Chapter 8

SUMMARY AND CONCLUSION

The continental shelves which make up 8% of the global ocean surface area play a significantly important role in the biogeochemistry and ecology of marine sediments. These regions inhabit a variety of microorganisms that account for more than 90% of the ocean biomass. Communities of bacteria, archaea and fungi are the primary catalysts of energy transformation, and are responsible for more than 98% of the carbon and nitrogen cycling in this ecosystem. In the last few decades several studies have shown that the role of microbes in benthic ecosystem is far more important than previously thought. And our knowledge about the diversity of microflora from the continental shelf regions of south east coast of India is still insufficient. Hence the present study was focused on the diversity and distribution of microflora in relation to the physico-chemical conditions in the shelf sediments of south western Bay of Bengal. In the present work we investigated the spatial and vertical distribution of benthic heterotrophic bacteria, actinomycetes, yeasts and fungi and their diversity in the shelf sediments. Sediment samples were collected from three discrete depths (50 m, 100 m and 200 m) covering 6 transects (18 stations). Hydrographical features and
sediment characteristics were studied besides the enumeration of the total heterotrophic bacteria (THB), actinomycetes, yeasts and fungal population through culture dependent studies. The isolates were identified based on molecular methods. Isolation of DNA, amplification of the 16S rRNA gene (for bacteria and actinomycetes) and ITS region (for yeasts and fungi), followed by restriction fragment pattern analysis (ARDRA) and sequencing of the representative strains helped to identify the isolates up to species level. The influence of physico-chemical factors on the microbial community has also been studied through statistical analysis. The salient findings of the study are as follows:

- The key hydrographical parameters such as temperature, salinity, dissolved oxygen and pH did not vary significantly between transects. Temperature noticed from the shelf regions of south western Bay of Bengal ranged between 13.10 - 27.52 °C. Salinity ranged between 34.41 - 34.95 psu. Dissolved oxygen ranged between 0.07 - 3.63 ml/l and was considerably low at 200 m (0.07 - 1.02 ml/l). Mean pH of the sediment was 7.71 ± 0.36.

- Sediment was olive green to grey in colour and the sediment types ranged from clayey silt to fine sand. The sediment was sandy at 50 and 100 m and was clayey silt at 200 m depth.

- Organic matter in sediment ranged from 0.95 - 3.76% of dry wt. and was significantly (p<0.05) higher at 200 m depth. Organic matter was found to be higher towards northern latitudes.
Average Total Carbon (TC), Total Hydrogen (TH), Total Nitrogen (TN) and Total Sulphur (TS) in the shelf sediments were 4.86 ± 2.83%, 0.72 ± 0.34%, 0.08 ± 0.04% and 0.08 ± 0.04% respectively. The concentration of TC, TH, TN and TS was much higher towards the greater depth (200 m).

Organic matter quality as measured by molar Carbon/Nitrogen (C/N) ratio was found to be 14.28 ± 5.47 (range: 8.44 – 28.38). Significantly low values (p<0.05) were sited at 200 m depth regions.

Concentration of protein, carbohydrate and lipid in sediment ranged from 0.09 to 3.08 mg/g, 0.62 to 2.55 mg/g and 4.64 to 9.83 mg/g respectively. A significant (p<0.05) difference in total protein and total carbohydrate between the inner and outer shelf was noticed. Total lipid did not showed any significant bathymetric variation though they varied significantly (p<0.05) between the northern and southern latitudes.

Biopolymeric carbon (BPC) determined as a relative measure of the amount of food potentially available for heterotrophic metabolism in the shelf sediments ranged from 4.10 to 9.64 mg C/g. BPC was found to be higher at 200 m depth.

Based on biochemical composition of the sediment, northern and southern latitudes of southeast coast of India could be clearly differentiated.
Total bacterial count in the shelf sediment ranged from $1.17 \times 10^8$ to $1.1 \times 10^9$ cells g$^{-1}$ dry wt. Total bacterial count in sediment also showed significant (p<0.05) depth wise variation and was found to be higher in 200 m depth.

ATP concentration ranged from 196.80 to 840.27 ng/g. ATP was found to be significantly (p<0.05) higher towards the northern latitudes. Total living microbial biomass (MBB) estimated from the concentration of ATP ranges from 49.20 to 210.06 µg C/g.

