Studies on identification of organic matter sources through lipid biomarkers

A Thesis submitted to Goa University for the degree of

DOCTOR OF PHILOSOPHY

in

Marine Sciences

By

Laxman Abhiman Gardade

Goa University, Goa – 403 206, India

March, 2021

Studies on identification of organic matter sources through lipid biomarkers

A Thesis submitted to Goa University for the degree of

DOCTOR OF PHILOSOPHY

in

Marine Sciences

By

Laxman Abhiman Gardade

Research Guide

Dr. Lidita D.S. Khandeparker

Principal Scientist

CSIR-National Institute of Oceanography

Dona Paula, Goa – 403 004, India

School of Earth, Ocean and Atmospheric Sciences

Goa University, Goa – 403 206, India

March, 2021

Dedicated to my family

Contents Page

Statement of the Candidate
Certificate of the Research Supervisor
Acknowledgments
Abbreviations
List of Figures and Tables

Chapter 1. General Introduction……..1-16

Chapter 2. Characterization of organic matter using lipid biomarkers in the selected study area…….17-60

2.1 Introduction……...17-19

2A. Assessment of spatio-temporal variations in the sedimentary organic matter composition in the Kolkata port ……....19
2A.2 Materials and methods……19
2A.2.1 Description of the study area……..19
2A.2.2 Sampling strategy…….....21
2A.2.3 Analyses of elemental components from the sediment……..22
2A.2.4 Analyses of biochemical components from the sediment.....22
2A.2.5 Analyses of fatty acids from the sediment…….....23
2A.2.6 Data analyses……..24
2A.3 Results……25
2A.3.1 Spatio-temporal variations in the elemental and biochemical composition of Kolkata port sediment…….....25
2A.3.2 Spatio-temporal variations in the fatty acids (FAs) in Kolkata port sediment……28
2A.3.3 Principal Component Analysis (PCA)……33

2B. Assessment of spatio-temporal variations in the sedimentary organic matter composition in the Kandla port ……36
2B.2 Materials and methods……36
2B.2.1 Description of the study area……..36
2B.2.2 Sampling strategy…….....37
2B.2.3 Analyses of elemental components from the sediment……..38
2B.2.4 Analyses of biochemical components from the sediment…..38
2B.2.5 Analyses of fatty acids from the sediment……..38
2B.2.6 Data analyses……..38
2B.3 Results……39
2B.3.1 Elemental and biochemical composition of Kandla port sediment……..39
2B.3.2 Spatio-temporal variations in the fatty acids (FAs)

in Kandla port sediment……..42
2B.3.3 Principal Component Analysis (PCA)……....46
2.4 Discussion……..49
2.4.1 Sources of OM in the sediment of Kolkata and Kandla ports indicated by elemental components and fatty acid biomarkers……..49
2.4.2 Benthic trophic status of Kolkata and Kandla ports……..54
2.5 Conclusions……..59-60

Chapter 3. Characterization of short-term variation in organic matter composition……...61-113

3.1 Introduction……...61-66

3A. Spatio-temporal variations in elemental, biochemical components, and bacterial populations in the surface sediment of the Zuari estuary: fortnightly observations……..66
3A.2 Materials and methods……66
3A.2.1 Description of the study area……...66
3A.2.2 Sampling strategy...……68
3A.2.3 Enumeration of Total Bacterial Count (TBC) in the surface sediment by using Flow Cytometry……..69
3A.2.4 Enumeration of Total Viable Count (TVC), *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria) in the surface sediment……70
3A.2.5 Analyses of elemental and biochemical components from the surface sediment……...71
3A.2.6 Data analysis…….....72
3A.3 Results……73
3A.3.1 Physico-chemical parameters of the near-bottom water…….73
3A.3.2 Spatio-temporal variations in TBC, TVC, *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria) in the surface sediment……73
3A.3.3 Elemental and biochemical composition of the surface Sediment……80
3A.4 Discussion…….....84
3A.5 Conclusions……89

3B. Evaluation of monthly variations in the sources of sedimentary OM in the Zuari estuary using FA biomarkers and elemental components...……90
3B.1 Introduction……90
3B.2 Materials and methods……91
3B.2.1 Description of the study area……91
3B.2.2 Sampling strategy…….....92

3B.2.3 Determination of the elemental composition of the sediment..93
3B.2.4 Analyses of fatty acids from the sediment…….....93
3B.2.5 Data analyses……...93
3B.3 Results…….95
3B.3.1 Elemental composition of the sediment……..95
3B.3.2 Spatio-temporal variations in the fatty acids (FAs)…….96
3B.4 Discussion…….....103
3B.5 Conclusions……112-113

Chapter 4. Evaluation of fate of sedimentary OM in the Zuari estuary through laboratory microcosm experiments…….....114-136

4.1 Introduction……...114-117
4.2 Materials and Methods…….117
4.2.1 Study area and sample collection……117
4.2.2 Culturing of diatoms for the microcosms…….....119
4.2.3 Collection of zooplankton…….....119
4.2.4 Microcosm set up…….119
4.2.5 Enumeration of total viable bacterial counts (TVC), *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria)……121
4.2.6 Analyses of fatty acids from the bulk and spiked sediment….121
4.3 Results……..121
4.3.1 Variations in total viable bacteria, *Vibrio* spp., coliforms, and bacteria-specific FAs from the sampling sites……121
4.3.2 Variations in the abundance of total viable bacteria, *Vibrio* spp., and coliforms in the microcosm experiments……121
4.3.3 Variations in the bacteria-specific fatty acids in the microcosm experiments…….....125
4.3.4 Variations in the diatoms-specific fatty acids during the incubation……...126
4.3.5 Variations in the zooplankton-specific fatty acids during the incubation……...128
4.3.6 Variations in the mangrove-specific fatty acids during the incubation……...129
4.4 Discussion……131
4.5 Conclusions……...135-136

Chapter 5. Summary……137-142

Bibliography……..143-174

Appendix……175-177

Publications……..178

STATEMENT OF THE CANDIDATE

As required under the University Ordinance OB-9A.5, I hereby state that the present thesis entitled "**Studies on identification of organic matter sources through lipid biomarkers**" is my original research work carried out in the CSIR-National Institute of Oceanography, Dona Paula, Goa and the same has not been submitted in part or in full elsewhere for any other degree or diploma.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Laxman Abhiman Gardade

सी एस आई आर - राष्ट्रीय समुद्र विज्ञान संस्थान
(वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद)
दोना पावला, गोवा 403 004 भारत
CSIR - National Institute of Oceanography
(Council of Scientific & Industrial Research)
DONA PAULA, GOA - 403 004, India

CERTIFICATE

This is to certify that the thesis entitled "**Studies on identification of organic matter sources through lipid biomarkers**", submitted by Mr. Laxman Abhiman Gardade for the award of the degree of Doctor of Philosophy in Marine Sciences is based on original studies carried out by him under my supervision. This thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

Dr. Lidita D.S. Khandeparker

Research Guide
Principal Scientist
Biofouling and Bioinvasion Laboratory
Biological Oceanography Division
CSIR-National Institute of Oceanography
Dona Paula, Goa 403004

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my guide, Dr. Lidita Khandeparker, for her valuable guidance, encouragement, and generous help during my Ph.D. work. I am very much grateful to her for constant unconditional support and motivating me for this work.

I would like to express my deep gratitude and respect to Dr. A. C. Anil, Chief Scientist and Head (retired), Biofouling and Bioinvasion Division, for providing all the facilities required for my work. I am grateful for his support and encouragement during the period of my work.

I would like to thank the Director, Council of Scientific and Industrial Research (CSIR)-National Institute of Oceanography (NIO), for giving me an opportunity to carry out this research in this institute.

I am thankful to the members of FRC committee, Dr. S. S. Sawant, my V.C.'s nominee for his thoughtful comments, valuable suggestions, and encouragement. I also acknowledge the other members of FRC committee, Prof. Janarthanam, Prof. C. U. Rivonker, Prof. Matta, Prof. P.K. Sharma, and Prof. H.B. Menon for their insightful comments and advice.

My special thanks to Dr. Dattesh Desai for his constructive suggestions during the scientific as well as personal difficulties. His readiness to help will always be valued.

I am very much thankful to Mr. K. Venkat for his immense help received during the Port Biological Baseline Surveys as well as in the lab.

I would like to thank Dr. Jagdish Patil, Dr. Temjensangba Imchen for their help during the field studies.

I also thank Mr. Kaushal Mapari for the help and support rendered during the work.

I would like to express my gratitude towards Dr. V. V. Gopalakrishna and Dr. D shankar for their support.

This work was supported by the Ballast Water Management Program, India (BAMPI) funded by the Directorate General, Ministry of Shipping, Government of India and CSIR funded Ocean Finder Program. The financial support from these sources is greatly acknowledged. I would like to thank all the crew members of boat and officers of ports for their invaluable help during the port surveys.

I wish to acknowledge the library, administrative, and HRM staff of NIO for their help during this work.

I would like to thank my lab mates Mr. Nishanth Kuchi, Mr. Ranjith E., Ms. Niyati Hede, Dr. Deodatta Gajbhiye, Dr. Gobardhan Sahoo, Mr. Sumit Charde, Mr. Dipesh Kale, Mrs. Sneha, Mrs. Akshaya, Dr. Chetan Gaonkar, Dr. Dhiraj Narale, Dr. Suchandan Bemal, Mr. Roy Rodrigues, Mr. Sathish Krishan, Dr. Rajaneesh Kolchar, Dr. Lalita Baragi, Mr. Atchuthan Puroshottam, Mr. Noyel Velayudham, Ms. Sangeeta Naik, Dr. Ravi Naik, Dr. Priya D'coasta, Dr. Shamina, Mr. Vijaykrishna, Mr. Amirthraj, Mrs. Deepti, Mrs. Sameeksha, Ms. Vivienne Godinho, Mr. Vinay Dethe, Ms. Karishma Prabhu, Mr. Aseem Rath, Mr. Siddhesh Shirodkar, Mrs. Vijayalaxmi, Mrs. Priya, Mrs. Harshal, Mr. Roshan Parab, Ms. Alisha Kumar, Ms. Swati Sutar, Mr. Sitam Mote, Mr. Nitin Gholkar, Mr. Yatin, Mr. Pandia Sudhan, Mr. Krishnasamy, Mrs. Sulaksha, Ms. Conchita Monteiro, Mrs. Dipti Verlekar, Mr. Sathish P, Mr. Dayakaran Pratap, Mrs. Manjitha,

Ms. Nikita Gadkar, Mr. Shailesh Nair, Ms. Samanta D'souza, Mr. Pranoy Paul, Mr. Wasim Ezaz, Mr. Manuel Esteves, Ms. Saili Naik, Mr. Vijay Rawat, Mr. Andrew, Mr. Sagar Khadke, and Mr. Roshan D'souza for their help and support which made my journey enjoyable.

I would like to thank my friends, Mr. Yuvraj Jadhav, Mr. Atul Dudhane, Mr. Santosh Yeware, Dr. Amar Yeware, Mr. Trinadha for their support and help in every possible way and situation.

I honestly thank all those who have helped me directly or indirectly in the successful completion of this thesis.

I am indebted to my parents for their love, care, and sacrifice done throughout their life for educating me and giving me whole hearted support. Although, bhau (father) you are not there among us now, I can always feel your presence and blessings. I am very much grateful to my aaie (mother), for her continuous support during this work. No words can express my appreciations and gratitude towards her. I sincerely thank my elder brother (Mr. Rambhau), sisters (Mrs. Pushpa, Manisha, Pramila, and Asha), and sister in-law (Mrs. Anita) for their untiring love and support during my education. I would like to thank my wife Mrs. Madhuri for her continuous support and understanding me. I have no words to express my gratitude towards her. I am warmed by the love, I got from my nephew Saurabh and niece Sayali.

The one above all of us, almighty God is thanked for giving me the strength and blessings.

Laxman Abhiman Gardade

Abbreviations

ANOVA:	Analysis of variance
BPC:	Biopolymeric carbon
CHO:	Carbohydrates
DO:	Dissolved oxygen
E:	Eutrophic
FAMEs:	Fatty acid methyl esters
FAs:	Fatty acids
GC-MS:	Gas Chromatography-Mass Spectrometry
IC:	Inorganic carbon
KPD:	Kidderpore Dock
LCFAs:	Long-chain fatty acids
LOM:	Labile organic matter
LPD:	Lipids
M:	Mesotrophic
MON:	Monsoon
MUFA:	Monounsaturated fatty acid
NSD:	Netaji Subhash Dock
O:	Oligotrophic

PCA: Principal component analysis

POM: Post-monsoon

PRM: Pre-monsoon

PRT: Proteins

PUFAs: Polyunsaturated fatty acids

RS: Riverine station

S: Station

SFAs: Saturated fatty acids

SH: *Shigella* spp.

SL: *Salmonella* spp.

TC: Total carbon

TFA: Total fatty acids

TN: Total nitrogen

TOC: Total organic carbon

TOM: Total organic matter

TS: Trophic status

TVC: Total Viable Counts

TBC: Total Bacterial Counts

VA: *Vibrio alginolyticus*

VC: *Vibrio cholerae*

VP: *Vibrio parahaemolyticus*

List of Figures

Figure 1.1 Schematic representation showing sources of organic matter and its role in the coastal and estuarine systems.

Figure 1.2 Schematic representation showing how biogeochemical tools differ in their ability to identify the specific sources of OM and the proportion of OM they represent.

Figure 1.3 Classification and structure of lipids**.**

Figure 1.4 Chemical structure of eicosapentaenoic acid (EPA).

Figure 2A.1 Map showing the location of stations in Kolkata port, north east coast of India.

Figure 2A.2 Average values of elemental and biochemical components in the surface sediment of Kolkata port during different seasons.

Figure 2A.3 Average values of detrital (ubiquitous) and source-specific fatty acids in the surface sediment of Kolkata port during different seasons.

Figure 2A.4 Spatio-temporal variations in the percentage contribution of source-specific FAs to detrital FAs in the Kolkata port.

Figure 2A.5 Principal Component Analysis (PCA) plots for fatty acids, elemental, and biochemical variables in the surface sediment of Kolkata port. a) Variables and factor loadings, b) Sample scores for sites.

Figure 2B.1 Map showing the location of stations in Kandla port, north west coast of India.

Figure 2B.2 Average values of elemental and biochemical components in the surface sediment of Kandla port during different seasons.

Figure 2B.3 Average values of detrital (ubiquitous) and source-specific fatty acids in the surface sediment of Kandla port during different seasons.

Figure 2B.4 Spatio-temporal variations in the percentage contribution of source-specific FAs to detrital FAs in Kandla port.

Figure 2B.5 Principal Component analysis (PCA) plots for fatty acids, elemental, and biochemical variables in the surface sediment of Kandla port. a) Variables and factor loadings, b) sample score for sites.

.

Figure 3A.1 Map showing the location of stations in the Zuari estuary, Goa, central west coast of India.

Figure 3A.2 Fortnightly variations in the (a) Total Bacterial Count, (b) Total Viable Count, (c) *V. cholerae*, (d) *V. alginolyticus*, (e) *V. parahaemolyticus*,

(f) Total coliforms, (g) *Shigella* spp., and (h) *Salmonella* spp. in the surface sediment of the Zuari estuary.

Figure 3A.3 Seasonal variations in the (a) Total Bacterial Count, (b) Total Viable Count, (c) *V. cholerae*, (d) *V. alginolyticus*, (e) *V. parahaemolyticus*, (f) Total coliforms, (g) *Shigella* spp., and (h) *Salmonella* spp. in the surface sediment of the Zuari estuary during different tidal phases.

Figure 3A.4 Fortnightly variations in the (a) total organic carbon (TOC), (b) total nitrogen (TN), and (c) TOC/TN ratio in the surface sediment of the Zuari estuary.

Figure 3A.5 Fortnightly variations in the (a) proteins (PRT), (b) carbohydrates (CHO), and (c) PRT/CHO ratio in the surface sediment of the Zuari estuary.

Figure 3B.1 Map showing sampling sites in the Zuari estuary, Goa, central west coast of India.

Figure 3B.2 Monthly variations in the distribution of elemental components in the surface sediment of the Zuari estuary.

Figure 3B.3 Monthly variations in the total, detrital (ubiquitous), and source-specific fatty acids (FAs) in the surface sediment of the Zuari estuary.

Figure 3B.4 Spatial variations in the contribution of source-specific fatty acids to total fatty acids in the Zuari estuary.

Figure 3B.5 Spatio-temporal variations in the percentage contribution of source-specific fatty acids to detrital fatty acids.

Figure 4.1 Map showing sampling sites in the Zuari estuary.

Figure 4.2 Schematic representation of the microcosm setup.

Figure 4.3a The content of FAs specific to different bacterial groups in the sediment of studied sites.

Figure 4.3b Variations in the abundance of total viable bacteria, *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria) in the sediment spiked with different organic materials.

Figure 4.3c Variations in the bacteria-specific FAs during the incubation in the spiked and control sediment.

Figure 4.4 Variations in the diatom-specific FAs during the incubation in the spiked and control sediment.

Figure 4.5 Variations in the zooplankton-specific FAs during the incubation in the spiked and control sediment.

Figure 4.6 Variations in the mangrove-specific FAs during the incubation in the spiked and control sediment.

List of Tables

Table 1.1 List of fatty acid biomarkers used to identify the sources of the organic matter in the sediment.

Table 2A.1 Summary of two-way ANOVA of elemental and biochemical components of surface sediment from the Kolkata port.

Table 2A.2 Summary of two-way ANOVA of fatty acids (FAs) of surface sediment from the Kolkata port.

Table 2A.3 Correlation matrix showing r values of fatty acid markers, elemental, and biochemical variables from the surface sediment of Kolkata port.

Table 2B.1 Summary of two-way ANOVA of elemental and biochemical variables of surface sediment from the Kandla port.

Table 2B.2 Summary of two-way ANOVA of fatty acids (FAs) of surface sediment from the Kandla port.

Table 2B.3 Correlation matrix showing r values of fatty acid markers, elemental, and biochemical variables from the surface sediment of Kandla port.

Table 2.4 Range of the biochemical components (PRT: Proteins; CHO: Carbohydrates; LPD: Lipids) in the sediment from the ports, estuaries, and coastal areas around the world.

Table 2.5 Average values of biochemical components of 4 seasons for the stations and benthic trophic status of the Kolkata port.

Table 2.6 Average values of biochemical components of 4 seasons for the stations and benthic trophic status of the Kandla port.

Table 3A.1a Details of sampling stations in the Zuari estuary.

Table 3A.1b Details of sampling and tidal height observed during the sampling period.

Table 3A.2 Correlation matrix showing r values of environmental parameters with bacterial populations in the surface sediment of the Zuari estuary.

Table 3A.3 Percentage (%) occurrence of different bacteria in the surface sediment of the Zuari estuary.

Table 3A.4 Correlation matrix showing r values of elemental, biochemical components, and bacterial populations in the surface sediment of Zuari estuary.

Table 3B.1 Details of sampling, tidal height, salinity, and temperature of the near-bottom water of the Zuari estuary.

Table 3B.2 Summary of two-way ANOVA of elemental components and fatty acid markers from the surface sediment of the Zuari estuary.

Table 3B.3 Correlation matrix showing r values of elemental components and fatty acid markers from the surface sediment of the Zuari estuary.

Table 4.1 The percentage (%) of diatoms-specific FAs utilized after 10 days of incubation in the sediment.

Table 4.2 The percentage (%) of zooplankton-specific FAs utilized after 10 days of incubation in the sediment.

Table 4.3 The percentage (%) of mangrove-specific PUFAs and LCFAs utilized after 10 days of incubation in the sediment.

Table 4.4 The percentage (%) of mangrove-specific long-chain FAs utilized after 65 days of incubation in the sediment spiked with mangrove leaves and a mixture of diatoms, zooplankton, and mangrove leaves.

Chapter 1

General Introduction

1. General Introduction

Coastal and estuarine areas are highly productive zones, which acts as nursery and breeding grounds for a wide range of organisms such as polychaetes, nematodes, gastropods, bivalves, lobsters, shrimps, oysters, crabs, fish, etc. (Blaber, 1997; Costanza et al., 1997). The organic matter (OM) forms the base of diverse food webs, acts as a source of energy and nutrients required for the growth, reproduction, and other activities of these organisms (McLusky and Elliot, 2004). The OM in the sediment of coastal and estuarine regions could be of autochthonous origin if it is formed in the system itself or of allochthonous origin if it is received in the system from external sources (Fig. 1.1). The autochthonous sources in these systems include remains of phytoplankton, macroalgae, zooplankton, bacteria, and allochthonous sources include inputs from the terrestrial plants, mangrove litter, riverine run-off, domestic waste, industrial discharge, etc. (Meyers, 1997; Mudge et al., 1998; Hu et al., 2006; Dai and Sun, 2007; Dunn et al., 2008; Harji et al., 2010).

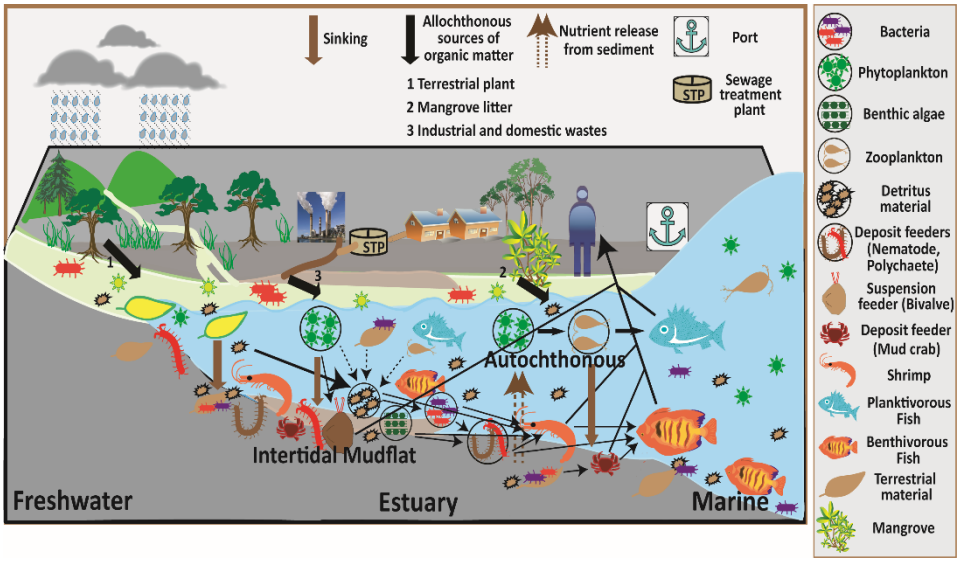


Fig. 1.1 Schematic representation showing sources of organic matter and its role in the coastal and estuarine systems.

The information on the sources and composition of OM is essential to understand the role of aquatic systems in the carbon cycle (Hedges and Keil, 1995; Wakeham and Canuel, 2006).

The autochthonous and terrestrial-derived OM in the system sinks through the overlying water column and finally gets preserved in the sediment (Meyers, 1994). Thus, surface sediment acts as a stock or the repository of the autochthonous and allochthonous derived materials (Fabiano and Danovaro, 1994; Dell'Anno et al., 2002). Characterization of OM from the sediment is useful to evaluate the changes in the productivity, nutritional quality, and the nature of food available in the sediment (Fabiano and Danovaro, 1994; Danovaro et al., 1999; Cividanes et al., 2002). Earlier studies have revealed that the distribution of benthic communities in the sediment is significantly influenced by the quantity of available food (Pearson and Rosenberg, 1978; Rosenberg, 1995; Wei et al., 2010). However, there are few studies that have recognized the importance of nutritional quality of the available food, and its influence on the distribution of benthic consumers (Wieking and Kroncke, 2005; Quintana et al., 2010). Muller-Navarra (2008) defined the term "food quality" as the degree of food which is accessible to satisfy the basic nutritional demand of the consumers, and this cannot be unraveled from quantity alone, since, large quantities of nutritionally-poor food may be equivalent to low quantities of nutritionally rich food. Therefore, to understand the influence of food on the benthic community structure, it is crucial to examine the quality and quantity of food along with other characteristics of the habitat. The juveniles of demersal fish are dependent upon the detrital pool derived from the sediment, and the

benthic standing crop sustains high resources of demersal fish and prawns in the coastal zones and estuaries (Harkantra and Parulekar, 1981; Ansari and Parulekar, 1994; Ansari et al., 1995). This indicates that OM, as a source of energy, plays an essential role in the trophic flow in the aquatic systems from sediment to benthic fauna to commercially exploitable fish. Thus, unraveling the origin of OM in the coastal and estuarine systems is useful to evaluate their ecosystem functioning (Careddu et al., 2015).

The total organic carbon to total nitrogen ratio (TOC/TN) has been used to identify the contribution of marine and terrestrial-derived OM in the sediment (Meyers, 1994). Marine OM mostly derived from the planktons is protein-rich and cellulose-poor, and has TOC/TN ratio values that range from 4 to 10, whereas vascular land plants, which are protein-poor and cellulose-rich, usually has TOC/TN ratio of 20 and greater. The selective decomposition of proteinaceous compounds has the potential to increase the TOC/TN ratio; however, microbial immobilization of nitrogen during remineralization of organic carbon can lower the TOC/TN ratio (Meyers, 1994). Stable isotopic composition ($\delta^{13}C$) is complementary to the TOC/TN ratio and is useful to distinguish the contribution between marine phytoplankton and different terrestrial plant types based on the carbon assimilation pathways (C_3, C_4) and isotopic composition of carbon source (Fry and Sherr, 1984; Meyers, 1994). Terrestrial plants that incorporate carbon into the OM using C_3 Calvin pathway are more depleted in ^{13}C ($\delta^{13}C$ = -31‰ to -26‰) and terrestrial plants that use C_4 Hatch-Slack pathway are enriched in ^{13}C ($\delta^{13}C$ = -16‰ to -12‰). Whereas, marine phytoplankton has

intermediate $\delta^{13}C$ (-22‰ to -20‰) values (Fry and Sherr, 1984; Meyers, 1994).

In the case of coastal areas, which receive OM from phytoplankton, and both C_3 and C_4 terrestrial vascular plants, the isotopic composition is not suitable due to overlap in the stable isotopic signatures of the source materials (Meyers, 1997; Cloern et al., 2002). Under such conditions, biomarkers are suitable tools to determine the OM composition because of their source specificity (Fig. 1.2).

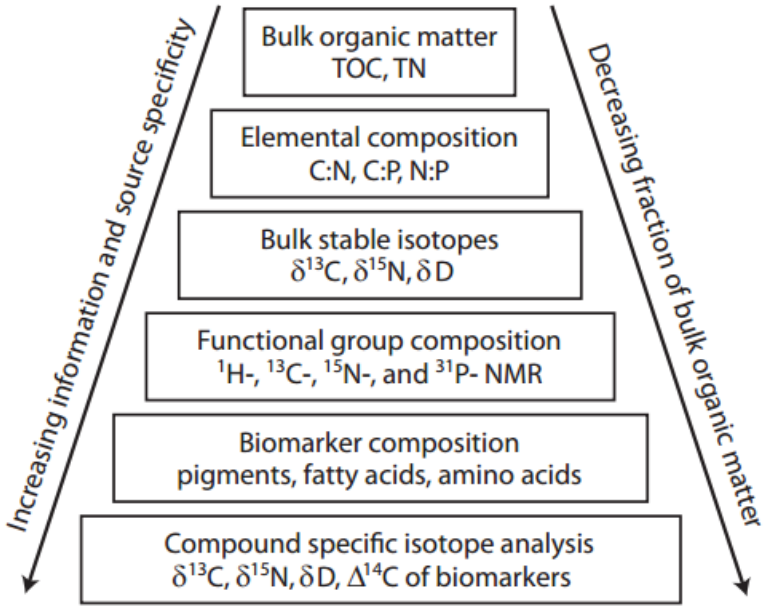


Fig. 1.2 Schematic representation showing how biogeochemical tools differ in their ability to identify the specific sources of OM and the proportion of OM they represent (Source: Bianchi and Canuel, 2011).

Biological markers, generally called as biomarkers, are the compounds that characterize specific biotic sources and retain their source information after burial in sediment, even after some alterations (Meyers, 2003). Lipid compounds such as fatty acids, *n*-alkanes, alcohols, and sterols have been widely used as biomarkers to identify the specific and diverse sources of OM

in the sediment of the aquatic systems (Canuel and Martens, 1996; Carrie et al., 1998; Mudge et al., 1998; Zimmerman and Canuel, 2001; Muri et al., 2004; Dai and Sun, 2007; Volkman et al., 2008; Dunn et al., 2008; Harji et al., 2010; Venturini et al., 2012b; Gireeshkumar et al., 2015; Guo et al., 2019). Lipids have been widely used as biomarkers because -

- Lipids are found in diverse sources and are essential components of living cells.
- Their relative stability, biological specificity, and structural features (carbon chain length, number, and position of double bonds) make them ideal biomarkers (Zimmerman and Canuel, 2001).
- Reactivity of compounds can be used to determine the quality of OM, e.g., polyunsaturated fatty acids (FAs) being labile; their presence in the sediment is indicative of freshness of OM. whereas saturated FAs are indicators of older and partially degraded OM (Canuel and Martens, 1993; Gong and Hollander, 1997; Carrie et al., 1998; Dunn et al., 2008).
- These compounds can characterize the contribution of autochthonous (in-situ produced) and allochthonous (terrestrial plant) derived OM.
- Some compounds have been used to determine the input of anthropogenic sources, e.g., short-chain FAs (C16:0, C18:0) and faecal sterols (coprostanol) have been used to determine the input of domestic wastes and sewage in the aquatic systems (Quéméneur and Marty, 1984; Mudge et al., 1998; Carreira et al., 2004; Shilla et al., 2011; Boechat et al., 2014).

Most importantly, these biomarkers have the ability to trace the OM derived from primary producers (diatoms, dinoflagellates, macroalgae), consumers

(zooplankton), decomposers (bacteria), and allochthonous materials (terrestrial plants, mangroves) simultaneously (Bianchi and Canuel, 2011).

Lipids are amphipathic or hydrophobic molecules that are insoluble in water and readily soluble in organic solvents. These molecules display a wide diversity in molecular structure and biological functions, like energy storage, membrane matrix, and signaling events. Lipids are the most critical component of cellular membranes and act as a structural matrix of the membrane (Dowhan et al., 2008). There are two major components of lipids, i.e., storage and membrane lipids. Storage lipids are also called as neutral lipids, and they act as energy storage molecules, which contain mainly triacylglycerols, having one glycerol molecule and three fatty acid molecules (Fig. 1.3).

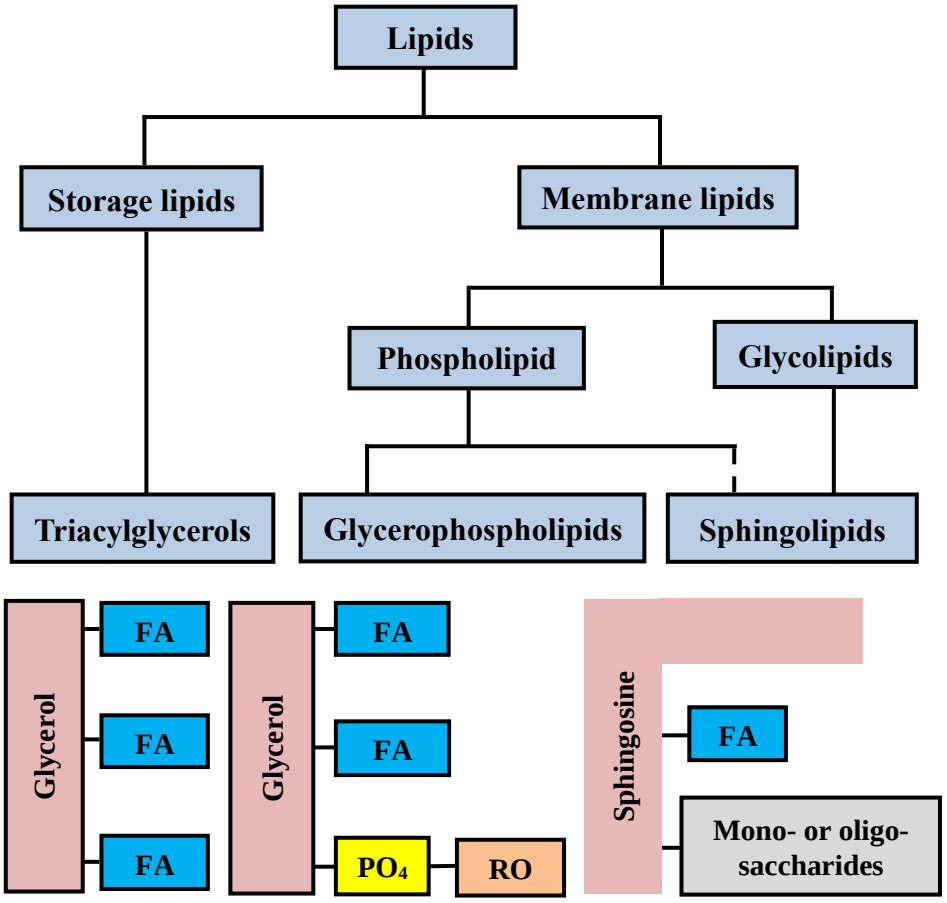


Fig. 1.3 Classification and structure of lipids (Source: Lehninger, 2013).

The membrane lipids are polar compounds and can be broadly categorized into phospholipids and glycolipids (Fig. 1.3). Furthermore, sphingolipids can be either glycosphingolipids or phosphospingolipids. Glycerophospholipids commonly referred to as phospholipids, are the predominant molecules in the cell membranes (Lehninger, 2013).

Fatty acids are the most versatile and fundamental component of lipids and are source-specific, diverse in structure, and hence commonly used as biomarkers to identify the sources, diagenetic alterations, and fate of OM in the sediment (Carrie et al., 1998; Mudge et al., 1998; Meziane and Tsuchiya, 2000; Dunn et al., 2008; Venturini et al., 2012b; Joseph et al., 2012). Fatty acids (FAs) are long-chain hydrocarbons with carboxyl and methyl groups. FAs can be saturated, having no double bonds in the carbon chain or unsaturated having one or more double bonds and are called monounsaturated or polyunsaturated FAs, respectively. The structure of FA is described as A: BωC, where A = number of carbon atoms, B = number of double bonds, and C = position of double bonds. Omega (ω) is the notation used for numbering the position of double bond from the methyl end of FA (Fig. 1.4). The delta (Δ) notation is also used for the nomenclature of FAs to specify the position of double bond from the carboxyl end of FA.

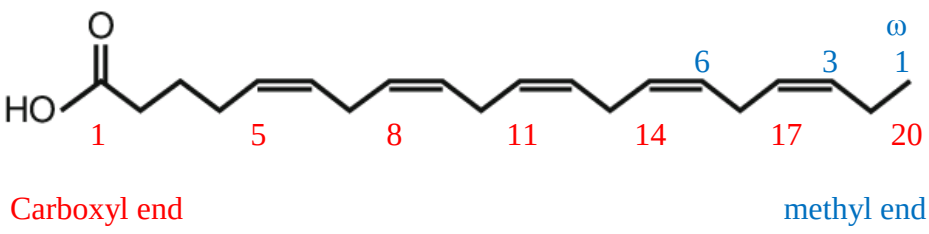


Fig. 1.4 Chemical structure of eicosapentaenoic acid (EPA) (Source: Blair and Dhillon, 2014).

Fig. 1.4 shows the chemical structure of eicosapentaenoic acid (EPA). EPA (C20:5ω3) is a polyunsaturated FA having 20 carbon atoms in the chain with five cis double bonds. This FA is called as ω-3 FA because it has the first double bond in "ω-3 position," i.e., third carbon from the methyl end. This FA is also named as C20:5($\Delta^{5,8,11,14,17}$), indicating the position of double bonds from the carboxyl end of FA (Fig. 1.4). There are two configurations of unsaturated FAs, i.e., cis and trans, based on the position of two hydrogen atoms adjacent to double bonds. The cis FA has hydrogen atoms located on the same side, and trans FA has hydrogen atoms on the opposite side. Branched FAs could be iso, when a methyl group is at the ω-1 position or anteiso, if methyl group is at ω-2 position (Zimmerman and Canuel, 2000).

Fatty acids are used to determine the contribution of certain groups, e.g., bacteria produce odd carbon-numbered, branched, and monounsaturated FAs (C15:0, C17:0, C18:1ω7, iC15:0, aC15:0, iC17:0, and aC17:0), diatoms are rich in C14:0, C16:1ω7, C20:4ω6, and C20:5ω3 FAs (Volkman et al., 1989, Carrie et al., 1998). Whereas, C18:1ω9, C22:6ω3 are found in the dinoflagellate, and C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3 in macroalgae (Table 1.1). The long-chain monounsaturated FAs (C22:1ω9, C24:1ω9) are dominant in the zooplankton (Table 1.1). Long-chain saturated FAs (C22:0 – C30:0) are present in waxy leaf coatings of vascular plants and are used as indicators of allochthonous terrestrial plant-derived materials (Canuel and Martens, 1993; Carrie et al., 1998). The short-chain saturated FAs such as C16:0, C18:0 are ubiquitous, found in the phytoplankton, bacteria, terrestrial plants (Rajendran et al., 1992; Carrie et al., 1998; Mudge et al., 1998; Canuel,

2001), and have been used as indicators of input of detrital materials (David et al., 2019; Zhukova et al., 2019).

Table 1.1 List of fatty acid biomarkers used to identify the sources of the organic matter in the sediment.

Fatty acids (FAs)	Sources of FAs	References
C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0, C18:1ω7	Bacteria	Volkman et al., 1989; Rajendran et al., 1995; Canuel and Martens, 1996
C14:0, C16:1ω7, C20:4ω6, C20:5ω3	Diatom	Volkman et al., 1989; Carrie et al., 1998; Mudge et al., 1998
C18:1ω9, C18:4ω3, C22:6ω3	Dinoflagellate	Carrie et al., 1998; Zimmerman and Canuel, 2001
C22:1ω9, C24:1ω9	Zooplankton	Carrie et al., 1998; Dalsgaard et al., 2003; Venturini et al., 2012b
C18:2ω6, C18:3ω3, C18:3ω6, C20:2ω6, C20:3ω3	Macroalgae	Volkman et al., 1989; Mudge et al., 1998; Meziane and Tsuchiya, 2000
Long chain fatty acids (C22:0 - C30:0)	Terrestrial plant	Meyers, 1997; Meziane and Tsuchiya, 2000
Ubiquitous fatty acids (C16:0, C18:0)	Detrital materials	Carrie et al., 1998; David et al., 2019; Zhukova et al., 2019

(i: iso; a: anteiso)

Fatty acids have been widely used to elucidate the sources of sedimentary OM from the coastal, estuarine, and riverine systems (Canuel and Martens, 1996; Carrie et al., 1998; Zimmerman and Canuel, 2001, Hu et al., 2006; Dai and Sun, 2007; Volkman et al., 2008; Venturini et al., 2012b; Guo et al., 2019), to evaluate the short-term changes in the quality and composition of suspended particulate organic matter (Bodineau et al., 1998; David et al., 2019) and sedimentary OM (Canuel and Martens, 1993). They have also been used to understand the degradation pattern and fate of algae,

diatoms, land grasses, salt marsh plants, and mangroves in the sediment through the different field and laboratory experiments (Sun et al., 2000, 2002; Mfilinge et al., 2003; Ding and Sun, 2005; Dai et al., 2009). FA biomarkers are also used for evaluating the impact of changes in land-use patterns and other human activities on the OM content of the sediment (Zimmerman and Canuel, 2000) and suspended particulate organic matter (Boechat et al., 2014). Some studies have utilized FA biomarkers to trace the pattern of OM delivery, its utilization, and preservation processes in the sediment. Shi et al. (2001) found deposition of terrestrial and marine phytoplankton derived particulate OM at different zones in the Altamaha estuarine sediment. Hu et al. (2006) identified the sources of OM by using FA biomarkers, and the $\delta^{13}C$ content of bacterial FAs confirmed the role of bacteria in the utilization of labile OM in the sediment of Pearl River estuary and adjacent shelf, Southern China.

In India, most of the studies on OM composition using FA biomarkers are carried out in the estuarine sediment (Ghosh et al., 1990; Harji, 2011; Joseph et al., 2012; Sanil Kumar and Nair, 2015; Gireeshkumar et al., 2015). FAs have also been used to trace the trophic interactions among producers and consumers in the mangrove ecosystem (Alikunhi et al., 2010). Joseph et al. (2012) reported nutritionally rich, and fresh OM dominated by diatoms, zooplankton, as well as terrestrial derived OM in the sediment of the mangrove ecosystem of Cochin. The combined bulk indices and FA biomarkers have also been used to elucidate the sources of sedimentary OM from the Cochin estuary (Gireeshkumar et al., 2015), and Chitrapuzha river estuary (Sanil Kumar and Naik, 2015). These studies suggested the effective

utilization of algal-derived labile OM by the bacteria during the settling and decomposition process in the sediment and selective preservation of terrestrial-derived OM.

The OM plays a significant role in the sustenance and ecological balancing of the food web dynamics. It has been reported that meio- and macrofauna obtain a large amount of energy (81% and 65%) from the sediment carbon and remaining (19% and 35%) from the water column (Warwick et al., 1979). Sediment is an active site of OM degradation and nutrient regeneration, which gets diffused and transported back to the overlying water column in shallow water systems (Hammond et al., 1984). The availability of sedimentary OM of high-quality supports the nutritional requirements of benthic organisms, their activities, and also helps in pelagic production. But, in recent years, the tremendous increase in the anthropogenic activities have resulted in an increase in the concentration of nutrients and organic materials which causes detrimental effects to the coastal, estuarine, and riverine ecosystems (Nixon, 1995; Zimmerman and Canuel, 2001; Cloern, 2001; Dell'Anno et al., 2002). An increase in OM input causes significant changes in the benthic trophic status which may affect assemblages of benthic organisms in the sediment, their ecological functions, as well as commercial fisheries (Moreno et al., 2008; Venturini et al., 2012a; Golubkov et al., 2019). Previously, the supply rate of organic carbon to the sediment was used as a tool to determine the trophic status of the coastal systems (Nixon, 1995). However, sedimentary organic carbon is more conservative and overestimates the labile fraction of the organic pool (Fabiano and Danovaro, 1994; Danovaro et al., 1999). Later on, Dell'Anno

et al. (2002) proposed a new approach to determine the benthic trophic status based on the content of proteins and carbohydrates in the sediment and has been widely used to determine the trophic state of the estuarine, coastal, and riverine systems (Vezzulli and Fabiano, 2006; Muniz et al., 2011; Venturini et al., 2012a; Joseph et al., 2012; Salas et al., 2015; Manju et al., 2016; Sanil Kumar et al., 2017; Rao et al., 2018). The aquatic systems can be classified into different trophic status such as oligotrophic (low productive), mesotrophic (intermediate productivity), and eutrophic (highly productive) (Salas et al., 2015). Pusceddu et al. (2011) reported that biopolymeric carbon content (BPC = sum of protein, carbohydrate, and lipid carbon equivalents) of sediment could be used to evaluate the benthic trophic status of the system, and systems can be classified as oligotrophic (BPC = < 1 mg C g^{-1}), mesotrophic (BPC = 1-3 mg C g^{-1}), and eutrophic (BPC = > 3 mg C g^{-1}). The assessment of the trophic status of the aquatic systems is essential to determine the health of water bodies, which helps in ecosystem management strategies (Vezzulli and Fabiano, 2006) and to prevent adverse environmental (eutrophication, hypoxia, harmful algal blooms) and economic impacts (mortality of fish and invertebrates, biodiversity loss) (Cloern, 2001).

Among different coastal ecosystems, the ports are important transport hubs. Port areas receive organisms, OM, and contaminants from the local sources as well as ballast water and sediment from the tanks of cargo ships, which could be of intra-costal, interoceanic, or transoceanic origin (McCarthy et al., 1991; Bailey et al., 2007; Renzi et al., 2009; Losi et al., 2013). A detailed evaluation of OM from the surface sediment of ports is crucial in delineating both the quality of food available for higher organisms

as well as organic enrichment since ports are the significant sites of shipping and industrial activities. It is also helpful to understand the ecosystem's health and to identify the factors influencing the accumulation of organic compounds. This information is helpful for the planning, development, and better management of the port ecosystems. However, there are only few reports are available about the sedimentary OM composition from the Indian ports (Harji et al., 2008, 2010), and these studies are limited with a one-time sampling event. It has been reported that sediment-water interface is a dynamic zone with a high rate of microbial activities and OM composition change on the short (days, weeks), intermediate (months, seasons), and long geochemical time scales, i.e., years, decades, and centuries (Canuel and Martens, 1993, 1996; Zimmerman and Canuel, 2000, 2001). Moreover, the processes occurring in the water column for a period of few days to months gets reflected in the surface sediment, which is helpful to understand short-time scale changes in sedimentary OM owing to recent natural and anthropogenic activities (Zimmerman and Canuel, 2001; Venturini et al., 2012b). Thus, detailed studies on the OM composition of the sediment from the ports with different morphological structures (enclosed vs. open), hydrographic conditions (stagnant vs. tidally flushed, and freshwater vs. seawater), and environmental settings are helpful in providing valuable insights into food web dynamics, and ecosystem functioning of such environments.

Other than the ports, which are mostly enclosed regions in the coastal ecosystems, estuaries are also coastal and continuously subjected to various forms of anthropogenic activities. They represent highly productive zones

and acts as nursery grounds for a wide variety of organisms that contribute to local and marine fisheries (Blaber, 1997). These ecosystems are influenced by the rapid and intense variability in the physicochemical conditions (McLusky and Elliot, 2004), which can change OM composition on spatial (within a few km) as well as on temporal scales (Bodineau et al., 1998; Zimmerman and Canuel, 2001; Palomo and Canuel, 2010). Moreover, the estuaries in the Indian subcontinent are monsoon-influenced (Vijith et al., 2009), and receive a large amount of allochthonous materials from the catchment area through the surface run-off. This has an influence on the functioning of such tropical estuaries, which can affect the OM composition and food web dynamics. Thus, evaluating the relative contribution of various sources of OM from monsoon-influenced estuaries is of crucial importance in understanding its influence on the biological resources of such a system. Taking the above points into consideration, the following objectives were laid down for the present study.

Objectives and overview of the thesis

Objective 1 Characterization of organic matter using lipid biomarkers in the selected study area

The organic matter (OM) present in the sediment of the coastal system is the outcome of the autochthonous production, terrigenous sources, and heterotrophic utilization (Fabiano and Danovaro, 1994). The quality and quantity of OM have a significant role in the structuring of benthic organisms (Fabiano and Danovaro, 1994; Wieking and Kroncke, 2005; Campanya-Llovet et al., 2017).

Ports in the coastal regions are of great economic importance (Bortone et al., 2004), and such areas are more prone to environmental pollution owing to increasing shipping activities, coastal urbanization, and limited water circulation (Marin et al., 2008; Renzi et al., 2009; Mestres et al., 2010). An increment in the OM input affects the energy flow of the system and food-web interactions (Moreno et al., 2008; Cheung et al., 2008; Xu et al., 2014; Golubkov et al., 2019). The information on the sedimentary OM composition from the port region of India is limited. In the present study, the spatio-temporal variations in the composition of sedimentary OM was evaluated from the two major ports of India, i.e., Kolkata **(Chapter 2A)** and Kandla **(Chapter 2B)** using biomarkers (fatty acids), elemental (TOC, TN), and biochemical components (proteins, carbohydrates, lipids). Such an assessment of the port system is important to determine the nature of food and the overall status of the ecosystem, which could be helpful in management practices.

Objective 2 Characterization of short-term variation in organic matter composition

It has been reported that the composition of particulate and sedimentary OM in the estuarine regime changes on the short (hours, days, weeks) as well as intermediate time scales (months, seasons) in response to the tidal cycle, primary productivity, bacterial activity, and the input of terrestrial-derived materials (Canuel and Martens, 1993, 1996; Bodineau et al., 1998; Zimmerman and Canuel, 2001; David et al., 2019). The present study was carried out to evaluate the variations in the sedimentary OM composition

from the Zuari estuary on the fortnightly and monthly basis **(Chapter 3A and 3B)**.

The field observations were carried out on a fortnightly basis to understand the population dynamics of bacteria and their influence on the sedimentary OM. The contribution of autochthonous and allochthonous derived materials and the nature of OM was elucidated using the TOC/TN ratio, and the content of proteins and carbohydrates (**Chapter 3A**). Furthermore, the OM composition was evaluated on a monthly basis using source-specific FA biomarkers and elemental components **(Chapter 3B**).

The OM received from the various sources undergoes degradation process through microbial activity in the sediment-water interface, which helps in the cycling of carbon (Sun et al., 2000). However, understanding these processes is a difficult task in the estuarine system due to complex and multiple sources of OM, differences in the reactivity of OM, and physical processes, which changes within a short period and on spatial scales (Palomo and Canuel, 2010; Bianchi and Canuel, 2011). In view of this, laboratory microcosm experiments were carried out **(Chapter 4)** to understand the mechanisms underlying the turnover of OM derived from the different sources (diatoms, zooplankton, and mangrove leaves) using FA biomarkers.

The overall summary is presented in **Chapter 5**.

Chapter 2

Characterization of organic matter using lipid biomarkers in the selected study area

2. Characterization of organic matter using lipid biomarkers in the selected study area

2.1 Introduction

Ports are characterized by heavy shipping traffic, industrial activities, and receive nutrients, organic materials, and contaminants from ship and boat traffic, fall out of cargo, vessel oil spills, discharge of industrial wastes, and sewage from the nearby urbanized areas (Ganapati and Raman, 1973, 1979; McCarthy et al., 1991; Bailey et al., 2007; Marin et al., 2008; Renzi et al., 2009; Luna et al., 2019). They are low-energy areas with high rates of accumulation of organic contaminants in the sediment (McCarthy et al., 1991). The navigable channel in the port region undergoes frequent dredging operations to maintain a certain depth for the movements of cargo ships. The re-suspension of such polluted sediment through dredging activities and changes in the hydrodynamic conditions results in the contamination of the overlying water column as well as other surrounding environments (McCarthy et al., 1991). Thus, port sediment act as a source as well as a sink for the nutrients, pollutants, and OM received from the various natural and anthropogenic sources (McCarthy et al., 1991; Bailey et al., 2007). The increasing coastal urbanization and shipping traffic worldwide in recent years have recommended the importance of assessment of port environments (Marin et al., 2008) to determine the environmental and chemical pollution (Renzi et al., 2009). A detailed assessment of OM composition from the port area is useful to evaluate the quality and quantity of food available for benthic organisms. The characterization of sediment in terms of biopolymers (proteins, carbohydrates, and lipids) also helps to evaluate the alteration of

such systems with organic enrichment associated with natural and anthropogenic perturbations (Dell'Anno et al., 2002; Cotano and Villate, 2006; Venturini et al., 2012a). The biopolymeric carbon content has been used as a tool to evaluate the benthic trophic status of the estuarine and coastal environments (Pusceddu et al., 2009, 2011; Venturini et al., 2012a; Hadlich et al., 2018). This is of utmost importance and helpful in management practices since ports are the significant sites of shipping activities and ballast water exchange.

The contribution of in-situ derived marine and allochthonous terrestrial-derived OM in the sediment can be analyzed using total organic carbon to total nitrogen (TOC/TN) ratio (Meyers, 1994). Fatty acids (FAs) are the main components of lipids and are source-specific, diverse in structure, and hence commonly used as molecular biomarkers to identify the sources, diagenetic alterations, and fate of OM in the sediment (Carrie et al., 1998; Mudge et al., 1998; Meziane and Tsuchiya, 2000; Dunn et al., 2008; Venturini et al., 2012b). The structurally diverse FA markers are important to obtain a snapshot of the overall community structure of sediment (Carrie et al., 1998 and references therein), also helps in efficient monitoring of the impact of urbanization on the ecosystem health (Boechat et al., 2014), and pollution stress on the microbial community (Harji et al., 2010).

India has a long coastline (~ 7500 km) dotted with 12 major ports located along the east and west coasts, adjoining the Bay of Bengal and the Arabian Sea, respectively. In the present study, two major ports, i.e., Kolkata and Kandla, were selected, which are distinct in the structural and hydrological characteristics. Kolkata is a freshwater, enclosed port located

on the bank of Hooghly River in the city of Kolkata whereas, Kandla is a tidally flushed seawater port located in the creek. The present study was carried out to elucidate the sources of sedimentary OM and to determine the factors affecting the distribution of OM in these two ports using fatty acid biomarkers, elemental (total organic carbon, total nitrogen), and biochemical components (proteins, carbohydrates, and lipids). It was expected that enclosed port with limited water flushing would have a higher content of OM of rich quality than tidally flushed port.

Chapter 2 is sub-divided into two chapters, i.e., 2A and 2B. In chapter 2A, the spatio-temporal variations in the sedimentary OM composition in the Kolkata port have been discussed, and chapter 2B discusses OM composition in the Kandla port.

2A Assessment of spatio-temporal variations in the sedimentary organic matter composition in the Kolkata port

2A.2 Materials and methods

2A.2.1 Description of the study area

Kolkata is a riverine port located at the bank of the Hooghly River in the state of West Bengal on the north east coast of India (22^{o} 32′ N; 88^{o} 18′ E). It has a pilotage channel of ~ 203 km from the mouth of Hooghly estuary (Bay of Bengal). This pilotage channel requires continuous dredging throughout the year to maintain a certain depth for the movement of cargo ships. Kolkata is an enclosed port and has lock entrance through which ships enter or exit in and out of the dock after crossing the pilotage channel. Hooghly estuary is tide-dominated with semi-diurnal tides. The tides are

macro-tidal with a tidal range of about 1.8 m during neap and 5.2 m during spring tide at the mouth, and tidal waves from the Bay of Bengal propagate up to ~ 250 km upstream (Mukhopadhyay et al., 2006). The catchment area of Hooghly River experiences about 80% of the annual precipitation during the southwest monsoon period (June - September) with an average rainfall of ~1700 mm $year^{-1}$ (Nath et al., 2004).

Kolkata port has two major docks: Kidderpore Dock (KPD), and Netaji Subhash Dock (NSD). KPD has 18 berths and 3 dry docks whereas, NSD has 10 berths and 2 dry docks. KPD is a narrow channel compared to NSD. KPD is further divided into outer KPD-I and inner KPD-II by a bascule bridge (Fig. 2A.1).

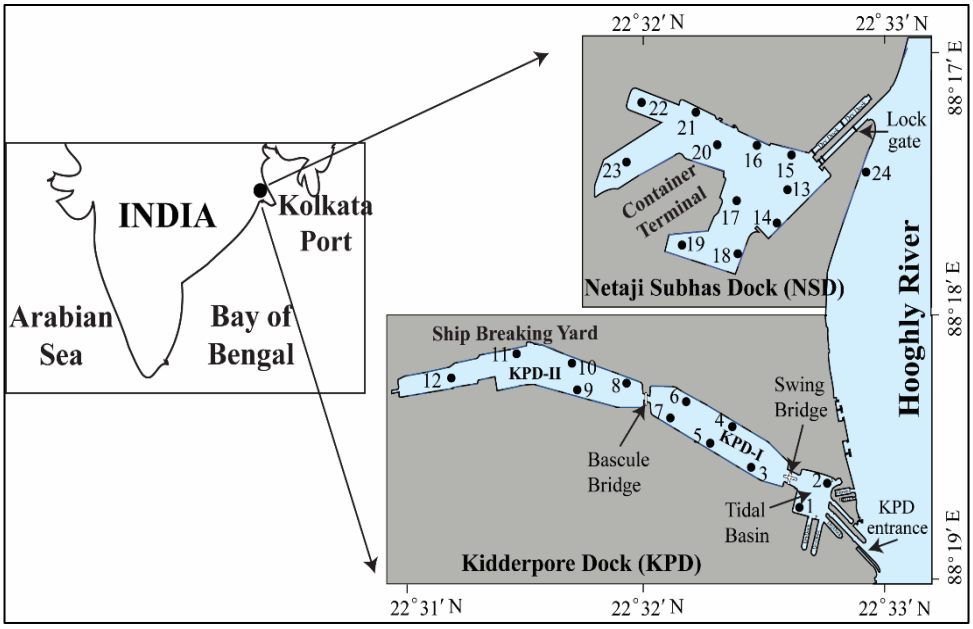


Fig. 2A.1 Map showing the location of stations in Kolkata port, north east coast of India. (**S1**: KPD Tidal basin-1, **S2**: KPD Tidal basin-2, **S3**: KPD Berth-3, **S4**: KPD Berth-6, **S5**: KPD Berth-7, **S6**: KPD Berth-10, **S7**: KPD Berth-11, **S8**: KPD Berth-15, **S9**: KPD Berth-24, **S10**: KPD Berth-17, **S11**: KPD Berth-19, **S12**: KPD Berth-28, **S13**: NSD Berth-1, **S14**: NSD Berth-2, **S15**: NSD Berth-14, **S16**: NSD Berth-13, **S17**: NSD Dolphin mooring-1, **S18**: NSD Berth-3, **S19**: NSD Berth-5 and 6, **S20**: NSD Berth-7, **S21**: NSD Ship breaking-1, **S22**: NSD Ship breaking-2, **S23**: NSD Dolphin mooring-2, **S24**: GR Jetty-4).

KPD-I and NSD are influenced by the riverine water during high tide when ships enter or exit through a lock gate. However, KPD-II is located at the inner side of the dock, characterized by restricted water circulation, and this was previously used for ship-breaking activities. The depth of the dock is around 8 - 12 m.

Kolkata port handles wide varieties of cargos, including coking coal, steam coal, pulses/peas, fertilizers, wooden logs, containers, rock phosphate, pet coke, limestone, raw petroleum liquids, carbon black, vegetable oil, rice, manganese, iron ore, etc. Kolkata Dock System handled 128.75 lakh tons of cargo during the year 2013-14 and 152.83 lakh tons during 2014-15 (http://www.kolkataporttrust.gov.in, Administrative Report, 2014-15).

2A.2.2 Sampling strategy

Sampling at the Kolkata port was carried out 4 times at 24 stations during September 2013 (MON), February 2014 (PRM-I), January-February 2015 (PRM-II), and December 2015 (POM). Sediment samples were collected from 7 stations (S1-S7) located in the KPD-I and 5 stations (S8-S12) in the KPD-II, 11 stations (S13-S23) in the NSD, and 1 station (S24) along the bank of Hooghly River outside the dock area (Fig. 2A.1). Sediment could not be sampled from S22 during MON, S14, and S24 during PRM-I, S21 to S24 during POM seasons.

The surface sediment samples from the docks and the riverine station were collected by using van Veen grab (0.04 m^2) operated from a trawler, and top ~ 5 cm of sediment was collected in zip lock bags and stored in the

icebox. These sediment samples were then transported to the laboratory on dry ice and kept frozen at -20°C after arrival to the laboratory until analyses.

2A.2.3 Analyses of elemental components from the sediment

The sediment samples were thawed, oven-dried (60°C for 48 h), and ground to a fine powder using mortar and pestle. Sediment was weighed (1-5 mg) in a tin boat and analyzed using a CHNS Analyzer (Vario MICRO Select, Germany) for the Total Carbon (TC) and Nitrogen (TN). These analyses were carried out in duplicate, and elemental concentration is expressed as a percentage dry weight of sediment (wt %). Sulfanilamide (Elemental composition: 41.81% C, 18.62% S, 16.25% N, and 4.65% H) was used as the standard for the calibration of the Elemental Analyzer. The sediment samples combusted at 500°C for 16 h in the muffle furnace were analyzed to determine the inorganic carbon (IC) content using CHNS Elementar Analyzer (Kristensen and Andersen, 1987). Total Organic Carbon (TOC) content of sediment was then obtained as the difference between TC and IC (TOC = TC – IC). The TOC content was converted into Total Organic Matter (TOM) by multiplying with factor 1.724 (Bhosle and Dhople, 1988).

2A.2.4 Analyses of biochemical components from the sediment

Proteins (PRT) were extracted from the sediment using 0.5 M NaOH for 4 h (Danovaro et al., 1993), and its content was determined according to the method described by Hartree (1972). Carbohydrates (CHO) were estimated using phenol and sulphuric acid, according to Dubois et al. (1956). Lipids (LPD) were extracted from the sediment by ultra-sonication with

chloroform: methanol (2:1 v/v) (Bligh and Dyer, 1959) for 20 min, and estimated following the method of Barnes and Blackstock (1973). Bovine serum albumin, glucose, and cholesterol were used as calibration standards for PRT, CHO, and LPD analyses, respectively. All these analyses were carried out in triplicate, and concentration is expressed as mg g^{-1} sediment dry weight. The blanks for each of the analysis were prepared by pre-combusting sediment samples at 450-500°C for 4 h in the muffle furnace. The sum of PRT, CHO, and LPD content is considered as labile organic matter (LOM) (Danovaro et al., 1993). The concentrations of PRT, CHO, and LPD were then converted to carbon equivalents using the conversion factor 0.49, 0.40, and 0.75, respectively (Fabiano and Danovaro, 1994; Danovaro et al., 1999). The sum of PRT, CHO, and LPD carbon is reported as biopolymeric carbon (BPC) (Danovaro et al., 1999), which has been used to determine the benthic trophic status of the system (Pusceddu et al., 2011).

2A.2.5 Analyses of fatty acids from the sediment

Fatty acids were extracted from the sediment by direct transesterification method, as described by Indarti et al. (2005), Nahon et al. (2010), and Bourgeois et al. (2011). Briefly, sediment samples (5 g) were homogenized with a mixture of methanol, sulphuric acid, and chloroform (1.7:0.3:2 v/v/v), vortexed, and incubated at 90°C for 90 min with occasional shaking. After incubation, samples were allowed to cool at room temperature and subsequently mixed with distilled water. The lower phases (organic solvent phases) containing fatty acid methyl esters (FAMEs) were transferred to clean tubes, and the upper phases were washed with hexane: chloroform

(4:1) for 2 times. The pooled organic phases were then evaporated to dryness in a rotary evaporator (Roteva, Medica Instrument Mfg. co., Mumbai), and FAMEs were resuspended in hexane. FAMEs were then analyzed by Gas chromatography-mass spectrometry (GC-MS, QP-2010, SHIMADZU), equipped with a capillary column (Stabilwax, 30 m × 0.25 mm internal diameter, 0.50 µm film thickness). One microliter of the sample was injected through autosampler with helium as carrier gas (column flow 1 mL min^{-1}). The temperature program of the oven was set to increase from 50°C (maintained for 2 minutes) to 200°C with a rate of 10°C min^{-1} and then from 200°C to 240°C at 5°C min^{-1}. The injector and detector temperatures were maintained at 240°C. The mass spectrometer was operated with ionization energy of 70 eV, and mass spectra were recorded in full scan mode (m/z 50 to m/z 500). The WILEY7 mass spectral library was used for the identification of individual FAME. FAME mix (Supelco 37 Component, 18919–1AMP, Sigma Aldrich, India) was used for the calibration and quantification of FAMEs from the samples. The concentration of FAMEs was then reported as µg g^{-1} of sediment.

2A.2.6 Data analyses

The data on stations located in the same dock, which are characterized by a similar type of water stagnation patterns and port activities, were pooled together and represented dock-wise, such as KPD-I (7 stations), KPD-II (5 stations), and NSD (11 stations). Data were then log (x+1) transformed to meet the assumption of normality and homogeneity. The elemental, biochemical components and fatty acid biomarkers were subjected to

analysis of variance (ANOVA) to evaluate the spatial and temporal variations. Correlation and Principal Component Analyses (PCA) were performed to determine the relationship between measured sedimentary variables and to identify the differences among the sampling sites and variables. All these analyses were performed by using STATISTICA software (version. 6.0, StatSoft, USA).

Appendix

Supplementary data related to Chapter 2 is available in Appendix I

2A.3 Results

2A.3.1. Spatio-temporal variations in the elemental and biochemical composition of Kolkata port sediment

The elemental and biochemical components were relatively higher inside the Kidderpore (KPD) and the Netaji Subhash dock (NSD) when compared to the outside riverine station (Fig. 2A.2a-h). The average TOM, TOC, and TN in the sediment ranged from 0.43 to 17.5%, 0.25 to 10.1%, and 0.01 to 0.46%, respectively (Fig. 2A.2a). Among the docks, average TOM was high in KPD-II (range: 11.9 – 17.5%), the inner dock with restricted water circulation, whereas in the case of KPD-I (3.88 – 5.13%) and NSD (2.86 – 3.61%), both of which get influenced by riverine water during entry and exit of ships, moderate TOM was observed, and it was low (0.43 – 1.08%) at the riverine station (Fig. 2A.2a). The ANOVA showed significant variation among the sites in TOM ($p \leq 0.00001$); however, seasonal variations were insignificant (Table 2A.1).

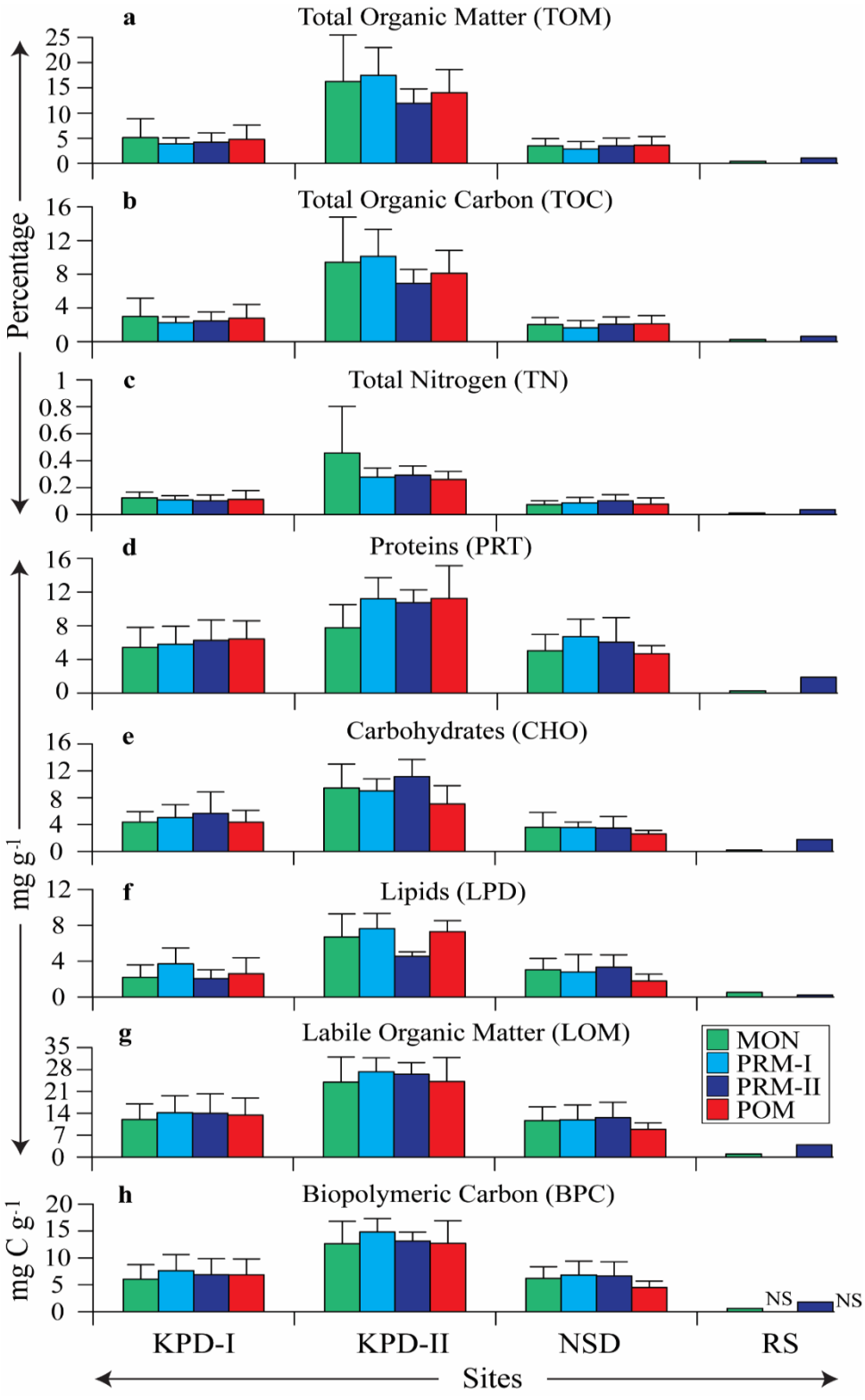


Fig. 2A.2 Average values of elemental and biochemical components in the surface sediment of Kolkata port during different seasons (Vertical lines indicate standard deviation (SD) from the mean; KPD: Kidderpore Dock; NSD: Netaji Subhash Dock; RS: Riverine Station; PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon; NS: No Samples).

A similar distribution trend was observed in TN, wherein, it was high in KPD-II (0.26 – 0.46%), moderate in KPD-I (0.10 – 0.12%), NSD (0.07 – 0.10%), and low (0.01 – 0.04%) at the riverine station (Fig. 2A.2c). There was significant site-wise variation in TN ($p \leq 0.00033$; Table 2A.1). The ratio of TOC/TN ranged from 17.9 to 36.6 in the surface sediment of Kolkata port. The average content of TOC/TN was low at the riverine station (21.4), medium in NSD (23.7), and high in KPD (25.8).

The average concentration of PRT, CHO, and LPD ranged from 0.27 to 11.3 mg g^{-1}, 0.22 to 11.1 mg g^{-1}, and 0.22 to 7.64 mg g^{-1}, respectively (Fig. 2A.2d-f). PRT, CHO, and LPD varied significantly among the sites with insignificant seasonal variations (Table 2A.1).

Table 2A.1 Summary of two-way ANOVA of elemental and biochemical components of surface sediment from the Kolkata port.

Factors	Parameters	df	MS	F	p-value
Sites	TOM	3	0.332753	90.2	**0.00001**
	TOC	3	0.332753	90.2	**0.00001**
	TN	3	0.007764	26.7	**0.00033**
	PRT	3	0.224440	37.9	**0.00011**
	CHO	3	0.252237	41.3	**0.00008**
	LPD	3	0.215313	32.3	**0.00018**
	LOM	3	0.35081	47.8	**0.00005**
	BPC	3	0.275770	75.2	**0.00001**
Seasons	TOM	3	0.000271	0.07	0.972
	TOC	3	0.000271	0.07	0.972
	TN	3	0.000215	0.74	0.561
	PRT	3	0.016983	2.86	0.114
	CHO	3	0.016990	2.78	0.119
	LPD	3	0.007695	1.15	0.392
	LOM	3	0.01330	1.81	0.233
	BPC	3	0.008133	2.22	0.174

(Bold value indicates significant p values ($p < 0.05$); df: degree of freedom; MS: mean square; TOM: Total Organic Matter; TOC: Total Organic Carbon; TN: Total Nitrogen; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; LOM: Labile Organic Matter; BPC: Biopolymeric Carbon).

Average PRT content was high in KPD-II (10.2 ± 1.67 mg g^{-1}), moderate in the KPD-I (5.97 ± 0.46 mg g^{-1}) and NSD (5.62 ± 0.93 mg g^{-1}) and low (1.08 ± 1.15 mg g^{-1}) at the riverine station (Fig. 2A.2d). CHO and LPD also showed a similar trend (KPD-II>KPD-I>NSD>riverine station) like PRT (Fig. 2A.2e and f). LOM and BPC in the sediment ranged from 1.01 to 27.9 mg g^{-1} and 0.61 to 14.8 mg C g^{-1}, respectively, with high content in KPD-II and low at the riverine station (Fig. 2A.2g and h).

2A.3.2 Spatio-temporal variations in the fatty acids (FAs) in Kolkata port sediment

The sources of OM in the port sediment were identified based on the FA biomarkers, which are given in Table 1.1. The total fatty acids (TFAs) in the Kolkata port ranged from 85.02 to 507.6 µg g^{-1}. The ubiquitous, short-chain fatty acids (C16:0+C18:0) were dominant, and their contribution was 36.2 - 80.8% to the TFAs. The C16:0 and C18:0 FAs are found in the phytoplankton, bacteria, terrestrial plants, and have been used as indicators of input of detrital material, hence hereafter referred to as detrital FAs. The content of detrital FAs varied from 30.8 – 288.3 µg g^{-1} (Fig. 2A.3a). These FAs showed significant variation between the sites (ANOVA; $p \leq 0.002$; Table 2A.2) with high content in the KPD-II (avg. 221.2 ± 47.2 µg g^{-1}) followed by KPD-I (190.6 ± 22.5 µg g^{-1}), NSD (91.8 ± 7.37 µg g^{-1}), and the riverine station (55.8 ± 35.5 µg g^{-1}). These detrital FAs showed a strong positive correlation with diatom-specific FAs ($r = 0.77$, $p = 0.001$), elemental and biochemical components, and were negatively correlated with zooplankton-specific FAs ($r = -0.62$, $p = 0.017$) (Table 2A.3).

The bacteria-specific FAs (C18:1ω7, C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0) ranged from 14.3 – 37.5 µg g^{-1} and their contribution to TFAs was 9.2 to 36.6%. These FAs followed a similar trend as that of detrital FAs (KPD-II>KPD-I>NSD>riverine station) with high (avg. 34.6 ± 2.11 µg g^{-1}) content in the KPD-II and low (21.7 ± 10.4 µg g^{-1}) in the riverine station (Fig. 2A.3b). Seasonally, it was high (avg. 32.7 ± 3.74 µg g^{-1}) during PRM-II and low (22.9 ± 8.84 µg g^{-1}) during the MON (Fig. 2A.3b). The ANOVA showed significant variation between the sites (ANOVA; $p \leq 0.026$) and the seasons (ANOVA; $p < 0.043$) in bacterial FAs (Table 2A.2).

The FAs specific to diatom (C14:0, C16:1ω7, C20:4ω6, C20:5ω3), macroalgae (C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3), and dinoflagellate (C18:1ω9, C22:6ω3) were relatively high in both the major docks than the riverine station (Fig. 2A.3c-e). The ANOVA showed variations between the sites in diatom (ANOVA; $p \leq 0.028$) and dinoflagellate-specific FAs (ANOVA; $p \leq 0.003$). The average content of diatom and dinoflagellate-specific FAs was high in KPD and macroalgae-specific FAs in NSD. When seasonally compared, KPD-II showed high content of diatom-specific FAs during PRM-I. Whereas, in the case of NSD and KPD-I, it was high during the MON season (Fig. 2A.3c), but these changes were non-significant. However, seasonal variation was significant in the case of macroalgae (ANOVA; $p \leq 0.001$) and dinoflagellate (ANOVA; $p \leq 0.005$) specific FA markers (Table 2A.2). Both macroalgae and dinoflagellate FAs were high during PRM-I but were low during the MON and POM seasons, respectively (Fig. 2A.3d and e).

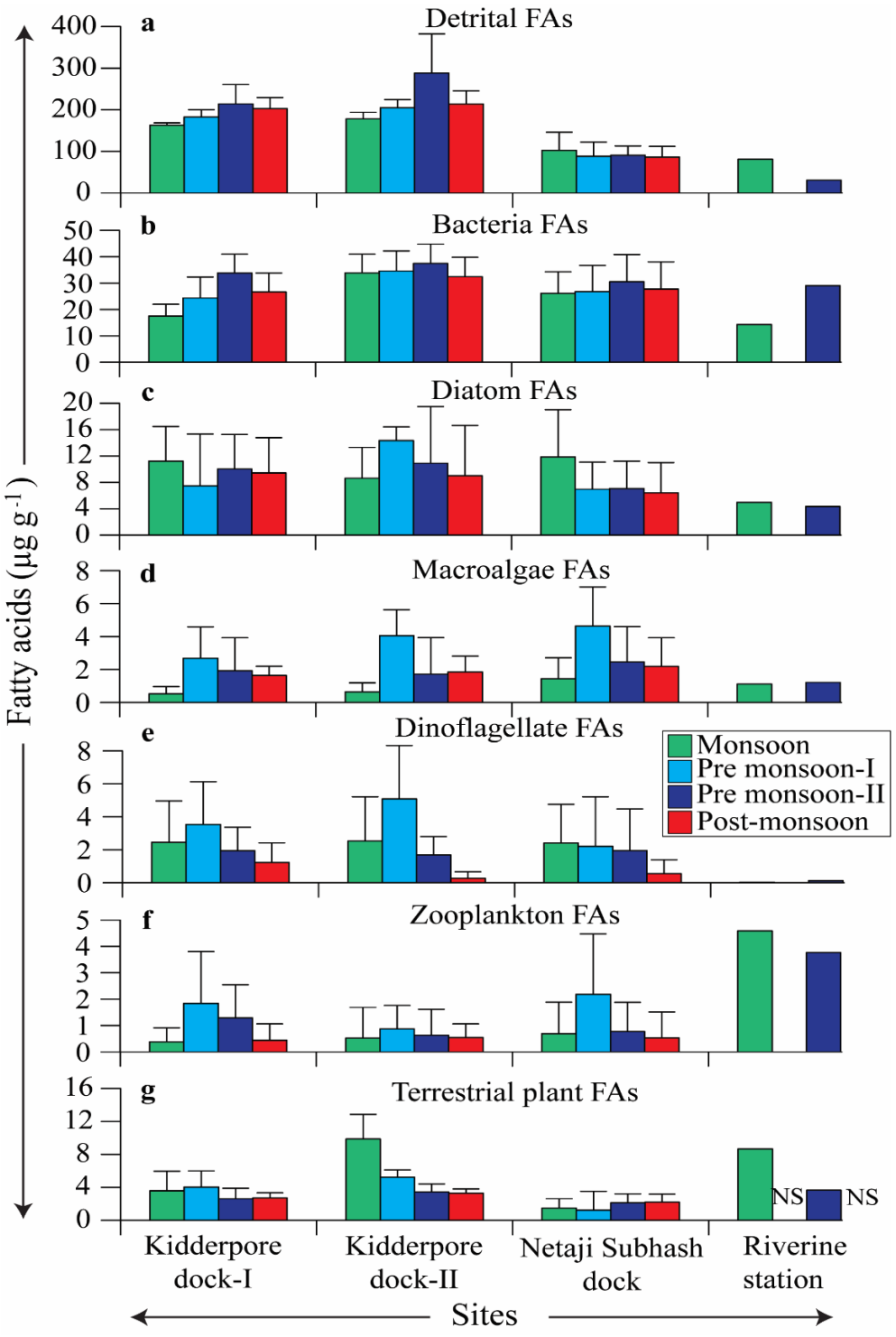


Fig. 2A.3 Average values of detrital (ubiquitous) and source-specific fatty acids in the surface sediment of Kolkata port during different seasons (Vertical lines indicate standard deviation (SD) from the mean; NS: No Samples).

Zooplankton FA markers (C22:1ω9, C24:1ω9) were significantly high in the riverine station sediment (avg. 4.18 ± 0.58 µg g^{-1}) than the dock stations (0.89 ± 0.58 µg g^{-1}) (Fig. 2A.3f). The ANOVA showed significant variation between the sites (ANOVA; $p \leq 0.001$) and the seasons (ANOVA; $p < 0.029$) in zooplankton-specific FAs (Table 2A.2). Zooplankton FA markers showed a strong negative correlation ($r = -0.72$, $p = 0.004$) with diatom-specific FAs (Table 2A.3).

Terrestrial plant-specific long-chain fatty acids (LCFAs: C22:0 and C24:0) ranged from 1.23 to 9.87 µg g^{-1} and were relatively higher in KPD-II and the riverine station during the MON season (Fig. 2A.3g). There was significant variation between the sites in the terrestrial plant-specific FAs (ANOVA; $p \leq 0.025$). The contribution of LCFAs to TFAs varied from 0.85 to 7.25% (avg. 2.17%) in the Kolkata port.

Table 2A.2 Summary of two-way ANOVA of fatty acids (FAs) of surface sediment from the Kolkata port.

Factors	Parameters	df	MS	F	p-value
Sites	Detrital FAs	3	0.23145	14.1	**0.002**
	Bacteria FAs	3	0.02380	5.77	**0.026**
	Diatom FAs	3	0.04880	5.61	**0.028**
	Macroalgae FAs	3	0.013490	3.40	0.086
	Dinoflagellate FAs	3	0.112813	12.4	**0.003**
	Zooplankton FAs	3	0.122124	20.5	**0.001**
	Terrestrial plant FAs	3	0.097653	5.82	**0.025**
Seasons	Detrital FAs	3	0.00142	0.09	0.965
	Bacteria FAs	3	0.01910	4.63	**0.043**
	Diatom FAs	3	0.00507	0.58	0.645
	Macroalgae FAs	3	0.079932	20.2	**0.001**
	Dinoflagellate FAs	3	0.100874	11.0	**0.005**
	Zooplankton FAs	3	0.032843	5.52	**0.029**
	Terrestrial plant FAs	3	0.023372	1.39	0.322

(Bold value indicates significant p values ($p < 0.05$); df: degree of freedom; MS: mean square).

Since detrital FAs were a dominant component of the FA composition of the Kolkata port sediment and are found in different OM sources, the

contribution of bacteria, phytoplankton, zooplankton, and terrestrial plants-specific FAs to detrital FAs was evaluated. The contribution of bacteria and macroalgae-specific FAs to detrital material was high during non-monsoon seasons compared to the monsoon (Fig. 2A.4). However, the contribution of diatom-specific FAs to detrital FAs was high during the monsoon in KPD-I, NSD, riverine station, and during the non-monsoon season in KPD-II.

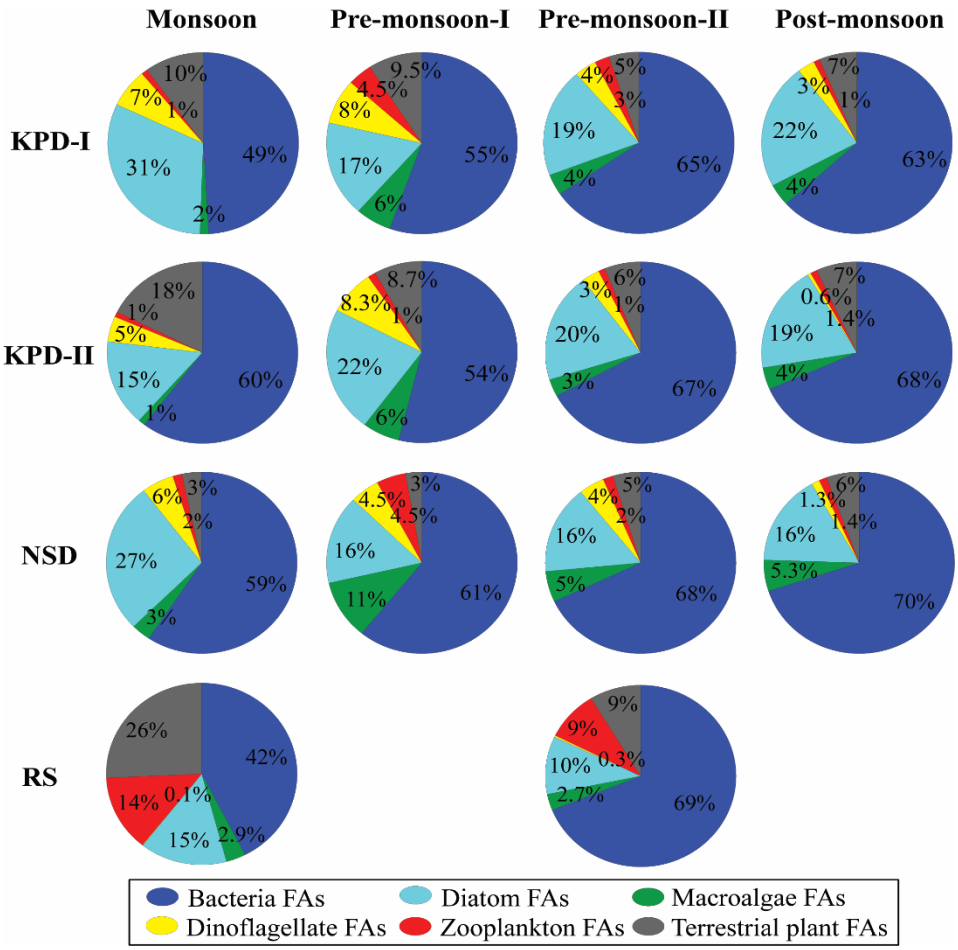


Fig. 2A.4 Spatio-temporal variations in the percentage contribution of source-specific FAs to detrital FAs in the Kolkata port (KPD: Kidderpore Dock; NSD: Netaji Subhash Dock; RS: Riverine Station).

Dinoflagellate-specific FAs contributed high inside docks during monsoon and pre-monsoon-I seasons. The contribution of zooplankton FAs was high in the riverine station than docks (Fig. 2A.4). Terrestrial plant-

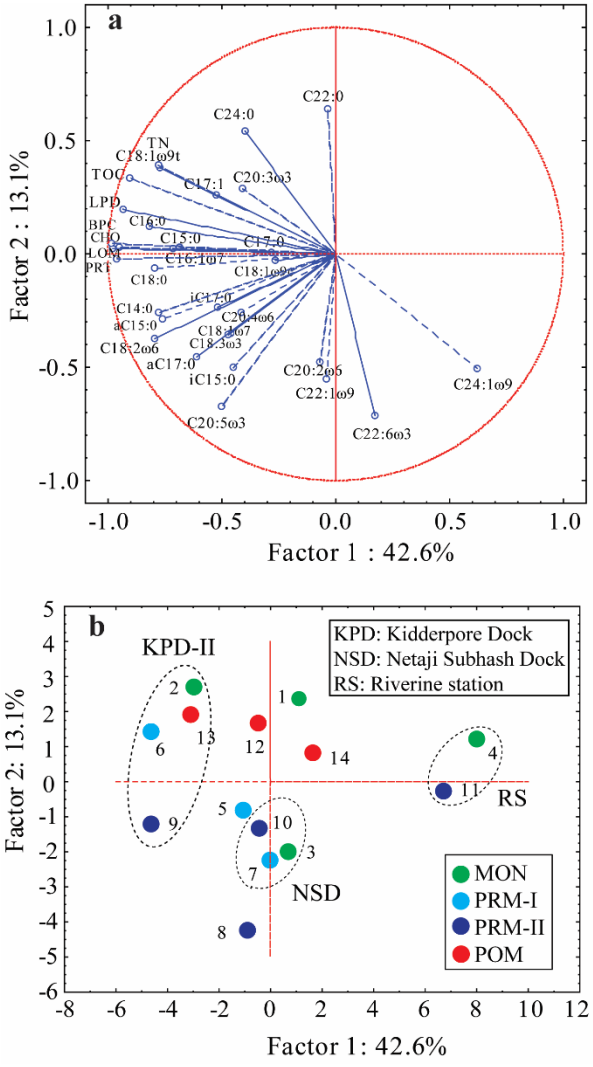
specific FAs showed the opposite trend to the bacteria FAs. There was not much variation in NSD in terrestrial plant materials (Fig. 2A.4). Overall, the contribution of terrestrial plant material to detrital FAs was high at the riverine station.

2A.3.3 Principal Component Analysis (PCA)

Factors 1 and 2 in the PCA plot of Kolkata port explained 42.6 and 13.1% of the variance, respectively (Fig. 2A.5a). Factor 1 showed strong negative loadings (-0.69 to -0.79) for C16:1ω7, C15:0, aC15:0, C18:1ω9t, C14:0, C18:2ω6, FAs and weak negative loadings (-0.50 to -0.61) for C20:5ω3, iC17:0, aC17:0 indicating input of diatom, macroalgae, and bacteria-derived OM. On the contrary, a weak positive loading (0.62) for C24:1ω9 was observed, indicating the input of zooplankton. Other variables with strong negative loadings (-0.78 to -0.99) for factor 1 were TOC, TN, LPD, CHO, PRT, and BPC (Fig. 2A.5a). Sample scores for factor 1 were most positive for the riverine station and more negative for KPD-II (Fig. 2A.5b), indicating that KPD-II sediment was relatively enriched in elemental and biochemical compounds (TOC, TN, PRT, CHO, LPD, and BPC) derived from the autochthonous materials (diatom, macroalgae, and bacteria).

Factor 2 revealed strong negative loadings (-0.67 to -0.72) for C20:5ω3 and C22:6ω3 FAs and sample scores were negative for NSD, and KPD-I representing phytoplankton derived fresh OM. Factor 2 also showed weak negative loadings (-0.51 to -0.55) for C24:1ω9 and C22:1ω9 and as well as for C18:3ω3, C20:2ω6 FAs (-0.36 to -0.48). This suggests the contribution of zooplankton and macroalgae derived materials. Factor 2 had positive

loading (0.54 to 0.64) for C24:0 and C22:0 FAs indicating the input of terrestrial plant materials. KPD and the riverine station showed a positive score for factor 2.



