.0001	0.03	0.63
	Z _m	Z _w	<0.0001	<0.0001	0.39
DOC	M _m	M _w	<0.0001	<0.0001	<0.0001
	Z _m	Z _w	<0.0001	0.001	<0.0001

Chl <i>a</i>	M _m	M _w	0.81	<0.0001	<0.0001
	Z _m	Z _w	<0.0001	<0.0001	<0.0001
BA	M _m	M _w	<0.0001	1	0.72
	Z _m	Z _w	1	0.002	0.001
PP	M _m	M _w	1	0.99	1
	Z _m	Z _w	1	1	0.99
BP	M _m	M _w	0.93	1	1
	Z _m	Z _w	0.44	1	1
CR	M _m	M _w	0.99	0.98	1
	Z _m	Z _w	0.99	1	1
BR	M _m	M _w	0.94	1	1
	Z _m	Z _w	1	0.99	1
BGE	M _m	M _w	1	0.95	1
	Z _m	Z _w	1	1	1

5.4. Discussion

Tropical mangrove-dominated estuaries are hotspots of biogeochemical activity sustained by high organic matter inputs from upstream rivers and impacted by several environmental parameters (Nittrouer et al. 1995; Brunskill 2010). Therefore, a comprehensive examination of the ambient environmental conditions is crucial for a holistic understanding of the ecosystem's processes and functioning. Variations in light intensity, temperature, and nutrient availability are known to greatly impact Chl *a* concentration (Perumal et al. 2009). The PM period, offers favorable conditions, such as low turbidity and

greater light penetration for phytoplankton growth. This aligns with our results, wherein the highest concentrations of Chl *a* were recorded during PM season in both estuaries.

In marine ecosystems, phytoplankton-derived carbon is considered a major carbon source for bacteria and thus, bacterial dynamics is tightly linked with phytoplankton (Cole et al. 1988; Ducklow 2000). However, the extent of this interdependence can exhibit variations influenced by temporal and spatial factors (Cho et al. 1994; Hyun and Kim 2003). Observations made in this study indicate that substantial organic matter influx from upstream waters and terrestrial runoff during Mo resulted in peak concentrations of DOC. These elevated DOC levels particularly impacted BA in both the M and Z estuaries. These findings align with previous studies by Rodrigues et al. (2011) and Khandeparker et al. (2017). Conversely, during PM and PoM periods, declining DOC concentrations led to a subsequent reduction in BA. The higher DOC and BA concentrations in M compared to Z were attributed to M receiving a greater runoff influx relative to Z (Qasim and Sengupta 1981). Moreover, increased DOC concentrations in M_m and Z_m were a consequence of additional organic matter inputs from decomposing mangrove leaf litter, actively fostering bacterial growth and reproduction (Kristensen et al. 2008).

The maximum PP in both the M and Z estuaries was observed during PM coinciding with optimal conditions that favored phytoplankton growth. With the onset of Mo, substantial nutrient influx from riverine sources altered the estuarine environment, resulting in increased turbidity and reduced light penetration. This environmental shift required phytoplankton to adapt to diminished nutrient availability (Devassy and Goes 1989), subsequently reducing their metabolic activity. Consistent seasonal variability in PP aligning with our findings was also reported by Thottathil et al. (2008a) in the Cochin estuary.

The balance between autotrophy and heterotrophy is crucial in determining how organic C is transferred to the higher trophic levels (Azam et al. 1983) where the BP/PP ratio

serves as a valuable indicator in assessing the trophic status (Thottathil et al. 2008b). When BP constitutes a substantial portion of PP ($BP/PP > 1$), it signifies a heterotrophic system subsidized by allochthonous C (Alongi 1989). Conversely, when $BP/PP < 1$, it indicates an autotrophic system relying on autochthonous (phytoplankton) C (Carlson and Ducklow 1996). Our study affirms the prior observations made by Ram et al. (2007) and Thottathil et al. (2008b), indicating a similar seasonal pattern in BP ($PM > Mo > PoM$). Predominantly heterotrophic ($BP/PP > 1$) ratios were consistently observed across all seasons in M and Z, with higher BP/PP ratios in the estuarine waters (M_w and Z_w) compared to their respective mangrove waters (M_m and Z_m). The low heterotrophic BP/PP ratios obtained during PoM are due to higher PP in comparison to BP. In contrast, the higher BP/PP ratios observed during the Mo and PM corresponded to relatively lower PP values and increased concentrations of DOC that stimulated BP (Revilla et al. 2000). Our results corroborate with a previous study by Ram et al. (2007) which documented lower heterotrophic ratios during PM and PoM in contrast to higher ratios observed during Mo. Furthermore, our study's recorded levels of DOC indicated an autotrophic system during PM, transitioning to a heterotrophic system during Mo and PoM. This observation is in line with the conclusions drawn by Prairie et al. (2002), who suggested that DOC concentrations below 4 to 6 mg/L indicate an autotrophic system. Additionally, the PCA analysis emphasized the substantial influence of seasonal variability on the trophic status in the estuarine and mangrove waters of M and Z. Furthermore, the autotrophic status of the PM was validated through PCA by the predominance of Chl *a*, PP, and CR; while loadings of nutrients, DO, DOC, BA, and BR were directed towards the heterotrophic status of Mo and PoM.

Respiration (community and bacterial) is an important C sink, making it vital for accurately estimating the C budget (Nguyen and Maranger 2011). High respiration rates are attributed to bacterial-mediated nitrification (Ram et al. 2007) and the presence of OM in the

water column (Bianchi et al. 2012). In our study, we observed that CR consistently exceeded PP, irrespective of the location and season, indicating heterotrophy similar to the reports of del Giorgio et al. (1997). Moreover, BR exceeds BP predominantly throughout the study period except during the PM in both estuaries. This indicates that the utilization of C for respiration is larger than its assimilation for bacterial biomass, affirming the role of respiration in determining the fate of bacterial C and influencing ecosystem productivity (Roland and Cole 1999; del Giorgio et al. 1997). The low ratios of PP/BR (< 1) during the Mo and PoM seasons are attributed to low PP values, as phytoplankton growth was restricted due to increased turbidity (Thottathil et al. 2008). Despite high phytoplankton biomass (Chl *a*) and PP values during the PM, the PP/BR ratios ranged between 0.51 to 1.42, suggesting that BR can occur concurrently with PP while being supported by external allochthonous carbon sources (del Giorgio et al. 1997). These findings are further supported by PCA which confirmed the interdependence of respiration and productivity parameters.

Bacterial growth efficiency refers to the amount of C substrates accessible to higher trophic levels (Azam 1998). The wide variability of BGE detected in our study during each season is attributed to a loose coupling between BP and BR, which may be due to the discrepancies in bacteria-mediated nitrification (Ram et al. 2007). BGE values from our study suggest that the PM season is the most favorable season for the transfer of bacterial C to the trophic food web. Low BGE aligned with elevated BR and increased concentrations of DOC, reaffirming that bacteria devote more energy to maintenance rather than metabolism (del Giorgio and Cole 1998). The average BGE reported from estuaries is 37 % with the prediction of a higher BGE in more productive ecosystems (del Giorgio and Cole 2000). Comparatively, our study indicated an average BGE of 30.35 % in M and 38.38 % in Z, implying that the Z estuary is potentially more productive than the M estuary. Additionally, higher BGE values in M_w and Z_w than in M_m and Z_m show that estuarine waters are more

productive than mangrove waters. The increase in BGE in the estuarine waters is likely a result of allochthonous C enhanced by inputs from mangroves. The increase in BGE in the estuarine waters is likely a result of allochthonous C enhanced by inputs from mangroves. Thus, the DOC derived from mangrove leaf litter decomposition acts as a substrate for in situ microbial activity, while concurrently, a fraction of this DOC is exported from the mangrove ecosystem to adjacent estuarine waters (Kristensen et al. 2008). In the estuary, this exported DOC continues to support bacterial growth and productivity, contributing to the overall productivity of the estuarine ecosystem.

The post hoc Tukey's HSD tests primarily emphasized the significant variations in the nutrients, DOC, Chl *a*, and BA between the estuarine and mangrove waters of both M and Z. This suggests that nutrients and OM play a significant role in shaping the microbial communities and overall ecosystem dynamics. Besides, the absence of significant variations in the productivity and respiration parameters indicates a remarkable ecological connectivity between the mangrove and estuarine waters. This highlights the crucial role of mangroves as transitional areas between terrestrial and aquatic realms facilitating the exchange of water, nutrients, and OM with a significant impact on the metabolic processes in estuarine waters.

Productivity and respiration show significant variability across aquatic ecosystems, from oligotrophic coral reefs and open oceans to eutrophic rivers, streams, and estuaries. For instance, a review by Amado et al. (2013) comparing tropical ecosystems (BP- average 4.65 $\mu\text{g C L}^{-1} \text{ h}^{-1}$; BR - average 41.40 $\mu\text{g C L}^{-1} \text{ h}^{-1}$; BGE - average 29 %) to temperate ecosystems (BP- average 4.24 $\mu\text{g C L}^{-1} \text{ h}^{-1}$; BR - average 25.00 $\mu\text{g C L}^{-1} \text{ h}^{-1}$; BGE - average 43 %) found that while BP and BR were higher in the tropics, BGE was lower. Similarly, Lee et al. (2009) reported higher BP and BR but lower BGE in Malaysian tropical coastal waters compared to temperate ecosystems. The present study also exhibited high BP and BR with lower BGE than those reported from the temperate zone. These differences have been attributed to a

combination of factors, including the higher temperatures, and increased sunlight exposure characteristic of tropical regions. The metabolic status even within a single aquatic system can vary temporally and spatially (Raymond et al. 2000). A comprehensive study conducted by Caffrey (2004) examined the metabolic status of 42 sites within 22 national estuarine reserves. It was found that sites adjacent to mangroves or in marsh creeks tended to exhibit heterotrophic conditions. Conversely, sites neighboring submerged aquatic vegetation, such as eelgrass or macroalgal beds, were mostly autotrophic. This phenomenon was attributed to variations in the nutrient and organic matter loadings. Thus, the outwelling of DOC from ecosystems such as mangroves and marsh creeks is a major contributor to the trophic dynamics.

5.5. Summary

We provide for the first time, insights into the trophic status within the mangroves of the M and Z estuaries. The impact of mangrove-derived organic matter OM on coastal ecosystems, supporting coastal heterotrophy during the Mo and PoM seasons in the M and Z estuaries aligned with Odum's (1968) "outwelling" concept. High BR values and low BGE in both estuaries indicated a dominant microbial loop with substantial bacterial carbon demand, suggesting that organic carbon is primarily used for respiration. The weak autoheterotrophic coupling in these estuaries caused the oscillation between autotrophy during the PM season and heterotrophy during Mo and PoM seasons. Using radio-labelled substrates, we quantified PP and BP, revealing that CR exceeds PP which indicated heterotrophy. Principal component analysis showed that variations in salinity, dissolved inorganic nutrients, and DOC significantly influence the trophic status of these ecosystems. Our study demonstrated that allochthonous carbon from mangroves subsidizes heterotrophic activity, with seasonal changes in estuarine dynamics significantly affecting carbon flux through bacteria.

Chapter 6

Bacterial Extracellular Enzyme

Activity in Organic Matter

Degradation

6.1. Introduction

The intricate interplay between microbial enzymatic activities and organic matter (OM) decomposition plays a pivotal role in shaping the dynamics and functioning of mangrove ecosystems (Bullock et al. 2017). Microbial communities, particularly bacteria, are key players in these processes, driving the degradation and transformation of organic matter through their enzymatic capabilities. The microbial loop, a critical component of the marine food web, is responsible for the transformation, repackaging, and respiration of 50 % of the primary production in surface waters (Azam et al. 1983; Arnosti et al. 2005; Azam and Malfatti 2007).

According to Luo et al. (2017), bacterial enzymes are essential for mediating the breakdown of complex organic molecules, thereby facilitating the recycling of nutrients and energy within these ecosystems. The ability of bacteria to secrete enzymes into their environment that can break down complex organic compounds into simpler forms is referred to as extracellular enzymatic activity (Amon and Benner 1996; Benner and Amon 2015). These extracellular enzymes, such as amylases, proteases, cellulases, and lipases, play a major role in the cycling of OM in marine environments by breaking down carbohydrates, proteins, cellulose, and lipids, respectively. In mangrove ecosystems, extracellular enzymatic activity is particularly significant due to the high input of organic matter from leaf litter, root exudates, and tidal exchanges. The enzymes secreted by microbial communities facilitate the decomposition of this organic matter, releasing nutrients that are then available for primary producers and other organisms within the ecosystem. This process not only supports the nutrient dynamics but also contributes to the overall productivity and resilience of the mangrove ecosystem. Thus, the role of microbial enzymatic activities in organic matter decomposition is fundamental to the ecological functioning of mangrove ecosystems. Understanding these processes and the factors influencing them is crucial for predicting how

these ecosystems respond to environmental changes and for developing strategies to conserve and manage these vital coastal habitats

6.2. Materials and Methods

6.2.1. Screening of bacterial isolates for enzymatic activity

The bacterial isolates, representing 31 different genera isolated from the water and sediment samples of Mandovi (M) and Zuari (Z) were further subjected to screening of extracellular enzyme activity for amylase, cellulase, lipase, and protease.

6.2.1.1. Amylase

Amylase activity was evaluated by cultivating selected strains on glucose yeast extract peptone (GYP) agar medium containing 2 % soluble starch (Kathiresan et al. 2011). Following incubation, 1 % iodine in 2 % potassium iodide was used to flood the plates. A positive amylase activity was indicated by the formation of a clear zone around the colony.

6.2.1.2. Cellulase

For cellulase activity, strains were cultured on yeast extract peptone (YP) agar medium supplemented with 0.5 % Na-carboxymethyl cellulose (CMC) (Singh et al. 2014). After incubation, plates were flooded with 0.2 % aqueous Congo red and destained with 1M NaCl for 15 minutes. The presence of a clear zone around the colony signified cellulase activity.

6.2.1.3. Lipase

Lipase activity was determined by growing strains on peptone agar medium supplemented with Tween 20 (separately sterilized and added at 1 ml per 100 ml medium) (Shelley et al. 1987). A lipase-positive strain was identified by the presence of a clear zone around the colony.

6.2.1.4. Protease

Protease assay involved cultivating strains on GYP agar medium amended with 0.4 % gelatin, adjusting the pH to 6. After incubation, plates were flooded with saturated aqueous

ammonium sulfate. The undigested gelatin precipitated with ammonium sulfate, and the clear area around the colony indicated protease activity (Mamangkey et al. 2019).

6.2.1.5. Index of relative enzyme activity

Enzyme activity on respective agar plates was recorded as the Index of Relative Enzyme Activity and was calculated using the below formula (Teather and Wood 1982):

Index of relative enzyme activity = Clear zone diameter (in cm) / Colony diameter (in cm)

6.2.2. Statistical analysis

The statistical software PAST 4.03 (Hammer and Harper 2001) was used to carry out the statistical analyses. The relationship between environmental parameters, bacterial diversity, and enzymatic activity was understood using a two-tailed Pearson's correlation analysis ($p < 0.001$) during PM, Mo, and PoM. Further, to reduce the intercorrelated variability, a principal component analysis (PCA) biplot was created by combining the sample scores plot and the vector loading plots to understand the relationship between the environmental parameters, bacterial abundance, diversity, and enzyme activity.

6.3. Results

6.3.1. Extracellular enzyme activity of bacterial isolates

Among the 31 bacterial isolates, positive activity for amylase was observed in 70.29 %, for protease in 61.29 %, for cellulase in 45.16 %, and lipase in 38.71 % of the isolates (**Fig. 6.1.**). Consequently, the order of enzyme activity was determined as follows: amylase > protease > cellulase > lipase.

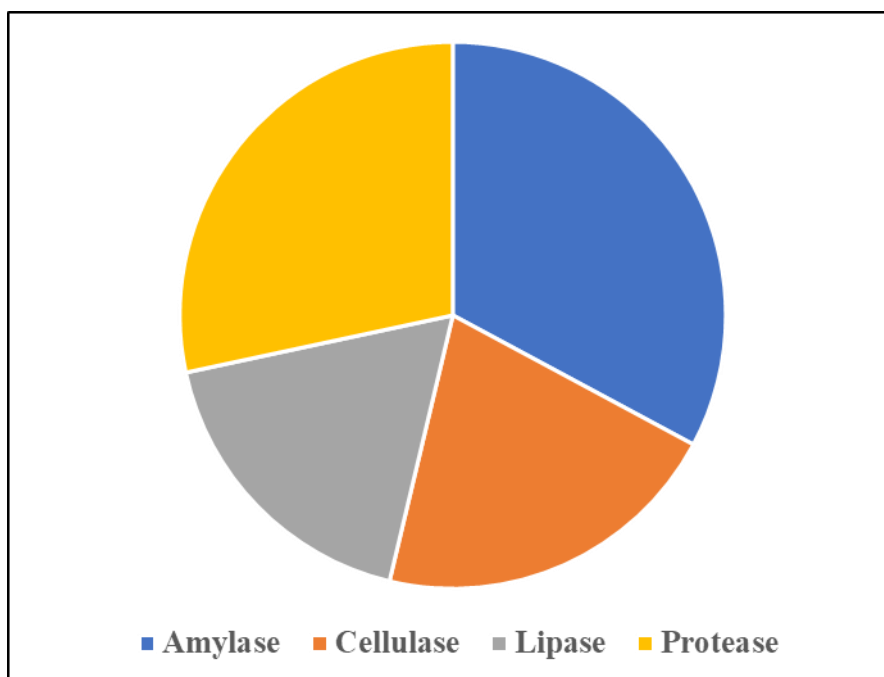


Fig. 6.1. Overall percentage variation in extracellular enzymatic activity in the Mandovi and Zuari estuaries.

Across all seasons, variability in enzyme activities was observed within and between the mangrove and estuarine sites (**Fig. 6.2.**). In M_m , amylase (34.27 %) remained prominent, followed by protease (25.87 %), cellulase (23.08%), and lipase (16.78 %), while in Z_m , amylase (36.42 %) maintained a high level, followed by protease (26.61 %), cellulase (19.21 %) and lipase (19.21 %) activities. Comparatively, M_w and Z_w sites displayed slightly different patterns, with amylase (37.10 % ; 38.98 %) consistently high, followed by protease (26.61 % ; 28.81 %), lipase (20.16 % ; 18.64 %), and cellulase (16.13 % ; 13.56 %).

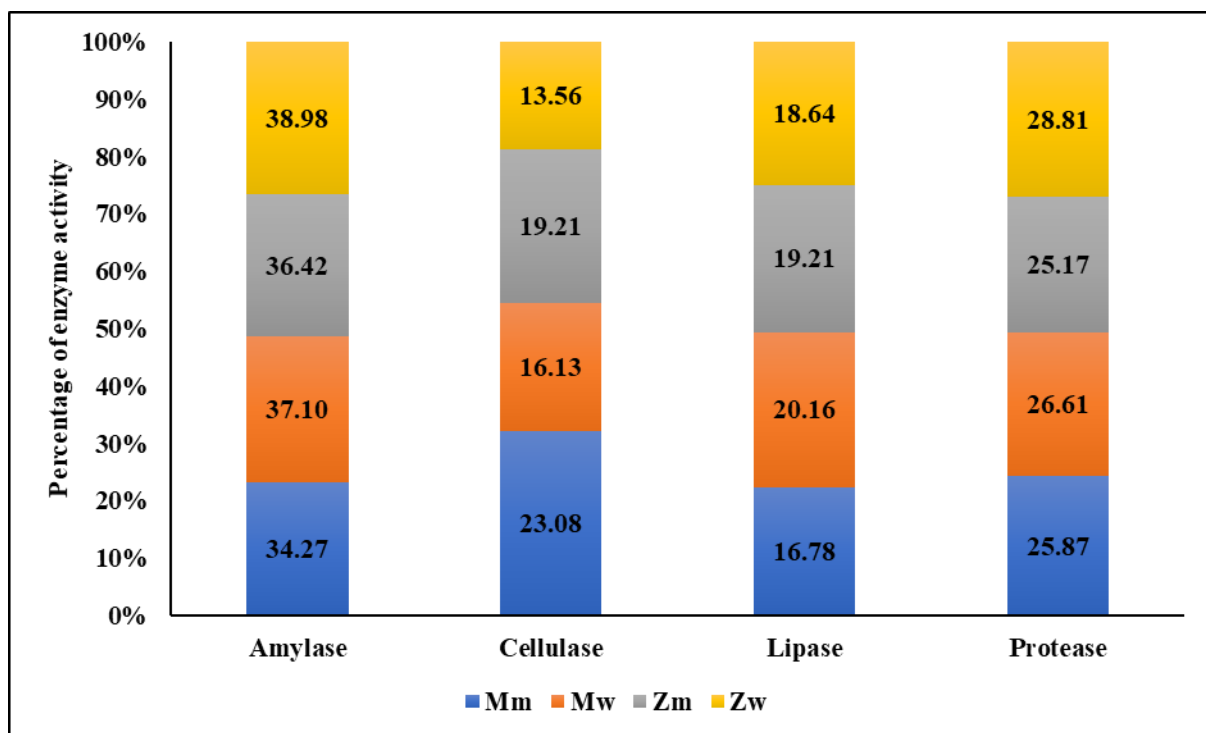


Fig. 6.2. Site-wise percentage variation of extracellular enzymatic activity in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

Throughout the PM, Mo, and PoM, the seasonal pattern of enzyme activity among bacterial isolates remained consistent in M and Z, with the highest activity observed for amylase, followed by protease, cellulase, and lipase (**Fig. 6.3. a., b.; Fig. 6.4. a., b.; Fig. 6.5. a., b.**). Additionally, amylase consistently exhibited the highest activity during Mo, followed by PoM and PM. Similarly, protease, cellulase, and lipase also displayed the highest activity during Mo, followed by PoM and PM, except lipase, which showed slightly higher activity during PM than PoM. In M_m, enzyme activities peaked during PoM, followed by Mo and PM. Conversely, in M_w, PM exhibited the highest activity, followed by Mo and PoM. For Z_m and Z_w, Mo consistently showed the highest activity, followed by PoM and PM.

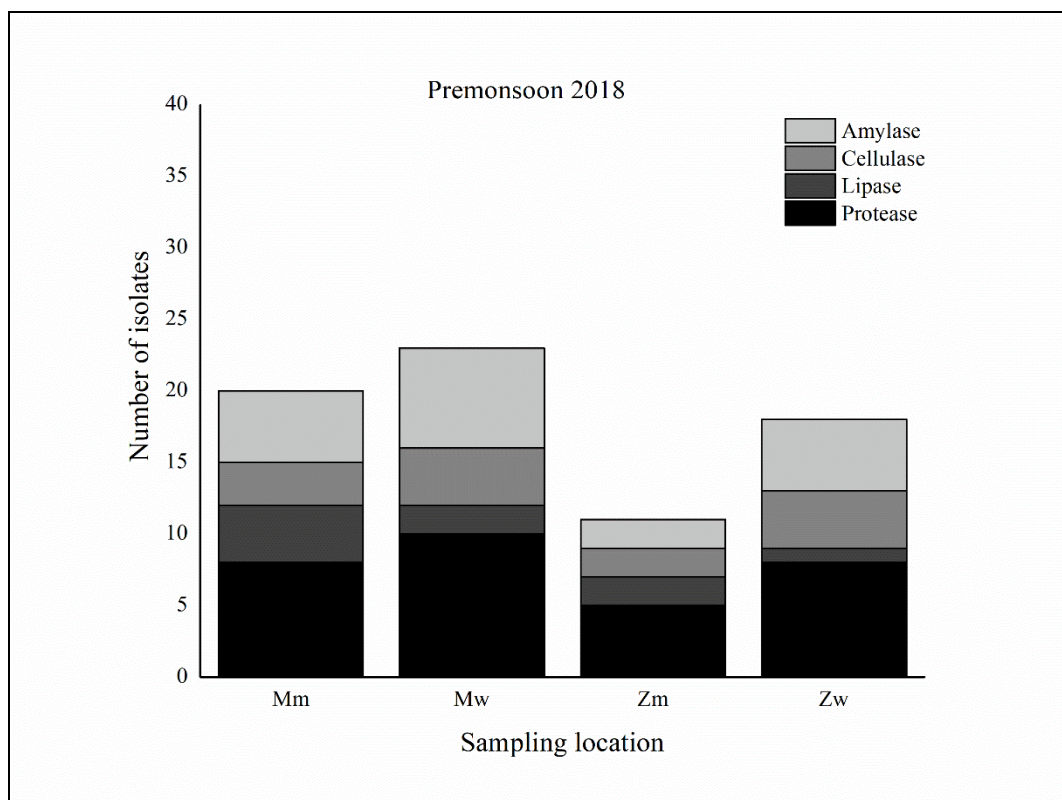


Fig. 6.3. a. Variation of extracellular enzymatic activity during Premonsoon 2018 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

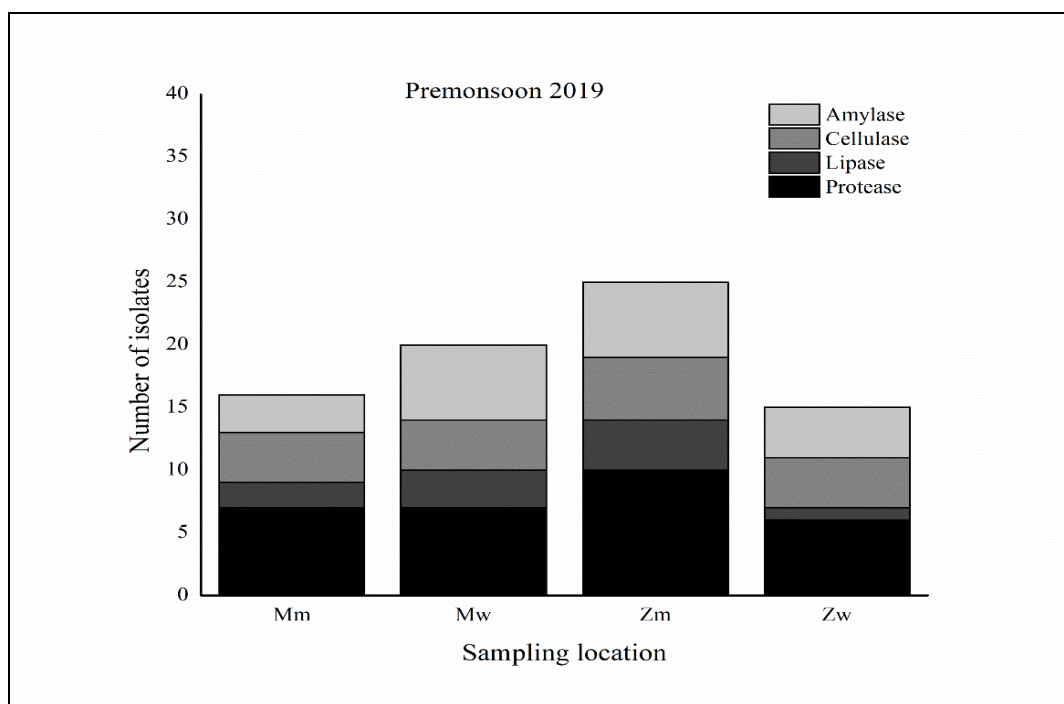


Fig. 6.3. b. Variation of extracellular enzymatic activity during Premonsoon 2019 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