Total heterotrophic bacterial population (THB) in the shelf sediments of Bay of Bengal ranged from $4.87 \times 10^3$ – $2.32 \times 10^5$ CFU g$^{-1}$ dry wt. Significant (p<0.05) differences between the northern and southern latitudes reflected in the bacterial population signifies its abundance towards northern latitudes. Bacterial abundance was generally higher in clayey and clayey silt sediments.

 Majority of the bacterial isolates were identified as Gram positive bacteria (59%) and remaining 41% as Gram negative bacteria.

16S rRNA gene sequencing of representative bacterial isolates having dissimilar ARDRA patterns resulted in 37 species representing 14 genera.

The bacterial communities identified from the shelf sediments comprised mostly of *Firmicutes* (59%), *Alpha-Proteobacteria*
(3%), *Beta-Proteobacteria* (1%), *Gamma-Proteobacteria* (28%) and *Bacteroidetes* (9%). The Shannon-Wiener diversity ($H'$) ranged from 3.13 to 3.65, clearly showing the diverse nature of heterotrophic bacteria at different stations. Bacterial communities in the shallower region (50 m) were more or less similar but at deeper regions (200 m) communities showed considerable differences in their occurrence at various stations.

- Actinomycetes population in the sediment was scanty. Population ranged from 0.019 to 1.34 CFU g$^{-1}$ dry wt.

- 16S rRNA gene sequencing of representative actinomycete isolates having dissimilar ARDRA patterns resulted in 18 species representing 5 genera.

- White spore forming actinomycetes colonised the shallow depths of the shelf and outnumbered the other spore forming groups.

- Most abundant species identified was *Streptomyces radiopugnans* (12%) and *Nocardiopsis alba* (12%) followed by *Micrococcus luteus* (11%), *Brevibacterium halotolerans* (9%), *Streptomyces variabilis* (9%), *Streptomyces exfoliates* (8%) and *Streptomyces acrimycin* (8%). The Shannon-Wiener diversity ($H'$) ranged from 1.58 to 3.16, showed the diverse nature of actinobacteria at different stations.

- In shelf sediments, the fungal population ranged between 0.13 to 3.1 CFU g$^{-1}$ dry wt. The population was found to be more at 200 m and lower at 50 m depth.
ITS sequencing of representative fungal isolates having dissimilar ARDRA patterns resulted in 26 species representing 16 genera.

Majority of the fungal species isolated from the south western Bay of Bengal coast belonged to the phylum Ascomycota.

The most prevalent fungal species identified from the shelf sediments were Penicillium citrinum (9%) and Aspergillus cristatus (9%) followed by Sagenomella sp. (8%) and Fusarium solani (8%). The Shannon-Wiener diversity (H') ranged from 2.32 to 3.43 and exhibited the diverse nature of fungi inhabiting in the shelf.

Yeast population in the sediment did not showed any depth wise and latitudinal variation. The population ranged from 0 to 2.2 CFU g\(^{-1}\) dry wt.

The marine yeasts in the shelf sediments belonged to 13 species representing 5 genera.

At all the three depths (50, 100 and 200 m), non-pigmented (68%) yeasts dominated over the pigmented ones (32%).

The dominant species found in the shelf sediments were Candida parapsilosis (18%) and Candida orthopsilosis (15%) followed by Hortaea wernickii (14%), Rhodotorula mucilagenosa (13%), Debaryomyces hansenii (9%) and Meyerozyma guilliermondii (6%). The Shannon-Wiener diversity (H') ranged from 1.58 to 2.57.
Summary and Conclusion

- Statistical analysis well explained the influence of physico-chemical parameters in the distribution and abundance of microflora inhabiting the shelf sediments.

- The total number of microbial species in the study area ranged between 23 - 35 (mean 29.16 ± 3.4), species richness varied from 6.69 – 9.09 (mean 7.93 ± 4.32), species diversity in the range of 4.48 – 5.08 (mean 4.81 ± 0.16), species evenness and species dominance was averaged to 0.991± 0.001 and 0.992 ± 0.001 respectively.