Factor loadings and eigenvalue

Variables	Factor 1	Factor 2
C14:0	**-0.78**	-0.26
C15:0	**-0.72**	0.02
C16:0	**-0.82**	0.12
C17:0	-0.28	0.01
C18:0	**-0.80**	-0.06
C22:0	-0.04	**0.64**
C24:0	-0.40	**0.54**
C16:1ω7	**-0.69**	0.03
C17:1	-0.53	0.26
C18:1ω7	-0.46	-0.35
C18:1ω9t	**-0.77**	0.38
C18:1ω9c	-0.26	-0.03
C22:1ω9	-0.04	**-0.55**
C24:1ω9	**0.62**	**-0.51**
C18:2ω6	**-0.79**	-0.37
C18:3ω3	-0.47	-0.36
C20:2ω6	-0.07	-0.48
C20:3ω3	-0.41	0.29
C20:4ω6	-0.42	-0.26
C20:5ω3	**-0.50**	**-0.67**
C22:6ω3	0.17	**-0.72**
iC15:0	-0.45	**-0.50**
aC15:0	**-0.76**	-0.29
iC17:0	**-0.52**	-0.23
aC17:0	**-0.61**	-0.45
PRT	**-0.96**	-0.02
CHO	**-0.95**	0.03
LPD	**-0.93**	0.20
LOM	**-0.98**	0.02
BPC	**-0.99**	0.05
TOC	**-0.90**	0.34
TN	**-0.78**	0.39
Eigenvalue	13.6	4.2
% Total Variance	42.6	13.1
Cumulative %	42.6	55.7

Sample scores

	Factor 1	Factor 2
1 (KPD-I; MON)	1.12	2.36
2 (KPD-II; MON)	-2.96	2.71
3 (NSD; MON)	0.68	-2.01
4 (RS; MON)	8.00	1.22
5 (KPD-I; PRM-I)	-1.05	-0.81
6 (KPD-II; PRM-I)	-4.63	1.42
7 (NSD; PRM-I)	-0.02	-2.24
8 (KPD-I; PRM-II)	-0.88	-4.24
9 (KPD-II; PRM-II)	-4.61	-1.22
10 (NSD; PRM-II)	-0.43	-1.32
11 (RS; PRM-II)	6.72	-0.28
12 (KPD-I; POM-II)	-0.48	1.67
13 (KPD-II; POM-II)	-3.11	1.90
14 (NSD; POM-II)	1.64	0.83

Fig. 2A.5 Principal Component Analysis (PCA) plots for fatty acids, elemental, and biochemical variables in the surface sediment of Kolkata port. a) Variables and factor loadings, b) Sample scores for sites (numbers correspond to sampling sites with different seasons).

These compound groupings suggest that factor 1 represents OM mostly of autochthonous origin, and factor 2 represents fresh, labile OM (rich in PUFAs of diatom and dinoflagellate) in KPD-I and NSD and terrestrial derived materials in KPD-II and the riverine station.

Table 2A.3 Correlation matrix showing r values of fatty acid markers, elemental, and biochemical variables from the surface sediment of Kolkata port.

	Bact. FAs	Dia. FAs	M.alg FAs	Dino. FAs	Zoo. FAs	TP. FAs	Det. FAs	PRT	CHO	LPD	LOM	BPC	TOC	TN
Bact. FAs	1.00													
Dia. FAs	0.35	1.00												
M.alg FAs	0.35	0.08	1.00											
Dino. FAs	0.29	**0.70**	0.33	1.00										
Zoo. FAs	-0.42	**-0.72**	0.11	-0.41	1.00									
TP FAs	-0.19	-0.11	-0.46	-0.09	0.21	1.00								
Det.FAs	0.32	**0.77**	0.05	0.50	**-0.62**	0.15	1.00							
PRT	**0.74**	**0.73**	0.35	**0.60**	**-0.76**	-0.27	**0.69**	1.00						
CHO	**0.75**	**0.74**	0.15	**0.63**	**-0.70**	-0.01	**0.76**	**0.94**	1.00					
LPD	**0.57**	**0.71**	0.28	**0.61**	**-0.68**	0.06	**0.75**	**0.87**	**0.85**	1.00				
LOM	**0.74**	**0.76**	0.25	**0.65**	**-0.77**	-0.15	**0.74**	**0.99**	**0.98**	**0.90**	1.00			
BPC	**0.72**	**0.76**	0.28	**0.66**	**-0.75**	-0.11	**0.76**	**0.98**	**0.97**	**0.94**	**0.99**	1.00		
TOC	**0.65**	**0.72**	0.07	0.48	**-0.71**	0.22	**0.75**	**0.86**	**0.91**	**0.91**	**0.90**	**0.92**	1.00	
TN	**0.60**	0.52	-0.10	0.39	**-0.55**	0.44	**0.64**	**0.69**	**0.83**	**0.82**	**0.77**	**0.79**	**0.93**	1.00

(r values marked as bold indicate that correlation is significant at the $p < 0.05$; Bact: Bacteria, Dia: Diatom; M. alg: Macroalgae; Dino: Dinoflagellate; Zoo: Zooplankton; TP: Terrestrial plant; Det: Detrital; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; LOM: Labile Organic Matter; TOC: Total Organic Carbon; TN: Total Nitrogen).

2B Assessment of spatio-temporal variations in the sedimentary organic matter composition in the Kandla port

2B.2 Materials and methods

2B.2.1 Description of the study area

Kandla is a tidal port located at the western bank of the Kandla creek in the state of Gujarat on the north west coast of India (23^{o} 01′ N; 70^{o} 13′ E) (Fig. 2B.1). The tides in the creek region are macro-tidal, with a tidal height of ~ 0.83 to 7.2 m. The current velocity ranges from 0.08 – 1.76 ms^{-1} during the flood and 0.09 – 2.0 ms^{-1} during ebb tide (Sinha et al., 2006). The eastern bank of this creek is dominated by the small tributaries, which get influenced by seawater influx from the Arabian Sea and are covered with vegetation and mangroves. The two other creeks, such as Sara and Phang, join at the inner part of Kandla creek, which finally opens into the inner Gulf of Kutch at a point about ~ 90 km from the mouth of the Gulf (Shirodkar et al., 2010; Dalal et al., 2010). The climate of this region is semi-arid with a rainfall of 400 - 500 mm year$^{-1,}$ and there is no direct riverine input (Kunte et al., 2003). Kandla creek is surrounded by fertilizer- and chemical manufacturing units as well as salt pans (Shirodkar et al., 2010).

This port has one navigable channel with a length of ~ 23 km, a width of ~ 200 to 1000 m, and depth is around 10 m. This port has 6 oil berths for the handling of petroleum, oil, lubricants (POL), edible oil, industrial chemicals, fertilizers, and 14 multipurpose cargo berths used for loading and unloading of dry bulk, food grains, mineral ore, etc. (Shirodkar et al., 2010). It has a bunder basin situated in the middle of the Kandla port to the north of the cargo berth area (Fig. 2B.1). Annual cargo traffic handled at the Kandla

port was 387.76 lakh tons and 469.42 lakh tons during the year 2014-15 and the year 2015-16, respectively (https://www.deendayalport.gov.in, Annual Administration Report, 2015-16).

2B.2.2 Sampling strategy

Sampling at the Kandla port was carried out for 4 times, i.e., during October 2014 (POM-I), July-August 2015 (MON), October 2015 (POM-II), and February 2016 (PRM), respectively. Sediment samples were collected from 15 stations, which are located along the oil jetties (S1-S5), dry dock and bunder basin (S6-S8), multipurpose cargo berths (S9-S12), as well as on the opposite side of berths (S13-S15) in the Kandla creek (Fig. 2B.1).

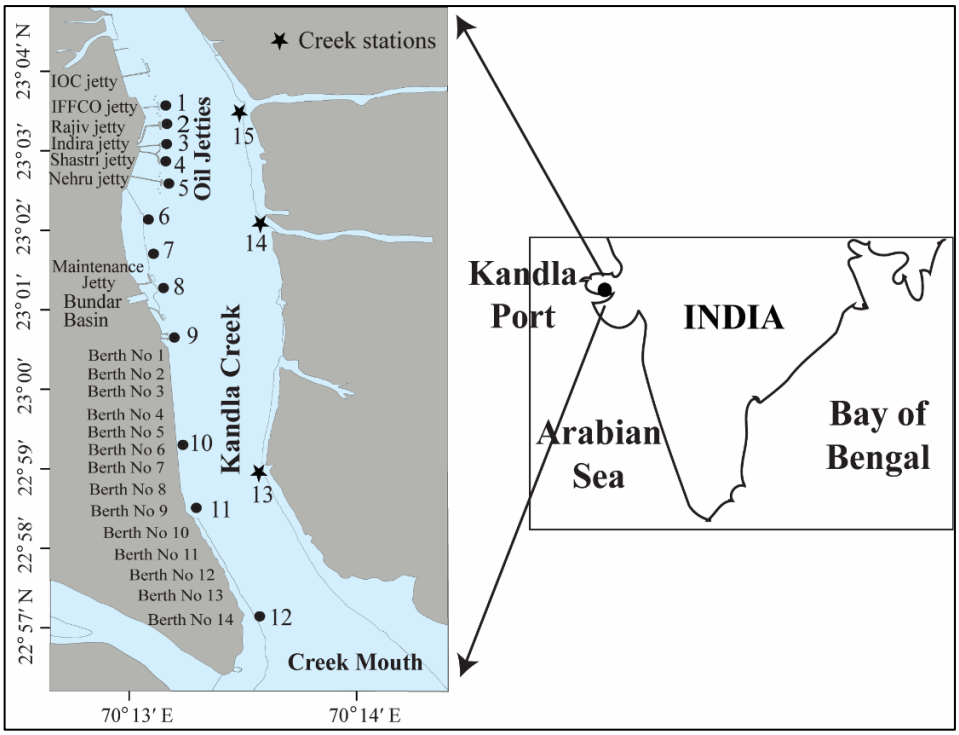


Fig. 2B.1 Map showing the location of stations in Kandla port, north west coast of India. (**S1**: IFFCO Jetty, **S2**: Rajiv Jetty, **S3**: Indira Jetty, **S4**: Shastri Jetty, **S5**: Nehru Jetty, **S6**: Fishing Anchorage-1, **S7**: Fishing Anchorage-2, **S8**: Bunder Basin, **S9**: Cargo Berth-1, **S10**: Cargo Berth-6, **S11**: Cargo Berth-9, **S12**: Cargo Berth-14, **S13**: Kandla Creek-2, **S14**: Kandla Creek-4, **S15**: Kandla Creek-5).

Sediment could not be sampled from S1, S2, and S3 during POM-II and S9 during PRM seasons.

The surface sediment samples from the selected study sites were collected by using van Veen grab (0.04 m^2) operated from a trawler, and top ~ 5 cm of sediment was collected in zip lock bags and kept in the icebox. These sediment samples were then transported to the laboratory on dry ice and kept frozen at -20°C after arrival to the laboratory until analyses.

2B.2.3 Analyses of elemental components from the sediment

Details of methodology for the analyses of elemental composition (TOC, TN) of the sediment have been given in chapter 2A, section 2A.2.3.

2B.2.4 Analyses of biochemical components from the sediment

Details of methodology for the estimation of proteins (PRT), carbohydrates (CHO), and lipids (LPD) from the sediment have been given in chapter 2A, section 2A.2.4.

2B.2.5 Analyses of fatty acids from the sediment

The detailed methodology for the extraction, transesterification, and analysis of fatty acids from the sediment is provided in chapter 2A, section 2A.2.5.

2B.2.6 Data analyses

The data on the stations located around the jetty/berth with a similar type of port activities were pooled together and represented as oil jetties (5

stations), dry docks (3 stations), cargo berths (4 stations), and creek stations (3 stations). Data were then log (x+1) transformed to meet the assumption of normality and homogeneity. The elemental, biochemical components and fatty acid biomarkers were subjected to analysis of variance (ANOVA) to evaluate the spatial and temporal variations. The relationship between the elemental components, biochemical variables, and fatty acids was determined using correlation analysis. Principal Component Analysis (PCA) was also carried out to identify the differences among the sampling sites and variables. All these analyses were performed by using STATISTICA software (version. 6.0, StatSoft, USA).

2B.3 Results

2B.3.1 Elemental and biochemical composition of Kandla port sediment

The average total organic matter (TOM) and total organic carbon (TOC) in the Kandla port sediment varied from 0.63 to 1.78% and 0.37 to 1.03%, respectively (Fig. 2B.2a and b). TOC was high (avg. 0.80 ± 0.16%) at cargo berths (located towards the mouth of Kandla creek) and creek stations, whereas, it was low (avg. 0.63 ± 0.2%) at oil jetty (located at the inner side of Kandla creek) and dry dock stations.

Total nitrogen (TN) ranged from 0.02 – 0.05% and showed opposite trend to TOC with high (0.032 ± 0.01%) content at oil jetty and low (0.018 ± 0.002%) at creek stations (Fig. 2B.2c). A significant variation in TN was observed between the sites (ANOVA; $p \leq 0.015$) and the seasons (ANOVA; $p \leq 0.024$) (Table 2B.1). The ratio of TOC/TN in the Kandla port ranged from 9.19 – 51.6. The average TOC/TN ratio was low at oil jetty stations (21.5),

medium at the dry dock (27.7), cargo berths (32.3), and high at creek stations (45.2).

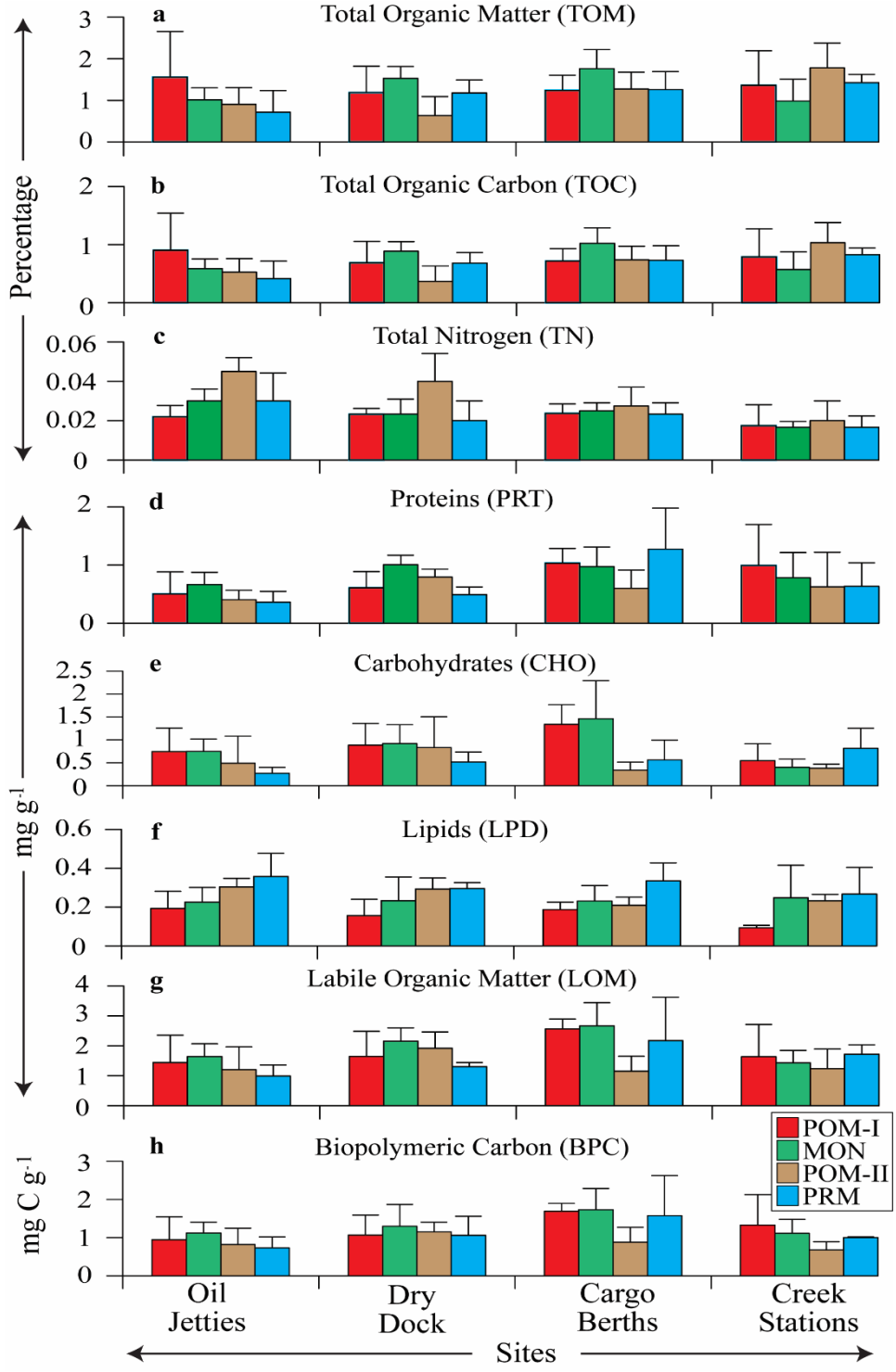


Fig. 2B.2 Average values of elemental and biochemical components in the surface sediment of Kandla port during different seasons (Vertical lines indicate standard deviation (SD) from the mean; PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon).

The average concentration of proteins (PRT) and carbohydrates (CHO) in the sediment of Kandla port varied from 0.36 to 1.27 mg g^{-1} and 0.27 to 1.46 mg g^{-1}, respectively (Fig. 2B.2d and e). Moreover, PRT showed spatial variations (ANOVA; $p < 0.042$) with high values (0.6 – 1.27 mg g^{-1}) at cargo berth stations and low (0.36 – 0.67 mg g^{-1}) at oil jetty stations (Fig. 2B.2d). CHO was also high (0.34 – 1.34 mg g^{-1}) at cargo berths but low at creek stations and oil jetty (0.27 – 0.82 mg g^{-1}) stations (Fig. 2B.2e). The content of lipids (LPD) ranged from 0.09 to 0.36 mg g^{-1} (Fig. 2B.2f). ANOVA revealed significant ($p \leq 0.001$) seasonal variation in LPD content (Table 2B.1), with high content during PRM and low during POM-I. LOM and BPC ranged from 0.99 to 2.66 mg g^{-1}, and 0.68 to 1.73 mg C g^{-1}, respectively (Fig. 2B.2g and h) and their content followed similar trends like PRT and CHO.

Table 2B.1 Summary of two-way ANOVA of elemental and biochemical variables of surface sediment from the Kandla port.

Factors	Parameters	df	MS	F	p-value
Sites	TOM	3	0.002778	0.98	0.445
	TOC	3	0.002778	0.98	0.445
	TN	3	0.000024	6.15	**0.015**
	PRT	3	0.0099	4.15	**0.042**
	CHO	3	0.0074	1.37	0.314
	LPD	3	0.0003	2.00	0.183
	LOM	3	0.0115	2.97	0.089
	BPC	3	0.008777	5.32	**0.022**
Seasons	TOM	3	0.00112	0.39	0.761
	TOC	3	0.00112	0.39	0.761
	TN	3	0.00002	5.11	**0.024**
	PRT	3	0.00321	1.34	0.321
	CHO	3	0.01019	1.87	0.204
	LPD	3	0.00212	14.2	**0.001**
	LOM	3	0.00749	1.93	0.195
	BPC	3	0.00638	3.87	**0.049**

(Bold value indicates significant p values ($p < 0.05$); df: degree of freedom; MS: mean square; TOM: Total Organic Matter; TOC: Total Organic Carbon; TN: Total Nitrogen; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; LOM: Labile Organic Matter; BPC: Biopolymeric Carbon).

2B.3.2 Spatio-temporal variations in the fatty acids (FAs) in Kandla port sediment

The sources of OM in the Kandla port sediment were identified based on the FA biomarkers (Table 1.1). Total fatty acids (TFAs) in the Kandla port sediment ranged from 18.3 – 60.9 µg g^{-1}. The sum of detrital FAs C16:0 and C18:0 ranged from 13.2 to 34.3 µg g^{-1} with high content at cargo berth and low at oil jetty stations (Fig. 2B.3a). Their contribution to TFAs ranged from 46.8 – 72.5%. Detrital FAs showed significant intra-seasonal variations (ANOVA; $p \leq 0.004$) (Table 2B.2), with high content in POM-I and low during POM-II. These FAs were positively correlated ($r = 0.52$, $p = 0.04$) with diatom FA markers (Table 2B.3).

Bacteria specific FAs (C18:1ω7, C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0) ranged from 4.4 – 11.9 µg g^{-1} with high content at oil jetty and low at creek stations (Fig. 2B.3b). The average bacterial FAs showed intra-seasonal variations in the contribution to TFAs with high (26.3%) contribution during POM-II and low (18.9%) during POM-I season.

FAs specific to diatom (C14:0, C16:1ω7, C20:5ω3), macroalgae (C18:2ω6, C18:3ω3), and dinoflagellate (C18:1ω9, C22:6ω3) varied from 0.44 to 3.2 µg g^{-1} (avg. 1.48 ± 0.66 µg g^{-1}), ND (Not Detected) to 0.65 µg g^{-1} (avg. 0.18 ± 0.24 µg g^{-1}), and ND to 1.12 µg g^{-1} (avg. 0.48 ± 0.31µg g^{-1}), respectively (Fig. 2B.3c-e). Diatom and macroalgae-specific FAs were high at cargo berths (mouth of Kandla creek) and low at oil jetty stations (Fig. 2B.3c and d). However, dinoflagellate FA markers showed the opposite trend (Fig. 2B.3e). Zooplankton FAs (C22:1ω9, C24:1ω9) were also high (0.15 ±

0.13 µg g^{-1}) at cargo berth and low (0.01 ± 0.01 µg g^{-1}) at oil jetty stations (Fig. 2B.3f).

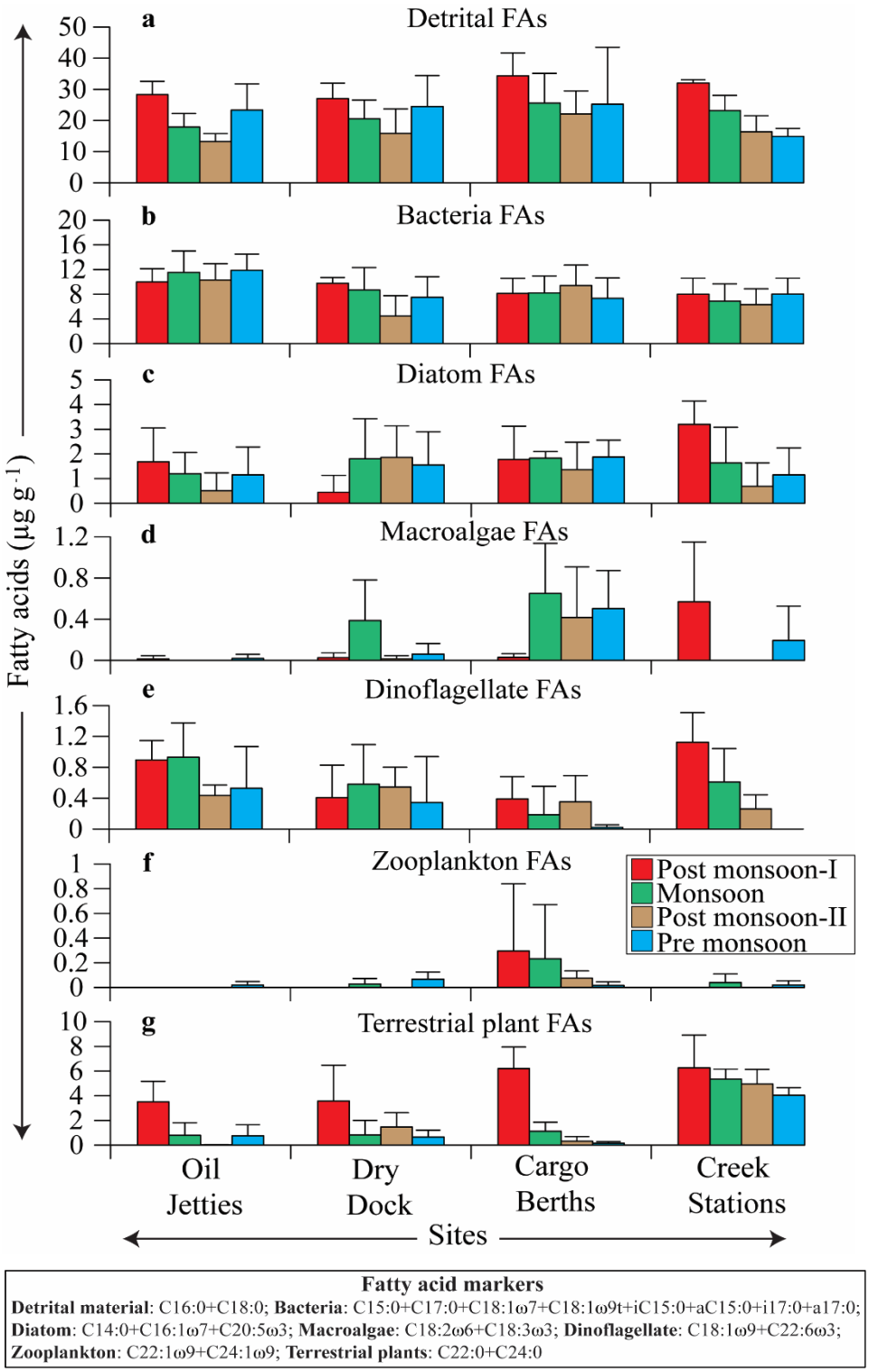


Fig. 2B.3 Average values of detrital (ubiquitous) and source-specific fatty acids in the surface sediment of Kandla port during different seasons (Vertical lines indicate standard deviation (SD) from the mean).

Terrestrial plant-derived FAs (C22:0 and C24:0) varied from ND to 6.27 µg g^{-1}. There was a significant spatial (ANOVA; $p \leq 0.005$) and temporal (ANOVA; $p \leq 0.008$) variation in the content of terrestrial plant FAs (Table 2B.2). Creek stations were characterized with a high contribution (avg. ~14%) of terrestrial plant FAs and were low (avg. ~3%) at oil jetty stations. Their content was high during POM-I season and low during PRM throughout the area (Fig. 2B.3g).

Table 2B.2 Summary of two-way ANOVA of fatty acids (FAs) of surface sediment from the Kandla port.

Factors	Parameters	df	MS	F	p-value
Sites	Detrital FAs	3	0.011	2.60	0.120
	Bacteria FAs	3	0.02180	3.92	**0.048**
	Diatom FAs	3	0.009105	0.54	0.665
	Macroalgae FAs	3	0.0389	0.01	2.075
	Dinoflagellate FAs	3	0.0126	2.82	0.099
	Zooplankton FAs	3	0.0030	4.04	**0.045**
	Terrestrial plant FAs	3	0.2061	8.87	**0.005**
Seasons	Detrital FAs	3	0.0420	9.70	**0.004**
	Bacteria FAs	3	0.0050	0.91	0.475
	Diatom FAs	3	0.00991	0.59	0.637
	Macroalgae FAs	3	0.00183	0.29	0.829
	Dinoflagellate FAs	3	0.01556	3.50	0.062
	Zooplankton FAs	3	0.00047	0.64	0.606
	Terrestrial plant FAs	3	0.17780	7.66	**0.008**

(Bold value indicates significant p values ($p < 0.05$); df: degree of freedom; MS: mean square).

The contribution of bacteria, phytoplankton, zooplankton, and terrestrial plants-specific FAs to detrital FAs was evaluated since detrital FAs were the dominant component of the FA composition of the Kandla port sediment. The contribution of bacteria and terrestrial plants-specific FAs to the detrital FAs was high in Kandla port. Moreover, at creek stations, terrestrial plant FAs' contribution to detrital FAs was high during all the

seasons and during the post-monsoon-I season at other sites (Fig. 2B.4). Zooplankton- specific FAs contributed at cargo berths stations, and diatom-specific FAs contributed high at dry dock and cargo berth stations compared to oil jetty stations (Fig. 2B.4).

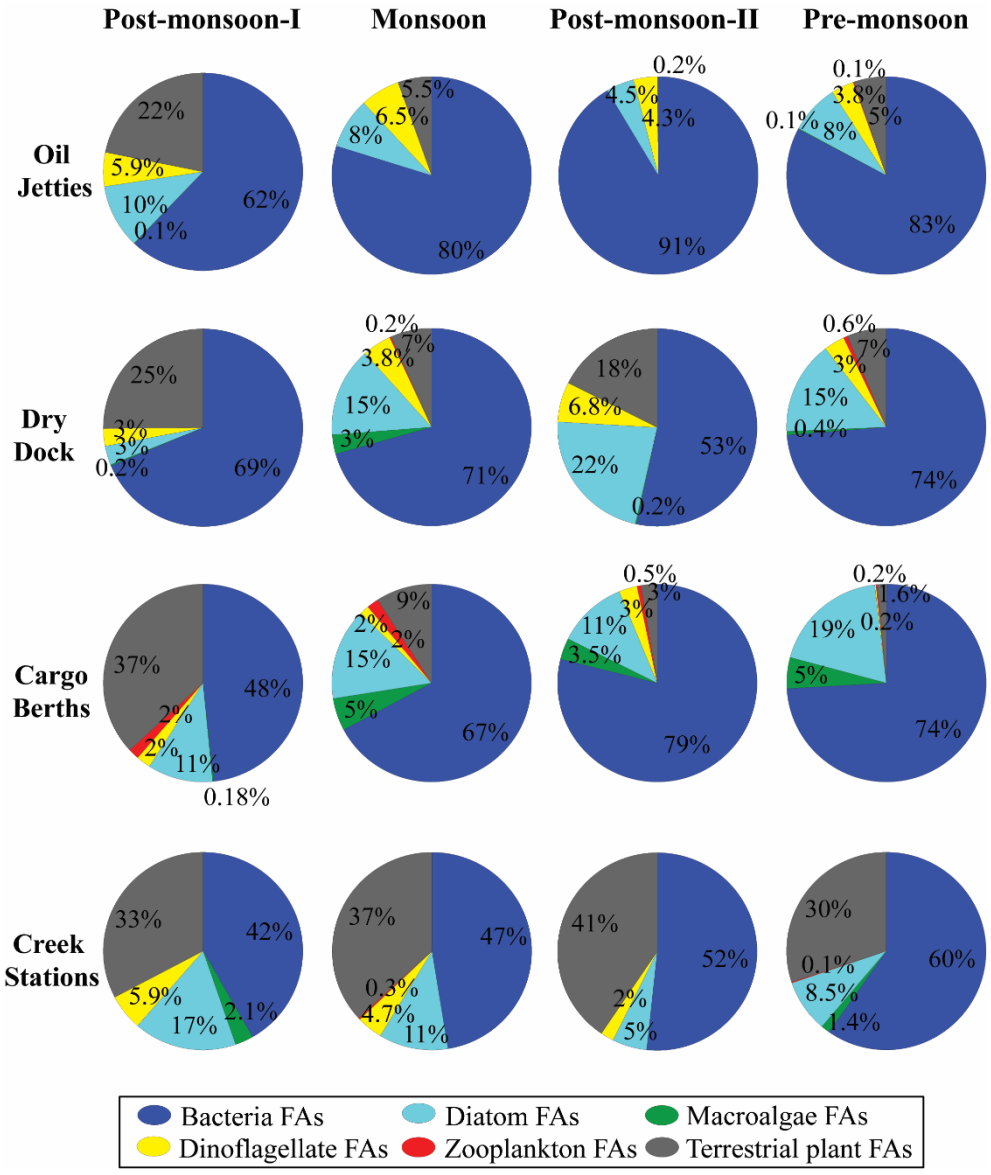
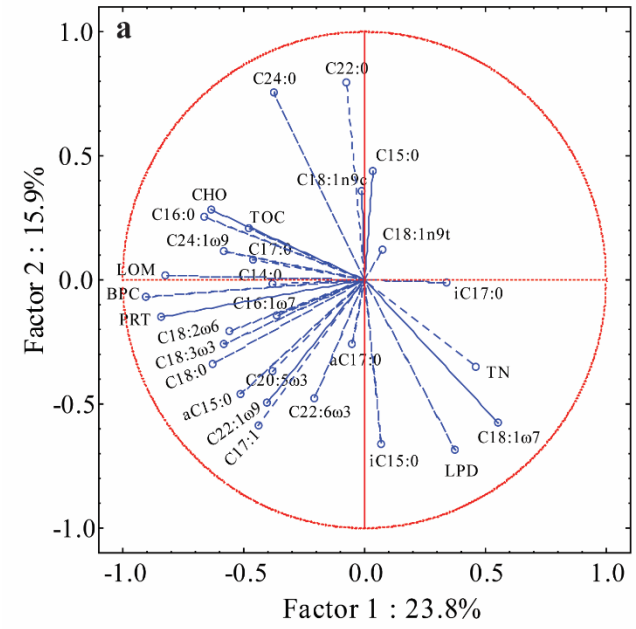


Fig. 2B.4 Spatio-temporal variations in the percentage contribution of source-specific FAs to detrital FAs in Kandla port.

2B.3.3 Principal Component Analysis (PCA)

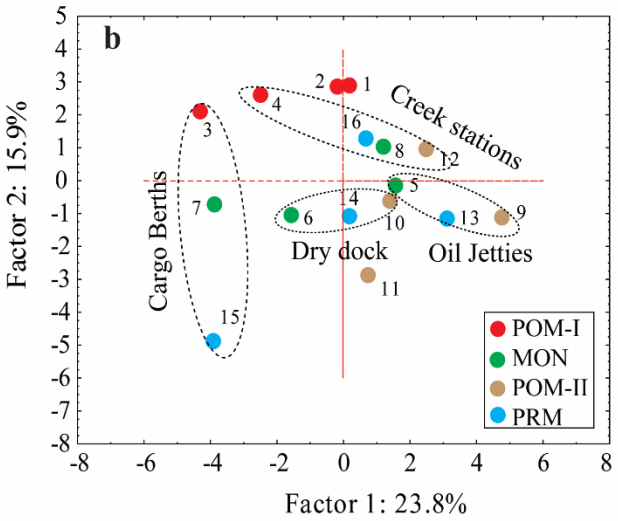
In the PCA plots, factors 1 and 2 explained 23.8 and 15.9% of the variance, respectively (Fig. 2B.5a). The variables with weak negative loadings (-0.51 to -0.58) for factor 1 were aC15:0, C18:2ω6, C18:3ω3, C24:1ω9, and positive (0.55) for C18:1ω7 representing the contribution of bacteria, macroalgae, and zooplankton derived OM. The negative loadings (-0.64 to -0.91) of CHO, PRT, LOM, and BPC for factor 1 indicated autochthonous sources of these organic compounds. Factor 1 score was more negative for the cargo berth stations and positive for oil jetty stations (Fig. 2B.5b).

Factor 2 was most positively loaded (0.76 to 0.80) on C24:0 and C22:0 FAs. This factor showed medium negative loadings (-0.58 to -0.69) for C18:1ω7, C17:1, iC15:0, and LPD and weak (-0.37 to -0.48) for C20:5ω3 and C22:6ω3 FAs. Creek stations showed a positive score for factor 2, representing the dominance of terrestrial plant-derived materials and cargo berth stations had the most negative score indicating input of bacteria and phytoplankton derived OM.



Factor loadings and eigenvalue

Variables	Factor 1	Factor 2
C14:0	-0.38	-0.02
C15:0	0.04	0.44
C16:0	**-0.66**	0.26
C17:0	-0.46	0.08
C18:0	**-0.63**	-0.34
C22:0	-0.07	**0.80**
C24:0	-0.37	**0.76**
C16:1ω7	-0.36	-0.14
C17:1	-0.44	**-0.59**
C18:1ω7	**0.55**	**-0.58**
C18:1ω9t	0.08	0.12
C18:1ω9c	-0.01	0.36
C22:1ω9	-0.40	**-0.50**
C24:1ω9	**-0.58**	0.11
C18:2ω6	**-0.56**	-0.21
C18:3ω3	**-0.58**	-0.26
C20:5ω3	-0.38	-0.37
C22:6ω3	-0.21	-0.48
iC15:0	0.07	**-0.66**
aC15:0	**-0.51**	-0.46
iC17:0	0.34	-0.01
aC17:0	-0.05	-0.26
PRT	**-0.84**	-0.15
CHO	**-0.64**	0.28
LPD	0.38	**-0.69**
LOM	**-0.83**	0.02
BPC	**-0.91**	-0.07
TOC	-0.48	0.21
TN	0.46	-0.35
Eigenvalue	6.89	4.62
% Total Variance	23.8	15.9
Cumulative %	23.8	39.7



Sample scores

	Factor 1	Factor 2
1 (Oil Jetties; POM-I)	0.17	2.87
2 (Dry Dock; POM-I)	-0.17	2.86
3 (Cargo Berths; POM-I)	-4.32	2.11
4 (Creek Stations; POM-I)	-2.50	2.61
5 (Oil Jetties; MON)	1.58	-0.17
6 (Dry Dock; MON)	-1.58	-1.03
7 (Cargo Berths; MON)	-3.88	-0.72
8 (Creek Stations; MON)	1.22	1.03
9 (Oil Jetties; POM-II)	4.76	-1.13
10 (Dry Dock; POM-II)	1.39	-0.63
11 (Cargo Berths; POM-II)	0.76	-2.87
12 (Creek Stations; POM-II)	2.49	0.97
13 (Oil Jetties; PRM)	3.13	-1.16
14 (Dry Dock; PRM)	0.17	-1.10
15 (Cargo Berths; PRM)	-3.91	-4.90
16 (Creek Stations; PRM)	0.69	1.26

Fig 2B.5 Principal Component analysis (PCA) plots for fatty acids, elemental, and biochemical variables in the surface sediment of Kandla port. a) Variables and factor loadings, b) sample score for sites (numbers correspond to sampling sites with different seasons).

Table 2B.3 Correlation matrix showing r values of fatty acid markers, elemental, and biochemical variables from the surface sediment of Kandla port.

	Bact. FAs	Dia. FAs	M.alg FAs	Dino. FAs	Zoo. FAs	TP. FAs	Det. FAs	PRT	CHO	LPD	LOM	BPC	TOC	TN
Bact. FAs	1.00													
Dia. FAs	-0.32	1.00												
M.alg FAs	-0.05	**0.56**	1.00											
Dino. FAs	0.25	0.29	-0.21	1.00										
Zoo. FAs	-0.03	0.26	0.24	-0.27	1.00									
TP FAs	-0.27	0.16	-0.19	0.22	0.13	1.00								
Det. FAs	0.15	**0.52**	0.30	0.24	0.47	0.36	1.00							
PRT	-0.42	**0.63**	**0.62**	-0.15	0.37	0.17	0.39	1.00						
CHO	-0.10	0.20	0.21	-0.12	**0.62**	0.21	0.26	0.49	1.00					
LPD	-0.08	-0.25	-0.23	-0.49	-0.12	**-0.63**	**-0.50**	-0.26	-0.30	1.00				
LOM	-0.30	0.44	0.45	-0.23	**0.58**	0.14	0.32	**0.83**	**0.88**	-0.20	1.00			
BPC	-0.22	**0.61**	**0.55**	-0.12	**0.62**	0.08	0.53	**0.86**	**0.74**	-0.24	**0.92**	1.00		
TOC	0.01	0.11	0.48	-0.23	0.26	0.30	0.25	0.32	0.32	-0.45	0.32	0.23	1.00	
TN	0.09	-0.32	-0.26	0.10	-0.12	**-0.61**	**-0.50**	-0.31	-0.03	0.40	-0.13	-0.20	**-0.61**	1.00

(r values marked as bold indicate that correlation is significant at the $p < 0.05$; Bact: Bacteria, Dia: Diatom; M. alg: Macroalgae; Dino: Dinoflagellate; Zoo: Zooplankton; TP: Terrestrial plant; Det: Detrital; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; LOM: Labile Organic Matter; TOC: Total Organic Carbon; TN: Total Nitrogen).

2.4 Discussion

2.4.1 Sources of OM in the sediment of Kolkata and Kandla ports indicated by elemental components and fatty acid biomarkers

Organic carbon and nitrogen are important components of the sediment, and the TOC/TN ratio has been used to distinguish the sources of OM in the aquatic systems. The accumulation of diverse source derived organic carbon and nitrogen in the sediment is influenced by the hydrodynamic condition of the system. It has been observed that sites subjected to low hydrodynamic conditions favor the deposition of OM rich fine particles in contrast to sites that are subjected to strong hydrodynamism (Cividanes et al., 2002). Thus, average OM content at Kolkata port with restricted water flushing was higher (TOC: 3.84%; TN: 0.15%) when compared to Kandla port (TOC: 0.72%; TN: 0.03%). The ratio of TOC/TN has been used to distinguish in-situ produced OM (TOC/TN: 4–10) from exogenous terrestrial derived OM (>20) (Meyers, 1994). Overall, the TOC/TN ratio in Kolkata (17.9 - 36.6) and Kandla ports (9.19 – 51.6) indicated a mixed contribution of exogenous and autochthonous produced OM. Average TOC/TN ratio was lower at the riverine station (21.4) when compared to the NSD (23.7) and KPD (25.8) of Kolkata port. The long-chain FAs (LCFAs), which are indicators of terrestrial plant input, were more in KPD than NSD. The short-chain saturated FAs such as C16:0 and C18:0, indicators of detrital material were also dominant in the sediment of KPD. Moreover, the contribution of LCFAs was high in the detrital FAs in KPD (Fig. 2A.4), owing to which the TOC/TN ratio was higher. Although the content of LCFAs was high at the riverine station, the contribution of

zooplankton-specific FAs to detrital FAs was high at the riverine station when compared to docks. This could be the possible reason for the low TOC/TN ratio at the riverine station. In the case of Kandla port, the average percentage contribution of LCFAs to TFAs was ~ 3 folds higher (6.43%) than Kolkata port (2.17%), also evidenced by high TOC/TN ratio. This could be attributed to the eastern bank of Kandla creek, which is covered with mangroves and vegetation on their banks. Moreover, a strong seasonal and spatial variation was observed in the terrestrial plant-specific FAs in the Kandla port, and their contribution was high in the creek stations and decreased towards oil jetty stations. Whereas, a significant site-wise variation was observed in the case of bacterial FAs. The contribution of bacteria-specific FAs to detrital FAs decreased from oil jetty stations to creek stations (Fig. 2B.4). This is also evident by the low TOC/TN ratio at oil jetty stations (21.5), medium at the dry dock (27.7), cargo berths (32.3), and high at creek stations (45.2).

A strong negative loadings for C14:0, C16:1ω7 FAs, which have been used as biomarkers of diatom (Volkman et al., 1989; Carrie et al., 1998; Mudge et al., 1998) as well as on C18:2ω6, a marker of macroalgae (Meziane and Tsuchiya, 2000) in PCA plot of Kolkata port indicate the high contribution of diatom and macroalgae in the sediment. However, in the case of Kandla port, PCA showed weak loading for diatom and macroalgae specific FAs. Overall, high content of phytoplankton specific FAs (including diatom, dinoflagellate, and macroalgae) in Kolkata port sediment than Kandla can be attributed to high primary production. The water column of Kolkata port showed higher content of nitrate (~ 4 times), ammonia (~ 1.5

times), and chlorophyll-*a* (~ 10 - 13 times) compared to Kandla (Sathish et al., 2020), which also supports the elevated primary production. Shirodkar et al. (2010) reported suppressed phytoplankton production in the Kandla creek due to high turbidity in the water column caused by the strong tidal currents and water flow from mudflats rich in the colloidal particles. Thus, high primary production and restricted water flushing could have resulted in a high accumulation of phytoplankton derived organic materials in the sediment of enclosed docks of Kolkata port. High biomass of phytoplankton and dominance of natural senescence over grazing as chlorophyll degradation pathway has been indicated using pheophorbide: pheophytin proxy in the water column of Kolkata port (Sathish et al., 2020), which also support the accumulation of phytoplankton derived OM. Zooplankton are the consumers of primary producers and play a significant role in the transfer of energy to higher trophic levels. Monounsaturated FAs (MUFAs) such as C22:1ω9 and C24:1ω9 have been commonly used as biomarkers of zooplankton (Wakeham et al., 1997; Carrie et al., 1998; Dalsgaard et al., 2003; Venturini et al., 2012b). An inverse relationship of diatom FA markers with zooplankton specific FAs in Kolkata port further indicates decoupling between them, which supports the surplus accumulation of OM derived from diatoms. The contribution of diatom-specific FAs to detrital material was also high inside the docks than the riverine station (Fig. 2A.4).

Diatom, dinoflagellate, and macroalgae are rich sources of PUFAs, which are essential nutrients for consumers such as zooplankton, mollusks, and fish (Canuel, 2001). PUFAs such as C20:4ω6 and C20:5ω3 are found in diatom (Volkman et al., 1989, Carrie et al., 1998), C22:6ω3 usually indicate

the contribution of dinoflagellate (Carrie et al., 1998), and C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3 have been used as markers of macroalgae (Volkman et al., 1989; Mudge et al., 1998; Meziane and Tsuchiya, 2000). The contribution of diatom-derived PUFAs (C20:4ω6+C20:5ω3; 0.15%) was high in Kolkata port compared to Kandla port (C20:5ω3; 0.03%). PUFAs such as C20:4ω6, C20:3ω3, C20:2ω6 were not detected in Kandla port sediment. PUFAs are more susceptible to degradation compared to MUFAs (Wakeham et al., 1997), and this could be the reason for the low contribution of PUFAs than MUFAs. The macroalgae-specific PUFAs such as C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3 were dominant in Kolkata (2.23 μg g^{-1}) sediment than Kandla (0.18 μg g^{-1}). PCA plots showed strong loadings on C20:5ω3 and C22:6ω3 FAs indicating input of fresh and labile OM derived from diatom and dinoflagellate especially in NSD and KPD-I, the average percentage contribution of LOM to TOM was more in NSD (38%) and KPD-I (33%) compared to KPD-II (20%). The dominance of saturated FAs (C16:0 and C18:0) in KPD-II indicates the presence of partially degraded detrital OM (Dunn et al., 2008). Overall, the average content of phytoplankton derived PUFAs was ~ 9 folds higher in Kolkata port (2.55 μg g^{-1}) than Kandla (0.19 μg g^{-1}) indicating high input of fresh OM in Kolkata port. Such an OM rich environment could be conducive for benthic organisms.

The present study indicates that bacterial FAs were the dominant contributors of TFAs than other source-specific FAs in both these ports. Bacteria have an important role in the degradation of OM received from diverse sources as well as contribute their own components to the newly synthesized materials (Canuel and Martens, 1996). It has been reported that

bacteria act as a food resource for benthic meiofauna (Danovaro et al., 1999; van der Heijden et al., 2019) as well as oxidizes organic carbon through the variety of pathways (Nealson, 1997). Bacteria produce odd carbon-numbered, iso, and anteiso-branched FAs within the range of C13 – C19 and the FAs such as iC15:0, aC15:0, C17:0, iC17:0, aC17:0 are well-known biomarkers of Gram-negative anaerobes and sulfate-reducing bacteria (Parkes and Taylor, 1983; Wakeham, 1995; Zhukova, 2005). The high content of these FAs in the sediment of Kolkata port indicates poor flushing of water and high accumulation of organic load, which was also evident from the high content of PRT, LPD, and BPC. The presence of trans-monounsaturated FA C18:1ω9t also suggests the impact of pollution stress on the bacterial community. This FA has been reported in the Visakhapatnam harbour and Cochin estuary and attributed to the input of municipal sewage, industrial wastes, crude oil, and petroleum products (Harji et al., 2010; Gireeshkumar et al., 2015). The high content of C18:1ω9t in the sediment of Kolkata docks (0.02 – 2.42 μg g^{-1}) than Kandla (0.03 – 0.23 μg g^{-1}) indicates that bacteria to be under stress owing to the impact of stagnation of water.

Though bacteria-specific FAs were low in the sediment of Kandla port than Kolkata, their average percentage contribution to the TFAs was high (23.6%) compared to Kolkata (16%), suggesting more bacterial re-working in Kandla port sediment. Vezzulli and Fabiano (2006) reported higher bacterial activity in the oligotrophic sediment with proper ecosystem functioning than eutrophic and hypertrophic sediment. In Kandla port, bacteria-derived FAs contribution was high at oil jetty stations suggesting more bacterial activities. IFFCO barge jetty, located near the oil jetties, and

used for the loading and unloading of fertilizers and raw materials, showed high content of TN. The fallout of fertilizers and raw materials during the transport process has been reported to be responsible for the contamination of Kandla creek water (Shirodkar et al., 2010). The contribution of bacteria in the sediment increases in response to the deposition of fresh OM or input of nutrients from run-off events (Carrie et al., 1988; Dunn et al., 2008). These could be the reasons for the spatial and seasonal variations in the contribution of bacteria-derived FAs in the Kandla port.

2.4.2 Benthic trophic status of Kolkata and Kandla ports

Port regions are influenced by extensive anthropogenic activities and are active sites of ballast water exchange, evaluation of the benthic trophic status of systems could be useful to determine the health of water bodies and to prevent adverse environmental and economic impacts (Cloern, 2001). The high content of PRT and CHO in the Kolkata port indicates high productivity. PRT and CHO have been used to describe the productivity of the systems (Danovaro et al., 1999; Dell'Anno et al., 2002). Areas with high input of anthropogenic wastes and nutrients result in the accumulation of recently produced PRT rich OM and alter the trophic condition of the system (Dell'Anno et al., 2002; Vezzulli and Fabiano, 2006; Hadlich et al., 2018). It has been reported that 25-50% of the organic carbon and nitrogen derived from phytoplankton sink to the benthos in nutritionally enriched systems (Cloern, 2001). The strong positive correlation of PRT and CHO with diatom and dinoflagellate-specific FAs support the accumulation of phytoplankton derived OM in the Kolkata port.

Table 2.4 Range of the biochemical components (PRT: Proteins; CHO: Carbohydrates; LPD: Lipids) in the sediment from the ports, estuaries, and coastal areas around the world.

Study Area	PRT (mg g^{-1})	CHO (mg g^{-1})	LPD (mg g^{-1})	References
Continental shelf, east coast of India	0.25 – 3.40	2.03 – 9.67	0.16 – 0.97	Bhosle and Dhople, 1988
Apulian coastal system, Italy	0.1 – 13.99	0.1 – 11.9	0.04 – 2.44	Dell'Anno et al., 2002
Alassio Harbour, Italy	0.01 – 1.28	0.09 – 5.59	0.02 – 1.36	Vezzulli et al., 2003
Sanremo Harbour, Italy	0.006 – 0.49	0.05 – 2.01	0.03 – 0.67	
Genoa-Voltri Harbour, Italy	0.63 – 14.4	1.06 – 44.5	–	Salvo et al., 2005
Visakhapatnam Harbour, east coast of India	–	–	0.3 – 14.9	Harji et al., 2010
Rio de la Plata estuary, Uruguay	2.37 – 15.3	0.83 – 8.49	0.65 – 8.35	Muniz et al., 2011
	1.08 – 16.4	0.29 – 8.86	0.5 – 8.35	Venturini et al., 2012a
Rapallo Harbour, Italy	0.1 – 3.0	0.3 – 23.5	–	Harriague et al., 2012
Cochin estuary, west coast of India	0.11 – 19.4	0.43 – 12.8	0.12 – 8.45	Salas et al., 2015
Montevideo Harbour, Uruguay	5.43 – 26.4	0.11 – 10.4	–	Muniz et al., 2015
Chitrapuzha River, west coast of India	5.51 – 11.0	5.48 – 9.93	3.08 – 7.42	Sanil Kumar et al., 2017
PiraqueAcu-Mirim estuary and Vitoria Bay, Brazil	0.2 – 5.5	0.3 – 18.8	0.1 – 10.4	Hadlich et al., 2018
Kolkata Port	**0.27 – 18.2***	**0.22 – 13.6***	**0.22 – 10.2***	**Present study**
Kandla Port	**0.12 – 2.08***	**0.08 – 2.70***	**0.08 – 0.53***	**Present study**

(*Values of the biochemical components (Proteins, Carbohydrates, and Lipids) for different stations and seasons are provided in Appendix I).

The high content of PRT and LPD in the sediment is also found to be associated with the input of anthropogenic wastes (Cotano and Villate, 2006; Muniz et al., 2011; Venturini et al., 2012a; Hadlich et al., 2018). The

concentration of PRT in the Kolkata port sediment is similar to that of Montevideo coastal zone-Rio de la Plata estuary (Uruguay), Cochin estuary (southwest coast of India), but lower than Montevideo harbour (Uruguay), which are reported as organically polluted (Table 2.4). The content of PRT, CHO, and LPD was ~ 9, 7, and 15 times higher in Kolkata port when compared to Kandla, indicating the influence of restricted water flushing.

Kolkata is an enclosed port influenced by the input of fresh water from the Hooghly River, which receives a large amount (1153.8 million L d^{-1}) of domestic and municipal wastes (68.95% of total discharge) as well as industrial (31.05%) effluents (Khan, 1995). It has also been reported that only 20% of waste is treated at sewage treatment plants, and the remaining is discharged directly into the Hooghly River (Sarkar et al., 2008). Domestic wastewaters are rich in PRT, CHO, and LPD (Samer, 2015), and their high content is expected in the areas characterized by large human settlements with tremendous anthropogenic activities (Golubkov et al., 2019). Thus, the contribution of biopolymers (PRT, CHO, and LPD) from these wastes into Kolkata dock sediment is possible through contaminated river water. Moreover, Rajaneesh (2018) reported that waste discharge from the anchored ships acts as a source of nutrients in Kolkata docks. A recent study on carbamazepine, the marker of wastewater discharge, revealed higher input of untreated wastewaters in the Hooghly river sediment (Chakraborty et al., 2019). Thus, the input of anthropogenic wastes, high primary production, and restricted water flushing could be the major reasons for the high content of PRT, CHO, and LPD in Kolkata port.

The systems can be classified into different trophic status on the basis of BPC as oligotrophic (BPC = < 1 mg C g^{-1}), mesotrophic (BPC = 1-3 mg C g^{-1}), and eutrophic (BPC = > 3 mg C g^{-1}) conditions (Pusceddu et al., 2011). All the stations of Kolkata port were eutrophic in condition based on the content of BPC (BPC = 3.11 – 16 mg C g^{-1}) except the outside riverine station (Table 2.5).

Table 2.5 Average values of biochemical components of 4 seasons for the stations and benthic trophic status of the Kolkata port.

Station number	PRT (mg g^{-1})	CHO (mg g^{-1})	LPD (mg g^{-1})	BPC (mg C g^{-1})	TS
Kidderpore dock					
S1	3.65	3.06	1.30	3.99	E
S2	3.38	2.37	0.67	3.11	E
S3	5.58	3.85	3.50	6.90	E
S4	6.43	5.86	2.71	7.53	E
S5	6.34	5.79	2.76	7.49	E
S6	7.51	5.66	3.20	8.34	E
S7	8.92	7.55	4.37	10.7	E
S8	8.63	6.42	5.99	11.3	E
S9	9.79	7.73	5.98	12.4	E
S10	9.76	9.74	4.46	11.2	E
S11	11.7	10.4	8.17	16.0	E
S12	11.3	11.6	7.45	15.8	E
Netaji Subhash Dock					
S13	3.68	2.06	2.13	4.23	E
S14	4.06	2.73	1.48	4.20	E
S15	4.18	3.16	2.04	4.84	E
S16	4.48	3.82	2.64	5.70	E
S17	4.94	2.99	2.96	5.28	E
S18	7.01	2.30	2.69	6.37	E
S19	6.07	3.41	1.88	5.40	E
S20	5.33	2.77	2.84	5.85	E
S21	6.81	4.98	3.18	7.72	E
S22	7.91	4.69	5.40	9.80	E
S23	10.1	5.59	5.23	11.1	E
Riverine Station					
S24	1.08	1.00	0.37	1.21	M

(PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; TS: Trophic Status; E: Eutrophic; M: Mesotrophic).

In Kolkata port, BPC was positively correlated with bacteria, diatom, dinoflagellate-specific FAs, and negatively with zooplankton-specific FAs suggesting accumulation of the surplus amount of autotroph originated OM inside the docks. High biomass of phytoplankton and dominance of natural senescence over grazing as the chlorophyll degradation pathway in the water column of Kolkata port (Sathish et al., 2020) also supports the accumulation of phytoplankton derived OM. In Kandla port, BPC was positively correlated with diatom, macroalgae, and zooplankton-specific FAs, and all the stations were found to be oligotrophic in conditions based on the BPC (0.59 - 1.27 mg C g^{-1}) content except S11 and S12, which were mesotrophic in conditions (Table 2.6).

Table 2.6 Average values of biochemical components of 4 seasons for the stations and benthic trophic status of the Kandla port.

Station number	PRT (mg g^{-1})	CHO (mg g^{-1})	LPD (mg g^{-1})	BPC (mg C g^{-1})	TS
Oil Jetties					
S1	0.47	0.62	0.18	0.61	O
S2	0.53	0.50	0.33	0.71	O
S3	0.48	0.49	0.32	0.67	O
S4	0.48	0.67	0.22	0.67	O
S5	0.52	0.57	0.27	0.69	O
Dry Dock					
S6	0.67	0.45	0.20	0.66	O
S7	0.81	0.97	0.22	0.91	O
S8	0.70	0.94	0.30	0.94	O
Cargo Berths					
S9	0.66	1.08	0.24	0.93	O
S10	0.85	0.55	0.27	0.84	O
S11	0.84	1.17	0.19	1.02	M
S12	1.38	1.03	0.24	1.27	M
Creek Stations					
S13	0.64	0.32	0.29	0.65	O
S14	0.39	0.63	0.20	0.59	O
S15	1.25	0.66	0.15	0.99	O

(PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; TS: Trophic Status; O: Oligotrophic; M: Mesotrophic).

It seems that strong tidal currents prevented the accumulation of organic compounds in the sediment, supporting the meso-oligotrophic condition of sediment. Vezzulli and Fabiano (2006) reported that bacterial density from oligotrophic sediment showed an inverse relationship with nutrients (proteins) and characterized with high bacterial activity, which enhances the utilization of resources, however eutrophic sediment though rich in bacteria, the high content of OM disturbs the further organization of the system. This corroborates with the present study, which also showed high bacterial contribution in meso-oligotrophic Kandla port compared to the Kolkata port. It has been reported that heterotrophic bacteria have high respiration activity (82-98% of planktonic respiration) in an oligotrophic environment; however, eutrophic systems are characterized with low bacterial respiration, exporting out a large amount of production (Biddanda et al., 2001).

Such information on sedimentary OM composition from the ports is helpful in understanding the trophic status of the environment and the quality and quantity of food available for the sustenance of benthic organisms.

2.5 Conclusions

This study revealed that the structure of the ports and the hydrodynamic conditions influenced the accumulation of diverse sources derived biochemical and elemental components (PRT, CHO, LPD, FAs, TOC, and TN) in the port sediment and had an impact on the benthic trophic status. Fatty acid biomarkers indicated that bacteria, diatom, dinoflagellate, and macroalgae were the major sources of OM in the Kolkata port sediment, whereas bacteria and terrestrial plant-derived OM was dominant in the

Kandla port. Spatial variations in the OM composition were prominent in the Kolkata port with increasing content towards inner areas of port owing to poor water flushing. There was also a seasonal variation in the autochthonous derived OM in the Kolkata port and allochthonous derived OM in the Kandla port. Kolkata being an enclosed port, was characterized by a high deposition of natural and anthropogenic derived OM, resulting in eutrophic conditions of the sediment; however, strong tidal currents in Kandla port resulted in low accumulation of OM in the sediment.

The information on the benthic trophic status of the sediment, obtained by combining different biomarkers is crucial to know the overall ecosystem health and is valuable to improve the port management activities.

Chapter 3

Characterization of short-term variation in organic matter composition

3. Characterization of short-term variation in organic matter composition

3.1 Introduction

Estuaries are transition zones between marine and freshwater systems and are characterized by rapid and intense variability in the physicochemical conditions (McLusky and Elliot, 2004). These are one of the most productive natural ecosystems sustaining diverse food webs and economically important organisms (Blaber, 1997; Costanza et al., 1997). The mixing of freshwater with seawater results in a deposition of materials to the sediment owing to flocculation and coagulation. The suspended organic matter (OM) received from different sources undergoes biological transformations at the sediment-water interface through the microbial activities (Canuel and Martens, 1993). Heterotrophic bacteria in the estuarine environment play a significant role in the biogeochemical processes and degradation of OM (Nealson, 1997). Bacteria help in the recycling of OM and nutrients in the system, which can be achieved through the generation of new bacterial biomass or degradation of larger macromolecules into smaller particles through ectoenzymes (Smith et al., 1992) and release of dissolved inorganic nutrients (Wakeham and Lee, 1993). It has been reported that benthic bacteria act as an important food resource for benthic meiofauna (Danovaro et al., 1999; van der Heijden et al., 2019) as well as macrofauna (Meziane and Tsuchiya, 2000; Meziane et al., 2002; Alfaro et al., 2006; Wang et al., 2015). Benthic bacteria also increase the quality of sedimentary OM by protein enrichment of detrital materials (Danovaro et al., 1999). The energy transfer role of bacteria makes

them central components of the food webs and, thus, plays a vital role in the ecosystem functioning (Azam et al., 1983).

Heterotrophic bacteria in the estuarine system could be of the autochthonous origin or derived from allochthonous sources. *Vibrios* such as *Vibrio cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* are found abundant in the marine and brackish environments and are considered as autochthonous bacteria of the estuarine environment (Bassler et al., 1991; Thompson et al., 2004). These autochthonous bacteria play a vital role in the processing of OM rich in chitin and in nutrient cycling (Huq et al., 1983; Thompson et al., 2004). Estuaries are subjected to various forms of anthropogenic activities such as industrial, municipal waste discharge, harbour activities, tourism, navigation, etc. (Rodrigues et al., 2011; Venturini et al., 2012a; Salas et al., 2015). The allochthonous bacteria, suspended solids, and OM into the estuarine waters are added mainly through the sewage discharge, domestic wastes, industrial effluents, agricultural, and urban runoff (Mudge et al., 1998; Malham et al., 2014; Pandey et al., 2014; Hassard et al., 2016). The attachment of allochthonous bacteria (coliforms) to the suspended particulate matter increases their viability by protecting themselves from other environmental factors and predators, and also increases the rate of transport of these bacteria to the sediment bed (Davis et al., 1995; Fries et al., 2006; Perkins et al., 2014; Hassard et al., 2016). Thus, sediment acts as a reservoir of autochthonous and allochthonous bacteria and OM in the estuarine systems (Davis et al., 1995; Dell'Anno et al., 2002; Craig et al., 2004). The sedimentary OM plays an important role in the survival and growth of bacteria and benthic organisms.

The estuaries in the Indian subcontinent experience distinct climatic variations due to the south-west monsoon rainfall during June to September period (Vijith et al., 2009), and Zuari is one such tropical monsoon-influenced estuary located along the west coast of India. Heavy precipitation and land runoff have marked effects on the physicochemical properties of the water, planktonic and benthic communities, detritus production, bacterial abundance, and occurrence of phytoplankton blooms, thus influencing the ecosystem functioning and food web dynamics of such a tropical estuary (Kumari et al., 1978; Qasim and Sen Gupta, 1981; Ansari and Parulekar, 1994; D'Souza and Bhosle, 2001; Patil and Anil, 2011, 2015; Bardhan et al., 2015; Khandeparker et al., 2015, 2017a). The monsoon season is followed by the post-monsoon season (October-January), which is the recovery period and then pre-monsoon season (February-May), characterized by stable marine conditions (Qasim and Sen Gupta, 1981).

Zuari estuary acts as a breeding and nursery ground for more than 200 species of fish, and approximately 1000 tons of fish are caught annually from this estuarine region (Sreekanth et al., 2018). The productive mudflats and mangrove vegetation support large numbers of commercially important finfish and shellfish (Sreekanth et al., 2018). The fin- and shell-fish resource from this estuary has revealed that 47 - 55% of fish species are demersal, and 24 - 32% species are pelagic. Moreover, crustaceans and mollusks contribute 8 - 9% and 12 - 13%, respectively, to the total fish catch. Among the fin- and shell-fish, 51.8% species are carnivorous, 30.8% omnivorous, 15.4% planktivorous, and 2% are herbivorous (Sreekanth et al., 2018). The juveniles of demersal fish are dependent upon the food derived from the sediment as

well as benthic standing crop sustain high resources of demersal fish and prawns in the coastal zones of Goa and Zuari estuary (Harkantra and Parulekar, 1981; Ansari and Parulekar, 1994; Ansari et al., 1995; Sreekanth et al., 2018). The meiobenthic community of Zuari estuary is dominated by nematodes and macrobenthos dominated by polychaetes, mollusks, and crustaceans (Ansari et al., 1994; Ingole and Parulekar, 1998; Sivadas et al., 2011). These organisms fulfill their food requirement by ingesting organic components present in the sediment. Thus, evaluating the relative contribution of various sources of OM from the estuaries is important in understanding the quality and quantity of available food for the organisms (Wieking and Kroncke, 2005).

The physical and biological processes found in the estuaries have a significant role in the estuarine food web dynamics (Bodineau et al., 1998; Canuel, 2001). Bodineau et al. (1998) reported that the composition of particulate OM in the estuary change over a tidal cycle at short time-scales, i.e., hours in response to tidal re-suspension or advection. The surface sediment as a recorder of the water column processes is helpful in understanding the short-time scale changes in the OM composition (Zimmerman and Canuel, 2001; Venturini et al., 2012b). It has been reported that the sedimentary OM composition changes on the short (days, weeks) as well as intermediate time scales, i.e., months, seasons (Canuel and Martens, 1993, 1996; Zimmerman and Canuel, 2001; Venturini et al., 2012b). The assessment of OM composition in terms of total organic carbon to total nitrogen (TOC/TN) ratio determines the contribution of in-situ and terrestrial-derived OM in the sediment (Meyers, 1994). The protein to

carbohydrate ratio has been used to evaluate the quality of sedimentary OM (Danovaro et al., 1993). However, fatty acids (FAs) are source-specific, diverse in structure and have been widely used as biomarkers to identify the OM sources in the sediment (Carrie et al., 1998; Meziane and Tsuchiya, 2002; Dunn et al., 2008; Venturini et al., 2012b).

An earlier study in the Zuari estuary has reported that the composition of suspended particulate matter changed within a few days (May 7 to June 2; sampled at 1 to 3-day intervals), and was attributed to phytoplankton blooms, pre-monsoon rain event, wind velocity, the input of terrestrial-derived materials, and biodegradation processes (D'Souza and Bhosle, 2001). However, not much is known about the quality of sedimentary OM from the Zuari estuary. An evaluation of sedimentary OM composition at different time scales, i.e., fortnightly, monthly, and seasonal basis, is crucial to understand the hindcast analysis of the events in the habitat and the nature of food available for the benthic organisms. An earlier study by Khandeparker et al. (2017a) through fine resolution sampling (1 to 3 h) reported the influence of spring and neap tides on the distribution of bacterial populations in the water column of the Zuari estuary. It was found that an increase in suspended particulate matter (SPM), mediated through the riverine discharge and tidal-amplitude regulates bacterial populations in the water column. However, short-time scale studies are not available on the sediment of this estuary. In view of this, the present study was carried out on a fortnightly and monthly basis to assess the distribution of bacteria and the nature of sedimentary OM.

Chapter 3 is subdivided into 3A and 3B. Chapter 3A describes the distribution of selected dominant autochthonous and allochthonous bacteria in the surface sediment of Zuari estuary, as heterotrophic bacteria are known to influence the nature of OM. The present study was carried out along the banks of the estuary on a fortnightly basis. The nature of OM was evaluated using the TOC/TN ratio and protein to carbohydrate ratio. The influence of tidal phases observed during the sampling period on the distribution of bacteria in the sediment was also addressed in the present study.

Chapter 3B discusses the evaluation of monthly variations in the OM composition using source-specific fatty acid biomarkers and elemental components.

3A Spatio-temporal variations in elemental, biochemical components, and bacterial populations in the surface sediment of the Zuari estuary: fortnightly observations

3A.2 Materials and methods

3A.2.1 Description of the study area

Zuari estuary is located in the state of Goa along the central west coast of India (15^{o} 27.5′ N, 73^{o} 48′ E) (Fig. 3A.1). Zuari River has a length of 92 km and an average depth of 5 m. The mouth of the estuary is funnel-shaped with a ~ 5 km width and narrows down towards the head of the estuary (Sheyte et al., 2007). Zuari River has a catchment area of about 550 km^2 and an annual run-off to be about 491×10^6 m^3 (Qasim and Sengupta, 1981; Shetye et al., 2007). The estuary remains well mixed during the non-monsoon season; however, it is stratified during the monsoon season. The tides are

semi-diurnal, with a height of ~ 2.3 m during spring tide and ~ 1.5 m during neap tide (Manoj and Unnikrishnan, 2009).

The surface sediment was collected from seven selected sites lining the banks and the mouth of the Zuari estuary (Fig. 3A.1). Station 1 (Odxel Beach – OB) and station 2 (Bambolim Beach – BB) are categorized as beach influenced stations. Stations 3 (Agacaim – AG) and 4 (Cortalim - CR) are influenced by the movement of ferry boats and fishing trawlers and are lower mid-estuarine stations (Fig. 3A.1).

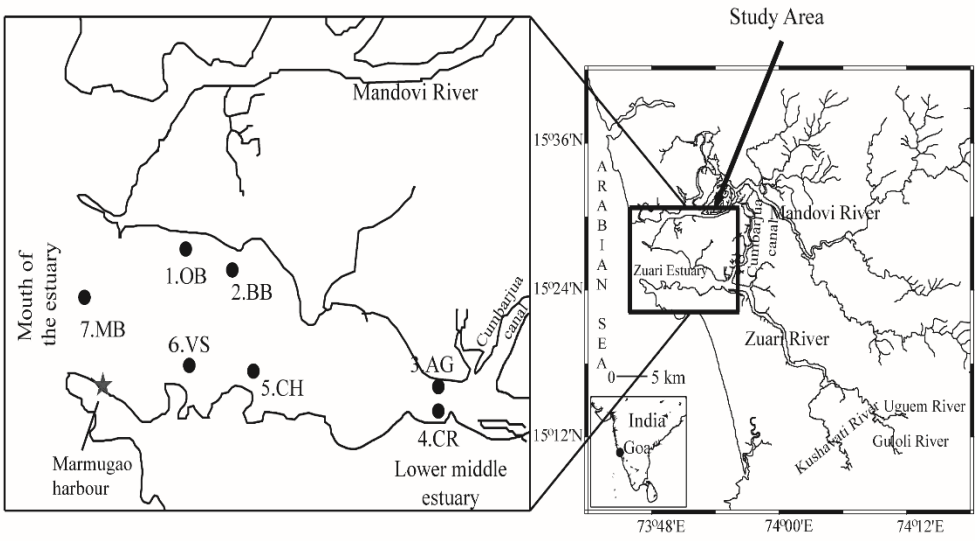


Fig. 3A.1 Map showing the location of stations in the Zuari estuary, Goa, central west coast of India (Station 1 - Odxel Beach (OB); station 2 - Bambolim Beach (BB); station 3 - Agacaim (AG); station 4 - Cortalim (CR); station 5 - Chicalim (CH); station 6 - Vasco (VS); station 7 - Marmugao Bay (MB)).

The anthropogenic and industrial influence is prominent at station 5 (Chicalim – CH). Station 6 (Vasco – VS) is situated close to the Marmugao port. Tidal influence is high at station 7 (Marmugao Bay – MB), which is located at the mouth of the estuary. The details of the sampling locations are given in Table 3A.1a.

Table 3A.1a Details of sampling stations in the Zuari estuary.

Stn.	Station Name	Station code	Latitude	Longitude
1	Odxel Beach	OB	N 15º 27′ 03.3″	E 73º 49′ 30.1″
2	Bambolim Beach	BB	N 15º 26′ 47.4″	E 73º 50′ 08.5″
3	Agacaim	AG	N 15º 24′ 48.0″	E 73º 54′ 17.5″
4	Cortalim	CR	N 15º 24′ 33.4″	E 73º 54′ 15.3″
5	Chicalim	CH	N 15º 24′ 48.2″	E 73º 50′ 27.4″
6	Vasco	VS	N 15º 24′ 49.5″	E 73º 49′ 28.9″
7	Marmugao Bay	MB	N 15º 26′ 13.6″	E 73º 47′ 54.4″

3A.2.2 Sampling strategy

Fortnightly sampling was carried out at the Zuari estuary from 30 November 2010 to 12 January 2012. The sampling was initiated at station 1 at ~7:00 am and concluded at station 7 at ~11:00 am. Different phases of tides were noticed during the sampling period (Table 3A.1b).

Table 3A.1b Details of sampling and tidal height observed during the sampling period.

Sr. No.	Sampling Date	Tide (m)
1	30 November 2010	0.67
2	14 December 2010	0.80
3	29 December 2010	0.55
4	17 January 2011	1.80
5	1 February 2011	1.86
6	17 February 2011	1.97
7	28 February 2011	1.65
8	16 March 2011	1.67
9	30 March 2011	1.63
10	18 April 2011	2.25
11	5 May 2011	2.09
12	11 May 2011	0.72
13	2 June 2011	2.08
14	15 June 2011	2.15
15	30 June 2011	1.99
16	13 July 2011	1.99
17	27 July 2011	1.74
18	18 August 2011	1.73
19	29 August 2011	2.19
20	14 September 2011	1.91
21	29 September 2011	2.23
22	19 November 2011	0.70
23	28 December 2011	0.91
24	12 January 2012	0.92

Surface sediment samples were collected by using a van Veen grab operated from a trawler and top ~ 2 cm of sediment was used for assessing the Total Viable Count (TVC), *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria), Total Organic Carbon (TOC), Total Nitrogen (TN), proteins, and carbohydrates. Sediment samples to be analyzed for the total bacterial count (TBC) were fixed with formaldehyde (final concentration 1-2 % v/v). The samples were transported to the laboratory in an icebox. Sediment could not be sampled from station 1 to station 7 on 30 November and 14 December 2010 for TBC, and from station 7 on 2 June 2011, 13 July 2011, 27 July 2011, 18 August 2011, as well as from station 1, station 2, station 6, and station 7 on 29 August 2011 for TBC, TVC, *Vibrio* spp., and coliforms. In addition to this, sediment could not be sampled from 17 January 2011 to 11 May 2011 and on 18 and 29 August 2011 for elemental and biochemical parameters.

The near-bottom water samples were also collected by using Niskin sampler operated from a trawler, for the analyses of dissolved oxygen (DO), salinity, and temperature. The Winkler titrimetric method was used for the estimation of DO (Grasshoff et al., 1983). Temperature and salinity of the seawater were measured using Digital Thermometer (EUROLAB) and Master Refractometer (ATAGO, Japan), respectively.

3A.2.3 Enumeration of Total Bacterial Count (TBC) in the surface sediment by using Flow Cytometry

Flow Cytometry (BD-Biosciences, USA) was used to analyze formaldehyde fixed sediment samples for TBC. Sediment samples (1 g)

suspended in 10 ml of autoclaved seawater were sonicated at 50% power in the water bath sonicator (Ultrasonic cleaner, Equitron) for 1 min and three times to separate the cells from sediment particles (Luna et al., 2002), further centrifuged at 3000 rpm for 1 min, and the supernatants were recovered. The supernatants (1 ml) were passed through BD cell strainers (pore size, 40 μm) to remove larger particles and subsequently stained with SYBR Green I (Molecular Probes, USA) at 1:10,000 final concentration, and incubated for 15 min at room temperature in the dark (Marie et al., 1999). Fluorescent beads (Polysciences) of 1 μm size were added as internal standards for the calibration. Then, the samples were analyzed using a BD FACSAria^{TM} II flow cytometer with a nuclear blue laser of 488 nm wavelength, which can differentiate green fluorescence excited by a blue laser. Emitted light was collected through the filter sets of 488/10 band pass (BP) for right-angle light scatter (RALS) and 530/30 BP for green fluorescence. Gating was done against RALS versus green fluorescence (FITC) using BD FACSDiva software, and results are expressed as cells g^{-1} of sediment.

3A.2.4 Enumeration of Total Viable Count (TVC), Vibrio spp. *(autochthonous bacteria), and coliforms (allochthonous bacteria) in the surface sediment*

The enumeration of bacteria in the sediment samples was carried out by using the standard spread plate technique. The different selective media were prepared in distilled water following the manufacturer's instructions (Hi-media, Mumbai). One gram of wet sediment was mixed in 10 ml of filtered, autoclaved seawater followed by vigorous shaking to dissociate the

bacteria from the sediment and left for 5-10 min for sediment particles to settle. Subsequently, 1 ml of supernatant was serially diluted in 9 ml of filtered, autoclaved seawater to get 10^1, 10^2, 10^3 dilutions. Then 0.1 ml from each of the dilutions were plated on Zobell Marine Agar (ZMA) for total viable (culturable) bacterial count (TVC) and incubated at room temperature (28 ± 2ºC) for 24 hrs. Thiosulphate-citrate-bile-salts sucrose (TCBS) agar was used to differentiate autochthonous *Vibrio* spp. depending on size and colour. *V. cholerae* grow as raised, yellow coloured colonies having diameter <2 mm, *V. alginolyticus* are bigger (>2 mm diameter) in size and produces yellow coloured colonies. However, *V. parahaemolyticus* develop as green coloured colonies on TCBS agar. Allochthonous bacteria (coliforms) were enumerated by spreading samples on different selective media like MacConkey agar, which differentiates *Escherichia coli* (pink/red coloured colonies) from *Shigella*/*Salmonella* species (transparent, colourless colonies). All colonies on MacConkey agar are reported as total coliforms (TC = *E. coli* and *Shigella*/*Salmonella* spp.). Xylose-Lysine Deoxycholate (XLD) agar was also used, which differentiates *Salmonella* (pink coloured colonies with a black centre) from *Shigella* (pink colonies) species. All selective media for autochthonous and allochthonous bacteria were incubated at 37ºC for 24 hrs, and the counts are expressed as Colony Forming Unit (CFU) g^{-1}. The abundance of *Shigella* and *Salmonella* spp. were determined later to November 2010-March 2011.

3A.2.5 Analyses of elemental and biochemical components from the surface sediment

Before analysis, the sediment samples stored at -20ºC were thawed, dried at 60ºC, and ground to a fine powder using mortar and pestle. The sedimentary TOC content was determined according to El Wakeel and Riley (1957) method and is expressed as a percentage dry weight of sediment (wt %). Total nitrogen (TN) was determined using a CHNS analyzer (Vario MICRO Select, Germany). Sulfanilamide was used as the standard, and results are expressed as a percentage dry weight of sediment (%). The carbohydrate (CHO) and protein (PRT) contents were estimated according to methods of Dubois et al. (1956) and Hartree (1972), respectively, by using a spectrophotometer (UV-1800 Spectrophotometer, Shimadzu). Glucose and bovine serum albumin were used as the calibration standards for CHO and PRT, respectively. The results are reported as mg g^{-1} sediment dry weight. Sediment samples previously combusted in a muffle furnace at 450ºC for 4 hours were used as the blanks for biochemical analyses.

3A.2.6 Data analysis

The relationship between different bacterial species (log-transformed) and near-bottom water salinity, dissolved oxygen, and tide was determined using correlation analysis. Correlation between elemental, biochemical components and bacterial populations in the surface sediment was also carried out. This analysis was done using Statistica 6.0 statistical package.

Appendix

Supplementary data related to Chapter 3A is available in Appendix II

3A.3 Results

3A.3.1 Physico-chemical parameters of the near-bottom water

The temperature and salinity of the near-bottom water varied from 25.7ºC - 31.1ºC and 4 – 37, respectively. Salinity was low (4) at Cortalim during the monsoon season while it was high (37) at Marmugao bay (mouth of the estuary) during the pre-monsoon season. Dissolved oxygen (DO) in the near-bottom water ranged from 1.55-7.83 mg L^{-1}. A strong correlation of DO with the tide ($r = 0.67$; $p = 0.001$) indicates the influence of tide on DO in the near-bottom water.

3A.3.2 Spatio-temporal variations in TBC, TVC, Vibrio spp. *(autochthonous bacteria), and coliforms (allochthonous bacteria) in the surface sediment*

The total bacterial count (TBC) in the surface sediment varied from 2.21×10^6 to 2.33×10^9 cells g^{-1}, with high abundance at Chicalim on 13 July 2011 (Fig. 3A.2a). Overall, the TBC was high during the monsoon season (Fig. 3A.3a). The TBC showed a positive correlation with near-bottom water DO ($r = 0.46$; $p = 0.001$) and tide ($r = 0.38$; $p = 0.001$), and a negative correlation ($r = -0.26$; $p = 0.006$) with salinity (Table 3A.2).

The TVC ranged from 5.50×10^2 - 3.75×10^7 CFU g^{-1}, with high abundance at Cortalim (lower mid-estuarine station) on 30 November 2010 (Fig. 3A.2b). The average TVC was high during the post-monsoon-I season (Fig. 3A.3b). The abundance of *V. cholerae* and *V. alginolyticus* ranged from Not Detected (ND) to 1.92×10^7 CFU g^{-1} and ND to 2.74×10^5, respectively (Fig. 3A.2c and d). The abundance of *V. cholerae* and *V. alginolyticus* was

high at Marmugao Bay (mouth of the estuary) on 30 November 2010 and 19 November 2011, respectively. The average abundance of *V. cholerae* and *V. alginolyticus* was high during the post-monsoon-I and post-monsoon-II season, respectively (Fig. 3A.3c and d). A positive correlation was found between *V. cholerae* and near-bottom water salinity ($r = 0.23$; $p = 0.015$). The abundance of *V. parahaemolyticus* varied from ND to 2.60×10^6 CFU g^{-1}, with high abundance at Agacaim (lower mid-estuarine station) on 30 November 2010 (Fig. 3A.2e). *V. parahaemolyticus* were positively influenced by tide ($r = 0.25$; $p = 0.009$) (Table 3A.2). Seasonal variation showed a high abundance of *V. parahaemolyticus* during the post-monsoon-I season (Fig. 3A.3e). The abundance of total coliforms ranged from ND - 8.87×10^6 CFU g^{-1}, with high abundance on 30 November 2010 (Fig. 3A.2f). The average abundance of total coliforms was high at Cortalim (lower mid-estuarine station). Total coliforms showed a positive correlation with near-bottom water DO ($r = 0.26$; $p = 0.006$), tide ($r = 0.25$; $p = 0.009$), and a negative correlation with the salinity ($r = -0.21$; $p = 0.030$) (Table 3A.2). The abundance of *Shigella* spp. and *Salmonella* spp. ranged from ND – 1.18×10^5 CFU g^{-1} and ND – 6.43×10^3 CFU g^{-1}, respectively (Fig. 3A.2g and h). *Shigella* spp. were high towards the mouth of the estuary on 19 November 2011 whereas, *Salmonella* spp. were high at Agacaim (lower mid-estuarine station) on 11 May 2011 (Fig. 3A.2g and h).

Overall, the TVC, *V. cholerae*, *V. parahaemolyticus*, and total coliforms were high during the slack period of tide observed during the post-monsoon-I season (Fig. 3A.3b, c, e, and f).

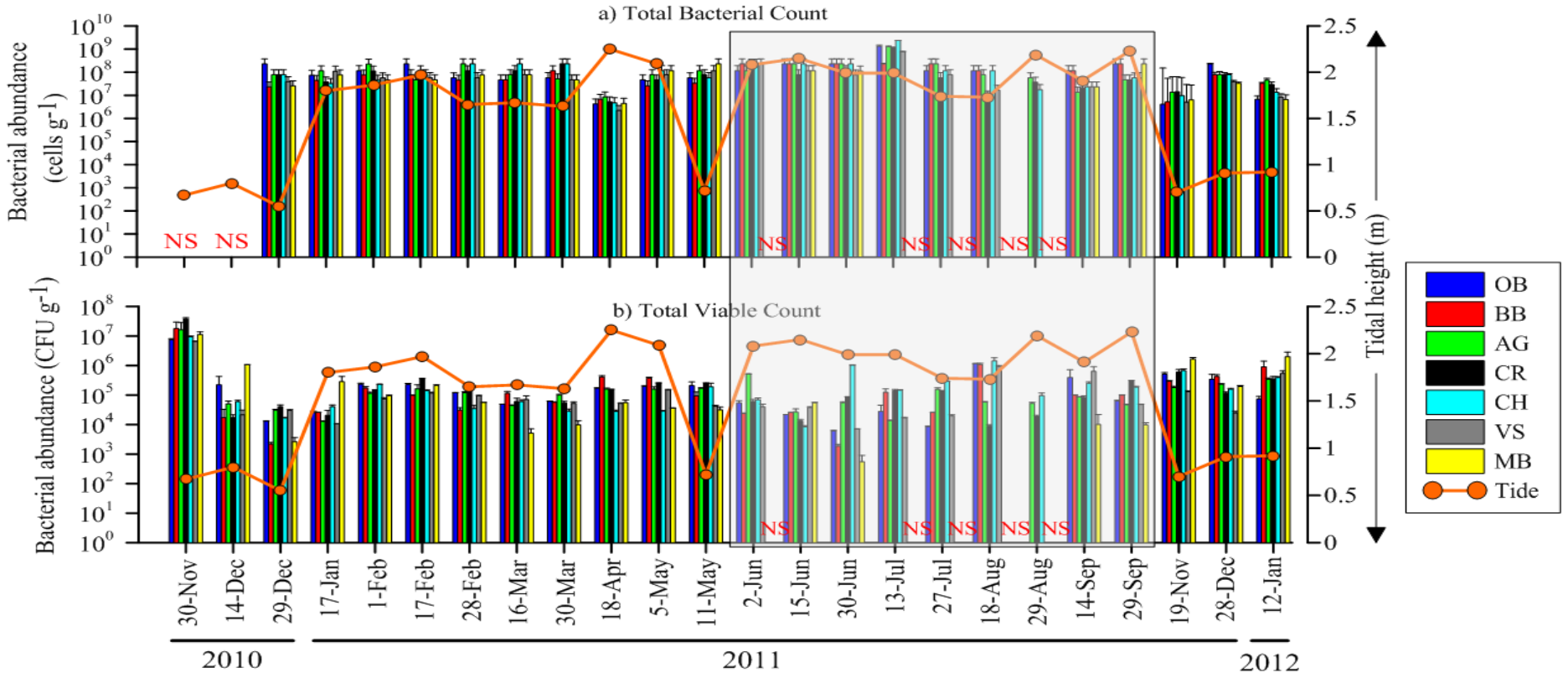


Fig. 3A.2 Fortnightly variations in the (a) Total Bacterial Count, (b) Total Viable Count, (c) *V. cholerae*, (d) *V. alginolyticus*, (e) *V. parahaemolyticus*, (f) Total coliforms, (g) *Shigella* spp., and (h) *Salmonella* spp. in the surface sediment of the Zuari estuary. (Vertical lines indicate standard deviation (SD) from the mean; OB-Odxel Beach; BB-Bambolim Beach; AG-Agacaim; CR-Cortalim; CH-Chicalim; VS-Vasco; MB-Marmugao bay; NS: No Samples; ND: Not detected; Shaded area indicate the monsoon season).