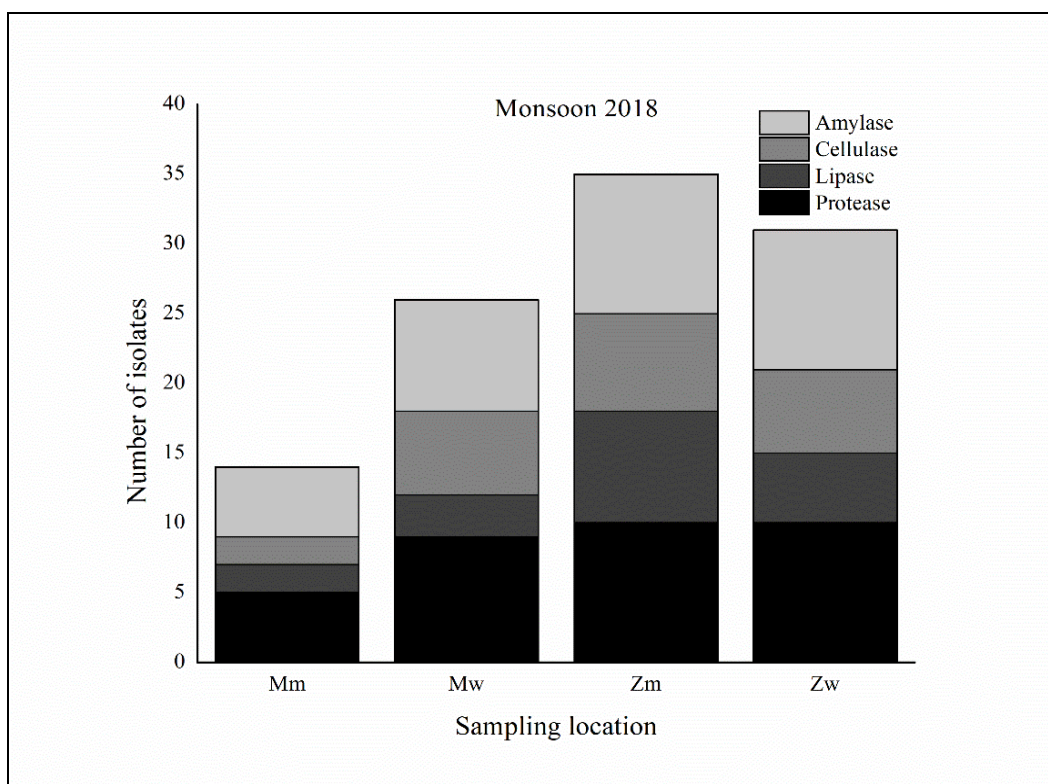


Fig. 6.4. a. Variation of extracellular enzymatic activity during Monsoon 2018 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

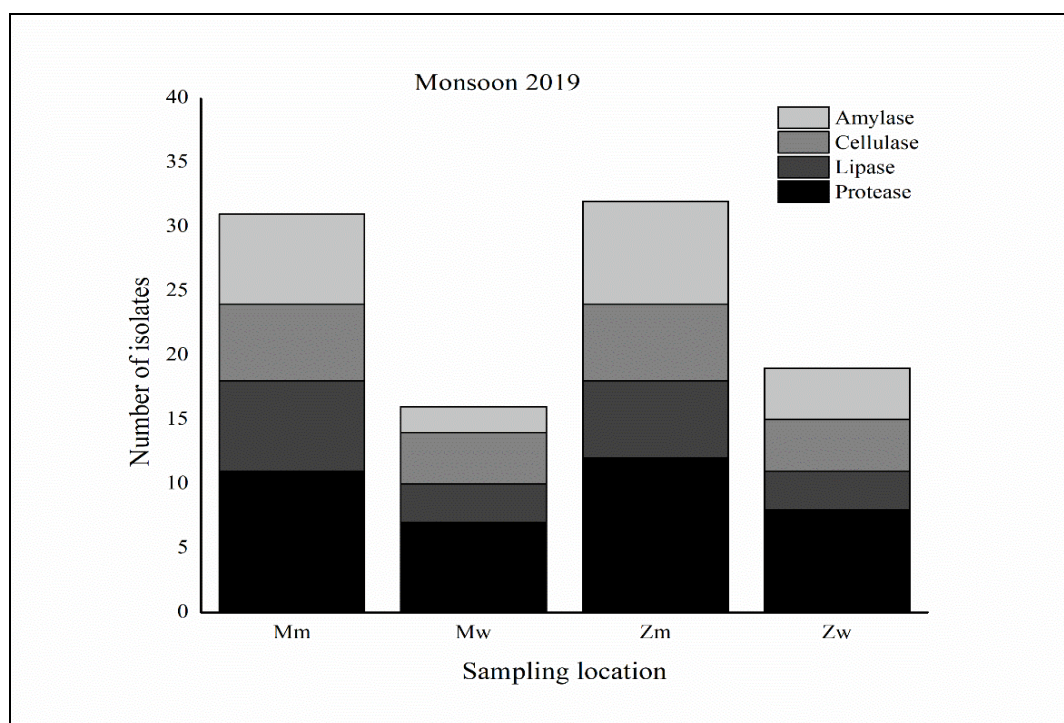


Fig. 6.4. b. Variation of extracellular enzymatic activity during Monsoon 2019 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

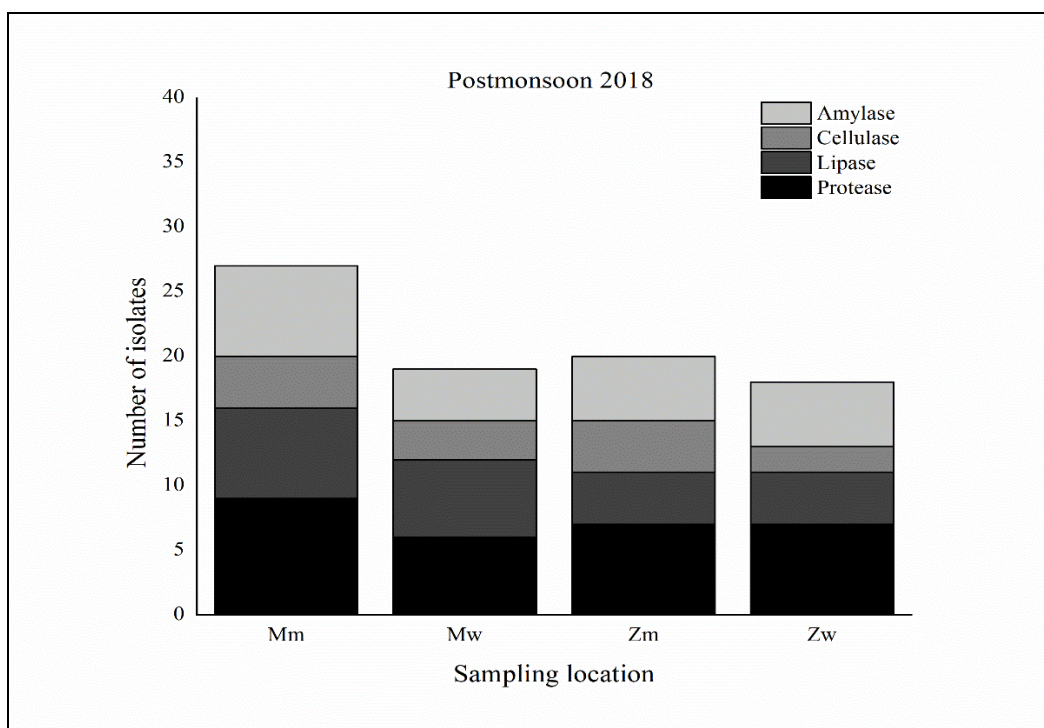


Fig. 6.5. a. Variation of extracellular enzymatic activity during Postmonsoon 2018 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

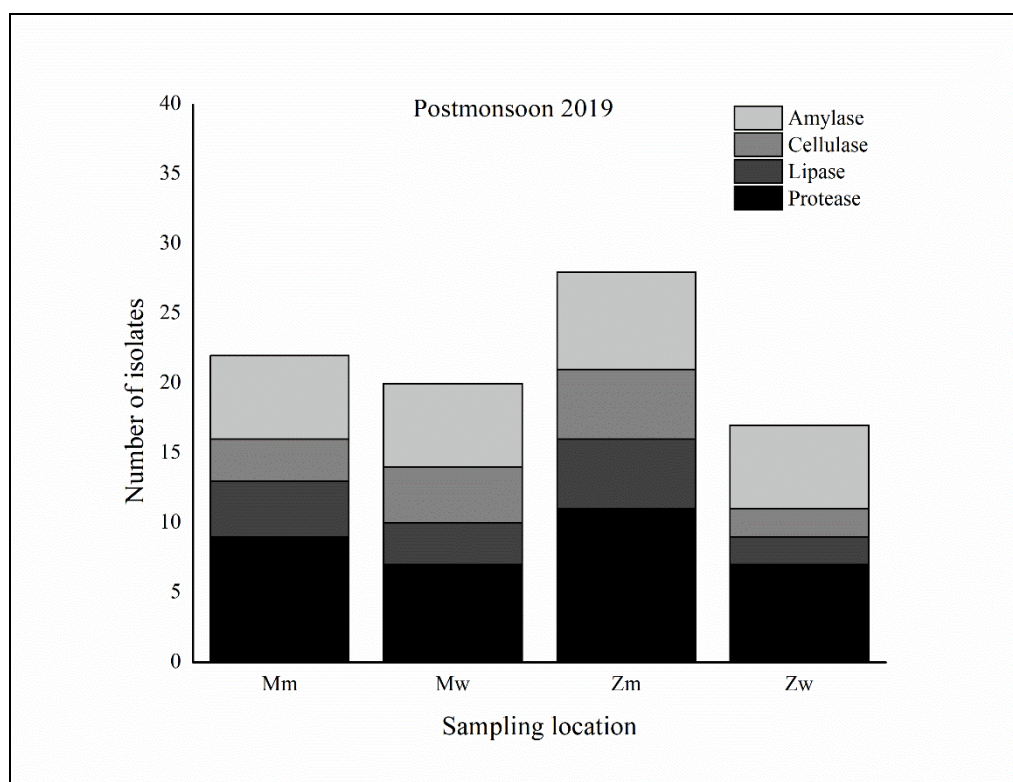


Fig. 6.5. b. Variation of extracellular enzymatic activity during Postmonsoon 2019 (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

The highest activity for amylase was detected in *Paenibacillus* sp. and *Staphylococcus* sp. (**Table 6.1.**). *Idiomarina* sp. demonstrated the highest cellulase activity, *Kosakonia* sp. showed the highest lipase activity and protease activity was maximum in *Ferrimonas* sp. *Oceanobacillus* sp. exhibited poly-enzyme activity, being positive for all the four enzymes viz. amylase, cellulase, lipase and protease. 11 isolates were positive for 3 enzymes and another 11 isolates were positive for only 2 enzymes. However, *Dermaococcus* sp. and *Alteromonas* sp. failed to show any activity for these enzymes.

Table 6.1. Index of Extracellular enzymatic activity of isolates for amylase, lipase, cellulase and protease (ND = Not Detected)

	Amylase	Cellulase	Lipase	Protease
<i>Pantoea</i> sp.	ND	1.46 ± 0.04	ND	ND
<i>Marimonas</i> sp.	ND	3.18 ± 0.38	ND	ND
<i>Oceanobacillus</i> sp.	1.24 ± 0.02	1.30 ± 0.11	1.55 ± 0.32	1.52 ± 0.22
<i>Paraclostridium</i> sp.	1.89 ± 0.19	ND	ND	ND
<i>Dermacoccus</i> sp.	ND	ND	ND	ND
<i>Paenibacillus</i> sp.	2.07 ± 0.51	ND	1.33 ± 0.21	ND
<i>Exiguobacterium</i> sp.	1.71 ± 0.69	ND	ND	ND
<i>Kosakonia</i> sp.	1.43 ± 0.09	ND	2.02 ± 0.34	4.49 ± 1.31
<i>Enterobacter</i> sp.	1.36 ± 0.13	1.21 ± 0.04	ND	1.27 ± 0.12
<i>Virgibacillus</i> sp.	1.46 ± 0.20	0.81 ± 0.06	ND	2.58 ± 0.40
<i>Bhargavaea</i> sp.	ND	ND	1.60 ± 0.04	2.75 ± 0.47
<i>Kangiella</i> sp.	ND	ND	ND	1.36 ± 0.13
<i>Klebsiella</i> sp.	ND	ND	1.19 ± 0.08	1.47 ± 0.06
<i>Lysinibacillus</i> sp.	1.68 ± 0.72	2.24 ± 0.48	ND	1.29 ± 0.20
<i>Solibacillus</i> sp.	1.32 ± 0.03	ND	1.33 ± 0.30	1.42 ± 0.14
<i>Curtobacterium</i> sp.	ND	2.06 ± 0.42	ND	1.21 ± 0.11
<i>Psychrobacter</i> sp.	ND	3.08 ± 1.38	1.49 ± 0.36	4.17 ± 1.61
<i>Alteromonas</i> sp.	ND	ND	ND	ND
<i>Paracoccus</i> sp.	1.61 ± 0.34	1.75 ± 0.46	ND	ND
<i>Microbacterium</i> sp.	1.74 ± 0.23	ND	ND	1.14 ± 0.06
<i>Idiomarina</i> sp.	1.58 ± 0.38	8.39 ± 2.30	ND	1.36 ± 0.13
<i>Vibrio</i> sp.	1.77 ± 0.09	ND	1.36 ± 0.13	ND
<i>Bacillus</i> sp.	1.58 ± 0.29	1.88 ± 0.30	ND	1.40 ± 0.06
<i>Pseudoalteromonas</i> sp.	1.67 ± 0.38	1.75 ± 0.46	ND	ND
<i>Acinetobacter</i> sp.	1.37 ± 0.11	ND	ND	4.11 ± 0.44
<i>Pseudomonas</i> sp.	1.40 ± 0.17	ND	1.62 ± 0.16	1.63 ± 0.32
<i>Shewanella</i> sp.	1.53 ± 0.06	ND	ND	4.37 ± 0.34
<i>Staphylococcus</i> sp.	1.99 ± 0.19	2.78 ± 0.38	1.40 ± 0.12	ND
<i>Marinobacter</i> sp.	1.49 ± 0.18	ND	1.60 ± 0.38	ND
<i>Ferrimonas</i> sp.	1.46 ± 0.12	ND	ND	8.44 ± 3.20
<i>Aeromonas</i> sp.	1.26 ± 0.02	1.13 ± 0.07	1.33 ± 0.03	ND

6.3.2. Relationship between physicochemical parameters, bacterial diversity, and extracellular enzymatic activity

Pearson's correlation coefficient was used to discern the relationship between the different variables across the PM, Mo, and PoM seasons. During PM, *Gammaproteobacteria* and *Alphaproteobacteria* were negatively correlated to all four enzymes – amylase, cellulase, protease, and lipase - while, *Bacilli* and *Actinobacteria* positively correlated (**Fig. 6.6. a.**). During Mo, *Gammaproteobacteria*, *Bacilli* and *Actinobacteria* exhibited positive correlations to the enzymes (**Fig. 6.6. b.**). The PoM season displayed similar correlation patterns as PM, with the addition of *Clostridia* exhibiting positive correlations to enzyme activities (**Fig. 6.6. c.**). PCA was employed to discern the influence of seasonal variability on the bacterial diversity and enzyme activities of M and Z. PCA analysis revealed that principal components 1 and 2 cumulatively represented 92.89% of the total variability with eigenvalue > 1 (**Fig. 6.7.**). Based on component 1 and 2, the PCA biplot was constructed revealing distinct clusters of the PM, Mo, and PoM seasons confirming that the seasonal variability had a significant influence on the estuarine and mangrove waters of M and Z. The environmental parameters such as salinity and pH primarily influenced the PM season with *Gammaproteobacteria* exhibiting maximum abundance. In the Mo season, OM and nutrient vectors, along with TVC_w and TVC_s were the primary drivers, with salinity exerting a negative influence. During the PoM season, salinity was the main environmental factor affecting bacterial communities and enzymatic activities. The remaining bacterial classes, including *Alphaproteobacteria*, *Bacilli*, *Actinobacteria*, and *Clostridia*, as well as the enzymes – amylase, protease, cellulase, and lipase, were clustered in the center of the plot, suggesting their ability to adapt to varying environmental gradients and seasonal changes.

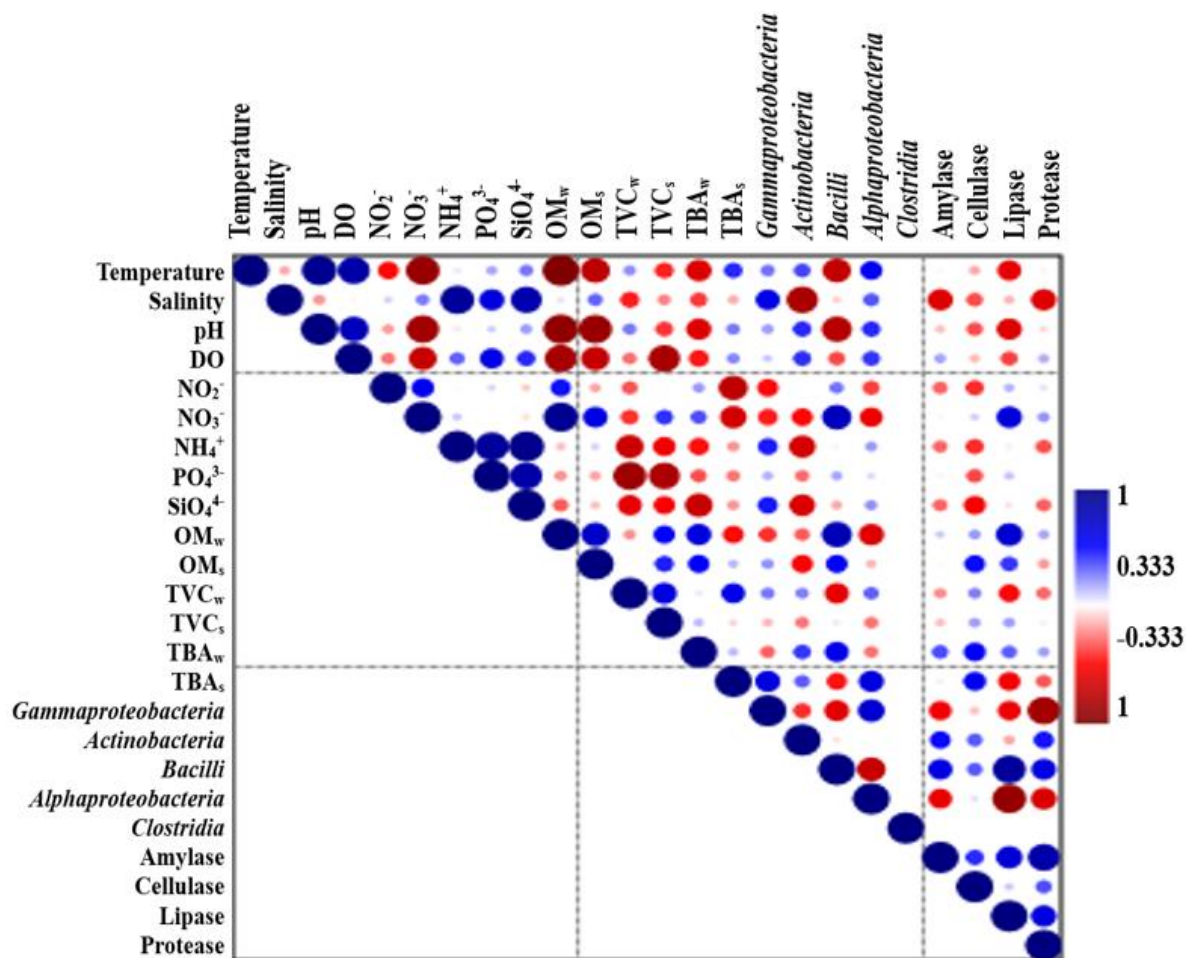


Fig. 6.6. a. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial abundance (TBA), bacterial classes and enzymes in the Mandovi and Zuari estuaries during Premonsoon.

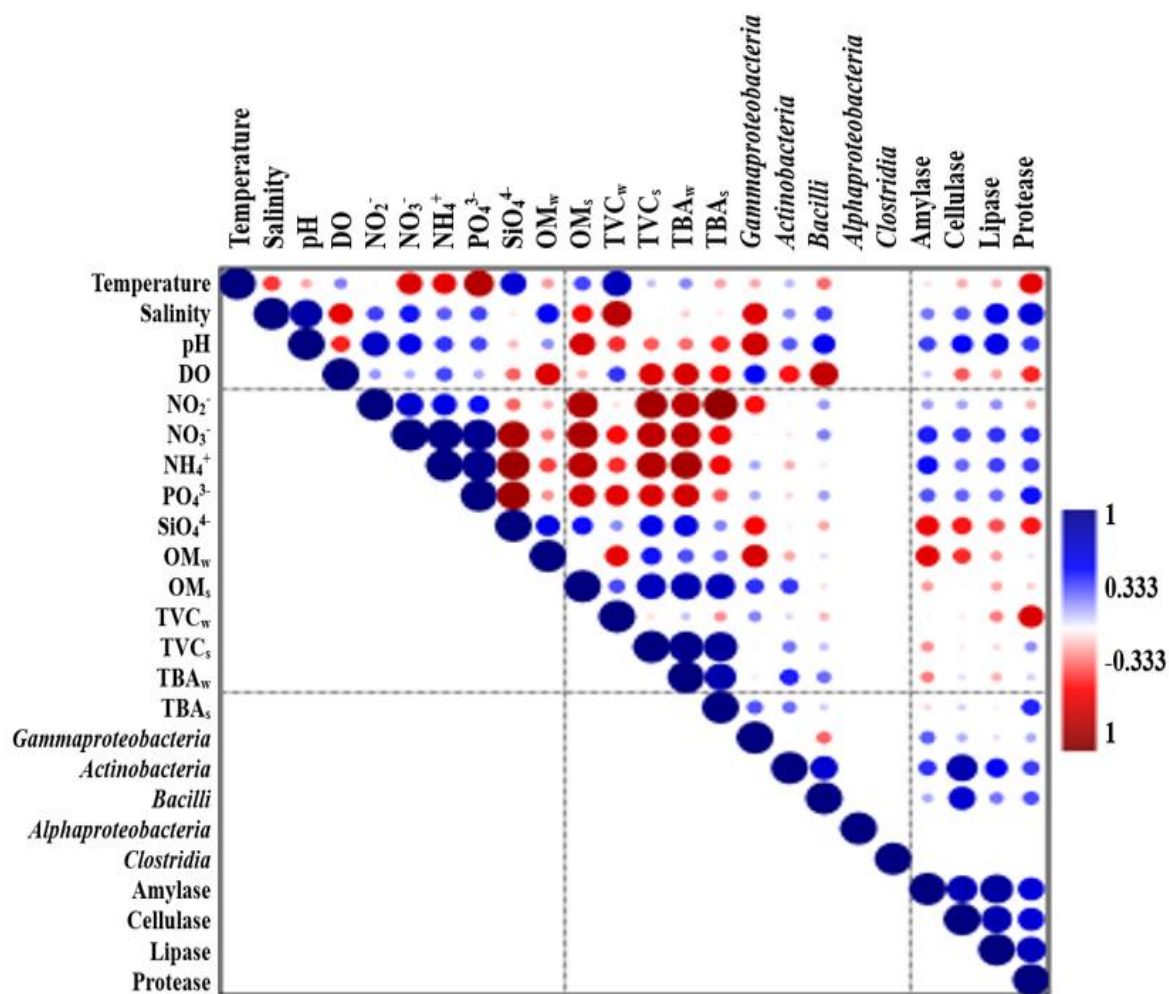


Fig. 6.6. b. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial abundance (TBA), bacterial classes and enzymes in the Mandovi and Zuari estuaries during Monsoon.

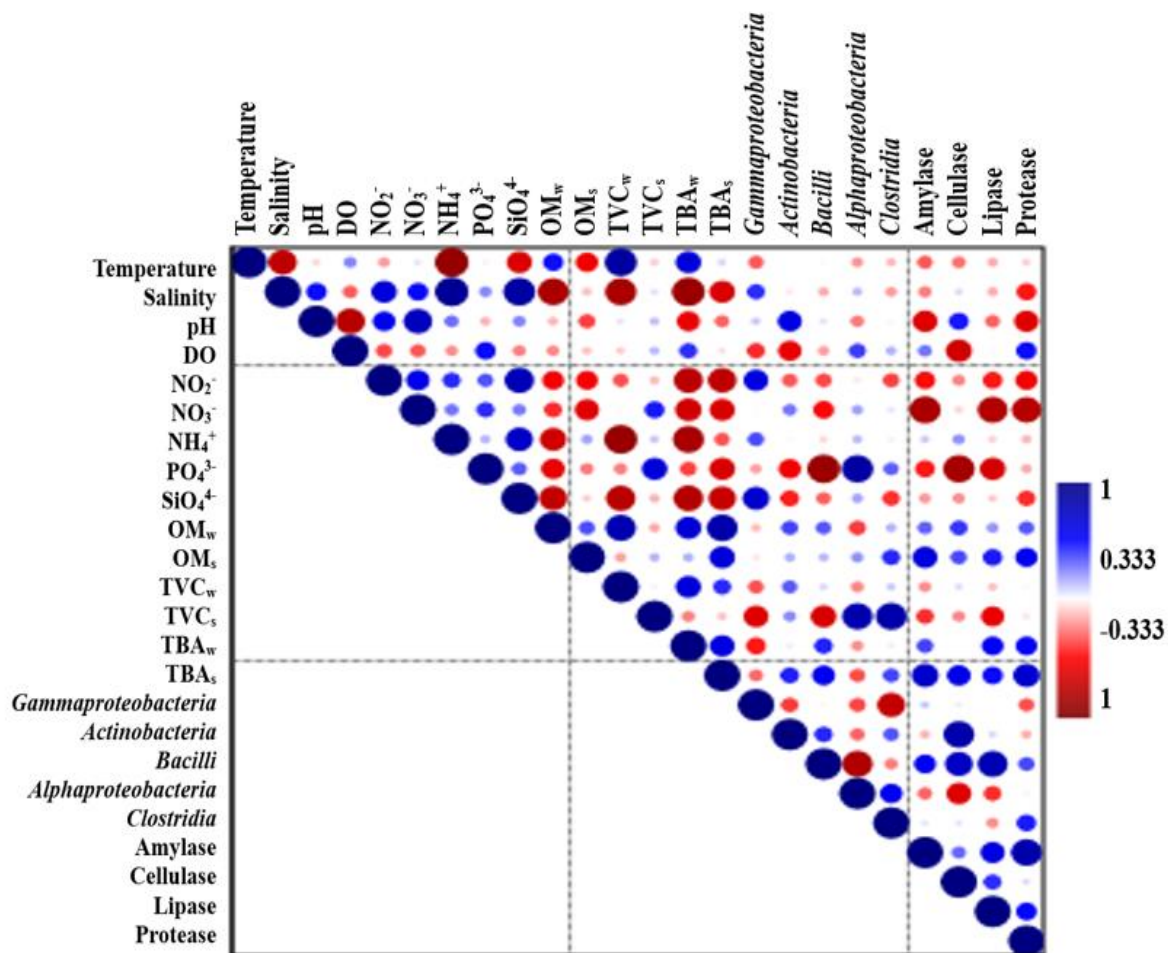


Fig. 6.6. c. Pearson's correlation coefficients between physicochemical parameters, Total viable counts (TVC), Total bacterial abundance (TBA), bacterial classes and enzymes in the Mandovi and Zuari estuaries during Postmonsoon.