- The influence of physical and biochemical properties of shelf sediment on microbial abundance was assessed by means of BIOENV. Results showed that the combination of environmental parameters which best explained the distribution patterns of culturable bacterial population was clay, silt, total protein, total lipid and total biopolymeric carbon (highest ρ value = 0.642). Similarly, the parameters that explained the distribution of fungi include salinity, clay, total organic matter, total protein and total sulphur (highest ρ value = 0.569).

- Similarly, correlation analysis showed that bacterial abundance showed significant positive correlation with the percentage of silt (r = 0.754, p< 0.001), clay (r = 0.777, p<0.001), total organic carbon (r = 0.612, p<0.001) total nitrogen (r = 0.733, p<0.001), total carbohydrate (r = 0.786, p<0.001), total protein (r = 0.692, p< 0.001), total lipid
(r = 0.721, p< 0.001) and biopolymeric carbon (r = 0.787, p<0.001).

- Bacterial diversity as measured by Shannon-Wiener index well explained the influence of salinity, total nitrogen, total organic carbon and biopolymeric carbon in its distribution. However, correlation analysis showed that the influence of environmental parameters on the diversity and distribution of culturable actinomycetes, fungi and yeasts were meagre.

- Cluster analysis delineates the microbial communities of the sampling stations into two main groups (A & B). The dichotomy includes the one that groups the microbial community of 50 m depth (group A) and another cluster that incorporates microbes of 100 m and 200 m depth (group B).

- Non-parametric MDS Ordination demarcated the shelf area into three based on the composition of microbial communities i.e., 50 m depth, 200 m and the third a mix of both 100 and 200 m stations.

- The Principal Component Analysis (PCA, normalised data) was carried out for ordination of the sample locations in relation to physico-chemical factors and microbial abundance. Principal components one and two (PC1 and PC2) together explained 92.2% of variance. Silt, sand and depth contribute significantly to the PC1 and accounted for 69.9% of variance with an eigen value of 24.8. PC 2 determined mainly by total
carbon (TC) and depth, accounted for 22.3% variance with eigen value 7.9.

- The Canonical Correspondence Analysis (CCA) was carried out to determine the role of environmental factors in the distribution of the dominant microorganisms in the shelf. The CCA axes 1 and 2 explained 50.18% and 34.2% of the species variation respectively. Analysis demonstrated the influence of concentration of organic matter and salinity on the abundance and distribution of Bacillus, influence of sediment texture and biopolymeric fractions on the Streptomyces and the effect of temperature and dissolved oxygen on the dispersal of Aspergillus.

- The biochemical variables such as concentration of proteins and carbohydrates were selected to determine the sediment trophic status. Protein to carbohydrate ratio in the shelf sediments grouped the study area into oligotrophic inner shelf (protein < 1.5 mg/g; PRT: CHO < 1) and eutrophic outer shelf (protein >1.5 mg/g; PRT: CHO > 1).

- The altered nutritional status indicates the abundance of microflora at deeper depths and confirms that the number of microorganisms in the shelf sediments varies with its trophic status.

The present study provides an account on the occurrence and diversity of marine microbes (culturable heterotrophic bacteria, actinomycetes, yeasts and fungi) in the shelf sediments of Bay of
Bengal. In terms of marine heterotrophic bacterial diversity, *Firmicutes* was found to be dominant in Bay of Bengal. Actinobacteria though considered as separate group, added the eubacterial abundance in the study area. Spore forming actinobacteria was higher compared to the nonspore formers. In the case of fungal communities, phylum ascomycota dominated in the shelf sediments of Bay of Bengal.

Further, the study provides an understanding on the abundance and diversity of marine microbes in the shelf sediments in relation to the prevailing physico-chemical parameters. The main factors influencing the distribution of microflora in the shelf sediments was the sediment grain size and organic matter concentration as well as composition. A hike in microbial abundance towards greater depths and to the northern latitudes of the study area substantiates this statement.

The result obtained from the present study on species abundance, distribution and diversity provided a benchmark data for the microbial wealth in the continental shelf sediments of Bay of Bengal. Majority of species identified from the study area proposed that, the continental shelf of Bay of Bengal sustains copious microbial population with high metabolic versatility. These different groups could act synergistically to degrade most of the autochthonous or allochthonous organic matter in the shelf sediments. In future, the isolates can be screened for bioactive compounds and the potential strains can be used for biotechnological and industrial applications.