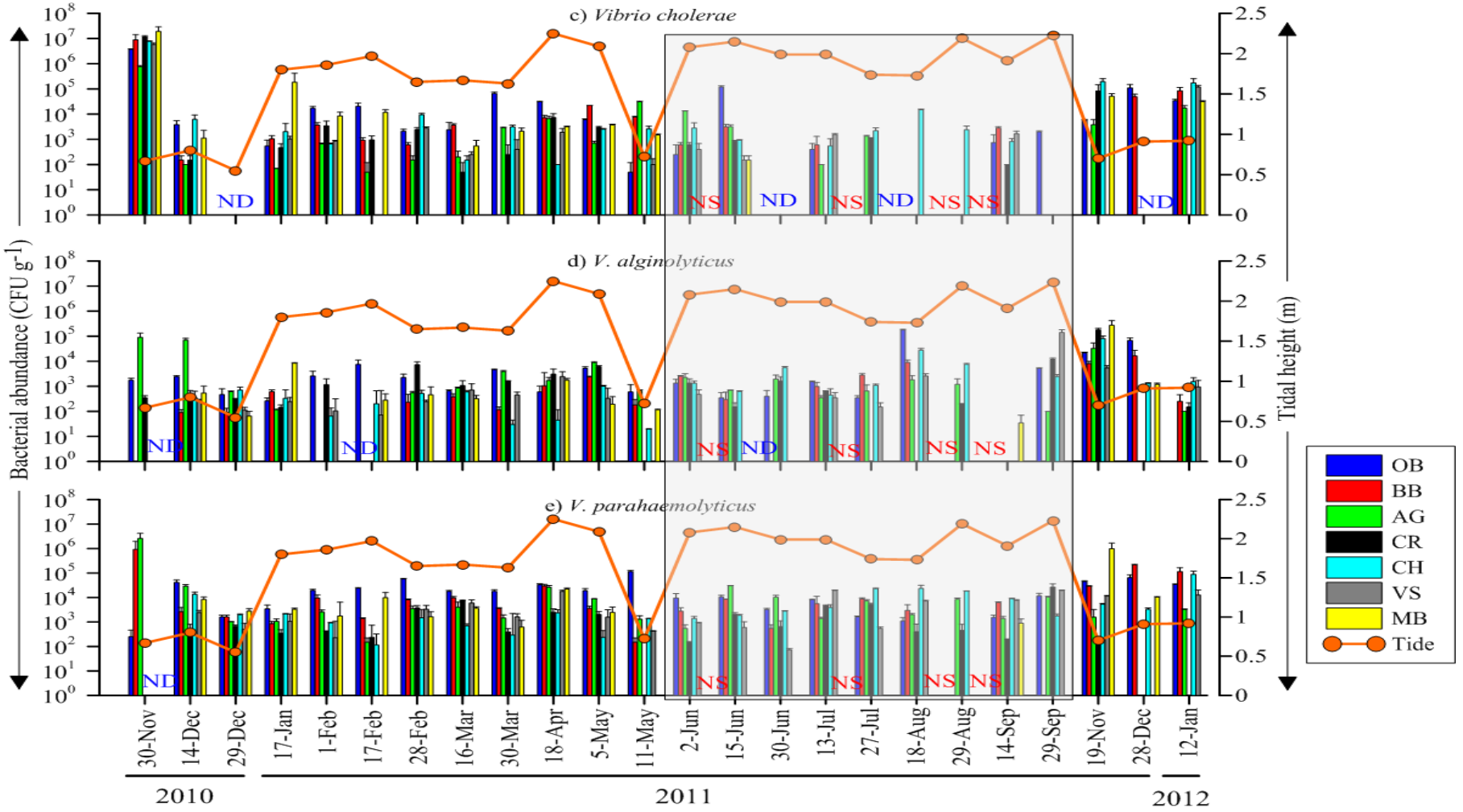


Fig. 3A.2 continued

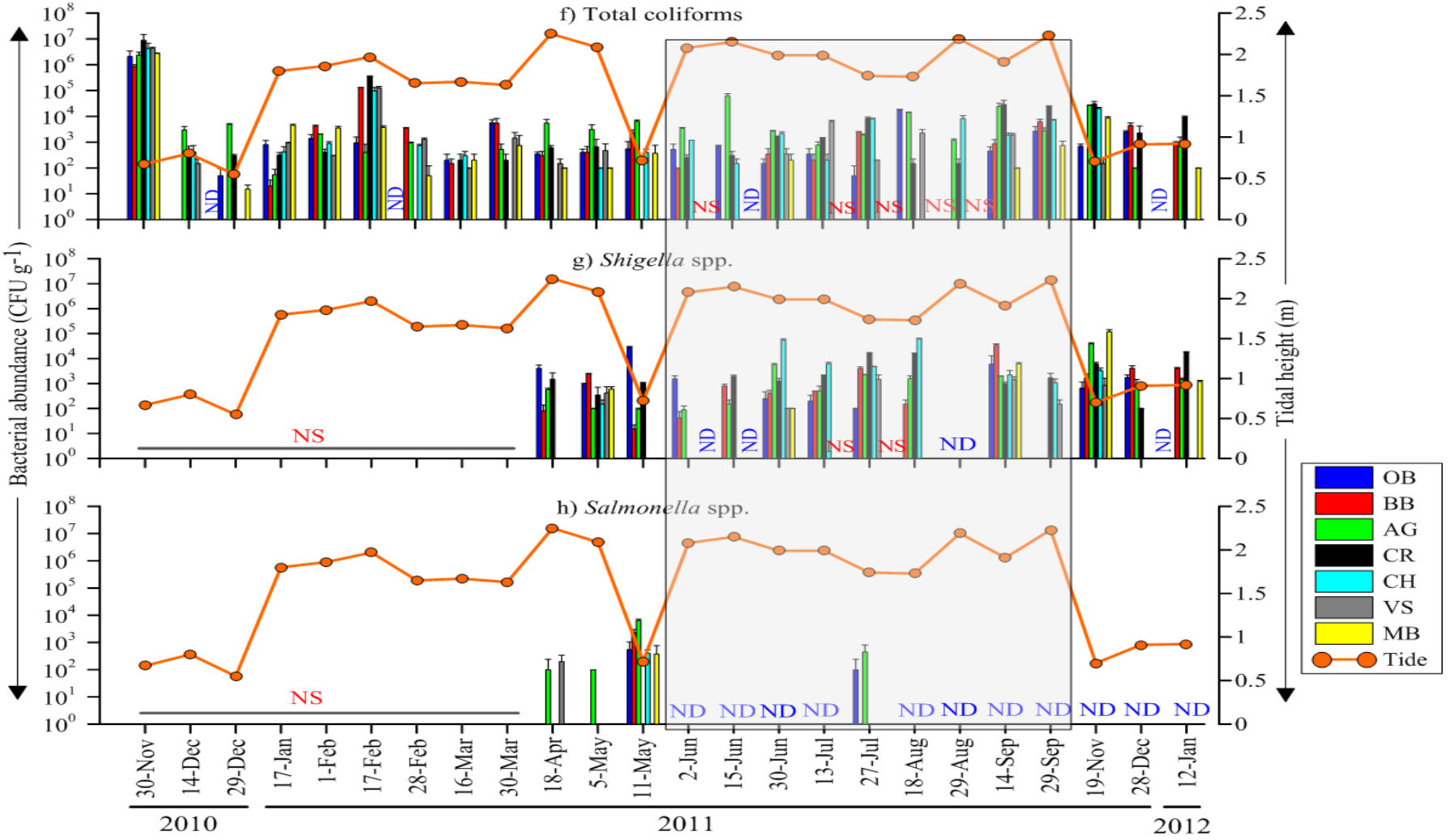


Fig. 3A.2 continued

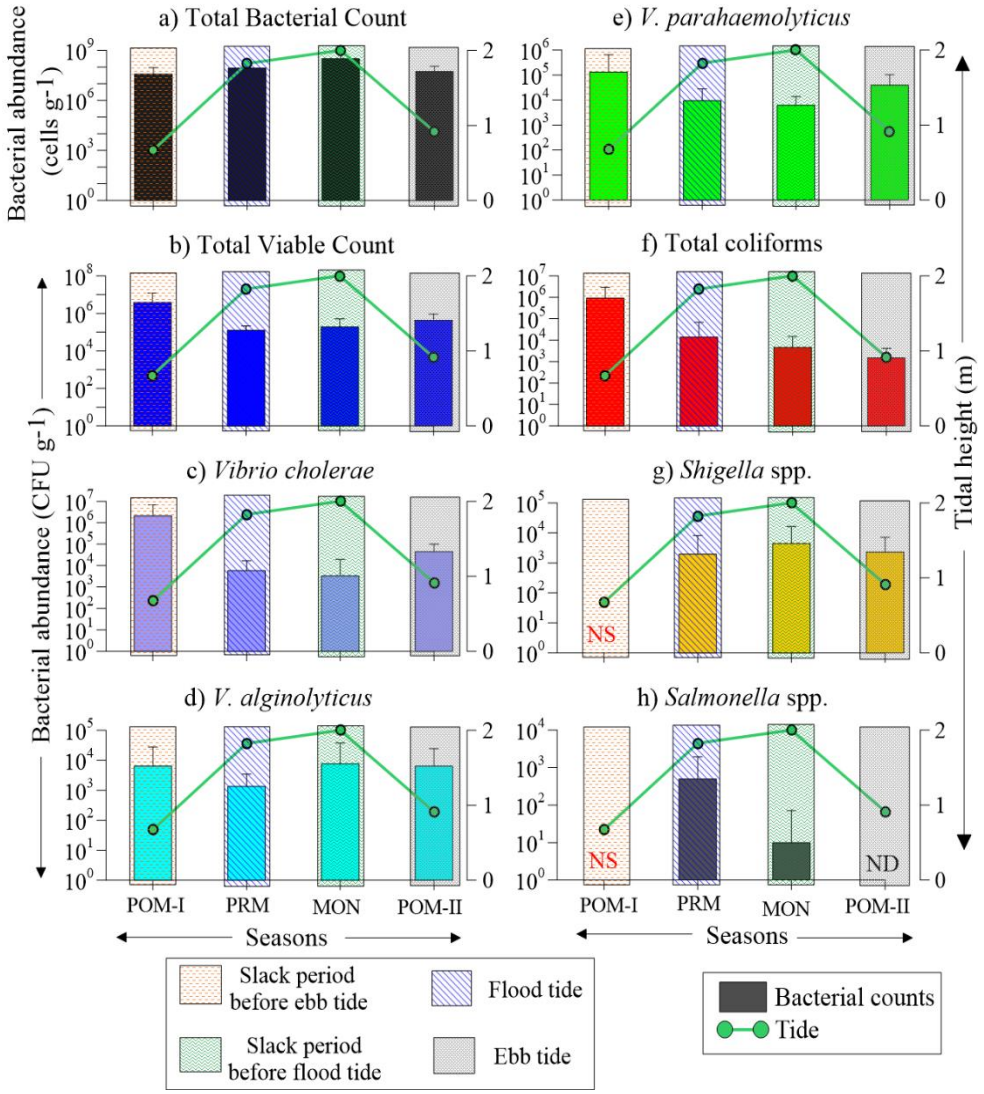


Fig. 3A.3 Seasonal variations in the (a) Total Bacterial Count, (b) Total Viable Count, (c) *V. cholerae*, (d) *V. alginolyticus*, (e) *V. parahaemolyticus*, (f) Total coliforms, (g) *Shigella* spp., and (h) *Salmonella* spp. in the surface sediment of the Zuari estuary during different tidal phases (Vertical lines indicate standard deviation (SD) from the mean; NS: No Samples; ND: Not detected; PRM: Pre-monsoon; MON: Monsoon; POM: post-monsoon).

Table 3A.2 Correlation matrix showing r values of environmental parameters with bacterial populations in the surface sediment of the Zuari estuary.

	TBC	TVC	TC	VC	VA	VP	SH	SL	DO	Salinity	Tide
TBC	1.00										
TVC	**-0.26**	1.00									
TC	0.03	**0.41**	1.00								
VC	-0.08	**0.21**	0.07	1.00							
VA	0.02	**0.35**	**0.30**	0.16	1.00						
VP	-0.04	**0.24**	0.17	**0.36**	**0.51**	1.00					
SH	-0.12	0.11	**0.19**	-0.10	-0.11	0.10	1.00				
SL	0.11	0.01	0.12	0.06	0.84	-0.02	-0.03	1.00			
DO	**0.46**	-0.02	**0.26**	-0.10	-0.01	0.16	0.12	**0.22**	1.00		
Salinity	**-0.26**	0.01	**-0.21**	**0.23**	-0.01	-0.09	-0.11	-0.12	**-0.44**	1.00	
Tide	**0.38**	-0.03	**0.25**	-0.03	-0.05	**0.25**	0.15	-0.15	**0.67**	**-0.34**	1.00

(r values marked as bold indicate that correlation is significant at the $p < 0.05$; TBC: Total Bacterial Count, TVC: Total Viable Count, TC: Total coliforms, VC: *V. cholerae*, VA: *V. alginolyticus*, VP: *V. parahaemolyticus*, SH: *Shigella* spp., SL: *Salmonella* spp., DO: Dissolved Oxygen).

The percentage occurrence of bacteria in the surface sediment showed dominance of *V. parahaemolyticus* (90%) and *V. alginolyticus* (79.4%) among autochthonous *Vibrio* spp. and total coliforms (83.1%) and *Shigella* spp. (70.1%) among allochthonous bacteria (Table 3A.3).

Table 3A.3 Percentage (%) occurrence of different bacteria in the surface sediment of the Zuari estuary.

Types of Bacteria	Number of samples analysed	Positive Occurrence	Overall Occurrence (%)
Vibrio cholerae	160	112	70.0
V. alginolyticus	160	127	79.4
V. parahaemolyticus	160	144	90.0
Total coliforms	160	133	83.1
Shigella spp.	97	68	70.1
Salmonella spp.	97	10	10.3

3A.3.3 Elemental and biochemical composition of the surface sediment

The high content of TOC and TN was observed at Agacaim, Cortalim, and Chicalim when compared to the station located at the mouth of the estuary (Fig. 3A.4a and b). The TOC and TN in the surface sediment of the Zuari estuary ranged from 0.05 to 4.05% and BDL (Below Detection Limit) to 0.20%, respectively (Fig. 3A.4a and b). The high content of TOC was found during the monsoon season (Fig. 3A.4a). Overall, the TOC/TN ratio ranged from 2.30 to 51.8 (Fig. 3A.4c), indicating the input of plankton and terrestrial derived OM. The TOC/TN ratio was low at Marmugao bay on 29 December 2010 (post-monsoon-I) and was high at Bambolim beach on 30 June 2011 (monsoon).

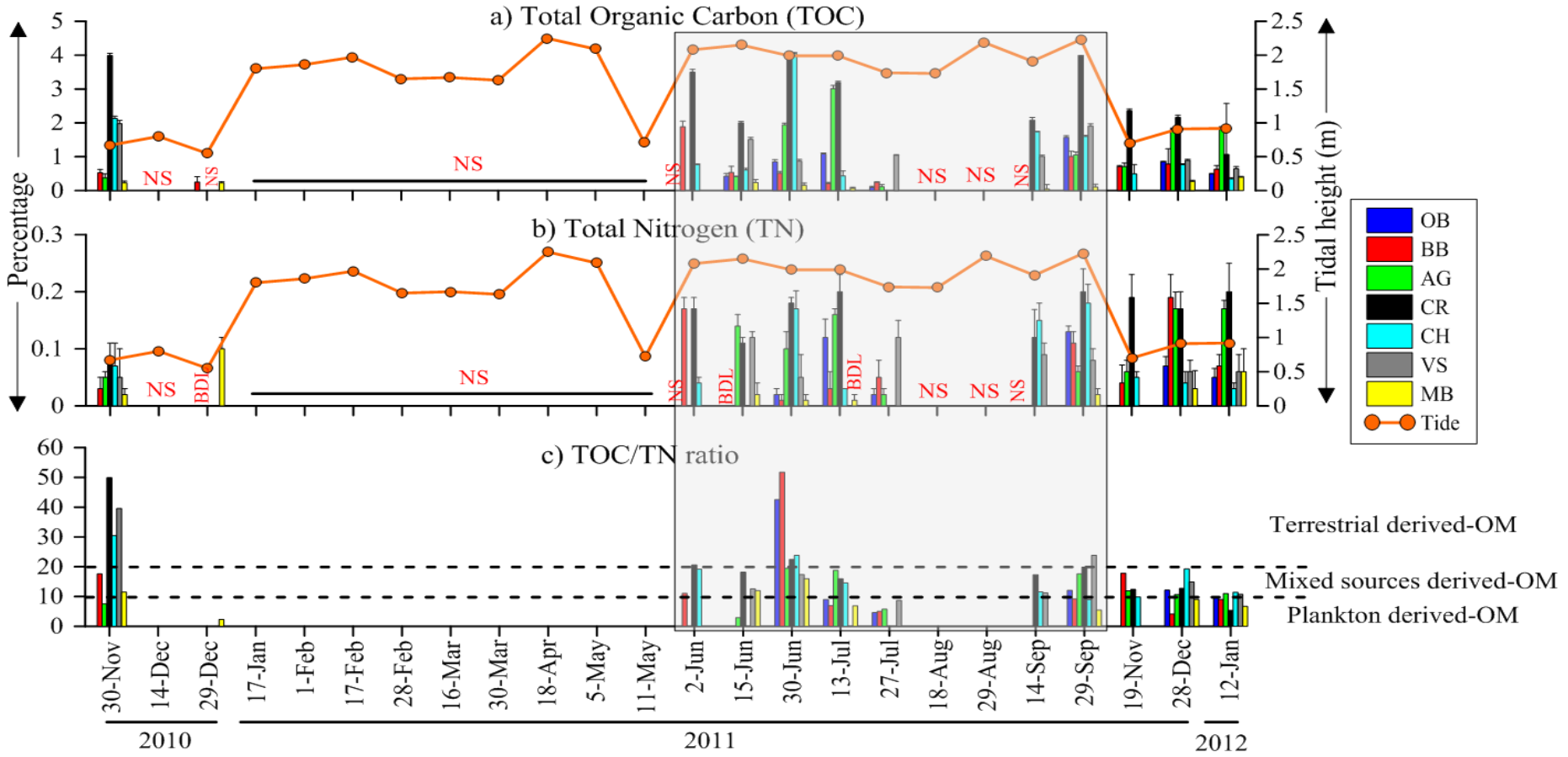


Fig. 3A.4 Fortnightly variations in the (a) total organic carbon (TOC), (b) total nitrogen (TN), and (c) TOC/TN ratio in the surface sediment of the Zuari estuary (Vertical lines indicate standard deviation (SD) from the mean; NS: No Samples; BDL: Below Detection Limit; Shaded area indicate the monsoon season).

The content of PRT and CHO varied from 0.13 to 16.1 mg g^{-1} and 0.45 to 18.6 mg g^{-1}, respectively (Fig. 3A.5a and b). The high content of PRT and CHO was observed at Agacaim, Cortalim, and Chicalim. The PRT/CHO ratio ranged from 0.30 to 1.51 (Fig. 3A.5c), indicating the presence of degraded, aged non-living as well as recently produced fresh OM. The PRT/CHO ratio was low at Marmugao bay on 30 June 2011 (monsoon) and was high at Bambolim beach on 27 July 2011 (monsoon).

The TVC showed a positive correlation with proteins (r = 0.24; p = 0.025) and carbohydrates (r = 0.25; p = 0.017) (Table 3A.4). A negative correlation of *V. parahaemolyticus* with proteins (r = - 0.25; p = 0.02) and TOC (r = - 0.32; p = 0.004) was evident. *Salmonella* spp. showed a negative correlation (r = - 0.23; p = 0.044) with carbohydrates (Table 3A.4).

Table 3A.4 Correlation matrix showing r values of elemental, biochemical components, and bacterial populations in the surface sediment of Zuari estuary.

	TBC	TVC	TC	VC	VA	VP	SH	SL
PRT	-0.05	**0.24**	0.17	-0.11	0.02	**-0.25**	0.15	-0.21
CHO	-0.06	**0.25**	0.17	-0.06	0.01	-0.18	0.15	**-0.23**
TOC	0.15	-0.03	0.08	-0.08	-0.04	**-0.32**	-0.01	-0.06
TN	0.05	0.17	0.06	-0.12	0.03	-0.14	-0.09	-0.19

(r values marked as bold indicate that correlation is significant at the $p < 0.05$; TBC: Total Bacterial Count, TVC: Total Viable Count, TC: Total Coliforms, VC: *V. cholerae*, VA: *V. alginolyticus*, VP: *V. parahaemolyticus*, SH: *Shigella* spp., SL: *Salmonella* spp.).

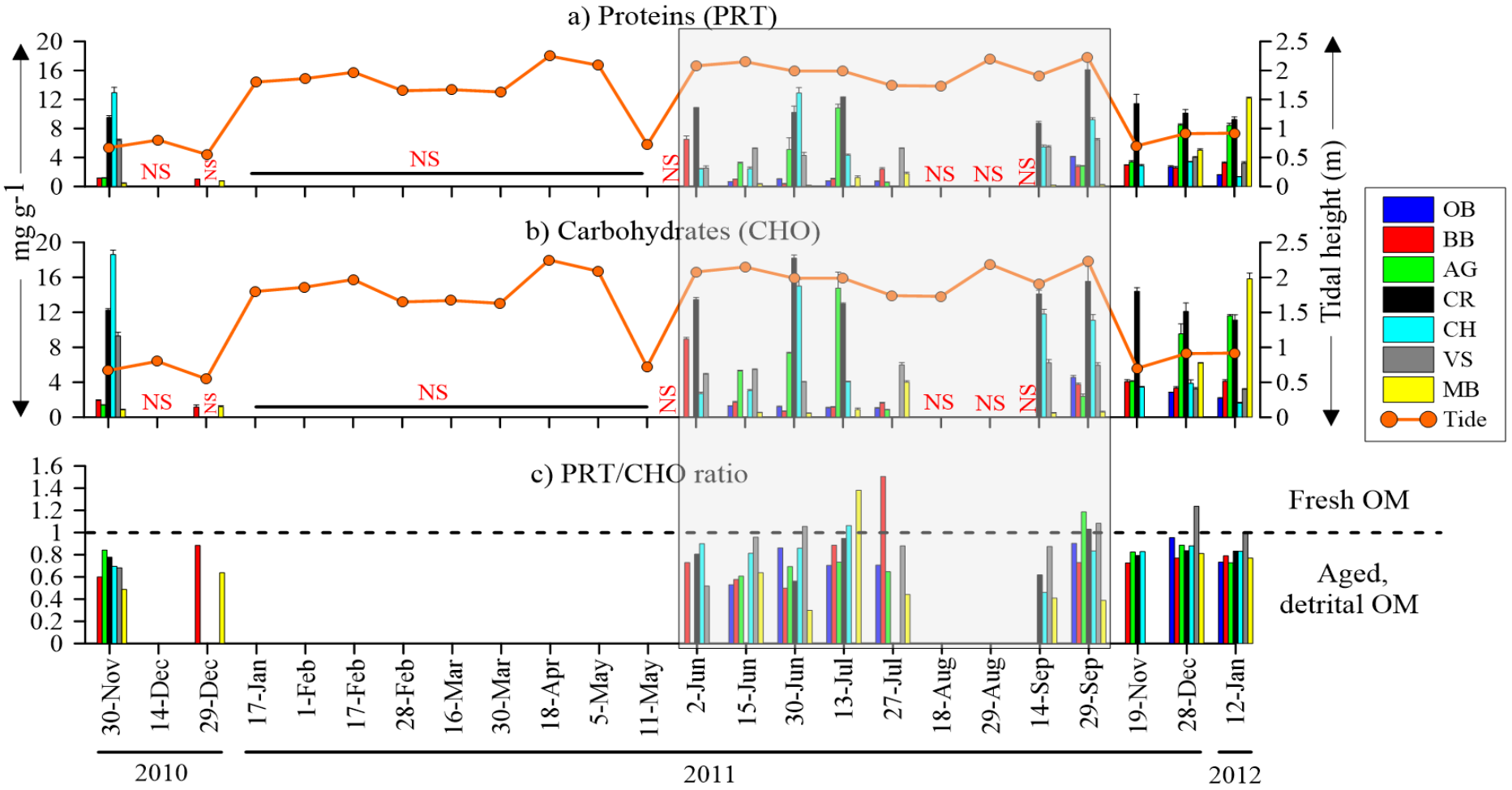


Fig. 3A.5 Fortnightly variations in the (a) proteins (PRT), (b) carbohydrates (CHO), and (c) PRT/CHO ratio in the surface sediment of the Zuari estuary (Vertical lines indicate standard deviation (SD) from the mean; NS: No Samples; Shaded area indicate the monsoon season).

3A.4 Discussion

The total bacterial count (TBC) was high in the sediment of Chicalim, which is influenced by anthropogenic activities. This site is subjected to various land-based anthropogenic activities and is actively used for shipbuilding workshops, yards, barge cleaning, and iron ore transportation (Shenai-Tirodkar et al., 2016). The present study showed moderate content of elemental and biochemical components at Chicalim with a mixed contribution of marine, and terrestrial derived OM during all the seasons as indicated by the average TOC/TN ratio of 16.56 (Appendix II). The ratio of TOC/TN has been used to distinguish the autochthonous or marine OM from allochthonous or terrestrial input in the sediment. The protein-rich algae and plankton are characterized by a low TOC/TN ratio (4-10) than terrestrial land plants (>20), which are rich in cellulose (Meyers, 1994). The ratio around 10-20 indicates mixed input of marine and terrestrial materials. Overall, TBC was high during the southwest monsoon season. During this season, a slack phase prior to flood tide was observed, which could be a possible reason for the high abundance of TBC (Fig. 3A.3). A negative correlation of TBC in the sediment with near-bottom water salinity supports their extraneous input. Zuari estuary receives high sediment discharge during peak rainfall (Qasim and Sen Gupta, 1981) owing to increased run-off, which is found to range from 100-400 m^3 s^{-1} (Shetye and Murty, 1987). Previously, Shynu et al. (2015) studied the $\delta^{13}C_{org}$ in the sediment and reported terrestrial OM to be the dominant component in the downstream region of the Zuari estuary during the monsoon season. Thus, monsoon season characterized with a large amount of freshwater discharge from the catchment area could be one of the

sources bringing in land run-off rich in bacterial load and anthropogenic input. The average TOC/TN ratio was 15.4 during the monsoon, which indicates mixed input of terrestrial and marine-derived OM in the sediment. It is expected that the TOC/TN ratio should be high during this season as it brings a large amount of terrestrial materials. But, low TOC/TN ratio during the monsoon indicates an increase in the protein derived OM (Bardhan et al., 2015, and references therein). High PRT/CHO ratio (>1) at Chicalim, Bambolim Beach, and Marmugao Bay during the monsoon (13 and 27 July 2011; Fig. 3A.5c) point out the presence of PRT rich, fresh OM, and can be attributed to the high bacterial abundance or phytoplankton blooms. The PRT/CHO ratio > 1 indicates newly or recently generated OM, and the ratio < 1 suggests the presence of non-living or aged detrital material in the sediment (Danovaro et al., 1993). Earlier studies have reported the occurrence of phytoplankton blooms in the Zuari estuary mostly during the monsoon season owing to increased input of nutrients (Patil and Anil, 2008, 2011, 2015). It has also been reported that colonization of bacteria on the terrestrial derived materials reduces the TOC/TN ratio, as it leads to an increase in the PRT content (Untawale et al., 1977; Wafar et al., 1997).

The total viable count (TVC) and total coliforms were high at Cortalim. This site is located near the junction, where the Zuari River channel merges into the Marmugao Bay and is influenced by tidal currents, waves, movement of fishing trawlers, ferry boats, is shallow in depth, and is a lower mid-estuarine station. This station was also characterized by a high content of TOC, TN, proteins, and carbohydrates, which possibly resulted in a high abundance of TVC and total coliforms. A positive correlation of TVC with

proteins and carbohydrates supports this observation. A recent study has reported that the stations towards the mid-estuarine region are rich in silt and clay content as compared to those located at the mouth of the estuary (Padalkar et al., 2019). The fine-grained sediment with the large surface area have a more adsorptive capacity for OM (Hedges and Keil, 1995) and can influence the accumulation of OM and bacteria. Dessai and Nayak (2007) also reported the dominance of silt and clay in the sediment of mid-estuarine region owing to deposition of particles during low energy conditions and colloidal aggregates during the estuarine mixing. Perkins et al. (2014) reported a high abundance of bacteria in the OM, silt, and clay-rich sediment at the Conwy estuary, UK. The attachment of coliforms to the fine particles increases their viability and transport to the sediment bed (Fries et al., 2006). The average TOC/TN ratio (19) indicated input of both autochthonous and terrestrial derived OM at Cortalim, which support a high abundance of allochthonous bacteria. The negative influence of near-bottom water salinity on the abundance of total coliforms in the sediment indicates their input from external sources.

The TVC, *V. parahaemolyticus*, and total coliforms numbers were significantly higher during the slack period before the ebb tide of post-monsoon-I than the ebb tide of post-monsoon-II (Fig. 3A.3b, e, and f). It has been reported that the re-suspended particles settle to the sediment bed during the slack period before starting of the ebb tide and get re-suspended from the sediment with ebb currents remain suspended for a certain degree and then settle to the bottom (Sanford et al., 2001). An earlier study from the Zuari estuary also reported a significant influence of tide on the distribution of

bacteria in the water column, regulated by an increase in the suspended particulate matter (Khandeparker et al., 2017a). The allochthonous and autochthonous bacteria attached to the suspended particles increase their downward flux to the bottom sediment (Droppo et al., 2009; Perkins et al., 2014). This could be the possible reason for the high abundance of coliforms and *V. parahaemolyticus* in the sediment towards the mid-estuarine station during the post-monsoon-I season. During this time, the dominance of carbohydrates over proteins and low PRT/CHO ratio (<1) indicates the detrital heterotrophic nature of the environment (Danovaro, 1996). The negative correlation of *V. parahaemolyticus* with proteins suggest high bacterial activity and utilization of labile OM.

High abundance of *V. cholerae* was found at the mouth of the estuary during the slack period prior to the ebb tide of post-monsoon-I. An earlier study by Khandeparker et al. (2017a) also reported high numbers of *V. cholerae* in the water column at the mouth of the Zuari estuary, and their abundance was influenced by suspended particulate matter (SPM), nutrients, and salinity. The average ratio of TOC/TN was low (8.7) at the mouth of the estuary, indicating the contribution of phytoplankton, which could be a possible reason for the high abundance of *V. cholerae*, as *Vibrio* spp. are associated with plankton population (Hsieh et al., 2007). However, *Salmonella* spp. were high towards the lower mid-estuarine station during flood tide of the pre-monsoon. The flood tide currents cause re-suspension of sediment bed and transfer particles towards land, whereas, during ebb currents, particles move towards the sea and settle to the bottom sediment (Sanford et al., 2001; Chant and Stoner, 2001). An earlier study in this estuary

has pointed out that the discharge of wastes from the ships in outer anchorage could be source of *Shigella* and *Salmonella* spp. (Rodrigues et al., 2011).

The present study showed that the percentage (%) of *V. parahaemolyticus* and *V. alginolyticus* was high in the sediment and is not surprising, as they are autochthonous to the estuarine and marine ecosystems (Thompson et al., 2004). High prevalence of coliforms and *Shigella* spp. among allochthonous bacteria can be attributed to their adaptability and survival in the sediment. Allochthonous bacteria adsorbed to sediment particles protect themselves from UV light, phage attack, and protozoan grazing and also survive for a longer time in the sediment (Davies et al., 1995; Craig et al., 2004; Perkins et al., 2014; Hassard et al., 2016). Allochthonous bacteria (coliforms) can even proliferate and re-grow in the OM and fine particle rich sediment (Desmarais et al., 2002). Moreover, *V. parahaemolyticus* emerged as a dominant bacterium in the sediment during the study period (Table 3A.3). *V. parahaemolyticus* can tolerate a wide range of salinities and is able to use large numbers of substrates for the growth (Kaneko and Colwell, 1975; Watkins and Cabelli, 1985). *V. parahaemolyticus* is found to be associated with copepods and play a vital role in the cycling of nutrient through the mineralization of chitinous material with the help of chitinase enzymes (Kaneko and Colwell, 1975). It has also been reported that *V. parahaemolyticus* are associated with phytoplankton, and their growth is influenced by decaying planktonic cells (Rehnstam-Holm et al., 2010). High prevalence of *V. parahaemolyticus* in the surface sediment and negative correlation with proteins and TOC suggests their role in the mineralization of OM derived from the marine and terrestrial inputs.

3A.5 Conclusions

The present study revealed a significant role of OM content, seasons, and tide on the distribution of heterotrophic bacteria in the surface sediment along the banks of the Zuari estuary. The OM content was high towards the lower mid-estuarine sites, rich in carbohydrates and proteins, and was of mixed origin (autochthonous and terrestrial). Whereas, it was of autochthonous origin at the mouth of the estuary and had a profound influence on the distribution of bacteria in the surface sediment. The nature of sedimentary OM changed even within a period of a fortnight, attributed to the bacterial response as well as the contribution of phytoplankton and terrestrial derived materials. High abundance of heterotrophic bacteria in the sediment during the slack period before ebb tide indicates their deposition in the sediment. *V. parahaemolyticus* and *V. alginolyticus* were the more frequently occurring bacteria among autochthonous whereas, total coliforms and *Shigella* spp. were dominant among allochthonous bacteria in the sediment. A negative correlation of total coliforms and TBC with salinity indicated the influence of land run-off. The present study highlighted that in addition to the nature of OM and seasons, the sampling time influenced by tidal condition also play an important role in the population dynamics of heterotrophic bacteria in the sediment.

3B Evaluation of monthly variations in the sources of sedimentary OM in the Zuari estuary using FA biomarkers and elemental components

3B.1 Introduction

Fatty acid biomarkers have been reported as suitable biomarkers to identify the OM sources. Fatty acids (FAs) are source-specific and diverse in structure (Carrie et al., 1998; Meziane and Tsuchiya, 2002; Dunn et al., 2008; Venturini et al., 2012b), and have been used to evaluate the short-term variations in the OM composition (Canuel and Martens, 1993; Bodineau et al., 1998; David et al., 2019). FAs have an advantage over the TOC/TN ratio because the TOC/TN ratio is not efficient in giving precise information on OM sources. The selective remineralization and decomposition of proteinaceous compounds and colonization of bacteria on the terrestrial-detrital materials have the potential to modify the TOC/TN ratio (Meyers, 1997; Bianchi and Canuel, 2011).

Thus, in the present study, monthly variability in the sources and composition of sedimentary OM was evaluated using source-specific FA biomarkers along with elemental components. The sediment samples were collected from the mouth of the estuary, mid-estuarine stations, and the banks of the estuary. The sampling area selected in the present study has been reported as breeding and nursery ground for economically important organisms such as fish (Sreekanth et al., 2018; 2019). The banks of Zuari estuary also act as an active habitat for the bivalves, which are of commercial value (Desai et al., 2020). It was hypothesized that the in-situ production, seasons (monsoon and non-monsoon), bacterial activity, and riverine input would influence the OM dynamics. The characterization of sedimentary OM

from the Zuari estuary will add information on the nature of food available to the benthic organisms.

3B.2 Materials and methods

3B.2.1 Description of the study area

Detailed information on the Zuari estuary has been given earlier in chapter 3A, section 3A.2.1.

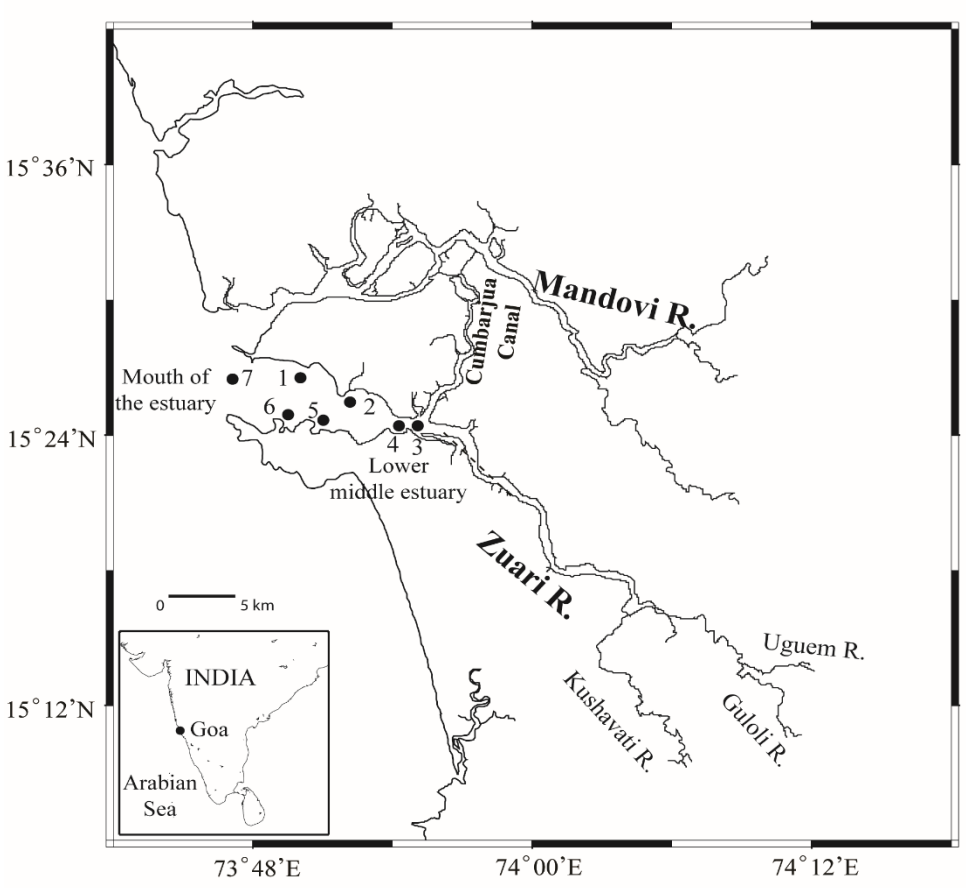


Fig. 3B.1 Map showing sampling sites in the Zuari estuary, Goa, central west coast of India. (Station 1 – Bambolim; station 2 - Siridao; station 3 - Cortalim; station 4 - Sancoale; station 5 - Chicalim; station 6 - Vasco; station 7 - Marmugao Bay).

Seven sampling sites were selected within the Zuari estuary (Fig. 3B.1). Station 1 (Bambolim) is characterized by foreshore beach and station

2 (Siridao) is located in the intertidal sand flat. Stations 3 (Cortalim) and 4 (Sancoale) are located ~ 5 km inside from station 2 in the estuarine mixing zone, where the Cumbharjua canal and river channel merges into the Marmugao Bay. The Cumbharjua canal is lined with dense mangroves on its banks. Sediment from the mid-estuarine region is found rich in silt (40-56%) and clay (30.4-41.8%) compared to sand (2.3-24.9%) particles (Padalkar et al., 2019). Station 4 is relatively shallow, characterized by detrital rich mudflat, and its southern side is covered with mangrove vegetation. Stations 5 (Chicalim) and 6 (Vasco) are close to the estuarine mouth and located ~ 6-8 km downstream from station 4 on the southern bank of the estuary. These stations are influenced by anthropogenic activities. Station 7 (Marmugao Bay) is located at the center of the mouth of the estuary, wherein sediment is rich in sand particles owing to high tidal energy (Rao and Rao, 1974).

3B.2.2 Sampling strategy

Surface sediment and near-bottom water samples were collected on a monthly basis during March 2016 – February 2017 from 7 sampling stations using van Veen grab (0.04 m^2) operated from a trawler, and top ~ 2 cm of sediment samples were packed in zip lock bags and kept in the icebox. These sediment samples were then transported to the laboratory on ice and stored at -20°C after arrival to the laboratory. Each sampling event was initiated at ~ 7:00 am (station 1) and completed at ~ 11:00 am (station 7). Sediment samples were not collected during the months of June and July due to rough weather conditions. The salinity and temperature of the overlying bottom water were measured using a refractometer (ATAGO, Japan) and digital

thermometer (EUROLAB), respectively. The details of the sampling are given in table 3B.1.

3B.2.3 Determination of the elemental composition of the sediment

Please refer chapter 2A, section 2A.2.3.

3B.2.4 Analyses of fatty acids from the sediment

Please refer chapter 2A, section 2A.2.5.

3B.2.5 Data analyses

Data on stations characterized with a similar type of sediment characteristics were pooled together and represented as the northern bank stations (NBS) of the Zuari bay (stations 1 and 2), lower mid-estuarine stations (LMES) (stations 3 and 4), representing brackish water conditions, southern bank stations (SBS) of the Zuari bay (stations 5 and 6), and the mouth of the estuary (station 7). Data were log (x+1) transformed to meet the assumption of normality and homogeneity. The elemental components and fatty acid biomarkers were subjected to analysis of variance (ANOVA) to evaluate the spatial and temporal variations. The relationship between measured sedimentary parameters was checked by correlation analysis. These analyses were performed by using STATISTICA software (version. 6.0, StatSoft, USA).

Table 3B.1 Details of sampling, tidal height, salinity, and temperature of the near-bottom water of the Zuari estuary.

		Tide prior to or at the start of sampling		Succeeding tide at the end of sampling		Salinity				Temperature (◦C)			
Sr. no.	Sampling dates	Time (h:m)	Tidal height (metre)	Time (h:m)	Tidal height (metre)	NBS	LMES	SBS	M	NBS	LMES	SBS	M
1	26 March 2016	06:21	0.50	13:00	1.98	35	31	35	36	31.1	31.6	31.0	31.0
2	25 April 2016	06:18	0.35	13:23	2.03	36	31	34	36	31.0	30.1	30.5	32.0
3	26 May 2016	07:06	0.35	14:18	2.05	34	26	32	35	32.1	32.7	32.4	32.2
4	30 August 2016	03:01	0.31	10:04	2.03	19	10	22	35	27.1	27.6	25.4	25.4
5	30 September 2016	04:14	0.58	10:30	2.01	29	15	28	36	25.5	27.1	26.3	25.0
6	25 October 2016	07:16	1.94	13:28	0.92	33	26	29	32	27.2	28.0	27.5	27.9
7	24 November 2016	07:02	1.94	13:48	0.80	35	33	35	35	27.2	27.4	27.3	27.8
8	24 December 2016	06:42	1.83	13:47	0.73	35	34	37	37	26.3	26.4	26.1	26.1
9	24 January 2017	07:27	1.77	14:33	0.60	32	33	33	35	26.7	27.3	26.9	26.7
10	28 February 2017	06:07	0.66	11:57	2.10	32	30	34	35	28.4	29.0	29.2	29.5

(NBS: Northern bank stations; LMES: Lower mid-estuarine stations; SBS: Southern bank stations; M: Mouth of the estuary).

3B.3 Results

3B.3.1 Elemental composition of the sediment

TOC and TN in the surface sediment ranged from 0.13 – 4.27% and 0.01 – 0.31%, respectively (Fig. 3B.2a and b). The content of TOC and TN varied significantly among the sites (ANOVA; $p \leq 0.0001$) (Table 3B.2). The high content of TOC and TN was observed at the lower mid-estuarine stations followed by southern bank stations, northern bank stations, and lower content was found at the mouth of the estuary (lower mid-estuarine stations>southern bank stations>northern bank stations> mouth of the estuary) (Fig. 3B.2a and b).

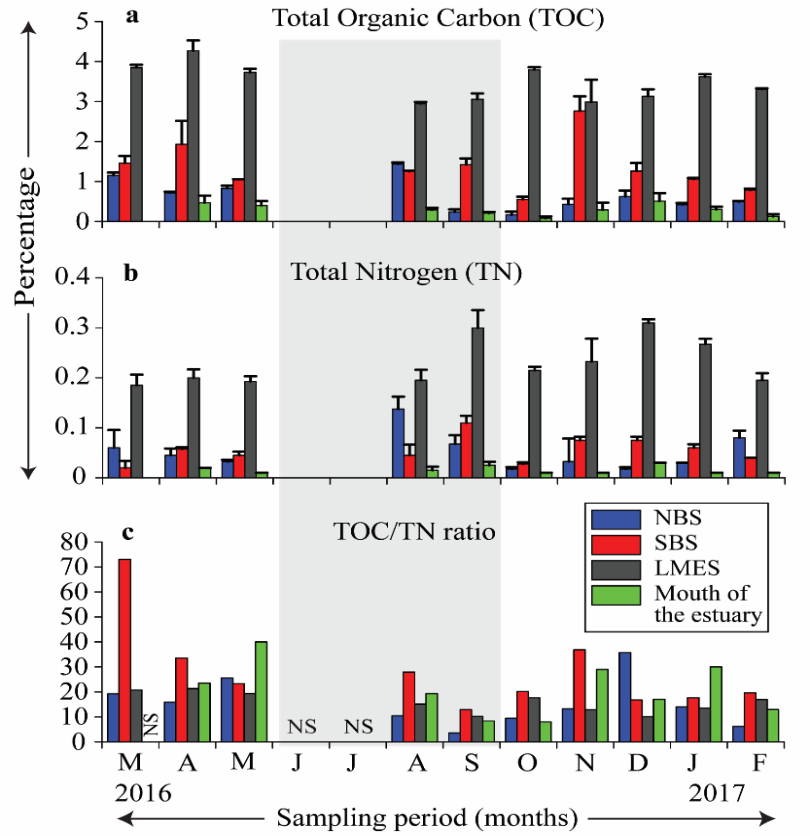


Fig. 3B.2 Monthly variations in the distribution of elemental components in the surface sediment of the Zuari estuary (Vertical lines indicate standard deviation (SD) from the mean; NBS: Northern bank stations; SBS: Southern bank stations; LMES: Lower mid-estuarine stations; NS: No Samples; Shaded area indicate monsoon season).

Overall, the ratio of TOC/TN ranged from 4.9 – 73 (Fig. 3B.2c). ANOVA showed significant variations in the TOC/TN ratio among the sites (ANOVA; $p \leq 0.0021$), and the months (ANOVA; $p \leq 0.0034$). This ratio was low at the northern bank (3.6 – 35.6; avg. 15.3) and lower mid-estuarine stations (10 – 21.4; avg. 15.8) when compared to the southern bank stations (12.9 - 73; avg. 28.2), and the mouth of the estuary (8 – 40; avg. 20.9). Pre-monsoon months were characterized with a high TOC/TN ratio (avg. 28.7 ± 16.2) when compared to the monsoon (avg. 13.6 ± 7.2) and post-monsoon (avg. 19.5 ± 8.7) months.

3B.3.2 Spatio-temporal variations in the fatty acids (FAs)

The sources of OM in the sediment were identified based on the FA biomarkers (Table 1.1). The total fatty acids (TFAs) in the Zuari estuary ranged from 29 to 122 µg g^{-1} (Fig. 3B.3a) and showed a significant spatial (ANOVA; $p \leq 0.0001$) and temporal (ANOVA; $p \leq 0.0015$) variation (Table 3B.2). TFAs were high at the lower mid-estuarine stations (101 ± 13.4 µg g^{-1}) followed by southern bank stations (73 ± 10.7 µg g^{-1}), northern bank stations (56 ± 8.1 µg g^{-1}) and low at the mouth (39 ± 7.4 µg g^{-1}) of the estuary (Fig. 3B.3a). The detrital FAs (C16:0+C18:0) followed a similar trend (Fig. 3B.3b) and were major contributors to TFAs. These detrital FAs showed a positive correlation with terrestrial plant ($r = 0.83$; $p = 0.0001$) and diatom-specific ($r = 0.67$; $p = 0.0001$) FAs (Table 3B.3).

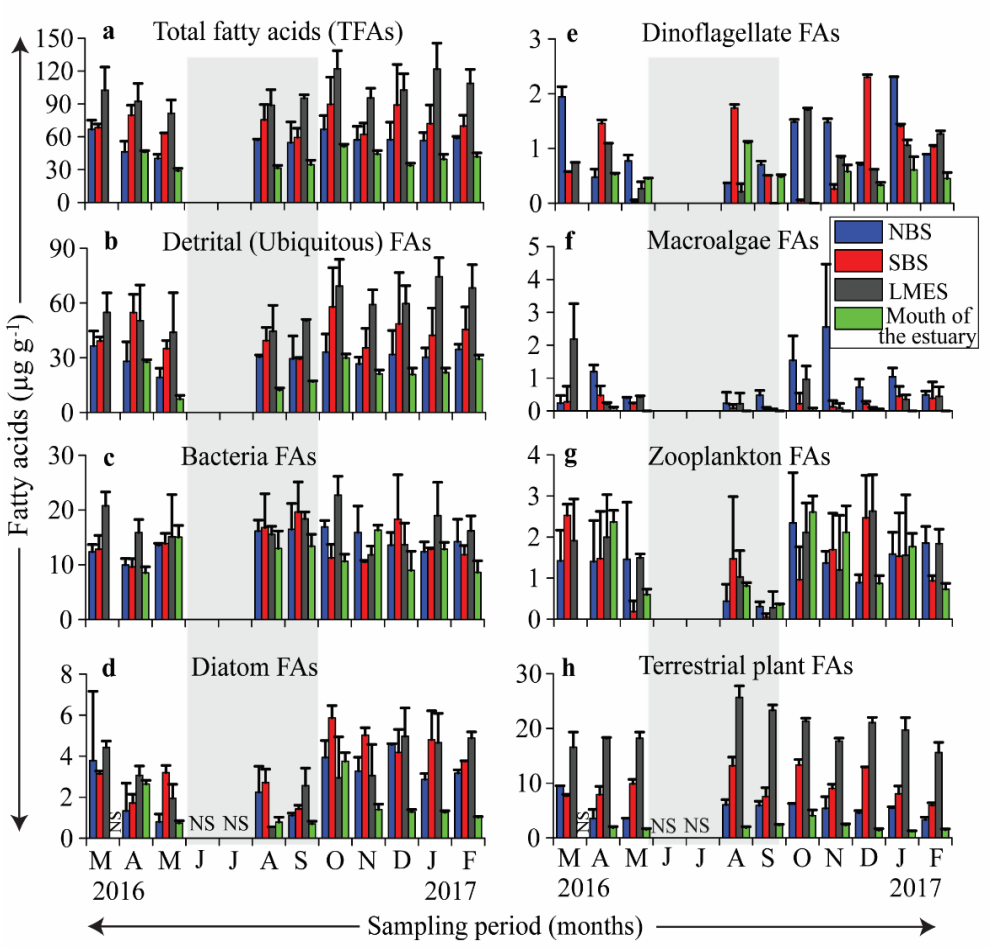


Fig. 3B.3 Monthly variations in the total, detrital (ubiquitous), and source-specific fatty acids (FAs) in the surface sediment of the Zuari estuary (Vertical lines indicate standard deviation (SD) from the mean; NBS: Northern bank stations; SBS: Southern bank stations; LMES: Lower mid-estuarine stations; NS: No Samples; Shaded areas indicate monsoon season).

Bacteria-specific FAs ranged from 8.1 – 22.9 µg g^{-1} (Fig. 3B.3c). These FAs showed significant spatial variation (ANOVA; $p \leq 0.0073$) (Table 3B.2), with high content at the lower mid-estuarine station and low content at the mouth of the estuary. But, their contribution to TFAs showed the opposite trend (Fig. 3B.4a and b). Although bacterial FAs were high during the monsoon and post-monsoon months compared to pre-monsoon months, these

differences were non-significant. Bacteria-specific FAs showed a positive correlation ($r = 0.52$; $p = 0.001$) with terrestrial plant-specific FAs and negative correlation ($r = - 0.39$; $p = 0.014$) with salinity irrespective of seasons (Table 3B.3).

The content of diatom, dinoflagellate, and macroalgae-specific FAs ranged from 0.5 – 5.9 µg g^{-1}, Not Detected (ND) – 2.3 µg g^{-1}, and ND – 2.6 µg g^{-1}, respectively (Fig. 3B.3d-f). Diatom and macroalgae-specific FAs showed significant variations among the sites (Table 3B.2). Diatom-specific FAs were high at the southern bank stations and macroalgae-specific FAs at the northern bank stations (Fig. 3B.3d and f). Moreover, there was significant monthly variation in diatom-specific FAs (ANOVA; $p \leq 0.0040$), with high content during the post-monsoon months, medium during pre-monsoon, and low during the monsoon months. Overall, the contribution of phytoplankton derived OM was high on the banks of the estuary (Fig. 3B.4a-d).

Zooplankton-specific FAs ranged from 0.06 – 2.6 µg g^{-1} (Fig. 3B.3g). Zooplankton FAs showed significant variations among the months (ANOVA; $p \leq 0.0007$), with high content during post-monsoon months and low during monsoon months (Table 3B.2). Zooplankton-specific FAs showed a positive correlation with diatom ($r = 0.44$; $p = 0.005$) and dinoflagellate-specific FA ($r = 0.39$; $p = 0.014$) markers (Table 3B.3).

Long-chain FAs (LCFAs), which are specific of terrestrial plant ranged from 1.20 to 25.7 µg g^{-1} (Fig. 3B.3h). These FAs varied significantly among the sites (ANOVA; $p \leq 0.0001$) and the months (ANOVA; $p \leq 0.0199$) (Table 3B.2). The contribution of terrestrial plant-derived OM was high at the lower mid-estuarine stations (avg. 19.8%) and low (avg. 5.2%) at the mouth of the

estuary (lower mid-estuarine stations>southern bank stations>northern bank stations >mouth of the estuary) (Fig. 3B.4a-d).

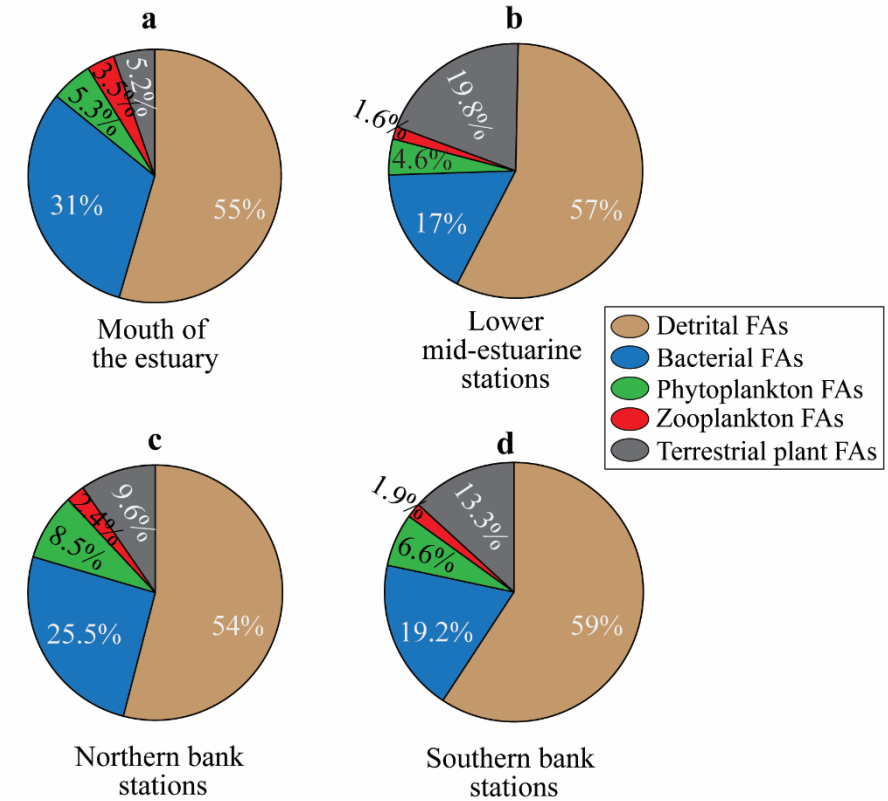


Fig. 3B.4 Spatial variations in the contribution of source-specific fatty acids to total fatty acids in the Zuari estuary.

Detrital FAs were the dominant component of the TFAs (Fig.3B.4). Detrital FAs are common in bacteria, phytoplankton, zooplankton, and terrestrial plant; thus, the contribution of group-specific FAs to detrital FAs was evaluated. The results indicated that the contribution of bacteria-specific FAs to detrital FAs was high at the mouth of the estuary and decreased towards the banks and lower mid-estuarine stations (Fig. 3B.5). Terrestrial plant-specific FAs showed the opposite trend to the bacteria-specific FAs. Phytoplankton-specific FAs contributed high to detrital FAs on the banks of the estuary during the non-monsoon season. Though the contribution of

bacteria-specific FAs to detrital FAs was high throughout the estuary, their signatures increased in the bank stations during the monsoon season (Fig. 3B.5).

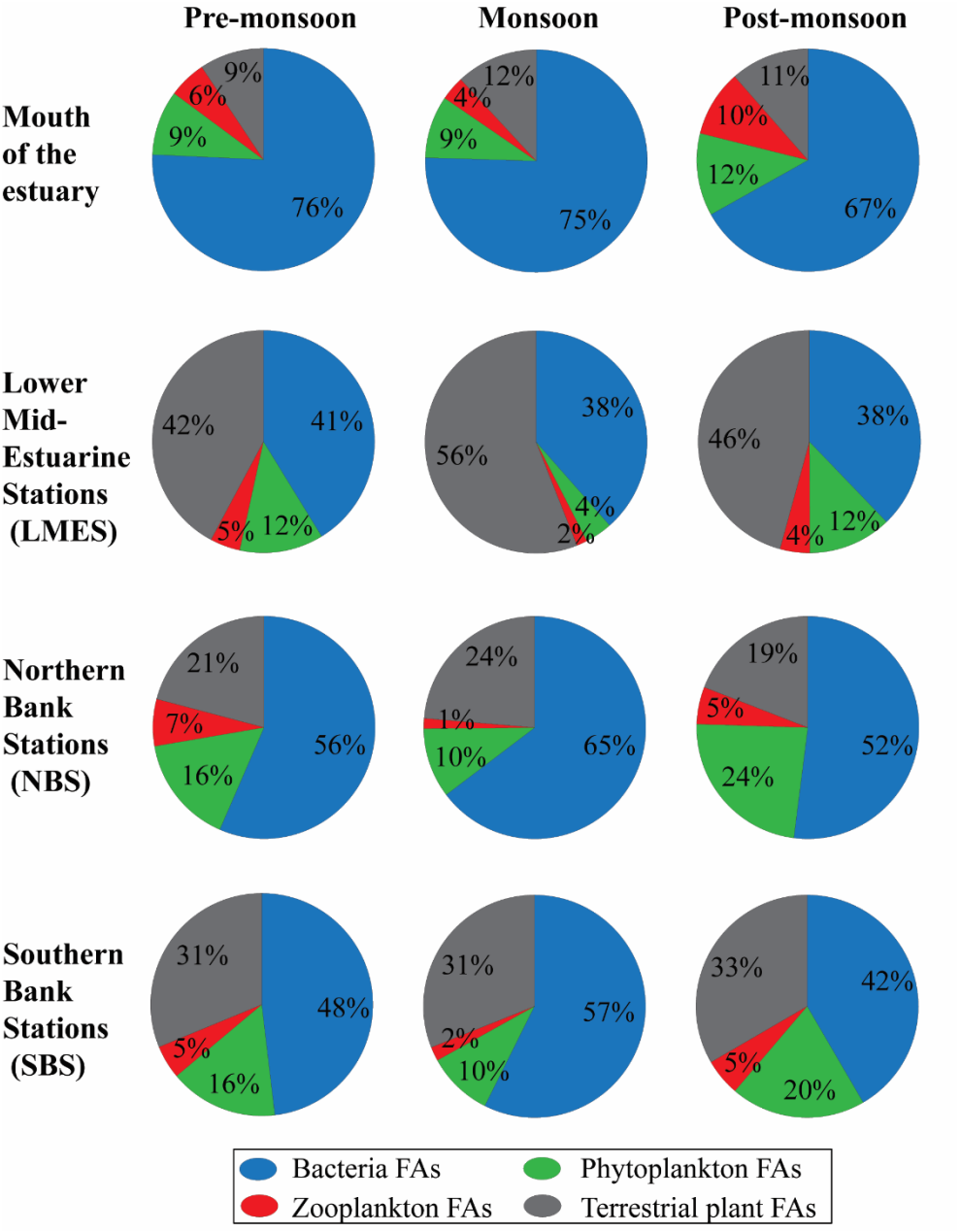


Fig. 3B.5 Spatio-temporal variations in the percentage contribution of source-specific fatty acids to detrital fatty acids.

Table 3B.2 Summary of two-way ANOVA of elemental components and fatty acid markers from the surface sediment of the Zuari estuary.

Factors	Parameters	df	MS	F	p-value
Sites	TOC	3	0.512	95.45	**0.0001**
	TN	3	0.013	95.06	**0.0001**
	TOC/TN	3	0.142	6.423	**0.0021**
	Total FAs	3	0.280	118.7	**0.0001**
	Detrital FAs	3	0.352	54.45	**0.0001**
	Bacteria FAs	3	0.033	4.983	**0.0073**
	Diatom FAs	3	0.120	7.689	**0.0008**
	Macroalgae FAs	3	0.092	7.355	**0.0010**
	Dinoflagellate FAs	3	0.028	1.463	0.25
	Zooplankton FAs	3	0.008	0.696	0.56
	Terrestrial plant FAs	3	1.189	167.5	**0.0001**
	Salinity	3	0.035	6.650	**0.0018**
Months	TOC	9	0.009	1.68	0.1458
	TN	9	0.002	1.46	0.2151
	TOC/TN	9	0.085	3.84	**0.0034**
	Total FAs	9	0.010	4.35	**0.0015**
	Detrital FAs	9	0.032	4.91	**0.0007**
	Bacteria FAs	9	0.009	1.47	0.2119
	Diatom FAs	9	0.058	3.73	**0.0040**
	Macroalgae FAs	9	0.011	0.92	0.5207
	Dinoflagellate FAs	9	0.017	0.89	0.5433
	Zooplankton FAs	9	0.054	4.90	**0.0007**
	Terrestrial plant FAs	9	0.020	2.78	**0.0199**
	Salinity	9	0.024	4.69	**0.0009**

(Bold value indicates significant p values ($p < 0.05$); df: degree of freedom; MS: mean square).

Table 3B.3 Correlation matrix showing r values of elemental components and fatty acid markers from the surface sediment of the Zuari estuary.

	TOC	TN	TFA	Detrital FAs	Bact. FAs	Dia. FAs	Dino. FAs	M. algae FAs	Zoo. FAs	TP. FAs	Salinity
TOC	1.00										
TN	**0.87**	1.00									
TFA	**0.80**	**0.76**	1.00								
Detrital FAs	**0.71**	**0.67**	**0.95**	1.00							
Bact. FAs	**0.42**	**0.49**	**0.47**	0.25	1.00						
Dia. FAs	**0.33**	0.26	**0.65**	**0.67**	0.12	1.00					
Dino. FAs	0.04	-0.04	0.22	0.18	0.25	0.22	1.00				
M. algae FAs	0.07	0.01	0.26	0.23	0.28	0.31	**0.40**	1.00			
Zoo. FAs	0.17	0.05	0.30	**0.32**	-0.06	**0.44**	**0.39**	0.25	1.00		
TP. FAs	**0.85**	**0.80**	**0.94**	**0.83**	**0.52**	**0.51**	0.10	0.18	0.14	1.00	
Salinity	**-0.40**	**-0.48**	**-0.36**	-0.27	**-0.39**	0.17	0.27	0.07	0.29	**-0.51**	1.00

(r values marked as bold indicate that correlation is significant at the $p < 0.05$; TOC: Total Organic Carbon; TN: Total Nitrogen; TFA: Total Fatty Acids; Bact: Bacteria, Dia: Diatom; Dino: Dinoflagellate; M. algae: Macroalgae; Zoo: Zooplankton; TP: Terrestrial plant).

3B.4 Discussion

The present study showed relatively high content of TOC, TN, and TFAs at the lower mid-estuarine stations, followed by the bank stations, and lower content at the mouth of the estuary attributed to hydrodynamic conditions and geomorphological structure of the estuary. Tides in the Zuari estuary are semi-diurnal with a height of ~2.3 m during spring and ~1.5 m during the neap (Shetye et al., 2007). The mouth region of the estuary is funnel-shaped, which causes churning of the sediment, enhancing the effective re-suspension of sedimentary OM. It has been reported that re-suspended particles have intense heterotrophic activity and are characterized by a high contribution of bacteria-specific FAs (David et al., 2019). In the present study, the contribution of bacteria-specific FAs to the TFAs was relatively high (31%) at the mouth of the estuary when compared to the other stations indicating an enhanced bacterial response. The contribution of bacterial FAs to detrital FAs was also high during all the seasons at the mouth of the estuary (Fig. 3B.5). The tidal mediated re-suspension resulting in high bacterial abundance could be responsible for the low content of OM components in the bottom sediment towards the mouth of the estuary. Among the banks, the southern bank was characterized by a high content of OM when compared to the northern bank and can be attributed to sediment grain size and geomorphological structure. Southern bank (Chicalim) with semi-enclosed nature and silty-sandy sediment results in more accumulation of detrital rich organic materials than the sand dominant northern bank (Siridao) (Desai et al., 2020). The TOC/TN ratio in the present study indicated mixed input of marine and terrestrial materials at the northern bank (avg. 15.3) and

dominance of terrestrial derived OM (avg. 28.2) at southern bank stations. This was also supported by the high contribution of long-chain FAs (LCFAs) at the southern bank stations (avg. 13.3%) when compared to the northern bank (avg. 9.6%) stations (Fig. 3B.4). LCFAs are found in the waxy leaf coatings of vascular plants and have been used as indicators of input of terrestrial plants (Meyers, 1997; Carrie et al., 1998; Meziane and Tsuchiya, 2000). Though the northern bank of Zuari estuary is lined with mangroves and vascular plants, it is characterized by flat, sandy and shallow inter-tidal area. Thus, the rate of accumulation of terrestrial materials could be lower. However, southern bank stations are semi-enclosed and relatively steep, enhancing the accumulation of terrestrial derived OM in the sediment with a low rate of dispersal from this site.

The lower mid-estuarine stations are located in the narrow junction, wherein Marmugao Bay, river channel, and Cumbarjua canal merge. The high content of sedimentary OM in this region could be attributed to the high deposition of suspended organic material owing to coagulation and flocculation processes. Previously, Rao et al. (2011) reported that the riverine channel of Zuari estuary was the major depositional center (40-80% of SPM) because particulate matter gets pushed from the bay owing to strong tidal and wind-driven currents through the funnel-shaped mouth of this estuary. Moreover, the lower mid-estuarine stations are lined with mangroves on its banks, supporting the high accumulation of allochthonous derived materials and mangrove leaf litterfall. Among all the sites, the contribution of terrestrial plant-derived OM was high (14 – 29%; avg. 19.8%) at the lower mid-estuarine stations and decreased (3 - 8%; avg. 5.2%) towards the mouth of

the estuary. Earlier studies on stable isotopic composition ($\delta^{13}C$, $\delta^{15}N$) of suspended particulate matter (SPM) and sediment also demonstrated a decrease in the content of terrestrial derived OM from the upstream towards the mouth of the estuary (Kessarkar et al., 2013; Bardhan et al., 2015; Shynu et al., 2015). Recently, Padalkar et al. (2019) reported that OM with high molecular weight predominated in the upstream region, moderate molecular weight OM at midstream region, and of low molecular weight towards the downstream region of this estuary.

Fatty acids derived from autochthonous sources, i.e., phytoplankton, zooplankton, and bacteria, showed spatio-temporal variations in the sediment. Phytoplankton such as diatoms, dinoflagellates, and macroalgae, are the producers of the estuarine environments and are characterized with distinct FAs. Diatoms are rich in FAs such as C14:0, C16:1ω7, C20:4ω6, C20:5ω3, dinoflagellates produce C18:1ω9, C22:6ω3 FAs, and C18:2ω6, C18:3ω3, C18:3ω6 are synthesized by macroalgae (Volkman et al., 1980, Volkman et al., 1989; Carrie et al., 1998; Harji, 2011). The average percentage contribution of primary producers to TFAs was determined for all the seasons and sites. The results indicated that among the primary producers, diatom-specific FAs were the major (4.11%) contributors to TFAs when compared to dinoflagellate (1.32%), and macroalgae (0.63%) specific FAs. Earlier studies have reported diatoms to be the dominant phytoplankton community in the water column of this estuary, followed by dinoflagellates, and blue-green algae (Krishna Kumari et al., 2002). It has been reported that phytoplankton has intra-seasonal and inter-annual variations, and on certain occasions (bloom conditions) dinoflagellates contributed more than diatoms

(Patil and Anil, 2015). High diatom-specific FAs at the lower mid-estuarine and southern bank stations can be attributed to the influence of grain size. It is known that the lower mid-estuarine and southern bank stations are characterized by silty-clayey sediment (Padalkar et al., 2019; Desai et al., 2020). It has also been reported that benthic diatoms flourish well in the fine particle rich sediment owing to the high availability of nutrients (Alfaro et al., 2006). Moreover, the occurrence of diatom blooms has been reported towards the southern bank of this estuary (Patil and Anil, 2008; 2011), supporting the high content of diatom derived FAs. Desai et al. (2020) has reported that the sedimentary chlorophyll *a* was almost similar (avg. 2.37 µg g^{-1}) at the southern (Chicalim) and northern (Siridao) bank (avg. 1.89 µg g^{-1}) stations. But, the high content of OM components at the southern bank than northern suggests the accumulation of detrital and fresh planktonic material from the water column at southern bank owing to semi-enclosed nature (Desai et al., 2020). A recent study on the partitioning of bivalves in the Zuari estuary reported dominance (57-183 no. m^{-2}; avg. 109 no. m^{-2}) of facultative deposit feeders, *Paphia malabarica* at the southern (Chicalim) bank of the estuary and suspension-feeding bivalve, *Meretrix casta* were found (53-104 no. m^{-2}; avg. 73 no. m^{-2}) at the northern bank station (Siridao) (Desai et al., 2020). The high content of diatom-specific FAs and low content of macroalgae-specific FAs at the southern bank indicate that the deposit feeder bivalves preferred macroalgal FAs since macroalgae are rich in polyunsaturated FAs (PUFAs). However, macroalgae and dinoflagellate-derived FAs were high in the surface sediment of northern bank stations with low content of diatom-specific FAs. The northern bank of the Zuari estuary

is shallow tidal sand flat with tightly packed sandy sediment suggesting a low rate of sediment re-suspension. This helps in maintaining the high photic depth of the water column and supports the production of dinoflagellate. Since *Meretrix casta* are suspension feeders, high content of dinoflagellate-specific FAs supports their dominance at the northern bank of the estuary. It has been reported that dinoflagellates are pelagic phytoplankton and are directly available for the suspension feeders (Alfaro et al., 2006). Thus, it seems that other than physical forcing and sediment characteristics, quality and the sources of OM are also important in determining the distribution of benthic communities in this estuarine region.

Zooplankton which are the consumers of primary producers, plays a significant role in the transfer of energy to the fish (Padmavati and Goswami, 1996). The FAs such as C22:1ω9 and C24:1ω9 are commonly found in zooplankton (Wakeham et al., 1997; Carrie et al., 1998; Dalsgaard et al., 2003; Venturini et al., 2012b). Zooplankton undergoes non-predatory mortality in the marine (11.6 to 59.8%) and freshwater (7.4 to 47.6%) environment caused by the senescence, quality and quantity of food, toxicity, winds, turbulence, and change in water temperature, salinity (Tang et al., 2014), which support the presence of zooplankton specific FAs in the estuarine sediment. A positive relationship of zooplankton specific FAs with diatom and dinoflagellate-specific FA markers (Table 3B.3) indicates their coupling.

It was found that diatom-specific FAs were high during post-monsoon months, and decreased from pre-monsoon to monsoon (Fig. 3B.3). The freshwater input declines after the monsoon, which lowers the stratification

and turbidity in the water column, and enhances the transfer of allochthonous-derived new nutrients along with suspended particles towards the bottom. This increases light penetration in the water column supporting high phytoplankton production during the post-monsoon season. Krishna Kumari et al. (2002) reported high primary production and the abundance of phytoplankton in the water column of this estuary during the non-monsoon seasons as compared to the monsoon. Subsequently, it has been reported that phytoplankton abundance shows inter-annual variations with a change in the phytoplankton abundance among the seasons due to phytoplankton blooms (Patil and Anil, 2011; 2015).

Earlier observations from the Zuari estuarine sediment have revealed that different tidal phases play an important role in determining the nature of OM, influencing the distribution of the bacterial populations (Gardade and Khandeparker, 2017). During the sampling period (7:00 am to 11:00 am), low tide was observed during the pre-monsoon months and high (flood) tide during the post-monsoon months (Table 3B.1). An earlier simulation study in the Zuari estuary reported that the increased abundance of barnacle larvae is favored by flood currents and high tides, pushing the larvae into the inshore area and vice-versa during the low tide and ebb currents (Gaonkar et al., 2012). A recent study by David et al. (2018) based on the FA markers in Can Gio mangrove creek (Vietnam) has reported that suspended particulate OM to be a nutritionally high quality and microalgae derived during flood tide and of poor quality of mangrove origin during ebb tide. The increase in the salinity in the present study at lower mid-estuarine stations and banks (especially southern bank) of the estuary during flood tide (Table 3B.1)

suggests intrusion of the Arabian Sea water into the estuarine region. This could be another possible reasons for high content of phytoplankton derived materials during the post-monsoon months on the banks.

It has also been reported that though photosynthesis is light-dependent, light intensity above the saturation level inhibits the photosynthesis (Krishna Kumari et al., 2002), attributed to the low content of phytoplankton FAs during pre-monsoon season. Recently, Patil and Anil (2019) reported that photosynthetic efficiency of phytoplankton was moderate-to-high during the non-monsoon period but reduced on few occasions (presence of photosynthetically inactive cells) owing to the combined effect of photoinhibition and low-nutrient concentration. The response of phytoplankton to the light, temperature, and nutrient concentration varies from species to species and has an impact on the physiological status and the content of biochemical components of phytoplankton (Renaud et al., 2002; Stehfest et al., 2005). Laboratory experiment on the phytoplankton from the tropical environment showed higher content of lipids and FAs over a temperature range of 27-30°C, the content then decreased at high temperatures (33°C and 35°C) (Renaud et al., 2002). A study by Harji (2011) on *Skeletonema* spp. showed high content of phospholipid FAs (PLFAs) at 27°C temperature, which decreased gradually at high temperature (30°C and 38°C), while in *Amphora* spp., it increased from 27°C to 30°C and then decreased at 38°C. However, PLFA content in both these phytoplankton increased with an increase in nutrients (Harji, 2011). In the present study, the bottom water temperature (28.4-32.7°C) was high during the pre-monsoon when compared to the post-monsoon (26.1-28°C) months (Table 3B.1). A

recent study has reported low content of sedimentary chlorophyll-*a* during the pre-monsoon season at southern (2.1 µg g^{-1}) and northern bank (1.7 µg g^{-1}) stations when compared to the post-monsoon (2.4 µg g^{-1}, 2.7 µg g^{-1}, respectively) season (Desai et al., 2020). Moreover, during pre-monsoon months of sampling, the ebb tide was experienced (Table 3B.1), which possibly transport suspended particles towards the sea side. These could be the reasons for the low content of phytoplankton-specific FAs during the pre-monsoon season. The contribution of phytoplankton-specific FAs to the detrital FAs was also low during the pre-monsoon compared to the post-monsoon season at the mouth of the estuary and bank stations (Fig. 3B.5).

The FA composition of the Zuari estuary sediment showed the dominance of short-chain, ubiquitous FAs such as C16:0 and C18:0. These FAs are found in phytoplankton, bacteria, and terrestrial plants (Rajendran et al., 1992; Carrie et al., 1998) and have been used as detrital FAs (David et al., 2019; Zhukova et al., 2019). Overall, a strong correlation of detrital FAs (C16:0+C18:0) with terrestrial plant-specific FAs than diatom-specific FAs indicate their source to be allochthonous. Previous studies from the estuaries of Goa have also reported that mangrove litter (25 g C m^{-2} d^{-1}) was the dominant source of detritus with a minor contribution from the autochthonous (1-2 g C m^{-2} d^{-1}) production (Pant et al., 1980; Qasim and Wafar, 1990). Wafar et al. (1997) found maximum litterfall from the leaves of mangroves during the months of March-May owing to dry, hot conditions and high wind speed prior to monsoon precipitation. The freshwater run-off, which is relatively low (< 10 m^3 s^{-1}) during dry (February to May) period, increases massively (100 to 400 m^3 s^{-1}) during the southwest monsoon (June

to September) season (Shetye and Murty, 1987). Thus, surface run-off through these mangrove litterfall and watershed areas during the monsoon precipitation transport large amounts of soil, litterfall materials, allochthonous bacteria, and anthropogenic wastes into the estuarine region. The relative increase in the contribution of terrestrial plant-derived FAs to detrital FAs at the lower mid-estuarine stations during the monsoon season (Fig. 3B.5), indicates input from the catchment area through the surface run-off.

During the monsoon, high input of terrestrial derived materials, an increase in the bacteria-specific FAs, and a decrease in the TOC/TN ratio suggests colonization of bacteria on the terrestrial derived materials and their role in the utilization of allochthonous inputs. The banks of Zuari and the neighboring Mandovi estuary are fringed with thick mangrove vegetation (1600 hectares), which produce large amounts (43-68%) of leaf litterfall (Wafar et al., 1997). Moreover, the leaves of mangroves contain PUFAs such as C18:2ω6 and C18:3ω3 (Meziane et al., 2007) as well as carbohydrates and proteins (Untawale et al., 1977). A positive correlation of bacteria-specific FAs with terrestrial plant-specific FAs (Table 3B.3) suggests that allochthonous derived materials support bacterial biomass in the sediment. Similar observations were reported by Bourgeois et al. (2011) from the mouth of the Rhone River (France), wherein, benthic bacteria were found to utilize the terrestrial-derived organic materials. Ram et al. (2007) has also reported that Zuari estuary switches to net heterotrophy during the monsoon season. Subsequently, a fine-scale sampling (1-day interval) study from this estuary has pointed out that 25% (30 days) of the total monsoon period (June

to September) is autotrophic owing to the occurrence of phytoplankton blooms (Patil and Anil, 2015). The process called as co-metabolism or priming effect plays a vital role in the degradation of terrestrial-derived carbon pool in the estuarine, coastal, and freshwater ecosystems stimulated in the presence of algal-derived labile OM (Canfield, 1994; Wakeham and Canuel, 2006; Guenet et al., 2010). Thus, it is possible that the phytoplankton rich labile OM could be available for the bacteria and possibly increases the utilization of terrestrial-derived materials. Previous studies on mangrove litter decomposition observed a gradual increase in the percentage of protein and nitrogen content of litter owing to the colonization of microbes on the surface of litter (Untawale et al., 1977; Wafar et al., 1997) which helps in sustaining high bacterial production in the Zuari-Mandovi estuaries (Wafar et al., 1997). Another study reported a high abundance of carbohydrate (starch, cellulose, hemicellulose) degrading bacteria in the water and sediment of the Zuari (77%) compared to neighbouring Mandovi (60%) estuary (Khandeparker et al., 2011). These studies support the active role of bacteria in the breakdown of allochthonous derived mangrove and terrestrial materials.

3B.5 Conclusions

The distribution and composition of sedimentary OM in the Zuari estuary was significantly regulated by the in-situ production, allochthonous input, and bacterial response. Low amount of OM at the mouth of the estuary can be attributed to the high bacterial contribution, which seems to be influenced by repetitive cycles of resuspension-deposition of sediment. The

estuarine bank stations were characterized with a high content of phytoplankton-derived OM, suggesting its role in the sustenance of the benthic community and economically important shellfish populations in this region. High deposition of terrestrial-plant derived materials towards the lower mid-estuarine stations located in the mixing zone can be attributed to the influence of flocculation and funnel-shaped mouth of the estuary. This study indicated the presence of relatively fresh plankton-derived OM during non-monsoon seasons. However, the input of terrestrial-plant and detrital rich OM at lower mid-estuarine stations and bacteria-derived OM on the banks increased during the monsoon season. The high content of terrestrial derived material during the monsoon and an increase in the bacteria-specific FAs suggest the assimilation of terrestrial material by the bacteria. This study also pointed out that estuarine morphology influences the deposition of the OM and plays an important role in the functioning of the ecosystem.

Chapter 4

Evaluation of fate of sedimentary OM in the Zuari estuary through laboratory microcosm experiments

4. Evaluation of fate of sedimentary OM in the Zuari estuary through laboratory microcosm experiments

4.1 Introduction

The organic material received from diverse sources undergoes various physicochemical and biological transformations during sinking through the water column (Meyers, 1994) as well as at the sediment-water interface (Canuel and Martens, 1993). Biological processes are important in the diagenesis of OM in the sediment and release of nutrients (Sun et al., 2000), which could be available for the pelagic compartment (Hammond et al., 1984; Snelgrove, 1998). The input of OM from various sources to the bottom sediment and its decomposition is a continuous process and changes with the nature of OM, microbial activity, rate of deposition of materials, and redox conditions (Canuel and Martens, 1993). The fate of OM in the sediment is also influenced by the sediment grain size (Rasheed et al., 2003). Sediment-water interface is a dynamic zone with a high rate of microbial activities (Canuel and Martens, 1993). Bacteria are dominant in the sediment, which plays an important role in the processing of sedimentary OM through the production of ectoenzymes (Chrost, 1990; Nealson, 1997).

The relationship between physical, biological processes, and composition of OM in the estuarine system is complex and varies within a short period as well as on spatial scales (Zimmerman and Canuel, 2001; Palomo and Canuel, 2010). Thus, understanding the relationship between bacterial response and fate of different sources derived OM in a closed system is helpful for elucidating the degradation pattern of OM in the estuarine environment. It is unclear that the reactivity and degradation of

plankton and terrestrial plant-derived OM in the sediment is dependent upon the molecular structure of the compounds or the nature of source material (Wakeham and Canuel, 2006). The process called co-metabolism wherein, the availability of labile OM is thought to enhance the degradation of refractory materials, i.e., terrestrial plant-derived OM (Wakeham and Canuel, 2006), plays an important role in the OM decomposition. Guenet et al. (2010) demonstrated that the co-metabolism process occurs across terrestrial, freshwater, and marine ecosystems and it can vary under different biogeochemical regimes (Canfield, 1994). This process could be positive and enhance the degradation of refractory OM or negative and retard the degradation process (Kuzyakov et al., 2000). Thus, it is important to understand the mechanisms underlying the turnover of OM of multiple sources within the surface sediment of the ecosystem (Ma et al., 2020) that can improve our understanding of carbon cycling (Zimmerman and Canuel, 2001).

Zuari is a tropical monsoon-influenced estuary located along the central west coast of India. The average primary (phytoplankton) and secondary (zooplankton) production in the Mandovi-Zuari estuaries is 186 g C m^{-2} yr^{-1} and 8.03 g C m^{-2} yr^{-1}, respectively (Qasim and Wafar, 1990). The occurrence of phytoplankton blooms has also been reported from the waters of Zuari estuary during the monsoon season (Patil and Anil, 2008, 2011, 2015). The mangrove litter yield, which is rich in leaves (43-68%) range from 10.2 – 17 tons ha^{-1} yr^{-1} (Wafar et al., 1997). The south-west monsoon rainfall during June to September period brings a large amount of terrestrial-derived OM in the estuarine region. It has been reported that bacterial carbon demand

is met by the allochthonous-derived OM during the monsoon period (De Souza et al., 2003). Eswaran and Khandeparker (2017) studied the bacterial community in the water column of Zuari estuary along the salinity gradient during different seasons. The results indicated a distinct shift in the bacterial community with time and space attributed to physicochemical parameters and organic pool. They reported an increase in bacterial production during the monsoon season, influenced by the riverine OM. Whereas, the emergence of distinct bacterial community during the non-monsoon seasons resulted in the utilization of the in-situ derived organic material. The OM in the sediment of the Zuari estuary is a mixture of autochthonous and allochthonous derived materials (Gardade and Khandeparker, 2017). Moreover, Zuari estuary is characterized by high heterotrophic activity in the water column as well as in the sediment. But, the fate of degradation of autochthonous and allochthonous derived materials in the sediment is unclear.

Fatty acids (FAs) have been reported as suitable tracers to evaluate the decomposition dynamics of single or diverse OM source materials in the sediment through microcosm experiments (Ding and Sun, 2005; Dai et al., 2009; Ma et al., 2020). Taking the above into consideration, in the present study, FA biomarkers were used to evaluate the degradation pattern of different organic material sources, i.e., diatoms (primary producers), zooplankton (consumers), and mangrove leaves (source of allochthonous OM) in the laboratory incubation microcosm experiments in the sediment, characterized with different grain size composition. The culturable bacterial abundance with an emphasis on *Vibrio* spp. (autochthonous bacteria), coliforms (allochthonous bacteria) and bacteria-specific FAs were used to

understand their role in the OM degradation. It was hypothesized that the degradation rate of different source materials in the estuarine sediment through heterotrophic activity is related to the structural composition of the materials, preferring the labile and simpler ones over refractory and complex molecules.

4.2 Materials and methods

4.2.1 Study area and sample collection

The surficial sediment (~ 2-3 cm) and seawater (40 L) samples were collected on 9th September 2018 during low tide from three sites, i.e., Chicalim, Cortalim, and Siridao located in the Zuari estuary (Fig. 4.1). Our previous study using FA biomarkers has indicated that Chicalim and Siridao are rich in phytoplankton and bacteria-derived OM whereas, Cortalim is dominated by terrestrial plant-derived OM. Moreover, these three sites are characterized by distinct sediment grain-size composition and are also influenced by different anthropogenic activities. The Chicalim and Siridao act as habitat for economically important organisms such as bivalves, oysters, and crabs. The harvesting of bivalves from the sediment is common practice at Chicalim and Siridao (Desai et al., 2020).

Chicalim is located on the southern bank of the Zuari estuary (Fig. 4.1). It is a semi-enclosed area and influenced by different anthropogenic activities such as shipbuilding workshops, barge cleaning, and transportation of ore materials. Sediment from this site is poorly sorted and characterized by silt and sand particles (Desai et al., 2020).

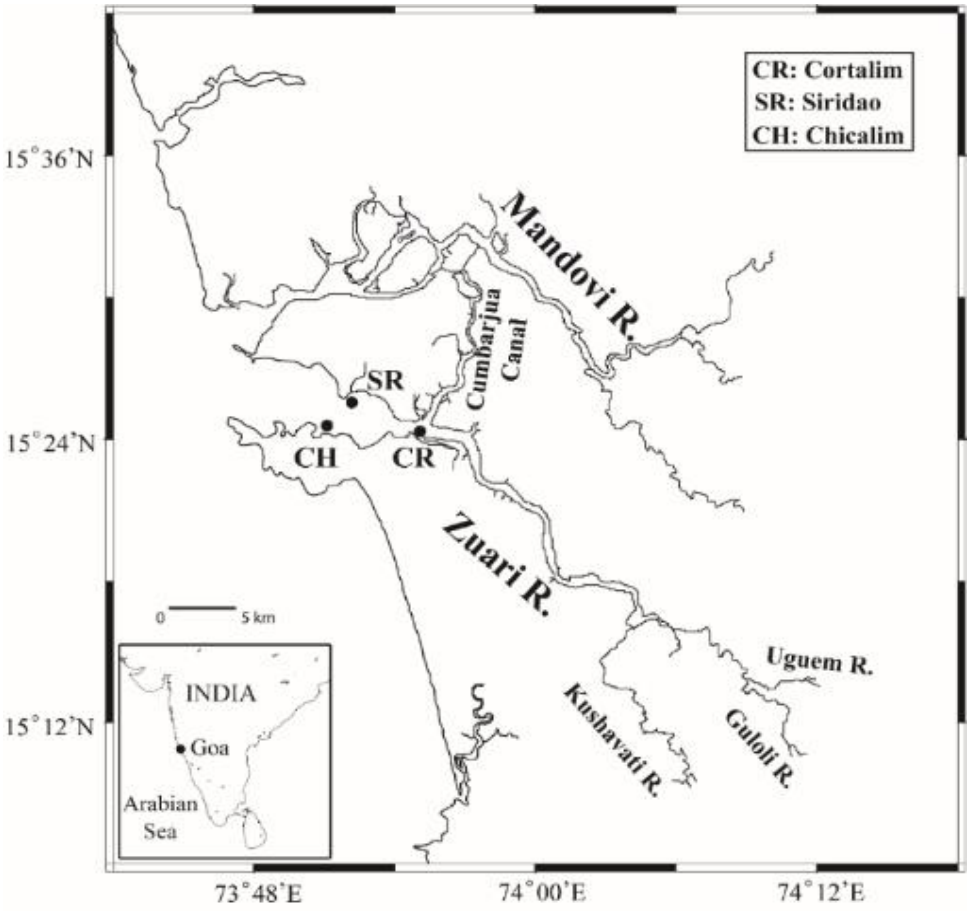


Fig. 4.1 Map showing sampling sites in the Zuari estuary.

Cortalim is a lower mid-estuarine station, situated in the estuarine mixing zone, where the Cumbharjua canal and river channel merges into the Marmugao Bay. The fishing trawler and ferry-boat activities are common in this region. Fine-grained (silty-clayey) sediment is dominant at this site (Padalkar et al., 2019).

Siridao is located on the northern bank of the estuary (Fig. 4.1) and relatively pristine when compared to the other two stations (Khandeparker et al., 2017b). Sediment from Siridao is dominated by tightly packed fine to medium sand particles (Desai et al., 2020).

4.2.2 Culturing of diatoms for the microcosms

The laboratory maintained cultures of diatoms (*Skeletonema costatum*, *Chaetoceros calcitrans*) were subcultured in 100 ml (n = 2; 2 flasks per culture) of f/2 medium (Guillard, and Ryther, 1962) and incubated for 10 days under light: dark (12:12) cycle. These 100 ml cultures were then transferred in two flasks (1.5 L each) containing f/2 medium and incubated for another 10 days for biomass production. The diatom cells were then harvested by centrifugation and used for the microcosm incubation experiments.

4.2.3 Collection of zooplankton

Zooplankton were collected from the mouth (Dona Paula Bay) of the Zuari estuary by horizontal hauls using Heron-Tranter zooplankton net with a mesh of 100 µm size. The suspended materials other than zooplankton (i.e., vascular plant and macroalgae fragments) were removed carefully. Subsequently, zooplankton were concentrated on 50 µm mesh, and wet biomass was used for the microcosm experiments.

4.2.4 Microcosm set up

The schematic representation of the microcosm set up is shown in Fig. 4.2. Sediment from each site was weighed (300 g) and then spiked (~1.5 g each) with different organic materials such as diatoms (wet biomass), zooplankton (wet biomass), and mangrove leaves (cut into pieces as 3×15 mm, W×L) separately, and mixed properly. Sediment was then placed as thin layers (~2.5-3 mm) in 24 containers (n = 24; 4 organic materials × 3 sites ×

2 replicates) of size 29×22×14 cm (L×B×H) and overlaid with seawater (3 L) collected from the same site. These containers were incubated under the dark condition and at 28 ± 2°C temperature for 65 days. In addition to this, six containers (n = 6; 3 sites × 2 replicates) with sediment were incubated with a mixture of diatoms, zooplankton, and mangrove leaves (0.7 - 0.8 g each), and 6 were control containers (n = 6; 3 sites × 2 replicates), without the addition of these organic materials.