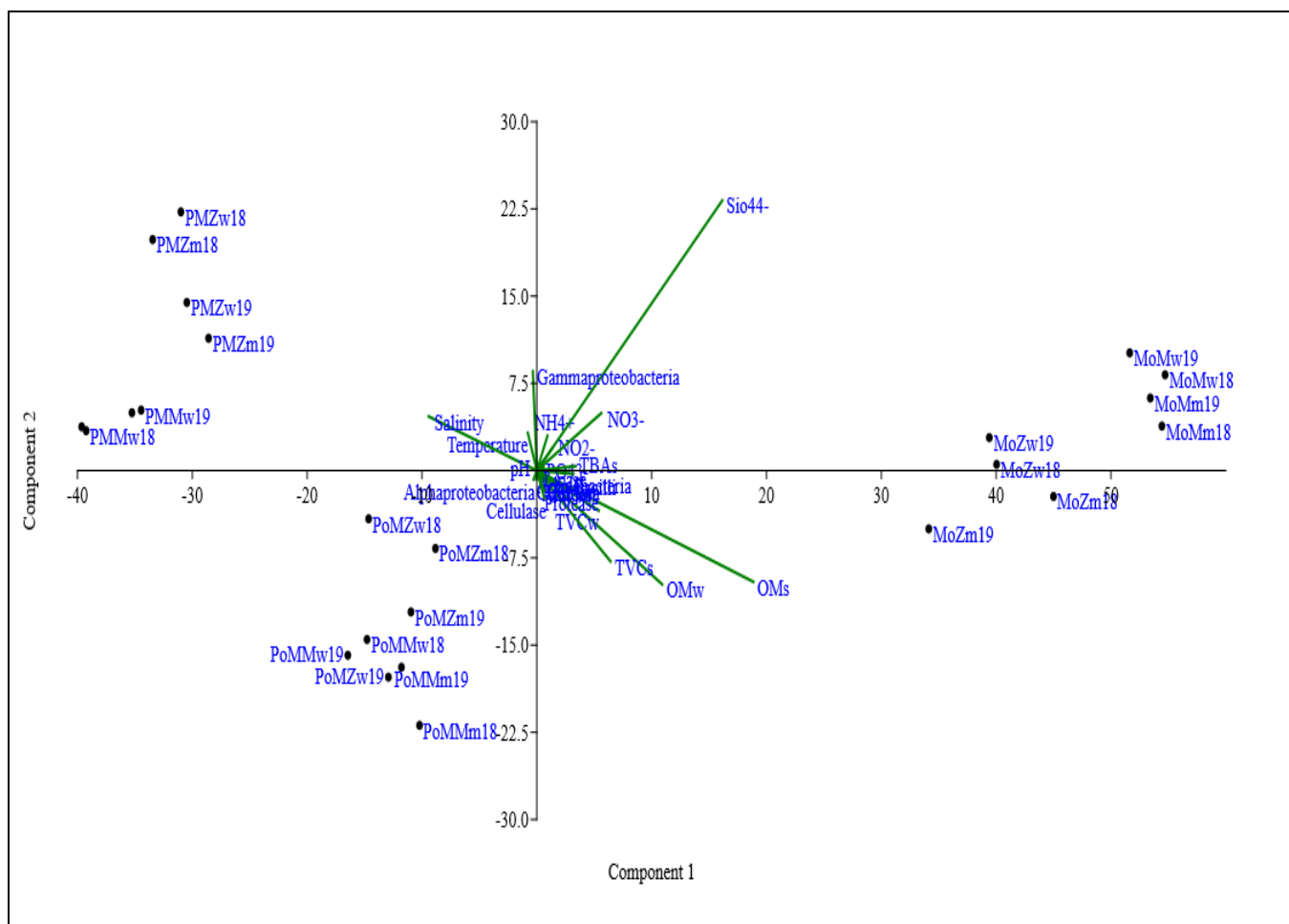


Fig. 6.7. Principal component analysis (PCA) biplot of the mean PC scores of physicochemical and biological parameters as vectors, together with the PC scores of each season.

6.4. Discussion

The bacterial community composition can significantly influence the patterns of enzyme activities (Rietl et al. 2016). The consistent seasonal pattern of enzyme activity observed among the bacterial isolates, with amylase exhibiting the highest activity followed by protease, cellulase, and lipase, across the PM, Mo, and PoM, reflect the complex interplay between environmental conditions, substrate availability, and bacterial metabolic responses (Koranda et al. 2013). The Mo season is characterized by increased moisture and organic matter input from rainfall and vegetation decomposition, leading to heightened bacterial enzyme activity (Seleyi et al. 2024). During this period, amylase, protease, cellulase, and

lipase exhibit their peak activities. This surge in enzyme production likely corresponds to the abundant availability of diverse organic substrates, promoting bacterial growth and metabolic activity. The PoM period, characterized by relatively stable environmental conditions and residual OM, sustains enzyme activity at moderate levels at a slightly lower level compared to the Mo. The PM season, marked by high temperatures and reduced organic input, exhibits the lowest enzyme activities.

The findings from a previous study by Haldar and Nazareth (2018), focusing solely on the PM season, revealed distinct enzyme activity patterns in M and Z mangroves. In the M mangroves, amylase (37.30 %) exhibited the highest activity, followed by lipase (22.8 %), and cellulase (17.14 %), with proteolytic activity notably absent. Conversely, in the Z mangroves, cellulase activity (68 %) was predominant, followed by amylase (56 %), lipase (48 %), and protease (16 %). However, in the present study, encompassing the PM, Mo, and PoM seasons, variability in enzyme activities was observed within and between the mangrove and estuarine sites, possibly reflecting the influence of substrate availability. Amylase, which breaks down starch into simpler sugars, may be favored when starch-rich substrates are abundant, such as during Mo and PoM when plant matter decomposes and releases starch (Luce et al. 2016). Protease, responsible for protein hydrolysis, likely follows amylase activity due to the concurrent availability of proteinaceous materials during these seasons. Cellulase activity may be influenced by factors such as temperature and humidity, which fluctuate seasonally, while lipase activity, catalyzing lipid breakdown, may be comparatively lower due to the limited availability of lipid-rich substrates or environmental conditions less conducive to lipid metabolism during these periods (Wei et al. 2021). Mangrove ecosystems, characterized by their proximity to the interface between land and sea, experience fluctuating environmental conditions such as tidal influence, salinity gradients, and nutrient availability. These factors can influence bacterial communities and substrate availability, affecting

enzyme production and activity. Additionally, differences in sediment composition, vegetation cover, and anthropogenic impacts between mangrove and estuarine sites further contribute to the observed variability in enzyme activities (Wei et al. 2021).

Furthermore, the varied enzyme activity profiles observed among different bacterial species reflect the diversity of metabolic capabilities and ecological niches within the microbial community. *Paenibacillus* sp. and *Staphylococcus* sp., exhibiting the highest amylolytic activity, possess genetic traits or regulatory mechanisms conducive to efficient starch hydrolysis, possibly reflecting their adaptation to starch-rich environments (Rajesh et al. 2013; Lakshmi et al. 2013). High cellulolytic activity of *Idiomarina* sp. suggests specialization in cellulose degradation (Al-Rubaye et al. 2017). The high lipolytic activity of *Kosakonia* sp. emphasizes its lipid degradation potential previously reported by Silva-Bedoya et al. (2016) and Kumar et al. (2020). The high proteolytic activity of *Ferrimonas* sp. may relate to its role in protein-rich substrate decomposition (Abbas and Mahmoud 2022). The poly-enzyme activity of *Oceanobacillus* sp. highlights its versatile metabolic capacity, advantageous for the breakdown of diverse organic substrates. The ability of *Oceanobacillus* sp. to produce amylases, proteases, cellulases, and lipases has been reported in various studies (Rohban et al. 2009; Kumar et al. 2012; Kiran et al. 2014; Moukrim et al. 2022). In general, strains with broad substrate preference and high utilization efficiency for inexpensive and widely accessible substrates are desirable features with promise for biotechnological and industrial applications (Xiao et al. 2018). These findings suggest that environmental factors associated with each habitat and seasonal variations play significant roles in shaping the enzyme activity profiles of bacterial isolates, highlighting the complexity of microbial dynamics within ecosystems (Lu et al. 2022; Wei et al. 2021).

The distribution of different types of enzymes varies temporally and spatially in response to environmental changes (Arnosti 2014). Pearson's correlation analyses revealed

distinct seasonal dynamics in the relationships between bacterial classes and enzyme activities, highlighting the intricate interplay between bacterial communities and their functional roles in OM degradation. During the PM season, the negative correlation between *Gammaproteobacteria* and *Alphaproteobacteria* with all four enzymes suggests a potential competitive exclusion or niche differentiation, where the involvement of these bacterial classes in enzyme-mediated degradation processes may be limited. Conversely, the positive correlations of *Bacilli* and *Actinobacteria* with amylase, cellulase, protease, and lipase indicate their active participation in breaking down complex OM. In the Mo season, the positive correlations of *Gammaproteobacteria*, *Bacilli*, and *Actinobacteria* with the enzymes suggest a synergistic relationship, possibly driven by increased availability of OM and nutrients. The PoM season mirrors that of the PM but with the addition of *Clostridia*, which also positively correlates with enzyme activities, indicating its role in OM degradation under specific environmental conditions. Furthermore, the PCA biplot effectively captures and summarizes the complex bacterial, enzymatic activity, and ecological interactions in the mangrove and estuarine sites across different sampling seasons. The PM, Mo and PoM observations are grouped into distinct clusters indicating their similarities. The clustering of Mo samples on the right side of the biplot suggests that bacteria are strongly influenced by higher OM, corroborating the observed highest abundance of TVC_w and TVC_s during the Mo season in both M and Z. The vectors for amylase, protease, cellulase, and lipase are clustered, indicating their co-occurrence and significant role across different seasons. Overall, the PCA biplot provides a comprehensive visualization that corroborates the observations on the variability of enzyme activities. It highlights the significant seasonal and temporal variations in bacterial communities and their metabolic potentials, providing an insight into the underlying environmental and biological interactions.

6.5. Summary

The extracellular enzyme activities exhibited distinct seasonal patterns, with amylase showing the highest activity, followed by protease, cellulase, and lipase. This variation reflects the interplay between environmental conditions, substrate availability, and bacterial metabolic responses. Notably, *Oceanobacillus sp.* exhibited poly-enzyme activity, being positive for all four enzymes, indicating varied metabolic capabilities and ecological niches within the microbial community. Higher enzyme activities in mangrove sites compared to estuarine sites further emphasize the significance of OM in stimulating bacterial enzymatic functions. Multivariate statistical analyses revealed significant correlations between bacteria and enzyme activities, highlighting the role of environmental factors such as salinity, pH, and nutrient availability in shaping bacterial communities and their functional roles in OM degradation. This study, being the first to assess multiple enzyme profiles across distinct monsoonal seasons from bacteria isolated from the M and Z estuaries, provides valuable insights into the mechanisms underlying OM degradation and nutrient cycling in mangrove ecosystems, contributing to ecosystem management and conservation efforts.

Chapter 7

Tracking Sources of OM outwelled into adjacent waters

7.1. Introduction

As mangroves inhabit transitional zones between marine and freshwater ecosystems, OM comprises a heterogeneous mixture of various dissolved and particulate matter. This mixture can be from allochthonous (e.g., riverine or terrigenous inputs) or autochthonous (e.g., mangrove litter) sources (Prasad et al. 2017). Large volumes of litter are produced by falling leaves, branches, and other debris in mangrove habitats. The process of decomposition recycles nutrients and produces OM in the mangroves and their adjacent ecosystems. The OM that is not exported by tides settles in the sediment and is subjected to microbial degradation, the byproducts of which supplement the food web (Kristensen et al. 2008). The outwelled OM and nutrients enrich the estuarine waters and, ultimately boost fishery resources (Saifullah et al. 2015). Thus, to validate the sources of OM, the signatures of carbon $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) and nitrogen $^{15}\text{N}/^{14}\text{N}$ ratio ($\delta^{15}\text{N}$) coupled with elemental C:N ratios in the mangrove and estuarine sediments were studied. The identification of the source(s) of OM has been carried out using dual isotopic tracers in different mangrove ecosystems (Hu et al. 2006; Zhang et al. 2007; Ranjan et al. 2011; Aschenbroich et al. 2015; Krishna et al. 2015; Chu et al. 2020). In the case of the Mandovi and Zuari estuaries, dual isotopic tracer analysis for the identification of OM origin has gained impetus only in the recent decade (Maya et al. 2011; Bardhan et al. 2015; Shynu et al. 2015). The use of stable isotopic studies to understand the outwelling of OM from mangroves and its implications on the M and Z estuarine productivity has not been addressed earlier. Thus, to test the outwelling hypothesis on the M and Z estuaries the identification of the sources of OM using isotopic ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ coupled with organic C and N elemental ratios was carried out.

7.2. Materials and Methods

7.2.1. Identification of organic matter sources

7.2.1.1. Sample processing

7.2.1.1.1. Mangrove leaves

Fresh leaves were collected from five dominant mangrove species of the M and Z estuaries. The leaves were thoroughly washed with de-ionized water. The leaves were oven-dried at 80 °C for five days and ground into a fine powder (Rao et al. 1994). Until further analysis, the samples were stored in tightly-screwed polycarbonate vials at room temperature.

7.2.1.1.2. Sediment

Sediment samples were decalcified with 1N HCl, and desalinated through multiple washes with de-ionized water. Each wash with de-ionized water was tested for Cl^- using a 0.5 M solution of silver nitrate (AgNO_3) (Fernandes et al. 2020). After ensuring the complete removal of Cl^- , the samples were dried to determine C and N isotopic and elemental ratios. The sediment samples were then powdered using a mortar and pestle to obtain a fine consistency. The samples were stored at room temperature in tightly screwed polycarbonate vials until further analysis.

7.2.1.2. Analysis of carbon and nitrogen isotopic and elemental ratios

The powdered samples were weighed (~ 5 mg) and packed into tin cups. The carbon $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) and nitrogen $^{15}\text{N}/^{14}\text{N}$ ratio ($\delta^{15}\text{N}$), along with C and N content, were determined in continuous-flow mode using an elemental analyzer (EURO3000 Eurovector) linked to an isotope ratio mass spectrometer (Thermo-Finnigan Delta V) (Owens and Rees 1989). The isotopic ratios are reported in the standard delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relative to the Pee Dee Belemnite (PDB) standard and atmospheric nitrogen, respectively. As established by the working standard repeatedly measured after every 5 samples, ϵ -Amino-n-Caproic Acid (ACA) ($^{13}\text{C} = 25.3$ and $^{15}\text{N} = 4.6$) and an in-house sediment standard COD ($^{13}\text{C} = 21$ and $^{15}\text{N} = 7.5$), the precision of C and N isotope readings was more than ± 0.2 ‰. The C

and N content of the sediment samples was determined using an ACA calibration curve with C and N standards ranging from 6 to 24 μM and 1 to 4 μM , respectively (Bardhan et al. 2015).

7.3. Results

7.3.1 Spatio-temporal variation of dual isotopic composition

7.3.1.1. Mangrove leaves

The $\delta^{13}\text{C}_{\text{mng}}$ values of mangrove leaves were recorded between -30.73 ‰ to -27.73 ‰; and the average $\delta^{13}\text{C}_{\text{mng}}$ was -28.39 ‰. In contrast, the $\delta^{15}\text{N}_{\text{mng}}$ values varied from 3.93 ‰ to 7.67 ‰ with an average of 5.33 ‰ (**Fig. 7.1.**).

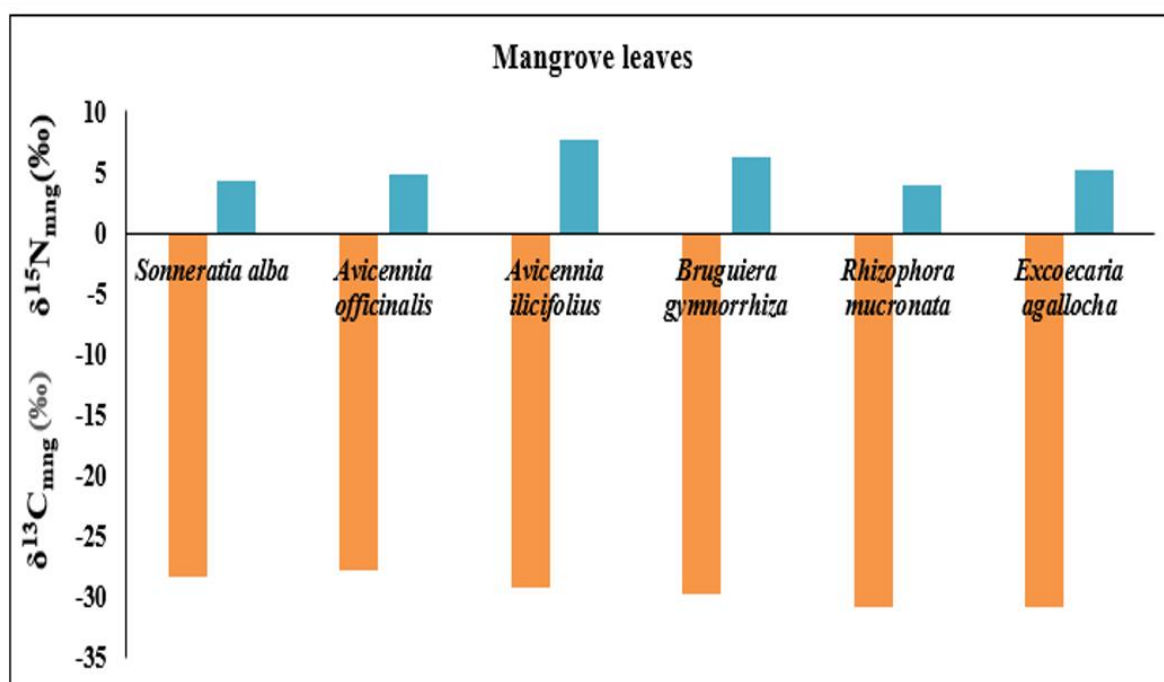


Fig. 7.1. Variations in $\delta^{13}\text{C}_{\text{mng}}$ and $\delta^{15}\text{N}_{\text{mng}}$ of mangrove leaves from the Mandovi and Zuari estuaries.

7.3.1.2. Sediment

The $\delta^{13}\text{C}_{\text{sed}}$ values recorded in M varied between -26.14‰ to -24.31‰ while in Z it ranged between -25.67‰ to -24.54‰ (**Fig. 7.2.**). The average $\delta^{13}\text{C}_{\text{sed}}$ in M_m was -25.76‰ and in M_w was -24.81‰ . While in Z_m and Z_w the average $\delta^{13}\text{C}_{\text{sed}}$ values were -24.76‰ and -25.23‰ , respectively.

The $\delta^{15}\text{N}_{\text{sed}}$ values ranged from 2.20‰ to 7.96‰ in M while it varied from 2.73‰ to 6.04‰ in Z (**Fig. 7.2.**). M_m (average 4.90‰) and Z_m (average 5.92‰) had depleted $\delta^{15}\text{N}_{\text{sed}}$ values than their respective estuarine sites M_w (5.34‰) and Z_w (average 4.80‰).

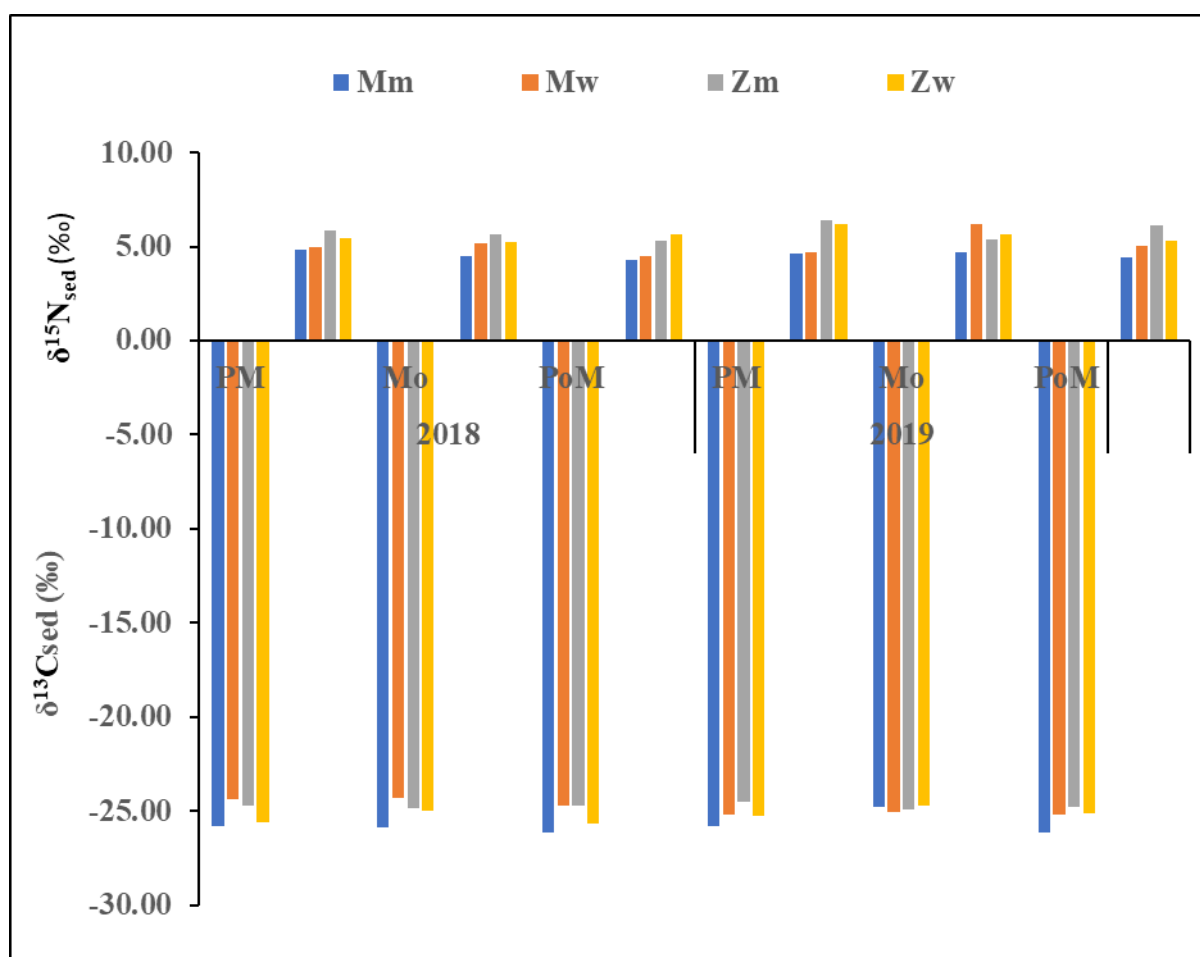


Fig. 7.2. Variations in $\delta^{13}\text{C}_{\text{sed}}$ and $\delta^{15}\text{N}_{\text{sed}}$ during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

7.3.2. Spatio-temporal variation of elemental ratio of carbon and nitrogen

7.3.2.1. Mangrove leaves

The C:N_{mng} ratios of mangrove leaves varied from 10.65 to 42.85 with an average of 24.62 (**Table 7.1.**). The highest C:N_{mng} ratio was found in leaves of *Rhizophora mucronata*, while the lowest C:N_{mng} belonged to *Bruguiera gymnorrhiza*.

Table 7.1. Spatio-temporal variation of C:N_{mng} ratios of mangrove leaves from the Mandovi and Zuari estuaries.

Species	C:N _{mng}
<i>Sonneratia alba</i>	29.85
<i>Avicennia officinalis</i>	24.76
<i>Avicennia ilicifolius</i>	15.88
<i>Bruguiera gymnorrhiza</i>	10.65
<i>Rhizophora mucronata</i>	42.85
<i>Excoecaria agallocha</i>	23.71

7.3.2.2. Sediment

The C:N_{sed} ratio in M was recorded between 10.01 to 18.71 and in Z between 7.54 to 15.69 (**Table 7.2.**). The average C:N_{sed} ratios obtained from this study were 13.44, 12.69, 11.90 and 11.27 in M_m, M_w, Z_m and Z_w, respectively. It was observed that the ratios in M_m > M_w and Z_m > Z_w. C:N_{sed} was highest during Mo (13.18 to 18.71) and least in PM (7.54 to 10.74).

Table 7.2. Spatio-temporal variation of C:N_{sed} ratios during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_m - Mandovi mangroves; M_w - Mandovi estuarine water; Z_m - Zuari mangroves; Z_w - Zuari estuarine water)

	2018			2019		
	PM	Mo	PoM	PM	Mo	PoM
M _m	10.74	18.71	13.58	10.38	15.73	11.51
M _w	10.05	16.31	13.52	10.01	15.14	11.10
Z _m	8.49	15.69	12.32	8.99	13.79	12.12
Z _w	7.54	15.42	11.19	8.52	13.18	11.77

7.4. Discussion

In general, the $\delta^{13}\text{C}$ signatures of mangroves is close to terrestrial C3 plants (-32‰ to -22‰) (Boutton 1996), indicating that mangroves follow the C3 photosynthetic pathway (Calvin-Benson cycle). The recorded $\delta^{13}\text{C}_{\text{mng}}$ (-30.73‰ to -27.73‰) and $\delta^{13}\text{C}_{\text{sed}}$ values of M_m and M_w (-26.14‰ to -24.31‰) coincided with the range typical for mangroves plants (-30‰ to -25‰ ; Muzuka and Shunula 2006) as well as that for marine phytoplankton (-18‰ to -24‰ ; Fry and Sherr 1989). In the case of Z_m and Z_w (-25.67‰ to -24.54‰), similar observations were noted. The observed $\delta^{13}\text{C}$ ranges has also been reported from other tropical mangrove dominated estuaries, like the Pichavaram estuarine-mangrove ecosystem of India (Prasad and Ramanathan 2009), and the Guayas River Estuary ecosystem of Ecuador (Cifuentes et al. 1996). The mixture of terrestrial derived material and marine derived material in the sedimentary OM has also been reported from the Hunts Bay, Forth estuary, Yangtze estuary, Pearl River estuary and Cochin estuary (Andrews et al. 1998; Graham et al. 2001; Zhang et al. 2007; Gireeshkumar et al. 2013). The concurrence of the $\delta^{13}\text{C}$ values suggests a mixed carbon input derived from both mangrove-derived OM and marine phytoplankton, indicating outwelling of OM from mangroves into the estuarine waters.