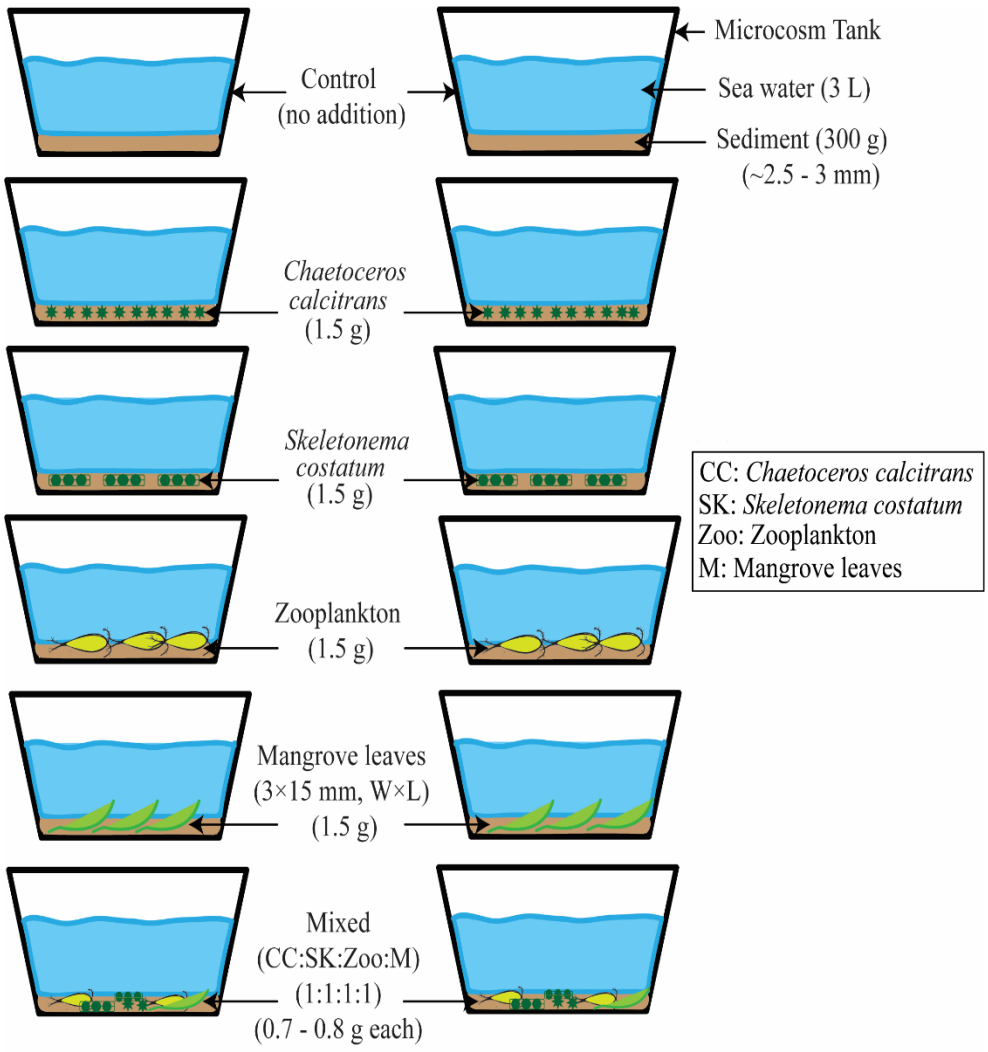


Fig. 4.2 Schematic representation of the microcosm setup.

Sediment samples (10 g) were collected on day 1, 3, 10, 17, 27, 37, 47, and 65 from each container and analysed immediately for total viable counts (TVC), *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria). The aliquots of sediment samples were stored at - 20°C for later analysis of the fatty acids.

4.2.5 Enumeration of total viable bacterial counts (TVC), Vibrio spp. *(autochthonous bacteria), coliforms (allochthonous bacteria)*

Please refer chapter 3A, section 3A.2.4.

4.2.6 Analyses of fatty acids from the bulk and spiked sediment

Please refer chapter 2A, section 2A.2.5.

4.3 Results

4.3.1 Variations in total viable bacteria, Vibrio spp. *(autochthonous bacteria), coliforms (allochthonous bacteria), and bacteria-specific FAs from the sampling sites*

The total viable bacteria count (TVC) was high in the Chicalim (southern bank station) sediment (from the field) ($2.61 \pm 0.21 \times 10^5$ CFU g^{-1}) followed by Cortalim (lower mid-estuarine station) ($6.93 \pm 1.24 \times 10^4$ CFU g^{-1}), and Siridao (northern bank station) ($2.0 \pm 0.42 \times 10^4$ CFU g^{-1}). The trend in the abundance of *Vibrio parahaemolyticus*, *V. alginolyticus*, and coliforms was also similar to TVC. Sediment samples were also analyzed for *Vibrio cholerae*, but they were not detected during the study period.

The FAs specific to Gram-positive, anaerobic bacteria were high in Cortalim (12.4 µg g^{-1}) sediment than Chicalim (8.74 µg g^{-1}), and Siridao (6.2

µg g^{-1}). Gram-negative, aerobic bacteria, and sulfate-reducing bacteria-specific FAs were high at Chicalim followed by Cortalim and Siridao (Fig. 4.3a).

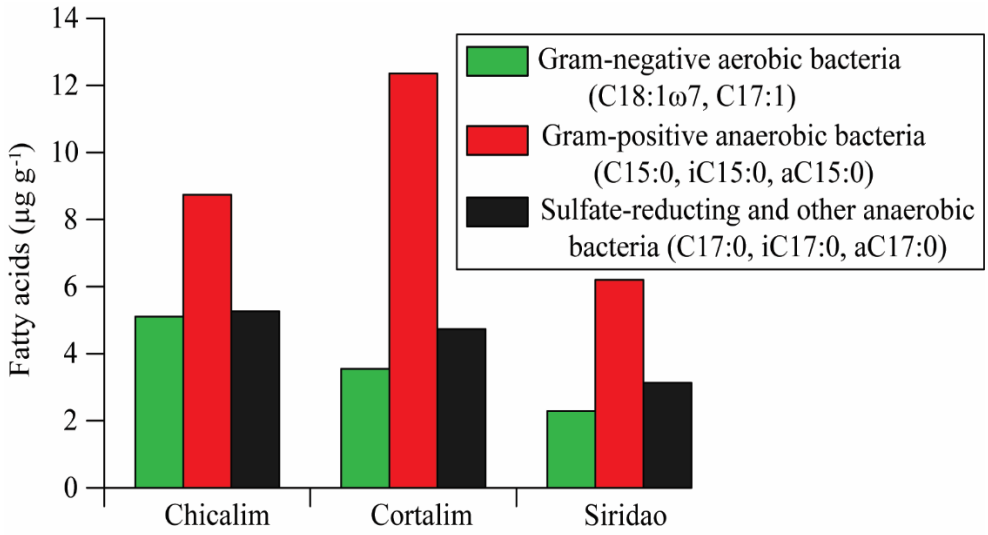


Fig. 4.3a The content of FAs specific to different bacterial groups in the sediment of studied sites.

4.3.2 Variations in the abundance of total viable bacteria, Vibrio spp.*, and coliforms in the microcosm experiments*

The total viable bacteria count (TVC) in the microcosm sediment was low (~10^4-10^5 CFU g^{-1}) on day 1, but subsequently increased significantly by two-three orders of magnitude (up to ~10^7 CFU g^{-1}) on day 3 irrespective of the sediment treatment (Fig. 4.3b), and this was predominant in the microcosms spiked with diatom cultures and mangroves. The second peak in the TVC was observed on day 10 in the mixed organic materials spiked sediment. In the later phase of incubation (day 27 – day 65), TVC decreased by 1-2 folds in all the microcosms.

The abundance of *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and coliforms increased during the early phase of incubation from day 3 - day 10 in the sediment spiked with different organic materials (Fig. 4.3b) irrespective of sites. A decline in these bacteria abundance was observed after day 10.

In the case of Chicalim microcosm (southern bank sediment), the abundance of *V. parahaemolyticus* was high in the sediment mixed with mangrove leaves between days 27 to day 65. In Cortalim microcosm (lower mid-estuarine sediment), the abundance was high in the case of mangrove leaves, and zooplankton spiked sediment. However, in Siridao microcosm (northern bank sediment), it was high in the sediment spiked with a mixture of organic materials and diatom cultures. *V. alginolyticus* was high in the sediment spiked with a mixture of organic materials and their abundance reached to not detectable level in control microcosms of Cortalim and Chicalim after 47 days of incubation.

Coliforms were high in the sediment spiked with *Chaetoceros*, mangrove leaves, and a mixture of organic materials from all three sites. But, they were not detected towards the end of the incubation period in all microcosm experiments of Chicalim and in control microcosms of Cortalim and Siridao.

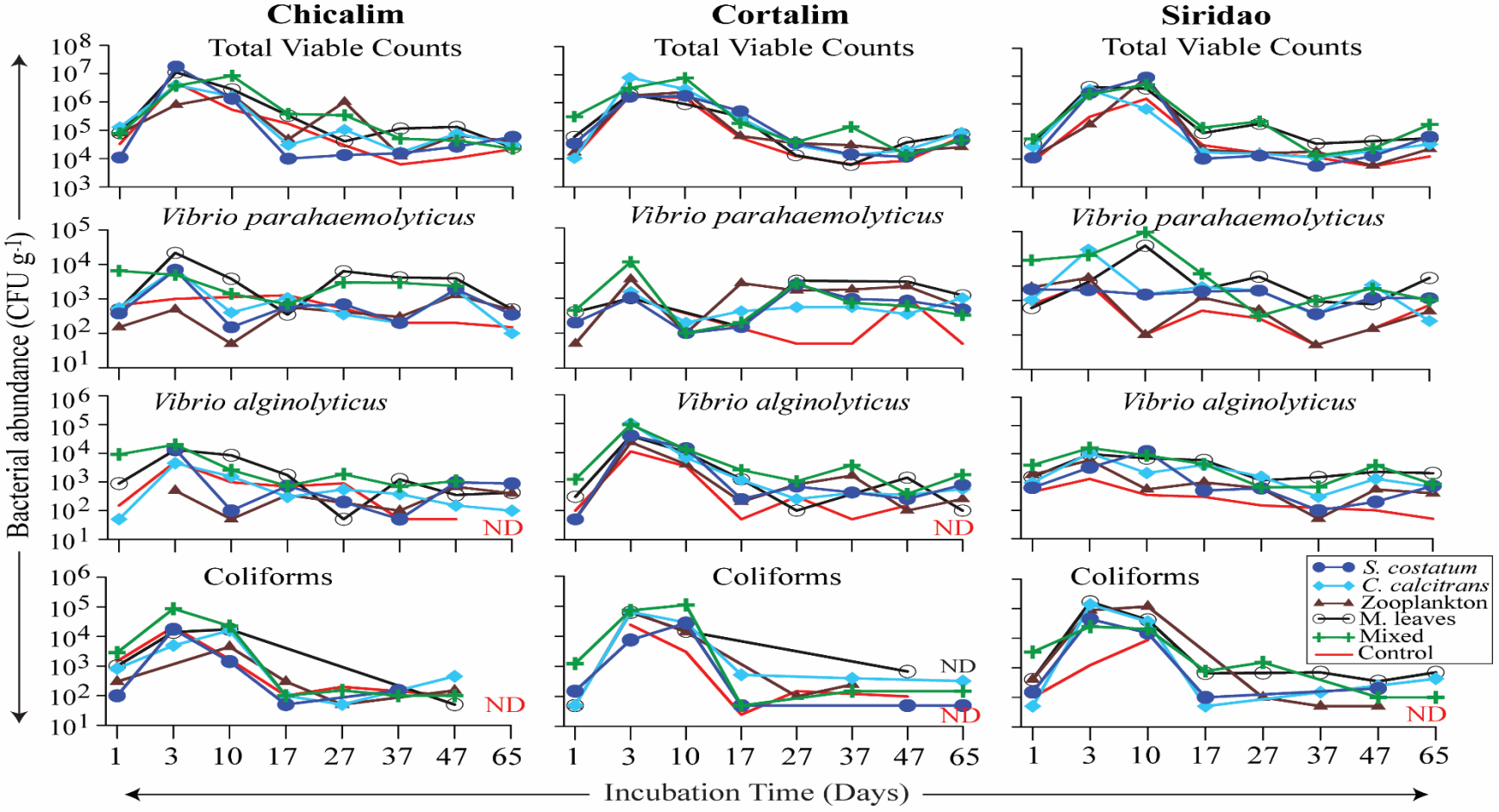


Fig. 4.3b Variations in the abundance of total viable bacteria, *Vibrio* spp. (autochthonous bacteria), and coliforms (allochthonous bacteria) in the sediment spiked with different organic materials (ND: Not Detected; M.: Mangrove).

4.3.3 Variations in the bacteria-specific fatty acids in the microcosm experiments

The bacteria-specific FAs increased substantially during day 3 - day 10 in all the treatments irrespective of the stations, then decreased slowly and reached to initial content (Fig. 4.3c).

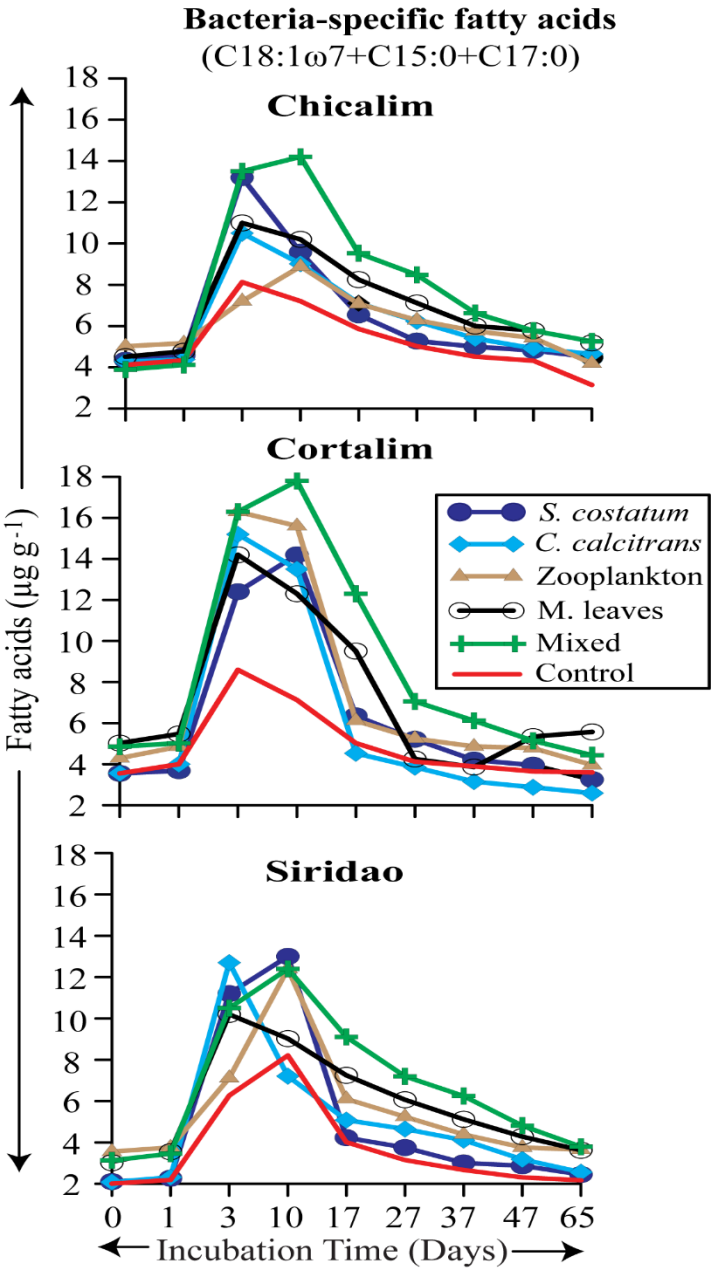


Fig. 4.3c Variations in the bacteria-specific FAs during the incubation in the spiked and control sediment (M.: Mangrove).

The diatoms and zooplankton spiked sediment showed higher content of bacteria-specific FAs during the initial incubation period (day 3 - day 10). During the later phase of incubation (after 10 days), though bacteria-specific FAs decreased in the sediment of all the microcosms, their content was high in the mangrove and mixed organic material spiked sediment than the other sediment. Among the stations, Cortalim sediment showed high content of bacterial FAs than Chicalim and Siridao (Fig. 4.3c).

4.3.4 Variations in the diatoms-specific fatty acids during the incubation

The percent utilization of fatty acids such as C14:0, C16:1ω7, and C20:5ω3, which are major components of diatoms, is indicated in Table 4.1. The fatty acids were utilized substantially during the early phase of incubation, i.e., during day 1 - day 10 (Fig. 4.4). The FA C14:0 was utilized up to 62 – 86% during this period (day 1 - day 10), C16:1ω7 was utilized up to 79 – 90% whereas, C20:5ω3 was utilized up to 86 – 97% irrespective of the stations (Table 4.1). The content of all these FAs decreased to zero after 65 days of incubation. In general, the utilization of FAs from *S. costatum* was marginally high when compared to that from *C. calcitrans*.

Siridao microcosm showed a marginally high rate of utilization for C20:5ω3 FA when compared to Chicalim and Cortalim. Overall, the rate of utilization was high for C20:5ω3 and low for C14:0 (C20:5ω3 > C16:1ω7 > C14:0) in both the diatom spiked sediment.

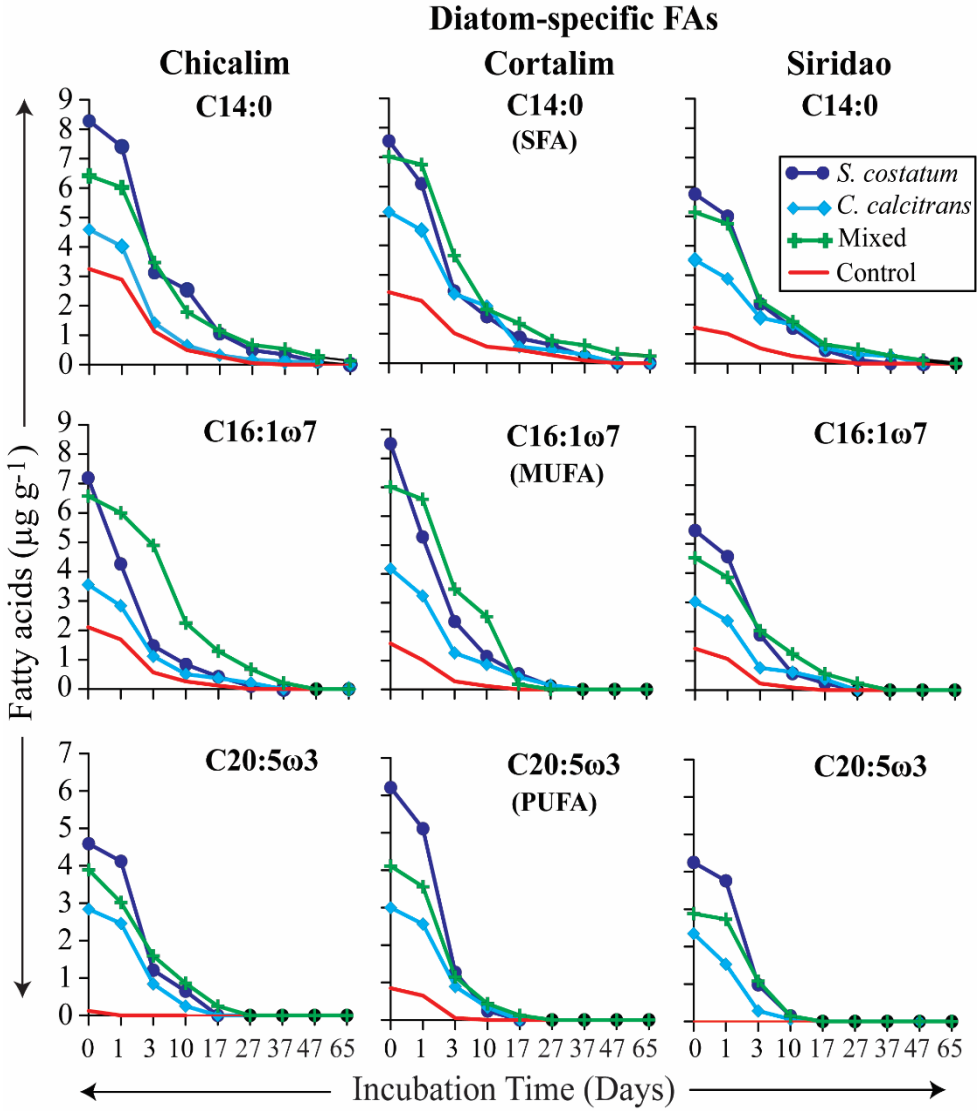


Fig. 4.4 Variations in the diatom-specific FAs during the incubation in the spiked and control sediment (SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid).

Table 4.1 The percentage (%) of diatoms-specific FAs utilized after 10 days of incubation in the sediment.

FAs	C14:0						C16:1ω7					
Diatom	*S. costatum*			*C. calcitrans*			*S. costatum*			*C. calcitrans*		
Sites	CH	CR	SR	CH	CR	SR	CH	CR	SR	CH	CR	SR
% Utilized after 10 days	69	**78**	**79**	**86**	62	63	88	87	**90**	**86**	85	79

FAs	C20:5ω3					
Diatom	*S. costatum*			*C. calcitrans*		
Sites	CH	CR	SR	CH	CR	SR
% Utilized after 10 days	86	96	**97**	88	89	**97**

(CH: Chicalim; CR: Cortalim; SR: Siridao).

4.3.5 Variations in the zooplankton-specific fatty acids during the incubation

The percent utilization of zooplankton-specific FAs such as C22:1ω9 and C24:1ω9 is indicated in Table 4.2. The FAs C22:1ω9 and C24:1ω9 were utilized up to 63 - 78% (Table 4.2) during the early phase of incubation, i.e., during day 1 - day 10 (Fig. 4.5).

The utilization of C22:1ω9 was marginally high in Siridao microcosm, and in the case of C24:1ω9, it was marginally high in Cortalim microcosm (Table 4.2).

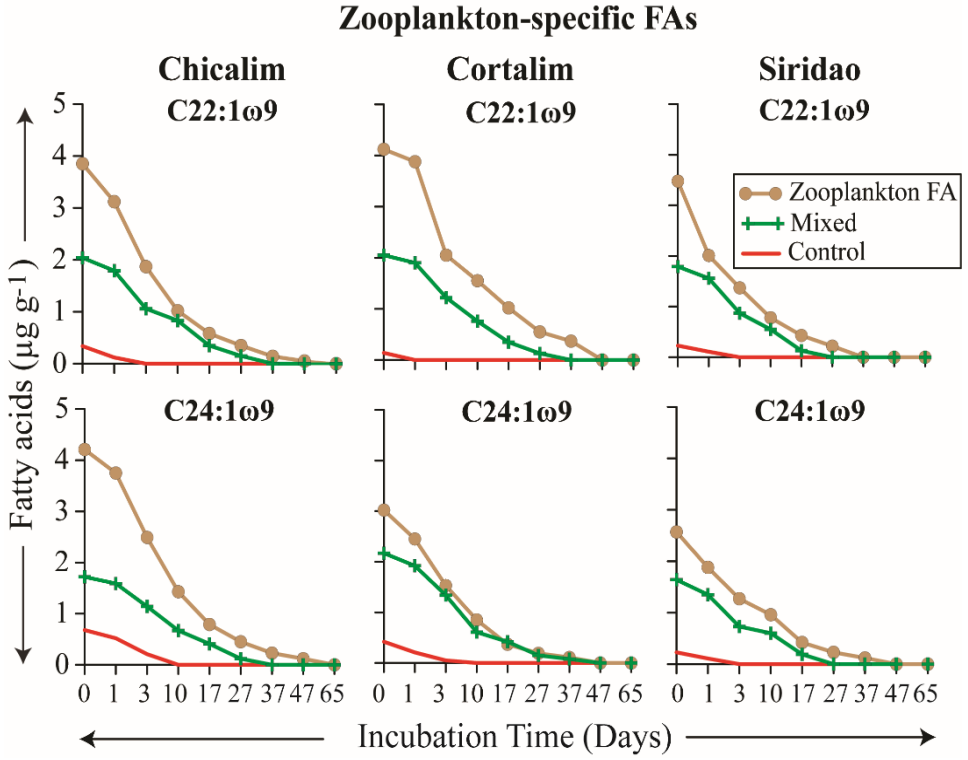


Fig. 4.5 Variations in the zooplankton-specific FAs during the incubation in the spiked and control sediment.

Table 4.2 The percentage (%) of zooplankton-specific FAs utilized after 10 days of incubation in the sediment.

FAs	C22:1ω9			C24:1ω9		
Sites	CH	CR	SR	CH	CR	SR
% Utilized after 10 days	73	74	**78**	66	**72**	63

(CH: Chicalim; CR: Cortalim; SR: Siridao).

4.3.6 Variations in the mangrove-specific fatty acids during the incubation

The percent utilization of mangrove-specific FAs, i.e., C18:2ω6, C18:3ω3, C22:0, and C24:0, is indicated in Table 4.3. The utilization of FAs C18:2ω6 and C18:3ω3, which are polyunsaturated FAs (PUFAs) was high in Cortalim (69% and 82%, respectively) microcosm when compared to Siridao (63%, 60%) and Chicalim (54%, 54%) during day 1 - day 10 (Table 4.3). However, utilization of long-chain FAs, i.e., C22:0 and C24:0 was slower when compared to PUFAs, i.e., C18:2ω6 and C18:3ω3 (Table 4.3). This trend was similar in the case of all the three sites (Fig. 4.6).

The utilization of long-chain FAs C22:0 (19 - 24%) and C24:0 (15 - 19%) was higher (~ 1.11 – 2.5 times) in the microcosms with mixed organic materials which included diatoms, zooplankton, and mangrove leaves when compared to the microcosms spiked only with mangrove leaves (8 - 17% for C22:0 and 8 - 10% for C24:0) (Table 4.4).

Among the sites, the rate of utilization of LCFAs in Cortalim sediment was marginally high than Chicalim and Siridao sediment (Table 4.4). A substantial fraction of LCFAs remained after an incubation period of 65 days.

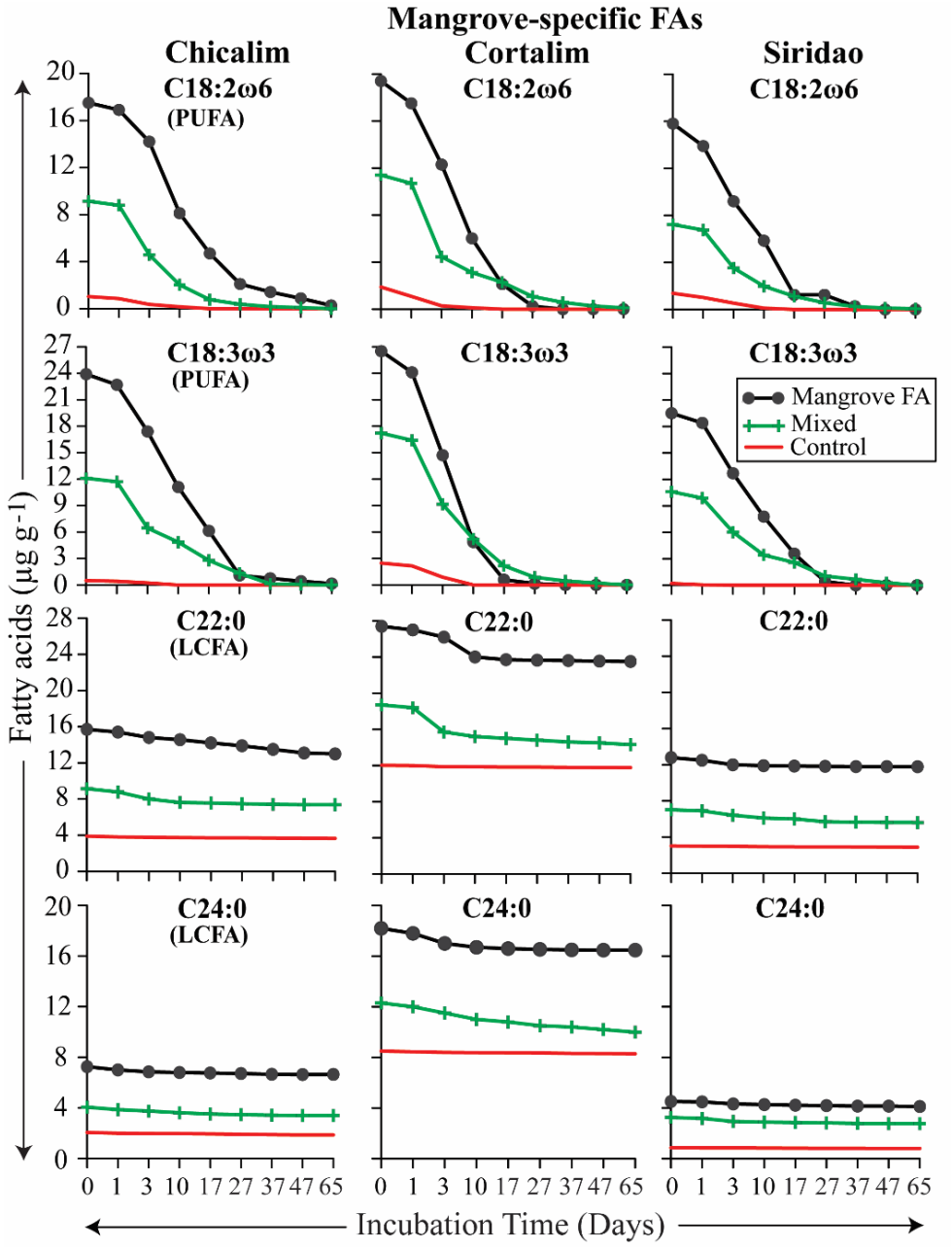


Fig. 4.6 Variations in the mangrove-specific FAs during the incubation in the spiked and control sediment (PUFA: Polyunsaturated fatty acid; LCFA: Long-chain fatty acid).

Table 4.3 The percentage (%) of mangrove-specific PUFAs and LCFAs utilized after 10 days of incubation in the sediment.

FAs	C18:2ω6 (PUFA)			C18:3ω3 (PUFA)		
Sites	CH	CR	SR	CH	CR	SR
% utilized after 10 days	54	**69**	63	54	**82**	60

FAs	C22:0 (LCFA)			C24:0 (LCFA)		
Sites	CH	CR	SR	CH	CR	SR
% utilized after 10 days	7	**12**	7	6	**8**	5

(CH: Chicalim; CR: Cortalim; SR: Siridao).

Table 4.4 The percentage (%) of mangrove-specific long-chain FAs utilized after 65 days of incubation in the sediment spiked with mangrove leaves and a mixture of diatoms, zooplankton, and mangrove leaves.

	FAs	C22:0			C24:0		
	Sites	CH	CR	SR	CH	CR	SR
% utilized after 65 days	Microcosms with mangrove leaves	17	14	8	8	10	9
	Microcosms with mixed sources	19	24	20	16	19	15

4.4 Discussion

Organic matter as the basic source of energy and nutrients for the organisms helps in sustaining diverse food webs (Moore et al., 2004). The present study showed that OM degradation was influenced by the source materials, structural features of the FAs, bacterial response, and the microbial community composition of the site.

The present incubation experiment involved three different abundant organic material sources in the study area, i.e., diatoms, zooplankton, and mangrove leaves. The viable bacterial counts and bacteria-specific FAs

increased substantially during the initial phase of incubation (day 3 - day 10) with a decrease in the content of source materials (diatoms, zooplankton, and mangrove leaves), irrespective of the stations indicating bacterial utilization of available source materials. The rate of decrease was high for diatom-specific FAs, medium for zooplankton-specific FAs, and low for mangrove-specific FAs (diatom > zooplankton > mangrove leaves). The variations in the degradation pattern could be attributed to the nature of source materials and their association with the material matrixes (Harvey and Macko, 1997; Dai et al., 2009). Earlier studies have revealed that algal-specific FAs have different rates of degradation, attributed to the structural composition, nature of compounds, their reactivity with enzymes and this can vary from species to species (Harvey and Macko, 1997; Sun et al., 2000; Ding and Sun, 2005). These could be the reasons for the variations in the degradation pattern of *Skeletonema costatum* and *Chaetoceros calcitrans* specific FAs. Moreover, the triacylglycerols are dominant in *C. calcitrans* (8.4%) than *S. costatum* (1.7%) (Volkman et al., 1989). Previously, it has been reported that triacylglycerols are hydrophobic in nature and are less reactive than amphipathic phospholipids (Ding and Sun, 2005), which could be another reason for variations in the degradation pattern of FAs from these two diatoms.

Zooplankton have chitinous exoskeleton which requires specific enzymes such as chitinases to degrade it after which the utilization of other materials from the zooplankton is possible. It has been reported that diatoms are rich in glycolipids and phospholipids (Volkman et al., 1989), which are amphipathic in nature whereas, vascular plant-specific FAs are partially

bound in the leaf waxes, which are hydrophobic in nature. The enzymes involved in the degradation of lipids such as lipases react more readily with amphipathic molecules than hydrophobic (Ding and Sun, 2005), increasing faster rate of decrease of diatom-specific FAs.

Overall, it was observed that the rate of utilization was influenced by the structure of FAs (polyunsaturated FAs > monounsaturated FAs > saturated FAs) irrespective of the source material. Such variations in the relative reactivity of FAs have also been reported from the earlier studies (Sun et al., 1997; Harvey and Macko, 1997; Dai et al., 2009). Monounsaturated FAs are characterized by the presence of one double bond, and polyunsaturated FAs have two or more double bonds in the carbon chain. Out of these two double bonds, one is sigma, and another is pi-bond, which is usually weaker and of lower energy than a sigma bond (Frankel, 2014). This resulted in a faster utilization of polyunsaturated FAs, followed by monounsaturated and saturated FAs.

The results indicated that the degradation of diatom and zooplankton-specific FAs was marginally high in the Siridao sediment when compared to Chicalim and Cortalim. However, mangrove-specific FAs showed a high rate of decrease in the Cortalim sediment than Chicalim and Siridao. This could be attributed to the microbial community structure and sediment grain size composition of that site. A study by Khandeparker et al. (2017b) on the microbial community from the surface sediment revealed the dominance of *Gammaproteobacteria* (91%) at Chicalim, (contaminated site) however, Siridao (more pristine) sediment were characterized with a diverse microbial community which includes *Gammaproteobacteria* (55.9%), *Actinobacteria*

(26.4%), *Acidobacteria* (7.76%), *Cyanobacteria* (5.72%) and *Bacteriodetes* (2.87%). *Gammaproteobacteria* and *Bacteriodetes* are involved in the degradation of phytoplankton, and algal derived particulate and dissolved matter in the seawater and sediment (Kirchman, 2002; Gihring et al., 2009; de Moraes et al., 2014; Fernandes et al., 2014) as well as in the degradation of cellulose (Edwards et al., 2010). *Actinobacteria* plays a significant role in the degradation of diverse macromolecules such as chitin, cellulose, hemicellulose, pectin in the soil and presence of these bacteria in the estuarine and marine environments (Mincer et al., 2002; Gihring et al., 2009; Fernandes et al., 2014; Eswaran and Khandeparker, 2017) suggest their role in the degradation of these complex polymers. The previous study on carbohydrate degrading culturable bacteria from the Zuari estuary have reported that Chicalim to be rich in both cellulose and hemicellulose degrading bacteria whereas, Cortalim was dominant only in hemicellulose degrading bacteria and majority of them were *Bacillus* and *Vibrio* spp. (Khandeparker et al., 2011). The present study showed high content of *Vibrio parahaemolyticus* and *V. alginolyticus* in the mangrove and zooplankton spiked microcosms of Chicalim and Cortalim and in diatoms and mixed materials spiked Siridao microcosms. This suggests their role in the degradation of these source materials. The presence of *Deltaproteobacteria* has been reported in the sediment of the inner estuarine region (near Cortalim) of Zuari estuary, which plays a role in the sulfur and organic carbon cycling through anaerobic processes (Loka bharathi et al., 1991). The present study showed high content of FAs specific to anaerobic, Gram-positive bacteria in Cortalim sediment than Chicalim and Siridao. FAs specific to

sulfate-reducing bacteria were high at Chicalim followed by Cortalim and Siridao. Thus, spatial variations in bacterial community structure, tightly coupled with the habitat characteristics mainly the composition of OM (Khandeparker et al., 2011), probably influenced the different rates of degradation of FAs of same source-material at three different sites. Moreover, sediment grain size composition is also an important factor affecting the rate of degradation of OM, sandy sediment being more permeable has high rates (~1.5 - 2 folds) of degradation of OM than less permeable silty-clayey sediment (Rasheed et al., 2003). Siridao sediment rich in the sand showed marginally high degradation of OM than Chicalim and Cortalim, which are characterized by silty-clayey sediment.

In the present study, it was observed that the rate of decrease of mangrove-specific long-chain FAs, i.e., C22:0 and C24:0 was ~ 1.11 – 2.5 times high in the microcosms sediment incubated with a mixture of organic materials (diatoms, zooplankton, and mangrove leaves) than the microcosms with only mangrove leaves. This indicates that positive co-metabolism possibly supported the utilization of allochthonous detrital rich OM by the bacteria in the presence of diatom-derived labile OM.

4.5 Conclusions

The laboratory microcosm experiments revealed that the degradation of OM in the sediment is influenced by the source material, structural features of FAs, and bacterial response. The substantial increase in the bacterial population during the initial phase of incubation period (10 days) indicates the utilization of source materials. The difference in the structural

composition of the source material was found to influence the degradation of FAs. The FAs specific to diatoms showed faster rate of utilization than zooplankton and mangrove leaves. The structural unsaturation of FAs increased their rate of utilization (polyunsaturated FAs > monounsaturated FAs > saturated FAs) irrespective of the sources. A substantial fraction of the saturated FAs persisted till the end of the incubation period (65 days). The site-specific microbial community and sediment grain size composition influenced the different rates of degradation of FAs of the same source-material in the sediment. The positive co-metabolism supported the utilization of mangrove-derived OM in the presence of labile OM. Understanding the fate of different sources of organic matter under different environmental conditions is useful in the benthic food web dynamics and elucidating different pathways by which the carbon is transferred to higher trophic levels in benthic-pelagic coupling is a step ahead.

Chapter 5

Summary

5. Summary

Sedimentary organic matter (OM) provides nutrients for the microbial metabolism, enhances bacterial biomass, and transfer energy to higher trophic levels through meio and macrofauna (Warwick et al., 1979; Carpenter et al., 2005). The organic fraction of sediment with a heterogeneous mixture of particles of living and non-living nature, i.e., bacteria, microalgae, fungi, dead materials of plants and animals fulfill the food requirement of wide variety of organisms and play a significant role in the ecological functioning of the food webs (Lopez and Levinton, 1987). Muller-Navarra (2008) has recognized that in order to elucidate the nature of accessible food available for benthic consumers, it is important to determine the quality of OM. In this context, the present study was carried out to evaluate the sedimentary OM composition from the ports and estuary using FA biomarkers, elemental, and biochemical components. Ports are the areas in the coastal ecosystems with intense shipping and industrial activities and are used as active sites for ballast water exchange. Thus, the characterization of OM from the sediment of ports is useful to understand the health of such systems and the nutritional quality of OM. Such studies from the estuarine sediment are helpful to determine the nature of food available for the economically important organisms such as bivalves, oysters, crabs, demersal fish, etc. and to evaluate the influence of seasons, tide, and biological processes (population dynamics of bacteria, primary production, and allochthonous input) on the OM composition.

The present study observed that the accumulation of diverse source derived biochemical compounds in the sediment was significantly influenced

by the primary productivity, bacterial response, morphological structure of the system, hydrodynamic conditions, seasonal fluctuations, and anthropogenic inputs. In the case of the enclosed freshwater Kolkata port, fatty acid biomarkers indicated that OM was derived from bacteria, diatoms, macroalgae, dinoflagellates, zooplankton, and terrestrial plant, with the dominance of autochthonous sources derived OM. The inner sites of docks with relatively poor flushing were characterized with high organic load rich in elemental and biochemical compounds leading to eutrophic conditions, imparting stress on the bacterial community with a lower bacterial contribution. In contrast, outside riverine station showed high bacterial contribution with lower content of OM and oligotrophic condition. The freshwater system with the higher content of labile and energy-rich OM in the sediment can facilitate the growth and sustenance of benthic organisms. However, the macrotidal, seawater Kandla port was characterized with bacteria and terrestrial plant-derived detrital-refractory OM of low quality, with a low contribution of diatoms, macroalgae, dinoflagellates, and zooplankton derived OM. The strong tidal currents in Kandla port resulted in a low accumulation of OM in the sediment with the oligotrophic condition. Thus, assessment of port areas in terms of OM composition gives information on the benthic food web dynamics and also evaluates the systems in terms of organic pollution. This could be helpful in port management, planning, and development activities, as shipping traffic and coastal urbanization are going to increase worldwide in recent years.

The fortnightly observations from the banks and the mouth of meso-tidal Zuari estuary sediment using elemental, biochemical components, and

bacterial populations revealed that seasons and tidal phases at the sampling time play an important role in the population dynamics of benthic bacteria and has an influence on the nature and quality of OM composition. The presence of detrital rich sedimentary OM with low protein/carbohydrate ratio and the high abundance of benthic bacteria suggested intense heterotrophic activity in the sediment. *Vibrio parahaemolyticus*, *V. alginolyticus*, *V. cholerae*, coliforms, and *Shigella* spp. were dominant among the bacterial populations and could have a role in the OM mineralization. *Vibrio* spp. are known to produce the chitinase enzymes, which helps in the utilization of chitinous material found abundant in the aquatic systems, and plays a vital role in OM cycling (Thompson et al., 2004). This study showed that the nature of sedimentary OM can change even within a period of a fortnight, attributed to the bacterial populations as well as the contribution of phytoplankton and terrestrial derived materials, indicated by TOC/TN and protein/carbohydrate ratios.

Elucidation of sedimentary OM from the Zuari estuary using source-specific FA biomarkers and elemental components on a monthly basis revealed spatio-temporal variations in the OM composition. OM was relatively fresh and plankton-rich during non-monsoon seasons and input of detrital-bacteria and terrestrial plant-derived OM during the monsoon season. The high content of elemental components, total FAs, terrestrial plant-specific FAs towards the lower mid-estuarine stations compared to the mouth of the estuary, points out this region as a major deposition site for OM. The mouth of the estuary was characterized by low content of the OM and high contribution of bacteria, indicating their active role in the degradation of the

organic pool through the resuspension-deposition cycle. The banks of the estuary were rich in phytoplankton and bacteria-derived OM, which forms a suitable nursery habitat for bivalves, oysters, and crabs. Among the banks, the southern bank with semi-enclosed nature showed a higher contribution of terrestrial plant-derived OM than the northern bank. This study indicated that the morphological structure of estuary has a significant role in the distribution of OM and the quality and quantity of food available for benthic organisms. Habitat characteristics in terms of OM composition play an important role in fulfilling the food requirement of economically important organisms in the Zuari estuary.

Overall, the present study showed that the nature and quality of OM changed even within a period of fortnight. This was related to primary productivity, allochthonous input, and heterotrophic bacterial abundance, which changes with the tidal phases, seasons, and sites. The diel-tidal changes of spring and the neap tide has an important role in the distribution and regulation of bacteria in the Zuari estuary (Khandeparker et al., 2017a). Fortnightly observations from the sediment also indicated that tidal phases play an important role in the distribution of bacteria in the sediment. Understanding the relationship between bacterial response and fate of different sources derived OM in the estuarine environments in a closed system is helpful for elucidating the degradation pattern of OM. In view of this, laboratory microcosm experiments were conducted, which revealed that the degradation of FAs in the sediment is influenced by the bacterial response, source material, and structural features of FAs. The site-specific microbial community and sediment grain size composition seem to influence

the different rates of degradation of FAs of the same source-material in the sediment. The polyunsaturated FAs degraded faster during the incubation period when compared to saturated FA attributed to the presence of weaker pi-bond in double bond position. The differences in the structural composition of the source material were found to influence the degradation of FAs. The present study showed a positive co-metabolism in the laboratory experiment, which supports the utilization of allochthonous detrital rich OM by the bacteria in the presence of diatom and zooplankton-derived labile OM. Understanding the fate of different sources of organic matter under different environmental conditions is useful in the assessment of benthic food webs and benthic-pelagic coupling.

Thus, it is clear through FA biomarkers, elemental, and biochemical components that the OM quantity and quality are influenced by the environmental settings and can vary in different coastal environments. Detailed information on the sedimentary OM composition from the ports is helpful in delineating both the nature of food available for the benthic organisms as well as the overall health of the ecosystem. Such study from the estuary on short-time and spatial scales is useful to elucidate the signatures of biological processes (bacterial response, primary production, and allochthonous input) on the quality and quantity of OM and to understand the benthic food web dynamics.

OM from the sediment plays a central role in carbon flow and have significant implication in the benthic food web and ecosystem functioning. Future studies should consider how OM is transferred to different trophic levels through the food chain. This could be of crucial importance in a better

understanding of the sustenance of food webs and the conservation of commercially important resources of the highly productive aquatic ecosystems.

Bibliography

Bibliography

Alfaro, A.C., Thomas, F., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coast. Shelf Sci. 70, 271-286.

Alikunhi, N.M., Narayanasamy, R., Kandasamy, K., 2010. Fatty acids in an estuarine mangrove ecosystem. Rev. Biol. Trop. 58(2), 577-587.

Ansari, Z.A., Chatterji, A., Ingole, B.S., Sreepada, R.A., Rivonkar, C.U., Parulekar, A.H., 1995. Community structure and seasonal variation of an inshore demersal fish community at Goa, west coast of India. Estuar. Coast. Shelf Sci. 41(5), 593-610.

Ansari, Z.A., Parulekar, A.H., 1994. Ecological energetics of benthic communities of an estuarine system of the West Coast of India. Proceedings-Indian National Science Academy Part B, 60, 99-106.

Ansari, Z.A., Sreepada, R.A., Kanti, A., Gracias, E.S., 1994. Macrobenthic assemblage in the soft sediment of Marmugao harbor, Goa (central west coast of India). Indian J. Mar. Sci. 23, 225 – 231.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. ecol. Prog. Ser. 257-263.

Bailey, S.A., Duggan, I.C., Nandakumar, K., MacIsaac, H.J., 2007. Sediments in ships: Biota as biological contaminants. Aquat. Ecosyst. Heal. Manag. 10, 93–100.

Bardhan, P., Karapurkar, S.G., Shenoy, D.M., Kurian, S., Sarkar, A., Maya, M.V., Naik, H., Varik, S. Naqvi, S.W.A., 2015. Carbon and nitrogen

isotopic composition of suspended particulate organic matter in Zuari Estuary, west coast of India. J. Mar. Syst. 141, 90-97.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for 'total' lipids. J. Exp. Mar. Biol. Ecol. 12, 103-118.

Bassler, B.L., Gibbons, P.J., Yu, C., Roseman, S., 1991. Chitin utilization by marine bacteria. J. Biol. Chem. 266, 24268–24275.

Bhosle, N.B., Dhople, V.M., 1988. Distribution of some biochemical compounds in the sediments of the Bay of Bengal. Chem. Geol. 67, 341–352.

Bianchi, T.S., Canuel, E.A., 2011. Chemical biomarkers in aquatic ecosystems. Princeton University Press. New Jersey.

Biddanda, B., Ogdahl, M., Cotner, J., 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol. Oceanogr. 46(3), 730-739.

Blaber, S.J.M., 1997. Fish and fisheries in tropical estuaries. Chapman and Hall, London.

Blair, H.A., Dhillon, S., 2014. Omega-3 carboxylic acids (epanova®): A review of its use in patients with severe hypertriglyceridemia. Am. J. Cardiovasc. Drugs, 14(5), 393-400.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Bodineau, L., Thoumelin, G., Béghin, V., Wartel, M., 1998. Tidal time-scale changes in the composition of particulate organic matter within the estuarine turbidity maximum zone in the macrotidal Seine Estuary,

France: the use of fatty acid and sterol biomarkers. Estuar. Coast. Shelf Sci. 47(1), 37-49.

Boechat, I.G., Krüger, A., Chaves, R.C., Graeber, D., Gücker, B., 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. Sci. Total Environ. 482–483, 62–70.

Bortone, G., Arevalo, E., Deibel, I., Detzner, H.D., de Propris, L., Elskens, F., Giordano, A., Hakstege, P., Hamer, K., Harmsen, J., Hauge, A., Palumbo, L., Veen, J.V., 2004. Sediment and dredged material treatment. J. Soil Sediments, 4, 225-232.

Bourgeois, S., Pruski, A.M., Sun, M.Y., Buscail, R., Lantoine, F., Kerhervé, P., Vétion, G., Rivière, B., Charles, F., 2011. Distribution and lability of land-derived organic matter in the surface sediments of the Rhône prodelta and the adjacent shelf (Mediterranean Sea, France): A multi proxy study. Biogeosciences, 8, 3107–3125.

Campanya-Llovet, N., Snelgrove, P.V., Parrish, C.C., 2017. Rethinking the importance of food quality in marine benthic food webs. Prog. Oceanogr. 156:240-251.

Canfield, D.E., 1994. Factors influencing organic carbon preservation in marine sediments. Chem. Geol. 114(3-4), 315-329.

Canuel, E.A., 2001. Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake Bays: a multivariate approach. Org. Geochem. 32, 563-583.

Canuel, E.A., Martens, C.S., 1993. Seasonal variations in the sources and alteration of organic matter associated with recently-deposited sediments. Org. Geochem. 20(5), 563-577.

Canuel, E.A., Martens, C.S., 1996. Reactivity of recently deposited organic matter: Degradation of lipid compounds near the sediment-water interface. Geochim. Cosmochim. Acta, 60, 1793-1806.

Careddu, G., Costantini, M.L., Calizza, E., Carlino, P., Bentivoglio, F., Orlandi, L., Rossi, L., 2015. Effects of terrestrial input on macrobenthic food webs of coastal sea are detected by stable isotope analysis in Gaeta Gulf. Estuar. Coast. Shelf Sci. 154, 158-168.

Carpenter, S.R., Cole, J.J., Pace, M.L., Van de Bogert, M., Bade, D.L., Bastviken, D., Gille, C.M., Hodgson, J.R., Kitchell, J.F., Kritzberg. E.S., 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology, 86:2737–2750.

Carreira, R.S., Wagener, A.L., Readman, J.W., 2004. Sterols as markers of sewage contamination in a tropical urban estuary (Guanabara Bay, Brazil): space–time variations. Estuar. Coast. Shelf Sci. 60(4), 587-598.

Carrie, R.H., Mitchell, L., Black, K.D., 1998. Fatty acids in surface sediment at the Hebridean shelf edge, west of Scotland. Org. Geochem. 29, 1583-1593.

Chakraborty, P., Mukhopadhyay, M., Sampath, S., Ramaswamy, B. R., Katsoyiannis, A., Cincinelli, A., Snow, D., 2019. Organic micropollutants in the surface riverine sediment along the lower stretch

of the transboundary river Ganga: Occurrences, sources and ecological risk assessment. Environ. Pollut. 249, 1071-1080.

Chant, R.J., Stoner, A.W., 2001. Particle trapping in a stratified flood-dominated estuary. J. Mar. Res. 59, 29-51.

Cheung, S.G., Lam, N.W.Y., Wu, R.S.S. Shin, P.K.S., 2008. Spatio-temporal changes of marine macrobenthic community in sub-tropical waters upon recovery from eutrophication. II. Life-history traits and feeding guilds of polychaete community. Mar. Pollut. Bull. 56(2), 297-307.

Chrost, R.J., 1990. Microbial ectoenzymes in aquatic environments. In Aquatic Microbial Ecology. Springer, New York, NY. 47-78.

Cividanes, S., Incera, M., López, J., 2002. Temporal variability in the biochemical composition of sedimentary organic matter in an intertidal flat of the Galician coast (NW Spain). Oceanol. Acta, 25, 1-12.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210, 223–253.

Cloern, J.E., Canuel, E.A., Harris, D., 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol. Oceanogr. 47(3), 713-729.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'neill, R.V. Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature, 387(6630), 253.

Cotano, U., Villate, F., 2006. Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: a biochemical approach. Mar. Pollut. Bull. 52, 404–414.

Craig, D.L., Fallowfield, H.J., Cromar, N.J., 2004. Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. J. Appl. Microbiol. 96, 922-930.

D'Souza, F., Bhosle, N.B., 2001. Variation in the composition of carbohydrates in the Dona Paula Bay (west of India) during May/June 1998. Oceanol. Acta, 24(3), 221-237.

Dai, J., Sun, M.Y., 2007. Organic matter sources and their use by bacteria in the sediments of the Altamaha estuary during high and low discharge periods. Org. Geochem. 38, 1-15.

Dai, J., Sun, M.Y., Culp, R.A., Noakes, J.E., 2009. A laboratory study on biochemical degradation and microbial utilization of organic matter comprising a marine diatom, land grass, and salt marsh plant in estuarine ecosystems. Aquat. Ecol. 43(4), 825-841.

Dalal, S.G., Shirodkar, P.V., Jagtap, T.G., Naik, B.G., Rao, G.S., 2010. Evaluation of significant sources influencing the variation of water quality of Kandla creek, Gulf of Katchchh, using PCA. Environ. Monit. Assess. 163: 49-56.

Dalsgaard, J., John, M.S., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225-340.

Danovaro, R., 1996. Detritus-Bacteria-Meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. Mar. Biol. 127, 1-13.

Danovaro, R., Fabiano, M., Della Croce, N., 1993. Labile organic matter and microbial biomasses in deep-sea sediments (Eastern Mediterranean Sea). Deep. Sea Res. Part I, 40, 953–965.

Danovaro, R., Marrale, D., Della Croce, N., Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: Trophic state and pelagic-benthic coupling. J. Sea Res. 42, 117–129.

David, F., Marchand, C., Taillardat, P., Thanh-Nho, N., Meziane, T., 2018. Nutritional composition of suspended particulate matter in a tropical mangrove creek during a tidal cycle (Can Gio, Vietnam). Estuar. Coast. Shelf Sci. 200, 126-130.

David, F., Marchand, C., Thiney, N., Nhu-Trang, T.T., Meziane, T., 2019. Short-term changes in the quality of suspended particulate matter in a human impacted and mangrove dominated tropical estuary (Can Gio, Vietnam). Cont. Shelf Res. 178, 59–67.

Davies, C.M., Long, J.A., Donald, M., Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. 61, 1888-1896.

de Moraes, P.C., Franco, D.C., Pellizari, V.H., Sumida, P.Y.G., 2014. Effect of plankton-derived organic matter on the microbial community of coastal marine sediments. J. Exp. Mar. Biol. Ecol. 461, 257-266.

De Souza, M.J.B.D., Nair, S., LokaBharathi, P.A., Chandramohan, D., 2003. Particle-associated bacterial dynamics in a tropical tidal plain (Zuari estuary, India). Aquat. Microb. Ecol. 33: 29-40.

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: A new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44, 611–622.

Desai, D.V., Gardade, L., Khandeparker, L., Anil, A.C., 2020. Habitat characteristics mediated partitioning of economically important bivalves in a tropical monsoon–influenced estuary. Environ. Sci. Pollut. Res.

Desmarais, T.R., Solo-Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Appl. Environ. Microbiol. 68, 1165-1172.

Dessai, D.V., Nayak, G.N., 2007. Seasonal distribution of surface sediments and hydrodynamic conditions in Zuari Estuary, Goa, central west coast of India. J. Indian Assoc. Sedimentologists, 26, 25-32.

Ding, H., Sun, M.Y., 2005. Biochemical degradation of algal fatty acids in oxic and anoxic sediment–seawater interface systems: effects of structural association and relative roles of aerobic and anaerobic bacteria. Mar. Chem. 93(1), 1-19.

Dowhan, W., Bogdanov, M., Mileykovskaya, E., 2008. Functional roles of lipids in membranes. In Biochemistry of lipids, lipoproteins and membranes. Elsevier, pp. 1-37.

Droppo, I.G., Liss, S.N., Williams, D., Nelson, T., Jaskot, C., Trapp, B., 2009. Dynamic existence of waterborne pathogens within river sediment compartments. Implications for water quality regulatory affairs. Environ. Sci. Technol. 43, 1737-1743.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Dunn, R.J., Welsh, D.T., Teasdale, P.R., Lee, S.Y., Lemckert, C.J., Méziane, T., 2008. Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. Cont. Shelf Res. 28, 2535-2549.

Edwards, J.L., Smith, D.L., Connolly, J., McDonald, J.E., Cox, M.J., Joint, I., Edwards, C., McCarthy, A.J., 2010. Identification of carbohydrate metabolism genes in the metagenome of a marine biofilm community shown to be dominated by Gammaproteobacteria and Bacteroidetes. Genes, 1(3), 371-384.

El Wakeel, S.K., Riley, J.P., 1957. The determination of organic carbon in marine muds. ICES. J. Mar. Sci. 22, 180-183.

Eswaran, R., Khandeparker, L., 2017. Seasonal variation in bacterial community composition and β-glucosidase expression in a tropical monsoon-influenced estuary. Aquat. Microb. Ecol. 80(3), 273-287.

Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia, 277, 71–84.

Fernandes, S.O., Kirchman, D.L., Michotey, V.D., Bonin, P.C., LokaBharathi, P.A., 2014. Bacterial diversity in relatively pristine and anthropogenically-influenced mangrove ecosystems (Goa, India). Braz. J. Microbiol. 45(4), 1161-1171.

Frankel, E.N., 2014. Lipid oxidation. Woodhead Publishing Limited, Cambridge, UK.

Fries, J.S., Characklis, G.W., Noble, R. T., 2006. Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, NC. J. Environ. Eng. 132, 1338-1345.

Fry, B., Sherr, E.B., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science, 27, 13 - 47.

Ganapati, P.N., Raman, A.V., 1973. Pollution in Visakhapatnam harbour. Curr. Sci. 42, 490-492.

Ganapati, P.N., Raman, A.V., 1979. Organic pollution and *Skeletonema* blooms in Visakhapatnam harbour. Indian J. Mar. Sci. 8, 184-187.

Gaonkar, C.A., Samiksha, S.V., George, G., Aboobacker, V.M., Vethamony, P., Anil, A.C., 2012. Numerical simulations of barnacle larval dispersion coupled with field observations on larval abundance, settlement and recruitment in a tropical monsoon influenced coastal marine environment. J. Mar. Syst. 94, 218-231.

Gardade, L., Khandeparker, L., 2017. Spatio-temporal variations in pathogenic bacteria in the surface sediments of the Zuari estuary, Goa, India. Curr. Sci. 113, 1729-1738.

Ghosh, P.B., Misra, S., Choudhury, A., Ghosh, A., 1990. Fatty acids and sterols in sediments of Hooghly estuary, northeast coast of India. Indian J. Mar. Sci. 19, 22-26.

Gihring, T.M., Humphrys, M., Mills, H.J., Huette, M., Kostka, J.E., 2009. Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. Limnol. Oceanogr. 54(4), 1073-1083.

Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., 2015. Distribution and sources of aliphatic hydrocarbons and fatty acids in surface sediments of a tropical estuary south west coast of India (Cochin estuary). Environ. Monit. Assess. 187, 56.

Golubkov, S.M., Golubkov, M.S., Tiunov, A. V., 2019. Anthropogenic carbon as a basal resource in the benthic food webs in the Neva Estuary (Baltic Sea). Mar. Pollut. Bull. 146, 190–200.

Gong, C., Hollander, D.J., 1997. Differential contribution of bacteria to sedimentary organic matter in oxic and anoxic environments, Santa Monica Basin, California. Org. Geochem. 26(9-10), 545-563.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Determination of oxygen. In Methods of Seawater Analysis. Wiley-Vch, Weinheim, pp. 75-89.

Guenet, B., Danger, M., Abbadie, L., Lacroix, G., 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology, 91(10), 2850-2861.

Guillard, R.R., Ryther, J.H. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8(2), 229-239.

Guo, W., Jia, G., Ye, F., Xiao, H., Zhang, Z., 2019. Lipid biomarkers in suspended particulate matter and surface sediments in the Pearl River Estuary, a subtropical estuary in southern China. Sci. Total Environ. 646, 416–426.

Hadlich, H.L., Venturini, N., Martins, C.C., Hatje, V., Tinelli, P., de Oliveira Gomes, L.E., Bernardino, A.F., 2018. Multiple biogeochemical indicators of environmental quality in tropical estuaries reveal contrasting conservation opportunities. Ecol. Indic. 95, 21-31.

Hammond, D.E., Giordani, P., Montanari, G., Rinaldi, A., Poletti, R., Rovatti, G., Astorri, M., Ravaioli, M., 1984. Benthic flux measurements in NW Adriatic coastal waters. Memoirs of the Society for Geology Italy, 27, 461-467.

Harji, R.R., 2011. Lipids in marine environment. PhD thesis, Goa University.

Harji, R.R., Bhosle, N.B., Garg, A., Sawant, S.S., Venkat, K., 2010. Sources of organic matter and microbial community structure in the sediments of the Visakhapatnam harbour, east coast of India. Chem. Geol. 276, 309–317.

Harji, R.R., Yvenat, A., Bhosle, N.B., 2008. Sources of hydrocarbons in sediments of the Mandovi estuary and the Marmugoa harbour, west coast of India. Environ. Int. 34(7), 959-965.

Harkantra, S.N., Parulekar, A.H., 1981. Ecology of benthic production in the coastal zone of Goa. Mahasagar, 14(2), 135-139.

Harriague, A.C., Albertelli, G., Misic, C., 2012. Macro-and meiofaunal community features in the critical environmental system of a tourist harbour (Rapallo, Ligurian Sea, NW Mediterranean). Mar. Env. Res. 74, 64-72.

Hartree, E.F., 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48, 422–427.

Harvey, H.R., Macko, S.A., 1997. Kinetics of phytoplankton decay during simulated sedimentation: changes in lipids under oxic and anoxic conditions. Org. Geochem. 27(3-4), 129-140.

Hassard, F., Gwyther, C.L., Farkas, K., Andrews, A., Jones, V., Cox, B., Brett, H., Jones, D. L., McDonald, J.E., Malham, S.K., 2016. Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments-a Review. Front. Microbiol. 7, 1-31.

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar. Chem. 49, 81-115.

Hsieh, J.L., Fries, J.S. and Noble, R.T., 2007. *Vibrio* and phytoplankton dynamics during the summer of 2004 in a eutrophying estuary. Ecol. Appl. 17, 102-109.

Hu, J., Zhang, H., Peng, P., 2006. Fatty acid composition of surface sediments in the subtropical Pearl River estuary and adjacent shelf, Southern China. Estuar. Coast. Shelf Sci. 66, 346–356.

Huq, A., Small, E.B., West, P.A., Huq, M.I., Rahman, R., Colwell, R.R., 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl. Environ. Microbiol. 45, 275–283.

Indarti, E., Majid, M.I.A., Hashim, R., Chong, A., 2005. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. J. Food Compos. Anal. 18, 161–170.

Ingole, B.S., Parulekar, A.H., 1998. Role of salinity in structuring the intertidal meiofauna of a tropical estuarine beach: Field evidence. Indian J. Mar. Sci. 27, 356 – 361.

Joseph, M.M., Renjith, K.R., Ratheesh Kumar, C.S., Chandramohanakumar, N., 2012. Assessment of organic matter sources in the tropical mangrove ecosystems of Cochin, Southwest India. Environ. Forensics, 13(3), 262-271.

Kaneko, T., Colwell, R.R., 1975. Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Appl. Microbiol. 29, 269-274.

Kessarkar, P.M., Shynu, R., Rao, V.P., Chong, F., Narvekar, T., Zhang, J., 2013. Geochemistry of the suspended sediment in the estuaries of the Mandovi and Zuari rivers, central west coast of India. Environ. Monit. Assess. 185(5), 4461-4480.

Khan, R.A., 1995. The pollution problem of Hooghly estuarine system; In: Estuarine Ecosystem Series, Zoological survey of India, part 2, pp. 497 - 542.

Khandeparker, L., Anil, A.C., Naik, S.D., Gaonkar, C.C., 2015. Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment. Mar. Pollut. Bull. 96(1-2), 337-343.

Khandeparker, L., Eswaran, R., Gardade, L., Kuchi, N., Mapari, K., Naik, S.D., Anil, A.C., 2017a. Elucidation of the tidal influence on bacterial populations in a monsoon influenced estuary through simultaneous observations. Environ. Monit. Assess. 189, 41.

Khandeparker, L., Kuchi, N., Kale, D., Anil, A.C., 2017b. Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. Ecol. Indic. 74, 172-181.

Khandeparker, R., Verma, P., Meena, R.M., Deobagkar, D.D., 2011. Phylogenetic diversity of carbohydrate degrading culturable bacteria from Mandovi and Zuari estuaries, Goa, west coast of India. Estuar. Coast. Shelf Sci. 95(4), 359-366.

Kirchman, D.L., 2002. The ecology of Cytophaga–Flavobacteria in aquatic environments. FEMS Microbiol. Ecol. 39(2), 91-100.

Krishna Kumari, L., Bhattathiri, P.M.A., Matondkar, S.G.P., John, J., 2002. Primary productivity in Mandovi-Zuari estuaries in Goa. J. Mar. Biol. Ass. India 44, 1-13.

Kristensen, E., Andersen, F.Ø., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Bio. Ecol. 109, 15-23.

Kumar, K.S., Nair, S.M., 2015. Predominance and Sources of Alkane and Fatty Acid Biomarkers in the Surface Sediments of Chitrapuzha River (South India). B. Environ. Contam. Tox. 94(4), 496-502.

Kumar, K.S., Nair, S.M., Salas, P.M., Cheriyan, E., 2017. Distribution and sources of sedimentary organic matter in Chitrapuzha, a tropical tidal river, southwest coast of India. Environ. Forensics, 18(2), 135-146.

Kumari, L.K., Wafar, M.V.M., Royan, J.P., Rajendran, A., 1978. Studies on detritus in a tropical estuary. Indian J. Mar. Sci. 7, 263-266.

Kunte, P.D., Wagle, B.G., 2003. Sediment transport and depth variation study of the Gulf of Kutch using remote sensing. Int. J. Remote Sensing, 24(11), 2253-2263.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32(11-12), 1485-1498.

LokaBharathi, P.A., Oak, S., Chandramohan, D., 1991. Sulfate-reducing bacteria from mangrove swamps II: Their ecology and physiology. Oceanol. Acta, 14:163-171.

Lopez, G.R., Levinton, J.S., 1987. Ecology of deposit-feeding animals in marine sediments. Q. Rev. Biol. 62:235-260.

Losi, V., Moreno, M., Gaozza, L., Vezzulli, L., Fabiano, M., Albertelli, G., 2013. Nematode biomass and allometric attributes as indicators of environmental quality in a Mediterranean harbour (Ligurian Sea, Italy). Ecol. Indic. 30, 80-89.

Luna, G. M., Manini, E., Danovaro, R., 2002. Large fraction of dead and inactive bacteria in coastal sediments: Comparison of protocols for determination and ecological significance. Appl. Environ. Microbiol. 68, 3509-3513.

Luna, G.M., Manini, E., Turk, V., Tinta, T., D'Errico, G., Baldrighi, E., Baljak, V., Buda, D., Cabrini, M., Campanelli, A. Cenov, A., et al., 2019. Status of faecal pollution in ports: A basin-wide investigation in the Adriatic Sea. Mar. Pollut. Bull. 147, 219-228.

Ma, J., Xu, X., Yu, C., Liu, H., Wang, G., Li, Z., Xu, B., Shi, R., 2020. Molecular biomarkers reveal co-metabolism effect of organic detritus in eutrophic lacustrine sediments. Sci. Total Environ. 698, 134328.

Malham, S.K., Rajko-Nenow, P., Howlett, E., Karen, E., Tuson, K.E., Perkins, L.P., Pallett, D.W., Wang, H., Colin, F., Jago, C.F., Jones,

D.L., McDonald, J.E., 2014. The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. Environ. Sci.: Process. Impacts. 16, 2145–2155.

Manju, M.N., Resmi, P., Kumar, C.R., Gireeshkumar, T.R., Chandramohanakumar, N., Joseph, M.M., 2016. Biochemical and stable carbon isotope records of mangrove derived organic matter in the sediment cores. Environ. Earth Sci. 75, 1-15.

Manoj, N.T., Unnikrishnan, A.S., 2009. Tidal circulation and salinity distribution in the Mandovi and Zuari estuaries: case study. J. Waterw. Port Coast. Ocean Eng. 135, 278-287.

Marie, D., Partensky, F., Vaulot, D., Brussaard, C., 2001. Enumeration of phytoplankton, bacteria and viruses in marine samples. Curr. Protoc. Cytom. 1-14.

Marin, V., Moreno, M., Vassallo, P., Vezzulli, L., Fabiano, M., 2008. Development of a multistep indicator-based approach (MIBA) for the assessment of environmental quality of harbours. ICES J. Mar. Sci. 65(8), 1436-1441.

McCarthy, J.F., Landrum, P.F., Palumbo, A. V, 1991. Organic contaminants in sediments: Biological processes. Org. Subst. Sediments Water, 3, 3–21.

McLusky, D.S., Elliott, M., 2004. The estuarine ecosystem: ecology, threats and management. OUP Oxford University Press.

Mestres, M., Sierra, J.P., Mösso, C., Sánchez-Arcilla, A., 2010. Sources of contamination and modelled pollutant trajectories in a Mediterranean harbour (Tarragona, Spain). Mar. Pollut. Bull. 60(6), 898-907.

Meyers, P.A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. 114, 289-302.

Meyers, P.A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Org. Geochem. 27, 213-250.

Meyers, P.A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Org. Geochem. 34(2), 261-289.

Meziane, T., Lee, S.Y., Mfilinge, P.L., Shin, P.K.S., Lam, M.H.W., Tsuchiya, M., 2007. Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. Mar. Biol. 150, 1103-1113.

Meziane, T., Tsuchiya, M., 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar. Ecol. Prog. Ser. 200, 49-57.

Meziane, T., Tsuchiya, M., 2002. Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: origin and utilization by two macrozoobenthic species. J. Sea Res. 47, 1-11.

Mfilinge, P.L., Meziane, T., Bachok, Z., Tsuchiya, M., 2003. Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality. Mar. Ecol. Prog. Ser. 265, 97-105.

Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W., 2002. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. Appl. Environ. Microbiol. 68(10), 5005-5011.

Moore, J.C., Berlow, E.L., Coleman, D.C., et al., 2004. Detritus, trophic dynamics and biodiversity. Ecol. Lett. 7(7), 584-600.

Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., Fabiano, M., 2008. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuar. Coast. Shelf Sci. 77(4), 565-576.

Mudge, S.M., East, J.A., Bebianno, M.J., Barreira, L.A., 1998. Fatty acids in the Ria Formosa lagoon, Portugal. Org. Geochem. 29, 963-977.

Mukhopadhyay, S.K., Biswas, H., De, T.K., Jana, T.K., 2006. Fluxes of nutrients from the tropical River Hooghly at the land-ocean boundary of Sundarbans, NE Coast of Bay of Bengal, India. J. Mar. Syst. 62, 9–21.

Muller-Navarra, D.C., 2008. Food web paradigms: the biochemical view on trophic interactions. Int. Rev. Hydrobiol. 93:489-505.

Muniz, P., Venturini, N., Hutton, M., Kandratavicius, N., Pita, A., Brugnoli, E., Burone, L., García-Rodríguez, F., 2011. Ecosystem health of Montevideo coastal zone: A multi approach using some different

benthic indicators to improve a ten-year-ago assessment. J. Sea Res. 65, 38–50.

Muniz, P., Venturini, N., Martins, C.C., Munshi, A.B., García-Rodríguez, F., Brugnoli, E., Dauner, A.L.L., Bicego, M.C., García-Alonso, J., 2015. Integrated assessment of contaminants and monitoring of an urbanized temperate harbor (Montevideo, Uruguay): a 12-year comparison. Braz. J. Oceanogr. 63, 311-330.

Muri, G., Wakeham, S.G., Pease, T.K., Faganeli, J., 2004. Evaluation of lipid biomarkers as indicators of changes in organic matter delivery to sediments from Lake Planina, a remote mountain lake in NW Slovenia. Org. Geochem. 35(10), 1083-1093.

Nahon, S., Charles, F., Lantoine, F., Vétion, G., Escoubeyrou, K., Desmalades, M., Pruski, A.M., 2010. Ultraviolet radiation negatively affects growth and food quality of the pelagic diatom *Skeletonema costatum*. J. Exp. Mar. Bio. Ecol. 383, 164–170.

Nath, D., Misra, R.N., Karmakar, H.C., 2004. The Hooghly estuarine system-ecological flux, fishery resources and production potential. Bulletin of the Central Inland Fisheries Research Institute, Kolkata, India, No. 130.

Nealson, K.H., 1997. Sediment bacteria: who's there, what are they doing, and what's new?. Annu. Rev. Earth Planet. Sci. 25, 403-434.

Nelson, D.L., Cox, M.M., 2013. Lehninger principles of biochemistry. 6., International ed. Freeman, New York.

Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia, 41(1), 199-219.

Padalkar, P. P., Chakraborty, P., Chennuri, K., Jayachandran, S., Sitlhou, L., Nanajkar, M., Tilvi, S., Singh, K., 2019. Molecular characteristics of sedimentary organic matter in controlling mercury (Hg) and elemental mercury (Hg0) distribution in tropical estuarine sediments. Sci. Total Environ. 668, 592-601.

Padmavati, G., Goswami, S.C., 1996. Zooplankton ecology in the Mandovi-Zuari estuarine system of Goa, west coast of India. Indian J. Mar. Sci. 25, 268-273.

Palomo, L., Canuel, E.A., 2010. Sources of fatty acids in sediments of the York River estuary: relationships with physical and biological processes. Estuar. Coast. 33(3), 585-599.

Pandey, P.K., Kass, P.H., Soupir, M.L., Biswas, S., Singh, V.P., 2014. Contamination of water resources by pathogenic bacteria. AMB Express, 4, 1-16.

Pant, A., Dhargalkar, V.K., Bhosle, N.B., Untawale, A.G., 1980. Contribution of phytoplankton photosynthesis to a mangrove ecosystem. Mahasagar, 13(3), 225-234.

Parkes, R.J., Taylor, J., 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuar. Coast. Shelf Sci. 16, 173-189.

Patil, J.S., Anil, A.C., 2008. Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. Cont. Shelf Res. 28, 2404-2416.

Patil, J.S., Anil, A.C., 2011. Variations in phytoplankton community in a monsoon-influenced tropical estuary. Environ. Monit. Assess. 182, 291-300.

Patil, J.S., Anil, A.C., 2015. Effect of monsoonal perturbations on the occurrence of phytoplankton blooms in a tropical bay. Mar. Ecol. Prog. Ser. 530, 77-92.

Patil, J.S., Anil, A.C., 2019. Assessment of phytoplankton photo-physiological status from a tropical monsoonal estuary. Ecol. Indic. 103, 289-300.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. 16:229-311.

Perkins, T.L., Clements, K., Baas, J.H., Jago, C.F., Jones, D.L., Malham, S.K., McDonald, J. E., 2014. Sediment composition influences spatial variation in the abundance of human pathogen indicator bacteria within an estuarine environment. PloS one, 9, e112951, 1-9.

Pusceddu, A., Bianchelli, S., Gambi, C., Danovaro, R., 2011. Assessment of benthic trophic status of marine coastal ecosystems: Significance of meiofaunal rare taxa. Estuar. Coast. Shelf Sci. 93, 420-430.

Pusceddu, A., Dell'Anno, A., Fabiano, M., Danovaro, R., 2009. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Mar. Ecol. Prog. Ser. 375, 41-52.

Qasim, S.Z., Sen Gupta, R.S., 1981. Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. Estuar. Coast. Shelf Sci. 13, 557-578.

Qasim, S.Z., Wafar, V.M., 1990. Marine resources in the tropics. In Tropical resources: ecology and development (pp. 141-169). Harwood Academic Publishers London.

Quéméneur, M., Marty, Y., 1994. Fatty acids and sterols in domestic wastewaters. Water Res. 28, 1217-1226.

Quintana, C.O., Yoshinaga, M.Y., Sumida, P.Y., 2010. Benthic responses to organic matter variation in a subtropical coastal area off SE Brazil. Mar. Ecol. 31:457-472.

Rajaneesh, K.M., 2018. Ecology of picophytoplankton from the waters surrounding India, PhD thesis, Goa University.

Rajendran, N., Matsuda, O., Imamura, N., Urushigawa, Y., 1992. Variation in microbial biomass and community structure in sediments of eutrophic bays as determined by phospholipid ester-linked fatty acids. Appl. Environ. Microbiol. 58, 562-571.

Rajendran, N., Matsuda, O., Imamura, N., Urushigawa, Y., 1995. Microbial community structure analysis of euxinic sediments using phospholipid fatty acid biomarkers. J. Oceanogra. 51, 21-38.

Ram, A.P., Nair, S., Chandramohan, D., 2007. Bacterial growth efficiency in a tropical estuary: seasonal variability subsidized by allochthonous carbon. Microb. Ecol. 53, 591-599.

Rao, D.G., Rao, T.C.S., 1974. Textural characteristics of sediments of Mormugao Bay, Central West coast of India. Indian J. Mar. Sci. 3, 93 – 98.

Rao, M.N., Ram, A., Pradhan, U.K., Siddaiah, V., 2018. Factors controlling organic matter composition and trophic state in seven tropical estuaries

along the west coast of India. Environ. Geochem. Health, 41(2), 545-562.

Rao, V.P., Shynu, R., Kessarkar, P.M., Sundar, D., Michael, G.S., Narvekar, T., Blossom, V., Mehra, P., 2011. Suspended sediment dynamics on a seasonal scale in the Mandovi and Zuari estuaries, central west coast of India. Estuar. Coast. Shelf Sci. 91, 78-86.

Rasheed, M., Badran, M.I., Huettel, M., 2003. Influence of sediment permeability and mineral composition on organic matter degradation in three sediments from the Gulf of Aqaba, Red Sea. Estuar. Coast. Shelf Sci. 57(1-2), 369-384.

Rehnstam-Holm, A.S., Godhe, A., Harnström, K., Raghunath, P., Saravanan, V., Collin, B., Karunasagar, I., 2010. Association between phytoplankton and *Vibrio* spp. along the southwest coast of India: a mesocosm experiment. Aquat. Microb. Ecol. 58, 127-139.

Renaud, S.M., Thinh, L.V., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture, 211(1-4), 195-214.

Renzi, M., Perra, G., Guerranti, C., Mariottini, M., Baroni, D., Volterrani, M., Graziosi, M., Focardi, S., 2009. Assessment of environmental pollutants in ten southern Italy harbor sediments. Toxicol. Ind. Health, 25(4-5), 351-363.

Rodrigues, V., Ramaiah, N., Kakti, S., Samant, D., 2011. Long-term variations in abundance and distribution of sewage pollution indicator

and human pathogenic bacteria along the central west coast of India. Ecol. Indic. 11, 318-327.

Rosenberg, R., 1995. Benthic marine fauna structured by hydrodynamic processes and food availability. Netherlands J. Sea Res. 34:303-317.

Salas, P.M., Sujatha, C.H., Kumar, C.R., 2015. Fate and source distribution of organic constituents in a river-dominated tropical estuary. J. Earth Syst. Sci. 124, 1265-1279.

Salvo, V.S., Gallizia, I., Moreno, M., Fabiano, M., 2005. Fungal communities in PAH-impacted sediments of Genoa-Voltri harbour (NW Mediterranean, Italy). Mar. Pollut. Bull. 50, 553-559.

Samer, M., 2015. Biological and chemical wastewater treatment processes. Wastewater Treatment Engineering, 1-50.

Sanford, L.P., Suttles, S.E., Halka, J.P., 2001. Reconsidering the physics of the Chesapeake Bay estuarine turbidity maximum. Estuaries, 24, 655-669.

Sarkar, S.K., Binelli, A., Riva, C., Parolini, M., Chatterjee, M., Bhattacharya, A.K., Bhattacharya, B.D., Satpathy, K.K., 2008. Organochlorine pesticide residues in sediment cores of Sunderban wetland, Northeastern part of Bay of Bengal, India, and their ecotoxicological significance. Arch. Environ. Contam. Toxicol. 55:358–371.

Sathish, K., Patil, J.S., Anil, A.C., 2020. Phytoplankton chlorophyll-breakdown pathway: Implication in ecosystem assessment. J. Environ. Manage. 258, 109989.

Shenai-Tirodkar, P.S., Gauns, M.U., Ansari, Z.A., 2016. Evaluation of surface water and sediment quality in Chicalim Bay, Nerul Creek, and

Chapora Bay from Goa coast, India-a statistical approach. Environ. Monit. Assess. 188, 1-13.

Shetye, S.R., Murty, C.S., 1987. Seasonal variation of the salinity in the Zuari estuary, Goa, India. Proc. Indian Acad. Sci. Earth Planet. Sci. 96, 249-257.

Shetye, S.R., Shankar, D., Neetu, S., Suprit, K., Michael, G.S., Chandramohan, P., 2007. The environment that conditions the Mandovi and Zuari estuaries, in: Shetye, S.R., Dileep Kumar, M., Shankar, D. (Eds.), The Mandovi and Zuari Estuaries. National Institute of Oceanography, Goa, India, pp. 3-27.

Shi, W., Sun, M.Y., Molina, M., Hodson, R.E., 2001. Variability in the distribution of lipid biomarkers and their molecular isotopic composition in Altamaha estuarine sediments: implications for the relative contribution of organic matter from various sources. Org. Geochem. 32(4), 453-467.

Shilla, D.J., Tsuchiya, M., Shilla, D.A., 2011. Terrigenous nutrient and organic matter in a subtropical river estuary, Okinawa, Japan: Origin, distribution and pattern across the estuarine salinity gradient. Chem. Ecol. 27, 523–542.

Shirodkar, P.V., Pradhan, U.K., Fernandes, D., Haldankar, S.R., Rao, G.S., 2010. Influence of anthropogenic activities on the existing environmental conditions of Kandla Creek (Gulf of Kutch). Curr. Sci. 98, 815–828.

Shynu, R., Rao, V.P., Sarma, V.V.S.S., Kessarkar, P.M., ManiMurali, R., 2015. Sources and fate of organic matter in suspended and bottom

sediments of the Mandovi and Zuari estuaries, western India. Curr. Sci. 108, 226-238.

Sinha, P.C., Jena, G.K., Rao, A.D., Jain, I., 2006. Numerical modeling of suspended sediment transport in the Gulf of Kachchh including Kandla creek, west coast of India. Mar. Geod. 29, 49–63.

Sivadas, S., Ingole, B., Nanajkar, M., 2011. Temporal variability of macrofauna from a disturbed habitat in Zuari estuary, west coast of India. Environ. Monit. Assess. 173(1-4), 65-78.

Smith, D.C., Simon, M., Alldredge, A.L., Azam, F., 1992. Intense hydrolytic enzyme-activity on marine aggregates and implications for rapid particle dissolution. Nature, 359:139–142.

Snelgrove, P.V., 1998. The biodiversity of macrofaunal organisms in marine sediments. Biodivers. Conserv. 7(9), 1123-1132.

Sreekanth, G.B., Chakraborty, S.K., Jaiswar, A.K., Zacharia, P.U., 2018. An inventory on the coastal finfish and shellfish species of Zuari estuary southwest coast of India. Indian J. Mar. Sci. 47(5), 945-958.

Sreekanth, G.B., Jaiswar, A.K., Zacharia, P.U., Pazhayamadom, D.G., Chakraborty, S.K., 2019. Effect of environment on spatio-temporal structuring of fish assemblages in a monsoon-influenced tropical estuary. Environ. Monit. Assess. 191(5), 305.

Stehfest, K., Toepel, J., Wilhelm, C., 2005. The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiol. Biochem. 43(7), 717-726.

Sun, M.Y., Aller, R.C., Lee, C., Wakeham, S.G., 2002. Effects of oxygen and redox oscillation on degradation of cell-associated lipids in surficial marine sediments. Geochim. Cosmochim. Acta. 66(11), 2003-2012.

Sun, M.Y., Shi, W., Lee, R.F., 2000. Lipid-degrading enzyme activities associated with distribution and degradation of fatty acids in the mixing zone of Altamaha estuarine sediments. Org. Geochem. 31(9), 889-902.

Sun, M.Y., Wakeham, S.G., Lee, C., 1997. Rates and mechanisms of fatty acid degradation in oxic and anoxic coastal marine sediments of Long Island Sound, New York, USA. Geochim. Cosmochim. Acta, 61(2), 341-355.

Tang, K.W., Gladyshev, M.I., Dubovskaya, O.P., Kirillin, G., Grossart, H.P., 2014. Zooplankton carcasses and non-predatory mortality in freshwater and inland sea environments. J. Plankton Res. 36, 597–612.

Thompson, F.L., Iida, T., Swings, J., 2004. Biodiversity of Vibrios. Microbiol. Mol. Biol. Rev. 68, 403-431.

Untawale, A.G., Bhosle, N.B., Dhargalkar, V.K., Matondkar, S.G.P., Bukhari, S., 1977. Biochemical changes in mangrove foliage during growth and decomposition. Indian J. Mar. Sci. 6, 104 -106.

van Der Heijden, L.H., Graeve, M., Asmus, R., Rzeznik-Orignac, J., Niquil, N., Bernier, Q., Guillou, G., Asmus, H.,Lebreton, B., 2019. Trophic importance of microphytobenthos and bacteria to meiofauna in soft-bottom intertidal habitats: A combined trophic marker approach. Mar. Environ. Res. 149, 50-66.

Venturini, N., Pita, A. L., Brugnoli, E., García-Rodríguez, F., Burone, L., Kandratavicius, N., Hutton, M., Muniz, P., 2012a. Benthic trophic

status of sediments in a metropolitan area (Rio de la Plata estuary): Linkages with natural and human pressures. Estuar. Coast. Shelf Sci. 112, 139-152.

Venturini, N., Salhi, M., Bessonart, M., Pires-Vanin, A.M.S., 2012b. Fatty acid biomarkers of organic matter sources and early diagenetic signatures in sediments from a coastal upwelling area (south-eastern Brazil). Chem. Ecol. 28, 221–238.

Vezzulli, L., Fabiano, M., 2006. Sediment biochemical and microbial variables for the evaluation of trophic status along the Italian and Albanian continental shelves. J. Mar. Biol. Assoc. United Kingdom, 86, 27–37.

Vezzulli, L., Fabiano, M., Granelli, V., Moreno, M., 2003. Influence of large-spectrum environmental contamination on the micro–meiobenthic assemblages in harbour sediments of the Ligurian Sea (W Mediterranean). Chem. Ecol. 19, 233-246.

Vijith, V., Sundar, D., Shetye, S.R., 2009. Time-dependence of salinity in monsoonal estuaries. Estuar. Coast. Shelf Sci. 85(4), 601-608.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Bio. Ecol. 128, 219–240.

Volkman, J.K., Johns, R.B., Gillan, F.T., Perry, G.J., Bavor, H.J., 1980. Microbial lipids of an intertidal sediment-I. Fatty acids and hydrocarbons. Geochim. Cosmochim. Acta, 44, 1133–1143.

Volkman, J.K., Revill, A.T., Holdsworth, D.G., Fredericks, D., 2008. Organic matter sources in an enclosed coastal inlet assessed using lipid biomarkers and stable isotopes. Org. Geochem. 39, 689–710.

Wafar, S., Untawale, A.G., Wafar, M., 1997. Litter fall and energy flux in a mangrove ecosystem. Estuar. Coast. Shelf Sci. 44(1), 111-124.

Wakeham, S.G., 1995. Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. Deep Sea Res. Part I: Oceanogr. Res. Pap. 42, 1749-1771.

Wakeham, S.G., Canuel, E.A., 2006. Degradation and preservation of organic matter in marine sediments. In: Volkman J.K. (eds) Marine Organic Matter: Biomarkers, Isotopes, and DNA. The Handbook of Environmental Chemistry, Vol. 2. Springer, Berlin, Heidelberg (pp. 295-321).

Wakeham, S.G., Hedges, J.I., Lee, C., Peterson, M.L., Hernes, P.J., 1997. Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. Deep Sea Res. Part II, 44, 2131-2162.

Wakeham, S.G., Lee, C., 1993. Production, transport, and alteration of particulate organic matter in the marine water column. Org. Geochem. 145-169.

Wang, S., Jin, B., Qin, H., Sheng. Q., W, J., 2015. Trophic dynamics of filter feeding bivalves in the Yangtze estuarine intertidal marsh: Stable isotope and fatty acid analyses. PloS One. 10:1-21.

Warwick, R.M., Joint, I.R., Radford, P.J., 1979. Secondary production of the benthos in an estuarine environment. In: Ecological processes in coastal environment, eds Jeffries, R.L. and Dary, A.J., Blackwell Scientific, Oxford, U.K. pp. 429-450.

Watkins, W.D., Cabelli, V.J., 1985. Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Appl. Environ. Microbiol. 49, 1307-1313.

Wei C.L., Rowe, G.T., Hubbard, G.F., Scheltema, A.H., Wilson, G.D., Petrescu, I., Foster, J.M., Wicksten, M.K., Chen, M., Davenport, R., Soliman, Y., Wang, Y., 2010. Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Mar. Ecol. Prog. Ser. 399:1-14.

Wieking, G., Kröncke, I., 2005. Is benthic trophic structure affected by food quality? The Dogger Bank example. Mar. Biol. 146(2), 387-400.

Xu, W.Z., Cheung, S.G., Shin, P.K., 2014. Structure and taxonomic composition of free-living nematode and macrofaunal assemblages in a eutrophic subtropical harbour, Hong Kong. Mar. Pollut. Bull. 85(2), 764-773.

Zhukova, N.V., 2005. Variation in microbial biomass and community structure in sediments of Peter the Great Bay (Sea of Japan/East Sea), as estimated from fatty acid biomarkers. Ocean Sci. J. 40, 34-42.

Zhukova, N.V., 2019. Fatty acids of marine mollusks: Impact of diet, bacterial symbiosis and biosynthetic potential. Biomolecules, 9(12), 857.

Zimmerman, A.R., Canuel, E.A., 2000. A geochemical record of eutrophication and anoxia in Chesapeake Bay sediments: anthropogenic influence on organic matter composition. Mar. Chem. 69(1-2), 117-137.

Zimmerman, A.R., Canuel, E.A., 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuar. Coast. Shelf Sci. 53, 319-341.

http://www.kolkataporttrust.gov.in.

https://www.deendayalport.gov.in.

Appendix

Appendix I

Table 1 Values of the biochemical components (Proteins, Carbohydrates, and Lipids) for different stations and seasons in the surface sediment of Kolkata port.

	Proteins (PRT)				Carbohydrates (CHO)				Lipids (LPD)			
Station	MON	PRM-I	PRM-II	POM	MON	PRM-I	PRM-II	POM	MON	PRM-I	PRM-II	POM
Kidderpore Dock (KPD)												
S1	3.17	4.18	3.37	3.88	3.53	2.89	3.13	2.68	0.67	2.60	1.19	0.75
S2	3.59	2.96	2.99	4.00	2.91	2.85	1.79	1.93	0.75	1.00	0.32	0.61
S3	5.43	6.11	5.25	5.52	3.95	5.87	2.14	3.42	2.51	5.94	2.54	3.03
S4	7.11	4.34	8.54	5.72	5.27	5.16	7.73	5.28	3.52	2.49	3.21	1.63
S5	4.53	5.89	6.65	8.28	6.03	3.94	7.88	5.32	1.69	3.83	2.50	3.02
S6	4.14	8.48	8.81	8.60	2.65	7.00	7.75	5.25	1.82	5.22	2.08	3.68
S7	9.97	8.59	8.14	8.99	6.46	7.66	9.27	6.79	4.42	4.89	2.57	5.60
S8	4.18	12.3	9.29	8.72	3.83	8.32	8.18	5.35	5.94	7.57	3.96	6.49
S9	6.23	13.2	10.2	9.58	8.48	9.34	8.53	4.56	5.28	7.28	5.33	6.02
S10	7.62	9.22	12.1	10.1	10.5	6.39	13.6	8.49	3.76	5.05	4.58	NS
S11	11.1	13.4	12.7	9.62	13.0	10.0	12.7	5.79	10.2	9.49	4.49	8.54
S12	9.67	7.87	9.62	18.2	11.4	11.0	12.7	11.2	8.46	8.79	4.40	8.14
Netaji Subhash Dock (NSD)												
S13	2.50	5.00	3.07	4.17	1.64	2.27	1.75	2.60	2.97	1.47	1.68	2.41
S14	4.44	NS	3.95	3.80	3.15	NS	2.77	2.26	0.81	NS	2.68	0.96
S15	5.97	4.12	2.49	4.15	4.75	3.17	2.78	1.94	3.08	0.94	2.85	1.29
S16	4.64	6.32	3.34	3.61	7.71	3.74	1.86	1.94	5.02	1.75	2.92	0.87
S17	4.91	5.48	4.60	4.79	2.82	2.73	3.26	3.16	4.20	2.47	2.20	NS
S18	4.94	6.53	11.8	4.77	1.58	3.29	1.27	3.04	4.28	1.99	2.41	2.06
S19	6.05	5.86	6.55	5.83	2.68	3.93	4.23	2.82	2.64	0.73	NS	2.26
S20	2.11	6.80	6.09	6.33	0.79	3.78	3.27	3.25	1.54	3.20	3.88	2.74
S21	5.97	7.41	7.06	NS	5.07	5.31	4.56	NS	2.60	3.42	3.53	NS
S22	NS	7.84	7.98	NS	NS	3.64	5.74	NS	NS	4.78	6.02	NS
S23	8.86	11.7	9.64	NS	5.99	3.92	6.87	NS	3.33	7.15	5.22	NS
Riverine Station (RS)												
S24	0.27	NS	1.89	NS	0.22	NS	1.78	NS	0.52	NS	0.22	NS

Table 2 Values of the biochemical components (Proteins, Carbohydrates, and Lipids) for different stations and seasons in the surface sediment of Kandla port.

	Proteins (PRT)				Carbohydrates (CHO)				Lipids (LPD)			
Station	POM-I	MON	POM-II	PRM	POM-I	MON	POM-II	PRM	POM-I	MON	POM-II	PRM
						Oil Jetties						
S1	0.68	0.51	NS	0.22	1.04	0.68	NS	0.13	0.13	0.16	NS	0.25
S2	0.14	0.76	NS	0.69	0.08	1.02	NS	0.39	0.19	0.28	NS	0.53
S3	0.32	0.87	NS	0.26	0.56	0.70	NS	0.20	0.28	0.26	NS	0.42
S4	0.30	0.81	0.52	0.31	0.59	0.98	0.91	0.21	0.08	0.13	0.33	0.34
S5	1.08	0.38	0.29	0.34	1.43	0.35	0.08	0.43	0.28	0.29	0.27	0.25
						Dry Dock						
S6	0.32	1.09	0.90	0.39	0.38	0.45	0.36	0.61	0.08	0.11	0.33	0.27
S7	0.63	1.11	0.84	0.64	0.94	1.09	1.60	0.27	0.14	0.24	NS	0.29
S8	0.88	0.82	0.64	0.45	1.33	1.21	0.54	0.67	0.25	0.35	0.25	0.33
						Cargo Berths						
S9	0.82	0.60	0.56	NS	1.86	0.95	0.44	NS	0.19	0.28	0.25	NS
S10	1.33	1.09	0.19	0.78	0.87	1.08	0.10	0.16	0.24	0.32	0.17	0.36
S11	0.84	0.82	0.74	0.96	1.15	2.70	0.31	0.51	0.16	0.18	0.18	0.23
S12	1.15	1.38	0.92	2.08	1.49	1.12	0.50	1.02	0.16	0.15	0.25	0.41
						Creek Stations						
S13	0.64	1.18	0.12	0.62	0.38	0.21	0.28	0.40	0.10	0.44	0.27	0.34
S14	0.54	0.32	0.47	0.23	0.30	0.55	0.41	1.27	0.08	0.14	0.21	0.36
S15	1.81	0.85	1.28	1.05	0.97	0.46	0.45	0.78	0.10	0.17	0.22	0.11

(NS: No Samples; PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon).

Appendix II

Table 1 Average values (± SD) of elemental and biochemical components in the surface sediment of different stations in the Zuari estuary.

Parameters	Odxel Beach	Bambolim Beach	Agacaim	Cortalim	Chicalim	Vasco	Marmugao Bay
TOC (%)	0.77 ± 0.48	0.67 ± 0.47	1.26 ± 0.96	2.83 ± 1.00	1.49 ± 1.35	1.23 ± 0.45	0.26 ± 0.61
TN (%)	0.07 ± 0.05	0.07 ± 0.06	0.10 ± 0.11	0.15 ± 0.04	0.09 ± 0.07	0.08 ± 0.03	0.03 ± 0.09
TOC/TN	11	9.57	12.6	19	16.56	15.37	8.7
PRT (mg g^{-1})	1.66 ± 1.30	2.27 ± 1.72	4.88 ± 3.58	10.9 ± 2.23	5.74 ± 4.37	4.76 ± 1.34	2.23 ± 3.79
CHO (mg g^{-1})	2.02 ± 1.30	2.95 ± 2.35	6.36 ± 4.83	13.8 ± 2.12	7.52 ± 6.05	5.38 ± 1.87	3.10 ± 4.86

(TOC: Total Organic Carbon; TN: Total Nitrogen; PRT: Proteins; CHO: Carbohydrates).

Table 2 Range and average concentration of elemental and biochemical components during different seasons in the surface sediment of the Zuari estuary.

	POM-I		PRM	MON		POM-II	
Parameters	Min – Max	Avg ± SD		Min - Max	Avg ± SD	Min - Max	Avg ± SD
TOC (%)	0.23 - 3.99	1.21 ± 1.37		0.05 - 4.05	1.39 ± 1.20	0.27 - 2.35	0.96 ± 0.64
TN (%)	BDL- 0.10	0.05 ± 0.03	No samples	BDL- 0.20	0.09 ± 0.06	0.03 - 0.19	0.10 ± 0.06
TOC/TN	-	24.2		-	15.4	-	9.6
PRT (mg g^{-1})	0.42 - 12.9	4.17 ± 4.84		0.13 - 16.1	4.41 ± 4.15	1.33 - 11.4	5.34 ± 3.57
CHO (mg g^{-1})	0.86 - 18.6	5.84 ± 6.75		0.45 - 18.2	5.62 ± 5.21	1.61 - 14.4	6.49 ± 4.59

(PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon; Min: Minimum; Max: Maximum; Avg: Average; SD: Standard Deviation; BDL: Below Detection Limit).

Publications

Marine Pollution Bulletin 160 (2020) 111679

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Sedimentary organic matter composition from tropical ports with distinct geographic and morpho-hydrodynamic characteristics: Evaluation through multiple biochemical markers

Laxman Gardade[a,b], Lidita Khandeparker[a,*]

[a] CSIR-National Institute of Oceanography, Dona Paula, Goa 403 004, India
[b] School of Earth, Ocean and Atmospheric Sciences, Goa University, Taleigao, Goa, India

ARTICLE INFO

Keywords:
Ports
Sedimentary organic matter
Biopolymeric carbon
Fatty acid biomarkers
Trophic status

ABSTRACT

Increasing coastal urbanization and shipping activity-related environmental pollution advocate the importance of assessment of port ecosystems. Fatty acid biomarkers, elemental components, and biopolymers were used to evaluate the composition of sedimentary organic matter and benthic trophic status of Kolkata (freshwater, enclosed docks) and Kandla (seawater, macrotidal) ports of India. The sediment fatty acid composition indicated relatively fresh and energy-rich organic matter of phytoplankton and bacterial origin inside Kolkata port than the outside riverine station and Kandla port. Biopolymeric carbon (BPC), used as an indicator of trophic status, revealed eutrophic condition in Kolkata port with high accumulation of organic matter of autochthonous origin, attributed to poor water flushing and input of anthropogenic wastes. In contrast, Kandla port was meso-oligotrophic, rich in bacteria, and terrestrial plant-derived materials. Such an assessment of ports' trophic status helps to evaluate the health of the ecosystem and in management practices.

1. Introduction

Coastal areas represent highly productive zones and act as nursery grounds for a wide variety of organisms that contribute to local and marine fisheries (Blaber, 1997). The organic matter (OM) is produced, transported, and transformed along the continuum of the river-estuary-coastal ocean. The organic pool present in the sediment of the coastal system is attributed to the dynamic equilibrium of autochthonous production, terrigenous sources, and heterotrophic utilization (Fabiano and Danovaro, 1994). The quality and quantity of sedimentary OM have a significant role in the structuring of the benthic organisms (Fabiano and Danovaro, 1994; Wieking and Kröncke, 2005). An increment in the OM input causes significant changes in the benthic trophic status, which may affect assemblages of benthic organisms in the sediment, their ecological functions as well as commercial fisheries (Moreno et al., 2008; Venturini et al., 2012a; Golubkov et al., 2019). Thus, characterization of OM from surface sediment is useful to evaluate the changes in the pelagic productivity, nutritional quality, and the nature of food which is available for benthic organisms (Fabiano and Danovaro, 1994; Danovaro et al., 1999; Cividanes et al., 2002) as well as to determine the benthic trophic status of the ecosystem (Pusceddu et al., 2009, 2011; Venturini et al., 2012a; Hadlich et al., 2018).

Harbours and ports are regions in the coastal ecosystems characterized by heavy shipping traffic and industrial activities. They receive nutrients, organic materials, and contaminants from various sources, including ship and boat traffic, fall out of cargo, vessel oil spills, discharge of sewage, and industrial wastes (Ganapati and Raman, 1973, 1979; McCarthy et al., 1991; Bailey et al., 2007). Moreover, there are reports on the dispersal of organisms of freshwater, brackish water, and seawater habitats through ship's ballast water and bottom sediment of the tank to the new environments such as ports, estuaries, lakes, river basins as well as inland areas (Carlton and Geller, 1993; Carlton, 1996; Anil et al., 2002; Grigorovich et al., 2003; Bailey et al., 2007; Drake et al., 2007; Gaonkar et al., 2010; Desai et al., 2018; Cabrini et al., 2019). The increasing coastal urbanization and shipping traffic worldwide in recent years have recommended the importance of assessment of port environments (Marin et al., 2008) to avoid the risk of unintentional introduction of organisms to new environments (Carlton and Geller, 1993; Anil et al., 2002). Ports are low-energy areas with high rates of accumulation of organic contaminants in the sediment (McCarthy et al., 1991). This demands the evaluation of the sediment quality in terms of organic contaminants such as persistent organic pollutants (POPs), which includes organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polycyclic aromatic

* Corresponding author.
E-mail address: klidita@nio.org (L. Khandeparker).

https://doi.org/10.1016/j.marpolbul.2020.111679
Received 14 March 2020; Received in revised form 10 September 2020; Accepted 11 September 2020
Available online 24 September 2020
0025-326X/

hydrocarbons (PAH). These organic contaminants are of global concern due to their long-range transport, bioaccumulation in organisms, adverse impact on the ecosystem, and human health (Jones and De Voogt, 1999). These POPs are very useful in identifying the level of organic chemical pollution in the systems, but these compounds are not suitable to elucidate the quality and quantity of food available for the benthic organisms. A detailed evaluation of OM from the surface sediment of ports using multiple biochemical markers is crucial in delineating both the quality of food as well as organic enrichment/pollution. This is of utmost importance and helpful in management practices since ports are the significant sites of shipping activities.

The composition of OM in terms of total organic carbon to total nitrogen (TOC/TN) ratio is useful to determine the contribution of in-situ derived and terrestrially derived OM in the sediment (Meyers, 1994, 1997). Biomarkers such as fatty acids, *n*-alkanes, alcohols, pigments, and sterols have been widely used to identify the specific and diverse sources of OM in the sediment of the aquatic systems (Canuel and Martens, 1996; Zimmerman and Canuel, 2001; Dai and Sun, 2007; Volkman et al., 2008; Dunn et al., 2008; Harji et al., 2010; Venturini et al., 2012b; Gireeshkumar et al., 2015; Guo et al., 2019). These biomarkers have an advantage over the TOC/TN ratio because the TOC/TN ratio is not efficient in giving precise information on OM sources. The selective decomposition of proteinaceous compounds has the potential to increase the TOC/TN ratio. Whereas, microbial immobilization of nitrogen during remineralization of organic carbon can lower the TOC/TN ratio (Meyers, 1994). Fatty acids (FAs) are the main components of lipids compared to other markers and are source-specific, diverse in structure, and hence commonly used as biomarkers to identify the sources, diagenetic alterations, and fate of OM in the sediment (Carrie et al., 1998; Mudge et al., 1998; Meziane and Tsuchiya, 2000; Dunn et al., 2008; Venturini et al., 2012b). The structurally diverse FA markers are important to obtain a snapshot of the overall community structure of sediment (Carrie et al., 1998 and references therein), also helps in efficient monitoring of the impact of urbanization on ecosystem health (Boechat et al., 2014), and pollution stress on the microbial community (Harji et al., 2010). Biopolymeric carbon (sum of protein, carbohydrate, and lipid carbon equivalents) is the labile fraction of OM and a sensitive tool to evaluate the benthic trophic status in the estuarine and coastal environments (Pusceddu et al., 2009, 2011; Venturini et al., 2012a). Studies on the trophic status are essential to determine the health of systems and to prevent alterations in the overall functioning of the ecosystems.

India has a long coastline (~ 7500 km) dotted with 12 major ports located along the east and west coasts, adjoining the Bay of Bengal and the Arabian Sea, respectively. Although there is plenty of information on the OM composition in port regions all over the world, in Indian ports, such information is scarce. In the present study, we have selected two ports (Kolkata and Kandla), distinct in the geographical, structural, and hydrological characteristics. Kolkata is a freshwater, enclosed port, and Kandla is a seaport, located along a tidally flushed creek. We hypothesized that dissimilarities in morpho-hydrodynamic characteristics of the ports and degree of input of anthropogenic materials from the nearby impacted areas in addition to port activities would influence the quantity, nature of OM, and benthic trophic status of the ports. We expected that an enclosed port with limited water flushing would have a higher content of OM of rich quality than a tidally-flushed one.

The objectives of the present study were to (i) assess the variations in elemental components (total organic carbon, total nitrogen) and biopolymers (proteins, carbohydrates, and lipids) in the sediment of Kolkata and Kandla ports, (ii) evaluate the benthic trophic status of the ports using biopolymeric carbon (BPC), and (iii) elucidate the sources of OM in the sediment of ports using fatty acid biomarkers.

2. Materials and methods

2.1. Study area

2.1.1. Kolkata port

Kolkata port is a riverine port which is situated (22°32′N, 88°18′E) on the bank of the Hooghly River in the state of West Bengal on the north east coast of India. It has a pilotage channel of ~203 km from the mouth of Hooghly estuary. This pilotage channel requires continuous dredging throughout the year to maintain a certain depth for the movement of cargo ships. Kolkata is an enclosed port and has a lock entrance through which ships enter or exit in and out of the dock after crossing the pilotage channel. Hooghly estuary is tide-dominated with semi-diurnal tides. The tides are macrotidal with a tidal range of about 1.8 m during neap and 5.2 m during spring tide at the mouth, and tidal waves from the Bay of Bengal propagate up to ~250 km upstream (Mukhopadhyay et al., 2006).

Kolkata port has two major docks: Kidderpore Dock (KPD) and Netaji Subhash Dock (NSD). KPD has 18 berths and 3 dry docks whereas, NSD has 10 berths and 2 dry docks. KPD is a narrow channel compared to NSD. KPD is further divided into outer KPD-I and inner KPD-II by a bascule bridge (Fig. 1). KPD-I and NSD get influenced by the riverine water during high tide when ships enter or exit through a lock gate. However, KPD-II is located at the inner side of the dock, characterized by restricted water circulation, and this was previously used for ship-breaking activities. The depth of the dock is around 8–12 m.

Kolkata port handles wide varieties of cargos, including coking coal, steam coal, pulses/peas, fertilizers, wooden logs, containers, rock phosphate, pet coke, limestone, raw petroleum liquids, carbon black, vegetable oil, rice, manganese, iron ore, etc. Kolkata Dock System handled 128.75 lakh tons of cargo during the year 2013–14 and 152.83 lakh tons during 2014–15 (http://www.kolkataporttrust.gov.in, Administrative Report, 2014–15).

2.1.2. Kandla port

Kandla port is located (23°01′N, 70°13′E) on the western bank of the Kandla creek in the state of Gujarat on the north west coast of India. The eastern bank of this creek is dominated by small tributaries, which get influenced by seawater influx from the Arabian Sea and are covered with vegetation and mangroves. This port has one navigable channel with a length of ~23 km, the width is ~200 to 1000 m, and the average depth is around 10 m (Shirodkar et al., 2010). The tides in the creek region are macrotidal, with a tidal height of ~0.83 to 7.2 m. The current velocity ranges from 0.08–1.76 ms^{-1} during the flood and 0.09–2.0 ms^{-1} during ebb tide (Sinha et al., 2006). The strong ebb currents cause flushing out of contaminants from the port region into the inner Gulf of Kutch (Shirodkar et al., 2010).

This port has 6 oil berths and 14 multipurpose cargo berths for the handling of petroleum, oil, lubricants (POL), industrial chemicals, fertilizers, food grains, mineral ore, etc. (Shirodkar et al., 2010). Annual cargo traffic handled at the Kandla port was 387.76 lakh tons and 469.42 lakh tons during the year 2014–15 and 2015–16, respectively (https://www.deendayalport.gov.in, Annual Administration Report, 2015–16).

2.2. Sampling strategy

The sampling at the Kolkata port was carried out 4 times at 24 stations during September 2013 (MON), February 2014 (PRM−I), January–February 2015 (PRM-II), and December 2015 (POM). Sediment samples were collected from 7 stations (S1-S7) located in the KPD−I, 5 stations (S8-S12) in the KPD-II, 11 stations (S13-S23) in the NSD, and 1 station (S24) along the bank of Hooghly River outside the dock area (Fig. 1). Sediment could not be sampled from S22 during MON, S14 and S24 during PRM−I, S21 to S24 during POM seasons.

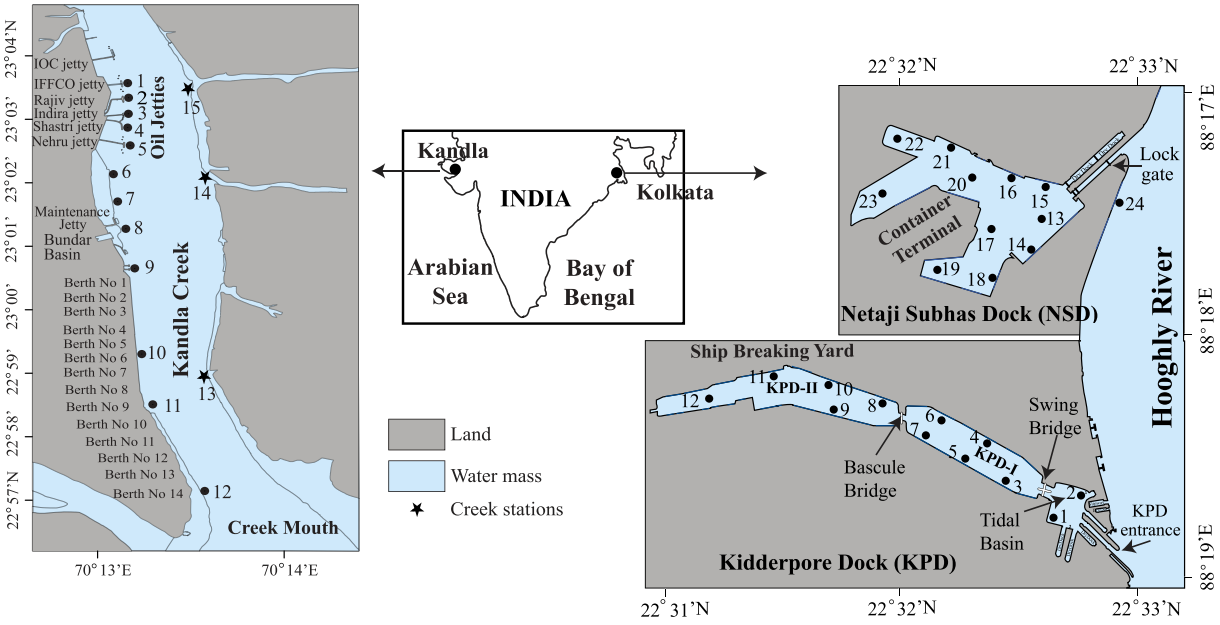


Fig. 1. Map showing location of sampling stations in and around the Kandla and Kolkata ports.

The sampling at the Kandla port was also carried out 4 times during October 2014 (POM−I), July–August 2015 (MON), October 2015 (POM-II), and February 2016 (PRM), respectively. Sediment samples were collected from 15 stations, which are located along the oil jetties (S1-S5), dry dock and bunder basin (S6-S8), multipurpose cargo berths (S9-S12) as well as on the opposite side of berths (S13-S15) in the Kandla creek (Fig. 1). Sediment could not be sampled from S1, S2, and S3 during POM-II and S9 during PRM seasons.

The surface sediment samples from both the ports were collected by using van Veen grab (0.04 m^2), and the top ~5 cm of sediment was collected in zip lock bags and stored in the icebox. These samples were then transported to the laboratory on dry ice and kept frozen at −20 °C after arrival to the laboratory until analyses.

2.3. Analyses of elemental components from the port sediment

The sediment samples were thawed, oven-dried, and ground to a fine powder using mortar and pestle. Sediment was weighed (1–5 mg) in a tin boat and analyzed using a CHNS Analyzer (Vario MICRO Select, Germany) for the Total Carbon (TC) and Nitrogen (TN). These analyses were carried out in duplicate, and elemental concentration is expressed as a percentage dry weight of sediment (wt%). Sulfanilamide (Elemental composition: 41.81% C, 18.62% S, 16.25% N, and 4.65% H) was used as the standard for the calibration of the Elemental Analyzer. The sediment samples were combusted at 500 °C for 16 h in the muffle furnace, and then analyzed to determine the inorganic carbon (IC) content using CHNS Elementar Analyzer (Kristensen and Andersen, 1987). Total Organic Carbon (TOC) content of sediment was then obtained as the difference between TC and IC (TOC = TC – IC). The TOC content was converted into the Total Organic Matter (TOM) by multiplying with factor 1.724 (Bhosle and Dhople, 1988).

2.4. Analyses of biochemical components from the port sediment

Proteins (PRT) were extracted from the sediment using 0.5 M NaOH for 4 h (Danovaro et al., 1993), and their content was determined according to the method described by Hartree (1972). Carbohydrates (CHO) were estimated using phenol and sulphuric acid, according to Dubois et al. (1956). Lipids (LPD) were extracted from the sediment by ultra-sonication with chloroform: methanol (2:1 *v*/v) (Bligh and Dyer, 1959) for 20 min, and estimated following the method of Barnes and Blackstock (1973). Bovine serum albumin, glucose, and cholesterol were used as calibration standards for PRT, CHO, and LPD analyses, respectively. All these analyses were carried out in triplicate, and concentration is expressed as mg g^{-1} sediment dry weight. The blanks for each of the analysis were prepared by pre-combusting sediment samples at 450–500 °C for 4 h in the muffle furnace. The sum of PRT, CHO, and LPD content is considered as labile organic matter (LOM) (Danovaro et al., 1993). The concentrations of PRT, CHO, and LPD were then converted to carbon equivalents using the conversion factor 0.49, 0.40, and 0.75, respectively (Fabiano and Danovaro, 1994; Danovaro et al., 1999). The sum of PRT, CHO, and LPD carbon is reported as biopolymeric carbon (BPC) (Danovaro et al., 1999), which has been used to determine the benthic trophic status of the system (Pusceddu et al., 2011).

2.5. Analyses of fatty acids from the port sediment

Fatty acids were extracted from the sediment by direct transesterification method, as described by Indarti et al. (2005), Nahon et al. (2010), and Bourgeois et al. (2011). Briefly, sediment samples (5 g) were homogenized with a mixture of methanol, sulphuric acid, and chloroform (1.7:0.3:2 v/v/v), vortexed, and incubated at 90 °C for 90 min with occasional shaking. After incubation, samples were allowed to cool at room temperature and subsequently mixed with distilled water. The lower phases containing fatty acid methyl esters (FAMEs) were transferred to clean tubes, and the upper phases were washed with hexane: chloroform (4:1) for 2 times. The pooled organic phases were then evaporated to dryness in a rotary evaporator (Roteva, Medica Instrument Mfg. co., Mumbai), and FAMEs were resuspended in hexane. Subsequently, FAMEs were then analyzed by Gas chromatography-mass spectrometry (GC–MS, QP-2010, SHIMADZU), equipped with a capillary column (Stabilwax, 30 m × 0.25 mm internal diameter, 0.50 μm film thickness). One microliter of the sample was injected through an autosampler with helium as carrier gas (column flow 1 mL min^{-1}). The temperature program of the oven was set to increase from 50 °C (maintained for 2 min) to 200 °C with a rate of 10 °C min^{-1} and then from 200 °C to 240 °C at 5 °C min^{-1}. The injector and detector temperatures were maintained at 240 °C. The mass spectrometer was operated with ionization energy of 70 eV, and mass spectra were recorded in full scan mode (*m/z* 50 to *m/z* 500). The WILEY7 mass spectral library was used for the identification of individual FAME.

FAME mix (Supelco 37 Component, 18919–1AMP, Sigma Aldrich, India) was used for the calibration and quantification of FAMEs from the samples. The concentration of FAME was then reported as μg g^{-1} of sediment.

2.6. Data analyses

The data on stations located in the same dock, which are characterized by a similar type of water stagnation patterns and port activities, were pooled together and represented dock wise, such as KPD-I (7 stations), KPD-II (5 stations), and NSD (11 stations). However, in the case of Kandla port, data on stations located around the jetty/berth with a similar type of port activities were pooled together and represented jetty/berth wise like oil jetties (5 stations), dry dock (3 stations), cargo berths (4 stations), and creek stations (3 stations). Data were then log (x + 1) transformed to meet the assumption of normality and homogeneity. The elemental and biochemical variables were subjected to analysis of variance (ANOVA) to evaluate the spatial and temporal variations. Correlation and Principal Component Analyses (PCA) were carried out to determine the relationship between measured sedimentary variables and to identify the differences among the sampling sites and variables. All these analyses were performed by using STATISTICA software (version. 6.0, StatSoft, USA).

3. Results

3.1. Elemental and biochemical composition of Kolkata port sediment

The elemental and biochemical components were relatively high inside Kidderpore (KPD) and Netaji Subhash dock (NSD) when compared to the outside riverine station (Fig. 2a-h). The TOM, TOC, and TN in the sediment ranged from 0.43 to 29.2%, 0.25 to 16.9%, and 0.01 to 0.97%, respectively (Supplementary Table 1a). Among the docks, the average TOM was high in KPD-II, the inner dock with restricted water circulation, and ranged from 11.9 to 17.5% (Fig. 2a). Whereas, in the case of KPD-I and NSD, both of which get influenced by riverine water during entry and exit of ships, moderate TOM was observed, ranging from 3.88–5.13% and 2.86–3.61%, respectively, and it was low (0.43–1.08%) at the riverine station (Fig. 2a). A similar distribution trend (KPD-II > KPD-I > NSD > riverine station) was observed in the TN, PRT, CHO, LPD, LOM, and BPC (Fig. 2c-h). ANOVA showed significant variations among the sites in elemental and biochemical components; however, seasonal variations were insignificant (Supplementary Table 2a). The TOC/TN ratio in the surface sediment of Kolkata port ranged from 17.9 to 36.6.

The concentration of PRT, CHO, LPD, and BPC ranged from 0.27 to 18.2 mg g^{-1}, 0.22 to 13.6 mg g^{-1}, 0.22 to 10.2 mg g^{-1}, and 0.61 to 19.5 mg C g^{-1}, respectively (Supplementary Table 1a). These biochemical components (PRT, CHO, and LPD) correlated significantly ($p > 0.0001$) with each other (Table 1a).

3.2. Elemental and biochemical composition of Kandla port sediment

The TOM and TOC in Kandla port sediment varied from 0.15 to 3.40% and 0.09 to 1.98%, respectively (Supplementary Table 1b) with high content at cargo berth stations (Fig. 2i & j). TN ranged from 0.017–0.045% and showed an opposite trend to TOC (Fig. 2k). The ratio of TOC/TN in the Kandla port ranged from 9.19–51.6.

The concentration of PRT, CHO, LPD, and BPC in the sediment varied from 0.12 to 2.08 mg g^{-1}, 0.08 to 2.70 mg g^{-1}, 0.08 to 0.53 mg g^{-1}, and 0.25 to 1.74 mg C g^{-1}, respectively (Supplementary Table 1b). PRT, CHO, and BPC were high at cargo berth stations and LPD at oil jetty stations (Fig. 2l-p).

3.3. Spatio-temporal variations in the fatty acids (FAs) in Kolkata port sediment

The sources of OM in the port sediment were identified based on the FA biomarkers (Table 2). The total fatty acids (TFAs) in the Kolkata port sediment ranged from 85.02 to 507.6 μg g^{-1}. The ubiquitous, short-chain FAs (C16:0 + C18:0) were dominant, and their contribution to the TFAs varied from 36.2–80.8%. These FAs showed significant variations (ANOVA; $p < 0.002$) among the sites (KPD-II > KPD-I > NSD > riverine station) (Supplementary Table 2a, Fig. 3a). A strong positive correlation was found between ubiquitous FAs ($r = 0.77$, $p = 0.001$) and diatom-specific FAs (Table 1a).

The bacteria-specific FAs (C18:1ω7, C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0) ranged from 14.3–37.5 μg g^{-1}, and their contribution to the TFAs was 9.2 to 36.6% and followed a similar trend as that of ubiquitous FAs (Fig. 3b). Seasonally, they were high (average, 32.7 ± 3.74 μg g^{-1}) during PRM-II and low (average, 22.9 ± 8.84 μg g^{-1}) during the MON (Fig. 3b). ANOVA showed significant variations among the sites (ANOVA; $p < 0.026$) and the seasons (ANOVA; $p < 0.043$) in bacterial FAs (Supplementary Table 2a).

The FAs specific to diatoms (C14:0, C16:1ω7, C20:4ω6, C20:5ω3), macroalgae (C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3), and dinoflagellates (C18:1ω9, C22:6ω3) were relatively high in both the major docks than the riverine station (Fig. 3c-e). Seasonal variations were significant in the macroalgae (ANOVA; $p < 0.001$) and dinoflagellate-specific FAs (ANOVA; $p < 0.005$) (Supplementary Table 2a, Fig. 3d & e).

Zooplankton-specific FA markers (C22:1ω9, C24:1ω9) were significantly high in the sediment of the riverine station (average, 4.18 ± 0.58 μg g^{-1}) than the dock stations (average, 0.89 ± 0.58 μg g^{-1}) (ANOVA; p < 0.001) (Fig. 3f, Supplementary Table 2a). Zooplankton FA markers showed a strong negative correlation ($r = -0.72$, $p = 0.004$) with diatom-specific FAs (Table 1a).

Terrestrial plant-specific long-chain fatty acids (LCFAs: C22:0 and C24:0) ranged from 1.23 to 9.87 μg g^{-1} and were relatively high in KPD-II and the riverine station during the MON season (Fig. 3g). The contribution of LCFAs to TFAs varied from 0.85 to 7.25% (average, 2.17%) in Kolkata port (Supplementary Fig. 1g).

Factors 1 and 2 in the PCA plot of Kolkata port explained 42.6 and 13.1% of the variance, respectively (Fig. 4a). Factor 1 showed strong negative loadings (−0.69 to −0.79) on C16:1ω7, C15:0, aC15:0, C18:1ω9t, C14:0, C18:2ω6 FAs, and weak loadings (−0.50 to −0.61) on C20:5ω3, iC17:0, aC17:0 indicating the input of diatoms, macroalgae, and bacteria-derived OM (Zimmerman and Canuel, 2001), and weak positive loading (0.62) on C24:1ω9 indicating the contribution of zooplankton.

Factor 2 was characterized by strong negative loadings (−0.67 to −0.72) on C20:5ω3 and C22:6ω3 FAs, and sample scores were negative for NSD and KPD-I (Fig. 4a & b) representing phytoplankton derived fresh OM (Zimmerman and Canuel, 2001). Factor 2 was also influenced by weak negative loadings (−0.51 to −0.55) on C24:1ω9 and C22:1ω9. Factor 2 had positive loading (0.54 to 0.64) on C22:0 and C24:0 FAs indicating the input of terrestrial plant materials (Carrie et al., 1998; Harji et al., 2010).

3.4. Spatio-temporal variations in the fatty acids (FAs) in Kandla port sediment

TFAs in Kandla port sediment ranged from 18.3–60.9 μg g^{-1}. The ubiquitous FAs (C16:0 + C18:0) varied from 13.2 to 34.3 μg g^{-1} (Fig. 3h) and contributed from 46.8–72.5% to TFAs (Supplementary Fig. 1h). These FAs showed weak positive correlation ($r = 0.52$, $p = 0.04$) with diatom-specific FAs (Table 1b).

Bacteria-specific FAs ranged from 4.4–11.9 μg g^{-1}, with high content at oil jetty and low at creek stations (Fig. 3i). The average bacterial FAs showed intra-seasonal variations in the contribution to TFAs with

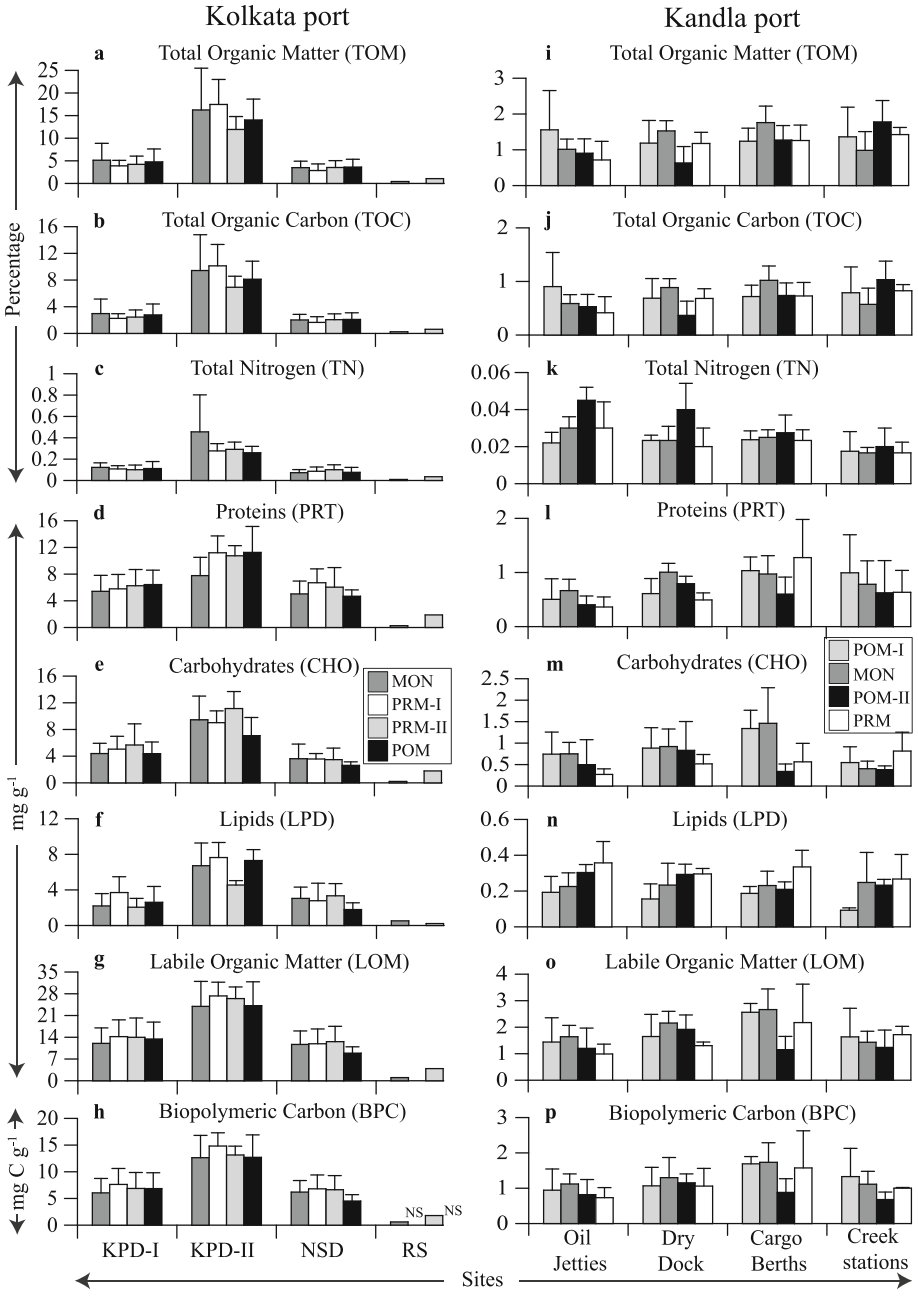


Fig. 2. Mean (± SD) values of elemental and biochemical components in the surface sediment of Kolkata and Kandla ports during different seasons (Note: KPD: Kidderpore Dock; NSD: Netaji Subhash Dock; RS: Riverine Station; PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon; NS: No Samples).

high (26.3%) contribution during POM-II and low (18.9%) during POM-I season.

Diatom, macroalgae, and dinoflagellate-specific FAs varied from 0.44 to 3.2 μg g^{-1} (average, 1.48 ± 0.66 μg g^{-1}), ND (Not Detected) to 0.65 μg g^{-1} (average, 0.18 ± 0.24 μg g^{-1}), ND to 1.12 μg g^{-1} (average, 0.48 ± 0.31 μg g^{-1}), respectively (Fig. 3j-l). Diatom and macroalgae-specific FAs were high at cargo berths and low at oil jetty stations (Fig. 3j & k). However, dinoflagellate FA markers showed the opposite trend (Fig. 3l). Zooplankton-specific FAs were also high (average, 0.15 ± 0.13 μg g^{-1}) at cargo berth and low (average, 0.01 ± 0.01 μg g^{-1}) at oil jetty stations (Fig. 3m).

Terrestrial plant-derived FAs varied from ND to 6.27 μg g^{-1} (Fig. 3n). There was significant spatial (ANOVA; $p < 0.005$) and temporal (ANOVA; $p < 0.008$) variations in the content of terrestrial plant FAs (Supplementary Table 2b). Creek stations were characterized with a high contribution (average, ~14%) of terrestrial plant FAs and were low (average, ~3%) at oil jetty stations (Supplementary Fig. 1n).

In the PCA plots, factors 1 and 2 explained 23.8 and 15.9% of the variance, respectively (Fig. 5a). The variables with weak negative loadings (−0.51 to −0.58) on factor 1 were aC15:0, C18:2ω6, C18:3ω3, C24:1ω9, and positive (0.55) on C18:1ω7 representing the contribution of bacteria, macroalgae, and zooplankton derived OM (Zimmerman and Canuel, 2001).

Factor 2 showed strong positive loadings (0.76 to 0.80) on C24:0 and C22:0 FAs. This factor showed medium negative loadings (−0.58 to −0.69) on C18:1ω7, C17:1, iC15:0, and LPD (Fig. 5a). Creek stations had a positive score for factor 2, representing the dominance of terrestrial plant-derived materials, and cargo berth stations had the most negative score (Fig. 5b), indicating the input of bacteria-derived OM.

Table 1a
Correlation matrix showing r values between fatty acid markers, elemental, and biochemical components from the surface sediment of Kolkata port.

	Bact. FAs	Dia. FAs	M. alg FAs	Dino. FAs	Zoo. FAs	TP. FAs	Ubi. FAs	PRT	CHO	LPD	LOM	BPC	TOC	TN
Bact. FAs	1.00													
Dia. FAs	0.35	1.00												
M. alg FAs	0.35	0.08	1.00											
Dino. FAs	0.29	**0.70**	0.33	1.00										
Zoo. FAs	−0.42	**−0.72**	0.11	−0.41	1.00									
TP FAs	−0.19	−0.11	−0.46	−0.09	0.21	1.00								
Ubi. FAs	0.32	**0.77**	0.05	0.50	**−0.62**	0.15	1.00							
PRT	**0.74**	**0.73**	0.35	**0.60**	**−0.76**	−0.27	**0.69**	1.00						
CHO	**0.75**	**0.74**	0.15	**0.63**	**−0.70**	−0.01	**0.76**	**0.94**	1.00					
LPD	**0.57**	**0.71**	0.28	**0.61**	**−0.68**	0.06	**0.75**	**0.87**	**0.85**	1.00				
LOM	**0.74**	**0.76**	0.25	**0.65**	**−0.77**	−0.15	**0.74**	**0.99**	**0.98**	**0.90**	1.00			
BPC	**0.72**	**0.76**	0.28	**0.66**	**−0.75**	−0.11	**0.76**	**0.98**	**0.97**	**0.94**	**0.99**	1.00		
TOC	**0.65**	**0.72**	0.07	0.48	**−0.71**	0.22	**0.75**	**0.86**	**0.91**	**0.91**	**0.90**	**0.92**	1.00	
TN	**0.60**	0.52	−0.10	0.39	**−0.55**	0.44	**0.64**	**0.69**	**0.83**	**0.82**	**0.77**	**0.79**	**0.93**	1.00

(Note: r values marked as bold indicate that correlation is significant at the $p < 0.05$; Bact: Bacteria, Dia: Diatom; M. alg: Macroalgae; Dino: Dinoflagellate; Zoo: Zooplankton; Ubi: Ubiquitous; TP: Terrestrial plants; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; LOM: Labile Organic Matter; TOC: Total Organic Carbon; TN: Total Nitrogen).

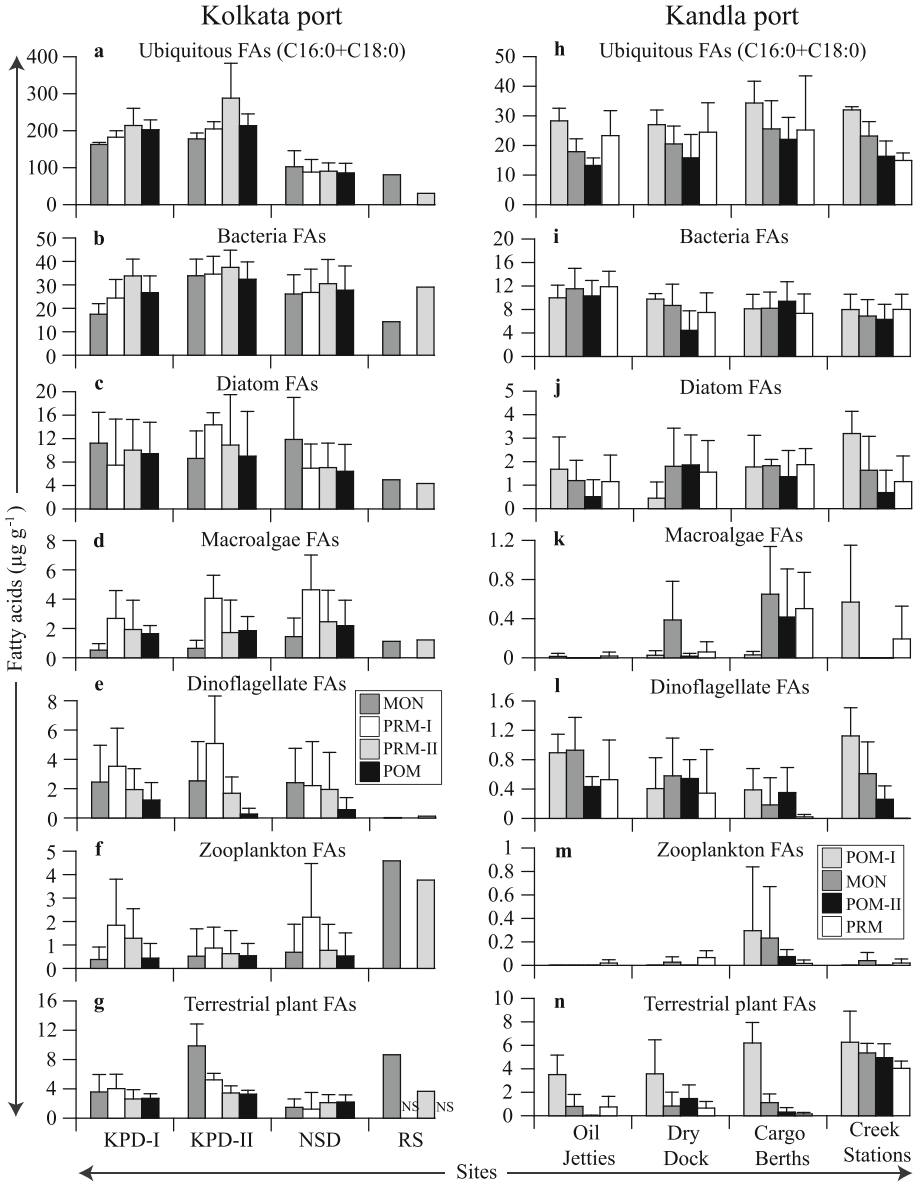
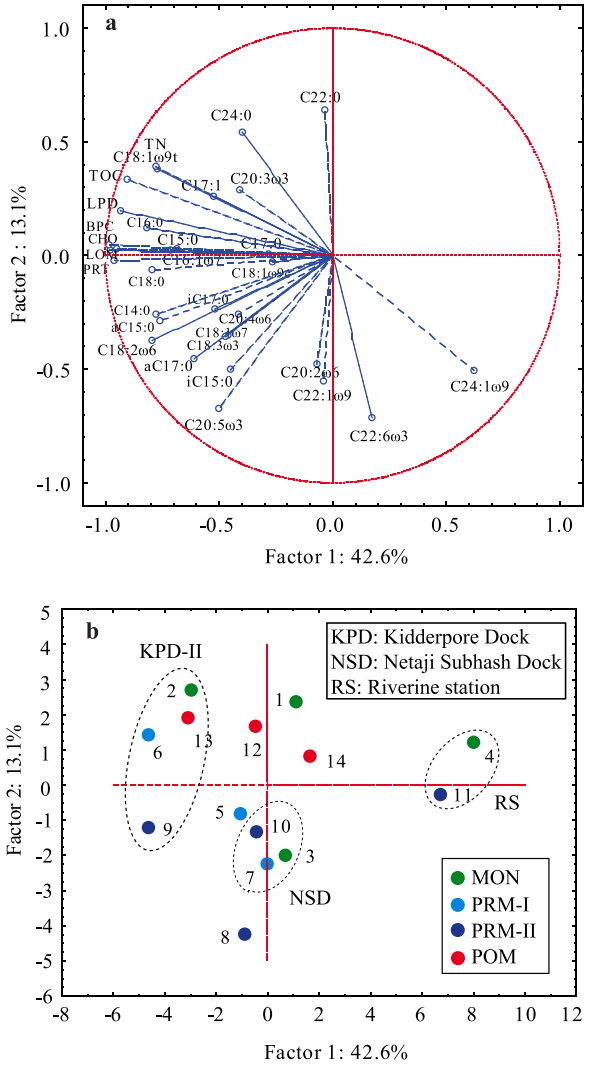


Fig. 3. Mean (± SD) values of ubiquitous and source-specific fatty acids in the surface sediment of Kolkata and Kandla ports during different seasons (Note: KPD: Kidderpore Dock; NSD: Netaji Subhash Dock; RS: Riverine Station; PRM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon; NS: No Samples).



Factor loadings and eigenvalue

Variables	Factor 1	Factor 2
C14:0	**-0.78**	-0.26
C15:0	**-0.72**	0.02
C16:0	**-0.82**	0.12
C17:0	-0.28	0.01
C18:0	**-0.80**	-0.06
C22:0	-0.04	**0.64**
C24:0	-0.40	**0.54**
C16:1ω7	**-0.69**	0.03
C17:1	-0.53	0.26
C18:1ω7	-0.46	-0.35
C18:1ω9t	**-0.77**	0.38
C18:1ω9c	-0.26	-0.03
C22:1ω9	-0.04	**-0.55**
C24:1ω9	**0.62**	**-0.51**
C18:2ω6	**-0.79**	-0.37
C18:3ω3	-0.47	-0.36
C20:2ω6	-0.07	-0.48
C20:3ω3	-0.41	0.29
C20:4ω6	-0.42	-0.26
C20:5ω3	**-0.50**	**-0.67**
C22:6ω3	0.17	**-0.72**
iC15:0	-0.45	**-0.50**
aC15:0	**-0.76**	-0.29
iC17:0	**-0.52**	-0.23
aC17:0	**-0.61**	-0.45
PRT	**-0.96**	-0.02
CHO	**-0.95**	0.03
LPD	**-0.93**	0.20
LOM	**-0.98**	0.02
BPC	**-0.99**	0.05
TOC	**-0.90**	0.34
TN	**-0.78**	0.39
Eigenvalue	13.6	4.2
% Total Variance	42.6	13.1
Cumulative %	42.6	55.7

Sample scores

	Factor 1	Factor 2
1 (KPD-I; MON)	1.12	2.36
2 (KPD-II; MON)	-2.96	2.71
3 (NSD; MON)	0.68	-2.01
4 (RS; MON)	8.00	1.22
5 (KPD-I; PRM-I)	-1.05	-0.81
6 (KPD-II; PRM-I)	-4.63	1.42
7 (NSD; PRM-I)	-0.02	-2.24
8 (KPD-I; PRM-II)	-0.88	-4.24
9 (KPD-II; PRM-II)	-4.61	-1.22
10 (NSD; PRM-II)	-0.43	-1.32
11 (RS; PRM-II)	6.72	-0.28
12 (KPD-I; POM-II)	-0.48	1.67
13 (KPD-II; POM-II)	-3.11	1.90
14 (NSD; POM-II)	1.64	0.83

Fig. 4. Principal Component Analysis (PCA) plots for fatty acids, elemental, and biochemical components in the surface sediment of Kolkata port. a) Variables and factor loadings, b) Sample scores for sites (numbers correspond to sampling sites with different seasons indicated by colors).

4. Discussion

Organic carbon and nitrogen are the important components of the sediment, and the TOC/TN ratio has been used to distinguish the sources of OM in the aquatic systems. The accumulation of diverse sources derived organic carbon and nitrogen (natural and anthropogenic origin) in the sediment is influenced by the hydrodynamic condition of the system. It has been reported that sites subjected to low hydrodynamic conditions favor the deposition of OM rich fine particles in contrast to sites subjected to strong hydrodynamism (Cividanes et al.,

Table 1b
Correlation matrix showing r values between fatty acid markers, elemental, and biochemical components from the surface sediment of Kandla port.

	Bact. FAs	Dia. FAs	M. alg FAs	Dino. FAs	Zoo. FAs	TP. FAs	Ubi. FAs	PRT	CHO	LPD	LOM	BPC	TOC	TN
Bact. FAs	1.00													
Dia. FAs	−0.32	1.00												
M. alg FAs	−0.05	**0.56**	1.00											
Dino. FAs	0.25	0.29	−0.21	1.00										
Zoo. FAs	−0.03	0.26	0.24	−0.27	1.00									
TP FAs	−0.27	0.16	−0.19	0.22	0.13	1.00								
Ubi. FAs	0.15	**0.52**	0.30	0.24	0.47	0.36	1.00							
PRT	−0.42	**0.63**	**0.62**	−0.15	0.37	0.17	0.39	1.00						
CHO	−0.10	0.20	0.21	−0.12	**0.62**	0.21	0.26	0.49	1.00					
LPD	−0.08	−0.25	−0.23	−0.49	−0.12	**−0.63**	**−0.50**	−0.26	−0.30	1.00				
LOM	−0.30	0.44	0.45	−0.23	**0.58**	0.14	0.32	**0.83**	**0.88**	−0.20	1.00			
BPC	−0.22	**0.61**	**0.55**	−0.12	**0.62**	0.08	0.53	**0.86**	**0.74**	−0.24	**0.92**	1.00		
TOC	0.01	0.11	0.48	−0.23	0.26	0.30	0.25	0.32	0.32	−0.45	0.32	0.23	1.00	
TN	0.09	−0.32	−0.26	0.10	−0.12	**−0.61**	**−0.50**	−0.31	−0.03	0.40	−0.13	−0.20	**−0.61**	1.00

(Note: r values marked as bold indicate that correlation is significant at the $p < 0.05$; See Table 1a for abbreviations).

Table 2
Fatty acid biomarkers used to identify the sources of the organic matter in the sediment.

Fatty acids (FAs)	Sources of FAs	References
C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0, C18:1ω7	Bacteria	Volkman et al., 1989; Canuel and Martens, 1996; Rajendran et al., 1995; Carrie et al., 1998; Meziane and Tsuchiya, 2000; Zhukova, 2005
C14:0, C16:1ω7, C20:4ω6, C20:5ω3	Diatom	Volkman et al., 1989; Carrie et al., 1998; Mudge et al., 1998
C18:1ω9, C18:4ω3, C22:6ω3	Dinoflagellate	Carrie et al., 1998; Zimmerman and Canuel, 2001
C20:1ω9, C22:1ω9, C24:1ω9	Zooplankton	Wakeham et al., 1997; Carrie et al., 1998; Venturini et al., 2012b
C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω3	Macroalgae	Volkman et al., 1989; Mudge et al., 1998; Meziane and Tsuchiya, 2000
Long-chain fatty acids (C22:0 - C30:0)	Terrestrial plant	Meyers, 1997; Meziane and Tsuchiya, 2000; Dunn et al., 2008; Guo et al., 2019
C16:0, C18:0	Ubiquitous FAs	Carrie et al., 1998; Mudge et al., 1998

2002). Thus, Kolkata being an enclosed port with restricted water flushing could have resulted in a higher buildup of natural and anthropogenic sources derived OM (average, TOC: 3.84%; TN: 0.15%) when compared to Kandla (average, TOC: 0.72%; TN: 0.03%). The ratio of TOC/TN has been used to distinguish in-situ produced OM (TOC/TN: 4–10) from exogenous terrestrial derived OM (> 20) (Meyers, 1994). Overall, the TOC/TN ratio in Kolkata (17.9–36.6) and Kandla ports (9.19–51.6) indicated the contribution of exogenous and autochthonous produced OM. FA composition also showed input from diverse sources, including in-situ produced and terrestrial plant-derived materials.

Among the FAs, short-chain saturated FAs such as C16:0 and C18:0, which are ubiquitous in a wide range of organisms, were dominant in the sediment of both these ports. These FAs showed a strong positive correlation with diatom-specific FAs, indicating their contribution from diatoms in the Kolkata port sediment. Factor 1 in the PCA plot of Kolkata port also showed strong negative loadings on C14:0, C16:1ω7 FAs, which have been used as biomarkers of diatoms (Volkman et al., 1989; Carrie et al., 1998; Mudge et al., 1998) as well as on C18:2ω6, a marker of macroalgae (Meziane and Tsuchiya, 2000), indicating the contribution of diatoms and macroalgae in the sediment. Higher content of these FAs in the sediment may act as a feast for consumers (Boechat et al., 2014). In the case of Kandla port, PCA showed weak loadings on diatom and macroalgae-specific FAs. Overall, high content of phytoplankton specific FAs (including diatoms, dinoflagellates, and macroalgae) in Kolkata port sediment than Kandla can be attributed to high primary production. The water column of Kolkata port showed higher content of nitrate (~4 times), ammonia (~1.5 times), and chlorophyll-*a* (~10–13 times) compared to Kandla (Sathish et al., 2020), which also supports the elevated primary production. Shirodkar et al. (2010) reported suppressed phytoplankton production in the Kandla creek due to high turbidity in the water column caused by the strong tidal currents and water flow from mudflats rich in the colloidal particles. Thus, high primary production and restricted water flushing could have resulted in an increased accumulation of phytoplankton derived OM in the sediment of enclosed docks of Kolkata port. Recently, Sathish et al. (2020) reported high biomass of phytoplankton and used pheophorbide: pheophytin ratio as a proxy to indicate the dominance of natural senescence as a chlorophyll degradation pathway in the water column of Kolkata port, and low biomass with a dominance of grazing in Kandla port. This could be another reason for the high content of phytoplankton derived OM in the Kolkata port. Zooplankton are the consumers of primary producers and play a significant role in the transfer of energy to higher trophic levels. Monounsaturated FAs

Table 3
Details of stations and mean values (4 seasons) of biochemical and elemental components in the surface sediment and benthic trophic status of the Kolkata port.

Stn. No.	Station Name	PRT (mg g^{-1})	TS	CHO (mg g^{-1})	TS	LPD (mg g^{-1})	BPC (mg C g^{-1})	TS	TOC (%)	TN (%)
Kidderpore Dock (KPD)										
S1	KPD Tidal basin-1	3.65	E	3.06	MO	1.30	3.99	E	1.23	0.08
S2	KPD Tidal basin-2	3.38	E	2.37	MO	0.67	3.11	E	1.41	0.07
S3	KPD Berth-3	5.58	H	3.85	MO	3.50	6.90	E	2.87	0.11
S4	KPD Berth-6	6.43	H	5.86	E	2.71	7.53	E	2.41	0.11
S5	KPD Berth-7	6.34	H	5.79	E	2.76	7.49	E	3.23	0.12
S6	KPD Berth-10	7.51	H	5.66	E	3.20	8.34	E	2.37	0.12
S7	KPD Berth-11	8.92	H	7.55	H	4.37	10.7	E	4.76	0.18
S8	KPD Berth-15	8.63	H	6.42	E	5.99	11.3	E	13.6	0.51
S9	KPD Berth-24	9.79	H	7.73	H	5.98	12.4	E	7.01	0.25
S10	KPD Berth-17	9.76	H	9.74	H	4.46	11.2	E	8.74	0.36
S11	KPD Berth-19	11.7	H	10.4	H	8.17	16.0	E	7.56	0.29
S12	KPD Berth-28	11.3	H	11.6	H	7.45	15.8	E	6.33	0.20
Netaji Subhash Dock (NSD)										
S13	NSD Berth-1-14	3.68	E	2.06	MO	2.13	4.23	E	1.30	0.05
S14	NSD Berth-2	4.06	H	2.73	MO	1.48	4.20	E	1.52	0.07
S15	NSD Berth-14	4.18	H	3.16	MO	2.04	4.84	E	2.30	0.07
S16	NSD Berth-13	4.48	H	3.82	MO	2.64	5.70	E	2.02	0.09
S17	NSD Dolphin mooring-1	4.94	H	2.99	MO	2.96	5.28	E	1.72	0.07
S18	NSD Berth-3	7.01	H	2.30	MO	2.69	6.37	E	1.46	0.07
S19	NSD Berth-5 and 6	6.07	H	3.41	MO	1.88	5.40	E	1.73	0.08
S20	NSD Berth-7	5.33	H	2.77	MO	2.84	5.85	E	1.50	0.07
S21	NSD Ship breaking-1	6.81	H	4.98	MO	3.18	7.72	E	2.25	0.10
S22	NSD Ship breaking-2	7.91	H	4.69	MO	5.40	9.80	E	2.27	0.14
S23	NSD Dolphin mooring-2	10.1	H	5.59	E	5.23	11.1	E	3.42	0.15
Riverine Station (RS)										
S24	GR Jetty-4	1.08	MO	1.00	MO	0.37	1.21	M	0.44	0.02

(Note: PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; TS: Trophic Status; MO: Meso-oligotrophic; E: Eutrophic; H: Hypertrophic, M: Mesotrophic; TOC: Total Organic Carbon; TN: Total Nitrogen).

Table 4
Range of the biochemical components (PRT: Proteins; CHO: Carbohydrates; LPD: Lipids) in the sediment from the ports, estuaries, and coastal areas around the world.

Study area	Sea/ocean/river /bay	PRT (mg g^{-1})	CHO (mg g^{-1})	LPD (mg g^{-1})	References
Continental shelf, east coast of India	Bay of Bengal	0.25–3.40	2.03–9.67	0.16–0.97	Bhosle and Dhople, 1988
Apulian coastal system, Italy	Adriatic Sea	0.1–13.99	0.1–11.9	0.04–2.44	Dell'Anno et al., 2002
Alassio Harbour, Italy	Ligurian Sea	0.01–1.28	0.09–5.59	0.02–1.36	Vezzulli et al., 2003
Sanremo Harbour, Italy		0.006–0.49	0.05–2.01	0.03–0.67	
Genoa-Voltri Harbour, Italy	Ligurian Sea	0.63–14.4	1.06–44.5	–	Salvo et al., 2005
		1.5–7.3*	1.6–16.6*	–	Moreno et al., 2008
Visakhapatnam Harbour, east coast of India	Bay of Bengal	–	–	0.3–14.9	Harji et al., 2010
Rio de la Plata estuary, Uruguay	Montevideo Bay	2.37–15.3	0.83–8.49	0.65–8.35	Muniz et al., 2011
		1.08–16.4	0.29–8.86	0.5–8.35	Venturini et al., 2012a
Rapallo Harbour, Italy	Ligurian Sea	0.1–3.0	0.3–23.5	–	Harriague et al., 2012
Cochin estuary, west coast of India	Arabian Sea	0.11–19.4	0.43–12.8	0.12–8.45	Salas et al., 2015
Montevideo Harbour, Uruguay	Montevideo Bay	5.43–26.4	0.11–10.4	–	Muniz et al., 2015
Chitrapuzha River, west coast of India	Arabian Sea	5.51–11.0	5.48–9.93	3.08–7.42	Sanil Kumar et al., 2017
Piraque Acu-Mirim estuary and Vitoria Bay, Brazil	Atlantic Ocean	0.2–5.5	0.3–18.8	0.1–10.4	Hadlich et al., 2018
Kolkata port, east coast of India	Hooghly River, Bay of Bengal	0.27–18.2	0.22–13.6	0.22–10.2	Present study
Kandla port, west coast of India	Arabian Sea	0.12–2.08	0.08–2.70	0.08–0.53	Present study

(Note: * indicates average values).

(MUFAs) such as C22:1ω9 and C24:1ω9 have been commonly used as biomarkers of zooplankton (Wakeham et al., 1997; Carrie et al., 1998; Venturini et al., 2012b). An inverse relationship of diatom FA markers with zooplankton-specific FAs in Kolkata port indicates decoupling between them, which supports the surplus accumulation of OM derived from unconsumed diatoms.

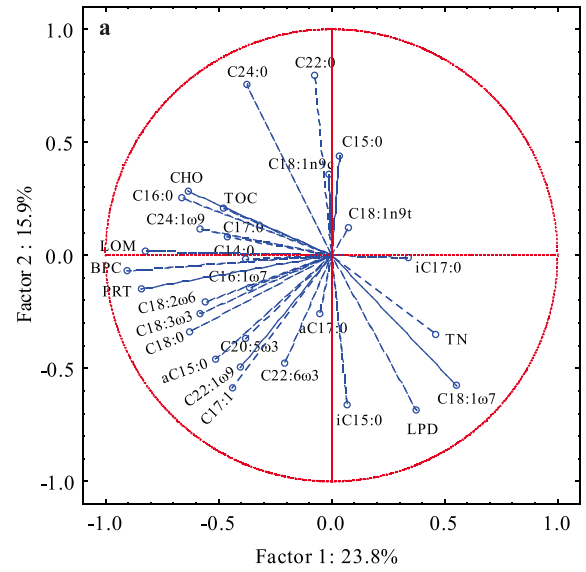
Diatoms, dinoflagellates, and macroalgae are rich sources of PUFAs, which are essential for consumers such as zooplankton, mollusks, and fish (Canuel, 2001). PUFAs such as C20:4ω6 and C20:5ω3 are found in diatoms (Volkman et al., 1989; Carrie et al., 1998), C22:6ω3 usually indicate a contribution of dinoflagellate (Carrie et al., 1998), and C18:2ω6, C18:3ω3, C20:3ω3, C20:2ω6 have been used as markers of macroalgae (Volkman et al., 1989; Mudge et al., 1998; Meziane and Tsuchiya, 2000). The diatom derived PUFAs (C20:4ω6 + C20:5ω3; 0.15%) were high in Kolkata port compared to Kandla port (C20:5ω3; 0.03%). PUFAs such as C20:4ω6, C20:3ω3, C20:2ω6 were not detected in Kandla port sediment. PUFAs are more susceptible to degradation compared to MUFAs (Wakeham et al., 1997), and this could be the reason for the low contribution of PUFAs than MUFAs. The macroalgae-specific PUFAs such as C18:2ω6 and C18:3ω3 were dominant in Kolkata (average, 2.23 μg g^{-1}) sediment than Kandla (average, 0.18 μg g^{-1}). In addition to this, C20:2ω6 and C20:3ω3 FAs were also dominant in the Kolkata port. PCA plots showed strong loadings on C20:5ω3 and C22:6ω3 FAs indicating input of fresh and labile OM derived from diatoms and dinoflagellates, especially in NSD and KPD−I. This was evidenced by high percentage contribution of LOM to TOM in NSD (average, 38%) and KPD-I (average, 33%) when compared to KPD-II (average, 20%). The average content of phytoplankton derived PUFAs was ~9 folds higher in Kolkata port (2.55 μg g^{-1}) than Kandla (0.19 μg g^{-1}), indicating high input of fresh OM in Kolkata port. Such an OM rich environment could be conducive for benthic organisms.

Factor 2 in the PCA plots showed loadings on long-chain fatty acids (LCFAs) such as C22:0 and C24:0, which are generally found in the waxy leaf coatings of terrestrial plants (Meyers, 1997; Carrie et al., 1998). The riverine station and KPD-II of Kolkata port showed high content of LCFAs during the MON season, suggesting the influence of surface run-off. However, the percentage contribution of LCFAs to TFAs was higher in Kandla (average, 6.43%) than Kolkata port (average,

Table 5
Details of stations and mean values (4 seasons) of biochemical and elemental components in the surface sediment and benthic trophic status of the Kandla port.

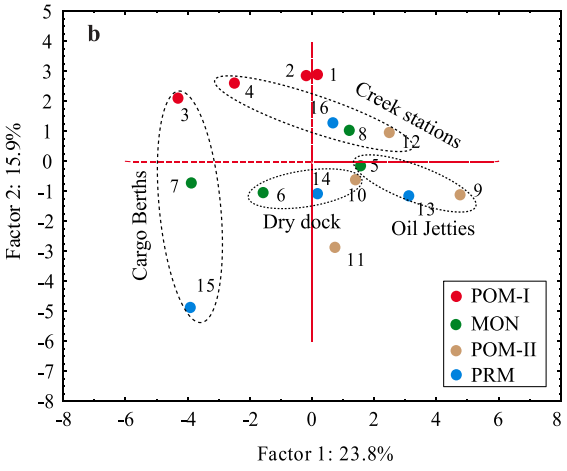
Stn. no.	Station name	PRT (mg g^{-1})	TS	CHO (mg g^{-1})	TS	LPD (mg g^{-1})	BPC (mg C g^{-1})	TS	TOC (%)	TN (%)
Oil Jetties (OJ)										
S1	IFFCO Jetty	0.47	MO	0.62	MO	0.18	0.61	O	0.39	0.03
S2	Rajiv jetty	0.53	MO	0.50	MO	0.33	0.71	O	1.12	0.04
S3	Indira jetty	0.48	MO	0.49	MO	0.32	0.67	O	0.59	0.03
S4	Shastri jetty	0.48	MO	0.67	MO	0.22	0.67	O	0.62	0.03
S5	Nehru jetty	0.52	MO	0.57	MO	0.27	0.69	O	0.46	0.03
Dry Dock (DD)										
S6	Fishing Anchorage-1	0.67	MO	0.45	MO	0.20	0.66	O	0.80	0.03
S7	Fishing Anchorage-2	0.81	MO	0.97	MO	0.22	0.91	O	0.57	0.02
S8	Bunder Basin	0.70	MO	0.94	MO	0.30	0.94	O	0.66	0.03
Cargo Berths (CB)										
S9	Cargo Berth-1	0.66	MO	1.08	MO	0.24	0.93	O	0.79	0.02
S10	Cargo Berth-7	0.85	MO	0.55	MO	0.27	0.84	O	0.75	0.02
S11	Cargo Berth-10	0.84	MO	1.17	MO	0.19	1.02	M	0.88	0.03
S12	Cargo Berth-14	1.38	MO	1.03	MO	0.24	1.27	M	0.81	0.03
Creek Stations (CS)										
S13	Kandla Creek-2	0.64	MO	0.32	MO	0.29	0.65	O	0.64	0.02
S14	Kandla Creek-4	0.39	MO	0.63	MO	0.20	0.59	O	0.70	0.01
S15	Kandla Creek-5	1.25	MO	0.66	MO	0.15	0.99	O	1.05	0.02

(Note: PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; BPC: Biopolymeric Carbon; TS: Trophic Status; MO: Meso-oligotrophic; O: Oligotrophic; M: Mesotrophic; TOC: Total Organic Carbon; TN: Total Nitrogen).



Factor loadings and eigenvalue

Variables	Factor 1	Factor 2
C14:0	-0.38	-0.02
C15:0	0.04	0.44
C16:0	**-0.66**	0.26
C17:0	-0.46	0.08
C18:0	**-0.63**	-0.34
C22:0	-0.07	**0.80**
C24:0	-0.37	**0.76**
C16:1ω7	-0.36	-0.14
C17:1	-0.44	**-0.59**
C18:1ω7	**0.55**	**-0.58**
C18:1ω9t	0.08	0.12
C18:1ω9c	-0.01	0.36
C22:1ω9	-0.40	**-0.50**
C24:1ω9	**-0.58**	0.11
C18:2ω6	**-0.56**	-0.21
C18:3ω3	**-0.58**	-0.26
C20:5ω3	-0.38	-0.37
C22:6ω3	-0.21	-0.48
iC15:0	0.07	**-0.66**
aC15:0	**-0.51**	-0.46
iC17:0	0.34	-0.01
aC17:0	-0.05	-0.26
PRT	**-0.84**	-0.15
CHO	**-0.64**	0.28
LPD	0.38	**-0.69**
LOM	**-0.83**	0.02
BPC	**-0.91**	-0.07
TOC	-0.48	0.21
TN	0.46	-0.35
Eigenvalue	6.89	4.62
% Total Variance	23.8	15.9
Cumulative %	23.8	39.7



Sample scores

	Factor 1	Factor 2
1 (Oil Jetties; POM-I)	0.17	2.87
2 (Dry Dock; POM-I)	-0.17	2.86
3 (Cargo Berths; POM-I)	-4.32	2.11
4 (Creek Stations; POM-I)	-2.50	2.61
5 (Oil Jetties; MON)	1.58	-0.17
6 (Dry Dock; MON)	-1.58	-1.03
7 (Cargo Berths; MON)	-3.88	-0.72
8 (Creek Stations; MON)	1.22	1.03
9 (Oil Jetties; POM-II)	4.76	-1.13
10 (Dry Dock; POM-II)	1.39	-0.63
11 (Cargo Berths; POM-II)	0.76	-2.87
12 (Creek Stations; POM-II)	2.49	0.97
13 (Oil Jetties; PRM)	3.13	-1.16
14 (Dry Dock; PRM)	0.17	-1.10
15 (Cargo Berths; PRM)	-3.91	-4.90
16 (Creek Stations; PRM)	0.69	1.26

Fig. 5. Principal Component Analysis (PCA) plots for fatty acids, elemental, and biochemical components in the surface sediment of Kandla port. a) Variables and factor loadings, b) Sample scores for sites (numbers correspond to sampling sites with different seasons indicated by colors).

2.17%) and could be attributed to the eastern bank of Kandla creek, which is covered with mangroves and vegetation on their banks.

Overall, the bacterial FAs were the dominant contributors of TFAs than other source-specific FAs in both these ports. Bacteria have a significant role in the degradation of OM received from diverse sources as well as contribute their components to the newly synthesized materials (Canuel and Martens, 1996). It has been reported that bacteria act as a food resource for benthic meiofauna (Danovaro et al., 1999; van der Heijden et al., 2019). It also oxidizes organic carbon through a variety of pathways (Nealson, 1997). Bacteria produce odd carbon-numbered, iso, and anteiso-branched FAs within the range of C13 – C19. The FAs such as iC15:0, aC15:0, C17:0, iC17:0, aC17:0 are well-known biomarkers of Gram-negative anaerobes and sulfate-reducing bacteria (Parkes and Taylor, 1983; Wakeham, 1995; Zhukova, 2005). The high content of these FAs in the sediment of Kolkata port indicates poor flushing of water and high accumulation of organic load, which was also evident from the high content of PRT, LPD, and BPC. Though bacteria-specific FAs were low in the sediment of Kandla port than Kolkata, their contribution to the TFAs was high (23.6%) compared to Kolkata (16%), suggesting more bacterial re-working in Kandla port sediment. Vezzulli and Fabiano (2006) reported higher bacterial activity in the oligotrophic sediment with proper ecosystem functioning than eutrophic and hypertrophic sediment. The contribution of bacteria in the sediment increases in response to the deposition of fresh OM or input of nutrients from run-off events (Carrie et al., 1998; Dunn et al., 2008). These could be the reasons for the spatial and seasonal variations in the contribution of bacteria-derived FAs in Kolkata and Kandla ports.

Port regions are influenced by extensive anthropogenic activities and are active sites of ballast water exchange. An evaluation of the benthic trophic status of such systems is useful to determine the health of water bodies and to prevent adverse environmental and economic impacts (Cloern, 2001). The trophic status of ecosystem can be estimated based on the sedimentary PRT and CHO content, and can be classified as meso-oligotrophic (PRT $<$ 1.5 mg g^{-1}; CHO $<$ 5.0 mg g^{-1}), eutrophic (PRT = 1.5–4.0 mg g^{-1}; CHO = 5.0–7.0 mg g^{-1}), and hypertrophic (PRT $>$ 4.0 mg g^{-1}; CHO $>$ 7.0 mg g^{-1}) conditions (Dell'Anno et al., 2002). Our study indicated that all the stations of Kolkata port were meso-oligotrophic to hypertrophic based on the content of PRT (PRT = 3.38–11.7 mg g^{-1}) and CHO (CHO = 2.06–11.6 mg g^{-1}), except for the riverine station (Table 3). PRT and CHO have been used to describe the productivity and the benthic trophic status of the systems (Danovaro et al., 1999; Dell'Anno et al., 2002). Areas with high input of anthropogenic wastes and nutrients result in the accumulation of recently produced PRT rich OM and alter the trophic condition of the system (Dell'Anno et al.,

2002; Vezzulli and Fabiano, 2006; Hadlich et al., 2018). It has been reported that 25–50% of the organic carbon and nitrogen derived from phytoplankton sink to the benthos in nutritionally enriched systems (Cloern, 2001). In the PCA plots, sample scores for factor 1 separated the riverine station from KPD-II, and variable loadings indicated the contribution of diatoms, macroalgae, and bacteria. A strong positive correlation of PRT and CHO with diatom and dinoflagellate-specific FAs support the accumulation of phytoplankton derived OM in Kolkata port.

The high content of PRT and LPD in the sediment is also associated with the input of anthropogenic wastes (Cotano and Villate, 2006; Muniz et al., 2011; Venturini et al., 2012a; Hadlich et al., 2018). The content of PRT, CHO, and LPD was ~9, 7, and 15 times higher in Kolkata port than in Kandla port. The range of these biopolymers observed in Kolkata port sediment is comparable with those reported in other ports, estuaries, and coastal areas affected by organic pollution (Table 4). Kolkata is an enclosed port influenced by the input of freshwater from the Hooghly River, which receives a significant amount (1153.8 million L d^{-1}) of domestic and municipal wastes (68.95% of total discharge) as well as industrial (31.05%) effluents (Khan, 1995). It has been reported that only 20% of waste is treated at sewage treatment plants, and the remaining is discharged directly into the Hooghly River (Sarkar et al., 2008). Thus, the contribution of biopolymers from these wastes into Kolkata dock sediment is possible through contaminated river water. Moreover, Rajaneesh (2018) reported that waste discharge from the anchored ships acts as a source of nutrients in Kolkata docks. A recent study on carbamazepine, the marker of wastewater discharge, revealed higher input of untreated wastewaters in the Hooghly river sediment (Chakraborty et al., 2019). Thus, high primary production influenced by the input of anthropogenic wastes and restricted water flushing could be the major reasons for the eutrophic condition in the Kolkata port.

We also used classification system of Pusceddu et al. (2011), which used BPC content to categorize the system into oligotrophic (BPC $\leq$ 1 mg C g^{-1}), mesotrophic (BPC = 1–3 mg C g^{-1}), and eutrophic (BPC $\geq$ 3 mg C g^{-1}) conditions of the sediment. All the stations of Kolkata port were eutrophic in condition based on the content of BPC (BPC = 3.11–16 mg C g^{-1}), except the outside riverine station (Table 3). BPC correlated positively with bacteria, diatom, dinoflagellate-specific FAs, and negatively with zooplankton-specific FAs suggesting accumulation of the surplus amount of autotrophs originated OM inside the docks. High biomass of phytoplankton and the dominance of natural senescence than grazing as the chlorophyll degradation pathway in the water column of Kolkata port (Sathish et al., 2020) also support the accumulation of phytoplankton derived OM. In Kandla port, BPC correlated positively with diatom, macroalgae, and zooplankton-specific FAs, and all the stations were found to be meso-oligotrophic in condition based on the PRT (0.39–1.38 mg g^{-1}), CHO (0.32–1.17 mg g^{-1}), and BPC (0.59–1.27 mg C g^{-1}) content (Table 5). It seems that strong tidal currents and high bacterial re-working caused the low accumulation of organic compounds in the sediment, supporting the meso-oligotrophic condition of sediment. Vezzulli and Fabiano (2006) reported that bacterial density from oligotrophic sediment shows an inverse relationship with nutrients (proteins) and characterized by high bacterial activity, which enhances the utilization of resources. However, in the case of eutrophic sediment, which is rich in bacteria, the high content of OM disturbs the further organization of the system. This corroborates well with the present study, which also showed high bacterial contribution in meso-oligotrophic Kandla port when compared to Kolkata. Thus, information on sedimentary OM composition and benthic trophic status of the ports is of prime importance for the planning, development, and port management activities.

5. Conclusions

This study revealed that the structure of the ports and the hydrodynamic conditions influenced the accumulation of diverse sources derived biochemical compounds (PRT, CHO, LPD, FAs, TOC, and TN) in the port sediment and had an impact on the benthic trophic status. Fatty acid biomarkers were used in the present study as they have a great potential to elucidate the specific sources of sedimentary OM as well as to validate the benthic trophic status. Kolkata being an enclosed port, resulted as a sink for natural (autochthonous) and anthropogenic derived OM, leading to a eutrophic condition of the sediment; however, strong tidal currents and high bacterial contribution in Kandla port caused low accumulation of OM in the sediment. Spatial variations in the OM composition were prominent in Kolkata port with increasing content towards the inner areas of port owing to poor water flushing. The assessment of the benthic trophic status of the ports using different biochemical markers is crucial to know the overall health of the ecosystem, and such information is valuable in port management practices.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111679.

CRediT authorship contribution statement

Laxman Gardade: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Lidita Khandeparker:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

We are grateful to the Director, CSIR-National Institute of Oceanography, Goa, for his support and encouragement. This work was carried out as a part of the “Ballast Water Management Program, India (BAMPI),” funded by the Directorate General, Ministry of Shipping, Government of India. We gratefully acknowledge Dr. A. C. Anil, Head of the Department, for providing lab resources during this work. We would like to thank Dr. Dattesh Desai, Mr. K. Venkat, and Mr. Kaushal Mapari for their support during Port Biological Baseline Surveys at Kandla and Kolkata ports. We are also thankful to the project staff for their help during sample collection. We acknowledge the help rendered by the officers of Kolkata and Kandla Port Trusts. The first author (LG) is a registered Ph.D. student in the School of Earth, Ocean and Atmospheric Sciences, Goa University. This is NIO contribution No. 6600.

References

Anil, A.C., Venkat, K., Sawant, S.S., Dileepkumar, M., Dhargalkar, V.K., Ramaiah, N., Harkantra, S.N., Ansari, Z.A., 2002. Marine bioinvasion: concern for ecology and shipping. Curr. Sci. 83, 214–218.

Bailey, S.A., Duggan, I.C., Nandakumar, K., MacIsaac, H.J., 2007. Sediments in ships: biota as biological contaminants. Aquat. Ecosyst. Health Manag. 10, 93–100.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for ‘total’ lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.

Bhosle, N.B., Dhople, V.M., 1988. Distribution of some biochemical compounds in the sediments of the Bay of Bengal. Chem. Geol. 67, 341–352.

Blaber, S.J.M., 1997. Fish and Fisheries in Tropical Estuaries. Chapman and Hall, London.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Boechat, I.G., Krüger, A., Chaves, R.C., Graeber, D., Gücker, B., 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. Sci. Total Environ. 482–483, 62–70.

Bourgeois, S., Pruski, A.M., Sun, M.Y., Buscail, R., Lantoine, F., Kerhervé, P., Vétion, G., Rivière, B., Charles, F., 2011. Distribution and lability of land-derived organic matter in the surface sediments of the Rhône prodelta and the adjacent shelf (Mediterranean Sea, France): a multi proxy study. Biogeosciences 8, 3107–3125.