$\delta^{15}\text{N}$ signatures of marine plankton typically have an average of 6‰ ranging from 4 to 10‰ , and terrestrial OM ranges from -10 to 10‰ averaging 2‰ (Alonso-Hernández et al.

2020). The recorded $\delta^{15}\text{N}_{\text{mng}}$ (3.93 ‰ to 7.67 ‰) exhibited overlapping values typical for both marine and terrestrial OM. The $\delta^{15}\text{N}_{\text{sed}}$ in M_m (4.08 ‰ to 5.31 ‰) and Z_m (5.60 ‰ to 6.40 ‰) indicated a consistent and narrower variation, implying a steady OM source predominantly originating from mangroves in these areas. Contrastingly, the wider variability in $\delta^{15}\text{N}_{\text{sed}}$ observed in M_w (2.20 ‰ to 7.96 ‰) and Z_w (2.73 ‰ to 5.97 ‰) suggests a more diverse and mixed origin for the sedimentary OM. This broader range indicates the potential contribution from multiple sources, leading to a varied nitrogen isotopic composition within the sediment, reflecting a more complex input of OM in these estuarine waters. The possible contribution of OM from both the terrestrial and marine sources has also been reported from the Bay of Bengal (Krishna et al. 2013) and the estuarine system of West Africa (Dan et al. 2019).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data are often insufficient for source identification of OM in dynamic systems like the M and Z estuaries, which experience major seasonal variations in hydrology and biogeochemistry. Hence, the C:N ratio is a useful indicator of OM origin. For terrestrial OM, higher ratios of 12 to 200 have been recorded (Hedges and Mann 1979), whereas marine OM is defined by an observed excess of C over N with ratios of 10 or less (Pocklington and Leonard 1979). The observed C:N_{mng} ratios (10.65 to 42.85) suggest a terrestrial origin of OM as they fall within the range characteristic of terrestrial OM, typically observed in plant material due to the high cellulose and lignin content (Fourqurean and Schrlau 2010).

Furthermore, the peak C:N_{sed} ratios recorded during Mo suggested a substantial terrigenous OM input originating from runoff events during this period. Moreover, the elevated C:N_{sed} ratios in M corroborated with its higher terrigenous influence, attributed to the greater number of freshwater tributaries flowing into this estuary. Conversely, the lower C:N_{sed} ratios in Z indicated a potentially higher proportion of marine-derived OM. Overall, the observed C:N ratios indicated a mixture of marine and terrestrial sources of OM, similar

to findings reported for the Yellow River estuary and the Beibu Gulf (Liu et al. 2015; Liao et al. 2018).

7.5. Summary

One of the important objectives of this study was to test the outwelling hypothesis on the M and Z estuaries by identifying the sources of OM using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ coupled with organic C and N elemental ratios. The use of stable isotopic studies and elemental ratios to understand the outwelling of OM from mangroves and its implications on the productivity of the M and Z estuaries has not been previously addressed in the literature. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in mangrove-dominated estuaries reveal a mixed carbon input from both mangrove-derived OM and marine phytoplankton, with $\delta^{13}\text{C}$ values in M_m , M_w , Z_m , and Z_w aligning with ranges typical for mangrove plants and marine phytoplankton. The $\delta^{15}\text{N}$ values also exhibit overlapping characteristics of both marine and terrestrial OM, with M_w and Z_w showing broader variability, indicating diverse OM sources. The C:N ratios further distinguish the OM origins, with higher ratios indicating terrestrial sources, especially evident during the Mo season due to increased runoff. The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios collectively suggest that these estuaries experience significant input from both terrestrial (including mangroves) and marine sources, with seasonal variations influencing the proportion and composition of OM. This complex interplay of OM sources highlights the dynamic nature of OM deposition in these ecosystems. It further confirms that the productivity in the M and Z estuaries is enhanced by mangrove-derived OM through the process of outwelling.

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PUBLICATIONS

1. **Seleyi, S.C.**, Ravindran, C., Mohandass, C., Patra, P. (2024). Seasonal shift in trophic status induced by bacterial metabolic activity in tropical mangrove dominated estuaries of Southwest India. *Wetlands Ecology and Management*. 1-16.
2. **Seleyi, S.C.**, Ravindran, C., Mohandass, C., Patra, P., Naik, T. (2024) Seasonal dynamics of mangrove outwelling and its impact on estuarine productivity: insights from multi-parameter analysis across tidal variations. *Aquatic Sciences*. 86 (3): 78.

Under Communication

1. **Seleyi, S.C.**, Ravindran, C., Mohandass, C., Patra, P. Extracellular Enzyme Activity and its implications on the degradation of organic matter in tropical mangrove-dominated estuaries. *Aquatic Microbial Ecology*. Under review.

CONFERENCE PRESENTATIONS

1. **Poster Presentation** at Conference on Conservation Science, Indian Institute of Science, Bengaluru, India, 9th – 12th October 2023, on the paper titled “*Blue carbon outwelling influences the trophic productivity in mangrove dominated tropical estuaries of Southwest India*”. **Seleyi, S.C.**, Ravindran, C., Mohandass, C.
2. **Oral Presentation** at International Conference on Emerging Trends in Microbiology M.L.K.(P.G.) College, Balrampur (U.P.) in association with Microbiologists Society, India, 28 February-1 March, 2023 on the paper titled “*Seasonal bacterial diversity in two mangrove dominated tropical estuaries and their possible influence on OM mineralization*”. **Seleyi, S.C.**, Ravindran, C., Mohandass, C.
3. **Oral Presentation** at 62nd Annual International Conference of Association of Microbiologists of India, University of Mysore, 21-23 September, 2022, on the paper titled “*Bacterial community mediates carbon outwelling in mangrove dominated tropical estuaries*”. **Seleyi, S.C.**, Ravindran, C., Mohandass, C.
4. **Workshop attended on INYAS-CUPB Lecture Workshop ‘tangled bank’** organized by Indian National Young Academy of Sciences and Department of Botany, Central University of Punjab, Bathinda, India in connection with World Environment Day, 31 May-5 June, 2020.
5. **Conference attended on Silver Jubilee Conference on Mangrove Ecosystem.** organized by Mangrove Society of India (MSI) and CSIR-National Institute of Oceanography (NIO), 26-27 July, 2017.



Seasonal shift in trophic status induced by bacterial metabolic activity in tropical mangrove-dominated estuaries of Southwest India

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Abstract Mangroves are highly productive ecosystems that supply carbon and nutrients to the coastal food web from autochthonous and allochthonous sources. The fluctuations in the quantity of these sources influence bacterial metabolic activity which is, in turn, directly linked to the trophic food web. Our study, reports the inter-annual variability of nutrients and dissolved organic carbon in addition to their impact on phytoplankton and bacterial biomass, respiration and production in the Mandovi and Zuari estuaries of southwest India. Radio-labeled substrates, $\text{NaH}^{14}\text{CO}_3$, and titrated thymidine (^3H -TdR) were employed to quantify primary productivity (PP) and bacterial productivity (BP), respectively. Our findings revealed that community respiration exceeded PP indicating a state of heterotrophy. Similarly, bacterial respiration (BR) exceeded BP suggesting that carbon is predominantly utilized for respiratory rather than assimilatory action. Analysis of BP/PP (Mandovi 0.48 to 4.46; Zuari 1.72 to 3.81) and PP/BR (Mandovi

0.02 to 1.42; Zuari 0.05 to 1.00) ratios showed considerable seasonal variability. Lower bacterial growth efficiency observed in Mandovi (average 30.35%) than Zuari (average 38.38%) suggests greater productivity in Zuari. Uncoupling of phytoplankton and bacteria during the monsoon and post-monsoon seasons implied heterotrophic conditions in both Mandovi and Zuari. Principal component analysis indicated distinct temporal variations in salinity, dissolved inorganic nutrients and dissolved organic carbon which had a significant influence on the trophic status of the mangrove and estuarine waters. This study highlights the critical role of allochthonous organic matter in driving coastal heterotrophy and shaping the seasonal trophic dynamics in estuarine ecosystems.

Keywords Bacterial productivity · Respiration · Allochthonous carbon · Trophic status · Tropical mangroves

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Introduction

Tropical mangroves are carbon (C) rich ecosystems that play a pivotal role in enhancing productivity in adjacent waters by facilitating the transfer of organic C and nutrients to higher trophic levels (Odum 1968; Abrantes et al. 2015). This transfer is mediated by heterotrophic bacteria which are the main consumers of organic C and nutrients responsible for the majority of the global marine metabolism by serving

as the link between dissolved organic carbon (DOC) and higher trophic levels (Azam et al. 1983; Pomeroy 1984). This link is sustained by bacteria through utilization of DOC for biomass production (i.e., bacterial production, BP) or by respiration of organic C to CO₂ (i.e., bacterial respiration, BR). The amount of C used for BP versus the total amount consumed for metabolism (both BP and BR) is referred to as bacterial growth efficiency (BGE) (del Giorgio and Cole 1998). BGE directly impacts the microbial food web which in turn, influences the phytoplankton-derived organic matter (Danger et al. 2007). The CO₂ production, the transfer of C to higher trophic levels as well as the export and storage of C, are all significantly impacted by this (García-Martín et al. 2019). Thus, the relationship between bacterial parameters (BR and BP) and phytoplankton productivity are prerequisite to determining the trophic status of the system (Thottathil et al. 2008a, b).

Understanding the dynamics of organic matter transformation and mineralization in mangrove ecosystems is essential, considering they receive organic material from both autochthonous (internally produced) and allochthonous (externally derived) sources which respond dynamically to environmental shifts. Highly productive systems are known to be fuelled by allochthonous C accelerating the loss of biogenic matter, while less productive systems are sustained by autochthonous C (Peinert et al. 1989). The metabolic balance of a system is said to be heterotrophic when the BP/PP and PP/BR ratios are > 1 and < 1 respectively, while the system is deemed autotrophic when the BP/PP and PP/BR ratios are < 1 and > 1 respectively (Thottathil et al. 2008a, b; Lagaria et al. 2010; Rahav et al. 2013, 2015; Robin et al. 2016). In an autotrophic estuary, organic matter can be harvested, buried, or exported to adjacent regions (Fisher and Likens 1973). However, when the estuarine system turns heterotrophic, its metabolic activities are supported by organic matter produced in situ or imported from land or coastal ocean (Odum 1968).

Limited studies on trophic status are reported from the tropical waters of South Asia belonging to Peninsular Malaysia (Lee and Bong 2006; Lee et al. 2009); Pearl River estuary, China (Xu et al. 2014; Li et al. 2019); Daya Bay, China (Song et al. 2015) and Gyeonggi Bay, South Korea (Kim et al. 2020). Despite India's significant mangrove cover, constituting more than 45% of South Asia's total, investigations into

the trophic dynamics within its mangrove ecosystems and their potential influence on estuarine water productivity remain unexplored (Forest Survey of India 2017). With reference to the tropical waters of India, the existing literature on trophic status studies remains relatively sparse and primarily focused on estuarine and coastal waters (Ram et al. 2003; 2007; Thottathil et al. 2008a, b; Robin et al. 2016; Gawade et al. 2017). These studies revealed shifts from autotrophy to heterotrophy driven by seasonal changes and allochthonous inputs, emphasizing the pivotal role of bacteria in carbon fluxes. However, the omission of the role of mangroves in these investigations despite harboring a substantial mangrove cover along the coast, highlights a significant gap in the existing literature.

The Mandovi (M) and Zuari (Z) estuaries are a part of the Northern Indian Ocean (southeastern Arabian Sea), harboring a mangrove cover of 700 and 900 ha, respectively (Wakle et al. 2018). These regions experience distinct seasonal patterns: the south-west monsoon (June–September) brings heavy precipitation (250–300 cm yr⁻¹), while the dry season (November–May) sees a drastic reduction to less than 10 cm yr⁻¹ (Shetye and Murty 1987). During post-monsoon, significant organic matter transport occurs, potentially impacting the ecosystem's metabolism (Shynu et al. 2015; Wakle et al. 2018). These estuaries foster a thriving fish population (154 fish taxa in M; 176 fish taxa in Z) which is a source of food and livelihood for the local population (Rodrigues et al. 2011; Sreekanth et al. 2020). Within the mangrove ecosystem, bacteria play a pivotal role in converting complex organic matter into simpler forms, contributing to the coastal food web, and ultimately influencing fish production (Chassot et al. 2010; Blanchard et al. 2012). Understanding the trophic status (autotrophic or heterotrophic), assessing BP/PP and PP/BR ratios, and examining BGE variability in such systems are imperative for comprehending the bacteria's role in sustaining an efficient coastal food web. Thus, the present study addresses three main questions: (1) what is the variation of bacterial and phytoplankton parameters within mangrove forests and adjacent estuaries in relation to environmental parameters?; (2) how do seasonal changes impact the trophic status of these estuaries? and (3) what is the influence of mangrove ecosystems on the productivity of estuarine waters?. Through addressing these questions,

this study aims to contribute valuable insights into the ecological dynamics of mangrove ecosystems and their broader implications for coastal productivity. Additionally, we provide for the first time, insights into the trophic status within the mangroves of the M and Z estuaries.

Materials and methods

Study sites

A total of six sites were selected for sampling from each estuary i.e., Mandovi and Zuari. Three sites selected in the Mandovi and Zuari mangrove forests were labeled as M_m1 , M_m2 , M_m3 and Z_m1 , Z_m2 , Z_m3 respectively. Another three estuarine water sites were selected directly opposite at a distance of ~300–400 m from the Mandovi and Zuari mangrove forests and were marked as M_w4 , M_w5 , M_w6 (Mandovi estuarine waters) and Z_w4 , Z_w5 , Z_w6 (Zuari estuarine waters) (Fig. 1) respectively. The samples collected from M_m1 , M_m2 , and M_m3 were treated as triplicates and represented as M_m . Similarly, samples

collected from M_w4 – M_w6 ; Z_m1 – Z_m3 , and Z_w4 – Z_w6 , represented M_w , Z_m , and Z_w respectively.

Sampling strategy

Sampling was carried out during the premonsoon (PM), monsoon (Mo), and postmonsoon (PoM) seasons for two consecutive years (2018 and 2019). Surface water samples (depth < 0.5 m), from mangrove sites were collected manually whereas estuarine water samples were collected using a Niskin sampler.

Hydrological parameters

Field measurements of in-situ surface water temperature and salinity were carried out using a portable thermometer and a handheld refractometer (ATAGO PAL-06S Pocket refractometer). pH was determined using a pH meter (Eutech pH 700). Dissolved oxygen (DO) concentrations of water samples were estimated by Winkler's iodometric method (Carpenter 1965). The concentrations of dissolved inorganic nutrients i.e., nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), and phosphate (PO_4^{3-}) were

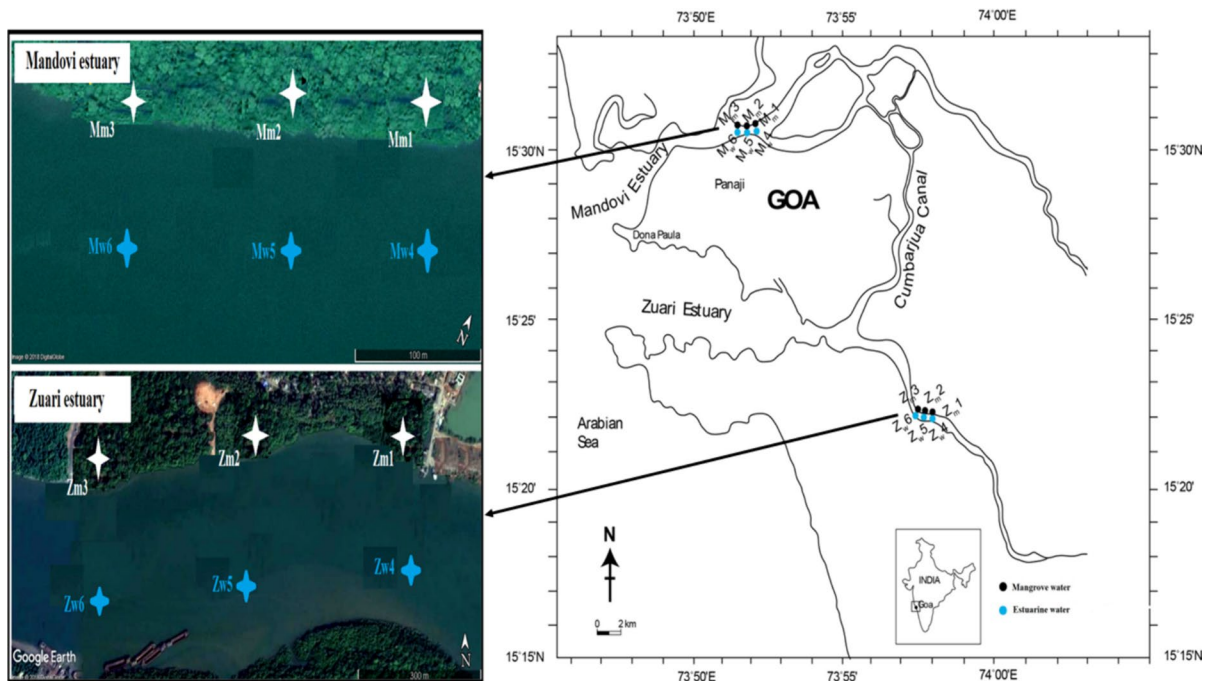


Fig. 1 Location of sampling sites in the Mandovi (M_m —Mandovi mangroves; M_w —Mandovi estuarine waters) and Zuari (Z_m —Zuari mangroves; Z_w —Zuari estuarine waters) estuaries, Goa, India

determined by standard colorimetric methods (Grashoff et al. 1983) using an Autoanalyser (Skalar San Plus). Dissolved organic carbon (DOC) was estimated using a total organic carbon analyzer (Shimadzu TOC LCPH) (Sugimura and Suzuki 1988). Chlorophyll *a* (Chl *a*) was measured using the Fluorometer (Turner 7200-000). Water sample (1L) was filtered through 0.7 µm GF/F Whatman filter paper, pigments were extracted in 90% acetone (UNESCO 1994), and fluorescence was measured using a fluorometer (Turner 7200-000). A standard, Chl *a* (Sigma) was used to calibrate the fluorometer.

Enumeration of bacterial abundance

Water samples were fixed with buffered formaldehyde (2% final concentration). Following the method of Porter and Feig (1980), 2 ml subsamples were incubated for 20 min with 40, 6-diamidino-2-phenylindole (DAPI; 20 µl of 1 mg ml⁻¹ working solution) and filtered through black polycarbonate membrane filters (0.22 µm, Millipore). The bacterial cell counts were determined by counting 20 observed fields to obtain a reliable mean using an epifluorescence microscope (Olympus BX-51 Japan).

Primary productivity

Primary productivity (PP) was measured using the ¹⁴C assimilation method (Strickland and Parsons 1972). Light and dark bottles (in duplicate) for each study location, were washed with 10% HCl and filled with 300 ml water samples from every site. Labeled NaH¹⁴CO₃ (Specific activity: 5 µCi ml⁻¹; Bhabha Atomic Research Centre, Mumbai, India) was added to each bottle and incubated at in situ temperature for 12 h. After incubation, water samples were immediately filtered through a 0.7 µm filter (Whatman) under diffused light and low pumping pressure (<50 mm Hg). The filters were then exposed to concentrate HCl for 1 min [to remove radiolabeled dissolved inorganic carbon (DI¹⁴C)] and placed in scintillation vials. Scintillation cocktail in dioxane (Spectrochem, Mumbai, India) was added right before the radioactivity was measured in a liquid scintillation counter (PerkinElmer Wallac 1409 DSA, USA).

Bacterial productivity

Bacterial productivity was determined by the incorporation of titrated thymidine (³H-TdR) into bacterial DNA (Fuhrman and Azam 1982). Water samples were filtered through a 0.7 µm GF/F filter (Whatman) at a low differential pressure (<50 mm Hg) to avoid rupture of fragile cells. Control (in duplicate) and water samples (in duplicate) of 20 ml were setup for each study site. Water samples were incubated with ³H thymidine (³H-TdR) (Specific activity: 18 Ci mmol⁻¹, Bhabha Atomic Research Centre) in situ conditions for 1 h (dark) and arrested by adding 2% formalin. The activity of the control was immediately terminated after the addition of ³H thymidine (³H-TdR) with 2% formalin. The samples were filtered through a 0.22 µm cellulose nitrate filter [presoaked with 5% trichloroacetic acid (TCA)], extracted in cold 5% TCA, and rinsed with 80% ethanol. The dried filters were placed in scintillation vials with dioxane-based scintillation cocktail (Spectrochem, Mumbai, India) and radioactivity was measured using a liquid scintillation counter (PerkinElmer Wallac 1409 DSA, USA). BP, calculated as mol TdR incorporated into DNA, was converted into the number of bacterial cells produced by applying a conversion factor (1.96 × 10¹⁸ cells mol⁻¹) (Ram et al. 2007) and to carbon by using a conversion factor of 2 × 10⁻¹⁴ g C cell⁻¹ (Lee and Fuhrman 1987).

Community respiration

Community respiration is the consumption of oxygen by the planktonic population when incubated in the dark for 24 h and is estimated by Winkler's titration method (Carignan et al. 2000). Water sample (125 ml) was collected in duplicates in biological oxygen demand (BOD) bottles, fixed immediately with Winkler's reagents and the initial DO (D1) was measured. Another duplicate set of BOD bottles (125 ml) containing water samples was incubated at in situ temperature in the dark for 24 h, fixed with Winkler's reagent, and DO (D2) was measured. The total respiration was calculated as, D1–D2. The oxygen consumed was converted to carbon respired by assuming a respiratory quotient of 1 (Biddanda et al. 1994).

Bacterial respiration

Bacterial respiration was estimated by filtering water samples through 0.7 µm GF/F Whatman filter paper, following which the samples were siphoned carefully into the BOD bottles (125 ml) to avoid the formation of air bubbles. For each site, controls were performed in duplicates and the initial DO (D1) concentration was measured by Winkler's method. Another set of duplicate bottles was incubated at in situ temperature in the dark for 24 h, fixed with Winkler's reagent, and DO (D2) was measured. The calculations for bacterial respiration are described in "Community respiration" section.

Bacterial growth efficiency

Bacterial growth efficiency (BGE) was derived as the slope of bacterial production versus the sum of bacterial production and bacterial respiration, and expressed as a percentage (Moran and Hodson 1990).

$$BGE(\%) = [BP / (BP + BR)] \times 100$$

Statistical analyses

The statistical software IBM® SPSS 23.0 was used to determine the statistical significance and each result was stated in mean ± standard deviation (SD). For reduction in the number of inter-correlated variables, principal components analysis (PCA) was performed with nutrients, DOC, Chl *a*, BA, PP, BP, CR, BR, and BGE. A PCA biplot based on correlation was obtained by combining the sample scores plot and the vector loading plots. One-way analysis of variance (ANOVA) was used to identify significant variations between the physicochemical and biological parameters. Subsequently, post hoc Tukey's HSD analysis compared estuarine and mangrove waters within M and Z for each season to determine the mutual significance of interactions between the variables.

Results

Environmental parameters

In Mandovi (M_m and M_w), the surface water temperatures varied between 27.5 and 31.9 °C while in Zuari (Z_m and Z_w) it ranged between 27.2 and 32.4 °C (Table 1). The highest temperatures were recorded during the PM, followed by PoM and Mo in both M and Z. The difference in temperatures in the mangrove and estuarine waters of M and Z were insignificant. The salinity was higher in Z (2–31 ppt) than in M (3–29 ppt), during all seasons with a significant drop during the Mo season (3–4 ppt in M and 2–6 ppt in Z) (Table 1). No substantial variations in salinity were noted in the mangrove and estuarine waters of M and Z, suggesting the water is well mixed. The pH remained relatively stable (average 7) in the mangrove and estuarine water sites of both M and Z during all seasons (Table 1). DO concentrations ranged from 4.35 (±0.29) to 6.94 (±0.18) mg/L and 4.82 (±0.19) to 7.98 (±0.01) mg/L in M and Z, respectively (Table 1). The highest DO concentrations were observed during the Mo, followed by PoM and PM with no notable difference between the mangrove and estuarine waters of M and Z.

In M, concentrations of NO_2^- , NO_3^- , NH_4^+ and PO_4^{3-} ranged from 0.07 (±0.02) to 3.69 (±0.04), 2.44 (±0.14) to 16.35 (±0.03), 0.32 (±0.23) to 2.50 (±0.03) and 0.06 (±0.02) to 1.89 (±0.01) µmol/L, respectively (Table 1). Seasonally, the concentration of nutrients was highest during Mo, followed by PM and PoM. In general, higher concentrations of nutrients were prevalent in M_w in comparison to M_m . In Z, NO_2^- concentrations varied between 0.06 (±0.00) to 5.28 (±0.09) µmol/L, while NO_3^- , NH_4^+ , and PO_4^{3-} concentrations varied between 2.17 (±0.04) to 24.23 (±0.25), 0.72 (±0.11) to 8.05 (±0.05) and 0.29 (±0.05) to 3.72 (±0.02) µmol/L, respectively (Table 1). In the case of Z, nutrient concentrations were maximum during Mo followed by PM and PoM; and nutrient concentrations were higher in Z_w than in Z_m . DOC concentration in M varied from 4.92(±0.04) to 40.53(±0.03) mg/L and in Z it ranged from 3.39(±0.01) to 40.01(±0.01) mg/L (Table 1). Higher concentrations of DOC were observed in mangrove waters than in estuarine waters in both M and Z estuaries. The maximum DOC concentrations were recorded during Mo, followed by PoM and PM

Table 1 Seasonal variation (mean $\pm$ standard deviation) of physicochemical and biological parameters in the mangrove and estuarine waters of Mandovi and Zuari

	2018				2019			
	M _m	M _w	Z _m	Z _w	M _m	M _w	Z _m	Z _w
Pre-monsoon								
Temperature (°C)	31.74 $\pm$ 0.02	31.90 $\pm$ 0.22	32.33 $\pm$ 0.09	32.41 $\pm$ 0.09	30.40 $\pm$ 0.61	30.80 $\pm$ 0.71	30.54 $\pm$ 0.08	30.23 $\pm$ 0.18
Salinity (ppt)	28	27	31	30	29	29	30	31
pH	7.63 $\pm$ 0.21	7.64 $\pm$ 0.20	7.65 $\pm$ 0.00	7.69 $\pm$ 0.03	7.50 $\pm$ 0.03	7.53 $\pm$ 0.04	7.43 $\pm$ 0.02	7.51 $\pm$ 0.01
DO (mg/L)	5.08 $\pm$ 0.04	5.18 $\pm$ 0.25	5.18 $\pm$ 0.01	5.27 $\pm$ 0.04	4.35 $\pm$ 0.29	4.60 $\pm$ 0.29	4.86 $\pm$ 0.03	4.82 $\pm$ 0.19
NO ₂ ⁻ (μmol/L)	1.32 $\pm$ 0.04	1.40 $\pm$ 0.05	1.06 $\pm$ 0.01	1.19 $\pm$ 0.04	1.30 $\pm$ 0.02	1.54 $\pm$ 0.03	1.01 $\pm$ 0.01	2.09 $\pm$ 0.01
NO ₃ ⁻ (μmol/L)	3.35 $\pm$ 0.10	4.12 $\pm$ 0.02	3.05 $\pm$ 0.04	3.46 $\pm$ 0.05	6.25 $\pm$ 0.21	8.90 $\pm$ 0.08	7.96 $\pm$ 0.02	8.85 $\pm$ 0.06
NH ₄ ⁺ (μmol/L)	0.75 $\pm$ 0.04	0.78 $\pm$ 0.02	4.65 $\pm$ 0.15	5.03 $\pm$ 0.03	1.26 $\pm$ 0.03	1.46 $\pm$ 0.03	7.74 $\pm$ 0.06	8.05 $\pm$ 0.05
PO ₄ ³⁻ (μmol/L)	0.54 $\pm$ 0.04	0.81 $\pm$ 0.04	1.15 $\pm$ 0.01	1.49 $\pm$ 0.10	0.12 $\pm$ 0.02	0.36 $\pm$ 0.08	0.72 $\pm$ 0.09	1.56 $\pm$ 0.28
DOC (mg/L)	4.92 $\pm$ 0.04	5.05 $\pm$ 0.01	3.39 $\pm$ 0.01	3.76 $\pm$ 0.01	7.46 $\pm$ 0.05	7.36 $\pm$ 0.02	7.61 $\pm$ 0.23	7.82 $\pm$ 0.12
Chl <i>a</i> (μg/L)	4.29 $\pm$ 0.02	5.08 $\pm$ 0.09	9.34 $\pm$ 0.02	9.23 $\pm$ 0.13	4.74 $\pm$ 0.13	4.78 $\pm$ 0.11	8.08 $\pm$ 0.05	8.26 $\pm$ 0.27
BA ($\times 10^6$ cells/ml)	4.84 $\pm$ 1.02	4.53 $\pm$ 1.09	3.49 $\pm$ 2.02	2.84 $\pm$ 2.13	4.78 $\pm$ 1.13	3.84 $\pm$ 2.11	4.84 $\pm$ 1.05	4.53 $\pm$ 1.27
Monsoon								
Temperature (°C)	28.11 $\pm$ 0.05	28.56 $\pm$ 0.04	27.88 $\pm$ 0.09	27.90 $\pm$ 0.06	29.10 $\pm$ 0.10	28.90 $\pm$ 0.36	28.11 $\pm$ 0.21	28.40 $\pm$ 0.10
Salinity (ppt)	3	4	5	6	3	3	2	3
pH	6.95 $\pm$ 0.03	7.06 $\pm$ 0.02	7.23 $\pm$ 0.02	7.28 $\pm$ 0.04	7.12 $\pm$ 0.01	7.16 $\pm$ 0.04	7.04 $\pm$ 0.30	7.11 $\pm$ 0.02
DO (mg/L)	6.30 $\pm$ 0.10	6.94 $\pm$ 0.18	5.83 $\pm$ 0.09	5.97 $\pm$ 0.01	6.49 $\pm$ 0.35	6.72 $\pm$ 0.01	7.69 $\pm$ 0.20	7.98 $\pm$ 0.01
NO ₂ ⁻ (μmol/L)	3.28 $\pm$ 0.03	3.69 $\pm$ 0.04	4.70 $\pm$ 0.03	4.87 $\pm$ 0.01	4.07 $\pm$ 0.02	5.04 $\pm$ 0.03	4.28 $\pm$ 0.09	5.28 $\pm$ 0.09
NO ₃ ⁻ (μmol/L)	14.79 $\pm$ 0.06	15.36 $\pm$ 0.16	22.49 $\pm$ 0.02	24.23 $\pm$ 0.25	15.56 $\pm$ 0.31	16.35 $\pm$ 0.03	22.19 $\pm$ 0.04	23.34 $\pm$ 0.04
NH ₄ ⁺ (μmol/L)	1.84 $\pm$ 0.19	3.07 $\pm$ 0.03	6.17 $\pm$ 0.06	7.38 $\pm$ 0.32	20.23 $\pm$ 0.02	21.50 $\pm$ 0.23	55.49 $\pm$ 0.05	52.84 $\pm$ 0.11
PO ₄ ³⁻ (μmol/L)	1.89 $\pm$ 0.01	1.62 $\pm$ 0.02	3.55 $\pm$ 0.05	3.64 $\pm$ 0.05	1.19 $\pm$ 0.06	1.15 $\pm$ 0.02	3.41 $\pm$ 0.35	3.72 $\pm$ 0.02
DOC (mg/L)	39.66 $\pm$ 0.17	40.53 $\pm$ 0.03	38.36 $\pm$ 0.01	40.01 $\pm$ 0.01	33.43 $\pm$ 0.03	38.69 $\pm$ 0.06	30.21 $\pm$ 0.03	34.63 $\pm$ 0.10
Chl <i>a</i> (μg/L)	2.12 $\pm$ 0.02	2.09 $\pm$ 0.02	1.51 $\pm$ 0.03	2.31 $\pm$ 0.02	2.54 $\pm$ 0.17	2.30 $\pm$ 0.22	2.25 $\pm$ 0.12	3.04 $\pm$ 0.10
BA ($\times 10^6$ cells/ml)	9.47 $\pm$ 1.22	8.48 $\pm$ 2.02	8.32 $\pm$ 1.03	7.76 $\pm$ 3.02	9.39 $\pm$ 1.17	7.91 $\pm$ 2.22	7.22 $\pm$ 3.12	6.68 $\pm$ 2.10
Post-monsoon								
Temperature (°C)	27.47 $\pm$ 0.04	27.80 $\pm$ 0.05	27.88 $\pm$ 0.06	27.40 $\pm$ 0.09	28.07 $\pm$ 0.15	28.50 $\pm$ 0.23	27.07 $\pm$ 0.15	27.56 $\pm$ 0.21
Salinity (ppt)	19	21	22	23	17	18	21	20
pH	7.44 $\pm$ 0.03	7.48 $\pm$ 0.04	7.40 $\pm$ 0.02	7.48 $\pm$ 0.01	7.28 $\pm$ 0.01	7.35 $\pm$ 0.02	7.25 $\pm$ 0.02	7.30 $\pm$ 0.02
DO (mg/L)	5.44 $\pm$ 0.10	5.45 $\pm$ 0.26	5.37 $\pm$ 0.04	5.75 $\pm$ 0.10	6.09 $\pm$ 0.06	6.74 $\pm$ 0.05	6.78 $\pm$ 0.04	7.06 $\pm$ 0.04
NO ₂ ⁻ (μmol/L)	0.07 $\pm$ 0.02	0.10 $\pm$ 0.02	0.19 $\pm$ 0.04	0.29 $\pm$ 0.01	0.09 $\pm$ 0.04	0.12 $\pm$ 0.03	0.06 $\pm$ 0.00	0.09 $\pm$ 0.02
NO ₃ ⁻ (μmol/L)	2.57 $\pm$ 0.08	3.18 $\pm$ 0.08	2.64 $\pm$ 0.05	3.22 $\pm$ 0.02	2.44 $\pm$ 0.14	2.59 $\pm$ 0.18	2.17 $\pm$ 0.04	2.92 $\pm$ 0.04
NH ₄ ⁺ (μmol/L)	0.68 $\pm$ 0.02	0.71 $\pm$ 0.01	0.80 $\pm$ 0.01	0.98 $\pm$ 0.02	0.41 $\pm$ 0.07	0.32 $\pm$ 0.04	0.91 $\pm$ 0.12	0.72 $\pm$ 0.12
PO ₄ ³⁻ (μmol/L)	0.06 $\pm$ 0.02	0.12 $\pm$ 0.05	0.29 $\pm$ 0.05	0.36 $\pm$ 0.03	0.16 $\pm$ 0.12	0.26 $\pm$ 0.03	0.12 $\pm$ 0.02	0.62 $\pm$ 0.02
DOC (mg/L)	24.55 $\pm$ 0.01	21.98 $\pm$ 0.01	21.08 $\pm$ 0.00	19.80 $\pm$ 0.01	25.52 $\pm$ 0.04	22.30 $\pm$ 0.03	20.87 $\pm$ 0.03	20.56 $\pm$ 0.04
Chl <i>a</i> (μg/L)	1.24 $\pm$ 0.07	1.19 $\pm$ 0.04	1.12 $\pm$ 0.05	1.10 $\pm$ 0.0	1.28 $\pm$ 0.20	1.42 $\pm$ 0.16	1.21 $\pm$ 0.04	1.13 $\pm$ 0.03
BA ($\times 10^6$ cells/ml)	5.91 $\pm$ 3.07	5.11 $\pm$ 2.04	4.52 $\pm$ 2.05	3.07 $\pm$ 2.07	6.63 $\pm$ 2.2	7.30 $\pm$ 1.16	5.91 $\pm$ 2.04	5.11 $\pm$ 2.03

M_m mandovi mangroves, *M_w* mandovi estuarine waters, *Z_m* zuari mangroves, *Z_w* zuari estuarine waters, *DO* dissolved oxygen, *NO₂⁻* nitrite, *NO₃⁻* nitrate, *NH₄⁺* ammonia, *PO₄³⁻* phosphate, *DOC* dissolved organic carbon, *Chl a* chlorophyll *a*, *BA* bacterial abundance

in both the estuaries. DOC concentrations were consistently higher in M than in Z, during all seasons.

Chlorophyll *a* (Chl *a*)

Chl *a* concentration ranged between $1.19(\pm 0.02)$ to $5.08(\pm 0.09)$ $\mu\text{g/L}$ in M and $1.10(\pm 0.02)$ to $9.34(\pm 0.02)$ $\mu\text{g/L}$ in Z (Table 1). In both the estuaries, the highest Chl *a* concentration, were recorded during PM, followed by Mo and PoM. The difference in Chl *a* concentration between the mangrove and estuarine waters of both M and Z was insignificant.

Bacterial abundance (BA)

The bacterial abundance in M ranged from $3.84 (\pm 2.1)$ to $9.47 (\pm 1.2) \times 10^6$ cells/ml and in Z it varied from $2.84 (\pm 2.1)$ to $8.32 (\pm 1.0) \times 10^6$ cells/ml (Table 1). In both estuaries, mangrove waters (M_m , Z_m) showed higher BA than estuarine waters (M_w , Z_w). Season-wise comparison revealed that BA was

highest in the Mo followed by PoM and PM in both M and Z. Overall, higher BA was observed in M in comparison to Z.

Primary productivity (PP) and bacterial productivity (BP)

Within M, PP fluctuated between $2.55 (\pm 1.22)$ and $17.64 (\pm 8.92)$ $\mu\text{g C L}^{-1} \text{h}^{-1}$ while in Z it ranged from $2.56 (\pm 2.22)$ to $17.33 (\pm 5.66)$ $\mu\text{g C L}^{-1} \text{h}^{-1}$ (Fig. 2. a, b). In both M and Z, the highest PP was recorded during PM and the lowest during PoM.

In M, BP ranged from $5.79 (\pm 1.90)$ to $32.54 (\pm 8.22)$ $\mu\text{g C L}^{-1} \text{h}^{-1}$ whereas in Z it varied from $8.62 (\pm 6.34)$ to $32.82 (\pm 2.87)$ $\mu\text{g C L}^{-1} \text{h}^{-1}$ (Fig. 2c, d). In general, BP in estuarine waters (M_w , Z_w) was higher than mangrove waters (M_m , Z_m). Across both M and Z (mangrove and estuarine) waters, BP was the maximum during PM and minimum during PoM.

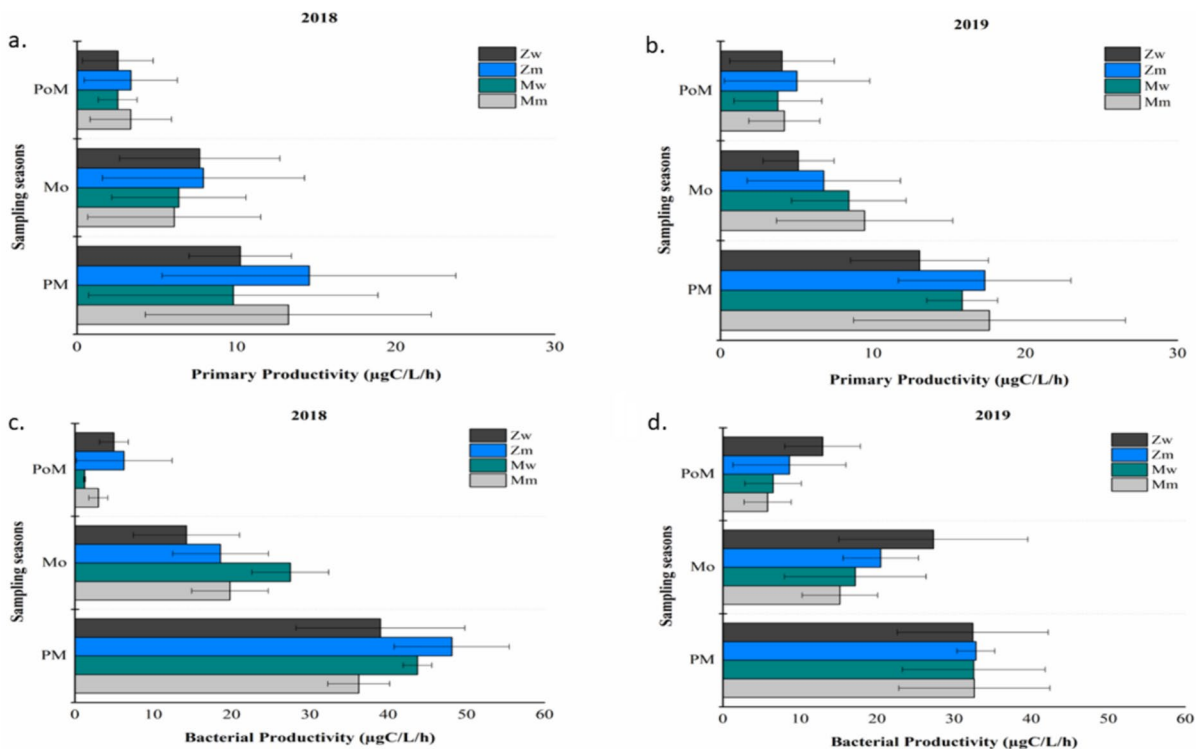


Fig. 2 Spatio-temporal variation in Primary productivity (a, b) and Bacterial productivity (c, d) in the Mandovi and Zuari estuaries during 2018 and 2019. M_m mandovi mangroves, M_w

mandovi estuarine waters, Z_m zuari mangroves, Z_w zuari estuarine waters, PM premonsoon, Mo monsoon, PoM postmonsoon

Community respiration (CR) and bacterial respiration (BR)

In M and Z, CR ranged from $16.95 (\pm 1.06)$ to $129.73 (\pm 2.37) \mu\text{g C L}^{-1} \text{h}^{-1}$ to $17.22 (\pm 0.95)$ to $207.72 (\pm 4.27) \mu\text{g C L}^{-1} \text{h}^{-1}$, respectively (Fig. 3a, b). The CR in the mangrove waters (M_m and Z_m) were higher than their respective estuarine waters (M_w and Z_w). In M, maximum values of CR were observed during PM and minimum during Mo. A similar pattern was observed in Z.

BR varied between $12.39 (\pm 0.82)$ to $159.62 (\pm 1.63) \mu\text{g C L}^{-1} \text{h}^{-1}$ in M and $13.00 (\pm 0.85)$ to $53.09 (\pm 2.69) \mu\text{g C L}^{-1} \text{h}^{-1}$ in Z (Fig. 3c, d). Higher BR were predominantly observed within M_m and Z_m in contrast to their corresponding estuarine sites (M_w and Z_w). Seasonally, BR in both M and Z was maximum during the PoM, followed by Mo and PM.

BP/PP and PP/BR ratio

BP/PP ratios were > 1 across all sampling locations throughout the three seasons, except, during PoM (2018) in M_m (0.89) and M_w (0.48) (Table 2). Higher ratios of BP/PP were observed in the M_w (average 2.51) and Z_w (average 3.10) than in M_m (average 1.95) and Z_m (average 2.36). Both the estuaries displayed their lowest BP/PP ratio was observed during PoM (average M 1.21, Z 2.18), with a steady increase in PM (average M 2.77, Z 2.87) and peaking during Mo (average M 2.80, Z 3.14).

With few exceptions, such as Z_m (PM, 2018) and M_m , M_w (PM, 2019), the PP/BR ratios recorded in all sampling sites were < 1 (Table 2). The ratios of M_m (average 0.36), M_w (average 0.31), Z_m (average 0.36), and Z_w (average 0.31) across all seasons demonstrated a comparable trend. In both the estuaries,

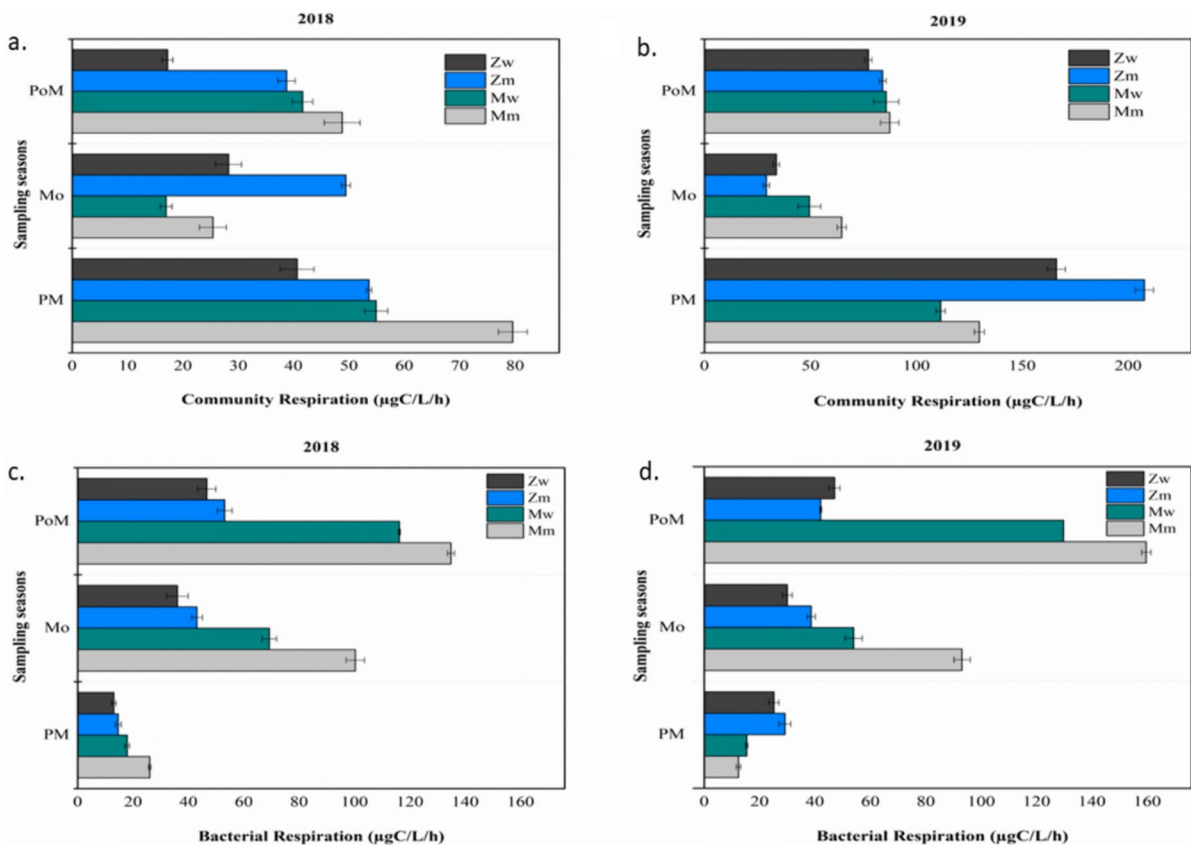


Fig. 3 Spatio-temporal variation in Community respiration (a, b) and Bacterial respiration (c, d) in the Mandovi and Zuari estuaries during 2018 and 2019. M_m mandovi mangroves, M_w

mandovi estuarine waters, Z_m zuari mangroves, Z_w zuari estuarine waters, PM premonsoon, Mo monsoon, PoM postmonsoon

Table 2 Variation in BP/PP and PP/BR ratios in the Mandovi and Zuari estuaries during 2018 and 2019

		2018				2019			
		M _m	M _w	Z _m	Z _w	M _m	M _w	Z _m	Z _w
<i>M_m</i> mandovi mangroves, <i>M_w</i> mandovi estuarine waters, <i>Z_m</i> zuari mangroves, <i>Z_w</i> zuari estuarine waters, <i>PM</i> premonsoon, <i>Mo</i> monsoon, <i>PoM</i> postmonsoon, <i>PP</i> primary productivity, <i>BP</i> bacterial productivity, <i>BR</i> bacterial respiration, <i>BGE</i> bacterial growth efficiency	BP/PP								
	PM	2.73	4.46	3.30	3.81	1.85	2.05	1.89	2.48
	Mo	3.24	4.30	2.34	1.85	1.60	2.04	3.02	5.34
	PoM	0.89	0.48	1.86	1.94	1.38	1.73	1.72	3.20
	PP/BR								
	PM	0.51	0.55	1.00	0.79	1.42	1.04	0.59	0.52
	Mo	0.06	0.09	0.18	0.21	0.10	0.16	0.18	0.17
	PoM	0.02	0.02	0.06	0.05	0.03	0.03	0.12	0.09
	BGE								
	PM	58.22	70.98	76.69	75.00	72.47	68.01	52.96	56.32
	Mo	16.48	28.42	30.16	28.33	14.01	24.14	34.67	47.69
	PoM	2.17	1.04	10.57	9.62	3.50	4.77	17.02	21.55

PP/BR ratios were lowest during PoM (average M 0.03, Z 0.08), with a gradual increase observed in Mo (average M 0.10, Z 0.19) and reaching maximum values during PM (average M 0.88, Z 0.72).

Bacterial growth efficiency (BGE)

BGE displayed a wide range of variation from 1.04% to 72.47% in M and 9.62% to 76.69% in Z (Table 2). In M, the BGE values in M_m (average 27.81%) were typically lower than M_w (average 32.90%) and in Z estuary, Z_w (average 39.75%) displayed higher values than in Z_m (average 37.00%). Seasonal fluctuations were observed, with the highest values of BGE recorded during the PM (average M 68.11%, Z 65.24%) and the lowest during the PoM (average M 2.87%, Z 14.68%) in both M and Z.

Statistical analyses

Principal component analysis (PCA) was employed to discern the influence of seasonal variability on the estuarine and mangrove waters of M and Z. PCA analysis revealed that principal components 1 and 2 cumulatively represented 68.39% of the total variability with eigenvalue > 1 (Fig. 4). Based on component 1 and 2, the PCA biplot was constructed. PCA projections revealed distinct clusters of the PM, Mo, and PoM seasons confirming that the seasonal variability had a significant influence on the estuarine and mangrove waters of Mandovi and Zuari. Notably, the primary drivers of seasonal variability were identified as

fluctuations in salinity, dissolved inorganic nutrients, and DOC. The PM season exhibited heightened levels of temperature, salinity, pH, Chl *a*, PP, BP, and BGE. Conversely, the Mo season depicted elevated nutrient concentrations, DOC, BA, and BR, in contrast, the PoM season and CR displayed minimal susceptibility to variations among the different vectors. Analysis via one-way ANOVA revealed significant differences in physicochemical and biological parameters across the study locations, except for NO₃⁻ (Table 3). Post hoc Tukey's HSD tests showed that nutrients, DOC, Chl *a*, and BA varied significantly ($p < 0.05$) between the estuarine and mangrove waters of both M and Z. However, the metabolic parameters such as PP, BP, BR, CR, and BGE did not exhibit significant variations (Table 4).

Discussion

Tropical mangrove-dominated estuaries are hot-spots of biogeochemical activity sustained by high organic matter inputs from upstream rivers and impacted by several environmental parameters (Nitttrouer et al. 1995; Brunskill 2010). Therefore, a comprehensive examination of the ambient environmental conditions is crucial for a holistic understanding of the ecosystem's processes and functioning. Our study documented surface temperatures consistent with previous findings in the M and Z estuaries as reported by Toraskar et al. (2022). Salinity exhibited marked fluctuations (2–31 ppt),

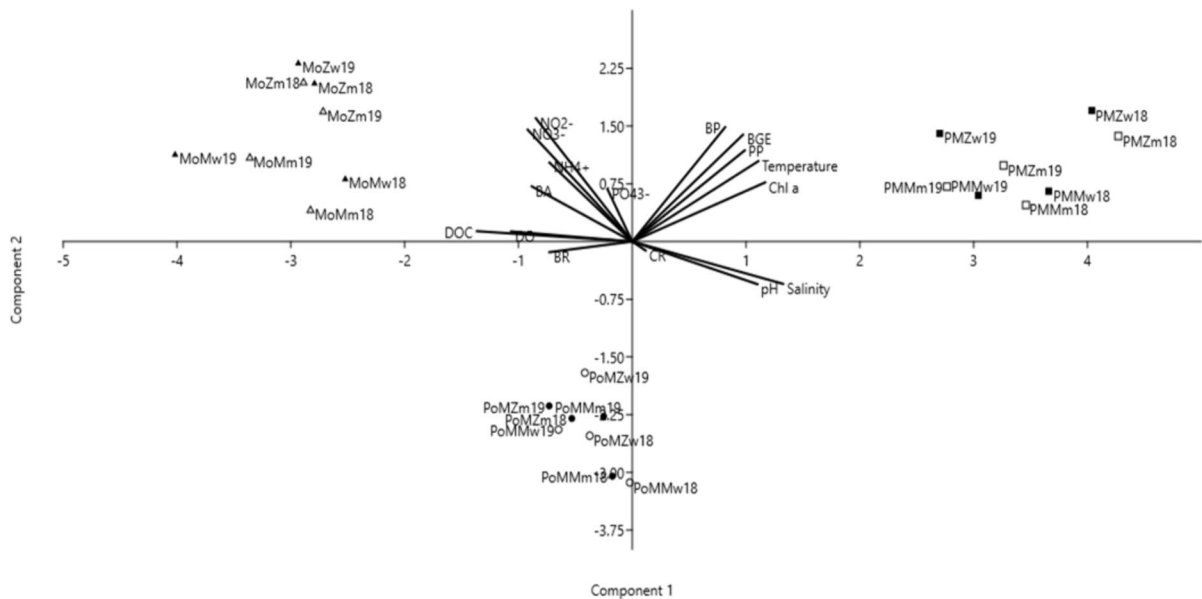


Fig. 4 Principal component analysis (PCA) biplot of the mean PC scores of each physico-chemical and biological parameter as vectors, together with the PC scores of each season

Table 3 Variation in physicochemical and biological parameters across the study sites and their significance ($p < 0.05$), analyzed by one-way ANOVA

	F value	<i>p</i>
Temperature	143.91	<0.0001
Salinity	3960.59	<0.0001
pH	9.09	<0.0001
DO	118.24	<0.0001
NO ₂ ⁻	8004.93	<0.0001
NO ₃ ⁻	1.04	0.438
NH ₄ ⁺	12,356.57	<0.0001
PO ₄ ³⁻	158,842.70	<0.0001
DOC	100,183.42	<0.0001
Chl <i>a</i>	1713.35	<0.0001
BA	2.32	0.007
PP	8.54	<0.0001
BP	13.44	<0.0001
CR	5.80	<0.0001
BR	4.99	<0.0001
BGE	4.953	<0.0001

DO dissolved oxygen, NO₂⁻ nitrite, NO₃⁻ nitrate, NH₄⁺ ammonia, PO₄³⁻ phosphate, DOC dissolved organic carbon, Chl *a* chlorophyll *a*, BA bacterial abundance, PP primary productivity, BP bacterial productivity, CR community respiration, BR bacterial respiration, BGE bacterial growth efficiency

notably decreasing during the Mo coinciding with heightened precipitation (460–1160 mm) and increasing during the PM with minimal precipitation (0.2 mm), suggesting salinities were primarily influenced by episodic influx of freshwater from rivers (Padmavati and Goswami 1997; Ghosh et al. 2021). Despite the dynamic nature of estuaries, pH values remained relatively stable (~7) across all seasons, largely attributable to the buffering capacity inherent in seawater that mitigates pH alterations (Pindar et al. 2021). DO concentrations indicated well-oxygenated conditions in both estuaries. The highest concentrations of nutrients (NO₂⁻, NO₃⁻, NH₄⁺, and PO₄³⁻) reported during Mo in this study (when freshwater supply increases by ~64%), indicate that the freshwater influx from rivers upstream into estuaries dictates the amount of dissolved inorganic nutrients to a large extent (Ghosh et al. 2021). Variations in light intensity, temperature, and nutrient availability are known to greatly impact Chl *a* concentration (Perumal et al. 2009). The PM period, offers favorable conditions, such as low turbidity and greater light penetration for phytoplankton growth. This aligns with our results, wherein the highest concentrations of Chl *a* were recorded during PM season in both estuaries.