Cabrini, M., Cerino, F., de Olazabal, A., Di Poi, E., Fabbro, C., Fornasaro, D., Goruppi, A., et al., 2019. Potential transfer of aquatic organisms via ballast water with a particular focus on harmful and non-indigenous species: a survey from Adriatic ports. Mar. Pollut. Bull. 147, 16–35.
Canuel, E.A., 2001. Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake bays: a multivariate approach. Org. Geochem. 32, 563–583.
Canuel, E.A., Martens, C.S., 1996. Reactivity of recently deposited organic matter: degradation of lipid compounds near the sediment-water interface. Geochim. Cosmochim. Acta 60, 1793–1806.
Carlton, J.T., 1996. Marine bioinvasions: the alteration of marine ecosystems by non-indigenous species. Oceanography 9, 36–43.
Carlton, J.T., Geller, J.B., 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Science 261, 78–82.
Carrie, R.H., Mitchell, L., Black, K.D., 1998. Fatty acids in surface sediment at the Hebridean shelf edge, west of Scotland. Org. Geochem. 29, 1583–1593.
Chakraborty, P., Mukhopadhyay, M., Sampath, S., Ramaswamy, B.R., Katsoyiannis, A., Cincinelli, A., Snow, D., 2019. Organic micropollutants in the surface riverine sediment along the lower stretch of the transboundary river Ganga: occurrences, sources and ecological risk assessment. Environ. Pollut. 249, 1071–1080.
Cividanes, S., Incera, M., López, J., 2002. Temporal variability in the biochemical composition of sedimentary organic matter in an intertidal flat of the Galician coast (NW Spain). Oceanol. Acta 25, 1–12.
Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210, 223–253.
Cotano, U., Villate, F., 2006. Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: a biochemical approach. Mar. Pollut. Bull. 52, 404–414.
Dai, J., Sun, M.Y., 2007. Organic matter sources and their use by bacteria in the sediments of the Altamaha estuary during high and low discharge periods. Org. Geochem. 38, 1–15.
Danovaro, R., Fabiano, M., Della Croce, N., 1993. Labile organic matter and microbial biomasses in deep-sea sediments (eastern Mediterranean Sea). Deep-Sea Res. I Oceanogr. Res. Pap. 40, 953–965.
Danovaro, R., Marrale, D., Della Croce, N., Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling. J. Sea Res. 42, 117–129.
Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44, 611–622.
Desai, D.V., Narale, D., Khandeparker, L., Anil, A.C., 2018. Potential ballast water transfer of organisms from the west to the east coast of India: insights through on board sampling. J. Sea Res. 133, 88–99.
Drake, L.A., Doblin, M.A., Dobbs, F.C., 2007. Potential microbial bioinvasions via ships' ballast water, sediment, and biofilm. Mar. Pollut. Bull. 55, 333–341.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Dunn, R.J., Welsh, D.T., Teasdale, P.R., Lee, S.Y., Lemckert, C.J., Méziane, T., 2008. Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. Cont. Shelf Res. 28, 2535–2549.
Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia 277, 71–84.
Ganapati, P.N., Raman, A.V., 1973. Pollution in Visakhapatnam harbour. Curr. Sci. 42, 490–492.
Ganapati, P.N., Raman, A.V., 1979. Organic pollution and *Skeletonema* blooms in Visakhapatnam harbour. Indian. J. Mar. Sci. 8, 184–187.
Gaonkar, C.A., Sawant, S.S., Anil, A.C., Venkat, K., Harkantra, S.N., 2010. Mumbai harbour, India: gateway for introduction of marine organisms. Environ. Monit. Assess. 163, 583–589.
Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., 2015. Distribution and sources of aliphatic hydrocarbons and fatty acids in surface sediments of a tropical estuary south west coast of India (Cochin estuary). Environ. Monit. Assess. 187, 56.
Golubkov, S.M., Golubkov, M.S., Tiunov, A.V., 2019. Anthropogenic carbon as a basal resource in the benthic food webs in the Neva estuary (Baltic Sea). Mar. Pollut. Bull. 146, 190–200.
Grigorovich, I.A., Colautti, R.I., Mills, E.L., Holeck, K., Ballert, A.G., MacIsaac, H.J., 2003. Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. Can. J. Fish. Aquat. Sci. 60, 740–756.
Guo, W., Jia, G., Ye, F., Xiao, H., Zhang, Z., 2019. Lipid biomarkers in suspended particulate matter and surface sediments in the Pearl River estuary, a subtropical estuary in southern China. Sci. Total Environ. 646, 416–426.
Hadlich, H.L., Venturini, N., Martins, C.C., Hatje, V., Tinelli, P., de Oliveira Gomes, L.E., Bernardino, A.F., 2018. Multiple biogeochemical indicators of environmental quality in tropical estuaries reveal contrasting conservation opportunities. Ecol. Indic. 95, 21–31.
Harji, R.R., Bhosle, N.B., Garg, A., Sawant, S.S., Venkat, K., 2010. Sources of organic matter and microbial community structure in the sediments of the Visakhapatnam harbour, east coast of India. Chem. Geol. 276, 309–317.
Harriague, A.C., Albertelli, G., Misic, C., 2012. Macro-and meiofaunal community features in the critical environmental system of a tourist harbour (Rapallo, Ligurian Sea, NW Mediterranean). Mar. Environ. Res. 74, 64–72.
Hartree, E.F., 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48, 422–427.
van der Heijden, L.H., Graeve, M., Asmus, R., Rzeznik-Orignac, J., Niquil, N., Bernier, Q., Guillou, G., Asmus, H., Lebreton, B., 2019. Trophic importance of microphytobenthos and bacteria to meiofauna in soft-bottom intertidal habitats: a combined trophic marker approach. Mar. Environ. Res. 149, 50–66.
Indarti, E., Majid, M.I.A., Hashim, R., Chong, A., 2005. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. J. Food Compos. Anal. 18, 161–170.
Jones, K.C., De Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science. Environ. Pollut. 100, 209–221.
Khan, R.A., 1995. The pollution problem of Hooghly estuarine system. In: Estuarine Ecosystem Series, Zoological Survey of India, Part 2, pp. 497–542.
Kristensen, E., Andersen, F.Ø., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109, 15–23.
Kumar, K.S., Nair, S.M., Salas, P.M., Cheriyan, E., 2017. Distribution and sources of sedimentary organic matter in Chitrapuzha, a tropical tidal river, southwest coast of India. Environ. Forensic 18, 135–146.
Marin, V., Moreno, M., Vassallo, P., Vezzulli, L., Fabiano, M., 2008. Development of a multistep indicator-based approach (MIBA) for the assessment of environmental quality of harbours. ICES J. Mar. Sci. 65, 1436–1441.
McCarthy, J.F., Landrum, P.F., Palumbo, A.V., 1991. Organic contaminants in sediments: biological processes. Org. Subst. Sediments Water 3, 3–21.
Meyers, P.A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. 114, 289–302.
Meyers, P.A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Org. Geochem. 27, 213–250.
Meziane, T., Tsuchiya, M., 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar. Ecol. Prog. Ser. 200, 49–57.
Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., Fabiano, M., 2008. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuar. Coast. Shelf Sci. 77, 565–576.
Mudge, S.M., East, J.A., Bebianno, M.J., Barreira, L.A., 1998. Fatty acids in the Ria Formosa lagoon, Portugal. Org. Geochem. 29, 963–977.
Mukhopadhyay, S.K., Biswas, H., De, T.K., Jana, T.K., 2006. Fluxes of nutrients from the tropical river Hooghly at the land-ocean boundary of Sundarbans, NE coast of Bay of Bengal, India. J. Mar. Syst. 62, 9–21.
Muniz, P., Venturini, N., Hutton, M., Kandratavicius, N., Pita, A., Brugnoli, E., Burone, L., García-Rodríguez, F., 2011. Ecosystem health of Montevideo coastal zone: a multi approach using some different benthic indicators to improve a ten-year-ago assessment. J. Sea Res. 65, 38–50.
Muniz, P., Venturini, N., Martins, C.C., Munshi, A.B., García-Rodríguez, F., Brugnoli, E., Dauner, A.L.L., Bicego, M.C., García-Alonso, J., 2015. Integrated assessment of contaminants and monitoring of an urbanized temperate harbor (Montevideo, Uruguay): a 12-year comparison. Braz. J. Oceanogr. 63, 311–330.
Nahon, S., Charles, F., Lantoine, F., Vétion, G., Escoubeyrou, K., Desmalades, M., Pruski, A.M., 2010. Ultraviolet radiation negatively affects growth and food quality of the pelagic diatom *Skeletonema costatum*. J. Exp. Mar. Biol. Ecol. 383, 164–170.
Nealson, K.H., 1997. Sediment bacteria: who's there, what are they doing, and what's new? Annu. Rev. Earth Planet. Sci. 25, 403–434.
Parkes, R.J., Taylor, J., 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuar. Coast. Shelf Sci. 16, 173–189.
Pusceddu, A., Dell'Anno, A., Fabiano, M., Danovaro, R., 2009. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Mar. Ecol. Prog. Ser. 375, 41–52.
Pusceddu, A., Bianchelli, S., Gambi, C., Danovaro, R., 2011. Assessment of benthic trophic status of marine coastal ecosystems: significance of meiofaunal rare taxa. Estuar. Coast. Shelf Sci. 93, 420–430.
Rajaneesh, K.M., 2018. Ecology of Picophytoplankton from the Waters Surrounding India. PhD thesis. Goa University.
Rajendran, N., Matsuda, O., Imamura, N., Urushigawa, Y., 1995. Microbial community structure analysis of euxinic sediments using phospholipid fatty acid biomarkers. J. Oceanogr. 51, 21–38.
Salas, P.M., Sujatha, C.H., Kumar, C.R., 2015. Fate and source distribution of organic constituents in a river-dominated tropical estuary. J. Earth Syst. Sci. 124, 1265–1279.
Salvo, V.S., Gallizia, I., Moreno, M., Fabiano, M., 2005. Fungal communities in PAH-impacted sediments of Genoa-Voltri harbour (NW Mediterranean, Italy). Mar. Pollut. Bull. 50, 553–559.
Sarkar, S.K., Binelli, A., Riva, C., Parolini, M., Chatterjee, M., Bhattacharya, A.K., Bhattacharya, B.D., Satpathy, K.K., 2008. Organochlorine pesticide residues in sediment cores of Sunderban wetland, northeastern part of Bay of Bengal, India, and their ecotoxicological significance. Arch. Environ. Contam. Toxicol. 55, 358–371.
Sathish, K., Patil, J.S., Anil, A.C., 2020. Phytoplankton chlorophyll-breakdown pathway: implication in ecosystem assessment. J. Environ. Manag. 258, 109989.
Shirodkar, P.V., Pradhan, U.K., Fernandes, D., Haldankar, S.R., Rao, G.S., 2010. Influence of anthropogenic activities on the existing environmental conditions of Kandla Creek (Gulf of Kutch). Curr. Sci. 98, 815–828.
Sinha, P.C., Jena, G.K., Rao, A.D., Jain, I., 2006. Numerical modeling of suspended sediment transport in the Gulf of Kachchh including Kandla creek, west coast of India. Mar. Geod. 29, 49–63.
Venturini, N., Pita, A.L., Brugnoli, E., García-Rodríguez, F., Burone, L., Kandratavicius, N., Hutton, M., Muniz, P., 2012a. Benthic trophic status of sediments in a metropolitan area (Rio de la Plata estuary): linkages with natural and human pressures. Estuar. Coast. Shelf Sci. 112, 139–152.
Venturini, N., Salhi, M., Bessonart, M., Pires-Vanin, A.M.S., 2012b. Fatty acid biomarkers

of organic matter sources and early diagenetic signatures in sediments from a coastal upwelling area (south-eastern Brazil). Chem. Ecol. 28, 221–238.
Vezzulli, L., Fabiano, M., 2006. Sediment biochemical and microbial variables for the evaluation of trophic status along the Italian and Albanian continental shelves. J. Mar. Biol. Assoc. United Kingdom 86, 27–37.
Vezzulli, L., Fabiano, M., Granelli, V., Moreno, M., 2003. Influence of large-spectrum environmental contamination on the micro–meiobenthic assemblages in harbour sediments of the Ligurian Sea (W Mediterranean). Chem. Ecol. 19, 233–246.
Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219–240.
Volkman, J.K., Revill, A.T., Holdsworth, D.G., Fredericks, D., 2008. Organic matter sources in an enclosed coastal inlet assessed using lipid biomarkers and stable isotopes. Org. Geochem. 39, 689–710.
Wakeham, S.G., 1995. Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. Deep Sea Res. Part I: Oceanogr. Res. Pap. 42, 1749–1771.
Wakeham, S.G., Hedges, J.I., Lee, C., Peterson, M.L., Hernes, P.J., 1997. Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. Deep-Sea Res. II Top. Stud. Oceanogr. 44, 2131–2162.
Wieking, G., Kröncke, I., 2005. Is benthic trophic structure affected by food quality? The Dogger Bank example. Mar. Biol. 146, 387–400.
Zhukova, N.V., 2005. Variation in microbial biomass and community structure in sediments of Peter the Great Bay (sea of Japan/East Sea), as estimated from fatty acid biomarkers. Ocean Sci. J. 40, 34–42.
Zimmerman, A.R., Canuel, E.A., 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuar. Coast. Shelf Sci. 53, 319–341.

Environmental Science and Pollution Research (2020) 27:29303–29326
https://doi.org/10.1007/s11356-020-09239-5

RESEARCH ARTICLE

Habitat characteristics mediated partitioning of economically important bivalves in a tropical monsoon–influenced estuary

Dattesh V Desai[1] · Laxman Gardade[1] · Lidita Khandeparker[1] · Arga Chandrashekar Anil[1]

Received: 23 December 2019 / Accepted: 11 May 2020 / Published online: 21 May 2020

Abstract

Bivalves are benthic organisms inhabiting coastal marine habitats, especially estuarine tidal and mudflats. Due to their high stocking density and rich protein content, they form a major part of the artisanal fishery resource around the world. A clear partitioning in the population of bivalves, *Paphia malabarica* (Chemnitz, 1782), and *Meretrix casta* (Gmelin, 1791) in southern (Chicalim) and northern (Siridao) bank of a tropical Zuari estuary influenced by the monsoon along the Indian west coast, is evidenced. This study unravels the reasons for their partitioning in this estuary. *Paphia malabarica* is an exclusive inhabitant of Chicalim which has silty-sandy sediment, whereas *M. casta* is exclusive to Siridao, a sandy habitat. Observations showed that this segregation is facilitated by the semi-enclosed nature of habitat at Chicalim with the high amount of degraded and aged sediment organic carbon, high chlorophyll *a*, elemental, and biochemical components, whereas Siridao experiences the high impact of tidal currents, low sediment organic carbon, and high water column chlorophyll *a*. The habitat in Siridao gets exposed to UV radiation during low tide, reducing the photosynthetic oxygen production, turning the habitat to periodic anoxia indicated by differences in the TOC:TS ratio. However, such conditions may not influence *M. casta*, which can derive oxygen from the water column. The fatty acids specific to diatoms, dinoflagellates, higher plants, and partially degraded organic matter in the tissues of *P. malabarica* indicate their ability to source the food from the sediment and water column, whereas in tissues of *M. casta*, higher dinoflagellate-specific fatty acids followed by diatom and bacteria indicate water column–derived food. Chicalim can be considered an actively coupled benthic-pelagic habitat, and Siridao as an uncoupled habitat. Thus, the diverse flux of food particles, species-specific feeding ecology, and local hydrodynamics operating at these habitats could be the determining factors in the partitioning of the bivalves.

Keywords *Paphia malabarica* · *Meretrix casta* · Zuari estuary · Resource partitioning · Sediment

Introduction

Estuaries are rich in biodiversity and inhabited by a wide range of benthic artisanal resources which include bivalves, oysters, crabs, etc. along with other macrobenthic organisms. The sand and mudflats within the estuaries on which a significant fraction of the artisanal fisheries are dependent often are anthropogenically stressed. The organisms inhabiting these sensitive ecosystems live close to their environmental tolerance limits, and subtle environmental changes can provoke a significant biological response in them, modifying the food web and affecting the stock of economically important artisanal resources. The important primary producers in shallow estuarine sediment are the microphytobenthos, which contribute considerably to the chlorophyll biomass and benthic ecology (MacIntyre et al. 1996; Cahoon 1999; Hope et al. 2019). The autochthonous biomass of microphytobenthos (dominated by benthic diatoms) acts as vital food resource for large numbers of consumers inhabiting in the sediment (Plante-Cuny and Plante 1986; MacIntyre et al. 1996; Miller et al. 1996; Cahoon 1999; Kanaya et al. 2005). Sediment characteristics, mainly texture, organic carbon, chlorophyll, and

Highlights
- Elucidated potential causes for bivalve habitat partitioning in an estuary.
- Short-neck bivalve *Paphia malabarica* preferred silty-sand substratum.
- Suspension feeding bivalve *Meretrix casta* preferred sandy substratum.
- Fatty acids are indicators of feeding habit in bivalves.
- Physical forcing, habitat characteristics, and quality and quantity of food influence bivalve partitioning.

Responsible editor: Vedula VSS Sarma

✉ Dattesh V Desai
ddattesh@nio.org

[1] CSIR-National Institute of Oceanography, Dona Paula, Goa 403004, India

microbial biomass, play an important role in the feeding ecology and biology of benthic consumers (Rhoads 1974; Lopez and Levinton 1987; Graf and Rosenberg 1997) especially bivalves (Kanaya et al. 2005).

The bivalves in the estuarine systems are important mediators of energy transfer from benthic and pelagic primary producers to the consumers (Heip et al. 1995; Carmichael et al. 2012; Wang et al. 2015) and also help in organic matter cycling. Organic matter (OM) is a vital component of the estuarine and coastal sediments and plays a significant role in the food web (Zimmerman and Canuel 2001). Estuarine sediment receives OM from different sources, including primary producers, consumers, decomposers, and allochthonous inputs through river discharge including land runoff, and industrial and domestic waste (Meziane and Tsuchiya 2002; Hu et al. 2006; Dunn et al. 2008). Fatty acids (FAs), the main components of lipids, act as energy source and nutrients for the organisms such as bivalves, snails, shrimps, and crabs and can be used to identify the diet of dominant fauna of the estuarine and coastal sediments (Meziane and Tsuchiya 2000; Kharlamenko et al. 2001; Meziane and Tsuchiya 2002; Bachok et al. 2003; Alfaro et al. 2006; Bachok et al. 2009; Wang et al. 2015). FAs are source-specific and diverse in structure and are commonly used to identify the OM sources and their fate in the sediments (Zimmerman and Canuel 2001; Meziane and Tsuchiya 2000, 2002; Dunn et al. 2008; Venturini et al. 2012b; Guo et al. 2019).

The benthic organisms might experience different responses based on the quantity of food, and competition would be greater between the consumers in food-limited environments (Wei et al. 2010; Campanya-Llovet et al. 2017). However, few studies have also recognized the importance of nutritional quality of the available food, and its influence on the distribution of benthic consumers (Wieking and Kröncke 2005; Quintana et al. 2010). Muller-Navarra (2008) defined the term "food quality" as the degree of the quantity and nutritional composition of food which is accessible to satisfy the basic nutritional demand of the consumers, and this cannot be unraveled from quantity alone, since large quantities of nutritionally poor food may be as valuable as low quantities of nutritionally rich food. Therefore, to understand the influence of food on the benthic community structure, it is important to examine the quality and quantity of food along with other characteristics of the habitat. The bivalves are filter-feeding organisms and generally found in the estuarine sediments and are commercially important food resources being harvested from the estuarine areas (Parulekar et al. 1984; Alexander et al. 1993; Narasimham and Laxmilatha 1966). In the present study area, Zuari estuary, Goa, two commercially important bivalves, namely, *Paphia malabarica* Chemnitz, 1791 (Sukumaran et al. 2019), and *Meretrix casta* Gmelin, 1971 (Huber 2010), inhabit. These two species are also marketed on a regular basis by the local fishermen during the harvesting season. The fishery of the bivalves has shown a declining trend in the state of Goa and also large inter-annual fluctuations in their stock. In the year 2012, the production of major bivalve resource, *Paphia malabarica*, was 442 t and in the year 2017 it was 82 t. From 2012 to 2015, the production of this species showed fluctuations; however, from 2015 onwards, it showed a sharp decline (2015–614 t; 2017–82 t) (Department of Fisheries, Goa 2018). However, such data is not available for other species (*Meretrix casta*).

The bivalve *P. malabarica* is a short-necked bivalve known to survive in diverse habitats in the subtidal zone and is a facultative deposit feeder, wherein it can take food both from the sediment and from the water column, whereas *M. casta* is a suspension feeder and take their food from the water column. These two bivalves have segregated habitats on either side of the banks of Zuari estuary. The bivalve *M. casta* is found at Siridao, the northern bank, and *P. malabarica* is found at Chicalim, which is the southern bank of the Zuari estuary. However, the reasons for their partitioning in this estuary are not known. We hypothesize that the possible reasons for the partitioning of these bivalve species could be the variations in the habitat characteristics, quality and the quantity of the organic matter, type of food, the physical processes, and local hydrodynamics acting in the bivalve habitat. In order to delineate this, we evaluated (a) the distribution and nature of sedimentary organic matter, (b) sources of fatty acids in the sediment, and (c) seasonal variation in the abundance of bivalves and their diet in order to understand the possible reasons for partitioning of the bivalve (*Paphia malabarica* and *Meretrix casta*) population. The results obtained through this study will be useful in managing the stocks of these bivalves in these habitats as they are the major spawning grounds of *Paphia malabarica* and *Meretrix casta*. The data obtained on the seasonal variation in the population of both the bivalves will be helpful in interlinking stock assessment and harvesting practices for the development of the conservation strategy. Inferences drawn from the scientific assessment of stocks would provide information on the life cycle strategies of these species and will help in understanding their production, leading the way for implementing harvest potential in a given area and their conservation for population replenishment through management of stocks.

Materials and methods

Study area

The study was conducted in the Zuari estuary, situated in the state of Goa, central west coast of India. The study region

experiences three main seasons in a year, which can be described as the pre-monsoon (February–May), south-west monsoon (June–September), and post-monsoon (October–January). Zuari River has a catchment area of about 550 km^2 and receives 491 × 10^6 m^3 of runoff annually (Qasim and Sen Gupta 1981; Shetye et al. 2007). The study site Chicalim (15° 24′ 10.92″ N and 73° 51′ 8.55″ E) is situated on the southern bank and is influenced by different anthropogenic activities such as shipbuilding and barge cleaning, whereas Siridao (15° 25′ 41.89″ N and 73° 52′ 38.84″ E) is situated on the northern bank of Zuari estuary (Fig. 1) and is relatively pristine.

Sampling and analytical methodology

Sampling was carried out in April (pre-monsoon, PreM), July (monsoon, MON), and November 2014 (post-monsoon, POM) during the low tide. Sediment samples were collected by using PVC cores of 20-cm length and 25-mm internal diameter. Each core was sliced into 4 segments (0–5, 5–10, 10–15, and 15–20 cm) and kept frozen at − 20 °C for further analysis. The bivalves were handpicked from the sediment, brought to the laboratory, and kept frozen at − 20 °C for not more than 2 weeks for further analysis of fatty acids. In the laboratory, the soft tissues of bivalves were removed carefully, washed thoroughly by filtered autoclaved seawater, and used for the extraction of lipids. The temperature and salinity of seawater were determined using digital thermometer and refractometer, respectively, during the sampling.

Analysis of sediment grain size and chlorophyll *a*

The sediment grain size was determined by the pipette analysis method (Buchanan 1984), and composition is expressed as the percentage of sand, silt, and clay. The chlorophyll *a* was extracted from sediment with 90% acetone in the dark at 4 °C (24 h), centrifuged on the next day, and the supernatant was estimated for the content of chlorophyll *a* and expressed as micrograms per gram of sediment. The chlorophyll *a* of the seawater was determined by filtering 500 ml of seawater through GF/F filter paper and followed the above procedure for the extraction of chlorophyll *a* and is expressed as micrograms per liter.

Estimation of the elemental and biochemical composition of the sediment core

The samples of sediment which were frozen at − 20 °C were thawed, oven-dried at 50 °C (Bhosle and Dhople 1988), and ground into fine powder for elemental and biochemical composition. Sediments were weighed in tin boat and the content of total carbon (TC), nitrogen (TN), and sulfur (TS) was determined using a Vario MICRO Select Elemental Analyzer (Germany) and expressed as percentage of sediment dry

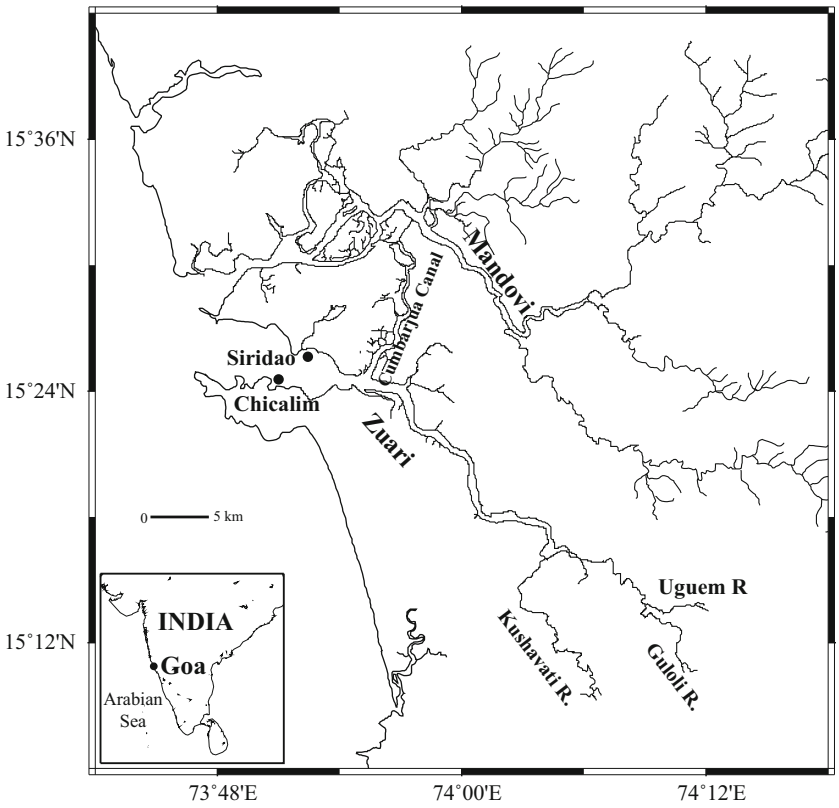


Fig. 1 Map showing location of sampling stations in the Zuari estuary

weight. Sulfanilamide was used as a standard for calibration. The analysis of total organic carbon (TOC) of the sediment was carried out according to El Wakeel and Riley (1957). The analyses were carried out in duplicate and the content is reported as milligrams per gram of sediment. TOC content was converted to the amount of total organic matter (TOM) by multiplying with factor 1.724 (Bhosle and Dhople 1988). Carbohydrates (CHO) in the sediment were estimated following the method by Dubois et al. (1956) using UV-1800 spectrophotometer (Shimadzu). The concentration of proteins (PRT) in the sediment was determined according to Hartree (1972). Lipids (LPDs) were extracted from the sediment using chloroform:methanol (2:1 v/v) mixture (Bligh and Dyer 1959) and estimated according to Barnes and Blackstock (1973). Glucose, bovine serum albumin, and cholesterol were used as calibration standards for CHO, PRT, and LPD, respectively. All these analyses were carried out in triplicate, and the concentration is expressed as milligrams per gram of sediment. The pre-combusted sediments (450–500 °C for 4 h) were used to prepare blanks. The sum of PRT, CHO, and LPD content of sediment was reported as labile organic matter (LOM).

Enumeration of total viable bacterial count in the sediment core

The sediment samples were analyzed by standard spread plate technique for the enumeration of total viable bacterial count (TVC) by using specific media. Wet sediment (1 g) was suspended in filtered, autoclaved seawater (10 ml) and diluted serially. Subsequently, 0.1 ml was spread on Zobell Marine Agar (ZMA) for the enumeration of total viable bacterial count, and these agar plates were incubated for 24 h at room temperature (28 ± 2 °C). The number of colonies were counted after 24 h and are expressed as colony-forming unit (CFU) per gram of sediment.

Enumeration of total bacterial count in the sediment core

Sediment sample (1 g) was suspended in filtered (0.22 μm), autoclaved seawater (10 ml), and fixed with formaldehyde (1–2%). The samples were then sonicated in the water bath sonicator (50% power) for 1 min. This step was repeated 3 times to separate bacterial cells from sediment particles (Luna et al. 2002) and then centrifuged at 3000 rpm for 1 min. Subsequently, the supernatant (1 ml) was mixed with 10 μL of SYBR Green I (Molecular Probes, USA) and incubated in the dark for 15 min at room temperature (Marie et al. 1999). The internal calibration of the instrument was carried out using fluorescent beads (1-μm size). The BD FACSAria™ II Flow cytometer (BD Biosciences, USA), with a nuclear blue laser (488-nm wavelength), was used for the analysis of samples. The events (10,000 nos.) were measured and were gated as SSC versus green fluorescence, and bacterial abundance is expressed as cells per gram of sediment. The BD FACS Diva software was used for data analysis.

Analysis of proteins using MALDI-TOF from the surface sediments

The extraction of proteins from the sediment was carried out using the method described by Wohlbrand et al. (2017) with some modifications. Briefly, 2 g (wet weight) sediment was mixed with 5 ml of SDS-Phenol buffer. This mixture was sonicated on ice at 90% pulsing and 40% energy for 60 s (Ultrasonic Probe Sonicator, Life Care, Mumbai) (Keiblinger et al. 2012). This treatment was repeated twice, followed by shaking for 1 h. Subsequently, homogenates were incubated at 90 °C (20 min) in the water bath with intermittent mixing followed by cooling down on the ice. The residual sediment was then removed by centrifugation at 12,000 rpm at 20 °C (10 min). Supernatants were transferred to another tube, combined, and proteins were precipitated by ammonium acetate (0.1 M) in methanol (fivefold volume), with overnight incubation at − 20 °C. On the next day, pellets were washed with ice-cold acetone, and proteins were recovered by centrifugation. The pellets were then digested overnight (37 °C) with trypsin (Promega). Subsequently, the pellets were resuspended in 100 μl of acetonitrile and stored at − 20 °C, and the aliquots were used for further analysis.

The aliquots (1 μl) were mixed with matrix (saturated solution of α-cyano-4-hydroxy cinnamic acid in 30% acetonitrile with 0.1% trifluoroacetic acid) and this mixture was then overlaid on the 384 target plate (stainless steel), and air-dried. The samples were analyzed using Bruker Ultra-flex MALDI TOF mass spectrometer (Bruker Daltonics, Germany). Peptide calibration standard II and bovine serum albumin (BSA) digest (Bruker Daltonics, Bremen, Germany) were used for calibration. Mass spectra were obtained by the accumulation of laser shots on the sample spot and were processed using Flex Analysis 3.0 software (Bruker Daltonics). Subsequently, selected parent masses were fragmented under the lift method. The BioTools 3.1 software (Bruker Daltonics) and MASCOT search engine (Matrix Science, London, UK) were used to identify the MS/MS spectra. The Mascot search database: NCBInr database in SwissProt, taxonomy: all entries, fixed modification: carbamidomethylation (C), variable modification: oxidation (M), mass tolerance: 100 ppm and digestion enzyme: trypsin.

Enumeration of *P. malabarica* and *M. casta* in the study area

An area of 1 m^2 was selected for the sampling of bivalves from both the stations. The collection of bivalves was carried out during low tide by placing the quadrat in the intertidal

area. The collection of bivalves was carried out in triplicate at each site during different seasons. The sediment samples were collected in a metal sieve (1-mm mesh size and 50-cm diameter), and the individual bivalves were collected, and enumerated, and their abundance is expressed as no. m^{-2}.

Analysis of fatty acids from the sediment core and bivalve tissues

Sediment samples and bivalve tissues were used for the extraction of lipids according to the Bligh and Dyer (1959) method. Samples were sonicated with chloroform:methanol (1:2 v/v) mixture for 20 min. Then, additional chloroform and water (1:1 v/v) were added, mixed well, and kept overnight in separation funnel. On the next day, the lower organic phase was separated and evaporated in a rotary vacuum evaporator. Subsequently, it was re-suspended in the mixture of methanol-toluene (1:1 v/v) and converted to fatty acids methyl esters (FAMEs) with mild alkaline methanolysis method (White et al. 1979). FAMEs were then analyzed using gas chromatography-mass spectrometry (GC-MS, QP-2010, SHIMADZU), with a capillary column (Stabilwax, 30 m × 0.25 mm internal diameter, 0.50-μm film thickness). One microliter of sample was injected by an autosampler and helium gas was used as a carrier. The temperature of the oven was started from 50 °C (maintained for 2 min) and increased to 200 °C with rate of 10 °C min^{-1} and then at 5 °C min^{-1} from 200 to 240 °C. The injector and detector temperature was maintained at 240 °C. FAME mix (Supelco 37 Component, 18,919–1AMP, Sigma-Aldrich, India) was used for the calibration of the instrument. The WILEY7 mass spectral library was used for the qualitative identification of individual FAME. The concentration of FAME has been reported as area percentage.

Data analyses

Spearman's correlation analyses were carried out between sedimentary biogeochemical components (TOC, TC, TN, TS, PRT, CHO, and LPD), grain size composition (sand, silt, and clay), and bacterial abundances (TVC and TBC; $\log x + 1$ transformed) at a significance level of ≤ 0.05. Two-way analysis of variance (ANOVA) was applied for the abundance of bivalves, bacteria (TVC, TBC), and the content of biogeochemical variables (TOC, PRT, CHO, LPD, LOM) of sediments (0–5 cm) with stations and seasons as independent factors. SPSS statistical software program (Version 16) was used to perform correlation and ANOVA. To determine the suitable multivariate analysis, data were processed using detrended correspondence analysis (DCA), and its results showed that the length of the gradient of the first axis was low (< 2.0), suggesting linearity in the data set (ter Braak and Smilauer 2002). The relationship between bivalves, bacteria, and biogeochemical variables was then evaluated using redundancy analysis (RDA) under a reduced model on the set of variables using a forward selection of environmental variables with 999 numbers of permutations. These analyses were carried out using CANACO version 4.5 (terBraak and Verdonschot 1995).

Results

The seawater temperature ranged from 25 to 32 °C at Chicalim and 27 to 31 °C at Siridao. At both the stations, the temperature was low during MON (25 °C and 27 °C at Chicalim and Siridao respectively), moderate during POM (29 °C at Chicalim and 30 °C at Siridao), and high during PreM (32 °C at Chicalim and 31 °C at Siridao). Salinity varied between 12 to 32 at Chicalim and 12 to 29 at Siridao, and it was low during MON at both the stations (12 at both Chicalim and Siridao). During PreM, the salinity was 32 and 29 at Chicalim and Siridao, respectively. The POM season was characterized with moderate salinity, i.e., 28 at Chicalim and 26 at Siridao.

Seasonal variation in the sediment texture

The sediment texture showed a significant (ANOVA; $p < 0.0001$) variation in their composition among Chicalim and Siridao (Fig. 2(a, b); Table 1). At Chicalim, the sediment was dominated by silt followed by sand and clay. The content of sand significantly varied with the seasons (Fig. 2(a); Table 1). The average percentage of sand, silt, and clay at Chicalim was 34.4 ± 9%, 64.9 ± 9%, and 0.7 ± 0.2% during PreM; 30.3 ± 7, 68.9 ± 7, and 0.78 ± 0.8% during MON; and 48.1 ± 2, 50.8 ± 1, and 1.1 ± 2% during POM respectively indicating an increase in the percentage of sand during POM. At Siridao, the sediment was dominated by sand (Fig. 2(b)) and the average percentage composition of sand, silt, and clay was 93.8 ± 2, 5.82 ± 2, and 0.41 ± 0.4% during PreM; 94.3 ± 0.6, 5.32 ± 0.9, and 0.38 ± 0.4% during MON; and 94.9 ± 2, 4.52 ± 2, and 0.6 ± 0.9% during POM respectively. The seasonal variation in the sediment texture was more pronounced in Chicalim than Siridao (Fig. 2(a, b)).

Seasonal variation in chlorophyll *a*

The sediment chlorophyll *a* was high in Chicalim than in Siridao, except during POM season. It was almost similar to both the stations during POM. At Chicalim, the chlorophyll *a* was high during MON (2.58 ± 0.43 μg g^{-1}) followed by POM (2.41 ± 0.75 μg g^{-1}) and PreM (2.11 ± 0.53 μg g^{-1}) (Fig. 3), whereas it was high during POM (2.66 ± 0.19 μg g^{-1}) when compared to PreM (1.73 ± 0.85 μg g^{-1}) and MON (1.27 ± 0.11 μg g^{-1}) seasons in Siridao (Fig. 3; Table 1, ANOVA; $p < 0.043$). The water column chlorophyll *a* was high in Siridao when compared to Chicalim. At Siridao, it was almost similar during PreM (1.54 μg l^{-1}) and POM (1.50 μg l^{-1})

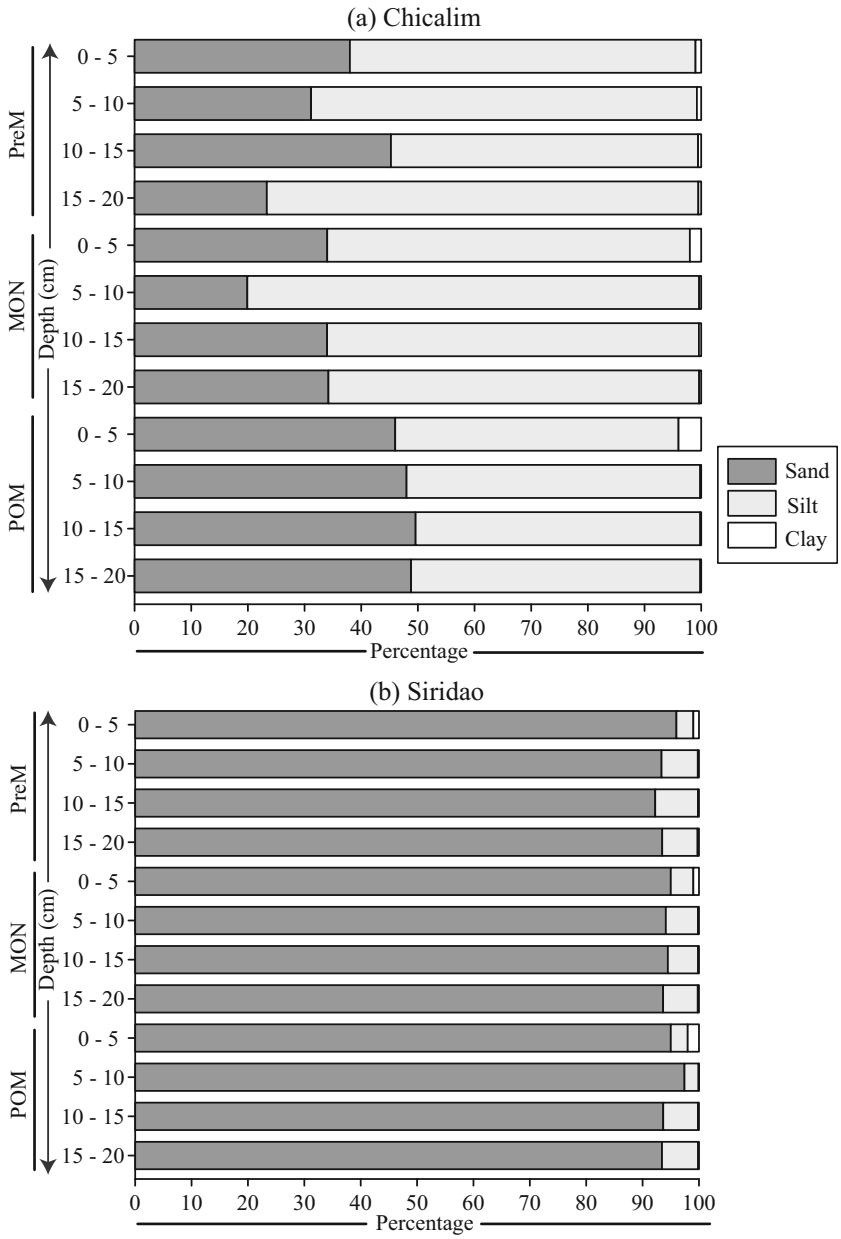


Fig. 2 Seasonal and vertical distribution of sediment texture at the study sites **a** Chicalim and **b** Siridao in the Zuari estuary. (PreM, pre-monsoon; MON, monsoon; POM, post-monsoon)

seasons and low during MON (0.76 μg l^{-1}) season. However, at Chicalim, the water column chlorophyll *a* was similar during POM (1.10 μg l^{-1}) and MON (1.00 μg l^{-1}) and was low during PreM (0.70 μg l^{-1}) season.

Seasonal variation in the elemental, biochemical, and bacterial abundance in the sediment core of Chicalim (southern bank)

The vertical distribution of TC, TN, TS, TOM, TOC, PRT, CHO, and LPD at different depths in Chicalim sediment core (total depth of 20 cm) is shown in Fig. 4(a–i). The elemental and biochemical components of sediments showed significant irregular downcore variations between the seasons (Table 2). The TC content was high during POM compared to other seasons (Fig. 4(a)). The TN was high during MON and it varied between 0.02 and 0.07% sediment dry weight during the study (Fig. 4(b)). The TS content (0.11–0.30%) was high at the surface during MON, whereas it was high at 15–20-cm depth during PreM (Fig. 4(c)). A sharp decline in the sulfur content from the surface to 5–10-cm depth of sediment was observed during MON. The TOC was high during the POM followed by MON and low during PreM, indicating significant seasonal variation in the surface sediments (Table 2). An increase in the TOC and TOM content was evident with depth (0–20 cm) during MON season (Fig. 4(d, e)).

The content of PRT in surface sediment (0–5 cm) was almost similar during all three seasons but, it varied with the

Table 1 Results of two-way ANOVA of biogeochemical parameters, sediment texture, bacterial, and bivalve abundance in the surface sediments (0–5 cm) at Chicalim and Siridao, Zuari estuary

Factors	Parameters	df	MS	*F*	*p* value
Chicalim					
Seasons	TOC	2	0.011	17.216	*0.023*
	PRT	2	0.001	0.627	0.592
	CHO	2	0.031	22.549	*0.016*
	LPD	2	0.006	18.200	*0.021*
	LOM	2	0.005	9.414	0.051
	TVC	2	0.349	235.045	*0.001*
	TBC	2	0.040	36.754	*0.008*
	Sand	2	0.015	33.500	*0.009*
	Silt	2	0.008	8.357	0.059
	Clay	2	0.064	0.997	0.465
	Sed. Chl a	2	0.001	1.147	0.427
	Paphia malabarica	2	0.123	60.648	*0.004*
Siridao					
Seasons	TOC	2	0.001	0.822	0.519
	PRT	2	0.001	1.058	0.449
	CHO	2	0.011	39.941	*0.007*
	LPD	2	0.002	37.333	*0.008*
	LOM	2	0.002	11.700	*0.038*
	TVC	2	1.387	101.710	*0.002*
	TBC	2	0.092	56.214	*0.004*
	Sand	2	0.000	4.500	0.125
	Silt	2	0.028	3.537	0.162
	Clay	2	0.029	3.769	0.152
	Sed. Chl a	2	0.018	10.762	*0.043*
	Meretrix casta	2	0.046	21.543	*0.017*
Stations (Chicalim × Siridao)	TOC	1	0.041	59.756	*0.0001*
	PRT	1	0.811	640.421	*0.0001*
	CHO	1	0.725	879.040	*0.0001*
	LPD	1	0.033	172.565	*0.0001*
	LOM	1	1.159	2.53 + 03	*0.0001*
	TVC	1	3.152	417.007	*0.0001*
	TBC	1	0.005	3.834	0.098
	Sand	1	0.426	1.82 + 03	*0.0001*
	Silt	1	3.597	822.189	*0.0001*
	Clay	1	0.180	5.029	0.066
	Sed. Chl a	1	0.003	2.674	0.153
	Bivalve	1	0.057	27.446	*0.002*
Seasons × stations (sites—Chicalim and Siridao)	TOC	2	0.005	7.402	*0.024*
	PRT	2	0.001	1.125	0.385
	CHO	2	0.029	35.525	*0.0001*
	LPD	2	0.006	32.870	*0.001*
	LOM	2	0.01	21.07	*0.002*
	TVC	2	0.347	45.936	*0.0001*
	TBC	2	0.010	7.387	*0.024*
	Sand	2	0.007	31	*0.001*
	Silt	2	0.014	3.126	0.117
	Clay	2	0.010	0.281	0.764

Table 1 (continued)

Factors	Parameters	df	MS	*F*	*p* value
	Sed. Chl a	2	0.011	9.363	*0.014*
	Bivalve	2	0.010	4.570	0.062

Italicized values indicate significant *p* values; *df* degree of freedom, *MS* mean square, *TOC* total organic carbon, *PRT* proteins, *CHO* carbohydrates, *LPD* lipids, *LOM* labile organic matter, *TVC* total viable count, *TBC* total bacterial count

depth, especially during PreM and POM (ANOVA; $p < 0.0001$, Table 2). During MON, it was almost similar from 0- to 20-cm depth of the core, whereas, during PreM and POM, it decreased sharply at 5–10-cm depth. The PRT content was almost similar between 15- and 20-cm depth during PreM and MON (Fig. 4(f)). The CHO concentration was maximum during MON followed by POM and PreM at the surface, and it was maximum in 15–20-cm depth during MON (Fig. 4(g)). The LPD content ranged from 0.29 to 1.37 mg g^{-1} of dry sediment (Fig. 4(h)). At the surface, the concentration of LPD was maximum during POM followed by MON and PreM. During POM and MON, the LPD content increased with the depth of the core, whereas during PreM, it increased (5–10 cm) and further decreased with the depth of the core (Fig. 4(h)). The PRT, CHO, and LPD showed significant variations between the seasons and depth at Chicalim (Table 2).

The TVC was high at 5–10-cm depth during PreM (1.14 ± 0.4 × 10^6 CFU g^{-1}) and MON (9.94 ± 0.14 × 10^5 CFU g^{-1}) season (Fig. 4(i)), and it varied with the depth between these seasons (Table 2). However, during POM, the TVC was low and almost similar from 0- to 20-cm depth (Fig. 4(i)). The total bacterial count (TBC) was higher during PreM season (Fig. 4(j)), and it was almost similar during MON and POM (Table 3) and did not vary with the depth during different seasons. A decreasing trend in the abundance of TVC and TBC was observed with seasons (PreM > MON > POM) in the Chicalim sediment.

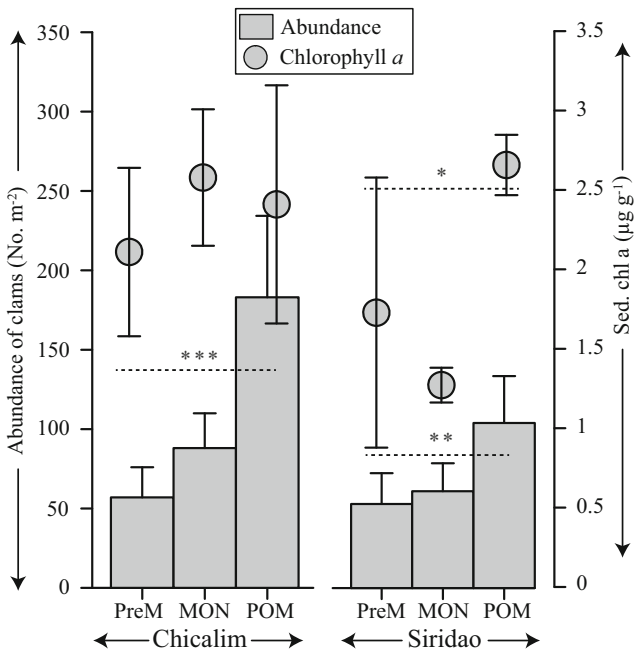


Fig. 3 Seasonal variations in the abundance of bivalves and sediment chlorophyll *a* at Chicalim and Siridao, Zuari estuary (PreM, pre-monsoon; MON, monsoon; POM, post-monsoon). Asterisk (*) and dashed line indicate significant differences among the seasons. (Significance levels—***$p < 0.004$, **$p < 0.017$, *$p < 0.043$)

Seasonal variation in the elemental, biochemical, and bacterial abundance in the sediment core at Siridao (northern bank)

The vertical distribution of TC, TN, TS, TOM, TOC, PRT, CHO, LPD, TVC, and TBC in Siridao sediment core is shown in Fig. 5(a–i). The TC was maximum at the surface during PreM and decreased with the depth; however, it was low at the surface during MON and POM and increased with the depth (Fig. 5(a)). The TS in the sediment varied from 0.06 to 0.26% (Fig. 5(b)), and it was maximum during POM at 10–15-cm depth. The TN content was below the detection level in the sediments at all the depths. The TOM and TOC content in the sediment was higher during POM (Fig. 5(c, d); Table 3) and ranged from 4.16 to 10.9 mg g^{-1} and 2.41 to 6.32 mg g^{-1}, respectively. A decrease in the TOM and TOC was observed during PreM. TOC showed a significant seasonal variation (ANOVA; $p < 0.002$, Table 2).

The concentration of PRT ranged from 0.33 to 0.80 mg g^{-1} of dry sediment weight (Fig. 5(e)), and it was maximum during MON (10–15-cm depth). At the surface, the PRT concentration was higher during PreM and POM, and it decreased with depth (up to 20 cm) (Fig. 5(e)). Significant depth-wise variability in the PRT was observed (ANOVA; $p < 0.024$); however, CHO varied significantly with the seasons (ANOVA; $p < 0.002$, Table 2). The concentration of CHO showed wide variation at the surface (0–5-cm depth) during all the seasons, and it was maximum during MON followed by PreM and POM (Fig. 5(f)). The average CHO content was high during MON (Table 3). The LPD content showed wide seasonal variation at the surface (0–5 cm) and was maximum during PreM followed by POM and MON season (Fig. 5(g); Table 3). It was observed that the LPD content gradually increased with depth during POM and MON (Fig. 5(g)). LPD showed significant seasonal and depth-wise variation at Siridao (Table 2).

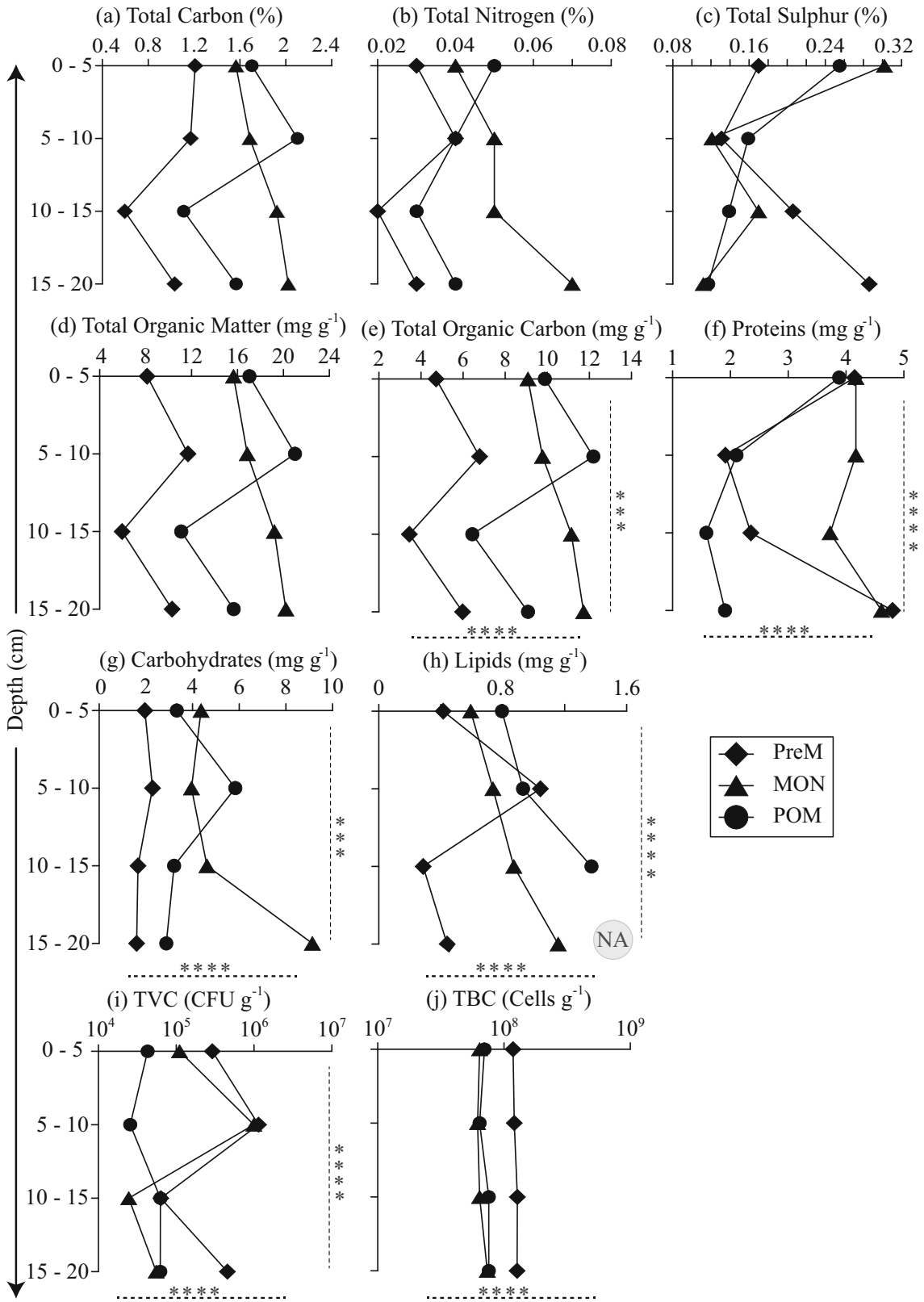


Fig. 4 Seasonal and vertical distribution pattern of sedimentary parameters at Chicalim, Zuari estuary. (TVC, total viable count; TBC, total bacterial count; NA, sample not available; PreM, pre-monsoon; MON, monsoon; POM, post-monsoon). Asterisk (*) indicate significant difference. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels—****$p < 0.0001$, ***$p < 0.001$)

Table 2 Results of two-way ANOVA of biogeochemical parameters and bacterial abundance in the sediment core (0–20 cm) collected from Chicalim and Siridao, Zuari estuary

Factors	Parameters	df	MS	*F*	*p* value
Chicalim					
Seasons	TOC	2	0.038	78.374	*0.0001*
	PRT	2	0.080	59.083	*0.0001*
	CHO	2	0.230	227.764	*0.0001*
	LPD	2	0.016	33.243	*0.0001*
	LOM	2	0.115	164.304	*0.0001*
	TVC	2	1.397	316.064	*0.0001*
	TBC	2	0.174	124.396	*0.0001*
Depth	TOC	3	0.005	10.026	*0.001*
	PRT	3	0.038	28.169	*0.0001*
	CHO	3	0.010	9.636	*0.002*
	LPD	3	0.015	31.707	*0.0001*
	LOM	3	0.008	11.976	*0.001*
	TVC	3	0.671	151.684	*0.0001*
	TBC	3	0.004	2.687	0.094
Seasons × depth	TOC	6	0.003	6.026	*0.004*
	PRT	6	0.021	15.635	*0.0001*
	CHO	6	0.030	29.368	*0.0001*
	LPD	6	0.029	60.768	*0.0001*
	LOM	6	0.024	34.685	*0.0001*
	TVC	6	0.441	99.838	*0.0001*
	TBC	6	0.001	0.428	0.847
Siridao					
Seasons	TOC	2	0.005	11.252	*0.002*
	PRT	2	0.001	0.952	0.413
	CHO	2	0.009	11.639	*0.002*
	LPD	2	0.003	72.444	*0.0001*
	LOM	2	0.003	4.917	*0.028*
	TVC	2	10.085	111.638	*0.0001*
	TBC	2	0.397	257.070	*0.0001*
Depth	TOC	3	0.001	2.628	0.098
	PRT	3	0.003	4.539	*0.024*
	CHO	3	0.000	0.326	0.806
	LPD	3	0.001	24.111	*0.0001*
	LOM	3	0.002	3.335	0.056
	TVC	3	0.119	1.314	0.315
	TBC	3	0.001	0.793	0.521
Seasons × depth	TOC	6	0.001	2.483	0.085
	PRT	6	0.002	3.847	*0.022*
	CHO	6	0.003	3.143	*0.043*
	LPD	6	0.000	9.333	*0.001*
	LOM	6	0.002	3.711	*0.025*
	TVC	6	1.649	18.253	*0.0001*
	TBC	6	0.003	1.850	0.172

Italicized values indicate significant *p* values; *df* degree of freedom, *MS* mean square; refer to Table 1 for abbreviations

The TVC was high in the bottom (15–20 cm) of the core during PreM and MON, whereas it was high at the surface during POM (Fig. 5(h)). The TBC was maximum at 0–5-cm depth during PreM and was almost similar throughout the core (Fig. 5(i)). A significant temporal variability (ANOVA; $p < 0.0001$, Table 2) was observed in the distribution of TVC and TBC, with a decreasing trend (PreM > MON > POM) from PreM to POM (Table 3).

Distribution of proteins in the surface sediments

The mass spectrum analysis of proteins from the surface sediments of Chicalim and Siridao showed seasonal variations. The MS/MS spectra revealed that the proteins in Chicalim sediments were predominantly of anthropogenic origin, whereas those in Siridao were of bacterial origin. The identified proteins were found to involve in different metabolic processes such as glycolysis, protein synthesis, flavonoid, amino acid, and nucleotide metabolism (Table 4).

Seasonal variation in the abundance of *P. malabarica* and *M. casta*

The abundance of bivalve *P. malabarica* at Chicalim was higher than *M. casta* at Siridao during all the seasons (Fig. 3). The higher abundance of *P. malabarica* (183 ± 80 no. m^{-2}) at Chicalim and *M. casta* (104 ± 78 no. m^{-2}) at Siridao was observed during POM season (Fig. 3), and their abundance was low during PreM season. ANOVA revealed a significant seasonal variation in the abundance of *P. malabarica* (ANOVA; $p < 0.004$) than *M. casta* (ANOVA; $p < 0.017$). A significant spatial (between stations) variation (ANOVA; $p < 0.002$) was also observed in the abundance of bivalves (Table 1).

Spatio-temporal and vertical distribution of fatty acids in the sediment

A seasonal variation in the fatty acid composition was observed in the sediments of both the regions, and their composition also varied with the depth of the core. The short-chain even carbon numbered saturated fatty acids (FAs) (SCFA: C12:0, C14:0, C16:0, C18:0, C20:0) were dominant in the sediments at both the sites (Table 5). Long-chain fatty acid (LCFA) C22:0 was also observed in the surficial (0–5-cm depth) sediment of the core during PreM and POM seasons at Chicalim (Table 5). However, at Siridao, C22:0 was dominant between 5- and 10-cm depth during PreM and recorded between 15–20-cm and 5–10-cm depth during MON and POM respectively. The LCFA C24:0 was also found at Chicalim at different depths of the core during PreM and MON (Table 5). At Siridao, C24:0 was dominant between 15 and 20 cm (40.9%) during PreM and was absent during MON and POM seasons (Table 5). Polyunsaturated fatty acid

Table 3 Seasonal variations in the mean (± SD) concentration of elemental and biochemical components of the sediments at the study sites along with two-way ANOVA results

	Chicalim			ANOVA *p* value			Siridao			ANOVA *p* value		
	PreM	MON	POM	S	D	S×D	PreM	MON	POM	S	D	S×D
TC (%)	1.00 ± 0.28	1.80 ± 0.21	1.62 ± 0.41	–	–	–	0.44 ± 0.09	0.54 ± 0.23	0.57 ± 0.11	–	–	–
TN (%)	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	–	–	–	BDL	BDL	BDL	–	–	–
TS (%)	0.20 ± 0.07	0.18 ± 0.09	0.17 ± 0.06	–	–	–	0.11 ± 0.03	0.11 ± 0.04	0.13 ± 0.09	–	–	–
TOM (mg g^{-1})	9.02 ± 2.52	18.0 ± 2.10	16.2 ± 4.09	–	–	–	5.15 ± 0.93	6.14 ± 1.07	8.13 ± 1.99	–	–	–
TOC (mg g^{-1})	5.23 ± 1.46	10.4 ± 1.22	9.40 ± 2.37	*0.0001*	*0.001*	*0.004*	2.99 ± 0.54	3.56 ± 0.62	4.71 ± 1.15	*0.002*	0.098	0.085
PRT (mg g^{-1})	3.30 ± 1.39	4.17 ± 0.36	2.37 ± 1.03	*0.0001*	*0.0001*	*0.0001*	0.49 ± 0.11	0.48 ± 0.21	0.50 ± 0.05	0.413	*0.024*	*0.022*
CHO (mg g^{-1})	1.88 ± 0.31	5.52 ± 2.43	3.81 ± 1.36	*0.0001*	*0.002*	*0.0001*	0.35 ± 0.05	0.48 ± 0.16	0.28 ± 0.14	*0.002*	0.806	*0.043*
LPD (mg g^{-1})	0.55 ± 0.34	0.84 ± 0.24	1.03 ± 0.30	*0.0001*	*0.0001*	*0.0001*	0.30 ± 0.05	0.19 ± 0.06	0.27 ± 0.04	*0.0001*	*0.0001*	*0.001*
LOM (mg g^{-1})	5.73	10.5	7.21	*0.0001*	*0.001*	*0.0001*	1.14	1.16	1.06	*0.028*	0.056	*0.025*
TOC/TN	17.5 ± 1.77	20.3 ± 2.76	23.6 ± 4.74	–	–	–	NA	NA	NA	–	–	–
PRT/CHO	1.76	0.76	0.62	*0.0001*	*0.0001*	*0.0001*	1.38	1.00	1.76	*0.0001*	0.136	*0.001*
LPD/CHO	0.29	0.15	0.27	*0.0001*	*0.0001*	*0.0001*	0.85	0.40	0.97	*0.0001*	0.094	*0.004*
PRT:LOM (%)	57.7	39.6	32.8	*0.0001*	*0.0001*	*0.0001*	43	41.8	47.2	*0.033*	0.087	*0.014*
CHO:LOM (%)	32.8	52.4	52.9	*0.0001*	*0.0001*	*0.0001*	30.7	41.6	26.8	*0.0001*	0.461	*0.003*
LPD:LOM (%)	9.5	8.0	14.3	*0.004*	*0.0001*	*0.0001*	26.3	16.6	26.0	*0.0001*	*0.006*	*0.038*
TVC (× 10^5 CFU g^{-1})	4.88 ± 4.65	2.96 ± 4.67	0.48 ± 0.18	*0.0001*	*0.0001*	*0.0001*	2.96 ± 1.87	0.15 ± 0.27	0.08 ± 0.11	*0.0001*	0.315	*0.0001*
TBC (× 10^8 cells g^{-1})	1.23 ± 0.05	0.66 ± 0.05	0.71 ± 0.06	*0.0001*	0.094	0.847	1.27 ± 0.01	0.06 ± 0.01	0.05 ± 0.04	*0.0001*	0.521	0.172
TVC:TBC	0.40	0.45	0.07	–	–	–	0.23	0.28	0.16	–	–	–
Bivalve density (No m^{-2})	57 ± 19	88 ± 22	183 ± 51	*0.004*	–	–	53 ± 19	61 ± 18	104 ± 29	*0.017*	–	–

TC total carbon, *TN* total nitrogen, *TS* total sulfur, *TOM* total organic matter, *TOC* total organic carbon, *PRT* proteins, *CHO* carbohydrates, *LPD* lipids, *LOM* labile organic matter, *TVC* total viable count, *TBC* total bacterial count, *BDL* below detection limit, *NA* not applicable, *PreM* pre-monsoon, *MON* monsoon, *POM* post-monsoon, *S* seasons, *D* depths; italicized values indicate significant *p* values

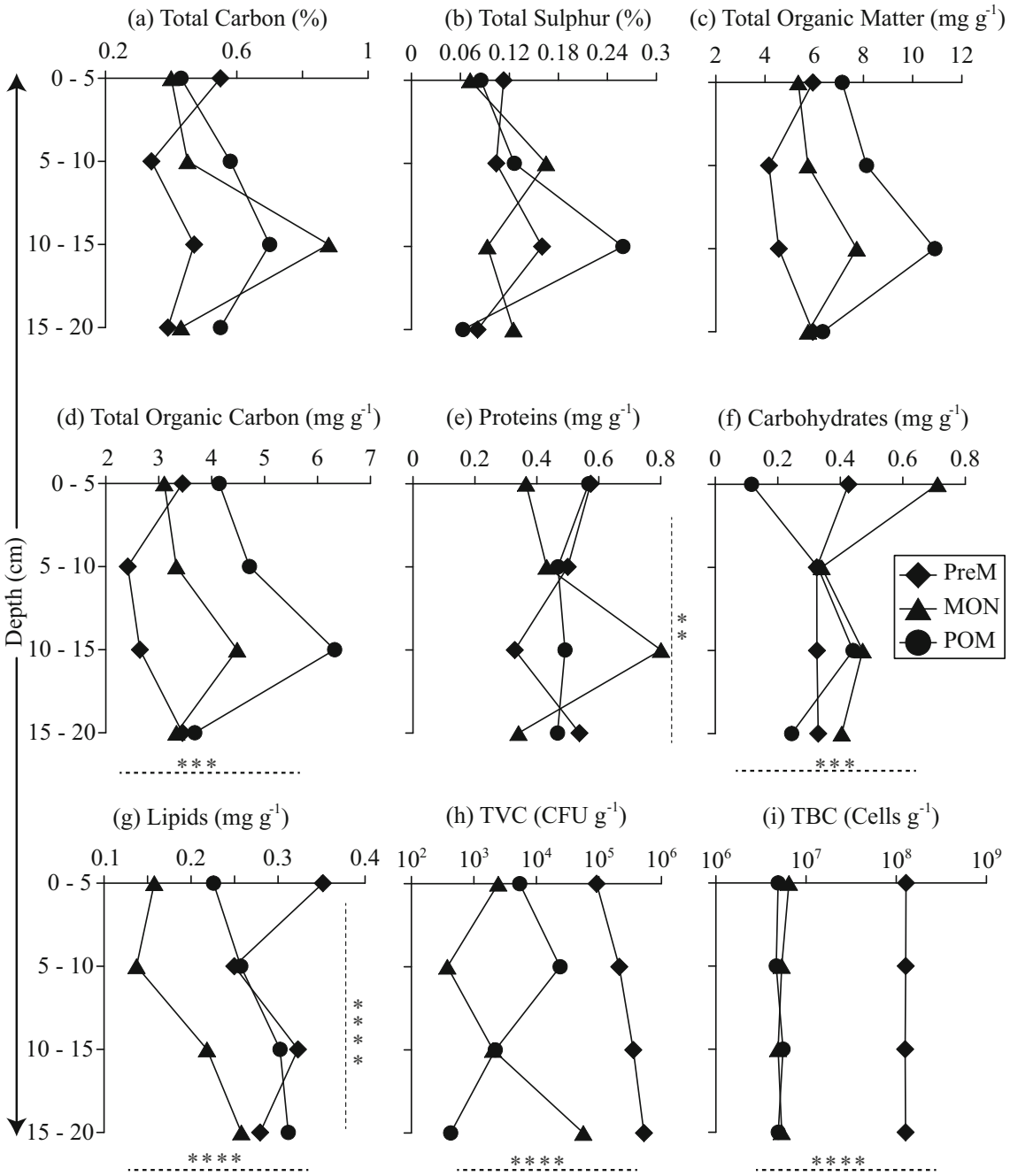


Fig. 5 Seasonal and vertical distribution pattern of sedimentary parameters at the Siridao, Zuari estuary. (TVC, total viable count; TBC, total bacterial count; PreM, pre-monsoon; MON, monsoon; POM, post-monsoon). Asterisk (*) indicate significant differences. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels—****p < 0.0001, ***$p < 0.002$, **$p < 0.024$)

(PUFA) C18:2ω6 was dominant between 0 and 10 cm at Chicalim, whereas in Siridao, it was dominant at 5–10 cm during PreM. The PUFA C18:3ω3 was observed at Siridao during PreM and at Chicalim during POM season (Table 5). Monounsaturated fatty acid (MUFA) C22:1ω9 was recorded in the surficial sediment at Chicalim and at Siridao from 5 to 10 cm during the PreM season. Another MUFA, C24:1ω9, was observed exclusively to Chicalim (Table 5).

Bacteria-specific FA such as C15:0 (6.4%) was observed between 10 and 15 cm at Chicalim during PreM and throughout the core except at surface during MON. It was also recorded at 5–15-cm depth during POM. While at Siridao, C15:0 was observed in the surface sediments during all the seasons and from 10 to 15 cm during MON. The FA C17:0 was observed during PreM, MON, and POM seasons, however, at different depths of the core (Table 5).

Table 4 Name of some proteins identified by MALDI TOF/TOF-MS, and their sources and functions

Source	Peptide ion	Name of protein	Source organisms	Function
SR-PreM	1336.343	50S ribosomal protein L7/L12	*Azorhizobium caulinodans*	Protein synthesis (Gudkov 1997; Smit et al. 2012)
SR-MON	1008.757	Putative quercetin 2,3-dioxygenase PA1205	*Pseudomonas aeruginosa*	Flavonoid metabolism (Pillai and Swarup 2002)
SR-POM	768.645	Amino acid acetyl transferase	*Yarrowia lipolytica*	Acetylation (Smit et al. 2012)
SR-POM	936.739	Serine hydroxymethyl transferase	*Mycoplasma genitalium*	Amino acid metabolism (Lin et al. 2015)
CH-PreM	877.395	Glutaminase	*Alkaliphilus oremlandii*	Amino acid metabolism (Smit et al. 2012)
CH-PreM	666.199	Heavy metal–dependent transcription regulator	*Rhizobium meliloti*	Transcriptional regulator (Reeve et al. 2002)
CH-PreM	877.395	Nucleoid-associated protein ybaB	*Salmonella paratyphi*A	Organization and compaction of DNA (Dame 2005)
CH-MON	954.310	Phosphoglycerate kinase	*Leptospira borgpetersenii*	Glycolysis (Lin et al. 2015)
CH-POM	877.188	Regulator of microtubule dynamics protein-2	*Macaca fascicularis*	Membrane protein (Riederer et al. 1997)
CH-POM	954.319	Uncharacterized protein ORF 47	*Ostereid herpervirus*1	*Ostereid herpervirus*1 involved in mass mortality of oyster *Crassostrea gigas* (Renault et al. 2014)

SR Siridao, *CH* Chicalim, *PreM* pre-monsoon, *MON* monsoon, *POM* post-monsoon

Seasonal variation of FAs in the tissues of *P. malabarica* and *M. casta*

The FAs reported in *P. malabarica* and *M. casta* differed between seasons, and some of the FAs were species-specific. During PreM season, FAs C14:0, C16:0, and C18:1ω9 were common to both the species, whereas the FAs C20:4, C18:0, C16:1ω7, and C24:0 were specific to *P. malabarica* and C12:0 and C20:0 were specific to *M. casta*. During MON season, C12:0 was observed in the tissues of *P. malabarica* and *M. casta*. However, FAs such as C15:0, C16:1ω7, and C18:2ω6 were specific to *P. malabarica* and C17:0, C18:1ω9, and C18:3ω3 were specific to *M. casta*. The POM season was characterized by the presence of short-chain FAs such as C12:0, C16:0, and C16:1ω7 in the tissues of both *P. malabarica* and *M. casta.* The FAs C18:0 and C20:0 were specific to *P. malabarica* and C17:0 and C18:1ω9 were specific to the tissues of *M. casta* (Table 5). However, the percentage of FAs varied with the species and seasons (Table 5).

Discussion

The distribution and nature of sedimentary OM

This study investigated the impact of different sedimentary parameters on the partitioning of two different bivalves on the banks of Zuari, a tropical monsoon-influenced estuary. A marked difference in the sediment grain size was evident between the two sites. At Chicalim, the sediment was rich in silt followed by sand with poorly sorted grains, whereas in Siridao, sediment was dominated by sand, and these differences could be attributed to tidal currents and water flow in the estuarine system. The tidal (ebb and flood) currents are reported to be stronger in the Zuari estuary (Manoj and Unnikrishnan 2009). Sundar et al. (2015) reported longitudinal flow in the channel and cross-shore flow at the estuarine mouth of the Zuari and the magnitude and duration of ebb and flood flow was nearly similar during pre- and post-monsoon seasons; however, during monsoon, an increase in the magnitude and duration of ebb flow is observed as compared to flood flow owing to discharge of a large amount of freshwater. It is also reported that the flow of coastal water is directed towards the northern bank of the estuary (Siridao) during high tide and towards the south side (Chicalim) when the water flushes out during low tide (Shirodkar et al. 2012). Such a flow pattern might possibly wash out fine particles, leaving behind the medium to fine sand particles in the sediment of Siridao. The habitat in Chicalim being sheltered, the impact of water flow during low tide will be comparatively less than that at Siridao, and this will not disturb the fine particles (silt and clay) in the sediment of Chicalim. The high TOC content at Chicalim compared to Siridao could be attributed to the accumulation of fine particles and detrital matter from the water column in the sediment. The fine-grain sediments with large surface areas (Meyers 1997) have high adsorptive capacity due to close hydraulic equivalence for the organic molecules (Cotano and Villate 2006). Rao et al. (2011), while studying the suspended sediment dynamics, indicated that suspended particulate matter (SPM) was high during high rainfall and low salinity and also during moderate/low rainfall peaks with high

Table 5 Seasonal variations in the composition and area percentage of fatty acids (FAs) in the sediment core and bivalve tissues collected from Chicalim and Siridao located in the Zuari estuary

Depth (cm)	Pre-monsoon				Monsoon				Post-monsoon			
	Chicalim		Siridao		Chicalim		Siridao		Chicalim		Siridao	
	FA	Area %	FA	Area %	FA	Area %	FA	Area %	FA	Area %	FA	Area %
0–5	C18:0	42.6	C12:0	15.2	C14:0	9.32	C12:0	27.8	C12:0	1.65	C12:0	13.2
	C18:2ω6	19.8	C15:0	9.01	C20:0	12.7	C15:0	9.94	C16:0	12.5	C15:0	6.16
	C22:0	4.19	C16:0	15.7	C24:0	31.8	C16:0	5.14	C20:0	6.78	C16:0	12.1
	C22:1ω9	19.9	C18:0	6.71	C24:1ω9	10.8	C18:0	3.01	C22:0	3.85		
5–10	C12:0	0.90	C16:0	0.30	C12:0	1.54	C12:0	14.9	C12:0	0.29	C12:0	0.75
	C16:0	2.22	C18:2ω6	4.55	C15:0	11.3	C16:0	3.76	C13:0	0.88	C16:0	3.24
	C18:0	64.3	C18:3ω3	0.69	C16:0	4.71	C17:0	5.18	C15:0	0.17	C18:0	11.8
	C18:2ω6	31.1	C22:0	38.8			C18:0	2.87	C20:0	0.48	C22:0	4.54
			C22:1ω9	21.0					C22:0	80.2		
			C23:0	19.8								
10–15	C12:0	9.67	C12:0	4.38	C12:0	13.7	C12:0	11.9	C12:0	0.49	C12:0	3.06
	C15:0	6.40	C16:0	10.5	C14:0	12.3	C15:0	11.4	C15:0	8.44	C17:0	0.88
	C16:0	2.86	C17:0	13.9	C15:0	12.9	C16:0	1.44	C16:0	2.44	C18:0	9.88
			C18:3ω3	4.39	C18:0	2.34	C18:0	2.93	C18:0	6.22	C20:0	4.01
											C21:0	7.53
15–20	C12:0	19.3	C12:0	1.80	C12:0	0.29	C12:0	1.02	C12:0	10.7	C12:0	6.53
	C14:0	9.52	C14:0	1.18	C15:0	1.96	C16:0	0.46	C15:0	1.56	C16:0	8.19
	C23:0	10.6	C16:0	3.06	C16:0	13.8	C17:0	1.20	C16:0	7.75	C17:0	5.42
	C24:0	22.3	C17:0	9.67	C18:0	3.98	C22:0	6.65	C18:2ω6	3.77	C18:0	7.52
	C24:1ω9	10.4	C24:0	40.9	C24:0	2.81					C20:0	6.02
Bivalve	PM		MC		PM		MC		PM		MC	
	C14:0	3.51	C12:0	23.5	C12:0	9.01	C12:0	13.4	C12:0	11.2	C12:0	6.90
	C16:0	22.6	C14:0	1.78	C15:0	3.54	C17:0	5.82	C16:0	14.9	C16:0	10.3
	C16:1ω7	4.82	C16:0	11.8	C16:1ω7	2.34	C18:1ω9	2.71	C16:1ω7	3.51	C16:1ω7	1.11
	C18:0	6.53	C18:1ω9	4.01	C18:2ω6	2.95	C18:3ω6	2.70	C18:0	8.20	C17:0	4.42
	C18:1ω9	2.70	C20:0	1.39					C200	4.20	C18:1ω9	1.73
	C20:4ω6	40.5										
	C24:0	1.66										

PM Paphia malabarica, *MC Meretrix casta*)

salinity during non-monsoon seasons. Overall, the content of SPM in the channel stations was lower during post and pre-monsoon when compared to monsoon season. However, SPM was maximum in the bay stations (stations located near the Siridao and Chicalim), and this might influence the OM distribution and availability. Thus, higher TOC at Chicalim as compared to Siridao could be attributed to sediment texture and the estuarine hydrodynamics. The water column depth at both the stations is shallow and range between 1 and 3 m. Since Zuari is a shallow estuary, combined effect of wind-generated and ebb currents during the neap tide may cause resuspension of sediments owing to shallow bottom (Rao et al. 2011) and such a phenomenon will influence the OM dynamics in the bivalve habitats which will benefit the deposit feeders (*P. malabarica*) rather than suspension feeders (*M. casta*). A significant negative correlation of TOC with sand ($r = -0.594$; $p < 0.002$) and positive correlation with silt ($r = 0.601$; $p < 0.002$) reveals higher accumulation of OM in Chicalim sediment which is rich in silt. The TC content was also high in Chicalim compared to Siridao, indicating that sediment grain size composition plays a significant role in the distribution of organic molecules in sediments, and such condition is conducive for deposit feeders as they obtain food from the sediment organic matter for their survival and growth. The specificity and higher abundance of *P. malabarica* at Chicalim can be attributed to such habitat characteristics.

The redox status of benthic habitat is also crucial as it provides information on the availability of oxygen for the benthic life, and can be determined using TOC to TS ratio (Raiswell et al. 1988). When TOC to TS ratio > 5, the sediments have been categorized as oxic, TOC:TS ratio between 1.5 and 5 indicates sediment under periodic anoxic condition, and TOC:TS < 1.5 indicate sediment is anoxic (Raiswell et al. 1988; Akhil et al. 2013; Salas et al. 2015). The sediment from Siridao showed periodic anoxic conditions with low TOC:TS ratio (PreM: 2.6, MON: 3.1 and POM: 3.5) when compared to Chicalim (PreM:2.6, MON: 5.9 and POM: 5.6). Rasheed et al. (2003) reported higher solute exchange and permeability to enhance the organic carbon decomposition and oxygen consumption rate (threefold) in the coarse sand when compared to the silt-clay sediments. Shallow water also enables UV radiation to reach the sediment surface, which causes damage to the aquatic organisms and reduces the photosynthetic uptake of atmospheric CO_2 and the productivity of sediments (Hader et al. 2007). The tides in the study area are also mixed type, ranging ~ 2.3 m during spring and 1.5 m during the neap tide (Manoj and Unnikrishnan 2009). The habitat at Siridao, being a shallow tidal mudflat, gets exposed to sunlight for a longer duration during low tide when compared to Chicalim. This might reduce the photosynthetic oxygen production. The Naval Hydrographic Chart of 2003 shows a well-developed mudflat that gets entirely exposed during the low tide. These could be the probable reasons for periodic anoxic conditions as indicated by the TOC:TS ratio at Siridao compared to Chicalim and can be attributed to habitat characteristics. However, such a condition (periodic anoxia in the sediment) may not impact the abundance of *M. casta* as it is a suspension feeder and can obtain dissolved oxygen from seawater during the high tide. At Chicalim, low TOC:TS ratio during PreM indicate periodic anoxic condition, whereas oxic condition during MON and POM seasons makes the habitat favorable for the deposit feeders. It has been reported that the breeding season commences in September, and peak spawning season is from October to December (POM) (Mohite and Mohite 2009) and during this season, the sediment in Chicalim being oxic seems to be optimum for breeding and/or spawning of bivalves.

The productivity of the benthic system has been described earlier using sedimentary PRT content (Danovaro et al. 1999; Dell'Anno et al. 2002), and PRTs are the most important source of nitrogen for benthic invertebrates (Danovaro et al. 1993; Fabiano et al. 1995). PRTs showed strong negative correlation with sand ($r = -0.792$; $p < 0.001$) and positive correlation with silt ($r = 0.779$; $p < 0.001$) and clay ($r = 0.434$; $p < 0.034$) suggesting an important role of sediment grain size in the accumulation of PRT. The dominance of PRT at Chicalim during PreM (Fig. 4(f)) indicates fresh OM, which could be contributed either by benthic primary production (Fabiano et al. 1995) or by deposition of phytoplankton from the water column (Pusceddu et al. 2003). Higher PRT content during PreM will benefit *P. malabarica*, as their peak somatic period is from February to April. Low protein content during POM coinciding with higher abundance can be attributed to assimilation by the bivalves as they attain the harvestable size during this season.

The chlorophyll *a* in the surface sediment was almost similar at both the sites, but the content of PRT was high at Chicalim which can be attributed to the accumulation of large amounts of OM derived from the water column phytoplankton. Diatoms are an important component of the microphytobenthos population, which account for nearly 50% of primary production in the estuarine benthic environments (Underwood and Kromkamp 1999). The annual microphytobenthos production in the Zuari-Mandovi estuarine system was found to range from 34.6 to 52.7 g C m^{-2} $year^{-1}$ (Ansari and Parulekar 1994). Krishna Kumari et al. (2002) reported high primary production and abundance of phytoplankton cells in the water column during PreM in the Zuari estuary. Chicalim is influenced by different anthropogenic activities such as shipbuilding workshops and yards, barge cleaning, and repairing, whereas Siridao is relatively pristine when compared to Chicalim (Khandeparker et al. 2017). Studies have pointed out high sediment PRT content

in the industrial and anthropogenically influenced sites in the estuarine and coastal systems (Cotano and Villate 2006; Muniz et al. 2011; Venturini et al. 2012a; Salas et al. 2015). Relatively high (~ 7 times higher) PRT concentration at Chicalim than Siridao sediment suggests anthropogenic inputs, and the results are also supported by MALDI-TOF mass spectrum analysis. However, irrespective of the source, higher availability of protein in the sediment in the case of Chicalim will benefit the deposit-feeding benthic invertebrates. The input of terrestrially derived materials in the Chicalim sediment is also supported by the predominance of CHO during MON and POM. This was also indicated by TOC/TN ratio, which has been used to discriminate the OM sources into the marine- and terrestrial-derived matter (Meyers 1994). The sedimentary OM characterized by low TOC/TN ratios (4–10) indicates a contribution from bacteria and plankton, whereas high TOC/TN ratios (> 20) suggests input from terrestrial plant material (Meyers 1994). The minimum TOC/TN ratio during PreM and maximum during POM at Chicalim indicate the contribution of OM from both marine and terrestrial origin to the sediments during PreM and further increase in terrestrial OM inputs during MON and POM. The surface runoff through the river basin during MON acts as a source of terrestrial materials in the estuarine sediment. The contribution of CHO to the labile organic matter (LOM) at Siridao also increased during MON (41.6%) compared to PreM (30.7%), indicating the input of terrestrial matter. However, relatively high CHO (~ 10-fold) content at Chicalim attributed to semi-enclosed nature and such a condition would support *P. malabarica* (Table 3).

During all the seasons, the PRT/CHO ratio was more than 1 at Siridao, and it was maximum during POM followed by PreM and MON. However, at Chicalim, the PRT/CHO ratio was high during PreM (1.76), and it was less than 1 during MON and POM. The PRT/CHO ratio has been used to differentiate the fresh or aged OM (Danovaro et al. 1993) in the sediment. PRT/CHO ratio > 1 indicates newly or recently generated OM and the ratio < 1 suggests the presence of non-living or aged detrital material (Danovaro et al. 1993). This indicates that at Siridao, the OM was newly generated, whereas at Chicalim, it was degraded-detrital and heterotrophic in nature. Low ratios also suggest their utilization as indicated by the higher abundance of *P. malabarica*. This was also supported by observations carried out at Dona Paula Bay, which is situated at the Zuari estuarine mouth, as sedimented particulate matter flux in the sediment trap was relatively fresh during summer, and detritus rich during the winter season (Bhaskar et al. 2000). The high percentage of TVC (viable heterotrophic bacteria) to TBC during MON compared to non-monsoon seasons at both the sites suggests their role in allochthonous OM processing. Studies on the water column primary production and bacterial carbon demand from the station located near Chicalim revealed a shift from an autotrophic system during PreM to the heterotrophy during MON and POM seasons (De Souza et al. 2003). A recent study by Eswaran and Khandeparker (2017) reported a high rate of bacterial production as well as the change in the bacterial community in the surface water in the inner region of this estuary during MON, which was found to be fuelled by the allochthonous material of riverine origin. These observations also support net heterotrophy during MON season in the Zuari estuary. The Chicalim being a low-energy area will benefit the deposition of OM of allochthonous origin, a condition favorable for the growth of *P. malabarica*.

Lipids (LPDs) are useful to indicate the detrital input derived from the water column and benthic production (Muniz et al. 2011), and LPD content has been used to determine the nutritional quality of sedimentary OM (Gremare et al. 2002). Although the PRT, CHO, and LPD were high in Chicalim sediment, the percentage contribution of LPD to the LOM was high at Siridao (on an average 23%) when compared to Chicalim (10.6%) indicating nutritionally rich detrital input at Siridao (Table 3). This probably helps the larger (harvestable size 21–37 cm) filter-feeding *M. casta* while siphoning the seawater from just above the sediment surface during ebb and flood tide. However, how much of this is actually available to the bivalves needs further validation. High PRT/CHO and LPD/CHO ratios at Siridao compared to Chicalim indicate labile and nutritionally rich OM. However, lower ratios during MON suggest lower productivity. Previous studies on the carbon and nitrogen isotopic composition of suspended particles from the Zuari estuary indicated terrestrial-derived OM during southwest monsoon (SWM) and in situ–produced OM during non-monsoon seasons (Bardhan et al. 2015; Shynu et al. 2015). The high flushing rate and high turbidity in the water column leading to lower light intensity were responsible for low autochthonous production during the period of SWM (Bardhan et al. 2015). The contribution of LOM to the TOM decreased during MON and POM seasons as compared to PreM, indicating an increase in the refractory OM in the sediments of Chicalim and Siridao. Recent study on TOC/TN ratio at the Zuari estuary also reported mixed input of marine and terrestrial OM at Chicalim (C/N ratio: 16.56) and autochthonous OM at Bambolim (C/N ratio: 8.7) as well as Agacaim (C/N ratio: 10.2), the stations located on the either side of Siridao along the northern bank of Zuari estuary (Gardade and Khandeparker 2017). Such autochthonous OM could be derived from microphytobenthos, which in turn benefit the suspension feeders.

Thus, relatively high content of elemental and biochemical components at Chicalim as compared to Siridao could be attributed to the semi-enclosed nature, sediment grain size, and the influence of anthropogenic input. It seems that the degraded-detrital and aged OM of low quality accumulated in Chicalim sediment are available for *P. malabarica* as compared to recently generated fresh OM with better quality and

high nutritional value found in Siridao sediment which possibly helps the suspension feeders during the high water.

Sources of fatty acids in the sediments

The study region is influenced by the tides, waves, and winds causing re-suspension of sediment and the churning of water, which could be attributed to the patchy distribution of FAs in the sediment core. The sources of organic matter were identified based on the FA biomarkers (Table 6). The short-chain saturated FAs (C12–C20) are the indicators of phytoplankton in the marine environment (Carrie et al. 1998; Hu et al. 2006; Guo et al. 2019); however, long-chain FAs (C22–C30) are indicators of vascular plants (Wakeham and Beier 1991; Meyers 1997; Meziane and Tsuchiya 2000; Dunn et al. 2008; Guo et al. 2019). The dominance of SCFAs at Chicalim and Siridao suggested input of phytodetritus in the sediment core, as SCFAs are less susceptible to microbial degradation and indicate deposition of partially degraded aged OM in sediments (Dunn et al. 2008). Sediment trap studies at the Dona Paula Bay have revealed ~ 61% of particulate matter of primary production gets mineralized in the water column and remaining ~ 39% reach to the sediment (Bhaskar et al. 2000). Further, large amount of sedimented carbon is either consumed (98%) at the sediment-water interface or re-suspended before burying into the sediment. A recent study on lipid biomarkers from the Pearl River estuary reported that phytoplankton-derived labile OM undergoes microbial degradation during sinking through the water column as well as in the surface of the sediment; however, terrestrial OM which is refractory in nature gets enriched in the sediments (Guo et al. 2019). The vascular plant-specific FAs (C22:0 or C24:0) were dominant during the PreM at Siridao and during MON and POM seasons at Chicalim, indicating deposition of plant material, which will add to the quality of food available for the deposit feeders. The banks of Mandovi-Zuari Rivers and the connecting canal (Cumbarjua) between these estuaries are lined with mangroves, and other terrestrial plants and Cumbarjua canal supplies additional water and sediment to the Zuari estuary during MON and ebb tides (Dessai et al. 2009). Wafar et al. (1997) reported the maximum litter fall of mangroves during the PreM and POM seasons in the Mandovi-Zuari estuarine systems. Though mangrove vegetation is found near Siridao, the material derived from the mangroves is expected to be exported out to other regions of the estuary due to the hydrodynamics operating at Siridao, resulting in lower content of LCFA during the MON and POM seasons compared to the PreM. The PUFAs C18:2ω6 and C18:3ω3 have been used as biomarkers of mangroves, which are found in the leaves of mangrove plants (Méziane et al. 2007). The detection of LCFAs and PUFAs confirmed the contribution of mangrove-derived OM in the sediments of the Zuari estuary, and this might benefit the benthic organisms. These PUFAs have also been used as biomarkers for green macroalgae, and their presence indicates fresh detritus inputs to the sediment (Carrie et al. 1998; Meziane and Tsuchiya 2000). The long-chain PUFAs C22:6ω3 and C20:5ω3 have been used as typical dinoflagellates and diatom biomarkers, respectively (Volkman et al. 1980, 1989; Carrie et al. 1998; Zimmerman and Canuel 2001). Although previous study has reported presence of these PUFAs in the surface sediments of the Zuari estuary (Harji 2011), these PUFAs were not detected in the present study indicating their degradation either by bacteria or by its utilization by the higher organisms via grazing (Carrie et al. 1998; Hu et al. 2006; Dunn et al. 2008).

The MUFAs are commonly found in algae, zooplankton, and bacteria. The FA C22:1ω9 is used as a specific biomarker of zooplankton (Venturini et al. 2012b) and was found at both the stations during the PreM, and the FA C24:1ω9, a zooplankton biomarker (Carrie et al. 1998), was reported at

Table 6 Fatty acid biomarkers used to identify the sources of the organic matter

Fatty acids (FAs)	Sources of FAs	References
C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0, C18:1ω7	Bacteria	Volkman et al. 1989; Canuel and Martens 1993; Rajendran et al. 1995 Carrie et al. 1998; Meziane and Tsuchiya 2000; Zhukova 2005
C14:0, C16:1ω7, C20:4ω6, C20:5ω3	Diatoms	Volkman et al. 1980, 1989; Carrie et al. 1998; Meziane and Tsuchiya 2000
C18:1ω9, C18:4ω3, C22:6ω3	Dinoflagellates	Carrie et al. 1998; Zimmerman and Canuel 2001
C20:1ω9, C22:1ω9, C24:1ω9	Zooplankton	Carrie et al. 1998; Venturini et al. 2012b
C16:4ω3, C18:2ω6, C18:3ω3, C18:3ω6	Macroalgae	Volkman et al. 1989; Meziane and Tsuchiya 2000
C18:3ω3, C18:2ω6	Mangroves	Bachok et al. 2003; Méziane et al. 2007
Short-chain fatty acids (C12:0–C20:0)	Phytoplankton	Meyers 1997; Hu et al. 2006; Dunn et al. 2008; Guo et al. 2019
Long-chain fatty acids (C22:0–C30:0)	Higher plants	Meyers 1997; Meziane and Tsuchiya 2000; Dunn et al. 2008; Guo et al. 2019

i iso, *a* anteiso)

Chicalim during PreM and MON seasons. The benthic zooplankton, Mysids, and Cumaceans were reported from the Mandovi-Zuari estuarine system (Padmavati and Goswami 1996), which are mostly benthic, living on the surface of the sediments or even burrow into the sediments and also found swimming in the water column (Porter 2016). These are generally omnivorous and can feed on algae, detritus, and zooplankton. The Cumaceans feed on microorganisms and organic material from the sediment and can compete for the food from the sediment with *P. malabarica*. The reduction in salinity due to a large amount of freshwater input into the estuary during the SWM may cause death and decay of the zooplankton, adding to the organic pool.