Table 4 Post hoc Tukey's HSD test for physicochemical and biological parameters during pre-monsoon (PM), monsoon (M) and post-monsoon (PoM) seasons. Mean difference is significant at $p < 0.05$ level

Dependent variables	Location (I)	Location (J)	Seasons		
			p (PM)	p (Mo)	p (PoM)
Temperature	M_m	M_w	0.99	0.99	0.13
	Z_m	Z_w	0.99	0.92	0.95
Salinity	M_m	M_w	<0.0001	0.12	1
	Z_m	Z_w	1	<0.001	<0.0001
pH	M_m	M_w	1	0.99	1
	Z_m	Z_w	0.99	0.99	0.99
DO	M_m	M_w	0.75	0.004	0.93
	Z_m	Z_w	1	0.50	0.93
NO_2^-	M_m	M_w	<0.0001	<0.0001	0.87
	Z_m	Z_w	<0.0001	<0.0001	0.27
NO_3^-	M_m	M_w	<0.0001	<0.0001	0.002
	Z_m	Z_w	<0.0001	<0.0001	<0.0001
NH_4^+	M_m	M_w	0.92	<0.0001	1
	Z_m	Z_w	<0.0001	<0.0001	0.006
PO_4^{-3}	M_m	M_w	<0.0001	0.03	0.63
	Z_m	Z_w	<0.0001	<0.0001	0.39
DOC	M_m	M_w	<0.0001	<0.0001	<0.0001
	Z_m	Z_w	<0.0001	0.001	<0.0001
Chl <i>a</i>	M_m	M_w	0.81	<0.0001	<0.0001
	Z_m	Z_w	<0.0001	<0.0001	<0.0001
BA	M_m	M_w	<0.0001	1	0.72
	Z_m	Z_w	1	0.002	0.001
PP	M_m	M_w	1	0.99	1
	Z_m	Z_w	1	1	0.99
BP	M_m	M_w	0.93	1	1
	Z_m	Z_w	0.44	1	1
CR	M_m	M_w	0.99	0.98	1
	Z_m	Z_w	0.99	1	1
BR	M_m	M_w	0.94	1	1
	Z_m	Z_w	1	0.99	1
BGE	M_m	M_w	1	0.95	1
	Z_m	Z_w	1	1	1

In marine ecosystems, phytoplankton-derived carbon is considered a major carbon source for bacteria and thus, bacterial dynamics is tightly linked with phytoplankton (Cole et al. 1988; Ducklow 2000). However, the extent of this interdependence can exhibit variations influenced by temporal and spatial factors (Cho et al. 2001; Hyun and Kim 2003). Observations made in this study indicate that substantial organic matter influx from upstream waters and terrestrial runoff during Mo resulted in peak concentrations of DOC. These elevated DOC levels particularly impacted BA in both the M and Z estuaries. These

findings align with previous studies by Rodrigues et al. (2011) and Khandeparker et al. (2017). Conversely, during PM and PoM periods, declining DOC concentrations led to a subsequent reduction in BA. The higher DOC and BA concentrations in M compared to Z were attributed to M receiving a greater runoff influx relative to Z (Qasim and Sengupta 1981). Moreover, increased DOC concentrations in M_m and Z_m were a consequence of additional organic matter inputs from decomposing mangrove leaf litter, actively fostering bacterial growth and reproduction (Kristensen et al. 2008).

The maximum PP in both the M and Z estuaries was observed during PM coinciding with optimal conditions that favored phytoplankton growth. With the onset of Mo, substantial nutrient influx from riverine sources altered the estuarine environment, resulting in increased turbidity and reduced light penetration. This environmental shift required phytoplankton to adapt to diminished nutrient availability (Devassy and Goes 1989), subsequently reducing their metabolic activity. Consistent seasonal variability in PP aligning with our findings was also reported by Thottathil et al. (2008a, b) in the Cochin estuary.

The balance between autotrophy and heterotrophy is crucial in determining how organic C is transferred to the higher trophic levels (Azam et al. 1983) where the BP/PP ratio serves as a valuable indicator in assessing the trophic status (Thottathil et al. 2008a, b). When BP constitutes a substantial portion of PP ($BP/PP > 1$), it signifies a heterotrophic system subsidized by allochthonous C (Alongi 1989). Conversely, when $BP/PP < 1$, it indicates an autotrophic system relying on autochthonous (phytoplankton) C (Carlson et al. 1996). Our study affirms the prior observations made by Ram et al. (2007) and Thottathil et al. (2008a, b), indicating a similar seasonal pattern in BP ($PM > Mo > PoM$). Predominantly heterotrophic ($BP/PP > 1$) ratios were consistently observed across all seasons in M and Z, with higher BP/PP ratios in the estuarine waters (M_w and Z_w) compared to their respective mangrove waters (M_m and Z_m). The low heterotrophic BP/PP ratios obtained during PoM are due to higher PP in comparison to BP. In contrast, the higher BP/PP ratios observed during the Mo and PM corresponded to relatively lower PP values and increased concentrations of DOC that stimulated BP (Revilla et al. 2000). Our results corroborate with a previous study by Ram et al. (2007) which documented lower heterotrophic ratios during PM and PoM in contrast to higher ratios observed during Mo. Furthermore, our study's recorded levels of DOC indicated an autotrophic system during PM, transitioning to a heterotrophic system during Mo and PoM. This observation is in line with the conclusions drawn by Prairie et al. (2002), who suggested that DOC concentrations below 4 to 6 mg/L indicate an autotrophic system. Additionally, the PCA analysis emphasized the substantial influence of seasonal variability on the trophic status in the estuarine and mangrove waters of M and Z. Furthermore, the autotrophic status of the

PM was validated through PCA by the predominance of Chl *a*, PP, and CR; while loadings of nutrients, DO, DOC, BA, and BR were directed towards the heterotrophic status of Mo and PoM.

Respiration (community and bacterial) is an important C sink, making it vital for accurately estimating the C budget (Nguyen and Maranger 2011). High respiration rates are attributed to bacterial-mediated nitrification (Ram et al. 2007) and the presence of organic matter in the water column (Bianchi et al. 2012). In our study, we observed that CR consistently exceeded PP, irrespective of the location and season, indicating heterotrophy similar to the reports of del Giorgio et al. (1997). Moreover, BR exceeds BP predominantly throughout the study period except during the PM in both estuaries. This indicates that the utilization of C for respiration is larger than its assimilation for bacterial biomass, affirming the role of respiration in determining the fate of bacterial C and influencing ecosystem productivity (Roland and Cole 1999; del Giorgio et al. 1997). The low ratios of PP/BR (< 1) during the Mo and PoM seasons are attributed to low PP values, as phytoplankton growth was restricted due to increased turbidity (Thottathil et al. 2008a, b). Despite high phytoplankton biomass (Chl *a*) and PP values during the PM, the PP/BR ratios ranged between 0.51 and 1.42, suggesting that BR can occur concurrently with PP while being supported by external allochthonous carbon sources (del Giorgio et al. 1997). These findings are further supported by PCA which confirmed the interdependence of respiration and productivity parameters.

Bacterial growth efficiency refers to the amount of C substrates accessible to higher trophic levels (Azam 1998). The wide variability of BGE detected in our study during each season is attributed to a loose coupling between BP and BR, which may be due to the discrepancies in bacteria-mediated nitrification (Ram et al. 2007). BGE values from our study suggest that the PM season is the most favorable season for the transfer of bacterial C to the trophic food web. Low BGE aligned with elevated BR and increased concentrations of DOC, reaffirming that bacteria devote more energy to maintenance rather than metabolism (del Giorgio and Cole 1998). The average BGE reported from estuaries is 37% with the prediction of a higher BGE in more productive ecosystems (del Giorgio and Cole 2000). Comparatively, our study indicated an average BGE of 30.35% in M and 38.38% in Z,

implying that the Z estuary is potentially more productive than the M estuary. Additionally, higher BGE values in M_w and Z_w than in M_m and Z_m show that estuarine waters are more productive than mangrove waters. The increase in BGE in the estuarine waters is likely a result of allochthonous C enhanced by inputs from mangroves. The increase in BGE in the estuarine waters is likely a result of allochthonous C enhanced by inputs from mangroves. Thus, the DOC derived from mangrove leaf litter decomposition acts as a substrate for in situ microbial activity, while concurrently, a fraction of this DOC is exported from the mangrove ecosystem to adjacent estuarine waters (Kristensen et al. 2008). In the estuary, this exported DOC continues to support bacterial growth and productivity, contributing to the overall productivity of the estuarine ecosystem.

The post hoc Tukey's HSD tests primarily emphasized the significant variations in the nutrients, DOC, Chl *a*, and BA between the estuarine and mangrove waters of both M and Z. This suggests that nutrients and OM play a significant role in shaping the microbial communities and overall ecosystem dynamics. Besides, the absence of significant variations in the productivity and respiration parameters indicates a remarkable ecological connectivity between the mangrove and estuarine waters. This highlights the crucial role of mangroves as transitional areas between terrestrial and aquatic realms facilitating the exchange of water, nutrients, and OM with a significant impact on the metabolic processes in estuarine waters.

Productivity and respiration show significant variability across aquatic ecosystems, from oligotrophic coral reefs and open oceans to eutrophic rivers, streams, and estuaries. For instance, a review by Amado et al. (2013) comparing tropical ecosystems (BP-average $4.65 \mu\text{g C L}^{-1} \text{h}^{-1}$; BR-average $41.40 \mu\text{g C L}^{-1} \text{h}^{-1}$; BGE-average 29%) to temperate ecosystems (BP-average $4.24 \mu\text{g C L}^{-1} \text{h}^{-1}$; BR-average $25.00 \mu\text{g C L}^{-1} \text{h}^{-1}$; BGE-average 43%) found that while BP and BR were higher in the tropics, BGE was lower. Similarly, Lee et al. (2009) reported higher BP and BR but lower BGE in Malaysian tropical coastal waters compared to temperate ecosystems. The present study also exhibited high BP and BR with lower BGE than those reported from the temperate zone. These differences have been attributed to a combination of factors, including the higher temperatures, and increased sunlight exposure characteristic

of tropical regions. The metabolic status even within a single aquatic system can vary temporally and spatially (Raymond et al. 2000). A comprehensive study conducted by Caffrey (2004) examined the metabolic status of 42 sites within 22 national estuarine reserves. It was found that sites adjacent to mangroves or in marsh creeks tended to exhibit heterotrophic conditions. Conversely, sites neighboring submerged aquatic vegetation, such as eelgrass or macroalgal beds, were mostly autotrophic. This phenomenon was attributed to variations in the nutrient and organic matter loadings. Thus, the outwelling of DOC from ecosystems such as mangroves and marsh creeks is a major contributor to the trophic dynamics.

Conclusion

The impact of mangrove-derived organic matter on coastal ecosystems is analogous to the "outwelling" concept proposed by Odum (1968). Our study complements the outwelling theory where allochthonous C supports coastal heterotrophy during Mo and PoM in both the M and Z estuaries and subsidizes the heterotrophic activity. Furthermore, the seasonal changes in the estuarine dynamics, greatly impact the carbon flux through bacteria. The high heterotrophic ratios established bacteria as the key players in the trophic cycle of mangrove and estuarine waters. Consistently high BR values in both the estuaries indicate a dominant microbial loop characterized by substantial bacterial C demand, indicating a significant portion of organic carbon is channeled towards respiration. Our findings suggest a relatively weak autoheterotrophic coupling exists in the mangrove and estuarine ecosystems that oscillates between autotrophy (during the PM season) and heterotrophy (during the Mo and PoM seasons). This switchover from autotrophy to heterotrophy is primarily influenced by allochthonous C sources notably those derived from mangroves. Our research indicates that estuarine waters exhibit higher productivity in both M and Z, highlighting that the mangroves augment the efficiency of the trophic cycle within adjoining estuarine waters. This finding underscores the crucial role of mangroves in elevating the overall productivity and trophic dynamics of tropical ecosystems, emphasizing their significance in sustaining biodiversity and ecological balance within estuarine environments.

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Author contributions Seyieleno C. Seleyi: Acquisition of data, Formal analysis, Writing—original draft, Writing—review and editing. C. Ravindran: Conceptualization, methodology, Writing—review and editing, Supervision. C. Mohandass: Writing—review and editing. Prantick Patra: Acquisition of data, Formal analysis.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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Seasonal dynamics of mangrove outwelling and its impact on estuarine productivity: insights from multi-parameter analysis across tidal variations

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Abstract

Tropical mangroves are subjected to extreme environmental conditions yet remain highly productive ecosystems. The Mandovi and Zuari estuaries of Goa on the western coast of India are lined by mangroves and are a rich source of fisheries that supports the coastal livelihood. Despite their ecological and economic significance, the nutrient and organic matter dynamics in tropical mangrove ecosystems remain poorly studied. The hydrographic conditions in the mangrove and adjacent estuarine waters are dictated by monsoonal precipitation, freshwater input, tides, and influx of seawater, resulting in subsequent changes in their physicochemical parameters and organic matter concentrations. The impact of these changes on the phytoplankton biomass and primary productivity in estuarine waters was investigated during the pre-monsoon, monsoon and post-monsoon seasons. Dissolved inorganic nutrients, such as nitrite, nitrate, ammonia, phosphate and silicate, displayed wide seasonal variability and were higher during the low tide than those in high tide. Isotopic and elemental ratios of carbon and nitrogen revealed that primary productivity in the estuaries was enhanced by the outwelling of mangrove-derived organic matter. The highest chlorophyll a and primary productivity were observed during the pre-monsoon season, with Zuari exhibiting higher levels compared with Mandovi. Multivariate statistical analyses confirmed that primary productivity was influenced mainly by temperature, salinity, pH, dissolved oxygen and organic matter. Results from our study emphasizes the role of outwelling of organic matter from mangroves and their influence on primary productivity in adjacent estuarine waters.

Keywords Primary productivity · Organic matter dynamics · Mangroves · Outwelling · Nutrients

Introduction

Mangroves are one of the most productive ecosystems in the world and support a great wealth of biodiversity (Meera et al. 2022). Odum attributed the biological productivity of coastal waters to the organic matter (OM) outwelled or exported from shallow water hotspots, such as, mangroves

(1968). Since then, there has been constant speculation regarding the contribution of mangroves to the productivity of adjacent ecosystems supporting fisheries, coral reefs, salt marshes, seagrass and seaweed beds. Several studies support this hypothesis (Dittmar et al. 2006; Jennerjahn and Ittekkot 2002; Granek et al. 2009; Abrantes et al. 2015) by highlighting mangroves as a vital source of (C) and nitrogen (N) to the adjacent coastal waters. While certain studies propose that mangroves serve as biofilters, thus mitigating eutrophic conditions (Marchand et al. 2014); others suggest they directly contribute nutrients to coastal waters (Tanaka and Choo 2000; Dittmar et al. 2006). Recent studies indicates that mangrove vegetation stores ambient CO₂, which is subsequently released into coastal waters through the porewater discharge of dissolved organic and inorganic C (Maher et al. 2018; Santos et al. 2019). Significant C inputs represented by the primary productivity (PP) of phytoplankton in mangrove dominated estuaries plays a pivotal role in ecosystem dynamics and global biogeochemical cycles (Sala

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and Austin 2000; Moore et al. 2013; Saifullah et al. 2015). Phytoplankton, as primary producers, are fundamental for sustaining the food web and providing energy for numerous organisms within mangrove estuarine ecosystems (Khattoon et al. 2010). Therefore, understanding the PP of mangrove dominated estuaries helps to assess the overall health and resilience of these fragile habitats (Sala and Austin 2000).

Unfortunately, there is a scarcity of studies focusing on PP of tropical mangrove-dominated estuaries compared with temperate estuaries worldwide (Navas-Parejo et al. 2020). Specifically, limited research from Indian tropical mangrove estuaries have been reported despite accounting for over 45% of South Asia's total mangrove cover (Singh and Rangarajan et al. 2023). Moreover, mangrove dynamics are shaped by a complex interplay of factors, such as local geomorphology (Vaslet et al. 2012), tidal variations (Ayukai et al. 1998), areal extent of forests (Guest et al. 2004), freshwater input and precipitation (Adame and Lovelock 2011) and physico-chemical conditions (Ylla et al. 2012). Given the variability of these factors, the role of mangroves in estuarine dynamics across various seasonal, tidal and spatial scales is still ambiguous and remains an ongoing challenge in coastal ecology.

As mangroves inhabit transitional zones between marine and freshwater ecosystems, OM comprises a heterogeneous mixture of various dissolved and particulate matter. This mixture can be from allochthonous (e.g. riverine or terrigenous inputs) or autochthonous (e.g. mangrove litter) sources (Prasad et al. 2017). Large volumes of litter are produced by falling leaves, branches, and other debris in mangrove habitats. The process of decomposition recycles nutrients and produces OM in the mangroves and its adjacent ecosystems. The OM that is not exported by tides settle in the sediment and is subjected to microbial degradation, the byproducts of which supplement the food web (Kristensen et al. 2008). The outwelled OM and nutrients enriches the estuarine waters and, ultimately boost fishery resources (Saifullah et al. 2015). Thus, to validate the sources of OM, the signatures of $\delta^{13}\text{C}_{\text{sed}}$ and $\delta^{15}\text{N}_{\text{sed}}$ coupled with elemental C:N ratios in the mangrove and estuarine sediments were studied. The identification of the source(s) of OM has been carried out using dual isotopic tracers in different mangrove ecosystems (Hu et al. 2006; Zhang et al. 2007; Ranjan et al. 2011; Aschenbroich et al. 2015; Krishna et al. 2015; Chu et al. 2020). The Mandovi (M) and Zuari (Z) estuaries harbour 80% of the mangrove cover in Goa, India (Jagtap 1985) with an area of 700–900 ha, respectively (Wakle et al. 2018). Both these estuaries serve as a rich source of fisheries sustaining the coastal food and economy (Rodrigues et al. 2011). In the case of the M and Z estuaries, dual isotopic tracer analysis for the identification of OM origin has only gained impetus in the recent decade (Maya et al. 2011; Bardhan et al.

2015; Shynu et al. 2015). The use of stable isotopic studies to understand the outwelling of OM from mangroves and its implications on the M and Z estuarine productivity has not been addressed earlier.

The objective of this study was to test the outwelling hypothesis on the M and Z estuaries by identifying the sources of OM using isotopic ratios of carbon $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) and nitrogen $^{15}\text{N}/^{14}\text{N}$ ratio ($\delta^{15}\text{N}$) coupled with organic C and N elemental ratios. The impact of physico-chemical parameters, tidal patterns and prevailing monsoon conditions on the variability of OM and PP in the M and Z estuaries was also investigated. By employing this comprehensive approach, our study seeks to elucidate the role of mangroves in enhancing estuarine productivity through the outwelling of OM and nutrients, thus contributing to a deeper understanding of tropical mangrove ecosystem dynamics.

Materials and methods

Sampling locations

Sampling was carried out at two different estuaries, Mandovi (M) and Zuari (Z). At each estuary, six sampling sites separated by a distance of ~400 m were chosen for sample collection. Locations M_m1 , M_m2 , M_m3 (M mangroves) and Z_m1 , Z_m2 and Z_m3 (Z mangroves) were selected within the mangrove swamps of M and Z, respectively. Similarly, locations M_w4 , M_w5 , M_w6 (M estuarine waters) and Z_w4 , Z_w5 and Z_w6 (Z estuarine waters) were stationed mid-river (~300 to 400 m away) opposite locations of M_m and Z_m , respectively (Fig. 1).

Sampling design

Water and sediment samples were obtained during the pre-monsoon (PM; March–May), monsoon (Mo; June–September) and postmonsoon (PoM; October–November) seasons for 2 years (2018–19). Surface water samples (depth < 0.5 m) from the mangrove and adjacent water sites were collected manually and using a Niskin sampler at low tide (LT) and high tide (HT), respectively. Sediment samples were collected using polyvinyl chloride (PVC) pipe corer (length 30 cm and diameter 7 cm) for the mangrove sites and Van Veen grab (area 1 m²) for adjacent water sites. The samples from M_m1 , M_m2 and M_m3 served as triplicates for the location M_m . Similarly, the samples from M_w4 , M_w5 and M_w6 ; Z_m1 , Z_m2 , Z_m3 and Z_w4 , Z_w5 and Z_w6 represented M_w , Z_m and Z_w , respectively.

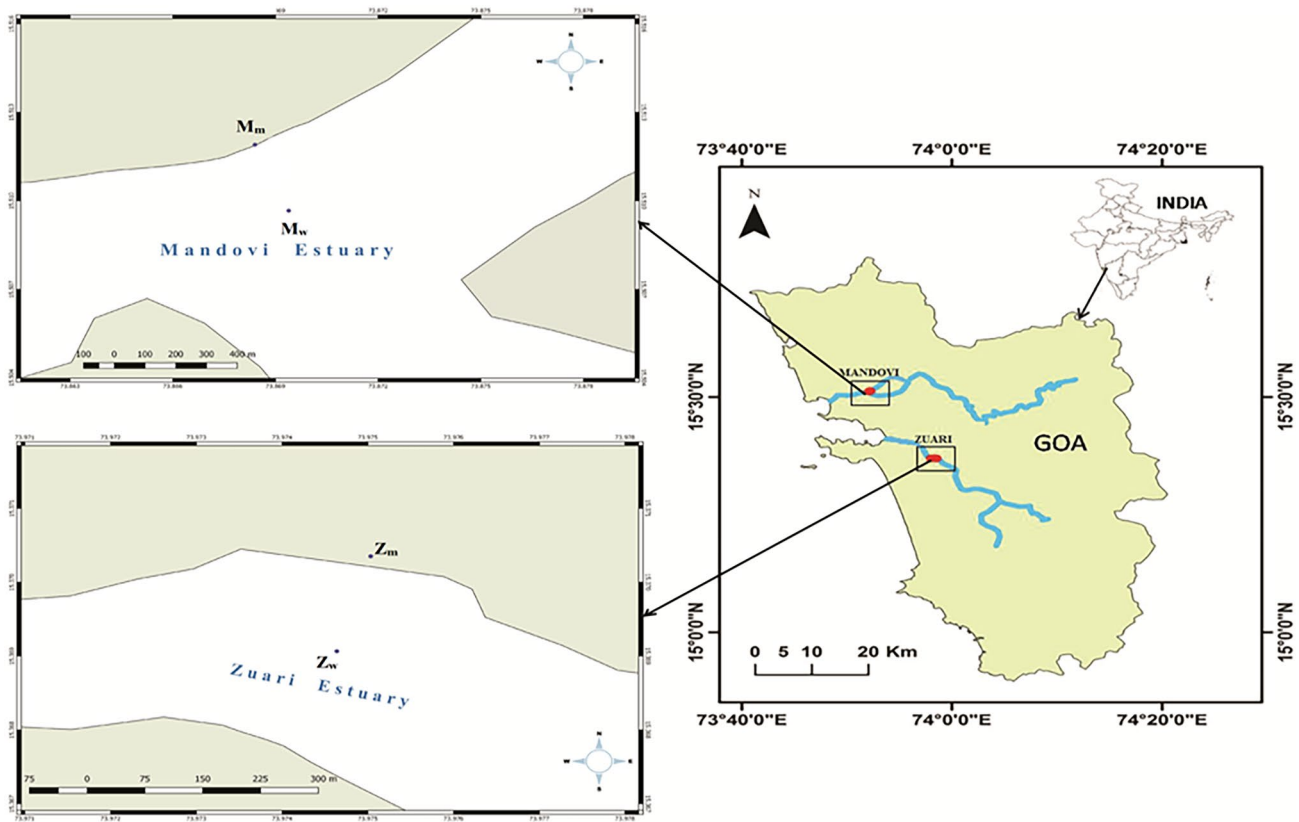


Fig. 1 Location of sampling sites in the Mandovi (M_m Mandovi mangroves; M_w Mandovi adjacent water) and Zuari (Z_m Zuari mangroves; Z_w Zuari adjacent water) estuaries, Goa, India

Determination of physico-chemical and biological parameters of water

A portable thermometer was used to measure the surface water temperature at each sampling site. Salinity was measured using a handheld refractometer (ATAGO PAL-06S Pocket refractometer). The pH was determined using a pH meter (Eutech pH 700). Dissolved oxygen (DO) concentrations were estimated by Winkler's iodometric method (Carpenter 1965; Trivedi and Goel 1986). Dissolved concentrations of nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) were determined using an Autoanalyser (Skalar San Plus) following standard colorimetric methods (Grashoff et al. 1983). Total organic carbon (TOC) was estimated using a TOC analyser (Shimadzu TOC LCPH) in which potassium hydrogen phthalate (KHP) was used as a quality control to check the performance of the TOC analyser. The organic matter (OM) content was obtained by multiplying the TOC by a factor of 1.724 (Trask 1939).