The studies on the microbial community from the surface sediment of Chicalim revealed the dominance of specific group, i.e., *Gammaproteobacteria* at Chicalim (91%) than Siridao (55.9%) indicating less diversity at the contaminated site than relatively pristine site (Khandeparker et al. 2017). The *Gammaproteobacteria* have been reported to play a significant role in the sulfur cycling as well as in the degradation of OM which is derived from phytoplankton in the marine sediments (Asami et al. 2005; Gihring et al. 2009; de Moraes et al. 2014) resulting in readily available food for the deposit feeders.

Seasonal variations in the diet of bivalves

Bivalves are filter feeders and are generally found in the estuarine sediments (Harkantra 1975; Nair et al. 1984; Parulekar et al. 1984; Alexander et al. 1993; Narasimham and Laxmilatha 1966). On examination of the gut content of *P. malabarica*, granules of sediment particles as well as microphytobenthos were observed, whereas *M. casta* take their food from the water column by filtering the seawater and such filter-feeding habit is responsible for the accumulation of particles inside their body (Al-Mossawi et al. 1983). The abundance of these bivalves in Siridao (*M. casta*) and Chicalim (*P. malabarica*) also coincided with the chlorophyll *a* in the sediment, further pointing towards the importance of food from the sediment for these bivalves. Though *Paphia malabarica* is dominant at Chicalim, their abundance was higher than *M. casta* which are dominant at Siridao. It can be noted that the harvestable size of *P. malabarica* is smaller (19–30 mm) than the harvestable size (21–37 mm) of *M. casta*. This indicates that the density of bivalves depends upon the space available for their growth, and will result in competition for food. Since *M. casta* is a suspension feeder and can filter their food, mainly phytoplankton from the water column, it might experience comparatively lower competition for food when compared to *P. malabarica*.

The FAs from the tissues of the bivalves varied with the seasons, and some were species-specific. During PreM, the FA C20:4ω6 was dominant (40.5%) in the tissue of *P. malabarica*. This FA is found dominant in the diatoms along with C20:5ω3, C14:0, and C16:1ω7, and are used as biomarkers of diatoms (Volkman et al. 1980; Carrie et al. 1998). Other diatom-specific FAs including C16:1ω7 (4.82%) and C14:0 (3.51%), and dinoflagellate-specific FA, C18:1ω9 (2.7%), were also found in the tissues of *P. malabarica* which might have contributed from the food. It has been reported that the diatoms and dinoflagellates form a major component in the diet of bivalves (Alfaro et al. 2006; Bachok et al. 2009; Wang et al. 2015). The diatoms are the dominant contributors to the phytoplankton community in the water column of this estuary, followed by dinoflagellates and a minor contribution from silicoflagellates and blue-green algae (Krishna Kumari et al. 2002; Patil and Anil 2011). Thus, in *P. malabarica*, being a facultative deposit feeder, the presence of diatom-specific FAs in their tissues can be attributed to their feeding on both sediment and water column phytoplankton and their assimilation. The availability of dead and degraded phytoplankton deposited in the sediment and the microphytobenthos as food for *P. malabarica* cannot be ruled out and requires further studies to specifically characterize the source of food. It has been reported that the FA C20:4ω6 plays an important role in the maturation of gametes of bivalves (Soudant et al. 1996; Bachok et al. 2009). The gametogenesis and gonad developmental activities of *P. malabarica* are high during PreM (Mohite and Mohite 2009). Nagvenkar and Jagtap (2013) found high content of proteins and lipids during PreM season in the *P. malabarica* from the Zuari estuary and suggested their role in the maturation of gametes. Thus, the presence of FA C20:4ω6 in *P. malabarica* during PreM season would provide energy required for gametogenesis. Earlier studies have reported that terrestrial plants also act as source of food for the bivalves (Bachok et al. 2009; Wang et al. 2015); however, their contribution was low (< 4%) in their tissues. The present observations corroborate with these findings, and content of LCFA (C24:0) in *P. malabarica* was low. TOC/TN ratio (17.5) indicated a mixed contribution of marine- and terrestrial-derived OM in the surface sediment of Chicalim. This also supports the presence of FAs derived from the diatoms, dinoflagellates, and higher plants in the tissues of *P. malabarica*.

At Siridao, the presence of short-chain saturated FAs (C12 + C14 + C16 + C20; 38.4%) and dinoflagellate-specific FA (C18:1ω9; 4.01%) suggests phytoplankton-derived material as an important source of food for *M. casta* during PreM. It has been reported that bivalve has diverse filtering activity which is found to be influenced by the concentration of planktons, and their size and shape as well as nutritive values (Rajesh et al. 2001; Arapov et al. 2010). The high contribution of dinoflagellates than diatoms was also reported in the diet of filter-feeding bivalves *Austrovenus stutchburyi* and *Paphies australis* from the Matapouri estuary (northern New

Zealand) (Alfaro et al. 2006) and it was attributed to the pelagic nature of dinoflagellates. Occurrence and dominance of diatoms and dinoflagellates varied with space and time in the water column of the Zuari estuary (Krishna Kumari et al. 2002; Patil and Anil 2008, 2011). Alkawri and Ramaiah (2010) found high abundance of dinoflagellates at low salinity estuarine station, i.e., off Siridao. The occurrences of diatom and dinoflagellate blooms were reported (Patil and Anil 2008, 2011) in the nearby region (~ 2–3 km) of the present study site (Chicalim). Though this phenomenon was found more prominent during the monsoon period, the diatom blooms are also reported during the non-monsoon seasons. The termination of blooms leads to the deposition of planktons to the sediment contributing to the sediment organic pool. Tamelander and Heiskanen (2004) reported that diatoms with intact cells settle faster in the coastal Baltic Sea, whereas vegetative cells of dinoflagellates undergo disintegration process in the water column leading to the formation of slower sinking phytodetritus during a spring bloom. Sara (2006) pointed out that hydrodynamic conditions of the regions have an impact on the origin and quality of OM available for the bivalves (mussels) while studying a lagoon which is open to flow and closed pond. It was observed that the mussels in the closed pond could be used in loco-produced OM by means of wind-driven resuspension, whereas in lagoon, the benthic and pelagic habitats were uncoupled with each habitat having distinct dynamics and production (Sara 2006). Similar are the conditions in Chicalim (actively coupled benthic and pelagic habitats) and Siridao (with uncoupled benthic and pelagic habitats). Thus, the OM available for the bivalves will have a profound influence on the secondary production. Sediment texture analysis also revealed that Siridao is hydrodynamically more energetic than Chicalim and the impact of this was observed on the content of OM at both the study sites. Although the content of sedimentary chlorophyll *a* was almost similar at both the sites, the PRT content was relatively high at Chicalim (PRT 4.15 mg g^{-1}; PRT/CHO 2.12; Chl*a* 2.11 μg g^{-1}) than at Siridao (PRT 0.43 mg g^{-1}; PRT/CHO 1.34; Chl*a* 1.73 μg g^{-1}), suggesting accumulation of planktonic particles from the water column. The diverse flux of food particles, species-specific feeding nature, and hydrodynamic condition of the study sites could be the possible reasons for the difference in the composition of food particles in the diet of *P. malabarica* and *M. casta*.

The MON season was characterized by the dominance of bacteria and mangrove-derived FAs over diatoms and dinoflagellates in the tissues of both the bivalves. It has been reported that mangrove detritus enriched with bacteria act as one of the important food sources for the suspension-feeding bivalve, *Geloina coaxans*, in the subtropical mangrove forest, Okinawa, Japan (Bachok et al. 2003). However, the two bivalves in the present study show differential feeding habits; benefits of such nutrition will differ from species to species along with the habitats. The consumption of mangrove-derived detritus by the filter-feeding bivalves (*Austrovenus stutchburyi* and *Paphies australis*) was also reported from the Matapouri estuary of northern New Zealand (Alfaro et al. 2006). Zuari estuary is characterized by high runoff (100 to 400 m^3 s^{-1}) during SWM and relatively low (< 10 m^3 s^{-1}) during the dry period (Shetye and Murty 1987). The surface runoff through catchment area and mangrove vegetation during MON precipitation could transport large amount of terrestrial materials into the estuarine area. The mangrove litter fall from the Mandovi-Zuari estuarine system acts as an important source of particulate and dissolved matter for the microbial food chain (Wafar et al. 1997). In Chicalim, being hydrodynamically less energetic, the sedimentation of particle-associated bacteria would favor the deposit feeders. The high contribution of viable heterotrophic bacteria to the TBC and low PRT/CHO (< 1) ratio in the surface sediment during MON at both the sites suggests role of bacteria in the processing of allochthonous OM and the presence of degraded-detrital OM.

During POM season, high (38.6%) content of short-chain saturated FAs indicate the presence of partially degraded materials in the tissues of *P. malabarica*. Diatom-specific FA (C16:1ω7; 3.51%) was also reported in *P. malabarica* during this season, but its contribution was low compared to PreM. However, diet of *M. casta* showed a dominance of bacteria-specific FA (C17:0; 4.42%) followed by dinoflagellate-specific (C18:1ω9; 1.73%) and diatom-specific (C16:1ω7; 1.11%) FA pointing towards the capture of food from the water column rather than from the sediment. The ratio of PRT/CHO showed detrital-heterotrophic nature of sedimentary OM at Chicalim (PRT/CHO: 1.17) and newly generated fresh OM at Siridao (PRT/CHO: 4.89). The high content of TOC/TN ratio (23.6) in the sediments of Chicalim also suggested input of allochthonous matter during this season. The seasonal changes in the source of food could be the reason for the variations in the food composition of bivalves, which in turn could be responsible for seasonal variation in the abundance of bivalves. Bachok et al. (2009) reported seasonality in the food sources of *Quidnipagus palatum*, a bivalve collected from the intertidal flat (Tomigusuku) of Okinawa Island, Japan, with dominance of vascular plants and bacteria during warm season, macroalgae, and phytoplankton during cold and diatoms during rainy season and these food components were derived from the sediment and the water column. Wang et al. (2015) revealed that the availability of food in the system and species-specific feeding habits of the bivalves are the major factors for the seasonal changes in their diet composition, as observed in the present study.

Factors influencing the partitioning of bivalves in the Zuari estuary

The bivalve *P. malabarica* is found dominant in the silty-sandy sediment of Chicalim, and *M. casta* in the sand-dominated sediment of Siridao. The distribution of these species was reported earlier in the estuaries of Goa (Nagvenkar 2014; Parulekar et al. 1984). Earlier studies have reported the occurrence of these bivalves (*Paphia* spp. and *Meretrix* spp.) along the Kali estuary, Karnataka (Alagarswami and Narasimham 1973; Nair et al. 1984), and Ashtamudi estuary, Kerala (Appukuttan et al. 2002).

The RDA analysis depicting the relationship between biotic and abiotic variables with respect to the abundance of bivalves indicated that the sedimentary PRT content was one of the determining ($p < 0.05$) factors in governing the distribution of these bivalves in their respective habitats (Fig. 6). The bivalve *P. malabarica* present in Chicalim found to be influenced by the high content of biochemical parameters, organic carbon, sediment with silt and clay, and higher sediment chlorophyll *a*. The sediment with higher percentage of fine particles hold higher content of food material, and such habitat is suitable for facultative deposit-feeding bivalves. However, *M. casta* was mainly influenced by sandy sediment and chlorophyll *a* from the water column, and this can be attributed to its suspension-feeding habit suggesting the utilization of food particles directly from the water column. It has been reported that bivalves change their feeding behavior with respect to sediment type, water flow, and hydrological conditions (Olafsson 1986). The bivalve *Macoma balthica* from exposed sandy habitat preferred suspension-feeding mode and bivalves from stagnant, sheltered, muddy sand sediment showed deposit-feeding habits (Olafsson 1986). Suspension feeding was mainly observed during the high amount of suspended materials, and deposit-feeding was a response of a decrease in the supply of these food particles.

Alexander et al. (1993) reported that the distribution and burrowing of bivalves are dependent upon the sediment characteristics and shell morphology and ornamentations. The morphological features of these two bivalves are comparatively distinct. The shell of *P. malabarica* is slightly inflated, triangularly ovate with a concentric sculpture on the surface, and the anterior and posterior margins are narrowly rounded. However, the shell of *M. casta* is thick, smooth, devoid of any sculpture, and triangularly ovate (Narasimham and Laxmilatha 1966). The bivalves with smooth and flattened shells get a chance to escape from the washout event of the high-energy waves, and currents during flood tide and thick shell facilitate them to cope up with high-temperature exposure for a longer duration of ebb tide period (Soon et al. 2017). Thus, *M. casta* with the smooth shell may be well adapted to the sandy substratum at Siridao, which may help in easy burrowing into the tightly packed sediment to avoid predation, and as this region gets exposed for a longer duration during low tide, the thick shell of *M. casta* is advantageous. Bivalve *M. casta*, being a suspension feeder, also gets a chance to use suspended particles during incoming high tide water as reflected in the composition of the diet, which showed the dominance of dinoflagellates, mangrove litter, and bacteria. However, *P. malabarica* used OM derived from

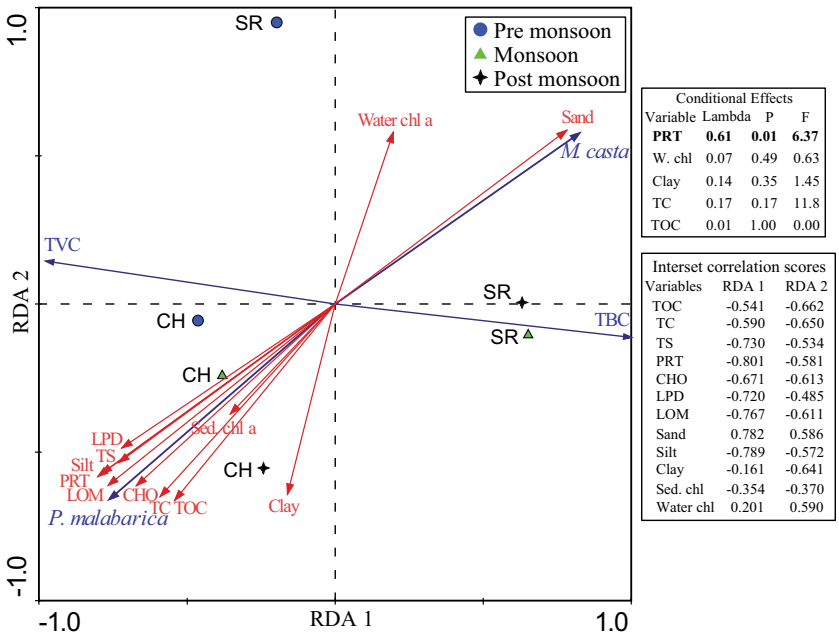


Fig. 6 Redundancy analysis (RDA) triplot depicting relation between biotic and abiotic variables during different seasons in the Zuari estuary. Arrows in red indicate abiotic variables and blue indicate species variables. Lambda is eigenvalue explained by the abiotic variables. Variable with $p < 0.05$ is highlighted in bold

diatoms as the major food source along with terrestrial materials, dinoflagellates, and bacteria. Dinoflagellates are mostly found as pelagic phytoplankton and are directly available to the suspension feeders (Alfaro et al. 2006), as observed in the case of *M. casta* and the RDA output also showed relation between *M. casta* population and water column chlorophyll *a* (Fig. 6). The sediment chlorophyll *a* content was almost similar at both the sites, but high content of OM at Chicalim compared to Siridao suggests the accumulation of either fresh or detritus planktonic matter from the water column which could be available for deposit-feeding *P. malabarica*. Siridao being shallow mudflat showed low accumulation of OM in the sediment from the water column. It seems that sediment characteristics driven by a complex interplay of physical processes and seasons resulting in variation in the quality and quantity of food determine the fate of these organisms.

Summary

The two economically important bivalves, *Paphia malabarica* and *Meretrix casta*, inhabit the Zuari estuary. A clear partitioning in their population has been observed as *Paphia malabarica* (Chemnitz, 1782) inhabit the southern bank (Chicalim), whereas *Meretrix casta* (Gmelin, 1791) inhabit the northern bank (Siridao) of the Zuari estuary. *Paphia malabarica* is an exclusive inhabitant of Chicalim with silty-sandy sediment, whereas *M. casta* is exclusive to Siridao, which is dominated by sand. The population of *P. malabarica* is facilitated by the semi-enclosed nature of the habitat at Chicalim with high amount of degraded and aged sediment organic carbon, high chlorophyll *a*, elemental, and biochemical components, whereas Siridao which experience high impact of tidal currents, low sediment organic carbon, and high water column chlorophyll *a* facilitated *M. casta*. The fatty acids in the tissues of *P. malabarica* indicate their ability to source the food from the sediment and water column, whereas *M. casta* can source their food from the water column. Chicalim can be considered an actively coupled benthic-pelagic habitat, and Siridao as an uncoupled habitat. Thus, the diverse flux of food particles, nature and quality of food, species-specific feeding ecology, and local hydrodynamics operating at these habitats could be the determining factors in the partitioning of the bivalves. Since habitats act as breeding grounds for the bivalves, they can be demarcated for population replenishment during the spat fall for successful recruitment of these commercially important bivalves and controlled harvest methods can be employed for conservation and management of the natural stocks.

Acknowledgments We thank the Director, CSIR-National Institute of Oceanography, Goa, for the support and encouragement. This work was carried out as a part of the XII plan project Ocean Finder with funding from CSIR, India award no. PSC0105. This is a NIO contribution 6541.

Funding information This study was funded by the Council of Scientific and Industrial Research, India, and award no. PSC0105.

Data availability All data generated or analyzed during this study are included in this article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No animal testing was performed during this study.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgments.

References

Akhil PS, Nair MP, Sujatha CH (2013) Core sediment biogeochemistry in specific zones of Cochin Estuarine System (CES). J Earth Syst Sci 122:1557–1570

Alagarswami K, Narasimham KA (1973) Clam, cockle and oyster resources of the Indian coasts.in: Proceedings of the symposium on living resources of the seas around India. Special publication Central Marine Fisheries Research institute, Cochin, India, pp. 648-658

Alexander RR, Stanton JRJ, Dodd JR (1993) Influence of sediment grain size on the burrowing of bivalves: correlation with distribution and stratigraphic persistence of selected neogene clams. Palaios 8:289–303

Alfaro AC, Thomas F, Sergent L, Duxbury M (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar Coast Shelf S 70:271–286

Alkawri AAS, Ramaiah N (2010) Spatio-temporal variability of dinoflagellate assemblages in different salinity regimes in the west coast of India. Harmful Algae 9:153–162

Al-Mossawi MJ, Kadri MH, Salem AA, Chugh TD (1983) The use of clams as bioindicator of fecal pollution in seawater. Water Air Soil Poll 20:257–263

Ansari ZA, Parulekar AH (1994) Ecological energetics of benthic communities of an estuarine system of the west coast of India. Proc Indian Nat Sci Acad Part B60:99–106

Appukuttan KK, Velayudhan TS, Laxmilatha P, Kripa V, Alloycious PS, Vinod M, Gayatri GS, Narasimham KA (2002) Clam resource in the Astamudi Lake, a tropical estuary in sourthern India: managment and conservation concerns, in: Marine Fisheries Information Service, Technical and Extension Series174:1–4

Arapov J, Ezgeta-Balic D, Peharda M, Nincevic Gladan Z (2010) Bivalve feeding—how and what they eat? Ribarstvo 68:105–116

Asami H, Aida M, Watanabe K (2005) Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. Appl Environ Microbiol 71:2925–2933

Bachok Z, Mfilinge PL, Tsuchiya M (2003) The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. J Exp Mar Biol Ecol 292:187–197

Bachok Z, Meziane T, Mfilinge PL, Tsuchiya M (2009) Fatty acid markers as an indicator for temporal changes in food sources of the bivalve *Quidnipagus palatum*. Aquat Ecosyst Health 12:390–400

Bardhan P, Karapurkar SG, Shenoy DM, Kurian S, Sarkar A, Maya MV, Naik H, Varik S, Naqvi SWA (2015) Carbon and nitrogen isotopic composition of suspended particulate organic matter in Zuari estuary, west coast of India. J Marine Syst 141:90–97

Barnes H, Blackstock J (1973) Estimsation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total'lipids. J Exp Mar Biol Ecol 12:103–118

Bhaskar PV, Cardozo E, Giriyan A, Garg A, Bhosle NB (2000) Sedimentation of particulate matter in the Dona Paula Bay, west coast of India during November to May 1995–1997. Estuaries 23: 722–734

Bhosle NB, Dhople VM (1988) Distribution of some biochemical compounds in the sediments of the Bay of Bengal. Chem Geol 67:341–352

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911–917

Buchanan JB (1984) Sediment analysis. In: Holme NA, McIntyre AD (eds) Methods for the study of marine benthos. Blackwell Scientific Publications, Oxford, pp 41–645

Cahoon LB (1999) The role of benthic microalgae in neritic ecosystems. In: Ansell A, Gibson RN, Barnes M (eds) Oceanography and marine biology, An Annual Review. Taylor and Francis, London, pp 37–86

Canuel EA, Martens CS (1993) Seasonal variations in the sources and alteration of organic matter associated with recently-deposited sediments. Org Geochem 20(5):563–577

Campanya-Llovet N, Snelgrove PV, Parrish CC (2017) Rethinking the importance of food quality in marine benthic food webs. Prog Oceanogr 156:240–251

Carmichael RH, Shriver AC, Valiela I (2012) Bivalve response to estuarine eutrophication: the balance between enhanced food supply and habitat alterations. J Shellfish Res 31(1):1–11

Carrie RH, Mitchell L, Black KD (1998) Fatty acids in surface sediment at the Hebridean shelf edge, west of Scotland. Org Geochem 29: 1583–1593

Cotano U, Villate F (2006) Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: a biochemical approach. Mar Pollut Bull 52:404–414

Dame RT (2005) The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin. Mol Microbiol 56: 858–870

Danovaro R, Fabiano M, Della Croce N (1993) Labile organic matter and microbial biomasses in deep-sea sediments (Eastern Mediterranean Sea). Deep Sea Res Pt I40:953–965

Danovaro R, Marrale D, Della Croce N, Parodi P, Fabiano M (1999) Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic–benthic coupling. J Sea Res 42:117–129

de Moraes PC, Franco DC, Pellizari VH, Sumida PYG (2014) Effect of plankton-derived organic matter on the microbial community of coastal marine sediments. J Exp Mar Biol Ecol 461:257–266

De Souza MJB, Nair S, Bharathi PL, Chandramohan D (2003) Particle-associated bacterial dynamics in a tropical tidal plain (Zuari estuary, India). Aquat Microb Ecol 33:29–40

Dell'Anno A, Mei ML, Pusceddu A, Danovaro R (2002) Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar Pollut Bull 44:611–622

Department of Fisheries, Goa (2018) Inland fish production of Goa 2001 to 2018. https://fisheries.goa.gov.in/wp-content/uploads/2019/05/Inland-Fish-Production-of-Goa-for-the-period-from-2001-to-2018.pdf

Dessai DV, Nayak GN, Basavaiah N (2009) Grain size, geochemistry, magnetic susceptibility: proxies in identifying sources and factors controlling distribution of metals in a tropical estuary, India. Estuar Coast Shelf S 85:307–318

Dubois M, Gilles KA, Hamilton JK, Rebers PAT, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Dunn RJK, Welsh DT, Teasdale PR, Lee SY, Lemckert CJ, Méziane T (2008) Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. Cont Shelf Res 28:2535–2549

El Wakeel SK, Riley JP (1957) The determination of organic carbon in marine muds. ICES J Mar Sci 22:180–183

Eswaran R, Khandeparker L (2017) Seasonal variation in bacterial community composition and β-glucosidase expression in a tropical monsoon-influenced estuary. Aquat Microb Ecol 80:273–287

Fabiano M, Danovaro R, Fraschetti S (1995) A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (northwestern Mediterranean). Cont Shelf Res 15:1453–1469

Gardade L, Khandeparker L (2017) Spatio-temporal variations in pathogenic bacteria in the surface sediments of the Zuari estuary, Goa, India. Curr Sci 113:1729–1738

Gihring TM, Humphrys M, Mills HJ, Huette M, Kostka JE (2009) Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. Limnol Oceanogr 54:1073–1083

Graf G, Rosenberg R (1997) Bioresuspension and biodeposition: a review. J Mar Syst 11:269–278

Gremare A, Medernach L, Amouroux JM, Vetion G, Albert P (2002) Relationships between sedimentary organics and benthic meiofauna on the continental shelf and the upper slope of the Gulf of Lions (NW Mediterranean). Mar Ecol Prog Ser 234:85–94

Gudkov AT (1997) The L7/L12 ribosomal domain of the ribosome: structural and functional studies. FEBS Lett 407:253–256

Guo W, Jia G, Ye F, Xiao H, Zhang Z (2019) Lipid biomarkers in suspended particulate matter and surface sediments in the Pearl River estuary, a subtropical estuary in southern China. Sci Total Environ 646:416–426

Hader DP, Kumar HD, Smith RC, Worrest RC (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol S6:267–285

Harji RR (2011) Lipids in marine environment. PhD thesis,Goa University

Harkantra SN (1975) Some observations on the clam beds of Kali estuary, Karwar. Mahasagar 8:101–108

Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48: 422–427

Heip CHR, Goosen NK, Herman PMJ, Kromkamp JC, Middelburg JJ, Soetaert KER (1995) Production and consumption of biological particles in temperate tidal estuaries. Oceanogr Mar Biol 33:1–149

Hope JA, Paterson DM, Thrush SF (2019) The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple services. J Ecol 108:815–830

Hu J, Zhang H, Peng PA (2006) Fatty acid composition of surface sediments in the subtropical Pearl River estuary and adjacent shelf, southern China. Estuar Coast Shelf S 66:346–356

Huber M (2010) Compendium of bivalves. A full-color guide to 3,300 of the world's marine bivalves. A status of Bivalvia after 250 years. Hackenheim:Concbooks, pp. 910

Kanaya G, Nobata E, Toya T, Kikuchi E (2005) Effects of different feeding habits of three bivalve species on sediment characteristics and benthic diatom abundance. Mar Ecol Prog Ser 299:67–78

Keiblinger KM, Wilhartitz IC, Schneider T, Roschitzki B, Schmid E, Eberl L, Riedel K, Zechmeister-Boltenstern S (2012) Soil

metaproteomics–comparative evaluation of protein extraction protocols. Soil Biol Biochem 54:14–24

Khandeparker L, Kuchi N, Kale D, Anil AC (2017) Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. Ecol Indic 74:172–181

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Mar Ecol Prog Ser 220:103–117

Krishna Kumari L, Bhattathiriv PMA, Matondkar SGP, John J (2002) Primary productivity in Mandovi-Zuari estuaries in Goa. J Mar Biol Ass India 44:1–13

Lin R, Lin X, Guo T, Wu L, Zhang W, Lin W (2015) Metaproteomic analysis of bacterial communities in marine mudflat aquaculture sediment. World J Microb Biot 31:1397–1408

Lopez GR, Levinton JS (1987) Ecology of deposit-feeding animals in marine sediments. Q Rev Biol 62:235–260

Luna GM, Manini E, Danovaro R (2002) Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. Appl Environ Microb 68:3509–3513

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries 19:186–201

Manoj NT, Unnikrishnan AS (2009) Tidal circulation and salinity distribution in the Mandovi and Zuari estuaries: case study. J Waterw Port Coast 135:278–287

Marie D, Partensky F, Vaulot D, Brussaard C (1999) Enumeration of phytoplankton, bacteria, and viruses in marine samples. Curr Protoc Cytom:11–11

Meyers PA (1994) Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem Geol 114:289–302

Meyers PA (1997) Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Org Geochem 27: 213–250

Meziane T, Tsuchiya M (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar Ecol Prog Ser 200:49–57

Meziane T, Tsuchiya M (2002) Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: origin and utilisation by two macrozoobenthic species. J Sea Res 47:1–11

Méziane T, Lee SY, Mfilinge PL, Shin PKS, Lam MHW, Tsuchiya M (2007) Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. Mar Biol 150: 1103–1113

Miller DC, Geider RJ, MacIntyre HL (1996) Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. II Role in sediment stability and shallow-water food webs. Estuaries 19:202–212

Mohite SA, Mohite AS (2009) Gonadal development in the shortneck clam *Paphia malabarica* in relation to hydrographic parameters in Kalbadevi estuary. J Mar Biol Ass India 51:164–172

Muller-Navarra DC (2008) Food web paradigms: the biochemical view on trophic interactions. Int Rev Hydrobiol 93:489–505

Muniz P, Venturini N, Hutton M, Kandratavicius N, Pita A, Brugnoli E, Burone L, García-Rodríguez F (2011) Ecosystem health of Montevideo coastal zone: a multi approach using some different benthic indicators to improve a ten-year-ago assessment. J Sea Res 65:38–50

Nagvenkar SS (2014) Studies on ecobiology of *Paphia malabarica* (Chemnitz) from estuarine habitats of Goa, PhD Thesis, Goa University

Nagvenkar SS, Jagtap TG (2013) Spatio-temporal variations in biochemical composition, condition index and percentage edibility of the clam, *Paphia malabarica* (Chemnitz) from estuarine regions of Goa. Indian J Mar Sci 42:786–793

Nair KN, Ramadoss K, Rajan CT, Sundaram N (1984) Molluscan resources of Kali river estuarine system in Karnataka. In: Marine Fisheries Information Service, Technical and Extension Series, vol 58. Central Marine Fisheries Research Institute, Cochin, pp 1–8

Narasimham KA, Laxmilatha P (1996) Clam culture, in: Artificial Reefs and Seafarming technologies, Bulletin Central Marine Fisheries Research Institute, Cochin, India, 48, pp. 76–87

Olafsson EB (1986) Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. J Anim Ecol 55:517–526

Padmavati G, Goswami SC (1996) Zooplankton ecology in the Mandovi-Zuari estuarine system of Goa, west coast of India. Indian J Mar Sci 25:268–273

Parulekar AH, Nair SA, Ansari ZA, Harkantra SN, Chatterji A, Ingole BS, Roy JM (1984) Ecology and culturing of edible bivalves in Goa. Indian J Mar Sci 13:190–192

Patil JS, Anil AC (2008) Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. Cont Shelf Res 28: 2404–2416

Patil JS, Anil AC (2011) Variations in phytoplankton community in a monsoon-influenced tropical estuary. Environ Monit Assess 182: 291–300

Pillai BV, Swarup S (2002) Elucidation of the flavonoid catabolism pathway in *Pseudomonas putida* PML2 by comparative metabolic profiling. Appl Environ Microb 68:143–151

Plante-Cuny MR, Plante R (1986) Benthic marine diatoms as food for benthic marine animals,in:Proceedings of the 8th international diatom symposium. Koeltz Scientific Books, Königstein, pp 525–537

Porter ML (2016) Collecting and processing mysids, stygiomysids, and lophogastrids. J Crustacean Biol 36:592–595

Pusceddu A, Dell'Anno A, Danovaro R, Manini E, Sara G, Fabiano M (2003) Enzymatically hydrolyzable protein and carbohydrate sedimentary pools as indicators of the trophic state of detritus sink systems: a case study in a Mediterranean coastal lagoon. Estuaries 26: 641–650

Qasim SZ, Sen Gupta RS (1981) Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. Estuar Coast Shelf S13: 557–578

Quintana CO, Yoshinaga MY, Sumida PY (2010) Benthic responses to organic matter variation in a subtropical coastal area off SE Brazil. Mar Ecol 31:457–472

Raiswell R, Buckley F, Berner RA, Anderson TF (1988) Degree of pyritization of iron as a paleoenvironmental indicator of bottom-water oxygenation. J Sediment Res 58:812–819

Rajendran N, Matsuda O, Imamura N, Urushigawa Y (1995) Microbial community structure analysis of euxinic sediments using phospholipid fatty acid biomarkers. J Oceanogr 51:21–38

Rajesh KV, Mohamed KS, Kripa V (2001) Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalves. Indian J Mar Sci 30:87–92

Rao VP, Shynu R, Kessarkar PM, Sundar D, Michael GS, Narvekar T, BlossomV MP (2011) Suspended sediment dynamics on a seasonal scale in the Mandovi and Zuari estuaries, central west coast of India. Estuar Coast Shelf S 91:78–86

Rasheed M, Badran MI, Huettel M (2003) Influence of sediment permeability and mineral composition on organic matter degradation in three sediments from the Gulf of Aqaba, Red Sea. Estuar Coast Shelf S 57:369–384

Reeve WG, Tiwari RP, Kale NB, Dilworth MJ, Glenn AR (2002) Act P controls copper homeostasis in *Rhizobium leguminosarum*bv. *viciae* and *Sinorhizobium meliloti* preventing low pH-induced copper toxicity. Mol Microbio l4:981–991

Renault T, Bouquet AL, Maurice JT, Lupo C, Blachier P (2014) Ostreid herpesvirus 1 infection among Pacific oyster (*Crassostrea gigas*)

spat: relevance of water temperature to virus replication and circulation prior to the onset of mortality. Appl Environ Microbiol 80: 5419–5426

Rhoads DC (1974) Organism-sediment relations on the muddy sea floor. Oceanogr Mar Biol 12:263–300

Riederer BM, Pellier V, Antonsson B, Di Paolo G, Stimpson SA, Lutjens R, Catsicas S, Grenningloh G (1997) Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10. P Natl Acad Sci USA 94:741–745

Salas PM, Sujatha CH, Kumar CR (2015) Fate and source distribution of organic constituents in a river-dominated tropical estuary. J Earth Syst Sci 124:1265–1279

Sara G (2006) Hydrodynamic effects on the origin and quality of organic matter for bivalves: an integrated isotopic, biochemical and transplant study. Mar Ecol Prog Ser 328:65–73

Shetye SR, Murty CS (1987) Seasonal variation of the salinity in the Zuari estuary, Goa, India. PIndian AS-Earth 96:249–257

Shetye SR, Shankar D, Neetu S, Suprit K, Michael GS, Chandramohan P (2007) The environment that conditions the Mandovi and Zuari estuaries. In: Shetye SR, Dileep Kumar M, Shankar D (eds) The Mandovi and Zuari estuaries. National Institute of Oceanography, Goa, pp 3–27

Shirodkar PV, Deepthi M, Vethamony P, Mesquita AM, Pradhan UK, Babu MT, Verlecar XN, Haldankar SR (2012) Tide dependent seasonal changes in water quality and assimilative capacity of anthropogenically influenced Mormugao harbour water. Indian J Mar Sci 41:314–330

Shynu R, Rao VP, Sarma VVSS, Kessarkar PM, ManiMurali R (2015) Sources and fate of organic matter in suspended and bottom sediments of the Mandovi and Zuari estuaries, western India. Curr Sci 108:226–238

Smit AM, Strabala TJ, Peng L, Rawson P, Lloyd-Jones G, Jordan TW (2012) Proteomic phenotyping of *Novosphingobium nitrogenifigens* reveals a robust capacity for simultaneous nitrogen fixation, polyhydroxyalkanoate production, and resistance to reactive oxygen species. Appl Environ Microbiol 78:4802–4815

Soon TK, Sing OF, Denil DJ, Ransangan J (2017) Distribution and fishing pressure of hard clam, *Meretrix meretrix* in Marudu Bay, Sabah. Int J Ocean Oceanogr 11:265–276

Soudant P, Moal J, Marty Y, Samain JF (1996) Impact of the quality of dietary fatty acids on metabolism and the composition of polar lipid classes in female gonads of *Pecten maximus* (L.). J Exp Mar Biol Ecol 205:149–163

Sukumaran S, Mohamed SK, Asokan PK, Sebastian W, Mukundan L, Gopalkrishnan A, Alloycious PS, Venkatesan V, Kavitha M (2019) Morphological and molecular investigations reveal that *Paphia malabarica* from Indian waters is not synonymous with *Paphia* (*Protapes*) *gallus*. Reg Stud Mar Sci 27:100549

Sundar D, Unnikrishnan AS, Michael GS, Kankonkar A, Nidheesh AG, Subeesh MP (2015) Observed variations in stratification and currents in the Zuari estuary, west coast of India. Environ Earth Sci 74: 6951–6965

Tamelander T, Heiskanen AS (2004) Effects of spring bloom phytoplankton dynamics and hydrography on the composition of settling material in the coastal northern Baltic Sea. J Mar Syst 52:217–234

ter Braak CJ, Smilauer P (2002) CANOCO reference manual and Cano Draw for Windows user's guide: software for canonical community ordination (version 4.5)

terBraak CJ, Verdonschot PF (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat Sci 57: 255–289

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. Adv Ecol Res 29: 93–153

Venturini N, Pita AL, Brugnoli E, García-Rodríguez F, Burone L, Kandratavicius N, Hutton M, Muniz P (2012a) Benthic trophic status of sediments in a metropolitan area (Rio de la Plata estuary): linkages with natural and human pressures. Estuar Coast Shelf S 112:139–152

Venturini N, Salhi M, Bessonart M, Pires-Vanin AMS (2012b) Fatty acid biomarkers of organic matter sources and early diagenetic signatures in sediments from a coastal upwelling area (south-eastern Brazil). Chem Ecol 28:221–238

Volkman JK, Johns RB, Gillan FT, Perry GJ, Bavor HJ (1980) Microbial lipids of an intertidal sediment-I. Fatty acids and hydrocarbons. Geochim Cosmochim Acta 44:1133–1143

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219–240

Wafar S, Untawale AG, Wafar M (1997) Litter fall and energy flux in a mangrove ecosystem. Estuar Coast Shelf S44:111–124

Wakeham SG, Beier JA (1991) Fatty acid and sterol biomarkers as indicators of particulate matter source and alteration processes in the Black Sea. Deep-Sea Res 38:S943–S968

Wang S, Jin B, Qin H, Sheng Q, Wu J (2015) Trophic dynamics of filter feeding bivalves in the Yangtze estuarine intertidal marsh: stable isotope and fatty acid analyses. PloSOne 10:1–21

Wei CL, Rowe GT, Hubbard GF, Scheltema AH, Wilson GD, Petrescu I, Foster JM, Wicksten MK, Chen M, Davenport R, Soliman Y, Wang Y (2010) Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Mar Ecol Prog Ser 399:1–14

White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51–62

Wieking G, Kröncke I (2005) Is benthic trophic structure affected by food quality? The Dogger Bank example. Mar Biol 146:387–400

Wohlbrand L, Feenders C, Nachbaur J, Freund H, Engelen B, Wilkes H, Brumsack H, Rabus R (2017) Impact of extraction methods on the detectable protein complement of metaproteomic analyses of marine sediments. Proteomics 17(22):1–12

Zhukova NV (2005) Variation in microbial biomass and community structure in sediments of Peter the Great Bay (Sea of Japan/East sea), as estimated from fatty acid biomarkers. Ocean Sci 40:1–9

Zimmerman AR, Canuel EA (2001) Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuar Coast Shelf S 53:319–341

Spatio-temporal variations in pathogenic bacteria in the surface sediments of the Zuari estuary, Goa, India

Laxman Gardade and Lidita Khandeparker*

CSIR-National Institute of Oceanography, Dona Paula, Goa 403 004, India

Estuaries are hotspots of anthropogenic activities. The deposition of pathogenic bacteria in the sediment and their re-suspension into the water column are influenced by riverine discharge and tides. The abundance of *Escherichia coli* O157:H7, *Shigella* spp., *Salmonella* spp., total coliforms (TC) and *Vibrio* spp. (*Vibrio cholerae* (VC), *Vibrio parahaemolyticus* (VP), *Vibrio alginolyticus* (VA)) was assessed along with the total bacterial count (TBC) and total viable count (TVC) in surface sediments along the banks of the Zuari estuary, Goa, India. The study was carried out fortnightly for a period of 17 months covering three seasons, i.e. pre-monsoon (PreM), monsoon (MON) and post-monsoon (POM). The spatial and temporal changes in the quality of organic matter were also assessed. The organic matter content was high and rich in carbohydrates and proteins towards upstream sites. The quality of organic matter was influenced by the seasons. *E. coli* O157:H7 was detected only during MON towards the upstream stations. A negative correlation between TC and TBC with salinity was evident indicating the influence of land run-off. The *Shigella* spp. and VA were high towards the mouth of the estuary during PreM. However, during POM, the TVC, TC and VP were abundant towards the upstream and VC were abundant at the mouth of the estuary. Among the *Vibrios*, VP and VA were the most frequently occurring bacteria whereas TC and *Shigella* spp. were dominant among allochthonous pathogens in the sediments irrespective of space and time. In addition to influence of seasons, the sampling time influenced by tidal condition also played an important role in the population dynamics of pathogenic bacteria in the sediments. Future studies should address the interaction of pathogenic bacteria with suspended particles, their transport and survival in the sediments.

Keywords: Coliforms, sediment, estuaries, pathogenic bacteria, *Vibrio* spp.

ESTUARIES and coastal regions are continuously subjected to natural as well as various forms of anthropogenic activities which can change the quality of coastal water rendering it unsafe for recreation or human use[1,2]. The sources of water pollution include point (sewage discharge, domestic wastes and industrial effluents) and non-point sources (agricultural and urban runoff) which bring pathogenic bacteria into the coastal and estuarine waters[3]. The presence of various pathogens (e.g. *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Pseudomonas* spp., *Enterococcus faecalis*, total coliforms, etc.) has been reported in the estuarine and coastal waters of India[4–10]. Most of these pathogens are responsible for waterborne diseases (gastroenteritis, diarrhea, dysentery, typhoid, cholera, food poisoning and wound infection) in humans[3], either due to consumption of contaminated sea food or contact with water[7]. The attachment of allochthonous pathogenic bacteria to the particulate matter in the water column protects them from other environmental and biotic factors. These bacteria are found in large numbers in the sediments rich in organic matter, silt and clay[11,12]. Sediment acts as a reservoir of pathogenic bacteria in estuarine systems[12,13]. There are reports on the re-suspension of coliforms and faecal indicators from sediment bed due to recreational activities, tidal currents and storms[14,15]. Other than anthropogenic activities, the re-suspension of sediment could be another source in adding pathogenic bacteria to the overlying water column. The transport of re-suspended sediment particles is land-ward during flood tide and bay-ward during ebb tide[16].

V. cholerae, *V. parahaemolyticus* and *V. vulnificus* are generally found in marine and brackish environments and are serious human pathogens[17]. Kanungo *et al.*[18] reported that high numbers of cholera outbreaks in India were found in West Bengal, Orissa, Maharashtra and Kerala from 1997 to 2006. According to World Health Organization (WHO), a total of 589,854 cholera cases were found in 58 countries worldwide during 2011 (ref. 19). As sediment acts as reservoir of pathogenic bacteria, monitoring the bacterial load in the sediment can provide stable indicator of their long-term abundance in the water column[20].

Zuari estuary is located along the central west coast of India (15°27.5′N, 73°48′E) and is influenced by the south west (SW) monsoon[21], hereafter referred to as the monsoon. The seasonality in this estuary can be categorized into three seasons: pre-monsoon (PreM; February–May),

*For correspondence. (e-mail: klidita@nio.org)

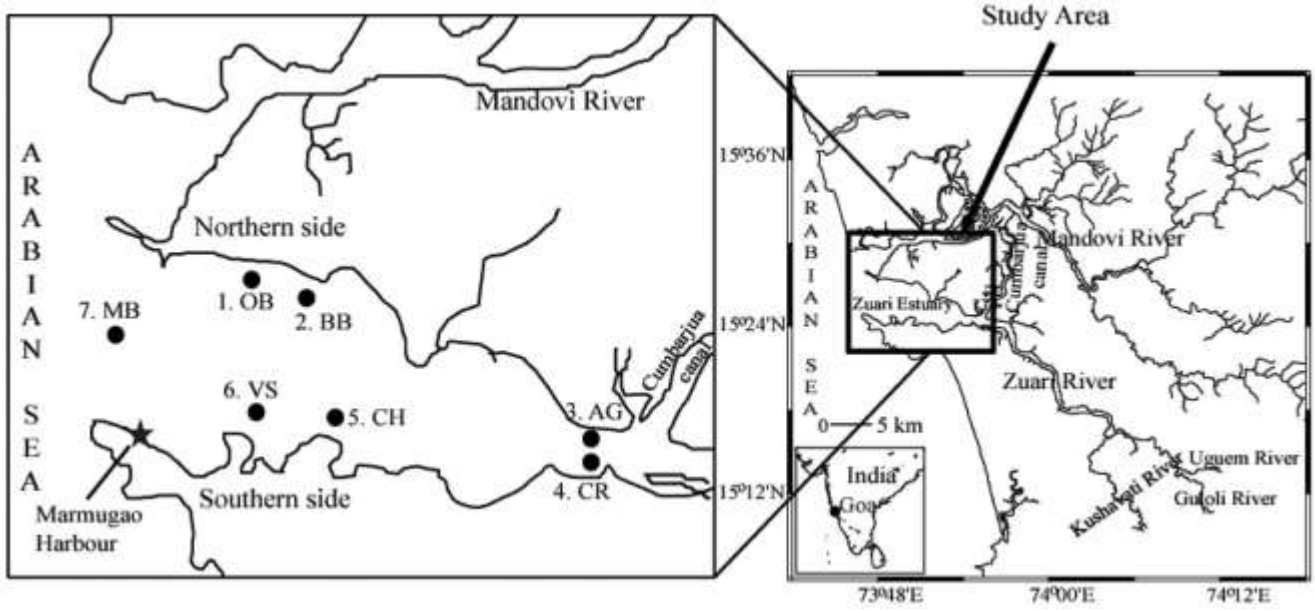


Figure 1. Location of stations in the Zuari estuary, Goa, central west coast of India (station 1, Odxel Beach (OB); station 2, Bambolim Beach (BB); station 3, Agacaim (AG); station 4, Cortalim (CR); station 5, Chicalim (CH); station 6, Vasco (VS); station 7, Marmugao bay (MB)).

Monsoon (MON; June–September) and post-monsoon (POM; October–January)[22]. During monsoon, the run-off is reported to range from 100 to 400 $m^3 s^{-1}$ in the Zuari estuary[23]. The amount of sewage discharged into the Mandovi–Zuari estuaries is estimated to be about 30 million litres per day[24]. Zuari estuary is used for navigation purpose, fishing, shellfish harvesting, transport of ores, discharge of wastes from transport ships, port- and tourist-related activities. Few studies have assessed the abundance of pathogens in the sediments in the midstream of the channel and the bay of the Zuari estuary[6,25–27]. The present study assesses the distribution of pathogenic bacteria in the surface sediments along the estuarine banks which are the active sites of anthropogenic activities. Fortnightly sampling was carried out in the Zuari estuary for 17 months from November 2010 to May 2012, covering all three seasons of the year. The present study also addresses the influence of tidal conditions observed during the sampling period on the distribution of pathogenic bacteria in the sediments.

Materials and methods

Study area

Sampling was carried out in the Zuari estuary located along the central west coast (Goa) of India (Figure 1). Zuari river has a length of 50 km and an average depth of 5 m. The mouth of the estuary is funnel-shaped with a width of ~5 km and narrows down towards the head of the estuary[28]. The estuary remains well mixed during PreM. However, it is stratified during monsoon season with less dense freshwater on the surface and more dense saline water at the bottom. The tides are semi-diurnal, with a height of ~2.3 m during spring tide and ~1.5 m during neap tide[21]. The surface sediment samples were collected from seven selected sites that line the banks of the Zuari estuary and are divided into five categories (Figure 1). Station 1 (Odxel Beach – OB) and station 2 (Bambolim Beach – BB) are categorized as beach influenced stations. Stations 3 (Agacaim – AG) and 4 (Cortalim – CR) are located towards upstream and influenced by the movement of ferry boats and fishing trawlers. The anthropogenic and industrial influence is prominent at station 5 (Chicalim – CH). Station 6 (Vasco – VS) is close to Marmugao port and is situated towards the mouth of the estuary. Tidal influence is high at station 7 (Marmugao Bay – MB) which is located at the mouth of the estuary. The details of the sampling locations are given in Table 1.

Collection of samples

Fortnightly sampling was carried out during post monsoon-I (November–December 2010 and January 2011; POM-I), pre monsoon-I (February–May, 2011; PreM-I), monsoon (June–September 2011; MON), post monsoon-II (December–January 2011/2012; POM-II) and pre monsoon-II (February–May 2012; PreM-II) seasons at the Zuari estuary. Sampling was initiated at station 1 at ~7 : 00 am and concluded at station 7 at ~11 : 00 a.m. The different phases of tides were noticed in the tide table during the sampling period. A slack period was observed before ebb tide during POM-I sampling and ebb tide

Table 1. Details of sampling locations at the Zuari estuary

Station no.	Station name	Station code	Latitude	Longitude
1	Odxel Beach	OB	15°27′03.3″N	73°49′30.1″E
2	Bambolim Beach	BB	15°26′47.4″N	73°50′08.5″E
3	Agacaim	AG	15°24′48.0″N	73°54′17.5″E
4	Cortalim	CR	15°24′33.4″N	73°54′15.3″E
5	Chicalim	CH	15°24′48.2″N	73°50′27.4″E
6	Vasco	VS	15°24′49.5″N	73°49′28.9″E
7	Marmugao bay	MB	15°26′13.6″N	73°47′54.4″E

during POM-II. Flood tide was observed during PreM-I and ebb tide during PreM-II. Monsoon sampling was done during the slack period before flood tide. Thus, except PreM-I season, all other samplings were done either during slack period before ebb or flood tide or during ebb tide. Surface sediment samples were collected by using a van Veen grab and the top ~2 cm of sediment was used to assess the TVC, pathogenic bacteria, total organic matter (TOM), total organic carbon (TOC), total nitrogen (TN), proteins and carbohydrates. Sediment samples to be analysed for total bacterial count (TBC) were fixed with formaldehyde (final concentration 1–2% v/v). The samples were transported to the laboratory in an ice box. The near bottom water samples were also collected using Niskin sampler for analysis of dissolved oxygen (DO), salinity and temperature. The Winkler titrimetric method was used in the estimation of DO[29]. Digital thermometer (EUROLAB) and master refractometer (ATAGO, Japan) were used to measure the temperature and salinity of sea water respectively.

Enumeration of TVC and pathogenic bacteria in the surface sediments: The bacteria in the sediment samples were enumerated using standard spread plate technique. The different selective media were brought from HiMedia (Mumbai) and prepared in distilled water following manufacturer's instructions. One gram of wet sediment was suspended in 10 ml of filtered, autoclaved sea water followed by vigorous shaking to dissociate the bacteria from the sediment. It was then left for 5–10 min for the sediment particles to settle. Subsequently, 1 ml of supernatant was serially diluted in 9 ml of filtered, autoclaved sea water to get 10^1, 10^2, 10^3 dilutions. From each of the dilutions 0.1 ml was plated on Zobell Marine Agar (ZMA) for total viable (culturable) bacteria and incubated at room temperature (28° ± 2°C) for 24 h. Pathogenic bacteria were enumerated by spreading samples on different selective media like MacConkey agar which differentiated *E. coli* (pink/red coloured colonies) from *Shigella*/*Salmonella* species (transparent, colourless colonies). All colonies on MacConkey agar were reported as total coliforms (TC = *E. coli* and *Shigella*/*Salmonella* spp.). Thiosulphate-citrate-bile-salts sucrose (TCBS) agar was used to differentiate *Vibrio* spp. depending on size and colour. *V. cholerae* grow as raised, yellow coloured colonies having diameter <2 mm; *V. alginolyticus* are bigger (>2 mm diameter) in size and produce yellow coloured colonies. However, *V. parahaemolyticus* develop as green coloured colonies on TCBS agar. Xylose-Lysine Deoxycholate (XLD) agar differentiates *Salmonella* (pink coloured colonies with black centre) from *Shigella* (pink colonies) species. Enterococcus confirmatory agar was used for *Enterococcus faecalis* (blue coloured colonies) and general *Streptococci* species (white colonies). Hichrome EC O157 : H7 selective agar was used for *E. coli* O157 : H7 (purple coloured colonies) in which Hichrome EC O157 : H7 selective supplement was added aseptically. All selective media for specific pathogens were incubated at 37°C for 24 h and the counts were expressed as colony forming unit (CFU) g^{-1}. The abundance of *Shigella* and *Salmonella* spp. was determined later to POM-I season (November–December 2010).

Enumeration of TBC in the surface sediments by using flow cytometry: Flow cytometry (BD-Biosciences, USA) was used to analyse formaldehyde fixed sediment samples for TBC. Sediment samples (1 g) suspended in 10 ml of autoclaved sea water were sonicated at 50% power in the water bath sonicator (ultrasonic cleaner, Equitron) for 1 min. This was done thrice to separate cells from sediment particles[30] and was further centrifuged at 3000 rpm for 1 min and the supernatants recovered. One ml of supernatant was passed through BD cell strainer cap to remove larger particles and was subsequently stained with SYBR Green I (Molecular Probes, USA) at 1 : 10,000 final concentration and incubated for 15 min at room temperature in the dark[31]. After incubation, the samples were analysed using a BD FACSAria™ II (BD-Biosciences, USA) flow cytometer equipped with a nuclear blue laser of 488 nm to differentiate green fluorescence excited by blue laser. Emitted light was collected through filter sets of 488/10 band pass (BP) for right angle light scatter (SSC) and 530/30 BP for green fluorescence. Fluorescent beads (1 μm, polysciences) were used as internal standards for calibration. Gating was done against SSC versus green fluorescence (FITC) using BD FACS Diva software and the results are expressed as cells g^{-1} of sediment.

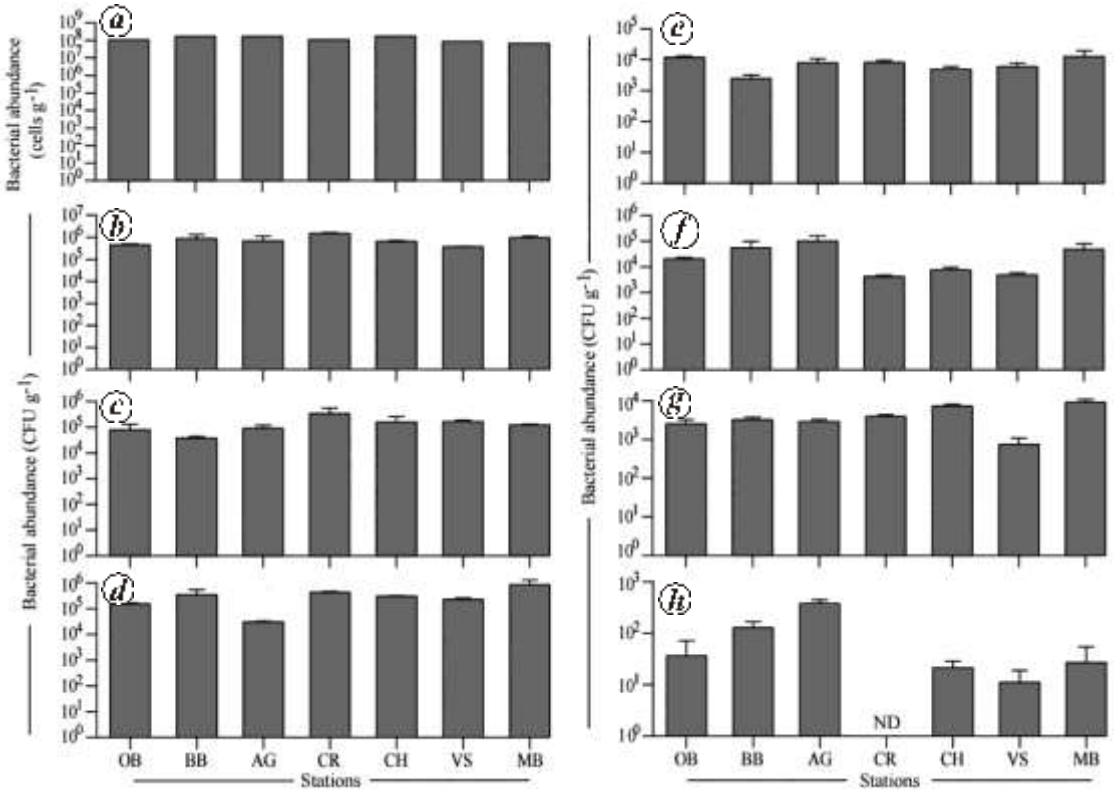


Figure 2. Spatial variations in the (***a***) total bacterial count, (***b***) total viable count, (***c***) total coliforms, (***d***) *V. cholerae*, (***e***) *V. alginolyticus*, (***f***) *V. parahaemolyticus*, (***g***) *Shigella* spp. and (***h***) *Salmonella* spp. in the surface sediments of the Zuari estuary. (These are mean values of bacterial abundance of all 28 fortnightly sampling events). OB, Odxel Beach; BB, Bambolim Beach; AG, Agacaim; CR, Cortalim; CH, Chicalim; VS-Vasco; MB, Marmugao bay; ND, none detected.

Analysis of biogeochemical parameters of surface sediments

Before analysis, the sediment samples stored at –20°C were thawed, dried at 50ºC and ground to fine powder using mortar and pestle. The sedimentary TOC content was determined by wet oxidation with chromic acid as described by El Wakeel and Riley[32]. It was then converted into TOM content by using a factor of 1.724 as described by Bhosle and Dhople[33] and expressed as dry weight percentage (%). Total nitrogen (TN) was determined using CHNS analyser (Vario MICRO Select Elementar) using sulphanilamide as standard and expressed as dry weight percentage (%). The carbohydrate (CHO) content was estimated according to Dubois *et al.*[34] method using spectrophotometer (UV-1800 spectrophotometer, Shimadzu) and glucose as standard. It was expressed as mg g^{-1} of sediment dry weight. The protein (PRT) content was estimated according to Hartree[35] method using bovine serum albumin as calibration standard and the results were reported as mg g^{-1} of sediment dry weight. Sediment samples previously combusted in a muffle furnace at 450°C for 4 h were used as the blanks for biochemical analysis.

Data analysis

A correlation analysis was performed to understand the relationship between different bacterial species (log transformed) and near bottom water salinity, dissolved oxygen and tide. This analysis was done using Statistica 8.0 statistical package.

Results

Physico-chemical parameters of the near bottom water

The near bottom water temperature during the study period varied from 25.7°C to 31.1°C, with maximum temperature during PreM-I. The average salinity fluctuated between 22 and 34. Salinity was low (4) towards the upstream at CR during MON and was high (37) at the mouth (MB) during the PreM-I season. DO in the near bottom water was high during PreM-I and low during POM-II and ranged from 1.55 to 7.83 mg l^{-1}. A strong correlation between DO and tide ($r = 0.67$; $P \leq 0.001$) indicates the influence of tide on DO in the near bottom water.

Table 2. Correlation matrix of environmental parameters with bacterial population in the surface sediments of the Zuari estuary

	TBC	TVC	TC	VC	VA	VP	GS	SH	SL	DO	Salinity	Tide
TBC	1.00											
TVC	**–0.26***	1.00										
TC	0.03	**0.41****	1.00									
VC	–0.08	**0.21***	0.07	1.00								
VA	0.02	**0.35****	**0.30****	0.16	1.00							
VP	–0.04	**0.24***	0.17	**0.36****	**0.51****	1.00						
GS	–0.11	**0.25***	0.05	0.09	**–0.19***	–0.05	1.00					
SH	–0.12	0.11	**0.19***	–0.10	–0.11	0.10	0.13	1.00				
SL	0.11	0.01	0.12	0.06	0.84	–0.02	0.17	–0.03	1.00			
DO	**0.46****	–0.02	**0.26***	–0.10	–0.01	0.16	0.00	0.12	**0.22***	1.00		
Salinity	**–0.26***	0.01	**–0.21***	**0.23***	–0.01	–0.09	0.10	–0.11	–0.12	**–0.44****	1.00	
Tide	**0.38****	–0.03	**0.25***	–0.03	–0.05	**0.25***	–0.12	0.15	–0.15	**0.67****	**–0.34****	1.00

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level. Significant correlations are highlighted in bold.
TBC, total bacterial count; TVC, total viable count; TC, total coliforms; VC, *V. cholerae*; VA, *V. alginolyticus*; VP, *V. parahaemolyticus*; GS, general *Streptococci* spp.; SH, *Shigella* spp.; SL, *Salmonella* spp.; DO, dissolved oxygen.

Spatio-temporal variations of the bacterial populations (TBC, TVC and pathogenic bacteria) in the surface sediments

The average abundance of TBC in the sediment was high (1.77×10^8 cells g^{-1}) at CH, a lower middle estuarine station and minimum (6.54×10^7 cells g^{-1}) at the mouth of the estuary (MB) (Figure 2 *a*). The abundance of TBC was high during MON, low during POM-I (Figure 3 *a*) and was influenced positively by near bottom water DO ($r = 0.46$; $P \leq 0.001$) and negatively by salinity ($r = –0.26$; $P \leq 0.006$). The tide showed a significant influence ($r = 0.38$; $P \leq 0.001$) on the distribution of TBC (Table 2). The TVC ranged from 3.64×10^5 to 1.48×10^6 CFU g^{-1} with maximum abundance towards the upstream at CR during POM-I season (Figures 2 *b* and 3 *b*).

The average abundance of TC (3.34×10^5 CFU g^{-1}) and general *Streptococci* spp. (GS) (1.22×10^3 CFU g^{-1}) was high during POM-I at CR (Figure 2 *c*). TC were influenced positively by bottom water DO ($r = 0.26$; $P \leq 0.006$), tide ($r = 0.25$; $P \leq 0.009$) and negatively by the salinity ($r = –0.21$; $P \leq 0.030$; Table 2). The average abundance of TC decreased from POM-I to POM-II season (POM-I > PreM-I > MON > POM-II; Figure 3 *c*). *E. coli* O157 : H7 was found towards upstream stations only during MON (Supplementary Table 1) and were high (3.90×10^3 CFU g^{-1}) at CR. The *Shigella* spp. and VA were high towards the mouth of estuary during PreM-II, whereas *Salmonella* spp. were high towards the upstream during PreM-I. The abundance of VC ranged from 3.02×10^4 to 8.48×10^5 CFU g^{-1} and was high at MB, the mouth of the estuary during POM-I (Figure 2 *d*) and influenced positively by near bottom water salinity ($r = 0.23$; $P \leq 0.015$). However, VP were high (9.85×10^4 CFU g^{-1}) towards upstream at AG (Figure 2 *f*) and influenced by tide ($r = 0.25$; $P \leq 0.009$; Table 2). Seasonal variation showed high abundance of VC and VP during POM-I (Supplementary Table 1). The percentage occurrence of pathogenic bacteria in the surface sediments showed dominance of VP (88.8%) and VA (80.9%) among autochthonous *Vibrio* spp. and TC (79.3%) and *Shigella* spp. (69.6%) among allochthonous pathogens (Table 3).

Biogeochemical composition of the surface sediments

The upstream stations (AG and CR) and anthropogenically influenced lower estuarine station (CH) were characterized by relatively high content of TOM, TOC and TN when compared to stations towards the mouth of the estuary (Table 4). TOM, TOC and TN in the surface sediments ranged from 0.08% to 6.98%, 0.05% to 4.05% and 0.00% to 0.35% respectively at the Zuari estuary (Table 5). High quantity of TOM and TOC was observed during MON. The spatial and seasonal distribution indicated that the concentration of CHO was more than PRT in the sediments. The concentration of PRT and CHO varied from 0.13 to 16.1 mg g^{-1} and 0.45 to 18.2 mg g^{-1} dry weight of sediment respectively (Table 5).

Discussion

Bacteria in the estuarine and marine environment play a significant role in biogeo-chemical processes, nutrient cycling and organic matter degradation[36]. The present study showed high abundance of TBC in sediments of CH, a lower middle estuarine station influenced by anthropogenic activities. This site is actively used for ship building workshops, yards and subjected to various land-based anthropogenic activities[37]. A recent study on microbial community structure in the surface sediment of CH in the Zuari estuary reported dominance of *Gammaproteobacteria* which includes members of potential pathogens such as *Enterobacteriaceae*, *Vibrionaceae* and

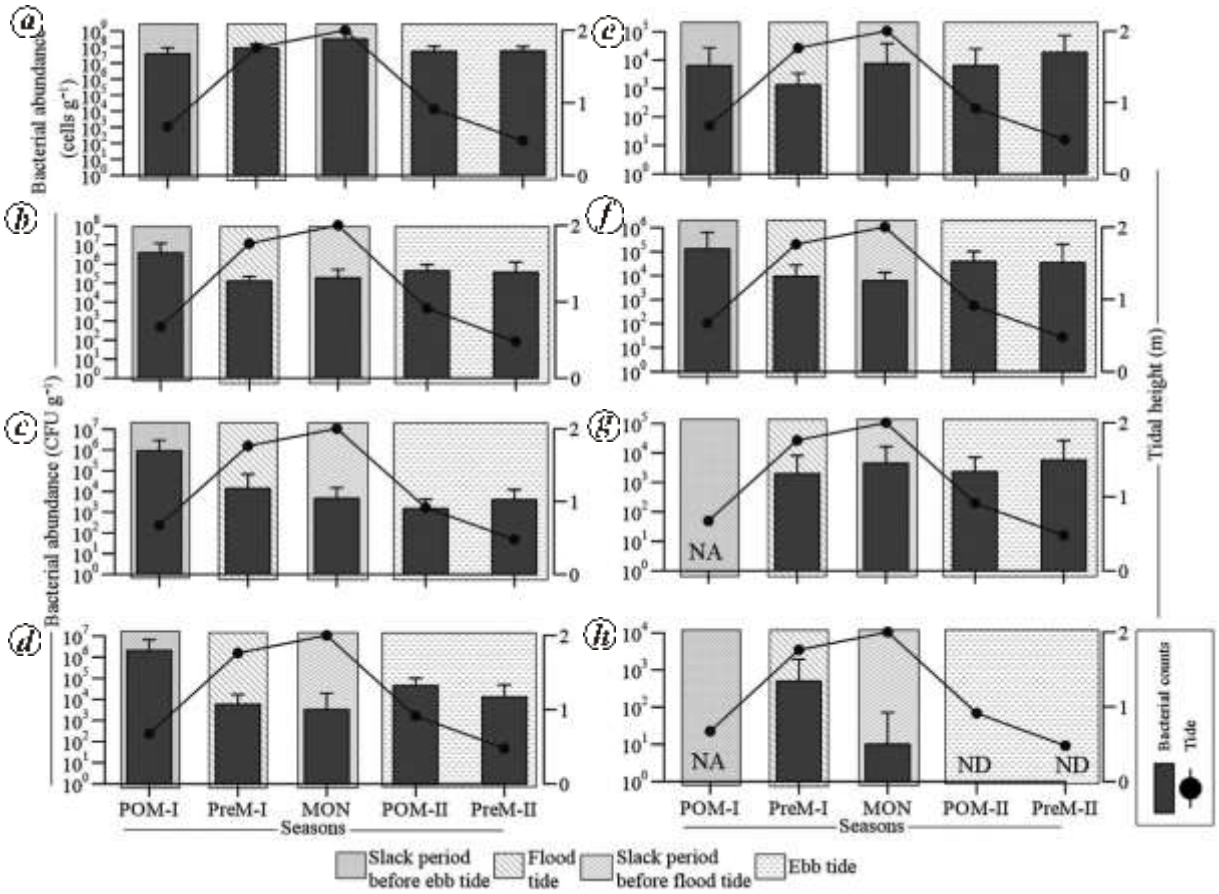


Figure 3. Seasonal variations in the (***a***) total bacterial count (TBC) (***b***) total viable count (TVC), (***c***) total coliforms (TC), (***d***) *V. cholerae*, (***e***) *V. alginolyticus*, (***f***) *V. parahaemolyticus*, (***g***) *Shigella* spp., (***h***) *Salmonella* spp. in the surface sediments of the Zuari estuary during different tidal phases. (NA, not analysed; ND, none detected). Tidal data for study period was obtained from tide table.

Table 3. Percentage (%) occurrence of different pathogens in the surface sediments of the Zuari estuary

Bacteria	Number of samples analysed	Positive occurrence	Overall occurrence (%)
TC	188	149	79.3
VC	188	128	68.1
VA	188	152	80.9
VP	188	167	88.8
EC	188	8	4.3
SH	125	87	69.6
SL	125	10	8.0
GS	188	39	20.7

TC, total coliforms; VC, *Vibrio cholerae*; VA, *V. alginolyticus*; VP, *V. parahaemolyticus*; EC, *E. coli* O157:H7; SH, *Shigella* spp.; SL, *Salmonella* spp. and GS, General *Streptococci* spp.

Pseudomonadaceae[38]. In general, high abundance of TBC was observed during MON. Large amount of fresh water discharge from catchment area during MON could be one of the sources that brings in land run-off rich in bacterial load and anthropogenic input. Zuari estuary receives maximum sediment discharge during peak rainfall[22]. Recently, Shynu *et al.*[39] studied $d^{13}C_{org}$ in the sediment and reported terrestrial organic matter to be the dominant component in the lower region of the Zuari estuary during MON. The ratio of TOC/TN in the sediment has been used to distinguish the autochthonous or marine organic matter from allochthonous or terrestrial input. The protein-rich algae and plankton are characterized by low C/N ratio (4–10) than terrestrial land plants (>20) which are rich in cellulose[40]. TOC/TN ratio in the present study was 15.44 during MON which indicates mixed input of terrestrial and marine derived organic matter in the surface sediments. This could be another possibility for high abundance of TBC. Mahalakshmi *et al.*[41] also observed high density of heterotrophic bacteria in the sediment and water column during the MON at the Uppanar estuary located on the south east coast of India.

The TVC, TC and general *Streptococci* spp. were high at CR which is located at the entrance of the channel near the junction between bay and upstream region of Zuari river, is shallow in depth and is influenced by tidal currents, waves, movement of fishing trawlers and ferry boats. Previous study by Dessai and Nayak[42] reported that sediments in this region are rich in silt and clay content due to deposition of fine particles during low energy

condition and colloidal aggregates during estuarine mixing. The mouth region is rich in sand owing to intense tidal currents which cause the transfer of fine grain particles away from the mouth. This station was also characterized by high TOM, TOC, TN, proteins and carbohydrates which possibly favoured high abundance of TVC, TC and general *Streptococci* spp. A parallel study on the sediment texture reported that the stations towards the upstream are rich in silt and clay (54.6%) content when compared to those located at the mouth (19.3%) of the estuary (Desai and Atchuthan; personal observation). Perkins *et al.*[11] also reported high abundance of pathogenic bacteria in organic matter, silt and clay rich sediments at the Conwy estuary, UK. The attachment of faecal coliforms to the fine particles increases their viability and transport to the sediment bed[43]. The fine grained sediments with large surface area have more adsorptive capacity for organic matter[44] and influence the accumulation of organic matter and bacteria. TOC/TN ratio indicated input of both autochthonous and terrestrial organic matter at CR which supported high abundance of allochthonous coliforms. The dominance of carbohydrates over proteins and low PRT/CHO ratio (<1) indicates the detrital heterotrophic nature of the environment[45]. The abundance of TC in the present study ranged from none detected (ND) to 88.7×10^5 CFU g^{-1} and was almost similar to the previous study (ND – 47.7×10^5 CFU g^{-1})[25]. The abundance of *E. coli* was 2-folds lower (ND – 3.90×10^3 CFU g^{-1}) than the previous study[25] (ND – 6.4×10^5 CFU g^{-1}). However, their study did not include the influence of tides. The flood tide and ebb tide to a certain extent cause decrease in the abundance of pathogenic bacteria from the sediment. A recent study reported that depending on the riverine discharge and tidal amplitude interplay sediment re-suspension, mediated increase in suspended particulate matter (SPM) significantly regulates bacterial population in the Zuari estuary[27]. The re-suspension of sediment affects the quality of overlying water by addition of SPM, organic matter and pathogens which are hazardous to human health. The assessment of pathogenic bacteria in the sediment can improve our understanding of the behaviour, fate and mitigation of potential pathogens in the sediment as well as water body[46].

The abundance of TVC, TC, general *Streptococci* spp. and VP was significantly higher during slack period before ebb tide of POM-I than ebb tide of POM-II. This variation in TVC and pathogenic bacterial abundance is attributed to the nature of sediment as well as tidal conditions observed during POM. It is well known that re-suspended particles settle to the sediment bed during slack period before starting of ebb tide. They get re-suspended from the sediment with ebb currents, remain suspended to a certain degree and then settle to the bottom[47]. The pathogenic bacteria attached to suspended particles increase their downward flux to the bottom sediment[11,48]. This is the possible reason for high abundance of coliforms, VP and general *Streptococci* spp. in the sediments towards the upstream station during POM. The negative influence of near bottom water salinity on the abundance of total coliforms in the sediment indicates their extraneous input into the sediment. The presence of the pathogenic strain, *E. coli* O157 : H7 in the sediment towards the upstream stations only during MON season can be attributed to land run-off and sewage input. Nagvenkar and Ramaiah[26] also reported the influence of land run-off and domestic sewage input on the abundance of fecal coliforms in the Zuari–Mandovi estuaries.

High abundance of *V. cholerae* was observed at the mouth of the estuary during slack period prior to ebb tide of POM. A recent study also reported high numbers of VC in the water column at the mouth of the Zuari estuary[27]. Their abundance was influenced by SPM, nutrients and salinity. The ratio of TOC/TN was low (8.71) at the mouth of the estuary, indicating contribution of phytoplankton which could be a possible reason for high abundance of VC, as *Vibrio* spp. are associated with plankton population[49]. The abundance of *Shigella* spp. and VA was high towards the mouth of the estuary during ebb tide of the PreM-II season indicating their deposition during ebb tide. However, *Salmonella* spp. were high towards the upstream during flood tide of PreM-I. The flood tide currents cause re-suspension of sediment bed and transfer particles towards land. During ebb currents particles move towards the sea and settle to the bottom sediment[47,50]. An earlier study in this estuary had also pointed out that the effluents containing high numbers of pathogenic bacteria accumulate towards downstream during low tide-period[6].

Overall, the present study showed that the percentage (%) occurrence of VP and VA was high in the sediments as they are autochthonous to the estuarine and marine ecosystems[17]. High prevalence of coliforms and *Shigella* spp. among allochthonous pathogens can be attributed to their adaptability and survival in the sediment. Pathogenic bacteria adsorbed to sediment particles protect themselves from UV light, phage attack and protozoan grazing and hence survive for longer time in the sediment[11–13,46]. The fecal indicator bacteria can even proliferate and regrow in organic matter and fine particle-rich sediments[51]. Moreover, VP emerged as a dominant bacterium among all pathogens in the sediment during the study period (Table 3). VP can tolerate wide range of salinities and is able to use large numbers of substrates for growth[52,53]. VP are found to be associated with copepods and play a significant role in nutrient cycling by mineralization of chitinous material with help of chitinase enzymes[17,53]. Also, it has been reported that VP are associated with phytoplankton and their growth is influenced by decaying planktonic cells[54]. Zuari estuary is productive during non-monsoon seasons and subjected to heterotrophy during monsoon due to large input of

Table 4. Spatial variations in organic matter parameters in the surface sediments of the Zuari estuary

Parameters	OB	BB	AG	CR	CH*	VS	MB
TOM (%)	1.03 ± 0.79	1.06 ± 0.76	2.64 ± 1.43	4.25 ± 1.73	2.57 ± 2.33	2.09 ± 0.77	1.05 ± 1.04
TOC (%)	0.60 ± 0.46	0.61 ± 0.44	1.53 ± 0.83	2.46 ± 1.00	1.49 ± 1.35	1.21 ± 0.45	0.61 ± 0.61
TN (%)	0.04 ± 0.04	0.07 ± 0.06	0.15 ± 0.11	0.18 ± 0.11	0.09 ± 0.07	0.07 ± 0.03	0.07 ± 0.09
TOC/TN	15.0	8.71	10.20	13.67	16.56	17.29	8.71
PRT (mg g^{-1})	1.37 ± 1.12	2.25 ± 1.60	5.97 ± 3.46	9.95 ± 3.22	5.32 ± 4.14	4.27 ± 1.36	1.92 ± 3.22
CHO (mg g^{-1})	1.91 ± 1.12	3.33 ± 2.54	8.12 ± 4.83	11.8 ± 4.67	7.06 ± 5.70	4.51 ± 2.17	2.87 ± 4.07
PRT/CHO	0.72	0.68	0.74	0.85	0.75	0.95	0.67

OB, Odxel Beach; BB, Bambolim beach; AG, Agacaim; CR, Cortalim; CH, Chicalim; VS, Vasco; MB, Marmugao bay; *Anthropogenically influenced; TOM: Total Organic Matter; TOC, total organic carbon; TN, total nitrogen; PRT, proteins; CHO, carbohydrates.
These are station wise average (± standard deviation) values of sedimentary parameters including different seasons (PreM, MON and POM).

Table 5. Range and average concentration of organic matter parameters during different seasons in the surface sediments of the Zuari estuary

	POM-I			MON		POM-II		PreM-II	
Parameters	Min–Max	Avg ± SD	PreM-I	Min–Max	Avg ± SD	Min–Max	Avg ± SD	Min–Max	Avg ± SD
TOM (%)	0.40–6.87	2.17 ± 2.36		0.08–6.98	2.39 ± 2.04	0.59–4.06	1.83 ± 1.16	0.18–4.72	1.81 ± 1.46
TOC (%)	0.23–3.99	1.26 ± 1.37		0.05–4.05	1.39 ± 1.18	0.34–2.35	1.12 ± 0.67	0.10–2.74	1.05 ± 0.85
TN (%)	0.00–0.05	0.02 ± 0.02	No samples	0.00–0.20	0.09 ± 0.07	0.03–0.19	0.10 ± 0.08	0.00–0.35	0.10 ± 0.10
PRT (mg g^{-1})	0.42–12.9	4.17 ± 4.84		0.13–16.1	4.41 ± 4.15	1.33–11.4	5.34 ± 3.57	0.28–10.9	3.98 ± 3.54
CHO (mg g^{-1})	0.86–18.6	5.84 ± 6.75		0.45–18.2	5.65 ± 5.21	1.61–14.4	6.49 ± 4.59	0.48–13.6	5.11 ± 4.54

Min, Minimum; Max, Maximum; Avg, average; SD, standard deviation.

terrestrial organic matter and nutrients through land runoff[22,55]. Estuaries play a significant role in trapping and modification of autochthonous and terrestrial organic matter[56]. The present study showed mixed input of marine and terrestrial organic matter in the surface sediments. Low PRT/CHO ratio (<1) indicates the presence of detrital organic matter. High prevalence of VP in the surface sediments suggests their role in the mineralization of detrital organic matter derived from marine and terrestrial inputs.

Conclusions

The present study revealed a significant role of organic matter content, seasons and tide on the distribution of pathogenic bacteria in surface sediments along the banks of the Zuari estuary. The organic matter was of mixed origin (autochthonous and terrestrial) towards upstream and autochthonous at the mouth of estuary and had profound influence on the distribution of pathogenic bacteria in surface sediments. The low (<1) PRT/CHO ratio suggests utilization of proteins and a heterotrophic environment in the sediment. High abundance of pathogenic bacteria in the sediment during slack period before ebb tide indicates their deposition in the sediment. VP and VA were the more frequently occurring bacteria among autochthonous, whereas TC and *Shigella* spp. were dominant among allochthonous pathogens in the sediment. Future studies should address the interaction of pathogenic bacteria with suspended particles, their transport and survival in the sediment which will help in understanding their ecology.

1. Vincy, M. V., Brilliant, R. and Pradeepkumar, A. P., Prevalence of indicator and pathogenic bacteria in a tropical river of Western Ghats, India. *Appl. Water Sci.*, 2015, 1–12.
2. Simeonov, V. *et al.*, Assessment of the surface water quality in Northern Greece. *Water Res.*, 2003, **37**, 4119–4124.
3. Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S. and Singh, V. P., Contamination of water resources by pathogenic bacteria. *AMB Express*, 2014, **4**, 1–16.
4. Clark, A., Turner, T., Dorothy, K. P., Goutham, J., Kalavati, C. and Rajanna, B., Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. *Ecotox. Environ. Safe*, 2003, **56**, 390–397.
5. Patra, A. K., Acharya, B. C. and Mohapatra, A., Occurrence and distribution of bacterial indicators and pathogens in coastal waters of Orissa. *Indian J. Mar. Sci.*, 2009, **38**, 474–480.
6. Rodrigues, V., Ramaiah, N., Kakti, S. and Samant, D., Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India. *Ecol. Indic.*, 2011, **11**, 318–327.
7. Robin, R. S., Vardhan, K. V., Muduli, P. R., Srinivasan, M. and Balasubramanian, T., Preponderance of enteric pathogens along the coastal waters of Southern Kerala, Southwest coast of India. *Mar. Sci.*, 2012, **2**, 6–11.
8. Borade, S., Dhawde, R., Maloo, A., Gajbhiye, S. N. and Dastager, S. G., Occurrence and seasonal variation in distribution of fecal indicator bacteria in Tapi estuary along the West coast of India. *Indian J. Mar. Sci.*, 2014, **43**, 340–347.
9. Khandeparker, L., Anil, A. C., Naik, S. D. and Gaonkar, C. C., Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment. *Marine Poll. Bull.*, 2015, **96**, 337–343.

10. Prasad, V. R., Srinivas, T. N. R. and Sarma, V. V. S. S., Influence of river discharge on abundance and dissemination of heterotrophic, indicator and pathogenic bacteria along the east coast of India. *Marine Poll. Bull.*, 2015, **95**, 115–125.
11. Perkins, T. L., Clements, K., Baas, J. H., Jago, C. F., Jones, D. L., Malham, S. K. and McDonald, J. E., Sediment composition influences spatial variation in the abundance of human pathogen indicator bacteria within an estuarine environment. *PLoS ONE*, 2014, **9**, e112951, 1–9.
12. Davies, C. M., Long, J. A., Donald, M. and Ashbolt, N. J., Survival of fecal microorganisms in marine and freshwater sediments. *Appl. Environ. Microbiol.*, 1995, **61**, 1888–1896.
13. Craig, D. L., Fallowfield, H. J. and Cromar, N. J., Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with *in situ* measurements. *J. Appl. Microbiol.*, 2004, **96**, 922–930.
14. Crabill, C., Donald, R., Snelling, J., Foust, R. and Southam, G., The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. *Water Res.*, 1999, **33**, 2163–2171.
15. An, Y. J., Kampbell, D. H. and Breidenbach, G. P., *Escherichia coli* and total coliforms in water and sediments at lake marinas. *Environ. Pollut.*, 2002, **120**, 771–778.
16. Shellenbarger, G. G., Downing-Kunz, M. A. and Schoellhamer, D. H., Suspended-sediment dynamics in the tidal reach of a San Francisco Bay tributary. *Ocean Dynm.*, 2015, **65**, 1477–1488.
17. Thompson, F. L., Iida, T. and Swings, J., Biodiversity of Vibrios. *Microbiol. Mol. Biol. Rev.*, 2004, **68**, 403–431.
18. Kanungo, S. *et al.*, Cholera in India: an analysis of reports, 1997–2006. *Bull. World Health Organ.*, 2010, **88**, 185–191.
19. World Health Organization (WHO), Water quality and health strategy 2013–2020. Geneva, Switzerland, 2013, 1–15.
20. Abhirosh, C., Sherin, V., Thomas, A. P., Hatha, A. A. M. and Abhilash, P. C., Potential exposure risk associated with the high prevalence and survival of indicator and pathogenic bacteria in the sediment of Vembanadu Lake, India. *Water Qual. Expo. Health*, 2010, **2**, 105–113.
21. Manoj, N. T. and Unnikrishnan, A. S., Tidal circulation and salinity distribution in the Mandovi and Zuari estuaries: case study. *J. Waterw. Port Coast. Ocean Eng.*, 2009, **135**, 278–287.
22. Qasim, S. Z. and Sen Gupta, R., Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuar. Coast. Shelf Sci.*, 1981, **13**, 557–578.
23. Shetye, S. R. and Murty, C. S., Seasonal variation of the salinity in the Zuari estuary, Goa, India. *Proc. Indian Acad. Sci. Earth Planet. Sci.*, 1987, **96**, 249–257.
24. Pangare, G., Pangare, V. and Das, B., Water at risk. In *Springs of Life: India's Water Resources*. Academic Foundation, New Delhi, India, 2006, pp. 207–210.
25. Ramaiah, N., Rodrigues, V., Alwares, E., Rodrigues, C., Baksh, R., Jayan, S. and Mohandass, C., Sewage-pollution indicator bacteria. In *The Mandovi and Zuari Estuaries* (eds Shetye, S. R., DileepKumar, M. D. and Shankar, D.), Research Publishing Services, Chennai, India, 2007, pp. 115–120.
26. Nagvenkar, G. S. and Ramaiah, N., Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex. *Environ. Monit. Assess.*, 2009, **155**, 245–256.
27. Khandeparker, L., Eswaran, R., Gardade, L., Kuchi, N., Mapari, K., Naik, S. D. and Anil, A. C., Elucidation of the tidal influence on bacterial populations in a monsoon influenced estuary through simultaneous observations. *Environ. Monit. Assess.*, 2017, **189**, 41.
28. Shetye, S. R., Shankar, D., Neetu, S., Suprit, K., Michael, G. S. and Chandramohan, P., The environment that conditions the Mandovi and Zuari estuaries. In *The Mandovi and Zuari Estuaries* (eds Shetye, S. R., DileepKumar, M. D. and Shankar, D.), Research Publishing Services, Chennai, India, 2007, pp. 3–27.
29. Grasshoff, K., Ehrhardt, M. and Kremling, K., Determination of oxygen. In *Methods of Seawater Analysis*, Wiley-Vch, Weinheim, 1983, pp. 75–89.
30. Luna, G. M., Manini, E. and Danovaro, R., Large fraction of dead and inactive bacteria in coastal sediments: comparison of protocols for determination and ecological significance. *Appl. Environ. Microbiol*., 2002, **68**, 3509–3513.
31. Marie, D., Partensky, F., Vaulot, D. and Brussaard, C., Enumeration of phytoplankton, bacteria and viruses in marine samples. *Curr. Protoc. Cytom*., 2001, 1–14.
32. El Wakeel, S. K. and Riley, J. P., The determination of organic carbon in marine muds. *ICES. J. Mar. Sci*., 1957, **22**, 180–183.
33. Bhosle, N. B. and Dhople, V. M., Distribution of some biochemical compounds in the sediments of the Bay of Bengal. *Chem. Geol.*, 1988, **67**, 341–352.
34. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. T. and Smith, F., Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 1956, **28**, 350–356.
35. Hartree, E. F., Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.*, 1972, **48**, 422–427.
36. Das, S., Lyla, P. S. and Khan, S. A., Marine microbial diversity and ecology: importance and future perspectives. *Curr. Sci.*, 2006, **90**, 1325–1335.
37. Shenai-Tirodkar, P. S., Gauns, M. U. and Ansari, Z. A., Evaluation of surface water and sediment quality in Chicalim Bay, Nerul Creek, and Chapora Bay from Goa coast, India-a statistical approach. *Environ. Monit. Assess.*, 2016, **188**, 1–13.
38. Khandeparker, L., Kuchi, N., Kale, D. and Anil, A. C., Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. *Ecol. Indic*., 2017, **74**, 172–181.
39. Shynu, R., Rao, V. P., Sarma, V. V. S. S., Kessarkar, P. M. and Mani Murali, R., Sources and fate of organic matter in suspended and bottom sediments of the Mandovi and Zuari estuaries, western India. *Curr. Sci.*, 2015, **108**, 226–238.
40. Meyers, P. A., Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem. Geol*., 1994, **114**, 289–302.
41. Mahalakshmi, M., Srinivasan, M., Murugan, M., Balakrishnan, S. and Devanathan, K., Isolation and identification of total heterotrophic bacteria and human pathogens in water and sediment from Cuddalore fishing harbour after the tsunami. *Asian J. Biol. Sci*., 2011, **4**, 148–156.
42. Dessai, D. V. and Nayak, G. N., Seasonal distribution of surface sediments and hydrodynamic conditions in Zuari Estuary, Goa, central west coast of India. *J. Indian Assoc. Sedimentologists*, 2007, **26**, 25–32.
43. Fries, J. S., Characklis, G. W. and Noble, R. T., Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, NC. *J. Environ. Eng.*, 2006, **132**, 1338–1345.
44. Manju, M. N., Resmi, P., Kumar, C. R., Gireeshkumar, T. R., Chandramohanakumar, N. and Joseph, M. M., Biochemical and stable carbon isotope records of mangrove derived organic matter in the sediment cores. *Environ. Earth Sci.*, 2016, **75**, 1–15.
45. Danovaro, R., Detritus-Bacteria-Meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. *Mar. Biol.*, 1996, **127**, 1–13.
46. Hassard, F. *et al.*, Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments – a review. *Front. Microbiol.*, 2016, **7**, 1–31.
47. Sanford, L. P., Suttles, S. E. and Halka, J. P., Reconsidering the physics of the Chesapeake Bay estuarine turbidity maximum. *Estuaries*, 2001, **24**, 655–669.
48. Droppo, I. G., Liss, S. N., Williams, D., Nelson, T., Jaskot, C. and Trapp, B., Dynamic existence of waterborne pathogens within

river sediment compartments. Implications for water quality regulatory affairs. *Environ. Sci. Technol.*, **43**, 1737–1743.
49. Hsieh, J. L., Fries, J. S. and Noble, R. T., *Vibrio* and phytoplankton dynamics during the summer of 2004 in a eutrophying estuary. *Ecol. Appl.*, 2007, **17**, 102–109.
50. Chant, R. J. and Stoner, A. W., Particle trapping in a stratified flood-dominated estuary. *J. Mar. Res.*, 2001, **59**, 29–51.
51. Desmarais, T. R., Solo-Gabriele, H. M. and Palmer, C. J., Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microbiol.*, 2002, **68**, 1165–1172.
52. Watkins, W. D. and Cabelli, V. J., Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. *Appl. Environ. Microbiol.*, 1985, **49**, 1307–1313.
53. Kaneko, T. and Colwell, R. R., Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. *Appl. Microbiol.*, 1975, **29**, 269–274.
54. Rehnstam-Holm, A. S., Godhe, A., Harnström, K., Raghunath, P., Saravanan, V., Collin, B. and Karunasagar, I., Association between phytoplankton and *Vibrio* spp. along the southwest coast of India: a mesocosm experiment. *Aquat. Microb. Ecol.*, 2010, **58**, 127–139.
55. Ram, A. S., Nair, S. and Chandramohan, D., Seasonal shift in net ecosystem production in a tropical estuary. *Limnol. Oceanogr.*, 2003, **48**, 1601–1607.
56. Minor, E. C., Boon, J. J., Harvey, H. R. and Mannino, A., Estuarine organic matter composition as probed by direct temperature-resolved mass spectrometry and traditional geochemical techniques. *Geochim. Cosmochim. Acta*, 2001, **65**, 2819–2834.