Organic matter sources and characterization

Sediment samples were decalcified with 1N HCl, rinsed completely with deionized water, and dried to determine C and N content and isotopic ratios. The $\delta^{13}\text{C}_{\text{sed}}$ and $^{15}\text{N}/^{14}\text{N}$ $\delta^{15}\text{N}_{\text{sed}}$ ratios, along with C and N content, were determined in continuous-flow mode using an elemental analyser (EURO3000 Eurovector) linked to an isotope ratio mass spectrometer (Thermo-Finnigan Delta V) (Owens and Rees 1989). Regarding Pee Dee Belemnite (PDB) and ambient N, isotopic ratios are stated in standard delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). As established by the working standard, -amino-n-caproic acid (ACA; $^{13}\text{C}=25.3$ and $^{15}\text{N}=4.6$) and an in-house sediment standard chemical oxygen demand (COD; $^{13}\text{C}=21$ and $^{15}\text{N}=7.5$), the precision of C and N isotope readings was more than $\pm 0.2\%$. The C and N content of the sediment samples was determined using an ACA calibration curve with C and N standards ranging from 6 μM to 24 μM and 1 μM to 4 μM , respectively.

Phytoplankton biomass and primary productivity

For Chlorophyll *a* (Chl *a*) analysis, a known volume of water sample was filtered through a 0.7 µm Whatman GF/F filter paper and pigments were extracted in 90% acetone (UNESCO 1994). The resultant fluorescence was then measured on a fluorometer (Turner 7200–000) which was calibrated using the Chl *a* standard (Sigma). PP was estimated in the adjacent water locations using the ^{14}C assimilation method (Strickland and Parsons 1972). Two light bottles and two dark bottle (300 ml capacity) containing water samples from each site were spiked with labelled $\text{NaH}^{14}\text{CO}_3$ (activity: 5 µCi ml $^{-1}$; Bhabha Atomic Research Centre, Mumbai, India) and incubated for 12 h under natural light and in situ temperature. After incubation, water samples were immediately filtered through a 0.45-µm filter (Millipore). The filter was then subjected to acid fumes for 1 min to remove radiolabelled dissolved inorganic carbon (DI^{14}C) and placed in scintillation vials with 5 ml of scintillation cocktail in dioxane (Spectrochem, Mumbai, India). Radioactivity was measured in a liquid scintillation counter (Hidex Triathler 425–034).

Statistical analyses

The statistical software PAST 4.03 (Hammer and Harper 2001) and IBM® SPSS 23.0 (IBM Corp., Armonk, NY, USA) were used to determine the statistical significance and each result was stated in mean $\pm$ standard deviation (SD). The relationship between physicochemical parameters, Chl *a* and PP was understood using a two-tailed Pearson's correlation analysis ($p < 0.05$). To minimize the number of inter-correlated variables, principal component analysis (PCA) was performed with physicochemical parameters, Chl *a* and PP. To improve the interpretation of factor loadings, the analysis used Varimax rotation with Kaiser Normalization. The factor coefficients obtained were then utilized to calculate factor scores. The components with eigen values > 1 were used as predictors to avoid multicollinearity in multiple regression analysis. One way analysis of variance (ANOVA) was performed to understand the impact of seasonal variations on the observed physical, chemical and biological parameters during LT and HT.

Results

Spatio-temporal variation of physico-chemical parameters

Temperature, salinity, pH, dissolved oxygen

During the sampling period, the average ambient water temperature ranged between 27 °C and 32 °C at LT and

27 °C and 30 °C at HT in both the M and Z estuaries (Fig. 2a, b). Tidal variations in the surface water temperatures were not prominent. The highest temperatures were observed during the PM (average 30.59 °C), followed by PoM (average 28.75 °C) and Mo (average 28.11 °C; Fig. 2a, b). Irrespective of seasons, higher temperatures were observed in the estuarine waters (i.e. M_w and Z_w) compared with the mangrove waters (i.e. M_m and Z_m). Distinct changes in the salinity were recorded varying from 2 ppt to 6 ppt in Mo, 17 ppt to 23 ppt in PoM and 27 ppt to 31 ppt in PM at LT (Fig. 2c). During HT, salinities of 1–8 ppt in Mo, 21–29 ppt in PoM and 29–33 ppt in PM were observed (Fig. 2d). A decrease in salinity was observed during Mo followed by a steady increase from PoM to PM. pH displayed variation over a very narrow range with the highest values during PM (7.50–7.69) and lowest during Mo in all our sampling sites (6.95–7.36). No variation was observed in the pH between LT (7.0–7.6) and HT (7.0–7.7; Fig. 2e, f). The average DO concentrations at LT were maximum during Mo (6.74 mg/L), followed by PoM (6.09 mg/L) and PM (4.92 mg/L) in both the estuaries (Fig. 2g). During HT, the highest DO concentrations were observed during Mo (6.29 mg/L), followed by PoM (5.39 mg/L) and PM (4.63 mg/L; Fig. 2h).

Dissolved inorganic nutrients

During LT, concentrations of NO_2^- varied from 0.07 µmol/L to 5.28 µmol/L, NO_3^- was between 2.17 µmol/L and 24.23 µmol/L, NH_4^+ fluctuated between 0.32 µmol/L and 8.05 µmol/L, PO_4^{3-} ranged from 0.06 µmol/L to 3.64 µmol/L and SiO_4^{4-} ranged between 14.75 µmol/L and 74.42 µmol/L (Table 1). In the case of HT, NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} and SiO_4^{4-} concentrations varied between 0.03 µmol/L and 4.25 µmol/L, 0.51 µmol/L and 19.72 µmol/L, 0.11 µmol/L and 37.39 µmol/L, 0.06 µmol/L and 6.06 µmol/L and 6.04 µmol/L and 125.66 µmol/L, respectively (Table 1).

Notably, the highest concentrations of NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} and SiO_4^{4-} were observed during Mo, followed by PM and PoM in both the estuaries (Table 1). Lower nutrient concentrations observed during PoM and PM coincided with increased salinity and lesser freshwater inflow (Table 1). Additionally, nutrient concentrations were higher in M_m and Z_m in comparison with their corresponding adjacent sites i.e. M_w and Z_w (Table 1).

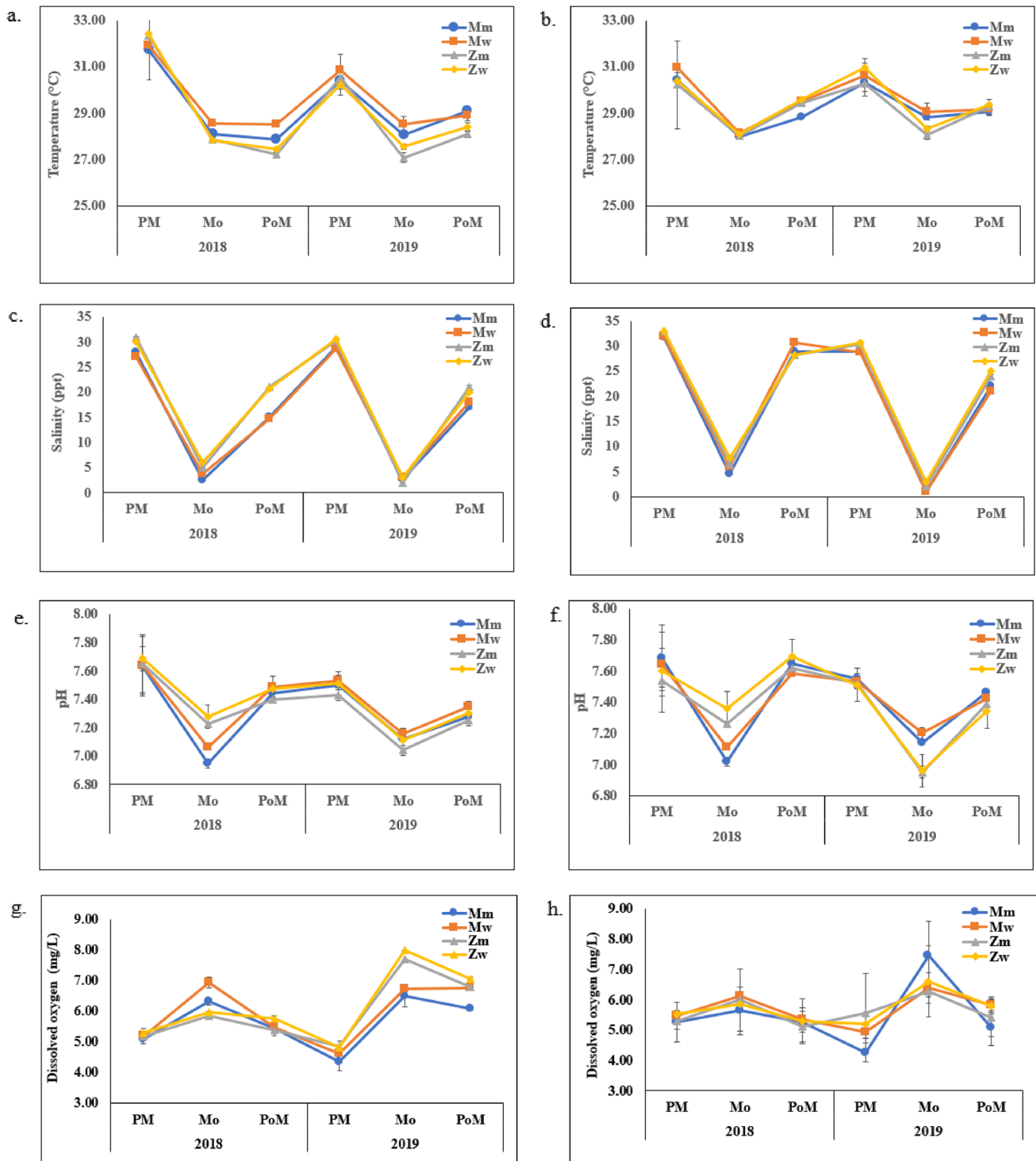


Fig. 2 Variations in physico-chemical parameters during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) seasons in the Mandovi and Zuari estuaries where (a, c, e, g) represent values dur-

ing low tide (Seleyi et al. 2024) and (b, d, f, h) represent values for high tide. M_m Mandovi mangroves; M_w Mandovi adjacent water; Z_m Zuari mangroves; Z_w Zuari adjacent water

Table 1 Variations (mean $\pm$ SD; $n=3$) of nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) in $\mu\text{mol/L}$ during pre-monsoon, monsoon and post-monsoon at low tide (Seleyi et al. (2024)) and high tide in the Mandovi and Zuari estuaries during 2018 and 2019

Nutrients	Location	PM		Mo		PoM	
		LT	HT	LT	HT	LT	HT
2018							
NO ₂ [−]	M _m	1.32±0.04	1.16±0.05	3.28±0.03	2.17±0.01	0.07±0.02	1.03±0.02
	M _w	1.4±0.05	1.25±0.05	3.69±0.04	3.6±0.02	0.10±0.02	1.04±0.02
	Z _m	1.06±0.01	1.02±0.01	4.7±0.03	2.38±0.06	0.19±0.04	0.14±0.10
	Z _w	1.19±0.04	0.93±0.12	4.87±0.01	2.36±0.06	0.29±0.01	0.16±0.04
NO ₃ [−]	M _m	3.35±0.1	0.51±0.12	14.79±0.06	5.13±0.07	2.57±0.08	2.46±0.10
	M _w	4.12±0.02	0.94±0.02	15.36±0.16	4.91±0.2	3.18±0.08	2.26±0.10
	Z _m	3.05±0.04	2.61±0.05	22.49±0.02	12.24±0.02	2.64±0.05	3.00±0.06
	Z _w	3.46±0.05	2.96±0.06	24.23±0.25	14.62±0.31	3.22±0.02	2.23±0.02
NH ₄ ⁺	M _m	0.75±0.04	2.72±0.05	1.84±0.19	1.08±0.2	0.68±0.02	1.66±0.37
	M _w	0.78±0.02	1.69±0.07	3.07±0.03	2.08±0.23	0.71±0.01	1.4±0.28
	Z _m	4.65±0.15	0.4±0.02	6.17±0.06	6.66±0.04	0.80±0.01	1.13±0.15
	Z _w	5.03±0.03	1.15±0.02	7.38±0.32	10.36±0.1	0.98±0.02	0.92±0.09
PO ₄ ^{3−}	M _m	0.54±0.04	0.18±0.05	1.89±0.01	0.55±0.04	0.06±0.02	0.05±0.02
	M _w	0.81±0.04	0.06±0.04	1.62±0.02	1.77±0.05	0.12±0.05	0.11±0.02
	Z _m	1.15±0.01	3.62±0.01	3.55±0.05	5.11±0.04	0.29±0.05	0.27±0.05
	Z _w	1.49±0.1	3.34±0.05	3.64±0.05	6.06±0.01	0.36±0.03	0.19±0.01
SiO ₄ ^{3−}	M _m	16.6±0.04	20.75±0.05	67.60±0.16	122.62±0.23	14.7±0.30	23.29±0.02
	M _w	18.52±0.06	16.22±0.02	72.61±0.19	120.35±0.04	17.98±0.23	20.35±0.01
	Z _m	32.74±0.02	51.25±0.18	55.19±0.02	111.18±0.07	26.87±0.12	31.71±0.01
	Z _w	37.64±0.02	56.49±0.04	57.04±0.08	125.66±0.39	25.79±0.07	33.42±0.02
2019							
NO ₂ [−]	M _m	1.30±0.02	0.13±0.06	4.07±0.02	3.81±0.07	0.09±0.04	0.09±0.02
	M _w	1.54±0.03	0.07±0.06	5.04±0.03	4.25±0.02	0.12±0.03	0.1±0.04
	Z _m	1.01±0.01	0.03±0.01	4.28±0.09	3.58±0.43	0.06±0	0.14±0.02
	Z _w	2.09±0.01	0.08±0.01	5.28±0.09	4.09±0.02	0.09±0.02	0.15±0.02
NO ₃ [−]	M _m	6.25±0.02	4.67±0.26	15.56±0.31	15.8±0.38	2.44±0.14	6.95±0.17
	M _w	8.90±0.08	4.88±0.1	16.35±0.03	18.83±0.04	2.59±0.18	8.23±0.22
	Z _m	7.96±0.02	5.68±0.02	22.19±0.04	18.26±0.05	2.17±0.04	8.51±0.05
	Z _w	8.85±0.06	6.83±0.07	23.34±0.04	19.72±0.05	2.92±0.04	9.94±0.05
NH ₄ ⁺	M _m	1.26±0.02	5.57±0.31	2.23±0.02	0.42±0.24	0.41±0.07	3.94±0.04
	M _w	1.46±0.03	4.5±0.06	2.50±0.23	0.27±0.1	0.32±0.04	5.93±0.2
	Z _m	4.49±0.06	28.13±0.21	7.74±0.05	0.13±0.02	0.91±0.12	31.74±0.07
	Z _w	5.04±0.05	37.39±0.07	8.05±0.11	0.11±0.02	0.72±0.12	32.34±0.18
PO ₄ ^{3−}	M _m	0.12±0.02	0.45±0.02	1.19±0.06	0.87±0.02	0.16±0.12	0.43±0.05
	M _w	0.36±0.08	0.06±0.04	1.15±0.02	0.83±0.04	0.26±0.03	0.63±0.04
	Z _m	1.38±0.09	1.24±0.01	3.41±0.35	0.61±0.11	0.12±0.02	3.08±0.01
	Z _w	1.56±0.02	1.41±0.01	3.72±0.02	0.77±0.11	0.62±0.02	0.49±0.02
SiO ₄ ^{4−}	M _m	18.73±0.25	7.94±0.04	69.29±0.2	61.27±0.02	15.89±0.03	29.93±0.09
	M _w	21.38±0.05	6.04±0.04	74.42±0.08	60.16±0.28	16.30±0.16	31.79±0.05
	Z _m	28.44±0.17	6.91±0.07	43.12±0.02	17.99±0.06	20.91±0.06	23.29±0.15
	Z _w	30.59±0.06	6.43±0.06	55.97±0.02	18.31±0.13	18.55±0.15	23.26±0.15

M_m Mandovi mangroves, M_w Mandovi estuarine water, Z_m Zuari mangroves, Z_w Zuari estuarine water, PM pre-monsoon, Mo monsoon, PoM post-monsoon, LT low tide, HT high tide

Organic matter

At LT, the concentration of organic matter in water (OM_w) varied from 8.48 mg/L to 69.87 mg/L in M and from 5.84 mg/L to 68.97 mg/L in Z (Fig. 3a). During HT, values of OM_w varied between 7.60 mg/L and 75.89 mg/L and 4.28 mg/L and 70.49 mg/L in M and Z, respectively (Fig. 3b). During LT, organic matter in sediment (OM_s) in M ranged between 2.29 mg/g and 62.61 mg/g and in Z from 4.71 mg/g to 57.36 mg/g (Fig. 3c). During HT, OM_s concentrations varied from 2.61 mg/g to 48.65 mg/g in M, whereas in Z concentrations were recorded from 4.04 mg/g to 57.48 mg/g (Fig. 3d). Higher concentrations were witnessed mostly during LT in comparison with HT. The seasonal variation of OM_w and OM_s were in the order: Mo > PoM > PM in both estuaries during both tides. The OM in the mangrove waters and sediment were higher than in its adjacent waters and sediments for both estuaries.

Spatiotemporal variability of isotopic and elemental ratios of carbon and nitrogen

The $\delta^{13}C_{sed}$ values recorded in M varied between -26.14% and -24.31% while in Z it ranged between -25.67% and -24.54% (Fig. 4). The average $\delta^{13}C_{sed}$ in M_m was -25.76%

and in M_w it was -24.81% . While in Z_m and Z_w the average $\delta^{13}C_{sed}$ values were -24.76% and -25.23% , respectively. The $\delta^{15}N_{sed}$ values ranged from 2.20% to 7.96% in M while, it varied from 2.73% to 6.04% in Z (Fig. 4). M_m (average 4.90%) and Z_m (average 5.92%) had depleted $\delta^{15}N_{sed}$ values compared with their respective estuarine sites M_w (5.34%) and Z_w (average 4.80%). The C:N_{sed} ratio in M was recorded to be between 10.01 and 18.71 and in Z it was between 7.54 and 15.69 (Table 2). The average C:N_{sed} ratios obtained from this study were 13.44, 12.69, 11.90 and 11.27 in M_m , M_w , Z_m and Z_w , respectively. It was observed that the ratios in $M_m > M_w$ and $Z_m > Z_w$. C:N_{sed} was highest during Mo (13.18–18.71) and lowest during PM (7.54–10.74).

Chlorophyll *a*

At LT, the average Chl *a* concentrations were found to be the highest during PM (6.73 $\mu\text{g/L}$), followed by Mo (2.27 $\mu\text{g/L}$) and PoM (1.21 $\mu\text{g/L}$) in both the estuaries (Fig. 5a). Comparatively, lower Chl *a* concentrations were observed at HT during PM (3.72 $\mu\text{g/L}$), Mo (1.57 $\mu\text{g/L}$) and PoM (0.83 $\mu\text{g/L}$) (Fig. 5b). The highest Chl *a* concentrations coincided with low rainfall intensity, high temperatures and high salinity. While the lower concentrations were prevalent during high water column turbidity and light inhibition owing to cloud cover. Interestingly, the

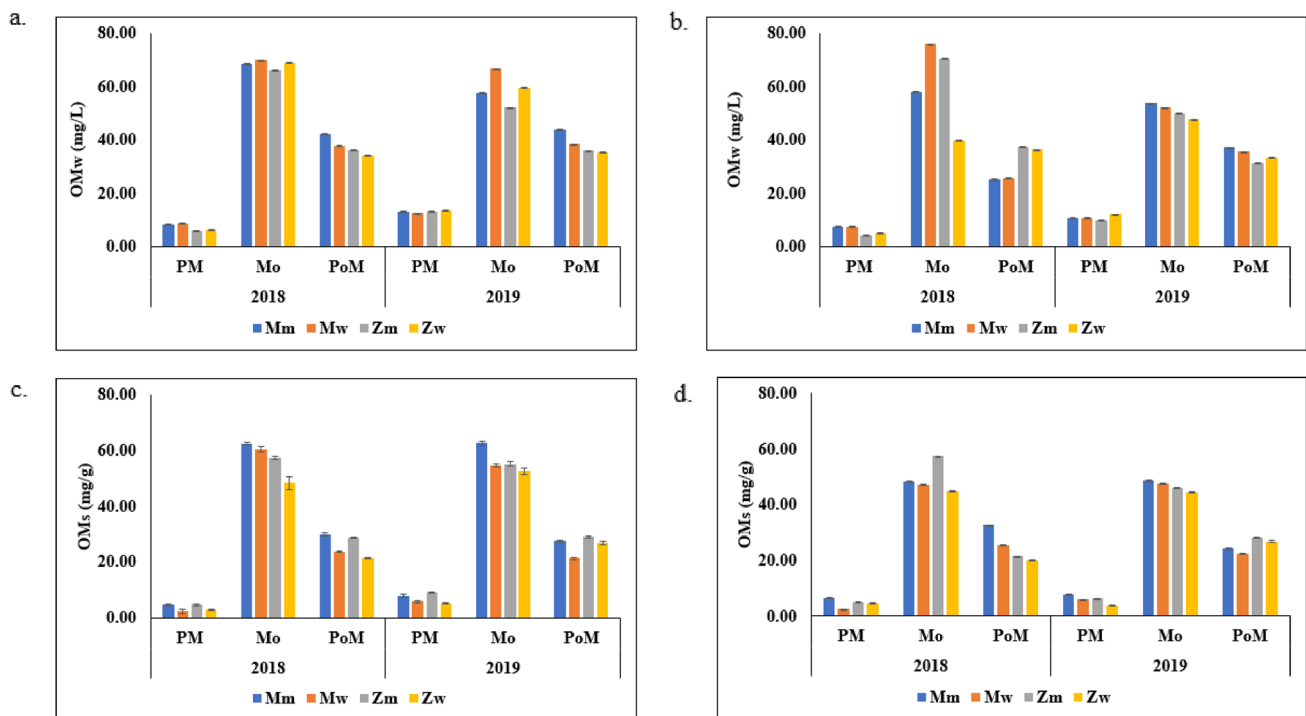


Fig. 3 Variation of organic matter in water (OM_w) (a low tide; b high tide) and organic matter in sediment (OM_s) (c low tide; d high tide) during pre-monsoon (PM), monsoon (Mo) and post-monsoon

(PoM) in the Mandovi and Zuari estuaries. (M_m Mandovi mangroves; M_w Mandovi adjacent water; Z_m Zuari mangroves; Z_w Zuari adjacent water)

Fig. 4 Variations in $\delta^{13}\text{C}_{\text{sed}}$ and $\delta^{15}\text{N}_{\text{sed}}$ during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_m Mandovi mangroves; M_w Mandovi adjacent water; Z_m Zuari mangroves; Z_w Zuari adjacent water)



Table 2 Spatio-temporal variation of C:N_{sed} ratios in the Mandovi and Zuari estuaries during 2018 and 2019

	2018			2019		
	PM	Mo	PoM	PM	Mo	PoM
M_m	10.74	18.71	13.58	10.38	15.73	11.51
M_w	10.05	16.31	13.52	10.01	15.14	11.10
Z_m	8.49	15.69	12.32	8.99	13.79	12.12
Z_w	7.54	15.42	11.19	8.52	13.18	11.77

M_m Mandovi mangroves, M_w Mandovi estuarine water, Z_m Zuari mangroves, Z_w Zuari estuarine water, PM pre-monsoon, Mo monsoon, PoM post-monsoon

concentration of Chl *a* was inversely proportional to the salinity in the two estuaries as the latter changed with the flood and ebb tides.

Primary productivity

PP in M_w ranged from 2.55 $\mu\text{gC/L/h}$ to 15.86 $\mu\text{gC/L/h}$ and in Z_w from 2.56 $\mu\text{gC/L/h}$ to 10.25 $\mu\text{gC/L/h}$ (Fig. 6). In both M_w and Z_w , the maximum PP was observed during the PM (9.81–15.86 $\mu\text{gC/L/h}$), intermediate during Mo (5.12–8.42 $\mu\text{gC/L/h}$) and least during the PoM (2.55–4.04 $\mu\text{gC/L/h}$). This pattern of seasonal variation of PP was in conjunction with that of Chl *a*. Although the nutrient enrichment was highest during the Mo, it was interesting to note that the PP maxima did not occur during Mo in both the estuaries. Alternatively, the maximum PP levels were observed during PM with lesser nutrient availability than the Mo.

Relationship between physico-chemical parameters, chlorophyll *a* and primary productivity

Pearson's correlation analysis revealed that there is a strong positive relationship between the productivity parameters

i.e. Chl *a* and PP with temperature, salinity, pH and DO irrespective of the tides (Fig. 7a, b). With regards to nutrients, the correlation with Chl *a* and PP is nominal and negative (Fig. 7a, b). Distinct negative correlations were found between OM_w and OM_s with Chl *a* and PP (Fig. 7a, b).