ACKNOWLEDGEMENTS. We acknowledge the facilities and support provided by the Director, National Institute of Oceanography. We thank Dr Dattesh Desai and other colleagues of Biofouling and Bioinvasion Division (BBD) for their help during sampling. This work was supported by grants under Ballast Water Management Programme, India (BAMPI-India), Ministry of Shipping and DG Shipping, Government of India and CSIR funded Ocean Finder Program. This is a NIO contribution No. 6051.

Received 18 November 2016; revised accepted 9 May 2017

doi: 10.18520/cs/v113/i09/1729-1738

Environ Monit Assess (2017) 189: 41
DOI 10.1007/s10661-016-5687-3

Elucidation of the tidal influence on bacterial populations in a monsoon influenced estuary through simultaneous observations

Lidita Khandeparker · Ranjith Eswaran · Laxman Gardade · Nishanth Kuchi · Kaushal Mapari · Sneha D. Naik · Arga Chandrashekar Anil

Received: 19 July 2016 / Accepted: 7 November 2016 / Published online: 29 December 2016

Abstract The influence of tides on bacterial populations in a monsoon influenced tropical estuary was assessed through fine resolution sampling (1 to 3 h) during spring and neap tides from mouth to the freshwater end at four stations during pre-monsoon, monsoon and post-monsoon seasons. Higher abundance of total bacterial count (TBC) in surface water near the river mouth, compared to the upstream, during pre-monsoon was followed by an opposite scenario during the monsoon When seasonally compared, it was during the post-monsoon season when TBC in surface water was highest, with simultaneous decrease in their count in the river sediment. The total viable bacterial count (TVC) was influenced by the depth-wise stratification of salinity, which varied with tidal fluctuation, usually high and low during the neap and spring tides respectively. The abundance of both the autochthonous *Vibrio* spp. and allochthonous coliform bacteria was influenced by the concentrations of dissolved nutrients and suspended particulate matter (SPM). It is concluded that depending on the interplay of riverine discharge and tidal amplitude, sediment re-suspension mediated increase in SPM significantly regulates bacteria populations in the estuarine water, urging the need of systematic regular monitoring for better prediction of related hazards, including those associated with the rise in pathogenic *Vibrio* spp. in the changing climatic scenarios.

Keywords Estuary · Simultaneous sampling · *Vibrio cholerae* · Total coliforms · Tides · Bacterial dynamics

Electronic supplementary material The online version of this article (doi:10.1007/s10661-016-5687-3) contains supplementary material, which is available to authorized users.

L. Khandeparker · R. Eswaran · L. Gardade · N. Kuchi · K. Mapari · S. D. Naik · A. C. Anil (✉)
CSIR-National Institute of Oceanography, Dona Paula, Goa, India
e-mail: acanil@nio.org

Introduction

Estuaries are transition zones between marine and freshwater environments, which are highly productive and experience continuous tidal forcing and mixing of the water column (Correll 1999; McLusky and Elliott 2004). The large amount of freshwater influx from different sources brings high amount of pollutants, untreated sewage and microbial contaminants which can alter the estuarine biological communities. Bacteria are an important component of the microbial loop that influence food web dynamics and ecosystem functioning, which are passive drifters, and their population is expected to be influenced by the physical processes and anthropogenic impact.

Bacteria play an important role in the biogeochemical cycling in an ecosystem (Azam et al. 1983). However, some bacteria are pathogenic which are either allochthonous or autochthonous and responsible for causing diseases in marine organisms and human beings (Neogi et al. 2014 and references therein). *Vibrio* spp. are autochthonous to estuaries and possesses chitinolytic enzymes, which can use crustacean exoskeleton made of chitin,

and thus play an important role in nutrient recycling (Bassler et al. 1991). Among *Vibrio's*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the most important pathogens known to cause diseases in humans, shellfish and shrimps (Daniels and Shafaie 2000; Aguirre-Guzmán et al. 2004; Oliver and Kaper 1997). *V. cholerae* is a causative agent of epidemic cholera in humans that results in rapid dehydration and even death of infected persons (Finkelstein 1996). More than 200 O-antigen serogroups of *V. cholerae* are identified till date, among which only two serogroups, O1 and O139, are known to cause epidemics and pandemics (Sack et al. 2003). Outbreak of cholera has been reported in various countries (Sur et al. 2000; Smirnova et al. 2004; CDC 2010; Hendriksen et al. 2011). In India, cholera epidemics crop up with seasonal regularity in the Gangetic delta (Colwell 1996). Their distribution and outbreaks in estuaries are often related to elevated water temperature and salinity (Blackwell and Oliver 2008; Oberbeckmann et al. 2012).

The allochthonous bacteria, especially fecal coliforms, are released directly through wastewater, raw sewage, human and animal waste to the rivers and the estuaries. *Escherichia coli* and intestinal *Enterococci* are used to check microbiological quality of water (Ouattara et al. 2011) and are crucial for understanding patterns of water quality impairment, establishing management planning and developing appropriate mitigation strategies (Stumpf et al. 2010). Evaluating the survival and persistence of such pathogens is of concern to human health and has implications in microbial ecology of such monsoon-influenced estuary.

Earlier studies have revealed that salinity changes with the tidal action are major factors in controlling the survival and viability of the bacterial populations in estuaries (Hyun et al. 1999; Devanathan et al. 2010). A study by Eldridge and Sieracki (1993) in the York River sub estuary also indicated that the bacterial abundance in the surface water was regulated by spring–neap tidal cycle. Generally, tides facilitate transport of organisms and nutrients in estuaries. Further, the tidal currents are also important in re-suspension of sediments at various estuaries (Lindsay et al. 1996; Morris and Howarth 1998). These bed sediments are known as reservoir for fecal bacteria and enteric pathogens in aquatic systems (Alm et al. 2003). Resuspension of such bed sediments to the water column increases persistence and transport of pathogens in the estuaries and risks to public health (Desmarais et al. 2002). Other than physical processes, enhancement of nutrient load via inflows may also trigger the growth of human pathogens. Role of nutrients in influencing the bacterial population is well known (Allen et al. 2002). Since bacteria are passive, their population is expected to be influenced by physical processes. Such influence of physical processes on pathogens in this estuary is poorly studied.

This study was undertaken in the Zuari estuary, a highly productive tide-dominated monsoonal estuary. A significant contamination from untreated sewage and effluent discharge has been reported in this estuary (Sawkar et al. 2003). Generally, low-saline estuarine areas have been recognized as region of severe physiological stress for both marine and freshwater organisms (Kinne 1971). Transition from fresh to brackish and marine conditions influence the biogeochemical process. Taking this into consideration, four sampling stations depicting the mouth of the estuary (M), lower middle estuary (LM), upper middle estuary (UM) and upstream (U) were simultaneously sampled using four trawlers anchored midstream in the study, which is first of its kind from the region. The sampling was repeated for three seasons in order to evaluate the temporal variations.

Previous studies have reported distribution and occurrence of different pathogens (such as *E. coli*, total coliforms, *Vibrios*, *Salmonella* spp., fecal indicators and *E. coli* O157:H7) (Nagvenkar and Ramaiah et al. 2009; Rodrigues et al. 2011; Khandeparker et al. 2015) and pollution (Shirodkar et al. 2012; Kessarkar et al. 2015) in this estuary. Earlier studies carried out in this estuary so far have provided snapshot views. In this study, the emphasis was on characterizing the bacterial distribution through high-resolution simultaneous observations. The present study was interdisciplinary during which the stratification and currents were also measured (Sunder et al. 2015). As the bacterial population is influenced by physical processes, in this study, we elucidate the differences in their population in the water column in relation to stratification in a monsoon-influenced estuary.

Materials and methods

Study sites and sampling

Zuari estuary (15° 27.5′ N, 73 ° 48′ E) is located along the west coast of India. The river source of this estuary originates in the Sahyadri Hills from Dighi Ghat and

experiences distinct climatic variations due to the impact of monsoon. The estuary remains well mixed in pre-monsoon (PreM) season, while it gets stratified during southwest monsoon (SW-MoN). Both ebb and flood currents are strong in Zuari estuary and receive high amount of freshwater during SW-MoN (Qasim and Sen Gupta 1981). Tides in this estuary are of mixed semidiurnal type normally occurring twice a day, and the tide ranges are approximately 2.5 and 1.5 m during spring and neap tides respectively (Shetye et al. 2007). The tidal influence is more at the mouth as compared to the upstream end of the estuary. These tides as well as monsoon runoff are responsible for the mixing and circulation. The main channel of Zuari estuary is about 50-km long and 5-km wide with approximate depth of 5 m. But their cross section area decreases towards the riverine end (0.5 km) (Shetye et al. 1995) and receives freshwater from a number of streams and rivers. During the SW-MoN (June–September), freshwater influence is comparatively more than the PreM summer season (March to May), wherein there is less river discharge and is vertically mixed throughout the estuary till the riverine region (Shetye et al. 1995).

The four sampling stations included were Dona Paula (DP; 15° 25′ 16.9″ N 73° 47′ 36.9″ E), bay station situated at the mouth of the estuary; Cortalim (CR; 15^0 24′ 32.0′ N, 73^0 54′ 50.2′ E), lower middle estuary; Borim (BR; 15° 25′ 03.6″ N, 73° 47′ 58.0″ E), upper middle estuary and Sanvordem (SV; 15^0 16′ 01.1′ N, 74^0 06′ 36.0′ E), upstream station (Fig. 1). No observations were carried out during the SW-MoN season due to rough weather only at DP station (mouth of the estuary). CR and BR are at the mid part of the estuary, and SV is at the head of the estuary with near zero salinity. Freshwater and human activities influence the environmental characteristics of the upstream station. The water is highly stratified at the lower middle estuary (CR) during the flood phase, whereas, at upper middle estuary (BR), it is well mixed. The upstream station (SV), being the river end, showed no such variation.

Observations were carried out every hour for total bacterial count (TBC) and every 3 h for total viable bacterial count (TVC) and pathogens, over a 24-h period. The study was carried out during the year 2011 for three different seasons, and details of the sampling are provided in Table 1. Surface and near bottom water samples were collected using a 5-L capacity Niskin sampler at an interval of 1 and 3 h, while sediment samples were collected at an interval of 5 h using Van Veen Grab from three different locations for 24 h. Water samples (surface and near bottom) were also collected for analysis of suspended particulate matter (SPM), temperature, salinity and dissolved nutrients. After collection, samples were stored in an icebox and transported to the laboratory for further analyses.

Physicochemical parameters

Temperature and salinity were recorded by using portable conductivity–temperature–depth (CTD) probe (Sea-Bird Electronics Inc., USA, of model 19 plus), having an accuracy of 0.005 °C in temperature and 0.005 psu in salinity measurements, and was operated from anchored boats every hour. Tide and flow were recorded using a current metre. SPM was obtained by filtering water through pre-weighed, ashed GF/F filter paper and dried in an oven at 60 °C for 48 h. After drying, filter papers were weighed using weighing balance and expressed as milligram per litre (mg l^{-1}) (Mettler Model AT20; Kumar et al. 2004). Dissolved nutrient concentrations, i.e. nitrate, nitrite, phosphate, ammonia and silicate were analyzed using autoanalyser (Skalar SAN PLUS 8505 Interface v3.331, Netherland; Khandeparker et al. 2015; Roy et al. 2015).

Enumeration of total bacterial count

For total bacterial count analysis, 5 ml of the samples were fixed with formaldehyde (2% final concentration) and 1 ml of this fixed sample was used in the analysis. In the case of sediment, 1 g of the sediment was weighed and diluted in 10 ml of sterile seawater and fixed as described above. For flow cytometric (FCM) analysis of sediment, samples were sonicated at 50% power in a water bath sonicator (Ultrasonic cleaner, Equitron) for 1 min three times to separate the sediment particles from the cells. Further, sonicated samples were centrifuged at 3000 rpm for 1 min and the supernatants were recovered (Luna et al. 2002). The clear supernatants of sediment and water samples were passed through BD cell strainer cap (BD Biosciences, USA; cat no.: 352235) to remove larger particles. Subsequently, the samples were stained with SYBR Green I (Molecular Probes, USA) at 1:10,000 final concentrations (Marie et al. 1999) and incubated for 15 min in the dark at room temperature. After incubation, the samples were analyzed using a BD FACSAria™ II (BD Biosciences, USA) flow cytometre equipped with a nuclear blue laser of 488 nm which can

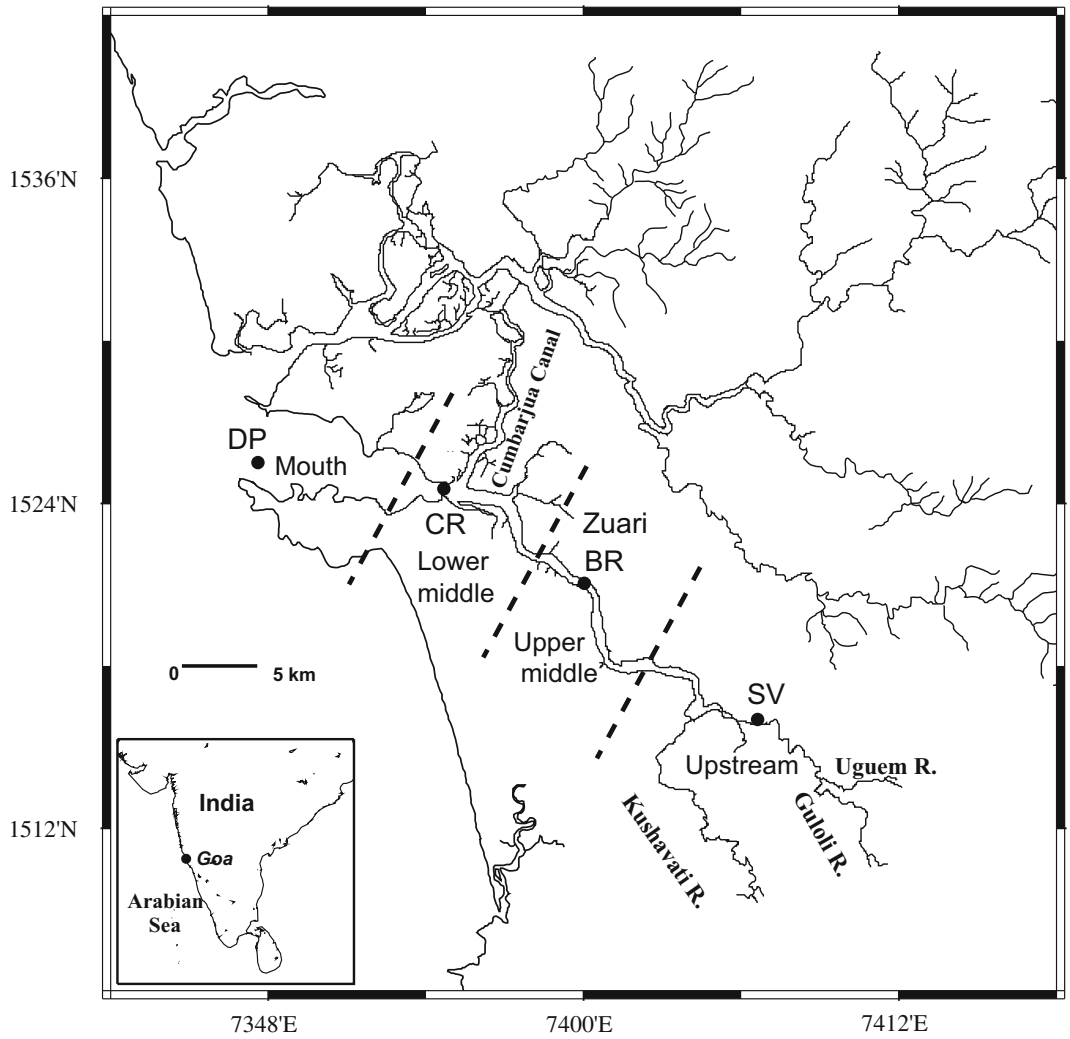


Fig. 1 Map showing the sampling locations along the Zuari estuary. Dona Paula (*DP*), mouth of the estuary; Cortalim (*CR*), lower middle estuary; Borim (*BR*), upper middle estuary and Sanvordem (*SV*), upstream station

differentiate green fluorescence excited by blue laser. Emitted light was collected through the filter sets, with 488/10 band pass (BP) for right angle light scatter (SSC) and 530/30 BP for green fluorescence. Fluorescent beads (1 μm, Polysciences) were used as internal standards and for calibration of the above parameters.

Table 1 Details of sampling seasons, tide, date and tidal height at the start and end time of sampling

Seasons	Tide	Date	Time (start time–end time)	Tidal height (at start–end time)
PreM	Spring	19–20 May 2011	11:00–11:00 AM	1.91–2.06 m
	Neap	27–28 April 2011	14:00–14:00 PM	1.08–1.01 m
SW-MoN	Spring	1–2 August 2011	9:00–9:00 AM	1.20–1.01 m
	Neap	9–10 August 2011	11:00–11:00 AM	1.56–2.08 m
PostM	Spring	29–30 October 2011	10:00–10:00 AM	1.72–1.32 m
	Neap	23–24 October 2011	11:00–11:00 AM	1.3−1.55 m

PreM premonsoon, *SW-MoN* southwest monsoon, *PostM* post-monsoon

Gating was done against SSC versus green fluorescence (FITC). Flow cytometry data were processed using the BD FACS Diva software (BD Biosciences, USA). The bacterial abundance is expressed as cells per millilitre (cells ml^{-1}) for water or per gram (cells g^{-1}) for sediment respectively.

Enumeration of viable and pathogenic bacteria

The samples were diluted as required and spread plated (0.1 ml) on Zobell Marine Agar 2216 (ZMA; HiMedia). ZMA has been extensively used to grow marine bacteria. Pathogenic bacteria were quantified using specific media following the manufacturer's instructions (HiMedia). Thiosulphate–citrate–bile salts (TCBS) medium was used for different *Vibrio* spp. (*V. cholerae*, *V. parahaemolyticus* and *Vibrio alginolyticus*). The presence of sucrose in TCBS allows differentiation of *Vibrio* species (Pfeffer and Oliver 2003). MacConkey agar was used for enumeration of total coliforms (TC). The samples were inoculated on selective agar plates and incubated at 37 °C for 24 h before colonies were enumerated. Cultivable and pathogenic bacterial abundance, thus quantified, is expressed as colony-forming unit per millilitre (CFU ml^{-1}) for water or per gram (CFU g^{-1}) for sediment respectively. Bacteria which were grown on TCBS and MacConkey agar were further confirmed as described by Khandeparker et al. (2015). In brief, bacterial colonies which were grown on selective agar were randomly picked and confirmed using appropriate biochemical tests (HiMedia, India). Subsequently, the bacterial species were also confirmed using MALDI-TOF MS biotyping (MTB) method.

Data analysis

Data were log $(X + 1)$ transformed in order to meet the normality assumptions. The relationship between the bacterial numbers (TVC, TBC, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus* and total coliforms) and the environmental parameters was investigated by means of canonical correspondence analysis (CCA), using the Canoco version 4.5 (ter Braak and Smilauer 2002). Prior to CCA analysis, Detrended Correspondence Analysis (DCA) was performed to determine the variability within species data set. The length of the first axis gradients for all data sets were >2 standard deviation unit (SD) indicating unimodal character of dataset. Due to the unimodal characteristics of the species data set CCA analysis was performed (ter Braak and Smilauer 2002). A forward selection was performed on the set of environmental variables, and statistical significance of each variable was tested using Monte Carlo permutation test under the reduced model with 999 number of permutations. Further, correlation analysis was performed between abiotic (temperature, salinity, dissolved nutrients and stratification factor (ΔS) and bacterial components. The above statistical analysis was performed using Statistica 6.0 statistical package at a significance level of ≤ 0.05 (StatSoft, OK, USA). The stratification factor ΔS, is computed as a difference between the near bottom and surface salinity (Sundar et al. 2015).

Results

Physicochemical parameters and dissolved nutrients

The details of the results of the physicochemical parameters and nutrient dynamics are provided in the supplementary Table 1a–f. Briefly, at the mouth of the estuary (DP), the variation in the mean salinity was minimal (~1 psu) in the surface water (34.97–35.43 during PreM and 31.96–32.82 during post-monsoon (PostM)). While, near bottom water salinity remained constant during the PreM (35.44 ± 0.1) and the PostM seasons (33.38 ± 0.20). The details of salinity variations are reported by Sundar et al. (2015). Large salinity variations were observed at the lower middle (CR) and upper middle (BR) estuarine stations during the PreM season which peaked during the SW-MoN; the influence was more during the high waters (Suppl. Table 1e). The stratification factor (ΔS) was high during neap tide irrespective of stations, except during the SW-MoN which showed strong stratification at the lower middle estuary irrespective of tides (Sundar et al. 2015).

Suspended particulate matter (SPM) was high during the spring tide of PostM season in the surface water (Suppl. Table 1f). During the PreM and the SW-MoN seasons, SPM was high at the lower middle estuarine station (CR; 178 ± 50 mg l^{-1}), followed by the mouth of the estuary (DP; 167 ± 52 mg l^{-1}), and BR (132 ± 35 mg l^{-1}) which is the upper middle estuarine station (CR > DP > BR). However, during the PostM, it was high at the mouth of the estuary (229.5 ± 61 mg l^{-1}). The lowest SPM was observed at the upstream station (SV; 21 ± 2.5 mg l^{-1}) during PreM season. A similar

trend was also observed in the near bottom water (CR (265.77 ± 72) > DP (215.49 ± 71) > BR (150.63 ± 56) > SV (21 ± 11) during PreM. In the case of dissolved nutrients, nitrate was high during PreM (18.27 μmol l^{-1}) and low during PostM (0.87 μmol l^{-1}) whereas an opposite trend was observed in the case of phosphate which was higher (13.03 μmol l^{-1}) during spring tide of PostM at the river mouth station. Dissolved nutrients in the surface water were higher during the spring tide and showed gradual decrease with the advection of neap tide. Nitrate and phosphate concentrations were higher in the upstream (SV) station and decreased towards the mouth of the estuary. The silicate concentration ranged from 0.21–222.7 μmol l^{-1} without any remarkable site-wise variation (Suppl. Table 1a–d).

Total bacterial count

During PreM season, TBC in surface water varied from 1.56×10^5- 2.1×10^6 cells ml^{-1} and was high at the mouth (DP; $2.1 \pm 0.002 \times 10^6$ cells ml^{-1}) followed by upper middle (BR; $3.11 \pm 0.023 \times 10^5$ cells ml^{-1}), lower middle (CR; $1.60 \pm 0.021 \times 10^5$ cells ml^{-1}) and upstream end (SV; $1.56 \pm 0.025 \times 10^5$ cells ml^{-1}) of the estuary during spring tide (DP > BR > CR > SV; Fig. 2a, c, f and i). TBC during neap tide ranged from 5.55×10^3–2.75×10^6 cells ml^{-1} and was low at all the stations.

In the case of SW-MoN, a reverse trend was observed, wherein abundance was high at the upstream end and decreased towards the mouth (Fig. 2d, g and j). Overall, abundance ranged from $4.04 \pm 0.10 \times 10^4$–$1.43 \pm 0.004 \times 10^5$ cells ml^{-1}. Whereas, the abundance of TBC in the near bottom water remained similar as PreM and decreased from lower middle to the upstream end (CR > BR > SV) of the estuary (Fig. 3d, g, and j).

During PostM, the TBC was high throughout the estuary (DP–SV) irrespective of tides in both surface and near bottom waters (Figs. 2 and 3b, e, h and k). It ranged from $1.16 \pm 0.003 \times 10^6$–$1.8 \pm 0.003 \times 10^6$ cells ml^{-1} in the surface and $4.75 \pm 0.01 \times 10^5$–$1.35 \pm 0.006 \times 10^6$ cells ml^{-1} in the near bottom water.

In the case of the sediment, TBC was high and almost similar during PreM and PostM seasons at the mouth and upper middle estuary (Table 2). On the other hand, lower middle estuary (CR) showed high TBC during the PreM followed by SW-MoN and least in the PostM season (Table 2). Sediment samples could not be collected at the upstream station.

Overall, during the PreM season, the TBC was high towards the mouth and low at the upstream end and vice versa was observed in the SW-MoN. During PostM, TBC was high irrespective of the stations and the tides in the surface water. TBC in near bottom water ranged from $9.9\ 1 \times 10^3$–4.12×10^6 cells ml^{-1} and was high throughout the estuary during PreM and PostM seasons. The bacterial numbers were low during SW-MoN season.

The CCA axis 1 and 2 (eigenvalues of 0.822 and 0.223 respectively) explained 90.07% of the cumulative variance of the relation between the species (TBC, TVC, total coliforms (TCs) and pathogens) and environmental variables (Salinity, SPM and nutrients; Fig. 4a). The abundance of TBC was separated along axis 1 and showed a positive relation with nitrate during spring tide in surface water ($r = 0.45$; $p \leq 0.05$). This relation was prominent during the PostM season (Fig. 4b).

Total viable count

During PreM and PostM seasons, TVC was high at the mouth ($2.82 \pm 0.2 \times 10^5$ and $8.96 \pm 0.003 \times 10^5$ CFU ml^{-1}) followed by lower middle ($1.34 \pm 0.06 \times 10^4$ and $8.37 \pm 1.53 \times 10^4$ CFU ml^{-1}), upper middle ($2.84 \pm 0.6 \times 10^3$ and $2.10 \pm 1.7 \times 10^4$ CFU ml^{-1}), and the upstream end ($1.27 \pm 0.3 \times 10^3$ and $3.10 \pm 0.4 \times 10^2$ CFU ml^{-1}) of the estuary irrespective of the tides. In the case of sediment, the trend remained the same and abundance steadily decreased from the mouth to the upstream end during PreM (DP > CR > BR; Table 2). In the present study, at the mouth and lower middle estuary, average TVC in near bottom water and sediment was 0.6 and 0.08% and 3.12 and 5.67% of TBC respectively during PreM and vice versa was observed during PostM spring tide (1.33 and 1.80% in near bottom water; 0.10 and 1.51% in sediments). Moreover, spring tide showed onefold–twofold high abundance than neap tide irrespective of the stations except at upper middle estuary (BR), where the trend was reversed (Table 2).

In the case of SW-MoN, also, TVC decreased from lower middle to the upstream end of the estuary (CR > BR > SV; Suppl. Table 2). However, not much variation in TVC numbers was observed between the tides as seen in other seasons (Suppl. Table 2).

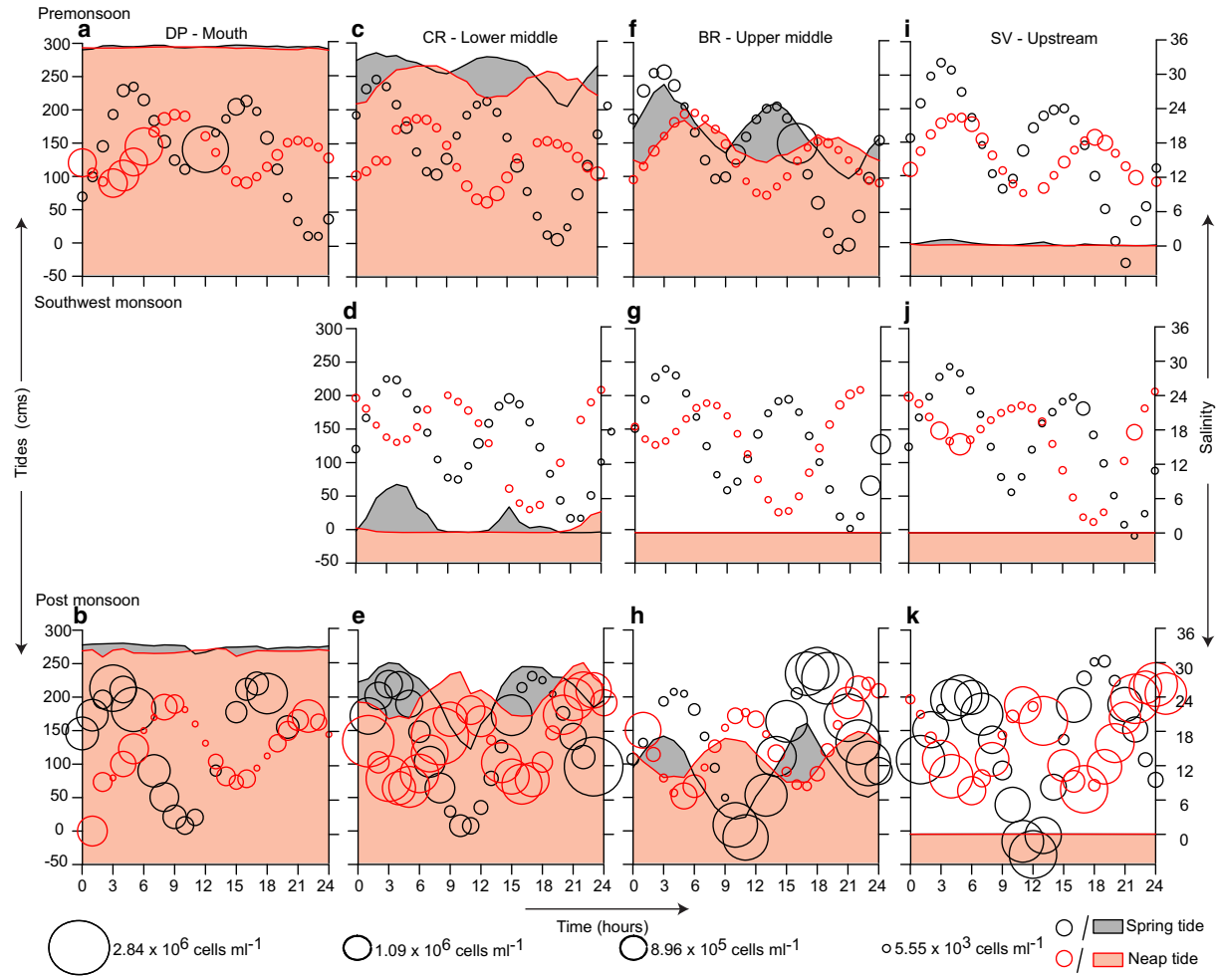


Fig. 2 Seasonal variation in bacterial abundance with respect to time, tide and salinity in surface water at Dona Paula (*DP*), mouth of the estuary; Cortalim (*CR*), lower middle estuary; Borim (*BR*), upper middle estuary and Sanvordem (*SV*), upstream station in Zuari estuary. Maximum bubble size corresponds to 2.84×10^6 cells ml^{-1} and minimum bubble size corresponds to 5.5×10^3 cells ml^{-1}

In the CCA analysis, TVC was located close to the centre of the axis and did not show a significant relation with any of the environmental variables (Figs. 4 and 5). However, correlation analysis showed that ΔS had inverse relationship with TVC abundance ($r = -0.73$, $p \leq 0.02$). In addition, SPM also showed a significant positive relation with TVC and this relation was more evident during PostM ($r = 0.672$; $p \leq 0.001$).

Pathogenic bacteria

Mean abundance of TC in the surface water decreased from the mouth to the upstream end of the estuary (DP > CR > BR > SV) irrespective of seasons during the spring tide (Fig. 6a, b). The abundance of TC was more at BR > SV > DP > CR during the neap tide. Similar trend was also observed for near bottom water (Fig. 6c, d). Overall, the abundance was high during the PostM season. TC abundance in sediment was also higher during PostM when compared to others. On an average, 0.14–0.24% of abundance increased from the mouth to the upstream end of the estuary (Table 2).

The variation in the *Vibrio* spp. along the estuarine stretch was influenced by tides and seasons (Fig. 6e–p). In the case of *V. alginolyticus*, abundance was high at the mouth (DP; $5.11 \pm 0.7 \times 10^2$ CFU ml^{-1}), followed by lower middle (CR; $2.78 \pm 0.5 \times 10^2$ CFU ml^{-1}), upper middle (BR; $1.02 \pm 0.3 \times 10^2$ CFU ml^{-1}) and upstream end (SV; 2.07 ± 2.3 CFU ml^{-1}) of the estuary during spring tide. The trend reversed during neap tide in both

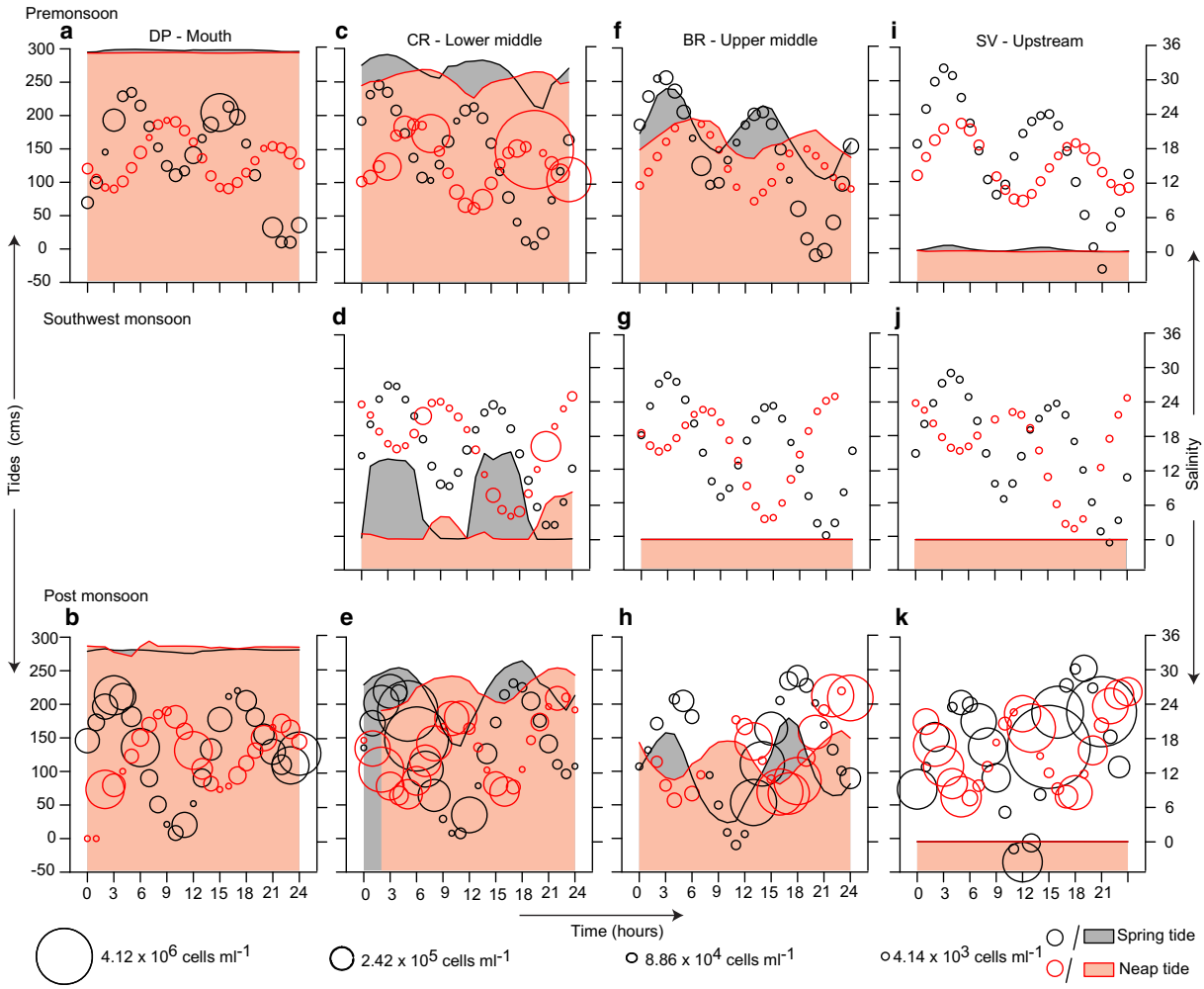
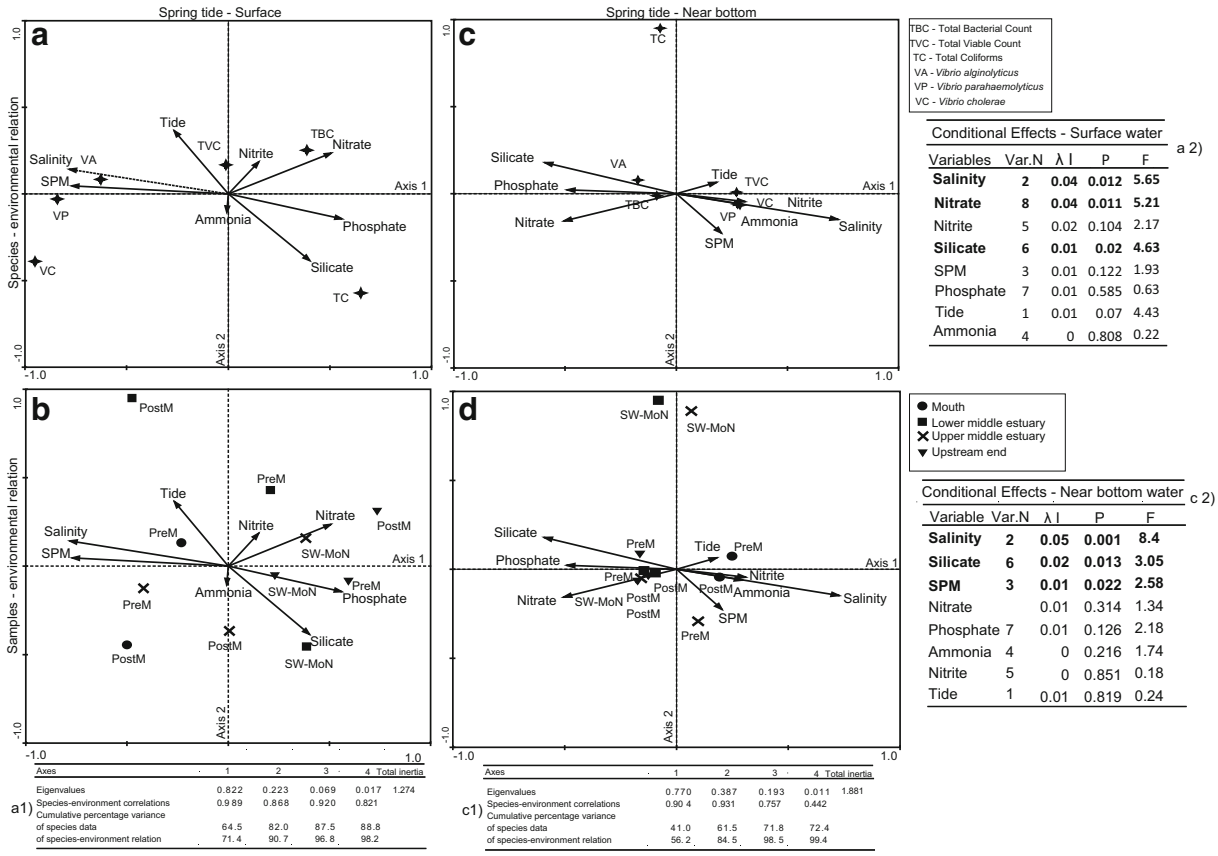


Fig. 3 Seasonal variation in bacterial abundance with respect to time, tide and salinity in near bottom water at Dona Paula (*DP*), mouth of the estuary; Cortalim (*CR*), lower middle estuary; Borim (*BR*), upper middle estuary and Sanvordem (*SV*), upstream station in Zuari estuary. Maximum bubble size corresponds to 4.12×10^6 cells ml^{-1} and minimum bubble size corresponds to 4.14×10^3 cells ml^{-1}

surface and near bottom waters (Fig. 6f, h). Similarly, *V. parahaemolyticus* and *V. cholerae* abundance also decreased from the mouth to upstream end (DP > CR > BR > SV) of the estuary during spring tide (Fig. 6 i–p). No such variation was observed during neap tide.

The CCA and correlation analysis indicated that the TC abundance was related to silicate (r = 0.515; $p \leq 0.05$), salinity (r = −0.655; $p \leq 0.01$) and SPM (r = -0.570; $p \leq 0.05$). The CCA biplot indicated that the association between silicate and TC was not direct and rather related to the monsoonal influences such as freshwater influx and runoff (Figs. 4 and 5b). For example, SW-MoN (irrespective of the stations) in the biplots separated along the axis 1 and closely associated with the increase of silicate and TC abundance (Figs. 4 and 5b) in surface and near bottom waters.

The abundance of *Vibrio* spp. showed significant positive relationship with the salinity ($r = 0.636$; $p \leq 0.05$) and SPM ($r = 0.757$; $p \leq 0.01$). The CCA biplots also revealed that PreM and PostM seasons were clearly separated from SW-MoN (Figs. 4 and 5b, d), indicating a possible influence of monsoon on *Vibrio* spp. abundance. Other than salinity and SPM, *V. cholerae* also showed a significant positive relation with nitrite during neap tide in near bottom water (Fig. 5c). This relation was prominent at the lower middle estuary (CR) during PreM where the mean abundance of *V. cholerae* was high (Fig. 6p) and could be related to high nitrite (39.76 μM l^{-1}).



Conditional Effects - Surface water (a 2)

Variables	Var.N	λ I	P	F
Salinity	**2**	**0.04**	**0.012**	**5.65**
Nitrate	**8**	**0.04**	**0.011**	**5.21**
Nitrite	5	0.02	0.104	2.17
Silicate	**6**	**0.01**	**0.02**	**4.63**
SPM	3	0.01	0.122	1.93
Phosphate	7	0.01	0.585	0.63
Tide	1	0.01	0.07	4.43
Ammonia	4	0	0.808	0.22

Conditional Effects - Near bottom water (c 2)

Variable	Var.N	λ I	P	F
Salinity	**2**	**0.05**	**0.001**	**8.4**
Silicate	**6**	**0.02**	**0.013**	**3.05**
SPM	**3**	**0.01**	**0.022**	**2.58**
Nitrate		0.01	0.314	1.34
Phosphate	7	0.01	0.126	2.18
Ammonia	4	0	0.216	1.74
Nitrite	5	0	0.851	0.18
Tide	1	0.01	0.819	0.24

a1)

Axes	1	2	3	4	Total inertia
Eigenvalues	0.822	0.223	0.069	0.017	1.274
Species-environment correlations	0.989	0.868	0.920	0.821	
Cumulative percentage variance of species data	64.5	82.0	87.5	88.8	
of species-environment relation	71.4	90.7	96.8	98.2	

c1)

Axes	1	2	3	4	Total inertia
Eigenvalues	0.770	0.387	0.193	0.011	1.881
Species-environment correlations	0.904	0.931	0.757	0.442	
Cumulative percentage variance of species data	41.0	61.5	71.8	72.4	
of species-environment relation	56.2	84.5	98.5	99.4	

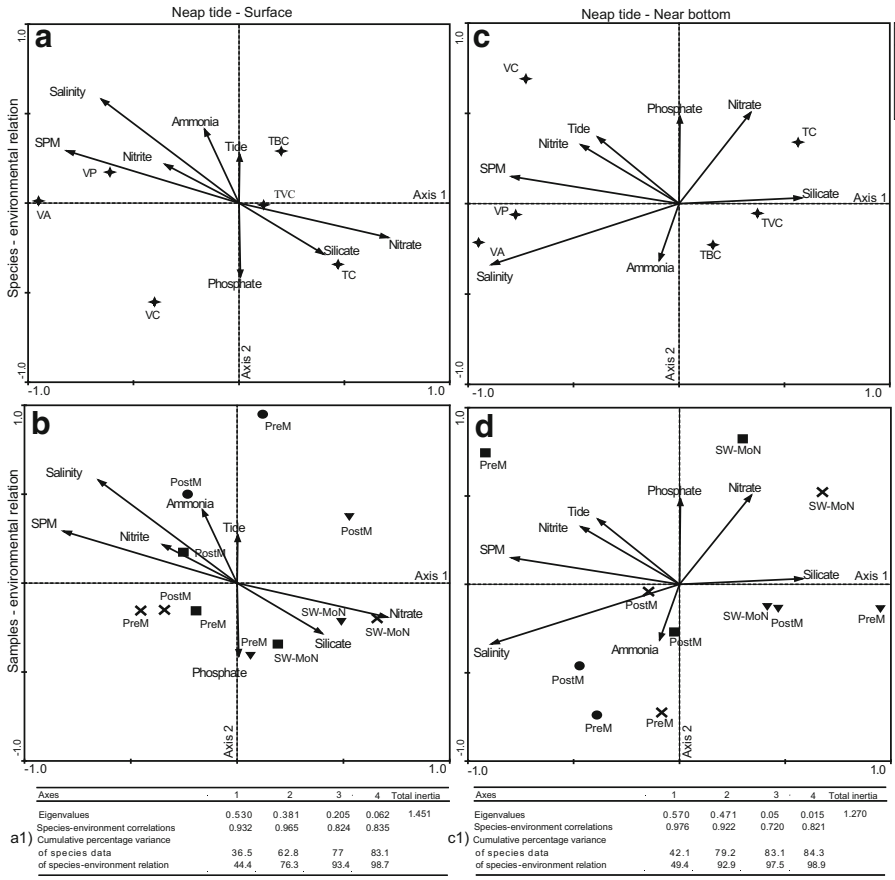
Fig. 4 Canonical correspondence analysis (CCA) for bacteria in water column together with associated environmental parameters during PreM, SW-MoN, and PostM spring tides. **a** Surface water species biplot. **b** Surface water station biplot where different stations are indicated by *symbols*. **c** Near bottom species biplot. **d** Near bottom station biplot where different stations are indicated by *symbols*. **a1** Eigenvalues for CCA axes of surface water analysis. **c1** Eigenvalues for CCA axes of near bottom water analysis. **a 2**, **c 2** Lambda (λ *I*) are the eigenvalues explained by the environment variables for surface and near bottom waters respectively. $P \leq 0.05$ are highlighted in *bold*

Discussion

The observations carried out simultaneously at fine resolution to elucidate spatial and temporal variations in the tropical Zuari estuary point out the bacterial population to be influenced by the physical processes.

Bacterial response to diel–tidal changes

The mouth of the estuary was supported by high total and viable bacterial abundance (TBC and TVC). The tidal range between spring–neap phases is about 1 m at the mouth, and it causes significant variability in ΔS (Sundar et al. 2015). ΔS were low during high waters at the mouth (0.5), indicating that the water mass was well mixed which possibly resulted in high bacterial abundance. A concurrent study carried out on the biofilm formation in Zuari estuary at the same location also showed high bacterial abundance in the biofilms developed at the mouth region during PostM (Khandeparker et al. 2015; personal observation). At the mouth, SPM was also high in the water column during PostM season. Tide-mediated mixing of the water column induces significant changes in physicochemical variables and suspended load; this variation has indirect effect on bacterial abundance. Average percentage of TVC in sediments with respect to TBC was also low (0.10%) as compared to the water (1.33%) which indicates the possible re-suspension of bacterial inoculum in the overlaying water column (Table 2). Onefold to threefold variations in TVC (~10^1–10^3) was evident between the tidal cycle (spring and neap tides) with high values during the spring phase. High bacterial abundance and SPM in the water column can be attributed to re-



a 2)

Conditional Effects - Surface water

Variables	Var.N	λ I	P	F
Salinity	**2**	**0.09**	**0.017**	**6.4**
SPM	**3**	**0.04**	**0.015**	**4.34**
Silicate	**6**	**0.02**	**0.028**	**3.42**
Tide	1	0.02	0.105	3.05
Nitrate	8	0.02	0.37	0.95
Phosphate	7	0.02	0.536	0.5
Nitrite	5	0.01	0.596	0.44
Ammonia	4	0.1	0.056	2.32

c 2)

Conditional Effects - Near bottom water

Variables	Var.N	λ I	P	F
Salinity	**2**	**0.07**	**0.001**	**8.2**
Nitrite	**5**	**0.01**	**0.041**	**2.85**
SPM	3	0.01	0.22	1.54
Ammonia	4	0.01	0.291	1.26
Tide	1	0.01	0.428	0.96
Phosphate	7	0.01	0.195	1.86
Nitrate	8	0	0.552	0.68
Silicate	6	0	0.963	0.07

a1)

Axes	1	2	3	4	Total inertia
Eigenvalues	0.530	0.381	0.205	0.062	1.451
Species-environment correlations	0.932	0.965	0.824	0.835	
Cumulative percentage variance of species data	36.5	62.8	77	83.1	
of species-environment relation	44.4	76.3	93.4	98.7	

c1)

Axes	1	2	3	4	Total inertia
Eigenvalues	0.570	0.471	0.05	0.015	1.270
Species-environment correlations	0.976	0.922	0.720	0.821	
Cumulative percentage variance of species data	42.1	79.2	83.1	84.3	
of species-environment relation	49.4	92.9	97.5	98.9	

Fig. 5 CCA for bacteria in water column together with associated environmental parameters during PreM, SW-MoN and PostM neap tides. **a** Surface water species biplot. **b** Surface water stations biplot where different stations are indicated by *symbols*. **c** Near bottom species biplot. **d** Near bottom station biplot where different stations are indicated by *symbols*. **a1** Eigenvalues for CCA axes of surface water analysis. **c1** Eigenvalues for CCA axes of near bottom water analysis. **a 2, c 2** Lambda (λ *I*) is the eigenvalue explained by the environment variables for surface and near bottom waters respectively. $P \leq 0.05$ are highlighted in *bold*

suspension events of bed sediments due to tidal currents. Tidal turbulence can inoculate detritus, bacteria and pigments from the sediment into the water column (Poremba et al. 1999). A study by Anand et al. (2014a) on sub hourly changes in biogeochemical properties in the surface water at the bay station of this estuary suggested that enhanced turbulence due to rising tide also enables the release of substances from the bottom sediments resulting in the increase in nutrients. Other than tidal turbulence, freshwater input also facilitates the sedimentary release and contaminants in the Zuari estuary (Shirodkar et al. 2012; Anand et al. 2014b). Additionally, south west monsoon carries bulk amount of fine-grained material to this estuary and alters the composition of SPM (Shetye et al. 1995; Kessarkar et al. 2009). Thus, it seems that other than ΔS, SPM also has a significant influence on the bacterial abundance. During SW-MoN, influence of salinity at lower middle estuary was also evident where TBC and TVC decreased. During monsoon, high stratification observed, during high waters at the lower middle estuary, weakens marginally at the peak of the high waters, during both spring and neap phases. Because of this, the salinity of the bottom layer remains constant for about 2–3 h while the surface salinity continues to increase up to the peak of high water, resulting in the weakening of stratification (Sunder et al. 2015). This could be a responsible factor for the decrease in TBC and TVC.

Bacterial response to nutrient dynamics

In the present study, nutrients such as nitrite, nitrate, phosphate and silicate showed seasonal and tidal variation and were low during PostM (mainly nitrate and

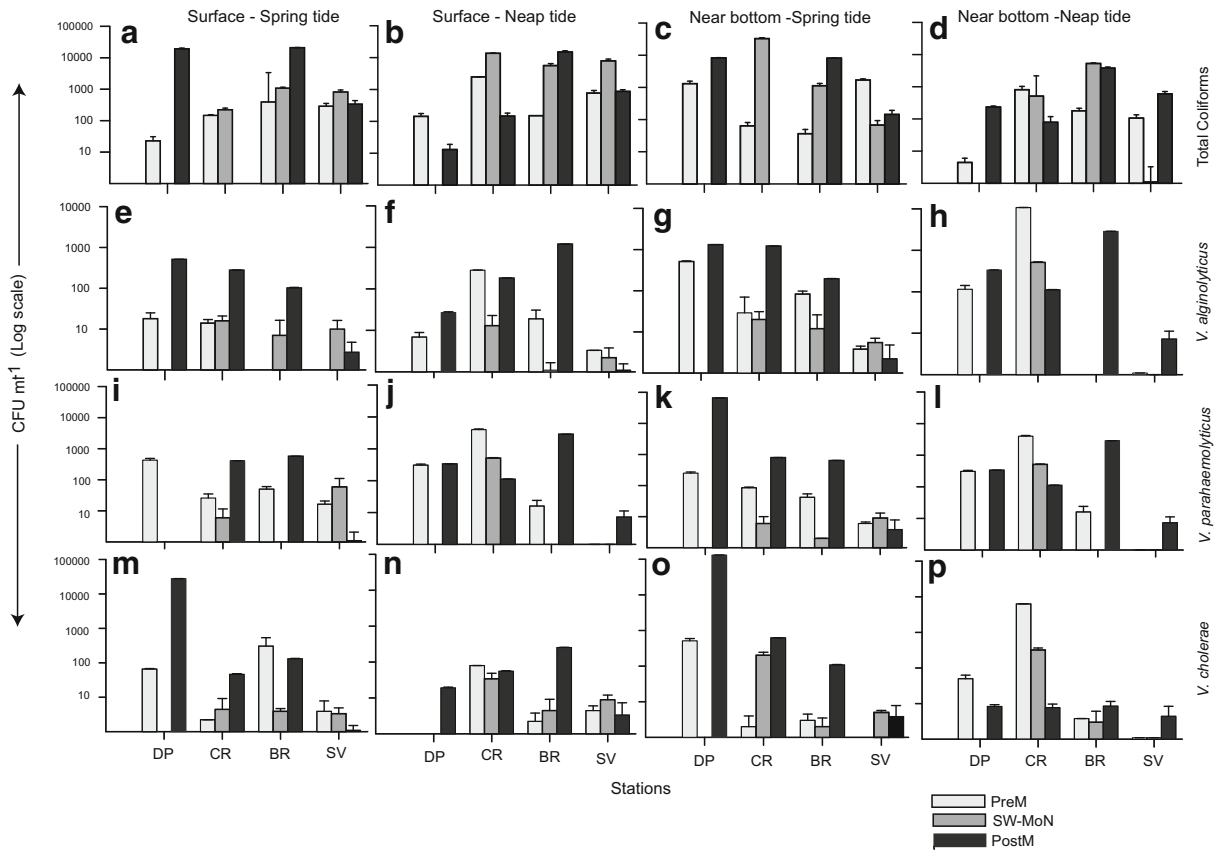


Fig. 6 Seasonal and tidal variations in total coliforms and *Vibrio* spp. abundance at Dona Paula (*DP*), mouth of the estuary; Cortalim (*CR*), lower middle estuary; Borim (*BR*), upper middle estuary and Sanvordem (*SV*), upstream station in Zuari estuary

nitrite). These nutrients showed a significant negative correlation with TBC and can be attributed to their active utilization. The most common forms of inorganic nitrogen in aquatic ecosystems are ammonium, nitrite and nitrate which originate from surface and groundwater runoff, N_2 fixation by certain prokaryotes and biological degradation of organic matter (Camargo and Alonso 2006). Three major factors control N and P limitation in an aquatic ecosystem: (1) nitrogen to phosphorus ratio in nutrients, (2) loss of N due to denitrification and (3) extent of nitrogen availability in an ecosystem through nitrogen fixation (Ward et al. 2009). The main limiting nutrient in terrestrial and marine ecosystem is nitrogen (Elser et al. 2007). Similarly, a study by Anand et al. (2014b) showed notable intraseasonal variation in nitrate and nitrite during PostM season in this estuary. Earlier study on daily variation in total and pathogenic bacteria abundance at the mouth of this estuary during 2009–2011 pointed out surge of nitrate during pre-monsoon. This correlated well with the increased abundance of total bacterial numbers and phosphate (Khandeparker et al. 2015). These observations were carried out at a fixed time (between 10:00 and 11:00 h) and did not bring out the variations related to the tidal cycle. The removal of nitrate could be linked with increase in total bacterial numbers and enhancement of phosphate.

Factors influencing the occurrence and distribution of coliforms in Zuari estuary

Estuaries are reservoir for disease—causing microorganisms due to interference of human waste. In the present study, allochthonous coliforms ranged from $<10^1$–10^5 CFU ml^{-1} in surface and near bottom waters. These values are consistent with the previous studies (Shirodkar et al. 2012; Khandeparker et al. 2015) and other estuaries (Blackwell and Oliver 2008; Neogi et al. 2012). TC are among the major groups of indicators of fecal bacteria stated in European standards regarding

Table 2 Average abundance of TBC and percentage abundance of viable and pathogenic bacteria (with respect to % abundance of TBC) in the sediments at Dona Paula, Cortalim and Borim during spring and neap tides

			TBC (avg. ± std. deviation)	TVC (% sed)	TVC (surf)	TVC (bott)	VC (%)	VA (%)	VP (%)	TCs (%)
Dona Paula (DP)	PreM	Spring	$4.17 \times 10^7 \pm 1.82 \times 10^7$	3.12	0.6	0.6	1.96	0	0.04	0.08
		Neap	$2.43 \times 10^7 \pm 4.52 \times 10^7$	0.10	0.001	0.001	0	0	0	0
	SW-MoN	Not sampled								
	PostM	Spring	$5.91 \times 10^7 \pm 1.84 \times 10^7$	0.10	1.5	1.33	0.01	0	0	0
		Neap	ND	ND	–	–	ND	ND	ND	ND
Cortalim (CR)	PreM	Spring	$3.79 \times 10^7 \pm 3.58 \times 10^7$	5.67	0.04	0.08	0.11	0.05	0.03	0.09
		Neap	$1.51 \times 10^9 \pm 3.21 \times 10^9$	0.03	0.002	0.0001	0	0	0	0
	SW-MoN	Spring	$6.75 \times 10^7 \pm 1.4 \times 10^7$	0.13	0.02	0.3	0.05	0	0.02	0.10
		Neap	ND	ND	–	–	ND	ND	ND	ND
	PostM	Spring	5.58×10^6	1.51	1.51	1.80	0.01	0.01	0.03	0.23
		Neap	ND	ND	–	–	ND	ND	ND	ND
Borim (BR)	PreM	Spring	$2.77 \times 10^6 \pm 1.13 \times 10^6$	2.48	0.1	0.10	0.02	0.03	0.06	0.03
		Neap	$1.99 \times 10^7 \pm 7.39 \times 10^6$	5	0.12	0.002	0	0.03	0.02	0.10
	SW-MoN	Spring	$6.18 \times 10^5 \pm 1.16 \times 10^5$	2.26	0.83	0.82	0	0	0	0.08
		Neap	$1.19 \times 10^7 \pm 4.38 \times 10^6$	2.88	0.032	0.036	0.04	0.28	0.64	0.59
	PostM	Spring	$3.87 \times 10^7 \pm 3.28 \times 10^7$	0.37	0.054	0.085	0	0	0.01	0.27
		Neap	ND	ND	–	–	ND	ND	ND	ND

TBC total bacterial count (cells gm^{-1}), *TVC* total viable count (CFU gm^{-1}), *VC Vibrio cholerae* (CFU gm^{-1}), *VA Vibrio alginolyticus* (CFU gm^{-1}), *VP Vibrio parahaemolyticus* (CFU gm^{-1}), *TC* total coliforms (CFU gm^{-1}), *ND* not done

microbiological quality of surface waters. We observed that mean abundance of TC was higher at the mouth of the estuary where TBC was high. Li et al. (2014) reported that TC distribution was mainly influenced by the total bacterial abundance at Jiahe River estuary. In the present study, occurrence and abundance of TC varied between the spring–neap phases. For example, during spring, the abundance was high towards the mouth. Whereas, it was high at the upper middle and upstream end of the estuary during the neap tide. Sediment re-suspension and high anthropogenic impact during high waters could be one of the possible reasons for high TC abundance at the mouth during spring tide. Sediment also showed low TC abundance during high waters indicating possible re-suspension. During flood tides, re-suspension of sediments tends to be maximal, and the sediment is rapidly transported towards landside and deposition of pathogenic estuarine bacteria is a risk to public health (Lee et al. 2006; Mallin et al. 2007). In addition, previous studies have pointed out that the load of sediment (SPM) in seawater can lead to an increase in the survival of fecal coliforms, mainly *E. coli* (Gerba and Mcleod 1976; Solo-Gabriele et al. 2000). Earlier studies have pointed out that release of particle-attached pathogens from bed sediments to the water column via re-suspension process play a vital role in the survival and transport of pathogens in the estuaries (Smith et al. 1978; Desmarais et al. 2002). A study by Shirodkar et al. (2012) also reported high TBC, TC and *Vibrio* spp. in this estuary and concluded that these pathogens could have been transported via waste discharge during high waters from the Mormugao sewage treatment plant (STP) which is located at the sides of the estuarine mouth. The tidal influence and different nutrient loads also regulate and enhance abundance of pathogens (Lafferty and Holt 2003; Johnson and Carpenter 2008). While, high abundance of TC at upstream station sediment could be due to its geographical location, as it is located at the entrance of the channel near the junction between the bay and the upstream region of Zuari river and influenced by tidal currents, waves, movement of fishing trawlers, ferry boat, shallow in depth (~1 m) and sediment is silty-clayey in nature. A recent observation on the sediment texture revealed that upstream station was rich in silt and clay (54.6%) content as compared to mouth (19.3%) (Desai and Atchuthan; personal

observation). A study by Perkins et al. (2014) reported a significant correlation between high abundance of pathogenic bacteria and sediment containing high amount of silt, clay and organic matter at the Conwy estuary, UK. Thus, the sediment texture could be one of the possible reasons for high abundance of TC in the sediment towards the upstream station. In the present study, TC abundance was influenced by high silicate irrespective of tides in both surface and near bottom waters during SW-MoN. There could be two possible sources for high silicate in this estuary especially during SW-MoN. Silicate is added in this estuary during SW-MoN at the upstream regions via land runoff and naturally occurring physical and chemical weathering (Shirodkar et al. 2012; Anand et al. 2014a). Another possible reason could be the silicate contributed by disintegrating freshwater phytoplankton which is transported to saline water (Morris et al. 1978). During SW-MoN, the fluctuation in salinity is maximal due to high river discharge in this estuary which can impact the phytoplankton community. Studies have also shown that pore water from marine sediments could also be a significant source for silicate especially during non-monsoon (Fanning and Pilson 1974; Balls et al. 1994). A study by Sarma et al. (2009) at the tropical monsoon-driven Godavari estuary also pointed out negative correlation between chl a and river discharge. But this was attributed to river-borne suspension during monsoon and clear water during non-monsoon.

Factors influencing the occurrence and distribution of *Vibrio* spp. in Zuari estuary

V. cholerae (VC) ranged from 0 to 2.79×10^4 CFU ml^{-1} and were high in marine water. Similarly, the other two *Vibrio* spp., i.e. *V. alginolyticus* (VA) and *V. parahaemolyticus* (VP), were also high in marine water and decreased towards the freshwater zone during spring tide. However, during neap tide, *Vibrio* spp. were high towards the freshwater end. This trend was more evident in the VA population. This observation points out survival of *Vibrio* spp. in a wide salinity range. *Vibrio* spp. are members of marine *Gamma proteobacteria* and they can survive in freshwater as well as marine systems (Hagstrom et al. 2000; Mookerjee et al. 2014). A recent study by Khandeparker et al. (2015) although observed that the abundance of *Vibrio* spp. was high towards the mouth of Zuari estuary, indicating that their growth is favored by saline conditions; tidal influence was not evaluated. A study carried out by Neogi et al. (2012) along the tropical Karnaphuli estuary which is located at the southern part of the Bangladesh too showed high *Vibrio* spp. abundance in saline water ($\sim 10^2$–10^4 CFU ml^{-1}) which declined towards the freshwater end ($<10^1$ CFU ml^{-1}). Also, the abundance was high during PostM compared to PreM, a trend which is similar to this estuary. Changes in abundance of *Vibrio* spp. have been linked to changing environmental conditions (Lipp et al. 2001; Louis et al. 2003). In an estuarine system, factors such as temperature, vertical mixing, tidal flushing, precipitation and nutrient load can change the environment and further alter microbial community structure, including *Vibrio* spp. (Hsieh et al. 2008). Sudden change or alteration in environment factors may also trigger the virulence genes in pathogens which are risk to nearby human populations.

The present study showed that viable *Vibrio* spp. were positively influenced by high SPM during PostM along the estuary. The increase was $\sim 10^1$–10^3-fold higher when compared to PreM and SW-MoN. The influence of SPM was more at the upper middle and upstream stations. The upper middle estuarine study area is fringed with thick mangroves which receive high amount of terrestrial derived organic matter in this estuary. In addition, it also receives a significant quantity of pathogen inoculum through many small streams and rivers located at the upstream. A study at the Karnaphuli estuary showed high cultivable *Vibrio* abundance to be associated with high suspended load (Lara et al. 2009). Suspended matter is often found densely packed by pathogens. Association of pathogens with sediment particles provides shelter from UV radiation, predation and environmental stress, and thus enhance their survival in aquatic systems (Davies et al. 2000; Craig et al. 2004). Thus, the changes in the SPM can influence the microorganisms (especially pathogens) associated with them. Members of the *Vibrio* spp. are known to form biofilms on plankton and suspended matter for their persistence (Islam et al. 2007). The interaction between microorganisms (pathogens) and particulate matter is most prevalent in estuarine environments due to the formation of suspended sediment (flocs) at the marine–freshwater interface (Malham et al. 2014). Generally, *Vibrio* spp. are autochthonous organisms in estuaries and are associated with zooplankton, shellfish (Novotny et al. 2004) and phytoplankton (Rehnstam-Holm et al. 2010), consisting of chitin as the

main structural polymer. *Vibrio* spp. are known as active consumers of chitin in various systems. Moreover, C/N ratio analysis of SPM derived from Sundarbans mangrove system showed significant contribution of chitin (~1–2 mg l^{-1}) to the total SPM pool (Lara et al. 2011).

In the present the study, VC abundance was strongly related with salinity and nitrite. A study by Batabyal et al. (2014) at the Ganges estuary pointed out that the influence of salinity variation with tidal cycles and sediment re-suspension from tidal flats can regulate the *Vibrio* spp. abundance in the aquatic systems. Nutrients can directly impact algae and zooplankton which may further affect the associated *V. cholerae* populations (Huq et al. 1996; Mourino-Pérez et al. 2003). Pathogens such as *V. cholerae* and *V. parahaemolyticus* preferentially attach to planktonic copepods and are important pathogens for fish (*V. alginolyticus*) and humans (*V. cholerae* and *V. parahaemolyticus*) and are closely linked with diatoms (Rehnstam-Holm et al. 2010). The present study also noted that VC abundance correlated with high nitrite in near bottom water and could be due to the reduction of nitrate. Similar relation was also reported by Lara et al. (2011) at the eastern Sundarbans mangrove forest, Bangladesh, after a storm event and concluded that it may be due to the increase of nitrate reducing *Vibrios*. The present study indicates a clear spatiotemporal variation within closely related species, which is controlled by nutrient load and tidal cycles in this estuary. Further, sediment re-suspension could be a significant source for pathogen inoculum (both allochthonous and autochthonous) in this estuary and thus has relevance to estuarine pathogen ecology.

The use of culture-dependent techniques in monitoring bacterial populations is reported to have some limitations like overestimation of a single taxa and non-detection of metabolically active but non-cultivable forms (Laiz et al. 2003; Dickson et al. 2014). The use of modern molecular tools to characterize the bacterial populations in this estuary would provide new insights on pathogens and their physiological changes and will be a step ahead.

Acknowledgments We are grateful to the director, National Institute of Oceanography, for his support and encouragement. The editor and reviewer's comments have been helpful in improving the manuscript and are gratefully acknowledged. We are thankful to Dr. A S Unnikrishnan for his valuable suggestions. We gratefully acknowledge Dr. Dattesh Desai, Dr. J.S. Patil and D Sundar for their support during the course of this work. We also thank the colleagues of the BBD department for their help. This work was supported by the Ballast Water Management Program, India (Ministry of Shipping and DG shipping) and the CSIR-funded Ocean Finder Program. This is a NIO contribution no. 5976.

References

Allen, A. E., Howard-Jones, M. H., Booth, M. G., Frischer, M. E., Verity, P. G., Bronk, D. A., & Sanderson, M. P. (2002). Importance of heterotrophic bacterial assimilation of ammonium and nitrate in the Barents Sea during summer. *Journal of Marine Systems, 38*, 93–108.

Alm, E. W., Burke, J., & Spain, A. (2003). Fecal indicator bacteria are abundant in wet sand at freshwater beaches. *Water Research, 37*, 3978–3982.

Anand, S. S., Anju, K. J., Mathew, D., & Kumar, M. D. (2014a). Sub-hourly changes in biogeochemical properties in surface waters of Zuari estuary, Goa. *Environmental Monitoring and Assessment, 186*, 719–724.

Anand, S. S., Muthukumar, S. C., Mangalaa, K. R., Sundar, D., Parab, S. G., & Kumar, M. D. (2014b). Intra-and inter-seasonal variability of nutrients in a tropical monsoonal estuary (Zuari, India). *Continental Shelf Research, 82*, 9–30.

Aguirre-Guzmán, G., Mejia Ruíz, H., & Ascencio, F. (2004). A review of extracellular virulence product of vibrio species important in diseases of cultivated shrimp. *Aquaculture Research, 35*, 1395–1404.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Estuaries, 10*, 257–263.

Balls, P. W. (1994). Nutrient inputs to estuaries from nine Scottish East Coast rivers; influence of estuarine processes on inputs to the North Sea. *Estuarine, Coastal and Shelf Science, 39*, 329–352.

Bassler, B. L., Gibbons, P. J., Yu, C., & Roseman, S. (1991). Chitin utilization by marine bacteria. *The Journal of Biological Chemistry, 266*, 24268–24275.

Batabyal, P., Einsporn, M. H., Mookerjee, S., Palit, A., Neogi, S. B., Nair, G. B., & Lara, R. J. (2014). Influence of hydrologic and anthropogenic factors on the abundance variability of enteropathogens in the Ganges estuary, a cholera endemic region. *Science of Total Environment, 472*, 154–161.

Blackwell, K. D., & Oliver, J. D. (2008). The ecology of Vibrio vulnificus, Vibrio cholerae, and *Vibrio parahaemolyticus* in North Carolina estuaries. *The Journal of Microbiology, 46*, 146–153.

Camargo, J. A., & Alonso, A. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International, 32*, 831–849.

Centers for Disease Control and Prevention (CDC) (2010). Update: cholera outbreak—Haiti, 2010. *MMWR. Morbidity and Mortality Weekly Report, 59*, 1473.

Colwell, R. R. (1996). Global climate and infectious disease: the cholera paradigm. *Science, 274*, 2025–2031.

Correll, D. L. (1999). Phosphorus: a rate limiting nutrient in surface waters. *Poultry Science, 78*, 674–682.

Craig, D. L., Fallowfield, H. J., & Cromar, N. J. (2004). Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. *Journal of Applied Microbiology, 96*, 922–930.

Daniels, N. A., & Shafaie, A. (2000). A review of pathogenic vibrio infections for clinicians. *Infections in Medicine Journal, 17*, 665–685.

Davies, C. M., & Bavor, H. J. (2000). The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. *Journal of Applied Microbiology, 89*, 349–360.

Desmarais, T. R., Solo-Gabriele, H. M., & Palmer, C. J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and Environmental Microbiology, 68*, 1165–1172.

Devanathan, K., Srinivasan, M., & Balakrishnan, S. (2010). Studies on the total heterotrophic bacterial population density from Uppanar estuary (harbour), Cuddalore coast. *Advanced Biomedical Research, 4*, 139–145.

Dickson, R. P., Erb-Downward, J. R., Prescott, H. C., Martinez, F. J., Curtis, J. L., Lama, V. N., & Huffnagle, G. B. (2014). Analysis of culture-dependent versus culture-independent techniques for identification of bacteria in clinically obtained Broncho alveolar lavage fluid. *Journal of Clinical Microbiology, 52*, 3605–3613.

Eldridge, P. M., & Sieracki, M. E. (1993). Biological and hydrodynamic regulation of the microbial food web in a periodically mixed estuary. *Limnology and Oceanography, 38*, 1666–1679.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Jennifer, J. B. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters, 10*, 1135–1142.

Fanning, K. A., & Pilson, M. E. (1974). The diffusion of dissolved silica out of deep-sea sediments. *Journal of Geophysical Research, 79*, 1293–1297.

Finkelstein, R. A. (1996). Cholera, Vibrio cholerae O1 and O139, and other pathogenic Vibrios. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 24.

Gerba, C. P., & Mcleod, J. S. (1976). Effect of sediments on the survival of *Escherichia coli* in marine waters. *Applied and Environmental Microbiology, 32*, 114–120.

Hagstrom, A., Pinhassi, J., & Zweifel, U. L. (2000). Biogeographical diversity among marine bacterioplankton. *Aquatic Microbial Ecology, 21*, 231–244.

Hendriksen, R. S., Price, L. B., Schupp, J. M., Gillece, J. D., Kaas, R. S., Engelthaler, D. M., Bortolaia, V., Pearson, T., Waters, A. E., Upadhyay, B. P., & Shrestha, S. D. (2011). Population genetics of vibrio cholerae from Nepal in 2010: evidence on the origin of the Haitian outbreak. *MBio, 2*, 00157–00111.

Hsieh, J. L., Fries, J. S., & Noble, R. T. (2008). Dynamics and predictive modeling of Vibrio spp. in the Neuse River Estuary, North Carolina, USA. *Environmental Microbiology, 10*, 57–64.

Huq, A., Xu, B., Chowdhury, M. A., Islam, M. S., Montilla, R., & Colwell, R. R. (1996). A simple filtration method to remove plankton-associated Vibrio cholerae in raw water supplies in developing countries. *Applied and Environmental Microbiology, 62*, 2508–2512.

Hyun, J. H., Choi, J. K., Chung, K. H., Yang, E. J., & Kim, M. K. (1999). Tidally induced changes in bacterial growth and viability in the macro tidal Han River estuary, Yellow Sea. *Estuarine, Coastal and Shelf Science, 48*, 143–153.

Islam, M. S., Jahid, M. I. K., Rahman, M. M., Rahman, M. Z., Islam, M. S., Kabir, M. S., Sack, D. A., & Schoolnik, G. K. (2007). Biofilm acts as a microenvironment for plankton-associated Vibrio cholerae in the aquatic environment of Bangladesh. *Microbiology and Immunology, 51*, 369–379.

Johnson, P. T. J., & Carpenter, S. R. (2008). Influence of eutrophication on disease in aquatic ecosystems: patterns, processes, and predictions. In: infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems. Chapter 4 Princeton. *NJ: Princeton University Press*, 71–99.

Kessarkar, P. M., Rao, V. P., Shynu, R., & Ahmad, I. M. (2009). Wind-driven estuarine turbidity maxima in Mandovi estuary, central west coast of India. *Journal of Earth System Science, 118*, 369–377.

Kessarkar, P. M., Suja, S., Sudheesh, V., Srivastava, S., & Rao, V. P. (2015). Iron ore pollution in Mandovi and Zuari estuarine sediments and its fate after mining ban. *Environmental Monitoring and Assessment, 187*, 1–17.

Khandeparker, L., Anil, A. C., Naik, S. D., & Gaonkar, C. C. (2015). Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment. *Marine Pollution Bulletin, 96*, 337–343.

Kinne, O. (1971). Marine ecology, a comprehensive, integrated treatise on life in oceans and coastal waters. Environmental factors Wiley Interscience, London. vol. 1, part 2.

Kumar, S., Ramesh, R., Bhosle, N. B., Sardesai, S., & Sheshshayee, M. S. (2004). Natural isotopic composition of nitrogen in suspended particulate matter in the bay of Bengal. *Biogeosciences, 1*, 63–70.

Lafferty, K. D., & Holt, R. D. (2003). How should environmental stress affect the population dynamics of disease? *Ecology Letters, 6*, 654–664.

Laiz, L., Piñar, G., Lubitz, W., & Saiz-Jimenez, C. (2003). Monitoring the colonization of monuments by bacteria: cultivation versus molecular methods. *Environmental Microbiology, 5*, 72–74.

Lara, R. J., Neogi, S. B., Islam, M. S., Mahmud, Z. H., Yamasaki, S., & Nair, G. B. (2009). Influence of catastrophic climatic events and human waste on Vibrio distribution in the Karnaphuli Estuary, Bangladesh. *EcoHealth, 6*, 279–286.

Lara, R. J., Neogi, S. B., Islam, M. S., Mahmud, Z. H., Islam, S., Paul, D., Demoz, B. B., Yamasaki, S., Nair, G. B., & Kattner, G. (2011). Vibrio cholerae in waters of the Sunderban mangrove: relationship with biogeochemical parameters and chitin in seston size fractions. *Wetlands Ecology and Management, 19*, 109–119.

Lee, M. C., Lin, T. Y., Lin, C. C., Kohbodi, G. A., Bhatt, A., Lee, R., & Jay, J. A. (2006). Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. *Water Research, 40*, 2593–2602.

Li, J., Wang, H., Liu, Y., Lin, M., Liu, X., & Hu, X. (2014). Distribution and diversity of coliform bacteria in estuary of Jiahe River, China. *International Journal of Environmental Research, 8*, 501–508.

Lindsay, P., Balls, P. W., & West, J. R. (1996). Influence of tidal range and river discharge on suspended particulate matter

fluxes in the Forth Estuary, Scotland. *Estuarine, Coastal and Shelf Science, 42*, 63–82.

Lipp, E., Rodriguez-Palacios, C., & Rose, J. B. (2001). Occurrence and distribution of the human pathogen Vibrio vulnificus in a subtropical Gulf of Mexico estuary. *Hydrobiologia, 460*, 165–173.

Louis, V. R., Russek-Cohen, E., Choopun, N., Rivera, I. N. G., Gangle, B., Jiang, S. C., Rubin, A., Jonathan, A. P., Huq, A., & Colwell, R. R. (2003). Predictability of Vibrio cholerae in Chesapeake Bay. *Applied and Environmental Microbiology, 69*, 2773–2785.

Luna, G. M., Manini, E., & Danovaro, R. (2002). Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. *Applied and Environmental Microbiology, 68*, 3509–3513.

Malham, S. K., Rajko-Nenow, P., Howlett, E., Karen, E., Tuson, K. E., Perkins, L. P., Pallett, D. W., Wang, H., Colin, F., Jago, C. F., Jones, D. L., & McDonald, J. E. (2014). The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. *Environmental Science: Processes & Impacts, 16*, 2145–2155.

Mallin, M. A., Cahoon, L. B., Toothman, B. R., Parsons, D. C., McIver, M. R., Ortwine, M. L., & Renee, N. (2007). Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary. *Marine Pollution Bulletin, 54*, 81–88.

Marie, D., Partensky, F., Vaulot, D., & Brussaard, C. (1999). Enumeration of phytoplankton, bacteria, and viruses in marine samples. *Current Protocols in Cytometry*, 11–11.

McLusky, D. S., & Elliott, M. (2004). The estuarine ecosystem: Ecology, threats and management. New York. Oxford University Press Inc.

Mookerjee, S., Jaiswal, A., Batabyal, P., Einsporn, M. H., Lara, R. J., Sarkar, B., Neogi, S. B., & Palit, A. (2014). Seasonal dynamics of Vibrio cholerae and its phages in riverine ecosystem of Gangetic West Bengal: cholera paradigm. *Environmental Monitoring and Assessment, 186*, 6241–6250.

Morris, A. W., & Howarth, M. J. (1998). Bed stress induced sediment resuspension. *Continental Shelf Research, 18*, 1203–1213.

Morris, A. W., Mantoura, R. F. C., Bale, A. J., & Howland, R. J. M. (1978). Very low salinity regions of estuaries: important sites for chemical and biological reactions. *Nature, 274*, 678–680.

Mourino-Pérez, R. R., Worden, A. Z., & Azam, F. (2003). Growth of Vibrio cholerae O1 in red tide waters off California. *Applied and Environmental Microbiology, 69*, 6923–6931.

Nagvenkar, G. S., & Ramaiah, N. (2009). Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex. *Environmental Monitoring and Assessment, 155*, 245–256.

Neogi, S. B., Islam, M. S., Nair, G. B., Yamasaki, S., & Lara, R. J. (2012). Occurrence and distribution of plankton-associated and free-living toxigenic Vibrio cholerae in a tropical estuary of a cholera endemic zone. *Wetlands Ecology and Management, 20*, 271–285.

Neogi, S. B., Yamasaki, S., Alam, M., & Lara, R. J. (2014). The role of wetland micro invertebrates in spreading human diseases. *Wetlands Ecology and Management, 22*, 469–491.

Novotny, L., Dvorska, L., Lorencova, A., Beran, V., & Pavlik, I. (2004). Fish: a potential source of bacterial pathogens for human beings. *A review, Veterinarni Medicina, 9*, 343–358.

Oberbeckmann, S., Fuchs, B. M., Meiners, M., Wichels, A., Wiltshire, K. H., & Gerdts, G. (2012). Seasonal dynamics and modeling of a Vibrio community in coastal waters of the North Sea. *Microbial Ecology, 63*, 543–551.

Oliver, J. D., & Kaper, J. B. (1997). Vibrio species. *Food microbiology: fundamentals and frontiers*, 228–264.

Ouattara, K. N., Passerat, J., & Servais, P. (2011). Faecal contamination of the water and sediment in the rivers of the Scheldt drainage network. *Environmental Monitoring and Assessment, 183*, 243–257.

Perkins, T. L., Clements, K., Baas, J. H., Jago, C. F., Jones, D. L., Malham, S. K., & McDonald, J. E. (2014). Sediment composition influences spatial variation in the abundance of human pathogen indicator bacteria within an estuarine environment. *PloS One, 9*, 112951.

Pfeffer, C., & Oliver, J. D. (2003). A comparison of thiosulphate-citrate-bile salts-sucrose (TCBS) agar and thiosulphate-chloride-iodide (TCI) agar for the isolation of vibrio species from estuarine environments. *Letters in Applied Microbiology, 36*, 150–151.

Poremba, K., Tillmann, U., & Hesse, K. J. (1999). Tidal impact on planktonic primary and bacterial production in the German Wadden Sea. *Helgoland Marine Research, 53*, 19–27.

Qasim, S. Z., & Sen Gupta, R. (1981). Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuarine, Coastal and Shelf Science, 13*, 557–578.

Rehnstam-Holm, A. S., Godhe, A., Härnström, K., Raghunath, P., Saravanan, V., Collin, B., Karunasagar, I., & Karunasagar, I. (2010). Association between phytoplankton and Vibrio spp. along the southwest coast of India: a mesocosm experiment. *Aquatic Microbial Ecology, 58*, 127–139.

Rodrigues, V., Ramaiah, N., Kakti, S., & Samant, D. (2011). Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India. *Ecological Indicators, 11*, 318–327.

Roy, R., Chitari, R., Kulkarni, V., Krishna, M. S., Sarma, V. V. S. S., & Anil, A. C. (2015). CHEMTAX-derived phytoplankton community structure associated with temperature fronts in the northeastern Arabian Sea. *Journal of Marine Systems, 144*, 81–91.

Sack, R. B., Siddique, A. K., Longini, I. M., Nizam, A., Yunus, M., Islam, M. S., Morris, J. G., Ali, A., Huq, A., Nair, B., Qadri, F., Faruque, S. H., Sack, D. A., & Colwell, R. R. (2003). A 4-year study of the epidemiology of Vibrio cholerae in four rural areas of Bangladesh. *Journal of Infectious Diseases, 187*, 96–101.

Sarma, V. V. S. S., Gupta, S. N. M., Babu, P. V. R., Acharya, T., Harikrishnachari, N., Vishnuvardhan, K., Rao, N. S., Reddy, N. P. C., Sarma, V. V., Sadhuram, Y., & Murty, T. V. R. (2009). Influence of river discharge on plankton metabolic rates in the tropical monsoon driven Godavari estuary, India. *Estuarine, Coastal and Shelf Science, 85*, 515–524.

Sawkar, K., Vethamony, P., Babu, M. T., Dias, C., Mesquita, A., Fernandes, B., Moses, S., Padmavati, M., & Naik, S. (2003). Measuring, modeling and grading the health of water bodies. In L. Noronha, N. Lourenco, J. P. Lobo-Fereira, A. Lleopart, E. Feoli, K. Sawkar, & A. Chachadi (Eds.), *Coastal tourism,*

environment and sustainable local development (pp. 179–210). India: Tata Energy Research Institute (TERI), New Delhi.

Shetye, S. R., Gouveia, A. D., Singbal, S. Y. S., Naik, C. G., Sundar, D., Michael, G. S., & Nampoorthiri, G. (1995). Propagation of tides in the Mandovi–Zuari estuarine network; proceedings of the Indian Academy of Sciences. *Earth and Planetary Science Letters, 104*, 667–682.

Shetye, S. R., Shankar, D., Neetu, S., Suprit, K., Michael, G. S., & Chandramohan, P. (2007). The environment that conditions the Mandovi and Zuari estuaries. In R. Shetye S, D. Kumar, & D. Shankar (Eds.), *The Mandovi and Zuari estuaries* (p. 3). Dona-Paula, Goa: National Institute of Oceanography.

Shirodkar, P. V., Deepthi, M., Vethamony, P., Mesquita, A. M., Pradhan, U. K., Babu, M. T., Verlecar, X. N., & Haldankar, S. R. (2012). Tide dependent seasonal changes in water quality and assimilative capacity of anthropogenically influenced Mormugao harbour water. *Indian Journal of Geo-Marine Sciences, 41*, 314–330.

Smirnova, N. I., Cheldyshova, N. B., Zadnova, S. P., & Kutyrev, V. V. (2004). Molecular–genetic peculiarities of classical biotype vibrio cholerae, the etiological agent of the last outbreak Asiatic cholera in Russia. *Microbial Pathogenesis, 36*, 131–139.

Smith, E. M., Gerba, C. P., & Melnick, J. L. (1978). Role of sediment in the persistence of enteroviruses in the estuarine environment. *Applied and Environmental Microbiology, 35*, 685–689.

Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R., & Palmer, C. J. (2000). Sources of *Escherichia coli* in a coastal subtropical environment. *Applied and Environmental Microbiology, 4*, 230–237.

Stumpf, C. H., Piehler, M. F., Thompson, S., & Noble, R. T. (2010). Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: hydrographic patterns and terrestrial runoff relationships. *Water Research, 44*, 4704–4715.

Sunder, D., & Shetye, S. R. (2005). Tides in the Mandovi and Zuari estuaries, Goa, west coast of India. *Journal of Earth System Science, 144*, 493–503.

Sundar, D., Unnikrishnan, A. S., Michael, G. S., Kankonkar, A., Nidheesh, A. G., & Subeesh, M. P. (2015). Observed variations in stratification and currents in the Zuari estuary, west coast of India. *Environmental Earth Sciences, 74*, 6951–6965.

Sur, D., Dutta, P., Nair, G. B., & Bhattacharya, S. K. (2000). Severe cholera outbreak following floods in a northern district of West Bengal. *Indian Journal of Medical Research, 112*, 178.

ter Braak, C.J., & Smilauer, P. (2002). CANOCO reference manual and Cano Draw for Windows user's guide: software for canonical community ordination (version 4.5).

Ward, B. B., Devol, A. H., Rich, J. J., Chang, B. X., Bulow, S. E., Naik, H., Pratihary, A., & Jayakumar, A. (2009). Denitrification as the dominant nitrogen loss process in the Arabian Sea. *Nature, 461*, 78–81.