Using PCA analysis, a total of four components were obtained with eigenvalues > 1 . To reduce multicollinearity, three components were selected, representing 71.95% of the total variance (Fig. 8). The first component contributed to 48.13% of the variance and consisted of temperature, salinity and pH. The second component showed a variance of 14.90% representing DO, NO_3^- , OM_w and OM_s . The third component had a variance of 8.93%, constituting NO_2^- , NH_4^+ , PO_4^{3-} and SiO_4^{4-} . The fourth component had a variance of 7.85% and was not considered for linear regression as they failed to represent any specific variable. For linear regression, factor scores of the first, second and third components were considered as the independent variable. The dependent variables were Chl *a* and PP (Table 3). It is noteworthy to state that component 1 showed the highest significance for both Chl *a* and PP ($p < 0.0001$) while component 2 was significant for PP only. One-way ANOVA aided us in further illustrating the impact of the seasonal variations on the observed parameters during LT

Fig. 5 Variation of chlorophyll *a* (Chl *a*) (a low tide (Seleyi et al., 2024); b high tide) during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_m Mandovi mangroves; M_w Mandovi adjacent water; Z_m Zuari mangroves; Z_w Zuari adjacent water)

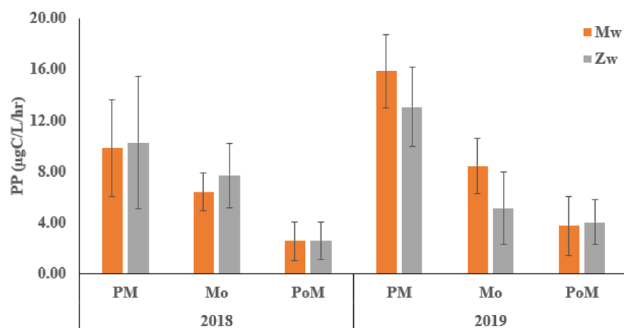
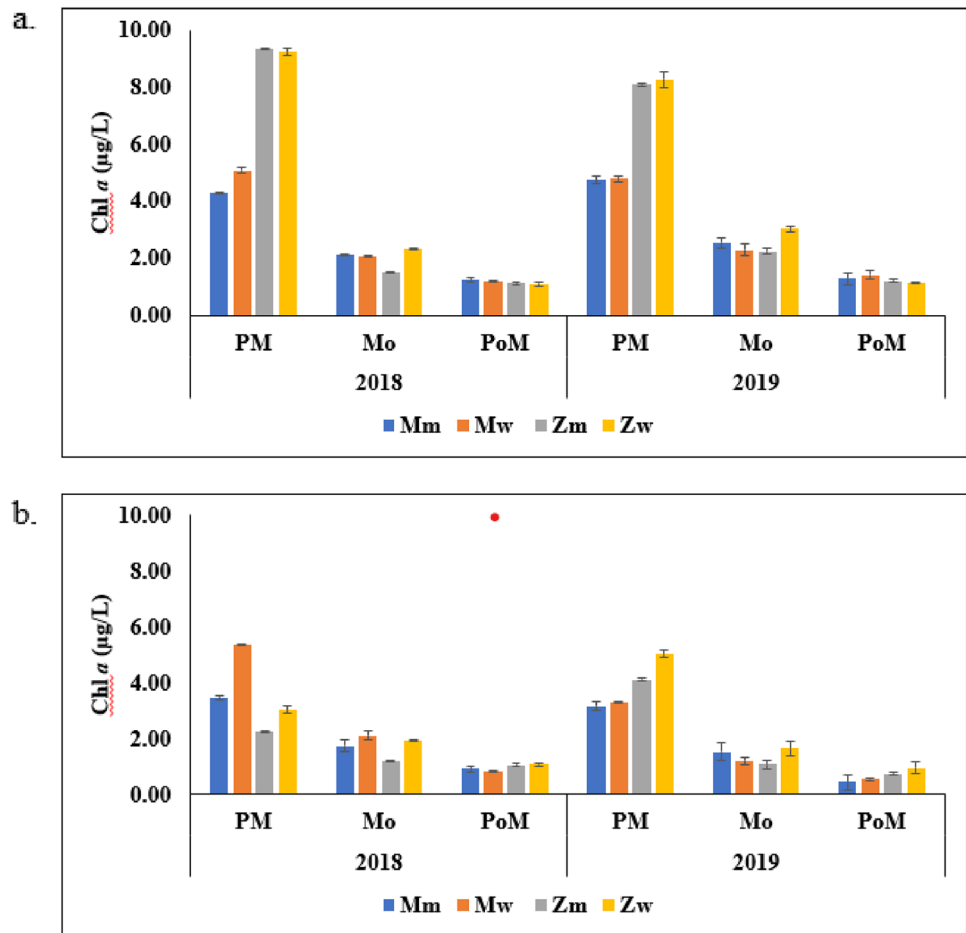


Fig. 6 Variation of primary productivity (PP) during pre-monsoon (PM), monsoon (Mo) and post-monsoon (PoM) in the Mandovi and Zuari estuaries. (M_w Mandovi adjacent water; Z_w Zuari adjacent water) (Seleyi et al., 2024).

and HT (Tables 4, 5). The spatio-temporal variations in temperature, salinity, pH, DO, NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} , SiO_4^{4-} , OM_w , OM_s , Chl *a* and PP were found to be significantly different ($p < 0.001$).

Discussion

Ambient environmental conditions

It is interesting to note that the M and Z estuaries are highly susceptible, yet remain remarkably adaptive to the continuous and dynamic changes observed throughout the study period. The surface water temperatures recorded were higher in M_w and Z_w than M_m and Z_m as a result of the dense mangrove canopy over the latter, thereby restricting the entry of sunlight. The seasonal variation in rainfall is an important contributor of freshwater flux exerting a considerable influence over the salinity patterns. These estuaries receive the highest amount of rainfall (460–1160 mm) during Mo (July–September), lesser rainfall (6.44–210 mm) during PoM (October–December) and almost no rainfall during the driest PM months of January and February (0.2 mm) (https://disc.gsfc.nasa.gov/datasets/GPM_3IMERGHH_06/ summary). Additionally, freshwater discharge from rivers upstream regulates the salinities in these estuaries. It has been estimated that the freshwater supply increases by ~64% during Mo, ~35% during PoM and ~7% during PM (Ghosh et al. 2021). While it is known that both these

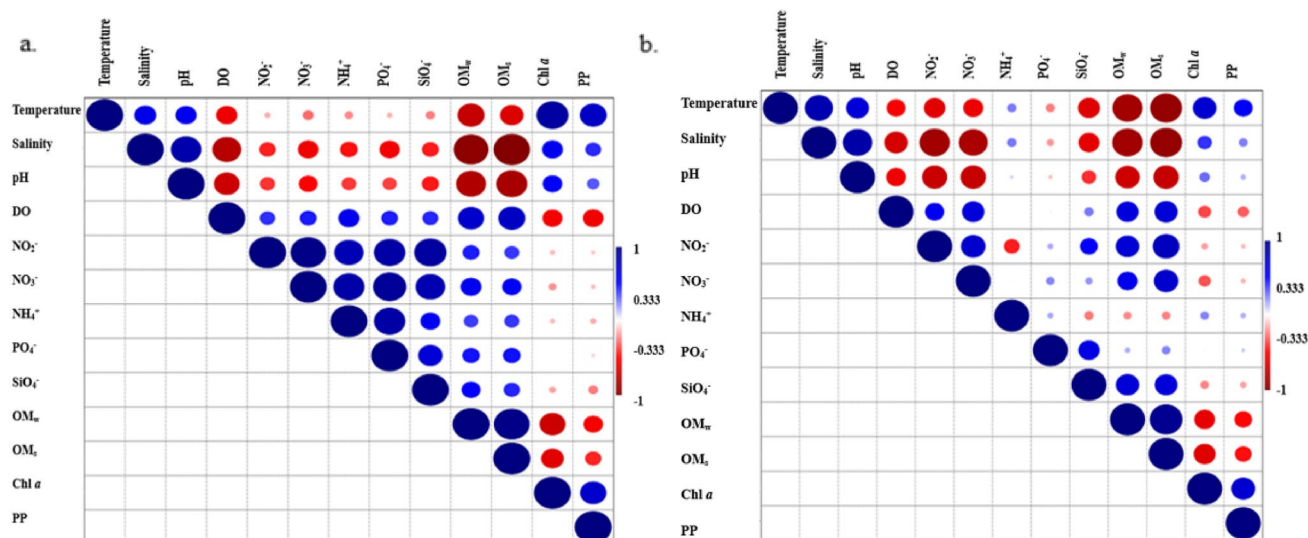
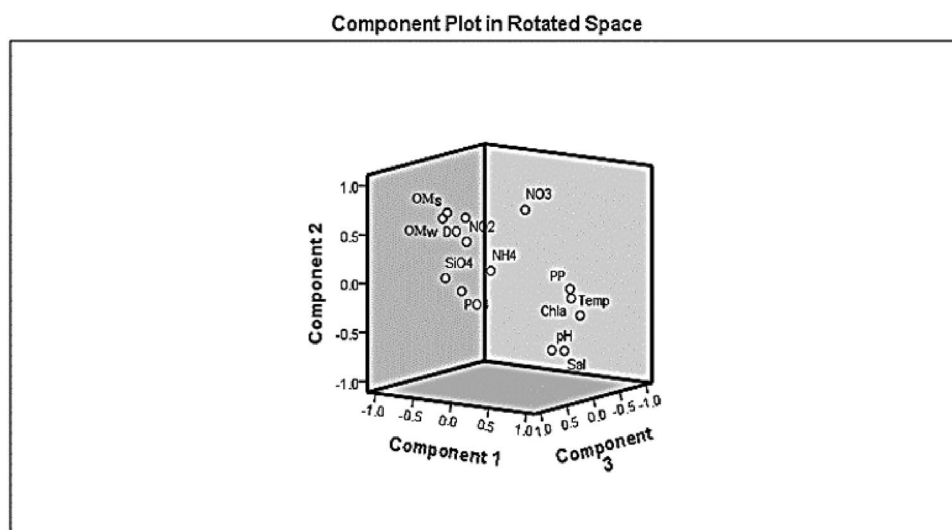


Fig. 7 Pearson's correlation coefficients between temperature, salinity, pH, dissolved oxygen (DO), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), organic mat-

ter in water (OM_w), organic matter in sediment (OM_s), chlorophyll a (Chl a) and primary productivity (PP) in the Mandovi and Zuari estuaries

Fig. 8 Component plot generated by principal component analysis (PCA): component 1 includes temperature, salinity and pH; component 2 represents dissolved oxygen (DO), nitrate (NO_3^-), organic matter in water (OM_w) and organic matter in sediment (TOC_s); component 3 includes nitrite (NO_2^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-})



Component	Eigen value	% of variance	Cumulative % of variance
1	6.25	48.13	48.13
2	1.93	14.89	63.02
3	1.16	8.92	71.95

estuaries originate from the Western ghats and share a common point of discharge, Z maintains more saline conditions in comparison with M almost throughout the year. This phenomenon is attributed to the wider mouth of Z connecting to the sea and lower number of tributaries resulting in higher salinities recorded at Z (Padmavati and Goswami

1997). In contrast, M receives a considerable amount of runoff through a larger tributary system (Qasim and Sengupta 1981) attaining an almost freshwater salinity range during Mo. This pattern of increasing salinity from Mo, PoM to PM seasons has also been documented in the tropical estuaries of Pondicherry, Sundarbans and Tamil Nadu (Prabu et al.

Table 3 Multiple regression model for predicting primary productivity (PP) and chlorophyll *a* (Chl *a*) using factor scores obtained from principal component analysis ($p < 0.05$)

Dependent variable	Predictors	Standardized Coefficients (β)	<i>t</i> -value	Significance (p)	R^2	Adjusted R^2
PP	Component 1	0.828	17.531	< 0.0001	0.688	0.681
	Component 2	−0.014	−0.297	< 0.0001		
	Component 3	−0.037	0.784	0.767		
Chl <i>a</i>	Component 1	0.857	20.255	< 0.0001	0.749	0.744
	Component 2	−0.104	−2.446	0.016		
	Component 3	0.056	1.324	0.188		

2008; Satheeshkumar and Khan 2009; Basu et al. 2021). The observed alterations in pH were not significant, consistent with findings reported by Basu et al. (2021) in the mangrove estuary of Tamil Nadu. The slightly elevated pH recorded during PM is caused by seawater intrusion which goes as far as 65 km into the estuarine zone, while lower pH observed during Mo is owing to limited seawater extending only upto 10 km upstream in M and 12 km upstream in Z (Qasim and Sengupta 1981). The DO concentrations suggested well aerated surface waters in all the sites during the sampling period. Relatively higher DO concentrations during the Mo and PoM were owing to the higher solubility of oxygen in freshwater (Mitra et al. 2018).

The nutrient profiles in the mangrove and estuarine waters are shaped by dual forces of the monsoonal pattern and the tidal inundation. The elevated concentrations of nutrients during LT in the mangrove swamps and its adjacent estuarine waters suggest a complex interplay of ecological dynamics. Unlike HT, where dilution effects might occur owing to greater water volumes, the reduced water levels during LT concentrate nutrients within a smaller volume, leading to higher observed concentrations. This phenomenon could signify localized nutrient accumulation, possibly from sediment resuspension or the retention of nutrients within the confined area of the mangrove swamps (Hasan et al. 2022). Moreover, it emphasizes the ecological importance of mangroves during tidal fluctuations, highlighting their role as nutrient reservoirs or hotspots within the larger estuarine ecosystem. It was consistently observed that the nutrient concentrations in the mangrove waters i.e. M_m and Z_m were higher than those of the estuarine waters i.e. M_w and Z_w , suggesting the outwelling of nutrients from the mangrove swamps. The nutrient outwelling arising from OM decomposition or root exudates within mangroves elucidates the role of mangroves as crucial sources of essential nutrients. The highest nutrient concentrations in the M and Z estuaries were observed during Mo when freshwater input was the maximum which has been recorded previously by Mochemadkar et al. (2013). This trend aligns with previous observations by Prabu et al. (2008) and Basu et al. (2021). Apart from the freshwater inputs, the surge in NO_2^- concentration during Mo arises owing to heightened biological

activity, such as phytoplankton excretion, ammonia oxidation, nitrate reduction, N recycling and bacterial decomposition of planktonic detritus (Govindasamy et al. 2000). The increased concentrations of NO_3^- in Z_m and Z_w during Mo has been reported previously by Maya et al. (2011), despite receiving lower freshwater input compared with M. This could also be a result of the greater expanse of mangrove forests in Z. Mangroves act as significant contributors of nitrogen compounds through biological processes like nitrogen fixation and decomposition of OM. The larger coverage of mangroves in Z likely enhances biological activities, such as microbial nitrogen transformations and nutrient recycling, resulting in elevated NO_3^- concentrations despite relatively lower freshwater input compared with M. Similarly, the higher NH_4^+ concentrations in Z might be attributed to the greater expanse of mangroves, leading to an accelerated rate of bacterial decomposition, mineralization, and ammonification of dissolved organic nitrogen originating from plant debris (Kristensen et al. 1988). Our results indicated a decrease in PO_4^{3-} concentrations as salinity increased, attributed to the tendency of higher salinity to cause phosphorus precipitation. This process results in the removal of phosphorus from the water column through sedimentation or its binding with other particles (De Jonge and Villerius 1989; Fang 2000). Reduced rainfall during PM and PoM led to a considerable decrease in SiO_4^{4-} concentrations as the SiO_4^{4-} supply becomes limited from natural weathering of rocks and erosion (Hessen 1999). Overall, the hydrographical conditions of the estuaries in our study were dictated primarily by the monsoon conditions, the freshwater input, nutrient outwelling and the proximity to the sea that regulates the ebb and flow of water.

Outwelling of organic matter

Higher OM observed in M than in Z can be attributed to the higher river runoffs in the former (Qasim and Sengupta 1981). The Mo season is characterized by maximum freshwater inputs and land runoffs; consequently, maximum OM concentrations were recorded during this season. Gireeshkumar et al. (2013) reported a similar seasonal peak in organic matter concentrations during the monsoon (Mo) period in

Table 4 Spatio-temporal variations of physico-chemical and biological parameters analysed through one-way ANOVA in the Mandovi and Zuari estuaries during low tide and their significance ($p < 0.001$)

	Sum of squares	df	Mean square	<i>F</i>	Significance
Temperature					
Between groups	204.196	23	8.878	143.914	0.000
Within groups	2.961	48	0.062		
Total	207.158	71			
pH					
Between groups	8373.284	23	364.056	3960.596	0.000
Within groups	4.412	48	0.092		
Total	8377.696	71			
Salinity					
Between groups	3.080	23	0.134	9.099	0.000
Within groups	0.707	48	0.015		
Total	3.787	71			
Dissolved oxygen					
Between groups	65.613	23	2.853	110.464	0.000
Within groups	1.240	48	0.026		
Total	66.852	71			
NO ₂ ⁻					
Between groups	244.973	23	10.651	8004.939	0.000
Within groups	.064	48	0.001		
Total	245.037	71			
NO ₃ ⁻					
Between groups	20,727.415	23	901.192	1.042	0.438
Within groups	41,520.778	48	865.016		
Total	62,248.193	71			
NH ₄ ⁺					
Between groups	17,792.996	23	773.609	66,475.491	0.000
Within groups	0.559	48	0.012		
Total	17,793.555	71			
PO ₄ ³⁻					
Between groups	101.133	23	4.397	395.590	0.000
Within groups	0.534	48	0.011		
Total	101.667	71			
SiO ₄ ⁴⁻					
Between groups	29,293.246	23	1273.619	69,840.515	0.000
Within groups	0.875	48	0.018		
Total	29,294.121	71			
OM _w					
Between groups	11,986.209	23	521.140	100,568.334	0.000
Within groups	0.249	48	0.005		
Total	11,986.458	71			
OM _s					
Between groups	11,117.166	23	483.355	962.425	0.000
Within groups	24.107	48	0.052		
Total	11,141.273	71			
Chl <i>a</i>					
Between groups	516.287	23	22.447	1713.351	0.000
Within groups	0.629	48	0.013		
Total	516.916	71			
Primary Productivity					
Between groups	1567.369	23	68.146	8.545	0.000
Within groups	382.782	48	7.975		
Total	1950.151	71			

Table 5 Spatio-temporal variations of physico-chemical and biological parameters analysed through one-way ANOVA in the Mandovi and Zuari estuaries during high tide and their significance ($p < 0.001$)

	Sum of squares	df	Mean square	<i>F</i>	Significance
Temperature					
Between groups	65.839	23	2.863	22.216	0.000
Within groups	6.185	48	0.129		
Total	72.024	71			
pH					
Between groups	10,351.953	23	450.085	1383.103	0.000
Within groups	15.620	48	0.325		
Total	10,367.573	71			
Salinity					
Between groups	3.776	23	0.164	10.563	0.000
Within groups	0.746	48	0.016		
Total	4.522	71			
DO					
Between groups	47.723	23	2.075	3.671	0.000
Within groups	27.129	48	0.565		
Total	74.852	71			
NO ₂ ⁻					
Between groups	151.812	23	6.601	659.413	0.000
Within groups	0.480	48	0.010		
Total	152.293	71			
NO ₃ ⁻					
Between groups	2434.579	23	105.851	5075.785	0.000
Within groups	1.001	48	0.021		
Total	2435.580	71			
NH ₄ ⁺					
Between groups	9433.715	23	410.162	15,101.830	0.000
Within groups	1.304	48	0.027		
Total	9435.018	71			
PO ₄ ³⁻					
Between groups	192.115	23	8.353	3956.612	0.000
Within groups	0.101	48	0.002		
Total	192.217	71			
SiO ₄ ⁴⁻					
Between groups	103,675.060	23	4507.611	256,154.707	0.000
Within groups	0.845	48	0.018		
Total	103,675.905	71			
OM _w					
Between groups	10,310.631	23	448.288	54,660.047	0.000
Within groups	0.394	48	0.008		
Total	10,311.025	71			
OM _s					
Between groups	7636.516	23	332.022	11,167.717	0.000
Within groups	1.427	48	0.030		
Total	7637.943	71			
Chl <i>a</i>					
Between groups	135.571	23	5.894	296.842	0.000
Within groups	0.953	48	0.020		
Total	136.524	71			
Primary Productivity					
Between groups	1567.369	23	68.146	8.545	0.000
Within groups	382.782	48	7.975		
Total	1950.151	71			

the Cochin estuary. Higher OM in M_m and Z_m than in M_w and Z_w may have resulted from the breakdown of mangrove leaf litter as reported by Kristensen et al. (2008), indicative of the outwelling of OM from the mangroves. This phenomenon of elevated organic matter in surface sediments was also documented in similar mangrove-dominated aquatic systems (Kristensen et al. 2008; Prasad and Ramanathan 2009).

In general, the $\delta^{13}C$ signatures of mangroves is close to terrestrial C3 plants (−32% to −22%) (Boutton 1996), indicating that mangroves follow the C3 photosynthetic pathway (Calvin–Benson cycle). The recorded $\delta^{13}C_{sed}$ values of M_m and M_w (−26.14% to −24.31%) coincided with the range typical for mangroves plants (−30 % to −25%; Muzuka and Shunula 2006) as well as that for marine phytoplankton (−18% to −24 %; Fry and Sherr 1989). In the case of Z_m and Z_w (−25.67% to −24.54%), similar observations were noted. The observed $\delta^{13}C_{sed}$ ranges have also been reported from other tropical mangrove dominated estuaries, such as the Pichavaram estuarine-mangrove ecosystem of India (Prasad and Ramanathan 2009), and the Guayas River Estuary ecosystem of Ecuador (Cifuentes et al. 1996). The mixture of terrestrial derived material and marine derived material in the sedimentary OM has also been reported from the Hunts Bay, Forth estuary, Yangtze estuary, Pearl River estuary and Cochin estuary (Andrews et al. 1998; Graham et al. 2001; Zhang et al. 2007; Gireeshkumar et al. 2013). The concurrence of the $\delta^{13}C_{sed}$ values suggests a mixed carbon input derived from both mangrove-derived OM and marine phytoplankton, indicating outwelling of OM from mangroves into estuarine waters.

$\delta^{15}N$ signatures of marine plankton typically have an average of 6‰ ranging from 4‰ to 10‰, and terrestrial OM ranges from −10‰ to 10‰, averaging 2‰ (Alonso-Hernández et al. 2020). The $\delta^{15}N_{sed}$ in M_m (4.08‰ to 5.31‰) and Z_m (5.60‰ to 6.40‰) indicated a consistent and narrower variation, implying a steady OM source predominantly originating from mangroves in these areas. Contrastingly, the wider variability in $\delta^{15}N_{sed}$ observed in M_w (2.20‰ to 7.96‰) and Z_w (2.73‰ to 5.97‰) suggests a more diverse and mixed origin for the sedimentary OM. This broader range indicates the potential contribution from multiple sources, leading to a varied nitrogen isotopic composition within the sediment, reflecting a more complex input of OM in these estuarine waters. The possible contribution of OM from both the terrestrial and marine sources has also been reported from the Bay of Bengal (Krishna et al. 2013) and the estuarine system of West Africa (Dan et al. 2019).

The $\delta^{13}C_{sed}$ and $\delta^{15}N_{sed}$ data are often insufficient for source identification of OM in dynamic systems, such as the M and Z estuaries, which experience major seasonal variations in hydrology and biogeochemistry. Hence, the C:N_{sed} ratio is a useful indicator of OM origin. For terrestrial OM,

higher ratios of 12 to 200 have been recorded (Hedges and Mann 1979), whereas marine OM is defined by an observed excess of C over N with ratios of 10 or less (Pocklington and Leonard 1979). In our study, the peak C:N_{sed} ratios recorded during Mo suggested a substantial terrigenous OM input originating from runoff events during this period. Moreover, the elevated C:N_{sed} ratios in M corroborated with its higher terrigenous influence, attributed to the greater number of freshwater tributaries flowing into this estuary. Conversely, the lower C:N_{sed} ratios in Z indicated a potentially higher proportion of marine-derived OM. Overall, the observed C:N ratios indicated a mixture of marine and terrestrial sources of OM, similar to findings reported for the Yellow River estuary and the Beibu Gulf (Liu et al. 2015; Liao et al. 2018).

Impact of organic matter outwelling on phytoplankton biomass and productivity

The relationship between OM and phytoplankton PP in estuaries is complex and multifaceted. OM serves as a crucial nutrient source for phytoplankton growth, providing essential elements, such as C and N (Gobler and Sañudo-Wilhelmy et al. 2001). As phytoplankton photosynthesize, they utilize these nutrients along with sunlight to produce organic compounds through PP. In particular reference to the present study, the source of OM has been attributed to both terrestrial (predominantly mangroves) as well as marine sources. Mangrove-dominated estuaries are acknowledged for their ecological richness, and the role of phytoplankton is believed to be a significant factor in sustaining and enhancing this productivity (Rahaman et al. 2013). The fluctuations in the phytoplankton biomass i.e. Chl *a* concentration observed across tidal variations in our study, influences the quantity of pigment as a result of varying phytoplankton stocks (Dehadrai 1970). Higher concentration of Chl *a* during LT in comparison with HT during PM was owing to the prevalence of lower turbidity and higher penetration of sunlight (Perumal et al. 2009). Conversely, lower concentrations during Mo were likely a consequence of light inhibition caused by cloud cover and heightened water column turbidity (Perumal et al. 2009).

The peak PP observed during the PM period is linked to maximal nutrient utilization by phytoplankton which is previously reported by Seleyi et al. (2024). However, during the Mo, the extensive water dilution and stratification create conditions of freshwater salinity, resulting in turbid waters with limited light penetration, constraining phytoplankton production (Perumal et al. 2009; Bardhan et al. 2015). Despite the high nutrient inputs characteristic of the Mo season, their effective utilization by phytoplankton is hindered (Devassy and Goes 1989). Conversely, the PoM period witnessed the lowest PP, potentially owing to insufficient N

proportions in adjacent waters, which is critical for optimal phytoplankton production (Mathew et al. 2021).

The negative correlations found between Chl *a* and PP with OM_w and OM_s indicate a notable increase in the rate at which phytoplankton utilize OM. Additionally, the positive correlations observed between temperature, salinity, pH and DO with both Chl *a* and PP signify strong interdependencies among these environmental factors. Collectively, Pearson's correlations suggest that phytoplankton abundance and productivity are intricately linked to the availability and utilization of OM and is influenced by temperature, salinity, pH and DO. The PCA clearly segregated the parameters into distinct components and further validated the correlational findings by the subsequent regression analysis which showed that Chl *a* and PP are significantly influenced by temperature, salinity, pH, DO and OM. This suggests a complex relationship where environmental conditions significantly influence the growth and activity of phytoplankton within the estuarine ecosystem. Moreover, the significance of the impact of monsoonal variations on all the observed parameters i.e. during LT as well as HT was established by one-way ANOVA, affirming that the monsoonal precipitation exerts a noteworthy influence on the estuarine hydrography and productivity.

In summary, our study revealed notable differences in the phytoplankton abundance and productivity owing to seasonal variation of hydrological parameters. The study also highlighted the substantial impact of tidal influences on the outwelling of OM and nutrients from mangroves. The $\delta^{13}\text{C}_{\text{sed}}$ values offered insights into the carbon sources, indicating contributions from both mangrove-derived and marine sources. Concurrently, $\delta^{15}\text{N}_{\text{sed}}$ variations hinted at the diversity of N origin, with narrower ranges suggesting more stable sources, notably from mangroves. The C:N_{sed} ratios complemented these findings, showcasing fluctuations in terrigenous inputs and OM composition across M and Z. Although the present study does not directly quantify the outwelling of OM from the M and Z estuaries, the dual isotopic and elemental ratios established the significance of mangroves as a valuable source of OM to the adjacent estuaries. These comprehensive insights highlight the complex interconnections among environmental parameters, nutrient availability, organic matter sources and the resulting impact on phytoplankton dynamics within these estuarine ecosystems, consequently influencing overall productivity.

Conclusions

Despite being valuable resource of fisheries, mangrove forests are often susceptible to anthropogenic pollution, land reclamation for construction and deforestation resulting in

habitat loss. Thus, it is critical to establish the indispensable role of mangroves in enhancing the productivity of the estuaries and surrounding coastal waters. Our study depicted the significance of ambient environmental conditions, tidal fluctuations and irregularity in freshwater inputs on the concentrations of OM and nutrients in M and Z mangrove and estuarine waters. The dual isotopic tracers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and C:N_{sed} ratios revealed that the source of OM was from a combination of terrestrial (including mangroves) and marine sources. This implied that the productivity in the M and Z estuaries is enhanced by mangrove-derived OM facilitated by the process of outwelling. Multivariate statistical analysis confirmed that temperature, salinity, pH, DO and OM are the primary environmental drivers of PP. Thus, the resultant productivity in mangrove dominated estuaries ecosystems is based on a complex network of physical, chemical and biological processes. These findings have implications for understanding the trophic dynamics in estuaries, as primary productivity directly impacts the availability of OM for organisms at higher trophic levels.

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Author contributions S.C.S. performed data acquisition, formal analysis, writing—original draft and writing—review and editing; C.R. performed study conceptualization, methodology, writing—review and editing and supervision; C. M. performed writing—review and editing; P.P. performed data acquisition and formal analysis; T.A.N. performed data acquisition and formal analysis.

Data Availability All data generated or analysed during this study are included in this manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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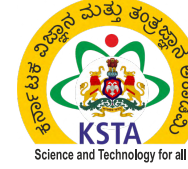
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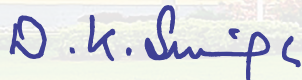
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